EFFECT OF COTTONSEED MEAL ON NUTRIENT UTILIZATION AND BLOOD BIOCHEMICAL PROFILE OF MALE BUFFALO CALVES

Thesis

Submitted to the Guru Angad Dev Veterinary and Animal Sciences University in partial fulfillment of the requirements for the degree of

MASTER OF VETERINARY SCIENCE
in
ANIMAL NUTRITION
(Minor Subject: Veterinary Biochemistry)

By

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CERTIFICATE – I

This is to certify that the thesis entitled **“Effect of cottonseed meal on nutrient utilization and blood biochemical profile of male buffalo calves”** submitted for the degree of **M.V.Sc.** in the subject of **Animal Nutrition** (Minor subject: **Veterinary Biochemistry**) of the Guru Angad Dev Veterinary and Animal Sciences University, Ludhiana, is a bonafide research work carried out by **Siliveru Srinath (L-2019-V-18-M)** under my supervision and that no part of this thesis has been submitted for any other degree.

The assistance and help received during the course of investigation have been fully acknowledged.

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CERTIFICATE – II

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ABSTRACT

The present study was conducted to evaluate the effect of replacement of soybean meal (SBM) with cottonseed meal (CSM) on nutrient utilization, blood profile and growth performance of male buffalo calves. The study was conducted in two phases. In phase I, *in vitro* evaluation of concentrate mixtures (containing CSM levels at 0, 3.75, 7.50, 11.25 and 15% replacing SBM @ 0, 25, 50, 75 and 100% on w/w basis) and TMRs (containing CSM as substrate) was carried out. In phase II, *in vivo* study was carried out in buffalo calves. *In vitro* study of concentrates and TMRs revealed that SBM could be replaced by CSM upto 100% without affecting the nutrient digestibility (OM, NDF and DM), and microbial mass production. NH_3-N showed a declining trend with increasing level of CSM in the concentrate mixtures. On the basis of digestibility of nutrients of TMRs observed in the *in vitro* study, two levels of CSM (75 and 100% replacement of SBM in concentrate) were selected for feeding to buffalo calves in the *in vivo* study. The intake of nutrients (OMI, CPI, EEI expressed as g/kg $W^{0.75}$) was higher ($P<0.05$) in treatment (T1 & T2) groups than control group. Digestibility of nutrients and nitrogen balance was similar in all the groups, indicating that CSM did not have any adverse effect on nutrient digestibility and nitrogen retention. CSM inclusion in the diet of buffalo calves did not have any adverse effect on blood profile, cell mediated immune response, ADG and FCR in male buffalo calves. Inclusion of CSM at 75% and 100% levels replacing SBM resulted in net saving of Rs. 11.67 and 16.65 per kg body weight gain respectively, in buffalo calves. It was concluded that CSM could economically replace soybean meal upto 100 % in the concentrate mixture of buffalo calves on w/w basis without any adverse effect on palatability, digestibility of nutrients, nitrogen balance and health of animals.

Keywords: Cottonseed meal, buffalo calves, *in vitro/ in vivo* evaluation, digestibility, blood parameters.

Signature of Major Advisor | **Signature of the Student**

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LIST OF ABBREVIATIONS USED

AA	:	Amino Acid
A: P	:	Acetate: Propionate
ADF	:	Acid Detergent Fibre
ADFI	:	Acid Detergent Fibre Intake
ADBWG	:	Average Daily Body Weight Gain
ADG	:	Average Daily Gain
ADICP	:	Acid Detergent Insoluble Crude Protein
ADIN	:	Acid Detergent Insoluble Nitrogen
ADL	:	Acid Detergent Lignin
ADS	:	Acid Detergent Soluble
AIA	:	Acid Insoluble Ash
ALP	:	Alkaline phosphatase
ALT	:	Alanine Aminotransferase
AST	:	Aspartate Aminotransferase
ATTD	:	Apparent Total Tract Digestibility
AV	:	Acidogenic Values
BAHS	:	Basic Animal Husbandry Statistics
BCSM	:	Black Cumin Seed Meal
BCVFA	:	Branched Chain Volatile Fatty Acid
BIN	:	Borate Phosphate Insoluble Nitrogen
Bt-CM	:	Bt-Cottonseed Meal
BUN	:	Blood Urea Nitrogen
BW	:	Body weight
C	:	Control
Ca	:	Calcium
$CaCl_2$	:	Calcium Chloride
CaCSM	:	Calcium hydroxide treated Cottonseed Meal
CC	:	Cassava Chip
C-CM	:	Conventional Cottonseed Meal
CF	:	Crude Fibre
CFM	:	Concentrate Feed Mixture
CGF	:	Corn Gluten Feed
CGM	:	Corn Gluten Meal
CH_4	:	Methane
CHO	:	Carbohydrates
CL	:	Cellulose
CLI	:	Cellulose Intake
CLR	:	Cottonseed Linter Residue
CMI	:	Cell Mediated Immunity

COCPC	:	Committee On Cotton Production and Consumption
CO_2	:	Carbon Dioxide
$CoCl_2$	:	Cobalt Chloride
CP	:	Crude Protein
CPI	:	Crude protein Intake
CRD	:	Completely Randomized Design
CR	:	Cassava chip + Rice bran
CSBP	:	Cotton Seed By Products
CS-B	:	Cottonseed Meal with Barley
CSC	:	Cotton Seed Cake
CSM	:	Cotton Seed Meal
CS-S	:	Cottonseed Meal with Sorghum
CTAB	:	Cetyl Trimethyl Ammonium Bromide
DBG	:	Dried Brewers Grains
DCSH	:	Delinted Cotton Seed Hulls
DCP	:	Digestible Crude Protein
DDGS	:	Dried Distiller Grains plus Solubles
DGNC	:	Deoiled Groundnut Cake
DLC	:	Differential Leucocyte Count
DM	:	Dry Matter
DMI	:	Dry Matter Intake
DMC	:	Deoiled Mustard Cake
DOC	:	Day Old Chicks
DTH	:	Delayed Type Hypersensitivity
EDTA	:	Ethylene Diamine Tetraacetic Acid
EB	:	Electron Beam
EE	:	Ether Extract
EEI	:	Ether Extract Intake
EMMP	:	Efficiency of Microbial Mass Production
ERCSM	:	Vitamin E supplemented Raw Cottonseed Meal
ES	:	Extruded Soybeans
FAO	:	Food and Agriculture Organisation
FCR	:	Feed Conversion Ratio
FCSM	:	Fermented Cottonseed Meal
FE	:	Fermentation Efficiency
$FeCl_2.6\ H_2O$	:	Iron chloride hexahydrate
FG	:	Free Gossypol
FI	:	Feed Intake
FM	:	Feed Mixture
GC	:	Gas Chromatography

GE	:	Gross energy
GGT	:	Gamma Glutamyl Transferase
GK	:	Guar Korma
GLC	:	Gas Liquid Chromatography
GN-B	:	Groundnut Meal with Barley
GNC	:	Groundnut Cake
GNM	:	Groundnut oil Meal
GN-S	:	Groundnut Meal with Sorghum
GR	:	Gamma Ray
H_2SO_4	:	Sulphuric Acid
HA	:	Hemagglutinin Antigen
Hb	:	Haemoglobin
HC	:	Hemicellulose
HCI	:	Hemicellulose Intake
HCM	:	High Cottonseed Meal
HDL	:	High Density Lipoprotein
HPDDG	:	High Protein Dried Distillers Grains
HR	:	Hydrogen recovery
IgG	:	Immunoglobulin G
IFCC	:	International Federation of Clinical Chemistry
IVDMD	:	In Vitro Dry Matter Digestibility
IVGP	:	In Vitro Gas Production
IVOMD	:	In Vitro Organic Matter Digestibility
IVPPD	:	In Vitro Pepsin Pancreatin Digestibility
IVTDDM	:	In Vitro True Dry Matter Digestibility
kGY	:	kilo gray
KH2PO4	:	Potassium Dihydrogen Phosphate
LCSH	:	Linted Cottonseed Hulls
LCM	:	Low Cottonseed Meal
LDL	:	Low Density Lipoprotein
LCSM	:	Low-Gossypol Cottonseed Meal
MC	:	Mustard cake
MCH	:	Mean Corpuscular Haemoglobin
MCHC	:	Mean Corpuscular Haemoglobin Concentration
MCV	:	Mean Corpuscular Volume
ME	:	Metabolizable energy
MGM	:	Maize Gluten Meal
$MgSO_4$	:	Magnesium Sulphate
MM	:	Mineral Mixture
MMP	:	Microbial Mass Production

MOC	:	Maize Oil Cake
MOL	:	Moringa Oleifera Leaves
MPV	:	Mean Platelet Volume
MUN	:	Milk Urea Nitrogen
MNS	:	Microbial Nitrogen Synthesis
$MnCl_2$	:	Manganese Chloride
N	:	Nitrogen
NH_4HCO_3	:	Ammonium Bicarbonate
$NaHCO_3$	:	Sodium Bicarbonate
NaOH	:	Sodium Hydroxide
Na_2HPO_4	:	Disodium Phosphate
Na_2S	:	Sodium Sulfide
NaN_3	:	Sodium Azide
NDF	:	Neutral Detergent Fiber
NDFI	:	Neutral Detergent Fiber Intake
NDFD	:	Neutral Detergent Fiber Digestibility
NDICP	:	Neutral Detergent Insoluble Crude Protein
NDIN	:	Neutral Detergent Insoluble Nitrogen
NDS	:	Neutral Detergent Solution
NE	:	Net Energy
NEFA	:	Non Esterified Fatty Acids
NFE	:	Nitrogen Free Extract
NGP	:	Net Gas Production
NH_3-N	:	Ammoniacal Nitrogen
NH_4HCO_3	:	Ammonium Hydrogen Carbonate
NPN	:	Non Protein Nitrogen
NSC	:	Niger Seed Cake
OM	:	Organic Matter
OMI	:	Organic Matter Intake
OMD	:	Organic Matter Digestibility
OMTD	:	Organic Matter True Digestibility
P	:	Phosphorus
PCV	:	Packed Cell Volume
PDWC	:	Platelet Distribution Width
PF	:	Partitioning Factor
PHA-P	:	Phytohemagglutinin-P
PIN	:	Protease Insoluble Nitrogen
PNC	:	Peanut cake
PUN	:	Plasma Urea Nitrogen
QT	:	Quebracho Tannin

RDWC	:	RBC Distribution Width
RCSM	:	Raw Cottonseed Meal
RGM	:	Rice gluten meal
RUP	:	Rumen Undegradable Protein
SBG	:	Spent Brewers Grain
SBM	:	Soybean meal
SSM	:	Sesame Seed Meal
SCFA	:	Short Chain Fatty Acids
SFC	:	Sun Flower Cake
SGOT	:	Serum Glutamic Oxaloacetic Transaminase
SGPT	:	Serum Glutamic Pyruvic Transaminase
SPSS	:	Statistical Package for Social Sciences
SRL	:	Strained Rumen Liquor
TA	:	Total Ash
TCA- N	:	Trichloroacetic acid Nitrogen
TCHO	:	Total Carbohydrates
TDDM	:	Truly Degradable Dry Matter
TDOM	:	Truly Degradable Organic Matter
TDN	:	Total Digestible Nutrients
TDS	:	Truly Degraded Substrate
TEC	:	Total Erythrocyte Count
VFA	:	Volatile Fatty Acid
VFA UI	:	Volatile Fatty Acid Utilization Index
TC	:	Total Cost
TLC	:	Total Leucocyte Count
TMR	:	Total Mixed Ration
TOMD	:	Total Organic Matter Digestibility
TP	:	Tomato Pomace
TR	:	Total Return
TVFA	:	Total Volatile Fatty Acids
TVC	:	Total Viable Count
UA	:	Uric Acid
VLDL	:	Very Low Density Lipoprotein
WCGF	:	Wet Corn Gluten Feed
WDGS	:	Wet Distillers Grains Plus Solubles
WSWLCS	:	Water Soaked Whole Linted Cotton Seed

CHAPTER I

INTRODUCTION

Livestock sector plays an immense role in the growth and sustainability of Indian economy. India has the largest livestock population in the world. In the 20th livestock census, (2019) total Livestock population in the country is 535.78 million showing an increase of 4.6% over preceding Livestock Census, 2012. Among them are 192.49 million cattle, 109.85 million buffaloes, 74.26 million sheep, 148.88 million goats, 9.06 million pigs, 0.38 million mithun, 0.06 million yak, 0.34 million horses and ponies, 0.08 million mules, 0.12 million donkeys and 0.25 million camels. There is distribution of 35.94%-cattle, 27.80%-goat, 20.45%-buffaloes, 13.87%-sheep, 1.69%-pigs among the livestock population. Improving the productivity of farm animals is one of the major challenges as livestock improves food and nutritional security by providing nutrient rich food products, generate income as well as employment and contribute to foreign exchange through exports. The scarcity and fluctuation of the quality and quantity of animal feed supply throughout the year is a major constraint to livestock production. To reach the potential productivity, animal nutrition is the main area that needs to be focused on various agro-industrial by products. Agro-industrial-by-products play a significant role in enhancing the nutritional status of different forms of rations and feeds of livestock as these contain various amounts of macro and micro nutrients that are essential for body growth and productivity.

