

**EFFECT OF FEEDING DIFFERENT SOURCES OF ENERGY
WITH FUNCTIONAL NUTRIENT SUPPLEMENTS DURING
PREPARTUM PERIOD ON POSTPARTUM NUTRITIONAL
AND METABOLIC STATUS OF COWS**



**THESIS SUBMITTED TO THE
ICAR-NATIONAL DAIRY RESEARCH INSTITUTE
(DEEMED UNIVERSITY)
KARNAL (HARYANA)**

DOCTOR OF PHILOSOPHY

IN

ANIMAL NUTRITION

BY

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M. V. Sc.

**DAIRY PRODUCTION SECTION
SOUTHERN REGIONAL STATION
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ADUGODI, BENGALURU- 560 030, INDIA**

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**IN PARTIAL FULFILMENT OF THE REQUIREMENTS
FOR THE AWARD OF THE DEGREE OF**


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IN

ANIMAL NUTRITION

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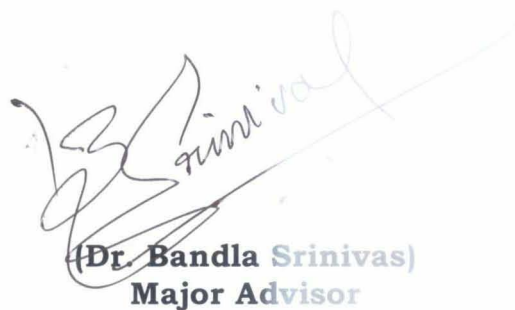
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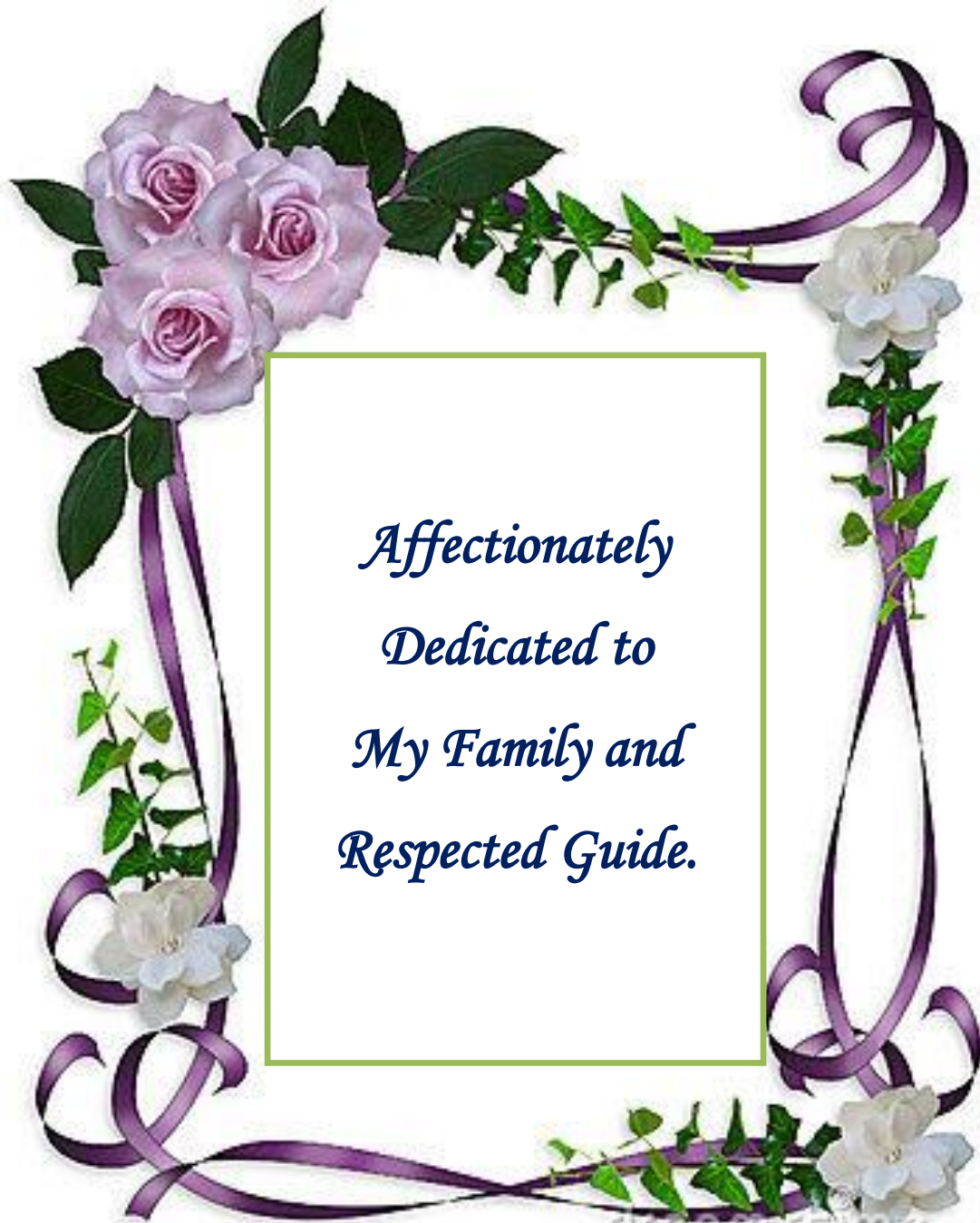
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This is to certify that the thesis entitled, **“EFFECT OF FEDING DIFFERENT SOURCES OF ENERGY WITH FUNCTIONAL NUTRIENT SUPPLEMENTS DURING PREPARTUM PERIOD ON POSTPARTUM NUTRITIONAL AND METABOLIC STATUS OF COWS”**, submitted by **Dr. KAMBHAM SUDHA RANI**, towards the partial fulfilment for the award of the degree of **DOCTOR OF PHILOSOPHY** in **ANIMAL NUTRITION** of the **ICAR-National Dairy Research Institute (Deemed University)**, Karnal (Haryana), India, is a bonafide research work carried out by her under my guidance, and no part of the thesis has been submitted for any other degree or diploma.

Date: 03/04/2021


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*Affectionately
Dedicated to
My Family and
Respected Guide.*

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K. Sudha Rani

ABSTRACT

The study was conducted to evaluate the effect of feeding glycerol (T1), jaggery (T2) or ground maize grain (T3) as 10% additional energy of different kind to prepartum Holstein Friesian crossbred (HFX) or Deoni cows without or with functional nutrient supplement (FNS) 30-days prior to calving and discontinued after calving to evaluate the calving day and postpartum performance of cows till 30-days of post-calving. *In vitro* fermentation kinetics of 3 kinds of energy was comparable with FNS.

In vivo experiments were carried under 4×2×2 factorial design with random distribution of 16 cows of each breed into 4 equal groups based on parity, and two cows in each group fed 1% FNS with energy. Digestible DM and TCHO intake (g/kg W^{0.75}) was higher (P< 0.001) in T1 with FNS than T2 and CG. Digestible CP (g/kg W^{0.75}) was high (P< 0.001) in TGs than CG. Energy +FNS had a significant effect on CC (P<0.05), NDF (P = 0.052) and ADF (P< 0.01). Energy × Breed ×FNS interaction effect on CC, HC and NFC intake in T1 was (P< 0.001) higher than CG, T2 and T3.

Colostrum quality was improved by 3% (P<0.001) on the day of calving and calf birth weight was 12% and 5% (P< 0.001) more in HFX and Deoni cows, respectively with FNS but FNS had no effect without energy fortification, thus both were complimentary to each other. Time taken for fetal membrane expulsion in Deoni cows was 0h38min lesser than HFX cows (P< 0.001) and Energy ×FNS (P = 0.08) interaction hastened the process.

The cow's appetite in postpartum was protected as evident from increased population (N=32) mean consumption of any nutrient was 2.4 times higher than the prepartum cows. Mean allantoin excretion in urine was 4.67 mM/L in prepartum than 4.58 mM/L in postpartum. Microbial protein (MBP) production was 35% and 43%, respectively higher in HFX cows than Deoni in prepartum and postpartum, respectively and energy with FNS had more impact on MBP. Mean residual CP and ME intake was +22% and -15%, respectively than recommended intake in postpartum however, up to 40% of energy during lactation are compensated by amino acids. Prepartum cow's protein requirements met (-0.2% in TGs & -6% in CG) and energy was even excess (+11% in TGs & -1% in CG) than requirement in TGs.

RBC and haemoglobin increased (P< 0.01) from -30 to +30 days of calving which was conspicuous with FNS (P< 0.001). ALT and ALT: AST were significantly different within the periods due to interaction of Energy × FNS × Breed. Serum NEFA concentration was less than the threshold levels of prepartum (<0.5mmol/L) and postpartum (<1.0mmol/L). BHBA concentration during the prepartum period was below 800mmol/L. Energy with FNS reduced BHBA concentration by 9.4%. Serum Cu²⁺ was 12.5% higher (P< 0.001) with FNS on day 14 after calving and serum Zn²⁺ was doubled 14 days prior to calving. There was depression in serum Ca²⁺, P³⁻, Mg²⁺, Mn²⁺, Cu²⁺ and Zn²⁺ on the day of calving but reversed to normal after calving which was indication to healthy cows to meet colostrum needs.

Study concluded that 10% energy fortification as glycerol second choice ground maize grain with 1% FNS during prepartum had beneficial effect on calf and dam parameters. Below threshold serum ketone bodies and 2.4 times more consumption of nutrients indicated no negative energy balance and protected appetite of cows in postpartum till 30 days of calving.

सार

यह अध्ययन पूर्वप्रसव होल्सटीन फ्रिसियन क्रॉस ब्रीड (एच एफ एक्स) या दियोनी गायों को जिन्हें कार्यात्मक पोषक तत्व पूरक के बिना या साथ, 30 दिन पूर्वप्रसव, ग्लिसरोल (टी 1), गुड (टी 2) या पीसी मक्का (टी 3) 10% विभिन्न प्रकार की अतिरिक्त ऊर्जा के रूप में खिलाने का प्रभाव, प्रसव के दिन और 30 दिन प्रसवोत्तर प्रदर्शन का मूल्यांकन करने के लिए किया गया। तीन प्रकार की ऊर्जा की कृत्रिम परिवेशीय किण्वन काइनेटिक, कार्यात्मक पोषक तत्व पूरक के साथ तुलनीय थी।

इन विद्यो प्रयोग यादृच्छिक वितरण के 4X2X2 भाज्य-संबंधी (फैक्टोरियल) डिजाइन के तहत प्रत्येक नस्ल की 16 गायों की चार समान समूहों में समानता के आधार पर किया गया, और प्रत्येक समूह में दो गायों को ऊर्जा के साथ 1% कार्यात्मक पोषक तत्व पूरक खिलाया गया। पाचन योग्य डीएम और टीसीएचओ का सेवन (ग्रा/किग्रा वजन^{0.75}) टी2 और सीजी की तुलना में कार्यात्मक पोषक तत्व पूरक के साथ टी1 में अधिक (पी <0.001) था। सीजी की तुलना में टीजीयों में पाचक सीपी (ग्रा/किग्रा वजन^{0.75}) उच्च (पी <0.001) था। ऊर्जा + कार्यात्मक पोषक तत्व पूरक का सीसी (पी <0.05), एनडीएफ (पी =0.052) और एडीएफ (पी <0.01) पर महत्वपूर्ण प्रभाव पड़ा। टी1 में सीसी, एचसी और एनएफसी सेवन पर ऊर्जा X नस्ल X कार्यात्मक पोषक तत्व पूरक परस्पर क्रिया प्रभाव सीजी, टी2 और टी 3 की तुलना में अधिक (पी <0.001) था।

कोलोस्ट्रम की गुणवत्ता में प्रसव के दिन 3% (पी <0.001) में सुधार हुआ और एच एफ एक्स और दियोनी गायों में क्रमशः कार्यात्मक पोषक तत्व पूरक के साथ बछड़े के जन्म का वजन 12% और 5% (पी <0.001) अधिक था, लेकिन कार्यात्मक पोषक तत्व पूरक अतिरिक्त ऊर्जा के बिना कोई प्रभाव नहीं था। दियोनी गायों में भ्रूण निष्कासन के लिए लिया गया समय एच एफ एक्स गाय (पी <0.001) से 38 मिनट कम था और ऊर्जा X कार्यात्मक पोषक तत्व पूरक (पी=0.08) परस्पर क्रिया ने क्रिया को टीजी में जरूरत से ज्यादा बढ़ाया।

प्रसवोत्तर में गायों की भूख बड़ी हुई थी जो कि औसतन किसी भी पोषक तत्वों की खपत की बढ़ी हुई आबादी (एन=32) से स्पष्ट था, और यह पूर्वप्रसव गायों की तुलना में 2.4 गुना अधिक थी। मूत्र में एलनटाइन का उत्सर्जन प्रसवोत्तर गायों में 4.58 मि.मोलर/लीटर की तुलना में पूर्वप्रसव गायों में 4.67 मि.मोलर/लीटर था। जीवाणु प्रोटीन का उत्पादन (एम बी पी) क्रमशः पूर्वप्रसव और प्रसवोत्तर में क्रमशः एच एफ एक्स और दियोनी गायों में 35% और 43% अधिक हुआ, और कार्यात्मक पोषक तत्व पूरक के साथ ऊर्जा का एम बी पी पर अधिक प्रभाव पड़ा। औसत अवशिष्ट सीपी और एम इ सेवन, प्रसवोत्तर में अनुशंसित सेवन की तुलना में क्रमशः 22% और 15% था, लेक्टेसन के दौरान 40% तक ऊर्जा की कमी पूर्वी अमीनो एसिड द्वारा हुई। पूर्वप्रसव गायों की प्रोटीन की जरूरतें पूरी हुई (-0.2% टीडी में और 6% सीजी में) और ऊर्जा भी अधिक (टीजी में +11% और सीजी में -1%) रही।

आरबीसी और हीमोग्लोबिन में वृद्धि (पी <0.01) प्रसव के -30 से +30 दिनों में हुई, जो कार्यात्मक पोषक तत्व पूरक (पी <0.001) के साथ सुस्पष्ट थी। ए एल टी और ए एल टी : ए एस टी, ऊर्जा X कार्यात्मक पोषक तत्व पूरक X नस्ल की अवधि के परस्पर क्रिया के कारण काफी भिन्न अनुपात में थे। सीरम गैर एस्टेरिफाइड फैटी एसिड सांद्रता पूर्वप्रसव (<0.5 मि. मोलर/ लीटर) और प्रसवोत्तर (<1.0 मि.मोलर/ लीटर) की दहलीज स्तर से कम थी। प्रसवोत्तर अवधि के दौरान बीएचबीए की सांद्रता 800 मि.मोलर/ लीटर से कम थी। कार्यात्मक पोषक तत्व पूरक के साथ ऊर्जा ने बी एच बी ए को सांद्रता में 9.4% तक कम कर दिया। प्रसव के 14 दिन बाद सिरम Cu^{+2} कार्यात्मक पोषक तत्व पूरक के साथ 12.5% अधिक (पी < 0.001) था और सिरम Zn^{+2} प्रसव के 14 दिन पूर्व दोगुना हुआ। सीरम Ca^{2+} , P^{3-} , Mg^{2+} , Mn^{2+} , Cu^{2+} और Zn^{2+} में प्रसव के दिन कमी आई लेकिन यह प्रसव उपरांत साधारण हो गया जो दर्शाता है कि स्वस्थ गाय कोलोस्ट्रम की जरूरत पूरी कर पाई है।

अध्ययन में निष्कर्ष निकाला गया कि पूर्व प्रसव दौरान ग्लिसरोल के रूप में 10% ऊर्जा फोर्टिफिकेशन के बाद 1% कार्यात्मक पोषक तत्व पूरक के साथ पीसी मक्का का सेवन बछड़े और गाय के मापदंडों पर लाभकारी प्रभाव पड़ा। सिरम कीटोन बॉडीज की दहलीज दर से नीचे और पोषक तत्वों का 2.4 गुना ज्यादा सेवन नकारात्मक ऊर्जा और गायों की 30 दिन प्रसवोत्तर संरक्षित भूख के संतुलन को दर्शाता है।

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LIST OF ABBREVIATIONS/ ACRONYMS

°C	: Degree Celsius
µeq/L	: Micro equivalents per liter
µg	: Micro gram
µg	: Microgram
A. I	: Artificial Insemination
ADF	: Acid detergent fibre
ADG	: Average daily gain
ADL	: Acid detergent lignin
AIA	: Acid insoluble ash
ALT	: Alanine transaminase
ANOVA	: Analysis of variance
AOAC	: Association of official analytical chemist
ARC	: Agricultural research council
AST	: Aspartate transaminase
ATP	: Adenosine tri phosphate
BCG	: Bromocresol green
BCS	: Body condition score
BHBA	: β-hydroxy butyric acid
BUN	: Blood urea nitrogen
BW	: Body weight
Ca	: Calcium
CB	: Crossbred
CC	: Cell content
CE	: Cholesterol esterase
CG	: Control group
Cl	: Chloride
CP	: Crude protein
Cr ₂ O ₃	: Chromic oxide
Cr	: Chromium
CS	: Concentrate supplement
CSIRO	: Commonwealth scientific and industrial research organisation
CTAB	: Cetyltrimethylammonium ammonium bromide

Cu	: Copper
CV	: Coefficient of variation
CWC	: Cell wall constituents
CWCHO	: Cell wall carbohydrates
DB	: Deoni bred
DCAD	: Dietary cation-anion difference
DCP	: Digestible crude protein
DE	: Digestible energy
DHA	: Docosahexaenoic acid
dL	: Deci liter
DM	: Dry matter
DMI	: Dry matter intake
DMRT	: Duncan multiple range test
DOMI	: Digestible organic matter intake
DOMR	: Digestible organic matter in rumen
DW	: Distilled water
EDTA	: Ethylene diamino tetra acetic acid
EE	: Ether extract
EEGF	: Ether extraction glass flask
ELISA	: Enzyme-linked immuno sorbent assay
EPA	: Eicosapentaenoic acid
ER	: Energy retention
FA	: Fatty acid
FAO	: Food and agricultural organization
FM straw	: Finger millet straw
FNS	: Functional nutrient supplement
g/dL	: Gram per deci litre
g/L	: Gram per litre
GE	: Gross energy
g	: Gram
GGT	: Gamma glutamyl transferase
GI	: Gastro intestinal
GOT	: Glutamic-oxaloacetic transaminase
hr	: Hour

H ₀	: Null hypothesis
Hb	: Haemoglobin
HC	: Hemicellulose
HF	: Holstein Friesian
HI	: Heat increment
HPLC	: High performance liquid chromatography
ICAR	: Indian council of agricultural research
Ig	: Immunoglobulin
I	: Iodine
IU	: International units
Kcal	: Kilocalorie
Kg	: Kilo gram
KJ	: Kilo joule
KMF	: Karnataka milk federation
K	: Potassium
L	: Litre
LW	: Live weight
LYP	: Lymphocytes
M	: Molar
mA	: Milli ampere
MBN	: Microbial nitrogen
MBP	: Microbial protein
MBW	: Metabolic body weight
M Cal	: Megacalorie
MCH	: Mean corpuscular haemoglobin
MCHC	: Mean corpuscular haemoglobin concentration
MCV	: Mean corpuscular volume
MCV	: Mean corpuscular volume
ME	: Metabolizable energy
meq/L	: Milli equalent/liter
mg	: Milligram
mg/dL	: Milli gram per deci liter
MGF	: Mixed green fodder
Mg	: Magnesium

mg	:	Milli gram
MJ	:	Megajoule
ml	:	Milliliter
mMol	:	Millimole
Mn	:	Manganese
MP	:	Microbial protein
Na	:	Sodium
NDF	:	Neutral detergent fibre
NE	:	Net energy
NEB	:	Negative energy balance
NEFA	:	Non esterified fatty acids
NE _L	:	Net energy for lactation
NE _m	:	Net energy for maintenance
NE _P	:	Net energy for pregnancy
NFC	:	Non fibrous carbohydrate
ng	:	Nanogram
nm	:	Nanometer
NPN	:	Non protein nitrogen
NR	:	Nutritive ratio
NRC	:	National research council
OD	:	Optical density
OM	:	Organic matter
P: E	:	Protein energy ratio
PCV	:	Packed cell volume
PD	:	Purine derivatives
PDC	:	Purine derivative to creatinine
PDI	:	Purine derivative index
pg	:	Pico gram
PLT	:	Platelet
P	:	Phosphorous
ppm	:	Parts per million
PTH	:	Para thyroid hormone
RBC	:	Red blood corpuscles
REI _{FSF}	:	Residual energy intake based on feeding standards

RFQ	: Relative feed quality
RPI _{FSF}	: Residual protein intake based on feeding standards
rpm	: Rotations per minute
SEM	: Standard error mean
Se	: Selenium
SGOT	: Serum glutamate oxaloacetate transaminase
SGPT	: Serum glutamate pyruvate transaminase
SPSS	: Software package for social science
S	: Sulphur
T _{1/2}	: Half rate of fermentation
TA	: Total ash
TCHO	: Total carbohydrate
TDN	: Total digestible nutrients
TEC	: Total erythrocyte count
TG	: Treatment group
TMR	: Total mixed ration
TMS	: Total mixed substrate
TP	: Total protein
UV	: Ultraviolet visible
v/v	: Volume by volume
W/V	: Weight by volume
W ^{0.75}	: Metabolic body weight
WBC	: White blood corpuscles

Introduction

1.0 INTRODUCTION

Dairy animals enter into cyclical life cycle comprising of oestrus cycle, conception, pregnancy, dry period and lactation from vertical growth phase from birth to sexual maturity. Lactation and conceptus growth may take place parallel to large extent with an optimum gap of 2 months from initiation of galactopoiesis. At the field level, nutrition management of lactating cattle is carried with precision attention than the young and dry cows. Nutritional management of dry cattle with advanced pregnancy though not taken as serious as lactating cows, livestock owners follow the advice of animal health services. Any negligence in the nutritional management of dry pregnant cow has long-lasting consequences involving the next production cycle of the dam and life-time performance of offspring. Required nutrient amounts for the dry pregnant cow are the sum of maintenance, pregnancy and reserve replenishment needs with additional requirements for growth during the first two pregnancies (Van Saun and Sniffen, 1996). Dry off period is divided into two phases such as far off dry period i.e. from -60 days to -21 days followed by close-up dry period i.e. from -21 days to calving day (zero-day).

Implementing an adequate nutrition program in pregnancy in last trimester in general, and close-up period in particular, have a cascade of benefits including metabolic health, immunity, milk production, reproductive cyclicality, neonatal incidences, calf growth, heifer age at maturity etc. Apart from higher growth of the foetus during the prepartum period, the physiological phase is also characterized by several changes in endocrine and immune system in preparation for colostrogenesis, parturition and lactogenesis. The highest growth of the foetus can observe in the last trimester of pregnancy that is approximately 70 % of calf birth weight wherein again 30% of foetus growth takes place in last month that is 9th month.

Foetus demand for energy is higher whereas protein and minerals demand is moderate. The foetus needs energy in the form of glucose than any other metabolite. As the close-up period advances, an inclination to dry matter intake (DMI) diminishes because of increased hepatic fuel oxidation which sends signals

to the hypothalamus to activate the satiety centre and translates into reduced DMI (Forbes, 1992; Allen *et al.*, 2005). The trade-off in DMI during the prepartum period, particularly 5 to 7 d before calving would lead to negative energy balance (NEB) which is the predisposing phase for many postpartum metabolic challenges (Dyk, 1996). Many research workers suggested an increased density of energy in the diet to balance the traded-off DMI (Trubenbach *et al.*, 2019). The increasing energy density of the total mixed ration (TMR) is likely to increase energy utilization efficiency (McLeod and Baldwin, 2000). Although the decline in DMI in pregnant cows as the close-up period advances is natural, the different energy sources have been tried to improve energy density in the diet and delay the setting of NEB (Goff, 2000; Ingvarlsen and Andersen, 2000; Hoedemaker *et al.*, 2004; Drackley *et al.*, 2005).

Apart from the decline in DMI, endocrinal changes during the prepartum period also affect nutrient partitioning. Nutrition partitioning during pregnancy involves homeorhetic controls arising from the conceptus thus, assures the growth of the fetus and fetal membranes. The major changes in the partitioning of available nutrients take place in maternal tissues, including coordinated shifts in the mammary, liver and adipose tissue metabolism that do not jeopardize maternal homeostasis or fetal and neonatal health (Bauman and Bruce currie, 1980; Baumgard *et al.*, 2017). The changes in homeostasis and homeorhesis regulations to various metabolic adaptations in peripartum cows are also induced stress (Tanritanir *et al.*, 2009).

The peripartum dairy cow experiences a state of reduced liver function coupled with increased inflammation and oxidative stress (Bionaz *et al.*, 2007; Trevisi *et al.*, 2012). Liver function as an indicator of gut health can be assessed by various enzymes e.g., gamma-glutamyl transferase (GGT), aspartate aminotransferase (AST) and alanine aminotransferase (ALT). Various nutritional limitations, metabolic impediments, fatty liver infiltration and liver cells degeneration during the prepartum period result in liver cell membrane damage and destruct cytoplasmic enzyme release thus, elevated concentration of liver enzymes can be seen in the blood (Lubojacka *et al.*, 2005). Serum levels of cholesterol, creatinine, blood urea nitrogen (BUN), triglycerides, ketone bodies etc., also reflect the gut health during the prepartum period.

Supplementation of various minerals to a pregnant cow in dry off period is one way to counter the oxidative stress. Macro mineral supplementation to peripartum cows needs a critical understanding of their regulation mechanism (Wilde, 2006). Trace mineral supplementation e.g., Cu^{+2} , Mn^{+2} , Zn^{+2} , Se^{+2} etc., during pre-partum period has a greater influence on postpartum colostrum yield, quality and immune competence of the dam. According to Bertoni *et al.* (2006), ω -3 fatty acid (Linolenic acid) feeding can reduce inflammatory consequences after calving. Despite numerous nutritional strategies adopted in past (Luc *et al.*, 2009; Bertoni *et al.*, 2006; Tienken *et al.*, 2015), still prepartum cow nutrition management to minimize the onset of metabolic diseases and impaired performance remain a challenge. Investigation of hemogram along with biochemical profiling helps to predict prepartum cow susceptibility to production diseases (Kevin and Ellen, 2012).

Consequences of different nutritional, physiological and metabolic precedence of the prepartum period have a spillover effect on postpartum and puerperium. The health and reproductive complications after parturition are the result of immune suppression. Supplementation of ω -3 fatty acids can have a beneficial effect on reproductive and immune health of dairy cattle (Moallem *et al.*, 2013). The homeostasis between helper T1 (Th1) and T2 (Th2) cells is important for immune regulation during pregnancy. The long-chain omega-3 fatty acids EPA (Eicosapentaenoic acid) and DHA (Docosahexaenoic acid) significantly benefit diverse inflammatory and autoimmune conditions without any specific Th1/Th2 effect (Kidd, 2003).

The said issues during prepartum also have a cumulative effect on the calf birth weight (Khan *et al.*, 2004) and colostrum quality (Barrington and Parish, 2001), postpartum metabolic disorders e.g., retention of placenta, milk fever, ketosis etc., and conception issues during puerperium (Zeng *et al.*, 2017). In all livestock species, including cow, offspring born at above-average body weight (BW) have a greater chance of survival rate compared to those born at below-average BW. The cows maintained on crude protein (CP) deficient diets in the prepartum period produce calves exhibiting weak calf syndrome (Bull *et al.*, 1979). This situation is further attenuated by the continuing decrease in DMI during postpartum and increased demand for nutrients for milk. As a result,

cows increasingly depended on body reserves. They lose their body condition score (BCS) and worsening NEB. The extent of NEB in the close-up period may also inhibit timing of 1st ovulation, return to cyclicity and oocyte quality (Zeng *et al.*, 2017).

Strategic nutritional management during the prepartum period not only is unavoidable but also essential for avoiding huge economic losses thereupon on the farm. The economic losses estimated due to milk fever soon after parturition is Rs. 1,068/cow and Rs. 665/buffalo and the prevalence rate in the population are 14 and 12%, respectively (Thirunavukkarasu *et al.*, 2010). Similarly, the cow which lost BW due to decreased DMI and NEB with a BCS ≤ 3.0 had a lower probability of conceiving at first insemination with odds ratio 0.64 ($P < 0.05$). Under such conditions, a cow requires additional expenditure on reproductive treatment (\$55.40) and other management (\$567.00) than cows that conceived at first insemination (Kim and Jeong, 2019). Depending upon milk price and milk yield, each 1% increase or decrease in pregnancy rate results in the gain or loss of approximately \$12 to \$25/cow/year (Overton, 2001). Metritis and endometritis, unfortunately, is very common disease complex observed in postparturient cattle, with a median lactational incidence risk of approximately 10 % but, with many herds in the 20% to 30 % range (Kelton *et al.*, 1998). According to Overton (2008), the total cost of metritis has been estimated to approximately \$358/diagnosed case, despite aggressive antibiotic therapy. The magnitude of this estimate may surprise, but the reality is that metritis is an expensive postpartum disease. The overall cost per case of clinical mastitis in the first 30 days in milking is \$444 and 71% of the costs are indirect costs accounting to \$316 (Rollin *et al.*, 2015). Direct costs included diagnostics (\$10), therapeutics (\$36), non-saleable milk (\$25), veterinary service (\$4), labor (\$21), and death loss (\$32). Indirect costs included future milk production loss (\$125), premature culling and replacement loss (\$182), and future reproductive loss (\$9). Ali (2011) accounted for an average per day loss suffered by the farmer due to delayed conception in heifers was Rs.292=81 with the average number of days delayed of 199. Mean services per conception in heifers were 3.81 and per day loss due to delayed conception per heifer was Rs.212=52. In the case of lactating animals, the average per day loss per farmer due to delayed conception was Rs.455=34 with the average numbers of days delayed of 214. Mean services per conception

in lactating animals were 5.25 and per day loss per lactating animal due to delayed conception was Rs.261=12.

In short, prepartum nutrition management should take care; decreased DMI, escalating NEB, disturbances in balance between Th1 and Th2 and Immune suppression. These measures are necessary to curtail the repercussion on the postpartum issues such as; metabolic disorders & susceptibility to diseases, escalating DMI & NEB, gut health disturbances, milk yield and quality. The huge economical loss estimate also warrants such attempts to functional corrections of puerperium such as uterine involution, postpartum temporary infertility, open period, estrus cyclicity and conception. The present research work was undertaken in the above backdrop with the following aim and objectives.

1.1 Aim

Identifying the effective energy source to cow during the prepartum period and developing integrated nutritional management to minimize postpartum metabolic complications.

1.2 Objectives

1. To evaluate the effect of different sources of isocaloric energy fed during the prepartum period on postpartum nutrient utilization and energy balance in cows.
2. To study the effect of functional nutrient supplements on energy efficiency and metabolic status in postpartum cows.
3. To study the prepartum nutritional management on rumen microbial protein, gut health and colostrum quality during the postpartum period

1.3 Hypothesis (H0)

Prepartum nutritional management is independent of postpartum nutrition and metabolic consequences in a cow.

Review of Literature

2.0 REVIEW OF LITERATURE

The conventional belief that the advance pregnancy or 2 months of the dry period before parturition is a rest period between two lactations. Such a belief reflected in poor to marginal feeding program during the period with least attention to declining cow appetite. Concepts like challenge feeding, *ad libitum* feeding etc., could not yield better results in controlling the postpartum metabolic and lactation issues. Thus, the advancement of cow sciences leads to contemporary belief that the prepartum period as a critical transitional period where the cow is set for lactation cycle that is complimentary to the gestation and birth of the calf. The review of literature is presented in two main parts such as cow nutrition during the pre and postpartum periods, metabolic and physiological issues, health etc., besides puerperium. The compiled review of the literature is given schematically (**Fig 2.1**).

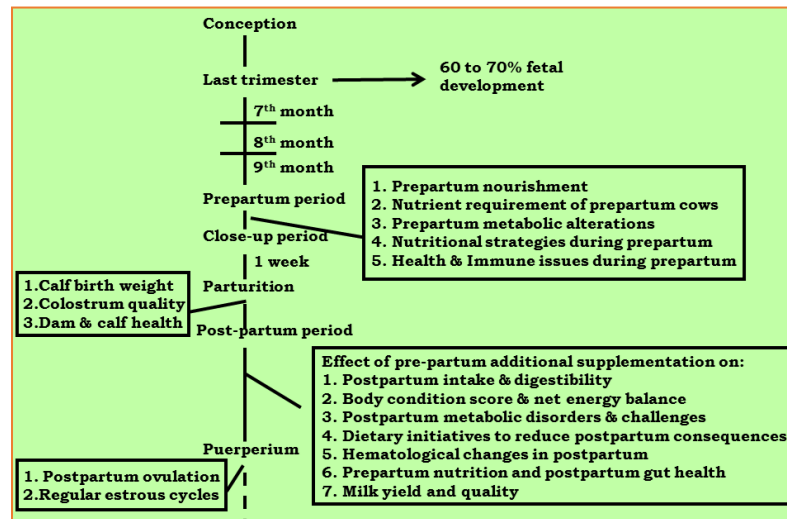


Fig. 2.1: Schematic map of review of literature

2.1 Prepartum Nourishment

Nutrition plays a balancing role between requirements and metabolic alterations during the prepartum period. During pregnancy, the fetus has priority over the dam for nearly all nutrients, with very few exceptions like vitamin A in the cow (McDonald, 2002), meaning that the dam sacrifices her own body to support fetal

growth. This sacrifice is likely to leave the dam malnourished, with visible effects during the postpartum. Hence, pregnancy is a nutritionally crucial period for the maintenance of cow, growth of the fetus and managing the gestational process to reduce peripartum deficiencies.

2.1.1 Nutrition

The prepartum nutrition should ensure that the dam is sufficiently nourished to express her genetic potential during lactation and quickly regain immunity after parturition. Pregnancy needs of water have little more importance over the basic physiological and metabolic functions right from the pre-implantation period because histotopic mode is special importance in providing nutrients to the conceptus (Martins *et al.*, 2018). The nutrient requirements of prepartum dairy cows are higher than maintenance (Van Saun and Sniffen, 1996). It was almost equivalent to the energy and protein required for the cow to produce 4.5 to 7.5 kg milk with 4% fat per day (NRC, 1988). Requirements for pregnancy signify nutrients necessary to support the growth and maintenance of foetus, placenta, uterus, mammary gland. Conceptus maintenance expenditure is a significant portion of the total pregnancy requirement because of low efficiencies of metabolizable energy (ME; 12.5%) and protein (50%) utilization in the growing fetus (Ferrell *et al.*, 1976). The exact requirements of the fetus nutrients depend on the birth weight, fetal membranes, and growth rate of the fetus.

2.1.2 Intake

DMI plays an important role in establishing the amount of nutrients required to the animal for health and production. In most of the situations, increase in energy requirements leads to increased feed intake (Woods *et al.*, 1998). This is, however, exceptional in the prepartum dairy cow where DMI normally diminishes to some degree (Bertics *et al.*, 1992). In most of the situations drive to eat feed increases with energy requirements (Woods *et al.*, 1998). This is however exceptional in the prepartum dairy cow. Average DMI for the prepartum transition period has been reported to range between 1.7 and 2.0% (Hayirli *et al.*, 1999) or, 2.25% of BW (NRC 2001; ICAR 2013). The DMI decreased 25% in first or second parity and 52% in the second parity during the final 14 days of gestation of the cow

(Marquardt *et al.*, 1977). The average DMI for the final 21 d before parturition was 1.88 and 1.69% of BW for cows and heifers, respectively in comparison to 2 to 2.5% by a normal cow. The decline in DMI is more gradual for cows than heifers. Marquardt *et al.* (1977) reported that DMI reduced to 25% and 52% in first and second parity, respectively during the final 14 days of gestation. Robinson and Garret (1999) reported that a decline in DMI was 30-35% in the prepartum period. Grant and Albright (1995), Grummer *et al.* (2004), Hayirli *et al.* (2002) were also observed 32% decreased DMI during the last 3 weeks of gestation among which 89% decline observed during last 5-7 days before calving (**Fig 2.2**). DMI and energy balance during late gestation leads to high or low body condition score (BCS) in the periparturient period and alter the bovine immune mechanism (Sordillo, 2016). DMI is the major element influencing water intake in prepartum cows. For each kg, DMI cow will consume 3.1 to 5.2 and 5.2 to 15.6 kg of water/day at the temperature of 4.4 to 26.7 °C and 26.7 to 37.8 °C, respectively. Pregnancy increases the daily requirement of water intake by 35-57% for cow ranging the BW 350 to 725 kg (Winchester and Morris,1956).

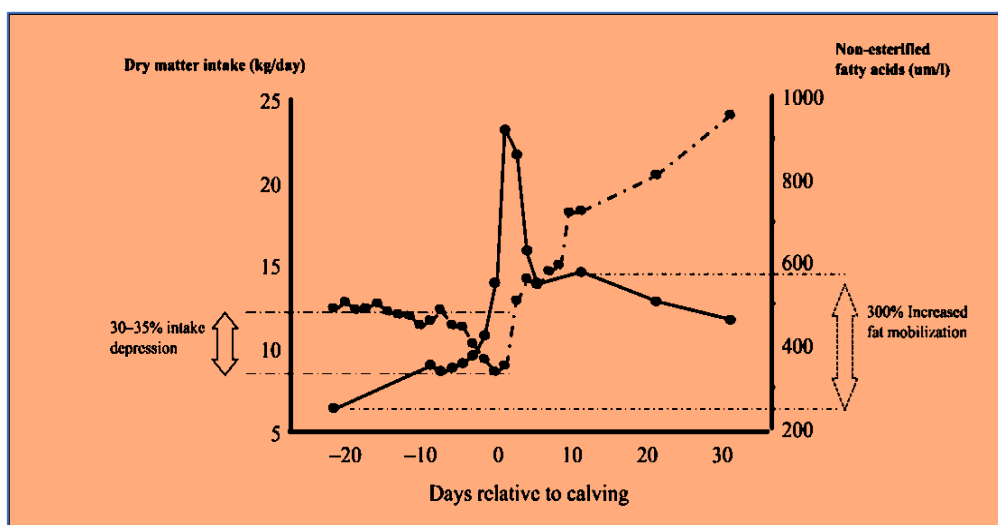


Fig. 2.2: DMI Vs. fat mobilization in peripartal cows (Grummer *et al.*, 1995)

Although fetal growth and prepartum DMI have been described as exponential functions, the physical capacity of the rumen is not the cause of depression in prepartum DMI. Park *et al.* (2011) reported that the expandability of the abdominal cavity is sufficient to accommodate the developing fetus without

unduly restricting rumen capacity. The difference in decline DMI differs from one to other animals of the same breed depending on metabolic body size, BCS, diet, quality of the ration, management practices, days to parturition and parity. Ingvarlsen *et al.*(2003) reviewed that fat mass, blood metabolites, corticosteroids, leptin, insulin, gut peptides, cytokines, neuropeptides, group changes, bunk space, overcrowding, diet changes and water quality may be the critical factors affecting prepartum DMI. Changes in blood concentrations of steroid hormones in the prepartum are more consistent with changes in feed intake. According to Grummer *et al.* (1990), the surge in blood estrogen might be responsible for the depression in feed intake before parturition.

2.1.3 Breed

In most of the breed's behaviour of DMI is more or less similar and any difference observed in the quantity consumed is because of body size and phenotypic plasticity of the digestive system (Singh and Srinivas, 2020). Since the physiological process of pregnancy is the same in all mammalian species to great extent, the animal DMI decreases in peripartal period irrespective of breed advantageous or limitations (**Fig 2.3**).

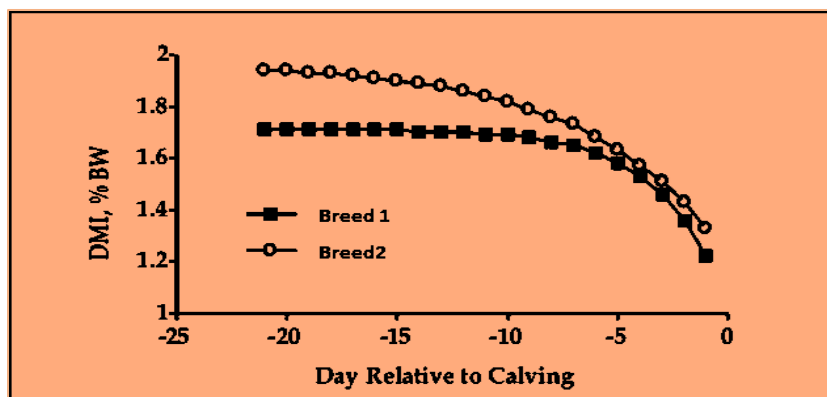


Fig 2.3: Breed effect of DMI in the prepartum period (Grummer *et al.*, 1994)

The decline in feed intake at peripartal period is not unique to the bovine and dairy cow but also common in other species during peripartal period (Grummer *et al.*, 2004). French (2002) reported that breed affects the DMI, where DMI at 21 days prepartum is approximately 2.35 % of BW for Jersey breed

compared to 1.98 % for Holstein Frisian (HF) cow. The DMI for Jersey cow ranged from 9 to 12 kg/day during the final 3 weeks of gestation which was 2.2% of BW. But some of the metabolic consequences such as the occurrence of milk fever were significantly associated with the breed, parity and level of milk production and highest incidences of milk fever i.e., 15%, occur in Jersey cow compared to just 5% in HF after 3rd lactation (Chiwome *et al.*, 2017). Goff (2000) attributed to this difference could be due to reduced concentration of intestinal receptors for calcitriol in Jersey cow when compared with HF cows. Chauhan *et al.* (1994) reported that crossbred or exotic breeds were resistant to milk fever and retention of the placenta by 71.4 and 63.3 %, respectively whereas, indigenous breeds were resistant 94.1%, 74.7%. More than a breed difference, the legitimate reason behind resistant to milk fever and retention of placenta in crossbred and indigenous breeds is the difference in the level of production and calf birth weight.

2.2 Nutrient requirement of prepartum cows

Protein, energy, macro and trace minerals, vitamins and ω -3 fatty acids requirement in prepartum cows for cow it-self and growing fetus are discussed in the light of pregnancy and reproductive health. Total nutrient requirements in the last month of pregnancy are about 75% greater than non-pregnant animals of the same weight. The requirements of different nutrients are summarized in

Table 2.1.

2.2.1 Protein

Amino acids play a vital role in the regulation of the production and functions of numerous hormones during the prepartum period. During the prepartum period, an amino acid required for fast-growing foetus for amino acids increases several folds beside the need for other tissues like muscle and liver, for protein synthesis (Bell ,1995). The shortage of amino acids contributes to change in the endocrine functions to affect DMI and nutrient partitioning.

Table 2.1: Prepartum nutrient requirements & tolerance levels in dairy cattle

Nutrient	Suggested range	Max tolerance	Reference
Dry Matter intake (%)	2.01-2.25	-----	NRC (2001), ICAR (2013)
Net Energy (NEL) MCal/kg	1.44 -1.55	-----	NRC (2001)
Crude Protein (% DM)	12 - 13	-----	NRC (2001)
Calcium (%)	0.4-0.6	2	NRC (2001), ICAR (2013), Lean <i>et al.</i> , (2003)
Phosphorus (%)	0.23-0.4	2	NRC (2001)
Magnesium (%)	0.12-0.45	0.45	NRC (2001)
Copper (mg/kg DM)	12-20	115	NRC (2001), Lean <i>et al.</i> , (2003), Mutoni <i>et al.</i> , (2012), Chandra <i>et al.</i> , (2013)
Manganese (mg/kg DM)	15	1000	NRC (2001)
Chromium (mg/d)	0.1-5		Hayirli <i>et al.</i> ,(2001), NRC (2001)
Zinc (mg/kg DM)	22-80	500	NRC (2001), Lean <i>et al.</i> , (2003), Mutoni <i>et al.</i> , (2012), Chandra <i>et al.</i> , (2013)
Cobalt (mg/kg DM)	0.11	5	NRC (2001)
Selenium (mg/kg DM)	0.3	2	NRC (2001), Lean <i>et al.</i> , (2003), LeBlanc <i>et al.</i> ., (2002)
Iodine (mg/kg DM)	0.25-0.4	50	Mee <i>et al.</i> , (1995), McCoy <i>et al.</i> , (1997)

This further affects the postpartum production performance of the animal directly or indirectly. Bell (1995) derived an equation to estimate metabolizable protein (MP) required for pregnancy which includes conceptus weight, calf birth weight and days of gestation as variables. MP for pregnancy = $[(0.69 \times \text{Days Pregnant}) - 69.2] \times (\text{Calf Birth Weight}/45) \div (\text{Efficiency of MP used for Pregnancy})$. Daily MP requirement for a prepartum HF cow weighing 620 kg was approximately 480 g for maintenance and 340 g for gravid uterus accretion (total 820 g/d) when the expected calf birth weight is 45 kg. This amount of MP could be easily achieved with the diet contains 11 to 13% CP, provided when DMI and energy declination is not noted. The meta-analysis of 27 experiments, 125 treatments and 1801 cows indicated that the mean \pm standard deviation and median (range) concentrations of dietary CP fed to prepartum cows were 14.4 ± 2.2 and 14.4% (8.9 to 20.9%), resulting in mean and median (range) intakes of CP prepartum of $1,720 \pm 432$ and 1,734 g/d (745 to 2,482 g/d). Predicted prepartum supply of MP averaged 822 ± 157 in nulliparous cows, ranging from 517 to 1,094 g/d, and $1,146 \pm 316$ in parous cows, ranging from 463 to 1,733 g/d. Increasing prepartum CP content increased postpartum DMI in nulliparous cows but the contrary was true for parous cows (Husnain and Santose, 2019). The NRC (2001) recommended 12 to 13% CP for prepartum cows. Increasing dietary CP above 14% had no beneficial impact on parous cows (Husnain and Santos, 2019). Estimated rates of accretion of CP in the gravid uterus or conceptus were 62 and 117 g/d at 190 and 270 d of gestation, respectively (Bell, 1995). According to Doepel *et al.* (2002), increased CP intake in the prepartum period did not appear to confer any advantages to cow productivity instead, accentuated the plasma urea, β -hydroxybutyric acid (BHBA) and decreased 3-methylhistidine.

2.2.2 Energy

Metabolizable energy (ME) requirement found to be an exponential function of days pregnant and to fetal growth. Energy demands of prepartum cows reach 1.5 to 3 times of maintenance as the animal approaching calving (Quigley and Drewry, 1998). NRC (2001) suggests maintenance requirement of 0.080 Mcal/kg $BW^{0.75}$ after adjusting breed variations, normal voluntary activities of cows housed in a dry lot or free stall system. Estimated rates of accretion of energy in

the gravid uterus or conceptus increased from 567 kcal/d at 190 d of gestation to 821 kcal/d at 270 d of gestation (Bell, 1995). Energy requirement during the gestation was 175% more than a non-pregnant cow of similar BW. Different models were suggested to calculate ME for pregnant cow by Moe and Tyrrell (1971) and net energy by NASEM (2016).

$$\text{ME, Kcal/kg } W^{0.75}/\text{d} = 100.8 + 0.567 e^{-0.174t}$$

$$\text{NE}_m = 0.077 \times \text{Empty BW}^{0.75} \quad (\text{EBW} = \text{Shrunk BW} \times 0.891 \ \& \ \text{SBW} = \text{BW} \times 0.96)$$

$$\text{NE}_G = [\text{CBW} \times (0.4504 - 0.000766t) \times e^{(0.03233 - 0.0000275t)t}] \div (1,000 \times \text{km})$$

$$\text{km} = \text{NE}_m / \text{ME}$$

where, t was number of days pregnant & CBW was calf birth weight which was considered as 36 kg under NASEM (2016).

The efficiency of ME utilization by gravid uterus is 14% which was far below its utilization above and below maintenance that is 60 to 84% (Moe and Tyrrell, 1971; Ferrell *et al.*, 1976). Most of the energy estimates for pregnancy are either factorial method or indirect calorimetry. The factorial approach considered accretion of tissue protein and fat and, the energy needed for the additional metabolism of added tissue including the fetus. According to King (1981), During 280 d of pregnancy of western woman require 300 kcal/d which ranges from 115 kcal/d in first-quarter, 380 and 420 kcal/d in the second and third quarter. The fourth quarter requirement is only 320 kcal/d because fetal tissue gain is greater but, the maternal gain is reduced or negative. Moran (2005) in his book stated that the ME requirement is 1 MJ or 4.184 Mcal/d for each month of pregnancy advancement till 5th month. Thereafter suggested 8, 10, 15 and 20 MJ/d in 6th, 7th, 8th and 9th months of pregnancy, respectively thus, energy component in the diet become significant only in the last 4 months of pregnancy. According to Doepel *et al.*, (2002), increased energy density of prepartum diets had beneficial effects on feed intake and lipid metabolism. Trubenbach *et al.* (2019) also suggested restricting the intake and increasing energy density in the diet to enhance energy efficiency. Glycerol feeding supplies sufficient energy to the rumen microbes to synthesize microbial protein (MBP) from nitrogen source (Lee *et al.*, 2011). Glycerol can be used by *Selenomonas ruminatium*, *Megasphaera*

elsdenii and *Streptococcus ruminantium* and promote the generation of acetate, propionate and butyrate to provide energy in the form of ATP during fermentation of the feed and enhance the energy efficiency in the rumen. However, Donkin *et al.* (2009) observed lowered fiber digestibility when maize was replaced with glycerol at the level of 5%, 10%, or 15% of DM intake in dairy cows. This may be due to a reduction in cellulolytic bacterial activity in *Ruminococcus flavifaciens* and *Fibrobacter succinogen* when crude glycerol administered through the diet (AbuGhazaleh *et al.*, 2011).

2.2.3 Minerals

Mineral supplementation in the gestation period is essential for metabolic pathways and to avoid metabolic consequences. Deficiencies of minerals in prepartum have an impact on postpartum metabolic disorders as they play a significant role in the activities of enzymes and hormones as constituents of body fluids and tissues. Macro mineral supplementation to peripartum cows needs a critical understanding of their regulatory mechanism (Wilde, 2006).

2.2.3.1 Macro minerals

Normal serum calcium (Ca^{+2}) level is 9-12 mg/dl. The decrease in Ca^{+2} concentration up to 5 mg/dl reduces abomasal motility by 70% and the strength of the contraction by 50% (Daniel, 1983). Reduction in muscle contractility will lead to a decrease in DMI because of decreased rumen function this is leading to severe NEB. Low Ca^{+2} concentrations also prevent insulin production and also further exacerbate NEB (Goff, 2000). At the beginning of lactation, the sudden demand of Ca^{+2} for milk production increases dramatically, leading to a fall in blood Ca^{+2} levels. If blood Ca^{+2} levels decrease to 4 mg/dL, it leads to milk fever. This stimulates the secretion of parathyroid hormone (PTH), which stimulates bone resorption. It takes 2–3 weeks for the PTH cycle to become fully functional. To avoid incidences of milk fever, the best feeding management practice is to provide low Ca^{+2} (<0.6% of kg DM) diet during the last 2–3 weeks of gestation. A meta-analysis of 137 published trials and 2545 calvings indicated that the milk fever risk was highest with a prepartum dietary concentration of 1.35% Ca^{+2} (Lean *et al.*, 2006) which was little higher than the critical level of 1.16%

suggested by Oetzel, (2000). Lean *et al.* (2003) suggested 60 g/d of Ca²⁺ intake during the prepartum period in contrast to Oetzel (2000) recommendation of 150 g/d that accounts to 1.1 to 1.5% of DM. Thilsing-Hansen *et al.* (2002) suggested <20 g/d Ca²⁺ for effective control of hypocalcemia. Goff (2000) concluded that 30 g/d feeding of Ca²⁺ that is just above the requirement had little influence on the incidence of milk fever.

A pre-calving diet high in phosphorus (P³⁻) can have a negative impact on Ca homeostasis (NRC, 2001). P³⁻ is also a significant predictor of milk fever and increasing P³⁻ concentrations increase milk fever risk. Increasing the P³⁻ concentrations from 0.3 to 0.4% in the pre-calving diet would increase the risk of milk fever by 18%. Ca²⁺ and P³⁻ are closely related to plasma PO₄³⁻ concentration that is regulated directly by 1, 25 (OH) vitamin D₃ and indirectly by the parathyroid hormone/Ca negative feedback loop (Goff, 2000). Supplementation of P³⁻ before calving should be less than 0.4% of kg DM to avoid the occurrence of milk fever (ICAR, 2013).

Magnesium (Mg²⁺) is known to be an important mineral in the homeostatic pathway for regulating blood Ca²⁺. Although preeclampsia restricted to women and higher apes, eclampsia/grass staggers is an acute and life-threatening emergency condition seen in animals shortly before or after birthing their offspring. Mg²⁺ intake below recommended levels may enhance fetal growth restrictions, eclampsia and many complications of pregnancy (Zarean and Tarjan, 2017). Lean *et al.* (2014) inferred from a meta-analysis that feeding higher concentrations of dietary Mg of 0.45 to 0.50% of kg DM during the prepartum has become common practice to prevent the incidences of hypocalcaemia following parturition. Theoretically, feeding higher concentrations of Mg²⁺ during the postpartum may help in the recovery of plasma Ca²⁺.

Increasing potassium (K⁺) in the diet causes hypocalcaemia due to metabolic alkalosis (Goff and Horst *et al.*, 1997). Rérat and Schlegel (2014) reported that feeding the prepartum diet with low K⁺ concentrations induces a mild metabolic acidosis before calving as indicated by higher urinary Ca²⁺. Feeding a low K⁺ diet moderately supplemented with anionic salts helps to

maintain dietary cation-anion difference (DCAD) close to zero without altering DMI. The DCAD theory found that diets high in Na^+ and K^+ and low in Cl^- and S^{2-} tended to increase the incidence of milk fever (Lean *et al.*, 2006). Block (2010) found a significant increase in the incidence of milk fever for cattle fed on diets that differed only in their quantities of Cl^- , S^{2-} and Na^+ . Phillipou *et al.* (1994) supported the earlier findings that feeding diets containing higher concentrations of chlorine and sulphur can reduce the risk of milk fever. Lean *et al.* (2006) suggested that $(\text{Na}^+ + \text{K}^+) - (\text{Cl}^- + \text{S}^{2-})$ is the best DCAD model for predicting the milk fever incidence.

2.2.3.2 Trace minerals

During last trimester of pregnancy, especially prepartum period, a balance between cow trace mineral needs and limits is needed in tune with increased physiological changes to support metabolism of cow and fetus (López-Alonso, 2012). Copper (Cu^{2+}), Manganese (Mn^{2+}), Zinc (Zn^{2+}), Iodine (I⁻) Iron (Fe^{2+}) and Selenium (Se^{2+}) supplementation during the prepartum period has a greater influence on postpartum colostrum yield, quality and immune competence of the dam. Trace minerals like Fe^{2+} , Cu^{2+} , Zn^{2+} etc., are essential for the successful pregnancy as they are crucial for the cell proliferation and their deficiency may have a teratogenic effect on the fetus (Delano *et al.*, 2002). This is likely driven by a reduction in the activity of key enzymes which require these metals structurally to function, as well as compromised oxidant defence systems henceforth, their requirement is important for mediating inflammation and immune response (Wilson *et al.*, 2018).

Castillo-Durán and Weisstaub (2003) summarized from animal experiments on the influence of Zn deficiency on fetal growth and birth weight. Plasma Zn^{2+} concentration decreases in dairy cows during pregnancy in general and at parturition in particular. During pregnancy, Zn^{2+} intake is essential for normal embryonic development. Plasma Zn^{2+} returns to baseline value within 3 days after parturition. Zn^{2+} plays a critical role in the repair and maintenance of the uterine lining following parturition and hastens the return of the uterus to normal reproductive functioning (Omur *et al.*, 2016). Zn^{2+} affects immunity via

its important role in cell replication and proliferation. Goff and Horst (1997) also reported that Zn^{2+} propionate may enhance the resistance to mastitis by enhancing the keratin synthesis in teats.

Chromium (Cr^{2+}) is attributed to reducing insulin resistance during the peripartum period. Cr^{2+} enhances the communication between insulin and its receptors located on the cell membrane of insulin, by increasing membrane fluidity and the rate of insulin internalization. Cr^{2+} supplementation during the pre-calving period may reduce insulin resistance and subsequently decrease plasma NEFA, liver triglyceride levels thus improve glucose tolerance (Hayirli *et al.* 2002). During mid-gestation, plasma glucose concentrations in cows fed Cr^{2+} decreased 0.282 mmol/L compared with cows fed control; however, by late gestation, glucose concentrations in cows fed Cr^{2+} increased 0.321 mMol/L compared with cows fed control. Regardless of Cr^{2+} supplementation, insulin and glucose concentrations, however, were reduced significantly as days in gestation increased (Kneeskern *et al.*, 2015). Positive metabolic and production responses have been demonstrated in response to Cr^{2+} supplementation during the prepartum period (Hayirli *et al.*, 2001). Rockwell and Allen (2016) reported that the diets top-dressed with 20 g/d Cr^{2+} propionate throughout the peripartum period along with corn starch source had a carryover effect on the postpartum production response.

Copper (Cu^{2+}) is essential for the activity of numerous enzymes, cofactors and reactive proteins (Suttle, 2010). Cu is involved in immune function phagocytic activity (Weiss and Gonzalo, 2006). Heifers fed with marginal Cu^{2+} during prepartum diets (6-7mg/kg diet) had a greater percentage (60% vs 30%) of infected quarters at calving than heifers supplemented with a relatively high amount of Cu @ 20 mg /kg diet (Harmon, 1994). Scaletti *et al.* (2003) reported that supplementation of 20 mg Cu^{2+} /kg DM from 60 days prepartum periods had lower somatic cell count in milk thus, reduced the incidences of mastitis. Although abnormal Cu^{2+} metabolism due to excess intake is a rare phenomenon, Perrin *et al.* (1990) documented experience of the miscalculated quantity of Cu supplementation of 400 to 500 mg /kg of the whole ration to pregnant cows lead to 50% calf mortality. Supplementation of Vit E 1000 I.U, Cu^{2+} 20 ppm, Zn^{2+} 80

ppm from 30 d before calving to 45 d after calving boost the mammary health and helpful in reducing the somatic cell count and % neutrophil secretion as observed in Sahiwal cows (Mutoni *et al.*, 2012). Other research workers also recommended Zn²⁺, Mn²⁺ and Co²⁺ supplements during the prepartum period to reduce postpartum incidences of mastitis (Griffiths *et al.*, 2007; Chandra *et al.*, 2013).

Manganese (Mn²⁺) is required at 15 mg/kg DM for normal function. It plays an important role in colostrum synthesis and maintains fetal skeletal muscle. Mn²⁺ deficiency in the prepartum diet of the heifer is said to cause neonatal chondrodystrophy (Underwood and Suttle, 1999). Hansen *et al.* (2006) fed pregnant heifers additional supplemental Mn²⁺ of 50 mg/kg of DM, in contrast, to control receiving just 15.8 mg/kg of DM. Calves born in the control group had lower whole blood Mn²⁺ and birth weight was lesser. Calves born to control group were suffered from brachygnathism, unsteadiness, disproportionate dwarfism, congenital skeletal abnormalities, swollen joints and joint laxity. Calves from first-calf heifers are more affected. Mn²⁺ deficiency also affects irregular estrus and poor conception rate (Campbell, 2016).

Pregnancy induces several major changes in thyroid physiology and Iodine (I) requirement during pregnancy increases due to increased maternal glomerular filtration rate which increases the loss of I in urine by accentuating passive mode (Yarrington and Pearce, 2011). Adverse effects of I- deficiency on fetus include congenital anomalies and neurological cretinism. Fetal thyroidogenesis occurs by approximately 12th week of gestation and I- deficiency can cause both maternal and fetal hypothyroidism (Yarrington and Pearce, 2011). Mild and transient hypothyroxinemia during pregnancy disturbs neuronal migration in the fetus, resulting in the ectopic neuron in different cortical layers (Auso *et al.*, 2004). Mee *et al.*, (1995) reported a significant reduction in thyroid weight in peri-natal calves from 22.9g to 15.8 g when the pregnant cow's I- intake from a grass silage diet containing 0.15– 0.21 mg / kg DM. McCoy *et al.* (1997) reported a 120% increase in thyroid weight in calves born to housed cows given experimental diets containing 0.06 rather than 0.27 mg I- / kg DM during late pregnancy. The intoxication of Iodine occurs when ruminants ingest more than

0.02 mg/kg BW (NRC, 2001). Mangkoewidjojo *et al.* (1980) induced Iodine intoxications in cattle with marked and mild clinical manifestations by daily I-doses of 2.2 mg/kg and 0.4 mg/kg, respectively. Iodism in cows is manifested by tachycardia, nervousness, loss of weight, naso-ocular discharges and respiratory diseases (Paulíková *et al.*, 2002).

Several studies have also indicated that prepartum Selenium (Se) supplementation along with vitamin E can reduce the incidence of retained placenta in dairy cows, the dairy animals receiving at the level of 0.3 ppm had a better immune response (Weiss and Gonzalo, 2006). Administering 0.1 mg Se²⁻/kg BW 21 days before calving reduced the duration of clinical mastitis by 46% but did not reduce the incidence. The nutritional requirements of Se²⁻ of beef and dairy cows are 0.1 and 0.3 mg/kg DM, respectively (Mehdi and Dufasne, 2016). Several trials have shown that Se²⁻ supplementation of Se²⁻ deficient diets reduced the incidence of retained placentas in dairy cows (Allison and Laven, 2000). According to Gunter *et al.* (2003) either inorganic or organic Se has no significant impact on birth weight and mortality rates in calves. The Se²⁻ supplementation may reduce the incidence of metritis and ovarian cysts during the postpartum period. The increase in fertility when adding Se²⁻ is attributed to the reduction of embryonic death during the first month of gestation (Mehdi and Dufasne, 2016). Pavlata *et al.* (2003) suggested sufficient saturation of Se²⁻ in the prepartum cows to prevent its deficiency in calves. Further, Khalili *et al.* (2020) recommended 0.5 ppm Se²⁻ in the diets of prepartum cows improved the post-partum reproductive health such as distance to the first estrus, insemination indexes and the number of open days.

2.2.4 Vitamins and ω -3 fatty acids

The effect of Vitamin A on the metabolism and physiology during pregnancy is limited. Data from studies of pregnant sheep suggest that the retinol and retinol-binding protein may vary between fetus and adult as a like difference in pregnant and non-pregnant animals (Bastos Maia *et al.*, 2019). Studies in vitamin A-sufficient pregnant sheep suggest that transport of vitamin A to the fetus increases but that efficiency of transfer decreases when high levels of vitamin A

are provided to the ewe suggesting that there may be some placental regulation of transport (Donoghue *et al.*, 1985). Vitamin D is critical for the growth and development of calves control of cellular differentiation and activates innate immune defences of cattle (Nelson *et al.*, 2016). These workers observed increased serum 25(OH)D of calves when cows in late pregnancy were supplemented with vitamin D and it is also recommended increased supplementation of vitamin D in the winter season. Calves are prone to Vitamin D deficiency in the absence of sufficient concentration of serum 25(OH)D hence, a supplementation 50000 IU bolus of vitamin D₃ at birth is recommended. Supplementation of cows with Vitamin D₃ well above the NRC (2001) recommendation of 21000 IU/d such as 50000 IU/d may be more than sufficient. Vitamin E is always supplemented to pregnant cows in combination with Se²⁺ (Kafilzadeh *et al.*, 2014), Cu²⁺ and Zn²⁺ (Dang *et al.*, 2013). A dairy cow requires 1,00,000 IU of Vitamin A, 50,000 IU of Vitamin D and 1,000 IU of Vitamin E (Moran, 2005).

Although the amount of B-vitamins provided by the diet and synthesized by the ruminal microflora are sufficient to prevent the emergence of deficiency symptoms in cattle, the animal suffers when the supply is inferior to the needs. Reports over the last two decades of beneficial effects of B-vitamin supplementation, such as thiamin, niacin, biotin, folic acid, and vitamin B12, suggested that, under some conditions, the need for B vitamins exceeds the supply from the diet and the synthesis by rumen microbes that may induce metabolic inefficiency in carbohydrate, protein and lipid metabolism (Girard, 2017). According to Beaudet *et al.* (2016), the apparent ruminal synthesis of thiamin (B1), niacin (B3) and Pyridoxine (B6) are negative on high starch or fiber diet but the duodenal balance was positive due to intake. Riboflavin (B2), Folic acid (B9) and cobalamin (B12) are fairly synthesized by rumen microflora. Tienken *et al.* (2015) showed that Jersey cows supplemented with 48 g/day of niacin from 30-day prepartum until calving had lower levels of plasma NEFA at calving and less DMI decline during the last week of gestation. Ruminal synthesis of Thiamin is account to 28 to 78 mg which meet or exceed metabolic requirements even with an estimated 48 per cent destruction of dietary thiamin in the rumen (Zinn *et al.*, 1987). Thiamin has no absolute requirement in

ruminates except when diets contain thiaminase activity which happens with the presence of *Fusarium* moulds in silages, stored beer industry waste etc. Niacin synthesized in the rumen when pyridoxine and tryptophan available but, ruminants are less efficient in this conversion than most species. Conversion efficiency is probably due to inherent differences in liver levels of picolinic acid carboxylase, the enzyme that diverts one of the intermediates (2-amino, 3-acroylelfumaric acid) to the picolinic acid pathway instead of allowing this compound to condense to quinolinic acid, the immediate precursor of nicotinic acid. Higher the levels of picolinic acid carboxylase activity in liver, the poorer is the conversion of tryptophan to Niacin. Even Nicotinamide and Folate are synthesized in the rumen; they are destructed before duodenum in the gastrointestinal tract (Santschi *et al.*, 2005).

Polyunsaturated fatty acids especially ω -3 fatty acids (FA) plays an important physiological role during pregnancy in cattle, as they are noted as critical for the development of the central nervous system and reproductive system (Koletzko *et al.*, 2007; Stoffel *et al.*, 2008) of the unborn calf. The ω -3 fatty acids are known as anti-inflammatory nutrients. According to Bertoni *et al.* (2006), ω -3 fatty acid (Linolenic acid) feeding reduced inflammatory consequences after calving that was evident from negative acute-phase proteins. Moallem *et al.* (2013) documented the beneficial effect of ω -3 fatty acids supplementation on reproductive and immune health of dairy cattle. The effects of ω -3 fatty acids supplementation in dairy cows have been investigated at a few levels such as ovaries and uterus, embryo survival and behavioural estrus. Feeding of extruded flaxseed to 276 multiparous cows fed at 4% during the prepartum period and 5% in the postpartum period had shown reduced days from first artificial insemination to conception and open days. Moallem *et al.* (2013) concluded that the beneficial effects obtained from feeding n-3 FA, at least for the reproductive system in cows and bulls, can be achieved with supplementation of ω -3 fatty acids from flaxseed, whereas no advantage was seen in feeding the longer n-3 fatty acids from fish oil EPA and DHA over feeding linseeds alone.

2.3 Prepartum nutritional alterations

The prepartum nutrition of pregnant cattle is not as simple as intake and fulfilment of the nutrient requirement. It must be integrated with the metabolic alterations and hormonal changes those control appetite-regulating centres in the hypothalamus. Cow metabolism during pregnancy is balanced with homeostasis and homeorhesis regulations. One maintains and controls physiological equilibrium (Homeostasis) while another coordinate metabolism of body tissues to support physiological state (Homeorhesis). Nutrition partitioning during pregnancy involves homeorhetic controls arising from the conceptus thus, assures the growth of the fetus and fetal membranes (**Fig 2.4**). Besides this nutrient demand and drainage, the hormonal changes during pregnancy would cause immune suppression, whose restoration immediately after parturition is also greatly dependent on nutrition (Bisinotto *et al.*, 2012).

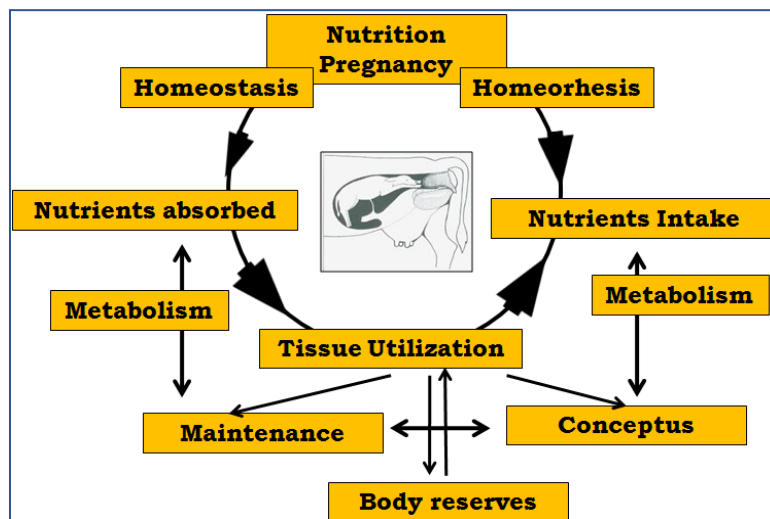


Fig. 2.4: Nutrition and Pregnancy: homeostasis and homeorhesis (Bauman and Bruce Currie, 1980)

2.3.1 Homeorhesis and nutrient demand

During pregnancy, the mother must adapt her body systems to support nutrient supply for growth of the baby *in utero* and subsequently lactation. These include changes in the cardiovascular, pulmonary, immune and metabolic systems of the mother. Failure to appropriately adjust maternal physiology to the pregnant state

may result in pregnancy complications (Napso *et al.*, 2018). The homeorhesis mechanism orchestrates or coordinates modifications during pregnancy. This notion describes how the physiological process is balanced during gestation, lactation and extended to the growth of the fetus (Bauman, 2000). This concept gives an idea about how the circulating nutrients utilization differs by tissue based on their physiological function. According to Bell and Bauman (1997), the key features of homeorhesis are;

1. Chronic in nature, regulatory criteria that take hours and days as against minutes and seconds for most instances of homeostatic control.
2. Simultaneous by influences on several tissues and systems resulting in an overall coordinated response.
3. Mediation involves altered tissue responses to homeostatic signals. This represents the key to understanding the mechanistic interrelationship between homeostasis and homeorhesis

Dynamic regulation of vital functions and metabolic coordination between the organs were the key features of homeostasis to maintain physiological equilibrium. During the last trimester of pregnancy, fetal demand for specific nutrients like glucose and amino acids are as high as a lactating cow which produces 3-6 kg milk/day. Glucose is the chief nutrient required by the mammary gland and gravid uterus for metabolism (Hay, 2006). Maternal adaptations to meet fetus needs are more critical and in turn, are expected to be regulated by the homeorhetic signal from conceptus. Conceptus oxidizes glucose, lactate and amino acids as a source of energy however glucose will be preferred. The glucose deficit for oxidation leads to an increased amino acid catabolism at the expense of protein synthesis and deposition in fetal tissue. The primary source of amino acids comes from skeletal muscle catabolism for gluconeogenesis (Bell, 1995).

Glucose and lactate are transported by facilitated diffusion, whereas amino acids by active transportation (Bauman and Bruce Currie, 1980). Complete oxidation of glucose and lactate provides 50-60%, amino acids account for 30-40% of fetus energy requirements (Bell and Ehrhardt, 2000). Increasing the

glucose availability to the fetus is accomplished by reducing the glucose utilization by the dam. Therefore NEFA, ketone bodies provide alternate energy to the maternal tissue. To provide NEFA lipolysis is stimulated than lipogenesis. In this condition, adipocytes are more sensitive to adrenogenic agonists than insulin (Baumgard *et al.*, 2017). During late pregnancy, there is a gradual increase in the insulin resistance in the maternal tissue to facilitate the glucose to the foetus due to homeorhesis. Inflated lipolysis results in a more elevated concentration of serum NEFA leads to lipid accumulation in the liver, which is a prerequisite to metabolic disorders. Placental lactogen which is similar to growth hormone may be involved in the homeorhetic control of maternal metabolism by coordinating maternal liver and adipose tissue metabolism to provide nutrients to the developing foetus (Kaplan and Grumbach, 1974; Napso *et al.*, 2018).

2.3.2 Metabolic changes for specific nutrients

Metabolic and reproductive hormones are highly inconsistent in the prepartum period. This leads to the greatest fluctuations in body metabolism (Ingvertsen and Anderson, 2000). In the prepartum period, the demands for nutrients increase quite considerably for, the fetus, fetal membranes, the supplementary tissue and mammogenesis. Metabolic changes may become exaggerated when maternal supplies of energy and protein or both are inadequate, thus leading to metabolic diseases. So, the metabolic adaptations occur in many non-reproductive tissues in a coordinated way. Important metabolic changes associated with prepartum were glucose, protein, fat and mineral metabolism.

The prepartum cow must alter her metabolic priorities for nutrients from reserve tissue deposits. The homeorhesis mechanism allows the cow to accommodate for the increased demand for pregnancy (Remppis *et al.*, 2011). Propionic acid from the rumen fermentation is the major substance for hepatic gluconeogenesis in ruminants. Propionate to net glucose release by gluconeogenesis is approximately 50-60% and other substrates for hepatic gluconeogenesis are lactate from the Cori cycle (Chung *et al.*, 2015, Koch *et al.*, 2016). Amino acids from protein catabolism make a sizable contribution to gluconeogenesis i.e 15-20%, among all amino acids, are the potential glucogenic

amino acids and glycerol released during lipolysis is about 15-20%, 2-4% respectively. In 2nd trimester of pregnancy, lipogenesis occurs in contrast to late gestation where the lipolytic activity is more (Zeng *et al.*, 2017).

Glucose is the major energy substrate for both gravid uterus and developing udder. The maternal metabolic process needs to designate more glucose for these purposes. Increasing the glucose availability to these reproductive tissues is accomplished by decreasing glucose utilization by maternal non-reproductive tissues and increasing the availability of glucogenic precursors. Reynolds *et al.* (2003) reported that across the portal drained viscera, the net flux of glucose was zero to slightly negative in the prepartum period and early lactation. The splanchnic output of glucose increases up to 267% before 9 days of the expected date of calving to 21 days of postpartum results in increased hepatic gluconeogenesis.

Decline DMI and insufficient glucose availability either favour or force the mobilization of body fat reserves from adipocytes. In mammals, insulin is involved in both up and down-regulation of feed intake. Postprandial insulin surge increases glucose uptake by peripheral tissue in mammals, but in late gestation adipose and muscle tissue develops resistance to insulin and sensitivity to lipolytic agents (León *et al.*, 2004). Oxidative stress-induced by NEFA is another reason to inhibit glucose uptake by peripheral tissue and interfere with insulin signal transduction pathway in the liver and peripheral tissues (Turk *et al.*, 2008). Low insulin concentrations favour gluconeogenesis and lipolysis (Herdt, 2000). Insulin is one of the putative mediators of nutritional status. Changes in insulin concentration play a major role in the body condition score of the animal (León *et al.*, 2004). As calving approaches, blood progesterone concentration decreases gradually from 8 to 0 ng/mL and estrogen increases from 100 to 600 pg/mL. The high circulating 17 β -estradiol is believed to be one major factor that contributes to diminished DMI around calving by acting predominantly on the paraventricular nucleus of the hypothalamus (Grummer *et al.*, 1990).

Ghrelin concentration in cattle increases at the time of NEB in dairy cows to stimulate the feed intake. Vargová *et al.*(2015) observed the lowest

concentration of ghrelin in the prepartum period (25pg/ml) and the highest concentration in the postpartum period (35.94 pg/ml) in the dairy cow. Pregnancy is considered to be physiological stressor which results in hyper adrenocortical activity. It causes a high level of circulating steroids like cortisol. At the time of parturition, cortisol concentration in blood of cow increases from 6 to 12 µg/dl due to severe stress.

2.3.3 Nutritional challenges during the close-up period

Homeostasis mechanisms, hormonal fluctuations, nutrient demand and nutrient partitioning push the cow into a cascade of metabolic challenges ascends with descending pregnancy. As the animal approaches parturition, decrease in DMI is reported despite increasing energy requirement for fast-growing foetus and lactogenesis (Bell, 1995). The increased demand for energy for the growth of the foetus, parturition and onset of lactation would lead to NEB (Ingvertsen and Andersen, 2000). This constitutes an enormous challenge for the liver to synthesize all of this glucose from propionate and amino acids (AA). The increased energy requirements can be partly met by increased hepatic gluconeogenesis, decreased glucose usage by peripheral tissues, increased fatty acids (FA) production from adipose tissues and increased AA mobilization from muscle in cows (White *et al.*, 2012). Despite all these changes, the cow enters into NEB. The massive mobilization of body fat is achieved by a combination of increased lipolysis and decreased rates of lipogenesis and FA re-esterification in adipose tissue. These changes are leading to the net release of NEFAs and glycerol into the bloodstream (Drackley *et al.*, 2001). A hormonal change associated with parturition alters the homeostasis of energy and protein metabolism including micronutrient utilization further leads to immunosuppression. As the cow approaches to calving, the demand for Ca increases in some degree of hypocalcemia at calving. In some cases, concentrations of plasma Ca become too low (<4mg/dL) to support nerve and muscle function, resulting in parturient paresis or milk fever (Goff and Horst, 1997).

2.4 Nutritional strategies during prepartum

The primary goal of prepartum dairy cow nutrition is orchestrating the nutrient supply to homeorhetic adaptations, reduce the incidences of the peripartum period and improve the immunity to support the postpartum production and reproduction. Four major strategies are outlined below:

2.4.1 Increasing the energy content of the diet

This concept referred to as steaming-up and typically consists of increasing the non-fibrous carbohydrate (NFC) content of the ration. Feeding high NFC diet before calving promotes the development of the ruminal papillae for adequate absorption of volatile fatty acids produced ruminal fermentation during postpartum. Dann and Grant (2009) replaced cracked corn diet with steam flaked corn (39% of total NFC) in the prepartum diet. They reported that there was no significant difference for both groups in prepartum DMI (13.6 kg/d vs. 14.6 kg/d). However, the steam flake corn group consumed more DM in prepartum resulted in less mobilization of adipose tissue and lowered plasma NEFA concentrations (145 vs. 251 $\mu\text{eq/L}$) as parturition approached. Vandehaar *et al.*(1999) reported that increasing the energy density of diets in the prepartum period from 1.3 to 1.6 Mcal of NE_L/kg diet with 16% CP did not decrease feed intake as calving approaches. Average DMI over the last 2 wk prepartum tended to be greater in animals fed the high energy high protein diet than those fed the low energy low protein diet (12.5 vs.11.0 kg/d). Decreased NEFA concentration before calving was observed in high energy high protein diet than low energy low protein diet (176 vs. 233 mM) but not associated with improvements in milk production, fertility, or body condition score after calving. Huang *et al.*(2019), fed prepartum cows fed the diet with low (1:1.07), medium (1:1.41) and high (1:2.08) NDF and NFC from -3 weeks to +5 weeks. DMI was decreased with increased NDF: NFC, increased daily feeding times but, helped in increasing the DMI in postpartum. NFC is also important for rumen MBP production whose increase during pregnancy help in improving cow resilience to various stressors because B complex vitamins. They secrete to meet host animal's requirement partially or completely depending on the type of B Complex vitamin (Ashwin and Srinivas, 2019).

2.4.2 Additional of dietary fat

This may help to decrease the concentration of NEFA and prevent the occurrence of ketosis. Fat provides additional energy availability to peripheral tissue and mammary gland thus decreases the mobilization of fat from adipose tissue. Grum *et al.* (1996) fed 6.5% more liquid fat to diet from drying off period until 7 days before expected calving. The high-fat treatment significantly increased blood levels of NEFA during the dry period (400 $\mu\text{eq/L}$ vs. 500 $\mu\text{eq/L}$). Immediately after calving the lipid content of the liver in the high-fat group was less than half when compared with the other two treatments (6% vs. 12%). It appears that elevated blood NEFA in the dry period primed the liver to cope with the increased mobilization. Cows fed with low (1.25 Mcal/kg), medium (1.44 Mcal/kg) or high (1.55 Mcal/kg) energy diets to Holstein cows 3 wks before calving resulted in higher DMI, gained more BW and body condition score and improved energy balance during but lost BW and body condition score during the first 8 wk of lactation (Zhang *et al.*, 2015).

2.4.3 Alternative energy source

Supplementation with glucogenic precursor; glycerol directly absorbed in the rumen epithelium of small intestine and transported to the liver where glycerol kinase enzyme converts glycerol to glycerol-3-phosphate used to drive gluconeogenesis. Carvalho *et al.* (2011) recommended feeding prepartum cows with glycerol by replacing the 11.5% DM in the corn-based rations without any adverse effect on DMI. However, DeFrain *et al.* (2004) reported that DMI in low glycerol fed groups was about 11 kg/d compared to 13 kg/d in control when the diet was top-dressed with glycerol in prepartum cows at the rate of 0, 430, or 860 g/d, respectively and fed for the last 22 d of calving. This reduction in feed intake can be explained by the hepatic oxidation theory (Allen *et al.*, 2005). According to Forbes and Barrio (1992), hepatic fuel oxidation increases adenosine triphosphate (ATP) concentration in hepatocytes which sends signals to nucleus tractus solitaries in the hypothalamus which activates the satiety centre that translates into reduced DMI. Contrary to DeFrain *et al.* (2004), Bodarski *et al.* (2005) reported no effect on prepartum feed intake when cows fed 300 or 500 ml of glycerol from -14 days to till calving. Propylene glycol is also supplemented in

place of glycerol to improve the energy status of prepartum cows. Indeed, propylene glycol is derived from glycerol in two stages such as dehydration (-H₂O) followed by hydrogenation (H₂). Oral administration of 300ml propylene glycol two days prior to the expected date of calving decreased plasma concentration of NEFA i.e. 0.533 vs.0.465 meq/L for control and propylene glycol supplemented group, respectively (Stokes and Goff, 2001).

2.4.4 Dietary cation and anion

Low Ca²⁺ diet during the prepartum period affects the Ca²⁺ absorption in the intestines and the mobilization mechanism. Negative dietary cation-anion balance (-10 to -15 meq/100g of DM) was more effective in preventing milk fever (Horst *et al.*, 1997). Goff and Koszewski (2018) fed cows with low Ca²⁺ i.e., 0.46%+167 mEq/kg of dietary cation and anion difference (DCAD) (low Ca²⁺), 0.46% Ca²⁺ and -13 mEq/kg of DCAD (low Ca²⁺ + anion), or 0.72% Ca²⁺ and -17 mEq/kg of DCAD (high Ca²⁺ + anion) for four weeks prior to calving. The results revealed that prepartum plasma Ca²⁺ concentration is decreased in all treatment groups, but clinical milk fever (plasma Ca less than 5 mg/dL) occurred in 3 of 20 cows fed the low Ca²⁺ diet, 1 of 19 cows in the high Ca²⁺ + anion diet while none of the 21 cows fed the low Ca²⁺ +anion group diet seldom showed clinical milk fever within the 28 h after calving. Low or negative DCAD increase the urinary Ca excretion before calving and increase plasma Ca²⁺ concentration after calving (Grünberg *et al.*, 2011). Razzaghi *et al.* (2012) fed the cows either a diet containing -100 DCAD or +100 DCAD for 21 d prepartum. Both anionic and cationic groups were divided into two groups, one received a +200 DCAD and the other +400 DCAD diet for 60 days postpartum. Prepartum reduction of DCAD decreased DMI, urinary and blood pH, urinary concentrations of Na⁺ or K⁺ and increased plasma and urinary Ca²⁺, Mg²⁺, Cl⁻ and S²⁻. Feeding low Ca²⁺ and anion diet during the prepartum period is strongly associated with improved Ca²⁺ status at calving and permitting more effective Ca²⁺ homeostasis (Goff and Koszewski, 2018).

2.5 Health and immunity issues during prepartum

The perimeter of the nutrition during the prepartum period did not end with balancing the nutrition and nutrient partitioning with the homeorhetic factors and metabolic changes, it should also be vigilant to the health challenges those spurt as close-up period approaches.

2.5.1 Nutrition & Hematology

Prepartum is a critical period where apart from the mammary gland, remarkable changes take place in the entire animal system. The haematological analysis is an effective tool in monitoring the health and nutritional status of the animal. Investigation of hemogram along with biochemical profiling helps predict prepartum cow susceptibility to production diseases (Kevin and Ellen, 2012). Anaemic conditions are inevitable in prepartum animals. RBC indices include mean corpuscular volume (MCV), mean corpuscular haemoglobin (MCH) and mean corpuscular haemoglobin concentration (MCHC) help diagnose different types of anaemia. Impaired function of white blood cells at the time of calving reduces the immunity (Klinkon and Zadnik, 1999). Joshi *et al.* (2018) reported total erythrocyte count (TEC) increases non-significantly ($6.77 \times 10^6/\mu\text{L}$ to $6.94 \times 10^6/\mu\text{L}$) on day 7 prepartum as compared to day 15 prepartum ($6.87 \times 10^6/\mu\text{L}$). Oliveira *et al.* (2019) reported that there was a non-significant increase in TEC ($8.78 \times 10^6/\mu\text{L}$ to $9.11 \times 10^6/\mu\text{L}$) of Nellore cows from prepartum to parturition. This high production of TEC may be due to higher metabolic demands of the foetus. Always in general TEC is associated with the need for the oxygen demand. As the oxygen demand increases the amount of RBC count also increases resulting in the release of erythropoietin by kidney tissue. Another elucidation that fits the situation for the increased TEC counts at calving is the possible hemo concentration not only by the reduction in water intake but also by the greater spleen contraction (Van Soest and Blosser, 1954).

Gāvan *et al.* (2010) reported that reduction of RBC count was due to deficiency of iron during the prepartum period. Number of WBC (White Blood Corpuscles) in the blood is a sign of health status of the animal thus it is an important subset of the complete blood count. Percentages of each WBC count

are also affected by mammary gland extensive influx of neutrophils into the colostrum around parturition. Kim *et al.* (2005) reported that a significant increase in total leucocyte count, lymphocyte and neutrophil percentage in late pregnancy. Elshahawy and Abdullaziz (2017) also reported that WBC count was significantly higher in the prepartum period than the postpartum period (12.25 ± 0.46 vs 10.93 ± 1.96). Joksimović and Davidović, (2012) stated that significantly higher leucocyte count on the day of parturition ($9.26 \pm 3.04 \times 10^9/L$) when compared to 15 days prior to calving ($7.16 \pm 1.87 \times 10^9/L$). The highest WBC count was due to elevation mediated by serum cortisol level around the partition, which stimulates agents for increased neutrophil per cent. Copper plays an important role in neutrophil activity, energy metabolism and lymphocyte proliferation.

The physiological mean value of Hb reported earlier in the literature was ranged between 8 and 15 g/dl in cattle (Feldman *et al.*, 2000). Oliveira *et al.* (2019) reported that Hb concentration was significantly higher in prepartum (14.43 g/L) compared to parturition or postpartum (13.45 g/L, 13.34 g/L). This increased haemoglobin level in prepartum is due to increased oxygen flow to gravid uterus for foetal growth (Abud *et al.*, 2016) and decreased rate of erythropoiesis (Klinkon, 1993). Mir *et al.* (2008) reported significantly decreased mean Hb (g/dl) in the prepartum period when compared to mid-stage of pregnancy (9.21 ± 0.28 vs. 10.01 ± 0.22). This was due to the dilution of blood which occurs as a consequence of the increase of plasma volume (Singh *et al.*, 1991). Găvan *et al.* (2010) reported that no significant difference was observed for erythrocyte indices during the prepartum period but iron deficiency might be the cause for a slight non-significant reduction in MCV, MCH.

Klinkon and Zadnik (1999) reported that the lowest mean absolute value for monocytes was constant during pregnancy and decrease slightly in the last month of pregnancy (213 ± 136 vs 224 ± 157). This was due to the migration of monocytes from circulation to tissues and undergo converting macrophages to provide immunity at the time of parturition stress. Schalm *et al.* (1986) reported that pregnancy did not affect the number of eosinophil (%), Basophil (%). Joshi

et al. (2018) reported that platelet count ($\times 10^6/\mu\text{L}$) non-significantly decreases at 15 d prior to calving to calving day (320.33 ± 21.69 vs 300.67 ± 34.81).

2.5.2 Nutrition & Gut health

The peripartum dairy cow experiences a state of reduced liver function coupled with increased inflammation and oxidative stress (Bionaz *et al.*, 2007; Trevisi *et al.*, 2012). Hence, gut health concept comprises a healthy upper and lower gastrointestinal tract along with organs like liver, pancreas, spleen. Gut health begins from the GI barrier, metabolic functions, mucosal immune system, the enteric nervous system and, nutrient and fluid uptake by the liver (Cummings *et al.*, 2004). Gut health can offer a new approach to nutrition where food acts as medicine to maintain health and healthy animals can perform their genetic potential of production traits (Bischoff, 2011). A healthy gut contributes to a strong immune system and improves the production of the animal. Bilirubin, glutamic-oxaloacetic transaminase (GOT), γ -glutamyltransferase (GGT) along with albumin and paraoxonase are commonly used biomarkers of liver status around calving (Bertoni *et al.*, 2006). The liver is responsible for clearance of bilirubin, and higher GOT and GGT is related to liver cell lysis and necrosis (Osorio *et al.*, 2014).

Liver function can be assessed by various enzymes like gamma-glutamyl transferase (GGT), aspartate aminotransferase (AST), alanine aminotransferase (ALT). All these liver enzymes suggest the process of lipid mobilization. AST is a hepatocellular leakage enzyme used as a marker efficiently for hepatic lipidosis and catalyzes the transamination of L-aspartate and 2-oxoglutarate to oxaloacetate and glutamate. The blood concentration of liver enzymes is positively correlated with the degree of liver infiltration (Pechova and Halouzka, 1997). AST concentration depends on the function of the liver, skeletal muscle and cardiac muscle of dairy cows. Van saun (2005) reported that the AST level after calving was significantly higher ($P < 0.0001$) in fresh cows than the prepartum period (75.7 vs. 123.2 IU/L). The highest activity of AST was documented during the first week after calving, as lactation progresses AST activity decreases (Elitok *et al.*, 2010). According to Fiore *et al.* (2014), the increased activity of AST during the

postpartum period than the prepartum period was considered as an indicator for hepatic steatosis (78.54 vs. 118.24 IU/L). During early lactation, 5-10% of high yielding dairy cows suffer from severe hepatosis and 30-40% cows suffer from mild hepatosis. Fat infiltration to liver or injury to the liver tissue increase the level of these enzymes (AST, GGT) that indicate the liver injury is generally augmented. When AST concentration reaches higher than 100 IU/L, it indicates hepatic lesions (Bobe *et al.*, 2004). González *et al.* (2011) observed negative correlation ($r=-0.46$) between AST activity and NEFA values ($P < 0.05$). Seifi *et al.* (2007) reported that AST level was lowest 22 d before calving (46.41 IU/L) when compared to 21 d postpartum (63.30 IU/L). Steen *et al.* (1997) observed that AST level in cows was significantly higher in fatty liver (400 IU/L) and ketosis group (240 IU/L) when compared with the control group (60 IU/L). This might be the fact that fatty liver infiltration and liver cells degeneration involve in liver cell membrane damage and destruction cytoplasmic enzyme release and elevated concentration in blood (Lubojacka *et al.*, 2005). Stojevic *et al.* (2005) observed decreased activity of ALT in the dry period but, it was statistically higher than the first period of lactation (11.84 vs. 8.91 IU/L). The role of ALT in predicting liver diseases was not significant (Stojevic *et al.*, 2005).