The buffalo plays an important role in Indian economy. India ranks first in the population of buffaloes. About 20.5% of total livestock is contributed by buffaloes in India. The population of buffaloes in Punjab is reported to be 4 million. The river buffaloes (*Bubalus bubalis*) of the Indian sub-continent are chiefly maintained for milk production. They are also dual-purpose animals, showing good meat characteristics, though their potential for meat still remains unexplored and unexploited. The buffalo forms the backbone of India's dairy industry and is rightly considered as the 'bearer cheque' of the rural flock considered as India's milking machine. Buffaloes play an important role in the economy of farmers, which is primarily based on agricultural production systems and they are well adapted to a hot and hot humid climate and They provide high quality milk and meat and are a source of draught power for smallholders in the country. Buffaloes serve as an insurance against the risk of crop failure due to natural calamities so they are considered as a financial asset (Dhanda, 2004).

Cotton is one of the most important fiber and cash crop of India and plays a dominant role in the industrial and agricultural economy of the country. After soybean, rapeseed and oil palm, cotton is the world's fourth largest oil crop. As per committee on cotton production and consumption (COCPC), 2020-21, an area of around 129.57 lakh hectares of cotton was sown with production of 371 lakh bales of 170 kg and yield of 487 kgs per hectare was observed. In the North zone, 5.01 lakh ha in Punjab, 7.37 lakh ha in Haryana & 6.72 lakh ha in Rajasthan was sown under cotton in the year 2020-21. In 2018, cottonseed production in India is 9.8 million metric tonnes (MMT) which ranked second after China (10.1 MMT) (FAO, 2018). The major products arising from cottonseed processing are cottonseed oil (16%), cottonseed hulls (26%), cottonseed meal (45.5%) and linters (8.5%) (Brien et al., 2005). Increase in cottonseed production in India has resulted in a greater availability of CSM which is the common protein source for livestock in cotton producing areas.

CSM is a by-product of the oil industry, i.e., oil extraction from cotton seeds. CSM is a relatively rich source of protein (30 to 50%) and amino acids (He et al., 2015). Cottonseed meal is a plant protein source, abundant in most parts of the world and is relatively high in protein and generally less expensive per unit of protein than soybean meal. CSM is rich in tryptophan and methionine. Nutritional value of cottonseed meal depends upon the method of extraction, proportion of husk, lint and degree of decortication. The price of conventional protein sources like soybean and peanut has increased markedly in recent years resulting in urgent need for exploring alternate protein sources (Sun et al., 2013). Due to increase in availability of cottonseed meal (CSM) in cotton-growing areas of India as compared to other oil seed meals, CSM is becoming one of the major components of concentrate mixture (CM) fed to animals. Ruminants can withstand the presence of gossypol in the diet so cottonseed meal is given to them which is used as a protein source. Due to increased price of soybean meal, dairy farmers are seeking suitable and viable alternate protein supplement to soybean meal. Therefore, keeping in view the above points, a comprehensive study was proposed with the following objectives:

a) To study *in vitro* nutritional worth of total mixed rations containing graded levels of cottonseed meal.

b) To study the effect of cottonseed meal as a replacement of soybean meal on nutrient utilization and blood biochemical profile in male buffalo calves.

CHAPTER II

REVIEW OF LITERATURE

2.1. Production of cotton seed meal

Cottonseed, is one of the richest sources of oil seeds which is available in many temperate and tropical countries. These are mostly processed to extract oil that is used as edible fat (Zhou et al., 2015). Cottonseed meal is produced mainly in cottonseed oil processing industry. After processing, cottonseeds yield 50% meal, 22% hulls, 16% oil and 7% linters, with a 5% loss (Hinze et al., 2015). The commonly used methods of lipid extraction from oil seeds are pressing and extraction with organic solvents (cold or hot).

There are several methods to extract cottonseed which result in cottonseed meal such as mechanical extraction, direct solvent extraction, pre-press solvent extraction. By these methods, gossypol is also extracted. The most commonly used method is solvent extraction by which seeds are subjected to dehulling, cooking, cracking and flaking, but by using solvent oil is removed. The commonly utilized commercial solvent for carrying out solvent extraction is hexane. To eliminate solvent, cake which is extracted was heated and then crushed into meal (Ash, 1992). The process has been schematically shown in the figure 1.

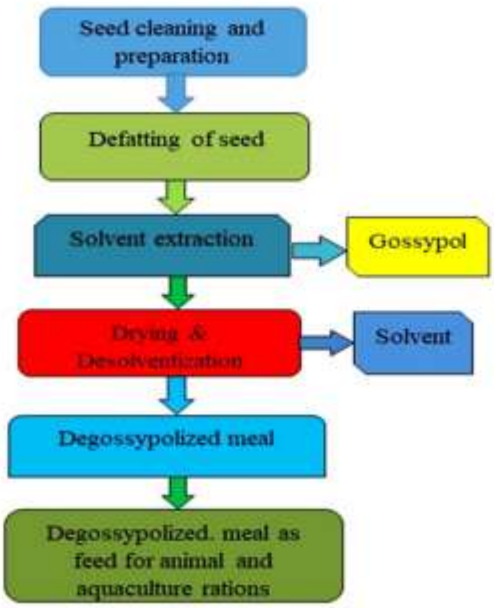

Fig. 1. Production of cottonseed meal by solvent extraction process from cottonseeds

2.2. Chemical composition of Cottonseed meal

Thirumalaisamy et al. (2015) performed an experiment on nutritive and feeding value of cottonseed meal for broilers. The content of crude protein, dry matter, total ash, crude fibre, free gossypol and total gossypol was 30.90%, 91.9%, 3.07%, 11.92%, 7.15%, 0.40% and 2.65%, respectively. Minerals were estimated like total phosphorous, calcium, zinc, magnesium, iron, copper and the values were 1.16%, 0.22%, 61.7mg/kg, 58.30mg/kg, 120mg/kg, 9.88 mg/kg, respectively.

Fadel and Ashmawy (2015) estimated chemical composition of cottonseed meal as % DM basis for DM, CP, CF, EE, NFE, Ash as 91, 45.05, 15.39, 1.65, 31.32, 6.59 %, respectively.

Zotte et al. (2013) studied the effect of adding cottonseed oilcake on chemical composition of meat and ostrich growth performance. It was reported that cotton seed meal contained dry matter, crude protein, crude fibre, crude fat, total ash, free gossypol, NDF and ADF was 91.9%, 30.9%, 26.2%, 0.6%, 6.03%,0.082%, 49.2 and 33.4, respectively.

Sun et al. (2013) reported that broiler chickens fed diets containing fermented cottonseed meal contained dry matter, crude protein, ether extract, calcium, phosphorus, lysine, methionine and threonine as 87.2%, 46.2%, 1.09%, 0.25%, 0.79%, 2.07%, 0.48% and 1.54%, respectively.

Salas et al. (2013) conducted a study in broilers on amino acid digestibility, amino acid content and proximate analysis of glandless and commercial cottonseed meal. The chemical composition of cotton seed meal was estimated as crude protein, crude fibre, total ash, total gossypol, free gossypol, calcium and phosphorus - 50.67%, 12.88%, 1.94%, 1.52%, 0.160%, 0.24% and 1.71%, respectively.

Wanapat et al. (2013) investigated the effect of levels of cottonseed meal on six months old four dairy bulls which were randomly allotted to receive four dietary treatments. They reported that CSM contained crude protein, organic matter, ether extract, acid detergent fiber, neutral detergent fiber as 435, 906, 19, 207, 323 g/kg DM, respectively.

Tang et al. (2012) analysed the chemical composition of cottonseed meal. The content of crude protein, ether extract, ash, crude fibre, dry matter, amino acids-lysine, methionine, cystine, methionine + cystine, threonine, arginine, isoleucine, valine, histidine, phenylalanine, total phosphorus, calcium, free gossypol was 465.2 g/kg , 10.8 g/kg, 60.2g/kg, 102.1g/kg, 882.3g/kg, 21.3g/kg, 5.6g/kg, 6.4g/kg, 1.2g/kg, 14.5g/kg, 49.8g/kg, 15.2g/kg, 26.8g/kg, 21.5g/kg, 12.6g/kg, 24.3g/kg, 11.1g/kg, 2.5g/kg, 0.82 g/kg as feed basis.

Silva et al. (2009) evaluated the replacement of soybean meal by cottonseed meal in the diet of dairy cows fed diets based on spineless cactus. They reported the chemical composition of cottonseed meal on %DM basis cottonseed meal contained ash, dry matter, ether extract, crude protein, acid detergent fiber, neutral detergent fiber, non-fibrous carbohydrates, total carbohydrates as 7.70, 90.98, 1.75, 40.90, 11.41, 28.21, 21.44, 49.65%, respectively.

Khanum et al. (2007) reported the chemical composition of cotton seed meal containing DM, ash, CF, CP, EE and ME as 86.3%, 8.2%, 12.2%, 34.3%, 4.05%, 9.74 MJ/kg ME, respectively.

Cheng and Hardy (2002) evaluated chemical composition of CSM from 4 places in southern part of USA. The nutritional content varied significantly among the samples by which crude protein ranged from 34.9% to 41.3%, phosphorus ranged from 9.95% to 1.10%, ether extract ranged from 1.43 to 3.70%, Threonine ranged from 1.17% to 1.30%, , Lysine ranged from 1.68% to 1.90%, Tryptophan ranged from 0.42% to 0.47% and Methionine ranged from 0.57% to 0.63%.

Church (1991) reported the chemical composition of 2 types of cottonseed meals i.e., mechanical extracted with 41% CP and solvent extracted with 41% CP having DM, CP, CF, NDF, ADF, Ca, P, ME, TDN as 93, 44.3, 13, 28, 20, 0.21, 1.16, 2.82 MJ/kg, 78 and 91, 45.2, 13, 26, 19, 0.18, 1.21, 2.75 MJ/kg, 76 %, respectively.

2.3. *In vitro* evaluation of cottonseed meal

2.3.1. Effect on gas production

DeVore (2018) evaluated the *in vitro* gas production from corn gluten feed (CGF), wheat middlings, solvent extracted cotton seed meal (CSM) and soyhulls in rumen fluid collected from steer (Bos taurus). Results showed that *in vitro* gas production over 24-hour period was highest ($P<0.05$) from the digestion of soyhulls. CGF and wheat middlings had intermediate ($P<0.05$) and CSM had lowest ($P<0.05$) in vitro total gas production.

Raji et al. (2017) conducted a study to determine chemical composition, *in vitro* gas production and post-incubation parameters of cottonseed (Gossypium spp) and its by-products. Cotton seed was processed using four different processing methods to produce four samples (treatments) i.e., raw cotton seed (A), parboiled cotton seed (B), roasted cottonseed(C) and cotton seed cake (D). Rumen liquor was collected from experimental goats, filtrated, dispensed into the artificial rumen with addition of differently processed cotton seed meal and incubated for 24, 48 and 72 hours. Cumulative gas production over 24 hr of incubation varied significantly ($P<0.05$) in all the treatments. The result revealed after 24 hr, that cotton seed cake had highest cumulative gas production.

Tripathi et al. (2014) studied the effect of Bt-cottonseed meal (Bt-CM) or conventional cottonseed meal (C-CM) in lambs which is fed for 120 days. Three feed mixtures (FM, forage: concentrate ration of 35: 65) contained groundnut oil meal (GNM), insect protected Bt-cottonseed meal (Bt-CM) or conventional whole cottonseed (C-CM) as protein source, were fed for 123 days to the control, C-CM and Bt-CM groups, respectively. Results showed that gas production (ml/ g DM incubated) was similar between Bt-CM (113.16) and C-CM (105.67) but less gas production per g DM fermented in Bt-CM was due to higher fermentation efficiency.

Abdalla et al. (2012) conducted *in vitro* studies with tanniniferous plants, herbal plant essential oils derived from thyme, fennel, ginger, lack seed, and eucalyptus oil added to the basal diet and cakes of oleaginous plants (cottonseed meal, palm, castor plant, turnip, and lupine), which were included in the basal diet to replace soybean meal. Gas production in cottonseed meal during 24 h of *in vitro* incubation was reported to be 115mL/g DM and was non-significant among other cakes like basal diet, castor plant, turnip, lupine having 118, 117, 121, 126 mL/g DM, respectively.