A Gamma-Glutamyltransferase (GGT) level estimate is a good biochemical test of hepatic minor and chronic injuries. GGT is considered a valuable biomarker of cholestasis or hepatic damage. Moreira *et al.* (2012) observed significantly ($P > 0.05$) higher concentration of GGT in the animals with hepatic histopathological injuries (23 ± 14 IU/L) compared to those without hepatic damage (18 ± 9 IU/L). The GGT values were significant increase ($P < 0.05$) on day 0 (18.4 IU/L) and at 1st wk after parturition (19.1 IU/L) when compared with 1 wk before parturition (17.2 IU/L).

The level of serum/plasma cholesterol was considered as an indirect index of liver function in periparturient cow (Alamouti *et al.*, 2009). In the prepartum cow, as a result of lipid mobilization, free fatty acids reach the liver and re-esterified into triglycerides. These triglycerides are subsequently transported as low-density lipoproteins. Cholesterol occurs in blood as a part of lipoproteins. Low-density lipoproteins are accountable for transporting the cholesterol to

peripheral tissue. The reduction in serum cholesterol causes the synthesis of low-density lipoproteins from hepatocytes (Bruss, 1997). Djoković *et al.* (2015) evaluated that serum cholesterol concentration was low during late pregnancy (3.3 mmol/L) compared to the mid-lactation (5.3 mmol/L). Bossaert *et al.*, (2012) reported that low cholesterol (1.7 mmol/L) at 9 d prior to calving, when compared with 21 d after calving (3.7 mmol/L), might be due to inflammatory changes and associated acute phase protein response during this period.

The serum triglyceride concentration significantly decreased ($P < 0.001$) from prepartum (14.83 mg/dL) to postpartum (9.83 mg/dL). Hormonal change, lipogenesis, and lipolysis are regulated to increase lipid reserve during pregnancy and these reserves are utilized for lactation (Piccione *et al.*, 2012). Seifi *et al.* (2007) reported that triglyceride concentration was significantly low ($P < 0.001$) in postpartum (0.02 mmol/L) when compared to prepartum (0.20 mmol/L). High serum cholesterol concentration in prepartum was probably due to decreased catabolism of triglycerides or increased lipolysis. Mammary gland uptake of triglycerides for milk production reduced the serum triglyceride concentration.

Sateesh *et al.* (2018) reported that total plasma protein in the prepartum period and at the time of calving was significantly lower (6.58 ± 0.22 , 6.75 ± 0.29 g/dl) when compared to the healthy control group (7.01 ± 0.14 g/dl). The average concentration of total protein (TP) during 7d before calving and on 60 d of lactation (69.59 ± 6.14 g/l to 67.71 ± 8.10) were significantly higher ($P < 0.001$) in comparison with the values (58.87 ± 3.29 g/l) obtained during 7d after calving (Prodanovic *et al.*, 2012). A sudden drop in TP as calving approaches may be due to foetus synthesize all its protein from the amino acids derived from the dam or due to drain of immune fraction in the form of colostrum. Yousuf *et al.* (2016) reported a high level of TP was observed after 2 months of calving when compared with the prepartum period (79.9 g/L vs. 60 g/L). The albumin concentration was a similar thorough prepartum period with a small increase in concentration at calving (2.95 g/DL to 3.15g/dL). The insignificant increase of albumin at calving could be due to higher albumin synthesis by the liver or to a decrease of plasma volume masked by hypoglobulinaemia (Piccione *et al.*, 2012). Physiological or pathologic conditions can cause shifts in albumin and globulin concentrations

and their evaluation is a valuable diagnostic tool (Alberghina *et al.*, 2011). Albumin is synthesized in the liver; lower serum albumin is indicative of the impaired function of the liver. The reference range of albumin concentration is approximately 2.1-3.6 g/DL. Seifi *et al.* (2007) reported that the lowest albumin was found on d 8 postpartum (29.2 g/L) when compared to 8d prepartum (35.5 g/L) in transition cow. This might be due to decreased synthesis of albumin by the liver or due to loss of albumin into the gut or through milk or increased catabolism of albumin. In contrast, Bossaert *et al.*(2012) stated that the concentration of albumin decreases around calving and increases in the postpartum period. This might be due to a decrease in the total pool of decreased Ca in prepartum partially linked to albumin concentration (Seifi *et al.*, 2007).

An accelerated rate of protein catabolism than the excretion rate of urea in cows causes an increase in urea concentration in blood (Kaneko *et al.*, 2008). During pregnancy, serum urea concentration remained nearly constant (1.67 - 1.94 mmol/L) but, increase after calving significantly (4.40 mmol/L) and return to previous prepartum range in the second month (Tainturier *et al.*, 1984). Macrae *et al.* (2006) collected blood samples from 35,500 cows and reported that on -10 d of calving 20.5% of cows had low blood urea concentration of <1.7 mmol/L that may be due to low DMI or deficit in effectively rumen degradable protein. In contrast, Piao *et al.*(2015) stated that urea level in blood was increased significantly ($P<0.05$) from early pregnancy (4 ~ 18.1 mg/dl) to late pregnancy (4.7~17.9 mg/dl) due to increased protein intake. Generally, the blood urea concentration was significantly lower in the first lactation than the second lactation in both pre and postpartum (Gerardo *et al.*, 2009).

Creatinine in the blood is an important marker for kidney function. It is the breakdown product of creatinine phosphate from muscle and protein metabolism. A significantly higher concentration of creatinine was observed in the serum of late pregnant heifers (1.16 mg/dl) compared with primiparous and multiparous dry cows (1.04 mg/dl) which were due to higher relative muscle mass in heifers considering that almost all creatinine is released by the striated muscle (Brscic *et al.*, 2015). Piccione *et al.* (2012) reported that a higher level of serum creatinine in late gestation (1.21 mg/dL) when compared to the second

week of lactation (0.99 mg/dL). The increased serum creatinine level in advanced pregnancy could be attributed to the development of foetal musculature (Roubies *et al.*, 2006).

2.5.3 Nutrition & Immune suppression

Immune suppression during the pregnancy as a state of immunological weakness and susceptible to disease is more a myth and actual reality behind it (Mor and Cardenas, 2010). In dairy cows, immune suppression has been reported around calving and lasted several weeks after calving that predisposes the dam to different health-associated problems with altered metabolism and function (Goff and Horst, 1997). The hormonal changes at calving are one of the reasons for immune suppression. NEB, shortage of proteins, vitamins and minerals in dam body are the major causes of metabolic stress contributing to reduced immunity during the prepartum period. Major trace minerals involved in immune competence are Fe, Zn, Cu and Se. Cu is the limiting element in the synthesis of ceruloplasmin which acts as anti-defence by removing free radicals (Saenko *et al.*, 1994). T-helper cells are classically divided into Th1 cells, characterized by IL-2, and IFN- γ production involved in cellular immunity and Th2 cells which are characterized by the production of IL-4, IL-5 and IL-13 which are involved in humoral immunity (**Fig 2.5**). Lower Cu concentration decreases humoral immunity and cell-mediated immunity. Deficiencies of Cu, Se, Vit E and Co in cattle reduce the ability of neutrophils for phagocytosis.

Th1 cells drive the type-1 pathway that is "cellular immunity" to fight viruses and other intracellular pathogens, eliminate cancerous cells, and stimulate delayed-type hypersensitivity (DTH) skin reactions. Th2 cells drive the type-2 pathway that is "humoral immunity" and up-regulate antibody production to fight extracellular organisms; type 2 dominance is credited with the tolerance of xenografts and of the fetus during pregnancy. The homeostasis between Th1 and Th2 is important for immune regulation during pregnancy (Kidd, 2003). Th17 cells are unique effector subsets that share the same lineage with Th1/Th2 and their levels in the blood should be stable for a healthy pregnancy (Nakashima *et al.*, 2010).

Metabolic stress-induced increase in plasma cortisol concentration (primarily of maternal origin) i.e. around 4-8 ng/ml at just 2-3 d before calving to 15 to 30 ng/ml on the day of parturition and postpartum. This increase impairs the immunity of the animal by decreasing proliferation and function of neutrophils and lymphocytes (Goff, 2006). Sex steroids like estradiol which is elevated at calving may also show alter immune function (Lamote *et al.*, 2004). Lipid mobilization during the prepartum period causes a significant change in cellular membrane phospholipid content, plasma fatty acid profile of hepatic and adipose tissue.

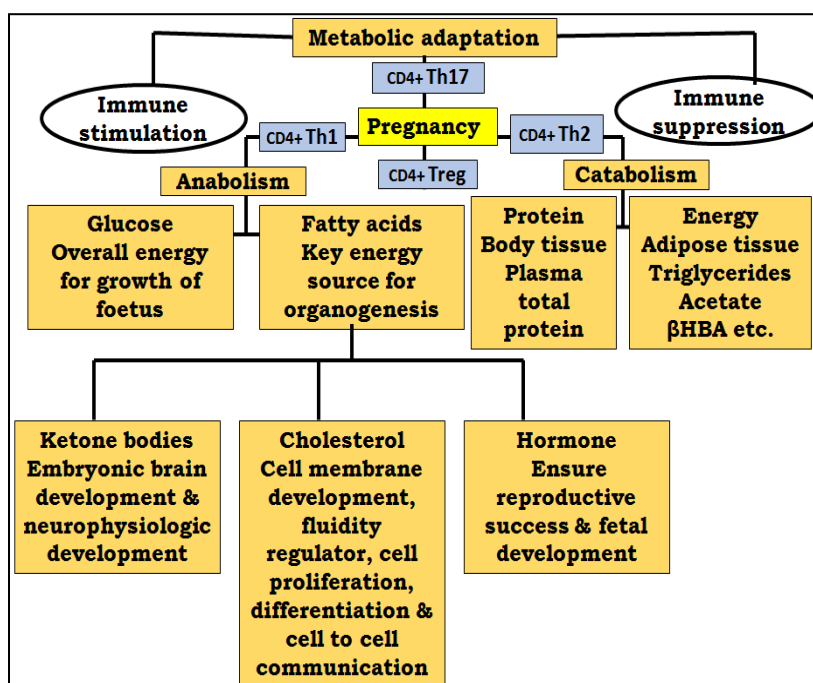


Fig. 2.5: Metabolic adaptation, nutrient partitioning and immune response during pregnancy (Kidd, 2003; Mor and Cardenas, 2010; Zeng *et al.*, 2017)

This results in increasing the palmitic acid concentration of hepatocyte cell membranes. The same changes observed in white blood cells of the lactating woman which causes an altered immune function (Otto *et al.*, 2001). Even though the increased fatty acid composition in bovine leucocyte or endothelial cell membrane has not been reported, the incremental changes in the fatty acid profile of other key cells contribute to the inflammatory processes which may

affect the cow during prepartum period. Increased concentrations of NEFA and ketone bodies have a multidimensional effect on reducing the activity of neutrophils, diminishing the mitogen inducing blastogenesis and secretion of interleukin-2 (Žekić-Stošić *et al.*, 2018). The factors responsible for reduced immunity in prepartum cows could be attributed to diminishing WBC activity, effects of metabolism associated with colostrogenesis, and stress impede immunity (Aleri *et al.*, 2016). Reduced neutrophil function further suppresses immunity due to change in the expression of transcripts involved in basic life processes like TCA cycle and DNA binding synthesis. A balance of Th1, Th2, Th17 and regulatory T (Treg) cells and their cytokines are important in the maintenance of health and immunity during pregnancy (Zhu and Paul, 2009). The long-chain omega-3 fatty acids EPA (eicosapentaenoic acid) and DHA (docosahexaenoic acid) significantly benefit diverse inflammatory and autoimmune conditions without any specific Th1/Th2 effect (Kidd, 2003).

2.6 Effect of prepartum nutrition on parturition

A pregnant cow's capacity to take care of her conception is largely determined by the way she partitions the nutrients to support embryonic, placental and fetal development together with her growth and maintenance and milk production (Banos *et al.*, 2007). Since, 75% of fetal growth occurs during the last trimester and nutrition during the last trimester can further impact organ system, calf birth weight and its long term growth. Soon after parturition, the birth weight of the calf affects the consequent growth rate. Even though adequate nutrition has been provided, the heavier birth weight calf gains weight at a faster rate than the calf with lower birth weight. Similarly, the quality of colostrum secreted by the cow is very important for the lifetime performance of the calf.

2.6.1 Prepartum nutrition of dam & calf birth weight

Fetal growth is considered as exponential by gestational age rather than linear with more than 60% of fetal weight gain being ensued during the final two months of gestation (Prior and Laster, 1979). This fetal growth pattern imposes the greatest nutritional burden on the dam in the last trimester of pregnancy. Consequently, the last 2 months of gestation needs special attention in terms of

provision of essential nutrients. Although the effects of nutrition and proper management like housing and health management are having greater importance, each difference of one kg in birth weight will expand to about 1.5 kg difference by twelve weeks of age (Mudgal *et al.*, 1995). Corah *et al.* (1975) reported that calves born to energy deficit diets (65% of NRC) were 2 kg lighter at birth (30.6 Vs. 28.6 kg), 13 kg lighter at weaning (160.6 Vs. 147.6 kg) in primiparous beef cows during last 100 days of pregnancy when compared to 100 % NRC recommended diet group. Loerch (1996) reported that limit feeding of high grain diet (corn) to beef cow during late gestation resulted in heavier birth weight calves i.e. 4.2 kg heavier (47.1 Vs 42.9 kg) compared to cows fed from isocaloric hay-based diets. This effect may be due to increased energy supplement to the dam during the fast-growing phase of the foetus.

Khan *et al.* (2004) reported that calves born from the cows fed with 30% less than recommended energy (32.6 ± 2.56 MJ ME/cow/day) for 3 months prior to calving were 2.8 kg lighter birth weight than cow fed with 30% more than recommended energy (50.57 ± 3.66 MJ ME/cow/day). They reported that low energy supplementation in the last trimester of pregnancy was not having any significant effect on the growth rate of the calf (0.29 ± 0.10 kg/calf/day vs 0.27 ± 0.10 kg/calf/day). Nowak *et al.* (2012) reported that mean calf birth weight (45.1 kg vs 45.4 kg) and daily weight gain (175 vs 143 g) were not different among the group of cows fed with *ad libitum* (2% BW) and restricted feeding (1.5% BW). Roche *et al.* (2005) also found that there is no relationship between different levels of DMI by a cow at 1.3%, 1.9%, 2.4%, 2.6% BW on calf birth weight of the calf. The outcome of various studies indicates that the calf birth weight is seldom influenced by the prepartum nutrition of the cow because it is compensated from maternal body reserves irrespective of different nutritional limitation such as NEB, less protein in the diet, minerals etc. The first weeks of life for neonates seem to have long-lasting consequences on the physiological function of the animal. Thus, nutritional management of pregnant cow not only has consequences for cow productivity but also the productivity of the next generation of the cow (Schoonmaker and Eastridge, 2013).

2.6.2 Prepartum nutrition of dam & colostrum quality

The process of colostrogenesis takes place in the prepartum period, during which immunoglobulins (Ig) are transferred from the dam's circulation to mammary secretions and subsequently the transfer of Ig ceases immediately prior to parturition (Barrington and Parish, 2001). Colostrum is the prime source of nutrients and immunity for the survival of a newborn (Quigley and Drewry, 1998). Colostrum contains bioactive components with immune-enhancing properties: Immunoglobulins, lactoferrin, lysozyme, lactoperoxidase, α -lactalbumin, β -lactoglobulin, or fat that carries important vitamins and polyunsaturated fatty acids (Kamila *et al.*, 2019). During this specific period, up to 500 grams of IgG/week is transferred into mammary secretions (Brandon *et al.*, 1971). The bovine dam has a syndesmochorial placenta, which averts transmission of antibodies to the foetus while it is in the gravid uterus. Therefore, the calf is born agammaglobulinemic and is exclusively dependent on colostrum for the passive transfer of immunoglobulins, immune cells, and nutrients that only minimally cross the placenta such as fat-soluble vitamins (Barrington and Parish, 2001). The transfer of passive immunity from cow to calf via colostrum is considered adequate when the IgG concentration in the serum of the calf exceeds 10 mg/mL in the 24-48 hr after birth (Godden, 2008; Logue and Mayne, 2014). Successful passive transfer of maternal immunoglobulin has been related to lower veterinary costs to the neonatal calf (Lorenz *et al.*, 2011). Dunn *et al.* (2017) reported that cows fed with grass silage with 3kg/d concentrate supplementation had a significant ($P < 0.05$) higher IgG concentration in the circulation of dam's blood in prepartum period than cows fed with only gross silage but had no effect on colostrum IgG concentration (55.2 vs 53.3 mg/mL) IgG yield in first milking (390 g vs 365 g) or colostrum composition. Westland *et al.* (2017) inferred that feeding 1.33% of mannan oligosaccharide to prepartum cow had no effect on IgG concentration and mass (53.7 IgG g/L vs 42.7 IgG g/L) but, cow yielded 7.5 kg/d colostrum compared to 5.6 kg in counterparts without mannan oligosaccharide supplementation. This increased quantity of colostrum may be due to enhancement of the dam immune system i.e., both specific and innate immunity leading to more efficient metabolism and enhanced colostrum production. Similar effects were observed when cows in the prepartum period received trace minerals

e.g., Mn, Zn, Cu (Roshanzamir *et al.*, 2019) and Se (Moeini *et al.*, 2011) because of their prominence in physiological functions (Suttle, 2010). Fat-soluble vitamins viz., vitamin E (Moeini *et al.*, 2011) and carotene (Kaewlamun *et al.*, 2011) supplementation also improved the colostrum quality but no effect on IgG. Supplementation of 1g beta carotene/cow/d in late gestation resulted in increased carotene concentration in colostrum (3.10 ± 0.23 mg/l to 1.44 ± 0.24 mg/l $P < 0.001$) than non-supplemented group (Kaewlamun *et al.*, 2011). This is due to carotene transferred from blood to colostrum at the time of colostrogenesis (Barrington and Parish, 2001). Aragona *et al.*, (2016) reported that supplementation of nicotinic acid 48 g/d prior to 4 weeks of the expected date of calving increased colostrum IgG concentration by 18 % when compared to the non-supplemented group. This may be due to facilitation of more blood flow to the mammary gland at the time of colostrogenesis due to vasodilation caused by activation of G protein-coupled receptor 109A.

Even the source of starch also appears to influence the colostrum quality. The total protein (13.70 vs. 12.99%), total solids (25.2 vs. 23.96%), total IgG concentration in colostrum (106.33 vs. 79.72mg/mL) were significantly higher for cows received wheat than maize starch in their diets (Fatahnia *et al.*, 2012). This increase in protein, total solids, total IgG might be related to a higher concentration of plasma TP in cow fed wheat which contains more protein than maize. The improved quality of colostrum cannot be implicated to starch because Kupczynski *et al.*, (2014) reported no effect on β -lactoglobulin (109.59 ± 12.68 g/L) when cows fed 500 ml of propylene glycol as an energy source compared to non supplemented group (106.34 ± 21.23 g/l). Research works suggested that colostrum IgG concentration not significantly affected by nutrition but had an effect on Ig absorption (Hough *et al.*, 1990; Swecker *et al.*, 1995)

2.6.3 Prepartum nutrition of dam & calf health

Dam health and also nutrition status affects calf survival. Calves born to the dams with inadequate nutrition at last trimester of pregnancy are mostly affected with prolonged anorexia, or septicemia and weakness. A calf should normally lift its head, attain sternal recumbency and attempt to stand and to stand

spontaneously, on average, 3, 5, 20 and 60-90 minutes after birth, respectively (Houwing *et al.*, 1990). In all livestock species, including cow, offspring born at an above-average BW have a greater chance of survival rate compared to those born at below-average BW. The cows maintained on crude protein-deficient diets in the prepartum period produced calves exhibiting weak calf syndrome (Bull *et al.*, 1979). Prepartum cow nutrition influences calf survival in both heifers and cows. The calves born to heifers and cows fed a ration with 65% of energy recommended by NRC were lost 7 and 10% at birth than those received 100% recommended energy (Corah *et al.*, 1975). The prepartum cow fed 70% of their calculated energy requirement during the last trimester produced calves with high morbidity and mortality rates (Corah *et al.*, 1975). Increased disease susceptibility observed in calves born to heifers mostly due to low volume of colostrum production by primigravid heifers. Calves born to thin heifers with BCS 3 and 4 had reduced serum Ig concentration at 24 h of calving. Morbidity in newly born calf not only increases the rate of mortality and reduces the performance. General morbidity in neonatal calf results in reducing the BW. Heifers restricted either energy or protein during the last trimester of pregnancy produce a calf with less ability to produce heat immediately after birth and are more susceptible to cold stress (Ridder *et al.*, 1991). According to Asmare and Kiros (2016) dam disease and poor body condition were significantly linked to high morbidity (83.3%) and mortality (22.2%) in dairy calves. Nutrient deprivation of the dam may increase serum cortisol and reduce serum triiodothyronine. Consequently, calves may be born with reduced metabolic rates, lower vigour, and, possibly, lower chances of survival (Hough *et al.*, 1990). Recent research also suggests that negative DCAD diets might increase the incidence of calves born in respiratory acidosis, which may impair the acquisition of passive immunity (Quigley and Drewry, 1998; Diehl *et al.*, 2018). At birth, calves born to -180 DCAD dams experienced a subtle and transient metabolic acidosis (pH = 7.33 ± 0.02 ; pCO₂ = 53.0 ± 2.4 mmHg; HCO₃⁻ = 27.6 ± 0.7 mmol/L) compared with the more evident metabolic acidosis observed in those calves born to -70 DCAD cows (pH = 7.28 ± 0.02 ; pCO₂ = 59.3 ± 2.4 mmHg; HCO₃⁻ = 27.8 ± 0.7 mmol/L). There was no relationship between measures of metabolic acidosis and measures of the efficiency of IgG absorption (Collazos *et al.*, 2017).

2.7 Parturition nutrition & postpartum performance of cows

The postpartum period is an entirely different physiological process of gradual upscaling to another reproductive cycle of cow life along with galactopoiesis. Parturition cow nutritional management transcribes the proclamation of the well-being of cow from parturition to resumption of ovarian cyclicity postpartum. How best the cow adapt to lactation and high milk production, sustain to metabolic and physiological imbalance and, returning to normal health, ovulation and conception are dependent on the nutrition management it receives during the parturition period (Drackley and Cardoso, 2014).

2.7.1 Postpartum intake & digestibility

It has been a well-known fact that postpartum feed intake is decreased in cows that are over-conditioned at parturition (Reid *et al.*, 1986). During the postpartum period, the mammary requirement of the same animal on 4d postpartum for energy was 3 times more than the parturition gravid uterus (Bell, 1995). Feeding increased amount of grain during the parturition period or close-up period results in increased propionate production during rumen fermentation which stimulates rumen papillae development and rumen microbes acclimatize to more fermentable diets in the postpartum period. DeFrain *et al.* (2004) replaced the glycerol with corn starch in diet from 14 d parturition to 21 d in milk and observed only decrease in DMI in cows replaced with glycerol during the parturition period but not in the postpartum period. However, a decline in DMI during the first 2 weeks of the postpartum period was experienced even when cows were force-fed during the parturition period (Bertics *et al.*, 1992). DMI (15.8 vs. 11.9 kg/d) was significantly higher ($P < 0.01$) for cows receiving the non-forage fiber supplement such as 28% of the forage in the diet with cottonseed hull and soy hulls when compared with those receiving 70% forage diets 60 d before calving (Pickett *et al.*, 2003). Non-fiber forage supplement influence positively digestion kinetics and NDF digestibility of parturition diets than forage fiber diets. Schröder and Südekum (1999) supplemented low starch concentration diets with different glycerol levels i.e. 48, 78, 131, 185 g/d on DM basis in sheep and stated that there was no positive effect of glycerol on organic matter, cell wall component

digestibility, but the similar level of glycerol with high starch diet resulted in cell wall digestibility reduction.

Dann *et al.* (1999) fed corn-based TMR diets from 28 d prior to calving to 63 d postpartum. The whole tract apparent DM, OM, CP, NDF and ADF, 58, 64, 55, 33 and 39% respectively, and same diets postpartum digestibility 61, 64, 59, 39 and 35, respectively. Vanzant *et al.*(1991) also reported that OM digestibility did not differ in heifers between the close-up period and 26 d postpartum. Okine and Mathison (1991) reported that mature dairy cows experienced postpartum depression in DM digestibility concomitant with increased DMI in dairy cattle. In contrast to prepartum cows which require only 12 to 14% CP, a postpartum cow requires 18 and 19 % CP to meet the protein requirement of milk production (Nocek, 1995; NRC, 1989). The estimated mammary requirement for energy is three times higher than that of the prepartum at 4-day postpartum (Bell, 1995). It was estimated that an HF cow producing 30 kg milk at day 4 postpartum requires 2.7 times of glucose, 2 times of fatty acids, 4.5 times of fatty acids than the requirement of the gravid uterus of the same animal in the prepartum period. Thus, there is an abrupt increase in requirements during the postpartum period for specific nutrients and energy compared to the nutrient requirement in the prepartum period (Bell, 1995).

2.7.2 Body condition score & net energy balance

The decreased DMI in postpartum and increased demand for nutrients for milk necessitates more loss to BCS to meet the nutritional demands. DeFrain *et al.* (2004) stated that feeding glycerol from 14 d prepartum to 21 d postpartum did not show any significant difference on postpartum DMI and BCS (3.28 vs. 3.30) and the BCS was negatively associated with energy requirement (Roche *et al.*, 2009). Avoiding over conditioning i.e., BCS > 4.0 on a 5-point scale during the dry period in the cow is of high importance for dairy farmers to prevent metabolic disease during early lactation (Ingvarsen *et al.*, 2003). Most of the researchers supported that a BCS of 3.25 at calving is optimal to allow for sufficient body fat reserves to support the energy requirements for prepartum maintenance, parturition and lactation while minimizing excessive adipose tissue energy

mobilization to the liver and risk of disease during early lactation (Herdt, 2000, Ingvarlsen *et al.*, 2003, DeFrain *et al.*, 2004, Dann *et al.*, 2006, Roche *et al.*, 2009). Overconsumption of energy during the dry period or prepartum period increases the severity of NEB, reduces the liver function and increases the risk of metabolic diseases during early lactation (Herdt, 2000, Dann *et al.*, 2006). Controlling or restricting prepartum energy intake yielded metabolic results that were strikingly similar both prepartum and postpartum, independent of the parity group. The use of a bulky diet controlled prepartum energy intake in multiparous and primiparous cows improved metabolic status postpartum and reduced the incidence of health problems. When metabolic profiles are considered collectively, cows overfed energy prepartum exhibited an "over nutrition syndrome" with characteristics of clinical symptoms displayed by diabetic or obese nonruminant subjects. This syndrome likely contributed to metabolic dysfunction postpartum (Janovick *et al.*, 2011).

The rapid increase in milk yield, concomitant physiological changes during the early postpartum period is often characterized by reduced DMI resulting in NEB and increased blood NEFA (Bell, 1995). The NEFA are taken up by the liver at ~26% of whole-body NEFA flux (Drackley *et al.*, 2001). Looor *et al.* (2006) fed the animal with 100% of NRC requirement of energy or 20% less or 30% more than NRC requirement from -65 days prepartum. They concluded that a higher or lower level than the required energy creates an overload on transcriptomic adaptation and expression. Either restricted or overfed energy had a strong effect on transcriptomic adaptation to lactation which will affect many metabolic pathways of the liver, mammary and adipose tissue (**Fig 2.6**).

The up-regulated genes cause more stress to the animal for adaptation. The enhanced density of energy causes a detrimental effect on animal health. This is one of the reasons for the failure of challenge feeding or steam up diet to the periparturient animals. Feeding protein and energy as per requirements are more practical and viable.

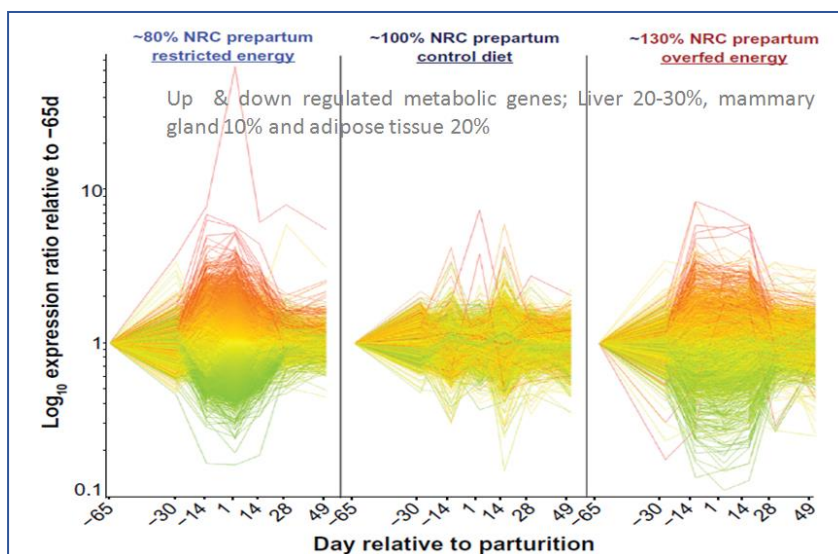


Fig. 2.6: Up and down-regulated genes for different levels of energy in diet during pre and postpartum (Loor *et al.*, 2006)

2.7.3 Postpartum metabolic disorders & challenges

The disproportion between the genetically determined ability for milk production and the limitations in improving the energy value of the ration may be the cause of metabolic disorders, e.g., displaced abomasum, ketosis, milk fever. Feeding an energy-dense diet 3 weeks before calving reduced the incidence of NEB (Drackley *et al.*, 2005) however, overconsumption of energy increased the risk of postpartum metabolic disease (Janovick *et al.*, 2011). Cows fed more propiogenic diets in early lactation (high starch or monensin) exhibited improved energy metabolism during early lactation. Reducing the number of diet changes before calving could facilitate ruminal adaptation to the lactation diet and improve energy balance postpartum. Accumulation of acetyl CoA and an increase in the synthesis of ketone bodies are major metabolic change observed soon after parturition due to increased demand on the protein and energy for galactopoiesis (Wieland *et al.*, 1964). Lack of carnitine and insufficient supply of B vitamins especially niacin are predisposing factors for the incomplete oxidation of acetyl CoA in the liver resulting in ketone body production (Longo *et al.*, 2016). However, feeding 87% of roughage by addition of straw in the diet for last 21 d of calving reduced the occurrence of subclinical ketosis and displaced abomasum in transition cows (Janovick *et al.*, 2011, Vickers *et al.*, 2013,) Hence, controlling

prepartum energy intake can reduce the occurrence of ketosis. The cows those develop ketosis often has lesser DMI (1.54 vs. 1.67 kg/100 kg BW) prior to calving than healthy cows (Pérez-Báez *et al.*, 2019). Each 1 Mcal decrease in the average of energy balance may reduce average DMI at the rate of 0.1% BW in last 3 d of calving and increase the occurrence of ketosis by 5% and 8%, respectively (Pérez-Báez *et al.*, 2019). Goldhawk *et al.*, (2009) reported that cows those with subclinical ketosis in the postpartum had shown an 18% reduction in DMI in the last week of calving compared to healthy cows. Clinical ketosis is accompanying with 2 fold increases the risk of clinical mastitis (Oltenuacu and Ekesbo, 1994) and the concentration of BHBA showed a strong positive correlation to the severity of mastitis induced by *E. coli* (Kremer *et al.*, 1993).

DeFrain *et al.*, (2004) evaluated glycerol supplementation in the diet of periparturient cows. Glycerol (0, 0.43, or 0.86 kg/d) was top-dressed with one-third of a TMR and fed to transition cows (n=10 per treatment) from 14 d prepartum to 21 d postpartum. Reported that glycerol treatments did not affect prepartum concentrations of glucose, insulin, NEFA or BHBA; however, postpartum concentrations of plasma glucose tended to be higher for the cows fed the control diet compared to that fed glycerol (65.8 vs. 63.0 and 60.1 mg/dL). Glycerol administration had little effect on postpartum blood metabolites. In a similar study, cows fed with glycerol at 11.5 and 10.8% ration DM for pre and postpartum respectively, from -28 to +56 d of calving had less than 1.4mmol/L of BHBA which has been considered as the threshold value for subclinical ketosis in lactation period (Carvalho *et al.*, 2011). Apart from metabolic disorders set in the postpartum period due to prepartum nutritional limitation, cows suffer oxidative stress from the release of free radicals in the bloodstream in the postpartum period (Sordillo, 2016). However, there is a normal response to the free radical formation during the postpartum period by the production of antioxidant enzymes which are regulated by minerals like Cu, Se.

2.7.4 Prepartum dietary initiatives to reduce postpartum consequences

Apart from the review of literature cited under 2.2 (Nutrient requirement of prepartum cows), supplementing 6 g niacin for 2 wk prepartum period and 12 g niacin for 4 wk postpartum period could reduce the plasma concentration of BHBA (5.94 vs. 8.54 mg/dl) and NEFA (11.59 vs. 15.23 mg/dl) during the postpartum period (Dufva *et al.*, 1983). Earlier research work showed reduced peak clinical response to *E. coli* mastitis and lowered somatic cell count when cows provided Cu supplementation during the prepartum period as Cu has a role in immune function by increasing capability of neutrophils to kill *Escherichia coli* (Scaletti *et al.*, 2003). Not only Cu but also Na, K, Mg and Se supplementation in the prepartum period affect somatic cell count in postpartum milk of transition cows and udder health (Warken *et al.*, 2018). These minerals minimize oxidative stress in the postpartum period. Warken *et al.* (2018) reported that Mg, P, K, Cu and Se supplemented cows had lower levels of reactive oxygen species and increased superoxide enzymes activity. Zn is a component in the structure of superoxide dismutase the enzyme involved in the antioxidant process. Kuczyńska *et al.* (2013) reported a positive correlation between Zn and keratin. Zn also involved in T-lymphocyte activity. Total protein, globulin and cytokine levels were higher in cows supplemented with mineral complexes. Therefore, it was concluded that subcutaneous mineral supplementation improved the immune response and minimized the oxidative stress in dairy cows during lactation. Cu and Se supplementation increase the interleukin -6 level to protect the animal against mastitis (Nakajima, 1997). Chandra *et al.* (2013) reported that supplementation of vitamin E (1000 IU/d/cow) along with Zn (60 ppm) from 60 d prepartum to 90 d postpartum reduced plasma thiobarbituric acid reactive substance (indicator for lipid peroxidation activity) in the postpartum group than non-supplemented group (3 μ M vs. 1.4 μ M). Both the nutrients neutralize the formation of reactive oxygen species during the postpartum period. The lower levels of the thiobarbituric acid reactive substance in Vitamin E treated group was also reported by Bouwstra *et al.* (2010).

2.7.5 Hematological changes in postpartum

Haematological parameters in postpartum period reflect the adaptability of the cow to physiological change i.e. from non-lactation to lactation and informative tool along with other diagnostic procedures (Ronald *et al.*, 2014). Haematological and biochemical profile with normal physiological range reflects a good health status of the animal and is highly correlated with milk production (Coroian *et al.*, 2017). Oliveira *et al.* (2019) reported TEC was significantly higher on 30day postpartum period when compared to calving day ($9.11 \times 10^6/\mu\text{L}$ vs. $11.19 \times 10^6/\mu\text{L}$). RBC count was significantly higher until two weeks after calving than before calving which was due to erythrocytosis at the time of uterine involution and circulatory endocrine changes (Stirnemann *et al.*, 1974). However, some workers reported lower values of erythrocyte count ($6.72 \times 10^6/\mu\text{L}$ vs. $5.90 \times 10^6/\mu\text{L}$) in the postpartum period non-significantly from the day of calving to four weeks postpartum (Meher *et al.*, 2002). This was due to the overproduction of adrenaline stress at the time followed by a higher level of estrogen concentration after parturition apart from galactopoiesis stress. Hb reduces (10.87 g/dL vs. 10.15 g/dL) significantly from the day of calving to 30 days postpartum (Joshi *et al.*, 2018). In early post-partum period, lower Hb is due to a decreased rate of erythropoiesis, anaemia or increased Hb requirement of mammary tissues for milk synthesis and the concomitant rise in blood flow to mammary glands (Kumar and Pachauri, 2000). The reduced RBC and Hb can predictably reduce the oxygen-carrying capacity and slow the metabolism. The postpartum period did not significantly affect the MCV, MCH and MCHC (Klinkon and Zadnik, 1999). Increased Total leucocyte count (12.45 vs. $7.49 \times 10^6/\mu\text{L}$) around the calving is mediated by a rise in cortisol hormone level whereas decreased leucocyte after calving is associated with the migration of leucocytes towards uterine lumen and mammary gland (Patel *et al.*, 2017). Kehrl *et al.* (1989) reported that during the first week after calving lymphocyte blastogenesis was markedly reduced in comparison to prepartum. Lymphocytes and neutrophils are directly affected by calving stress and neuroendocrine changes associated with the postpartum period. This is also reflected in differential leucocyte count. According to Schalm *et al.* (1986) mean absolute value of the neutrophils significantly increase to 55.4% on the day of calving and decreases to a mean of

47.3% after 24 h. The decreased mean value of eosinophil on the day of calving and continued decreasing for 2 weeks after calving has been reported (Pomsel, 1980). Joshi *et al.* (2018) reported a significantly higher level of platelet count on day 7 and 15 after parturition as compared to the day of parturition (457.33 ± 67.79 , 572.33 ± 66.89 and $300.67 \pm 21.69 \times 10^6/\mu\text{L}$). The decrease in platelet count at the time of parturition could be due to the utilization of a large number of platelets while controlling the haemorrhage during parturition (Joshi *et al.*, 2018).

2.7.6 Prepartum nutrition & Postpartum gut health

Cows fed 300 ml of glycerine/d in TMR from -7 to +21 d of parturition showed no difference in AST and GGT but ALT activity was increased significantly (Kupczynski *et al.*, 2011) Although Ballard *et al.* (2001) also reported no change in AST activity, Mikula *et al.* (2008) observed significantly low ($P < 0.01$) AST activity in postpartum with 400g of glycerol supplementation from 14 d prior calving to till calving. However, Mikula *et al.* (2008) reported no significant difference in GGT. No change in AST activity during postpartum was also reported when cows fed Propylene glycerol in prepartum period (Hoedemaker *et al.*, 2004) but, its supplementation from 10 prior to calving increased total cholesterol level significantly ($P < 0.05$) at d 25 and 50 postpartum (Formigoni *et al.*, 1996). Kupczynski *et al.* (2011) also observed significantly increased total cholesterol level ($P < 0.05$) in cows fed 300ml/d glycerol from 7d prior to calving until 3 wk postpartum period compared to non-supplemented group (3.02 vs. 1.95 mmol/L) during the postpartum period. Significantly high total cholesterol level in the treatment group suggests that energy balance was improved by propylene glycol supplementation. Lower DMI depressed the hepatic total cholesterol synthesis (Reid *et al.*, 1983).

Any marginal increase within physiological range in AST activity during first 2 wks of postpartum can be possible due to adjustment of the liver cells turnover rate to the increased metabolic demands during early lactation (Bostedt, 1974). Total cholesterol concentration was not affected during the postpartum period (3.55 mmol/L vs. 3.48 mmol /L) by top dressing the diet with 400 g

powered propylene glycol from 14 days before parturition to till parturition (Mikula *et al.*, 2008). Bhimte *et al.* (2018) reported that TP (5.8 vs.4.7 mg/dL) and albumin (3.7 vs. 3.4 mg/dL) concentration was significantly more in minerals (Se, Zn, Cu) and vitamin E supplemented cows from 28 d prepartum to 56 d postpartum because of reduced protein catabolism by mitigating oxidative stress. Even AST and ALT activity also reduced in the postpartum period which indicates decreased transaminase enzyme activity which otherwise, indicate stress and liver damage status (Sattler and Fürll, 2004).

2.7.7 Milk yield and quality

Chandra *et al.* (2013) conducted a study to evaluate the effect of vitamin E (1,000 IU/d/cow) or Zn (60 ppm) or both together supplementation to 36 Sahiwal cows from 60 d prepartum to 90 d postpartum on milk production and observed milk yield was significantly higher ($p < 0.05$) in cows supplemented vitamin E plus Zn together. A similar type of study conducted by Roshanzamir *et al.* (2019) by supplementing $MnSO_4$, $Zn\ So_4$ and $Cu\ So_4$, glycine and methionine salts and noticed significantly higher milk yield from 22 to 100 d. Supplementation with Cr-Methionine during the periparturient period also increase milk yield but at the cost of milk protein (Sadri *et al.*, 2009). Besides, Hayiril *et al.* (2001) reported quadratic increases in the pre- and postpartum DMI by supplementation of Cr-Methionine. Milk yield for multiparous cows fed with anionic diets prepartum was significantly higher ($P < 0.05$) when compared with cows fed with a control diet (43.5 vs. 36.6 kg/d). However, no significant difference was observed in the milk fat and milk protein % for both groups. The increase in milk production in the anion supplemented group may be due to increasing DMI in the postpartum period (DeGroot *et al.*, 2010) evaluated the effect of prepartum anion supplementation on milk production.

DeFrain *et al.* (2004) studied the effects of feeding glycerol (430 or 860 g/d) 2 wk prior to 3 wk after calving on lactation performance and observed no significant difference in milk yield without or with glycerol. The other studies of Linke *et al.* (2004) and, Osborne *et al.* (2009) also reported no effect ($P > 0.05$) of glycerol supplementation at the rate of 20 g/l of drinking water or drenching 800

g/d on milk yield and composition. However, glycerol increased molar proportions of plasma propionate, butyrate with decreased proportions of acetate. Ballard *et al.* (2001) supplemented dry energy diet consisted of 22% sugar cane molasses, 45% beet pulp, 17% propylene glycol and, 16% Ca propionate in 50:50 ratio to ground corn or corn alone (906 g) or dry energy alone (908 g) along with basal diet and observed that milk yield in ground corn plus dry energy supplemented group was 2.7 and 1.8 kg/d higher than supplementation of exclusively either of the source. This indicated that a combination of moderate and rapidly fermenting carbohydrate source is better to avoid reduced ruminal pH and reduced microbial protein besides lower milk yield. Other research workers opined that no significant effect of feeding the corn irrespective of its form such as cracked, steam flaked or gluteal fed during the dry period on lactation performance (Dann *et al.*, 2009).

2.8 Effect of prepartum nutrition on puerperium

The puerperal period is the period of postpartum in which uterine tissue involutes to normal size and becomes re-epithelialized, the hypothalamo-hypophyseal-ovarian axis resumes cyclical secretions of gonadotrophs, leading to first postpartum ovulation and regular oestrous cycles (Kruip *et al.*, 2001). In general, the whole process completes within 6 weeks of and from this time, inseminations can result in the cow becoming pregnant by 90 days postpartum.

Salehi *et al.* (2016) supplemented rolled sunflower seed (high in linoleic acid), canola seed (high in olic acid) and no oilseed in the control group at 8% on DM basis from 35 days before calving till calving. They reported that prepartum dietary treatment of oilseed has no significant difference on the formation of a dominant follicle after calving was 8.9 ± 0.52 , 9.2 ± 0.47 and 10 ± 0.44 d; the interval from calving to first ovulation was 20.7 ± 1.59 , 22.9 ± 1.5 , 20.6 ± 1.41 d and, pregnancy to first A.I % was 22.6, 25.8 and 33.3 in control, canola seed and sunflower seed, respectively. Hoedemaker *et al.* (2004) supplemented peripartum cows with 10% of propylene glycol i.e., 150 ml of propylene glycol on day 13 prepartum, 300 ml on day 12 prepartum til parturition and 100 ml from the day of calving to 12 d postpartum and reported non-significant difference on the first

service conception rate % (39.4 vs. 39.6), the mean interval from calving to first A.I (89.2 +3.3 vs. 88.6+3.3 days) and interval from A.I to conception (26.2+ 4.0 vs. 28.9+4.7 days) in propylene glycol supplemented and control groups, respectively. Probably, the effect of propylene glycol supplementation was not conspicuous since the cows considered under study were provided with good nutrition and recouped to NEB. Bayril *et al.* (2015) conducted a study on the effect of parenteral supplementation of Se and vitamin E during late pregnancy and inferred Vitamin E and selenium supplementation during peripartum period hastens the expulsion of foetal membranes further promoting involution and uterine health.

Formigoni *et al.* (1996) conducted a research in 39 multiparous HF cows by supplementing 300 g of propylene glycol directly mix with diet from day 10 prior to calving until parturition and diluting in the water on days 3, 6, 9 and 12 after calving. They reported that there was no difference in the percentage of acyclic cows from parturition to day 40 in the treatment and control groups (80% of cows acyclic in both groups). Thereafter, resumption of ovarian function in propylene glycol supplemented group continued and only 30% of animals in the treatment group were acyclic on day 90 whereas, 58% of the cows remain acyclic in control group ($P < 0.001$). Resumption of an oestrous cycle after parturition is a complex relation between hypothalamus, pituitary and ovary which are influenced by external (nutrition) internal (endocrine) signals. The resumption of the oestrous cycle in propylene glycol supplemented cows may be associated with stimulating luteal cells for progesterone production and greater quantities of cholesterol observed (1.63 + 0.07 mg/ml vs. 1.90+0.08 mg/ml in plasma) than the control group on day 25 and 50 of postpartum. The results imply that energy balance in the postpartum period was improved by administration of propylene glycol in the periparturient period. The cows supplemented with high energy through cracked corn grain 3.5 kg/day during 3 weeks before the expected date of calving were ovulated 12 days earlier ($P < 0.05$) than the 37.4 ± 3.7 d in the control group (Cavestany *et al.*, 2009). Higher energy supplementation in the treatment group increased the insulin level significantly ($P < 0.05$) in the postpartum period thereby reducing the interval from calving to first ovulation. Any increase in insulin concentration promotes the maturation and

differentiation of the dominant follicle, thereby increasing the chance of dominant follicle, further response to LH surge (Butler, 2013). Roth *et al.* (2001) reported that acute stress due to NEB affects the follicular steroidogenesis and follicular size because low insulin and IGF-1 levels during the postpartum period reduced the follicular diameter. Apart from insulin, leptin also plays an important role in the activation of the hypothalamus-pituitary axis and reinitiating of the ovarian activity (Meikle *et al.*, 2004). Supplementation of 8 mg of Cr propionate from 21 days before the expected date of calving to 63 d post-calving had a non-significant increase in the neutrophils per cent in uterine lavage on day 7 postpartum than control (41.1% vs. 32.8%) and the incidences of cytological endometritis between 40 and 60 d postpartum were also decreased significantly ($P < 0.02$) to 50 % (Yasui *et al.*, 2014).

Materials and Methods

3.0 MATERIALS AND METHODS

3.1 Selection of problem

Late gestation diet plays a major role in modulating the cow's predisposition to prepartum health disorders as well as, postpartum production potential. Specific nutrient imbalance in the diet of the late pregnant cow is related to various metabolic problems like; milk fever, retained placenta, displaced abomasum, udder edema, mastitis, ketosis, fatty liver etc. Anyone metabolic problem during the transition phase may lead to another issue to affect subsequent production and reproduction. The research area was identified in the field of nutrition on a thorough examination of field problems in transition cow. Literature was reviewed on the issue and discussions at the time of synopsis approval. Energy and mineral supplementation in prepartum nutrition was considered as the nucleus of the issue and the basic question was; how to improve the postpartum metabolic health of cow during prepartum nutrition?

Many studies were conducted on individual nutrient supplements in peripartum cows and observed their effect in post-partum feed intake, negative energy balance (NEB) or metabolic disorders. There is a paradigm shift in the concept of nutrition for past 3 to 4 decades by departing from the conventional concept of animal requirements based on physiological stage and level of production to means of health, productivity and whole-life performance. Published literature on the effect of interactive nutritional supplements on energy efficiency and metabolic health of peripartum cows is awaited. Contemporary nutritional management does not only focus on the physiological stage but also take care of consequential impacts on the productive life of the cows. The following are some of the facts focused while designing experiments and the nutritional regime of peripartum cows for tuning with their homeostasis and homeorhesis changes:

1. Minimising ruminal disruption to reduce metabolic acidosis/alkalosis.
2. Countering the diminished DMI.

3. Improving prepartum nutrition to reduce NEB.
4. Improving the rumen and liver functional health (gut health).
5. Minimising lipid mobilization and metabolic disorders to reduce the occurrence of ketosis and fatty liver.
6. Minimising mineral deficiencies to avoid pre and postpartum metabolic complications.

3.2 Place and Weather

3.2.1 Topography

The experiment was conducted at the Livestock Research Centre, Southern Regional Station, ICAR-NDRI, Bengaluru. The latitude (Φ), longitude (λ) and elevation of the experimental place were 12.947014°N (12° 56' 49.2504" N), 77.607679 (77° 36' 27.6444" E) and 921 m from mean sea level, respectively. The tropical climate in Bengaluru is considered to be Aw (Savanna, wet) according to the Köppen-Geiger climate classification with the maximum ambient temperature in summer goes up to 32°C and minimum temperature in winter comes down to about 10°C with diurnal variation to the order of 17-26°C. The average annual rainfall is 859 mm, most of which is received from June to September and second spell in November to December.

3.2.2 Weather

Weather conditions like the monthly average of the minimum and maximum temperature, relative humidity, heat index, wind velocity and average rainfall during the study period (November 2018 to March 2020) is shown in **Table 3.1**. The heat index was calculated based on the polynomial equation (Srinivas *et al.*, 2008). As per the cattle heat index chart based on temperature and humidity, is normal $\leq 75^\circ\text{F}$, alert between 75 to 78°F , danger $\geq 79^\circ\text{F}$ and emergency $\geq 84^\circ\text{F}$.

$$\text{HI } (^\circ\text{F}) = -42.379 + (2.0490 \times \text{T}^\circ\text{F}) + (10.143 \times \text{RH}) - (0.22476 \times \text{T}^\circ\text{F} \times \text{RH}) - (6.8378 \times 10^{-3} \times \text{T}^2^\circ\text{F}) - (5.4817 \times 10^{-2} \times \text{RH}^2) + (1.2287 \times 10^{-3} \times \text{T}^2^\circ\text{F} \times \text{RH}) + (8.5282 \times 10^{-4} \times \text{RH}^2 \times \text{T}^\circ\text{F}) - (1.99 \times 10^{-6} \times \text{T}^2^\circ\text{F} \times \text{RH}^2)$$

Table 3.1: Weather forcing parameters during experimental period and heat index

Parameter	Year & Month																
	2018			2019											2020		
	Nov	Dec	Jan	Feb	Mar	Apr	May	June	July	Aug	Sep	Oct	Nov	Dec	Jan	Feb	March
Ambient Temperature (°C)																	
Max	28	28	15	31	34	34	33	31	29	21	21	21	20	19	18	17	17
Min	19	18	28	18	22	23	23	22	21	27	28	29	28	29	29	30	30
Mean	22.5	25	21	25	27	28	26.5	25.5	24	23	24	23	23.5	23	23	23.5	23.5
Wind speed (kmph)																	
Max	20	18	26	28	21	20	22	26	28	28	29	17	17	18	16	17	16
Min	0	0	0	0	0	0	0	9	0	0	0	0	0	0	0	0	0
Mean	10	9	13	14	10.5	10	11	13	14	14	14.5	8.5	8.5	9	8	8.5	8
Humidity (%)																	
Max	100	94	100	100	94	94	100	100	100	100	100	100	100	100	100	100	100
Min	22	28	3	9	11	15	23	21	43	34	25	22	27	42	11	22	22
Mean	61	61	51.5	54.5	52.5	54.5	61.5	60.5	71.5	67	62.5	61	63.5	71	55.5	61	61
Rain Fall (mm)																	
Mean	20	5	0	0	0	0	0	57	53	136	137	231	24	0	0	0	0
Heat Index (°F)																	
Max	112	108	142	150	163	163	173	139	120	112	112	120	120	112	139	129	129
Min	56	54	49	55	61	67	68	67	65	66	66	66	62	59	55	58	58
Mean	84	81	96	103	112	115	121	103	93	89	89	93	91	86	97	94	94

Where,

HI = Heat index in °Fahrenheit

T²°F = Temperature in °Fahrenheit

RH = Relative humidity

3.3 *In vitro* experiments

In vitro experiments were conducted to evaluate the change in substrate fermentation after 10% fortification of mono, di or polysaccharide source of energy without or with functional supplementation using *in-vitro* Gas Production (IVGP) technique (Menke and Steingass, 1988). Gases accumulated during incubation were the end products of anaerobic fermentation and the pattern of their production at different intervals was subjected to kinetic model for understanding the substrate fermentation pattern.

3.3.1 Substrate and fortification

The substrate consisted of mixed green fodder (MGF), dry fodder and concentrate supplement. MGF consisted of Para grass (*Bracharia mutica*), maize (*Zea maize*), Hybrid Napier (*Pennisetum glaucum* × *Pennisetum purpureum*) in the proportion of 25:25:50. Dry roughage fed was Finger millet (Ragi) straw (*Elusine coracana*). The ingredient composition of concentrate supplement (CS) fed is presented in

Table 3.2.

Table 3.2: Ingredient composition of concentrate pellets

Sl. No.	Ingredient	Proportion (%)
1.	Maize grain	38
2.	Rapeseed meal deoiled	3
3.	Cottonseed meal deoiled	7
4.	Coconut meal deoiled	12
5.	Rice bran deoiled	20
6.	Rice polish	5
7.	Molasses	10
8.	Common salt	1
9.	Calcite powder	2
10.	Mineral mixture	1
11.	Urea	1

Supplied as per the composition by M/s Karnataka Milk Federation, Bengaluru

The ratio of mixed green fodder, dry roughage and CS in the total mixed substrate (TMS) was 50:20:30 where roughage to CS ratio was 70:30. TMS was taken as a basal substrate. TMS was fortified by the energy in the form of Glycerol as monosaccharide derivative (Polyol) or Jaggery as disaccharide (Fructose + Glucose) or Maize as a starch polysaccharide (Glucose polymers) and balanced calorific value (Isocaloric) of the sources to 10% of gross energy of the TMS.

In addition to the source of energy as the main factor, TMS and TMS with a different source of energy were also fortified with functional supplement consisted of selective minerals, niacin (vitamin B3) and ω -3 fatty acids. The requirement of these functional nutrients was calculated as mg/kg DM of the total ration with consideration to *in vivo* experiments on HF graded crossbred cows and Deoni cows.

3.3.2 *In vitro* experimental groups

In vitro Fermentation rate kinetics (IVFK) of TMS fortified with the source of energy without or with functional nutritional supplementation was designed in 2×2 factorial experimental design having the source of energy fortified as the main factor and functional supplement as subfactor with 10 replicates each for main treatment and sub treatment (**Table 3.3**).

Table 3.3: Control and treatments under *in vivo* 2×2 factorial design

Energy (Main Factor)	Suppl. (S) (Sub Factor)	Replicates
Blank*	No substrate	10
Total mixed substrate (TMS)	Without	10
	With	10
TMS + 10% Glycerol	Without	10
	With	10
TMS + 10% Jaggery	Without	10
	With	10
TMS + 10% Maize	Without	10
	With	10

*Only inoculum and buffer

3.3.3 *In vitro* gas production

Rumen liquor was collected once in 4 days at 8.00 AM from cattle fixed with flexible rumen cannula (Large cannula #1C, M/s Bar Diamond, USA) and fed on similar diet as described for TMS described in **3.3.1**. Rumen liquor was drawn before offering feed at 8.00 AM with a minimum of 6 h gap from the last offering of drinking water, collected in the thermos and brought to the laboratory. Immediately, rumen liquor was strained (SRL) through a double layer muslin cloth. SRL was used as inoculum and taken 10 ml in each syringe along with 20 ml of buffer media (Srinivas *et al.*, 2008). Reagents used in the preparation of buffer were;

Main element Solution: 5.7 g of Na_2HPO_4 , 6.2 g KH_2PO_4 , 0.6 g $\text{MgSO}_4 \cdot 7\text{H}_2\text{O}$ and made a volume to 1 L with distilled water.

1. Trace element Solution: 13.2 g $\text{CaCl}_2 \cdot 2\text{H}_2\text{O}$, 10.0 g $\text{MnCl}_2 \cdot 4\text{H}_2\text{O}$, 1.0 g $\text{CoCl}_2 \cdot 6\text{H}_2\text{O}$, 0.8 g $\text{FeCl}_2 \cdot 6\text{H}_2\text{O}$ and made volume to 100 ml with DW.
2. Buffer Solution: 35.0 g NaHCO_3 , 4.0 g $(\text{NH}_4) \text{HCO}_3$ and made volume to 1L with DW.
3. Resazurin Solution: 100 mg Resazurin weighed dissolved in DW and made volume to 100 ml.
4. Reduction Solution: 2.0 ml 1N NaOH, 285.0 mg $\text{Na}_2\text{S} \cdot 7\text{H}_2\text{O}$ and 47.5 ml DW. The reduction solution was freshly prepared each time during the experiment. The other solutions were prepared previously and stored.
5. Preparation of Buffer media: the solutions are mixed in the following order, 474.00 ml DW, 0.12 ml trace element solution, 237.00 ml buffer solution, 237.00 ml main element solution, 1.22 ml resazurin solution and warm to 39 °C.

CO_2 gas was passed through the solutions while the reduction solution was added simultaneously. The slightly bluish solution first turned to reddish colour and then became colourless. The SRL was added only when the buffer media changed to colourless. The ratio of SRL to buffer medium was 1:2 (v/v). CO_2 was flushed continuously for 15 min. The piston of the syringes lubricated

with petroleum jelly was fixed into the cylinder while removing any excess air. Syringes were then incubated in BOD incubator at 39 °C. Gas production in the syringes was measured at 1/2, 1, 2, 4, 8, 12, 18, 24, 36, 48 and 72 h of fermentation intervals (FI). IVGP values at different intervals were adjusted to blank which contained only buffer and inoculum. Blank corrected IVGP values of different time intervals were fitted to the first-order kinetic model using two rate constants (Equation-1) and fermentation rate (Equation-2) according to France *et al.* (1993). These mathematical calculations were carried out using MS Excel worksheets (MS Office, 2007).

$$\text{Kinetic rate of gas production: } Y = A [1 - e^{-k(t-L)-d(\sqrt{t} - \sqrt{L})}] \text{ -----Equation-1}$$

$$\text{Fermentation rate: } \mu^{1/2} = c + (d/2\sqrt{t^{1/2}}) \text{ -----Equation-2}$$

Where,

Y = Total gas production

A = Upper asymptote for total gas production

k = Rate of gas production = $[0.693 \div t^{1/2}]$

d = Rate of gas production at $t^{1/2}$ time $[0.693 \div t^{1/2}\text{-half}]$

t = Time and,

L = Lag time for initiation of gas production

3.4 *In vivo* experiments prepartum

In vivo experiments during the prepartum period were conducted like *in vitro* studies but, additional ‘Breed’ was incorporated as another sub-factor besides the source of energy and supplement.

3.4.1 Experimental animals

The study was undertaken in indigenous (*Bos indicus*) and graded HF crossbred (*Bos taurus*) cattle. A total of 32 prepartum cows (16 indigenous and 16 crossbred cattle) those are at the least 35 d prior to their expected date of calving were selected from Livestock Research Centre of ICAR-National Dairy Research Institute, Southern Regional Station (SRS), Bengaluru. Apart from the date of calving, BW (BW) and parity (**Table 3.4**) were also recorded.

Table 3.4: Graded HF crossbred and Deoni cattle selected for the experiment

Group	Breed	Sl. No.	Animal Id	Date of calving	Parity (No)	BW (kg)
Control	Graded HF crosses	1	C-110	23.01.20	3	325
		2	C-41	14.02.20	3	512
	Deoni	1	D-377	29.01.20	3	330
		2	D-761	08.01.20	3	335
Control + Supplement	Graded HF crosses	1	C-52	10.03.20	5	540
		2	C-39	14.03.20	4	402
	Deoni	1	D-729	09.03.20	3	357
		2	D-745	12.03.20	3	301
10% Maize Fortification	Graded HF crosses	1	C-51	05.12.18	3	504
		2	C-23	18.12.18	5	443
	Deoni	1	D-616	23.12.18	3	350
		2	D-678	23.12.18	4	376
10% Maize Fortification + Supplement	Graded HF crosses	1	C-31	15.10.19	6	555
		2	C-62	16.10.19	3	400
	Deoni	1	D-689	17.10.19	2	350
		2	D-718	22.10.19	3	325
10% Jaggery Fortification	Graded HF crosses	1	C-55	04.12.18	2	444
		2	C-47	20.02.19	4	547
	Deoni	1	D-566	09.04.19	6	385
		2	D-457	01.04.19	3	398
10% Jaggery Fortification + Supplement	Graded HF crosses	1	C-14	02.09.19	3	520
		2	C-54	31.08.19	8	510
	Deoni	1	D- 661	15.10.19	3	375
		2	D-397	05.10.19	8	415
10% Glycerol Fortification	Graded HF crosses	1	C-29	07.03.19	3	475
		2	C-49	01.04.19	5	400
	Deoni	1	D-418	18.03.19	3	365
		2	D-710	09.04.19	4	310
10% Glycerol Fortification + Supplement	Graded HF crosses	1	C-58	14.09.19	3	537
		2	C-28	08.10.19	5	513
	Deoni	1	D-764	28.09.19	3	329
		2	D-713	14.10.19	4	300

3.4.2 Grouping

3.4.2.1 Main factor - Energy

Source of energy was taken as the main factor. Different sources of energy were fortified 10% in addition to the total dietary energy of prepartum cattle. The energy source was identified based on the complexity of their chemical structure. Glycerol is a simple polyol compound that was selected under the category of rapidly released energy source. It has a calorific value of 3990 kcal/kg. Jaggery was selected under the category of disaccharide containing up to 50- 70% sucrose and $\leq 20\%$ invert sugar with a calorific value of 3571 kcal/kg. Maize grain was selected as the third source of energy which is a polysaccharide with the calorific value of 3248 kcal/kg.

3.4.2.2 Sub factor - Supplement

Supplement consisted of micronutrients, niacin (vitamin B3) and ω -3 fatty acid was taken as subfactor to 3 different sources of energy. Micronutrient requirement of prepartum cows was fixed based on the earlier recommendations. Composition of the micronutrient supplements was formulated after deducting the respective mineral element available through the diet (**Table 3.5**). Amount of each mineral required in the supplement was calculated based on the following formula;

$$\text{Mineral salt (mg/kg DM)} = \left(\frac{A \times B}{C \times P} \times 100 \right)$$

Where,

A= Molecular weight of mineral salt

B= Balance amount of mineral elements to be fed

C= Atomic weight of the mineral

P= Purity of mineral salt

3.4.2.3 Sub factor - Breed

Deoni and graded HF crossbred cattle were two different breeds with morphological and production distinction and taken as another sub-factor because pregnancy is a physiological phenomenon resembles in all physiological

Table 3.5: Supplementation of micronutrient and other nutrients to HFX and Deoni cows

Functional Nutrient	(mg/kg DM)
Minerals	90-100mg/d
Vitamin	10-15g/d
Fatty acids	40g/d
The FNS details are undisclosed as per the procedures and mandatory requirements of the patent filed	

* All requirement was calculated based on the purity of the source and gap in the supply for optimum performance

criteria, however, breed variations are morphological. The salient production features were detailed below in nutshell.

- Deoni is a dual purpose cattle breed prevails in Balaghat region of Sahyadri hills, mainly in Maharashtra, India. It is a medium-sized cattle breed and 12 cows in advanced pregnancy were selected. In general, the mean birth weight of Deoni calves is 20 to 22 kg and reaches maturity when they attain a BW of 220 to 240 kg. Their mean milk yield is 770 to 820 kg during mean lactation length of 150 to 190 d. (Basak and Das, 2018).
- HF is a milk purpose cattle breed originating in northern Holland and Friesland. It is a large-sized cattle breed. Pure HF calf mean birth weight is 40 to 50 kg. They reach maturity when attaining a BW of 315 to 340 kg. Their mean milk yield is 6000 to 10000 kg during mean lactation length of 300 to 310 days. In contrast to pure HF, graded HF crossbred calf BW is 26 to 35 kg and mature BW is 280 to 300 kg. Their mean milk yield is 3000 to 4500 kg during mean lactation length of 300 to 310d.

3.5 Animal care

3.5.1 Ethical committee approval

The study was conducted after obtaining the approval from the Institutional Animal Ethics Committee, SRS, NDRI, Bengaluru (CPCSEA/IAEC/LA/SRS-ICAR-NDRI-2019/NO.10).

3.5.2 Housing

Experimental cows were housed in well-ventilated calving shed consisting of 6 × 6 ft calving pens partitioned with 5 ft wall. Calving shed had an arrangement for individual feeding, well-maintained drainage channels and ample air circulation to provide evaporative cooling. Calving pens were cleaned once in the forenoon and afternoon. Experimental cows were bathed every day by splashing the water. Cows were groomed twice in a day in the afternoon and evening. Proper hygienic conditions and healthy surroundings were maintained until 5 d of parturition. Dung was removed periodically as and when required to maintain hygienic

condition. Drinking water is provided at frequent intervals by polypropylene buckets of 30 litres capacity at least 6 times in a day.

3.5.3 Prepartal period management and calving

Relaxation of the pelvic ligament near the pin bones and/or sunken appearance on each side of tail head which were generally observed 2-3 days prior to calving as were taken as symptoms to identify the closer day of the calving and animals were provided with hourly monitoring and care. Apart from these symptoms, few behavioural changes observed 6 h prior to calving were elevation and switching of the tail, mucous discharges from vagina, edematous vulval lips and apathetic feeding behaviour. Soft bedding was arranged with paddy straw at the time of calving. After parturition, the cows were under constant monitoring for recording the time of the expulsion of foetal membranes. If any cow failed to shed foetal membranes within 12 h after parturition, was considered as a case of retention of placenta.

3.6 Nutrient Requirement

Nutrient requirement of the experimental cows was calculated as per the ICAR (2013) recommendations and compared with NRC (2001) recommendations (**Table 3.6**). Control group was fed basal diet as per the requirement without or with supplement. Energy as the main factor was additionally supplemented by 10% to basal diet as Glycerol (G) or Jaggery (J) or Maize (M) to HF graded crossbred (CB) or Deoni breed (DB) of cows where the breed was sub-factor. Another set of both the breeds along with anyone energy source in addition to basal diet were also provided supplement. The diets fed to CB or DB with a different energy source and without or with the supplement is summarized in **table 3.7**.

The supplement consisted of minerals, niacin (vitamin B3) and ω -3 fatty acids. Additional energy provided to CB or DB was balanced for energy content and made isocaloric. Basal diet consisted of MGF, ragi straw (*Elusina Coracona*) and concentrate supplement (CS) pellet. Mixed green fodder consisted of para grass (*Brachiariamutica*), Hybrid Napier (Bajra \times Napier) and Maize (*Zea mays*)

Table 3.6: Nutrient requirement prepartum cows

Allowance	NRC (2001)	ICAR (2013)	The requirement for maintenance & pregnancy
DM (kg)	8.4 ^B	9.73 ^A	A. Based on 8.6 kg DM/400 kg BW and 1.13 kg for pregnancy allowance B. Based on 8.4 kg DM/400 kg bodyweight dry cow @ 270 days of pregnancy
Protein (g)	544.7 ^B	699 ^A	A. Based on crude protein required @ 436g/400 kg BW and 263 g crude protein for pregnancy allowance B. Based on metabolizable protein for prepartum cows @ 605 g/ 400 kg bodyweight dry cow @ 270 days of pregnancy
Energy (Mcal)	60.24 ^B	49.28 ^A	A. Metabolizable Energy (ME) for maintenance and pregnancy @11.82 Mcal/ 400 kg BW and 3.05 MCal for pregnancy allowance B. NE _L for dry cow @14.4 MCal/day for dry cow @ 270 days of pregnancy

Table 3.7: Grouping of animals based on diets

Breed (B)	Suppl. (S)	Group	Diet
Crossbred (CB)	Without	Control	Diet
		CBG	Diet + 10% Glycerol
		CBJ	Diet + 10% Jaggery
		CBM	Diet + 10% Maize
	With	Control	Diet + Suppl.
		CBGS	Diet + 10% Glycerol + Suppl.
		CBJS	Diet + 10% Jaggery + Suppl.
		CBMS	Diet + 10% Maize + Suppl.
Deoni Breed (DB)	Without	Control	Diet
		DBG	Diet + 10% Glycerol
		DBJ	Diet + 10% Jaggery
		DBM	Diet + 10% Maize
	With	Control	Diet + Suppl.
		DBGS	Diet + 10% Glycerol + Suppl.
		DBJS	Diet + 10% Jaggery + Suppl.
		DBMS	Diet + 10% Maize + Suppl.

in the ratio of 25:25:50. Ingredient composition of concentrate supplement was as per the suggested formula supplied by the M/s Karnataka Milk Federation (KMF), Bangalore (**Table 3.6**). All animals were fed with respective diets from 35 ± 5 days prior to calving. Weighed quantities of CS along with energy source with or without functional nutrient supplement fed once only at 8:00 AM. MGF and ragi straw were weighed and fed at 10:00 AM and 7:00 P.M, respectively.

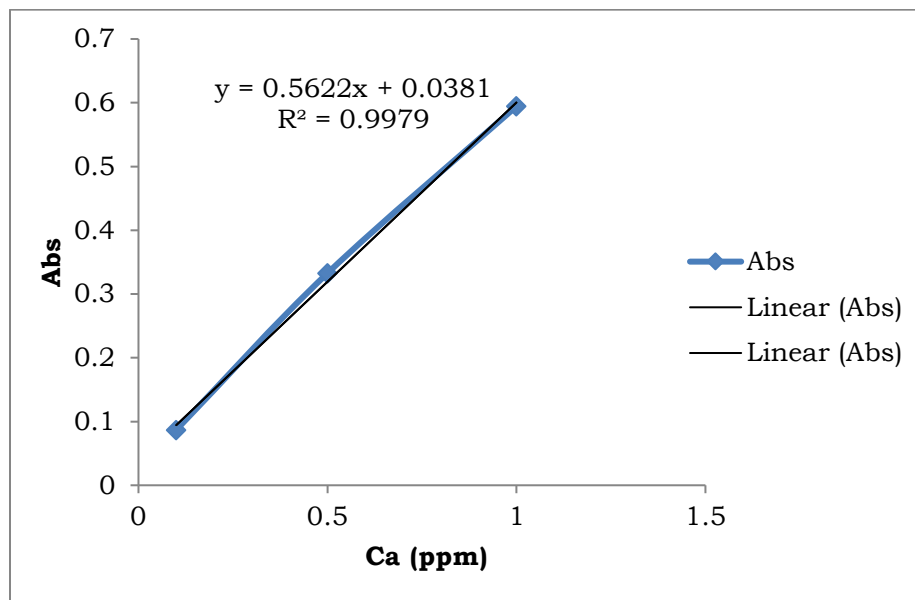
3.7 Mineral estimation in the feed samples

3.7.1 Processing of the sample for mineral estimation by using AAS

About 1 g of feed sample was digested by 9 ml of tri acid mixture consisted of HNO₃:HClO₄: H₂SO₄ in the ratio of 3:2:1 in 100 ml Kjeldahl flask. Glass beads were added to the flask avoid bumping. Contents were heated at very low temperature initially and gradually increased to moderate to a high temperature until the Perchloric acid (HClO₄) fumes ceases and, the contents in the flask were clear. Then the digested sample was diluted with distilled water and filtered through Whatman filter paper No.42 and final volume made up to 25 ml. The contents of different minerals in the samples were analyzed by using optical double beam atomic absorption spectrophotometer (AAS Model Shimadzu-7880, M/s Toshniwal Instruments Pvt., Ltd., India) fitted with 8 hallow cathode lamp on motorized turret. The calibration curve of the mineral element was prepared using blank and working standards solutions of respective minerals. The calibration was periodically verified by analyzing a standard at the frequency of 10 readings. Stock solutions of 1000 ppm of Ca, Mg, Mn, Cu, Zn and Co of AAS grade were procured from M/s Merck Life Sciences Pvt., Ltd., Bengaluru.

3.7.1.1 Calcium (Ca) estimation

Ca element working standards of 0.5, 1, 2, 5 ppm were prepared from the stock solution of 1000 PPM and, the standard curve was prepared (**Figure 3.1**).

Figure 3.1: Standard curve for Calcium estimation in samples

The operating parameters for estimation of Ca were;

- Wavelength - 324 nm
- Slit width - 0.5 nm
- Lamp current - 3.0 mA
- Vapor type - Air/Acetylene

3.7.1.2 Phosphorous (P) estimation

P in the feed sample was estimated by using Ammonium molybdate ($[(\text{NH}_4)_6\text{Mo}_7\text{O}_{24}]$) method by precipitating the Phosphorus present in the ash solution as Ammonium phosphomolybdate ($[(\text{NH}_4)_3\text{PMo}_{12}\text{O}_{40}]$). Ash solution of 10 ml was taken into a beaker and added 10 ml each concentrated Nitric acid (HNO_3) and Ammonium molybdate reagent slowly and simultaneously into the beaker while constantly stirring the contents with a glass rod. Contents of the flask were incubated overnight. At the end of incubation, contents were filtered through No.42 filter paper by decantation without disturbing the precipitate. The precipitate was washed only once with 2% HNO_3 solution followed by, washing 5 to 6 times with 3% neutral Potassium nitrate (KNO_3) solution until the filtrate was free from acid. Acid-free filtrate was confirmed by persisting pink colour of the filtrate that was tested by adding 1 drop of Phenolphthalein indicator and 2 to 3 drops of N/10 Sodium hydroxide (NaOH) to approximately 10 ml of filtrate. After thorough washing, the precipitate along with filter paper was transferred to the same flask. The contents in the flask were thoroughly homogenized with glass

rod by slowly adding 20 ml of DDW followed by adding known quantities of N/10 NaOH using burette until all the homogenate was dissolved. The alkaline contents of the flask were reverse titrated using N/10 Hydrochloric acid (HCl) and the P content in the sample was calculated as below.

$$\text{Phosphorus (\%)} = (X-Y) \times 0.1347 \times \frac{\text{Total vol. of ash sol.}}{\text{Aliquot of ash solution}} \times \frac{100}{\text{Wt. of sample}} \times \frac{1}{1000}$$

Where,

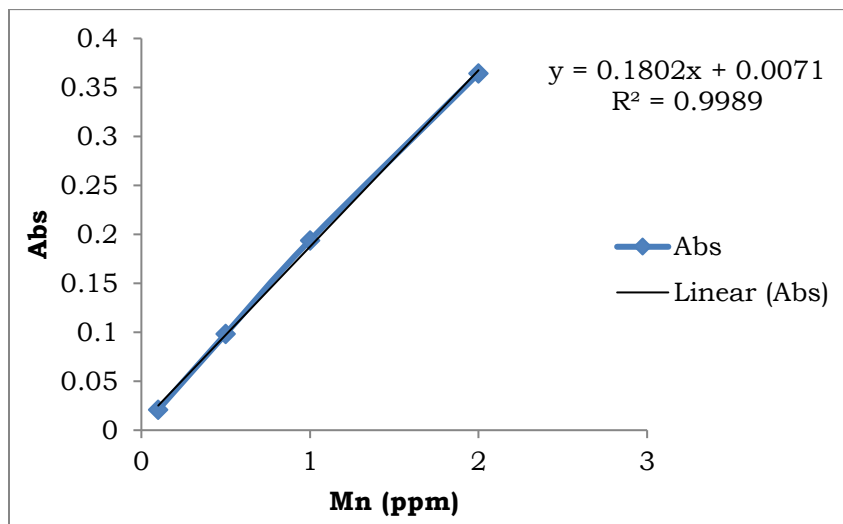
X was ml of standard N/10 NaOH added and,

Y was ml of standard N/10 HCl required for reverse titration

3.7.1.3 Manganese (Mn) estimation

Mn element working standards of 0, 0.1, 0.5, 1, 2 ppm were prepared from the stock solution of 1000 ppm and a standard curve was prepared (**Figure 3.2**).

Figure 3.2: Standard curve for Manganese Mn estimation in samples

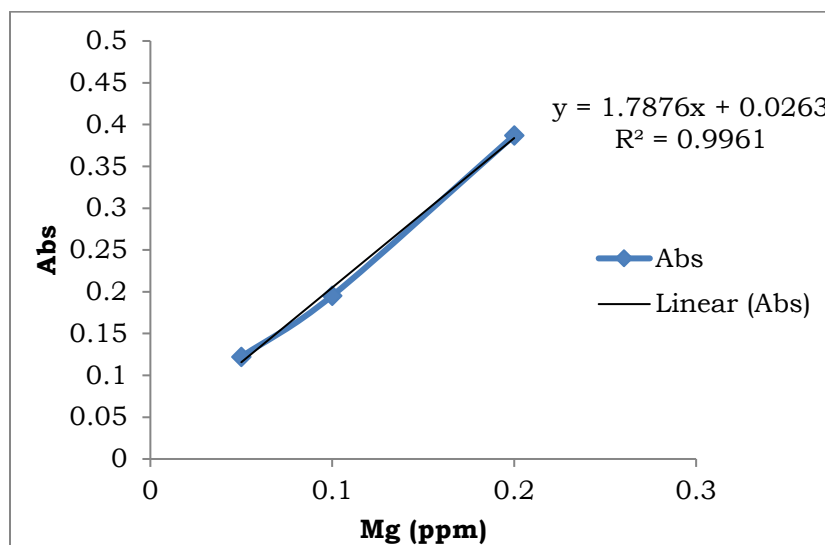


The operating parameters for the estimation of Mn were;

- Wavelength - 279.5 nm
- Slit width - 0.2 nm
- Lamp current - 10.0 mA
- Vapour type - Air/Acetylene

3.7.1.4 Magnesium (Mg) Estimation

Mg element working standards of 0, 0.1, 0.5, 1, 2 ppm were prepared from the stock solution of 1000 ppm and a standard curve was prepared (**Figure 3.3**).

Figure 3.3: Standard curve for Magnesium (Mg) estimation in samples

The operating parameters for estimation of Mg were;

- Wavelength - 285.2 nm
- Slit width - 0.7 nm
- Lamp current - 8.0 mA
- Vapour type - Air/Acetylene.

3.7.1.5 Copper (Cu) estimation

Cu element working standards of 0, 0.1, 0.5, 1, 2 ppm were prepared from the stock solution of 1000 ppm and a standard curve was prepared (**Figure 3.4**).

The operating parameters for the estimation of Cu were;

- Wavelength - 324.8 nm
- Slit width - 0.7 nm
- Lamp current - 6.0 mA
- Vapour type - Air/Acetylene.

3.7.1.6 Zinc (Zn) estimation

Zn element working standards of 0, 0.1, 0.5, 1 ppm were prepared from the stock solution of 1000 ppm and a standard curve was prepared (**Figure 3.5**).

The operating parameters for the estimation of Zn were;

- Wavelength - 213.9 nm
- Slit width - 0.7 nm
- Lamp current - 8.0 mA
- Vapour type - Air/Aacetylene

Figure 3.4: Standard curve for Cu estimation

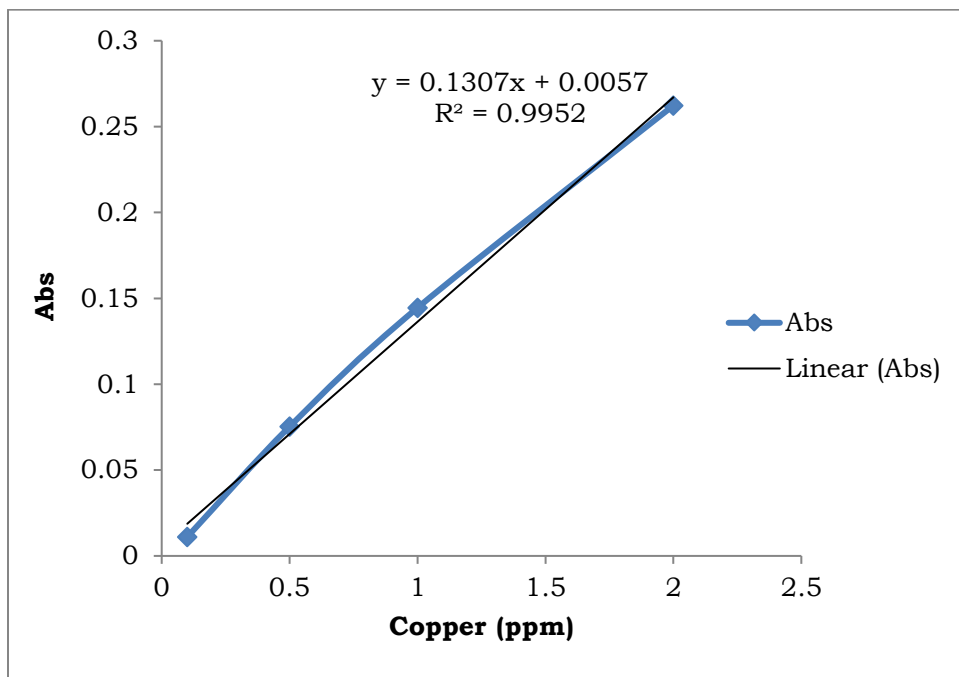
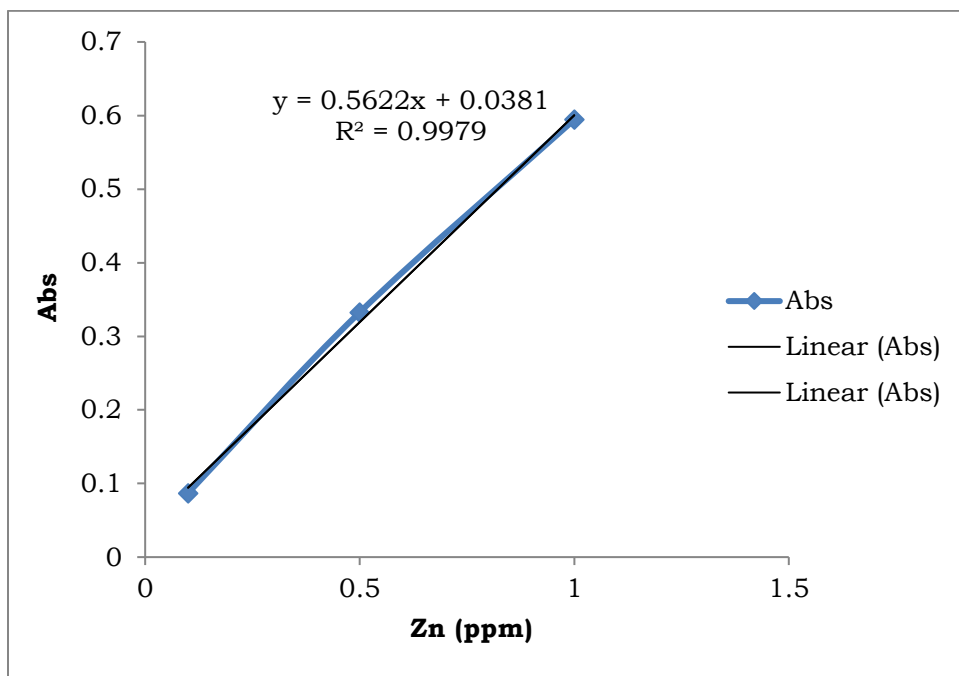


Figure 3.5: Standard curve for Zn estimation



- Wave length - 240.7 nm
- Slit width - 0.2 nm
- Lamp current - 12.0 mA
- Vapour type - Air/Acetylene.

3.8 Digestibility Trial

Digestibility trial was carried out in prepartum period 1 week prior to the expected date of calving by marker method. DMI and fecal output were quantified by double indicator method (Krishna *et al.*, 1981) where lignin was used as an internal indicator for feed intake while Chromic oxide (Cr_2O_3) was used as an external indicator to determine the fecal output. However, real-time daily dietary intake during the pre-partum period was also recorded. Animals dosed with 10 g of Cr_2O_3 for 5 days as a cellulose paper capsule daily at 9.00 AM. Faecal samples were collected from the rectum in the morning and evening hours for 7 days from the second day of dosing Cr_2O_3 . Faecal samples were pooled and sub-sample of 50 g dried in drought oven at 60-70^o C for 24 h. About 1 g of oven-dried fecal samples were transferred to a 100 ml Kjeldahl flask and 10 ml of concentrated HNO_3 was added. Flasks was kept overnight and afterwards added 10 ml of digestion mixture consisted of 10 g Sodium molybdate (Na_2MoO_4) dissolved in 150 ml DDW, 150 ml of concentrated sulphuric acid (H_2SO_4) and 200 ml of 70% Perchloric acid (HClO_4). Digestion continued till a light yellowish tint appeared. After Digestion, contents transferred to 100 ml volumetric flask and volume made with DDW. A stock solution of 10 mg Cr_2O_3 prepared in a similar procedure and final volume made to 100 ml. Standard curve of Cr_2O_3 was drawn using 20, 30, 40, 50, 60, 70 and 80 μg per ml. Cr_2O_3 concentration in the samples was determined by the colorimetric method by reading the absorbance at 430nm using UV-visible spectrometer (Model LMSP-UV1200, M/s Labman Scientific Instruments Pvt. Ltd., Chennai). Depending on the concentration of Cr_2O_3 in the faeces, faecal output was estimated from a dose of 1 g of Cr_2O_3 administered using the following formula.

$$\text{Faecal output (g/day)} = \frac{\text{Cr}_2\text{O}_3 \text{ fed (g/d)} \times \text{Weight of dried fecal sample (g)}}{\text{Cr}_2\text{O}_3 \text{ concentration in fecal sample}}$$

DMI and digestibility were estimated using lignin as an internal marker. Ingested lignin from feed supposedly passed in faeces without any enzymatic digestion. Sample of diet offered to pre-partum cows was collected and preserved for the estimation of lignin. Lignin in the feed and faecal samples estimated as per the methods of Van Soest *et al.* (1991). Based on the amount of lignin present

in the faeces and feed, DMI and digestibility of nutrients in cows were calculated according to the formulae given below:

$$\text{Dry matter intake (g/day)} = \frac{\% \text{ Lignin in faeces} \times \text{Fecal output}}{\% \text{ Lignin in feed}}$$

$$\text{Digestibility coefficient (\%)} = \frac{\text{Nutrient (g) in total feed} - \text{Nutrient (g) in faeces}}{\text{Nutrient (g) in feed}} \times 100$$

3.9 Chemical analysis

Feed (offered and residue) and faecal samples collected during the digestibility trial were analyzed for proximate and cell wall constituents (CWC).

3.9.1 Proximate constituents

Feedstuffs were analyzed for proximate constituents according to AOAC (2012).

3.9.1.1 Moisture (AOAC, 2012, Method No. 934.01)

Fresh samples (feed or faeces) of 2 to 3 g was taken in an aluminium tray and dried overnight in an oven at 95 to 100 ± 1 °C under pressure ≤ 100 mm Hg to constant weight. Loss on drying was taken as an estimate of moisture content in the sample.

$$\text{Moisture \%} = (\text{Wt. Loss on drying} / \text{Wt. of the fresh sample}) \times 100$$

$$\text{Dry matter (DM), \%} = 100 - \text{Moisture}$$

3.9.1.2 Total ash (TA: AOAC, 2012, Method No. 942.05)

Oven-dried sample of 2 to 3 g was taken into porcelain crucible and placed in a temperature-controlled furnace at 600 ± 5 °C and hold for 2 h at this temperature. Crucible was transferred directly to desiccators, cooled and weighed immediately. Per cent of ash was calculated as below.

$$\text{Total ash \%} = \frac{\text{Wt. of the crucible with ash} - \text{Wt. of empty crucible}}{\text{Wt. of sample on DM basis}} \times 100$$

3.9.1.3 Total Nitrogen (AOAC, 2012, Method No. 988.05)

Nitrogen (N) content of feedstuffs was estimated by mixed catalyst Kjeldahl method for which 0.5 to 1 g of sample was digested in 25 ml of concentrated H₂SO₄ along with 0.25 g digestion mixture consisting of Copper sulfate (CuSO₄) and Potassium sulfate (K₂SO₄) in the ratio of 1:10. Digestion continued until clear transparent colour obtained to the content in the digestion tube. Contents were made to 250 ml in a volumetric flask. A sample of 50 ml was steam distilled and the distillate was collected in a conical flask containing 10 ml of 2% (w/v) Boric acid (H₃BO₃) and mixed indicator consisted of Bromocresol green and Methyl red in 2:1 ratio. About 50 to 60 ml of distillate was collected and titrated against N/10 H₂SO₄. Total N was determined and the crude protein (CP) content of the sample was calculated by multiplying the N content with the factor 6.25.

$$\text{Total N \%} = \frac{\text{ml. of standard acid used} \times \text{normality} \times 0.014 \times \text{volume}}{\text{Wt of sample on DM basis} \times \text{Aliquot taken}} \times 100$$

3.9.1.4 Ether extract (EE: AOAC, 2012, Method No. 920.39)

Ground, moisture-free sample of 2 g was taken in Whatman thimble and extracted for 6 h with Petroleum ether (C₆H₁₄) at a temperature of 60 °C in 50ml pre-weighed EE glass flask (EEGF). At the end, flasks removed, excess of ether was dried slowly in the oven at 100 ± 0.5 °C for 30 min., taken out from oven and cooled in the desiccator. Weight of the flask with ether was recorded at room temperature to a constant weight. EE in the sample was quantified as the difference in the weights between pre and post-extraction weight of EEGF and expressed as a percent on DM basis.

$$\text{EE \%} = \frac{\text{Pre - wt. of EEGF} - \text{Wt. of EEGF after extraction}}{\text{Wt of sample on DM basis}} \times 100$$

3.9.1.5 Acid Insoluble Ash (AIA: ISO 776:2011[en])

After determination of ash, the crucible was filled with 20 ml of HCl (1:1) covered with a watch glass and digested for 30 min and, filtered through Whatman filter paper No. 40 into the volumetric flask. The residue was washed with DW. Residue with filter paper was transferred into the same crucible, dried and ignited in

muffle furnace at 600 °C. Crucible was removed when the temperature of the muffle furnace was dropped to 40 °C, cooled in desiccators and recorded the weight.

$$\text{AIA \%} = \frac{\text{Wt. of crucible with AIA} - \text{Wt. of empty crucible}}{\text{Wt. of sample on DM basis}} \times 100$$

3.9.1.6 Total carbohydrates (TCHO)

TCHO was calculated mathematically by subtracting the sum CP, EE and TA from 100

$$\text{TCHO \%} = 100 - (\text{Total of CP} + \text{EE} + \text{TA})$$

3.9.1.7 Organic Matter (OM)

OM was calculated by subtracting inorganic portion such as the ash content from 100.

$$\text{OM \%} = 100 - \text{TA}$$

3.9.2 Cell wall constituents (CWC)

Feedstuffs were analyzed for CWC as per AOAC (2012).