Nasser (2009) conducted an *in vitro* experiment by adding jojoba meal to a concentrate diet at 6%, 9% and 18% levels, substituting for cottonseed meal. A gas production experiment was performed by collecting rumen liquor from three fistulated Santa Ines sheep. Cumulative gas production (ml/200mg DM) was recorded at 3, 6, 9, 12, 24, 48, 72 and 96 h of incubation time. Volatile fatty acids (VFA), ammonia-N (NH_3-N) concentrations, true dry and organic matter degradability was determined at

24 h of incubation time. Gas produced at 96 h incubation ranged between 45.03 and 48.10 ml/200mg DM. The volume of gas production (ml/200mg DM) for control was significantly (P< 0.05) lower than those for both level 1 and 2 of jojoba meal at 24, 48, 72 and 96 h incubation; but there was no significant (P >0.05) difference between both control and level 3 of jojoba meal at 72 and 96 h of incubation.

2.3.2. Effect on Total volatile fatty acids

Raji et al. (2017) conducted a study to determine chemical composition, *in vitro* gas production and post-incubation parameters of cottonseed (Gossypium spp) and its by-products. Cotton seed was processed using four different processing methods to produce four samples (treatments) i.e. raw cotton seed (A), parboiled cotton seed (B), roasted cottonseed(C) and cotton seed cake (D). Rumen liquor was collected from experimental goats, filtrated, dispensed into the artificial rumen with addition of differently processed cotton seed meal and incubated for 24, 48 and 72 hours. Results revealed that cottonseed cake produced higher (P<0.05) SCFA (0.62 µ mol) when compared to raw cottonseed (0.29 µ mol), parboiled cotton seed (0.26 µ mol), roasted cottonseed (0.20 µ mol).

Lamba et al. (2014) evaluated in-*vitro* methane and total volatile fatty acids (TVFAs) production; *in sacco* degradability and metabolizable energy (ME) availability from conventional and non-conventional protein supplements like mustard cake (MC), deoiled mustard cake (DMC), deoiled groundnut cake (DGNC), soybean meal (SBM), cottonseed cake (CSC), corn gluten meal (CGM), maize oil cake (MOC), tomato pomace (TP) and spent brewers' grains (SBG). The TVFA of cottonseed cake was 7.89 mM/dl. Acetate, Propionate, Butyrate, A:P ratio of CSC was 4.64, 2.51, 0.58, 1.85 mM/dl, respectively.

Bo et al. (2012) conducted *in vitro* experiment on cottonseed by-products (CSBPs), which are linted cottonseed hulls (LCSH), delinted cottonseed hulls (DCSH) and cottonseed linter residue (CLR), were assessed by chemical analysis, an *in situ* nylon bag technique, an *in vitro* cumulative gas production technique and *in vitro* enzyme procedure. Results revealed that highest concentration of total volatile fatty acids was observed in CLR (103.7 mM) after a 72 h incubation (p<0.05). Molar proportions of acetate, butyrate, valerate and Branched chain volatile fatty acid (BCVFA) were similar among the three CSBPs, but molar propionate proportion is highest (p<0.05) in CLR (30.4 mM) and similar in LCSH (23.6) and DCSH (21.2).

Nasser (2009) conducted *in vitro* experiment by adding jojoba meal to a concentrate diet at 6% (L1), 9% (L2) and 18% (L3) levels, substituting for cottonseed meal. Cumulative gas production (ml/200mg DM) was recorded at 3, 6, 9, 12, 24, 48, 72 and 96 h of incubation time. volatile fatty acids (VFA), ammonia-N (NH_3-N) concentrations, true dry and organic matter degradability was determined at 24 h of incubation time. The TVFA concentration (mM) was significantly ($P<0.05$) increased when jojoba meal was added at L2 and L3 levels but not in L1 ($P > 0.05$). The average acetate and propionate concentrations for all substrates which contained jojoba meal were higher than control although the results were not statistically significant.

2.3.3. Effect on *in vitro* digestibility

DeVore (2018) evaluated *in vitro* true digestibility of wheat middlings, corn gluten feed (CGF), soyhulls and solvent extracted CSM. *In vitro* true dry matter digestibility ($IVTD_{DM}$) of CSM and soyhulls was more ($P<0.05$) than CGF and wheat middlings. *In vitro* true dry matter digestibility for wheat middlings, CGF, soyhulls and solvent extracted CSM was 62.82%, 60.24%, 75.44% and 72.64%, respectively.

Raji et al. (2017) conducted a study to determine chemical composition, *in vitro* gas production and post-incubation parameters of cottonseed (Gossypium spp) and its by-products. Cotton seed was processed using four different processing methods to produce four treatments i.e., raw cotton seed (A), parboiled cotton seed (B), roasted cotton seed (C) and cotton seed cake (D). Rumen liquor was collected from experimental goats, filtrated, dispensed into the artificial rumen with addition of differently processed cotton seed and incubated for 24, 48 and 72 hours. Study revealed that cottonseed cake produced more organic matter digestibility (OMD) (52.94%) compared to other by products like raw cotton seed, parboiled cotton seed, roasted which had 35.65, 36.37, 35.24% OMD, respectively.

Tripathi et al. (2014) studied the effect of Bt-CM or conventional cottonseed meal in lambs which is fed for 120 days. Three feed mixtures (FM, forage: concentrate ration of 35: 65) contained groundnut oil meal (GNM), insect protected Bt-cottonseed meal (Bt-CM) or conventional whole cottonseed (C-CM) as protein source, were fed to the control, C-CM and Bt-CM groups, respectively. *In-vitro* fermentation characteristics like truly degradable dry matter (TDDM), truly degradable organic matter (TDOM) was higher ($p<0.05$) in Bt-CM as compared to C-CM. TDDM of Bt-CM and C-CM was 692.6 and 578.6 g/kg respectively and TDOM of BT-CM and C-CM was 662.9 and 550.5 g/kg respectively.

Abdalla et al. (2012) conducted *in vitro* studies with tanniniferous plants, herbal plant essential oils derived from thyme, fennel, ginger, lack seed, and eucalyptus oil added to the basal diet and cakes of oleaginous plants (cottonseed meal, palm, castor plant, turnip, and lupine), which were included in the basal diet to replace soybean meal. Organic matter true digestibility (OMTD) of cottonseed meal during 24 h of *in vitro* incubation was reported to be 496g/kg and varied non significantly with basal diet and castor plant which had 557 and 511 g/kg of OMTD.

Ghanbari et al. (2012) conducted a study to compare the effects of electron beam (EB) and gamma ray (GR) treatments at doses of 25, 50 and 75 kGy on ruminal degradation kinetics of crude protein (CP), amino acid (AA), and *in vitro* digestibility of cottonseed meal (CSM). EB and GR treatments at a dose of 75 kGy increased *in vitro* digestibility of CSM numerically

Nasser (2009) conducted *in vitro* experiment by adding jojoba meal to a concentrate diet at 6%, 9% and 18% levels, substituting for cottonseed meal. Cumulative gas production (ml/200mg DM) was recorded at 3, 6, 9, 12, 24, 48, 72 and 96 h of incubation time. Volatile fatty acids (VFA), ammonia-N (NH_3-N) concentrations, true dry and organic matter degradability was determined at 24 h of incubation time. Results showed that there were no significant ($P > 0.05$) differences among all substrates in true degradability of dry matter (TDDM) and organic matter (TOMD) at 24 h of incubation time.

2.3.4. Effect on pH

Nasser (2009) conducted *in vitro* experiment by adding jojoba meal to a concentrate diet at 6% (level 1), 9% (level 2) and 18% (level 3), substituting for cottonseed meal. Cumulative gas production (ml/200mg DM) was recorded at 3, 6, 9, 12, 24, 48, 72 and 96 h of incubation time. The average values of pH ranged from 6.60 to 6.70 at 24 h of incubation time. pH value was decreased significantly ($P<0.05$) at the third level of jojoba meal (6.60) compared to that of control (6.70), level 1 (6.68) and level 2 (6.70) of jojoba meal.

2.3.5. Effect on NH_3-N

Nasser (2009) conducted *in vitro* experiment by adding jojoba meal to a concentrate diet at 0%(C), 6%(L1), 9%(L2) and 18%(L3) levels, substituting for cottonseed meal. Cumulative gas production (ml/200mg DM) was recorded at 3, 6, 9, 12, 24, 48, 72 and 96 h of incubation time. Ammonia-N (NH3-N) concentrations, was determined at 24 h of incubation time. There was no significant difference among the NH_3-N values among all substrates at 24 h of incubation time. The NH_3-N values in C, L1, L2, L3 were 18.55, 18.03, 18.43, 18.17 mg/l, respectively

2.3.6. Effect on methane production

Raji et al. (2017) conducted a study to determine chemical composition, *in vitro* gas production and post-incubation parameters of cottonseed (Gossypium spp) and its by-products. Cotton seed was processed using four different processing methods to produce four treatments i.e., raw cotton seed (A), parboiled cotton seed (B), roasted cotton seed (C) and cotton seed cake (D). Rumen liquor was collected from experimental goats, filtrated, dispensed into the artificial rumen with addition of differently processed cotton seed meal and incubated for 24, 48 and 72 hours. Methane production was highest ($P<0.05$) in cottonseed cake and lowest in roasted cottonseed comparatively.

Abdalla et al. (2012) conducted *in vitro* studies with tanniniferous plants, herbal plant essential oils derived from thyme, fennel, ginger, lack seed, and eucalyptus oil added to the basal diet and cakes of oleaginous plants (cottonseed meal, palm, castor plant, turnip, and lupine), which were included in the basal diet to replace soybean meal. Methane production of cottonseed meal during 24 hr of *in vitro* incubation was 70.3 (ml/g of organic matter true digestibility) which was similar to basal diet, palm, castor plant having 63.9, 73.5, 67.6 ml/g OMTD.

Nasser (2009) conducted *in vitro* experiment by adding jojoba meal to a concentrate diet at 0% (C), 6% (L1), 9% (L2) and 18% (L3) levels, substituting for cottonseed meal. Methane emission values of C, L1, L2, L3 were 6.70, 7.94, 6.92, 6.85 ml/g DM respectively. There was no significant difference ($P>0.05$) in methane emission among all treatments.

2.4. *In vivo* evaluation of CSM

2.4.1. Effect of CSM on feed intake

Nomeary et al. (2021) conducted a study on 20 Farafra male lambs divided randomly into four similar groups of five animals each for a period of 66 days. At 3% of their body weight, all the groups were given concentrate feed mixture (CFM) and *ad lib* wheat straw. Soybean meal (SBM) as a source of protein was replaced 100% with cottonseed meal (CSM), black cumin seed meal (BCSM), and sesame seed meal (SSM) which were integrated into the concentrate feed mixture. The results showed that feed intake in treatment groups is having no difference. Cottonseed meal was found to be the most effective treatment in the terms of feed conversion ratio, average daily gain and economic efficiency when compared to soybean meal, black cumin seed meal and sesame seed meal treatments. When economics was taken into account, cottonseed meal might completely replace 100% soybean meal in sheep rations.

Kannan et al. (2013) studied the effect of feeding calcium hydroxide treated or vitamin E-supplemented cottonseed meal (CSM). Twenty four 6-7 months old male Bikaneri lambs were divided into 4 groups of 6 animals each were fed concentrate mixture containing same protein and energy comprising control diet of 20 % soybean meal, 40 % raw CSM (RCSM), 40 % raw CSM supplemented with 500 IU of vitamin E per head per day (ERCSM), and 40 % CSM treated with 1.5 % calcium hydroxide (CaCSM) along with *ad libitum* wheat straw throughout 510 days of experimental feeding. The daily intake of nutrients (DM, CP, DCP, TDN and total gossypol) were similar among the groups. However, free gossypol intake was higher ($P<0.05$) significantly in RCSM, ECSM than CaCSM group.

Tripathi et al. (2012) studied feeding value of conventional and Bt cottonseed meal as replacement of groundnut oil meal for the feeding of lamb (90±5 day of age; 15.5±0.89 kg). Diet fed to control lambs contained groundnut oil meal as source of CP while other two groups received diet containing either conventional cottonseed meal (CSM) or Bt-cottonseed meal (Bt-CSM) at 18% level. Total and average daily BW gain, feed intake and its conversion efficiency were similar among groups. However, Bt-CSM diet fed lambs had higher ($P>0.05$) ADG (111g/ day) and better feed efficiency (8.0 kg feed/ kg gain) than CSM fed lambs with 89 g/day ADG and feed efficiency of 9.1 kg feed/kg gain, respectively. DM intake ranged from 706 to

861 g/day, which was similar among groups, but Bt-CSM diet fed lambs had higher DMI by 8.6 per cent. Intake and digestibility of OM, CP and NDF were similar. Therefore, genetically modified cottonseed can be used in lamb feeding in replacement of conventional cottonseed or groundnut oil meal as protein supplement up to 18% for mutton production.

Solomon et al. (2008) conducted experiment in twenty-four yearling intact male Sidama goats with a mean body weight (BW) of 16.8±1.14 kg. The experiment consisted of 90 days of feeding trial and 10 days of digestibility trial followed by evaluation of carcass parameters at the end of the experiment. The treatments included *ad libitum* feeding of hay (T1, control) and daily supplementation of CSM at 200 g (T2), 300 g (T3) and 400 g (T4) per head on dry matter (DM) basis. Hay DM, CP and NDF intake were higher ($P<0.01$) in the non-supplemented than in the supplemented goats. Total DM intake was higher ($P<0.01$) for goats supplemented with the high level of CSM than those on the control treatment. Among CSM supplemented goats, the intake of CSM DM, CP, NDF and total CP were in the order of T4>T3>T2.

Ojewola et al. (2006) performed a trail for six weeks on broiler birds by feeding cotton seed meal in 0, 25, 50, 75 and 100% by substituting soybean meal. Results showed that the mean daily feed intake was higher ($P<0.05$) in diet 3 (150.93) and diet 4 (15.93). There were no significant variations ($P<0.05$) in mean daily weight gain and feed-to-gain ratio. The nutrient utilization of diet 5 has highest ($P<0.05$) percent mean values and diet 1 has lowest ($P<0.05$) percent mean values. Diet with 100% CSM had highest percent mean values in nitrogen, crude fibre, ether extract, ash and dry matter 81.45, 60.81,95.57, 66.79 and 85.72 respectively, whereas all these values are least for diet containing 0% CSM.

Azman and Yilmaz (2005) estimated the effect of diets containing lysine supplemented with cottonseed meal (CSM) in place of soybean meal (SBM) on the growth performance of 300, 7-day-old broilers which were divided into 3 groups. During 15 days, the chicks were fed control diet of corn-soy-based starter meals and experimental groups were fed with 20 percent cottonseed meal based diets supplemented with 1.5 percent or 3 percent lysine. During the trial period, no significant variations in body weight, daily body weight growth, or daily feed intake were detected between control and treated birds. Although slight but not significant increase of daily weight gain and feed intake was noticed in CSM-1.5% lys group. In conclusion, the study reported that cottonseed meal could be included in broiler diets with enough lysine supplementation without affecting growth performance.

Nagalakshmi et al. (2002) evaluated feeding of raw and processed cottonseed meals (CSM) on 20 male lambs of uniform body weight allotted to 5 treatments and fed control diet of 30 percent deoiled groundnut cake (DGNC) in isonitrogenous and isocaloric concentrate mixtures, 40% of raw CSM, 45 minutes cooked CSM, 1% calcium hydroxide treated CSM and iron treated cottonseed meal. Feeding of lambs with raw CSM amounting to 17.9% of total DM intake (i.e., 303 mg free gossypol intake per day) adversely affected utilisation of nutrients and rumen fermentation. This depression was alleviated to the greatest extent by 45 min cooking and 1% calcium hydroxide treatment. Iron treatment of CSM had little overall benefit.

2.4.2. Effect of CSM on nutrient digestibility and utilization

Nomeary et al. (2021) conducted a study on 20 Farafra male lambs divided randomly into four similar groups of five animals each for a period of 66 days. At 3% of their body weight, all the groups were given concentrate feed mixture (CFM) and *ad lib* wheat straw. Soybean meal (SBM) as a source of protein was replaced 100% with cottonseed meal (CSM), black cumin seed meal (BCSM), and sesame seed meal (SSM) which were integrated into the concentrate feed mixture. Digestibility and nitrogen (N) balance trials are conducted. The digestible crude protein content of the CSM ration was higher than that of the BCSM and SBM rations. CSM fed animals had greater ($P<0.05$) crude protein, ether extract, feed conversion, average daily weight gain, and crude fibre digestibility than those fed the other rations. When compared to alternative protein sources, the CSM ration had the best relative economic efficiency and nitrogen balance value. CSM could replace 100% soybean meal in sheep ration when economics was considered.

Fadel and Ashmawy (2015) conducted experiment on 32 Zaraibi dairy goats and observed their offspring performance in which goats are divided into four similar groups (8 does/ each) in which R1 and R3 groups were fed 60% concentrate feed mixture (CFM) containing untreated linseed meal or cotton seed meal respectively along with 40% rice straw. R2 and R4 groups were fed 60% CFM contained treated linseed meal or cotton seed meal by quebracho tannin (QT) at the rate of 2% plus 40% rice straw on DM basis for all rations. Digestibility coefficient of CP was significantly higher ($P<0.05$) for R4 (78.21) and R2 (77.88%) groups and lowest ($P<0.05$) in R1 (66.91). There was no significant difference in dry matter, organic matter, crude fiber, ether extract, nitrogen free extract digestibility among the treatments.

Wanapat et al. (2013) investigated the effect of levels of cottonseed meal on six months old four dairy bulls randomly allotted to receive four dietary treatments. Factor A was carbohydrate source; cassava chip (CC) and cassava chip+rice bran in the ratio of 3:1 (CR3:1), and factor B was cotton seed meal levels in the concentrate; 109 g CP/kg (low cotton meal) and 328 g CP/kg (high cotton meal) having similar CP levels (490 g CP/kg). Bulls were fed with urea-lime treated rice straw on *ad libitum* basis and were supplemented with 10 g of concentrate/kg BW. Using CR3:1 as a carbohydrate source resulted in higher DM and OM digestibility when compared with CC treatments ($P<0.05$). The protein digestibility was higher in LCM received Bulls than those receiving HCM.

Tripathi et al. (2012) studied feeding value of conventional and Bt cottonseed meal as replacement of groundnut oil meal for the feeding of lamb (90±5 day of age; 15.5±0.89 kg). Diet fed to control lambs contained groundnut oil meal as source of CP while other two groups received diet containing either conventional cottonseed meal (CSM) or Bt-cottonseed meal (Bt-CSM) at 18% level. Lambs of three groups had similar DM intake, daily gain and nutrient digestibility. Intake and digestibility of OM, CP and NDF were similar, whereas CSM diet had higher ($P=0.022$) digestibility of ADF whereas cellulose digestibility was lower ($P=0.002$), however hemicellulose digestibility was higher ($P=0.047$) in Bt-CM diet fed lambs. Thus, Bt-CM could be incorporated at 180 g/kg in lamb diet.

Lorena-Rezende et al. (2012) studied the digestibility of cottonseed meal with or without addition of enzymes (phytase and protease) in 18 growing pigs (Landrace x Large White), housed in metabolism cages having average body weight 25.8 ± 3.6 kg. which were distributed in three treatments and six repetitions. The treatment were T1 – reference diet (based on corn and soybean meal); T2 – diet with replacement of 30% of the reference diet by cottonseed meal without enzyme supplementation, and T3 – diet with 30% of the reference diet replaced by cottonseed meal with enzyme supplementation (20 g protease and 15 g phytase per 100 kg of ration). They determined the digestible energy, digestible protein, digestibility of dry matter, protein and energy and reported no effect on the digestibility of dry matter, gross energy and crude protein in growing barrows fed with the diet containing cottonseed meal with addition of phytase enzymes. The diets containing 30% replacement of reference diet with cotton seed meal gave better results in terms of digestible protein with values of 15.3 and 16.3 %, with and without enzymes, respectively.

Barros et al. (2011) conducted a study on beef heifers (25 Nellore heifers and 10 crossbred heifers with predominance of Zebu breed at 16 months of age) in Brachiaria decumbens pasture. Supplements having approximately 30% of crude protein (CP) and soybean meal were replaced by cottonseed meal (38%CP) at the levels 0; 33; 67 and 100%, (CS0, CS33, CS67 and CS100, respectively) in relation to a control group, which contained only mineral mixture (MM) *ad libitum* basis. Coefficients of total apparent digestibility of CP and NFC were greater in animals fed supplements in relation to the control group. It was reported that coefficients of total apparent digestibility of DM, CP and organic matter showed a positive linear effect on increasing the replacement level of soybean meal by cottonseed meal. The use of cottonseed meal (38%CP) in the diet did not harm the performance of grazing beef heifers.

Silva et al. (2009) conducted a study in dairy cows to evaluate the effect of replacement of soybean meal with cottonseed meal in the diets based on spineless cactus. Five Girolando lactating cows were, distributed in a 5 × 5 Latin square design (5 animals, 5 treatments and 5 experimental periods), having average live weight of 490 kg and average production of 11.5 kg of milk/day with 15 days of each experimental period with 10 days adaptation period to the diet and 5 days for data collection. The experimental diet was formulated with spineless cactus (53%), sorghum silage (32%) and concentrate (15%). The soybean meal was replaced with 0, 25, 50, 75 and 100% of cottonseed meal in the concentrate. The nutrients intake and digestibility were not affected by the treatments, with an average intake and digestibility of 15.55 and 56.05; 1.79 and 48.14; 13.8 and 59.31, 0.37 and 49.40, 5.32 and 30.95, 9.94 and 54.31, 4.43 kg/day and 80.99%, for the dry matter, crude protein, organic matter, ether extract, neutral detergent fiber, total carbohydrates and non-fibrous carbohydrates, respectively. The total digestible nutrients were also not affected with an average of 8.30 kg/day. The replacement of soybean meal with cottonseed meal did not alter nutrient digestibility and nutrient intake. Thus, the replacement of soybean meal with cottonseed meal is recommended for low-production cows fed with spineless cactus-based diets.

2.4.3. Effect of CSM on nitrogen balance

Lorena-Rezende et al. (2012) evaluated the digestibility of cottonseed meal with or without addition of enzymes (phytase and protease) by using 18 barrows with three treatments and six repetitions. The treatments consisted of a control diet based on corn and soybean meal, the second treatment with replacement of 30% of the control diet by cottonseed meal without enzymes, and the third with 30% of the control diet replaced by cottonseed meal with added enzymes. No significant difference between the treatments with cottonseed meal was observed, concerning the ingested content of nitrogen. There was a higher intake of nitrogen because the diets with cottonseed meal provided a slight increase of CP in the diet. The diets with cottonseed meal resulted in higher amount of retained nitrogen. The nitrogen excreted in the faeces was greater in the treatments with cottonseed meal (7.26 g/day) compared to control (4.06 g/day). No significant difference was observed among treatments in urinary nitrogen.

Li et al. (2012) conducted experiment on 12 growing barrows which were allotted to two 6*6 Latin square designs, with six barrows and six periods and six diets for each. A corn-dehulled soybean meal diet was used as the basal diet, and the other ten diets (10 cottonseed meal (CSM) samples collected from different provinces of China) were formulated with corn, dehulled soybean meal and 19.20% CSM. Although the amount of N intake was highest ($P<0.0001$) in diet 9 (59.62g) and lowest ($P<0.0001$) in diet 2 (46.39) and N excreted from faeces was highest ($P<0.05$) in diet 9 (8.92g), diet 6 (8.83g), diet 7 (8.69g) and lowest in diet 1 (7.27g), diet 2 (7.14). No differences in the amount of N excreted from the urine and N retention were observed. However, pigs fed basal diet had lower ($p<0.0001$) N excretion from faeces than that of pigs fed CSM diets, and they also had a tendency to lower ($P=0.0577$) N excretion from urine and greater ($P = 0.0535$) ATTD (apparent total tract digestibility) of N.

Tripathi et al. (2012) studied feeding value of conventional and Bt cottonseed meal as replacement of groundnut oil meal for the feeding of lamb (90±5 day of age; 15.5±0.89 kg). Diet fed to control lambs contained groundnut oil meal as source of CP while other two groups received diet containing either conventional cottonseed meal (CSM) or Bt-cottonseed meal (Bt-CSM) at 18% level. The N intake, its excretion in faeces and urine and its retention were similar among control and treatment diets.

Barros et al. (2011) conducted a study on beef heifers (25 Nellore heifers and 10 crossbred heifers with predominance of Zebu breed at 16 months of age) in Brachiaria decumbens pasture. Supplements having approximately 30% of crude protein (CP) and soybean meal were replaced by cottonseed meal (38% CP) at the levels 0; 33; 67 and 100%, (CS0, CS33, CS67 and CS100, respectively) analysed in relation to a control group, in which animals received only mineral mixture *ad libitum*. There was difference in ($P<0.10$) apparent nitrogen balance among supplemented and no supplemented animals. This variable followed the behaviour of variables of nitrogen consumption and the nitrogen faecal excretion, which were lower for non-supplemented animals. There was a reduction ($P<0.10$) in the nitrogen faecal excretion as the level of cottonseed meal 38% increase in the supplements. Cottonseed meal (38%) showed positive linear effect ($P<0.10$) on the nitrogen balance in which there is more retention of dietary nitrogen with increased CSM in supplements.