3.9.2.1 Neutral detergent fiber (NDF: AOAC, 2012, Method No. 2002:04)

Feed, Orts and faecal samples were analyzed for NDF was estimated according to AOAC (2012; Method. 2002:04). Neutral Detergent solution (NDS) consisted of Sodium Lauryl sulphate ($\text{NaC}_{12}\text{H}_{25}\text{SO}_4$) 30.90, Disodium ethylene diamine tetraacetic acid (EDTA) dehydrate 18.61, Sodium borate decahydrate (Borax) 6.81, Disodium hydrogen phosphate (Na_2HPO_4) 4.56 g/L and 2-Ethoxyethanol ($\text{C}_4\text{H}_{10}\text{O}_2$) 10.00 ml/L was prepared as follows; EDTA and Borax were added together in a large beaker with some DW. The contents were heated until dissolved. Na_2HPO_4 was taken in another beaker and dissolved in DW by heating. Both the solutions were then added together in a volumetric flask. Sodium lauryl sulfate and 2-Ethoxyethanol were also added in the flask and finally, the volume was made to one liter with DW.

Approximately 1g sample was taken in a sprout less beaker along with 100 ml NDS, 2 ml decalin and 0.5 g sodium sulfite and the contents boiled on a hot plate for exactly 1 h. After boiling, the samples were filtered through a pre-weighed sintered glass crucible (Grade G1) using vacuum pump. Samples were dried overnight in a drought oven ($90\pm 5^{\circ}\text{C}$) and weighed. NDF was calculated as follows;

$$\text{NDF \%} = \frac{(\text{Wt. of crucible with residue} - \text{Wt. of empty crucible})}{\text{Wt. of sample on DM basis}} \times 100$$

3.9.2.2 Acid detergent fiber (ADF: AOAC, 2012, Method No. 973:18)

Approximately 1 g of sample was taken in a spoutless beaker of 1 L capacity. To this, 100 ml acid detergent solution was added (Acid detergent solution consists of 20 g Cetyl trimethyl ammonium bromide (CTAB) dissolved in one liter 1 N H_2SO_4). The contents were refluxed for exactly 1 h and at the end, the residue was filtered through pre-weighed sintered glass crucible (Grade-I) using the vacuum pump, washed with hot water 2 to 3 times and finally flushed with Acetone ($\text{C}_3\text{H}_6\text{O}$) to remove residual acid. The crucible containing residue was dried in hot air oven ($100 \pm 1^{\circ}\text{C}$) and weighed again. The ADF was calculated as follows;

$$\text{ADF \%} = \frac{(\text{Wt. of crucible with residue} - \text{Wt. of empty crucible})}{\text{Wt. of sample on DM basis}} \times 100$$

3.9.2.3 Cellulose

After the determination of ADF, the crucible was placed in a shallow glass tray and filled with cooled (15°C) 72% H_2SO_4 (v/v) and contents stirred with glass rod intermittently to smooth paste while smoothly breaking the lumps. Crucible was refilled half-way with H_2SO_4 at 1 h interval. After 3h, the crucible was removed from the tray and filtered completely with 2 to 3 hot water washing using a vacuum pump. Crucible along with leftover contents was dried in an oven at $100 \pm 1^{\circ}\text{C}$ and weighed. Cellulose content was calculated as follows (Van Soest *et al.*, 1991);

$$\text{Cellulose \%} = \frac{(\text{Wt of crucible} + \text{ADF}) - (\text{Wt of crucible after acid wash})}{\text{Wt. of sample on DM basis}} \times 100$$

3.9.2.4 Hemicelluloses (HC)

Hemicellulose (HC) was calculated as the difference between NDF and ADF.

$$\text{HC \%} = \% \text{NDF} - \% \text{ADF}$$

3.9.2.5 Acid Detergent Lignin (ADL)

After treating the contents with 72% H₂SO₄ (v/v), the crucible was dried in an oven at 100±1°C, weighed and ignited in the furnace at 500±5 °C at 2 h. Crucible was removed from the furnace when the temperature reduced to 60°C. Crucible was then placed in forced drought oven at 100±1°C for 1 h followed by cooling to a constant temperature in the desiccator. The difference in the weight of the crucible before (BI) and after (AI) ignition was taken as ADL.

$$\text{ADL \%} = \frac{(\text{Wt of crucible BI} - \text{Wt of crucible AI})}{\text{Wt. of sample on DM basis}} \times 100$$

3.9.2.6 Non-fibrous carbohydrates (NFC)

NFC was calculated by adopting the formula proposed by Hall (2003) as follows;

$$\text{NFC} = 100 - \% \text{CP} - \% \text{NDF} - \% \text{EE} - \% \text{Ash}$$

3.10 Digestibility of nutrients and nutritive value of diet

Digestibility coefficient of nutrients was determined from the pooled samples of feed, orts and faces obtained during the digestibility trial.

3.10.1 Digestibility coefficient of nutrients

Digestibility of proximate principles and CWC was determined as the difference between total intake and faecal output and calculated the digestibility coefficient of nutrient over its total intake as below.

$$\text{Digestibility coefficient} = [(\text{Total intake} - \text{Fecal output}) \div \text{Total intake}] \times 100$$

3.10.2 Nutritive value

- Digestible crude protein % (DCP) = (Digestible CP intake ÷ Total DM intake) × 100

- Total Digestible nutrients % (TDN) = [(Digestible CP intake + Digestible EE intake \times 2.25 + Digestible TCHO intake) \div (Total DM intake)] \times 100
- Nutritive ratio (NR) = (TDN% - DCP %) / (DCP %)

3.10.3 Energy value

Energy value of the diet was predicted from the amounts of digested nutrients viz., CP, EE and TCHO using the East German System for cattle. Empirical formulae used for different energy parameters were as below (ARC, 1984).

- Gross energy (GE; kcal) = $4Y_1 + 9Y_2 + 4Y_3$
- Digested energy (DE; kcal) = $5.79X_1 + 8.15X_2 + 4.24X_3$
- Metabolizable energy (ME; kcal) = $4.32X_1 + 7.73X_2 + 3.61X_3 - 6.26X_4$
- Energy retention (ER; kcal) = $(1.72X_1 + 7.35X_2 + 1.96X_3) \times (-0.513 + 0.03962X_5 - 0.0002596 [X_5]^2) - 57.4X_4$
- Q-Value (Metabolizability of the gross energy of feed): ME/GE
- Digestibility of energy (%): $(DE \div GE) \times 100$
- Heat Increment (HI; MJ): ME - ER
- Energy efficiency (%): $(ER \div ME) \times 100$
- $ME_{\text{Gestation}}$ (Mcal/d) = $[(0.00318 \times D - 0.0352) \times (CBW/45)]/0.14$ (NRC, 2001)
- $NE_{\text{Maintenance}}$ (Mcal/d) = $1.37 ME - 0.138 ME^2 + 0.0105 ME^3 - 112$ (NRC, 2001)
- $NE_{\text{Pregnancy}}$ (Mcal/d) = $[(0.00318 \times D - 0.0352) \times (CBW/45)]/0.218$ (NRC, 2001)

Where,

Y_1 = CP (g)

Y_2 = EE (g)

Y_3 = TCHO (g)

X_1 = Digested CP (g)

X_2 = Digested EE (g)

X_3 = Digested TCHO (g)

X_4 = Metabolic BW ($\text{kg W}^{0.75}$)

X_5 = Digestibility of energy (%)

D = Gestation between 190 to 279 d

CBW = Calf birth weight in kg

The above values used for the DE and ME were digestible fraction of X_1 , X_2 and X_3 . All values were expressed in Mcal or 1 Mcal is equivalent to 4.184 MJ.

3.11 Estimation of MBN production in the rumen

MBN production was estimated from the spot urine samples using purine derivatives (PD) and creatinine as markers in the urine (Chen *et al.*, 1995).

3.11.1 Collection of urine samples

Spot urine sample was collected 2-3 times a day for 3-4 d at different time. 20 ml of sample was collected from each animal every time and mixed with 1/10th quantity of dilute sulphuric acid (10% H₂SO₄) and stored at -20°C. Finally, one sample of each cow is pooled from all the samples of the same animal and was stored at -20°C and preserved until further analysis. Urine samples were thawed and 20 ml aliquots were taken, centrifuged and filtered through a Millipore filter of 0.2 µm pore size (M/s Sigma Aldrich, Cat no. F-1387). One ml of the filtrate was taken and diluted 10-fold with water after adjusting the pH to 7.0 using 0.01 N NaOH and 0.01 N H₂SO₄. The processed sample was ready to inject into HPLC (High performance liquid chromatography).

3.11.2 Estimation of purine derivatives using HPLC

Allantoin, creatinine and uric acid in the urine were estimated using the HPLC method (Ashwin and Srinivas, 2019). Standard solutions of allantoin (M/s Sigma Aldrich, Cat no. 93791), creatinine (M/s Sigma Aldrich, Cat no. C4255) and uric acid (M/s Sigma Aldrich, Cat no. U0081) each containing 50µg/ml (w/v) of respective standards were separately prepared in HPLC grade water. HPLC (M/s Waters India Pvt., Ltd.) was run in isocratic condition using C-18 reverse-phase column (M/s Waters India Pvt., Ltd., and 4.5 × 260 mm I.D., 5µm) with 10mM potassium dihydrogen phosphate (pH 4.7) as mobile phase. The flow rate was fixed at 1 ml/min and reading was taken at 220 nm wavelength (Model 2489 UV/visible detector, M/s Waters India Pvt., Ltd.). Peaks were standardized for allantoin, uric acid and creatinine by injecting 20 µl of respective standard.

3.11.3 Calculation of MBN from PD and creatinine estimation

The concentration of allantoin, creatinine and uric acid was calculated by comparing the area of characteristic peaks in the sample with the corresponding area in the standard solution. 20 μ L of the sample and standard (50 μ g/ml) was used in injecting to HPLC.

$$\text{Allantoin} \left(\frac{\text{mg}}{\text{L}} \right) = \frac{\text{area of allantoin peak in the sample}}{\text{area of allantoin peak in the standard}} \times 50 \times 10$$

$$\text{Creatinine} \left(\frac{\text{mg}}{\text{L}} \right) = \frac{\text{area of creatinin peak in the sample}}{\text{area of creatinin peak in the standard}} \times 50 \times 10$$

$$\text{Uric acid} \left(\frac{\text{mg}}{\text{L}} \right) = \frac{\text{area of uric acid peak in the sample}}{\text{area of uric acid peak in the standard}} \times 50 \times 10$$

Concentrations of allantoin, creatinine and uric acid in mM/L are arrived by dividing their concentrations in mg/L by their gram molecular weight; that is 158.12, 113.12, and 168.11 respectively.

Excretion of total PD was calculated as below (Chen *et al.*, 1995):

1. PDC index: It was calculated as the ratio of the concentrations of PD (mM/L) to creatinine (mM/L) times the metabolic body weight (MBW)
2. PDC Index = [(PD mM/L) / (Creatinine mM/L)] \times MBW
3. The following equation was used to determine the quantitative relationship between the absorption of purines and excretion of PD (Y mM/d) in the urine. $Y = 0.85X + (0.147W^{0.75})$. Where $W^{0.75}$ represents the MBW (kg) of the experimental animal. The slope of 0.85 in equation represented the recovery of absorbed purines as PD in urine and X was PDC index. In zebu cattle, the endogenous contribution was taken as a constant at $0.147W^{0.75}$ per day (Chen and Orskov, 2003). Thus, the daily purine absorption was back-calculated as $X = (Y - 0.147 \times W^{0.75}) \div 0.85$.
4. The intestinal flow of MBN was calculated from the excretion of PD as $(X \times 70) / (0.83 \times 0.116 \times 1000)$. Where the digestibility of microbial PD was 0.83, N concentration in purine was 70 % and, 0.116 was the ratio between N in

PD to total N in mixed rumen microbes. The efficiency of MBN production was expressed as g N kg⁻¹ of OM digested in the rumen (DOMR) by multiplying digestible OM by 0.65.

3.12 Health indicators

3.12.1 Blood analysis

Blood samples were collected from the jugular vein using vacutainer needle into 10ml capped EDTA coated tubes at -30, -15, and 0 days of parturition. Plasma was separated by centrifuging the samples at 3000 rpm (Rotations per minute) for 10 minutes and stored at -20°C for biochemical analysis. Approximately 2-3 ml of fresh blood in EDTA coated tubes were taken for haematological analysis. Tubes were labelled, kept in a flask with an ice gel pack and stored at 4°C. Samples were thawed and complete blood count was analyzed using auto haematology analyzer (Model: BC-2800 Vet, M/s Mindray Medical International Ltd., Shenzhen, China). Blood parameters analyzed and their normal range was presented in **Table 3.8**.

Table 3.8: Blood parameters and normal range

Parameter	Range	Parameter	Range
Lymphocytes	45-75 %	Red blood corpuscles	5-10 (X10 ⁶ /μl)
Monocytes	2-7%	Hemoglobin	8-15 g%
Eosinophils	2-20%	White blood corpuscles	4-12 (X10 ³ /μl)
Basophols	2-10%	Platelet	175-620 (X10 ⁵ /μl)
Neutrophils	15-45%	Packed cell volume (PCV)	24-45%
Mean Copuscular Volume	40-60 fL	Mean Corpuscular Haemoglobin	11-17 pg

3.12.2 Blood metabolites

3.12.2.1 Estimation of blood glucose

Plasma glucose was estimated using GOD-POD kit (93LS100-75, M/s Span Diagnostics Ltd., India). In this procedure, glucose oxidase oxidized glucose to gluconic acid and hydrogen peroxide. In the presence of enzyme peroxidase, hydrogen peroxide released was coupled with phenol and form 4-amino antipyrine (4-AAP) which is proportional to glucose concentration in the sample. 10 µl of plasma aliquots were pipetted in 10x75 mm tubes (in duplicate), to which 1000 µl of working glucose reagent was added. The contents were mixed well. The tubes were then incubated at room temperature (15-30°C) for 30 min. Along with the unknown sample, blank and standard tubes (in duplicate) containing 10 µl of double distilled water and 10 µl of standard (100 mg/dl), respectively were processed identically. The absorbance of standard and the unknown sample was measured by the light UV-Spectrophotometer (LMSP-UV1200, M/s Labman Scientific Instruments Pvt. Ltd., Chennai) after setting to zero with reagent blank and concentration was calculated as per formula and expressed as mg/dl

$$\text{Plasma glucose (mg/dl)} = \frac{\text{absorbance of test}}{\text{absorbance of standard}} \times 100$$

$$\text{Concentration of standard} = 100 \text{ mg/dl}$$

3.12.2.2 Estimation of plasma triglycerides

Triglycerides were estimated in the blood plasma sample using GPO-PAP liquid gold kit (72LS100-60, M/s Span Diagnostic Ltd., India). It is based on the principle that triglycerides present in plasma are catabolized into glycerol and free fatty acids by lipoprotein lipase. Liberated glycerol is converted to glycerol-3-phosphate in presence of glycerol Kinase and ATP. Glycerol-3-phosphate is acted upon by glycerol-3-phosphate oxidase to form dihydroxyacetone phosphate and hydrogen peroxide. In presence of peroxidase, hydrogen peroxide couples with 4-Aminoantipyrine and 4-Chlorophenol to produce red quinoneiminedye. Pipetted 1ml triglycerides reagent in blank, standard and test sample in tubes. 10 µl standard solution of triglycerides were added in standard tubes while 10 µl

plasma was added to test tubes, mixed well and incubated for 10 minutes at 37°C. Absorbance was measured at 505 nm which is proportional to triglycerides concentration in the sample.

$$\text{Plasma triglycerides (mg/dl)} = \frac{\text{absorbance of test}}{\text{absorbance of standard}} \times 200$$

Concentration of standard = 200 mg/dl

3.12.2.3 Total Protein

Total protein was estimated in blood plasma samples using the kit supplied by M/s Span Diagnostic Ltd.(83LS100-60), India. The principle of the assay is that peptide bonds of proteins react with cupric ions in alkaline solution to form a coloured chelate and the absorbance is measured at 578nm. The absorbance of the final colour is proportional to the concentration of TP in the sample. The kit reagent was prepared and stored as per the instruction provided with the assay kit. 10 µl of plasma aliquots were pipetted in 10 x 75 mm tubes in duplicate, to which 1000 µl of working Biuret reagent was added. Similarly, blank (10 µl distilled water) and standard (10 µl from protein standard 6.5 g/dl) was pipetted in duplicates, to which 1000 µl of working Biuret reagent was added. The contents were mixed well and incubated at room temperature for 1 minute. UV-Spectrophotometer was set zero with reagent blank and the absorbance of standard and test sample was measured at 578nm. Total protein concentration was calculated as per formula and expressed in g/dl.

$$\text{Plasma total protein(g/dl)} = \frac{\text{absorbance of test}}{\text{absorbance of standard}} \times 6.5$$

Concentration of standard = 6.5 g/dl

3.12.2.4 Albumin

Albumin was estimated in blood plasma samples using the kit supplied by M/s Span Diagnosis Ltd.(84LS100-60), India. It is based on the principle that albumin binds with anionic dye bromocresol green (BCG) to form green colour complex, which is measured at 630nm. The kit reagent was prepared and stored as per the

instruction provided with the assay kit. 10 µl of plasma aliquots were pipetted in 10 x 75 mm tubes in duplicate, to which 1000 µl of working albumin reagent was added. Similarly, blank (10 µl distilled water) and standard (10 µl from standard 4 g/dl) were pipetted in duplicates, to which 1000 µl of working albumin reagent was added. The content was mixed well and incubated at room temperature for 1 minute. UV-Spectrophotometer was set zero with reagent blank and the absorbance of standard and test sample was measured at 630nm. The albumin concentration was calculated as per the formula and expressed in g/dl.

$$\text{Plasma albumin(g/dl)} = \frac{\text{absorbance of test}}{\text{absorbance of standard}} \times 4$$

Concentration of standard = 4 g/dl

3.12.2.5 Estimation of blood urea nitrogen (BUN)

BUN was estimated in plasma samples by urea kits obtained from M/s Span Diagnostics Ltd.(81DP300-72), India. It is based on the principle that Urea hydrolyses to Ammonia in presence of Urease enzyme which reacts with Hypochlorite and Phenolic chromogen in alkaline medium to form a coloured complex which is measured at 578nm. Kit reagents were prepared and stored as per the instructions provided with the assay kit. 10 µl plasma aliquots were pipetted in 10 x 75 mm tubes in duplicate, to which 1000 µl of working BUN solution-1 was added. Similarly, blank (10 µl distilled water) and standard (10 µl of standard 50 mg/dl) pipetted in duplicate to which 1000 µl of working BUN solution-1 was added. The contents were mixed well and incubated at 37°C for 3 min. 1000 µl of working BUN solution-2 was added to each tube. The contents were mixed well and incubated at 37°C for 5 minutes. UV-Spectrophotometer was set zero with reagent blank and the absorbance of standard and test sample was measured. Plasma BUN levels were calculated as per formula and expressed in mg/dl.

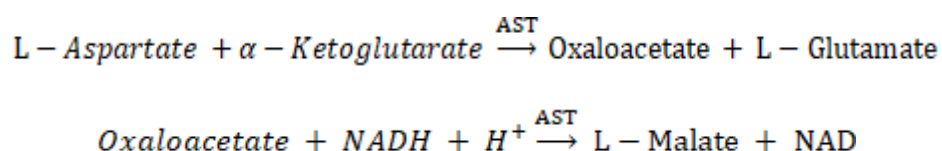
$$\text{Plasma Urea(mg/dl)} = \frac{\text{Absorbance of test}}{\text{Absorbance of standard}} \times \text{Concentration of standard}$$

Concentration of standard = 50 mg/dl

$$\text{Plasma BUN(Mg/dl)} = \text{Urea concentration in mg/dl} \times 0.467$$

3.12.2.6 Estimation of Aspartate transaminase (AST)

It is also called serum glutamate oxaloacetate transaminase (SGOT). It was estimated in plasma samples by liquid gold AST test kits (M/s Span Diagnostics Ltd., India, 77LS200-60) by modified UV (IFCC) kinetic assay. The principle of analysis is that AST catalyses the transamination of L-Aspartate and α -Ketoglutarate to form L-Glutamate and Oxaloacetate. In a subsequent reaction, Malate Dehydrogenase (MDH) reduces oxaloacetate to Malate with simultaneous oxidation of Nicotinamide Adenine Dinucleotide (reduced i.e. NADH) to Nicotinamide Adenine Dinucleotide (NAD). The rate of oxidation of NADH is measured kinetically by monitoring the decrease in absorbance at 340 nm and is directly proportional to AST activity in the Sample. Lactate Dehydrogenase (LD) is added to the enzyme system to prevent endogenous Pyruvate interference, which is normally present in the plasma.



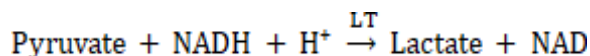
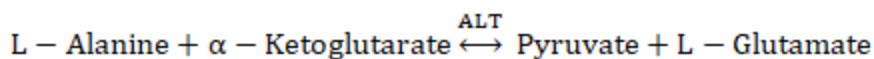
AST was estimated by taking 100 μl plasma aliquots were pipetted in 10 x 75 mm tubes, and added 1000 μl of working AST reagent. Contents are mixed well and absorbance was taken after 60 seconds and repeated the readings at every 30 seconds up to 120 seconds at 340 nm. Mean absorbance change per minute was determined and AST activity is calculated by a formula using the kinetic factor of 1768.

$$\text{AST activity (U/L)} = \text{change in absorbance per minute} \times \text{Kinetic factor}$$

3.12.2.7 Estimation of alanine transaminase (ALT)

ALT was estimated in plasma samples by liquid gold ALT test kits (M/s Span Diagnostics Ltd., 76LS200-60) by modified UV (IFCC) kinetic assay. ALT catalyses the transamination of L-Alanine and α -Ketoglutarate to form L-Glutamate and Pyruvate. In a subsequent reaction, Lactate Dehydrogenase (LD) reduces Pyruvate to Lactate with simultaneous oxidation of Nicotinamide Adenine Dinucleotide (reduced i.e. NADH) to Nicotinamide Adenine Dinucleotide (NAD). The rate of oxidation of NADH is measured kinetically by monitoring the decrease

in absorbance at 340 nm. LD rapidly and completely reduces endogenous sample Pyruvate during the initial incubation period, so that it does not interfere with the assay chemical equation and procedure followed were presented below;



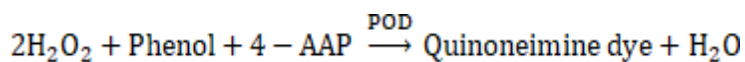
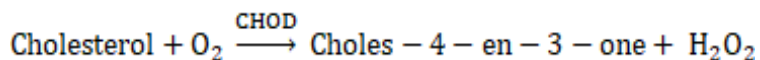
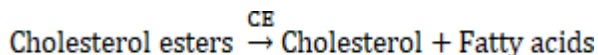
ALT was estimated by taking 100 μl plasma aliquots were pipette in 10 x 75 mm tubes, and added 1000 μl of working ALT reagent. Contents are mixed well and absorbance was taken after 60 seconds and repeated the readings at every 30 seconds up to 120 seconds at 340 nm. Mean absorbance change per minute was determined and ALT activity is calculated by the formula.

$$\text{ALT activity (U/L)} = \text{change in absorbance per minute} \times \text{Kinetic factor}$$

$$\text{Kinetic factor} = 1768$$

3.12.2.8 Total Cholesterol

Total cholesterol was estimated in the blood plasma sample using CHOD-PAP enzymatic end-point assay liquid gold kit purchased from M/s Span Diagnostic Ltd.(71LS200-60). It is based on the principle that cholesterol esters are hydrolysed by cholesterol esterase (CE) to give free cholesterol and fatty acids. In a subsequent reaction, Cholesterol oxidase (CHOD) oxidises the 3-OH group of free cholesterol to liberate cholest-4-en-3-one and hydrogen peroxide. In presence of peroxidase (POD), hydrogen peroxide couples with 4-amino antipyrine (4-AAP) and phenol to produce red Quinoneimine dye. The absorbance of coloured dye is measured at 505 nm and is proportional to the amount of total cholesterol concentration in the sample. Chemical equation and procedure followed were presented below;



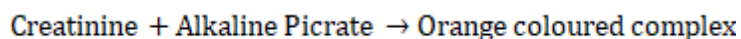
Cholesterol in the sample was estimated by taking 10 µl of plasma aliquots were pipetted in 10 x 75 mm tubes in duplicate. Similarly, blank (10 µl distilled water) and standard (10 µl from standard 200 mg/dl) were also taken in duplicates. 1000 µl of cholesterol mono reagent was added to all the tubes. The content was mixed well and incubated at 37°C for 10 minutes. UV-Spectrophotometer was set to zero with reagent blank and the absorbance of standard and test sample was measured at 505 nm. The cholesterol concentration was calculated as per the formula and expressed in mg/dl.

$$\text{total cholesterol(mg/dl)} = \frac{\text{absorbance of test}}{\text{absorbance of standard}} \times 200$$

$$\text{Concentration of standard} = 200 \text{ mg/dl}$$

3.12.2.9 Estimation of Creatinine

Serum creatinine was estimated by using modified Jaffa's kinetic method (Tulip Diagnostics Ltd.). The principle of analysis is that picric acid in an alkaline medium reacts with creatinine to form an orange coloured complex with the alkaline Picrate. The intensity of the colour formed during the fixed time is directly proportional to the amount of Creatinine present in the sample.



A mixture was prepared using 500µl of picric acid reagent and 500µl buffer reagent to which 100µl of creatinine standard was added. Similarly, for estimation of creatinine in samples, the standard was replaced by serum. The contents were mixed well and incubated for 30 sec after which Initial absorbance (A1) was noted. Another absorbance was taken exactly after 60 sec (A2) after the first reading by using UV-Spectrophotometer. Serum creatinine levels were calculated as per formula and expressed in mg/dl.

$$\text{Standard } \Delta AS = A_2S - A_1S$$

$$\text{Sample/Test } \Delta AS = A_2T - A_1T$$

$$\text{Creatinine(mg/dl)} = \frac{\Delta AT}{\Delta AS} \times 2.0$$

3.12.2.10 Estimation of Gamma-glutamyltransferase (GGT)

Gamma-glutamyltransferase was estimated in blood serum sample using GGT kinetic test (Lab-care Diagnostics Ltd., India) which uses the principle that the enzyme GGT transfers the **Gamma-glutamyl** in gamma-glutamyl-p-nitroanilide to Glycylglycine forming p-Nitroaniline. The amount of p-Nitroaniline formed is directly proportioned to Gamma-GT activity. The reaction mixture consisting of 100µl of serum and 1000µl of working reagent were mixed well and incubated for 60 sec at 37°C. Absorbance was noted at different intervals viz. 1, 2, 3, 4 min at 405nm. The GGT concentration was calculated by using the following formula;

$$\text{Gamma Glutamyl Transferase (U/l)} = \Delta \text{ Abs/min} \times 1640$$

3.12.3 Ketone body Estimation

3.12.3.1 Non-Esterified Fatty Acid (NEFA)

NEFA was estimated by the competitive enzyme immunoassay technique utilizing a monoclonal anti-NEFA antibody and a NEFA-HRP (Horse Radish Peroxidase) conjugate using ELISA kit (M/s Shanghai Korain Biotech Co., Ltd, China)

The standards, samples of about 100 µl were added to the appropriate well.

1. Blank control well was filled with Milli-Q water of 100 µl.
2. NEFA-HRP conjugate about 50 µl was added to each well, except blank control well, mixed thoroughly, covered and incubated the plate for 1 hour at 37 °C with sunlight protection.
3. After the incubation period, the wells are decanted and washed 5 times after complete removal of the liquid at each step.
4. The wells were then incubated with a substrate and HRP enzyme and the contents of the well were turned into the blue coloured enzyme-substrate complex.
5. Substrate A and B both of 50 µl were added to each well including blank control well. Subsequently covered and incubated for 10-15 minutes at 37 °C

6. Stop solution of 50 μ l was added to each well including blank control well, mixed well and again incubated for 10-15 minutes at 37 °C. At the end of incubation, contents in the well were turned yellow.
7. The intensity of the colour was measured immediately at 450 nm using a microplate reader.
8. The intensity of the colour was inversely proportional to the NEFA concentration since NEFA from samples and NEFA-HRP conjugate compete for anti- NEFA antibody binding site.

3.12.3.2 Beta Hydroxy Butyric Acid (β HBA)

β HBA was estimated using the quantitative sandwich ELISA kit where the microtiter plate provided in the kit was pre-coated with an antibody specific to antigen β HBA (M/s My Biosource, Catalog No. MBS046814).

1. 50 μ l of standard in duplicates and samples were added to respective well coated with a biotin-conjugated antibody specific to antigen β HBA.
2. Subsequently, 50 μ l sample diluents was added to the respective well.
3. 100 μ l of HRP-conjugate Reagent was added to each well, covered with a plate sealer and incubated for 60 minutes at 37 °C.
4. The plate was washed 4 times using a washing buffer solution.
5. Chromogen solution A (50 μ l) and chromogen solution B (50 μ l) were added to each well successively. Then protected from light and incubated for 15 minutes at 37 °C.
6. Acid stop solution of 50 μ l was added to each well to terminate the enzyme-substrate reaction.
7. The optical density (OD) was measured at 450 nm using a microplate reader within 5 minutes.

3.12.3.3 Calculation of NEFA and β HBA concentration

The average value of the duplicate readings for each NEFA and β HBA standard was calculated. The concentration of unknown samples was calculated according

to the fitted curve using the OD. The average zero standards/blank OD was subtracted from standards and unknown samples OD before calculation of results. The standard curve was constructed by plotting the mean OD and concentration for each standard and draw a best fit curve through the points on the graph and then the concentration of unknown samples was calculated. If samples have been diluted Eg. Hp, the final concentration are multiplied by the dilution factor. The final results based on OD were calculated using SkanIt software 3.2.1.4 and 4.1 for M/s Thermo scientific Multi skan GO (en).

3.13 Serum mineral estimation

As described in 3.7

3.13.1 Serum calcium estimation

As described in 3.7.1.1

3.13.2 Serum Phosphorus estimation

Phosphorus estimation in serum samples was analyzed using Mindray autoanalyzer (Model: BS-240Vet, M/s Mindray Medical International Ltd., Shenzhen, China).

3.13.3 Serum magnesium estimation

As described in 3.7.1.4

3.13.4 Serum manganese estimation

As described in 3.7.1.3

3.13.5 Serum copper estimation

As described in 3.7.1.5

3.13.6 Serum Zinc estimation

As described in 3.7.1.6

3.14 *In vivo* experiments postpartum

During the postpartum period, irrespective of groups such as control or treatment groups, all cows were kept on common and general feeding regime on the farm. All the parameters carried during the pre-partum period were also carried during the postpartum period to evaluate the impact of nutrition they received during the pre-partum period such as the different source of energy with or without supplement in HF graded crossbred and Deoni cows. Apart from dams, observations were also carried on calves.

3.14.1 Postpartum Calf component

Birth weight of calf was recorded immediately soon after calving using a weighing balance (M/s Krupa Instruments Pvt. Ltd, India). The colostrum was fed to the calf within 30 min of birth. Colostrum quality was measured based on specific gravity using colostrometer (**Fig 3.7**).

Figure 3.7: Colostrum meter



Colostrometer consisted of a bulb and calibrated upper portion which indicates the direct reading of immunoglobulins-G (IgG) concentration that was directly proportional to the specific gravity of Colostrum. After cooling the colostrum to 20°C/68°F, it was taken in a calibrated measuring cylinder. The

colostrometer was slowly immersed without touching the walls of the cylinder. The reading on the stalk of the cylinder was recorded as per colour code zone given in **Table 3.9**. The reading on the stalk of the cylinder which varies with specific gravity of colostrum was directly proportional to the concentration of IgG in the colostrum (Fleenor and Stott, 1980). Calves were shifted to neonatal shed after 5 d of birth and no further follow-up observations on calf was carried.

Table 3.9 Interpretation of Colostrum quality using colostrometer

Specific gravity	Zone	Interpretation
<1035	Red zone	Poor quality
1035-1045	Light green zone	Intermediate quality
>1045	Dark green zone	Good quality

3.14.2 Postpartum Dam component

3.14.2.1 Parturition

During parturition, the time lapse between the appearance of the water bag and expulsion of the foetus was recorded besides recording of time taken for the expulsion of the fetal membrane. The dam was kept under observation for subsequent 5 days along with calf (Described in **3.14.1**) and shifted to milking herd thereafter.

3.14.2.2 Follow-up nutrition of dam in the postpartum period

All the dams, irrespective of treatment groups in the prepartum period were kept on the general feeding schedule on the farm during the postpartum period. General health and production performance were observed for 30 d. Energy and protein requirement in the ration for all experimental cows was calculated for maintenance plus milk production. Animals were provided with weighed quantities of CS (**Table 3.2**) twice a day during milking at 5 AM and 5 PM, respectively. After milking, cows were sent for 4 h grazing from 8 AM to 12 PM. The spot graze samples were collected thrice in a week for 4 weeks. The herbal cover in graze area consisted of Dub grass (*Cynodon dactylon*), Para grass (*Brachiariamutica*), Hybrid Napier (Bajra × Napier), Rhodes grass (*Chloris*

gayana) and Guinea grass (*Guinea grass*). After returning from grazing, cows were housed in an open paddock and offered MGF at 2 PM. Mixed green fodder consisted of Para grass, Hybrid Napier, and Maize (*Zea mays*). Animals had free access to water as per the standard management practice on the farm.

After 2 weeks of parturition, the digestibility trial was conducted by the marker method using lignin as an internal indicator for feed intake and Chromic oxide (Cr_2O_3) as an external indicator (Described in **3.8**). Feed and faecal samples collected during the digestibility trial were analyzed for proximate and CWC (Described in **3.9**). Digestibility coefficient of nutrients (Described in **3.10.1**), nutritive value (Described in **3.10.2**) and energy value of the feed were calculated (Described in **3.10.3**). MBN production was estimated from the spot urine samples by using purine derivatives and creatinine as markers in urine samples (Described in **3.11**).

3.14.2.3 Health and production

Blood samples during the postpartum period were collected from the jugular vein using vacutainer needle into 10ml caped EDTA coated tubes on +15 and +30 days from parturition (Described in **3.12.1**). All blood metabolites analysed during the prepartum period such as; like blood glucose, plasma triglycerides, TP, albumin, BUN, AST, ALT, Total Cholesterol, Creatinine, GGT were analyzed (Described in **3.12.2**). Ketone bodies (NEFA and BHBA) in the serum samples were estimated by using ELISA kits (Described in **3.12.3**). Serum minerals viz., Ca, P, Mg, Mn, Cu and Zn were also estimated (Described in **3.13**). Besides, the following parameters were also recorded during the postpartum period;

- Milk yield up to 60 d of post-calving
- Any metabolic or systemic disorders

3.15 Measurement of Feed Efficiency

A positive $\text{RPI}_{\text{FSF}} / \text{REI}_{\text{FSF}}$ means that the animal protein/energy intake exceeds than the standard requirements recommended for maintenance and production. Actual intake was compared with the recommended intake of particular

nutrients. A negative RPI_{FSF}/REI_{FSF} indicated that animal was consuming less than the standard requirement for maintenance and production (Herd and Arther, 2009).

3.15.1 Residual protein intake

Actual CP intake from the diet in prepartum and postpartum were compared with the recommended standards of the ICAR (2013) and residual protein intake was calculated as below:

Residual Protein Intake (RPI_{FSF}) = Observed CP (kg/d) – Expected CP intake as per feeding standards (kg/d)

3.15.2 Residual metabolizable energy intake

Actual metabolizable (ME) intake from the diet in prepartum and postpartum were compared with the recommended standards of the ICAR (2013) and residual ME intake was calculated as below:

Residual Energy Intake (REI_{FSF}) = Observed Energy intake (kcal/d) – Expected Energy intake (kcal/d) as per feeding standards

3.16 Statistical analysis

In vitro and *in vivo* data were subjected to central tendency, dispersion of data, variance, correlation and regression using Statistical Package for Social Science (SPSS, Ver.17.0; M/S, IBM India Pvt. Ltd.).

3.16.1 *In vitro* experiment

Experimental design followed for *in vitro* studies was 2×2 factorial design where energy (Described in 3.4.2.1) and functional nutrient (Described in 3.4.2.2) fortification were 2 factors (See Table 3.4.2.3). Data were subjected to statistical analysis using the following ANOVA model.

$$Y_{ijk} = \mu m + \alpha_i + \beta_j + (\alpha\beta)_{ij} + e_{ijk}, i = 1, 2, 3, 4; j = 1, 2; k = 1, 2, \dots, n$$

Where, Y_{ijk} was any observation for which, μ was general mean, α_i was control (Basal diet) and 3 treatment effects (Basal diet fortified with 10% of mono or, di or polysaccharide iso-caloric energy), B_j was sub-factor at two levels such as with or without functional nutrient supplements to the main factor i.e., fortified energy, $(\alpha\beta)_{ij}$ was an interaction effect of main and subfactor and e_{ijk} was random error component (Montgomery, 1976). According to the above model, the degrees of freedom for the factors is shown in **Table 3.11**. The variance between treatments was compared by Duncan Multiple Range Test (DMRT) for the main factor and significance between mean values at the probability of $\alpha \leq 0.001$ to 0.05 was noted with different alpha superscripts.

3.16.2 *In vivo* studies

Experimental design followed for *in vivo* studies was also factorial design like in the *in vitro* studies but, an additional factor such as ‘Breed’ (Described in **3.4.2.3**) was added as another sub-factor besides energy (Described in **3.4.2.1**) functional supplement (Described in **3.4.2.2**). Data were subjected to statistical analysis using the following ANOVA model.

$$Y_{ijkl} = \mu + \alpha_i + \beta_j + \gamma_k + (\alpha\beta)_{ij} + (\beta\gamma)_{jk} + (\alpha\gamma)_{ik} + e_{ijkl}, i = 1, 2, 3, 4; j = 1, 2; k = 1, 2; l = 1, 2, 3 \dots n$$

Where, Y_{ijkl} was any observation for which, μ was general mean, α_i was main factor effects at 4 levels, B_j (Supplement) and γ_k (Breed) were sub-factors at each 2 levels. The other components of the models such as $(\alpha\beta)_{ij}$, $(\beta\gamma)_{jk}$ and $(\alpha\gamma)_{ik}$ were interaction effect between main and respective sub-treatments and e_{ijkl} was random error component (Montgomery, 1976). According to the above model, the degrees of freedom for the factors is shown in **Table 3.12**. The variance between treatments was compared by Duncan Multiple Range Test (DMRT) for the main factor and significance between mean values at probability of $\alpha \leq 0.001$ to 0.05 was noted with different alpha superscripts.

Table 3.10: Standard recommendation of protein and energy for lactating cows

Standard	Breed	Dietary Protein Requirement (g/day)			Daitary Energy Requirement (MJ/day)		
		Maintenance	Lactation	Total	Maintenance	Lactation	Total
ICAR (2013)	HFX Cows	476	1632	2108	54.56	75.3	129.86
	Deoni Cows	394	434	828	44.56	22.59	67.15
CSIRO, Australia (Moran, 2005)	HFX Cows	450	1080	1530	54.00	88.40	142.40
	Deoni Cows	350	360	710	40.00	24.75	64.75
FAO (1993)	HFX Cows	391	1394	1785	46.90	85.00	131.90
	Deoni Cows	324	369	693	38.80	22.50	61.30
NRC (2001)	HFX Cows	472	1351	1823	56.72	72.84	129.56
	Deoni Cows	367	252	620	41.91	27.61	69.52
ARC (1980)	HFX Cows	354	1020	1374	56.9	80.41	137.31
	Deoni Cows	275	270	545	44.3	21.28	65.58

Table 3.11: Anova model and degrees of freedom for *in vitro* experiments

Factors	Degrees of freedom
Energy	3
Supplement	1
Energy × Supplement	3
Error (By subtraction)	72
Total	79

Table 3.12: Anova model and degrees of freedom for *in vivo* experiments

Factors	Degrees of freedom
Energy	3
Supplement	1
Breed	1
Energy × Supplement	3
Energy × Breed	3
Supplement × Breed	1
Error (By subtraction)	19
Total	31

Repeat measure analysis of variance (rANOVA) was used for multiple measures of attribute at different time intervals, for example, blood parameters, blood metabolites etc., based on the following model:

$$F = \text{MSS}_{\text{Treatment}} \div \text{MS}_{\text{Error}} = (\text{SS}_{\text{Treatment}}/\text{df}_{\text{Treatment}}) \div (\text{SS}_{\text{Error}}/\text{df}_{\text{Error}})$$

Between subjects effects: $\text{SS}_{\text{Total}} = \text{SS}_{\text{Treatment}} + \text{SS}_{\text{Error}}$ (df = n-1)

Within subjects effects: $\text{SS}_{\text{Total}} = \text{SS}_{\text{Treatment (excluding individual difference)}} + \text{SS}_{\text{Subjects}} + \text{SS}_{\text{Error}}$ where, $\text{df}_{\text{Total}} = \text{df}_{\text{Treatments (within subjects)}} + \text{df}_{\text{between subjects}} + \text{df}_{\text{error}} = (k-1)+(n-1)+((n-k)(n-1))$.

Result and Discussion

4.0 RESULT AND DISCUSSION

4.1 *In vitro* studies

Proximate (**Table 4.1**) and the cell wall (**Table 4.2**) chemical composition of the substrate without or with a different source of energy showed a significant difference in respective parameters except for gross energy ($P = 0.08$) and AIA. Total mixed substrate CP content was about 8%, NDF was 62%, and NFC was 18%. TCHOs in the substrate was 79%, to which 10% or 20 mg energy source from glycerol (T1) or jaggery (T2) or ground maize grain (T3) was fortified in treatment groups (TGs). Energy fortification (10%) to the substrate in TGs increased the GE by 0.02 Mcal to 0.04 Mcal because of an increase in TCHO by 1% to 2% and increased calorific value (Silveira *et al.*, 2015). Glycerol is a simple polyol compound while jaggery is a disaccharide. Both are readily soluble and hardly contain any other chemical constituents. But, maize being a rich starch source also contains other proximate and cell wall constituents. CP% in CG and T3 was comparable but, a significantly differ with T1 and T2. NFC% was increased significantly in TGs because substrate fortified by 10% with different sources of energy. NFC% was comparable between T1 and T2 but, 4% higher to T3 and 7% to CG. Glycerol and jaggery were instantly soluble than maize starch. Their calorific value also differed (Section- **3.4.2.1**). NFC present in the substrate is important for the lag fermentation and progression of fermentation at least for 6 to 8 h from initiation of the incubation (Srinivas and Krishnamoorthy, 2005).

Further, CG and TGs were added functional nutrients (FNS) supplement 2 mg to 200 mg of the substrate that was 1% of the substrate and compared to without supplement to understand any interaction effect of the FNS supplement on the energy fermentation thus, the main affect on the substrate fermentation was the main affect of fortified energy and the sub affect was FNS supplement. Hemicelluloses were comparable between CG and T3 (26%) or T2 and T3 (24%) but, cellulose was significantly more in CG (28%) than TGs (26%) and since energy sources are different, CWC in substrates was significantly less in T1, T2, and T3 than CG thus, the calorific value was more and bulkiness was less.

Table 4.1: Proximate composition of substrate and substrate fortified with different sources of energy for *in vitro* fermentation studies (% on DM basis)

	Replicate (R)	DM	OM	CP	EE	TA	AIA	TCHO	GE (Mcal)
Substrate (Control/ Reference)	1	91.26	89.12	8.34	1.73	10.88	3.32	79.05	3.65
	2	90.74	89.82	8.32	1.81	10.18	3.65	79.69	3.68
	3	91.07	89.29	8.41	1.64	10.71	3.28	79.24	3.65
	Mean	91.02^c	89.41^a	8.36^b	1.73^{ab}	10.59^b	3.42	79.33^a	3.66
Substrate + Glycerol (T1)	1	82.97	90.11	7.58	1.57	9.89	3.02	80.95	3.68
	2	82.45	90.75	7.56	1.65	9.25	3.32	81.54	3.71
	3	82.78	90.26	7.65	1.49	9.74	2.98	81.13	3.69
	Mean	82.73^a	90.37^b	7.60^a	1.57^a	9.63^a	3.11	81.21^b	3.69
Substrate + Jaggerey (T2)	1	91.03	89.79	7.78	1.57	10.21	3.12	80.43	3.67
	2	90.51	90.43	7.76	1.65	9.57	3.42	81.02	3.70
	3	90.84	89.94	7.85	1.49	10.06	3.09	80.61	3.67
	Mean	90.79^{bc}	90.05^{ab}	7.80^b	1.57^a	9.95^a	3.21	80.69^{ab}	3.68
Substrate + Ground maize (T3)	1	90.66	90.03	8.35	1.80	9.97	3.04	79.88	3.69
	2	90.14	90.67	8.33	1.88	9.33	3.34	80.46	3.72
	3	90.47	90.19	8.41	1.72	9.81	3.00	80.05	3.69
	Mean	90.42^b	90.30^b	8.36^c	1.80^b	9.70^a	3.13	80.13^b	3.70
	SEM	0.152	0.198	0.027	0.047	0.198	0.109	0.178	0.010
	P value (TG)	0.001	0.032	0.001	0.019	0.032	0.248	0.001	0.079
	P value (R)	0.983	0.142	0.947	0.194	0.142	0.014	0.577	0.075

Values with different lower alpha superscripts in the column for a parameter differ significantly; ***P≤ 0.001, *P≤ 0.05

Table 4.2: Cell wall composition of substrate and substrate fortified with different sources of energy for *in vitro* fermentation studies (% on DM basis)

	Replicate (R)	CC	NDF	ADF	HC	Cellulose	ADL	NFC
Substrate	1	38.54	61.46	35.32	26.14	28.51	4.70	17.59
	2	37.74	62.26	34.87	27.39	27.46	4.48	17.43
	3	38.82	61.18	35.77	25.41	28.45	4.38	18.06
	Mean	38.37^a	61.63^c	35.32^b	26.31^b	28.14^b	4.52^b	17.69^a
Substrate +Glycerol	1	44.13	55.87	32.11	23.76	25.92	4.27	25.08
	2	43.40	56.60	31.70	24.90	24.96	4.07	24.94
	3	44.38	55.62	32.52	23.10	25.86	3.98	25.51
	Mean	43.97^c	56.03^a	32.11^a	23.92^a	25.58^a	4.11^a	25.18^c
Substrate + Jaggerey	1	44.13	55.87	32.11	23.76	25.92	4.27	24.56
	2	43.40	56.60	31.70	24.90	24.96	4.07	24.42
	3	44.38	55.62	32.52	23.10	25.86	3.98	24.99
	Mean	43.97^c	56.03^a	32.11^a	23.92^a	25.58^a	4.11^a	24.66^c
Substrate +Ground maize	1	41.47	58.53	32.60	25.93	25.92	4.27	21.36
	2	40.75	59.25	32.19	27.06	24.96	4.07	21.21
	3	41.73	58.27	33.01	25.26	25.86	3.98	21.78
	Mean	41.32^b	58.68^b	32.60^a	26.08^b	25.58^a	4.11^a	21.45^b
	SEM	0.301	0.301	0.243	0.539	0.318	0.088	0.176
	P value (TG)	0.001	0.001	0.001	0.019	0.001	0.024	0.001
P value (R)	0.861	0.861	0.750	0.191	0.504	0.170	0.970	

Values with different lower alpha superscripts in the column for a parameter differ significantly; ***P≤ 0.001, *P≤ 0.05

Fermentation between different grains was significantly varied due to difference in chemical composition (Singh and Srinivas, 2012).

IVGP (mL/200 mg) in CG and TGs at different incubation intervals is presented in **Table 4.3**. During the initial 4 hr, IVGP was significantly higher ($P < 0.001$) in CG, T2, and T3 than T1. IVGP was significantly ($P < 0.001$) different between groups until 48 hr of fermentation because of differences in solubility of energy sources, particularly glycerol and jaggery. The difference in fermentation was due to their simple to complex nutrient differences in their chemical composition. IVGP was not significant from 48 to 72 hr of incubation between groups indicated reaching a critical point of digestible part of the substrate by 48 hr. Concentrate feedstuffs incubation is mostly complete by 24 hr while roughages fermentation was evaluated till 96 hr of incubation (Makar, 2004; Srinivas and Krishna moorthy, 2005; Sallem, 2002). Though glycerol is polyol compound and soluble as rapidly as disaccharide of jaggery, IVGP was significantly ($P < 0.001$) higher in T2 than CG, T1, and T3 between 2 to 6 hr of fermentation. This indicated that jaggery solubility and fermentation were faster than glycerol and the starch I maize grains was also utilized by 18 hr of incubation. Acceleration in the IVGP was significantly ($P < 0.001$) more in T1 at 12 hr of incubation than CG, T2, and T3. At 18 hr of incubation, IVGP in TGs was significantly higher ($P < 0.001$) than CG for the main factor. The substrate fortification with jaggery, glycerol, or maize grain could accelerate fermentation at 6, 12, and 18 hr, respectively. An increase in the IVGP from 24 hr to 48 hr of incubation was significant ($P < 0.001$; $P < 0.01$) in-ground maize fortified group (T3) than CG, T1, and T2. Compared to glycerol and jaggery, protein matrix surrounded by starch granules may limit microbial access to starch and solubility may take at a slower phase than other two energy sources (Cheng *et al.*, 1991). Further fortification of the substrate besides energy, with FNS supplement (**Table 3.5**) significantly ($P < 0.001$) increased IVGP than without supplement. Although FNS supplement (sub-factor) increased significantly ($P < 0.001$) IVGP in CG, it was lesser than TGs with FNS supplement until 60 hr. This indicated that the substrate fermentation was extended by another 12 hr because of FNS supplement to energy. Some minerals are essential for the enzymatic process in rumen microbes hence; FNS supplement imparted

Table 4.3: IVGP on different source of energy with or without supplement at different intervals of fermentation (ml/200 mg substrate)

Fermentation Interval	Group	Energy source	Supplement		SEM			P-Value		
			Without	With	Energy	Suppl.	Interaction	Energy	Suppl.	Interaction
0.5 hr	Control	4.13 ^d	2.99 ^A	5.26 ^B	0.11	0.07	0.15	0.001	0.001	0.001
	Glycerol	2.01 ^a	1.17 ^A	2.86 ^B						
	Jaggery	2.74 ^b	1.54 ^A	3.94 ^B						
	Maize	1.87 ^a	1.66 ^A	2.09 ^B						
	Mean	2.69^{***}	1.84^{***}	3.54^{***}						
1 hr	Control	1.21 ^a	0.40 ^A	2.01 ^B	0.14	0.10	0.20	0.001	0.001	0.001
	Glycerol	1.84 ^b	1.01 ^A	2.66 ^B						
	Jaggery	1.91 ^b	1.89 ^A	1.93 ^B						
	Maize	1.54 ^{ab}	1.47 ^A	1.61 ^B						
	Mean	1.62^{***}	1.19^{***}	2.06^{***}						
2 hr	Control	3.34 ^b	2.69 ^A	3.98 ^B	0.21	0.15	0.30	0.001	0.001	0.04
	Glycerol	2.49 ^a	1.27 ^A	3.72 ^B						
	Jaggery	4.12 ^c	4.16 ^A	4.88 ^B						
	Maize	3.18 ^b	2.37 ^A	3.98 ^B						
	Mean	3.38^{***}	4.18^{***}	4.93^{***}						
4 hr	Control	4.78 ^b	3.81 ^A	5.67 ^B	0.18	0.13	0.25	0.001	0.001	0.02
	Glycerol	3.66 ^a	3.44 ^A	3.87 ^B						
	Jaggery	5.67 ^c	5.43 ^A	5.91 ^B						
	Maize	4.09 ^a	3.93 ^A	4.25 ^B						
	Mean	4.55^{***}	4.17^{***}	4.93^{***}						
6 hr	Control	3.91 ^a	2.63 ^A	5.19 ^B	0.22	0.32	0.32	0.001	0.001	0.02
	Glycerol	4.40 ^a	3.63 ^A	5.17 ^B						
	Jaggery	6.93 ^c	6.52 ^A	7.34 ^B						
	Maize	5.13 ^b	4.74 ^A	5.52 ^B						
	Mean	5.09^{***}	4.38^{***}	5.80^{***}						
12 hr	Control	8.06 ^b	5.09 ^A	11.04 ^B	0.37	0.26	0.53	0.001	0.001	0.001
	Glycerol	11.10 ^c	5.98 ^A	16.22 ^B						
	Jaggery	5.44 ^a	4.80 ^A	6.07 ^B						
	Maize	7.52 ^b	5.46 ^A	9.59 ^B						
	Mean	8.03^{***}	5.33^{***}	10.73^{***}						

Fermentation Interval	Group	Energy source	Supplement		SEM			P-Value		
			Without	With	Energy	Suppl.	Interaction	Energy	Suppl.	Interaction
18 hr	Control	5.10 ^a	4.05 ^A	6.17 ^B	0.24	0.17	0.34	0.002	0.001	0.001
	Glycerol	5.86 ^b	4.90 ^A	6.82 ^B						
	Jaggery	6.34 ^b	6.34 ^A	6.36 ^B						
	Maize	6.26 ^b	3.84 ^A	8.68 ^B						
	Mean	5.89^{***}	4.78^{***}	7.00^{***}						
24 hr	Control	1.85 ^a	1.51 ^A	2.18 ^B	0.17	0.12	0.24	0.001	0.001	0.001
	Glycerol	2.64 ^b	1.97 ^A	3.31 ^B						
	Jaggery	3.47 ^c	3.39 ^A	3.54 ^B						
	Maize	4.16 ^d	3.70 ^A	4.61 ^B						
	Mean	3.03^{***}	2.64^{***}	3.41^{***}						
36 hr	Control	2.68 ^a	1.99 ^A	3.36 ^B	0.20	0.14	0.28	0.001	0.003	0.05
	Glycerol	2.16 ^a	1.87 ^A	2.45 ^B						
	Jaggery	2.41 ^a	2.09 ^B	2.73 ^B						
	Maize	3.51 ^b	3.61 ^B	3.41 ^B						
	Mean	2.69^{***}	2.39^{***}	2.99^{***}						
48 hr	Control	1.37 ^c	0.69 ^B	2.04 ^B	0.14	0.10	0.20	0.001	0.001	0.003
	Glycerol	0.65 ^a	0.47 ^B	0.83 ^B						
	Jaggery	1.91 ^c	1.40 ^B	2.43 ^B						
	Maize	4.03 ^d	3.09 ^B	4.98 ^B						
	Mean	1.99^{***}	1.41^{***}	2.57^{***}						
60 hr	Control	1.89	1.30 ^B	2.47 ^B	0.26	0.18	0.36	0.12	0.04	0.003
	Glycerol	2.27	2.45 ^B	2.08 ^B						
	Jaggery	1.73	0.83 ^B	2.63 ^B						
	Maize	2.53	2.79 ^B	2.36 ^B						
	Mean	2.10	1.84[*]	2.36[*]						
72 hr	Control	1.83	1.62	2.05	0.18	0.13	0.26	0.17	0.40	0.15
	Glycerol	1.77	1.72	1.81						
	Jaggery	1.31	1.01	1.61						
	Maize	1.52	1.78	1.27						
	Mean	1.61	1.53	1.69						

'n' for each main treatment (Energy source) was 20 (N= 80) & 'n' for sub-treatment (Supplement) was 10 (N = 40)

Values with different lower alpha superscripts in the row for a parameter differ significantly; ***P≤ 0.001

Values with different upper alpha superscripts with or without supplement for a respective energy source in a row under each parameter differ significantly; ***P≤ 0.001, *P≤ 0.05

a positive effect on IVGP and interaction effect between energy and FNS supplement had a positive effect on till 60 hr. Pino and Heinrichs (2016) also concluded increased total gas production on organic trace mineral supplementation to substrate.

During initial hours CG had significantly ($P < 0.001$) higher cumulative IVGP than TGs (**Table 4.4**). Cumulative IVGP in T2 was higher ($P < 0.001$) than T1 and T3 till 24 hr (**Fig 4.1a**). Further, FNS supplementation in TGs significantly ($P < 0.001$) increased IVGP compared to without FNS supplement. Interaction between energy and FNS supplementation significantly ($P < 0.001$) influenced IVGP until 72 hr. Cumulative IVGP was beyond 50% in either CG or T1 and T2 at 12 hr but, 18 hr in T3 (**Table 4.5**).

Substrate fermentation was completed 75% by 24 hr of incubation in CG and TGs. The effect of FNS supplement had a greater influence on CG and T1 that indicated the need of FNS supplement for efficient fermentation of glycerol than either jaggery or maize grain (**Fig 4.1b**). The interaction effect of energy without and with supplement was significant ($P < 0.001$) until 60 hr of fermentation, but not significant at/after 60 hr of fermentation interval.

4.1.1 Fermentation kinetics

Fermentation kinetics were significantly ($P < 0.001$) different between CG and TGs (**Table 4.5**). Total gas production (TGP) in TGs at 72 hr was ranged between 43 mL (T1) to 49 mL (T2) compared to 43 mL in CG. Although TGP in T1 without FNS supplement was comparable to CG and lesser than T2 and T3, TGP in T1 was higher than T2 with FNS supplement. This indicated that substrate fermentation with glycerol needed FNS supplements because rumen microbes may require minerals for phosphorylation of glycerol to utilize (Blötz and Stülke, 2017). The interaction effect of energy and FNS supplement was significant ($P < 0.001$) between CG and TGs.

Lag time (λ) was significantly ($P < 0.001$) more in T3 than CG, T1, and T2. Higher λ for fermentation of T3 substrate indicated delayed initiation of fermentation because of the complexity of maize starch with α -zein protein and

Table 4.4: Cumulative IVGP (%) of energy source associated with or without supplement at different intervals of fermentation (%/200 mg substrate)

Fermentation Interval	Group	Energy source	Energy & Supplement Interaction		SEM			P-Value		
			Without	With	Energy	Suppl.	Interaction	Energy	Suppl.	Interaction
0.5 hr	Control	9.63 ^d	9.61 ^A	9.66 ^B	0.23	0.16	0.33	0.001	0.001	0.001
	Glycerol	4.50 ^b	3.71 ^A	5.28 ^B						
	Jaggery	5.63 ^c	3.69 ^A	7.57 ^B						
	Maize	3.90 ^a	3.75 ^A	4.04 ^B						
	Mean	5.91	5.20	6.62						
1 hr	Control	12.12 ^d	10.96 ^A	13.28 ^B	0.35	0.25	0.49	0.001	0.001	0.001
	Glycerol	8.48 ^b	6.80 ^A	10.16 ^B						
	Jaggery	9.80 ^c	8.31 ^A	11.29 ^B						
	Maize	7.06 ^a	6.62 ^A	7.50 ^B						
	Mean	9.36	8.17	10.55						
2 hr	Control	20.10 ^a	19.64 ^A	20.54 ^B	0.47	0.33	0.66	0.001	0.001	0.001
	Glycerol	13.90 ^a	10.82 ^A	16.97 ^B						
	Jaggery	19.43 ^b	18.23 ^A	20.62 ^B						
	Maize	13.49 ^b	13.25 ^A	13.72 ^B						
	Mean	16.73	15.48	17.97						
4 hr	Control	31.58 ^b	30.09 ^A	32.71 ^B	0.58	0.41	0.82	0.001	0.05	0.05
	Glycerol	22.95 ^a	21.75 ^A	24.15 ^B						
	Jaggery	31.66 ^b	31.28 ^A	32.03 ^B						
	Maize	22.00 ^a	21.38 ^A	22.62 ^B						
	Mean	27.05	26.78	27.43						
6 hr	Control	40.59 ^b	40.39	40.79 ^B	0.73	0.52	1.03	0.001	0.05	0.05
	Glycerol	33.48 ^a	33.21	33.74 ^B						
	Jaggery	46.57 ^c	46.11	47.04 ^B						
	Maize	32.62 ^a	31.18	34.05 ^B						
	Mean	38.31	37.82	38.57						
12 hr	Control	58.91 ^b	57.40	60.42 ^B	0.74	0.52	1.04	0.001	0.001	0.001
	Glycerol	57.80 ^b	52.05	63.55 ^B						
	Jaggery	58.20 ^b	57.75	58.64 ^B						
	Maize	47.76 ^a	47.09	48.43 ^B						
	Mean	55.67	53.80	57.54						

Fermentation Interval	Group	Energy source	Energy & Supplement Interaction		SEM			P-Value		
			Without	With	Energy	Suppl.	Interaction	Energy	Suppl.	Interaction
18 hr	Control	71.05 ^b	70.47	71.64 ^B	0.78	0.55	1.11	0.001	0.001	0.001
	Glycerol	71.82 ^b	67.56	76.09 ^B						
	Jaggery	71.98 ^b	73.99	69.97 ^B						
	Maize	60.17 ^a	56.34	64.01 ^B						
	Mean	68.76	67.09	70.43						
24 hr	Control	75.48 ^b	75.34	75.63 ^B	0.84	0.60	1.19	0.001	0.003	0.001
	Glycerol	78.00 ^c	73.79	82.22 ^B						
	Jaggery	79.47 ^c	82.19	76.75 ^B						
	Maize	68.79 ^a	65.35	72.24 ^B						
	Mean	75.44	74.17	76.71						
36 hr	Control	88.03 ^b	88.21	87.85 ^B	0.65	0.46	0.92	0.001	0.05	0.001
	Glycerol	88.34 ^b	85.36	91.32 ^B						
	Jaggery	89.70 ^b	91.20	87.19 ^B						
	Maize	83.33 ^a	82.29	84.37 ^B						
	Mean	87.35	85.76	88.68						
48 hr	Control	91.13 ^a	90.50	91.76 ^B	0.65	0.46	0.91	0.001	0.002	0.001
	Glycerol	89.86 ^a	86.90	92.83 ^B						
	Jaggery	93.71 ^b	95.57	91.85 ^B						
	Maize	91.44 ^a	89.05	93.82 ^B						
	Mean	91.53	90.51	92.87						
60 hr	Control	95.50 ^a	94.76	96.24 ^B	0.46	0.33	0.65	0.03	0.008	0.12
	Glycerol	95.63 ^a	94.62	96.64 ^B						
	Jaggery	97.22 ^b	96.89	97.56 ^B						
	Maize	96.65 ^{ab}	95.68	97.73 ^B						
	Mean	96.25	95.49	97.04						
72 hr	Control	100.00	100.00	100.00	-	-	-	-	-	-
	Glycerol	100.00	100.00	100.00						
	Jaggery	100.00	100.00	100.00						
	Maize	100.00	100.00	100.00						
	Mean	100.00	100.00	100.00						

'n' for each main treatment (Energy source) was 20 (N = 80) & 'n' for sub-treatment (Supplement) was 10 (N = 40)

Values with different lower alpha superscripts in the row for a parameter differ significantly; ***P ≤ 0.001, **P ≤ 0.01, *P ≤ 0.05

Values with different upper alpha superscripts with or without supplement for a respective energy source in a row under each parameter differ significantly; ***P ≤ 0.001

slow soluble sugars than glycerol or jaggery. Soluble sugars in different maize varieties is <4.5% in TCHO (Zilic *et al.*, 2011) yet λ fermentation of T3 was higher than CG. The amount of soluble sugars available in the substrate is important for the chemotactic attraction of microbes for colonization on the feed particles (Diaz *et al.*, 2014). Either glycerol or jaggery is soluble rapidly in the inoculum. Even though glycerol solubility in water is faster than jaggery yet, later contains glucose and fructose monosaccharides. Most of the bacteria inherently can utilize glucose as a preferential energy source. The glucose moieties available from jaggery are first to attract microflora. However, some of the bacteria have a preference for fructose due to the control of glucose-6-phosphate dehydrogenase (Conrod and Schlegel, 1978). In the case of glycerol, it is transported into microbial cells and then becomes phosphorylated and catabolized in Embden–Meyerhoff pathway (Blötz and Stülke, 2017). Hence, there will be a difference due to different catabolic pathways in the utilization of jaggery and glycerol in the rumen inoculum. Contrary to glycerol or jaggery, maize starch is a complex polysaccharide (75% amylopectin and 25% amylose) and takes a long time for its breakdown to glucose molecules. Henceforth, there was a difference in the λ fermentation between CG and TGs. The λ fermentation was further improved ($P < 0.001$) with FNS supplementation to the substrate, particularly in T1 and T2 but, less in T3. According to Nguyen and Tran (2018), some divalent metal cations e.g., Mg^{2+} , Mn^{2+} , Co^{2+} , etc., have been proved to optimize fermentation. The improved λ fermentation with FNS supplementation proved its catalyst role in the utilization of energy and augmented the anaerobic fermentation.

$T_{1/2}$ fermentation was below 11 hr in CG, T1, and T2 in contrast to 12 hr in T3 ($P < 0.001$). The complexity in the maize starch not only influenced the λ fermentation but also $T_{1/2}$ fermentation. Supplementation of FNS supplement undoubtedly had a significant ($P < 0.001$) influence on the improving $T_{1/2}$ fermentation. If λ fermentation, TGP, or kinetic rate of gas production (Y_G) explains the microbial characteristics (Srinivas and Krishnamoorthy, 2005), $T_{1/2}$ time and rate constant of fermentation represent the substrate characteristics (Srinivas and Swain, 2009). The rate constant of fermentation at $T_{1/2}$ time and rate constant for TGP was significantly ($P < 0.001$) higher in CG, T1, and T2 than T3. According to Singh and Srinivas (2012), if the rate of fermentation is variable

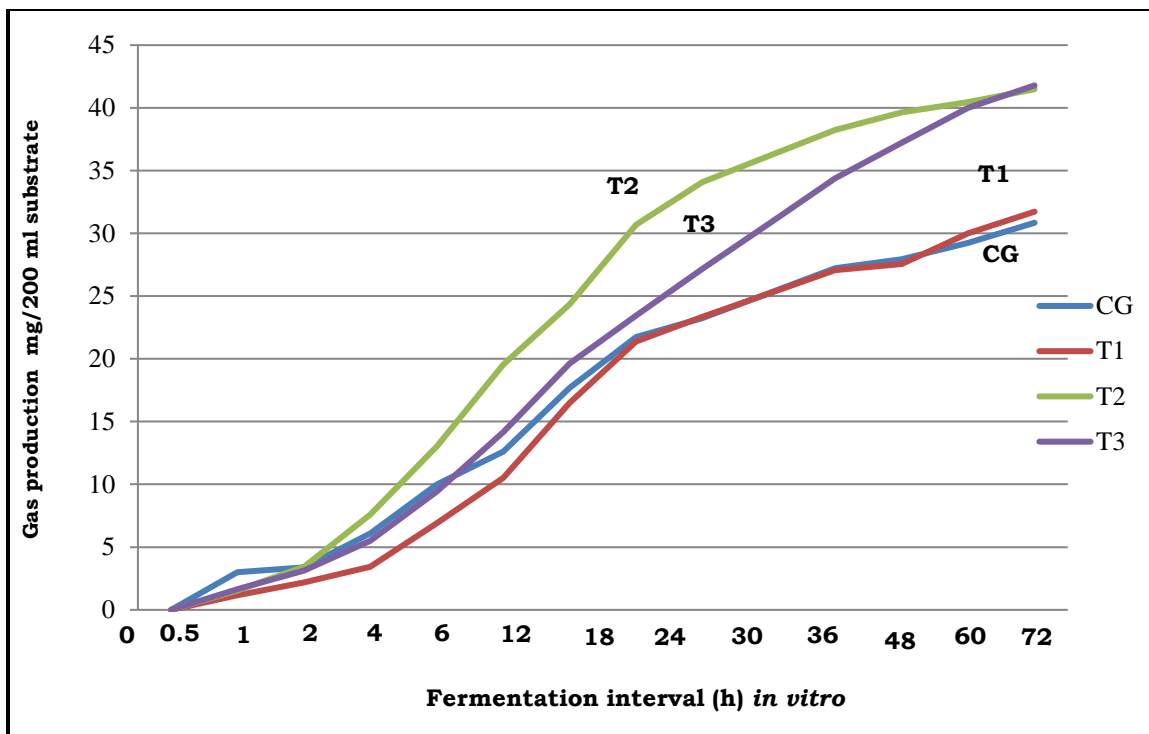


Fig. 4.1a: Cumulative gas production in different groups without supplement

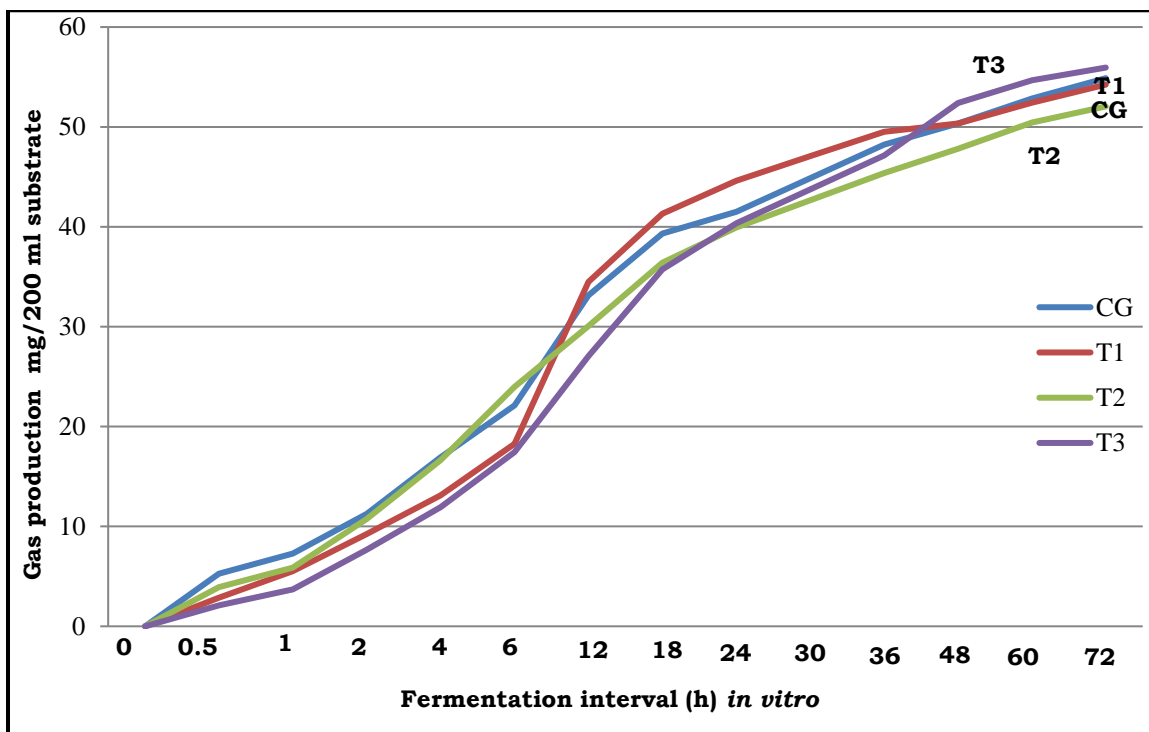


Fig. 4.1b: Cumulative gas production in different groups with supplement

Table 4.5: Gas production and fermentation kinetics on different source of energy with or without supplement

Kinetic parameter	Group	Energy source	Supplement		SEM			P-Value		
			Without	With	Energy	Suppl.	Interaction	Energy	Suppl.	Interaction
Total gas production (ml/200mg)	Control	42.87 ^a	30.85 ^A	54.88	0.88	0.62	1.24	0.001	0.001	0.001
	Glycerol	42.98 ^a	31.73 ^A	54.23						
	Jaggery	46.77 ^b	41.48 ^A	52.05						
	Maize	48.87 ^b	41.79 ^A	55.94						
	Mean	45.37^{***}	34.46^{***}	54.28^{***}						
Fermentation lag time (h)	Control	0.12 ^a	0.13 ^B	0.12 ^A	0.002	0.001	0.003	0.001	0.001	0.001
	Glycerol	0.13 ^a	0.14 ^B	0.11 ^A						
	Jaggery	0.12 ^a	0.12 ^B	0.11 ^A						
	Maize	0.15 ^b	0.15 ^B	0.14 ^A						
	Mean	0.13^{***}	0.14^{***}	0.13^{***}						
Fermentation T _{1/2} (h)	Control	10.61 ^a	10.84 ^B	10.36 ^A	0.12	0.08	0.16	0.001	0.001	0.001
	Glycerol	10.86 ^a	11.69 ^B	10.02 ^A						
	Jaggery	10.77 ^a	10.79 ^B	10.74 ^A						
	Maize	12.43 ^b	12.57 ^B	12.29 ^A						
	Mean	11.16^{***}	11.46^{***}	10.87^{***}						
Rate constant of T _{1/2} (%/h)	Control	6.56 ^b	6.41 ^A	6.70 ^B	0.06	0.05	0.09	0.001	0.001	0.001
	Glycerol	6.43 ^b	5.94 ^A	6.92 ^B						
	Jaggery	6.44 ^b	6.43 ^A	6.46 ^B						
	Maize	5.59 ^a	5.53 ^A	5.65 ^B						
	Mean	6.25^{***}	6.08^{***}	6.43^{***}						
Rate constant of TGP (%/h)	Control	13.61 ^b	13.26 ^A	13.96 ^B	0.17	0.12	0.24	0.001	0.001	0.001
	Glycerol	13.35 ^b	12.02 ^A	14.68 ^B						
	Jaggery	13.44 ^b	13.34 ^A	13.55 ^B						
	Maize	11.03 ^a	10.88 ^A	11.19 ^B						
	Mean	12.86^{***}	12.43^{***}	13.29^{***}						
Kinetic rate of gas production (ml/200mg)	Control	42.73 ^a	30.73 ^A	54.72 ^B	0.87	0.61	1.23	0.001	0.001	0.001
	Glycerol	42.83 ^a	31.55 ^A	54.11 ^B						
	Jaggery	46.60 ^b	41.34 ^A	51.86 ^B						
	Maize	48.47 ^b	41.43 ^A	55.51 ^B						
	Mean	45.16^{***}	36.26^{***}	54.05^{***}						
Fermentation rate (%/h)	Control	7.02 ^b	7.01 ^A	7.03 ^B	0.08	0.06	0.11	0.001	0.001	0.001
	Glycerol	7.06 ^b	6.46 ^A	7.65 ^B						
	Jaggery	7.07 ^b	7.05 ^A	7.09 ^B						
	Maize	6.04 ^a	5.96 ^A	6.11 ^B						
	Mean	6.84^{***}	6.64^{***}	7.05^{***}						

'n' for each main treatment (Energy source) was 20 (N= 80) & 'n' for sub-treatment (Supplement) was 10 (N = 40)

Values with different lower alpha superscripts in the row for a parameter differ significantly; ***P≤ 0.001

Values with different upper alpha superscripts with or without supplement for a respective energy source in a row under each parameter differ significantly; ***P≤ 0.001

with NFC% in the substrate, CG had lesser NFC (18%) than T3 (22%) or other TGs (25%), it should have been greater in T3 than CG. NFC is made-up of starch, simple sugars, and soluble fiber hence, how much of soluble sugars are present in NFC is important than NFC% *in toto*. FNS supplementation to substrates in CG and TGs had a positive effect on all the kinetic parameters. The fermentation rate of substrate fortified with maize grain without or with FNS supplementation had a 1% hr⁻¹ lesser (P< 0.001) rate or 24% slower fermentation in 24 hr than CG or T1 and T2. It was concluded from the *in vitro* studies that among different energy sources, jaggery followed by glycerol were accentuated fermentation than maize grain. Substrate fermentation with maize grain fortification at 72 hr of incubation was comparable to other energy sources but, slower by 24%. FNS supplementation had an absolute effect on accentuating the overall fermentation kinetics. Jaggery with FNS supplement was best, followed by glycerol and least was maize in improving the substrate fermentation.

4.2 In vivo studies

The live BW (kg), or metabolic BW (kg W^{0.75}) of 32 prepartum cows belonging 16 to the HFX and, 16 to the Deoni breed, were randomly allocated for the energy and FNS supplement and their mean BW are presented in **Table 4.6**. The Deoni cows' BW was significantly (P< 0.001) lesser than the HFX cows because they belonged to medium size breeds whereas Holstein Friesian or its crosses belongs to large breeds.

The Deoni breed of cows was genetically not well developed compared to the HFX cows. The variance in live or metabolic BW between the HFX and the Deoni cows was because of the lesser genetic diversity in the population of HF crosses that is < 2% σ^2 (Betz, 2007). The higher population variation in the body size of indigenous cows could be due to lack of selection intensity, generation interval, genetic variation and accuracy of selection (Betz, 2007). Since metabolic BW had a larger variance in the Deoni cows, their metabolic active tissue is also highly variable (Singh and Srinivas, 2020). The conception, conceptus growth and the process of gestation is a physiological phenomenon, therefore, we contemplated that breed effects are null and void for biochemical changes

Table 4.6: Live and metabolic body weight of prepartum cows of the experiment

Parameter	Group	Energy × Breed		Energy × Suppl.		HFX × Suppl.		Deoni × Suppl.		E × B × S
		HFX	Deoni	Without	With	Without	With	Without	With	
Live body weight (kg)	Control	444.75	330.75	375.50	400.00	418.50	471.00	332.50	329.00	387.75
	Glycerol	481.25	326.00	387.50	419.75	437.50	525.00	337.50	314.50	403.60
	Jaggery	505.25	393.25	443.50	455.00	495.50	515.00	391.00	395.00	449.25
	Maize	475.50	350.25	418.25	407.50	477.50	473.50	363.00	337.50	413.00
	Mean	476.70	350.00	406.20	420.60	456.25	497.10	356.10	344.00	413.40
	SEM	14.58		14.58		20.61		20.61		
	P Value	0.001***		0.496		0.217		0.229		
Metabolic body weight(kg)	Control	96.49	77.53	84.98	89.05	92.09	100.90	77.87	77.20	87.01
	Glycerol	102.63	76.68	87.15	92.17	95.60	109.67	78.70	74.67	89.66
	Jaggery	106.51	88.30	96.47	98.35	104.92	108.11	88.02	88.59	97.41
	Maize	101.68	80.94	92.31	90.31	101.47	101.90	83.16	78.73	91.31
	Mean	101.68	80.86	90.22	92.47	98.52	105.41	81.93	79.79	91.35
	SEM	4.778		4.778		3.378		3.378		
	P Value	0.001***		0.516		0.213		0.206		

Level of significance; ***P≤ 0.001, **P≤ 0.01, *P≤ 0.05

but, physical changes may possible for e.g., DMI, calf birth weight etc., because of difference in their live or metabolic BW. Grummer *et al.* (2004) also suggested that the decline in feed intake at the peripartal period is not unique to bovine and dairy cow but also common in other species. The live BW was 36% higher ($P < 0.001$) in the HFX cows compared to the Deoni cows with a mean BW of 350 kg. Except breed difference, the live or metabolic BW of prepartum cows randomized in groups for energy, energy and FNS supplement, or any breed and FNS supplement, or triangular interaction between energy, breed and supplement were insignificant. Therefore, any of the tested parameter *in vivo* should logically free from the effect of metabolic BW except for breed difference. Generally, 4 yr cows attain 86% of mature BW while 96% of height was achieved by 3 yr and 100% growth was attained by 3rd parity (Pilarczyk and Wojcik, 2008). All the cows on experiment were between 3rd to 5th parity and attained their full BW. Many of the reproductive caveats are associated with 1st and 2nd parity but, no differences observed in BW or calving parameters in 3rd parity (Pilarczyk and Wojcik, 2008). But, different breeds express differently for the same feeding management (Albertí *et al.*, 2008).

4.2.1 Nutrient composition of the diet

Nutrient composition of MGF, CS, FM straw, glycerol, jaggery, ground maize grain, and flaxseed are presented in **Table 4.7**. DM and CP of MGF (*Section- 3.3.1 and 3.5*) was 25% and 5%, respectively. Moisture content in green roughages may vary from 60% to 90% depending on the season and stage of maturity. Srinivas *et al.* (2018) reported CP% of 4 to 11% in fresh perennial and cultivated grasses and about 6% in MGF. The CP% in the MGF fed is non-maintenance type roughage in contrast, roughages with 9% to 11% CP cater the maintenance requirements. Seasonal and spatial variations in the nutritive value of native pastures also may vary from 32 to 140 g CP/kg DM (Onyango *et al.*, 2019). The average DM and CP of the CS was 90% and 18%, respectively. CS with protein from 14 to 18% is good for the health of dairy cattle (Hynes *et al.*, 2016). Various possible caveats associated with high protein CS are reduced fertility, increased ammonia excretion in urine, acid load on kidneys, metabolic diseases, etc., (Pesta and Samuel, 2014; Salo, 2018). FM straw had 5%

Table 4.7: Nutrient composition of dietary ingredients (%DM basis) offered to experimental cows

Nutrient	Green fodder	Concentrate supplement	Ragi straw	Glycerol	Jaggery	Maize	Flaxseed
DM	25.07 ± 0.08	89.51 ± 0.17	94.34 ± 0.06	97.48 ± 0.07	91.14 ± 0.12	87.04 ± 0.24	93.18 ± 0.08
OM	91.43 ± 0.05	87.06 ± 0.10	91.71 ± 0.07	99.86 ± 0.00	96.56 ± 0.13	99.16 ± 0.01	95.95 ± 0.01
Crude protein	5.19 ± 0.02	17.48 ± 0.07	4.82 ± 0.20	-	2.21 ± 0.04	8.43 ± 0.03	21.06 ± 0.11
Ether Extract	1.49 ± 0.01	1.91 ± 0.04	2.82 ± 0.02	-	-	2.54 ± 0.03	35.36 ± 0.13
Total Ash	8.56 ± 0.06	12.94 ± 0.10	8.29 ± 0.07	-	3.51 ± 0.06	0.83 ± 0.01	4.04 ± 0.01
AIA	3.46 ± 0.02	2.64 ± 0.05	4.60 ± 0.05	-	1.14 ± 0.03	0.23 ± 0.00	0.40 ± 0.00
TCHO	84.76 ± 0.06	67.66 ± 0.11	84.07 ± 0.07	99.87 ± 0.00	94.30 ± 0.13	88.18 ± 0.05	39.54 ± 0.17
Cell content	33.93 ± 0.14	54.57 ± 0.32	26.59 ± 0.12	100 ± 0.00	100 ± 0.00	70.81 ± 0.12	63.76 ± 0.18
NDF	66.07 ± 0.14	45.06 ± 0.23	73.41 ± 0.12	-	-	29.19 ± 0.12	36.24 ± 0.18
ADF	43.53 ± 0.13	11.96 ± 0.12	47.18 ± 0.12	-	-	5.39 ± 0.06	20.28 ± 0.07
HC	22.54 ± 0.15	33.10 ± 0.31	26.22 ± 0.17	-	-	23.80 ± 0.16	13.99 ± 0.08
Cellulose	35.86 ± 0.20	7.02 ± 0.11	41.01 ± 0.07	-	-	-	15.97 ± 0.16
ADL	5.04 ± 0.04	3.85 ± 0.05	3.38 ± 0.04	-	-	-	4.02 ± 0.01
NFC	18.69 ± 0.16	22.60 ± 0.27	10.66 ± 0.12	99.87 ± 0.00	94.28 ± 0.08	59.00 ± 0.15	3.30 ± 0.28
Gross energy (Kcal/kg DM)	3732 ± 2.42	3577 ± 0.45	3809 ± 2.77	3994 ± 0.02	3860 ± 0.57	4094 ± 0.18	5606 ± 0.66

All values are average of triplicates

CP that was fairly higher than many cereal straws like wheat, paddy, sorghum, etc. Similar like *in vitro* studies, glycerol, jaggery, and maize grain were fortified to the diets (TG) as additional energy and compared to diets without any energy fortification (CG). TCHO in the 3 energy sources were 99.9%, 94.3%, and 88.2%, respectively. Apart from starch energy in maize grain, it was also marginally provided CP compared to either jaggery or glycerol.

Among the 3 energy sources fortified, glycerol cannot be considered under carbohydrates as it is a polyol compound that required phosphorylation before use as an energy source by either rumen microbes or their host animal. Glycerol can either phosphorylated by glycerol kinase and then oxidized by glycerol-3-phosphate oxidase that is called in short the *glpK* pathway or oxidized by glycerol dehydrogenase and then phosphorylated by dihydroxyacetone kinase that is called in short the *dhaK* pathway. Both pathways lead to the formation of dihydroxyacetone phosphate that is an intermediate of glycolysis. It was assumed that the *glpK* pathway operates during aerobiosis and that the *dhaK* pathway operates under anaerobic conditions.

CC of glycerol and jaggery was 100%, and it was 71% in ground maize. NFC in glycerol was 5.6% higher than jaggery and 41% higher than maize. The GE content of the maize was 2.5% higher than glycerol and 5.7% higher than jaggery. Flaxseed (*Linum usitatissimum*) was offered to provide ω -3 fatty acids to prepartum cows which contain 35.6% of EE. Flaxseed is the oilseed richest in ω -3 fatty acids, averaging 18% of the total seed weight and consisting of 53% of the flaxseed fatty acids (USDA, 1986; Chow and Jesse, 1992). CP and NFC in flaxseed were 21.1% and 3.3 %, respectively.

4.2.2 Nutrient composition of leftover dry fodder

Sorting or selective consumption behaviour of ruminants for certain portions of plants caused the difference in chemical composition between offered and leftover MGF and FM straw (**Table 4.8**).

CP% of the MGF offered and orts were comparable but marginally higher in the FM straw leftover than offered. NDF in the roughages was higher because

ruminants selectively consume more tender leafy parts and thin stems thus, leaving behind the thick stems with nodes and internodes (Antongiovanni and Sargentini, 1991).

Table 4.8: Nutrient composition of leftover dry fodder (%DM basis)

Nutrient	Green fodder	Finger millet straw
Dry Matter	34.21 ± 0.11	95.73 ± 0.04
Organic Matter	90.39 ± 0.06	89.50 ± 0.06
Crude protein	5.05 ± 0.02	5.07 ± 0.01
Ether Extract	1.25 ± 0.00	1.85 ± 0.01
Total Ash	9.60 ± 0.06	10.50 ± 0.06
AIA	3.83 ± 0.03	4.43 ± 0.04
TCHO	83.69 ± 0.07	82.58 ± 0.06
Cell content	30.34 ± 0.07	18.47 ± 0.05
NDF	69.67 ± 0.07	81.53 ± 0.05
ADF	48.65 ± 0.06	47.24 ± 0.01
Hemicellulose	21.02 ± 0.09	34.29 ± 0.05
Cellulose	39.26 ± 0.04	42.20 ± 0.13
ADL	5.60 ± 0.07	3.56 ± 0.04
NFC	14.03 ± 0.08	1.05 ± 0.06
Gross energy (Kcal/kg DM)	3698 ± 2.53	3673 ± 2.37

All values are average of triplicates

4.2.3 Nutrient intake from the diet

4.2.3.1 Total diet intake of prepartum cows

DMI during 21 d before parturition in the HFX and the Deoni breed was presented in **Figure 4.2**. The average DMI for the prepartum cows was ranged between 1.7% and 2% of the BW that was in the general range of 1% to 3% DMI by the mature cows deepening on the quality of diet. The DMI from 21 days before calving to until 3 days before calving was almost constant. The overall mean DMI reduction in HFX cows was lesser than the Deoni cows 2 days before calving to till calving.

Diminished DMI in either the HFX or the Deoni cows is a physiological phenomenon irrespective of breed characteristics (Simianer, 2000).

DMI difference between both the breed with and without supplementation was presented in **(Figure 4.2)**. One week before calving, DMI was noticeably higher with the FNS supplemented group than the prepartum cows of any breed without it, however, differences were statistically insignificant. Among both breed, DMI reduction in the last 3 days before calving day was 10% and 5%, respectively in the Deoni and the HFX cows compared to DMI 21 days before calving. Roshanzamir *et al.* (2020) also reported that prepartum DMI was higher ($P < 0.05$) for the cows receiving supplemental Mn, Zn, and Cu source of the mineral than control animals in the close-up transition period.

DMI decline differed in the same breed without or with FNS supplement **(Figure 4.2)**. The HFX cows without FNS supplement showed more decline in the DMI. The response of the HFX for DMI with FNS supplement was constant till calving without any decline. The pattern of DMI by the Deoni cows without or with FNS supplementation was more or less similar. DMI reduction in the Deoni cows from 3 days before calving was not much. The prepartum HFX cows response to DMI for 3 weeks before calving thus, indicated they required FNS supplements while the Deoni cows pattern of DMI indicated that it was not emphatic but, recommended to counter physical and biochemical stress cows undergo before calving as functional nutrients act as anti-oxidative and anti-inflammatory (Omur *et al.*, 2016, Wilson *et al.*, 2018).