Khan et al. (2000) conducted a study on sixteen male lambs given four total mixed rations containing A (40% untreated solvent extracted CSM), B (40% formaldehyde treated solvent extracted meal), C (40% untreated mechanical extracted meal) and D (40% formaldehyde treated mechanical extracted meal) for 90 days. Nitrogen intake of rations A and B did not differ ($P>0.05$) 46.45, 46.29 g/day respectively, however, the N intake was less ($P<0.05$) on rations C (40.00g/day) and D (43.70g/day) compared to rations A and B. Nitrogen excretion through faeces was similar for all the rations. Lambs fed rations C and D excreted less ($P<0.05$) nitrogen through urine than lambs fed rations A and B. Nitrogen retention as of % nitrogen intake was higher ($P<0.05$) in lambs fed rations B (22.12%) and D (24.80%) having formaldehyde treated cottonseed meals compared to rations A (20.88%) and C

(19.30%) having untreated solvent and mechanical extracted cottonseed meal, respectively.

2.4.4 Effect of CSM on blood parameters

Thirumalaisamy et al. (2016) conducted an experiment in 400 broiler chicks divided to 9 experimental diets with 6 replicates, 8 chicks each. Based on amino acids (total and digestible) with or without iron supplementation with 2% and 4% levels of CSM experimental diets were formulated along with maize-soybean diets (control). The serum cholesterol in terms of total cholesterol HDL, LDL, and triglycerides was not statistically significant between the dietary treatments. Inclusion of CSM at 4% level without iron supplementation showed lower PCV value (33.86-35.54%), red blood cell (RBC) numbers (2.78-2.87×106/μl), and Hb (10.30-10.70%) when compared to control diet.

He et al. (2015) conducted an experiment in 432 40-week old Hy-line W36 laying hens to evaluate feeding of low-gossypol cottonseed meal (LCSM) for 12 weeks in layers diets which were given to one of the 6 dietary treatments with 6 replicates of 12 birds each. Corn-soybean meal basal diet (control), and the 4 experimental diets consisted of a basal diet with 50, 98.3, 144.2, or 189 g/kg LCSM, respectively in which 25%, 50%, 75%, 100% soybean meal were replaced by LCSM. Free gossypol (FG group) was added with basal diet in 6^{th} group. The serum parameters like alanine aminotransferase (ALT), aspartate aminotransferase (AST), total protein (TP), albumin (ALB), blood urea nitrogen (BUN), and uric acid (UA) levels in the serum were not different ($P > 0.05$) in all experiment and control groups.

Tripathi et al. (2012) studied feeding value of conventional and Bt cottonseed meal as replacement of groundnut oil meal for the feeding of lamb (90±5 day of age; 15.5±0.89 kg). Diet fed to control lambs contained groundnut oil meal as source of CP while other two groups received diet containing either conventional cottonseed meal (CSM) or Bt-cottonseed meal (Bt-CSM) at 18% level. The results showed that serum total cholesterol was higher ($P=0.032$) in Bt-CSM diet than CSM and control diets, while HDL cholesterol had no significant difference between CSM and Bt-CSM fed lambs, but it was lower ($P<0.001$) in control diet with quadratic increase ($P<0.001$) at 90 days of feeding. The diet had significant effect on HDL level. The IgG level was not different among diets and remained unchanged during experimental feeding period.

Kannan et al. (2013) performed a study on blood parameters, plasma gossypol and animal performance by feeding calcium hydroxide treated or vitamin E-supplemented cottonseed meal (CSM) diets in twenty four 6-7 months old male Bikaneri lambs which are divided into 4 groups of 6 animals each were fed with diet containing 20 % soybean meal (CON) or 40 % raw CSM (RCSM), 40 % raw CSM supplemented with 500 IU of vitamin E per head per day (ERCSM), and 40 % CSM treated with 1.5 % calcium hydroxide (CaCSM) along with *ad libitum* wheat straw for 510 day. ALT activity was significantly ($P<0.05$) higher on RCSM group as compared to other groups and serum iron and blood hemoglobin levels were significantly ($P<0.05$) lower in RCSM group as compared to CaCSM and CON groups. Plasma gossypol and osmotic fragility of erythrocytes in RCSM group were raised significantly ($P<0.05$) when compared to CaCSM and ERCSM groups. The concentration of other blood or serum biochemical constituents has no significant difference among the groups. It was concluded that there is no adverse effect on blood parameters and animal performance by feeding 40 % CSM in the concentrate mixture in diet of lambs. As a result, supplementing with calcium hydroxide or vitamin E provided no significant additional benefits.

Tang et al. (2012) conducted an experiment to examine the effects of partially replacing soybean meal (SBM) by solid-state fermented cottonseed meal (FCSM) on immune response, growth performance and serum biochemical parameters of 600, day old male yellow-feathered broilers randomly distributed into four groups with three replicates of 50 chicks each. Control group was fed with corn-SBM based diet and the rest three experimental diets included 4, 8 or 12% FCSM, replacing SBM. In both growth phases, there were no changes ($P>0.05$) between treatments regarding the concentrations of total protein, albumin, phosphorous, calcium. No significant differences ($P>0.05$) were observed among treatments regarding the serum biochemical parameters and the relative weights of immune organs. Thus, it was concluded, FCSM could be fed to broilers up to 12% of the total diet by replacement of SBM with FCSM and it might improve growth performance and immunity in broilers.

Adeyemo and Longe (2007) evaluated the haematological parameters on 180 day old chicks (from day old to 8 weeks) were fed five experimental diets i.e., 0, 25, 50, 75 and 100% of CSM replacing soybean meal (SBM). When CSM completely replaced SBM, there was no change in haemoglobin, serum protein, packed cell volume (PCV), albumin or globulin.

Matondi et al. (2007) studied the effect of cottonseed meal in goat rations on erythrocyte membrane osmotic fragility at 0, 8, 16 and 24% graded level. 16 goats were randomly assigned to four diets according to age groups. Blood samples were collected at the start of the experiment and for every two weeks and upto next six weeks. Erythrocyte membrane osmotic fragility increased ($P<0.001$) in diets containing cottonseed meal (24%>16%>8%) and increased with exposure time. Erythrocyte fragility for diets with 0% and 8% CSM was less than 10% and ranged between 14.6 and 26.43% for diets with 16 and 18% CSM in week six. During first week, Erythrocyte fragility was less than 5% for all the diets.

Oguz et al. (2006) supplemented iron sulphate in 15% CSM broiler diet. The results showed that levels of haematocrit and haemoglobin level were not significant among control and treatment groups, but mean corpuscular haemoglobin concentration (MCHC) was higher ($P=0.02$) in treatment group (28.45 %) than control group (25.59 %). Iron sulphate supplementation has a significant effect on MCHC for diet containing cottonseed meal.

Saijpaul et al. (2006) conducted an experiment of 120 d duration to study the effect of feeding water soaked whole linted cotton seed (WSWLCS) on the feed intake, lactation performance, blood biochemical profile and conception rate of high yielding lactating crossbred cows during the hot and humid climate (June-September). Twenty-two crossbred milch cows were randomly divided into two groups of eleven each on the basis of the average body weight and milk production as control (C) and treatment (T) groups. In the T group, supplementation of WSWLCS @ 5% of their milk yield was made replacing equal quantity of control concentrate mixture. In addition, all the experimental animals were provided *ad libitum* non-leguminous fodder. The values of the total milk yield, related variables, DM intake and blood biochemical profile were comparable among the groups. The levels of haemoglobin, glucose, cholesterol, creatinine, urea nitrogen, uric acid, chloride, calcium, and phosphorus in the blood plasma of the experimental animals did not differ significantly between the groups and were well within the normal physiological range.

2.5. Effect of CSM on *in vivo* rumen fermentation parameters

2.5.1 Effect on ruminal pH

Nomeary et al. (2021) studied the comparison between various protein sources in 20 Farafra male lambs which are divided randomly into four groups of five animals each for a period of 66 days. At 3% of their body weight, all the groups were given concentrate feed mixture (CFM) and *ad lib* wheat straw. Soybean meal (SBM) as a source of protein was replaced 100% with cottonseed meal (CSM), black cumin seed meal (BCSM), and sesame seed meal (SSM) which were integrated into the concentrate feed mixture. The pH (5.92) of lambs fed SSM was reported to be lowest ($P<0.05$). Ruminal pH levels in lambs fed CSM, SBM, or BCSM diets were unaffected ($P>0.05$). The pH of the rumen ranged from 5.92 to 6.47.

Tripathi et al. (2014) studied the effect of the Bt or conventional cottonseed meal on the fermentation in lambs. Three feed mixture (FM, forage: concentrate ration of 35: 65) containing groundnut oil meal (GNM), conventional whole cottonseed (C-CM) or insect protected Bt cottonseed meal (Bt-CM) as protein source, were fed for 123 days to the control, C-CM and Bt-CM group of lambs, respectively. Mean rumen fluid pH was not different among the three diets which ranged from 6.68 to 6.71. Rumen fluid pH was lowest ($P<0.001$) at 4 h post feeding in C-CM and Bt-CM diet fed lambs whereas control diet fed lambs had lowest ($P<0.001$) pH at 8 h post feeding. Rumen fluid pH increased linearly ($P<0.001$) after 4 h post feeding, which had ($P<0.001$) a quadratic ($P<0.001$) decrease at 4 h post feeding; interactions between treatment and post feeding time were also significant ($P=0.008$).

2.5.2. Effect on ruminal VFA

Nomeary et al. (2021) conducted a study to observe economical feed efficiency and highest production performance among various protein sources in sheep ration. 20 Farafra male lambs were divided randomly into four groups of five animals each for a period of 66 days. At 3% of their body weight, all the groups were given concentrate feed mixture (CFM) and *ad lib* wheat straw. Soybean meal (SBM) as a source of protein was replaced 100% with cottonseed meal (CSM), black cumin seed meal (BCSM), and sesame seed meal (SSM) which were integrated into the concentrate feed mixture followed by digestibility and nitrogen (N) balance trials. Results showed that TVFAs were higher in SBM when compared with other diets.

TVFA concentrations increased significantly ($P<0.05$) 3 hours after feeding, followed by 6 hours after feeding. The lowest readings, on the other hand, were obtained prior to feeding.

Tripathi et al. (2014) studied the effect of the Bt or conventional cottonseed meal on the fermentation in lambs. Three feed mixture (FM, forage: concentrate ration of 35: 65) containing groundnut oil meal (GNM), conventional whole cottonseed (C-CM) or insect protected Bt cottonseed meal (Bt-CM) as protein source, were fed for 123 days to the control, C-CM and Bt-CM group of lambs, respectively. Mean total volatile fatty acid concentrations (mmol/l) were similar among the three diet fed lambs. The TVFA concentration were the highest at 4 h in C-CM and Bt-CM. The post feeding period had significant influence on NH_3-N and TVFA concentration, which increased linearly ($P<0.001$) with increasing post feeding time.

Wanapat et al. (2013) conducted study on early lactation in crossbred cows (82.5 % Holstein) to investigate the effect of feeding cottonseed meal level and carbohydrate source in the concentrate on rumen fermentation and milk production. According to 2×2 factorial arrangement in a 4×4 Latin square design, cows were assigned randomly to receive 4 dietary treatments. Factor A was carbohydrate source: cassava chip (CC) and CC + rice bran at a ratio 3:1 (CR3:1), and factor B was variation in the level of cottonseed meal (CM): 109 g CP/kg (LCM) and 328 g CP/kg (HCM) at similar overall CP levels (490 g CP/kg). The concentration of total volatile fatty acids was more ($P<0.05$) in HCM than LCM treatments, while the concentration of butyric acid was higher ($P<0.05$) in LCM than HCM treatments.

Nagalakshmi et al. (2002) conducted a study on twenty crossbred 3–4 month-old male lambs which were allotted to five dietary treatments in a completely randomized design and fed control diet of 30 percent deoiled groundnut cake (DGNC) in isonitrogenous and isocaloric concentrate mixtures, 40% of raw CSM, 45 minutes cooked CSM, 1% calcium hydroxide treated CSM and iron treated cottonseed meal, with *ad libitum* access to chopped maize hay (*Zea mays*) for 135 days. Total volatile fatty acid concentration and rumen pH had no difference among diets. The concentration of TVFA in SRL was influenced ($P<0.05$) by the interaction between dietary treatment and time of collection. In control group and raw CSM fed groups, the TVFA concentration increased from 0 to 2 h and peaked at 4 h of feeding to decrease by 6 h of feeding, which were similar to the levels observed at 2 h of feeding. In processed CSM fed lambs, the TVFA concentration increased from 0 to 2 h after feeding, and then maintained until 6 h after feeding. Average TVFA concentrations among diets did not differ.