Interestingly, reduced DMI was observed in HFX cows those were calved in the afternoon while DMI of HFX cows those calved after dusk was meager. No such behavioural pattern was observed in Deoni cows. It was also noticed that the DMI in the Deoni cows was more in the night hours that may aptly be described as the behaviour of vespertine animals. Foraging habits of higher animals was wider as an adaptation to antipredation that has been the basis for the crepuscular feeding habits of many mammals in general, foraging animals in particular. Prepartum cows were maintained under stall feeding hence, their DMI pattern would relate to diurnal variation. Diurnal variation in the feeding occurs

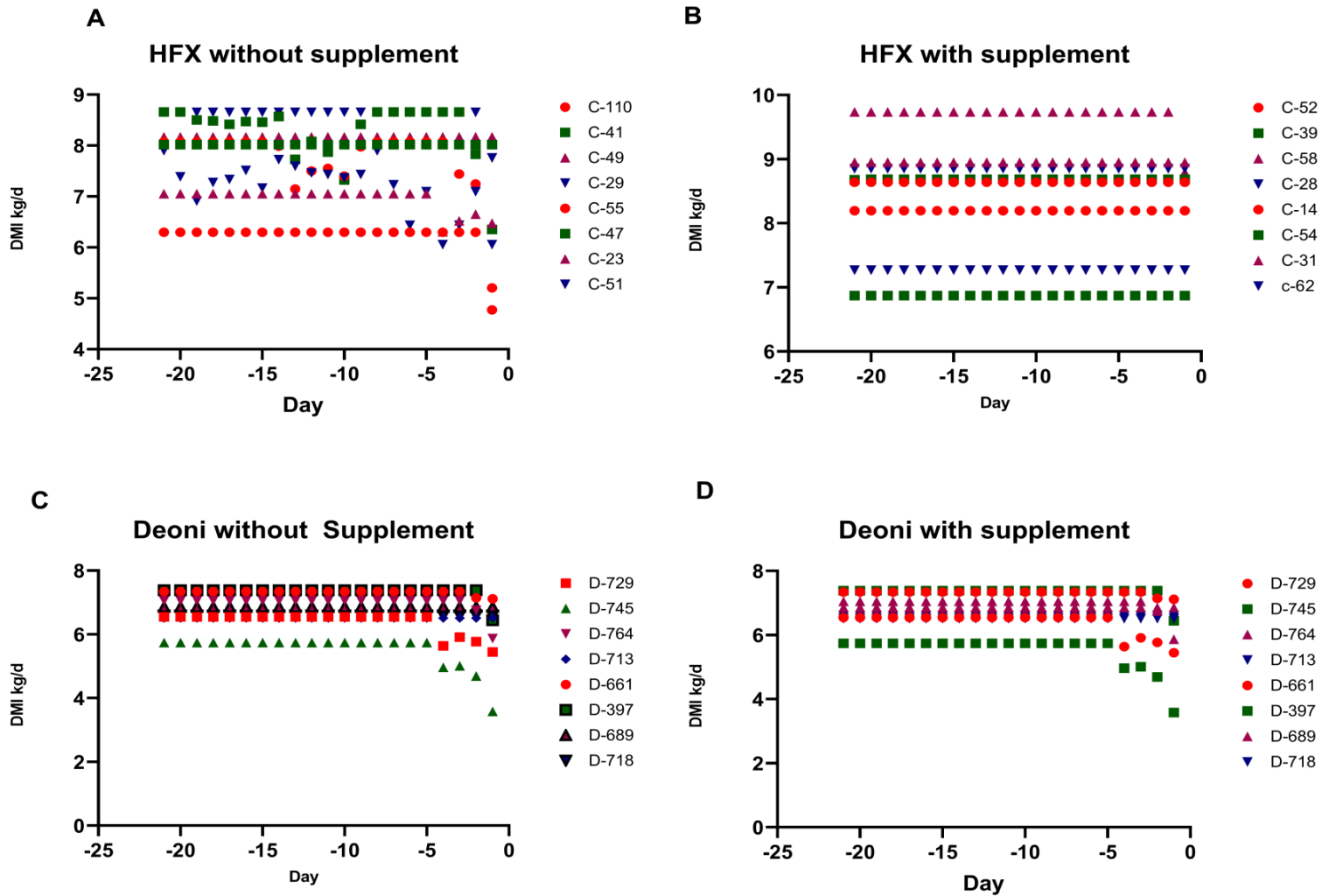


Fig. 4.2: DMI during the prepartum period in different groups (A) HFX cows without FNS (B) HFX cows with FNS (C) Deoni cows without FNS (D) Deoni cows with FNS

depending upon the diet component they consume such as; green forage, dry roughage, or CS (DeVris *et al.*, 2003).

All prepartum cows consumed CS without any leftover but as the calving time zeroing, both green and dry roughage were left in the Deoni at least from 12 hours. The HFX cows preferred green roughage and left dry roughage when calved in night hours. These behavioural patterns in cows indicated difference in the both breed for the stress encountered with greater hormonal fluxes when calving time is zeroing. Henceforth, the DMI changes when calving time is zeroing it was entirely due to physical and biochemical stress that cows undergo at least 6 h prior to calving and also subsequently. The smaller quantity decline in the DMI among the cows supplemented with FNS was due to the anti-oxidative and anti-inflammatory response of the ingredients.

4.2.3.2 Nutrient intake from the prepartum diet

The DM, OM, CP, EE, and TCHO intake from the MGF were significantly higher ($P < 0.001$) in the HFX cows by 1/3rd than the Deoni cows (**Table 4.9**) that could be attributed to the difference in body size (Srinivas and Ramesha, 2014). FNS supplements to TGs significantly increased ($P < 0.01$) DMI from MGF in contrary to without FNS supplement. Machado *et al.* (2019) recommended protein supplements for growing HF heifers after grazing the green fodder rather than the energy which is contrary to our observation in dry prepartum cows. Improvement in the DMI of MGF was 15% and 21% ($P = 0.97$), in the HFX and the Deoni cows, respectively.

Same was observed in the OM, CP, TA and TCHO intake except in EE ($P = 0.57$). Interaction between energy, breed, and supplement for DM, OM, CP, EE, and TCHO intake from MGF was not significant ($P > 0.60$).

NDF of the MGF was 66% hence, CWC intake was also significantly higher ($P < 0.001$) in the HFX cows than the Deoni cows in association with DMI (**Table 4.10**). CC and NDF intake in the HFX cows of TGs was on average 33%

Table 4.9: Nutrient intake from mixed green fodder (kg/d) in prepartum cows

Parameter	Group	Energy × Breed		Energy × Suppl.		HFX × Suppl.		Deoni × Suppl.		E × B × S
		HFX	Deoni	Without	With	Without	With	Without	With	
DM	Control	4.54	3.70	4.14	4.11	4.51	4.58	3.76	3.64	4.12
	Glycerol	4.75	3.54	3.52	4.77	3.98	5.52	3.07	4.01	4.14
	Jaggery	4.89	3.88	3.95	4.83	4.51	5.27	3.38	4.39	4.39
	Maize	5.17	3.67	4.10	4.73	5.01	5.33	3.20	4.14	4.42
	Mean	4.84	3.70	3.93	4.61	4.50	5.17	3.35	4.04	4.27
	SEM	0.294		0.294		0.416		0.208		
	P Value	0.001***		0.005**		0.965		0.646		
OM	Control	4.15	3.38	3.78	3.75	4.13	4.18	3.44	3.33	3.77
	Glycerol	4.34	3.24	3.22	4.36	3.63	5.04	2.81	3.67	3.79
	Jaggery	4.47	3.55	3.61	4.41	4.13	4.81	3.09	4.02	4.01
	Maize	4.73	3.36	3.75	4.33	4.58	4.87	2.93	3.79	4.04
	Mean	4.42	3.38	3.59	4.21	4.12	4.73	3.07	3.70	3.90
	SEM	0.270		0.270		0.381		0.191		
	P Value	0.001***		0.005**		0.954		0.644		
CP	Control	0.24	0.20	0.22	0.21	0.24	0.24	0.20	0.19	0.22
	Glycerol	0.25	0.19	0.18	0.25	0.21	0.29	0.16	0.21	0.22
	Jaggery	0.26	0.20	0.21	0.25	0.24	0.28	0.18	0.23	0.23
	Maize	0.27	0.19	0.21	0.25	0.26	0.28	0.17	0.22	0.23
	Mean	0.25	0.19	0.21	0.24	0.23	0.27	0.18	0.21	0.22
	SEM	0.015		0.015		0.022		0.011		
	P Value	0.001***		0.006**		0.955		0.698		

Parameter	Group	Energy × Breed		Energy × Suppl.		HFX × Suppl.		Deoni × Suppl.		E × B × S
		HFX	Deoni	Without	With	Without	With	Without	With	
EE	Control	0.07	0.06	0.06	0.06	0.07	0.07	0.06	0.05	0.06
	Glycerol	0.07	0.05	0.05	0.07	0.06	0.08	0.05	0.06	0.06
	Jaggery	0.07	0.06	0.06	0.07	0.07	0.08	0.05	0.07	0.06
	Maize	0.08	0.06	0.06	0.07	0.07	0.08	0.05	0.06	0.07
	Mean	0.07	0.06	0.06	0.07	0.06	0.08	0.05	0.06	0.06
	SEM	0.005		0.005		0.006			0.003	
	P Value	0.001***		0.011**		0.572			0.606	
TA	Control	0.39	0.32	0.35	0.35	0.39	0.39	0.32	0.31	0.35
	Glycerol	0.41	0.30	0.30	0.41	0.34	0.47	0.27	0.34	0.35
	Jaggery	0.42	0.33	0.34	0.41	0.39	0.45	0.29	0.38	0.38
	Maize	0.44	0.31	0.35	0.40	0.43	0.46	0.28	0.35	0.38
	Mean	0.41	0.32	0.34	0.39	0.38	0.44	0.29	0.34	0.37
	SEM	0.026		0.026		0.036			0.018	
	P Value	0.001***		0.007**		1.000			0.641	
TCHO	Control	3.85	3.14	3.51	3.48	3.83	3.88	3.19	3.08	3.49
	Glycerol	4.02	3.00	2.99	4.04	3.37	4.67	2.61	3.40	3.51
	Jaggery	4.14	3.29	3.35	4.09	3.83	4.46	2.87	3.72	3.72
	Maize	4.38	3.11	3.48	4.01	4.25	4.52	2.71	3.51	3.75
	Mean	4.10	3.13	3.33	3.90	3.82	4.38	2.84	3.43	3.62
	SEM	0.249		0.249		0.352			0.018	
	P Value	0.001***		0.005**		0.964			0.642	

Values with different superscripts for absolute interaction for respective energy source in a row under each parameter differ significantly; ***P≤ 0.001, **P≤ 0.01, *P≤ 0.05

Table 4.10: Cell content and cell wall constituent intake from green fodder (kg/d) in prepartum cows

Parameter	Group	Energy × Breed		Energy × Suppl.		HFX × Suppl.		Deoni × Suppl.		E × B × S
		HFX	Deoni	Without	With	Without	With	Without	With	
CC	Control	1.54	1.26	1.41	1.39	1.53	1.55	1.28	1.24	1.40
	Glycerol	1.61	1.20	1.19	1.62	1.35	1.87	1.04	1.37	1.41
	Jaggery	1.66	1.32	1.34	1.64	1.53	1.79	1.15	1.49	1.49
	Maize	1.75	1.25	1.39	1.61	1.70	1.81	1.09	1.41	1.50
	Mean	1.64	1.26	1.33	1.56	1.53	1.75	1.14	1.38	1.45
	SEM	0.100		0.100			0.142			0.071
	P Value	0.001***		0.005**			0.952			0.648
NDF	Control	3.00	2.44	2.73	2.72	2.98	3.02	2.48	2.41	2.72
	Glycerol	3.13	2.34	2.33	3.15	2.63	3.64	2.03	2.65	2.74
	Jaggery	3.23	2.57	2.61	3.19	2.98	3.48	2.24	2.90	2.90
	Maize	3.42	2.42	2.71	3.13	3.31	3.52	2.11	2.74	2.92
	Mean	3.19	2.44	2.59	3.04	2.97	3.41	2.22	2.67	2.82
	SEM	0.195		0.195			0.276			0.138
	P Value	0.001***		0.005**			0.950			0.642
ADF	Control	1.98	1.61	1.80	1.79	1.97	1.99	1.64	1.59	1.80
	Glycerol	2.07	1.54	1.53	2.08	1.73	2.40	1.34	1.75	1.80
	Jaggery	2.13	1.69	1.72	2.10	1.97	2.29	1.47	1.91	1.91
	Maize	2.25	1.60	1.79	2.06	2.18	2.32	1.39	1.81	1.92
	Mean	2.11	1.61	1.71	2.01	1.96	2.25	1.46	1.76	1.86
	SEM	0.128		0.128			0.182			0.091
	P Value	0.001***		0.005**			0.941			0.651
HC	Control	1.02	0.84	0.93	0.93	1.02	1.03	0.85	0.82	0.93
	Glycerol	1.07	0.80	0.80	1.07	0.90	1.24	0.70	0.91	0.93
	Jaggery	1.10	0.88	0.89	1.09	1.02	1.19	0.76	0.99	0.99
	Maize	1.17	0.83	0.93	1.07	1.13	1.20	0.72	0.93	1.00
	Mean	1.09	0.83	0.89	1.04	1.01	1.16	0.76	0.91	0.96
	SEM	0.067		0.067			0.094			0.047
	P Value	0.001***		0.005**			0.958			0.659

Parameter	Group	Energy × Breed		Energy × Suppl.		HFX × Suppl.		Deoni × Suppl.		E × B × S
		HFX	Deoni	Without	With	Without	With	Without	With	
Cellulose	Control	1.63	1.33	1.49	1.47	1.62	1.64	1.35	1.31	1.48
	Glycerol	1.70	1.27	1.26	1.71	1.43	1.98	1.10	1.44	1.49
	Jaggery	1.76	1.39	1.42	1.73	1.62	1.89	1.21	1.58	1.57
	Maize	1.86	1.32	1.47	1.70	1.80	1.91	1.15	1.49	1.59
	Mean	1.74	1.33	1.41	1.65	1.62	1.86	1.20	1.45	1.53
	SEM	0.106		0.106		0.151		0.075		
	P Value	0.001***		0.005**		0.941		0.651		
ADL	Control	0.23	0.19	0.21	0.21	0.23	0.23	0.19	0.18	0.21
	Glycerol	0.24	0.18	0.18	0.24	0.20	0.28	0.16	0.20	0.21
	Jaggery	0.25	0.20	0.20	0.24	0.23	0.27	0.17	0.22	0.22
	Maize	0.26	0.18	0.21	0.24	0.25	0.27	0.17	0.22	0.22
	Mean	0.24	0.19	0.20	0.23	0.23	0.26	0.17	0.20	0.21
	SEM	0.015		0.015		0.021		0.010		
	P Value	0.001***		0.005**		0.860		0.665		
NFC	Control	0.85	0.69	0.77	0.77	0.85	0.86	0.70	0.68	0.77
	Glycerol	0.89	0.66	0.66	0.89	0.75	1.04	0.57	0.75	0.78
	Jaggery	0.92	0.73	0.74	0.90	0.85	0.99	0.63	0.83	0.82
	Maize	0.97	0.69	0.77	0.89	0.94	1.00	0.60	0.78	0.83
	Mean	0.92	0.70	0.74	0.86	0.84	0.97	0.63	0.76	0.80
	SEM	0.056		0.056		0.079		0.040		
	P Value	0.001***		0.006**		0.926		0.645		

Values with different superscripts for absolute interaction for a respective energy source in a row under each parameter differ significantly; ***P ≤ 0.001, **P ≤ 0.01, *P ≤ 0.05

higher than the Deoni cows of TGs. Their intake even in CG of HFX cows was 22% higher than the CG of Deoni cows. CC and NDF intake among the HFX cows was 9% higher in TGs than CG but, the contrary was true for Deoni cows where their intake was higher in CG than TGs. The higher energy density in the diet affected CWC intake in Deoni cows because genetically underdeveloped breed suffer inefficiency of energy utilization. There is a dispute among research worker on the need of 10% less maintenance ME in *Bos indicus* and *Bos taurus* (Tedeschi *et al.*, 2002; Cardenas-Medina *et al.*, 2010) but, our studies on Deoni cows in past showed energy inefficiency compared to crossbred cows (Singh and Srinivas, 2016, Mohanavel and Srinivas, 2016, Singh and Srinivas, 2020). CC or NDF intake was increased by 17% ($P < 0.01$) with FNS supplement to energy than without supplement.

Breed response to NDF intake with FNS Supplement was 15% in the HFX cows than 20% in Deoni cows however, supplement and breed interaction was statistically comparable ($P = 0.95$). The green fodder anatomy and rate of digestion of succulent fodder result in large digestible NDF fraction although, it has high NDF concentration. This is true for perennial and cultivated non-leguminous fodder crops rather leguminous fodder crops. Further, particle buoyancy in the rumen due to green fodder is lesser and the indigestible fraction of NDF also rapidly diminishes to free the rumen fill (Jung and Allen, 1995).

All cows were fed only 1 kg FM straw to meet the nutrient requirement in addition to MGF and also to accommodating rumen fill for the CS (**Table 4.11**). The standard lactation length is 305 days and a cow should conceive usually between 60 and 90 days after calving, but below 100 days. This enables a dry period of 60 days in lactation cycle and high forage diet should be replaced with high concentrate diet from 3 to 5 weeks before calving that has many advantages of supplying more nutrients to the growing calf and the cow. As the cow approaches parturition, DMI declines. In close up cows, the typical DMI control was based on energy intake than either oxygen consumption based intake regulation in the early lactation or NDF based intake regulation in the mid and late lactation (Erickson and Kalscheur, 2020).

Table 4.11: Nutrient intake from finger millet straw (kg/d) in prepartum cows

Parameter	Group	MBW	Energy × Breed		Energy × Suppl.		HFX × Suppl.		Deoni × Suppl.		E × B × S
			HFX	Deoni	Without	With	Without	With	Without	With	
DM	Control	87.01	0.94	0.94	0.94	0.94	0.94	0.94	0.94	0.94	0.94
	Glycerol	89.66	0.94	0.94	0.94	0.94	0.94	0.94	0.94	0.94	0.94
	Jaggery	97.41	0.94	0.94	0.94	0.94	0.94	0.94	0.94	0.94	0.94
	Maize	91.31	0.94	0.94	0.94	0.94	0.94	0.94	0.94	0.94	0.94
	Mean	91.34	0.94	0.94	0.94	0.94	0.94	0.94	0.94	0.94	0.94
	SEM	3.38	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	
	P Value	0.06	-	-	-	-	-	-	-	-	
OM	Control	87.01	0.87	0.87	0.87	0.87	0.87	0.87	0.87	0.87	0.87
	Glycerol	89.66	0.87	0.87	0.87	0.87	0.87	0.87	0.87	0.87	0.87
	Jaggery	97.41	0.87	0.87	0.87	0.87	0.87	0.87	0.87	0.87	0.87
	Maize	91.31	0.87	0.87	0.87	0.87	0.87	0.87	0.87	0.87	0.87
	Mean	91.34	0.87	0.87	0.87	0.87	0.87	0.87	0.87	0.87	0.87
	SEM	3.38	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	
	P Value	0.06	-	-	-	-	-	-	-	-	
CP	Control	87.01	0.05	0.05	0.05	0.05	0.05	0.05	0.05	0.05	0.05
	Glycerol	89.66	0.05	0.05	0.05	0.05	0.05	0.05	0.05	0.05	0.05
	Jaggery	97.41	0.05	0.05	0.05	0.05	0.05	0.05	0.05	0.05	0.05
	Maize	91.31	0.05	0.05	0.05	0.05	0.05	0.05	0.05	0.05	0.05
	Mean	91.34	0.05	0.05	0.05	0.05	0.05	0.05	0.05	0.05	0.05
	SEM	3.38	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	
	P Value	0.06	-	-	-	-	-	-	-	-	

Parameter	Group	MBW	Energy × Breed		Energy × Suppl.		HFX × Suppl.		Deoni × Suppl.		E × B × S
			HFX	Deoni	Without	With	Without	With	Without	With	
EE	Control	87.01	0.03	0.03	0.03	0.03	0.03	0.03	0.03	0.03	0.03
	Glycerol	89.66	0.03	0.03	0.03	0.03	0.03	0.03	0.03	0.03	0.03
	Jaggery	97.41	0.03	0.03	0.03	0.03	0.03	0.03	0.03	0.03	0.03
	Maize	91.31	0.03	0.03	0.03	0.03	0.03	0.03	0.03	0.03	0.03
	Mean	91.34	0.03	0.03	0.03	0.03	0.03	0.03	0.03	0.03	0.03
	SEM	3.38	0.000		0.000		0.000		0.000		0.000
	P Value	0.06		-		-		-		-	
TA	Control	87.01	0.08	0.08	0.08	0.08	0.08	0.08	0.08	0.08	0.08
	Glycerol	89.66	0.08	0.08	0.08	0.08	0.08	0.08	0.08	0.08	0.08
	Jaggery	97.41	0.08	0.08	0.08	0.08	0.08	0.08	0.08	0.08	0.08
	Maize	91.31	0.08	0.08	0.08	0.08	0.08	0.08	0.08	0.08	0.08
	Mean	91.34	0.08	0.08	0.08	0.08	0.08	0.08	0.08	0.08	0.08
	SEM	3.38	0.000		0.000		0.000		0.000		0.000
	P Value	0.06		-		-		-		-	
TCHO	Control	87.01	0.80	0.80	0.80	0.80	0.80	0.80	0.80	0.80	0.80
	Glycerol	89.66	0.80	0.80	0.80	0.80	0.80	0.80	0.80	0.80	0.80
	Jaggery	97.41	0.80	0.80	0.80	0.80	0.80	0.80	0.80	0.80	0.80
	Maize	91.31	0.80	0.80	0.80	0.80	0.80	0.80	0.80	0.80	0.80
	Mean	91.34	0.80	0.80	0.80	0.80	0.80	0.80	0.80	0.80	0.80
	SEM	3.38	0.000		0.000		0.000		0.000		0.000
	P Value	0.06		-		-		-		-	

MGF and limited FM straw fed to prepartum cows were able to meet the protein requirement of 300 g to the HFX cows and 250 g to the Deoni cows as against the protein requirement of 699 g as per ICAR (2013), or 545 g as per NRC (2001) thus, accounting to only 43% and 36% of the recommendation of ICAR (2013), respectively whereas 55% and 45%, respectively according to NRC (2001) recommendation. Prepartum cows energy or protein requirement is almost equivalent to lactating cow producing 4% fat corrected milk yield of 4½ to 7½ kg/day (NRC, 2001). GE in either MGF or FM straw was 3.7 Mcal. Litherland *et al.* (2013) reported a positive impact of wheat straw compared to grass hay when fed to sixty multiparous Holstein and Holstein crosses on postpartum health and energy. The high fiber content of forages is the main nutritional factor that differentiates them from CS and results in a relatively low energy value. The ME value of dry roughages may range from 1 to 1.5 Mcal in contrast to 1.9 to 2.2 Mcal in cultivated forages (Kumar *et al.*, 2015) and such variation is mainly due to characteristics of plant cell walls (Jung and Allen, 1995) as well as heat increment.

The NDF intake by prepartum cows from FM straw was 3 to 5 times lesser than MGF yet fiber intake from dry roughages is important in cows for production, gut health and wellbeing (**Table 4.12**). In the present experiment, the FM straw was restricted to 1 kg/d keeping in mind the decrease in DMI, rumen fill and also, a good strategy to avoid heat increment as parturition approaches (Kanjapruithipong *et al.*, 2010). There are greater chances of metabolizing more body fat on high forage diets than medium or low forage diets (Kanjapruithipong *et al.*, 2010).

The roughage was accounting to 60% of the total ration that was within the recommended forage levels of 40 to 100% of the ration and 60% is vital for maintaining the health (Bargo *et al.*, 2002). The balance of protein or energy requirement of the prepartum cows were met from the CS. Proximate nutrient intake from total CS is presented in **Table 4.13**. By restricting the roughages to 60% in the total ration, there was enough rumen fill for the consumption of CS in CG and TGs. All the prepartum cows consumed CS without leaving any residues.

Table 4.12: Cell and cell wall constituents intake from finger millet straw (kg/d) in different groups of prepartum cows

Parameter	Group	MBW	Energy × Breed		Energy × Suppl.		HFX × Suppl.		Deoni × Suppl.		E × B × S
			HFX	Deoni	Without	With	Without	With	Without	With	
CC	Control	87.01	0.25	0.25	0.25	0.25	0.25	0.25	0.25	0.25	0.25
	Glycerol	89.66	0.25	0.25	0.25	0.25	0.25	0.25	0.25	0.25	0.25
	Jaggery	97.41	0.25	0.25	0.25	0.25	0.25	0.25	0.25	0.25	0.25
	Maize	91.31	0.25	0.25	0.25	0.25	0.25	0.25	0.25	0.25	0.25
	Mean	91.34	0.25	0.25	0.25	0.25	0.25	0.25	0.25	0.25	0.25
	SEM	3.38	0.000		0.000		0.000		0.000		0.000
	P Value	0.06		-		-		-		-	
NDF	Control	87.01	0.69	0.69	0.69	0.69	0.69	0.69	0.69	0.69	0.69
	Glycerol	89.66	0.69	0.69	0.69	0.69	0.69	0.69	0.69	0.69	0.69
	Jaggery	97.41	0.69	0.69	0.69	0.69	0.69	0.69	0.69	0.69	0.69
	Maize	91.31	0.69	0.69	0.69	0.69	0.69	0.69	0.69	0.69	0.69
	Mean	91.34	0.69	0.69	0.69	0.69	0.69	0.69	0.69	0.69	0.69
	SEM	3.38	0.000		0.000		0.000		0.000		0.000
	P Value	0.06		-		-		-		-	
ADF	Control	87.01	0.45	0.45	0.45	0.45	0.45	0.45	0.45	0.45	0.45
	Glycerol	89.66	0.45	0.45	0.45	0.45	0.45	0.45	0.45	0.45	0.45
	Jaggery	97.41	0.45	0.45	0.45	0.45	0.45	0.45	0.45	0.45	0.45
	Maize	91.31	0.45	0.45	0.45	0.45	0.45	0.45	0.45	0.45	0.45
	Mean	91.34	0.45	0.45	0.45	0.45	0.45	0.45	0.45	0.45	0.45
	SEM	3.38	0.000		0.000		0.000		0.000		0.000
	P Value	0.06		-		-		-		-	
HC	Control	87.01	0.25	0.25	0.25	0.25	0.25	0.25	0.25	0.25	0.25
	Glycerol	89.66	0.25	0.25	0.25	0.25	0.25	0.25	0.25	0.25	0.25
	Jaggery	97.41	0.25	0.25	0.25	0.25	0.25	0.25	0.25	0.25	0.25
	Maize	91.31	0.25	0.25	0.25	0.25	0.25	0.25	0.25	0.25	0.25
	Mean	91.34	0.25	0.25	0.25	0.25	0.25	0.25	0.25	0.25	0.25
	SEM	3.38	0.000		0.000		0.000		0.000		0.000
	P Value	0.06		-		-		-		-	

Parameter	Group	MBW	Energy × Breed		Energy × Suppl.		HFX × Suppl.		Deoni × Suppl.		E × B × S
			HFX	Deoni	Without	With	Without	With	Without	With	
Cellulose	Control	87.01	0.39	0.39	0.39	0.39	0.39	0.39	0.39	0.39	0.39
	Glycerol	89.66	0.39	0.39	0.39	0.39	0.39	0.39	0.39	0.39	0.39
	Jaggery	97.41	0.39	0.39	0.39	0.39	0.39	0.39	0.39	0.39	0.39
	Maize	91.31	0.39	0.39	0.39	0.39	0.39	0.39	0.39	0.39	0.39
	Mean	91.34	0.39	0.39	0.39	0.39	0.39	0.39	0.39	0.39	0.39
	SEM	3.38	0.000		0.000		0.000		0.000		0.000
	P Value	0.06		-		-		-		-	
ADL	Control	87.01	0.03	0.03	0.03	0.03	0.03	0.03	0.03	0.03	0.03
	Glycerol	89.66	0.03	0.03	0.03	0.03	0.03	0.03	0.03	0.03	0.03
	Jaggery	97.41	0.03	0.03	0.03	0.03	0.03	0.03	0.03	0.03	0.03
	Maize	91.31	0.03	0.03	0.03	0.03	0.03	0.03	0.03	0.03	0.03
	Mean	91.34	0.03	0.03	0.03	0.03	0.03	0.03	0.03	0.03	0.03
	SEM	3.38	0.000		0.000		0.000		0.000		0.000
	P Value	0.06		-		-		-		-	
NFC	Control	87.01	0.10	0.10	0.10	0.10	0.10	0.10	0.10	0.10	0.10
	Glycerol	89.66	0.10	0.10	0.10	0.10	0.10	0.10	0.10	0.10	0.10
	Jaggery	97.41	0.10	0.10	0.10	0.10	0.10	0.10	0.10	0.10	0.10
	Maize	91.31	0.10	0.10	0.10	0.10	0.10	0.10	0.10	0.10	0.10
	Mean	91.34	0.10	0.10	0.10	0.10	0.10	0.10	0.10	0.10	0.10
	SEM	3.38	0.000		0.000		0.000		0.000		0.000
	P Value	0.06		-		-		-		-	

Table 4.13: Nutrient intake by prepartum cows from CS, kg/d (CG) or CS fortified with 3 different energy sources (TGs)

Parameter	Group	MBW	Energy × Breed		Energy × Suppl.		HFX × Suppl.		Deoni × Suppl.		E × B × S
			HFX	Deoni	Without	With	Without	With	Without	With	
DM	Control	87.01	1.86	1.55	1.62	1.79	1.70	2.02	1.53	1.57	1.70 ^a
	Glycerol	89.66	2.53	2.20	2.59	2.14	2.62	2.44	2.57	1.84	2.37 ^{bc}
	Jaggery	97.41	2.68	2.33	2.76	2.25	2.91	2.46	2.62	2.04	2.50 ^c
	Maize	91.31	2.34	2.07	2.48	1.93	2.45	2.23	2.50	1.64	2.20 ^b
	Mean	91.34	2.35	2.04	2.36	2.03	2.42	2.29	2.30	1.77	2.19
	SEM	3.38	0.115		0.115		0.163		0.08		
	P Value	0.06	0.001***		0.001***		0.026*		0.001***		
OM	Control	87.01	1.66	1.39	1.45	1.60	1.52	1.80	1.37	1.40	1.52 ^a
	Glycerol	89.66	2.31	2.00	2.36	1.95	2.39	2.23	2.33	1.68	2.16 ^{bc}
	Jaggery	97.41	2.43	2.10	2.50	2.04	2.64	2.23	2.37	1.85	2.27 ^c
	Maize	91.31	2.14	1.89	2.26	1.77	2.24	2.04	2.28	1.50	2.01 ^b
	Mean	91.34	2.14	1.84	2.14	1.84	2.20	2.07	2.09	1.60	1.99
	SEM	3.38	0.103		0.103		0.146		0.07		
	P Value	0.06	0.001***		0.001***		0.03*		0.001***		
CP	Control	87.01	0.32	0.27	0.28	0.31	0.30	0.35	0.27	0.27	0.30 ^a
	Glycerol	89.66	0.37	0.33	0.39	0.31	0.39	0.35	0.39	0.27	0.35 ^b
	Jaggery	97.41	0.40	0.35	0.42	0.33	0.44	0.36	0.40	0.30	0.37 ^b
	Maize	91.31	0.37	0.33	0.40	0.31	0.39	0.35	0.40	0.26	0.35 ^b
	Mean	91.34	0.36	0.32	0.37	0.31	0.38	0.35	0.37	0.27	0.34
	SEM	3.38	0.020		0.020		0.029		0.01		
	P Value	0.06	0.01**		0.001***		0.04*		0.001***		

Parameter	Group	MBW	Energy × Breed		Energy × Suppl.		HFX × Suppl.		Deoni × Suppl.		E × B × S
			HFX	Deoni	Without	With	Without	With	Without	With	
EE	Control	87.01	0.04	0.03	0.03	0.03	0.04	0.04	0.03	0.03	0.03 ^a
	Glycerol	89.66	0.04	0.04	0.04	0.04	0.04	0.04	0.04	0.03	0.04 ^b
	Jaggery	97.41	0.04	0.04	0.04	0.03	0.05	0.04	0.04	0.03	0.04 ^b
	Maize	91.31	0.05	0.04	0.05	0.04	0.05	0.05	0.05	0.03	0.04 ^b
	Mean	91.34	0.04	0.04	0.04	0.03	0.04	0.04	0.04	0.03	0.04
	SEM	3.38	0.002		0.002		0.003		0.002		0.002
	P Value	0.06	0.01**		0.001***		0.026*		0.001***		0.001***
TA	Control	87.01	0.24	0.20	0.21	0.23	0.22	0.26	0.20	0.20	0.22 ^a
	Glycerol	89.66	0.28	0.25	0.29	0.23	0.29	0.26	0.29	0.20	0.26 ^b
	Jaggery	97.41	0.31	0.27	0.32	0.26	0.34	0.28	0.30	0.23	0.29 ^b
	Maize	91.31	0.28	0.25	0.30	0.23	0.30	0.27	0.31	0.20	0.27 ^b
	Mean	91.34	0.28	0.24	0.28	0.24	0.29	0.27	0.27	0.21	0.26
	SEM	3.38	0.015		0.015		0.022		0.01		0.01
	P Value	0.06	0.001***		0.001***		0.048*		0.001***		0.001***
TCHO	Control	87.01	1.20	1.05	1.09	1.21	1.15	1.36	1.04	1.06	1.15 ^a
	Glycerol	89.66	1.85	1.60	1.87	1.57	1.90	1.79	1.85	1.35	1.72 ^{bc}
	Jaggery	97.41	1.93	1.67	1.98	1.63	2.09	1.78	1.87	1.48	1.80 ^c
	Maize	91.31	1.68	1.47	1.76	1.38	1.75	1.60	1.77	1.17	1.57 ^b
	Mean	91.34	1.68	1.45	1.68	1.45	1.72	1.63	1.63	1.26	1.56
	SEM	3.38	0.078		0.078		0.111		0.06		0.06
	P Value	0.06	0.01**		0.001***		0.024*		0.001***		0.001***

Values with different superscripts for absolute interaction for a respective energy source in a row under each parameter differ significantly; ***P ≤ 0.001, **P ≤ 0.01, *P ≤ 0.05

CS fed at the rate of 2 kg or 2.5 kg to the prepartum cows is reported to be important for postpartum production and reproduction (Prima *et al.*, 2018). CS supplied an average CP of about 350 g to the HFX cows and 300 g to the Deoni cows. The NDF intake from CS by the prepartum cows was about 800 to 900 g/d that was marginally higher than its intake from the FM straw (**Table 4.14**). Unlike roughages, principal nutrient intake from CS was significant at all levels of interactions viz., energy and breed, energy and supplement, the HFX cows and supplement, the Deoni cows and supplement, or energy, breed and supplement. Protein or energy requirement of the HFX cows was higher than the Deoni cows owing to the difference in metabolic body size. CS intake in the prepartum cows of the Deoni breed was 13% lesser ($P < 0.01$) than the HFX that could be due to limited plasticity of the digestive system in the Deoni breed compared to genetically developed HFX cows (Singh and Srinivas, 2020). The DMI by Deoni breed of cows, unlike the HFX, can be hampered by the diet quality (Simianer, 2000) or diet protein% as well as GE (Singh and Srinivas, 2020). Interestingly, FNS supplementation increased the intake of MGF by 15 to 21% while, the contrary was observed for CS intake in prepartum cows belongs to the HFX (-6%) or the Deoni (-30%). The decrease in CS after FNS in the HFX or the Deoni may probably due to any variation in the BW and energy regulated DMI.

CS intake was compromised more in the TGs rather than CG either in the HFX or the Deoni cows. CS in CG without any energy fortification indeed increased with FNS supplements. The difference could be due to an increased density of energy in TGs. The NDF or NFC intake in the FNS supplemented group of the HFX cows was comparable among the HFX cows without FNS supplement but, distinct in the Deoni. Compared to green or dry forage, the effect of the FNS supplement on NDF or its component intake from CS was significant because of interaction between main factor and sub-factors.

The total principal nutrient intake (kg/d) (**Table 4.15**) from the diet was significantly different between breeds, between energy sources, and with or without FNS supplement.

Table 4.14: Cell wall constituents intake from concentrate supplement (kg/d) by prepartum cows in CG and fortified with 3 different sources of energy

Parameter	Group	MBW	Energy × Breed		Energy × Suppl.		HFX × Suppl.		Deoni × Suppl.		E × B × S
			HFX	Deoni	Without	With	Without	With	Without	With	
CC	Control	87.01	1.01	0.85	0.88	0.98	0.93	1.10	0.84	0.86	0.93 ^a
	Glycerol	89.66	1.57	1.35	1.57	1.34	1.60	1.53	1.55	1.15	1.45 ^c
	Jaggery	97.41	1.67	1.44	1.69	1.41	1.79	1.54	1.60	1.28	1.55 ^c
	Maize	91.31	1.35	1.18	1.42	1.12	1.41	1.29	1.43	0.94	1.27 ^b
	Mean	91.34	1.40	1.20	1.39	1.21	1.43	1.37	1.35	1.05	1.30
	SEM	3.38	0.063		0.063		0.089				0.044
	P Value	0.06	0.001***		0.001***		0.019**				0.001***
NDF	Control	87.01	0.84	0.70	0.73	0.81	0.77	0.91	0.69	0.71	0.77 ^a
	Glycerol	89.66	0.96	0.85	1.01	0.80	1.01	0.91	1.01	0.69	0.91 ^b
	Jaggery	97.41	1.01	0.88	1.06	0.83	1.11	0.91	1.01	0.75	0.95 ^b
	Maize	91.31	0.99	0.88	1.05	0.81	1.04	0.94	1.07	0.69	0.93 ^b
	Mean	91.34	0.95	0.83	0.96	0.81	0.98	0.92	0.94	0.71	0.89
	SEM	3.38	0.052		0.052		0.074				0.037
	P Value	0.06	0.01**		0.001***		0.033*				0.01**
ADF	Control	87.01	0.22	0.18	0.19	0.21	0.21	0.24	0.18	0.19	0.20 ^a
	Glycerol	89.66	0.26	0.23	0.27	0.21	0.27	0.25	0.27	0.18	0.24 ^b
	Jaggery	97.41	0.27	0.23	0.28	0.22	0.30	0.24	0.28	0.20	0.25 ^b
	Maize	91.31	0.25	0.23	0.27	0.21	0.27	0.24	0.28	0.18	0.24 ^b
	Mean	91.34	0.25	0.22	0.25	0.21	0.26	0.24	0.25	0.19	0.23
	SEM	3.38	0.014		0.014		0.02				0.01
	P Value	0.06	0.01**		0.001***		0.032*				0.02*
HC	Control	87.01	0.61	0.51	0.53	0.59	0.56	0.67	0.51	0.52	0.56 ^a
	Glycerol	89.66	0.70	0.62	0.74	0.58	0.74	0.67	0.74	0.50	0.66 ^b
	Jaggery	97.41	0.74	0.64	0.78	0.61	0.82	0.67	0.74	0.55	0.69 ^b
	Maize	91.31	0.74	0.65	0.78	0.61	0.77	0.70	0.74	0.52	0.69 ^b
	Mean	91.34	0.70	0.61	0.71	0.60	0.72	0.67	0.79	0.52	0.65
	SEM	3.38	0.03		0.038		0.054				0.027
	P Value	0.06	0.01**		0.001***		0.032*				0.01**

Parameter	Group	MBW	Energy × Breed		Energy × Suppl.		HFX × Suppl.		Deoni × Suppl.		E × B × S
			HFX	Deoni	Without	With	Without	With	Without	With	
Cellulose	Control		0.13	0.11	0.11	0.13	0.12	0.15	0.11	0.11	0.12 ^a
	Glycerol		0.15	0.14	0.16	0.13	0.16	0.14	0.16	0.11	0.14 ^{bc}
	Jaggery		0.16	0.14	0.17	0.13	0.18	0.15	0.16	0.12	0.15 ^c
	Maize		0.14	0.12	0.15	0.11	0.15	0.13	0.15	0.09	0.13 ^{ab}
	Mean		0.14	0.13	0.15	0.12	0.15	0.14	0.15	0.11	0.14
	SEM		0.008		0.008		0.012		0.006		
	P Value		0.006**		0.001***		0.024*		0.009**		
ADL	Control		0.07	0.06	0.06	0.07	0.07	0.08	0.06	0.06	0.06 ^a
	Glycerol		0.09	0.08	0.09	0.07	0.09	0.08	0.09	0.06	0.08 ^{bc}
	Jaggery		0.09	0.08	0.09	0.07	0.10	0.08	0.09	0.07	0.08 ^c
	Maize		0.08	0.07	0.08	0.06	0.08	0.07	0.09	0.05	0.07 ^{ab}
	Mean		0.08	0.07	0.08	0.07	0.08	0.08	0.08	0.06	0.08
	SEM		0.005		0.005		0.007		0.003		
	P Value		0.008**		0.001***		0.018*		0.010**		
NFC	Control		0.42	0.35	0.37	0.40	0.39	0.46	0.35	0.35	0.38 ^a
	Glycerol		0.49	0.43	0.51	0.40	0.51	0.46	0.51	0.34	0.46 ^{bc}
	Jaggery		0.51	0.44	0.54	0.41	0.56	0.46	0.51	0.37	0.47 ^c
	Maize		0.43	0.39	0.47	0.35	0.46	0.40	0.49	0.30	0.41 ^{ab}
	Mean		0.46	0.40	0.47	0.39	0.48	0.44	0.46	0.34	0.43
	SEM		0.027		0.027		0.038		0.019		
	P Value		0.007**		0.001***		0.035*		0.015*		

Values with different superscripts for absolute interaction for respective energy source in a row under each parameter differ significantly; ***P≤ 0.001, **P≤ 0.01, *P≤ 0.05

Table 4.15: Nutrient intake from the total diet (kg/d) in prepartum cows

Parameter	Group	Energy × Breed		Energy × Suppl.		HFX × Suppl.		Deoni × Suppl.		E × B × S
		HFX	Deoni	Without	With	Without	With	Without	With	
DM	Control	7.35	6.19	6.70	6.84	7.16	7.54	6.24	6.15	6.77 ^a
	Glycerol	8.22	6.69	7.06	7.85	7.54	8.90	6.58	6.79	7.45 ^b
	Jaggery	8.51	7.15	7.65	8.01	8.36	8.67	6.94	7.36	7.83 ^b
	Maize	8.46	6.68	7.53	7.61	8.41	8.50	6.64	6.72	7.57 ^b
	Mean	8.13	6.68	7.23	7.58	7.87	8.40	6.60	6.75	7.40
	SEM	0.319		0.319		0.451		0.225		
	P Value	0.001^{***}		0.148		0.413		0.026[*]		
OM	Control	6.68	5.63	6.09	6.22	6.51	6.85	5.68	5.59	6.16 ^a
	Glycerol	7.51	6.11	6.44	7.17	6.88	8.14	6.01	6.21	6.81 ^b
	Jaggery	7.77	6.53	6.98	7.32	7.63	7.91	6.33	6.73	7.15 ^b
	Maize	7.73	6.10	6.88	6.96	7.69	7.78	6.06	6.14	6.92 ^b
	Mean	7.42	6.09	6.60	6.92	7.18	7.67	6.02	6.17	6.76
	SEM	0.291		0.291		0.411		0.206		
	P Value	0.001^{***}		0.140		0.422		0.022[*]		
CP	Control	0.61	0.51	0.55	0.57	0.58	0.64	0.51	0.51	0.56 ^a
	Glycerol	0.67	0.56	0.62	0.60	0.65	0.69	0.60	0.52	0.61 ^{ab}
	Jaggery	0.70	0.60	0.67	0.63	0.72	0.68	0.62	0.57	0.65 ^b
	Maize	0.69	0.57	0.66	0.60	0.70	0.68	0.62	0.52	0.63 ^b
	Mean	0.66	0.56	0.62	0.60	0.66	0.67	0.59	0.53	0.61
	SEM	0.025		0.025		0.036		0.018		
	P Value	0.001^{***}		0.205		0.090		0.017[*]		

Parameter	Group	Energy × Breed		Energy × Suppl.		HFX × Suppl.		Deoni × Suppl.		E × B × S
		HFX	Deoni	Without	With	Without	With	Without	With	
EE	Control	0.13	0.11	0.12	0.12	0.13	0.13	0.11	0.12	0.12 ^a
	Glycerol	0.14	0.12	0.12	0.13	0.13	0.15	0.12	0.12	0.13 ^{ab}
	Jaggery	0.14	0.12	0.13	0.13	0.14	0.14	0.12	0.12	0.13 ^{ab}
	Maize	0.15	0.12	0.14	0.14	0.16	0.15	0.13	0.12	0.14 ^b
	Mean	0.14	0.12	0.13	0.13	0.14	0.14	0.12	0.12	0.13
	SEM	0.005		0.005		0.007				0.004
	P Value	0.001^{***}		0.410		0.410				0.028[*]
TA	Control	0.71	0.60	0.64	0.66	0.69	0.73	0.60	0.60	0.65 ^a
	Glycerol	0.76	0.63	0.67	0.72	0.71	0.82	0.64	0.62	0.70 ^{ab}
	Jaggery	0.80	0.68	0.73	0.74	0.80	0.81	0.67	0.68	0.74 ^b
	Maize	0.80	0.65	0.73	0.72	0.80	0.81	0.66	0.63	0.72 ^b
	Mean	0.77	0.64	0.69	0.71	0.75	0.79	0.64	0.63	0.70
	SEM	0.029		0.029		0.041				0.020
	P Value	0.001^{***}		0.454		0.227				0.044[*]
TCHO	Control	5.90	4.97	5.39	5.48	5.77	6.04	5.02	4.93	5.44 ^a
	Glycerol	6.66	5.39	5.65	6.40	6.06	7.26	5.24	5.54	6.02 ^b
	Jaggery	6.87	5.76	6.12	6.51	6.71	7.04	5.53	5.99	6.31 ^b
	Maize	6.85	5.37	6.03	6.19	6.80	6.91	5.27	5.47	6.11 ^b
	Mean	6.57	5.37	5.80	6.14	6.33	6.81	5.26	5.48	5.97
	SEM	0.265		0.265		0.375				0.187
	P Value	0.001^{***}		0.083		0.500				0.026[*]

Values with different superscripts for absolute interaction for a respective energy source in a row under each parameter differ significantly; ***P≤ 0.001, *P≤ 0.05

Total diet intake in the HFX cows was 23% higher than the Deoni cows. The total DMI (kg/d) between TGs were significantly higher ($P < 0.001$) in T2 than either T1 or T3 but, The interaction between energy, breed, and supplement for CP intake in T2 and T3 was significantly higher ($P < 0.05$) than CG or T1. OM, EE, and TCHO intake were also significantly ($P < 0.001$) high in the HFX than the Deoni due to the significant difference in LW. But, the intake in terms of metabolic BW was higher ($P < 0.05$) in the Deoni cows than the HFX cows.

Dry matter, OM, CP, EE, and TCHO intakes per kg of MBW are presented in **Table 4.16**. DMI ($\text{g/kg W}^{0.75}$) was significantly higher ($P < 0.05$) in the Deoni cows than HFX cows. It means that DMI (g) per kg of metabolically active tissue was 3.4% higher in the Deoni cows than HFX cows. Glycerol generally considered as an appetite stimulant when fed with a concentrate diet hence, there was observably higher DMI, $\text{g/kg W}^{0.75}$ in T1 than either other TGs or CG (Fisher *et al.*, 1971). CP, EE, and TCHO intake were significantly 5.7%, 5.8%, and 3.1% higher in the Deoni cows than HFX cows. Although either glycerol or jaggery was free of CP, its intake in T1 was comparable to T3 that could be attributed to the appetizing effect of glycerol. GE intake ($\text{g/kgW}^{0.75}$) in the Deoni cows was 306 ± 6 Kcal compared to 297 ± 6 Kcal in HFX cows that accounted for 3% higher GE availability. This was contrary to the observations of Singh and Srinivas (2020) in the growing HFX or the Deoni heifers. Among 16 Deoni cows under this study, 15 were above 3rd parity and only 1 cow was in 2nd parity. Dairy animals do not attain their full-body or organ growth at the age of maturity but, continue to accrete body protein during their lactating lives at least up to 3 lactations (Coffey *et al.*, 2006).

The age at maturity of Deoni cows is much later than the HFX cows and fully expresses their milk potential in the 3rd parity only (Basak and Das, 2018). Any metabolic differences in the Deoni calf or heifers or cows before 3rd parity were appeared to be nullified thereafter and their metabolic intakes were higher than the HFX cows and hardly any difference exists for the gestation period (Mamun *et al.*, 2015). Irrespective of the physical state of the jaggery or glycerol, both are highly water-soluble. Dietary intakes were improved more on glycerol and maize than jaggery. Intake of EE was significantly higher ($P < 0.05$) in T3 compared to CG because other energy sources in T1 or T2 hardly contained any EE while maize had 2.54% germ oil (*Table 4.8*).

Table 4.16: Nutrient intake from total diet (g/kgW^{0.75}/d) in different groups

Parameter	Group	Energy × Breed		Energy × Suppl.		HFX × Suppl.		Deoni × Suppl.		E × B × S
		HFX	Deoni	Without	With	Without	With	Without	With	
DM	Control	76.61	79.83	79.22	77.22	78.38	74.84	80.07	79.60	78.22 ^a
	Glycerol	80.05	87.40	81.40	86.06	78.94	81.17	83.87	90.94	83.73 ^b
	Jaggery	80.07	81.05	79.43	81.68	79.80	80.14	78.87	83.23	80.56 ^{ab}
	Maize	83.08	82.61	81.44	82.24	82.98	83.18	79.91	85.32	82.85 ^b
	Mean	79.95	82.72	80.37	82.30	80.07	79.83	80.68	84.77	81.34
	SEM	1.472		1.472		2.082		1.041		
	P Value	0.017*		0.083		0.054		0.008**		
OM	Control	69.70	72.64	72.10	70.24	71.34	68.07	72.09	74.22	71.17 ^a
	Glycerol	73.15	79.83	74.30	78.68	72.09	74.22	76.52	83.15	76.49 ^c
	Jaggery	73.04	73.92	72.40	74.57	72.93	73.15	71.87	75.98	73.48 ^{ab}
	Maize	75.96	75.48	74.40	77.03	75.85	76.07	72.96	78.00	75.72 ^{bc}
	Mean	72.96	75.47	73.30	75.13	73.05	72.88	73.55	77.38	74.21
	SEM	1.346		1.346		1.904		0.952		
	P Value	0.018*		0.073		0.051		0.005**		
CP	Control	6.29	6.57	6.40	6.46	6.28	6.30	6.53	6.61	6.43 ^a
	Glycerol	6.49	7.28	7.17	6.60	6.74	6.24	7.60	6.97	6.88 ^b
	Jaggery	6.60	6.75	6.98	6.37	6.91	6.30	7.05	6.45	6.68 ^{ab}
	Maize	6.73	6.99	7.12	6.61	6.84	6.62	7.39	6.60	6.86 ^b
	Mean	6.53	6.90	6.92	6.51	6.69	6.36	7.14	6.65	6.71
	SEM	0.181		0.181		0.256		0.128		
	P Value	0.010**		0.060		0.550		0.049*		

Parameter	Group	Energy × Breed		Energy × Suppl.		HFX × Suppl.		Deoni × Suppl.		E × B × S
		HFX	Deoni	Without	With	Without	With	Without	With	
EE	Control	1.36	1.44	1.41	1.38	1.39	1.33	1.44	1.44	1.40 ^{ab}
	Glycerol	1.35	1.51	1.41	1.45	1.35	1.35	1.47	1.55	1.43 ^b
	Jaggery	1.34	1.38	1.35	1.36	1.35	1.33	1.36	1.40	1.36 ^a
	Maize	1.49	1.52	1.49	1.51	1.49	1.49	1.50	1.54	1.50 ^c
	Mean	1.38	1.46	1.42	1.42	1.39	1.37	1.44	1.48	1.42
	SEM	0.028		0.028		0.040		0.020		
	P Value	0.001^{***}		0.642		0.158		0.001^{***}		
TA	Control	7.37	7.68	7.58	7.47	7.49	7.26	7.68	7.68	7.53 ^a
	Glycerol	7.41	8.16	7.73	7.84	7.42	7.40	8.04	8.28	7.78 ^{ab}
	Jaggery	7.55	7.66	7.64	7.57	7.65	7.45	7.62	7.70	7.60 ^a
	Maize	7.90	7.96	7.93	7.94	7.94	7.87	7.92	8.01	7.93 ^b
	Mean	7.56	7.87	7.72	7.70	7.62	7.49	7.81	7.92	7.71
	SEM	0.142		0.142		0.200		0.100		
	P Value	0.007^{**}		0.893		0.258		0.047[*]		
TCHO	Control	61.59	64.14	61.82	61.91	63.22	59.96	64.43	63.86	62.87 ^a
	Glycerol	64.81	70.45	65.09	70.17	63.43	66.20	66.75	74.15	67.63 ^b
	Jaggery	64.58	65.26	63.46	66.38	64.08	65.08	62.84	67.69	64.92 ^{ab}
	Maize	67.28	66.44	65.23	68.49	67.03	67.54	63.44	69.44	66.86 ^b
	Mean	64.56	66.57	64.40	66.74	64.44	64.49	64.36	68.78	65.57
	SEM	1.237		1.237		1.749		0.874		
	P Value	0.006^{**}		0.017[*]		0.030[*]		0.006^{**}		

Values with different superscripts for absolute interaction for a respective energy source in a row under each parameter differ significantly; ***P ≤ 0.001, **P ≤ 0.01, *P ≤ 0.05

FNS supplementation to CG or TGs was non-significant for ether breed or energy. Breed and FNS supplementation interaction was also non-significant. The interaction effect of energy, breed and FNS supplement on DMI and CP intake (g/kg $W^{0.75}$) was significantly higher in TGs compared to CG. DMI, CP, and TCHO intake (g/kg $W^{0.75}$) were significantly higher ($P < 0.05$) in T1 and T3, while CG and T2 were comparable. EE intake (g/kg $W^{0.75}$) was highest ($P < 0.05$) in T3 followed by T1 and T2. However, EE in CG was comparable with the T1 and T2 and significantly lesser than T3.

The interaction effect of energy, breed and supplement on Total DMI (g/kg $W^{0.75}$) in T1 and T3 were significantly higher than T2. Diet intake expression in terms of kg/d by the animal is an inappropriate expression because it is related to the relative size of the body hence, %BW or g/kg $W^{0.75}$ is uniform of expression balancing the difference in LW. Henceforth, total diet nutrient intake either proximate or cell wall were higher in the Deoni than HFX cows.

Cell content, NDF, HC, cellulose and NFC intake of HFX cows from the total diet was significantly ($P < 0.05$) higher by 21.4%, 21.9%, 20.7%, 22.8%, 22.6%, respectively than Deoni cows (**Table 4.17**). Indigenous cows are inclined to more cell wall diets than quality diets (Simianer, 2000). The difference in the NDF intake by HFX cows compared to Deoni cows was 31% and 15% more than its intake through green fodder and CS, respectively. The green fodder consumption by both the breed was very selective and limited compared to dry fodder, but CS intake was complete without leaving any residue. Henceforth, the difference in the total diet NDF intake was selective by the cows and that could be related to available rumen space and fill that may decline with the rapidly growing fetus at this period.

FNS supplementation impact on CG or TGs was significant ($P < 0.001$) only for ADF intake. Breed and supplement interaction was non-significant. CC intake from total diet (kg/d) was significantly high ($P < 0.01$) in T2 which is comparable with the T1 and higher than T3. NFC intake from the total diet was significantly ($P < 0.001$) higher by 34.1%, 41.2% and 20% in T1, T2, and T3, respectively in comparison with CG.

Table 4.17: Cell content and cell wall intake from total diet (kg/d) in prepartum cows

Parameter	Group	Energy × Breed		Energy × Suppl.		HFX × Suppl.		Deoni × Suppl.		E × B × S
		HFX	Deoni	Without	With	Without	With	Without	With	
CC	Control	2.81	2.35	2.54	2.62	2.71	2.90	2.37	2.34	2.58 ^a
	Glycerol	3.43	2.80	3.02	3.20	3.21	3.65	2.84	2.76	3.11 ^{bc}
	Jaggery	3.58	3.01	3.28	3.30	3.57	3.58	3.00	3.02	3.29 ^c
	Maize	3.36	2.68	3.07	2.97	3.37	3.35	2.77	2.60	3.02 ^b
	Mean	3.29	2.71	2.98	3.02	3.21	3.37	2.74	2.68	3.00
	SEM	0.121		0.121			0.171		0.085	
	P Value	0.001^{***}		0.595			0.216		0.01^{**}	
NDF	Control	4.53	3.83	4.15	4.21	4.44	4.62	3.87	3.80	4.18
	Glycerol	4.78	3.88	4.03	4.63	4.33	5.24	3.73	4.03	4.33
	Jaggery	4.93	4.14	4.36	4.71	4.78	5.08	3.94	4.34	4.53
	Maize	5.10	3.99	4.46	4.63	5.05	5.15	3.87	4.12	4.55
	Mean	4.83	3.96	4.25	4.55	4.65	5.02	3.85	4.07	4.40
	SEM	0.200		0.200			0.283		0.142	
	P Value	0.001^{***}		0.053			0.592		0.247	
ADF	Control	2.65	2.24	2.44	2.45	2.61	2.68	2.27	2.22	2.44
	Glycerol	2.77	2.21	2.25	2.73	2.45	3.09	2.05	2.37	2.49
	Jaggery	2.84	2.37	2.45	2.77	2.71	2.98	2.19	2.55	2.61
	Maize	2.95	2.27	2.50	2.71	2.89	3.00	2.11	2.43	2.61
	Mean	2.80	2.27	2.41	2.66	2.66	2.94	2.15	2.39	2.54
	SEM	0.129		0.129			0.182		0.91	
	P Value	0.001^{***}		0.013[*]			0.839		0.490	
HC	Control	1.89	1.59	1.72	1.77	1.83	1.95	1.60	1.59	1.74 ^a
	Glycerol	2.02	1.67	1.78	1.91	1.89	2.16	1.68	1.66	1.84 ^{ab}
	Jaggery	2.09	1.77	1.92	1.94	2.08	2.10	1.75	1.79	1.93 ^b
	Maize	2.15	1.73	1.96	1.92	2.15	2.15	1.77	1.69	1.94 ^b
	Mean	2.04	1.69	1.84	1.88	1.99	2.09	1.70	1.68	1.86
	SEM	0.076		0.076			0.107		0.053	
	P Value	0.001^{***}		0.465			0.278		0.043[*]	

Parameter	Group	Energy × Breed		Energy × Suppl.		HFX × Suppl.		Deoni × Suppl.		E × B × S
		HFX	Deoni	Without	With	Without	With	Without	With	
Cellulose	Control	2.15	1.82	1.98	1.99	2.13	2.17	1.84	1.80	1.98
	Glycerol	2.24	1.79	1.81	2.22	1.97	2.51	1.65	1.93	2.01
	Jaggery	2.30	1.92	1.97	2.25	2.18	2.42	1.76	2.08	2.11
	Maize	2.38	1.83	2.01	2.20	2.33	2.43	1.69	1.97	2.10
	Mean	2.26	1.84	1.94	2.16	2.15	2.38	1.73	1.94	2.05
	SEM	0.105		0.105		0.149		0.075		
	P Value	0.001***		0.090		0.895		0.573		
ADL	Control	0.33	0.28	0.30	0.31	0.33	0.34	0.28	0.28	0.31
	Glycerol	0.36	0.29	0.30	0.34	0.32	0.39	0.28	0.30	0.32
	Jaggery	0.37	0.31	0.32	0.35	0.36	0.38	0.29	0.32	0.34
	Maize	0.37	0.29	0.32	0.33	0.37	0.37	0.28	0.30	0.33
	Mean	0.36	0.29	0.31	0.33	0.34	0.37	0.28	0.30	0.32
	SEM	0.015		0.015		0.022		0.011		
	P Value	0.001***		0.053		0.569		0.293		
NFC	Control	1.37	1.14	1.24	1.28	1.33	1.41	1.15	1.14	1.26 ^a
	Glycerol	1.88	1.51	1.62	1.76	1.74	2.02	1.51	1.51	1.69 ^{bc}
	Jaggery	1.94	1.62	1.76	1.80	1.93	1.96	1.60	1.65	1.78 ^c
	Maize	1.76	1.38	1.58	1.56	1.75	1.76	1.40	1.35	1.57 ^b
	Mean	1.73	1.41	1.55	1.60	1.68	1.79	1.41	1.41	1.57
	SEM	0.066		0.066		0.094		0.046		
	P Value	0.001***		0.303		0.268		0.001***		

Values with different superscripts for absolute interaction for a respective energy source in a row under each parameter differ significantly; ***P ≤ 0.001, **P ≤ 0.01, *P ≤ 0.05

Table 4.18: Cell content and cell wall constituents intake from total diet (g/kgW^{0.75}/d) in prepartum cows

Parameter	Group	Energy × Breed		Energy × Suppl.		HFX × Suppl.		Deoni × Suppl.		E × B × S
		HFX	Deoni	Without	With	Without	With	Without	With	
CC	Control	29.97	29.59	29.97	29.59	29.60	28.82	30.34	30.35	29.78 ^a
	Glycerol	33.89	35.10	34.89	35.10	33.56	33.28	36.22	36.93	34.99 ^c
	Jaggery	34.12	33.60	34.12	33.60	34.21	33.09	34.04	34.11	33.86 ^{bc}
	Maize	33.19	32.89	33.19	32.89	33.15	32.81	33.22	32.97	33.04 ^b
	Mean	32.31	33.52	33.04	32.79	32.63	32.00	33.45	33.59	32.91
	SEM	0.592		0.592			0.837			0.418
	P Value	0.011*		0.563			0.373			0.001***
NDF	Control	49.18	47.56	49.18	47.56	48.70	45.95	49.66	49.17	48.37 ^{ab}
	Glycerol	46.42	50.88	46.42	50.88	45.30	47.83	47.55	53.94	48.65 ^{ab}
	Jaggery	45.22	48.01	45.22	48.01	45.70	46.98	44.74	49.05	46.62 ^a
	Maize	48.17	51.30	48.17	51.30	49.75	50.31	46.60	52.29	49.74 ^b
	Mean	47.56	49.12	47.25	49.44	47.36	47.77	47.14	51.11	48.34
	SEM	0.981		0.981			1.387			0.693
	P Value	0.011*		0.006**			0.020*			0.041*
ADF	Control	28.90	27.61	28.90	27.61	28.71	26.63	29.09	28.60	28.26
	Glycerol	25.84	29.96	25.84	29.96	25.56	28.16	26.12	31.77	27.90
	Jaggery	25.31	28.22	25.31	28.22	25.79	27.55	24.84	28.88	26.76
	Maize	26.99	30.02	26.98	30.02	28.56	29.28	25.41	30.77	28.50
	Mean	27.58	28.19	26.76	28.95	27.15	27.90	26.37	30.01	27.86
	SEM	0.710		0.701			1.004			0.502
	P Value	0.208		0.001***			0.011*			0.111
HC	Control	20.28	19.95	20.28	19.95	19.99	19.33	20.57	20.57	20.11 ^{ab}
	Glycerol	20.58	20.92	20.58	20.92	19.74	19.67	21.42	22.17	20.75 ^{bc}
	Jaggery	19.91	19.80	19.91	19.78	19.91	19.43	19.90	20.17	19.85 ^a
	Maize	21.19	21.28	21.19	21.28	21.19	21.03	21.19	21.52	21.23 ^c
	Mean	20.04	20.94	20.49	20.48	20.21	19.86	20.77	21.11	20.49
	SEM	0.382		0.382			0.540			0.270
	P Value	0.004**		0.991			0.227			0.010

Parameter	Group	Energy × Breed		Energy × Suppl.		HFX × Suppl.		Deoni × Suppl.		E × B × S
		HFX	Deoni	Without	With	Without	With	Without	With	
Cellulose	Control	23.51	22.41	23.52	22.41	23.36	21.57	23.67	23.25	22.96
	Glycerol	20.79	24.36	20.79	24.36	20.60	22.86	20.97	25.87	22.57
	Jaggery	20.37	22.93	20.37	22.93	20.77	22.36	19.98	23.49	21.65
	Maize	21.61	24.29	21.61	24.29	22.98	23.62	20.25	24.96	22.95
	Mean	22.26	22.80	21.57	23.50	21.93	22.60	21.22	24.39	22.53
	SEM	0.605		0.605			0.837			0.428
	P Value	0.226		0.001***			0.010**			0.144
ADL	Control	3.58	3.47	3.53	3.47	3.56	3.38	3.61	3.56	3.53
	Glycerol	3.41	3.73	3.47	3.73	3.34	3.54	3.48	3.92	3.67
	Jaggery	3.33	3.53	3.41	3.53	3.39	3.47	3.28	3.58	3.43
	Maize	3.44	3.61	3.48	3.66	3.58	3.61	3.31	3.71	3.55
	Mean	3.48	3.55	3.41	3.60	3.46	3.50	3.42	3.69	3.52
	SEM	0.068		0.068			0.096			0.048
	P Value	0.039*		0.005**			0.025*			0.220
NFC	Control	14.65	14.36	14.78	14.48	14.53	14.01	14.77	14.70	14.50 ^a
	Glycerol	18.68	19.29	18.79	19.43	18.14	18.37	19.22	20.22	18.99 ^d
	Jaggery	18.24	18.36	18.35	18.50	18.39	18.10	18.10	18.64	18.31 ^c
	Maize	17.07	17.20	17.19	17.34	17.29	17.24	16.85	17.16	17.13 ^b
	Mean	17.00	17.46	17.28	17.44	17.09	16.93	17.23	17.68	17.23
	SEM	0.283		0.283			0.200			0.200
	P Value	0.145		0.483			0.152			0.001***

Values with different superscripts for absolute interaction for a respective energy source in a row under each parameter differ significantly; ***P ≤ 0.001, **P ≤ 0.01, *P ≤ 0.05

CC, NDF, HC intake (g/kg $W^{0.75}$) for metabolically active tissue was higher ($P < 0.05$) in the Deoni cows than HFX cows (**Table 4.18**). Cattle have a higher metabolic rate per unit of metabolic BW than do most other domestic ruminant and CWC intake was more in Deoni than HFX cows because the indigenous breed of cows are low yielders and intake of low quality feeds is more and quality forages are lesser (Silanikove, 1992; Simianer, 2000). NDF, ADF, and Cellulose intake (g/kg $W^{0.75}$) was significantly higher ($P < 0.01$) with FNS supplement than without supplement to CG and TGs. NDF, ADF, and cellulose intake (g/kg $W^{0.75}$) in HFX ($P < 0.01$) and Deoni ($P < 0.05$) cows were significantly higher in FNS supplemented than without it. The interaction between energy, breeds and supplement for CC intake was significantly ($P < 0.001$) higher in TGs than CG. The interaction between energy, breed and supplement for CC intake from the total diet (g/kg $W^{0.75}$) was highest in T1, followed by T3 and T2. The difference was significant ($P < 0.001$). NDF intake from the total diet (g/kg $W^{0.75}$) was significantly higher in the T3 by 6.3% than T2, while T2 was comparable to CG and T1 ($P < 0.05$).

HC intake from the total diet (g/kg $W^{0.75}$) was significantly higher ($P < 0.01$) in the T3 that was statistically comparable to CG and T1 but, 11.2% higher than T2. NFC intake (g/kg $W^{0.75}$) was significantly high in TGs than CG because of 10% more inclusion of glycerol, jaggery and maize grain in TG diets. NFC intake (g/kg $W^{0.75}$) in T1 was 3.7%, 10.9%, and 31% higher than T2, T3, and CG, respectively.

4.2.4 Digestible Nutrient intake

Digestible DM ($P = 0.339$), OM ($P = 0.245$), CP ($P = 0.188$), EE ($P = 0.147$) and TCHO ($P = 0.44$) intake per metabolic BW was statistically comparable (**Table 4.19**) between HFX and Deoni cows. Energy or breed interaction with FNS supplement on digestible DM, OM, CP, EE and TCHO intake (g/kg $W^{0.75}$) were non-significant. The interaction between energy, breed and FNS supplement for digestible DM, OM, CP and TCHO intake (g/kg $W^{0.75}$) was significantly ($P < 0.005$) different.

The digestible DM and TCHO intake (g/kg $W^{0.75}$) was significantly ($P < 0.01$) higher in T1 with FNS supplement compared with T2 and CG but, T1 and T3 were comparable. Digestible DM (g/kg $W^{0.75}$) was 10.5%, 5.5% higher in T1 with

FNS supplement than CG and T3. This indicated that glycerol which is water-soluble polyol compound are preferred and instantly available to the rumen microbes and ferment after phosphorylation in the presence of FNS supplement. Such preferential fermentation, as also observed in the *in vitro*, may provide greater energy to the rumen microbes. Although the additional energy fortified was about 3-5 % on total DMI in TGs, its net impact on the digestible nutrients intake was null. Contrast to it, Schröder and Südekum (1999) reported a negative effect on nutrient digestibility in dairy cattle fed glycerol at the rate of 10% of total DM. The rapidly soluble carbohydrates like molasses in diet must restrict to 20% of the CS to avoid inefficient usage of the basal diet (Preston *et al.*, 1986). Digestible OM intake marginally higher in CG and TGs between breeds or energy and FNS supplement or any breed and FNS supplement interaction. Digestible OM or CP intake was significantly higher in T1 and T3 than CG or T1. Digestible CP (g/kg W^{0.75}) was found significantly (P< 0.001) high in TGs than CG. Compared to instantly fermentable carbohydrates like jaggery, medium and slow fermenting polyol or maize starch carbohydrates have better influence on N utilization efficiency and such controlled release of energy is reported helpful in supporting production in dairy cows (Oba, 2011). Unlike in younger Deoni calves, no limitation in digestible CP intake was observed in prepartum Deoni cows as reported by Singh and Srinivas (2018).

The digestible CC, NDF, ADF, Cellulose and NFC intake (g/kg W^{0.75}) was non-significant between HFX and Deoni cows in prepartum (**Table 4.20**). Digestible HC intake (g/kg W^{0.75}) in prepartum was higher (P< 0.05) in Deoni than HFX cows although total dietary HC intake (g/kg W^{0.75}) was also higher. Energy with FNS supplement had a significant effect on CC (P<0.05), NDF (P = 0.052) and ADF (P< 0.01).

Probably minerals presented in FNS supplement augmented microbial activity in rumen as they are essential for some microbial enzymes. Breed and supplement interaction for digestible cellulose intake (g/kgW^{0.75}) was found significantly higher (P< 0.05) and its effect were better in Deoni cows and FNS supplement thus, ascertaining the positive impact of FNS supplement on rumen cellulolytic digestibility in Deoni cows.

Table 4.19: Digestible nutrient intake (Proximate composition) from total diet (g/kgW^{0.75}/d) in prepartum cows

Parameter	Group	Energy × Breed		Energy × Suppl.		HFX × Suppl.		Deoni × Suppl.		E × B × S
		HFX	Deoni	Without	With	Without	With	Without	With	
DM	Control	42.92	43.81	43.85	42.87	43.52	42.32	44.19	43.43	43.36 ^a
	Glycerol	46.16	49.82	47.36	48.62	46.63	45.69	48.09	51.55	47.99 ^c
	Jaggery	45.19	45.78	45.46	45.51	45.13	45.25	45.79	45.77	45.48 ^{ab}
	Maize	47.31	45.20	45.53	46.98	47.34	47.27	43.72	46.68	46.35 ^{bc}
	Mean	45.39	46.15	45.55	46.00	45.65	45.13	45.44	46.86	45.77
	SEM	1.088		1.088		1.539		0.769		
	P Value	0.339		0.571		0.207		0.005**		
OM	Control	41.01	41.95	41.97	40.98	41.63	40.39	42.32	41.58	41.48 ^a
	Glycerol	43.56	47.49	44.77	46.29	43.77	43.35	45.76	49.22	45.53 ^b
	Jaggery	42.80	43.77	43.08	43.49	42.53	43.07	43.63	43.92	43.28 ^{ab}
	Maize	45.53	43.27	43.62	45.17	45.51	45.55	41.76	44.79	44.40 ^b
	Mean	43.22	44.12	43.36	43.98	43.36	43.09	43.37	44.88	43.67
	SEM	1.052		1.051		1.486		0.743		
	P Value	0.245		0.416		0.249		0.009**		
CP	Control	3.32	3.34	3.28	3.38	3.12	3.53	3.44	3.24	3.30 ^a
	Glycerol	3.89	4.28	4.36	3.81	4.10	3.68	4.61	3.95	4.08 ^b
	Jaggery	3.98	4.00	4.26	3.71	4.19	3.77	4.34	3.66	3.99 ^b
	Maize	3.85	4.00	4.12	3.81	3.87	3.83	4.37	3.79	3.96 ^b
	Mean	3.76	3.92	4.00	3.68	3.82	3.70	4.19	3.66	3.84
	SEM	0.142		0.142		0.201		0.100		
	P Value	0.188		0.055		0.057		0.001***		
EE	Control	0.80	0.80	0.77	0.83	0.84	0.77	0.71	0.89	0.80
	Glycerol	0.79	0.88	0.84	0.83	0.82	0.77	0.87	0.90	0.84
	Jaggery	0.77	0.82	0.82	0.76	0.79	0.76	0.86	0.77	0.79
	Maize	0.87	0.91	0.88	0.90	0.85	0.89	0.92	0.91	0.89
	Mean	0.81	0.85	0.83	0.83	0.82	0.79	0.84	0.87	0.83
	SEM	0.041		0.041		0.058		0.029		
	P Value	0.147		0.983		0.370		0.121		
TCHO	Control	36.41	37.32	37.45	36.27	37.22	35.66	37.69	36.95	36.86 ^a
	Glycerol	38.79	41.73	39.26	41.27	38.94	38.65	39.59	43.88	40.26 ^c
	Jaggery	37.53	38.41	37.38	38.56	36.97	38.09	37.80	39.03	37.97 ^{ab}
	Maize	40.36	37.77	38.08	40.04	40.32	40.40	35.85	39.69	39.06 ^{bc}
	Mean	38.27	38.81	38.04	39.03	38.36	38.12	37.73	39.89	38.54
	SEM	0.963		0.963		1.362		0.681		
	P Value	0.444		0.165		0.106		0.017*		

Values with different superscripts for absolute interaction for a respective energy source in a row under each parameter differ significantly; ***P ≤ 0.001, **P ≤ 0.01, *P ≤ 0.05

Table 4.20: Digestible Cell content and cell wall constituents intake from the total diet (g/kgW^{0.75}/d) in prepartum cow

Parameter	Group	Energy × Breed		Energy × Suppl.		HFX × Suppl.		Deoni × Suppl.		E × B × S
		HFX	Deoni	Without	With	Without	With	Without	With	
CC	Control	19.22	19.62	19.45	19.39	19.27	19.18	19.63	19.61	19.42 ^a
	Glycerol	23.24	25.07	24.47	23.85	23.73	22.76	25.22	24.93	24.16 ^d
	Jaggery	23.03	23.09	23.88	22.25	23.86	22.20	23.89	22.29	23.06 ^c
	Maize	21.46	21.64	21.92	21.18	21.88	21.05	21.96	21.32	21.55 ^b
	Mean	21.74	22.35	22.43	21.67	22.18	21.30	22.67	22.04	22.05
	SEM	0.462		0.462		0.654		0.327		
	P Value	0.078		0.333		0.706		0.001^{***}		
NDF	Control	23.63	24.11	24.34	23.40	24.19	23.07	24.49	23.74	23.87
	Glycerol	22.84	24.65	22.79	24.70	22.82	22.86	22.76	26.55	23.75
	Jaggery	22.08	22.61	21.49	23.19	21.18	22.98	21.81	23.41	22.34
	Maize	25.78	23.48	23.53	25.73	25.39	26.17	21.67	25.30	24.63
	Mean	23.58	23.71	23.04	24.26	23.39	23.77	22.68	24.75	23.65
	SEM	0.823		0.823		1.164		0.582		
	P Value	0.820		0.052		0.165		0.081		
ADF	Control	11.80	11.72	12.11	11.40	12.35	11.25	11.88	11.56	11.76
	Glycerol	10.65	11.27	9.44	12.48	9.67	11.65	9.23	13.32	10.96
	Jaggery	10.73	9.58	9.17	11.14	9.77	11.69	8.57	10.60	10.16
	Maize	11.90	11.72	9.96	12.16	11.42	12.38	8.51	11.94	11.06
	Mean	11.27	10.70	12.31	13.01	10.80	11.74	9.55	11.85	10.98
	SEM	0.690		0.690		0.976		0.488		
	P Value	0.260		0.004^{**}		0.182		0.187		

Parameter	Group	Energy × Breed		Energy × Suppl.		HFX × Suppl.		Deoni × Suppl.		E × B × S
		HFX	Deoni	Without	With	Without	With	Without	With	
HC	Control	11.83	12.39	12.22	12.00	11.84	11.83	12.61	12.18	12.11 ^a
	Glycerol	12.19	13.38	13.35	12.22	13.16	11.21	13.53	13.23	12.78 ^a
	Jaggery	11.34	13.02	12.32	12.04	11.41	11.28	13.24	12.81	12.18 ^a
	Maize	13.87	13.27	13.57	13.57	13.97	13.78	13.17	13.37	13.57 ^b
	Mean	12.31	13.01	12.86	12.50	12.59	12.02	13.13	12.89	12.66
	SEM	0.357		0.357		0.506				0.253
	P Value	0.013*		0.129		0.525				0.003**
Cellulose	Control	23.51	22.41	23.52	22.41	23.36	21.57	23.67	23.25	22.96
	Glycerol	20.79	24.36	20.79	24.36	20.60	22.86	20.97	25.87	22.57
	Jaggery	20.37	22.93	20.37	22.93	20.77	22.36	19.98	23.49	21.65
	Maize	21.61	24.29	21.61	24.29	22.98	23.62	20.25	24.96	22.95
	Mean	22.26	22.80	21.57	23.50	21.93	22.60	21.22	24.39	22.53
	SEM	0.605		0.605		0.837				0.428
	P Value	0.226		0.001***		0.010**				0.144
NFC	Control	14.65	14.36	14.78	14.48	14.53	14.01	14.77	14.70	14.50 ^a
	Glycerol	18.68	19.29	18.79	19.43	18.14	18.37	19.22	20.22	18.99 ^d
	Jaggery	18.24	18.36	18.35	18.50	18.39	18.10	18.10	18.64	18.31 ^c
	Maize	17.07	17.20	17.19	17.34	17.29	17.24	16.85	17.16	17.13 ^b
	Mean	17.00	17.46	17.28	17.44	17.09	16.93	17.23	17.68	17.23
	SEM	0.283		0.283		0.200				0.200
	P Value	0.145		0.483		0.152				0.001***

Values with different superscripts for absolute interaction for a respective energy source in a row under each parameter differ significantly; ***P ≤ 0.001, **P ≤ 0.01, *P ≤ 0.05

Interaction effect of energy, breed and FNS supplement on CC (g/kg W^{0.75}) intake in T1 was significantly (P< 0.001) higher by 24.4%, 4.7% and 12% than CG, T2 and T3, respectively. Digestible HC in T3 was significantly higher (P< 0.05) than CG and T1 and T2. Probably starch in maize support non-cellulolytic or amylolytic bacteria (Kotarski *et al.*, 1992). Digestible NFC intake (g/kg W^{0.75}) was significantly higher (P< 0.001) in TGs than CG. Among the TGs digestible NFC intake (g/kg W^{0.75}) in T1 was 19 g/kgW^{0.75} in comparison to 15 g/kgW^{0.75} in CG (P< 0.001). Digestible NFC intake in T1 was even higher than T2 or T3 which was evidence for better utilization of glycerol being polyol compound than di or polysaccharides.

4.2.5 Digestibility coefficient of nutrients

Digestibility of proximate principles viz., DM, OM, CP, EE and TCHO (**Table 4.21**) and CWC viz., CC, NDF, ADF, HC, Cellulose and NFC (**Table 4.22**) were comparable between prepartum HFX and Deoni cows and there was no breed difference. The effect of FNS supplementation had a comparable impact on the digestibility of nutrients between CG and TGs. Breed and supplement interaction also had no significant impact on the digestibility of cellular or cell wall nutrients. Dermauw *et al.* (2013) also reported no effect of trace mineral supplementation (Cu, Zn, Mn, Se, I and Co) on the nutrient digestibility when fed for 28 days to grass-fed zebu cattle. Digestibility is a relative comparison of digestible nutrient intake and apparent retention of nutrients in the gastrointestinal tract. Except for the significant difference in CP (P< 0.001) and NFC (P< 0.01) digestibility due to interaction between energy, breed and FNS supplement, the interaction effect of energy × breed, energy × FNS supplement or HFX/Deoni cows × FNS supplement was none on the digestibility of nutrients. The significant difference in CP and NFC digestibility due to interaction effect of energy, breed and FNS supplement was only between CG and TGs but comparable within TGs, hence the difference could be attributed to intake, nature and type of energy fed to TGs compared to CG.

The increased CP digestibility with energy fortification in TGs also suggested that there is more chances of N trapping by the rumen microflora for MBP synthesis in utilizing the central NH₃ pool (Hoover and Stokes, 1991).

Table 4.21: Digestible coefficient (Proximate composition) of the total diet in prepartum cows

Parameter	Group	Energy × Breed		Energy × Suppl.		HFX × Suppl.		Deoni × Suppl.		E × B × S
		HFX	Deoni	Without	With	Without	With	Without	With	
DM (%)	Control	56.00	54.87	55.34	55.54	55.50	56.51	55.19	54.56	55.43
	Glycerol	57.63	57.01	58.19	56.48	59.50	56.28	57.33	56.69	57.33
	Jaggery	56.42	56.54	57.23	55.74	56.39	56.88	58.07	55.01	56.48
	Maize	56.98	54.71	55.86	55.80	57.08	56.53	54.70	54.72	55.84
	Mean	56.87	55.91	56.66	56.12	57.00	56.53	56.31	55.24	56.27
	SEM		0.826		0.830		1.174			0.587
	P Value		0.113		0.207		0.615			0.157
OM (%)	Control	58.81	57.74	58.20	58.36	58.32	59.31	58.08	57.41	58.28
	Glycerol	59.55	59.49	60.24	58.81	60.70	58.41	59.78	59.20	59.52
	Jaggery	58.58	59.26	59.49	58.35	58.27	58.88	60.71	57.83	58.92
	Maize	59.97	57.33	58.63	58.67	60.62	59.92	57.23	57.43	58.65
	Mean	59.23	58.46	59.14	58.55	59.33	59.13	58.95	57.97	58.84
	SEM		0.825		0.824		1.165			0.510
	P Value		0.204		0.326		0.511			0.509
CP (%)	Control	52.84	50.84	51.19	52.48	49.68	55.99	52.70	48.98	51.84 ^a
	Glycerol	59.78	58.68	60.69	57.77	60.70	58.86	60.69	56.68	59.23 ^b
	Jaggery	60.16	59.17	61.03	58.29	60.53	59.79	61.53	56.80	59.66 ^b
	Maize	57.15	58.22	57.77	57.60	56.40	57.90	59.13	57.31	57.68 ^b
	Mean	57.48	56.73	57.67	56.54	56.83	58.13	58.51	54.94	57.10
	SEM		0.908		0.908		1.284			0.642
	P Value		0.258		0.326		0.174			0.001^{***}
EE (%)	Control	59.32	55.69	55.18	59.84	60.78	57.86	49.58	61.81	55.57
	Glycerol	59.71	58.46	59.55	57.62	60.36	57.07	58.75	58.18	57.44
	Jaggery	57.37	59.35	60.63	56.08	57.86	56.88	63.41	55.29	56.59
	Maize	59.27	59.82	58.91	59.71	56.77	59.77	61.05	58.58	55.96
	Mean	58.42	58.33	58.56	58.17	58.94	57.89	58.20	58.46	58.37
	SEM		2.867		2.867		4.055			2.027
	P Value		0.966		0.097		0.750			0.958
TCHO (%)	Control	59.10	58.16	58.67	58.59	58.83	59.36	58.50	57.83	58.63
	Glycerol	59.87	59.23	60.32	58.78	61.36	58.38	59.28	59.19	59.55
	Jaggery	58.10	58.93	58.91	58.11	57.67	59.87	60.61	57.69	58.51
	Maize	60.02	56.82	58.33	58.51	60.16	57.69	56.49	57.15	58.42
	Mean	59.27	58.29	59.06	58.50	59.51	59.03	58.61	57.96	58.78
	SEM		0.832		0.832		1.176			0.588
	P Value		0.114		0.358		0.886			0.518

Values with different superscripts for absolute interaction for a respective energy source in a row under each parameter differ significantly; ***P≤ 0.001

Table 4.22: Digestibility coefficient of cell and cell wall constituents of total diet in different prepartum groups

Parameter	Group	Energy × Breed		Energy × Suppl.		HFX × Suppl.		Deoni × Suppl.		E × B × S
		HFX	Deoni	Without	With	Without	With	Without	With	
CC (%)	Control	65.81	64.69	64.90	65.60	65.10	66.52	64.71	64.68	66.25 ^a
	Glycerol	69.54	68.60	70.19	67.95	70.70	68.39	70.70	68.39	69.07 ^b
	Jaggery	68.42	67.76	69.98	66.20	69.68	67.51	69.75	67.09	68.09 ^b
	Maize	65.10	65.39	66.06	64.43	70.20	65.32	66.02	64.68	65.24 ^a
	Mean	67.22	66.61	67.78	66.04	67.78	66.54	67.67	65.55	66.91
	SEM	0.974		0.974		1.377				0.689
	P Value	0.392		0.203		0.581				0.002^{**}
NDF (%)	Control	49.87	48.75	49.44	49.18	49.58	50.16	49.31	48.20	49.31
	Glycerol	49.07	48.52	49.09	48.50	50.35	47.80	47.82	49.21	48.79
	Jaggery	47.61	48.27	47.54	48.34	46.33	48.89	48.75	47.79	47.94
	Maize	51.55	47.42	48.75	50.21	51.06	52.04	46.45	48.39	49.48
	Mean	49.52	48.24	48.71	49.06	49.33	49.72	48.08	48.40	48.88
	SEM	1.124		1.124		1.589				0.794
	P Value	0.125		0.663		0.964				0.532
ADF (%)	Control	42.59	40.48	41.89	41.17	42.95	42.22	40.84	40.12	41.53
	Glycerol	39.57	38.57	36.52	41.63	37.78	41.36	35.26	41.89	39.07
	Jaggery	40.14	35.58	36.19	39.53	37.88	42.40	34.50	36.67	37.86
	Maize	41.18	36.01	36.59	40.59	39.95	42.40	33.24	38.79	38.59
	Mean	40.87	37.66	37.80	40.73	39.64	42.10	35.96	39.37	39.26
	SEM	1.924		1.924		2.720				1.360
	P Value	0.057		0.057		0.730				0.288

Parameter	Group	Energy × Breed		Energy × Suppl.		HFX × Suppl.		Deoni × Suppl.		E × B × S
		HFX	Deoni	Without	With	Without	With	Without	With	
HC (%)	Control	60.11	60.28	60.20	60.20	59.12	61.12	61.29	59.28	60.20
	Glycerol	61.81	61.42	64.89	58.34	66.64	57.000.	63.15	59.69	61.62
	Jaggery	57.69	65.08	61.93	60.84	57.32	58.04	66.53	63.64	61.38
	Maize	65.72	62.15	64.06	63.81	65.95	65.49	62.18	62.12	63.93
	Mean	61.33	62.23	62.77	60.80	62.26	60.41	63.28	61.18	61.78
	SEM	1.474		1.473		2.084		1.042		
	P Value	0.401		0.076		0.903		0.122		
Cellulose (%)	Control	53.94	52.20	54.39	51.75	54.93	52.95	53.85	50.55	53.07
	Glycerol	53.12	50.62	49.96	53.78	52.28	53.96	47.64	53.61	51.87
	Jaggery	50.75	49.37	49.70	50.42	50.18	51.32	49.22	49.52	50.06
	Maize	50.64	48.88	47.67	51.85	50.92	50.37	44.41	53.34	49.76
	Mean	52.11	50.26	50.43	51.95	52.07	52.15	48.78	51.75	51.18
	SEM	1.480		1.479		2.091		1.046		
	P Value	0.096		0.165		0.184		0.124		
NFC (%)	Control	81.05	81.54	80.93	81.67	80.94	81.17	80.92	82.17	81.30 ^a
	Glycerol	87.51	86.80	88.32	85.98	88.96	86.05	87.68	85.91	87.15 ^b
	Jaggery	84.81	86.17	87.20	83.78	85.94	83.68	88.46	83.88	85.49 ^b
	Maize	84.57	84.18	85.42	83.44	86.51	82.84	84.32	84.03	84.43 ^b
	Mean	84.57	84.67	85.47	83.71	85.59	83.43	85.34	84.00	84.59
	SEM	1.292		1.292		1.828		0.904		
	P Value	0.862		0.071		0.662		0.002^{**}		

Values with different superscripts for absolute interaction for a respective energy source in a row under each parameter differ significantly; **P ≤ 0.01

According to Hoover and Stokes (1991), the major factor controlling the energy available for the microbial growth is proportional to the presence of starches and soluble sugars besides their rate of fermentation. Paiva *et al.* (2016) also reported greater digestibility of CP when lactating cows fed 210 g/kg of glycerin due to increased digestibility of DM.

4.2.6 Nutritive value of the diet

The DCP% of <5% in the diets in this study was safer for prepartum cows because the maximum DCP% of the ration safer for dairy rations is $\leq 12\%$. Nutritive value of the diet is presented in **Table 4.23**. DCP% of the total diet between prepartum HFX and Deoni cows was comparable and no breed effect was observed on energy fortification. DCP% in FNS supplement group was lesser ($P < 0.001$) than the non supplemented group. The interaction effect of breed and supplement on DCP% of the diet was higher ($P < 0.01$) without FNS supplement in either HFX or Deoni cows.

Energy, breed and supplement interaction also had a positive effect on DCP% ($P < 0.01$) in the diet between TGs than CG. The NRC (2001) recommended 12% to 13% CP for prepartum cows. Dietary CP, EE and DM intake had no direct impact on the fecal loss of CP, whereas CF content, the proportion of CS and forage type influence the fecal CP but magnitude fluctuates. According to Doepel *et al.* (2002), increased CP intake in the prepartum period was not having any advantages to cow productivity instead, various caveats associated with high protein diets include fertility problems, increased ammonia excretion in urine, acid load on kidneys, metabolic diseases, increased BHBA, decreased 3-methylhistidine etc., (Pesta and Samuel, 2014). The TDN (%) of the diet was found comparable between energy interaction with breed or supplement and both together.