2.5.3. Effect on ruminal ammonia nitrogen

Nomeary et al. (2021) conducted a study on production performance in sheep fed various protein sources. 20 Farafra male lambs are divided randomly into four groups of five animals each for a period of 66 days. At 3% of their body weight, all the groups were given concentrate feed mixture (CFM) and *ad lib* wheat straw. Soybean meal (SBM) as a source of protein was replaced 100% with cottonseed meal (CSM), black cumin seed meal (BCSM), and sesame seed meal (SSM) which were integrated into the concentrate feed mixture followed by digestibility and nitrogen (N) balance trials. NH_3-N value of BCSM group increased significantly ($P<0.05$) than diets containing SBM or CSM. The results also showed that 3 hours after feeding, NH_3-N and concentrations increased significantly ($P<0.05$), followed by 6 hours after feeding. The lowest readings, on the other hand, were obtained prior to feeding.

Tripathi et al. (2014) studied the effect of the Bt or conventional cottonseed meal on the fermentation in lambs. Three feed mixture (FM, forage: concentrate ration of 35: 65) contained groundnut oil meal (GNM), conventional whole cottonseed (C-CM) or insect protected Bt cottonseed meal (Bt-CM) as protein source, were fed for 123 days to the control, C-CM and Bt-CM group of lambs, respectively. Ammonia-N (mg/l) was higher ($P<0.001$) in control lambs and lower ($P<0.001$) in C-CM diet fed lambs. Rumen NH_3-N was lowest ($P<0.001$) at 4 h post feeding in C-CM and at 8 h in the control, while Bt-CM diet fed lamb had increased NH_3-N up to 8 h post feeding.

Nagalakshmi et al. (2002) conducted a study on twenty crossbred 3–4 month-old male lambs which were allotted to five dietary treatments in a completely randomised design and fed control diet of 30 percent deoiled groundnut cake (DGNC) in isonitrogenous and isocaloric concentrate mixtures, 40% of raw CSM, 45 minutes cooked CSM, 1% calcium hydroxide treated CSM and iron treated cottonseed meal, with *ad libitum* access to chopped maize hay (*Zea mays*) for 135 days. NH_3–N was lower ($P<0.05$) on the cooked CSM (23.74 mg/100 ml) compared to control (30.54

mg/100 ml). The concentration of NH_3–N was highest ($P<0.01$) 4 h after feeding but decreased by 6 h. Average NH_3–N concentrations were lower ($P<0.01$) in lambs fed cooked CSM compared to those fed control or raw CSM containing diets.

2.6. Effect of cottonseed meal on growth performance

Nomeary et al. (2021) studied the comparison between various protein sources in 20 Farafra male lambs which are divided randomly into four groups of five animals each for a period of 66 days. At 3% of their body weight, all the groups were given concentrate feed mixture (CFM) and *ad lib* wheat straw. Soybean meal (SBM) as a source of protein was replaced 100% with cottonseed meal (CSM), black cumin seed meal (BCSM), and sesame seed meal (SSM) which were integrated into the concentrate feed mixture. Results revealed that lambs fed CSM diet had highest ($P<0.05$) daily BW gain when compared to lambs fed BCSM, SSM and SBM diets. Cottonseed meal was found to be the most effective treatment in terms of average daily gain, feed conversion ratio, and economic efficiency when compared to soybean meal, black cumin seed meal, and sesame seed meal treatments.

Fadel and Ashmawy (2015) conducted experiment on 32 Zaraibi dairy goats and observed their offspring performance in which goats are divided into four similar groups (8 does/ each) in which R1 and R3 groups were fed 60% concentrate feed mixture (CFM) containing untreated linseed meal or cotton seed meal respectively along with 40% rice straw. R2 and R4 groups were fed 60% CFM containing treated linseed meal or cotton seed meal by quebracho tannin (QT) at the rate of 2% plus 40% rice straw on DM basis for all rations. Daily gain for kids from birth up to weaning was significantly ($P<0.05$) higher for treated rations (R2 and R4) than kids fed untreated ones by tannins (R1 and R3).

Tripathi et al. (2014) studied the effect Bt or conventional cottonseed meal in lambs which for 120 days. Three feed mixtures (FM, forage: concentrate ration of 35: 65) containing groundnut oil meal (GNM), insect protected Bt-cottonseed meal (Bt-CM) or conventional whole cottonseed (C-CM) as protein source, were fed for 123 days to the control, C-CM and Bt-CM groups, respectively. Initial BW, final BW, total BW gain and ADG of lambs during the experiment had no significant difference among control, CSM and Bt-CSM lamb's groups. The live weight change of the lambs had an Average daily gain (ADG) of 102, 89 and 111 g, respectively in the control, C-CM and Bt-CM diet fed lambs.

Hassanabadi et al. (2009) conducted an experiment on 42 days old broilers chicks which were fed with 5, 10, 15, 20 % CSM with and without lysine and ferrous sulfate compared with control group and were fed with corn and soybean meal-based diets. Results revealed that weight gain and FCR improved (P<0.05) by addition of lysine at all levels when compared to the unsupplemented and control groups.

Solomon et al. (2008) conducted experiment in twenty-four yearling intact male Sidama goats with a mean body weight (BW) of 16.8±1.14 kg. The experiment consisted of 90 days of feeding trial and 10 days of digestibility trial followed by evaluation of carcass parameters at the end of the experiment. The treatments included *ad libitum* feeding of hay (T1, control) and daily supplementation of CSM at 200 g (T2), 300 g (T3) and 400 g (T4) per head on dry matter (DM) basis. Daily BW gain, final BW, total BW change were higher (P<0.01) at medium than at low level of supplementation and all these variables were lower (P<0.01) in the non-supplemented than in the supplemented animals. The BW of animals supplemented with the medium and high level of CSM supplementation increased (P<0.01) in all the treatments through time.

Ojewola et al. (2006) conducted a 6-day feeding trial in which cottonseed meal was substituted for soybean meal at 0, 25, 50, 75 and 100% and designated these 5 diets in completely randomized design. The results showed that there was no significant (P>0.05) in birds mean daily weight gain and feed to gain ratio. Highest weight gain (2666.6g) was observed in birds fed with diet 3 and lowest (2443.27g) was observed in diet 1.

Azman and Yilmaz (2005) evaluated the effect of diets having lysine supplemented cottonseed meal (CSM) in place of soybean meal (SBM) on the growth performance of 300, 7-day-old broilers which were divided into 3 groups. During 15 days, the chicks were fed control diet of corn-soy-based starter meals and experimental groups were fed with 20 percent cottonseed meal based diets supplemented with 1.5 percent or 3 percent lysine. During the trial period, no significant variations in body weight, daily body weight growth, or daily feed intake were detected between control and treated birds. Although slight but non significant increases of daily weight gain were noticed in CSM-1.5% lys group. In conclusion, the study reported that cottonseed meal could be included in broiler diets with enough lysine supplementation without affecting growth performance.

Henry et al. (2001) conducted 3 experiments on performance of one week old broiler chicks which were fed with diets containing lysine supplemented 20% feed-grade or extruded CSM and was compared with corn and soybean meal based control diet. According to the findings, body weight gain was reduced, feed intake was raised, and feed utilization was inefficient. The body weight gains of chicks were not significantly affected by adding 2% lysine to feed-grade or extruded CSM when compared to control group.

Worrell et al. (1990) conducted a study on CSM and rye grass based grazing system with 96 crossbred yearling steers which were given diets with or without CSM 0.45 kg/day + lasalocid 150 mg/day. In the study it was found that CSM increased ($P<0.05$) average daily gain (ADG) early in the grazing season but not later. Addition of lasalocid increased ($P<0.05$) ADG during the spring grazing period containing lasalocid. There was improvement in seasonal weight gain in steers supplemented with CSM.

2.7. Effect of cottonseed meal on immunity

Pattanaik et al. (2003) evaluated the effect of feeding 9.95 mg free gossypol/kg live weight through CSM on immune response in 20 intact male calves fed barley and sorghum as a source of cereal for 120 days. Calves were arranged in four groups of 5 animals each. Control groups were given concentrate supplement containing groundnut meal (GN) along with barley (B) and sorghum (S), i.e., GN-B and GN-S. Treatment groups were given same barley and sorghum as cereal but a part of the groundnut meal in each was replaced by cottonseed meal, i.e., CS-B and CS-S. Humoral immune response was measured through antibody titre against *Brucella abortus* S99 inoculation. The antibody titre was detected in calves fed GN diets as early as 7 d post-inoculation (PI) and showed a steady rise till 28 d PI. The CS fed calves failed to respond by 14 d PI, and antibody was detected only after 21 d PI. It was concluded that seroreactivity of the CS fed calves was poor compared to GN fed calves.

Nagalakshmi et al. (2001) evaluated feeding of raw and processed cottonseed meals (CSM) on 20 male lambs of uniform body weight allotted to 5 treatments in a completely randomised design and fed control diet of 30 percent deoiled groundnut cake (DGNC) in isonitrogenous and isocaloric concentrate mixtures, 40% of raw

CSM, 45 minutes cooked CSM, 1% calcium hydroxide treated CSM and iron treated cottonseed meal along with *ad lib* maize hay for 180 days. After 140 days *brucella abortus* S_{99} was used to sensitize lambs and subjected to ELISA and delayed type hypersensitivity (DTH). Lambs fed raw CSM had a decreased ($P<0.05$) humoral immune response and DTH reactivity. Raw CSM fed lambs had a better cell mediated immunity (CMI) response upon cooking, Ca(OH)2, and iron treatment than raw CSM fed lambs.

2.8. Effect of cottonseed meal on economics

Nomeary et al. (2021) conducted a study on 20 Farafra male lambs which are divided randomly into four groups of five animals each for a period of 66 days. At 3% of their body weight, all the groups were given concentrate feed mixture (CFM) and *ad lib* wheat straw. Soybean meal (SBM) as a source of protein was replaced 100% with cottonseed meal (CSM), black cumin seed meal (BCSM), and sesame seed meal (SSM) which were integrated into the concentrate feed mixture. Economic evaluation revealed that CSM has higher values of revenue (284.45 L.E) (L.E-Egyptian pound) and relative efficiency (153.18%) (Relative efficiency- ratio of cost input and revenue outcome) compared to other protein sources.

Attanayaka et al. (2016) conducted a study on commercial day old broilers to investigate whether soybean meal (SBM) could be replaced with cottonseed meal (CSM) in dietary treatments, with CSM replacing SBM at levels of 0, 5, 10, and 15%. The diet containing 15% CSM had the lowest cost of production per bird, and there is no difference in feed cost per kg live weight gain between treatments. Thus, CSM can be utilized to replace up to 10% of SBM in the diet safely, which would be significant from a commercial aspect, especially when the price of SBM is remarkably high.

Fadel and Ashmawy (2015) conducted experiment on 32 Zaraibi dairy goats and observed their offspring performance in which goats are divided into four similar groups (8 does/ each) in which R1 and R3 groups were fed 60% concentrate feed mixture (CFM) which contained untreated linseed meal or cotton seed meal respectively along with 40% rice straw. R2 and R4 groups were fed 60% CFM which contained treated linseed meal or cotton seed meal by quebracho tannin (QT) at the rate of 2% plus 40% rice straw on DM basis for all rations. The protected linseed meal and cotton seed meal at 2% resulted in better economic evaluation expressed as economic return i.e., R2 (4.30 L.E) (L.E-Egyptian pound) and R4 (4.75 L.E), and the lowest value was recorded for R1 (3.59 L.E) and R3 (3.89 L.E) groups. So, a beneficial effect was seen in protected linseed meal and cotton seed meal diets.

Ojewola et al. (2006) conducted a 6-day feeding trial in which cottonseed meal was substituted for soybean meal at 0, 25, 50, 75 and 100% and designated these 5 diets in completely randomized design. Diet 1 was highest in cost per kg (62.02 N) (N- Nigerian naira) followed by diet 2 (57.80 N), 3 (53.61 N), 4 (50.21 N), 5 (49.00 N). least cost was observed in diet 5 because soybean meal was excluded completely and also revenue realized from sale of birds was observed to be highest for diet 5 (359.97 N) and least in diet 1 (285.98 N). It was indicated that cottonseed meal could be a suitable substitute for soybean meal.

Khan et al. (2002) conducted a study on fifteen 14-21 days old cross bred cow calves which were randomly allotted to 3 experimental diets. Diet A comprised of milk replacer and concentrate feed whereas two isocaloric and iso-nitrogenous early weaning diets viz., B and C were prepared without and with lysine and methionine supplemented cottonseed meal, respectively for 60 days. The economic efficiency (Cost of feed (Rs.) to gain per kg of live body weight) was noticed to be better on diets B (19.19) and C (17.92) compared to diet A (33.39).

CHAPTER III

MATERIALS AND METHODS

The present study was conducted in the Department of Animal Nutrition, Guru Angad Dev Veterinary and Animal Sciences University (GADVASU), Ludhiana.

A brief description of experimental techniques and procedures of analysis adopted during the study are reported in this chapter.

3.1 Chemical analysis

The finely ground feed ingredients (Cottonseed meal, soybean meal, groundnut cake, mustard cake and deoiled mustard cake), concentrate mixtures (Table 3.1) and complete feeds (TMRs) were analyzed for proximate and cell wall constituents as mentioned below.

3.1.1 Proximate principles

3.1.1.1 Dry matter (DM)

For this, a known quantity of the well mixed sample was taken in an aluminum tray and then it was dried in an oven for 24 h at 100°C. The weight of the dried sample was noted and used for dry matter estimation of the sample.

3.1.1.2 Total ash

Finely ground sample (2 g) was taken in duplicate in tarred crucibles over a hot plate and then ignited in muffle furnace for 3 hours at 600°C. After that the crucibles were taken out, put in desiccator and weighed. The difference between initial weight of empty crucible and final weight of crucible with ash gave the total ash content in the sample and was expressed as percent of DM (AOAC, 2007). The loss in weight after ignition in muffle furnace was taken as organic matter.

3.1.1.3 Crude protein (CP)

The N content was estimated by Macro-Kjeldahl method (AOAC, 2007). Finely ground sample (0.25 g) or 2 ml of urine or 5 g of fresh faeces was digested with 15 ml (25 ml for faeces) of concentrated sulphuric acid and 5-6 g of digestion mixture (potassium sulphate and copper sulphate, 9:1). The material after digestion was distilled in presence of 40% sodium hydroxide. The ammonia liberated was collected in 20 ml of 4% boric acid-mixed indicator solution. Mixed indicator was prepared by taking Bromocresol green and methyl red in 5:1 ratio in 95% ethanol.

Ammonium borate thus formed was titrated against standard (0.1 N) sulphuric acid. The reading for blank was also recorded. The CP (%) content was calculated by multiplying the nitrogen by 6.25.

$$\text{Nitrogen (\%)} = \frac{\text{(Normality of acid)} \times \text{(Vol. of acid, ml)} \times 0.014}{\text{Wt. of sample (g)}} \times 100$$

3.1.1.4 Ether extract

About 3 g of the ground sample was weighed and quantitatively transferred into a thimble made of Whatman filter paper No.1. The sample with thimble was placed in the extraction beaker of SOCS PLUS® (M/S Pelican Equipments) six place automatic solvent extraction systems (AOAC, 2007). The extraction was carried out for 2 hours with 80 ml petroleum ether (BP 60- 80°C). After completion of the extraction process, the beaker was dried in the hot air oven at 100°C.

$$\text{EE (\%)} = \frac{\text{Wt. of beaker with ether extract} - \text{Wt. of empty beaker}}{\text{Wt. of sample (g)}} \times 100$$

3.2 Cell wall constituents

3.2.1 Acid detergent fibre (ADF)

One gram of sample was taken into spoutless beaker and add 100 ml of acid detergent solution (20 g CTAB dissolved in one liter of l N H_2SO_4). For 1 hour, contents were refluxed. The components were filtered through a sintered glass crucible (G-l) that had been previously weighed and rinsed in hot water and then washed once with acetone. The residue was dried at 80°C in a hot air oven for overnight. The difference in initial (empty crucible) and final (crucible + residue) weight of crucible gave ADF content. It was expressed as % on DM basis (Van Soest et al., 1991).

3.2.2 Neutral detergent fibre (NDF)

Half gram of sample was taken into spoutless beaker and added 50 ml of neutral detergent solution (NDS). The NDS was prepared by weighing 18.61 g disodium salt of EDTA and 6.81 g sodium borate in a beaker, added some distilled water and heated till dissolved. Then 4.56 g disodium hydrogen ortho-phosphate was taken in another beaker, added distilled water and heated till dissolved. Then, added 30 g sodium lauryl sulphate and 10 ml of ethoxy ethanol in 850 ml distilled water.

Then added contents of two previous beakers to it and mixed. The volume was made to one litre.

After the boiling had begun, the sample in NDS was refluxed for one hour. Filtration of contents was done with sintered glass crucible (G-1) and washed with hot water until it was free from NDS and then finally washed with acetone. The residue was dried at 80°C in a hot air oven for overnight. The difference in initial weight (empty crucible) and final (crucible + residue) weight of crucible gave the NDF content. It was expressed as percent NDF on DM basis (Van Soest et al., 1991).

Amylase treated Neutral detergent fibre (Mcqueen & Nicholson, 1979)

Apparatus: Spoutless beaker, sintered crucible, vacuum pump, hot air oven, muffle furnace, weighing balance, desiccator.

Reagents:

Neutral detergent solution (NDS), Amylase solution, acetone, hot boiling water.

Preparation of Neutral detergent solution (NDS)

Same as discussed under 3.2.2

Preparation of Amylase solution

Reagents:

Diastase/Amylase, thermolabile (Thomas baker-12095) - Activity (130 U/mg)

1. Phosphate buffer (0.067M)
   a. KH_2PO_4—3.56g
   b. Na_2HPO_4. $2H_2O$—7.22g
   c. NaN_3—0.2g

   Mix the above in 1L distilled water and adjust the pH to 7.

2. Amylase solution—8 g amylase in 1L phosphate buffer

Procedure

0.5 g of sample of CSM and other oilseed cakes was taken in a 500 ml spoutless beaker. 30 ml amylase solution was added into the beaker and mixed with swirling action. Covered the beaker (to avoid evaporation) and incubated at 40^{o}C over night (12-18h). Next day, 50 ml NDS and 0.5g sodium sulphite were added and boiling for 1 hour was carried out during which the contents in the beaker were refluxed. The contents in the beaker were filtered using a vacuum pump with 50 ml sintered glass crucible (G-I) and washed with hot water then finally washed with acetone to clear salts. The crucible with residue was placed in hot air oven (100 ± 5°C) for drying overnight then cooled and weighed. The crucibles were kept in a muffle furnace for ashing at 500ºC for 2- 3 h and crucible was weighed again containing ash.

$$\text{NDF (\%)} = \frac{\text{Wt. of crucible with residue} - \text{Wt. of crucible with residual ash}}{\text{Weight of sample (g)}} \times 100$$

3.2.3 Hemicellulose

Hemicellulose was soluble in ADS and thereby calculated by subtraction of ADF from NDF as follows:

$$\text{Hemicellulose (\%)} = \text{NDF (\%)} - \text{ADF (\%)}$$

3.2.4 Cellulose

0.5 g sample was taken in 15 ml of digestion mixture solution (650 ml glacial acetic acid, 80 ml nitric acid and 150 ml distilled water) in plastic tubes. The tubes were placed in boiling water bath for 30 minutes. Then contents were filtered through sintered glass crucible (G-l), washed with hot water repeatedly and then final washing with ethanol. The residue was dried at 80°C for overnight in a hot air oven. Then crucibles were weighed and ignited at 500°C in a muffle furnace for half an hour. The loss in weight on ignition represented cellulose content which was expressed as per cent cellulose on DM basis (Crampton & Maynard, 1938).

3.2.5 Acid detergent lignin (ADL)

Sulphuric acid (72%, w/v) was added to the sintered crucibles containing ADF and kept at room temperature for 3h. After draining the acid, the residue was washed with water till it became acid free, dried in hot air oven for overnight and then weighed. It was then ignited at 500ºC in a muffle furnace for 3 hr. The loss in weight upon ignition represented ADL, which was expressed as % on DM basis (Van Soest et al., 1991).

3.2.6 Total carbohydrates (TCHO)

It was estimated according to Sniffen et al. (1992). Total carbohydrates in the feedstuff was estimated by difference:

$$\text{TCHO (\%)} = 100 - (\text{CP\%} + \text{Ash\%} + \text{EE\%})$$

3.3 Estimation of nitrogen fractions

It was done according to Licitra et al. (1996).

3.3.1 Determination of acid detergent insoluble nitrogen (ADIN)

As per Van Soest et al. (1991) the procedure was same as that of ADF estimation. By following standard Kjeldahl method the ADF residues were recovered and nitrogen in residue was estimated. ADIN of the sample was expressed as percent of total nitrogen or as acid detergent insoluble crude protein (ADICP). ADICP was expressed as % of DM (Licitra et al., 1996).

3.3.2 Determination of neutral detergent insoluble nitrogen (NDIN)

As per Van Soest et al. (1991) the procedure was same as that of NDF estimation. By following standard Kjeldahl method, the NDF residues were recovered and nitrogen in residue was estimated. NDIN of sample was expressed as percent of total nitrogen or as neutral detergent insoluble crude protein (NDICP). NDICP was either expressed as % of DM (Licitra et al., 1996).

3.4 *IN VITRO* EVALUATION OF FEEDS

3.4.1 *In vitro* gas production

The *in vitro* gas production was assessed according to Menke and Steingass (1988).

3.4.1.1 Preparation of samples

About 375±5mg of sample (ingredients, concentrates and TMRs) in triplicate was weighed in a weighing boat (with removable stem) and the sample was put at the bottom of the 100 ml calibrated glass syringe taking caution that it did not stick to the walls of syringe. Then the piston, greased with petroleum jelly (Vaseline) was pushed into the cylinder. The syringes containing sample in triplicate were kept in an incubator at 39°C for 24h.

3.4.1.2 Collection of rumen liquor

Rumen fluid collected from male buffaloes with rumen fistulae which were fed on 2 kg conventional concentrate mixture (38% maize, 15% mustard cake, 15% soybean meal, 12% deoiled rice bran, 10% wheat bran, 7% rice polish, 2% mineral mixture, 1% salt), 17 kg green fodder, 3 kg wheat straw and *ad lib* urea molasses mineral block (UMMB) were used as a donor for rumen liquor. The rumen contents were collected in thermos flask, then flushed with CO_2 and maintained at 39°C. By using blender, the rumen contents were blended for 2 to 3 minutes which was maintained at 39°C and then strained over 4-ply muslin cloth.

3.4.1.3 Preparation of solutions

Following solutions were prepared well in advance:

1) Micro mineral solution

$CaCl_2.2H_2O$ = 13.2 g

$MnCl_2.4H_2O$ = 10.2 g

$CoCl_2.6H_2O$ = 1 g

$FeCl_2.6H_2O$ = 8 g

Dissolved in distilled water and made the volume 100 ml.

2) Macro-mineral solution

Na_2HPO_4 = 5.7 g

KH_2PO_4 = 6.2 g

$MgSO_4.7H_2O$ = 0.6 g

Dissolved in distilled water and made the volume 1000 ml.

3) Buffer solution

$NaHCO_3$ = 35.0 g

NH_4HCO_3 = 4.0 g

Dissolved in distilled water and made volume 1000 ml.

4) **Resazurine solution:** Dissolved 100 mg of resazurine in distilled water and made volume 100 ml and kept in refrigerator.

5) **Reducing solution (This solution is to be prepared fresh before each incubation)**

$Na_2S.H_2O$ = 373.0 mg

1N NaOH = 2.6 ml

Distilled water = 62.0 ml

The above solutions were mixed in following ratio in a Woulff flask (3 litre capacity) mixed with magnetic stirrer in a water bath at 39°C.

1. Distilled water = 960 ml
2. Micro mineral solution = 0.16 ml

3. Buffer = 660 ml

4. Macro mineral solution = 330 ml

5. Resazurine = 1.6 ml

Add 50 ml of reducing solution.

3.4.1.4 Procedure

While the reducing solution was added, CO_2 was flushed through a submerged tube. The slightly bluish color first turned pinkish then became colorless. The strained rumen liquor (SRL) was added to the buffer media in 1:2 ratio only when solution was colorless. The flushing of CO_2 was continued till the last syringe was filled. For filling up of syringes, the tube on the capillary attachment to the syringe was firmly fixed on to the bottle top dispenser. 30 ml of SRL: buffer solution from the flask kept in a water bath was pumped in each syringe. The contents in the syringe were mixed by gentle shaking. Air bubbles were brought to the surface and removed through the capillary by careful upward movement of the piston. The clip was closed immediately and exact volume of the contents in the syringe was noted and kept in a water bath maintained at 39°C. The contents in all the syringes were swirled at 1 hour interval for first few hours. If at 8h, the gas exceeded 70 ml, the gas was removed after recording volume of gas. The volume of gas produced in each syringe was measured after 24 hours. Blanks and standard hay in triplicate were also run with each set of incubation. After 24 hr, the NH_3-N, TVFA and NDF of residue were determined. ME was calculated by using amount of gas production. The partitioning factor (PF) defined as the ratio of substrate truly degraded *in vitro* (mg) to the volume of gas (ml) produced by it was calculated (France et al., 1993).