Nutritive ratio (NR) which is also called as albuminoid ratio was found statistically similar between breeds. NR in energy fortified with FNS supplemented group was significantly ($P < 0.01$) lesser than the non supplemented groups. Breed and FNS supplement interaction significantly ($P < 0.01$) higher in cows without FNS supplement than with FNS supplement. Energy, breed and

Table 4.23: Nutritive value of the diet in different prepartum groups

Parameter	Group	Energy × Breed		Energy × Suppl.		HFX × Suppl.		Deoni × Suppl.		E × B × S
		HFX	Deoni	Without	With	Without	With	Without	With	
DCP (%)	Control	4.33	4.17	4.14	4.36	3.99	4.69	4.29	4.04	4.25 ^a
	Glycerol	4.84	4.91	5.34	4.41	5.19	4.50	5.50	4.31	4.88 ^b
	Jaggery	4.94	4.94	5.36	4.53	5.22	4.68	5.50	4.38	4.95 ^b
	Maize	4.63	4.94	5.07	4.50	4.66	4.59	5.47	4.41	4.78 ^b
	Mean	4.68	4.74	4.98	4.45	4.76	4.62	5.19	4.29	4.71
	SEM	0.152		0.152		0.215				1.108
	P Value	0.662		0.001^{***}		0.003^{**}				0.001^{***}
TDN (%)	Control	54.06	53.00	53.61	53.46	53.95	54.28	53.36	52.64	53.53
	Glycerol	55.41	54.74	55.90	54.26	56.81	54.02	54.99	54.49	55.08
	Jaggery	53.86	54.50	54.76	53.60	53.62	54.10	55.89	53.11	54.18
	Maize	55.45	52.95	54.22	54.18	55.55	55.34	52.89	53.11	54.20
	Mean	54.69	53.80	54.62	53.87	54.96	54.43	54.28	53.32	54.24
	SEM	0.802		0.802		1.135				0.567
	P Value	0.132		0.208		0.701				0.326
NR	Control	11.55	11.79	11.98	11.37	12.52	10.58	11.43	12.16	11.67 ^b
	Glycerol	10.49	10.32	9.50	11.32	9.97	11.00	9.00	11.64	10.41 ^a
	Jaggery	9.97	10.15	9.24	10.88	9.31	10.63	9.16	11.13	10.06 ^a
	Maize	11.04	9.85	9.83	11.06	10.98	11.09	8.68	11.03	10.44 ^a
	Mean	10.76	10.53	10.13	11.6	10.70	10.82	9.57	11.49	10.64
	SEM	0.416		0.416		0.589				0.294
	P Value	0.445		0.003^{**}		0.008				0.007^{**}
RFQ	Control	74.22 ^A	80.92 ^B	79.06	76.08	76.77	71.67	81.35	80.50	77.57 ^a
	Glycerol	77.37 ^A	91.99 ^B	83.81	85.54	79.92	74.82	87.72	96.26	84.68 ^b
	Jaggery	74.26 ^A	80.82 ^B	77.38	77.70	74.19	74.33	80.57	81.07	77.54 ^a
	Maize	80.55 ^A	82.55 ^B	79.57	83.53	80.36	80.75	78.78	86.32	81.55 ^{ab}
	Mean	76.60	84.07	79.96	80.71	77.81	75.39	82.10	86.03	80.33
	SEM	2.948		2.948		4.169				2.084
	P Value	0.002^{**}		0.720		0.147				0.077

Values with different superscripts for absolute interaction for a respective energy source in a row under each parameter differ significantly; ***P< 0.001, **P< 0.01, *P< 0.05

Table 4.24: Nutrient intake (%) in prepartum cows in comparison to standard requirements of prepartum cows

Parameter	Group	Energy × Breed		Energy × Suppl.		HFX × Suppl.		Deoni × Suppl.		E × B × S
		HFX	Deoni	Without	With	Without	With	Without	With	
DM (%)	Control	74.03	86.42	84.22	76.23	81.99	66.07	86.45	86.38	80.22
	Glycerol	75.35	90.64	80.64	85.34	75.14	75.56	86.15	95.13	82.99
	Jaggery	75.18	75.62	76.03	74.77	74.46	75.89	77.60	73.64	85.40
	Maize	78.14	93.79	79.91	92.03	78.03	78.26	81.80	105.80	85.97
	Mean	75.67	86.62	80.20	82.09	77.40	73.94	83.00	90.24	81.15
	SEM	3.523		3.523		4.983		4.983		2.491
	P Value	0.001		0.459		0.048		0.048		0.409
DCP (%)	Control	97.28	90.92	94.28	93.91	98.09	96.47	90.48	91.35	94.10 ^a
	Glycerol	99.29	100.50	103.35	96.46	100.80	97.80	105.89	95.11	99.90 ^b
	Jaggery	101.72	98.82	103.47	97.08	105.23	97.75	101.23	96.42	100.27 ^b
	Maize	97.77	102.17	100.53	99.41	99.64	95.89	101.42	102.92	99.97 ^b
	Mean	99.01	98.01	100.40	96.71	101.60	96.97	99.76	96.45	98.56
	SEM	1.989		1.989		2.813		2.813		1.406
	P Value	0.53		0.02*		0.788		0.788		0.02**
ME (%)	Control	97.99	98.00	100.57	97.35	101.08	94.92	100.08	99.79	98.96 ^a
	Glycerol	112.36	11.82	110.12	114.05	110.01	114.70	110.23	113.40	112.08 ^b
	Jaggery	11.73	106.64	110.08	108.30	110.62	112.84	109.53	103.75	109.19 ^b
	Maize	112.00	111.48	111.80	111.69	113.97	110.04	109.63	113.33	111.74 ^b
	Mean	108.52	107.47	108.14	107.84	108.92	108.02	107.37	107.57	108.00
	SEM	1.439		1.439		2.035		2.035		1.018
	P Value	0.532		0.775		0.630		0.630		0.001***
RPI _{FSF} (%)	Control	-2.72	-9.08	-5.71	-6.09	-1.914	-3.53	-9.52	-8.65	-5.90 ^a
	Glycerol	-7.03	0.507	3.35	-3.54	0.79	-2.20	5.90	-4.89	-0.09 ^b
	Jaggery	1.72	-1.17	3.47	-2.92	5.70	-2.25	1.23	-3.59	0.27 ^b
	Maize	-2.23	2.17	0.53	-0.60	-0.36	-4.10	1.42	2.91	-0.03 ^b
	Mean	-0.98	-1.897	0.41	-3.29	1.06	-3.02	-0.24	-3.55	-1.43
	SEM	1.989		1.989		2.813		2.813		1.406
	P Value	0.53		0.02*		0.788		0.788		0.02*

Parameter	Group	Energy × Breed		Energy × Suppl.		HFX × Suppl.		Deoni × Suppl.		E × B × S
		HFX	Deoni	Without	With	Without	With	Without	With	
REI _{FSF} (%)	Control	-2.0	-0.07	0.57	-2.65	1.08	-5.08	0.07	-0.21	-1.04 ^a
	Glycerol	12.36	11.82	10.12	14.05	10.01	14.70	10.23	13.41	12.08 ^b
	Jaggery	11.73	6.64	10.08	8.30	10.62	12.84	9.53	3.75	9.91 ^b
	Maize	12.00	11.48	11.80	11.69	13.97	10.04	9.63	13.33	11.74 ^b
	Mean	8.52	7.46	8.14	7.84	8.92	8.12	7.37	7.57	7.99
	SEM	1.439	1.439	1.439	1.439	2.035	2.035	2.035	2.035	1.018
	P Value	0.32	0.32	0.78	0.78	0.630	0.630	0.630	0.630	0.001

Values with different superscripts for absolute interaction for a respective energy source in a row under each parameter differ significantly; ***P ≤ 0.001, **P ≤ 0.01, *P ≤ 0.05

supplement interaction was significantly higher ($P < 0.01$) in CG than TGs indicated higher energy available for unit of digested protein at tissue level for CP utilization. Energy and protein deprivation of dam may increase the serum cortisol and serum tri-iodothyronine consequently calves may bear with less metabolic BW and low chances of survival (Hough *et al.*, 1990). The concept of energy and protein ratio has great nutritional and economical importance and it is considered the rations with wider ratio (1:9) are suitable for cattle, medium ratio (1:6) suitable for early fattening and lactating cows (Reddy, 2001).

Relative feed quality (RFQ) index that uses feed digestibility to estimate intake, as well as the total digestible nutrients of the feed, were significantly higher ($P < 0.01$) in prepartum Deoni than HFX cows. It might be due to more intake of digestible DM as %BW (Jeranyama and Garcia, 2004). Interaction effect of energy, supplement or/and breed \times supplement was on RFQ was statistically comparable. Residual protein intake during prepartum was less than 1%, 2% respectively in HFX cows and Deoni cows. The ME requirement of HFX cows and Deoni cows was 8% higher than the ICAR(2013) recommendations (**Table 4.24**). All T1, T2, T3 group animals consumed 12%, 9% and 11.7%, respectively higher energy than recommended ME (ICAR, 2013).

4.2.7 Energy Value of the diet

The increased demand of energy for foetus and onset of lactation during the close-up period in prepartum cows obviously push the cows into NEB (Ingvertsen *et al.*, 2003, Kidd, 2003, Mor and Cardenas, 2010, Zeng *et al.*, 2017). Energy values of the prepartum diet are presented in **Table 4.25 and Table 4.26**. GE (Kcal/kg $W^{0.75}$) intake was higher ($P < 0.05$) in Deoni cows than HFX cows. FNS supplement effect was also higher ($P < 0.05$) in Deoni cows. Energy, breed and FNS supplement interaction effect was statistically significant ($P < 0.01$) in T1 than CG, T2 and T3. Although energy digestibility was more or less comparable across all the interaction effects, energy, breed and FNS supplement interaction effect on DE (Kcal/kg $W^{0.75}$) was significantly higher in T1 than CG, T2 and T3. A similar trend observed for the ME (Kcal/kg $W^{0.75}$) intake also. Although glycerol (T1) is not a carbohydrate, the ME was better in the polyol compound than di (T2) or polysaccharides (T3). The energy requirement and the ME intake indicated balance between the requirement and intake in prepartum HFX and Deoni cows.

Table 4.25: Energy value of the diet in different prepartum groups

Parameter	Group	Energy × Breed		Energy × Suppl.		HFX × Suppl.		Deoni × Suppl.		E × B × S
		HFX	Deoni	Without	With	Without	With	Without	With	
GE (Mcal)	Control	27.20	22.94	24.81	25.32	26.52	27.88	23.11	22.77	25.07 ^a
	Glycerol	30.53	24.82	26.16	29.18	27.96	33.10	24.37	25.27	27.67 ^b
	Jaggery	31.56	26.51	28.32	29.75	30.97	32.15	25.68	27.34	29.03 ^b
	Maize	31.50	24.85	27.99	28.36	31.31	31.68	24.67	25.03	28.17 ^b
	Mean	30.19	24.78	26.82	28.15	29.19	31.20	24.45	25.10	27.49
	SEM	1.183		1.183			1.164			0.837
	P Value	0.001^{***}		0.132			0.427			0.023[*]
DE (Mcal)	Control	17.25	14.30	15.55	16.00	16.66	17.84	14.45	14.15	15.77 ^a
	Glycerol	19.81	15.98	17.22	18.57	18.63	20.98	15.81	16.15	17.89 ^b
	Jaggery	20.02	17.01	18.25	18.78	19.57	20.48	16.93	17.08	18.51 ^b
	Maize	20.37	15.45	17.83	17.99	20.32	20.43	15.35	15.55	17.91 ^b
	Mean	19.36	15.68	17.21	17.83	18.79	19.93	15.63	15.73	17.52
	SEM	0.690		0.690			0.976			0.488
	P Value	0.001^{***}		0.222			0.302			0.006^{**}
ME (Mcal)	Control	13.95	11.58	12.59	12.94	13.49	14.40	11.70	11.47	12.76 ^a
	Glycerol	16.04	12.98	13.94	15.08	15.09	17.60	2.80	13.16	14.51 ^b
	Jaggery	16.19	13.77	14.75	15.21	15.81	16.58	13.69	13.84	14.98 ^b
	Maize	16.54	12.51	14.44	14.61	16.49	16.59	12.39	12.63	14.52 ^b
	Mean	15.68	12.71	13.93	14.46	15.22	16.14	12.64	12.77	14.19
	SEM	0.559		0.559			0.790			0.395
	P Value	0.001^{***}		0.200			0.331			0.006^{**}
Q Value	Control	0.52	0.50	0.51	0.51	0.51	0.52	0.51	0.50	0.51
	Glycerol	0.53	0.52	0.53	0.52	0.54	0.52	0.53	0.52	0.53
	Jaggery	0.51	0.52	0.52	0.51	0.51	0.52	0.53	0.53	0.52
	Maize	0.53	0.51	0.52	0.52	0.53	0.53	0.51	0.51	0.52
	Mean	0.52	0.51	0.52	0.51	0.52	0.52	0.51	0.51	0.52
	SEM	0.008		0.008			0.011			0.006
	P Value	0.146		0.291			0.668			0.289

Parameter	Group	Energy × Breed		Energy × Suppl.		HFX × Suppl.		Deoni × Suppl.		E × B × S
		HFX	Deoni	Without	With	Without	With	Without	With	
ER (Mcal)	Control	2.05	1.79	1.93	1.91	2.05	2.06	1.82	1.76	1.92 ^a
	Glycerol	2.85	2.63	2.62	2.86	2.81	2.89	2.43	2.84	2.74 ^b
	Jaggery	2.63	2.40	2.47	2.56	2.49	2.77	2.46	2.35	2.51 ^b
	Maize	3.17	2.07	2.52	2.72	3.17	3.18	1.88	2.26	2.62 ^b
	Mean	2.67	2.22	2.38	2.51	2.63	2.72	2.14	2.30	2.45
	SEM	0.176	0.176	0.176	0.176	0.249	0.249	0.249	0.249	0.125
P Value	0.002**	0.002**	0.323	0.323	0.824	0.824	0.824	0.824	0.001***	
HI (Mcal)	Control	11.90	9.79	10.66	11.03	11.45	12.35	9.88	9.71	10.84 ^a
	Glycerol	13.20	10.35	11.33	12.22	12.28	14.11	10.37	10.32	11.77 ^{ab}
	Jaggery	13.56	11.37	12.28	12.65	13.32	13.81	11.24	11.50	12.46 ^b
	Maize	13.36	10.44	11.92	11.89	13.32	13.41	10.51	10.37	11.90 ^{ab}
	Mean	13.00	10.48	11.54	11.94	12.59	13.42	10.50	10.47	11.74
	SEM	0.518	0.518	0.518	0.518	0.733	0.733	0.733	0.733	0.366
P Value	0.001***	0.001***	0.290	0.290	0.261	0.261	0.261	0.261	0.045*	
Energy Dig. (%)	Control	63.49	62.23	62.70	63.02	62.87	64.12	62.52	61.93	62.86
	Glycerol	65.06	64.39	65.79	63.66	66.72	63.40	64.87	63.91	64.72
	Jaggery	63.47	64.21	64.59	63.08	63.24	63.71	65.95	62.46	63.84
	Maize	64.81	62.17	63.53	63.45	64.84	64.79	62.23	62.12	63.49
	Mean	64.21	63.25	64.15	63.30	64.41	64.00	63.89	62.60	63.73
	SEM	0.918	0.918	0.918	0.918	1.298	1.298	1.298	1.298	0.649
P Value	0.159	0.159	0.209	0.209	0.509	0.509	0.509	0.509	0.269	
Energy Eff. (%)	Control	14.91	15.18	15.46	14.63	15.39	14.23	15.52	14.84	15.04 ^a
	Glycerol	17.82	20.25	18.82	19.26	18.64	17.00	19.00	21.51	19.04 ^b
	Jaggery	16.24	17.44	16.87	16.81	15.79	16.69	17.95	16.94	16.84 ^{ab}
	Maize	19.25	16.50	17.16	18.59	19.18	19.31	15.14	17.87	17.87 ^b
	Mean	17.05	17.34	17.07	17.32	17.25	16.86	16.90	17.79	17.20
	SEM	1.232	1.232	1.232	1.232	1.742	1.742	1.742	1.742	0.871
P Value	0.742	0.742	0.771	0.771	0.473	0.473	0.473	0.473	0.032*	

Values with different superscripts for absolute interaction for a respective energy source in a row under each parameter differ significantly; ***P< 0.001, **P< 0.01, *P< 0.05

Table 4.26: Energy value of the diet (kcal/kgW^{0.75}/d) in different prepartum groups

Parameter	Group	Energy × Breed		Energy × Suppl.		HFX × Suppl.		Deoni × Suppl.		E × B × S
		HFX	Deoni	Without	With	Without	With	Without	With	
GE (Kcal/kg MBW)	Control	283.71	295.76	293.59	285.88	290.47	276.96	296.72	294.81	289.74 ^a
	Glycerol	297.29	324.46	301.69	320.06	292.78	301.79	310.60	338.88	310.87 ^b
	Jaggery	296.76	300.43	293.94	303.25	296.11	297.41	291.78	309.09	298.60 ^{ab}
	Maize	309.42	307.42	302.84	313.99	308.88	309.96	296.80	318.03	308.42 ^{ab}
	Mean	296.79	307.02	298.02	305.79	297.06	296.53	298.97	315.06	301.90
	SEM	5.50		5.50		7.779		3.889		
	P Value	0.02*		0.063		0.048*		0.01**		
DE (Kcal/kg MBW)	Control	180.16	184.06	184.09	180.13	182.69	177.64	185.50	182.63	182.11 ^a
	Glycerol	193.38	208.90	198.50	203.79	195.43	191.34	201.57	216.24	201.14 ^b
	Jaggery	188.42	192.68	189.89	191.20	187.37	189.46	192.42	192.94	190.55 ^{ab}
	Maize	200.42	191.14	192.46	199.10	200.18	200.67	184.75	197.53	195.78 ^{ab}
	Mean	190.60	194.19	191.24	193.55	191.42	189.78	191.06	197.33	192.39
	SEM	4.682		4.682		6.221		3.311		
	P Value	0.29		0.494		0.28		0.01**		
ME (Kcal/kg MBW)	Control	145.74	149.05	149.09	145.70	148.04	143.45	150.15	147.96	147.40 ^a
	Glycerol	156.64	169.69	160.75	165.59	158.27	155.02	163.23	176.16	163.17 ^b
	Jaggery	152.34	156.00	153.47	154.87	151.33	153.35	155.60	156.39	154.17 ^{ab}
	Maize	162.73	154.71	155.79	161.64	162.50	162.96	146.69	160.32	158.72 ^{ab}
	Mean	154.36	157.36	154.77	156.95	155.03	153.69	154.52	160.21	155.86^b
	SEM	3.964		3.964		5.606		2.803		
	P Value	0.301		0.449		0.228		0.01**		
Q Value	Control	0.52	0.50	0.51	0.51	0.51	0.52	0.51	0.50	0.51
	Glycerol	0.53	0.52	0.53	0.52	0.54	0.52	0.53	0.52	0.53
	Jaggery	0.51	0.52	0.52	0.51	0.51	0.52	0.53	0.53	0.52
	Maize	0.53	0.51	0.52	0.52	0.53	0.53	0.51	0.51	0.52
	Mean	0.52	0.51	0.52	0.51	0.52	0.52	0.52	0.51	0.52
	SEM	0.008		0.008		0.011		0.006		
	P Value	0.146		0.291		0.668		0.289		

Parameter	Group	Energy × Breed		Energy × Suppl.		HFX × Suppl.		Deoni × Suppl.		E × B × S
		HFX	Deoni	Without	With	Without	With	Without	With	
ER (Kcal/kg MBW)	Control	21.92	22.82	23.15	21.59	23.00	20.85	23.30	22.34	22.37 ^a
	Glycerol	28.01	34.57	30.43	32.14	29.64	26.37	31.23	37.92	31.29 ^b
	Jaggery	24.85	27.21	26.02	26.04	24.11	25.59	27.92	26.50	26.03 ^{ab}
	Maize	31.32	25.65	26.91	30.06	31.17	31.48	22.65	28.65	28.48 ^b
	Mean	26.53	27.56	26.63	27.46	26.98	26.07	26.27	28.85	27.04
	SEM	2.497		2.497			3.532		1.766	
	P Value	0.566		0.643			0.339		0.02*	
HI (Kcal/kg MBW)	Control	123.82	126.23	125.94	124.11	125.04	122.60	126.84	125.62	125.02
	Glycerol	128.64	135.12	130.32	133.44	128.64	128.65	132.01	138.24	131.88
	Jaggery	127.49	128.79	127.45	125.83	127.22	127.76	127.68	129.89	128.14
	Maize	131.40	129.07	128.89	131.58	131.33	131.48	126.45	131.68	130.23
	Mean	127.84	129.80	128.15	129.49	128.06	127.62	128.24	131.36	128.81
	SEM	1.579		1.579			2.233		1.117	
	P Value	0.10		0.248			0.132		0.01	
Energy Dig. (%)	Control	63.49	62.23	62.70	63.02	62.87	64.12	62.52	61.93	62.86
	Glycerol	65.06	64.39	65.79	63.66	66.72	63.40	64.87	63.91	64.72
	Jaggery	63.47	64.21	64.59	63.08	63.24	63.71	65.95	62.46	63.84
	Maize	64.81	62.17	63.53	63.45	64.84	64.79	62.23	62.12	63.49
	Mean	64.21	63.25	64.15	63.30	64.41	64.00	63.89	62.60	63.73
	SEM	0.918		0.918			1.298		0.649	
	P Value	0.159		0.209			0.509		0.271	
Energy Eff. (%)	Control	14.91	15.18	15.46	14.63	15.39	14.23	15.52	14.84	15.04 ^a
	Glycerol	17.82	20.25	18.82	19.26	18.64	17.00	19.00	21.51	19.04 ^b
	Jaggery	16.24	17.44	16.87	16.81	15.79	16.69	17.95	16.94	16.84 ^{ab}
	Maize	19.25	16.50	17.16	18.59	19.18	19.31	15.14	17.87	17.87 ^b
	Mean	17.05	17.34	17.07	17.32	17.25	16.86	16.90	17.79	17.20
	SEM	1.232		1.232			1.742		0.871	
	P Value	0.742		0.771			0.473		0.032*	

ME was spent possibly in 3 ways viz. during rest, obligatory process (digestion and ingestion process) and on activity (Hall *et al.*, 2012) and ME intake was sufficient to balance the requirement. Q-value indicated comparable wastage of energy in faeces, urine and fermentation which was almost 50% due to the interaction of energy with the breed, HNS supplement or all the 3 factors together. A Q-value of 0.60 and above for the diet (ME/GE) indicate efficient utilization of energy. Higher nutrient intakes need not translate into better energy retention rather increase energy losses and 20% less birth weight of lambs (Srinivas and Sankhyan, 2010), and seems that excess N and energy intake disturbed the upper limit of nutrient intake by the foetus when the efficiency of ME use by the gravid uterus is hardly 14% (McNeil *et al.*, 1998).

HI in prepartum HFX cows was 19.4% higher than Deoni cows. Interaction effect of energy \times supplement, and breed \times supplement on HI was statistically comparable. Energy retention (ER) in prepartum HFX cows was 16.8% higher than Deoni cows. The energy efficiency of Deoni cows has been reported lesser than crossbred cows (Singh and Srinivas, 2016; Mohanavel and Srinivas, 2016, Sigh and Srinivas, 2020). HI was non-significantly higher in T2 than other TGs.

The interaction effect of energy, breed and supplement for ER in T1, T2 and T3 were significantly higher ($P < 0.001$) than CG. Energy efficiency in T1 and T3 was significantly ($P < 0.05$) higher than CG and T1. This could be attributed to diet composition, intake and digestibility of CP and NFC in terms of metabolically active tissue.

4.2.8 Microbial Protein Production

Measurement of urinary PD excretion is an indirect, non-invasive method for estimating microbial CP (MCP) supply to cattle fed different diets (Chen and Gomes, 1992). Excretion of PD in urine and their index is presented in **Table 4.27**. Urinary allantoin excretion was significantly ($P < 0.01$) higher by 14% in prepartum HFX than Deoni cows. MBP production is proportional to rumen size and higger in HFX cows (Srinivas and Krishnamoorthy, 2013, Singh and Srinivas, 2018, Srinivas *et al.*, 2018). This in turn leads to higher metabolism of MBP derivatives at small intestine in HFX cows. The major metabolite of PD; allantoin

Table 4.27: Excretion of total purine derivatives and their index in prepartum cows

Parameter	Group	Energy × Breed		Energy × Suppl.		HFX × Suppl.		Deoni × Suppl.		E × B × S
		HFX	Deoni	Without	With	Without	With	Without	With	
Allantoin (Mm/L)	Control	5.19	4.56	4.84	4.91	5.16	5.23	4.51	5.00	4.47
	Glycerol	4.83	4.25	4.53	4.55	4.69	4.98	4.38	4.13	4.54
	Jaggery	4.87	4.33	4.44	4.86	5.06	4.68	3.81	4.85	4.60
	Maize	5.00	4.34	4.48	4.86	5.23	4.76	3.72	4.95	4.67
	Mean	4.97	4.37	4.57	4.77	5.03	4.91	4.11	4.63	4.67
	SEM		0.286		0.286		0.405		0.202	
P Value		0.01**		0.335		0.128		0.190		
Creatinine (Mm/L)	Control	2.43	2.20	2.33	2.29	2.54	2.31	2.12	2.28	2.31
	Glycerol	2.30	1.82	2.33	1.79	2.69	1.91	1.96	1.67	2.06
	Jaggery	1.98	1.80	1.78	2.00	1.93	2.02	1.62	1.99	1.89
	Maize	1.80	1.75	1.94	1.61	2.02	1.57	1.85	1.64	1.77
	Mean	2.12	1.89	2.09	1.92	2.30	1.95	1.89	1.89	2.00
	SEM		0.230		0.230		0.325		0.162	
P Value		0.17		0.307		0.300		0.993		
Uric acid (Mm/L)	Control	0.39	0.34	0.37	0.35	0.46	0.31	0.29	0.39	0.36
	Glycerol	0.45	0.87	0.48	0.83	0.39	0.51	0.57	1.16	0.66
	Jaggery	0.24	0.87	0.85	0.25	0.21	0.26	1.49	0.25	0.55
	Maize	0.67	0.53	0.31	0.88	0.32	1.03	0.32	0.74	0.60
	Mean	0.43	0.65	0.51	0.58	0.34	0.52	0.67	0.63	0.54
	SEM		0.222		0.22		0.313		0.157	
P Value		0.190		0.649		0.505		0.237		
Total PD (Mm/L)	Control	5.58	4.89	5.21	5.26	5.62	5.53	4.80	4.98	5.23
	Glycerol	5.28	5.12	5.01	5.39	5.08	5.49	4.95	5.28	5.20
	Jaggery	5.10	5.20	5.29	5.01	5.28	4.93	5.30	5.10	5.15
	Maize	5.66	4.86	4.79	5.74	5.54	5.79	4.04	5.69	5.26
	Mean	5.40	5.17	5.08	5.35	5.38	5.44	4.77	5.26	5.21
	SEM		0.279		0.279		0.395		0.198	
P Value		0.066		0.185		0.287		0.563		

Values with different superscripts for absolute interaction for a respective energy source in a row under each parameter differ significantly; ***P< 0.001, **P< 0.01, *P< 0.05

in HFX cows was 91% and uric acid was 9% in contrast to 85% and 15% in Deoni cows. PD excretion was only significant due to breed difference but the interaction effect of energy and FNS supplement, or breed and FNS supplement, or energy, breed, and FNS supplement had no effect.

PD hydrolysis in the small intestine is allantoin 80-85% and rest is uric acid in cattle (Chen *et al.*, 1995). Although digestible DM or OM intake (g/kg $W^{0.75}$) was higher in the Deoni cows, the mean metabolic BW of HFX cows in the present experiment was 26% higher. In absolute terms, digestible DM or OM intake were higher in HFX cows than Deoni cows hence, it was the main driver for more urinary excretion of allantoin by HFX cows (Südekum *et al.*, 2006). The allantoin concentration in plasma or urine can also vary with the physiological condition of the animals (Matsumoto and Yonai, 1996). The PD excretion in the prepartum HFX or Deoni cows was higher than the earlier reports (Singh and Srinivas, 2016; Mohanavel and Srinivas, 2016) because nucleic acids may also originate from the whole body nucleic acid anabolism including fetal tissues during the last period of gestation apart from rumen microorganism,. Such a phenomenal increase in allantoin has been reported by Matsumoto and Yonai (1996) two weeks prior to parturition but sharp reduction thereafter.

Creatinine excretion is proportional to muscle mass and constant per metabolic BW but the season (Srinivas and Swain, 2011), breed and physiological stage (Chen and Ørskov, 2004) affect its excretion, but age has no relation (Whittet *et al.*, 2019). Singh and Srinivas (2016) reported excretion of 3.0 to 3.4 mM/L creatinine in lactating Deoni cows but in the present experiment, a maximum of 2 mM/L observed in prepartum cows. Creatinine excretion due to the interaction of energy with the breed, or FNS supplement, or breed and FNS supplement were not significant. Whittet *et al.* (2019) also reported no effect ($P = 0.42$) of gestation on urinary creatinine excretion or PD: creatinine ratio in crossbred heifers and cows.

PDC index was significantly different between breeds ($P < 0.001$) being higher in prepartum HFX than Deoni cows because of higher allantoin excretion and metabolic BW (**Table 4.28**).

PDC index was not significant at any other interaction levels of energy like in creatinine excretion. PD excretion rate was higher ($P < 0.001$) in prepartum HFX than Deoni cows and breed is one of the factors that affect PD excretion rate besides species, and glomerular filtration rate (Chen *et al.* 1995, Thanh and Ørskov, 2006). Higher urinary PD and duodenal flow of purine difference were observed in cattle than buffalo (Chen *et al.*, 1995) due to higher xanthine oxidase presence in the intestinal epithelium of buffalo than cattle. The difference in PD excretion within the species is also possible due to the physiological stage (Liang *et al.*, 1994; Matsumoto and Yonai, 1996; Srinivas and Krishnamoorthy, 2013).

Daily MBP production was found significantly ($P < 0.001$) higher by 35% in prepartum HFX than Deoni cows with energy fortification (**Fig 4.3**). Bach *et al.* (2005) demonstrated a quadratic relationship between the efficiency of MBP synthesis and N utilization. MBP production in ruminants is influenced by various biological, physical and chemical factors of animal, diet and environment in the rumen (Srinivas and Krishnamoorthy, 2013), but in the resent study we observed a significant effect of energy source on MBP production only because breed but not because the FNS supplement or their *inter se* interaction. Qualitative improvement of MBP production in the rumen has nutritional significance while quantitative improvement in MBP production has nutritional and health benefits, particularly B-complex vitamins production to relieve the stress (Singh and Srinivas, 2020). During the prepartum period which is characterized by the dramatic change in physiology and nutrient metabolism may alter the rumen fermentation characteristics thereby affect the feed efficiency of the cow (Wang *et al.*, 2001). MBP production in prepartum HFX cows was 350 g/d and prepartum Deoni cows 260 g/d compared 80g/d to 270g/d on straw-based diets with or without CS (Srinivas and Gupta, 1997). Even MBP production observed in Deoni cows was higher than earlier reports (Singh and Srinivas, 2016; Mohanavel and Srinivas, 2016). There might be a possibility of overestimation of MBP during the prepartum period due to increased PD excretion in the last 8 weeks of pregnancy (Matsumoto and Yonai, 1996).

Table 4.28: Microbial Nitrogen production and efficiency in prepartum cows

Parameter	Group	Energy × Breed		Energy × Suppl.		HFX × Suppl.		Deoni × Suppl.		E × B × S
		HFX	Deoni	Without	With	Without	With	Without	With	
PD index	Control	537.01	379.53	443.43	472.11	515.39	558.62	373.46	385.59	458.27
	Glycerol	543.96	392.16	437.91	498.21	486.98	600.94	388.84	395.47	468.06
	Jaggery	543.08	458.12	509.28	491.92	552.84	533.30	465.71	450.54	500.60
	Maize	571.22	391.17	448.40	513.99	561.37	581.07	335.43	446.92	481.20
	Mean	548.82	405.25	460.00	494.06	529.15	568.49	390.86	419.63	477.03
	SEM	30.057		30.057		42.507		21.254		
	P Value	0.001***		0.45		0.509		0.543		
PD excretion (Mm/d)	Control	470.64	333.99	390.26	414.38	451.62	489.66	328.89	339.10	402.32
	Glycerol	477.46	344.61	385.04	437.03	427.99	526.93	342.08	347.13	411.03
	Jaggery	477.27	402.39	447.06	432.59	485.33	469.20	408.79	395.98	439.83
	Maize	500.49	344.40	394.71	450.17	492.08	508.89	297.34	391.45	422.24
	Mean	481.46	356.34	404.27	433.54	464.26	498.67	344.28	368.42	418.90
	SEM	25.973		25.973		36.732		18.366		
	P Value	0.001***		0.46		0.524		0.523		
Duodenal flow of MBP (g/d)	Control	342.18	242.83	283.73	301.27	328.35	356.01	239.12	246.54	292.50
	Glycerol	347.13	250.54	279.94	317.74	311.17	383.10	248.71	252.38	298.84
	Jaggery	347.00	292.55	325.04	314.51	352.86	341.13	297.21	287.89	319.77
	Maize	363.88	250.39	286.97	327.30	357.77	369.99	216.18	284.60	307.13
	Mean	350.05	259.08	293.92	315.21	337.54	362.56	250.31	267.86	297.56
	SEM	18.884		18.884		26.706		13.353		
	P Value	0.001***		0.46		0.524		0.523		
MBP efficiency (g/kg of DOMI)	Control	86.86	74.66	79.40	82.12	86.23	87.49	72.57	76.75	80.76
	Glycerol	77.52	66.89	71.87	74.53	74.44	80.16	69.31	68.46	73.20
	Jaggery	76.32	77.79	78.43	73.69	79.38	73.27	77.47	74.10	76.05
	Maize	79.19	71.60	69.95	80.84	77.59	80.79	62.30	80.90	75.40
	Mean	79.97	72.73	74.91	77.79	79.41	80.54	70.42	75.05	76.35
	SEM	3.938		3.938		5.570		2.785		
	P Value	0.02*		0.53		0.304		0.308		
MBP Efficiency (g /Mcal of ME)	Control	24.44	21.02	22.35	23.11	24.25	24.64	20.46	21.58	22.73
	Glycerol	21.56	19.28	20.01	20.83	20.59	22.53	19.44	19.31	20.42
	Jaggery	21.45	21.27	22.02	20.70	22.31	20.58	21.72	20.81	21.36
	Maize	22.16	20.30	19.60	22.60	21.74	22.58	17.46	22.60	21.09
	Mean	22.40	20.40	21.00	21.81	22.22	22.58	19.77	21.03	21.40
	SEM	1.104		1.104		1.562		0.781		
	P Value	0.02*		0.54		0.317		0.242		

Values with different superscripts for absolute interaction for a respective energy source in a row under each parameter differ significantly; ***P≤ 0.001, *P≤ 0.05

The efficiency of MBP production was 80g/kg DOM which was higher than optimum suggested 30g/kg DOM in the rumen under continuous culture fermenter studies when dietary N usage by ruminal bacterial was 69% Bach *et al.* (2005). Optimum MBP production for kg DOM intake would be about 50g/kg.

Theoretical growth efficiency of mixed rumen microflora *in vivo* ranges from 1/3 to 2/3 of the theoretical maximum of 34 to 66 g microbial DM for a mole of ATP calculated from biochemical pathways (Hackmann and Firkins, 2015). The MBP production efficiency in the present study was 12 g/Mcal of GE or 22 g/Mcal of ME. A mole of ATP gives 7.3 Kcal thus, 16 g of MBP was produced per a mole of ATP. ATP requirement for protein synthesis was 36.5 mmol/g while RNA and DNA synthesis requires 14.6 and 18 mmol/g. Glycogen and lipid synthesis require 12.4 and 1.5 mmol/g of cellular macromolecules (Hackmann and Firkins, 2015). MBP production efficiency as digestible OM or ME was much higher than the reported values that may due to an overestimation of MBP production in prepartum cows during last 8 weeks of pregnancy because of more allantoin in plasma and urine (Matsumoto and Yonai,1996). Singh and Srinivas (2016) reported 50% efficiency based on grain-based diets in Deoni cows. Mohanavel and Srinivas (2016) reported 30-39% on oil seed-based CS. The interaction effect of energy and FNS supplement (**Fig 4.5 B**) or Breed and FNS supplement (**Fig 4.6 B**) on the efficiency of MBP production was comparable. The efficiency of MBP production g/kg DOMI was non-significantly higher by 10%, 4% and 3% in CG, T2 and T3 supplemented group, respectively in comparison with T1.

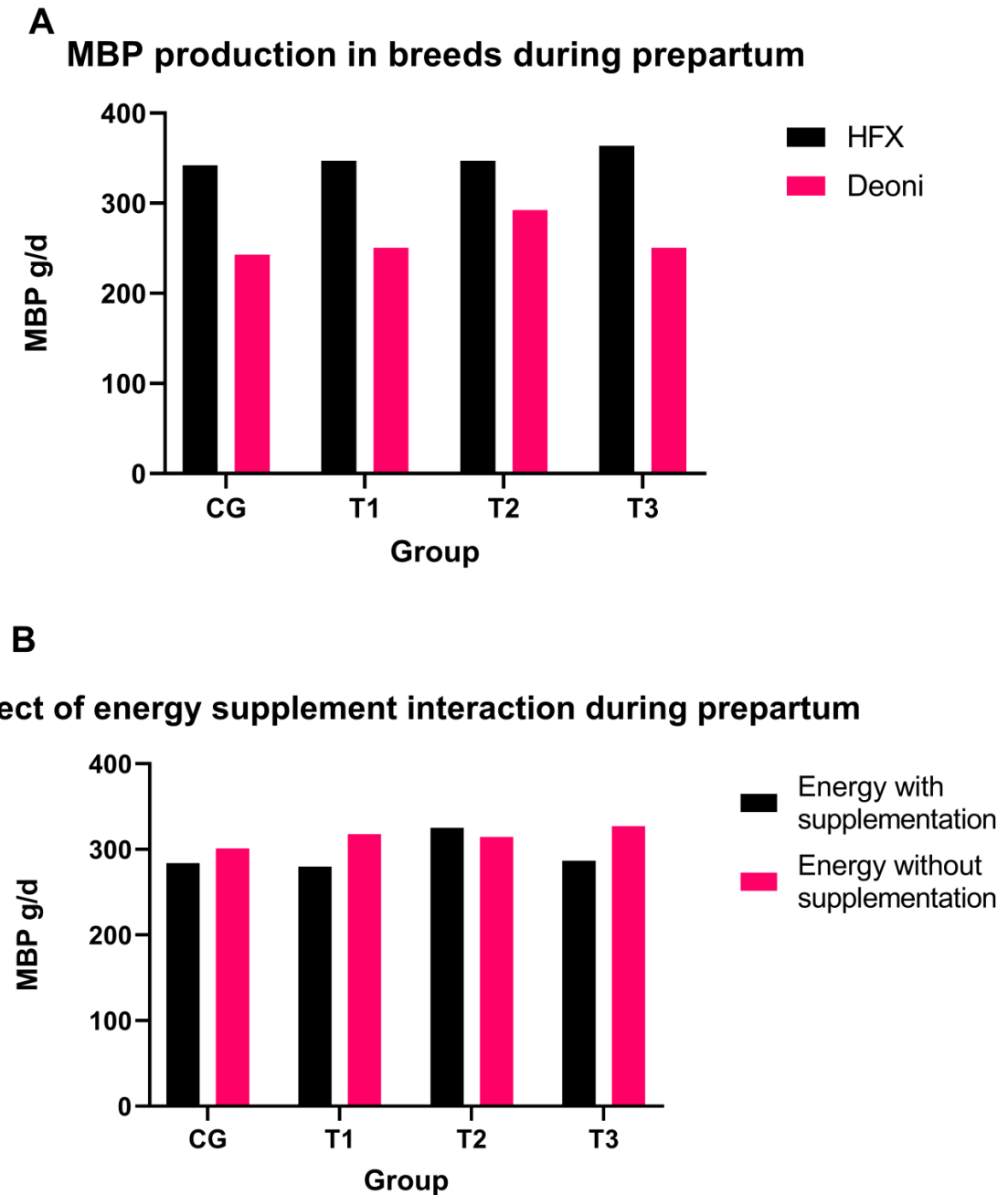


Fig. 4.3a: MBP production during prepartum period (A) MBP production in breeds during prepartum period (B) Effect of energy and supplement interaction during prepartum period

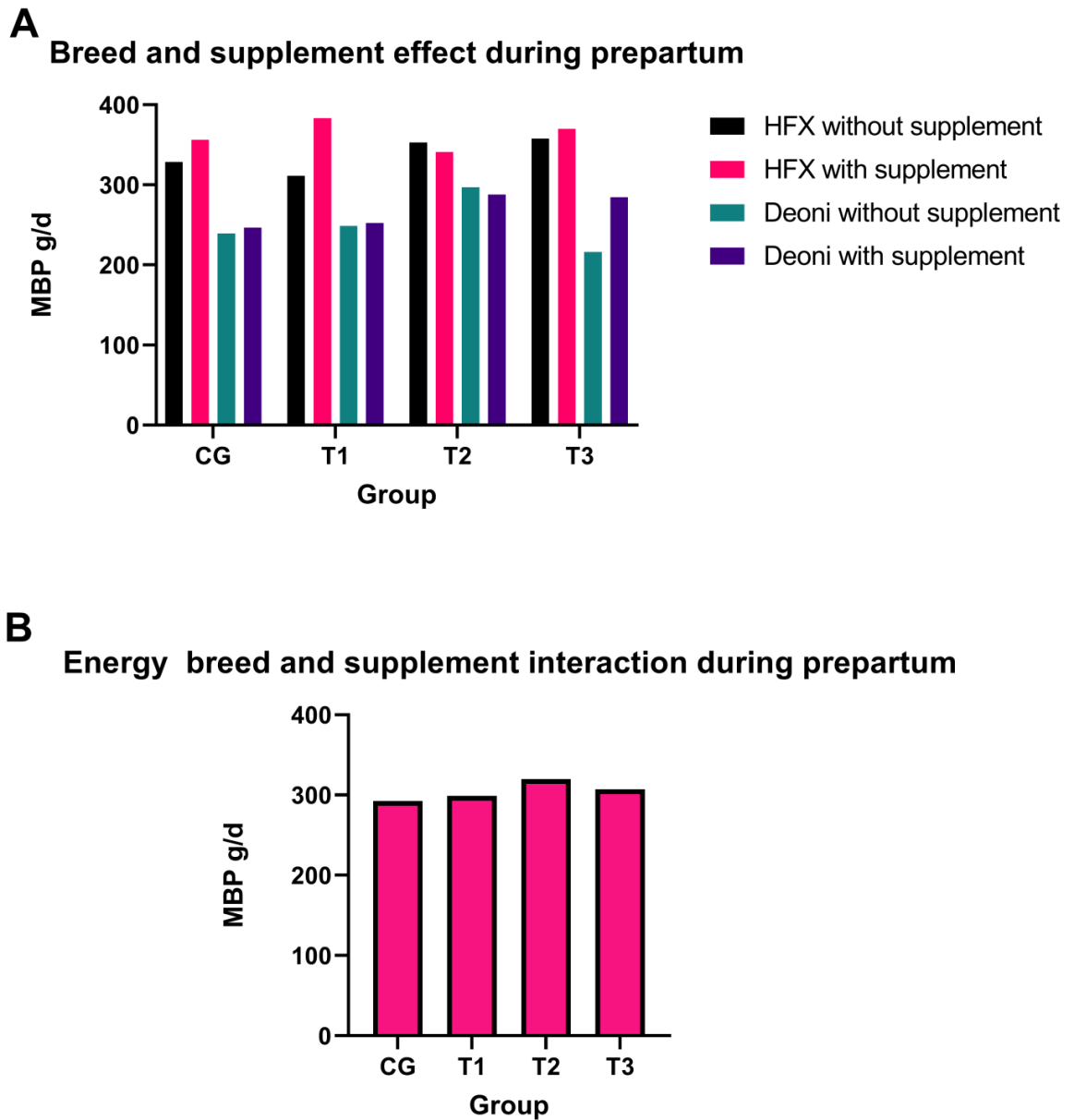


Fig. 4.3b: MBP production in with B×FNS and B×E×FNS during prepartum period (A) Breed and supplement interaction in different groups (B) Energy breed and supplement interaction in different groups of prepartum cows

4.3 Effect of prepartum nutrition on parturition

4.3.1 Prepartum nutrition of the dam on colostrum quality

Colostrum quality was measured by using colostrometer is presented in **Table 4.29**. Colostrum quality was measured based on the specific gravity. According to the colostrometer colour scale (**Section 3.9**), the mean Ig concentration on the day of calving or day zero in HFX and Deoni cows were > 50 g/L whereas, the threshold level on day 1 was 50 g/L Ig concentration. The digestibility of lactose and fat has been reported to be >95% in comparison to protein digestibility on average 92% till 2 d of age (Kertz *et al.*, 2017). The mean HFX cows Ig concentration were significantly ($P < 0.001$) higher than Deoni cow on day zero (**Table 4.30**). The colostrum quality is variable between individuals and environmental reasons those include parity, prepartum diet, season, breed, dry days, delayed colostrum collection, any specific drug or vaccine executed prior to parturition etc., (McGrath *et al.*, 2016; Silva-del-Rio *et al.*, 2017). The Ig values observed in either HFX or Deoni cows on day zero are in agreement to Vaz *et al.* (2004) who reported total Ig levels were > 50 mg/dL in HF cows. The levels of Ig at zero hours of birth and 6 h after birth are 13 and 8 g% out of 17 and 12% protein present in colostrum, respectively but, reduce to 3% out of 6% protein present after 12 h of calf birth (Puppel *et al.*, 2019). The quality of colostrum on day 4 was only $\frac{1}{3}$ of it was on day zero. The quality of colostrum almost decreased by 50% on day one it-self due to decrease in Ig concentration to a threshold level on day 5 to 0.6% of the 3.6% milk protein (McGrath *et al.*, 2016; Puppel *et al.*, 2019).

Interactive effect of prepartum FNS supplement shown a significant influence on the colostrum quality on the day of calving only by 3%. The results were in support of the Aragona *et al.* (2016) who reported 18% improvement in IgG concentration with 48 g/d nicotinic acid supplement during the prepartum period. Although FNS supplemented in the present study contained niacin, it was only 12 g. We opine that increasing niacin supplementation may helpful inactivation of G protein-coupled receptor 109A to have an additive effect on adiponectin secretion as well as augmenting glucose and fat metabolism those

Table 4.29: Colostrum quality in different groups of experimental cows

Parameter	Group	Energy × Breed		Energy × Supplement		E×B×S
		HF	Deoni	without	with	
Colostrum density on 0 day	Control	1074	1071	1072	1073	1072
	Glycerol	1075	1071	1072	1074	1073
	Jaggery	1074	1072	1072	1074	1073
	Maize	1074	1071	1072	1075	1073
	Mean	1076	1071	1072	1074	1073
	SEM	0.196		0.201		0.173
P Value	0.106		0.951		0.436	
Colostrum density on 1st day	Control	1047	1047	1046	1048	1047
	Glycerol	1051	1043	1048	1045	1047
	Jaggery	1047	1044	1045	1046	1045
	Maize	1044	1046	1046	1045	1045
	Mean	1047	1045	1046	1046	1046
	SEM	0.101		1.109		0.108
P Value	0.235		0.376		0.087	
Colostrum density on 2nd day	Control	1037	1036	1036	1037	1036
	Glycerol	1036	1037	1037	1036	1036
	Jaggery	1037	1036	1036	1036	1036
	Maize	1036	1037	1037	1036	1036
	Mean	1036	1036	1036	1036	1036
	SEM	0.133		0.141		0.143
P Value	0.111		0.063		0.083	
Colostrum density on 3rd day	Control	1036	1035	1036	1035	1035
	Glycerol	1036	1035	1035	1036	1035
	Jaggery	1036	1035	1036	1035	1035
	Maize	1035	1035	1035	1035	1035
	Mean	1035	1035	1035	1035	1035
	SEM	0.097		0.131		0.111
P Value	0.801		0.780		0.141	
Colostrum density on 4th day	Control	1035	1033	1034	1034	1034
	Glycerol	1034	1034	1034	1035	1034
	Jaggery	1034	1033	1032	1035	1034
	Maize	1034	1034	1033	1035	1034
	Mean	1034	1034	1033	1035	1034
	SEM	0.104		0.101		0.719
P Value	0.081		0.368		0.111	

*Ex: 1074 Delaval colostrometer reading is equivalent to specific gravity of 1.074

are necessary to improve IgG secretion at the time of colostrogenesis (Aragona *et al.*, 2016).

Interactive effect of prepartum diet energy, breed and FNS supplement interaction effect was also found non-significant on the colostrum quality. According to Westland *et al.* (2017), feeding mannan oligosaccharides in addition to the diet may increase colostrum yield (5.6 kg to 7.5 kg) but not IgG concentration (43 g to 54 g/L with SEM 5 g/L). In the present study, we did not quantify the colostrum yield as it was directly fed to calf from the dam. Further, it also appeared that either protein or energy fed to cows in the prepartum period has little to influence Ig quality of colostrum (Hough *et al.*, 1990). Any insignificant improvement in IgG concentration, particularly on day zero, due to additional energy or FNS supplementation in HFX or Deoni cows could be attributed to improved immunity of the cows.

4.3.2 Effect of prepartum nutrition of dam on calf birth weight

Nutrition management of the pregnant cows, particularly in last trimester not only has consequences for the productivity of the individual but also productivity of the next generation of the cow (Prior and Laster, 1979). The birth weight and metabolic BW of calves born to HFX and Deoni cows are presented in **Table 4.28**. Birth weight of HFX calves was 10 kg or 49% higher ($P < 0.001$) than the Deoni calves. Birth weight of crossbred calves compared to indigenous calves is generally higher by 8 to 12 kg (Singh and Srinivas, 2020). Effect of lower birth weight of calves in Deoni breed can be seen till 2nd lactation. Hence, the growth of the calves for sexual maturity, as well as their lifetime performance, is influenced by the birth weight and nutrition in the form of colostrum and milk (Singh and Srinivas, 2020). The metabolic BW of the HFX calves was 26% higher than the Deoni calves and same difference existed between the metabolic BW of their dams which was 20% (**Table 4.6**) hence, the calf birth weight is directly proportional to dam's BW (Paputungan and Makarechian, 2000).

Energy fortification not only showed a significant difference between the breeds but FNS supplementation was also had a significant impact on the calf birth weight in either HFX or Deoni dams.

Table 4.30: Colostrum IgG concentration (mg/ml) in different groups of experimental cows

IgG (mg/ml)	Group	Energy × Breed		Energy × Suppl.		HFX × Suppl.		Deoni × Suppl.		E × B × S
		HFX	Deoni	Without	With	Without	With	Without	With	
0 th day	Control	59.89	56.54	57.74	58.70	58.93	60.85	56.54	56.54	58.22
	Glycerol	61.09	57.02	57.98	60.14	59.42	62.77	56.54	57.50	59.06
	Jaggery	60.13	57.50	58.22	59.42	59.42	60.85	57.02	57.98	58.82
	Maize	61.33	56.54	57.50	60.38	59.89	62.77	55.10	57.98	58.94
	Mean	60.61	56.90	57.86	59.66	59.41	61.81	56.30	57.50	58.76
	SEM		0.464		0.464		0.656		0.328	
P Value		0.001***		0.001***		0.087		0.310		
1 st day	Control	34.03	33.79	33.31	34.50	33.07	34.98	33.55	34.03	33.91
	Glycerol	37.38	29.72	34.74	32.35	39.77	34.99	29.72	29.72	33.55
	Jaggery	33.55	31.15	32.11	32.59	32.59	34.51	31.63	30.68	32.35
	Maize	31.39	32.83	32.59	31.63	29.72	33.07	35.46	30.19	32.11
	Mean	34.09	31.87	33.19	32.77	33.79	34.39	32.59	31.15	32.98
	SEM		1.834		1.834		2.593		1.297	
P Value		0.107		0.751		0.444		0.712		
2 nd day	Control	23.73	23.73	23.49	24.21	23.49	24.45	23.49	23.97	23.85
	Glycerol	23.97	23.97	23.97	23.73	23.97	23.49	23.97	23.97	23.85
	Jaggery	23.49	23.73	23.73	23.73	23.97	23.97	23.49	23.49	23.73
	Maize	23.97	23.97	23.97	23.73	23.97	23.97	24.45	23.49	23.85
	Mean	23.79	23.79	23.79	23.73	23.97	23.97	23.85	23.73	23.82
	SEM		0.224		0.224		0.159		0.159	
P Value		0.710		0.710		0.933		0.933		
3 rd day	Control	23.01	22.77	23.01	22.77	23.49	22.53	22.53	23.01	22.89
	Glycerol	23.25	22.53	22.77	23.01	23.01	23.49	22.53	22.53	22.89
	Jaggery	22.53	22.53	22.53	22.53	22.53	22.53	22.53	22.53	22.53
	Maize	22.29	23.01	22.29	22.53	22.05	22.53	22.53	22.53	22.41
	Mean	22.77	22.59	22.65	22.71	22.77	22.77	22.53	22.65	22.68
	SEM		0.281		0.281		0.398		0.199	
P Value		0.379		0.379		0.767		0.801		
4 th day	Control	22.53	20.86	21.81	21.57	22.53	22.53	21.10	20.62	21.69
	Glycerol	21.81	21.81	21.09	22.53	21.09	22.53	20.62	22.53	21.81
	Jaggery	21.57	20.86	19.90	22.53	20.62	22.53	19.18	22.53	21.21
	Maize	21.33	21.81	20.62	22.53	20.14	22.53	21.10	22.53	21.57
	Mean	21.81	21.33	20.86	22.29	21.09	22.53	20.62	22.53	21.57
	SEM		0.573		0.573		0.811		0.405	
P Value		0.256		0.303		0.998		0.751		

*Values with different superscripts for absolute interaction for a respective energy source in a row under each parameter differ significantly; ***P ≤ 0.001

**IgG concentration in colostrum (mg/ml) = (958 × specific gravity of colostrum) - 969 (Mechor *et al.*, 1992)

Table 4.31: Calf birth weight in different groups of animals

Parameter	Group	Energy × Breed		Energy × Suppl.		HFX × Suppl.		Deoni × Suppl.		E × B × S
		HFX	Deoni	Without	With	Without	With	Without	With	
Calf birth weight (kg)	Control	27.95	19.93	23.60	24.28	26.15	29.75	21.05	18.80	23.94
	Glycerol	32.19	19.48	23.75	27.91	29.25	35.13	18.25	20.70	25.83
	Jaggery	29.48	21.37	24.74	26.11	28.69	30.28	20.79	21.95	25.43
	Maize	31.03	20.38	24.54	26.86	29.93	32.13	19.16	21.60	25.70
	Mean	30.16	20.29	24.16	26.29	28.50	31.82	19.81	20.76	25.22
	SEM	0.685	0.685	0.685	0.685	0.485	0.485	0.485	0.485	
	P Value	0.001***	0.001***	0.001***	0.001***	0.027*	0.027*	0.027*	0.049*	
Calf metabolic body weight (kg)	Control	12.15	9.43	10.69	10.88	11.56	12.73	9.83	9.03	10.79
	Glycerol	13.50	9.27	10.71	12.07	12.58	14.43	8.83	9.70	11.38
	Jaggery	12.67	9.94	11.07	11.52	12.40	12.91	9.74	10.14	11.29
	Maize	13.14	9.59	10.98	11.75	12.80	13.50	9.16	10.01	11.37
	Mean	12.86	9.57	10.86	11.56	12.33	13.39	9.39	9.72	11.21
	SEM	0.227	0.227	0.227	0.227	0.161	0.161	0.161	0.161	
	P Value	0.001***	0.001***	0.001***	0.001***	0.038*	0.038*	0.038*	0.056	

Values with different superscripts for absolute interaction for a respective energy source in a row under each parameter differ significantly; ***P≤ 0.001, *P≤ 0.05

The interactive effect of FNS supplementation in diets fed to prepartum cows resulted in 8% more birth weight of calves ($P < 0.001$) however, this interactive effect was greater on calf birth weight in HFX than Deoni dams ($P < 0.027$). The calf birth weight was 12% more for those born to HFX cows and only 5% more in those born to Deoni cows with FNS supplementation compared to without supplementation.

Apart from the genetic difference in the BW of the breeds, the limit on the calf birth weight between breeds with energy and FNS supplement was also observed. Although the overall impact of FNS supplement to energy was 2 kg difference in calf birth weight, it was 2 kg in Deoni calves and 3 kg in HFX calves. Hence, the FNS supplementation helped not only increase in the calf birth weight but also supported to maximize the genetic potential to express calf birth weight. These result indicated the interactive effect of energy and FNS supplement during prepartum was positive on the calf birth weight and breed genetic potential. Khan *et al.* (2004) reported 2.8 kg higher birth weight in cows fed 30% more than recommended energy for last trimester of gestation while Loerch (1996) reported 4.2 kg heavier birth weight when cows fed high grain diet during late gestation in comparison with isocaloric hay-based diets. In the absence of 10% additional energy fortification in the CG, the calf birth weight due to FNS supplement was 2.25 kg or 11% lesser than without FNS supplement ($P < 0.001$). Effect of FNS supplement without energy fortification (CG) on calf birth weight was appeared negative to none that was contrary to TGs where 11% and 10.4% more calf birth weight observed in HFX and Deoni calves, respectively.

Energy, breed and FNS supplement interaction had a significant impact ($P < 0.05$) on the calf birth weight by 1.7 kg or 7% more than CG. Generally, the efficiency of energy metabolism in the pregnancy is only 20% thus, 10% fortification of energy along with FNS supplement in TGs yielded better result on calf birth weight.

4.3.3 Prepartum nutrition effect on dam components

The significant difference in the calf birth weight was over ruled to any difference to parity because all the cows were within 3rd to 5th parity (**Table 4.32**). The retained placental membrane is one of the most common disorders which have negative effects on subsequent reproduction as well as production in cows after parturition and its retention for more than 12 hours post-calving is considered abnormal. The time taken for foetal membrane expulsion process with uterine muscular contractions in Deoni cows was 38 min lesser than HFX cows ($P < 0.001$). Such a significant difference in foetal membrane expulsion between HFX and Deoni cows would also facilitate early involution of the uterus (McDowell, 1989). Deoni cows were aggressive and restless at least 2 hours prior to until 24 hours after parturition. This behaviour noticed only in Deoni cows but not in HFX cows. HFX cows were docile and approachable by caretakers.

The energy with FNS supplementation hastened the expulsion of foetal membranes with probability levels of 8% ($P = 0.08$). Such affect could be due to the inclusion of Se in FNS supplement at the rate of 0.3 ppm. Selenium supplement has been reported to increase myometrial contractility and help in early expulsion of the placenta (Allison and Laven, 2000).

Energy breed and FNS supplement interaction effect on the time taken to expel the fetal membranes was significant at 10% probability ($P = 0.099$) because Se supplementation at the rate of 0.3 ppm reported to elevate the plasma antioxidants, reduce the oxidative stress and its immune suppressive consequences (Weiss and Gonzalo, 2006). Although there was breed differences observed in foetal membrane expulsion in CG and TGs due to energy fortification, the breed interaction with FNS supplementation effect was insignificant due to difference of only 5 to 10 min. Since all the cows were between 3rd and 5th parity, the incidence of retention of foetal membranes was none. Usually, the incidence of retention of the foetal membrane increases linearly with parity. Sarder (2008) reported 9, 20 and 29% chances of retention of the foetal membranes in 3rd, 4th, 5th and beyond 6th parity, respectively. The significant difference in milk yield on day 5th after calving was observed between HFX and Deoni cows.

Table 4.32: Dam observations in different groups of animals

Parameter	Group	Energy × Breed		Energy × Suppl.		HFX × Suppl.		Deoni × Suppl.		E × B × S
		HFX	Deoni	Without	With	Without	With	Without	With	
Parity	Control	3.00	3.75	3.75	3.00	3.00	3.00	4.50	3.00	3.38
	Glycerol	3.75	3.50	4.25	3.00	4.00	3.50	4.50	2.50	3.63
	Jaggery	3.75	5.50	4.25	5.00	3.00	4.50	5.50	5.50	4.63
	Maize	3.75	3.75	4.00	3.50	4.00	3.50	4.00	3.50	3.75
	Mean	3.56	4.13	4.06	3.63	3.50	3.63	4.63	3.63	3.84
	SEM	0.834		0.834		1.179				0.590
	P value	0.354		0.469		0.354				0.487
Fetal membrane expulsion time (min)	Control	206	175	194	188	218	195	170	180	190
	Glycerol	211	154	189	176	213	210	165	143	183
	Jaggery	194	159	179	174	200	188	158	160	177
	Maize	191	163	181	173	195	188	168	158	177
	Mean	201	163	186	178	206	195	165	160	181
	SEM	2.997		2.997		4.239				4.239
	P value	0.001***		0.08		0.472				0.099
Milk yield on 5th day after calving (kg/d)	Control	8.20	3.18	5.18	6.20	6.25	10.15	4.10	2.25	5.69
	Glycerol	9.83	2.50	5.95	6.38	9.15	10.50	2.75	2.25	6.16
	Jaggery	11.13	2.50	6.40	7.23	11.00	11.25	1.80	3.20	6.81
	Maize	10.50	2.40	7.53	5.38	13.25	7.75	1.80	3.0	6.45
	Mean	9.91	2.64	6.26	6.29	9.91	9.91	2.61	2.68	6.27
	SEM	1.406		1.406		1.988				0.994
	P value	0.001***		0.975		0.975				0.875

Values with different superscripts for absolute interaction for a respective energy source in a row under each parameter differ significantly; ***P ≤ 0.001

HFX cows produced 3.75 times ($P < 0.001$) more milk than Deoni cows because genetic potential for milk production in crossbred cows was more than indigenous cows. The milk production potential of HFX cows is unparalleled to indigenous cows (Sreenath and Srinivas, 2011; Mohanavel and Srinivas, 2016). Hence, milk yield can't be an yardstick for understanding any interactive effect of nutrition received by cows in different groups during prepartum on the health and production in postpartum. Milk yield on day 5th was least influenced ($P = 0.98$) by the FNS supplement cows received in the prepartum. The milk production in HFX cows or Deoni cows on day 5th with or without FNS supplement was more or less comparable. Gillah *et al.* (2014) observed increased milk yield but did not affect milk composition, while Warken *et al.* (2018) reported improved immune response and minimized the oxidative stress in cows during the lactation. Apart from these quantitative observations, we noticed that all the dams and calves were healthy until day 5th of parturition and no metabolic diseases like milk fever, calf scores or calf mortality were observed.

4.3.4 Nutrient intake in relation to milk production in postpartum

Calf hood, growth and maturity are vertical developmental process but, pregnancy, prepartum and postpartum are cyclical physiological stages repeatedly happens in the lifetime of a cow. Some of the issues when cows physiological state changes from prepartum to postpartum or close-up to the fresh cow are the NEB, reduced DMI and nutrient imbalance, poor nutrition, immunity, metabolic disorder etc. The target of prepartum nutrition provided in the experiment was sustaining the milk production potential and nutrient balance in postpartum.

Cow's milk production at weekly intervals is presented in **Table 4.33**. Mean milk yield during in the first week in HFX cows was 103 kg/week while Deoni cows were 27 kg/wk that accounts to about 15 kg and 4 kg/d only. Milk yield of HFX cows was 3 to 4 times higher ($P < 0.001$) than Deoni cows. The milk yield in HFX cows varied between 114 to 118 kg/week or 16 to 17 kg/d till 8th week whereas in Deoni cows it was 30 to 32 kg/week or 4.2 to 4.7 kg/d.

Interactive effect of prepartum energy in the diet on postpartum milk production was found non-significant till 8 weeks, however, numerically a consistent improvement in the milk yield was observed in cows those fed FNS supplement along with diets including CG until 3rd week (**Fig 4.6**). Milk production in HFX cows those received FNS supplement in their prepartum diets was 5 to 7% (P=0.64) higher in the 1st two weeks but, became comparable (P=0.95) from the 3rd week of lactation with milk yield of those cows without FNS supplement in prepartum (**Fig 4.7**). Probably, the follow-up effect of prepartum FNS supplement on the postpartum performance of cows was observed until 3rd week from the calving. In Deoni cows, feeding prepartum FNS supplementation did not show any significant difference in milk production in comparison to those cows without FNS supplement in prepartum. Energy, breed and supplement interaction effect of prepartum diets was none on the postpartum milk production in CG and TGs. In the 1st week of postpartum, T3 cows produced non significantly higher milk than CG and other TGs. The impact of prepartum dietary factors on postpartum milk production was null. DeFrain *et al.* (2004) also reported that feeding glycerol 2 week prior to 3 week after calving had no significant effect on milk yield. Osborne *et al.* (2009) also suggested that the glycerol supplementation 800 g/day had no significant effect on milk production. DM, CP, and ME consumption in postpartum was statistically comparable in cows those received FNS supplement or without FNS supplement along with energy in their diet during prepartum.

Table 4.33: Total milk yield (kg/week/cow) of different groups till 8th week of postpartum

Week	Group	Energy × Breed		Energy × Suppl.		HFX × Suppl.		Deoni × Suppl.		E × B × S
		HFX	Deoni	Without	With	Without	With	Without	With	
I	Control	15.19	3.70	8.43	10.47	12.34	18.04	4.51	2.90	9.45
	Glycerol	14.38	3.34	8.58	9.14	13.65	15.11	3.51	3.16	8.86
	Jaggery	14.82	3.66	9.04	9.45	14.64	15.00	3.43	3.89	9.24
	Maize	14.71	4.46	9.85	9.33	16.39	13.04	3.31	5.61	9.59
	Mean	14.78	3.79	8.97	9.59	14.26	15.30	3.69	3.89	9.28
	SEM	1.252		1.252		1.771		0.885		
P Value	0.001***		0.495		0.633		0.946			
II	Control	18.29	4.14	10.26	12.17	15.47	21.11	5.04	3.23	11.21
	Glycerol	17.50	4.06	10.32	11.23	16.63	18.36	4.01	4.10	10.78
	Jaggery	15.96	4.61	10.67	9.90	16.43	15.50	4.91	4.31	10.29
	Maize	15.91	4.20	10.91	9.20	17.61	14.21	4.21	4.19	10.06
	Mean	16.92	4.25	10.54	10.63	16.53	17.30	4.55	3.96	10.58
	SEM	1.208		1.208		1.709		0.854		
P Value	0.001***		0.914		0.437		0.770			
III	Control	18.48	4.17	10.07	12.58	15.36	21.59	4.77	3.57	11.33
	Glycerol	16.66	4.15	9.91	10.89	15.96	15.96	3.86	4.43	10.44
	Jaggery	16.32	4.30	10.62	10.00	17.32	17.32	3.91	4.68	10.31
	Maize	16.30	4.43	11.55	9.20	18.96	18.96	4.14	4.75	10.37
	Mean	16.94	4.26	10.54	10.67	16.90	16.90	4.17	4.36	10.60
	SEM	1.247		1.247		1.763		0.882		
P Value	0.001***		0.900		0.955		0.834			
IV	Control	16.82	4.52	10.35	10.99	15.71	17.93	4.98	4.06	10.67
	Glycerol	15.76	4.05	9.52	10.29	15.14	16.38	3.90	4.19	9.90
	Jaggery	16.38	4.51	10.92	9.98	17.68	15.09	4.16	4.87	10.45
	Maize	16.38	4.53	11.80	9.10	19.32	13.44	4.29	4.76	10.45
	Mean	16.34	4.40	10.65	10.09	16.96	15.71	4.33	4.47	10.37
	SEM	0.940		0.940		1.329		0.664		
P Value	0.001***		0.400		0.316		0.868			

Week	Group	Energy × Breed		Energy × Suppl.		HFX × Suppl.		Deoni × Suppl.		E × B × S
		HFX	Deoni	Without	With	Without	With	Without	With	
V	Control	18.06	4.60	10.14	12.51	15.37	20.76	4.94	4.26	11.33
	Glycerol	15.77	4.07	9.24	10.60	14.89	16.64	3.58	4.56	9.92
	Jaggery	16.16	4.61	11.08	9.70	17.46	14.86	4.69	4.54	10.39
	Maize	15.77	4.65	11.56	8.86	18.93	12.61	4.19	5.11	10.21
	Mean	16.44	4.48	10.51	10.42	16.66	16.22	4.35	4.62	10.46
	SEM	0.991		0.991		1.401		0.700		
	P Value	0.001***		0.916		0.600		0.534		
VI	Control	18.70	4.83	10.22	13.30	15.43	21.96	5.01	4.64	11.76
	Glycerol	16.21	4.43	10.07	10.58	16.29	16.14	3.86	5.01	10.32
	Jaggery	15.95	4.46	10.91	9.49	17.75	14.14	4.08	4.84	10.20
	Maize	15.77	4.62	11.55	8.84	18.79	12.76	4.31	4.92	10.19
	Mean	16.66	4.58	10.69	10.55	17.06	16.25	4.31	4.85	10.62
	SEM	1.01		1.01		1.432		0.716		
	P Value	0.001***		0.843		0.373		0.356		
VII	Control	18.92	4.88	10.34	13.46	15.72	22.12	4.96	4.79	11.90
	Glycerol	16.04	4.56	10.22	10.38	16.68	15.39	3.76	5.36	10.30
	Jaggery	15.38	4.19	10.55	9.02	17.09	13.75	4.02	4.36	9.79
	Maize	15.88	4.56	11.11	9.33	18.01	16.24	4.21	4.90	10.22
	Mean	16.56	4.55	10.56	10.54	16.88	16.24	4.24	4.85	10.55
	SEM	1.10		1.10		1.558		0.779		
	P Value	0.001***		0.987		0.434		0.272		
VIII	Control	19.29	4.66	10.73	13.22	16.61	21.96	4.85	4.48	11.98
	Glycerol	15.41	4.50	9.98	9.88	16.18	14.64	3.79	5.11	9.93
	Jaggery	15.75	4.49	11.07	9.17	17.46	14.04	4.68	4.30	10.12
	Maize	14.70	4.56	10.43	8.83	16.68	12.71	4.18	4.95	9.63
	Mean	16.29	4.54	10.55	10.28	16.73	15.84	4.37	4.71	10.41
	SEM	1.072		1.072		1.515		0.758		
	P Value	0.001***		0.734		0.431		0.161		
Average milk/Cow/ 2 months	Control	17.08	4.22	9.57	11.73	14.50	19.66	4.64	3.81	10.65
	Glycerol	15.15	3.93	9.24	9.85	14.88	15.43	3.60	4.27	9.54
	Jaggery	15.04	4.14	10.08	9.10	16.14	13.95	4.02	4.26	9.59
	Maize	14.87	4.28	10.51	8.64	17.12	12.62	3.91	4.66	9.57
	Mean	15.53	4.14	9.85	9.83	15.66	15.41	4.04	4.25	9.83
	SEM	0.999		0.999		1.413		0.707		
	P Value	0.001***		0.978		0.755		0.633		

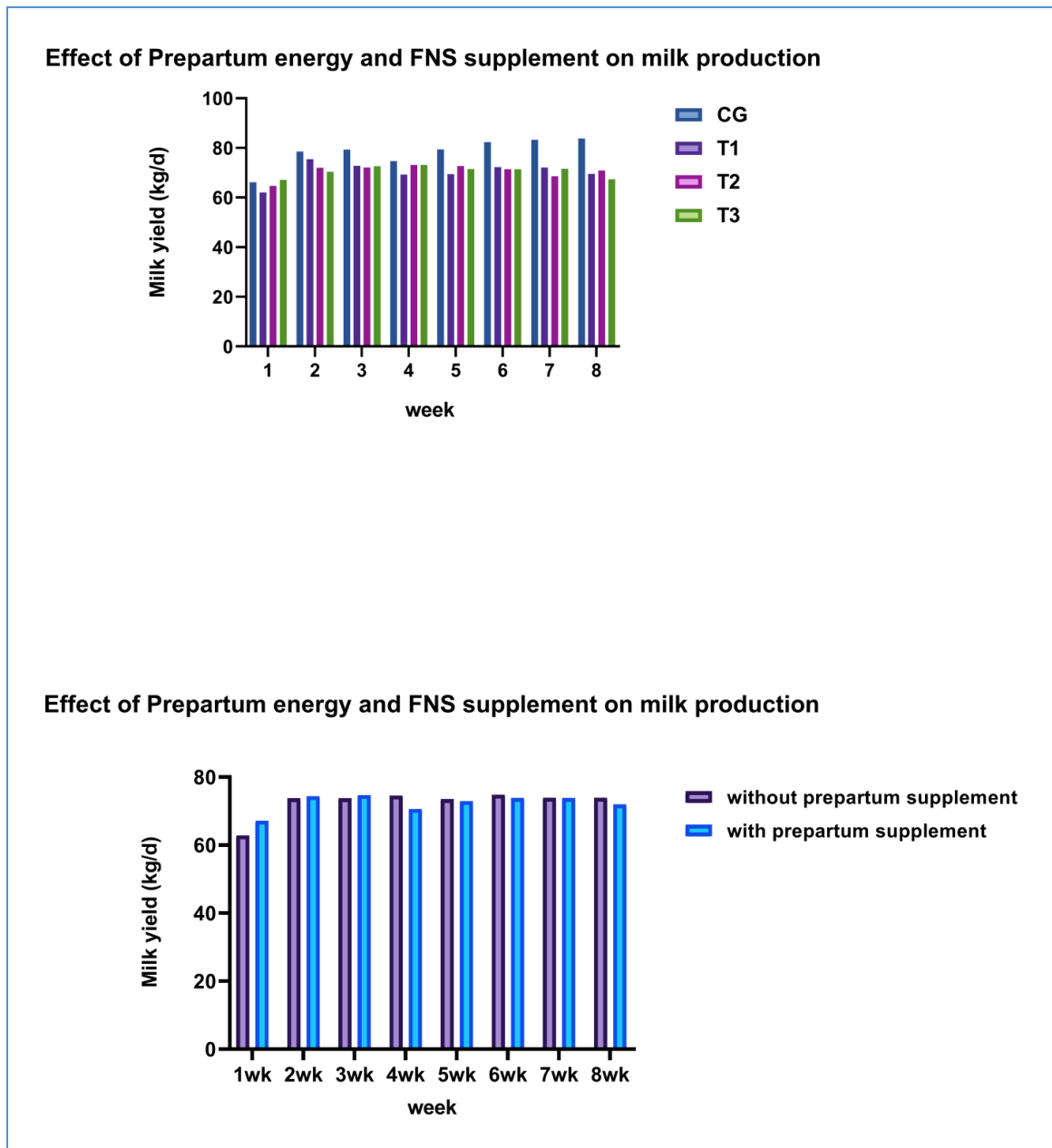


Fig. 4.5: Effect of prepartum energy and FNS effect on milk production

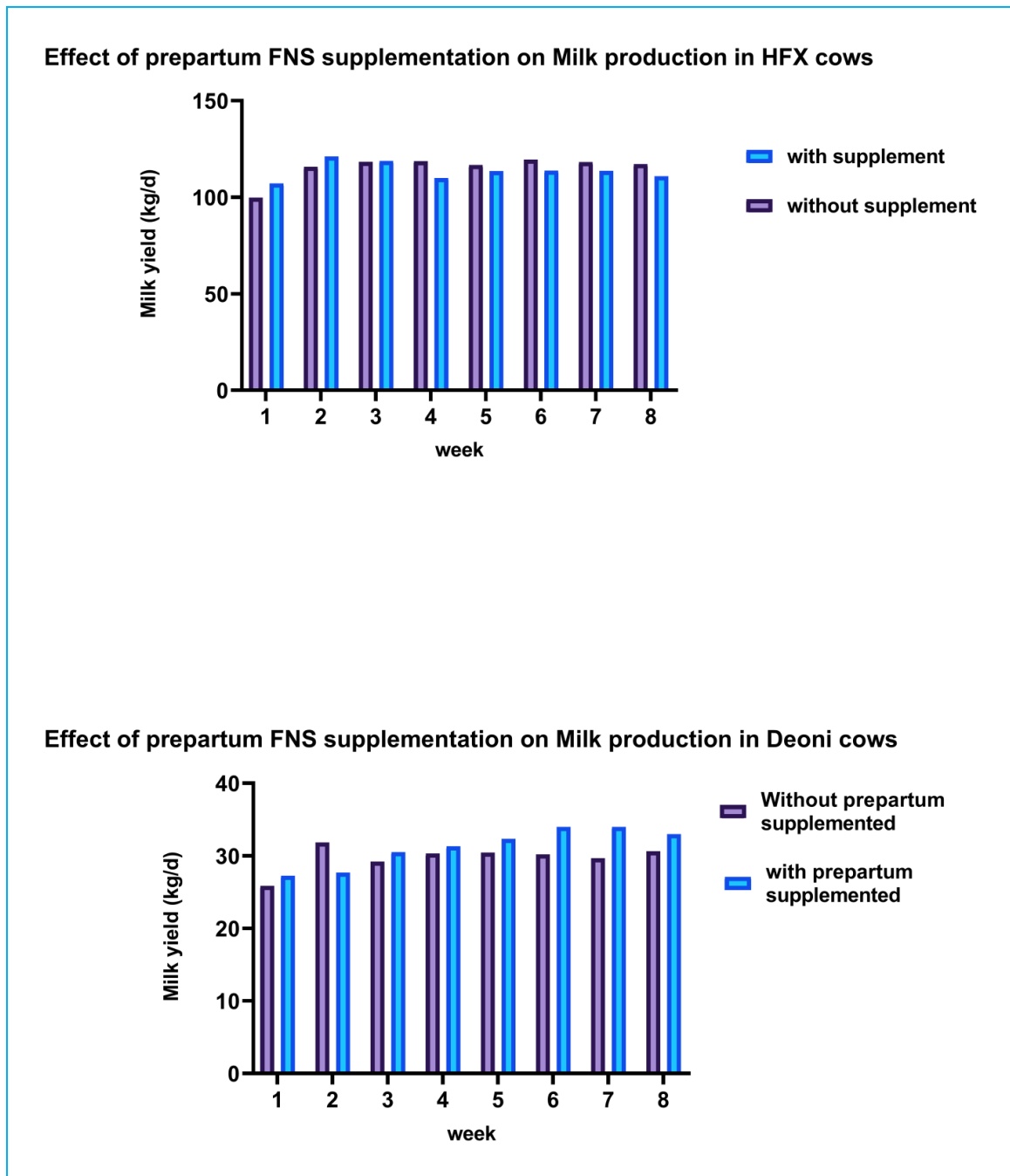


Fig. 4.6: Effect of prepartum energy and FNS on milk production in breeds

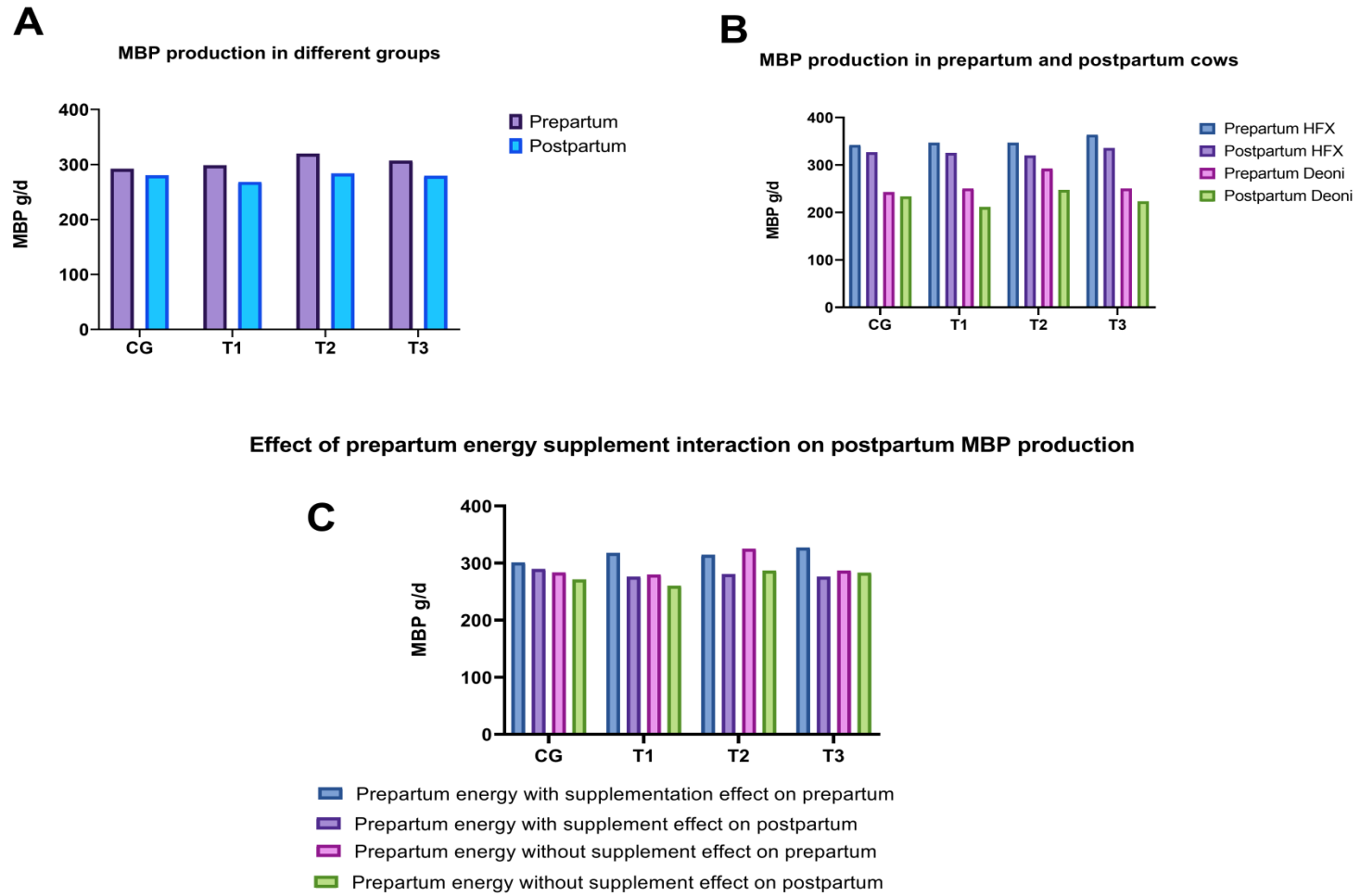


Fig 4.7 (A) MBP production in different groups (B) MBP production in prepartum and postpartum cows (C) Effect of prepartum energy supplement interaction on MBP production

Fig. 4.7: Postpartum MBP production in comparison with prepartum MBP production

4.3.5 Effect of prepartum nutrition on postpartum digestibility

The cows of different groups in the prepartum period were shifted to the general herd after 5th day of calving. The cows belonging to different groups in the prepartum were monitored for interactive effect of nutrition they received during prepartum on the postpartum; Nutrition, health and milk production when all cows were under uniform feeding management on the farm (*Section 3.14.2.2*). All the parameters measured during the prepartum were evaluated in the postpartum to deduce any follow-up effect of planned prepartum energy and FNS supplements (FNS) provided to HFX and Deoni cows. All the parameters in the postpartum period were compared with prepartum and expressed positive or negative change.

4.3.5.1 Postpartum nutrition and metabolism *In vivo*

The live BW (kg) or metabolic BW ($\text{kgW}^{0.75}$) of cows in postpartum as per their grouping in prepartum is presented in **Table 4.34**. The BW of HFX and Deoni cows was one of the major difference ($P < 0.001$) in the both breed (Srinivas and Ramesha, 2014). Ríos-Utrera *et al.* (2013) also observed BW difference in HF and Brown Swiss cows at the time of calving. The HFX cows live BW in prepartum and postpartum were 36% and 37% higher ($P < 0.001$) than Deoni cows, respectively. This indicated proportionate BW change in both the breeds after calving although the mean calf birth weight was 30 kg in HFX compared to 20 kg in Deoni breed. Both the breed proportionately maintained the BW between prepartum and postpartum which is important for the normal purpureum (Lopez *et al.*, 2003). Putnam and Henderson (1946) reported Ayrshire females BW in last month of pregnancy in 1st, 2nd and 3rd parity was 495, 547 and 576 kg that was changed to 450, 489 and 512 kg soon after calving. The weight change in cow BW before and after calving was 45, 58 and 64 kg, respectively. The BW in the HFX cows ranged from 349 kg to 503 kg except one cow which was only 284 kg thus, the coefficient of variation (CV) was 21 kg in comparison to Deoni cows with a range of 291 kg to 372 kg and CV of 20 kg. Absolute change in BW in HFX and Deoni cows in comparison to prepartum BW was similar.

Table 4.34: Live and metabolic body weight of postpartum cows of the experiment

Parameter	Group	Energy × Breed		Energy × Suppl.		HFX × Suppl.		Deoni × Suppl.		E × B × S
		HFX	Deoni	Without	With	Without	With	Without	With	
Live body weight	Control	396.75	291.25	331.50	356.50	371.50	422.00	291.50	291.00	344.00
	Glycerol	426.75	287.50	343.75	37.50	387.00	466.50	300.50	274.50	357.13
	Jaggery	454.25	350.50	396.00	408.75	444.00	464.50	348.00	353.00	402.38
	Maize	424.50	308.50	373.00	360.00	423.00	426.00	323.00	294.00	366.50
	Mean	425.56	309.44	361.06	373.94	406.38	444.75	315.75	303.12	367.50
	SEM	28.506		28.506			40.314			20.157
	P Value	0.001***		0.532			0.224			0.243
Metabolic body weight	Control	88.53	70.47	77.36	81.66	84.18	92.88	70.55	70.39	79.50
	Glycerol	93.78	69.77	79.64	83.90	87.17	100.38	72.13	67.42	81.77
	Jaggery	98.33	80.99	88.59	90.73	96.60	100.05	80.57	81.42	89.66
	Maize	93.35	73.59	84.71	82.24	93.23	93.48	76.19	70.99	83.47
	Mean	93.49	73.71	82.58	84.63	90.29	96.70	74.86	72.55	83.60
	SEM	4.805		4.805			6.795			3.398
	P Value	0.001***		0.555			0.218			0.219

The seasonal variation in the weather was not much in the study regions, and similarly, all the cows were also in 3rd to 5th parity therefore, their influences on postpartum BW were also nullified (Pugashetti and Kulkarni, 2009). The parity difference in cows may exert some influence on the BW gain during pregnancy (Putnam and Henderson, 1946) which was 64, 67 and 73 kg, respectively in 1st, 2nd and 3rd parity in Aarshyre cows with a mean calf birth weight of 33 kg. The alike BW changes observed in both the breeds from prepartum to postpartum irrespective of calf birth weight was a good indicator for post-calving reproductive performance (Burke *et al.*, 1998).

4.3.5.2 Chemical composition of the diet

Nutrient composition of MGF and CS provided to lactating cows on the farm during postpartum was presented in **Table 4.7** (Described in 4.2.1). The diets fed to cows in the postpartum were only MGF and CS but avoided FM straw. All the cows were under semi-open system of feeding for MGF but, CS was measured and fed at the time of milking 5h00 and 17h00, respectively. The intake was estimated by indirect marker method.

4.3.5.3 Nutrient intake from the diet

The breed difference ($P < 0.001$) in DM, OM, CP, EE and TCHO consumption from the roughage was similar to their intake in the prepartum. The HFX cows consumed 25% lesser DM (**Table 4.35**) from MGF in postpartum than its intake in prepartum i.e., 3.65 kg/d. Deoni cows in postpartum consumed 2.34 kg/d MGF compared to 3.70 kg/d in the prepartum which accounted to -37%. DMI from MGF during postpartum was reduced by 39% compared to its intake during prepartum when cows fed FNS supplement in CG or TGs. However, the reduction in DM intake from MGF in postpartum in cows without FNS supplement in prepartum was only 10% (**Table 4.35A**). This indicated that discontinuation of FNS supplementation in the postpartum might be partially hampered the MGF consumption during this period. FNS supplementation impact on DMI from MGF was declined more in Deoni (42%) compared to HFX cows (37%). The overall cow breed, energy and FNS supplementation interaction effect in the prepartum on DMI from MGF was 30% more compared to postpartum.

Table 4.35: Postpartum nutrient intake (Proximate composition) from green fodder (kg/d) in cows

Parameter	Group	Energy × Breed		Energy × Suppl.		HFX × Suppl.		Deoni × Suppl.		E × B × S
		HFX	Deoni	Without	With	Without	With	Without	With	
DM	Control	3.89	1.84	3.14	2.59	4.45	3.34	1.84	1.84	2.86 ^{ab}
	Glycerol	3.40	1.81	2.79	2.42	3.82	2.98	1.76	1.86	2.60 ^a
	Jaggery	3.81	2.71	3.58	2.95	4.21	3.41	2.95	2.48	3.26 ^b
	Maize	3.49	2.99	3.26	3.21	3.71	3.27	2.82	3.16	3.24 ^b
	Mean	3.65	2.34	3.19	2.79	4.04	3.25	2.34	2.33	2.99
	SEM	0.181		0.181		0.256		0.128		0.128
	P Value	0.001***		0.01**		0.01**		0.01**		0.01**
OM	Control	3.56	1.68	2.87	2.37	4.07	3.06	1.68	1.68	2.62 ^{ab}
	Glycerol	3.11	1.66	2.55	2.22	3.49	2.73	1.61	1.70	2.38 ^a
	Jaggery	3.48	2.48	3.27	2.69	3.85	3.12	2.69	2.27	2.98 ^b
	Maize	3.19	2.73	2.98	2.94	3.39	2.99	2.58	2.89	2.96 ^b
	Mean	3.34	2.14	2.91	2.55	3.70	2.97	2.14	2.13	2.74
	SEM	0.165		0.165		0.233		0.116		0.116
	P Value	0.001		0.01**		0.01**		0.01**		0.01**
CP	Control	0.20	0.10	0.16	0.14	0.23	0.18	0.10	0.10	0.15 ^{ab}
	Glycerol	0.18	0.09	0.14	0.13	0.20	0.16	0.09	0.10	0.13 ^a
	Jaggery	0.20	0.14	0.19	0.15	0.22	0.18	0.16	0.13	0.17 ^b
	Maize	0.18	0.16	0.17	0.17	0.20	0.17	0.15	0.17	0.17 ^b
	Mean	0.19	0.12	0.17	0.14	0.21	0.17	0.12	0.12	0.16
	SEM	0.01		0.01		0.025		0.007		0.007
	P Value	0.001***		0.01**		0.922		0.01**		0.01**

Parameter	Group	Energy × Breed		Energy × Suppl.		HFX × Suppl.		Deoni × Suppl.		E × B × S
		HFX	Deoni	Without	With	Without	With	Without	With	
EE	Control	0.06	0.03	0.05	0.04	0.07	0.05	0.03	0.03	0.04 ^{ab}
	Glycerol	0.05	0.03	0.05	0.04	0.06	0.05	0.03	0.03	0.04 ^a
	Jaggery	0.06	0.04	0.05	0.04	0.06	0.05	0.05	0.04	0.05 ^b
	Maize	0.05	0.05	0.04	0.05	0.06	0.05	0.05	0.05	0.05 ^b
	Mean	0.05	0.04	0.05	0.04	0.06	0.05	0.04	0.05	0.05
	SEM	0.003		0.003		0.005		0.005		0.002
	P Value	0.001***		0.03*		0.02*		0.05*		0.05*
TA	Control	0.33	0.16	0.27	0.22	0.38	0.29	0.16	0.16	0.25 ^{ab}
	Glycerol	0.29	0.15	0.24	0.21	0.33	0.26	0.15	0.16	0.22 ^a
	Jaggery	0.33	0.23	0.31	0.25	0.37	0.29	0.25	0.22	0.28 ^b
	Maize	0.30	0.26	0.28	0.28	0.32	0.28	0.24	0.27	0.28 ^b
	Mean	0.31	0.20	0.27	0.24	0.34	0.28	0.20	0.20	0.26
	SEM	0.016		0.016		0.041		0.041		0.011
	P Value	0.001***		0.01**		0.01**		0.05*		0.05*
TCHO	Control	3.30	1.56	2.67	2.20	3.77	2.83	1.56	1.56	2.43 ^{ab}
	Glycerol	2.88	1.54	2.36	2.05	3.23	2.53	1.50	1.58	2.21 ^a
	Jaggery	3.22	2.30	3.04	2.49	3.57	2.89	2.50	2.10	2.76 ^b
	Maize	2.96	2.54	2.77	2.73	3.14	2.77	2.39	2.68	2.75 ^b
	Mean	3.01	1.98	2.71	2.37	3.43	2.75	1.99	1.98	2.54
	SEM	0.153		0.153		0.217		0.217		0.108
	P Value	0.001		0.01**		0.01**		0.01**		0.01**

Values with different superscripts for absolute interaction for a respective energy source in a row under each parameter differ significantly; ***P ≤ 0.001

The other reason for the reduction in MGF intake was group feeding in the semi-open system. In semi-open system of feeding, the size of the cow, the space occupied by each cow and even spreading of the green fodder to provide equal opportunity to each cow for ingestion may also affect the MGF intake (DeVries *et al.*, 2004).

The other nutrients consumption in postpartum from MGF viz., OM, CP, TCHO was in the same pattern like DM consumption. The contrasting feature of MGF intake between post and prepartum for the experimental cows was significant difference between CG and TGs during prepartum for different factors or their interaction. It was observed that DM, OM, CP, EE, TCHO and TA consumption during the postpartum from MGF was better in T2 and T3 than CG but, T1 was comparably lesser than CG. This indicated that the MGF consumption during postpartum was better with jaggery or maize grain as a source of energy was better than cows fed glycerol as an energy source in the prepartum period.

NDF, ADF, hemicelluloses, and celluloses consumption from MGF during the postpartum was reduced by 32% in HFX cows compared to prepartum (**Table 4.36**). It was 58% lesser ($P < 0.001$) in Deoni cows. The NDF consumption from MGF in postpartum was decreased by 65% compared to prepartum where these cows received FNS supplements in the diet in CG and TGs. The decrease in NDF consumption from MGF in the postpartum due to FNS supplementation compared to prepartum period was more in Deoni (75%) than HFX (59%) cows (**Table 4.36A**). The hemicelluloses and celluloses consumption from MGF during postpartum was also same like NDF consumption pattern during prepartum. It was observed that either proximate nutrient or CWC consumption from MGF in cows was reduced in postpartum compared to prepartum because MGF was non-maintenance type roughage. The reduction in nutrient consumption from MGF during the postpartum is determinantal to the health of the cows because green fodder acts as buffer for the high CS requirement during lactation (Montañez-Valdez *et al.*, 2013). The total roughage intake in HFX cows and Deoni cows during postpartum was 25% and 35%, respectively which was lesser than the recommended level of 40% of the total ration for better health of the cows (Bargo *et al.*, 2002).

Table 4.36: Cell and cell wall intake from green fodder (kg/d) in postpartum cows

Parameter	Group	Energy × Breed		Energy × Suppl.		HFX × Suppl.		Deoni × Suppl.		E × B × S
		HFX	Deoni	Without	With	Without	With	Without	With	
CC	Control	1.32	0.63	1.07	0.88	1.51	1.31	0.63	0.63	0.97 ^a
	Glycerol	1.16	0.61	0.95	0.82	1.30	1.02	0.60	0.63	0.88 ^a
	Jaggery	1.29	0.92	1.22	1.00	1.43	1.16	1.00	0.84	1.11 ^b
	Maize	1.19	1.02	1.11	1.09	1.26	1.11	0.96	1.08	1.10 ^b
	Mean	1.24	0.79	1.08	0.95	1.37	1.10	0.80	0.79	1.02
	SEM	0.06		0.06		0.086				0.043
	P Value	0.001***		0.01**		0.01**				0.01**
NDF	Control	2.57	1.22	2.08	1.71	2.94	2.21	1.22	1.22	1.89 ^{ab}
	Glycerol	2.25	1.20	1.84	1.60	3.52	1.98	1.16	1.23	1.72 ^a
	Jaggery	2.52	1.79	2.36	1.95	2.78	2.26	1.95	1.64	2.15 ^b
	Maize	2.31	1.98	2.16	2.12	2.45	2.16	1.87	2.09	2.14 ^b
	Mean	2.41	1.54	2.11	1.84	2.67	2.15	1.55	1.54	1.98
	SEM	0.119		0.119		0.168				0.08
	P Value	0.001***		0.01**		0.01**				0.01**
ADF	Control	1.69	0.80	1.37	1.13	1.93	1.46	0.80	0.80	1.25 ^{ab}
	Glycerol	1.48	0.79	1.22	1.05	1.66	1.30	0.77	0.81	1.13 ^a
	Jaggery	1.66	1.18	1.56	1.28	1.84	1.49	1.29	1.08	1.42 ^b
	Maize	1.52	1.30	1.42	1.40	1.61	1.42	1.23	1.38	1.41 ^b
	Mean	1.59	1.02	1.39	1.22	1.76	1.41	1.02	1.02	1.30
	SEM	0.079		0.079		0.111				0.05
	P Value	0.001***		0.01**		0.01**				0.01**
HC	Control	0.88	0.42	0.71	0.58	1.00	0.75	0.42	0.42	0.65 ^{ab}
	Glycerol	0.77	0.41	0.63	0.54	0.86	0.67	0.40	0.42	0.59 ^a
	Jaggery	0.86	0.61	0.81	0.66	0.95	0.77	0.67	0.56	0.74 ^b
	Maize	0.79	0.67	0.74	0.73	0.84	0.74	0.64	0.71	0.73 ^b
	Mean	0.82	0.53	0.72	0.63	0.91	0.73	0.53	0.52	0.67
	SEM	0.041		0.041		0.058				0.029
	P Value	0.001***		0.01**		0.01**				0.01**

Parameter	Group	Energy × Breed		Energy × Suppl.		HFX × Suppl.		Deoni × Suppl.		E × B × S
		HFX	Deoni	Without	With	Without	With	Without	With	
Cellulose	Control	1.40	0.66	1.13	0.93	1.60	1.20	0.66	0.66	1.03 ^{ab}
	Glycerol	1.22	0.65	1.00	0.87	1.37	1.07	0.63	0.67	0.93 ^a
	Jaggery	1.37	0.97	1.28	1.06	1.51	1.23	1.06	0.89	1.17 ^b
	Maize	1.25	1.07	1.17	1.15	1.33	1.17	1.01	1.13	1.16 ^b
	Mean	1.31	0.84	1.14	1.00	1.45	1.17	0.84	0.83	1.07
	SEM	0.065	0.065	0.091	0.046					
	P Value	0.001***	0.01**	0.01**						
ADL	Control	0.20	0.09	0.16	0.13	0.23	0.17	1.00	0.90	0.14 ^{ab}
	Glycerol	0.18	0.09	0.14	0.13	0.20	0.16	0.09	0.10	0.13 ^a
	Jaggery	0.19	0.14	0.18	0.15	0.22	0.17	0.15	0.13	0.17 ^c
	Maize	0.17	0.15	0.16	0.16	0.19	0.16	0.14	0.16	0.16 ^{bc}
	Mean	0.18	0.12	0.16	0.14	0.21	0.16	0.12	0.12	0.15
	SEM	0.009	0.009	0.012	0.006					
	P Value	0.001***	0.003**	0.004**	0.01**					
NFC	Control	0.74	0.35	0.60	0.49	0.85	0.63	0.35	0.35	0.54 ^{ab}
	Glycerol	0.64	0.34	0.53	0.46	0.73	0.57	0.34	0.35	0.50 ^a
	Jaggery	0.72	0.52	0.68	0.56	0.80	0.65	0.56	0.47	0.62 ^b
	Maize	0.66	0.58	0.62	0.61	0.71	0.62	0.54	0.60	0.62 ^b
	Mean	0.70	0.44	0.61	0.53	0.77	0.62	0.45	0.44	0.57
	SEM	0.035	0.031	0.049	0.024					
	P Value	0.001***	0.01**	0.01**						

Values with different superscripts for absolute interaction for a respective energy source in a row under each parameter differ significantly; ***P ≤ 0.01, **P ≤ 0.001

Table 4.35A: Difference (%) in proximate nutrient intake from roughage in cows between pre and postpartum periods

Parameter	Energy × Breed		Energy × Suppl.		HFX × Suppl.		Deoni × Suppl.		E × B × S
	HFX	Deoni	Without	With	Without	With	Without	With	
DM	-25	-37	-19	-39	-10	-37	-30	-42	-30
OM	-24	-37	-19	-39	-10	-37	-30	-42	-30
CP	-24	-37	-19	-42	-9	-37	-33	-43	-27
EE	-29	-33	-17	-43	0	-38	-20	-17	-17
TA	-24	-38	-21	-38	-11	-36	-31	-41	-30
TCHO	-27	-37	-19	-39	-10	-37	-30	-42	-30
Min	-29	-38	-21	-43	-11	-38	-33	-43	-30
Max	-24	-33	-17	-38	0	-36	-20	-17	-17
Mean	-25	-36	-19	-40	-8	-37	-29	-38	-27
CV	-7	-4	-7	-4	-50	-1	-16	-28	-19

Table 4.36A: Difference (%) in CWC intake from green fodder in cows between pre and postpartum periods

Parameter	Energy × Breed		Energy × Suppl.		HFX × Suppl.		Deoni × Suppl.		E × B × S
	HFX	Deoni	Without	With	Without	With	Without	With	
CC	-32	-59	-23	-64	-12	-59	-43	-75	-42
NDF	-32	-58	-23	-65	-11	-59	-43	-73	-42
ADF	-33	-58	-23	-65	-11	-60	-43	-73	-43
HC	-33	-57	-24	-65	-11	-59	-43	-75	-43
Cellulose	-33	-58	-24	-65	-12	-59	-43	-75	-43
ADL	-33	-58	-25	-64	-10	-63	-42	-67	-40
NFC	-31	-59	-21	-62	-9	-56	-40	-73	-40
Min	-33	-59	-25	-65	-12	-63	-43	-75	-43
Max	-31	-57	-23	-64	-10	-59	-42	-67	-40
Mean	-33	-58	-24	-65	-11	-60	-43	-73	-42
CV	-1	-2	-3	-1	-7	-2	-1	-4	-3

The disturbing proportion of roughage ratio in the total diet can a predisposing factor to sub-acute rumen acidosis (SARA), laminitis, diminished immunity, udder infection etc., (Nagaraja and Titgemeyer, 2007; Enemark, 2013). We observed laminitis in 12 out of 16 postpartum HFX cows whereas no laminitis observed in Deoni cows. Not only breed effect, but also attributed to the level of milk production, nutrient requirement, non-maintenance type of MGF, roughage% in diet etc (Enemark, 2013). Postpartum NFC consumption from MGF was also reduced by 30 and 59%, respectively in HFX and Deoni cows. The reduction in NFC consumption in postpartum from MGF was 59% in those HFX cows received FNS supplements during prepartum. The discontinued additional nutrition received by Deoni cows during prepartum resulted in reduced NFC consumption by 70% in postpartum. It was observed that NDF, ADF, hemicelluloses, celluloses and NFC consumption from MGF in postpartum except CC were better in cows belongs to T2 and T3 than CG and T1 during prepartum. CC consumption of Cows in CG and T1, and T2 and T3 of prepartum were comparable for their intake during postpartum too. This indicated that CWC intake from MGF in cows fed with jaggery or maize grain than glycerol as a source of energy in prepartum was better during postpartum.

The breed variation ($P < 0.001$) in DM, OM, CP, EE and TCHO consumption from CS between postpartum HFX and Deoni cows was similar to their intake during the prepartum. The consumption of CP from CS during postpartum was 1560 g/d by HFX cows, and 760 g/d by Deoni cows. Unlike in the prepartum, the maximum of CP and energy requirement of the cows during postpartum were met from the CS. The CS proportion in the diet during prepartum and postpartum was 40% and 70%, respectively. In early lactation, cows require more CS owing to positive curvilinear relationship exist with milk production (Kellaway and Harington, 2004). Since MGF was of non-maintenance type, nutrient requirements for maintenance partially and milk production completely were met through CS. CS consumption in postpartum HFX cows was 1.12 times higher than Deoni cows ($P < 0.001$) because their maintenance and milk production requirements were higher. Compared to close-up cows, fresh cows DM intake from CS was 291% (9.2 kg/d) and 120% (4.48 kg/d) more in HFX and Deoni cows **(Table 4.37)**.

Table 4.37: Nutrient intake (Proximate composition) from concentrate supplement (kg/d) in postpartum cows

Parameter	Group	Energy × Breed		Energy × Suppl.		HFX × Suppl.		Deoni × Suppl.		E × B × S
		HFX	Deoni	Without	With	Without	With	Without	With	
DM	Control	8.39	4.48	6.15	6.72	7.83	8.95	4.48	4.48	6.44
	Glycerol	9.40	4.48	6.72	7.16	8.95	9.85	4.48	4.48	6.94
	Jaggery	9.51	4.48	6.94	7.05	9.40	9.63	4.48	4.48	7.00
	Maize	9.51	4.48	7.39	6.60	10.30	8.73	4.48	4.48	7.00
	Mean	9.20	4.48	6.80	6.88	9.12	9.29	4.48	4.48	6.84
	SEM	0.162		0.162		0.458				0.229
	P Value	0.001***		0.720		0.720				0.28
OM	Control	7.51	4.01	5.51	6.01	7.01	8.01	4.01	4.01	5.76
	Glycerol	8.41	4.01	6.01	6.41	8.01	8.81	4.01	4.01	6.21
	Jaggery	8.51	4.01	6.21	6.31	8.41	8.61	4.01	4.01	6.26
	Maize	8.51	4.01	6.61	5.91	9.22	7.88	4.01	4.01	6.26
	Mean	8.23	4.01	6.09	6.16	8.16	8.31	4.01	4.01	6.12
	SEM	0.145		0.145		0.410				0.21
	P Value	0.001***		0.722		0.722				0.28
CP	Control	1.47	0.78	1.08	1.17	1.37	1.56	0.78	0.78	1.12
	Glycerol	1.64	0.78	1.17	1.25	1.56	1.72	0.78	0.78	1.21
	Jaggery	1.66	0.78	1.21	1.23	1.64	1.68	0.78	0.78	1.22
	Maize	1.66	0.78	1.29	1.15	1.80	1.53	0.78	0.78	1.22
	Mean	1.61	0.78	1.19	1.20	1.59	1.62	0.78	0.78	1.19
	SEM	0.029		0.029		0.081				0.04
	P Value	0.001***		0.715		0.715				0.29

Parameter	Group	Energy × Breed		Energy × Suppl.		HFX × Suppl.		Deoni × Suppl.		E × B × S
		HFX	Deoni	Without	With	Without	With	Without	With	
EE	Control	0.16	0.09	0.12	0.13	0.15	0.17	0.09	0.09	0.13
	Glycerol	0.18	0.09	0.13	0.14	0.17	0.19	0.09	0.09	0.14
	Jaggery	0.18	0.09	0.14	0.14	0.18	0.19	0.09	0.09	0.14
	Maize	0.18	0.09	0.14	0.13	0.20	0.17	0.09	0.09	0.14
	Mean	0.18	0.09	0.13	0.13	0.17	0.18	0.09	0.09	0.13
	SEM	0.006		0.006		0.009		0.009		0.004
	P Value	0.001***		0.680		0.680		0.680		0.29
TA	Control	1.09	0.58	0.79	0.87	1.02	1.16	0.58	0.58	0.83
	Glycerol	1.21	0.58	0.87	0.93	1.16	1.28	0.58	0.58	0.90
	Jaggery	1.23	0.58	0.90	0.91	1.22	1.25	0.58	0.58	0.91
	Maize	1.24	0.58	0.96	0.86	1.34	1.13	0.58	0.58	0.91
	Mean	1.20	0.58	0.88	0.89	1.18	1.20	0.58	0.58	0.89
	SEM	0.042		0.109		0.059		0.059		0.029
	P Value	0.001***		0.723		0.715		0.715		0.27
TCHO	Control	5.68	3.30	4.16	4.55	5.30	6.06	3.30	3.30	4.35
	Glycerol	6.36	3.03	4.55	4.85	6.06	6.67	3.03	3.03	4.70
	Jaggery	6.44	3.03	4.70	4.77	6.36	6.51	3.03	3.03	4.73
	Maize	6.44	3.03	5.00	4.47	6.97	5.91	3.03	3.03	4.73
	Mean	6.23	3.03	4.60	4.66	6.17	6.29	3.03	3.03	4.63
	SEM	0.219		0.219		0.309		0.309		0.155
	P Value	0.001***		0.715		0.723		0.723		0.28

Values with different superscripts for absolute interaction for a respective energy source in a row under each parameter differ significantly; ***P ≤ 0.001, **P ≤ 0.01

FNS supplement provided during prepartum had significant influence on the postpartum CS consumption (240%) compared to those cows without FNS supplement (188%) in the prepartum (**Table 4.37A**). Interactive effect of FNS supplement during prepartum on postpartum DM consumption from CS was higher in HFX (300%) than Deoni (150%) cows. Even the interaction effect of dietary energy and FNS supplementation received by both breed on the DM consumption from CS in postpartum was 2 folds more. Increased appetite and consumption of CS is necessary to sustain cow genetic potential of milk production (Gillah *et al.*, 2014). Other nutrients consumption viz., OM, CP and TCHO of cows between postpartum and prepartum were increased with higher CS intake. No significant difference observed in the consumption of DM, OM, CP, EE and TCHO by the cows during postpartum those belonging to CG or TGs of prepartum. More or less all cows consumption of proximate nutrient were comparable during postpartum.

NDF, ADF, hemicellulose, and cellulose consumption from CS during postpartum was increased by 3.40 times and CC consumption increased by 2.59 times in HFX cows compared to the prepartum (**Table 4.38**) but, this difference ($P < 0.001$) was lesser in Deoni cows being only 2 folds. The NDF consumption from CS in postpartum increased 283% in cows those received energy and FNS supplement during the prepartum, where greater consumption was observed in HFX (354%) than Deoni (185%) cows (**Table 4.38A**). The hemicellulose and cellulose consumption from CS was also same as NDF. It was observed that either proximate nutrient or CWC consumption from CS in fresh cows was 2.5 times more than the close-up cows. NFC consumption from CS which is important in the light of gluconeogenesis during lactation (Aschenbach *et al.*, 2010). NFC consumption in postpartum in HFX and Deoni was 3.5 and 1.5 times more than its consumption in respective cows. The no significant difference in the CG and TGs for CWC consumption from CS in the postpartum indicated no follow-up effect of experimental diets fed to CG and TGs during the prepartum period.

Total diet consumption in postpartum (**Table 4.39**) was increased by 58% than prepartum in HFX cows while it was only 2% in Deoni cows. The mean increase in DM, OM, EE, TCHO in postpartum compared to prepartum was about

Table 4.38: Cell and cell wall intake from concentrate supplement (kg/d) in different groups of postpartum cows

Parameter	Group	Energy × Breed		Energy × Suppl.		HFX × Suppl.		Deoni × Suppl.		E × B × S
		HFX	Deoni	Without	With	Without	With	Without	With	
CC	Control	4.58	2.44	3.36	3.66	4.28	4.88	2.44	2.44	3.51
	Glycerol	5.13	2.44	3.66	3.91	4.88	5.37	2.44	2.44	3.78
	Jaggery	5.19	2.44	3.78	3.85	5.13	5.25	2.44	2.44	3.81
	Maize	5.19	2.44	4.03	3.60	5.62	4.76	2.44	2.44	3.81
	Mean	5.02	2.44	3.71	3.75	4.97	5.07	2.44	2.44	3.73
	SEM	0.177		0.177		0.250				0.125
	P Value	0.001***		0.720		0.720				0.28
NDF	Control	3.78	2.02	2.78	3.03	3.53	4.03	2.02	2.02	2.90
	Glycerol	4.23	2.02	3.03	3.23	4.03	4.44	2.02	2.02	3.13
	Jaggery	4.29	2.02	3.13	3.18	4.24	4.34	2.02	2.02	3.15
	Maize	4.28	2.02	3.33	2.98	4.64	3.93	2.02	2.02	3.15
	Mean	4.15	2.02	3.06	3.10	4.11	4.18	2.02	2.02	3.08
	SEM	0.146		0.146		0.207				0.103
	P Value	0.001***		0.721		0.721				0.28
ADF	Control	1.01	0.54	0.74	0.81	0.94	1.07	0.54	0.54	0.77
	Glycerol	1.12	0.54	0.81	0.86	1.07	1.18	0.54	0.54	0.83
	Jaggery	1.14	0.54	0.83	0.85	1.13	1.15	0.54	0.54	0.84
	Maize	1.14	0.54	0.89	0.79	1.23	1.05	0.54	0.54	0.84
	Mean	1.10	0.54	0.82	0.83	1.09	1.11	0.54	0.54	0.82
	SEM	0.039		0.039		0.055				0.027
	P Value	0.001***		0.735		0.735				0.28
HC	Control	2.78	1.48	2.04	2.22	2.59	2.96	1.48	1.48	2.12
	Glycerol	3.11	1.48	2.22	2.37	2.96	3.26	1.48	1.48	2.30
	Jaggery	3.15	1.48	2.30	2.33	3.11	3.19	1.48	1.48	2.31
	Maize	3.15	1.48	2.44	2.18	3.41	2.89	1.48	1.48	2.31
	Mean	3.04	1.48	2.25	2.28	3.02	3.07	1.48	1.48	2.26
	SEM	0.108		0.108		0.152				0.076
	P Value	0.001***		0.716		0.716				0.27

Parameter	Group	Energy × Breed		Energy × Suppl.		HFX × Suppl.		Deoni × Suppl.		E × B × S
		HFX	Deoni	Without	With	Without	With	Without	With	
Cellulose	Control	0.59	0.31	0.43	0.47	0.55	0.63	0.31	0.31	0.45
	Glycerol	0.66	0.31	0.47	0.50	0.63	0.69	0.31	0.31	0.49
	Jaggery	0.67	0.31	0.49	0.49	0.66	0.68	0.31	0.31	0.49
	Maize	0.67	0.31	0.52	0.46	0.73	0.62	0.31	0.31	0.49
	Mean	0.65	0.31	0.48	0.48	0.64	0.65	0.31	0.31	0.48
	SEM	0.023		0.023		0.032		0.016		
	P Value	0.001***		0.730		0.730		0.28		
ADL	Control	1.90	1.01	1.39	1.52	1.77	2.02	1.01	1.01	1.45
	Glycerol	2.12	1.01	1.52	1.62	2.02	2.23	1.01	1.01	1.57
	Jaggery	2.15	1.01	1.57	1.59	2.13	2.18	1.01	1.01	1.58
	Maize	2.15	1.01	1.67	1.49	2.33	1.97	1.01	1.01	1.58
	Mean	2.08	1.01	1.54	1.55	2.06	2.10	1.01	1.01	1.54
	SEM	0.074		0.074		0.104		0.052		
	P Value	0.001***		0.724		0.724		0.281		
NFC	Control	0.32	0.17	0.24	0.26	0.31	0.34	0.17	0.17	0.25
	Glycerol	0.36	0.17	0.26	0.27	0.34	0.38	0.17	0.17	0.26
	Jaggery	0.37	0.17	0.27	0.27	0.36	0.37	0.17	0.17	0.27
	Maize	0.37	0.17	0.28	0.25	0.40	0.34	0.17	0.17	0.27
	Mean	0.35	0.17	0.26	0.26	0.35	0.36	0.17	0.17	0.26
	SEM	0.013		0.013		0.018		0.009		
	P Value	0.001***		0.789		0.789		0.33		

Values with different superscripts for absolute interaction for respective energy source in a row under each parameter differ significantly; ***P ≤ 0.001, **P ≤ 0.01, *P ≤ 0.05

Table 4.37A: Difference (%) in proximate nutrient intake from CS in cows between pre and postpartum periods

Parameter	Energy × Breed		Energy × Suppl.		HFX × Suppl.		Deoni × Suppl.		E × B × S
	HFX	Deoni	Without	With	Without	With	Without	With	
DM	291	120	188	239	277	306	95	153	212
OM	285	118	185	235	271	301	92	151	208
CP	347	144	222	287	318	363	111	189	250
EE	350	125	225	333	325	350	125	200	225
TA	329	142	214	271	307	344	115	176	242
TCHO	271	573	576	221	259	286	86	140	197
Min	271	118	185	221	259	286	86	140	197
Max	350	573	576	333	325	363	125	200	250
Mean	312	204	268	264	293	325	104	168	222
CV	11	89	57	16	9	10	15	14	9

Table 4.38A: Difference (%) in CWC intake from CS in cows between pre and postpartum periods

Parameter	Energy × Breed		Energy × Suppl.		HFX × Suppl.		Deoni × Suppl.		E × B × S
	HFX	Deoni	Without	With	Without	With	Without	With	
CC	259	103	167	210	248	270	81	132	187
NDF	337	143	219	283	319	354	115	185	246
ADF	340	145	228	295	319	363	116	184	257
HC	334	143	217	280	319	358	87	185	248
Cellulose	364	138	220	300	327	364	107	182	243
ADL	338	143	225	271	338	350	113	183	225
NFC	352	153	228	297	329	377	120	197	258
Min	259	103	167	210	248	270	81	132	187
Max	364	145	228	300	338	364	116	185	257
Mean	332	136	213	273	312	343	103	175	234
CV	11	12	11	12	10	11	15	12	11

81% but, increase in CP and TA consumption was 173% and 95%, respectively in HFX cows.

In contrary; DM, OM, EE, TCHO consumption in Deoni cows during postpartum was almost same as prepartum with only difference of 1 or 2% however, the difference in CP and TA consumption was 61% and 22%, respectively. Difference in the total diet consumption in HFX and Deoni cows between postpartum and prepartum was significant. A lot of difference exists in the nutrient requirement of close-up and fresh cows. According to Douglas *et al.* (2004) the DMI would increase 30 to 50% over the prepartum intake in the first week of lactation due to increase nutrient requirement.

Except for EE; DM, OM, CP, TCHO and TA consumption from the total diet during postpartum in HFX cows was 17% to 19% more than their consumption in the prepartum. In Deoni cows, only CP consumption was more by 19% while all other nutrients intake was either reduced or marginally increased compared to prepartum irrespective of whether these cows fed FNS supplementation or not during prepartum. The CP consumption in postpartum was increased by 19% in cows those provided FNS supplement during the prepartum. There was 118% and 125% higher CP consumption between postpartum and prepartum in cows due to interaction of diet energy and FNS supplement in the prepartum.

DM, OM, CP, EE and TCHO consumption in postpartum per kg metabolic BW are presented in **Table 4.40**. The HFX and Deoni cows consumed 74% and 12% higher total DMI (g/kg $w^{0.75}$), respectively in postpartum compared to prepartum. The increase in DM or OM was about 74% but, CP intake was increased by 197%, total ash by 115%, and TCHO by 56% in HFX cows (**Table 4.40A**). But, in Deoni cows the CP consumption in postpartum was higher by 79% compared to prepartum. The cow's DM and CP consumption from the total diet during postpartum was increased by 37% and 140%, respectively compared to prepartum when these cows provided FNS supplement along with energy. Compared to DM and CP consumption (g/kg $w^{0.75}$) from total diet during the prepartum because of interaction between energy and FNS supplement, their postpartum consumption was also increased by 37% and 140%, respectively in

Table 4.39: Nutrient intake (Proximate composition) from total diet (kg/d) in post-partum cows

Parameter	Group	Energy × Breed		Energy × Suppl.		HFX × Suppl.		Deoni × Suppl.		E × B × S
		HFX	Deoni	Without	With	Without	With	Without	With	
DM	Control	12.28	6.32	9.30	9.30	12.28	12.29	6.32	6.32	9.30 ^a
	Glycerol	12.80	6.29	9.50	9.58	12.77	12.83	6.24	6.34	9.54 ^{ab}
	Jaggery	13.32	7.19	10.52	10.00	13.61	13.03	7.42	6.96	10.25 ^b
	Maize	13.00	7.47	10.65	9.82	14.00	12.00	7.30	7.64	10.23 ^b
	Mean	12.85	6.81	9.99	9.67	13.16	12.53	6.82	6.81	9.83
	SEM	0.352		0.352		0.498				0.249
	P Value	0.001***		0.222		0.231				0.03*
OM	Control	11.07	5.69	8.38	8.38	11.08	11.07	5.68	5.69	8.38 ^a
	Glycerol	11.52	5.66	8.56	8.62	11.50	11.54	5.62	5.71	8.59 ^{ab}
	Jaggery	12.00	6.49	9.48	9.00	12.27	11.73	6.70	6.27	9.24 ^b
	Maize	11.70	6.74	9.59	8.85	12.60	10.80	6.59	6.89	9.22 ^b
	Mean	11.57	6.14	9.00	8.71	11.86	11.28	6.15	6.14	8.86
	SEM	0.316		0.316		0.447				0.224
	P Value	0.001***		0.208		0.224				0.03*
CP	Control	1.67	0.88	1.24	1.31	1.60	1.74	0.88	0.88	1.27
	Glycerol	1.82	0.88	1.32	1.38	1.77	1.88	0.88	0.88	1.35
	Jaggery	1.86	0.88	1.40	1.39	1.87	1.86	0.94	0.92	1.40
	Maize	1.84	0.94	1.46	1.32	1.99	1.70	0.93	0.95	1.39
	Mean	1.80	0.90	1.35	1.35	1.08	1.79	0.90	0.91	1.35
	SEM	0.056		0.056		0.08				0.04
	P Value	0.001***		0.902		0.877				0.15

Parameter	Group	Energy × Breed		Energy × Suppl.		HFX × Suppl.		Deoni × Suppl.		E × B × S
		HFX	Deoni	Without	With	Without	With	Without	With	
EE	Control	0.22	0.11	0.17	0.17	0.22	0.22	0.12	0.11	0.17
	Glycerol	0.23	0.11	0.17	0.17	0.23	0.23	0.12	0.11	0.17
	Jaggery	0.24	0.13	0.19	0.18	0.24	0.24	0.13	0.13	0.18
	Maize	0.23	0.13	0.19	0.17	0.25	0.22	0.13	0.13	0.18
	Mean	0.23	0.12	0.18	0.17	0.23	0.23	0.12	0.12	0.17
	SEM	0.007		0.007			0.010			0.005
	P Value	0.001***		0.258			0.524			0.072
TA	Control	1.42	0.74	1.07	1.09	1.40	1.45	0.74	0.74	1.08
	Glycerol	1.51	0.73	1.11	1.13	1.49	1.53	0.73	0.74	1.12
	Jaggery	1.56	0.81	1.20	1.17	1.58	1.54	0.83	0.80	1.18
	Maize	1.53	0.84	1.24	1.13	1.65	1.41	0.82	0.85	1.18
	Mean	1.50	0.78	1.15	1.13	1.53	1.48	0.78	0.78	1.14
	SEM	0.042		0.042			0.06			0.03
	P Value	0.001***		0.465			0.441			0.07
TCHO	Control	8.98	4.59	6.83	6.74	9.07	8.89	4.59	4.59	6.78 ^a
	Glycerol	9.24	4.56	6.91	6.90	9.29	9.19	4.52	4.61	6.90 ^a
	Jaggery	9.67	5.33	7.73	7.27	9.93	9.40	5.53	5.13	7.50 ^b
	Maize	9.39	5.56	7.76	7.19	10.11	8.68	5.42	5.71	7.48 ^b
	Mean	9.32	5.01	7.31	7.02	9.60	9.04	5.01	5.01	7.16
	SEM	0.253		0.253			0.357			0.179
	P Value	0.001***		0.133			0.141			0.02*

Values with different superscripts for absolute interaction for a respective energy source in a row under each parameter differ significantly; ***P ≤ 0.001, **P ≤ 0.01, *P ≤ 0.05

Table 4.40: Nutrient intake (Proximate composition) from total diet (g/kgW^{0.75}/d) in different groups

Parameter	Group	Energy × Breed		Energy × Suppl.		HFX × Suppl.		Deoni × Suppl.		E × B × S
		HFX	Deoni	Without	With	Without	With	Without	With	
DM	Control	141.19	89.89	118.96	118.12	148.40	133.99	89.52	90.26	115.54
	Glycerol	137.58	90.56	117.17	110.96	147.35	127.80	86.99	94.13	114.07
	Jaggery	135.93	88.74	116.85	107.82	141.63	130.24	92.08	85.41	112.34
	Maize	141.30	101.76	123.68	119.38	151.52	131.08	95.83	107.68	121.53
	Mean	139.00	92.74	119.16	112.57	147.22	130.77	91.10	94.37	115.87
	SEM	7.554		7.554		10.683				5.342
	P Value	0.001***		0.235		0.084				0.650
OM	Control	127.24	80.97	107.25	100.96	133.86	120.63	80.64	81.30	104.10
	Glycerol	123.85	81.56	105.54	99.87	132.74	114.97	78.34	84.78	102.71
	Jaggery	122.43	80.07	105.37	97.13	127.62	117.23	83.12	77.03	101.25
	Maize	127.20	91.87	111.44	107.62	136.39	118.01	86.49	97.24	109.53
	Mean	125.18	83.62	107.40	101.40	132.65	117.71	82.15	85.09	104.40
	SEM	6.801		6.801		9.618				4.809
	P Value	0.001***		0.230		0.081				0.646
CP	Control	19.16	12.50	15.89	15.77	19.33	18.98	12.45	12.55	15.83
	Glycerol	19.51	12.61	16.26	15.87	20.34	18.68	12.17	13.06	16.06
	Jaggery	18.97	11.40	15.48	14.89	19.36	18.59	11.61	11.20	15.19
	Maize	20.06	12.77	16.89	15.94	21.58	18.54	12.20	13.35	16.42
	Mean	19.42	12.32	16.13	15.62	20.15	18.70	12.10	12.54	15.87
	SEM	1.098		1.098		1.552				0.776
	P Value	0.001***		0.519		0.241				0.724

Parameter	Group	Energy × Breed		Energy × Suppl.		HFX × Suppl.		Deoni × Suppl.		E × B × S
		HFX	Deoni	Without	With	Without	With	Without	With	
EE	Control	2.51	1.61	2.11	2.01	2.61	2.41	1.61	1.62	2.06
	Glycerol	2.48	1.62	2.10	2.00	2.63	2.32	1.56	1.68	2.05
	Jaggery	2.43	1.56	2.06	1.93	2.52	2.35	1.61	1.51	1.99
	Maize	2.54	1.77	2.20	2.11	2.73	2.36	1.68	1.87	2.16
	Mean	2.49	1.64	2.12	2.01	2.62	2.36	1.61	1.67	2.06
	SEM	0.136		0.136			0.192			0.096
	P Value	0.001***		0.294			0.114			0.691
TA	Control	16.3	10.49	13.64	13.14	16.85	15.76	10.44	10.53	13.39
	Glycerol	16.19	10.57	13.66	13.1	17.14	15.23	10.18	10.97	13.38
	Jaggery	15.88	10.02	13.36	12.54	16.4	15.36	10.32	9.73	12.95
	Maize	16.63	11.38	14.32	13.69	17.86	15.4	10.78	11.98	14.01
	Mean	16.25	10.61	13.75	13.12	17.06	15.44	10.43	10.8	13.43
	SEM	0.894		0.894			1.264			0.632
	P Value	0.001***		0.336			0.133			0.706
TCHO	Control	103.22	65.30	87.32	81.20	109.60	96.84	65.04	65.57	84.26
	Glycerol	99.40	65.75	85.16	79.99	107.23	91.57	63.08	68.41	82.57
	Jaggery	98.64	65.76	85.94	78.46	103.35	93.94	68.55	62.98	82.20
	Maize	102.06	75.83	90.26	87.63	109.35	94.78	71.18	80.48	88.94
	Mean	100.83	68.16	87.17	81.82	107.38	94.28	66.96	69.36	84.49
	SEM	5.465		5.465			7.729			3.865
	P Value	0.001***		0.185			0.062			0.599

Values with different superscripts for absolute interaction for a respective energy source in a row under each parameter differ significantly; ***P ≤ 0.001

Table 4.39A: Difference (%) in proximate nutrient intake from Total diet in cows between pre and postpartum periods

Parameter	Energy × Breed		Energy × Suppl.		HFX × Suppl.		Deoni × Suppl.		E × B × S
	HFX	Deoni	Without	With	Without	With	Without	With	
DM	58	2	38	28	67	49	3	1	33
OM	56	1	36	26	65	47	2	0	31
CP	173	61	118	125	64	167	53	72	121
EE	64	0	38	31	64	64	0	0	31
TA	95	22	67	59	104	87	22	24	63
TCHO	42	-7	26	14	52	33	-5	-9	20
Min	42	-7	26	14	52	33	-5	-9	20
Max	173	61	118	125	104	167	53	72	121
Mean	81	13	54	47	69	75	13	15	50
CV	59	192	63	87	26	66	173	206	76

Table 4.40A: Difference (%) in proximate intake in cows (g/kgW^{0.75}) from Total diet between pre and postpartum periods

Parameter	Energy × Breed		Energy × Suppl.		HFX × Suppl.		Deoni × Suppl.		E × B × S
	HFX	Deoni	Without	With	Without	With	Without	With	
DM	74	12	48	37	84	64	13	11	42
OM	72	11	47	35	82	62	12	10	41
CP	197	79	133	140	201	194	69	89	137
EE	80	12	49	42	88	72	12	13	45
TA	115	35	78	70	124	106	34	36	74
TCHO	56	2	35	23	67	46	4	1	29
Min	56	2	35	23	67	46	4	1	29
Max	197	79	133	140	201	194	69	89	137
Mean	99	25	65	58	108	91	24	27	61
CV	52	112	56	75	46	60	102	122	65

contrast to without FNS supplementation which was 48% and 133%. Thus, the interaction effect between diet energy and FNS supplement in the prepartum period translated into 9% and 7% more DM and CP consumption during postpartum.

The difference in total diet DM consumption among HFX cows in postpartum due to FNS supplement they received in prepartum was 20% more than without supplement while CP consumption was -7%. In contrary, DM consumption in Deoni during postpartum because of FNS supplementation in prepartum resulted in only 2% more, while CP consumption was 20% more. Thus, FNS supplementation appeared to have more influence in stimulating the total diet DM consumption in HFX however, DM consumption in Deoni cows during postpartum was limited by the CP consumption because limitation in carboxypeptidase enzyme in their GI tract as explained by Singh and Srinivas (2020).

The total diet CC and HC consumption in postpartum were increased by 90%, and NFC consumption increased by 60% in HFX cows compared to prepartum, while it was 19% for CC or HC, and only 4% for NFC in Deoni cows (**Table 4.41**). NDF consumption during postpartum in comparison to prepartum was increased by 36% in HFX cows but, reduced by 10% in Deoni cows. Prepartum FNS supplementation had negative affect on postpartum CC, NDF, ADF, HC, Cellulose and NFC consumption on some cows and positive affect on some cows (**Table 4.41A**). CC, HC and NFC consumption in postpartum for the cows belonging to T2 and T3 during prepartum was significantly higher ($P < 0.02$) than cows in CG which was comparable to T1.

CC, NDF, HC, and NFC consumption ($\text{g/kg W}^{0.75}$) for metabolic tissue was significantly higher in ($P < 0.001$) postpartum HFX than Deoni cows (**Table 4.42**). CWC consumption ($\text{g/kgW}^{0.75}$) from the total diet was higher in prepartum Deoni than HFX cows. CC and HC consumption ($\text{g/kg W}^{0.75}$) in HFX cows during postpartum was increased by 109% and, NDF and NFC consumption was increased by 49% and 76%, respectively than prepartum. Contrarily, the NDF consumption by Deoni cows during postpartum was decreased by 1%. However, CC and HC consumption in Deoni cows was increased by 31% and NFC

Table 4.41: Cell and cell wall constituents intake from the total diet (kg/d) in different groups of postpartum cows

Parameter	Group	Energy × Breed		Energy × Suppl.		HFX × Suppl.		Deoni × Suppl.		E × B × S
		HFX	Deoni	Without	With	Without	With	Without	With	
CC	Control	5.90	3.07	4.42	4.54	5.78	6.02	3.07	3.07	4.48
	Glycerol	6.28	3.06	4.61	4.73	6.18	6.39	3.04	3.07	4.67
	Jaggery	6.48	3.36	5.00	4.84	6.56	6.41	3.44	3.28	4.92
	Maize	6.37	3.46	5.14	4.70	6.88	5.87	3.40	3.52	4.75
	Mean	6.26	3.23	4.79	4.70	6.35	6.17	3.24	3.23	4.75
	SEM	0.181		0.181		0.255		0.128		
	P Value	0.001***		0.491		0.509		0.08		
NDF	Control	6.35	3.23	4.85	4.74	6.47	6.24	3.23	3.23	4.79 ^a
	Glycerol	6.48	3.21	4.87	4.83	6.56	6.41	3.18	3.25	4.85 ^a
	Jaggery	6.80	3.81	5.49	5.12	7.02	6.89	3.96	3.66	5.30 ^b
	Maize	6.59	3.99	5.48	5.10	7.09	6.09	3.88	4.10	5.29 ^b
	Mean	6.56	3.56	5.17	4.94	6.78	6.33	3.56	3.56	5.06
	SEM	0.178		0.178		0.251		0.126		
	P Value	0.001***		0.10		0.095		0.01**		
ADF	Control	2.70	1.34	2.10	1.93	2.87	2.53	1.34	1.34	2.02 ^a
	Glycerol	2.60	1.32	2.02	1.91	2.73	2.48	1.30	1.35	1.96 ^a
	Jaggery	2.80	1.72	2.39	2.13	2.96	2.64	1.82	1.62	2.26 ^b
	Maize	2.66	1.84	2.30	2.19	2.85	2.47	1.76	1.91	2.25 ^b
	Mean	2.69	1.55	2.20	2.04	2.85	2.53	1.55	1.55	2.12
	SEM	0.083		0.083		0.117		0.059		
	P Value	0.001***		0.01**		0.014		0.004**		
HC	Control	3.65	1.90	2.74	2.81	3.59	3.72	1.90	1.90	2.78
	Glycerol	3.88	1.89	2.85	2.92	3.83	3.94	1.88	1.90	2.89
	Jaggery	4.01	2.09	3.11	3.00	4.07	3.96	2.14	2.04	3.05
	Maize	3.94	2.16	3.18	2.91	4.25	3.63	2.12	2.20	3.05
	Mean	3.87	2.01	2.97	2.91	3.93	3.81	2.01	2.01	2.94
	SEM	0.111		0.111		0.156		0.078		
	P Value	0.001***		0.436		0.445		0.06		

Parameter	Group	Energy × Breed		Energy × Suppl.		HFX × Suppl.		Deoni × Suppl.		E × B × S
		HFX	Deoni	Without	With	Without	With	Without	With	
Cellulose	Control	1.98	0.98	1.56	1.40	2.14	1.83	0.98	0.98	1.48 ^a
	Glycerol	1.88	0.96	1.47	1.37	2.00	1.78	0.95	0.98	1.42 ^a
	Jaggery	2.04	1.29	1.77	1.55	2.17	1.90	1.37	1.21	1.66 ^b
	Maize	1.92	1.39	1.69	1.62	2.06	1.79	1.33	1.45	1.65 ^b
	Mean	1.95	1.15	1.62	1.49	2.09	1.82	1.15	1.15	1.55
	SEM		0.066		0.066		0.094			0.047
	P Value		0.001***		0.01**		0.011			0.004**
ADL	Control	0.52	0.27	0.40	0.39	0.53	0.52	0.27	0.27	0.39 ^a
	Glycerol	0.53	0.26	0.40	0.40	0.54	0.53	0.26	0.27	0.40 ^a
	Jaggery	0.56	0.31	0.45	0.42	0.58	0.55	0.32	0.30	0.43 ^b
	Maize	0.54	0.32	0.45	0.42	0.59	0.50	0.32	0.33	0.43 ^b
	Mean	0.54	0.29	0.42	0.41	0.55	0.52	0.29	0.29	0.41
	SEM		0.015		0.015		0.021			0.01
	P Value		0.001***		0.126		0.154			0.02*
NFC	Control	2.64	1.36	1.99	2.01	2.62	2.66	1.36	1.36	2.00 ^a
	Glycerol	2.77	1.36	2.05	2.08	2.75	2.79	1.35	1.37	2.06 ^{ab}
	Jaggery	2.87	1.53	2.25	2.15	2.92	2.82	1.57	1.49	2.20 ^b
	Maize	2.81	1.58	2.29	2.10	3.03	2.59	1.55	1.61	2.19 ^b
	Mean	2.77	1.46	2.14	2.08	2.83	2.71	1.46	1.46	2.11
	SEM		0.077		0.077		0.109			0.054
	P Value		0.001***		0.312		0.312			0.05*

Table 4.42: Cell content and cell wall constituents intake from total diet (g/kgW^{0.75}/d) in postpartum cows

Parameter	Group	Energy × Breed		Energy × Suppl.		HFX × Suppl.		Deoni × Suppl.		E × B × S
		HFX	Deoni	Without	With	Without	With	Without	With	
CC	Control	67.77	43.65	56.68	57.74	69.98	65.66	43.47	43.83	55.71
	Glycerol	67.45	44.02	56.84	54.63	71.31	63.59	42.37	45.66	55.73
	Jaggery	66.13	41.52	55.46	52.20	68.21	94.05	42.71	40.34	53.83
	Maize	69.13	47.10	59.55	56.87	74.45	64.18	44.65	49.56	58.21
	Mean	67.67	44.07	57.13	54.61	70.96	64.37	43.30	44.85	55.87
	SEM	3.727		3.727		5.270				2.635
	P Value	0.001		0.353		0.142				0.711
NDF	Control	73.07	46.01	61.99	57.08	78.17	67.97	45.82	46.19	59.54
	Glycerol	69.75	46.31	60.02	56.04	75.65	63.85	44.39	48.22	58.03
	Jaggery	69.45	47.01	61.11	55.35	73.06	65.84	49.16	44.86	58.23
	Maize	71.60	54.43	63.82	62.22	76.66	66.55	50.97	57.89	63.02
	Mean	70.97	48.44	61.73	57.67	75.88	66.05	47.59	49.29	59.71
	SEM	3.847		3.847		5.441				2.720
	P Value	0.001		0.155		0.05				0.554
ADF	Control	31.08	19.02	26.83	23.26	34.73	27.43	18.94	19.10	25.05
	Glycerol	28.11	19.09	24.87	22.34	31.54	24.69	18.21	19.98	23.60
	Jaggery	28.57	21.17	26.69	23.06	30.83	26.32	22.55	19.80	24.87
	Maize	28.82	25.06	26.94	26.94	30.73	26.92	23.16	26.96	26.94
	Mean	29.15	21.08	26.33	23.90	31.95	26.34	20.17	21.46	25.12
	SEM	1.650		1.650		2.334				1.167
	P Value	0.001		0.053		0.02				0.282
HC	Control	41.99	26.99	35.16	33.82	43.44	40.54	26.88	27.10	34.49
	Glycerol	41.64	27.21	35.15	33.70	44.12	39.16	26.18	28.24	34.42
	Jaggery	40.87	25.85	34.43	32.29	42.23	39.52	26.63	25.07	33.36
	Maize	42.78	29.37	36.87	35.28	45.94	39.63	27.81	30.93	36.08
	Mean	41.82	27.35	35.40	33.77	43.93	39.71	26.87	27.83	34.59
	SEM	2.297		2.297		3.248				1.624
	P Value	0.001		0.330		0.13				0.704

Parameter	Group	Energy × Breed		Energy × Suppl.		HFX × Suppl.		Deoni × Suppl.		E × B × S
		HFX	Deoni	Without	With	Without	With	Without	With	
Cellulose	Control	22.88	13.86	19.87	16.87	25.93	19.83	13.81	13.92	18.37
	Glycerol	20.31	13.90	18.14	16.07	23.06	17.57	13.23	14.58	17.11
	Jaggery	20.80	15.87	19.82	16.85	22.63	18.96	17.00	14.75	18.34
	Maize	20.81	18.92	19.75	19.95	22.15	19.48	17.42	20.42	19.87
	Mean	21.20	15.64	19.40	17.44	23.44	18.96	15.36	15.91	18.42
	SEM	1.236		1.236		1.748				0.874
	P Value	0.001		0.039		0.011				0.214
ADL	Control	5.97	3.78	5.06	4.69	6.35	5.59	3.76	3.79	4.87
	Glycerol	5.74	3.80	4.92	4.62	6.20	5.28	3.64	3.96	4.77
	Jaggery	5.70	3.82	4.98	4.52	5.98	5.42	3.98	3.65	4.76
	Maize	5.89	4.40	5.22	5.07	6.31	5.47	4.13	4.68	5.15
	Mean	5.82	3.95	5.04	4.73	6.21	5.44	3.87	4.02	4.89
	SEM	0.315		0.315		0.446				0.223
	P Value	0.001		0.177		0.06				0.587
NFC	Control	30.27	19.36	25.43	24.20	31.58	28.97	19.28	19.44	24.82
	Glycerol	29.75	19.51	25.23	24.03	31.70	27.80	18.76	20.27	24.63
	Jaggery	29.30	18.84	24.94	23.20	30.41	28.20	19.48	18.20	24.07
	Maize	30.56	21.51	26.55	25.52	32.79	28.33	20.31	22.72	26.04
	Mean	29.97	19.81	25.54	24.24	31.62	28.32	19.46	20.15	24.89
	SEM	1.635		1.635		2.312				1.156
	P Value	0.001		0.277		0.104				0.679

Values with different superscripts for absolute interaction for a respective energy source in a row under each parameter differ significantly; ***P≤ 0.001

Table 4.41A: Difference (%) in proximate nutrient intake from Total diet in cows between pre and postpartum periods

Parameter	Energy × Breed		Energy × Suppl.		HFX × Suppl.		Deoni × Suppl.		E × B × S
	HFX	Deoni	Without	With	Without	With	Without	With	
CC	90	19	61	56	98	83	18	21	58
NDF	36	-10	22	9	46	26	-8	-13	15
ADF	-4	-32	-9	-23	7	-14	-28	-35	-17
HC	90	19	61	55	97	82	18	20	58
Cellulose	-14	-38	-16	-31	-3	-24	-34	-41	-24
ADL	50	0	35	24	62	41	4	-3	28
NFC	60	4	38	30	68	51	4	4	34
Min	-14	-38	-16	-31	-3	-24	-34	-41	-24
Max	90	19	61	56	98	83	18	21	58
Mean	44	-7	26	15	51	32	-5	-9	20
CV	94	-419	112	203	74	120	-570	-355	151

Table 4.42A: Difference (%) in CWC intake from Total diet(G/kgW^{0.75}) in cows between pre and postpartum periods

Parameter	Energy × Breed		Energy × Suppl.		HFX × Suppl.		Deoni × Suppl.		E × B × S
	HFX	Deoni	Without	With	Without	With	Without	With	
CC	109	31	73	67	117	101	29	34	70
NDF	49	-1	31	17	60	38	1	-4	24
ADF	6	-25	-2	-17	18	-6	-24	-28	-10
HC	109	31	73	65	117	100	29	32	69
Cellulose	-5	-31	-10	-26	7	-16	-28	-35	-18
ADL	67	11	48	31	79	55	13	9	39
NFC	76	13	48	39	85	67	13	14	44
Min	-5	-31	-10	-26	7	-16	-28	-35	-18
Max	109	31	73	67	117	101	29	34	70
Mean	59	3	35	23	67	46	4	1	29
CV	77	607	89	146	64	96	466	882	113

consumption was increased by 13%. In any case, their consumption in Deoni cows were comparably lesser than postpartum HFX (g/kg $W^{0.75}$) cows (**Table 4.42 A**). The CC, NDF, HC and NFC consumption during the postpartum was increased by 67%, 17%, 65% and 39%, respectively for cows those received energy and FNS supplement during prepartum. Consumption (g/kg $W^{0.75}$) of CC, NDF, HC and NFC was increased by 73%, 31%, 73% and 48%, respectively during postpartum in those cows provided additional energy but without FNS supplement during prepartum. This was higher by 5%, 17%, 6% and 9% respectively in CC, NDF, HC and NDF consumption (g/kg $W^{0.75}$) during postpartum in cows those did not provide FNS supplement along with energy during prepartum. The difference in CC, NDF, HC and NFC consumption (g/kg $W^{0.75}$) in HFX cows during the postpartum period was -6%, -14%, -8% and -9%, respectively in cows those fed because FNS supplement than HFX cows without FNS supplement. During prepartum contrarily, Deoni cows CC, HC and celluloses consumption during postpartum was increased by 5%, 3% and 1% respectively, because prepartum FNS supplement they received than cows without FNS supplement. No significant difference in the CG and TGs for CWC consumption from the total diet (g/kg $W^{0.75}$) in the postpartum period indicated no follow-up effect of experimental diets they received during the prepartum period.

4.3.5.4 Digestible nutrient intake

Though digestible DM, OM, CP, EE and TCHO consumption in terms of MBW was comparable during the prepartum the same was found significantly higher in ($P < 0.001$) in HFX cows than Deoni cows during the postpartum due to 50% increase in the DM consumption (g/kg $W^{0.75}$) from total diet (**Table 4.43**). During the postpartum, the digestible DM consumption (g/kg $W^{0.75}$) in HFX cows and Deoni cows were more by 84% and 21%, respectively than their intake during prepartum.

The digestible nutrient intake from DM, OM, TCHO was decreased in postpartum by 11%, 12% and 15% in cows provided energy and FNS supplement during prepartum than those cows provided only energy but without FNS

supplement. But, digestible CP intake in postpartum was increased (169%) in cows those provided FNS supplement than without FNS supplement (152%) in prepartum (**Table 4.43A**). Postpartum digestible DM, TCHO and CP consumption (g/kg $W^{0.75}$) in HFX cows due to prepartum FNS supplementation lead to a difference of -21%, -23% and -8% respectively, than cows without supplement during this period. Contrary to this, Deoni cows prepartum supplementation lead to increase the digestible CP and EE consumption in postpartum by 34% and 20% respectively, compared to cows without prepartum FNS supplement. Energy alone fortification in diets during prepartum had no interactive effect on the postpartum digestible nutrient intake between CG and TGs.

The digestible CC, NDF, Cellulose and NFC consumption (g/kg $W^{0.75}$) was found significantly ($P < 0.001$) higher during the postpartum in HFX cows than Deoni cows (**Table 4.44**). During postpartum, the digestible CC, NDF and NFC consumption were increased by 119%, 50% and 59% respectively, in HFX cows. But, postpartum CC and NDF digestible nutrient consumption in Deoni cows increased by only 39% and 5%, respectively. During the postpartum, NDF and NFC digestible nutrient consumption in prepartum energy without FNS supplement (g/kg $W^{0.75}$) was increased by 18% and 24% than those cows received energy and FNS supplement (**Table 4.44A**). These were increased by 37% and 33% during the postpartum in the cows those provided energy with FNS supplement during prepartum. The digestible CC, NDF and NFC intake from HFX cows during the postpartum were 113%, 35% and 50% respectively, because prepartum FNS supplement. These were however, 8%, 30% and 17%, respectively was lesser than without prepartum FNS supplement. In Deoni cows, digestible CC consumption was increased by 9% and digestible NDF consumption was decreased by 7% in cows provided FNS supplement during prepartum. The no significant difference in CG and TGs for digestible nutrient intake from CWC due to prepartum energy and FNS supplement, and breed interaction indicated that interactive influence of experimental diets fed to CG and TG during the prepartum.

Table 4.43: Digestible nutrient intake (Proximate composition) from total diet (g/kgW^{0.75}/d) in postpartum cows

Parameter	Group	Energy × Breed		Energy × Suppl.		HFX × Suppl.		Deoni × Suppl.		E × B × S
		HFX	Deoni	Without	With	Without	With	Without	With	
DM	Control	82.85	54.79	71.07	66.57	86.85	78.86	55.30	54.28	68.82
	Glycerol	83.24	54.58	70.75	67.07	89.09	77.40	52.41	56.75	68.91
	Jaggery	81.25	53.12	70.21	64.16	85.84	76.66	54.59	51.66	67.19
	Maize	85.89	61.54	75.56	71.87	93.11	78.67	58.01	65.07	73.71
	Mean	83.31	56.01	71.90	67.42	88.72	77.89	55.07	56.94	69.66
	SEM	4.768		4.768			6.742			3.371
	P Value	0.001***		0.202			0.078			0.567
OM	Control	78.63	51.57	67.37	62.82	82.66	74.59	52.08	51.05	65.10
	Glycerol	78.26	51.06	66.66	62.66	84.04	72.49	49.28	52.84	64.66
	Jaggery	76.70	49.86	66.18	60.39	80.91	72.50	51.44	48.29	63.28
	Maize	80.99	57.73	71.19	67.53	87.85	74.13	54.53	60.93	69.36
	Mean	78.64	52.55	67.85	63.35	83.86	73.43	51.83	53.28	65.60
	SEM	4.397		4.397			6.219			3.109
	P Value	0.001***		0.167			0.074			0.559
CP	Control	11.96	7.72	9.69	10.00	11.59	12.34	7.79	7.66	9.84
	Glycerol	12.17	8.27	10.18	10.25	12.33	12.01	8.04	8.50	10.22
	Jaggery	12.05	6.82	9.56	9.31	12.46	11.64	6.65	6.98	9.43
	Maize	13.14	7.78	10.89	10.03	14.31	11.97	7.47	8.09	10.46
	Mean	12.33	7.64	10.08	9.90	12.67	11.99	7.49	7.81	9.43
	SEM	0.759		0.759			1.073			0.537
	P Value	0.001***		0.737			0.362			0.566
EE	Control	1.63	1.08	1.40	1.32	1.70	1.57	1.10	1.06	1.36
	Glycerol	1.74	1.13	1.47	1.40	1.83	1.64	1.10	1.17	1.43
	Jaggery	1.64	1.02	1.36	1.30	1.70	1.58	1.03	1.02	1.33
	Maize	1.79	1.22	1.55	1.47	1.93	1.65	1.16	1.29	1.51
	Mean	1.70	1.11	1.44	1.37	1.79	1.61	1.10	1.31	1.41
	SEM	0.098		0.098			0.139			0.069
	P Value	0.001***		0.297			0.094			0.454
TCHO	Control	62.67	41.20	54.35	49.53	67.06	58.29	41.64	40.77	51.94
	Glycerol	61.89	40.11	53.04	49.06	67.35	56.44	38.62	41.55	51.05
	Jaggery	60.64	40.67	53.38	47.93	64.36	56.92	42.41	38.94	50.65
	Maize	63.55	47.26	56.70	54.11	68.91	58.20	44.49	50.03	55.40
	Mean	62.21	42.31	54.37	50.15	66.93	57.49	41.80	42.84	52.26
	SEM	3.518		3.518			4.975			2.487
	P Value	0.001***		0.110			0.051			0.535

Values with different superscripts for absolute interaction for a respective energy source in a row under each parameter differ significantly; ***P≤ 0.001

Table 4.44: Digestible cell content and cell wall intake from the total diet (g/kg W^{0.75}/d) in postpartum cows

Parameter	Group	Energy × Breed		Energy × Suppl.		HFX × Suppl.		Deoni × Suppl.		E × B × S
		HFX	Deoni	Without	With	Without	With	Without	With	
CC	Control	47.27	30.66	39.39	38.55	48.09	46.46	30.69	30.63	38.97
	Glycerol	48.10	30.98	40.09	39.98	50.50	45.69	29.69	32.28	39.54
	Jaggery	46.18	29.19	38.82	36.54	47.91	44.44	29.73	28.64	37.68
	Maize	48.88	33.09	41.96	40.01	52.69	45.08	31.23	34.94	40.98
	Mean	47.61	30.98	40.07	38.52	49.80	45.42	30.33	31.62	39.29
	SEM	2.659		2.659		3.760				1.880
	P Value	0.001***		0.423		0.151				0.668
NDF	Control	35.23	23.90	31.40	27.73	38.42	32.04	24.38	23.42	29.56
	Glycerol	34.78	23.36	30.35	27.79	38.21	31.35	22.49	24.23	29.07
	Jaggery	34.72	23.73	31.11	27.34	37.57	31.87	24.65	22.82	29.22
	Maize	36.62	28.23	33.28	31.57	44.01	33.24	25.56	29.90	32.42
	Mean	35.33	24.80	31.53	28.60	38.55	32.12	24.52	25.09	30.07
	SEM	2.211		2.211		3.127				1.564
	P Value	0.001***		0.079		0.04				0.407
ADF	Control	11.28	7.43	10.16	8.55	12.61	9.94	7.70	7.16	9.35
	Glycerol	9.84	6.42	8.75	7.50	11.43	8.24	6.08	6.76	8.13
	Jaggery	10.60	8.75	11.36	7.99	13.01	8.19	9.71	7.78	9.67
	Maize	10.59	10.18	11.13	9.70	13.29	8.03	8.98	11.38	10.42
	Mean	10.57	8.19	10.35	8.43	12.59	8.60	8.12	8.27	9.39
	SEM	0.829		0.829		1.172				0.586
	P Value	0.001***		0.01**		0.01**				0.084

Parameter	Group	Energy × Breed		Energy × Suppl.		HFX × Suppl.		Deoni × Suppl.		E × B × S
		HFX	Deoni	Without	With	Without	With	Without	With	
HC	Control	23.95	16.47	21.24	19.18	25.80	22.10	16.68	16.26	20.21
	Glycerol	24.94	16.94	21.59	20.29	26.78	23.11	16.41	17.48	20.94
	Jaggery	24.11	14.98	19.75	19.35	24.56	23.67	14.94	15.03	19.55
	Maize	25.97	18.05	22.15	21.87	26.73	25.22	17.58	18.52	22.01
	Mean	24.74	16.61	21.18	20.17	25.96	23.52	16.40	16.82	20.68
	SEM	1.677		1.677		2.372				1.186
	P Value	0.001***		0.407		0.245				0.516
Cellulose	Control	10.19	6.16	9.23	7.12	12.27	8.12	6.21	6.12	8.18
	Glycerol	9.20	6.10	8.48	6.82	11.04	7.36	5.92	6.29	7.65
	Jaggery	9.44	8.11	10.06	7.50	11.28	7.61	8.83	7.39	8.78
	Maize	9.48	10.01	10.03	9.46	10.84	8.12	9.22	10.81	9.75
	Mean	9.58	7.60	9.45	7.72	11.35	7.80	7.54	7.65	8.59
	SEM	0.847		0.847		1.198				0.599
	P Value	0.01**		0.01**		0.01**				0.121
NFC	Control	25.57	17.38	23.06	21.88	28.79	26.35	17.34	17.42	22.47
	Glycerol	27.30	16.83	22.78	21.35	29.32	25.28	16.24	17.42	22.06
	Jaggery	26.03	17.03	22.38	20.68	26.91	25.15	17.85	16.21	21.53
	Maize	27.03	19.14	23.52	22.65	29.01	25.06	18.03	20.25	23.08
	Mean	26.98	17.59	22.93	21.64	28.50	25.46	17.36	17.82	22.29
	SEM	1.403		1.403		1.984				0.992
	P Value	0.001***		0.211		0.096				0.728

Values with different superscripts for absolute interaction for a respective energy source in a row under each parameter differ significantly; ***P ≤ 0.001, **P ≤ 0.01

Table 4.43 A: Difference (%) in proximate digestible nutrient intake (g/kg $W^{0.75}$) in cows between pre and postpartum periods

Parameter	Energy × Breed		Energy × Suppl.		HFX × Suppl.		Deoni × Suppl.		E × B × S
	HFX	Deoni	Without	With	Without	With	Without	With	
DM	84	21	58	47	94	73	21	22	52
OM	82	19	56	44	93	70	20	19	50
CP	228	95	152	169	232	224	79	113	146
EE	110	31	73	65	118	104	31	51	70
TCHO	63	9	43	28	74	51	11	7	36
Min	63	9	43	28	74	51	11	7	36
Max	228	95	152	169	232	224	79	113	146
Mean	113	35	77	71	122	104	32	42	71
CV	59	98	57	80	51	67	84	101	62

Table 4.44 A: Difference (%) in CWC digestible nutrient intake (g/kg $W^{0.75}$) in cows between pre and postpartum periods

Parameter	Energy × Breed		Energy × Suppl.		HFX × Suppl.		Deoni × Suppl.		E × B × S
	HFX	Deoni	Without	With	Without	With	Without	With	
CC	119	39	79	78	125	113	34	43	78
NDF	50	5	37	18	65	35	8	1	27
ADF	-6	-23	-16	-35	17	-27	-15	-30	-14
HC	101	28	65	61	106	96	25	30	63
Cellulose	-57	-67	-56	-67	-48	-65	-64	-69	-62
NFC	59	1	33	24	67	50	1	1	29
Min	-57	-67	-56	-67	-48	-65	-64	-69	-62
Max	119	39	79	78	125	113	34	43	78
Mean	44	-4	22	11	53	30	-3	-5	18
CV	149	-1232	217	423	114	205	-1776	-1080	254

4.3.5.5 Digestibility coefficient of nutrients

Digestibility is a relative comparison of digestible nutrient intake and apparent retention of nutrients in the alimentary canal. Digestibility of Proximate principles viz., DM, OM, CP, EE and TCHO (**Table 4.45**) and CWC viz., CC, NDF, ADF, HC, Cellulose and NFC (**Table 4.46**) were comparable between postpartum HFX and Deoni cows and there was no breed difference. CP and EE digestibility in postpartum cows were 10% and 15% higher in both HFX and Deoni cows than their digestibility in prepartum. Prepartum energy with FNS supplement increased the CP digestibility by 4% in postpartum than prepartum energy without FNS supplement. Only in Deoni cow's, FNS supplementation during prepartum augmented CP digestibility by 13% in postpartum compared to cows those without FNS supplement (6%) in prepartum (**Table 4.45 A**). Though digestible nutrient intake per kg MBW was higher in HFX cows than Deoni cows during the postpartum no significant change was observed in postpartum DM, OM, CP, EE and TCHO digestibilities because of prepartum energy and FNS supplement. The feed passage rate through the gastrointestinal tract was more quicker in HFX cows in comparison to Deoni cows owing to higher DM requirement and intake. The postpartum NDF and NFC digestibility was increased by 6% and 5% in Deoni cows. Whereas no difference was observed in NDF digestibility in postpartum HFX cows in comparison to the prepartum period. The NFC digestibility in HFX cows during postpartum was higher by 7% than prepartum (**Table 4.46 A**). Prepartum energy with FNS supplementation provided to cows was improved CC digestibility by 4% but decreased NDF digestibility by 4% during postpartum. Overall no significant difference in CG and TG because of interaction of dietary energy and FNS supplement with the breed of cows in prepartum indicated that no adverse effect on postpartum nutrient digestibility when close-up cows moved to lactation which is a good indication to retained appetite and gut health.

4.3.5.6 Nutritive value of the diet

Nutritive value of the postpartum diet is presented in **Table 4.48**. The DCP% in postpartum diets was 8 to 9% in contrast to 4 to 5% in prepartum.

Table 4.45: Digestibility coefficient (Proximate composition) of the total diet in postpartum cows

Parameter	Group	Energy × Breed		Energy × Suppl.		HFX × Suppl.		Deoni × Suppl.		E × B × S
		HFX	Deoni	Without	With	Without	With	Without	With	
DM	Control	58.67	60.95	60.12	59.50	58.52	58.83	61.73	60.18	59.81
	Glycerol	60.46	60.24	60.27	60.43	60.5	60.56	60.19	60.30	60.35
	Jaggery	59.73	59.88	59.94	59.67	60.61	58.86	59.27	60.49	59.81
	Maize	60.72	60.48	60.95	60.25	61.37	60.07	60.53	60.43	60.60
	Mean	59.89	60.39	60.32	59.96	60.21	59.58	60.43	60.35	60.14
	SEM		0.419		0.419		0.593		0.593	0.296
P Value		0.116		0.246		0.364		0.364	0.118	
OM	Control	61.82	63.69	63.17	62.33	61.78	61.85	64.56	62.82	62.75
	Glycerol	63.13	62.58	63.02	62.69	63.20	63.05	62.85	62.32	62.85
	Jaggery	62.16	62.28	62.63	62.26	63.85	61.84	61.87	62.69	62.44
	Maize	63.65	62.84	63.69	62.80	64.35	62.96	63.04	62.64	63.25
	Mean	62.80	62.85	63.13	62.52	63.18	62.43	63.08	62.61	62.82
	SEM		0.423		0.423		0.599		0.599	0.299
P Value		0.884		0.059		0.636		0.636	0.335	
CP	Control	62.61	61.91	61.32	63.19	60.07	65.16	62.58	61.23	62.26
	Glycerol	62.31	65.58	63.34	64.55	60.61	64.02	66.06	65.09	63.94
	Jaggery	63.49	59.83	60.86	62.46	64.41	62.57	57.31	62.35	61.66
	Maize	65.30	60.98	63.60	62.68	65.90	64.70	61.30	60.67	63.14
	Mean	63.43	62.07	62.28	63.22	62.75	64.11	61.82	62.33	62.75
	SEM		1.148		1.148		1.632		1.632	0.812
P Value		0.115		0.263		0.612		0.612	0.247	
EE	Control	65.19	67.10	66.53	65.76	64.84	65.54	68.21	63.98	66.14
	Glycerol	66.68	67.73	67.27	67.14	67.08	66.28	67.45	68.01	67.20
	Jaggery	67.38	65.68	65.57	67.49	67.46	67.30	63.68	67.68	66.53
	Maize	69.06	67.45	68.37	68.14	69.49	68.64	67.26	67.64	68.26
	Mean	67.08	66.99	66.93	67.13	67.22	66.94	66.65	67.32	67.03
	SEM		1.458		1.458		2.062		2.062	1.031
P Value		0.932		0.850		0.650		0.650	0.508	
TCHO	Control	60.69	63.07	62.59	61.16	61.20	60.18	63.99	62.15	61.88
	Glycerol	62.24	60.97	61.96	61.25	62.73	61.78	61.20	60.75	61.61
	Jaggery	61.42	61.83	62.04	61.21	62.25	60.59	61.83	61.83	61.62
	Maize	62.29	62.30	62.75	61.84	63.01	61.56	62.48	62.13	62.29
	Mean	61.66	62.04	62.33	61.37	62.30	61.02	62.37	61.71	61.85
	SEM		0.532		0.532		0.752		0.752	0.376
P Value		0.320		0.20		0.425		0.425	0.550	

Table 4.46: Digestibility coefficient of cell and cell wall constituents of the total diet in postpartum cows

Parameter	Group	Energy × Breed		Energy × Suppl.		HFX × Suppl.		Deoni × Suppl.		E × B × S
		HFX	Deoni	Without	With	Without	With	Without	With	
CC	Control	68.81	70.28	69.72	70.37	68.81	70.18	70.63	69.93	70.04
	Glycerol	71.27	70.35	70.37	71.26	70.73	71.82	70.00	60.69	70.81
	Jaggery	69.82	70.31	69.94	70.19	70.26	69.39	69.62	71.00	70.06
	Maize	70.47	70.27	70.47	70.27	70.93	70.02	70.02	70.52	70.37
	Mean	70.34	70.30	70.12	70.52	70.18	70.51	70.07	70.53	70.32
	SEM	0.546		0.546		0.772				0.386
	P Value	0.911		0.319		0.861				0.481
NDF	Control	48.10	51.87	51.07	48.89	49.14	47.06	53.01	50.73	49.98
	Glycerol	49.73	50.42	50.48	49.68	50.37	49.10	50.59	50.26	50.08
	Jaggery	49.89	50.46	50.73	49.62	51.38	48.41	50.09	50.83	50.17
	Maize	51.10	51.82	51.98	50.93	51.94	50.26	52.02	51.61	51.46
	Mean	49.70	51.14	51.07	49.78	50.71	48.70	51.42	50.85	50.42
	SEM	1.017		1.017		1.438				0.719
	P Value	0.063		0.092		0.334				0.449
ADF	Control	36.36	38.83	38.26	36.93	36.22	36.50	40.29	37.36	37.59
	Glycerol	34.68	33.20	34.37	33.51	36.03	33.33	32.71	33.70	33.94
	Jaggery	36.57	41.13	42.60	35.10	42.18	30.96	43.03	39.24	38.85
	Maize	36.16	40.48	40.90	36.62	43.17	30.90	38.62	42.34	38.76
	Mean	35.94	38.41	39.03	35.54	39.40	39.92	38.66	38.16	37.28
	SEM	2.092		2.092		2.958				1.479
	P Value	0.148		0.031*		0.061				0.104

Parameter	Group	Energy × Breed		Energy × Suppl.		HFX × Suppl.		Deoni × Suppl.		E × B × S
		HFX	Deoni	Without	With	Without	With	Without	With	
HC	Control	56.80	61.03	60.69	57.14	59.46	54.14	61.92	60.14	58.92
	Glycerol	59.85	62.37	61.71	60.51	60.62	59.08	62.81	61.94	61.11
	Jaggery	58.99	58.04	57.08	59.95	58.10	59.89	56.07	60.01	58.51
	Maize	60.60	61.44	60.49	61.56	57.81	63.39	63.17	59.72	61.02
	Mean	59.06	60.72	59.99	59.79	58.99	59.13	60.99	60.45	59.89
	SEM		1.581		1.581		2.235		1.118	
	P Value		0.157		0.858		0.768		0.253	
Cellulose	Control	44.22	44.16	45.81	42.58	47.32	41.12	44.29	44.04	44.19
	Glycerol	44.51	43.60	45.95	42.15	47.56	41.45	44.34	42.85	44.05
	Jaggery	44.78	50.82	50.56	45.03	49.57	39.98	51.56	50.08	47.80
	Maize	45.89	52.68	50.99	45.57	49.26	42.51	52.72	52.64	49.28
	Mean	44.85	47.81	48.33	44.33	48.43	41.26	48.23	47.40	46.33
	SEM		2.696		2.696		3.813		1.907	
	P Value		0.139		0.054		0.116		0.172	
NFC	Control	91.17	89.76	90.64	90.29	91.22	91.13	90.07	89.44	90.46
	Glycerol	91.66	86.14	89.38	88.42	92.35	90.97	86.41	85.87	88.90
	Jaggery	88.83	90.35	90.06	89.12	88.48	89.19	91.65	89.05	89.59
	Maize	88.69	89.00	89.00	88.69	89.13	88.26	88.87	89.13	88.85
	Mean	90.08	88.81	88.77	89.13	90.29	89.89	89.25	88.37	89.45
	SEM		1.417		1.417		2.004		1.002	
	P Value		0.221		0.531		0.817		0.643	

Table 4.45A: Difference (%) in proximate digestibility in cows between pre and postpartum periods

Parameter	Energy × Breed		Energy × Suppl.		HFX × Suppl.		Deoni × Suppl.		E × B × S
	HFX	Deoni	Without	With	Without	With	Without	With	
DM	5	8	6	7	6	5	7	9	7
OM	6	8	7	7	6	6	7	8	7
CP	10	9	8	12	10	10	6	13	10
EE	15	15	14	15	14	16	15	15	15
TCHO	4	6	6	5	5	3	6	6	5
Min	4	6	6	5	5	3	6	6	5
Max	15	15	14	15	14	16	15	15	15
Mean	8	9	8	9	8	8	8	10	9
CV	55	36	43	47	47	61	44	35	44

Table 4.46A: Difference (%) in CWC digestibility in cows between pre and postpartum periods

Parameter	Energy × Breed		Energy × Suppl.		HFX × Suppl.		Deoni × Suppl.		E × B × S
	HFX	Deoni	Without	With	Without	With	Without	With	
CC	5	6	3	7	4	6	4	8	5
NDF	0	6	5	1	3	-2	7	5	3
ADF	-12	2	3	-13	-1	-5	8	-3	-5
HC	-4	-2	-4	-2	-5	-2	-4	-1	-3
Cellulose	-14	-5	-4	-15	-7	-21	-1	-8	-9
NFC	7	5	4	6	5	8	5	5	6
Min	-14	-5	-4	-15	-7	-21	-4	-8	-9
Max	7	6	5	7	4	6	8	8	5
Mean	-3	1	1	-4	-1	-5	3	0	-2
CV	-281	245	373	-391	-2971	-371	150	708	-1035

Postpartum dietary DCP% is optimum for lactating cows because the maximum DCP% of the rations for dairy cow recommended is $\leq 12\%$. DCP% of diet in postpartum between HFX and Deoni breeds was significantly different ($P < 0.01$) but comparable in prepartum diet ($P = 0.66$). TDN% in diet consumed by HFX and Deoni cows either postpartum ($P = 0.47$) or prepartum ($P = 0.14$) were statistically comparable. The difference in postpartum and prepartum diets was conspicuous for DCP which was two folds more than TDN. The NR of postpartum and prepartum diets was 1:6 and 1:11. It appears that energy intake was lesser than DCP in the postpartum where the recommended NR for lactating cows was 1:9 (Reddy, 2001) while ME was suggested $> 48\%$ (Gabler and Heinrichs, 2003). The NR of the diets was decreased by 48% in HFX and 43% in Deoni cows during postpartum due to higher CP consumption that was necessary for lactation.

RFQ index of diets was 84% and 72% higher in HFX and Deoni cows, respectively compared to prepartum diets. The RFQ of postpartum diets of Energy \times HFX cows and HFX cows \times without FNS supplement during the prepartum period was more than 140% and above the suggested range of 140% to 160% for lactating cows up to 200 d of lactation (Undersander and Moore 2002). The improvement in RFQ for said groups was two fold between postpartum and prepartum. The RFQ of postpartum diets of Energy \times Deoni cows and Deoni cows \times without FNS supplement during the prepartum period was below 125% that was below the quality recommended for late lactation cows (125% to 150%) i.e., beyond 200 days of lactation (Undersander and Moore, 2002). The RFQ value of the postpartum diets of Deoni cows with FNS supplement in the prepartum was improved by 5% compared to without FNS supplement in the prepartum while contrary was true in case of HFX cows where about 25% lesser RFQ value was observed. Such a difference in HFX and Deoni cows due to interaction of dietary energy or FNS supplement during prepartum could be due to consumption difference in digestible DM and its requirement (Jeranyama and Garcia, 2004). Breed and FNS supplement interaction effect of diet in prepartum and discontinuation of energy fortification or FNS supplement or both together was more in HFX cows than Deoni cows owing to the difference in BW and level of milk production.

Nutrients (%) consumption during postpartum in comparison to standard requirements (ICAR, 2013) of lactating cows is presented in **Table 4.47**. The HFX and Deoni cows consumed 25% and 29% lesser DM, respectively during postpartum than the lactation requirement and such decline in DMI cannot be avoided even during the first 2 weeks when cows force-fed during prepartum (Bertics *et al.*, 1992). The decrease in DMI in fresh cows related to shift in homeorhetic factors, changes in gene regulation in liver, mammary gland and adipocyte regulation (Loore *et al.*, 2006). Grummer *et al.* (2004) reported that the rate of increase or decrease in DMI during lactation is a primary factor that dictates both energy intake and balance. DMI is important however, if protein and energy requirement are fulfilled in less than stipulated quantities, such diet is good for minimal total discomfort of the cows (Forbes and Provenza, 2000). The decreased DMI in early lactation is challenged by compensating the energy density of the diet by replacing the forage with more CS. Roughage consumption by HFX and Deoni cows was 3.65 kg/day and 2.68 kg/day, respectively (**Table 4.35**). The decreased roughage by the postpartum cow allow to consume more energy and that leads to decrease rumination, rumen buffering, rumen pH and eventually leads to rumen acidosis that is a predisposing factor to laminitis, particularly in HFX cows where the requirement of CS was more. The pain of laminitis compounds the problems of NEB because food intake decreases as cow spend more time in rest and less time to eat. The ratio of roughage to CS was 30:70 in fresh HFX cows and 40:60 in fresh Deoni cows. The increased nutrient requirement during the fresh period was compensated by CS in both breed. One of the reason for laminitis in HFX cows was not only the CS to roughage ratio but also the quantity of CS consumed also important because HFX cows consumed CS of 9.20 kg/day compared to 4.48 kg/day by Deoni cows (**Table 4.37**). The DMI of a cow weighing 450, 550 and 650 kg and 10 kg/day milk yield consumes 2.6%, 2.3% and 2.1% of the live BW, respectively (Trupa *et al.*, 2015). Lead feeding suggests, feeding grains 1% of cows' BW from 2 weeks before calving to improve appetite in postpartum and must avoid bicarbonates in diets.

The ME intake in prepartum was 15.68 Mcal/day (65.61 MJ/day) by HFX cows and 12.71 Mcal/day (53.18 MJ/day) by Deoni cows. In postpartum, ME intake was 28.92 Mcal/day (121.02 MJ/day) in HFX cows and 15.20 Mcal/day

(63.60 MJ/day) in Deoni cows which was almost 83% and 20% higher than prepartum ME intake, respectively. This was short of 16% and 13% of their recommended intake for lactation (ICAR, 2013) but, as per the recommended requirements of the cow in the humid tropics (NRC, 2001). the Deoni cows requirements (64.75 MJ/d) was less by only 1.15 MJ/day or 1.8% but, HFX cows requirement was short of 21 MJ/day (121.02 MJ/day Vs. 142.40 MJ/day) that was equal to 15%. According to FAO (1993), HFX cows ME intake for lactation was 10.88 MJ/day or 8% less but, in Deoni cows, it was 2.30 MJ/day or 3.8% more than required. As per different feeding requirements, the ME intake in postpartum HFX cows was ranged from -8 to -16% and Deoni cows was -13% to +4%.

CP intake in prepartum (**Table 4.15**) was 660 g/d and 560 g/d in HFX and Deoni cows, respectively which according to NRC (2001) is almost equivalent to lactating cow producing 4% fat corrected milk yield of 4½ to 7½ kg/day. In such a case, the same prepartum diet is sufficient to the Deoni cows in postpartum also to meet only lactation requirements but not for HFX cows. The CP consumption in postpartum was 1800 g/d and 900 g/d in HFX and Deoni cows (**Table 4.37**), respectively. This CP consumed in postpartum by HFX cows was 10% more than ICAR (2013) recommendation. In the case of Deoni cows, CP consumption in postpartum was just 1% more than the recommended requirement (ICAR, 2013). CP consumption in postpartum by HFX cows is 0.84% higher than recommended 1788 g/day (NRC, 2001). According to FAO (1993), the CP requirement for the HFX cows based on the observed milk yield is 1785 g/d which was 1% lesser than the actual consumption but, Deoni cows' intake was 30% higher than the requirement that is 693 g/day.

The positive value of residual DP_{FSF} indicated that the cows consumed more CP than the recommended requirements discussed above. Contrary to CP, negative residual ME_{FSF} indicated that the energy consumption was less than the maximum recommended. It was -16 and -13% lesser in HFX and Deoni cows, respectively on the upper side or maximum NEB was 15%.

4.3.5.7 Energy Value of the diet

The increased demand for energy for milk synthesis leads to NEB in fresh cows (Ingvartsen *et al.*, 2003). Postpartum energy values of the diets are presented in **Table 4.49**. GE, DE, ME, and ER (kcal/kgW^{0.75}) were significantly higher in HFX cows than Deoni cows. Postpartum HFX cows and Deoni cows consumed 70% and 10% more GE than its intake in the prepartum. During postpartum, DE and ME in HFX cows were higher by 83 to 84% and in Deoni cows by 19 to 20% than their intake during prepartum. The Q-value of the diet in postpartum and prepartum were more or less comparable because there was proportionate increase in ME of the diets in postpartum with increased nutrient demand. ER in the postpartum period was higher by 261% in HFX cows and 64% in Deoni cows (**Table 4.45A**) because of the change in physiological state from close-up to fresh cows. HI of HFX cows and Deoni cows in postpartum was about 60 kcal/kgW^{0.75} and 15 kcal/kgW^{0.75}, respectively higher than HI in prepartum. Heat losses are more in pregnancy than lactation (Srinivas and Sankhyan, 2010). Energy efficiency during pregnancy is $\leq 20\%$ in comparison to 60% in lactation. Energy efficiency observed for cows during prepartum was 17% in HFX or Deoni cows but in postpartum, it was 35% for HFX cows and only 23% in Deoni cows. Saha *et al.* (2012) reported 35% efficiency in lactating *Bos Indicus* between early to mid-lactation. Even the efficiency of Deoni cows was only 35% compared to 60% or more in crossbred cows in the later stages of lactation (Srinivas and Sreenath, 2011). In 1st month of lactation, the energy efficiency for lactation is lesser than above 60 days of lactation. During pregnancy a lot of energy lost in heat increment from cow and gravid uterus (Srinivas and Snakhyan 2008) while in early lactation NEB is the issue (Dębski *et al.*, 2017). The energy efficiency in HFX cows was higher than Deoni cows in postpartum (Singh and Srinivas, 2020).

FNS supplement during the prepartum had no significant improvement in GE, DE, ME, ER and HI except for little numerically higher values, however, these energy terms in postpartum were lesser with supplement than without FNS supplement that accounted to 6% to 7%.

Table 4.47: Nutrient intake (%) in postpartum cows in comparison to standard requirements of lactating cows

Parameter	Group	Energy × Breed		Energy × Suppl.		HFX × Suppl.		Deoni × Suppl.		E × B × S
		HFX	Deoni	Without	With	Without	With	Without	With	
DM	Control	83.61	72.83	80.12	76.32	91.09	76.12	69.14	76.51	78.22
	Glycerol	70.57	72.30	76.62	70.24	75.23	65.90	70.01	74.58	71.43
	Jaggery	72.11	66.29	71.20	67.20	77.28	66.93	65.12	67.46	69.20
	Maize	73.80	75.67	72.87	76.61	77.46	70.14	68.27	83.08	74.74
	Mean	75.02	71.77	74.20	72.59	80.27	69.78	68.14	75.41	73.39
	SEM	7.127		7.127		10.081		5.04		
	P Value	0.528		0.753		0.977		0.618		
DCP	Control	112.85	138.76	118.35	133.26	106.65	119.04	130.05	147.47	125.80
	Glycerol	104.08	148.97	122.29	130.76	102.97	105.19	141.61	156.37	126.53
	Jaggery	107.88	125.54	115.44	117.97	114.33	101.43	116.56	134.52	116.71
	Maize	115.83	128.04	119.42	124.46	115.36	116.31	123.48	132.60	121.94
	Mean	110.16	135.33	118.88	126.16	109.83	110.49	127.93	142.73	122.74
	SEM	12.635		12.635		17.869		8.94		
	P Value	0.01**		0.399		0.956		0.858		
ME	Control	88.44	86.71	89.59	85.57	94.80	82.09	84.37	89.05	87.57
	Glycerol	78.77	87.33	83.00	83.10	81.30	76.25	84.71	89.95	83.05
	Jaggery	82.64	80.71	84.41	78.93	89.26	76.02	79.57	81.84	81.67
	Maize	85.79	91.79	86.88	90.69	88.22	88.22	85.55	98.03	88.79
	Mean	83.91	86.63	85.97	84.57	88.39	88.39	83.55	89.72	85.27
	SEM	7.535		7.535		10.656		5.328		
	P Value	0.616		0.796		0.995		0.743		
RPI _{FSF}	Control	12.85	38.76	18.35	33.26	6.65	19.05	30.05	47.47	25.80
	Glycerol	4.08	48.97	22.29	30.76	2.97	5.19	41.61	56.34	26.53
	Jaggery	7.88	28.54	15.44	17.97	14.33	1.43	16.56	34.52	16.71
	Maize	15.83	20.04	19.42	24.46	15.36	16.31	23.48	32.60	21.94
	Mean	10.16	33.33	18.88	26.61	9.83	10.49	27.92	42.73	22.74
	SEM	12.635		7.794		17.869		8.935		
	P Value	0.012*		0.399		0.440		0.858		
REI _{FSF}	Control	-11.56	-13.29	-10.41	-14.43	-5.20	-15.63	-15.63	-10.95	-12.42
	Glycerol	-21.23	-12.67	-17.00	-16.90	-18.70	-15.29	-15.29	-10.06	-16.95
	Jaggery	-17.36	-19.30	-15.59	-21.07	-10.75	-20.43	-20.43	-18.16	-18.33
	Maize	-14.21	-8.21	-13.12	-9.31	-11.79	-14.45	-14.45	-1.97	-11.21
	Mean	-16.09	-13.37	-14.03	-15.43	-11.61	-16.45	-16.45	-10.28	-14.73
	SEM	7.535		7.535		10.656		5.328		
	P Value	0.616		0.796		0.175		0.743		

Table 4.48: Nutritive value of the diet in different postpartum groups

Parameter	Group	Energy × Breed		Energy × Suppl.		HFX × Suppl.		Deoni × Suppl.		E × B × S
		HFX	Deoni	Without	With	Without	With	Without	With	
DCP (%)	Control	8.52	8.62	8.27	8.87	7.83	9.22	8.72	8.52	8.57
	Glycerol	8.87	9.15	8.28	9.20	8.37	9.37	9.27	9.03	9.01
	Jaggery	8.88	7.70	8.02	8.56	8.81	8.94	7.23	8.18	8.29
	Maize	9.26	7.67	8.59	8.33	9.37	9.15	7.81	7.52	8.46
	Mean	8.80	8.28	8.42	8.74	8.59	9.17	8.26	8.31	8.58
	SEM	0.252		0.252		0.357		0.178		
	P Value	0.004**		0.096		0.164		0.063		
TDN (%)	Control	55.48	57.12	56.75	55.84	55.59	55.37	57.92	56.32	56.30
	Glycerol	56.53	56.13	56.53	56.13	56.71	56.34	56.34	55.92	56.33
	Jaggery	56.14	56.10	56.34	55.90	56.92	55.36	55.76	56.45	56.12
	Maize	57.06	56.72	57.27	56.51	57.68	56.44	56.86	55.59	56.89
	Mean	56.30	56.52	56.72	56.10	56.73	55.88	56.72	56.32	56.40
	SEM	0.408		0.418		0.591		0.295		
	P Value	0.472		0.05		0.460		0.316		
NR	Control	5.56	5.65	5.89	5.32	6.11	5.01	5.67	5.63	5.60
	Glycerol	5.43	5.14	5.42	5.14	5.78	5.08	5.09	5.20	5.29
	Jaggery	5.34	6.32	6.09	5.56	5.47	5.22	6.72	5.91	5.83
	Maize	5.18	6.41	5.73	5.85	5.18	5.18	6.29	6.53	5.79
	Mean	5.38	5.88	5.79	5.47	5.63	5.12	5.94	5.81	5.63
	SEM	0.195		0.195		0.276		0.138		
	P Value	0.002**		0.03*		0.180		0.054		
RFQ	Control	144.51	101.22	128.50	117.23	154.92	134.11	102.09	100.36	122.87
	Glycerol	140.02	100.70	125.11	115.61	154.09	125.95	96.14	105.27	120.36
	Jaggery	134.83	93.55	120.02	108.35	143.39	126.27	96.66	90.44	114.19
	Maize	145.53	112.17	131.01	126.69	157.46	133.60	104.56	119.79	128.85
	Mean	141.22	101.91	126.16	116.97	152.46	129.98	99.86	103.96	121.57
	SEM	10.242		10.242		14.484		7.242		
	P Value	0.001***		0.223		0.085		0.564		

Table 4.48A: Difference (%) in nutritive value of prepartum and postpartum period diets

Parameter	Energy × Breed		Energy × Suppl.		HFX × Suppl.		Deoni × Suppl.		E × B × S
	HFX	Deoni	Without	With	Without	With	Without	With	
DCP	83	72	64	91	72	93	58	88	77
TDN	3	5	4	4	3	3	5	6	5
NR	-48	-43	-41	-51	-44	-51	-37	-47	-45
RFQ	84	21	58	45	96	73	22	21	51
Min	-48	-43	-41	-51	-44	-51	-37	-47	-45
Max	84	72	64	91	96	93	58	88	77
Mean	31	14	21	22	32	29	12	17	22
CV	211	342	233	271	203	225	338	332	244

Table 4.49A: Difference (%) in energy value of prepartum and postpartum period diets

Parameter	Energy × Breed		Energy × Suppl.		HFX × Suppl.		Deoni × Suppl.		E × B × S
	HFX	Deoni	Without	With	Without	With	Without	With	
GE	70	10	45	33	80	60	11	9	39
DE	83	20	57	45	94	72	20	20	51
ME	84	19	58	45	96	73	20	19	52
Q-Value	10	10	8	-2	10	8	8	10	8
ER	261	64	182	140	294	227	67	61	161
HI	48	10	32	25	54	41	10	10	29
Energy Dig	8	9	8	9	8	8	9	10	9
Energy EFF.	96	37	70	62	101	90	39	35	66
Min	10	10	8	-2	10	8	8	9	8
Max	261	64	182	140	294	227	67	61	161
Mean	92	25	70	52	115	88	25	24	62
CV	94	95	96	101	94	95	99	94	95

During prepartum, response to FNS supplement by HFX cows and Deoni cows was none or statistically comparable with or without FNS supplement. GE, DE or ME consumption in postpartum HFX cows with FNS supplement in prepartum had negative influence accounting to 13% to 15% compared to those HFX cows without any FNS supplement in prepartum. In contrary, GE, DE and ME consumption in Deoni cows between without FNS supplement or with the supplement in prepartum, the energy terms in postpartum was increased by 3% which is statistically insignificant. Q-value of the diet in postpartum was statistically same for cows these diets with or without FNS supplement in prepartum or source of energy. HI or energy efficiency in postpartum was least influenced by the respective diets fed to cows in the prepartum. Prepartum dietary factors such as energy, breed and FNS supplement interaction effect on postpartum GE, DE, ME, Q-value of the diet, HI, ER, energy digestibility and efficiency was insignificant.

4.3.6 Microbial Protein Production

Urinary allantoin excretion was significantly higher in HFX cows than Deoni cows by 15% during the postpartum (**Table 4.50**). In ruminants, most of the PD excreted in urine comes from the metabolism of nucleic acids absorbed into the duodenum as most of the feed nucleic acids are metabolized in the rumen (Razzaque *et al.*, 1981). Among the PD, the allantoin accounts to 90% and uric acid accounts to 10% in cattle because there was no xanthine plus hypoxanthine in cattle like in buffaloes, sheep, goat and camel that may vary between 10% to 20% (Moscardini *et al.*, 1999) however, Chen *et al.* (1995) suggested 80 to 85% of allantoin as a metabolite of PD hydrolysis in small intestine and rest is uric acid in cattle (Chen *et al.*, 1995). Uric acid excretion was 65% higher in postpartum HFX cows and 8% higher in Deoni cows and its proportion was 13% to 14% in total PD.

Allantoin excretion in urine in HFX and Deoni cows were decreased by 4 to 5% respectively, in postpartum compared to prepartum.

Table 4.49: Energy value of the diet in different postpartum groups

Parameter	Group	Energy × Breed		Energy × Suppl.		HFX × Suppl.		Deoni × Suppl.		E × B × S
		HFX	Deoni	Without	With	Without	With	Without	With	
GE (Kcal/kg MBW)	Control	512.06	325.63	431.75	405.95	539.19	484.93	324.30	326.97	418.85
	Glycerol	497.88	328.02	424.49	401.41	533.94	461.82	315.30	341.00	412.95
	Jaggery	492.34	322.64	424.27	390.71	513.48	471.19	335.06	310.22	407.49
	Maize	511.33	370.34	448.40	433.27	548.24	474.42	348.56	392.12	440.83
	Mean	503.40	336.66	432.22	407.23	533.71	473.09	330.74	342.58	420.03
	SEM	27.34		27.34		38.661				19.33
	P Value	0.001		0.225		0.079				0.641
DE (Kcal/kg MBW)	Control	348.26	228.17	297.87	278.56	365.21	331.32	230.54	228.80	288.22
	Glycerol	346.65	226.91	295.31	278.25	371.57	321.73	219.05	234.77	286.78
	Jaggery	340.20	220.21	292.72	267.67	358.86	321.55	226.63	213.80	280.21
	Maize	359.08	255.17	315.77	299.24	390.46	329.22	241.07	269.29	307.50
	Mean	348.74	232.61	300.42	280.93	371.52	325.95	229.32	235.91	290.68
	SEM	19.48		19.48		27.543				13.771
	P Value	0.001		0.176		0.077				0.550
ME (Kcal/kg MBW)	Control	284.26	184.15	242.55	225.86	298.96	269.55	186.14	182.16	234.20
	Glycerol	282.77	182.76	240.06	225.46	303.99	261.56	176.17	189.35	232.77
	Jaggery	277.34	177.88	238.23	216.99	293.02	261.66	183.45	172.32	227.61
	Maize	293.49	207.19	257.14	243.54	318.96	268.02	195.33	219.06	250.34
	Mean	284.46	187.99	244.50	227.96	303.73	265.20	185.27	190.72	236.23
	SEM	16.24		16.24		22.961				11.48
	P Value	0.001		0.169		0.073				0.548
Q Value	Control	0.56	0.57	0.57	0.56	0.56	0.56	0.58	0.56	0.56
	Glycerol	0.57	0.56	0.57	0.56	0.57	0.57	0.56	0.56	0.56
	Jaggery	0.56	0.55	0.56	0.56	0.57	0.56	0.55	0.56	0.56
	Maize	0.57	0.56	0.57	0.56	0.58	0.57	0.56	0.56	0.57
	Mean	0.57	0.56	0.56	0.50	0.57	0.56	0.56	0.56	0.56
	SEM	0.004		0.004		0.006				0.003
	P Value	0.02		0.059		0.675				0.184

Parameter	Group	Energy × Breed		Energy × Suppl.		HFX × Suppl.		Deoni × Suppl.		E × B × S
		HFX	Deoni	Without	With	Without	With	Without	With	
ER (Kcal/kg MBW)	Control	95.01	43.35	73.91	64.45	103.06	86.96	44.77	41.94	69.18
	Glycerol	95.10	42.04	72.66	64.48	106.68	83.51	38.64	45.45	68.57
	Jaggery	91.93	39.71	71.62	60.02	100.72	83.15	42.52	36.90	65.82
	Maize	100.94	55.61	82.07	74.47	114.80	87.08	49.35	61.86	78.27
	Mean	95.74	45.18	75.06	65.85	106.31	85.17	43.82	46.54	70.46
	SEM	8.608		8.608		12.714		6.807		0.517
P Value	0.001		0.150		0.068		0.517		0.517	
HI (Kcal/kg MBW)	Control	189.25	140.80	168.64	161.41	195.90	182.60	141.38	140.23	165.03
	Glycerol	187.68	140.72	167.42	160.97	197.31	178.05	137.54	143.90	164.20
	Jaggery	185.41	138.18	166.61	156.97	192.30	178.52	140.92	135.43	161.79
	Maize	192.55	151.59	175.05	169.07	204.17	180.94	145.98	157.20	172.07
	Mean	188.72	142.82	169.43	162.11	197.42	180.03	141.45	144.19	165.77
	SEM	7.64		7.64		10.805		5.402		0.583
P Value	0.001		0.194		0.081		0.583		0.583	
Energy Dig. (%)	Control	68.06	70.08	69.42	68.73	67.77	68.35	71.06	69.11	69.07
	Glycerol	69.57	69.16	69.47	69.25	69.48	69.66	69.47	68.84	69.36
	Jaggery	69.06	68.27	68.75	68.58	69.88	68.25	67.63	68.92	68.67
	Maize	70.34	68.91	70.14	69.10	71.12	69.55	69.16	68.65	69.62
	Mean	69.26	69.10	69.44	68.91	69.56	68.95	69.33	68.88	69.18
	SEM	0.464		0.464		0.656		0.328		0.238
P Value	0.648		0.125		0.813		0.238		0.238	
Energy Eff. (%)	Control	33.19	23.40	29.12	27.47	34.31	32.07	23.93	22.86	28.29
	Glycerol	33.39	22.70	28.17	27.92	34.86	31.93	21.49	23.92	28.05
	Jaggery	33.04	22.26	28.71	26.59	34.30	31.78	23.13	21.40	27.65
	Maize	33.80	26.66	30.33	30.12	35.50	32.10	25.17	28.15	30.23
	Mean	33.35	23.75	29.08	28.02	34.74	31.97	23.43	24.08	28.55
	SEM	1.400		1.400		1.980		0.990		0.238
P Value	0.001		0.300		0.103		0.238		0.238	

The main driver of MBP synthesis is the availability of fermentable substrate in the rumen. Although feed intake during postpartum was 20 to 75% higher than the prepartum, plasma allantoin was lower in the postpartum than prepartum. Urinary excretion of allantoin has been reported to increase with increased intake of digestible DM or OM (Singh and Srinivas, 2016; Mohanavel and Srinivas, 2016). The major metabolite of PD; allantoin in HFX cows during prepartum was 91% and reduced to 86% during postpartum but unchanged in Deoni cows that were 85%. The decrease in allantoin in postpartum HFX cows might be due to reduction of gestational allantoin contribution and no change in Deoni cows illustrated lower levels of gestational allantoin (Matsumoto and Yonai, 1996).

Total PD excretion during postpartum was 7% lesser in HFX cows but 12% lesser in Deoni cows compared to prepartum (**Table 4.51 A**). Singh and Srinivas (2016) reported breed difference in PD excretion ($P < 0.001$). Creatinine concentration depends on glomerular filtration of plasma creatinine resulting from muscle creatinine break down. Singh and Srinivas (2016) reported creatinine excretion of 3.0 mM/L to 3.4 mM/L in lactating cows but in the present study, we observed 2.82 mM/L in fresh cows. Creatinine excretion was increased by 26% in HFX cows and 8% in Deoni cows during postpartum than prepartum. Maltz and Silanikove (1996) reported higher plasma and urinary creatinine in Holstein cows 2 wk before calving (prepartum) than after 2 wk of calving (postpartum) with creatinine in urine declined from 10.5 mM/L in prepartum to 5.0 mM/L in postpartum. Cattle have a higher metabolic rate per unit of metabolic BW than do most other domestic ruminants, and cattle also have a poorly developed mechanism in the kidney and gut for water retention (Silanikove, 1992). This indicated that more MBP hydrolyzed at small intestine in comparison to metabolically active tissue in HFX cows or vice-versa with Deoni cows.

PD index, PD excretion and MBN production in postpartum HFX cows were decreased by 7% than prepartum cows and it was decreased by 12% in Deoni cows.

Table 4.50: Excretion of total purine derivatives and their index in postpartum cows

Parameter	Group	Energy × Breed		Energy × Suppl.		HFX × Suppl.		Deoni × Suppl.		E × B × S
		HFX	Deoni	Without	With	Without	With	Without	With	
Allantoin (Mm/L)	Control	5.05	4.44	4.75	4.75	5.25	4.85	4.24	4.65	4.75 ^b
	Glycerol	4.68	3.99	4.29	4.37	4.68	4.67	3.91	4.07	4.33 ^a
	Jaggery	4.45	4.15	4.39	4.21	4.84	4.05	3.94	4.37	4.30 ^a
	Maize	5.01	4.08	4.50	4.58	5.11	4.90	3.90	4.26	4.54 ^{ab}
	Mean	4.79	4.17	4.48	4.48	4.97	4.62	4.00	4.34	4.48
	SEM	0.136		0.136		0.192				0.096
	P Value	0.001***		0.94		0.002**				0.02*
Creatinine (Mm/L)	Control	2.74	2.15	2.35	2.54	2.66	2.82	2.04	2.26	2.44
	Glycerol	2.56	2.08	2.30	2.36	2.61	2.55	1.98	2.17	2.33
	Jaggery	2.68	2.02	2.41	2.30	2.71	2.66	2.11	1.94	2.35
	Maize	2.68	1.96	2.41	2.23	2.78	2.59	2.03	1.88	2.32
	Mean	2.67	2.05	2.37	2.36	2.69	2.65	2.04	2.06	2.36
	SEM	0.078		0.078		0.110				0.055
	P Value	0.001		0.90		0.598				0.38
Uric acid (Mm/L)	Control	0.78	0.75	0.74	0.80	0.71	0.85	0.76	0.74	0.77
	Glycerol	0.78	0.75	0.81	0.72	0.74	0.82	0.88	0.63	0.77
	Jaggery	0.67	0.62	0.64	0.64	0.70	0.63	0.58	0.65	0.64
	Maize	0.63	0.67	0.68	0.63	0.65	0.61	0.71	0.64	0.65
	Mean	0.71	0.70	0.72	0.70	0.70	0.73	0.73	0.67	0.71
	SEM	0.068		0.068		0.096				0.048
	P Value	0.744		0.694		0.345				0.149
Total PD (Mm/L)	Control	5.83	5.19	5.48	5.54	5.96	5.70	4.99	5.38	5.51 ^b
	Glycerol	5.45	4.74	5.10	5.09	5.42	5.49	5.79	4.70	5.10 ^a
	Jaggery	5.11	4.77	5.03	4.84	5.55	4.68	4.52	5.02	4.94 ^a
	Maize	5.64	4.75	5.18	5.21	5.76	5.52	4.60	4.90	5.19 ^a
	Mean	5.51	4.86	5.20	5.17	5.67	5.34	4.73	5.00	5.19
	SEM	0.138		0.138		0.196				0.098
	P Value	0.001***		0.790		0.01**				0.01**

These results show that the plasma allantoin level is affected by not only rumen MBP production but also by the physiological conditions of the host animals (Matsumoto and Yonai, 1996). During postpartum, which was characterized by the dramatic change in physiology and nutrition metabolism that may alter the rumen fermentation characteristics and affect the feed efficiency of the cow (Wang *et al.*, 2001).

The efficiency (g/kgDOMI) of MBP production was decreased by 43% and 18% in HFX cows and Deoni cows, respectively than prepartum MBP production but, higher than optimum suggested 30g/kg DOM in rumen under continuous culture fermentation. The MBP production efficiency was 21g/Mcal of ME in HFX cows and 18g/Mcal of ME in Deoni cows during the postpartum period which was 7% and 11% lesser than prepartum (**Table 4.51**) in HFX and Deoni cows, respectively (Hackmann and Firkins, 2015).

Creatinine and uric acid concentration in postpartum in cows provided energy with FNS supplement was increased by 10% and 20% than the cows provided prepartum energy without FNS supplement. Mean MBP production was increased by 7% during postpartum in cows those received FNS supplement along with energy in prepartum compared to those without FNS supplement (**Table 4.51A**). MBP production due to the interaction of energy and FNS supplement ($P = 0.026$) or energy alone ($P = 0.085$) between post and prepartum were significantly different and lesser in postpartum than prepartum due to physiological change (Maltz and Silanikove, 1996; Wang *et al.*, 2001). Postpartum MBP production in CG increased by 18 g/d in cows where control diet was supplemented with FNS supplement in prepartum, while it was 38 and 40 g/d, in T1 and T3, respectively. Only in T2, MBP production was reduced in cows received FNS supplement. FNS supplement appeared to have a beneficial effect with glycerol and maize grain than jaggery. Based on the evidence of the data in the present study and literature, such difference between jaggery and other energy sources was because rapid solubility, glucose or fructose utilization by rumen microflora was simple and straight whereas glycerol needs ATP molecules for phosphorylation to join in the metabolic path while maize polysaccharides take time for breakdown to simple saccharides.

Table 4.51: Microbial Nitrogen production and efficiency in postpartum cows

Parameter	Group	Energy × Breed		Energy × Suppl.		HFX × Suppl.		Deoni × Suppl.		E × B × S
		HFX	Deoni	Without	With	Without	With	Without	With	
PD index	Control	513.84	366.00	425.33	454.51	498.42	529.25	332.24	379.77	439.92
	Glycerol	510.53	329.94	407.49	432.98	471.09	549.98	343.90	315.98	420.24
	Jaggery	501.00	386.42	449.18	438.21	534.02	467.98	364.34	408.50	443.71
	Maize	527.03	349.26	443.75	432.54	536.87	517.19	350.63	347.89	438.15
	Mean	513.10	357.90	431.44	439.57	510.10	516.10	352.78	363.03	435.50
	SEM	26.150		26.150		36.981		18.491		
P Value	0.001***		0.666		0.910		0.812			
PD excretion (Mm/d)	Control	449.77	321.46	372.90	398.33	436.03	463.51	309.77	333.15	385.62
	Glycerol	447.74	290.71	358.08	380.37	413.24	482.24	302.92	278.50	369.22
	Jaggery	440.31	340.37	394.83	385.84	468.12	412.49	321.54	359.19	390.34
	Maize	461.70	307.69	389.64	379.75	470.05	453.35	309.24	306.14	384.69
	Mean	449.88	315.06	378.86	386.07	446.86	452.90	310.87	319.24	382.47
	SEM	22.859		22.859		32.327		16.164		
P Value	0.001***		0.662		0.943		0.810			
Duodenal flow of MBP (g/d)	Control	327.01	233.71	271.12	289.60	317.02	337.00	225.22	242.21	280.36
	Glycerol	325.53	211.36	260.34	276.54	300.45	350.61	220.24	202.48	268.44
	Jaggery	320.12	247.46	287.06	280.53	340.34	299.90	233.78	261.15	283.79
	Maize	335.68	223.70	283.29	276.09	341.75	329.61	204.83	222.58	279.69
	Mean	327.08	229.06	275.45	280.69	324.87	329.28	226.01	232.10	278.07
	SEM	16.620		16.620		23.504		11.752		
P Value	0.001***		0.662		0.943		0.810			
MBP efficiency (g/kg of DOMI)	Control	47.62	64.70	53.87	58.46	46.11	49.13	61.63	67.79	56.16
	Glycerol	44.87	59.76	51.98	52.64	41.41	48.33	62.55	56.96	52.31
	Jaggery	42.56	61.43	50.07	53.93	43.76	41.35	56.37	66.50	52.33
	Maize	45.72	52.95	48.59	50.07	42.91	48.53	54.28	51.62	49.33
	Mean	45.19	59.71	51.13	53.78	43.55	46.83	58.71	60.72	52.45
	SEM	2.984		2.984		4.220		2.110		
P Value	0.001***		0.228		0.766		0.192			
MBP Efficiency (g /Mcal of ME)	Control	23.36	20.23	21.34	22.25	23.42	23.31	19.27	21.19	21.80 ^b
	Glycerol	20.27	16.32	18.56	18.02	19.91	20.63	17.21	15.42	18.29 ^a
	Jaggery	19.81	17.97	19.30	18.48	21.53	18.09	17.07	18.87	18.89 ^a
	Maize	20.24	17.89	19.43	18.70	20.71	19.77	18.15	17.64	19.07 ^a
	Mean	20.92	18.10	19.66	19.36	21.39	20.45	17.93	18.27	19.51
	SEM	0.510		0.510		0.721		0.361		
P Value	0.001***		0.425		0.092		0.001***			

Table 4.51 A: Difference (%) in total purine derivatives and MBP in the prepartum and postpartum period

Parameter	Energy × Breed		Energy × Suppl.		HFX × Suppl.		Deoni × Suppl.		E × B × S
	HFX	Deoni	Without	With	Without	With	Without	With	
Allantoin	-4	-5	-2	-6	-1	-6	-3	-6	-4
Creatinine	26	8	13	23	17	36	8	9	18
Uric acid	65	8	41	21	106	40	9	6	31
Total PD	2	-6	2	-3	5	-2	-1	-5	0
PD index	-7	-12	-6	-11	-4	-9	-10	-13	-9
PD excretion	-7	-12	-6	-11	-4	-9	-10	-13	-9
Duodenal flow of MBP	-7	-12	-6	-11	-4	-9	-10	-13	-7
MBP efficiency (g/ kg of DOMI)	-43	-18	-32	-31	-45	-42	-17	-19	-31
MBP efficiency (g/ Mcal of ME)	-7	-11	-6	-11	-4	-9	-9	-13	-9
Min	-43	-6	-2	-6	-1	-6	-3	-6	-4
Max	65	20	41	23	106	40	20	21	31
Mean	5	5	15	11	30	18	7	5	13
CV	553	-420	727	-2146	392	1080	-829	-349	1547

Difference between the cows MBP production due to energy and FNS supplement or without FNS supplement was -0.60 ($P = 0.94$). The difference in MBP production in postpartum due to FNS supplements to prepartum diets of CG and T1 was 18 and 16 g/d, respectively but, 7 g/d reduced in T2 and T3, respectively. MBP production during postpartum when HFX cows provided FNS supplement in prepartum increased by 11 to 16 g/d and lowest in CG and T1 and highest in T3. Prepartum FNS supplement impact on Deoni cows on postpartum MBP production was greater than HFX cows ($P = 0.94$). The increase in postpartum MBP production due to prepartum FNS supplement to Deoni cows was 4 g/d, 50 g/d, 27 g/d and 62 g/d in CG, T1, T2, T3 and T4, respectively ($P = 0.036$). Similarly, the difference in MBP production between postpartum and prepartum Deoni cows without FNS supplement in prepartum was 14 g/d, 28 g/d, 63 g/d and 18 g/d, in CG, T1, T2 and T3, respectively ($P = 0.002$). In the CG and T2 group of cows, the prepartum FNS supplement effect on postpartum MBP production was none. MBP production in postpartum was increased over the prepartum ($P = 0.014$) due to the diet interaction effect of breed, energy and FNS supplement. However, the significant difference in close-up and fresh cows for MBP production may also include increased diet intake in postpartum.

MBP production efficiency (g/kgDOMI) in cows was 31 to 32% decreased in postpartum than prepartum with or without FNS supplementation probably the overestimation of allantoin in prepartum due to gestational allantoin could also be a reason (Maltz and Silanikove, 1996). MBP production efficiency during postpartum was decreased by 5% in cows provided energy with FNS supplement than without FNS supplement during prepartum which is statistically insignificant. MBP production efficiency in Deoni cows with prepartum FNS supplement was 60 g/kg DOMI which was higher than optimum suggested 30 g/kg DOMR where DOMR was taken as 65% of digestible OM intake (ARC, 1980). Optimum MBP production for kg DOM intake was about 50 g/kg which was in agreement to Singh and Srinivas (2016) who reported 50% efficiency on grain-based diets in Deoni cows. MBP production efficiency in postpartum was high in CG than TGs. MBP production efficiency (g/kg DOMI) was significantly higher in CG and is comparable with T3, but higher than T1 and T2 groups.

4.4 Effect of prepartum nutrition on pre and postpartum metabolic health

4.4.1 Hematological parameters

The haematological parameter in the postpartum period reflects the adaptability of the cow to physiological change i.e., from non-lactating to lactation and informative tool along with other diagnostic procedures (Ronald *et al.*, 2014). Haematological parameters 30 days and 14 days before (**Table 4.52**) and after calving (**Table 4.53**) and on the day of calving (**Table 4.54**) were evaluated. WBC count increased from $7.98 \times 10^3/\mu\text{L}$ to $9 \times 10^3/\mu\text{L}$. Physiological leukocytosis is seen in parturition and not significant between breeds and also insignificant without or with FNS supplement or breed interaction without or with FNS supplement (Ronald *et al.*, 2014). Physiological leukocytosis is also seen during stress, excitation or fear which are common behaviours during parturition result in secretion of adrenalin, ACTH and cortisol. This is in support with Kim *et al.* (2005) who reported that the WBC count increases significantly during the prepartum. Elshahawy and Abdullaziz (2017) also reported that WBC count was significantly higher in the prepartum than postpartum (12.25 ± 0.46 vs $10.93 + 1.96$). After calving or lactating cows, WBC count decreased from $9 \times 10^3/\mu\text{L}$ to $8 \times 10^3/\mu\text{L}$ due to migration of WBC towards uterus lumen and mammary gland (Patel *et al.*, 2017). Energy \times breed interaction was found non-significant during pre and post partum as well as on calving day. The interaction effect of energy \times FNS supplement, or Energy \times breed \times FNS supplement also found non-significant during prepartum and postpartum as well as on calving day. Irrespective of prepartum and post partum as well as on calving day, WBC was within the physiological range of 4 to $12 \times 10^3/\mu\text{L}$. Lymphocyte (%) during the study was stable till 30 days after calving (50-51%). Contrary to this, Kehrl *et al.*, (1989) reported that during the first week after calving, lymphocyte blastogenesis was markedly reduced in comparison to prepartum. Lymphocytes, and granulocytes were significantly ($P < 0.05$) different between periods among HFX and Deoni cows. Physiological lymphocytosis was more in prepartum than postpartum in Deoni cows or during both periods in HFX cows.

Table 4.52: Whole blood profile in different groups of cows during the prepartum experiment period

Parameter	Group	30 d before calving					14 d before calving				
		Energy × Breed		Energy × Supplement		E × B × S	Energy × Breed		Energy × Supplement		E × B × S
		HFX	Deoni	Without	With		HFX	Deoni	Without	With	
WBC (× 10 ³ /μL)	Control	7.93	7.85	7.60	8.18	7.89	7.93	8.05	7.58	8.40	7.99
	Glycerol	7.85	7.95	7.83	7.98	7.90	8.00	8.03	8.03	8.00	8.01
	Jaggery	8.43	7.95	8.58	7.80	8.19	8.73	7.85	8.68	7.90	8.29
	Maize	7.85	8.18	8.28	7.75	8.01	8.13	8.15	8.25	8.03	8.14
	Mean	8.01	7.98	8.07	7.93	8.00	8.19	8.02	8.13	8.08	8.11
	SEM	0.180		0.205		0.175	0.200		0.225		0.179
P Value	0.865		0.495		0.280	0.396		0.829		0.291	
RBC (× 10 ⁶ /μL)	Control	5.20	5.36	5.30	5.26	5.53	5.59	5.73	5.35	5.97	5.66
	Glycerol	4.79	5.82	5.01	5.60	5.25	5.26	5.83	4.90	6.20	5.55
	Jaggery	5.15	5.81	5.52	5.44	5.91	5.50	5.83	5.04	6.29	5.66
	Maize	5.35	5.72	5.23	5.84	5.90	5.27	6.71	4.83	7.15	5.99
	Mean	5.12	5.68	5.26	5.54	5.65	5.40	6.03	5.03	6.40	5.71
	SEM	0.145		0.149		0.152	0.201		0.219		0.219
P Value	0.01**		0.084		0.108	0.01**		0.001***		0.206	
Hb (g %)	Control	9.55	9.03	9.05	9.53	9.29	9.95	10.03	8.80	11.18	9.99
	Glycerol	8.73	9.05	8.58	9.20	8.89	8.88	9.68	8.70	9.85	9.28
	Jaggery	9.20	9.85	9.28	9.78	9.53	9.60	9.83	9.05	10.38	9.71
	Maize	9.08	8.94	9.24	8.78	9.01	9.48	10.53	9.45	10.55	10.00
	Mean	9.14	9.22	9.03	9.32	9.18	9.48	10.01	9.00	10.49	9.74
	SEM	0.319		0.330		0.213	0.200		0.187		0.198
P Value	0.802		0.408		0.064	0.02*		0.001***		0.342	
Platelets (× 10 ⁵ /μL)	Control	344	403	372	375	374	355	377	354	378	366
	Glycerol	370	375	337	409	373	450	465	436	479	457
	Jaggery	337	426	400	363	381	431	461	428	465	446
	Maize	431	382	439	374	406	482	398	481	399	440
	Mean	370	397	387	380	383	429	425	424	430	427
	SEM	31.05		30.07		35.21	36.70		35.04		31.75
P Value	0.412		0.814		0.543	0.908		0.876		0.075	
PCV (%)	Control	29.2	27.9	27.5	29.7	28.6	29.5	28.9	27.8	30.6	29.2
	Glycerol	27.2	27.7	26.5	28.4	27.5	28.4	28.7	27.5	29.6	28.5
	Jaggery	27.1	29.3	27.3	29.1	28.2	30.1	29.0	29.5	29.6	29.5
	Maize	25.5	28.5	28.0	26.0	27.0	26.5	29.9	28.4	27.9	28.2
	Mean	27.3	28.3	27.3	28.3	27.8	28.6	29.1	28.3	29.4	28.9
	SEM	0.822		0.915		0.894	0.777		0.988		0.771
P Value	0.209		0.313		0.274	0.510		0.135		0.261	

Parameter	Group	30 d before calving					14 d before calving				
		Energy × Breed		Energy × Supplement		E × B × S	Energy × Breed		Energy × Supplement		E × B × S
		HFX	Deoni	Without	With		HFX	Deoni	Without	With	
Lymphocyte (%)	Control	54.7	55.4	54.2	55.8	55.0	49.6	52.2	51.1	50.7	50.9
	Glycerol	54.4	55.6	55.8	54.2	55.0	53.6	50.7	53.2	51.1	52.1
	Jaggery	55.0	54.9	56.4	53.5	54.9	50.0	49.3	49.3	50.1	49.7
	Maize	57.0	53.6	57.3	53.4	55.3	52.5	47.3	50.7	49.2	49.9
	Mean	55.3	54.9	55.9	54.2	55.1	51.4	49.9	51.0	50.3	50.7
	SEM	1.351		1.592		1.521	1.250		1.060		1.132
P Value	0.761		0.315		0.867	0.229		0.472		0.172	
Monocyte (%)	Control	7.4	5.6	6.8	6.2	6.5	9.3	7.2	8.0	8.6	8.3
	Glycerol	7.2	6.4	7.0	6.5	6.8	6.6	7.6	6.8	7.4	7.1
	Jaggery	6.5	6.8	6.7	6.6	6.6	7.6	7.8	7.9	7.5	7.7
	Maize	5.9	8.1	6.5	7.5	7.0	7.6	7.7	7.3	8.0	7.6
	Mean	6.7	6.7	6.7	6.7	6.7	7.8	7.6	7.5	7.9	7.7
	SEM	0.453		0.296		0.440	0.470		0.384		0.391
P Value	0.903		0.884		0.494	0.686		0.337		0.066	
Granulocyte (%)	Control	38.0	39.0	39.0	38.0	38.5	41.1	40.6	40.9	40.7	40.8
	Glycerol	38.5	38.0	37.3	39.2	38.2	39.8	41.7	40.0	41.5	40.8
	Jaggery	38.5	38.4	36.9	39.9	38.4	42.3	43.0	42.9	42.4	42.6
	Maize	37.0	38.3	36.2	39.1	37.7	39.9	45.0	42.0	42.9	42.5
	Mean	38.0	38.4	37.4	39.1	38.2	40.8	42.6	41.5	41.9	41.7
	SEM	1.291		1.578		1.473	0.968		0.848		0.918
P Value	0.722		0.300		0.726	0.087		0.654		0.199	
MCV (%)	Control	55.2	47.8	52.0	51.0	51.5	52.7	52.5	47.5	57.7	52.6
	Glycerol	55.4	48.5	56.0	47.9	52.0	51.3	50.8	53.8	48.3	51.1
	Jaggery	53.2	42.3	48.0	47.4	47.7	56.9	45.9	55.3	47.5	51.4
	Maize	57.7	48.7	61.5	44.8	53.2	50.9	52.5	57.7	45.7	51.7
	Mean	55.3	46.8	54.4	47.8	51.1	53.0	50.4	53.6	49.8	51.7
	SEM	1.292		1.578		2.578	0.968		0.848		2.972
P Value	0.722		0.300		0.187	0.087		0.654		0.743	
MCH (%)	Control	18.4	16.8	17.1	18.1	17.6	13.9	14.3	12.6	15.6	14.1
	Glycerol	18.3	15.7	17.4	16.6	17.0	17.1	16.7	16.7	17.1	16.9
	Jaggery	17.8	17.0	16.8	18.0	17.4	17.6	15.5	16.7	16.4	16.6
	Maize	17.1	15.7	17.8	15.0	16.4	18.0	14.5	18.2	14.3	16.3
	Mean	17.9	16.3	17.3	16.9	17.1	16.7	15.3	16.1	15.8	16.0
	SEM	0.566		0.600		0.565	0.626		0.896		0.803
P Value	0.01*		0.609		0.189	0.04*		0.792		0.593	

Values with different superscripts for absolute interaction for respective energy source in a row under each parameter differ significantly; ***P ≤ 0.001, **P ≤ 0.01, *P ≤ 0.05

While monocytes were higher at the time of calving than prepartum or postpartum in Deoni and HFX cows due to interaction of period, energy and FNS supplement (**Table 4.53**). Monocyte numbers are variable in cattle and not sensible indicator for any specific physiological, etiological or behavioural indicators (Ronald *et al.*, 2014). Monocyte was reduced from 7.5% to 6% on day 14 before calving to calving day. This could be due to the migration of monocytes from circulation to tissues and undergo converting macrophages to provide immunity at the time of parturition stress. Klinkon and Zadnik (1999) also reported that the lowest mean absolute value for monocytes was constant during pregnancy and decrease slightly in the last month of pregnancy (213±136 vs. 224±157). Granulocytes were also significantly different between breeds for prepartum and postpartum, and on the day of calving. Granulocyte (%) was increased as calving day approached and later decreased in the postpartum. Granulocytes were higher in both the breed on the day of calving than other periods ($P < 0.05$).

RBC count in cows 30 days before calving was significantly higher by 18% ($P < 0.001$) in Deoni than HFX cows. It was 12% higher ($6.03 \times 10^6/\mu\text{L}$) in Deoni ($P < 0.01$) than HFX cows ($5.40 \times 10^6/\mu\text{L}$) during 14 days prior to calving and further narrowd to only 5% more ($P < 0.001$) in Deoni cows ($5.91 \times 10^3/\mu\text{L}$) than HFX cows ($5.63 \times 10^6/\mu\text{L}$) on the day of calving. RBC was again increased to 16% more in Deoni cows during 14 days and 30 days of postpartum ($P < 0.01$) than HFX cows. Based on the result of this study and study conducted by Adam *et al.* (2015), Deoni cattle had a high number of erythrocytes than HFX cows. Adam *et al.* (2015) reported that cattle breed also influenced the number of erythrocytes. Petrera and Abeni (2018) reported that dual-purpose Modenese cattle showed greater RBC than Italian Friesian cow during all physiological periods i.e. antepartum, early postpartum and lactation. On the day of calving, RBC count in FNS supplemented cows ($6.37 \times 10^6/\mu\text{L}$) was higher by 23% ($P < 0.05$) than the cows without FNS supplement ($5.17 \times 10^6/\mu\text{L}$). FNS supplementation during prepartum period improved the RBC count significantly by 17.5% and 19%, respectively ($P < 0.01$) on day 14 and 30 after calving in comparison to without supplement cows.

Table 4.53: Whole blood profile in different groups of cows on the day of calving

Parameter	Group	Calving day				E × B × S
		Energy × Breed		Energy × Supplement		
		HFX	Deoni	Without	With	
WBC (× 10 ³ /μL)	Control	9.53	8.80	9.00	9.33	9.16
	Glycerol	8.83	8.50	8.63	8.70	8.66
	Jaggery	9.90	8.20	8.90	9.20	9.05
	Maize	9.23	9.60	9.68	9.15	9.41
	Mean	9.37	8.78	9.05	9.09	9.07
	SEM	0.261		0.238		0.257
	P Value	0.135		0.857		0.070
RBC (× 10 ⁶ /μL)	Control	5.83	5.74	5.35	6.21	5.78
	Glycerol	5.59	6.11	5.27	6.42	5.85
	Jaggery	5.69	6.21	5.32	6.58	5.95
	Maize	5.42	5.59	4.74	6.27	5.50
	Mean	5.63	5.91	5.17	6.37	5.77
	SEM	0.114		0.099		0.117
	P Value	0.03*		0.001***		0.354
Hb (g %)	Control	10.70	8.85	8.75	10.80	9.78
	Glycerol	9.03	9.85	8.55	10.33	9.44
	Jaggery	10.03	10.73	9.63	11.13	10.38
	Maize	10.05	10.55	9.90	10.70	10.30
	Mean	9.95	9.99	9.21	10.74	9.97
	SEM	0.365		0.202		0.319
	P Value	0.906		0.001***		0.069
Platlets (× 10 ⁵ /μL)	Control	306	308	304	310	302
	Glycerol	308	315	302	293	324
	Jaggery	332	319	344	351	312
	Maize	344	378	310	368	320
	Mean	323	330	315	331	314
	SEM	21.14		20.11		19.27
	P Value	0.496		0.435		0.215
PCV (%)	Control	29.7	30.0	28.9	30.8	29.8
	Glycerol	30.4	31.7	29.7	32.4	31.1
	Jaggery	31.2	31.0	30.9	31.2	31.1
	Maize	30.8	31.8	31.9	30.6	31.3
	Mean	30.5	31.1	30.3	31.2	30.8
	SEM	0.988		1.085		0.932
	P Value	0.545		0.420		0.330

Parameter	Group	Calving day				E × B × S
		Energy × Breed		Energy × Supplement		
		HFX	Deoni	Without	With	
Lymphocyte (%)	Control	49.9	51.9	50.4	51.5	50.9
	Glycerol	51.5	50.0	51.0	50.5	50.8
	Jaggery	51.1	48.6	49.6	50.1	49.8
	Maize	51.7	49.5	52.1	49.1	50.6
	Mean	51.0	50.0	50.8	50.3	50.5
	SEM	0.833		0.716		0.797
	P Value	0.229		0.511		0.393
Monocyte (%)	Control	6.20	5.90	6.40	5.70	6.10
	Glycerol	5.60	6.60	5.70	6.50	6.10
	Jaggery	6.20	6.60	6.50	6.40	6.40
	Maize	6.10	5.60	5.90	5.80	5.80
	Mean	6.00	6.20	6.10	6.10	6.10
	SEM	0.398		0.379		0.228
	P Value	0.712		0.948		0.188
Granulocyte (%)	Control	43.9	42.2	43.2	42.8	43.0
	Glycerol	42.9	43.4	43.3	43.0	43.1
	Jaggery	42.7	44.7	43.9	43.5	47.7
	Maize	42.3	44.9	42.0	45.2	43.6
	Mean	42.9	43.8	43.1	43.6	43.4
	SEM	0.956		0.867		0.790
	P Value	0.368		0.563		0.567
MCV (%)	Control	53.7	60.5	54.4	59.8	57.1
	Glycerol	48.4	53.8	52.4	49.9	55.1
	Jaggery	56.8	47.7	55.0	49.5	52.2
	Maize	51.8	44.7	55.3	41.2	48.2
	Mean	52.7	51.7	54.3	50.1	52.2
	SEM	0.956		0.867		3.394
	P Value	0.368		0.563		0.106
MCH (%)	Control	15.1	14.4	13.4	16.1	14.8
	Glycerol	15.9	14.3	15.9	14.3	15.1
	Jaggery	16.7	13.3	15.2	14.8	15.0
	Maize	13.9	12.5	15.9	10.5	13.2
	Mean	15.4	13.6	15.1	13.9	14.5
	SEM	0.828		1.132		0.901
	P Value	0.052		1.308		0.197

Since erythrocytes in cattle have an average diameter of 5 to 6 μm and a life span of 130 to 160 days, any improvement in prepartum have an impact on postpartum also (Ronald *et al.*, 2014).

RBC count is associated with the need for the oxygen in the body as the demand for oxygen increases erythropoiesis in the kidney also increases (Oliveira *et al.*, 2019). Cu^{2+} being embedded in ceruloplasmin which used in the fortified nutrient supplementation is necessary to mobilize the Fe^{2+} from the liver to bone marrow, where it is used for erythropoiesis (Abramowicz *et al.*, 2019). This might be the reason for increased RBC count in the FNS supplemented cows than non-supplemented cows. Salama *et al.* (2011) also reported that 60 mg Zn^{2+} , 20 mg Cu^{2+} , 0.3 mg Se^{2+} for 30 days prepartum to calving was improved the RBC count than the control group. Although significant variation in breed was observed for the RBC count ($5.12 \times 10^6 / \mu\text{L}$ – $6.03 \times 10^6 / \mu\text{L}$) during prepartum and postpartum and on the day of calving, it was within the physiological ($5 - 10 \times 10^6 / \mu\text{L}$) range (Rodostits *et al.*, 2007) however, the energy \times breed \times supplement interaction was found non-significant during all the periods. The data revealed that prepartum supplementation had positive effect on postpartum RBC count in Deoni and HFX cows with FNS supplementation.

On 14 d before calving, Hb content in Deoni cows (10.01g %) was significantly ($P < 0.05$) higher than HFX cows (9.48 g %), but both the values of Hb are within the reference range (Feldman *et al.*, 2000). In FNS supplemented cows, Hb was significantly ($P < 0.001$) higher (16.5%) than the non-supplemented cows on day 14 prior to calving however, energy \times breed \times FNS supplement interaction were non-significant during same period. On the day of calving, FNS supplemented cows (10.74 g%) had significantly higher ($P < 0.001$) Hb (g%) than the non-supplemented group (9.21 g%).

On the day of calving breed \times FNS supplement, and energy \times breed \times FNS supplement interaction were found non-significant (**Table 4.55**). FNS supplementation during prepartum period enhanced the Hb content 16.6 g% on the day of calving and 12 g% ($P < 0.001$) subsequently till 30 days postpartum compared to cows without FNS supplement. The energy, breed and supplement interaction were non-significant in the postpartum. The higher value of Hb might be attributed to high erythrocyte count in Deoni cattle.

Table 4.54: Whole blood profile in different groups of cows during the postpartum period

Parameter	Group	14 d after calving					30 d after calving				
		Energy × Breed		Energy × Supplement		E × B × S	Energy × Breed		Energy × Supplement		E × B × S
		HFX	Deoni	Without	With		HFX	Deoni	Without	With	
WBC (× 10 ³ /μL)	Control	8.83	8.25	8.20	8.88	8.54	8.53	8.28	8.05	8.75	8.40
	Glycerol	8.33	8.10	8.05	8.38	8.21	8.43	8.00	8.05	8.38	8.21
	Jaggery	8.45	8.00	8.38	8.08	8.23	8.38	8.20	8.18	8.40	8.29
	Maize	8.23	8.10	8.30	8.03	8.16	8.65	8.10	8.63	8.13	8.38
	Mean	8.46	8.11	8.23	8.34	8.28	8.49	8.14	8.23	8.41	8.32
	SEM	0.177		0.214		0.202	0.200		0.263		0.229
	P Value	0.072		0.627		0.242	0.101		0.487		0.603
RBC (× 10 ⁶ /μL)	Control	5.02	5.93	5.35	5.59	5.47	4.99	6.07	5.31	5.75	5.53
	Glycerol	4.85	5.67	4.99	5.53	5.26	4.57	5.60	4.54	5.63	5.09
	Jaggery	5.52	5.79	5.18	6.13	5.66	5.83	5.80	5.56	6.06	5.81
	Maize	5.09	6.47	4.84	6.72	5.78	5.20	6.39	4.86	6.73	5.79
	Mean	5.12	5.96	5.09	5.99	5.54	5.15	5.96	5.07	6.04	5.56
	SEM	0.177		0.242		0.206	0.273		0.300		0.263
	P Value	0.001***		0.01**		0.112	0.01**		0.01**		0.087
Hb (g %)	Control	9.63	8.80	8.35	10.08	9.21	9.30	8.53	8.48	9.35	8.91
	Glycerol	8.70	8.80	8.13	9.38	8.75	8.40	8.88	7.90	9.38	8.64
	Jaggery	9.60	9.95	9.20	10.35	9.78	9.20	9.55	8.88	9.88	9.38
	Maize	9.20	9.08	9.03	9.25	9.14	9.08	8.93	8.65	9.35	9.00
	Mean	9.28	9.16	8.68	9.76	9.22	8.99	8.97	8.48	9.49	8.98
	SEM	0.231		0.221		0.247	0.308		0.225		0.281
	P Value	0.597		0.001***		0.097	0.936		0.001***		0.102
Platelets (× 10 ⁵ /μL)	Control	430	428	422	436	429	383	382	399	366	382
	Glycerol	471	443	464	451	457	373	373	355	392	373
	Jaggery	429	480	479	431	455	393	401	396	398	397
	Maize	538	459	513	485	499	500	328	338	490	414
	Mean	467	453	469	451	460	412	371	372	411	392
	SEM	25.36		19.73		20.55	28.34		25.98		29.22
	P Value	0.573		0.363		0.165	0.165		0.149		0.376
PCV (%)	Control	27.3	27.4	26.6	28.1	27.3	27.5	27.7	27.0	28.3	27.6
	Glycerol	26.5	28.9	27.9	27.5	27.7	26.6	29.0	28.3	27.3	27.8
	Jaggery	28.4	29.1	28.4	29.1	28.8	27.4	27.6	27.2	27.8	27.5
	Maize	26.9	27.3	26.7	27.5	27.1	26.9	27.7	26.7	27.9	27.3
	Mean	27.3	28.2	27.4	28.0	27.7	27.1	28.0	27.3	27.8	27.6
	SEM	0.533		0.540		0.545	0.519		0.563		0.519
	P Value	0.099		0.247		0.060	0.099		0.354		0.561

Parameter	Group	14 d after calving					30 d after calving				
		Energy × Breed		Energy × Supplement		E × B × S	Energy × Breed		Energy × Supplement		E × B × S
		HFX	Deoni	Without	With		HFX	Deoni	Without	With	
Lymphocyte (%)	Control	56.7	52.3	54.9	54.2	54.5	51.9	58.1	58.3	51.7	55.0
	Glycerol	49.6	50.8	50.2	50.2	50.2	48.1	54.2	49.7	52.6	51.2
	Jaggery	51.1	50.7	49.9	52.0	50.9	53.2	53.6	52.8	54.0	53.4
	Maize	53.3	48.8	52.7	49.4	51.0	50.5	48.1	48.6	50.0	49.3
	Mean	52.7	50.7	51.9	51.4	51.7	50.9	53.5	52.3	52.1	52.2
	SEM	1.479		1.440		1.404	1.838		1.766		1.661
P Value	0.186		0.746		0.059	0.187		0.881		0.135	
Monocyte (%)	Control	8.20	7.30	6.90	8.60	7.80	9.40	8.60	8.90	9.10	9.00
	Glycerol	7.60	8.30	7.70	8.20	8.00	8.40	9.60	8.00	10.0	9.00
	Jaggery	7.80	7.50	7.90	7.40	7.60	8.70	9.00	8.80	8.90	8.90
	Maize	7.90	7.60	8.10	7.40	7.70	9.70	8.40	9.10	9.00	9.00
	Mean	7.90	7.70	7.60	7.90	7.80	9.10	8.9	8.70	9.20	9.00
	SEM	0.324		0.344		0.331	0.329		0.305		0.368
P Value	0.571		0.436		0.532	0.590		0.102		0.762	
Granulocyte (%)	Control	35.1	40.3	38.2	37.2	37.7	38.7	33.3	32.8	39.2	36.0
	Glycerol	42.7	40.9	42.1	41.6	41.8	43.5	36.2	42.2	37.4	39.8
	Jaggery	41.1	41.8	42.2	40.7	41.4	38.1	37.5	38.4	37.1	37.8
	Maize	38.8	43.6	39.2	43.2	41.2	39.8	43.6	42.4	41.0	41.7
	Mean	39.4	41.7	40.4	40.7	40.5	40.0	37.6	39.0	38.7	38.8
	SEM	1.508		1.423		1.422	1.956		1.860		1.804
P Value	0.160		0.894		0.074	0.246		0.890		0.062	
MCV (%)	Control	55.7	51.8	54.7	52.8	53.8	60.9	52.4	60.7	52.6	56.7
	Glycerol	58.3	51.0	54.7	54.6	54.6	59.3	55.2	62.4	52.1	57.2
	Jaggery	68.7	45.5	56.7	57.5	57.1	60.7	46.3	52.3	54.7	53.5
	Maize	61.3	51.9	61.2	52.1	56.6	68.3	52.5	68.3	52.5	60.4
	Mean	61.0	50.1	56.8	54.2	55.5	62.3	51.6	60.9	53.0	56.9
	SEM	1.508		1.423		2.729	1.956		1.860		2.950
P Value	0.160		0.894		0.435	0.246		0.890		0.143	
MCH (%)	Control	14.9	14.4	14.0	15.4	14.7	14.5	14.4	15.2	13.7	14.5
	Glycerol	16.0	14.8	16.8	14.1	15.4	16.0	15.0	17.8	13.2	15.5
	Jaggery	17.6	12.7	14.7	15.5	15.1	18.4	14.1	15.8	16.7	16.2
	Maize	16.9	13.5	17.4	13.0	15.2	19.3	14.3	19.1	14.5	16.8
	Mean	16.3	13.8	15.7	14.5	15.1	17.0	14.5	17.0	14.5	15.7
	SEM	6.999		0.843		0.789	0.662		0.778		0.860
P Value	0.003**		0.161		0.549	0.002**		0.006**		0.092	

Values with different superscripts for absolute interaction for respective energy source in a row under each parameter differ significantly; ***P ≤ 0.001, **P ≤ 0.01

Table 4.55: Repeat measures extracted table for complete blood count

	WBC ($\times 10^3/\mu\text{L}$)	RBC ($\times 10^6/\mu\text{L}$)	Hb (g%)	Platelets ($\times 10^5/\mu\text{L}$)	PCV (%)	Monocytes (%)	Lymphocytes (%)	Granulocytes (%)	MCV (%)	MCH (%)
Within-subject effects										
Period \times Energy	0.38	0.001***	0.08	0.65	0.01**	0.23	0.15	0.188	0.04*	0.01**
Period \times Breed	0.17	0.001***	0.046*	0.25	0.80	0.85	0.049*	0.042*	0.001***	0.422
Period \times FNS	0.65	0.001***	0.001***	0.28	0.76	0.41	0.92	0.786	0.09	0.03*
Period \times Energy \times Breed	0.14	0.001***	0.03*	0.97	0.003**	0.001***	0.43	0.287	0.008	0.14
Period \times Energy \times FNS	0.57	0.03*	0.38	0.06	0.002**	0.01**	0.21	0.125	0.043*	0.15
Period \times Breed \times FNS	0.13	0.001***	0.001***	0.83	0.14	0.042*	0.56	0.393	0.049*	0.11
Period \times Energy \times Breed \times FNS	0.65	0.03*	0.18	0.23	0.002**	0.15	0.054	0.029*	0.523	0.47
Between subject effects										
Energy	0.69	0.21	0.02*	0.049*	0.78	0.94	0.19	0.227	0.843	0.69
Breed	0.06	0.001***	0.51	0.40	0.18	0.53	0.46	0.418	0.002**	0.06
Supplement	0.85	0.001***	0.001***	0.96	0.16	0.14	0.29	0.494	0.021*	0.85
Energy \times Breed	0.36	0.29	0.03*	0.01**	0.61	0.003**	0.08	0.137	0.144	0.36
Energy \times FNS	0.10	0.01**	0.14	0.39	0.58	0.2	0.62	0.66	0.03**	0.09
Breed \times FNS	0.17	0.11	0.08	0.06	0.06	0.003**	0.88	0.58	0.764	0.17
Energy \times Breed \times FNS	0.77	0.13	0.12	0.001***	0.16	0.02*	0.07	0.202	0.063	0.75

*Values with different superscripts for absolute interaction in a row under each parameter differ significantly; *** $P \leq 0.001$, ** $P \leq 0.01$, * $P \leq 0.05$

Increased oxygen demand during peripartum stimulates the release of erythropoietin in the kidney to further increasing the production of erythrocytes, in turn increasing the Hb concentration (Pittman, 2011; Klein *et al.*, 2013). Lawrence *et al.* (2017) also correlated the increase in Hb with an increase in the number of erythrocytes. Salama *et al.*, (2011) also opined that trace mineral supplementation with 20 mg Cu, 60 mg Zn and 0.3 mg Se/Kg diet from 30 days before calving to calving day improved Hb Concentration by 12% ($P < 0.05$) than the control group. HFX platelet count in the present study decreased from 14 days before calving to calving day from $429 \times 10^5/\mu\text{L}$ to $323 \times 10^5/\mu\text{L}$. It was $425 \times 10^5/\mu\text{L}$ to $330 \times 10^5/\mu\text{L}$ in Deoni cows. This decrease in platelet count at the time of calving might be due to the utilization of a large number of platelets to control the haemorrhage during parturition. Joshi *et al.* (2018) also reported that a significantly higher level of platelet count on day 7 and day 15 after parturition as compared to the day of parturition (457.33 ± 67.79 , 572.33 ± 66.89 and $300.67 \pm 21.69 \times 10^6/\mu\text{L}$). However, energy \times FNS supplement interaction was found non-significant throughout the experiment period. Energy \times FNS supplement effect, and energy \times breed \times FNS supplement effect were found non-significant throughout the experiment period.

The data revealed that there was no effect between breed, supplement and energy, breed and FNS supplement interaction on PCV and MCV during postpartum due to prepartum planned feeding in experiment cows. Energy \times FNS supplement and energy \times breed \times FNS supplement was also non-significant for PCV and MCV. All the values were very much within the physiological range. MCH (%) was found significantly high ($P < 0.05$) in HFX cows than Deoni cows on day 30 and day 14 prior to calving until day 30 after calving. FNS supplemented cows on the day of calving had significantly higher ($P < 0.01$) MCH (%) than cows with FNS supplementation. But the values were found in the physiological range 13-18 pg (Radostits *et al.*, 2000). Găvan *et al.* (2010) also reported that no significant difference was observed for erythrocyte indices during prepartum. The blood profile from 30 days prior to calving to 30 days after calving showed significant increase in RBC and Hb (g%) due to energy and FNS supplement as well as breed impact. Generally, glycolysis is reduced in RBC during prepartum like reduction in β -oxidation in WBC but improves in postpartum (Dębski *et al.*,

2017) hence, any improvement in WBC and RBC in prepartum reduce NEB in postpartum because increased expression of genes involved in fatty acid transport and oxidation after parturition in dairy cow (Schäff *et al.*, 2013). Based on the blood count observed during prepartum and postpartum hardly and prognosis of pathological or metabolic disease or sickness noticed in cows (Ronald *et al.*, 2014). Since, there was no abnormal morphology or blood cell impairment noticed. The preliminary screening i.e., total blood count as certain health of all the experimental cows.

4.4.2 Serum parameters

Serum parameters during 30 days prior to calving (**Table 4.56**), on calving day (**Table 4.57**) and the 30 days postpartum (**Table 4.58**) were evaluated. Blood glucose in HFX or Deoni cows due to effect of energy was 65 mg/dL ($P = 0.81$) and comparable with normal range. The lower levels of blood glucose i.e., 60 mg/dL has been reported in repeat breeders than normal cows with 79 mg/dL irrespective of primiparous and multiparous cows. This vital source of energy for many of the cells in the body are available from the breakdown of the carbohydrate, and blood glucose level will be maintained but not from the endogenous production by gluconeogenesis and glycolysis. Glycerol and inverted sugar in jaggery enter the glucogenic pathway at triose phosphate level which is metabolically closer to glucose (Leng, 1970). In case of glycerol, glycerol kinase converts glycerol to glycerol 3-phosphate which enters either gluconeogenesis or glycolysis. On day 14 prior to calving, the mean glucose concentration in FNS supplemented group although non-significant was lesser by 1.3 % than the CG. Such a small reduction may occur when Cr supplemented because of increased insulin function and increased glucose uptake by the cells. The mean glucose value during the prepartum, on the day calving and postpartum was found within the physiological range i.e., 42 mg/dL to 75 mg/dL (Latimer *et al.*, 2003). DeFrain *et al.* (2004) also supported that prepartum supplementation of either corn starch or glycerol did not significantly increase prepartum glucose (73.3 vs. 70.5 mg/dL). In contrast, Goff and Horst (1997) reported that drenching of 0.83, 1.66, and 2.49 kg of glycerol via oesophageal pump increased concentrations of blood glucose by 16, 20, and 25%,

respectively, 30 min after dosing. Linke *et al.* (2004) compared delivery methods of glycerol (feeding vs. drenching 800 g) and found drenching to be more efficacious at increasing plasma glucose and insulin concentrations. But in the present experiment, blood samples were collected before feeding the glycerol. No significant difference was found in blood glucose of cows due to interaction of breed × FNS supplement, energy × FNS supplement and energy × breed × FNS supplement. Blood glucose was improved up to 70 mg/dl after calving in postpartum on day 14 and day 30. Although all the values were found within the physiological range, such increase is important for lactation as well as first heat after calving. Kappal *et al.* (1984) observed such increase after 25 days of calving in normal cows unlike our observations in the present study. Probably, the energy and FNS supplement fortification to diet might advanced the blood glucose increase.

Serum triglycerides were not significantly different between HFX or Deoni cows fortified with different energy sources or different energy sources supplemented with FNS supplement or breed impact with or without FNS supplement or triangular interaction of energy, breed and FNS supplement. Serum triglycerides were 12 mg/dL to 14 mg/dL during prepartum and reduced to 10 mg/dL to 11 mg/dL on the day of calving or postpartum up to 30 days. Radostits *et al.* (2000) suggested 13.57 ± 0.73 mg/dL in dairy animals including cattle and buffaloes with a reference range of 0 to 14 mg/dL. Triglycerides, which are present within adipose tissue are the body's major fuel reserve (Hall *et al.*, 2005) were found lesser in the present study than many other reports (Nejad and Cheraghi, 2003; Nozad *et al.*, 2012). Guretzky *et al.* (2006) reported improvement in triglycerides in periparturient cows receiving the rumen protected choline from 14.7 mg/dL to 17.0 mg/dL where these values were comparable to serum triglycerides in prepartum but not on 30 days of postpartum. Triglycerides are fatty acid esters of glycerol and represent the main lipid component of dietary fat and fat depots of animals. According to Hall *et al.*, (2005) each adipocyte contains 0.4 to 0.6 μ g of triglycerides and 1000 adipocytes yield 3.71 cal of energy. Triglycerides, cholesterol and high density lipoproteins are important constituents of the lipid fractions in the body thus, their lower levels in serum

explains less export of triglycerides from liver into the blood and within normal physiological range.

Total cholesterol was considered as an indirect index of liver function. Serum total cholesterol was found significant ($P < 0.05$) only on day 14 after calving in cows due to energy, breed and FNS supplement interaction. However, glycerol fortified T1 cows total cholesterol (118 mg/dL) was comparable to jaggery fortified T2 cows (112 mg/dL) and significantly higher ($P < 0.05$) than maize fortified T3 cows (103.8 mg/dL). T2 and T3 were comparable to CG (110.4 mg/dL) but not with T1 ($P < 0.03$). This indicated that glycerol fortification in prepartum had advantage in improving the serum cholesterol levels during postpartum which can also comparably noticed on day 30 ($P = 0.36$). Nejad and Cheraghi (2003) reported 82 mg/dL to 88 mg/dL in normal and repeat breeder HF cows in contrast to above 100 mg/dL observed in the present study except on the day of calving that was 92 mg/dL to 95 mg/dL. The postpartum increase in total cholesterol is required for lactating cows (Schweigert and Eisele, 1990). Kappal *et al.*, (1984) suggested that postpartum serum cholesterol increase up to 88 days after calving and the normal range was 125 ± 29 mg/dL. Increased serum total cholesterol concentration is a result of increased secretion of VLDL (Very Low-Density Lipoproteins) and the higher level of cholesterol with the advancement of lactation might be a physiological adjustment to meet the lactation requirements (Neama, 2015). No significant difference was found in postpartum due to breed \times dietary energy, and breed \times FNS supplement in cows during prepartum.

TP concentration was decreased from 7.7 g/dl to 7.3 g/dl as the calving day approached. This could be due to synthesis of foetus all its protein from maternal tissue of the dam or due to drain of immune fraction in the form of colostrum (Yousuf *et al.*, 2016). TP are heterogenous groups of thousands of individual proteins and any change its concentration affect the oncotic pressure, transportation of substances and in coagulation etc. Sateesh *et al.* (2018) also reported that prepartum TP was significantly ($P < 0.01$) lesser than the healthy control group. TP in prepartum was irrespective of different groups and factors was 7.1 g/dL to to 7.7 g/dL with little decrease of 0.1 g/dL to 0.3 g/dL TP on the day of calving.

Table 4.56: Serum parameters in different groups of cows during the prepartum experiment period

Parameter	Group	30 d before calving					14 d before calving				
		Energy × Breed		Energy × Supplement		E × B × S	Energy × Breed		Energy × Supplement		E × B × S
		HF	Deoni	Without	With		HF	Deoni	Without	With	
Glucose (mg/dL)	Control	64.2	69.5	65.3	68.4	66.9	65.0	59.8	62.2	62.7	62.4
	Glycerol	64.2	56.9	56.1	65.0	60.5	62.4	62.1	64.2	60.3	62.2
	Jaggery	64.6	67.6	64.7	67.5	61.1	63.0	63.8	62.0	64.8	63.4
	Maize	66.5	68.3	66.9	67.9	67.4	65.9	63.7	66.0	63.7	64.8
	Mean	64.9	65.6	63.2	67.2	65.2	64.1	62.4	63.6	62.8	63.2
	SEM	2.820		2.259		2.534	1.354		1.447		1.135
	P Value	0.806		0.100		0.092	0.225		0.618		0.225
BUN (mg/dL)	Control	15.4	16.9	16.9	15.5	16.2	17.7	18.5	19.3	16.9	18.1
	Glycerol	14.8	13.9	14.3	14.4	14.3	17.0	16.9	16.1	17.8	16.9
	Jaggery	15.2	16.9	18.5	13.6	16.1	16.0	17.5	16.9	16.7	16.8
	Maize	13.9	14.0	13.2	14.8	14.0	16.8	18.2	18.8	16.2	17.5
	Mean	14.8	15.4	15.7	14.6	15.1	16.9	17.8	17.8	16.9	17.3
	SEM	1.071		1.155		1.019	0.658		0.811		0.732
	P Value	0.576		0.340		0.176	0.183		0.288		0.263
Total Cholesterol (mg/dL)	Control	109.5	99.4	107.5	101.3	104.4	117.2	114.4	117.9	113.7	115.8
	Glycerol	108.8	125.6	111.2	123.2	117.2	114.8	123.9	116.9	121.8	119.3
	Jaggery	101.2	105.2	96.6	109.8	103.2	118.4	114.7	124.2	109.0	116.6
	Maize	130.0	105.8	118.8	117.0	117.9	114.0	112.6	115.9	110.8	113.3
	Mean	112.4	109.0	108.5	112.8	110.7	116.1	116.4	118.7	113.8	116.2
	SEM	6.094		4.367		5.691	3.414		3.548		3.391
	P Value	0.589		0.340		0.106	0.930		0.188		0.266
Triglyceride (mg/dL)	Control	14.3	11.7	12.3	13.7	13.0	15.3	13.5	15.2	13.6	14.4
	Glycerol	13.4	11.6	12.8	12.3	12.5	12.5	12.8	12.8	12.4	12.6
	Jaggery	10.7	10.9	10.3	11.4	10.8	12.5	11.6	12.2	12.0	12.1
	Maize	12.1	11.9	12.5	11.5	12.0	12.6	12.5	13.1	12.0	12.5
	Mean	12.6	11.5	11.9	12.2	12.1	13.2	12.6	13.3	12.5	12.9
	SEM	0.690		0.731		0.727	0.475		0.394		0.449
	P Value	0.128		0.737		0.067	0.199		0.057		1.000

Parameter	Group	30 d before calving					14 d before calving				
		Energy × Breed		Energy × Supplement		E × B × S	Energy × Breed		Energy × Supplement		E × B × S
		HF	Deoni	Without	With		HF	Deoni	Without	With	
Total protein (g/dL)	Control	7.1	6.7	6.9	6.9	6.9	7.1	6.9	6.9	7.1	7.0
	Glycerol	8.1	7.9	7.9	8.1	8.0	7.3	7.1	7.4	7.0	7.2
	Jaggery	7.8	8.1	7.8	8.1	7.9	6.8	7.5	7.3	7.0	7.2
	Maize	7.6	7.9	7.8	7.7	7.8	7.3	7.7	7.7	7.3	7.5
	Mean	7.6	7.7	7.6	7.7	7.6	7.1	7.3	7.3	7.1	7.2
	SEM	0.175		0.176		0.149	0.261		0.142		0.243
	P Value	0.916		0.555		0.252	0.498		0.194		0.207
Albumin (g/dL)	Control	2.6	3.5	2.5	3.6	3.0	3.0	3.6	3.2	3.4	3.3
	Glycerol	3.0	3.3	3.0	3.3	3.2	3.4	3.2	3.1	3.4	3.3
	Jaggery	3.3	3.3	3.2	3.3	3.3	3.1	3.1	3.2	3.0	3.1
	Maize	3.0	3.3	3.1	3.2	3.1	3.1	3.3	3.2	3.3	3.2
	Mean	3.0	3.3	3.0	3.3	3.1	3.1	3.3	3.2	3.3	3.2
	SEM	0.167		0.199		0.179	0.148		0.129		0.128
	P Value	0.054		0.071		0.400	0.310		0.371		0.294
Creatinine (mg/dL)	Control	1.2	1.2	1.1	1.3	1.2	1.5	1.3	1.4	1.4	1.4
	Glycerol	1.3	1.3	1.4	1.2	1.3	1.5	1.2	1.5	1.3	1.4
	Jaggery	1.3	1.3	1.4	1.1	1.3	1.4	1.3	1.4	1.3	1.4
	Maize	1.3	1.3	1.4	1.2	1.3	1.5	1.2	1.4	1.3	1.3
	Mean	1.3	1.2	1.3	1.2	1.3	1.5	1.3	1.4	1.3	1.4
	SEM	0.066		0.076		0.054	0.041		0.062		0.054
	P Value	0.780		0.079		0.150	0.001***		0.227		0.384
AST (U/L)	Control	76.0	72.0	74.4	73.6	74.0	73.1	71.9	73.1	71.9	72.5
	Glycerol	70.3	73.6	68.3	75.5	71.9	69.4	73.3	71.4	71.3	71.3
	Jaggery	78.0	62.8	70.6	70.2	70.4	74.0	65.0	67.2	71.8	69.5
	Maize	60.5	78.3	68.7	70.2	69.4	65.6	77.1	71.0	71.7	71.3
	Mean	71.2	71.7	70.5	72.4	71.4	70.5	71.8	70.7	71.6	71.2
	SEM	3.922		2.467		3.336	2.705		2.076		2.378
	P Value	0.909		0.463		0.387	0.643		0.647		0.424

Parameter	Group	30 d before calving					14 d before calving				
		Energy × Breed		Energy × Supplement		E × B × S	Energy × Breed		Energy × Supplement		E × B × S
		HF	Deoni	Without	With		HF	Deoni	Without	With	
ALT (U/L)	Control	21.8	19.3	23.3	17.9	20.6	22.7	24.5	24.4	22.8	23.6
	Glycerol	19.9	24.7	22.6	22.0	22.3	22.6	24.7	21.7	25.6	23.6
	Jaggery	21.8	22.0	21.7	22.1	21.9	19.8	24.5	22.7	21.7	22.2
	Maize	18.4	25.6	23.8	20.1	22.0	21.2	26.6	23.9	23.9	23.9
	Mean	20.5	22.9	22.8	20.5	21.7	21.6	25.1	23.2	23.5	23.3
	SEM	1.398		1.191		1.350	1.534		1.623		1.454
	P Value	0.102		0.071		0.428	0.038		0.850		0.449
AST:ALT	Control	3.55	4.06	3.27	4.33	3.80	3.34	3.00	3.05	3.30	3.17
	Glycerol	3.61	3.03	3.06	3.58	3.32	3.10	3.12	3.33	2.89	3.11
	Jaggery	3.64	2.92	3.35	3.21	3.28	3.79	2.66	3.11	3.35	3.23
	Maize	3.36	3.09	2.90	3.55	3.23	3.17	2.94	3.10	3.00	3.05
	Mean	3.54	3.27	3.15	3.67	3.41	3.35	2.93	3.15	3.14	3.14
	SEM	0.206		0.181		0.199	0.204		0.237		0.234
	P Value	0.220		0.011*		0.078	0.080		0.960		0.630
GGT (IU/L)	Control	13.3	13.0	12.0	14.3	13.1	14.6	13.8	13.7	14.7	14.2
	Glycerol	13.5	13.4	13.1	13.8	13.4	15.0	12.6	13.8	13.8	13.8
	Jaggery	12.4	14.2	13.1	13.5	13.3	13.8	12.8	13.6	13.0	13.3
	Maize	11.8	14.0	14.6	11.2	12.9	13.5	13.3	14.5	12.3	13.4
	Mean	12.7	13.6	13.2	13.2	13.2	14.2	13.1	13.9	13.4	13.7
	SEM	0.853		0.954		0.977	0.531		0.516		0.533
	P Value	0.314		1.000		0.738	0.056		0.360		0.268

Values with different superscripts for absolute interaction for respective energy source in a row under each parameter differ significantly; ***P≤ 0.001, *P≤ 0.05

It was recovered to prepartum levels thereafter till 30 days of post calving. Nozad *et al.* (2012) reported serum portien content of 10.40 mg/dL and 9.80 mg/dL in high and low yielding cows. Albumin concentration throughout the experiment was similar except a small increase in concentration at the time of calving (3.0g/dl to 3.1 g/dl). Generally, increased albumin concentration in serum is due to higher synthesis of albumin by the liver (Piccione *et al.*, 2012). TP, albumin and BUN were not having any significant differences between breeds during prepartum or on the day of calving, and postpartum. Energy fortification or energy \times breed or/and FNS supplement interaction was also found non-significant on serum albumin from -30 days from calving to +30 days after calving. All the values during this period were within physiological range i.e. 5.7g/dL to 8.1 g/dL for TP, 2.1 g/dL to 3.6 g/dL for albumin, and 6 mg/dL to 27 mg/dL for BUN. Nozad *et al.* (2012) reported 24 mg/dL of BUN in cows irrespective of milk yield such as high or low. No differences in BUN values in the experiment period indicated that the ammonia produced in the rumen from dietary protein was utilized efficiently (Vongsamphan and Wanapat, 2004). Nejad and Cheraghi (2003) reported that difference in serum levels of glucose, cholesterol, triglycerides, TP, and albumin in repeat breeders and native cows was because deficiency in energy and protein in diets.

Creatinine concentration in HFX cows (1.5 mg/dL) was found significantly higher ($P < 0.001$) than Deoni cows (1.3 mg/dL) 14 days prior to calving because elevated level of creatinine on day 14 before calving is because foetal maternal circulation and a load of organic waste of the foetus (Ghanem *et al.*, 2012; Ferrell, 1991) where HF calf birth weight was 10 kg higher than the Deoni calves. On the day of calving and during the postpartum, there was no significant difference in serum cretinie between HFX and Deoni cows with source of energy or FNS supplement to the energy. Energy, breed and FNS supplement interaction was also found non-significant in prepartum and postpartum. FNS supplementation in prepartum had non-significant effect on serum creatinine between fortnights starts from 30 days prior to 30 days after calving. All the mean values for serum creatinine levels in this study for different factors interaction were below 1.5 mg/dL. which is specified as an upper limit to the serum creatinine level

(Radostits *et al.*, 2000), while Nozad *et al.*(2012) reported 0.86 and 0.75 mg/dL in high and low producing cows with a mean of 0.81 mg/dL .

The assay of AST, ALT and GGT concentration in plasma or serum can predict the condition of hepatocytes (Steen *et al.*, 1997). AST is known as a serum biomarker for liver health. In the case of exacerbating liver damage, AST is more sensitive biomarker (Kew *et al.*, 2000). Serum AST was 71 IU/L to 74 IU/L in prepartum and on the day of calving, but 85 IU/L to 95 IU/L in postpartum. Different periods (**Table 4.59**) had significant affect from periods \times dietary energy ($P < 0.001$), and periods \times dietary energy \times breed ($P < 0.001$) but not significantly different due to diet energy \times breed interaction affect in prepartum till 30 days after postpartum. The AST concentration in fresh cows was higher than prepartum cows. The normal AST physiological range in healthy cows is 70 IU/L to 132 IU/L (Invartsen, 2006). Higher concentration of AST adversely affect lactation progress (Elitok *et al.*, 2010). FNS supplementation had no influence on the serum AST. Energy, breed and supplement interaction was also found non-significant on serum AST. Sun *et al.* (2014) found the correlation between AST and ketosis. They reported that the ketosis prediction threshold for AST was >104 IU/L which was higher than AST level observed in postpartum. Sheth *et al.* (1998) reported that in severe hepatitis and chronic hepatitis cases, AST will be significantly increased than the normal range. In this study, all the mean values for AST (70.5- 93.5 U/L) were found within the physiological range. In the case of acute liver damage, ALT is a more sensitive indicator (Kew, 2000) than AST but, ALT activity is not associated with ketosis Sun *et al.*, (2014). ALT was less than 25 IU/L irrespectve of dietary or breed factors interaction in prepartum, on the day of calving or postpartum till 30 days after calving. Period \times breed, or period \times FNS supplement, or Period \times energy \times breed \times FNS supplement interactions had significant affect on ALT levels in serum. There would be doubling of ALT activity and AST increases continue to exceed the value of ALT in case of 1% hepatocytes are damaged. This condition indicates that damage in hepatic parenchyma (Giannini *et al.*, 1999). ALT was found significant in the breed during postpartum period i.e., on day 14 before calving as well as on the day of calving. On day 14 before calving, ALT concentration in Deoni cow serum (25.1 IU/L) was significantly higher ($P < 0.05$) than HFX cows (21.6 IU/L). On the

day of calving also, Deoni cows ALT concentration in serum (23.4 IU/L) was found significantly higher ($P < 0.05$) than HFX cows (18.7 IU/L). In these two conditions, ALT concentration in both breeds was mid value of normal physiological range 11 IU/L to 44 IU/L (Rodostits *et al.*, 2000). Whenever mild hepatic damage occurs the increase in ALT was higher than AST i.e. AST/ALT is < 1.0 (Sheth *et al.*, 1998).

The ratio between AST to ALT was 1:3 to 1:4 in the present study. AST:ALT was not significant between breeds or diet with FNS supplementation. Energy, breed and supplement interaction was also found non-significant. AST:ALT is a biomarker for liver health. Although ALT and AST is similar, the former is predominantly found in liver and later is found in liver, heart, kidney, brain and RBC. Increased AST activity in the serum is a sensitive marker of liver damage, even if the damage is of a subclinical nature (Meyer and Harvey, 1998). Unlike AST, horse, pig, and ruminant liver cells do not show high ALT activity, and the increased activity of that enzyme in the serum during liver damage, even in necrosis, is insignificant (Forenbacher, 1993). According to Stojević *et al.* (2005) seasons, circadian changes, age of the animal and energy status have an influence on ALT, AST and AST:ALT values and constant monitoring has been suggested in the point of nutrition, health and production.

Breed, supplementation, energy breed and supplement interaction were found non-significant for GGT. It is considered a valuable biomarker of cholestasis or hepatic damage. We observed 12 IU/L to 14 IU/L during prepartum and increased to 14 IU/L to 15 IU/L on the day of calving, and 15 IU/L to 16 IU/L subsequently in postpartum but, within the normal physiological range of 6.1- 17.4 IU/L (Rodostits *et al.*, 2000). The summary of significance levels of different serum metabolites is shown in (**Table 5.11**). Repeat measure analysis confirmed that the variation in BUN, TG, and ALT was observed throughout the experiment period and the difference was significant among the energy fortified groups (**Table 4.59**). The variation in creatinine, and ALT was observed between periods thus, indicating influence of physiological stage transformation from close-up to fresh cows. The variation in TP and AST concentration in FNS supplement cows was observed over 60 days of period spreading from 30 days in prepartum and 30 days in postpartum.

Serum glucose, triglycerides, total cholesterol, TP, albumin, BUN, creatinine, AST, ALT, AST:ALT ratio and GGT were within normal physiological range thus, indicated healthy liver and kidney in the postpartum due the prepartum dietary intervention. Although dietary CP intake was 10% more than the requirement, neither BUN nor triglycerides showed above critical physiological range. Hence, it could be presumed that the excess AAs metabolized in the liver to cater the any deficiency in energy which found sufficiently to fulfill 15% of the total energy in lactating cows (Chung *et al.*, 2015; Zeng *et al.*, 2017).

4.4.3 Prepartum nutrition effect on postpartum energy metabolites

Serum energy metabolites during the prepartum period (**Table 4.60**), on the day of calving (**Table 4.61**) and the postpartum period (**Table 4.62**) were analysed to confirm any NEB within different periods because postpartum CP intake was higher than the requirement for lactating cows, but ME intake was 8% to 15% lesser in HFX cows and 2% to 4% lesser in Deoni cows. A rough estimate of 3 Mcal NE/day is used for mammary requirements in the periparturient cows (Van Den *et al.*, 1995). Bertics *et al.* (1992) suggested that the prepartum decline in DMI may lead to lipid mobilization from adipose tissue and became a cause for lipid-related metabolic disorders such as fatty liver and ketosis. Lipids, released as NEFA from adipose tissue are substrate for oxidation and are used by many tissues as an energy source during periods of NEB. NEFA concentration in prepartum was maximum of 0.57 mmol/L which was below the concentration of threshold levels of ≥ 0.3 mmol/L to 0.5 mmol/L (Kaufmann *et al.*, 2010). NEFA concentration of 0.52 mmol/L in 14 days prior to calving in HFX cows increased to 0.77 mmol/L or ranged from 0.75 mmol/L to 0.77 mmol/L only on the day of calving, but back to the maximum concentration of 0.57 mmol/L on day 14 and on day 30 after calving or postpartum which was also lesser than the threshold levels of NEFA suggested during postpartum that is, ≥ 0.7 mmol/L to 1.0 mmol/L (Kaufmann *et al.*, 2010). Plasma NEFA began increasing in prepartum were peaked at calving (0.5 meq/L to 1.2 meq/L), but declined during postpartum (< 0.6 meq/L) and such phenomena are common for healthy cows and a good sign. It appears that only at the time of calving, cows faced the lesser energy hence, adipose tissue might be mobilized to meet out the demands.

Table 4.57: Serum parameters in different groups of cows on the day of calving

Parameter	Group	Calving day				E × B × S
		Energy × Breed		Energy × Supplement		
		HF	Deoni	Without	With	
Glucose (mg/dL)	Control	62.7	60.0	62.2	60.6	61.4
	Glycerol	61.7	64.5	62.8	63.4	63.1
	Jaggery	64.6	62.6	62.5	64.7	63.6
	Maize	61.2	60.8	62.8	59.7	61.0
	Mean	62.5	62.0	62.6	61.9	62.2
	SEM	1.467		1.667		1.554
P Value	0.704		0.721		0.298	
BUN (mg/dL)	Control	18.2	22.7	21.6	19.3	20.4
	Glycerol	21.1	21.3	21.1	21.3	21.2
	Jaggery	20.4	19.0	19.3	20.1	19.7
	Maize	19.6	21.8	19.8	21.5	20.7
	Mean	19.8	21.2	20.4	20.5	20.5
	SEM	1.144		0.993		0.913
P Value	0.263		0.916		0.304	
Total Cholesterol (mg/dL)	Control	93.3	95.4	97.1	91.7	94.4
	Glycerol	93.6	93.3	98.1	88.8	93.5
	Jaggery	95.8	87.0	92.5	90.3	91.4
	Maize	87.7	99.9	92.3	95.3	93.8
	Mean	92.6	93.9	95.0	91.5	93.2
	SEM	3.199		3.021		3.032
P Value	0.696		0.266		0.535	
Triglyceride (mg/dL)	Control	15.2	12.4	14.0	13.6	13.8
	Glycerol	9.1	9.5	9.9	8.7	9.3
	Jaggery	9.6	9.1	9.2	9.5	9.4
	Maize	9.0	10.1	9.7	9.5	9.6
	Mean	10.7	10.3	10.7	10.3	10.5
	SEM	0.761		0.554		0.684
P Value	0.563		0.495		1.000	
Total protein (g/dL)	Control	6.8	6.7	6.5	6.9	6.7
	Glycerol	6.9	7.6	7.3	7.2	7.2
	Jaggery	6.7	7.2	6.9	7.0	6.9
	Maize	7.3	6.7	6.6	7.4	7.0
	Mean	6.9	7.0	6.8	7.1	7.0
	SEM	0.316		0.341		0.189
P Value	0.218		0.123		0.099	
Albumin (g/dL)	Control	2.7	3.5	2.7	3.5	2.7
	Glycerol	3.0	3.1	3.0	3.1	3.0
	Jaggery	2.9	3.1	3.3	2.7	3.0
	Maize	3.3	2.9	3.1	3.1	3.1
	Mean	3.0	3.1	3.0	3.1	2.9
	SEM	0.214		0.187		0.195
P Value	0.511		0.768		0.597	

Parameter	Group	Calving day				E × B × S
		Energy × Breed		Energy × Supplement		
		HF	Deoni	Without	With	
Creatinine (mg/dL)	Control	1.5	1.5	1.5	1.5	1.5
	Glycerol	1.5	1.4	1.6	1.3	1.5
	Jaggery	1.3	1.4	1.4	1.4	1.4
	Maize	1.4	1.3	1.4	1.3	1.3
	Mean	1.4	1.4	1.5	1.4	1.4
	SEM	0.070		0.060		0.057
P Value	0.930		0.196		0.130	
AST (IU/L)	Control	83.7	77.7	84.4	77.0	80.7
	Glycerol	64.1	65.2	61.9	67.5	64.7
	Jaggery	67.4	78.8	74.6	71.6	73.1
	Maize	69.1	73.7	71.4	71.5	71.4
	Mean	71.1	73.9	73.1	71.9	72.5
	SEM	2.251		2.286		2.225
P Value	0.235		0.619		1.000	
ALT (IU/L)	Control	20.0	24.6	21.6	23.0	22.3
	Glycerol	19.9	21.0	17.2	23.7	20.4
	Jaggery	17.0	24.4	20.4	21.0	20.7
	Maize	17.9	23.5	20.2	21.2	20.7
	Mean	18.7	23.4	19.8	22.2	21.0
	SEM	1.797		1.828		1.369
P Value	0.021*		0.214		0.383	
AST:ALT	Control	4.37	3.57	4.15	3.78	3.97
	Glycerol	3.30	3.24	3.61	2.92	3.27
	Jaggery	4.18	3.29	3.76	3.71	3.73
	Maize	3.90	3.16	3.55	3.51	3.53
	Mean	3.94	3.31	3.77	3.48	3.63
	SEM	0.346		0.344		0.263
P Value	0.094		0.412		0.099	
GGT (U/L)	Control	14.5	14.9	14.2	15.1	14.7
	Glycerol	15.8	13.1	14.8	14.1	14.4
	Jaggery	13.2	14.2	14.0	13.3	13.7
	Maize	14.6	13.7	14.2	14.1	14.1
	Mean	14.5	14.0	14.3	14.2	14.2
	SEM	0.516		0.375		0.502
P Value	0.325		0.719		0.216	

Values with different superscripts for absolute interaction for a respective energy source in a row under each parameter differ significantly; *P≤ 0.05

Table 4.58: Serum parameters in different groups of cows during the postpartum period

Parameter	Group	14 d after calving					30 d after calving				
		Energy ×Breed		Energy × Supplement		E × B × S	Energy ×Breed		Energy × Supplement		E × B × S
		HF	Deoni	Without	With		HF	Deoni	Without	With	
Glucose (mg/dL)	Control	63.6	67.2	68.1	62.7	65.4	66.6	67.6	67.3	66.9	67.1
	Glycerol	71.5	64.2	66.5	69.2	67.9	69.1	65.1	66.9	67.3	67.1
	Jaggery	70.0	66.5	66.8	69.7	68.3	65.1	70.7	60.6	75.2	67.9
	Maize	69.4	68.1	69.3	68.2	68.7	69.6	72.8	70.9	71.5	71.2
	Mean	68.6	66.5	67.7	67.4	67.6	67.6	69.0	66.4	70.2	68.3
	SEM	2.148		2.285		2.164	1.977		2.592		2.769
	P Value	0.387		0.925		0.331	0.471		0.163		0.348
BUN (mg/dL)	Control	21.7	20.4	20.9	21.2	21.2	21.2	23.3	22.5	21.9	22.2
	Glycerol	21.3	21.2	19.4	23.1	21.3	23.4	21.8	21.1	24.1	22.6
	Jaggery	23.5	19.0	20.8	21.8	21.3	21.1	15.6	17.0	19.7	18.3
	Maize	22.5	24.5	23.8	23.2	23.5	23.0	25.0	23.6	24.4	24.0
	Mean	22.2	21.3	21.2	22.3	21.8	22.0	21.4	21.0	22.5	21.8
	SEM	0.795		0.707		0.833	1.289		1.089		1.066
	P Value	0.247		0.147		0.070	0.573		0.183		0.278
Total Cholesterol (mg/dL)	Control	109.2	111.5	111.7	109.1	110.4 ^{ab}	123.9	111.5	120.8	114.7	117.7
	Glycerol	107.5	128.4	122.8	113.1	118.0 ^b	119.6	132.3	136.7	115.2	125.9
	Jaggery	112.3	112.1	112.7	111.7	112.2 ^{ab}	120.1	124.0	117.8	126.3	122.0
	Maize	101.0	106.6	99.2	108.4	103.8 ^a	123.4	115.1	121.1	117.3	119.2
	Mean	107.5	114.7	111.6	110.6	111.1	121.8	120.7	124.1	118.4	121.2
	SEM	4.215		3.837		3.871	5.820		6.262		5.681
	P Value	0.110		0.797		0.025*	0.860		0.378		0.361
Triglyceride (mg/dL)	Control	11.9	9.6	10.5	11.0	10.7	11.4	9.2	10.2	10.4	10.3
	Glycerol	10.1	11.6	10.2	11.4	10.8	9.7	10.0	9.9	9.8	9.8
	Jaggery	10.9	9.5	9.1	11.3	10.2	9.8	8.4	8.7	9.5	9.1
	Maize	10.2	11.1	10.8	10.5	10.6	9.3	10.3	10.4	9.2	9.8
	Mean	10.8	10.4	10.2	11.0	10.6	10.0	9.5	9.8	9.7	9.8
	SEM	0.619		0.576		0.520	0.436		0.478		0.415
	P Value	0.594		0.161		0.437	0.198		0.877		0.070

Parameter	Group	14 d after calving					30 d after calving				
		Energy × Breed		Energy × Supplement		E × B × S	Energy × Breed		Energy × Supplement		E × B × S
		HF	Deoni	Without	With		HF	Deoni	Without	With	
Total protein (g/dL)	Control	7.0	7.1	7.1	7.0	7.0	7.1	7.3	7.2	7.2	7.2
	Glycerol	7.2	7.4	7.8	6.7	7.3	7.4	7.2	7.5	7.1	7.3
	Jaggery	7.4	7.3	8.0	6.8	7.4	7.1	7.6	7.5	7.2	7.3
	Maize	6.9	7.7	7.7	6.9	7.3	8.0	7.6	8.1	7.4	7.8
	Mean	7.1	7.4	7.6	6.9	7.2	7.4	7.4	7.6	7.2	7.4
	SEM	0.246		0.294		0.230	0.279		0.244		0.239
	P Value	0.125		0.057		0.371	0.792		0.257		0.132
Albumin (g/dL)	Control	3.1	3.1	3.1	3.1	3.1	3.2	3.2	3.3	3.2	3.2
	Glycerol	2.9	2.8	2.8	2.9	2.9	3.2	3.2	3.1	3.3	3.2
	Jaggery	3.1	3.1	3.1	3.1	3.1	3.2	3.2	3.4	3.0	3.2
	Maize	3.1	3.2	3.3	2.9	3.1	3.2	3.1	3.3	3.0	3.1
	Mean	3.0	3.1	3.1	3.0	3.0	3.2	3.2	3.2	3.1	3.2
	SEM	0.077		0.104		0.099	0.054		0.093		0.088
	P Value	0.749		0.414		0.115	0.652		0.247		0.587
Creatinine (mg/dL)	Control	1.3	1.4	1.3	1.3	1.3	1.1	1.3	1.1	1.3	1.2
	Glycerol	1.2	1.2	1.3	1.2	1.2	1.1	1.1	1.0	1.1	1.1
	Jaggery	1.3	1.4	1.4	1.2	1.3	1.3	1.2	1.4	1.1	1.3
	Maize	1.3	1.2	1.3	1.1	1.2	1.0	1.1	1.0	1.1	1.1
	Mean	1.3	1.3	1.3	1.2	1.3	1.1	1.2	1.1	1.2	1.1
	SEM	0.056		0.062		0.063	0.052		0.079		0.068
	P Value	0.741		0.149		0.351	0.293		0.700		0.069
AST (U/L)	Control	87.9	81.2	84.8	84.3	84.6	97.7	92.3	95.1	94.8	95.0
	Glycerol	84.9	85.9	83.9	87.0	85.4	85.2	90.9	87.6	88.5	88.1
	Jaggery	86.2	84.4	87.8	82.9	85.3	92.9	92.8	96.3	89.5	92.9
	Maize	85.2	88.5	88.5	85.2	86.8	91.7	96.6	95.1	93.2	94.1
	Mean	86.1	85.0	86.2	84.8	85.5	91.9	93.1	93.5	91.5	92.5
	SEM	1.479		1.098		1.518	1.589		1.324		1.519
	P Value	0.489		0.222		0.346	0.435		0.147		0.369

Parameter	Group	14 d after calving					30 d after calving				
		Energy ×Breed		Energy × Supplement		E × B × S	Energy ×Breed		Energy × Supplement		E × B × S
		HF	Deoni	Without	With		HF	Deoni	Without	With	
ALT (U/L)	Control	24.0	23.4	23.6	23.9	23.7	25.0	22.4	24.8	22.7	23.7
	Glycerol	22.9	21.9	20.8	23.9	22.4	22.6	24.5	23.3	23.8	23.5
	Jaggery	21.4	23.4	21.6	23.2	22.4	24.5	25.0	23.8	25.7	24.7
	Maize	20.1	25.6	21.5	24.2	22.8	24.1	24.0	25.5	22.6	24.1
	Mean	22.1	23.6	21.9	23.8	22.8	24.1	24.0	24.3	23.7	24.0
	SEM	1.414		1.293		1.139	0.745		0.905		0.806
	P Value	0.309		0.162		0.444	0.895		0.467		0.352
AST:ALT	Control	3.70	3.57	3.69	3.58	3.63	3.93	4.14	3.87	4.20	4.03
	Glycerol	3.75	3.94	4.04	3.66	3.85	3.79	3.73	3.78	3.75	3.76
	Jaggery	4.06	3.71	4.08	3.69	3.88	3.81	3.76	4.05	3.53	3.79
	Maize	4.49	3.47	4.42	3.54	3.98	3.85	4.05	3.76	4.14	3.95
	Mean	4.00	3.67	4.06	3.62	3.84	3.84	3.92	3.86	3.90	3.88
	SEM	1.066		1.035		0.232	0.448		0.659		0.121
	P Value	0.236		0.111		0.347	0.486		0.811		0.162
GGT (U/L)	Control	16.4	15.0	16.0	15.5	15.7	15.7	14.4	14.4	15.7	15.0
	Glycerol	15.1	14.2	14.1	15.1	14.6	16.0	13.8	14.9	14.9	14.9
	Jaggery	13.3	15.2	14.0	14.5	14.2	14.4	17.0	15.5	15.9	15.7
	Maize	15.6	15.0	15.5	13.7	15.3	15.3	15.6	15.0	16.0	15.5
	Mean	15.1	14.8	14.9	14.7	15.0	15.3	15.2	14.9	15.6	15.3
	SEM	0.589		0.336		0.477	0.779		0.695		0.758
	P Value	0.685		0.622		0.056	0.868		0.342		0.503

Values with different superscripts for absolute interaction for a respective energy source in a row under each parameter differ significantly; **P≤ 0.01

Table 4.59: Repeat measures extracted table for serum metabolites

	Glucose (mg/dL)	BUN (mg/dL)	TC (mg/dL)	TG (mg/dL)	TP (mg/dL)	Albumin (g/dL)	Creatinine (g/dL)	AST (U/L)	ALT (U/L)	AST:ALT	GGT (U/L)
Within-subject effects											
Period × Energy	0.76	0.003**	0.34	0.02*	0.18	0.88	0.32	0.001***	0.68	0.28	0.80
Period × Breed	0.73	0.166	0.46	0.90	0.86	0.27	0.02*	0.60	0.004**	0.07	0.14
Period × FNS	0.30	0.08	0.43	0.31	0.002**	0.053	0.31	0.42	0.002**	0.002**	0.65
Period × Energy × Breed	0.60	0.10	0.20	0.89	0.19	0.32	0.89	0.001***	0.06	0.19	0.60
Period × Energy × FNS	0.72	0.20	0.22	0.97	0.81	0.31	0.68	0.54	0.534	0.35	0.32
Period × Breed × FNS	0.97	0.40	0.52	0.84	0.13	0.48	0.17	0.55	0.001***	0.002**	0.74
Period × Energy × Breed × FNS	0.58	0.80	0.72	0.91	0.98	0.23	0.69	0.45	0.002**	0.003**	0.77
Between subject effects											
Energy	0.34	0.10	0.13	0.001***	0.09	0.96	0.40*	0.03*	0.96	0.49*	0.80
Breed	0.67	0.58	0.66	0.04*	0.39	0.07	0.37	0.39	0.002**	0.02*	0.55
Supplement	0.25	0.75	0.29	0.91	0.20	0.29	0.04*	0.74	0.633	0.76	0.90
Energy × Breed	0.51	0.03*	0.04*	0.02*	0.70	0.06	0.42	0.002**	0.129	0.34	0.07
Energy × FNS	0.17	0.12	0.62	0.27	0.65	0.02*	0.03*	0.27	0.173	0.56	0.31
Breed × FNS	0.87	0.006**	0.04*	0.51	0.49	0.42	0.05	0.73	0.023*	0.04*	0.20
Energy × Breed × FNS	0.63	0.57	0.31	0.17	0.76	0.49	0.80	0.34	0.071	0.06	0.29

*Values with different superscripts for absolute interaction in a row under each parameter differ significantly; ***P≤ 0.001, **P≤0.01,

*P≤ 0.05

On the day of calving it was observed that those cows calved at night hr was more stressed than those calved on the next day in the day time. Cows were visibly stressed with absolute off feed at least 4hr prior to calving. This indeed, not only a natural phenomenon but also help in judging the “parturition” ready cow. NEFA concentration was reduced by 5.8% in FNS supplemented cows than without supplemented cows. Below threshold levels of NEFA from 30 days before to 30 days after calving revealed that the HFX or Deoni cows were adapted to maintain the homeostasis in NEB (Roche *et al.*, 2013). Although the estimated mammary requirement of energy in 4th day of postpartum is 3 times more than prepartum (Bell, 1995), the NEFA concentration was not more than 0.57 mmol/L and as same as its level in prepartum. This clearly enumerated that the cows were least suffered to the NEB in postpartum probably due to diet energy and FNS supplement they received in prepartum. BHBA concentration on 14 days before calving was 7.6% higher in HFX cows than Deoni cows. FNS supplementation reduced BHBA concentration by 9.4% than non-FNS supplemented cows. BHBA concentration during the prepartum period was below 800 $\mu\text{mol/L}$. According to Nydam *et al.* (2013) threshold concentration of BHBA is $>800 \mu\text{mol/L}$ during the prepartum period to evaluate the individual cow level associated with a negative outcome. Kaufmann *et al.* (2010) suggested $\geq 0.6 \text{ mmol/L}$ to 0.8 mmol/L as the threshold levels for BHBA thus, BHBA concentration in the present study was ranged from $657 \mu\text{mol/L}$ to $737 \mu\text{mol/L}$. BHBA was $737 \mu\text{mol/L}$ in HFX cows than $681 \mu\text{mol/L}$ in Deoni cows 14 days before calving ($P < 0.02$). BHBA was $744 \mu\text{mol/L}$ without FNS supplementation to energy compared to $674 \mu\text{mol/L}$ in cows with FNS supplement. BHBA in HFX cows and Deoni cows on the day of calving was $910 \mu\text{mol/L}$ and $833 \mu\text{mol/L}$, respectively. It was $735 \mu\text{mol/L}$ and $631 \mu\text{mol/L}$ in HFX and Deoni cows in day 14 of postpartum, respectively while, it was $969 \mu\text{mol/L}$ and $738 \mu\text{mol/L}$ in HFX and Deoni cows, respectively in day 30 of postpartum. During prepartum or on the day of calving or postpartum, BHBA was reduced in FNS supplemented cows than without supplemented cows about 30 to $60 \mu\text{mol/L}$. The BHBA concentration on the day of calving or 30 days after calving was below threshold levels of 1.0 to $1.4 \mu\text{mol/L}$ (Kaufmann *et al.*, 2010).

Table 4.60: Energy metabolites in different groups of cows during the prepartum period

Parameter	Group	30 d before calving					14 d before calving				
		Energy × Breed		Energy × Supplement		E × B × S	Energy × Breed		Energy × Supplement		E × B × S
		HF	Deoni	Without	With		HF	Deoni	Without	With	
NEFA (mmol/L)	Control	0.35	0.35	0.35	0.35	0.35	0.57	0.47	0.52	0.52	0.52
	Glycerol	0.33	0.41	0.38	0.37	0.37	0.48	0.50	0.51	0.47	0.49
	Jaggery	0.38	0.37	0.38	0.37	0.38	0.51	0.49	0.50	0.51	0.50
	Maize	0.34	0.34	0.33	0.35	0.34	0.53	0.49	0.53	0.48	0.51
	Mean	0.35	0.37	0.36	0.36	0.36	0.52	0.49	0.52	0.49	0.51
	SEM	0.012		0.10		0.012	0.02		0.02		0.023
	P Value	0.332		0.510		0.059	0.079		0.421		0.409
BHBA (µmol/L)	Control	750.1	773.8	783.2	740.7	761.9	908.8	776.7	872.3	813.2	842.7
	Glycerol	685.7	693.5	767.3	611.9	689.6	677.5	684.3	735.9	625.9	680.9
	Jaggery	687.8	634.7	662.2	660.3	661.3	733.1	647.2	737.8	642.5	690.2
	Maize	559.8	680.3	625.1	615.0	620.1	629.2	615.9	630.5	614.7	622.6
	Mean	670.8	695.6	709.4	657.0	683.2	737.2	681.0	744.1	674.1	709.1
	SEM	31.17		29.86		28.934	21.345		18.132		19.325
	P Value	0.44		0.100		0.09	0.02		0.002**		1.000

Values with different superscripts for absolute interaction for a respective energy source in a row under each parameter differ significantly; **P≤ 0.01

Table 4.61: Energy metabolites in different groups of cows on the day of calving

Parameter	Group	Calving day				
		Energy × Breed		Energy × Supplement		E × B × S
		HF	Deoni	Without	With	
NEFA (mmol/L)	Control	0.87	0.76	0.81	0.82	0.81
	Glycerol	0.74	0.75	0.76	0.72	0.74
	Jaggery	0.69	0.73	0.71	0.71	0.71
	Maize	0.78	0.70	0.75	0.73	0.74
	Mean	0.77	0.73	0.76	0.75	0.75
	SEM	0.03		0.03		0.030
	P Value	0.326		0.725		0.127
BHBA (µmol/L)	Control	1087.6	888.8	1031.3	945.1	988.2
	Glycerol	875.4	778.4	857.1	796.7	826.9
	Jaggery	928.2	888.9	912.7	904.4	908.5
	Maize	748.4	774.1	752.2	770.3	761.3
	Mean	909.9	832.5	888.3	854.1	871.2
	SEM	33.298		31.140		30.722
	P Value	0.04*		0.29		0.08

Values with different superscripts for absolute interaction for a respective energy source in a row under each parameter differ significantly; *P ≤ 0.05

Table 4.62: Energy metabolites in different groups of cows during the postpartum period

Parameter	Group	14 d after calving					30 d after calving				
		Energy × Breed		Energy × Supplement		E × B × S	Energy × Breed		Energy × Supplement		E × B × S
		HF	Deoni	Without	With		HF	Deoni	Without	With	
NEFA (mmol/L)	Control	0.53	0.45	0.48	0.51	0.49	0.54	0.51	0.51	0.54	0.53
	Glycerol	0.56	0.58	0.58	0.55	0.57	0.47	0.47	0.47	0.47	0.47
	Jaggery	0.47	0.51	0.46	0.52	0.49	0.41	0.39	0.40	0.39	0.40
	Maize	0.47	0.46	0.47	0.45	0.46	0.37	0.45	0.40	0.42	0.41
	Mean	0.51	0.50	0.50	0.51	0.50	0.45	0.45	0.44	0.45	0.45
	SEM	0.02		0.02		0.018	0.02		0.02		0.021
	P Value	0.701		0.466		0.307	0.751		0.619		0.082
BHBA (μmol/L)	Control	806	614.8	828.2	592.6	710.4	1106	783.8	993.5	897.0	945.2
	Glycerol	933.8	746.3	803.5	876.6	840.1	1024	826.7	925.1	925.1	925.1
	Jaggery	623.8	648.1	625.9	645.9	635.9	815.6	722.5	774.4	763.8	769.1
	Maize	576.9	515.9	517.2	575.6	546.4	931.9	620.0	805.0	746.9	775.9
	Mean	735.1	631.3	693.7	672.7	683.2	969	738.3	874.5	833.2	853.8
	SEM	58.70		60.64		57.28	59.87		56.13		56.37
	P Value	0.100		0.734		0.124	0.002**		0.47		0.054

Values with different superscripts for absolute interaction for a respective energy source in a row under each parameter differ significantly; **P< 0.01

Table 4.63: Repeat measures extracted table for energy metabolites

	NEFA	BHBA
Within subject effects		
Period × Energy	0.001***	0.001***
Period × Breed	0.27	0.001***
Period × FNS	0.71	0.88
Period × Energy × Breed	0.40	0.19
Period × Energy × FNS	0.98	0.33
Period × Breed × FNS	0.02*	0.08
Period × Energy × Breed × FNS	0.18	0.45
Between subject effects		
Energy	0.01**	0.001***
Breed	0.24	0.003**
Supplement	0.76	0.10
Energy × Breed	0.03*	0.34
Energy × FNS	0.40	0.50
Breed × FNS	0.37	0.14
Energy × Breed × FNS	0.62	0.67

Values with different superscripts for absolute interaction for a respective energy source in a row under each parameter differ significantly; *** $P \leq 0.001$, ** $P \leq 0.01$

Since, NEFA and BHBA levels were below threshold level from 30 days prior and 30 days after calving overrules any disease incidence like displaced abomasums, clinical ketosis, metritis, clinical endometritis, fatty liver syndrome etc., which otherwise increase 2 to 4 times more risk of these disease incidence (Huzzey *et al.*, 2014). Ospina *et al.* (2010) conducted a large study to establish the cutoff or threshold value of NEFA and BHBA to predict metabolic diseases like displacement of abomasum, ketosis, metritis and retained placenta. They established threshold level for NEFA concentration in prepartum as 0.3 mEq/L and postpartum is 0.6 mEq/L; whereas postpartum BHBA 10 mg/dL. There are 4 possible distinctive metabolisms of NEFAs; (1) efficiently used for the synthesis of mammary triglyceride (TG), (2) completely oxidized to carbon dioxide to provide energy for the liver, (3) partially oxidized to produce ketone bodies that are released into the blood and serve as fuels for other tissues or (4) reconverted to storage fat as TG (Kleppe *et al.*, 1988). Energy breed and FNS supplement interaction were also found non-significant during the prepartum and postpartum. During the first week post-calving, due to shortage of glucose covered by body fat mobilization, the contribution of propionate to gluconeogenesis has been estimated to be 32–73% (Drackley *et al.*, 2001).

Repeated measure analysis confirmed that BHBA concentration reduction throughout the experiment period due to prepartum energy and FNS supplementation. Breed effect was observed in BHBA concentration due to the difference in maintenance body size, calf birth weight and milk yield (**Table 4.63**).

The peak disease incidences in dairy cows is correspondence with the time of NEB, the peak blood concentration of NEFA and the greatest acceleration of milk yield (Ingvarstsen *et al.*, 2003). We observed 10% to 15% NEB but NEFA was hardly beyond 0.60 mmol/L in postpartum and the milk production was peaked to 16 to 20 kg/d from the 2nd wk in HFX cows and 4 to 4.5 kg/d in Deoni cows from the 2nd wk of postpartum. These yield either marginally persisted or same levels until 8th week. Thus, indicated no impact of NEB that could be due to improved appetite lead to 10% more CP consumption which was even 30% in Deoni cows. The presence of NEFA at < 0.60 mmol/L in the serum postpartum may also because complete inhibition of lipogenesis which also decrease re-

esterification of NEFA within adipocyte. Even with 15% NEB and physiological inhibition of lipogenesis when cow shifted from close-up to lactation. The below threshold levels of either NEFA or BHBA went least influenced DMI, hepatic lipid accumulation as evident from significantly improved IgG in colostrum. This suggested that there was no observed problem during transition in the cows due to nutritionally strategy adopted in prepartum.

4.4.4 Prepartum nutrition effect on postpartum serum mineral profile

Deficiencies of mineral during the prepartum period have an impact on postpartum metabolic disorders as they play a vital role in the activities of enzymes and hormones as constituents of body fluids and tissues. Serum/Plasma mineral concentration during the prepartum such as 30 and 14 days before calving (**Table 4.64**), on the day of calving (**Table 4.65**) and 14 and 30 days of postpartum (**Table 4.66**) were estimated. In the present experiment, mean serum Ca^{2+} level in prepartum HFX and Deoni cows were 8.81 mg/dL and 8.48 mg/dL, respectively before 30 days to calving which was little below the minimum levels of normal serum range of 9 mg/dL to 12 mg/dL and remained same on 14 days before calving in HFX and Deoni cows (8.49 mg/dL) and (8.37 mg/dL). Where diets were fortified with different energy sources FNS supplementation had no impact on the serum Ca^{2+} and remained little less than the lower limits of the normal range. Radostits *et al.* (2000) suggested a normal range of 9.7 mg/dL to 12.4 mg/dL and in such case prepartum Ca^{2+} in HFX and Deoni cows was 1 to 1.5 mg/dL lesser than the normal range in prepartum. Jhambh *et al.* (2016) estimated various serum metabolites in pregnant and lactating cows from field and reported 7.05 ± 0.38 mg/dL or 7.48 ± 0.12 mg/dL in total dairy animals ($n = 146$) which is lesser than what was observed in prepartum cows but somewhat nearer to postpartum cows. In any case, serum Ca levels were less than the normal range in prepartum and postpartum (1.5 mg/dL to 2mg/dL). The decrease in serum Ca^{2+} during prepartum could be anticipated for Ca requirement by the foetus particularly in the last month of the pregnancy, while in postpartum, obviously due to increase demand for the milk production since milk is a good source of Ca^{2+} . The requirement of Ca^{2+} of a cow in late gestation is 10 g/d while it was 30 g/d to 50 g/d for colostrum production (Horst *et al.*, 1997). These values decreased gradually and reached to 7.73 mg/dL and

7.5 mg/dL respectively, on the day of calving in both HFX and Deoni cows. Colostrum contains 0.26% of Ca^{2+} in comparison to 0.13% in bovine milk hence, the decline in serum Ca^{2+} on the day of calving could be due to its drain to colostrum (Gupta *et al.*, 1995; Puppel *et al.*, 2019). Dietary Ca^{2+} was 0.6% of kg DM during the prepartum to activate the PTH secretion that in turn stimulates the bone resorption. Since in prepartum and postpartum serum Ca^{2+} was above 8 mg/dL, there was no chance of milk fever that may occur when serum Ca^{2+} is going below 4 mg/dL. There was no significant difference in Ca^{2+} concentration either in energy \times breed or energy \times FNS supplement, or Energy \times breed \times FNS supplement interaction.

Pre calving diets which are high in phosphorous (P^{3-}) may have a negative impact on Ca^{2+} homeostasis (NRC, 2001). The mean serum P^{3-} level during the prepartum period was 5.10 mg/dL to 5.25 mg/dL which is little below the minimum levels i.e., 5.6mg/dL to 6.5 mg/dL (Rodostits *et al.*, 2000). Before 14 days to calving, serum P^{3-} (5.53 mg/dL) was significantly higher ($P < 0.001$) with FNS supplementation than without supplementation (4.77 mg/dL). In ruminants, the transfer of inorganic P^{3-} from the maternal pool is through passive diffusion. Calcitrol produced by foetal kidney increases both Ca^{2+} and P^{3-} transfer across the placenta (Durand *et al.*, 1983). On the day of calving, P^{3-} was decreased to 4.72 mg/dL. The decreased mean P^{3-} level at the time of calving (4.63- 4.72 mg/dL) could be due to increased utilization of P^{3-} during advanced pregnancy, enhanced carbohydrate metabolism (Padodara *et al.*, 2012) and drain of P^{3-} in colostrum secretion (Rook and Thomas, 1983). Colostrum contains 0.235% of P^{3-} compared to 0.113% in bovine milk (Puppel *et al.*, 2019). Phosphorous concentration during the postpartum was increased in HFX cows by 5% and maintained at the same level as prepartum in Deoni cows. The concentration of P^{3-} in milk is 0.9 g/kg milk (NRC, 2001). A cow producing 4500 kg milk secretes about 4.28 kg P^{3-} into milk in the lactation period. To make up the loss in P^{3-} in milk, cow has to consume 14.5 g/day P^{3-} from the diet, but bone reabsorption of P^{3-} occurs in the peak period in case of dietary P^{3-} is not sufficient. The trend of mean plasma inorganic P^{3-} levels obtained from calving to early postpartum concurred with the findings of Dhama *et al.*, (2006), Ram (2006), Sutaria (2010), and Patel (2017). On day 14 postpartum, the interaction between energy \times breed

× FNS supplement was found significant ($P < 0.05$). In T3 (4.73 mg/dL) P^{3-} was found significantly lesser ($P < 0.05$) than T1 (5.10 mg/dL) and T2 (5.10mg/dL). T3 was comparable with CG (4.86 mg/Dl). The P^{3-} content in T2 and T3 were comparable to serum inorganic P^{3-} of 5.08 ± 0.38 in field cattle (Jhambh *et al.*, 2016). This might be due to non-significant/comparable higher milk production in maize and CG than glycerol and jaggery supplemented group. Ambica and Rao (2012) also reported that postpartum decrease of plasma P^{3-} could be due to increased excretion in milk and increased activity of Para Thyroid Hormone. On day 30 of postpartum, although milk yield of HFX cows was significantly higher ($P < 0.05$) than Deoni cows, serum P^{3-} was more in HFX cows (5.07 mg/dL) than Deoni cows (4.83 mg/dL).

Magnesium (Mg^{2+}) is an important mineral to maintain the homeostatic pathway for regulating blood Ca^{2+} . The mean Mg^{2+} concentration on day 30 (2.39 mg/dL and 2.38 mg/dL) and day 14 (2.30 mg/dL and 2.37 mg/dL) before calving in HFX cows (2.39 mg/dL) were comparable to Deoni cows. The normal level of Mg^{2+} recommended in the plasma of cows is 0.75 mmol/L to 1.00 mmol/L or 1.8 mg/dL to 2.92 mg/dL (NRC, 2001). Ca^{2+} , P^{3-} and Mg^{2+} heritabilities (0.20 to 0.43) in milk are moderate to high and influenced by the genetic make-up of cows. No significant difference was found in serum Mg^{2+} in cows with or without FNS supplement. Energy × breed × FNS supplement interaction was also found non-significant. The mean Mg^{2+} concentration was found significantly lesser ($P < 0.001$) on the day of calving with the FNS supplemented (2.52 mg/dL) cows compared to cows without FNS supplement (2.80 mg/dL). Mg^{2+} was increased on the day of calving in HFX (2.62 mg/dL) and Deoni (2.70 mg/dL) cows. Although Mg^{2+} in colostrum was 0.037% (0.4 g/kg) compared to 0.011% in bovine milk, serum Mg^{2+} was increased (NRC, 2001; Puppel *et al.*, 2019) unlike Ca^{2+} or P^{3-} .

Serum Mg^{2+} was increased during day 14 and 30 of postpartum like on the day of calving compared to prepartum in HFX (2.80 mg/dL and 2.97 mg/dL) than Deoni (2.84 mg/dL and 3.03 mg/dL). Unlike Ca^{2+} or P^{3-} , serum Mg^{2+} was decreased with FNS supplement to energy in cows (2.68 mg/dL and 2.91 mg/dL) than without supplement (2.96 mg/dL and 3.10 mg/dL) on day 14 and day 30 of postpartum.

Table 4.64: Serum /Plasma minerals in different groups of cows during the prepartum experiment period

Parameter	Group	30 d before calving					14 d before calving				
		Energy × Breed		Energy × Supplement		E × B × S	Energy × Breed		Energy × Supplement		E × B × S
		HFX	Deoni	Without	With		HFX	Deoni	Without	With	
Calcium (mg/dL)	Control	8.90	8.15	8.38	8.68	8.53	8.05	8.15	8.10	8.10	8.10
	Glycerol	8.73	8.48	8.60	8.60	8.60	8.48	8.30	8.38	8.48	8.39
	Jaggery	8.85	8.58	9.00	8.43	8.71	8.80	8.55	8.68	8.68	8.68
	Maize	8.78	8.70	8.63	8.85	8.74	8.65	8.48	8.38	8.75	8.56
	Mean	8.81	8.48	8.65	8.64	8.64	8.49	8.37	8.38	8.48	8.43
	SEM	0.196		0.201		0.173	0.101		0.110		0.133
	P Value	0.106		0.951		0.436	0.235		0.376		0.111
Phosphorous (mg/dL)	Control	5.35	5.13	5.18	5.18	5.24	5.25	5.13	5.00	5.38	5.19
	Glycerol	5.00	5.28	5.08	5.08	5.14	5.18	5.00	4.83	5.35	5.09
	Jaggery	5.43	4.83	4.83	4.83	5.13	5.18	5.13	4.90	5.40	5.15
	Maize	4.63	5.73	5.73	4.95	5.20	4.75	4.80	4.35	5.20	4.78
	Mean	5.10	5.25	5.25	5.01	5.18	5.09	5.01	4.77	5.33	5.05
	SEM	0.234		0.171		0.220	0.119		0.120		0.127
	P Value	0.531		0.067		0.744	0.539		0.001***		0.607
Magnesium (mg/dL)	Control	2.44	2.14	2.34	2.25	2.29	2.40	2.35	2.42	2.33	2.37
	Glycerol	2.41	2.34	2.23	2.52	2.37	2.27	2.34	2.28	2.33	2.30
	Jaggery	2.29	2.43	2.27	2.45	2.36	2.26	2.46	2.42	2.31	2.36
	Maize	2.42	2.60	2.57	2.45	2.51	2.28	2.33	2.27	2.34	2.31
	Mean	2.39	2.38	2.35	2.41	2.38	2.30	2.37	2.35	2.33	2.34
	SEM	0.656		0.071		0.070	0.049		0.043		0.036
	P Value	0.867		0.375		0.055	0.190		0.642		0.227

Parameter	Group	30 d before calving					14 d before calving				
		Energy × Breed		Energy × Supplement		E × B × S	Energy × Breed		Energy × Supplement		E × B × S
		HF	Deoni	Without	With		HF	Deoni	Without	With	
Manganese (ng/ml)	Control	4.96	4.96	5.01	4.91	4.96	4.75	4.55	4.88	4.41	4.65
	Glycerol	5.99	5.27	5.18	6.08	5.63	5.55	4.55	4.88	5.22	5.05
	Jaggery	5.31	4.64	5.27	4.68	4.97	5.95	6.29	5.20	7.05	6.12
	Maize	4.92	4.94	5.25	4.61	4.93	5.87	4.95	5.42	5.40	5.41
	Mean	5.30	4.95	5.18	5.07	5.12	5.53	5.09	5.10	5.52	5.31
	SEM	0.263		0.278		0.280	0.236		0.235		0.222
	P Value	0.211		0.120		0.120	0.049		0.567		0.093
Copper (mg/L)	Control	0.90	0.83	0.84	0.89	0.87	0.78	0.77	0.71	0.84	0.77
	Glycerol	0.82	0.91	0.81	0.92	0.86	0.74	0.76	0.66	0.83	0.75
	Jaggery	0.87	0.85	0.83	0.89	0.86	0.91	0.64	0.73	0.82	0.77
	Maize	0.86	0.74	0.80	0.80	0.80	0.85	0.76	0.71	0.90	0.88
	Mean	0.86	0.83	0.82	0.87	0.85	0.82	0.73	0.70	0.85	0.77
	SEM	0.034		0.026		0.030	0.043		0.038		0.042
	P Value	0.391		0.060		0.163	0.067		0.002		0.414
Zinc (mg/L)	Control	0.36	0.36	0.37	0.35	0.36	0.36	0.24	0.18	0.41	0.30
	Glycerol	0.41	0.38	0.39	0.41	0.40	0.49	0.51	0.16	0.84	0.50
	Jaggery	0.23	0.58	0.38	0.42	0.40	0.48	0.51	0.18	0.82	0.50
	Maize	0.38	0.31	0.33	0.36	0.35	0.44	0.54	0.19	0.79	0.49
	Mean	0.34	0.41	0.37	0.38	0.38	0.44	0.45	0.18	0.72	0.45
	SEM	0.046		0.014		0.041	0.039		0.058		0.054
	P Value	0.196		0.221		0.362	0.751		0.001***		0.867

Values with different superscripts for absolute interaction for a respective energy source in a row under each parameter differ significantly; ***P ≤ 0.001

Table 4.65: Serum /Plasma minerals in different groups of cows on the day of calving

Parameter	Group	Calving day				E × B × S
		Energy × Breed		Energy × Supplement		
		HF	Deoni	Without	With	
Calcium (mg/dL)	Control	7.28	7.30	7.28	7.30	7.29
	Glycerol	7.75	7.63	7.40	7.98	7.69
	Jaggery	7.93	7.60	7.58	7.95	7.76
	Maize	7.95	7.48	7.58	7.85	7.71
	Mean	7.73	7.50	7.46	7.77	7.61
	SEM	0.133		0.141		0.143
P Value	0.111		0.04*		0.730	
Phosphorous (mg/dL)	Control	5.15	4.83	4.80	5.18	4.99
	Glycerol	4.73	4.83	4.95	4.60	4.78
	Jaggery	4.63	4.90	4.68	4.85	4.76
	Maize	4.53	4.53	4.10	4.50	4.53
	Mean	4.76	4.77	4.63	4.78	4.76
	SEM	0.104		0.143		0.129
P Value	0.906		0.087		0.256	
Magnesium (mg/dL)	Control	2.61	2.63	2.85	2.39	2.62
	Glycerol	2.59	2.75	2.82	2.53	2.67
	Jaggery	2.65	2.66	2.71	2.60	2.65
	Maize	2.63	2.76	2.81	2.58	2.69
	Mean	2.62	2.70	2.80	2.52	2.66
	SEM	0.062		0.069		0.053
P Value	0.201		0.001***		0.383	
Manganese (ng/dL)	Control	4.27	4.27	4.14	4.40	4.27
	Glycerol	4.17	3.68	4.00	3.84	3.92
	Jaggery	3.86	4.28	4.21	3.93	4.07
	Maize	5.29	4.25	4.73	4.81	4.77
	Mean	4.40	4.12	4.27	4.24	4.26
	SEM	0.200		0.183		0.197
P Value	0.659		0.261		0.135	
Copper (mg/L)	Control	0.42	0.71	0.48	0.66	0.57
	Glycerol	0.72	0.78	0.68	0.82	0.75
	Jaggery	0.71	0.67	0.62	0.76	0.69
	Maize	0.74	0.62	0.58	0.78	0.68
	Mean	0.65	0.70	0.59	0.76	0.67
	SEM	0.060		0.045		0.051
P Value	0.456		0.01**		0.363	
Zinc (mg/L)	Control	0.24	0.12	0.10	0.26	0.18
	Glycerol	0.26	0.39	0.11	0.54	0.33
	Jaggery	0.26	0.43	0.13	0.56	0.35
	Maize	0.50	0.38	0.09	0.79	0.44
	Mean	0.32	0.33	0.11	0.54	0.32
	SEM	0.056		0.062		0.054
P Value	0.802		0.001***		0.867	

Values with different superscripts for absolute interaction for a respective energy source in a row under each parameter differ significantly; *P≤ 0.05, ***P≤ 0.001

Table 4.66: Serum /Plasma minerals in different groups of cows during the postpartum experiment period

Parameter	Group	14 d after calving					30 d after calving				
		Energy × Breed		Energy × Supplement		E × B × S	Energy × Breed		Energy × Supplement		E × B × S
		HF	Deoni	Without	With		HF	Deoni	Without	With	
Calcium (mg/dL)	Control	7.83	8.03	7.70	8.15	7.93	8.05	8.28	8.13	8.20	8.16
	Glycerol	8.00	8.03	7.93	8.10	8.01	8.28	8.00	8.20	8.08	8.14
	Jaggery	8.33	8.05	8.30	8.08	8.19	8.35	7.93	8.18	8.10	8.14
	Maize	8.05	8.00	8.15	7.90	8.03	8.25	7.95	8.23	7.98	8.14
	Mean	8.05	8.03	8.02	8.60	8.04	8.25	8.04	8.18	8.09	8.13
	SEM	0.097		0.132		0.111	0.103		0.101		0.111
	P Value	0.801		0.781		0.141	0.081		0.368		0.719
Phosphorous (mg/dL)	Control	4.90	4.83	4.68	5.05	4.86 ^{ab}	5.18	5.03	4.90	5.30	5.10
	Glycerol	5.09	5.10	5.16	5.03	5.09 ^b	5.08	5.23	5.20	5.10	5.15
	Jaggery	5.23	4.98	5.13	5.08	5.10 ^b	5.13	4.93	5.00	5.05	5.03
	Maize	4.83	4.63	4.53	4.93	4.73 ^a	4.90	4.13	4.53	4.50	4.51
	Mean	5.01	4.88	4.87	5.02	4.95	5.07	4.83	4.91	4.99	4.95
	SEM	0.066		0.078		0.077	0.106		0.070		0.095
	P Value	0.071		0.075		0.05*	0.036*		0.262		0.389
Magnesium (mg/dL)	Control	2.75	2.80	3.01	2.54	2.77	2.93	2.95	3.09	2.79	2.94
	Glycerol	2.74	2.85	2.96	2.62	2.79	2.93	2.93	3.04	2.82	2.93
	Jaggery	2.84	2.81	2.91	2.75	2.83	3.01	2.93	3.04	2.90	2.97
	Maize	2.86	2.93	2.95	2.83	2.89	3.02	3.30	3.22	3.11	3.16
	Mean	2.80	2.84	2.96	2.68	2.82	2.97	3.03	3.10	2.91	3.00
	SEM	0.045		0.047		0.044	0.061		0.047		0.056
	P Value	0.288		0.001***		0.095	0.362		0.001***		0.683

Parameter	Group	14 d after calving					30 d after calving				
		Energy × Breed		Energy × Supplement		E × B × S	Energy × Breed		Energy × Supplement		E × B × S
		HF	Deoni	Without	With		HF	Deoni	Without	With	
Manganese (ng/mL)	Control	5.60	4.84	5.20	5.25	5.22	5.69	5.42	5.51	5.60	5.55
	Glycerol	4.79	4.12	4.60	4.31	4.66	5.45	5.68	5.50	5.63	5.56
	Jaggery	4.77	4.45	4.58	4.63	4.61	5.43	5.05	5.76	4.72	5.24
	Maize	4.88	4.76	5.52	4.12	4.82	5.70	6.07	6.41	5.36	5.88
	Mean	5.01	4.54	4.97	4.58	4.78	5.57	5.55	5.79	5.33	5.56
	SEM	0.186		0.240		0.225	0.273		0.344		0.310
	P Value	0.033		0.190		0.157	0.966		0.193		0.195
Copper (mg/L)	Control	0.82	0.83	0.79	0.87	0.83	0.88	0.87	0.91	0.84	0.87
	Glycerol	0.71	0.74	0.66	0.80	0.73	0.79	0.77	0.71	0.84	0.78
	Jaggery	0.78	0.68	0.67	0.79	0.73	0.80	0.73	0.71	0.82	0.77
	Maize	0.75	0.72	0.71	0.76	0.73	0.76	0.71	0.73	0.76	0.73
	Mean	0.77	0.74	0.71	0.80	0.75	0.81	0.77	0.76	0.82	0.79
	SEM	0.030		0.210		0.031	0.270		0.291		0.028
	P Value	0.443		0.001***		0.875	0.193		0.069		0.301
Zinc (mg/L)	Control	0.36	0.17	0.17	0.36	0.27	0.27	0.44	0.33	0.39	0.36
	Glycerol	0.69	0.62	0.24	1.07	0.65	0.47	0.74	0.28	0.93	0.60
	Jaggery	0.51	0.46	0.18	0.79	0.48	0.66	0.76	0.65	0.78	0.71
	Maize	0.55	0.46	0.29	0.72	0.50	0.77	0.38	0.47	0.68	0.57
	Mean	0.53	0.43	0.22	0.73	0.48	0.54	0.58	0.43	0.69	0.56
	SEM	0.062		0.082		0.082	0.117		0.108		0.114
	P Value	0.146		0.001***		0.186	0.737		0.03*		0.056

Values with different superscripts for absolute interaction for a respective energy source in a row under each parameter differ significantly; *P ≤ 0.05, ***P ≤ 0.001

The decreased Mg^{2+} in FNS supplemented cows might be due to non-significant/comparatively higher milk production in the first and second fortnight. This lead to a higher degree of mobilization of Mg^{2+} from bone and utilization of Mg^{2+} by mammary gland during early lactation (Kupczynski and Chudoba-Drozdowska, 2002). Energy \times breed \times supplement interaction throughout the experiment was found non-significant. All the experiment cows during the prepartum period were provided with 50 mg Manganese (Mn^{2+}) per kg DM which is optimal for normal foetal development (Hansen *et al.*, 2006). Serum Mn^{2+} before 30 days to calving was found non-significant between breeds ($P = 0.211$), either with or without FNS supplement to the cows ($P=0.701$). Energy, breed and supplement interaction was found non-significant ($P=0.120$). Before 14 d to calving, HFX cows mean serum Mn^{2+} was 5.53 ppb was significantly higher ($P < 0.05$) than 5.09 ppb in Deoni cows whereas, FNS supplement to energy had no significant effect on serum Mn^{2+} . Energy, breed and supplement interaction had non-significant effect on serum Mn^{2+} before 14 days to calving. However, serum Mn^{2+} in CG was significantly low (4.65 ppb) compared to TGs >5 ppb ($P < 0.10$).

As calving day approached, mean serum Mn^{2+} concentration was decreased gradually and reached 4.40 ppb in HFX cows and 4.12 ppb in Deoni cows ($P = 0.66$). Mn^{2+} deficiency any in prepartum may affect the fetal development and impart skeletal deformities (Hidiroglou and Knipfel, 1981). Energy, breed and FNS supplement interaction were also found non-significant for serum Mn^{2+} . During the postpartum period, no significant difference was found between without and with FNS supplement to energy. Energy, breed and supplement interaction was also found insignificant during postpartum. Only 14 d after calving, HFX cows had significantly higher ($P < 0.05$) serum Mn^{2+} (5.01 ng/mL) than Deoni cows (4.54 ng/mL). Mn^{2+} is also a part of Mn-superoxide dismutase, which is involved with limiting the build-up of highly reactive oxide molecules in cells. Mn^{2+} is involved in the biosynthesis of choline and thus a deficiency of Mn^{2+} may result in a deficiency of choline. Choline deficiency limits the liver's ability to metabolize fats and use them for energy and may result in the build-up fatty liver. This situation would be especially important in the early postpartum dairy cow with NEB and is relying mainly on the liver to metabolize body fat to meet her energy needs for milk production. Both prepartum and

postpartum cows are having a marginal concentration of Mn^{2+} i.e., 5 ppb to 6 ppb in serum whereas adequate serum Mn^{2+} concentration was 6 ppb to 70 ppb (Radostits *et al.*, 2000). The decrease in serum Mn^{2+} concentration during prepartum may be due to mobilization of Mn^{2+} from dam maternal source to the foetus for skeleton development which is very important for healthy calf (Hidioglou and Knipfel, 1981).

Cu^{2+} is actively transported across the placenta from the dam to the foetus and gradual serum Cu^{2+} concentration reduction was observed during the prepartum. The mean serum Cu^{2+} concentration during the prepartum period 30 days before calving was non-significant between HFX or Deoni cows \times FNS supplement, and between energy \times supplement. Energy, breed and FNS supplement interaction were also found non-significant. During 14 day before calving, Cu^{2+} supplementation was found non-significant ($P = 0.70$) between HFX and Deoni cows. However, FNS supplementation (0.85 ppm) had 21% higher ($P < 0.001$) serum Cu^{2+} concentration than cows without FNS supplement (0.70 ppm). As the day of calving approaches serum Cu^{2+} was decreased in both FNS supplemented and non-supplemented cows. However, FNS supplemented cows (0.76 ppm) had significantly higher (29%) Cu^{2+} concentration ($P < 0.01$) in serum than cows without FNS supplement (0.59 ppm). On day 14 of postpartum FNS supplemented cows had 12.5 % higher ($P < 0.001$) serum Cu^{2+} than non-supplemented group. No significant difference was found on the day 30 of postpartum. Energy, breed and FNS supplement interaction also found non-significant on plasma during the postpartum. Plasma Cu^{2+} was significantly lesser in cows without FNS supplement than cows with the supplement, thus prepartum Cu^{2+} supplementation had positive impact on the postpartum also. During prepartum homeostatic mechanism of the dam also plays a major role to maintain plasma Cu^{2+} concentration at a reasonable level and conserves much-needed Cu^{2+} by minimizing the loss via bile (Gooneratne *et al.*, 1986). The variation in serum concentration during the present study was a reflection of net demand for foetus/lactation. However, all the animal's serum Cu^{2+} concentration during the experiment period was within the physiological range i.e. 0.49 ppm to 1.5 ppm (Auza *et al.*, 1999). Cu^{2+} in the supplemented cows played an effective role than non-supplemented cows. The absorption, mobilization and transport of

iron to the bone marrow which is necessary for the production and maturation of RBC in the bone marrow was high in the supplemented cows. Supplemented Cu^{2+} was also played a major role in the synthesis of heme through the activation of ferrochelatase hence, haemoglobin and RBC were also significantly higher in postpartum.

Trace minerals like Cu^{2+} and Zn^{2+} are very essential during pregnancy as they are having crucial role in cell proliferation and their deficiency may have a teratogenic effect on foetus (Delano *et al.*, 2002). Castillo-Durán and Weisstaub (2003) reported that Zn^{2+} influences foetal growth and birth weight. The mean prepartum Zn^{2+} level before 30 days to calving in HFX and Deoni cows were 0.34 ppm and 0.41 ppm, respectively. Prepartum FNS supplementation doubled the serum Zn^{2+} on day 14 prior to calving. The mean Zn^{2+} concentration was decreased as the animal was approached calving.

However, FNS supplementation (0.54 ppm) had significantly higher ($P < 0.001$) serum Zn^{2+} than the cows without its supplementation. On day 14 after calving, FNS supplemented cows in prepartum (0.72 ppm) had significantly higher ($P < 0.001$) Zn^{2+} compared to cows (0.18 ppm) without supplementation in the prepartum period. Reduced mean Zn^{2+} concentration around parturition was due to the foetus accumulation of the Zn^{2+} at the rate of 12 mg Zn^{2+} /day as reported by House and bell (1993) apart from colostrum synthesis (Kincaid and Conrath, 1992). During the postpartum period on day 14 and day 30, FNS supplemented cows in prepartum had significantly higher ($P < 0.001$; $P < 0.05$) serum Zn^{2+} concentration than the cows without supplement during prepartum. Serum Zn^{2+} were within the physiological range of 0.7 ppm to 1.3 ppm (Rodostits *et al.*, 2000) during the peripartum period. During the postpartum period, Zn^{2+} plays an important role in the repair and maintenance of uterus lining followed by calving and hasten the uterus involution to normal reproductive functioning (Omur *et al.*, 2016). Zn^{2+} also enhances the resistance to mastitis by enhancing the keratin synthesis in teats (Goff and Horst, 1997). In the present study, the prepartum FNS supplemented group was not affected by mastitis whereas in non-supplemented cows 3 HFX cows were affected by mastitis. Repeat measure analysis indicated (**Table 4.67**) that P^{3-} ($P < 0.02$) and Mg^{2+} ($P < 0.001$) had

significant difference between periods due to FNS supplementation. Diet energy and breed such as HFX or Deoni cows interaction effect was also significantly different within the periods for P^{3-} ($P < 0.001$) and Mg^{2+} ($P < 0.01$). Within period effect on Mg^{2+} was significant due to interaction between energy and FNS supplement.

Table 4.67: Repeat measures extracted table for serum minerals

	Ca (mg/dL)	P (mg/dL)	Mg (mg/dL)	Mn (ng/mL)	Cu (mg/L)	Zn (mg/L)
Within subject effects						
Period × Energy	0.40	0.06	0.02*	0.005	0.001	0.001
Period × Breed	0.37	0.09	0.45	0.75	0.43	0.001
Period × FNS	0.12	0.02*	0.001***	0.14	0.001	0.88
Period × Energy × Breed	0.41	0.001***	0.01**	0.51	0.10	0.19
Period × Energy × FNS	0.14	0.12	0.01**	0.08	0.11	0.33
Period × Breed × FNS	0.37	0.68	0.33	0.57	0.96	0.08
Period × Energy × Breed × FNS	0.25	0.42	0.052	0.34	0.86	0.45
Between subject effects						
Energy	0.09	0.01**	0.18	0.37	0.01**	0.001***
Breed	0.04*	0.41	0.20	0.01**	0.24	0.01**
Supplement	0.40	0.001***	0.002**	0.28	0.76	0.10
Energy × Breed	0.69	0.42	0.36	0.58	0.03	0.34
Energy × FNS	0.65	0.11	0.22	0.08	0.40	0.50
Breed × FNS	0.30	0.91	0.11	0.94	0.37	0.14
Energy × Breed × FNS	0.22	0.44	0.51	0.004**	0.62	0.67

Summary and Conclusion

5.0 SUMMARY AND CONCLUSION

5.1 Summary

The study on “**Effect of feeding different sources of energy with functional nutrient supplements during prepartum period on postpartum nutritional and metabolic status of cows**”, was undertaken with the following objectives;

1. To evaluate the effect of different sources of energy fed during the prepartum period on postpartum nutrient utilization and energy balance in cows.
2. To study the effect of functional nutrient supplements (FNS) on energy efficiency and metabolic status in postpartum cows.
3. To study the prepartum nutritional management on rumen microbial protein, gut health and colostrum quality during the postpartum period.

The study was concluded in 3 phases

Phase I: In vitro fermentation rate kinetics of substrate fortified with the source of energy without or with FNS

Phase II: Prepartum in vivo nutrition management

Phase III: Prepartum nutrition effect on postpartum digestibility, parturition, energy balance, metabolic status and gut health

5.1.1. *In vitro* studies

IVGP studies were conducted to test the efficacy of source of energy and functional nutrient (FNS) supplement thus, consisted of energy as main factor and FNS supplement as sub-factor. Substrate or total mixed substrate (TMS) consisted of MGF, finger millet straw and concentrate supplement (CS) in the ratio of 50:20:30 where roughage to CS ratio was 70:30 (CG). The TMS in treatment groups (TG) was fortified 10% with energy source from Glycerol (T1), Jaggery (T2), and Ground maize (T3) and replicates used were 20 each thus, total

N=80. Again half of the replicates of CG and TGs were fortified with functional nutrients (FNS) supplement at the rate of 1% of TMS or 2 mg/200 mg of TMS and compared against without FNS supplement. CG and TGs along with blanks were incubated up to 72 hours and measured the gases release pattern at 1/2, 1, 2, 4, 8, 12, 18, 24, 36, 48, 60 and 72 h of fermentation intervals. These gases release patterns were subjected to first order kinetic models with 2 rate constants and analysed the additive effect of the energy fortification and FNS supplement.

1. FNS supplementation to substrates in CG and TGs had an absolute positive effect on accentuating the overall kinetic parameters.
2. It was concluded from the *in vitro* studies that among different energy sources, jaggery followed by glycerol were accentuated fermentation better than maize grain.
3. Substrate fermentation with maize grain fortification at 72 hr of incubation was comparable to other energy sources but, slower by 24%.
4. Jaggery with FNS supplement was best, followed by glycerol and least was maize in improving the substrate fermentation.

5.1.2 *In vivo* studies during the prepartum period

Significant and non-significant difference in various parameters studied under *in vivo* observation during prepartum, on the day of calving and postpartum are summarized in **Table 5.1** and **Table 5.2**, respectively.

5.1.2.1 Prepartum total and digestible nutrient intake and digestibility

In vivo experiments were conducted on non-lactating HFX and Deoni cows in the last month of the pregnancy by selecting 16 for each breed with a total of 32 cows. The cows of either HFX or Deoni were divided randomly into 4 equal groups based on parity thus, each group had 4 Deoni cows and 4 HFX cows. All cows were fed on MGF, limited finger millet straw and CS as per the nutrient requirement (Control). The cows in treatment groups (TG) were fortified the diet with 10% glycerol (T1) or jaggery (T2) or ground maize grain (T3) as energy source from simple polyol compound, disaccharides or polysacchrides, respectively. Again, 2

cows from each breed in CG or TG were given the FNS supplements consisted of selective minerals, vitamin B-complex and essential fatty acids in CG or TGs. Hence, the designed experiment had energy as main factor, breed and FNS supplement as sub-factors. The sample size for energy x breed had 4 in each group for HFX or Deoni cows and similar for energy x FNS supplement, or breed x FNS supplement. The total sample size for the interaction effect of energy, breed and FNS supplement was 8 in each group thus, N = 32.

The cows fed the designed feeding regimes only in the prepartum period and discontinued soon after calving. Our aim was to prepare the cows for the postpartum, and what would be the follow-up affect of the diets fed in prepartum on the day of calving and postpartum performance of cows? In the contemporary context it is integration of past feeding regime on the future performance. Hence, the postpartum feeding regime was common to all the 32 cows but, performance was analysed as per the prepartum groups such as CG, T1, T2 and T3.

Energy × Breed effect

1. Green fodder intake was 31% higher in the HFX than the Deoni cow's
2. CS intake was 17% higher in the HFX than the Deoni cow's
3. Total diet intake, g/kg $W^{0.75}$ was 4% higher in Deoni than the HFX (**Table 5.2**) cows.
4. Total diet intake g/kg $W^{0.75}$ was higher in T1 or T3 than T2.
5. Digestible proximate principles and CWC intake, as well as their digestibility (%) between breeds, were comparable (**Table 5.3**).

Energy × Supplement effect

1. Green fodder intake was 17% higher with the FNS supplemented groups than without supplement.
2. CS intake was 16% lesser with the FNS supplemented groups than without supplement.

3. Total diet intake, g/kg $W^{0.75}$ was with the FNS supplemented groups were comparable to without supplement. FNS showed more response in T1 than other TGs however, the difference was non-significant.
4. Total diet intake, g/kg $W^{0.75}$ was noticeably more in T1 and T3 because energy, breed and supplement interaction.
5. Digestible DM, OM, and CP intake was 1% and TCHO intake was 2.5% higher in supplemented than the non-supplemented group but, statistically not significant. Only ADF (5.6%) and cellulose (11.2%) digestibility in the supplemented group was significantly ($P < 0.01$ & $P < 0.001$, respectively) improved than the non-supplemented group.
6. Digestibility % of proximate principles and CWC were statistically comparable.

Breed × Supplement effect

1. The Deoni cows' green fodder intake after FNS supplement was 25% in comparison to 15% in HFX cows.
2. Either CS intake of the Deoni or HFX cows was decreased with FNS supplementation. It was reduced by 30% and 6%, respectively in Deoni and HFX cows with FNS supplement than without supplement.
3. Total diet intake, g/kg $W^{0.75}$ in Deoni cows was 5% more with FNS supplement than without supplement however, it was comparable in HFX cows.
4. Total diet intake was noticeably more in T1 with FNS supplements than T2 and T3.
5. Digestible cellulose intake, g/kg $W^{0.75}$ was increased by 3% in HFX cows and 22% in Deoni cows supplemented group ($P < 0.01$) than the non-supplemented group.
6. Digestibility % of proximate principles or CWC was statistically comparable.

Breed × Energy × Supplement effect

1. Green fodder intake was 6% higher in T1 or T3 than CG or T2 (4.14 kg/d) however, differences were insignificant.

2. CS intake was 14% higher in T2 followed by 8% in T1 compared to T3 where it was 2.20 kg/d, while it was only 1.70 kg/d in CG.
3. Total diet intake was 7%, 6%, and 3%, respectively higher than $78.22 \text{ g/kg } W^{0.75}$ in CG.
4. Except for EE, digestible DM, OM, CP, TCHO, CC, HC and NFC were significantly different.
5. Digestibility of DM, OM and TCHO intake, $\text{g/kg } W^{0.75}$ was statistically significant in T1 while digestible CP intake was comparable between T1, T2 and T3 but, 20 to 24% higher than CG because 15% higher digestibility coefficient.
6. Digestible CC and NFC intake were significantly higher in the T1 (24%) than either T2 (12%) or T3 (5%). Apart from CP digestibility, CC and NFC digestibility (%) was significant ($P < 0.01$) in T1 and T2 than CG or T3.

5.1.2.2 Nutritive value and energy value of the prepartum diets

Energy × Breed effect

1. DCP, TDN and NR was 5%, 55% and 1:9 ratio and comparable but, RFQ was 10.5% higher ($P < 0.01$) in Deoni cows than HFX cows (**Table 5.4**).
2. Except for GE ($P < 0.05$); DE, ME, Q-value of diet, HI, ER and energy efficiency were comparable between prepartum HFX and Deoni cows.
3. PD, creatinine, PDC index and PD excretion in urine was higher in prepartum HFX than Deoni cows. The duodenal flow of MBP (g/d) was 35% higher and its efficiency of production was 10% in prepartum HFX than Deoni cows

Energy × supplement effect

1. DCP% was lesser by 12% ($P < 0.001$) in the FNS supplemented group than without supplement. However, TDN and RFQ were comparable with or without FNS supplement.
2. Except for GE ($P < 0.05$); DE, ME, Q-value of diet, HI, ER and energy efficiency were comparable.

3. FNS supplementation observably increased MBP production by 7% and its efficiency of production by 4% but P values were non-significant.

Breed × supplement effect

1. Either HFX or Deoni cows DCP% ($P < 0.01$) of the diet and NR ($P < 0.01$) were significantly better without FNS supplement but, TDN and RFQ were comparable with or without FNS supplement to prepartum HFX or Deoni cows.
2. Except for GE ($P < 0.05$); DE, ME, Q-value of diet, HI, ER and energy efficiency were comparable
3. MBP production and its efficiency in prepartum HFX cows was improved with FNS supplementation 7.5% and 1.5%, respectively while in Deoni cows improvement in both the parameters was 7%.

Energy × Breed × Supplement

1. DCP% in TGs was higher by 14.8%, 16.4%, and 12.4% in T1, T2 and T3 than CG. NR was narrowed ($P < 0.01$) in TGs (1:10) than CG (1:11) but, TDN and RFQ were statistically comparable. RFQ was little better in T1 ($P = 0.08$) than CG and other TGs.
2. GE, DE, ME and ER were significantly prominent in T1 followed by T3.
3. MBP production was observably higher in T2 while the efficiency of MBP production was observably higher in CG than TGs, but the variance was statistically insignificant.

5.1.3 Effect of prepartum nutrition on parturition

5.1.3.1 Prepartum nutrition effect on colostrum quality & calf birth weight

1. The mean HFX cows IgG concentration were significantly ($P < 0.001$) higher than Deoni cow on day zero (**Table 5.8**).
2. Prepartum FNS supplementation shown a significant difference in colostrum quality IgG on the day of calving by 3%.

3. The overall impact of FNS supplementation to prepartum cows resulted in 8% more birth weight of calves ($P < 0.001$). The calf birth weight was 12% more ($P < 0.001$) in HFX cows and only 5% ($P < 0.001$) more in Deoni cows with FNS supplementation compared to without supplementation.
4. Energy, breed and FNS supplement interaction had a significant impact ($P < 0.05$) on the calf birth weight by 1.7 kg or 7% more than CG.

5.1.3.2. Prepartum nutrition effect on dam components

1. The time taken for foetal membrane expulsion process with uterine muscular contractions in Deoni breed was 38 min lesser than HFX breed ($P < 0.001$).
2. The energy with FNS supplementation hastened the expulsion of foetal membranes with probability levels of 8% ($P = 0.08$) because the inclusion of Se in FNS supplement at the rate of 0.3 ppm.
3. Breed interaction with FNS supplementation on the expulsion of foetal membranes effect was insignificant with the only difference of 5 to 10 min.
4. The significant difference in milk yield on day 5th after calving was observed between HFX and Deoni cows. HFX cows produced 3.75 times ($P < 0.001$) more milk than Deoni cows which could be attributed to crossbreeding rather than prepartum nutrition these cows received.
5. Milk yield on day 5th was least influenced ($P = 0.98$) by the FNS supplement cows received during prepartum. Breed and FNS supplement interaction was comparable to without FNS supplement in Deoni and HFX cows for milk yield on day 5th after calving.
6. All the dams and calves were healthy until day 5th of parturition and no milk fever, calf scores or calf mortality were observed.
7. The HFX and Deoni cows consumed 25% and 29% lesser DM, respectively during postpartum than the lactation requirement.
8. The ratio of CS to roughage was 70:30 in fresh HFX cows and 60:40 in fresh Deoni cows and increased nutrient requirement during the fresh period was compensated by CS. HFX cows consumed CS of 9.20 kg/day compared to 4.48 kg/day by Deoni cows during the fresh period.

9. We also observed 12 of 16 HFX cows suffered laminitis but, no such symptoms observed in Deoni cows.
10. Milk yield of HFX cows was 3 to 4 times higher ($P < 0.001$) than Deoni cows. The milk yield in HFX cows varied between 114 to 118 kg/ wk or 16 to 17 kg/d till 8 wk whereas in Deoni cows it was 30 to 32 kg/wk or 4.2 to 4.7 kg/d.
11. In postpartum, ME intake was 28.92 Mcal/day (121.02 MJ/day) in HFX cows and 15.20 Mcal/day (63.60 MJ/day), respectively which was almost 83% and 20% higher than prepartum ME intake. This was short of 16% and 13% of their recommended intake for lactation (ICAR, 2013) but, as per the recommended requirements of the cow in the humid tropics, the Deoni cows requirements (64.75 MJ/d) was less by only 1.15 MJ/day or 1.8% but, HFX cows requirement was short of 21 MJ/day (121.02 MJ/day Vs. 142.40 MJ/day) that was equal to 15%.
12. According to FAO (1993), HFX cows ME intake for lactation was 10.88 MJ/day or 8% less but, in Deoni cows, it was 2.30 MJ/day or 3.8% more than required. Negative residual ME_{FSF} indicated that the energy consumption was less than the recommended. It was -16% and -13% lesser in HFX and Deoni cows, respectively.
13. The CP consumption in postpartum was 1800 g/d and 900 g/d in HFX and Deoni cows, respectively. This CP consumption in postpartum by HFX cows was 10% more than the recommendation of ICAR (2013). In the case of Deoni cows, CP consumption in postpartum was just 1% more than the recommended requirement (ICAR, 2013). The positive value of residual CP_{FSF} indicated that the cows consumed more CP than the recommended requirements.
14. Prepartum energy supplement interaction of diet on postpartum milk production was found non-significant till 8 weeks, however, numerically a consistent improvement in the milk yield was observed in cows those provided FNS supplement along with energy during prepartum energy until 3rd week.

15. Prepartum FNS supplemented HFX cow's milk production was higher by 5 to 7% ($P=0.64$) for 1st fort night but, became comparable ($P=0.95$) from the 3rd week of lactation in comparison with prepartum without FNS supplemented HFX cows.
16. In Deoni cows prepartum FNS supplementation does not show a significant difference in milk production in comparison with those cows without FNS supplement.
17. Energy, breed and supplement interaction effect of prepartum diets was none on postpartum milk production in CG and TGs.
18. In the 1st week of postpartum, T3 cows produced non significantly higher milk than CG and other TGs. The impact of prepartum dietary factors on postpartum milk production was null.
19. RPI_{FSF} in postpartum was +19% and +27% higher than the recommended intake (ICAR, 2013), respectively when prepartum diets energy was without or with FNS supplement (**Table 5.4**).
20. FNS supplement impact on RPI_{FSF} was about +10% in HFX cows without or with FNS supplement but, it was +28% and +43% higher in Deoni cows, respectively.
21. The overall interaction effect of breed, energy and supplement in the prepartum diet on postpartum RPI_{FSF} was +23% higher dietary protein intake than standard recommendation (ICAR, 2013). This appears to be a good response in cows to sync with comparable ($P > 0.50$) more improvement in milk production in cows those received FNS supplement along with energy in TGs or even in CG.
22. REI_{FSF} was negative in postpartum, irrespective of main factor (Energy) or subfactors (Breed and/or FNS supplement) or interaction of all the 3 factors. The deficiency ranged from 10 to 17% in cows irrespective of interactions effect of main and/or sub-factors.
23. Positive RPI_{FSF} may be contributed to the negative REI_{FSF} thus, the additional 10% DCP intake in HFX may contribute partially to the 17% deficiency in ME

while 30% excess DCP intake in Deoni cows may sufficiently meet the deficiency of 14% ME in the Deoni cows.

24. The additional protein intake in fresh cows was also advantageous to cater deficient ME or protein where AA contribute upto 40% of energy needs and major source of gluconeogenesis in lactating cows.

5.1.4 Effect of prepartum nutrition on postpartum digestibility

5.1.4.1 Effect of prepartum nutrition on postpartum consumption

1. MGF consumption in cows fed with jaggery or maize grain as a source of energy was better than cows fed glycerol as an energy source in the prepartum.
2. The influence of FNS supplement provided during prepartum on the postpartum CS consumption (240%) was significant compared to those cows without FNS supplement (188%) in the prepartum.
3. Total diet consumption in postpartum was increased by 58% than prepartum in HFX cows while it was only 2% in Deoni cows (**Table 5.1**).
4. Except for EE, DM, OM, CP, TCHO and TA consumption from the total diet during postpartum in HFX cows was 17% to 19% more than their consumption in the prepartum.
5. In Deoni cows, only CP consumption was more by 19% while all other nutrients intake was either reduced or marginally increased compared to prepartum irrespective of these cows fed FNS supplementation or not.
6. CP consumption in postpartum was increased by 19% in cows those were fed FNS supplement during the prepartum.
7. There was 118% and 125% higher CP consumption between postpartum and prepartum in cows due to the interaction of diet energy and FNS supplement in the prepartum.
8. The HFX and Deoni cows consumed 74% and 12% higher total DMI (g/kg w^{0.75}), respectively in postpartum compared to prepartum. The increase in

DM or OM was about 74% but, CP intake was increased by 197%, total ash by 115%, and TCHO by 56% in HFX cows **(Table 5.2)**.

9. Compared to DM and CP consumption (g/kg $w^{0.75}$) from total diet during the prepartum because of interaction between energy and FNS supplement, their postpartum consumption was increased by 37% and 140%, respectively in contrast to without FNS supplementation which were 48% and 133%.
10. The interaction effect between diet energy and FNS supplement in the prepartum period translated into 9% and 7% more DM and CP consumption during postpartum.
11. The difference in total diet DM consumption among HFX cows in postpartum due to FNS supplement they received in prepartum was 20% more than without supplement while CP consumption was -7%.
12. DM consumption in Deoni during postpartum because of FNS supplementation in prepartum resulted in only 2% more, while CP consumption was 20% more.
13. NDF consumption during postpartum was increased by 36% in HFX cows but, reduced by 10% in Deoni cows compared to prepartum.
14. CC, HC and NFC consumption in postpartum for the cows belonging to T2 and T3 group during prepartum was significantly higher ($P < 0.02$) than CG which was comparable to T1.
15. CC, NDF, HC, and NFC consumption (g/kg $W^{0.75}$) for metabolic tissue was significantly higher in ($P < 0.001$) HFX breed than Deoni breed.
16. In prepartum cows fed with energy and without FNS supplemented group CC, NDF, HC and NFC consumption (g/kg $W^{0.75}$) was increased by 73%, 31%, 73% and 48% respectively during the postpartum. This was higher by 5%, 17%, 6% and 9% respectively in CC, NDF, HC and NFC consumption (g/kg $W^{0.75}$) in energy without FNS supplemented group than prepartum energy with the supplemented group.
17. The difference in CC, NDF, HC and NFC consumption (g/kg $W^{0.75}$) was -6%, -14%, -8% and -9% respectively in prepartum with FNS supplemented HFX cows than without supplemented cows during the postpartum period.

18. In Deoni cows CC, HC and celluloses consumption was increased by 5%, 3% and 1%, respectively in prepartum FNS supplemented cows in comparison to prepartum without FNS supplemented cows.
19. No significant difference in the CG and TGs for CWC consumption from the total diet (g/kg $W^{0.75}$) in the postpartum indicated no follow-up effect of experimental diets fed to CG and TGs during the prepartum.

5.1.4.2 Effect of prepartum nutrition on postpartum digestible nutrient consumption & digestibility

1. During the postpartum period, the digestible DM consumption (g/kg $W^{0.75}$) in HFX cows and Deoni cows were higher by 84% and 21% respectively, than those in the prepartum period. CP digestible nutrient intake was increased (169%) in prepartum FNS supplemented group than prepartum without FNS supplemented group (152%) during the postpartum period (**Table 5.3**).
2. In HFX cows digestible DM, TCHO and CP nutrient consumption (g/kg $W^{0.75}$) due to prepartum supplementation lead to a difference of -21%, -23% and -8%, respectively than without supplementation cows.
3. In Deoni cows, prepartum supplementation lead to increase the digestible CP and EE consumption by 34% and 20% respectively, in comparison without prepartum FNS supplemented cows during the postpartum.
4. No significant difference in CG and TGs for digestible nutrient intake from proximate nutrient indicated that no follow-up effect of experimental diets fed to CG and TG during the prepartum.
5. The digestible CC, NDF, Cellulose and NFC consumption (g/kg $W^{0.75}$) was found significantly ($P < 0.001$) higher during the postpartum in HFX than Deoni cows.
6. The no significant difference in CG and TGs for digestible nutrient intake from CWC indicated that no follow-up effect of experimental diets fed to CG and TGs during the prepartum.
7. CP and EE digestibility in postpartum cows were 10% and 15% higher in HFX and Deoni cows than the prepartum.

8. Deoni cow's prepartum FNS supplementation improved CP digestibility by 13% than without FNS supplementation group where improvement was only 6%.
9. Overall no significant difference in CG and TGs for proximate and CWC nutrient digestibility during postpartum indicated that no follow-up effect of prepartum experimental diets fed to CG and TGs.

5.1.4.3 Nutritive value, energy value and MBP production:

1. The difference in postpartum and prepartum diets was conspicuous for DCP which was two folds rather than TDN (**Table 5.4**). The NR of postpartum and prepartum diets was 1:6 and 1:11. It appears that energy intake was lesser than DCP which is very important in the postpartum where the recommended NR for lactating cows was 1:9.
2. Breed × FNS supplement interaction effect of diet in prepartum and discontinuation of energy fortification or FNS supplement or both together was more in HFX than Deoni cows owing to the difference in BW and level of milk production.
3. Postpartum HFX and Deoni cows consumed 70% and 10% more GE than their intake in the prepartum. During postpartum, DE and ME in HFX cows were higher by 83 to 84% and in Deoni cows by 19 to 20% than their intake on prepartum diets.
4. GE, DE or ME consumption in HFX cows with FNS supplement in prepartum had negative influence accounting to 13% to 15% compared to those HFX cows without any FNS supplement in prepartum.
5. GE, DE and ME consumption in Deoni cows without or with FNS supplement in prepartum was increased by 3% which was statistically insignificant.
6. Prepartum dietary factors such as energy, breed and FNS supplement interaction effect on postpartum GE, DE, ME, Q-value of the diet, HI, ER, energy digestibility and efficiency was insignificant.

7. Mean MBP production was increased by 7% during postpartum in cows those received FNS supplement with energy in prepartum compared to those without FNS supplement.
8. Postpartum MBP production in CG increased by 18 g/d in cows where control diet was supplemented with FNS in prepartum, while it was 38 g/d and 40 g/d, in T1 and T3, respectively. Only in T2, MBP production was reduced in cows received FNS supplement.
9. The efficiency (g/kgDOMI) of MBP production was decreased by 43% and 18% in HFX and Deoni cows, respectively than prepartum MBP production but, higher than optimum suggested (30g/kgDOM) in rumen under continuous culture fermentation.
10. The MBP production efficiency was 21g/Mcal of ME in HFX cows and 18g/Mcal of ME in Deoni cows during the postpartum which was 7% and 11% lesser than the prepartum in HFX and Deoni cows respectively.
11. MBP production efficiency (g/ kgDOMI) was 31 to 32% decreased in postpartum cows than prepartum cows either with or without FNS supplementation
12. MBP production efficiency was high in CG than TGs. MBP production efficiency (g/ kgDOMI) was significantly higher in CG and is comparable with T3, but higher than T1 and T2 cows.

5.1.5 Effect of prepartum nutrition on pre and postpartum metabolic health

5.1.5.1: Hematological parameters

1. FNS supplementation cows RBC count was significantly higher by 23% higher than without the supplemented cows on the day of calving (**Table 5.10**).
2. FNS supplementation during the prepartum enhanced the RBC count significantly by 17.5% and 19%, respectively on 14 day and 30 day after calving than cows without FNS supplement.

3. Prepartum FNS supplementation enhanced the Hb by 16.6 g% on the day of calving and 12 g% significantly on day 14 and day 30 after calving than non-supplemented group.
4. Repeat measure analysis showed significant increase in RBC during different periods (from -30 days to +30days of calving) in pre and postpartum, and on the day of calving for energy × FNS mainly due to serum Cu²⁺.
5. The significant increase in RBC and Hb (g %) during prepartum had cascading effect on postpartum also thus, such increase help in compensate the reduced glycolysis of RBC during periparturient cows as proved in the earlier experiments.

5.1.5.2 Serum metabolites

1. Serum metabolites revealed that serum total cholesterol was found significant (P< 0.05) only on day 14 after calving for energy breed and supplement interaction. The postpartum increase in total cholesterol may be due to lactogenesis and milking.
2. Creatinine concentration in HFX cows (1.5 mg/dL) was found significantly higher (P< 0.001) than Deoni cow (1.3 mg/dL). This elevated level of creatinine on day 14 before calving may be due to foetal maternal circulation and a load of organic waste of the foetus.
3. On the day of calving also Deoni cows serum ALT concentration (23.4 U/L) was found significantly higher (P<0.05) than HFX cows (18.7 U/L).
4. Repeat measure analysis confirmed that the variation in BUN, TG, and ALT was observed throughout the experiment period and the difference was significant among the energy fortified cows. The variation in Creatinine, ALT was observed throughout the period between groups might be due to variation in metabolism.
5. The variation in TP and AST concentration in FNS supplement cows was observed throughout the period. Variation in AST concentration was not only due to FNS supplementation to energy but also due to huge physiological changes observed in the periparturient period.

6. The data revealed that energy fortification does not have any significant effect on serum biochemical parameters because all the values were within the physiological range.
7. Prepartum energy with FNS supplement reduced BHBA concentration significantly ($P < 0.002$) in fortified energy supplemented cows on day14 before calving in comparison with energy fortified non-supplemented group.
8. On the day of calving and day30 after calving BHBA concentration was significantly ($P < 0.04$) less in Deoni cows in comparison with HFX cows.
9. Repeated measure analysis confirmed that BHBA concentration reduction throughout the experiment period mainly due to prepartum energy and FNS supplementation.
10. Breed effect was observed in BHBA concentration which might be due to the physiological changes within the breed to meet the milk production requirements.
11. REI_{FSF} was negative in postpartum, irrespective of main factor (Energy) or subfactors (Breed and/or FNS supplement) or interaction of all the 3 factors. The deficiency ranged from 10 to 17% in cows irrespective of interactions in the postpartum. However, 30% excess DCP intake in Deoni cows may sufficiently meet out the deficiency of 14% ME in the Deoni cows as evident from the reduced BHBA concentration during the postpartum period.
12. The prepartum energy with FNS supplement reduced BHBA concentration significantly ($P < 0.002$) in cows on day14 before calving in comparison to non-supplemented cows (**Table 5.12**).
13. On the day of calving and 30 d after calving BHBA concentration was significantly ($P < 0.04$) less in Deoni cows in comparison with HFX cows.
14. Repeated measure analysis confirmed that BHBA concentration reduction throughout the experiment period mainly due to prepartum energy and FNS supplementation.
15. Breed effect was observed in BHBA concentration which might be due to the physiological changes and level of milk production. REI_{FSF} was negative in postpartum, irrespective of main factor (Energy) or subfactors (Breed and/or

FNS supplement) or interaction of all the 3 factors. The deficiency ranged from 10 to 17% in cows irrespective of interactions in the postpartum. However, 30% excess DCP intake in Deoni cows was sufficiently meet the deficiency of 14% ME in the Deoni cows as evident from the reduced BHBA concentration during the postpartum.

5.1.5.3 Serum minerals

1. The serum Ca^{2+} and P^{3-} were little below the minimum normal levels and decreased with advancement of calving and postpartum because of need for colostrum and milk production. However, FNS supplement received by the cows in prepartum had positive impact on the serum Ca^{2+} and P^{3-} during postpartum (**Table 5.13**).
2. Serum P^{3-} in T3 where maize energy fortified (4.73 mg/dL) was comparable to control without any additional energy fortification but lesser ($P < 0.05$) than T1 and T2 (5.10 mg/dL) where additional energy sources were glycerol and Jaggery, respectively.
3. Unlike Ca^{2+} and P^{3-} , Mg^{2+} in serum was less in prepartum and increased more in postpartum, probably due to increased intake of green fodder and CS. FNS supplementation to energy also showed vice-versa impact on serum Mg^{2+} compared to Ca^{2+} and P^{3-} where FNS supplementation to cows along with energy during prepartum reduced the serum Mg^{2+} 2.52 mg/dL compared to cows without FNS supplement i.e., 2.80 mg/dL.
4. Due to prepartum FNS supplementation, serum Cu^{2+} enhanced by 1.3 times at the time of calving compared to cows without FNS supplement.
5. Serum Zn^{2+} supplementation during prepartum had positive effect on the serum Zn^{2+} in postpartum.

Table 5.1: Summary of significant parameters for nutrient intake (kg/d) from total diet

Parameter	Energy × Breed		Energy × Supplement		HFX × Supplement		Deoni × Supplement		E×B×S
	HFX	Deoni	Without	With	Without	With	Without	With	
Prepartum period									
DM	0.001***								0.03*
OM	0.001***								0.02*
CP	0.001***								0.02*
EE	0.001***								0.03*
TA	0.001***								0.04*
TCHO	0.001***								0.03*
CC	0.001***								0.01**
NDF	0.001***								
ADF	0.001***								0.05*
HC	0.001***								
Cellulose	0.001***								
NFC	0.001***								0.001***
Postpartum period									
DM	0.001***								0.03*
OM	0.001***								0.03*
CP	0.001***								
EE	0.001***								
TA	0.001***								
TCHO	0.001***								0.02*
CC	0.001***								
NDF	0.001***								0.01**
ADF	0.001***								0.01**
HC	0.001***								
Cellulose	0.001***								0.01**
NFC	0.001***								0.05*

Values with different superscripts for absolute interaction for a respective energy source in a row under each parameter differ significantly; ***P≤ 0.001, **P≤ 0.01, *P≤ 0.05

Table 5.2: Summary significance parameters for nutrient intake (g/kgW^{0.75}/d) from total diet

Parameter	Energy × Breed		Energy × Supplement		HFX × Supplement		Deoni × Supplement		E×B×S
	HFX	Deoni	Without	With	Without	With	Without	With	
Prepartum period									
DM		0.02*							0.01**
OM		0.02*							0.01**
CP		0.01*							0.049*
EE		0.001***							0.001***
TA		0.01**							0.047*
TCHO		0.01**							0.01**
CC		0.01**							0.001***
NDF		0.01**							0.04*
ADF									
HC		0.01**							0.01**
Cellulose									
NFC									0.001***
Postpartum period									
DM		0.001***							
OM		0.001***							
CP		0.001***							
EE		0.001***							
TA		0.001***							
TCHO		0.001***							
CC		0.001***							
NDF		0.001***							
ADF		0.001***							
HC		0.001***							
Cellulose		0.001***							
NFC		0.001***							

Values with different superscripts for absolute interaction for a respective energy source in a row under each parameter differ significantly; ***P≤ 0.001, **P≤ 0.01, *P≤ 0.05

Table 5.3: Summary of significant parameters for Digestible nutrient intake (g/kgW^{0.75}/d) from total diet

Parameter	Energy × Breed		Energy × Supplement		HFX × Supplement		Deoni × Supplement		E×B×S
	HFX	Deoni	Without	With	Without	With	Without	With	
Prepartum period									
DM									0.01**
OM									0.01**
CP									0.001***
EE									
TCHO									0.02*
CC									0.001***
NDF									
ADF			0.01**						
HC	0.01**								0.003**
Cellulose			0.001***				0.01**		
NFC									0.001***
Postpartum period									
DM	0.001***								
OM	0.001***								
CP	0.001***								
EE	0.001***								
TA	0.001***								
TCHO	0.001***								
CC	0.001***								
NDF	0.001***						0.04*		
ADF	0.001***						0.01**		
HC	0.001***								
Cellulose	0.01**		0.01**				0.01**		
NFC	0.001***								

Values with different superscripts for absolute interaction for a respective energy source in a row under each parameter differ significantly; ***P ≤ 0.001, **P ≤ 0.01, *P ≤ 0.05

Table 5.4: Summary of significant parameters for nutritive and energy value of the diets

Parameter	Energy × Breed		Energy × Supplement		HFX × Supplement		Deoni × Supplement		E×B×S
	HFX	Deoni	Without	With	Without	With	without	with	
Prepartum period									
DCP (%)			0.001***				0.01**		0.001***
NR			0.01**						
RFQ	0.01**								
RPI _{FSF}			0.02*						0.02*
REI _{FSF}									0.001***
GE	0.001***								0.02*
DE	0.001***								0.01**
ME	0.001***								0.01**
ER									0.001***
HI	0.001***								0.045*
Energy Effi.(%)									0.03*
Postpartum									
DCP (%)	0.01**								
TDN (%)			0.05*						
NR	0.01**		0.03*						
RFQ	0.001***								
RPI _{FSF}	0.02**								
REI _{FSF}									
GE	0.001***								
DE	0.001***								
ME	0.001***								
Q Value	0.02**								
ER	0.001***								
HI	0.001***								
Energy Effi.(%)	0.001***								

Values with different superscripts for absolute interaction for a respective energy source in a row under each parameter differ significantly; ***P≤ 0.001, **P≤ 0.01, *P≤ 0.05

Table 5.5: Summary of significant parameters for excretion of total purine derivatives and their index

Parameter	Energy ×Breed		Energy × Supplement		HFX× Supplement		Deoni× Supplement		E×B×S
	HFX	Deoni	Without	With	Without	With	without	with	
Prepartum period									
Allantoin(mM/L)	0.01**								
Creatinine(mM/L)									
Uric acid(mM/L)									
Total PD(mM/L)									
Postpartum period									
Allantoin(mM/L)	0.001***						0.01**		0.02*
Creatinine(mM/L)	0.001***								
Uric acid(mM/L)									
Total PD(mM/L)	0.001***						0.01**		0.01**

Values with different superscripts for absolute interaction for a respective energy source in a row under each parameter differ significantly; ***P≤ 0.001, **P≤ 0.01, *P≤ 0.05

Table 5.7: Summary of significant parameters for colostrum IgG concentration (mg/ml)

Parameter	Energy ×Breed		Energy ×Supplement		HFX×Supplement		Deoni×Supplement		E×B×S
	HFX	Deoni	Without	With	Without	With	Without	With	
Prepartum period									
0 th day	0.001***		0.001***						
1 st day									
2 nd day									
3 rd day									
4 th day									

Table 5.8: Summary of significance parameters for calf birth weight

Parameter	Energy ×Breed		Energy ×Supplement		HFX×Suppl.		Deoni×Suppl.		E×B×S
	HFX	Deoni	Without	With	Without	With	Without	With	
Prepartum period									
Calf birth weight (kg)	0.001***		0.001***				0.03*		0.049*
Calf metabolic BW (kg)	0.001***		0.001***				0.04*		

Table 5.9: Summary significance parameters for dam observations

Parameter	Energy × Breed		Energy × Supplement		HFX × Suppl.		Deoni × Suppl.		E×B×S	
	HFX	Deoni	Without	With	Without	With	Without	With		
Prepartum period										
Fetal membrane expulsion time	0.001***									0.09
Milk yield on 5 th day after calving (kg/d)	0.001***									

Values with different superscripts for absolute interaction for a respective energy source in a row under each parameter differ significantly; ***P≤ 0.001, **P≤ 0.01, *P≤ 0.05

Table 5.10: Summary of significant parameters for complete blood count

Parameter	30 d before parturition			14 d before parturition			On the day of calving			14 d after parturition			30 d after parturition		
	E×B	E×S	E×B×S	E×B	E×S	E×B×S	E×B	E×S	E×B×S	E×B	E×S	E×B×S	E×B	E×S	E×B×S
WBC															
RBC	0.01**			0.01***	0.001***			0.001***		0.001***	0.01**		0.01***	0.01**	
Hb				0.02*	0.001***			0.001***			0.001***			0.001***	
Platelets															
PCV															
Lymphocytes															
Monocytes															
Granulocytes															
MCV															
MCH	0.01**														

Values with different superscripts for absolute interaction for a respective energy source in a row under each parameter differ significantly; ***P≤ 0.001, **P≤ 0.01, *P≤ 0.05

Table 5.11: Summary of significant parameters for serum metabolites

Parameter	30 d before parturition			14 d before parturition			On the day of calving			14 d after parturition			30 d after parturition		
	E×B	E×S	E×B×S	E×B	E×S	E×B×S	E×B	E×S	E×B×S	E×B	E×S	E×B×S	E×B	E×S	E×B×S
Glucose															
BUN															
TC															
TG															
TP															
Albumin															
Creatinine						0.001***									
AST															
ALT															
AST:ALT															
GGT															

Values with different superscripts for absolute interaction for a respective energy source in a row under each parameter differ significantly; ***P≤ 0.001, **P≤ 0.01, *P≤ 0.05

Table 5.12: Summary significant parameters for energy metabolites

Parameter	30 d before parturition			14 d before parturition			On the day of calving			14 d after parturition			30 d after parturition		
	E×B	E×S	E×B×S	E×B	E×S	E×B×S	E×B	E×S	E×B×S	E×B	E×S	E×B×S	E×B	E×S	E×B×S
NEFA (mmol/L)															
BHBA (μmol/L)					0.02*			0.04*							0.002**

Values with different superscripts for absolute interaction for a respective energy source in a row under each parameter differ significantly; ***P≤ 0.001, **P≤ 0.01, *P≤ 0.05

Table 5.13: Summary of significant parameters for serum minerals

Parameter	30 d before parturition			14 d before parturition			On the day of calving			14 d after parturition			30 d after parturition		
	E×B	E×S	E×B×S	E×B	E×S	E×B×S	E×B	E×S	E×B×S	E×B	E×S	E×B×S	E×B	E×S	E×B×S
Ca (mg/dL)								0.04*							
P (mg/dL)					0.001***								0.05*	0.04*	
Mg (mg/dL)								0.001***				0.001***			
Mn (ng/mL)											0.03*				
Cu (ppm)					0.01**			0.01**				0.001***			
Zn (ppm)					0.001***			0.001***				0.001***			0.03*

Values with different superscripts for absolute interaction for a respective energy source in a row under each parameter differ significantly; ***P≤ 0.001, **P≤ 0.01, *P≤ 0.05

6. Repeat measure analysis indicated that P^{3-} ($P < 0.02$) and Mg^{2+} ($P < 0.001$) had significant difference between periods due to FNS supplementation. Diet energy and breed interaction effect was also significantly different within the periods for P^{3-} ($P < 0.001$) and Mg^{2+} ($P < 0.01$).

5.2 Conclusion

5.2.1 The questions answered

1. Is there any difference in the source of energy additionally fortified in prepartum diets on the postpartum performance, particularly energy balance?

Yes, glycerol supplementation during prepartum had more positive impact on the nutrition, energy, MBP production in rumen and serum metabolites.

2. Did FNS supplementation to energy source help to improve the energy efficiency in pre and postpartum?

FNS supplement improved digestible nutrient intake, RBC count and hemoglobin in cows during prepartum and postpartum and maintained the energy needs of the prepartum cows and manageable negative energy levels i.e., only about 12% in postpartum cows.

3. Did FNS supplement has any influence on serum metabolites during postpartum?

Yes, FNS supplement maintained serum Ca^{2+} , P^{3-} , Mn^{2+} , Cu^{2+} and Zn^{2+} but not Mg^{2+} in postpartum and their concentration decreased on the day of calving indicated more accretion into colostrum which later revert back to marginally below the lower limit of the physiological range but far above the level predisposing to milk fever.

4. Is there any breed effect on energy fortification and FNS supplementation?

HFX cows were responded more to the FNS supplement for nutrition, energy balance, serum metabolites and minerals status. Although the Deoni cows response to FNS supplement on nutrition and energy was less but, better for serum metabolites and minerals status.

5. Is there any affect of prepartum nutrition on the parturition and colostrum?

Energy and FNS supplementation during prepartum improved calf birth weight, the time of expulsion of foetal membranes and improved the IgG concentration in colostrum. No indication of any retained placenta cases.

6. Is there any interactive affect of prepartum diets on the postpartum nutrition, energy balance and milk produciton?

The prepartume diets and management had positive response on the diet intake, CP and energy consumption. CP intake in postpartum was +19% and +27% higher than the recommended intake while ME deficiency in cows was ranged from 10 to 17% (ICAR, 2013). However, CP also used as fuel and contributes 10% to 15% energy in the system, the higher CP intake compensate the phenomenal NEB in postpartum as evident from the lower serum NEFA and BHBA concentration.

7. Did prepartum nutrition has influence on the postpartum serum NEFA, BHBA and other metabolites?

NEFA concentration in prepartum, on the day of calving and post partum was much less than the threshold levels (prepartum < 0.5 mmol/L & postpartum < 1.0 mmol/L). Plasma NEFA was peaked at calving but declined during postpartum that was good phenomena common for healthy cows. Repeated measure analysis confirmed that BHBA concentration reduction throughout the experiment period mainly due to prepartum energy and FNS supplementation.

8. If prepartum nutrition had iinteractive affect on the postpartum, how long the follow-up affect observed?

The interative affect of prepartum diets on postpartum was limited to health and rumen MBP till 30 d of postpartum studied in terms of blood profile, serum metabolites including NEFA and BHBA and serum minerals status. The prepartum diets had no interactive affect on the milk yield and nutrition of fresh cows except protecting the appetite.

5.2.2 Conclusion as for the objective of the study

1. To evaluate the effect of different sources of energy fed during the prepartum period on postpartum nutrient utilization and energy balance in cows.

Glycerol, jaggery and ground maize grain as source of energy was fortified to diets and observed glycerol had more effect on prepartum nutrient utilization and energy balance followed by maize grain and the least was jaggery.

2. To study the effect of functional nutrient supplements on energy efficiency and metabolic status in postpartum cows.

Functional nutrient supplements consisted of selective minerals, vitamins and fatty acids had positive affect on the cell wall digestibility, prepartum energy, RBC, hemoglobin, blood glucose, reduced NEFA, reduced BHBA and serum Ca^{2+} , P^{3-} , Mn^{2+} , Cu^{2+} , and Zn^{2+} .

3. To study the prepartum nutritional management on rumen microbial protein, gut health and colostrum quality during the postpartum period.

Rumen MBP production was significantly improved with energy and FNS supplements during prepartum and postpartum, colostrum quality, improved the time of expulsion of foetal membrane and calf birth weight.

5.3 Recommendation

Prepartum supplementation of 300 ml of glycerol and 1% of FNS supplement including 40g flax seed to cows at least 30 days prior to calving irrespective of breed improved prepartum performance, colostrum quality and calf birth weight and had follow-up effect on health till 30 days of postpartum.

5.4 Future research and thrust

The above suggested prepartum supplement had effect only on the appetite of the cows but very limited impact on postpartum milk yield hence, developing a suitable energy source and FNS supplement plan at least upto 60 days of postcalving or lactation may protect cows reproductive cyclicity and peak lactation needs.

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ASSISTANT PROFESSOR, COLLEGE OF VETERINARY SCIENCE,
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OBJECTIVE

To be a Researcher in a challenging environment for mutual growth and development

SKILLS

Technical Skills

- *In vitro* fermentation studies
- Ration formulation for poultry and dairy animals
- Handling and operation of feed mill equipment

Instrument Handling

- AAS
- HPLC

Computer Skills

- SPSS
- GraphPad Prism
- MS Office

RESEARCH SUMMARY

- Research Publications: 3
- Total Abstracts: 5
- Popular articles: 10
- Conference Attended: 4

EDUCATION

Degree	University/Board	Year Completed	Grade
Ph.D.	ICAR- NDRI, Karnal	Ongoing	8.36/10.0
P.G. Dip. Rural Dev	MANAGE	2011	A grade
M. V. Sc.	S.V.V.U TIRUPATI	2010	8.63/10.0
B. V. Sc. & AH	S.V.V.U TIRUPATI	2007	6.99/10.0

RESEARCH EXPERIENCE

- Effect of Feeding Different Sources of Energy with Functional Nutrient Supplements During Prepartum Period on Postpartum Nutritional
- A Study on Release of Minerals in Groundnut Haulm Based Complete Diets in Ruminants Supplemented with Organic and Inorganic Mineral Sources by In Sacco And In Vivo Methods

AWARDS AND HONOURS

- Nestle Purina Gold Medal during M. V. Sc programme
- INCAN Best Poster presentation Award -2019



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PROFESSIONAL TRAININGS

- Foundation Course for Newly Recruited Faculty Members of SVVU, 2014
 - Orientation Course Organized by NSS Empaneled Institute Sponsored by Ministry of youth affairs and sports, Govt. of India
 - ICAR sponsored winter school on nutritional strategies to enhance livestock productivity and farm economy- 2018
-

LAB MANUAL PREPARATION

- A Laboratory Manual of Animal Nutrition-AN 211, SVVU, TPT.
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PUBLICATIONS

- **Rani, K. S.,** and Ramana, J. V. **2009.** Managemental Practices for Dairy Calves-An Overview. *Intas Polivet*, **10**: 158-164.
- **Rani, K. S.,** Ramana, J. V., Devasena, B., Gowda, N. K. S., and Reddy, P. S. **2015.** *In vivo* evaluation of mineral bioavailability in groundnut haulm based complete diets supplemented with organic and inorganic mineral sources in goats. *Indian Journal of Animal Sciences*, **85**: 1098-1103.
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