3.4.2 *In vitro* true OM digestibility of substrate

The content of syringes was transferred to spoutless beaker by repeated washing with 20 ml neutral detergent solution. The flask content was refluxed for one hour and filtered through pre weighed Gooch crucibles (grade G-I). The dry matter content of the residue was weighed and *in vitro* true digestibility of feeds was calculated (Van Soest & Robertson, 1988).

$$\text{True OM digestibility (TOMD\%)} = \frac{\text{Initial OM of feed taken for incubation - OM residue}}{\text{Initial OM of feed taken for incubation}} \times 100$$

Fig. 2. ***In vitro*** **syringes incubated for 24h in water bath**

Fig. 3. Recording ***in vitro*** **reading**

3.4.3 Estimation of volatile fatty acids

Cottyn and Boucque (1968) described a method which is equipped with flame ionization detector using Netchrom 9100 gas chromatograph for estimating Volatile fatty acids. The estimation of VFA was done using a gas column (6 feet long and 1/8 inch in diameter) packed with chromosorb 101. Nitrogen, hydrogen and zero air gas fluxes were 15, 30, and 300 ml/min, respectively. Injector oven, column and detector temperatures were 250°C, 175°C and 270°C, respectively.

Samples were made by adding 0.2 ml of 25% metaphosphoric acid per ml of rumen liquor/*in vitro* syringes contents, allowing it to sit for 2 hours, then centrifuging it at 4000 rpm for 7 minutes. The VFA was calculated using the supernatant. The VFA standard mixture was made by combining stock solutions (each at a concentration of 25 mg/ml) of standard VFAs and distilled water in the proportion of acetic acid 1.68 ml, propionic acid 0.48 ml, isobutyric acid 0.12ml, butyric acid 0.24 ml, isovelaric acid 0.12ml, valeric acid 0.12ml and made the volume to 10 ml to obtain final concentration of acetic acid 7.0mM/100 ml, propionic acid 1.62mM/100 ml, butyric acid 0.68mM/100 ml, isobutyric acid 0.34mM/100 ml, valeric acid 0.29 mM/100 ml, isovaleric acid 0.29mM/100 ml. The standard was stored in deep freeze until further use.

3.4.4 ME availability

The ME value of the substrate was calculated by using the following equation developed by Menke et al. (1979).

$$ME\ (kg) = 1.24 + 0.146\ G\ (ml/200\ mg\ DM) + 0.007\ CP + 0.0244\ EE$$

where,

ME = Metabolizable energy, MJ/kg DM

G =Net gas production, ml/200mg DM

CP = Crude protein, g/ kg

EE = Ether extract, g/kg.

3.4.5 Hydrogen balance

Hydrogen recovery (%) was estimated as $(4M+2P+2B) / (2A+P+4B) \times 100$, the ratio of hydrogen consumed via CH_4/VFA was estimated as $4M/(2P+2B)$, where acetate (A), propionate (P), butyrate (B) and methane (M) production was expressed in mmol by Demeyer (1991).

3.4.6 Fermentation efficiency

This was derived using Orskov (1975) equation, which was later revised by Baran and Zitnan (2002).

$$FE = (0.622a + 1.092p + 1.56b)\ 100 / (a+p+2b)$$

Where: a, p, and b denote the concentrations (µmol) of acetic, propionic and butyric acids in the total concentration of VFA produced, respectively. The equation's final results have been expressed as a percentage and the amount of energy stored in VFAs is shown as a percentage of the share from the initial energy.

3.4.7 VFAs utilization index

According to Orskov (1975) this was stated as the non-glucogenic VFAs/glucogenic VFAs ratio (NGGR).

$$NGGR = (A + 2B + V) / (P+V)$$

Where A, P, B and V denote the concentrations (µmol) of acetic, propionic, butyric, and valeric acids, respectively. Valeric acid on oxidation produces 1 mole of acetic acid and 1 mole of the propionic acid so it is classified as both glucogenic and non-glucogenic VFA. Extremely high NGGR suggests a lot of energy is being lost in the form of gases.

3.5 Effect of cottonseed meal on performance of buffalo calves *in vivo*

A 120-day growth trial was conducted on 6 to 12 month old buffalo calves (average initial body weight 149.88±9.93kg) to see the effect of supplementation of different levels of cottonseed meal on nutrient utilization, blood profile and growth performance. The buffalo calves were fed total mixed ration with roughage to concentrate ratio 60:40 on DM basis. All the TMRs contained mixed (berseem, oats, mustard) fodder.

3.5.1 Selection, distribution and maintenance of animals

Twelve buffalo calves were randomly distributed into three groups of four animals each. On the basis of *in vitro* study, two levels of CSM (75 and 100% replacement of soybean meal in concentrate) were selected for feeding to buffalo calves in the *in vivo* study. The animals in each group were fed as per ICAR (2013) feeding standard. The animals in control group were fed with basal diet consisting of mixed (berseem, oats, mustard) fodder and SBM based conventional concentrate mixture. The animals in experimental groups 2 and 3 were fed green fodder, wheat straw and concentrate mixtures in which soybean meal was replaced with CSM at 75% and 100% levels, respectively. The inclusion level of CSM was 11.25 % and 15%, respectively in the concentrate mixtures for the *in vivo* study. The daily record of feed intake and orts was maintained. The animals were weighed for 3 consecutive days at every fortnight interval and the feeding schedule was revised accordingly.

3.5.2 Housing

The animals were housed in a concrete shed and were stall fed individually at 9:00 AM daily. The animals had free access to water twice daily. The ingredient composition of concentrate mixtures used in the present study is given in Table 3.1.

Table 3.1. Ingredient composition of concentrate mixtures (parts/100 parts)

Ingredient	**CONC 1 (0% CSM)**	**CONC 2 (25% CSM)**	**CONC 3 (50% CSM)**	**CONC 4 (75% CSM)**	**CONC 5 (100% CSM)**
Maize	34	34	34	34	34
Soybean meal	15	11.25	7.5	3.75	0
Cottonseed meal	0	3.75	7.5	11.25	15
Mustard cake	15	15	15	15	15
Wheat bran	10	10	10	10	10
Deoiled rice bran	17	17	17	17	17
Rice polish	6	6	6	6	6
Mineral mixture	2	2	2	2	2
Salt	1	1	1	1	1

3.6 Conduction of metabolic trial

A 7-day metabolic trial was conducted on all the animals towards the end of growth trial. During metabolic trial, the animals were kept in specially designed metabolic cages, where a metallic pipe led the excreted urine into a narrow mouth plastic container (5 litres capacity) containing 100 ml of 20% H_2SO_4. The collection of faeces and urine was done for 7 days. The faeces voided were collected manually for 24 h. Faeces were collected manually by trained persons, who were put on duty round the clock. However, the collection of urine was automatic. The combined residue of green and concentrate mixture, if any, was weighed every morning at 9:00 AM in the morning before offering the next day's ration.

3.6.1 Sampling of feed, orts, faeces and urine

3.6.1.1 Feed/ orts

Samples of feedstuffs and orts were collected at 24 h interval and dried in hot air oven in duplicates at 100^o C. The materials were pooled for seven days before being finely ground and tested for proximal principles and cell wall constituents.

3.6.1.2 Faeces

After thoroughly mixing, the 24 h faecal material of each animal, one by two hundredth parts in duplicate was weighed in circular aluminium tray for DM determination. The samples were dried at 100^o C in a hot air oven overnight and weighed. The 7 days dried faeces were pooled, and finely ground to pass through 1mm sieve and preserved in airtight glass sampling bottles. For nitrogen estimation, 30 grams faeces was preserved in previously tarred wide mouth plastic bottles daily, containing 25 ml of 20% sulphuric acid solution added on the first day of collection. Every day, after placing the faeces in the bottles, it was thoroughly mixed to prevent loss of ammonia and possible infestation with fungi. Faecal samples were stored in a refrigerator till analyzed. Samples of feed, faeces and orts were analyzed for total ash, N as per AOAC (2007), cellulose as per Crampton and Maynard (1938) and cell wall constituents as per Van Soest et al. (1991).

3.6.1.3 Urine

An aliquot of urine equal to one by tenth part of the total urine voided was preserved in narrow mouth glass bottles (1000 ml capacity) daily, which were kept in a refrigerator till analyzed for nitrogen content.

3.6.1.4 Analysis of feeds, faeces and urine

Samples of different concentrate mixtures, green fodder, feed residue and faeces were analyzed for their proximate constituents and cell wall constituents as mentioned in the previous section.

3.7 Blood biochemical profile

To study the effect of CSM replacing SBM feeding on nutrient (carbohydrates, fat and protein) metabolism, monitoring of blood parameters was carried out. Blood samples were drawn from all buffalo calves at the end of experimental feeding.

Fig. 4. Feeding of animals during 120 day growth trial

Fig. 5. Animals kept in metabolic cages during metabolic trial

3.7.1 Collection of blood, plasma separation and preservation

The blood sample was collected in heparin vials from the jugular vein of animals before feeding. Vials were centrifuged at 3000 rpm for 10 min and plasma was separated. Plasma was preserved at -20^{o}C to analyze the following parameters

1. Total protein
2. AST
3. ALT
4. Albumin
5. Cholesterol
6. Triglycerides
7. BUN
8. Glucose
9. GGT

3.7.1.1 Total protein

It was estimated by Biuret method (Henry et al., 1974) by using diagnostic kits supplied by Transasia-Biomedicals Ltd.

Procedure

To 10 µl distilled water, standard and plasma, 1ml of reagent was added. The contents were mixed thoroughly and left for 10 minutes at room temperature. Concentration was noted by using Erba (Mannheim) Chem 5X (Transasia) at 546nm.

3.7.1.2 AST/ SGOT

It was estimated by using diagnostic kits supplied by Transasia-Biomedicals Ltd. Based on International federation of Clinical Chemistry (IFCC).

Procedure

To 100 µl of plasma, 1 ml reagent was added and mixed at room temperature. Concentration was noted by using Erba (Mannheim) Chem 5X (Transasia) at 340 nm.

3.7.1.3 ALT/ SGPT

It was estimated by using diagnostic kits supplied by Transasia-Biomedicals Ltd. Based on International federation of Clinical Chemistry (IFCC).

Procedure

To 100 µl of plasma, 1 ml reagent was added and mixed at room temperature. Concentration was noted by using Erba (Mannheim) Chem 5X (Transasia) at 340 nm.

3.7.1.4 Albumin

It was estimated by Bromocresol green (BCG) dye binding method (Doumas et al., 1971) by using diagnostic kits supplied by Recombigen Laboratories Pvt. Ltd.

Procedure:

To 10µl distilled water, standard and plasma, 1ml of reagent was added. The contents were mixed at 37°C. Concentration was noted by using Global 240 analyzer at 630 nm.

3.7.1.5. Cholesterol

It was estimated by Roeschlau et al. (1974) method using diagnostic kits supplied by Transasia-Biomedicals Ltd.

Procedure

To 10 µl distilled water, standard and plasma, 1 ml of reagent was added. The contents were mixed thoroughly and left for 5 minutes at room temperature. Concentration was noted by using Erba (Mannheim) Chem 5X (Transasia) at 505 nm.

3.7.1.6. Triglycerides

It was estimated by enzymatic colorimetric method (Bucolo & David, 1973) by using diagnostic kits supplied by Transasia-Biomedicals Ltd.

Procedure:

To 10 µl distilled water, standard and plasma, 1 ml of reagent was added. The contents were mixed thoroughly and left for 10 minutes at room temperature. Concentration was noted by using Erba (Mannheim) Chem 5X (Transasia) at 546 nm.

3.7.1.7 Blood urea nitrogen

It was estimated by urease method (Talke & Schubert, 1965) by using diagnostic kits supplied by Transasia-Biomedicals Ltd.

Procedure

To 25 µl distilled water, standard and plasma, 1 ml of reagent was added. The contents were mixed thoroughly. Concentration was noted by using Erba (Mannheim) Chem 5X (Transasia) at 546 nm immediately.

Fig. 6. Marked area on neck for injecting antigen (PHA-P)

Fig. 7. Injecting PHA-P antigen in marked area of neck

3.7.1.8 Blood glucose

It was estimated by glucose oxidase/peroxidase method (Trinder, 1969) by using diagnostic kits supplied by Transasia-Biomedicals Ltd.

Procedure:

To 10 µl distilled water, standard and plasma, 1ml of reagent was added. The mixture was mixed thoroughly and left for 15 minutes at 37º C temperature. Concentration was noted by using Erba (Mannheim) Chem 5X (Transasia) at 505nm.

3.7.1.9 Gamma Glutamyl Transferase

It was estimated by using diagnostic kits supplied by Transasia-Biomedicals Ltd. Based on International federation of Clinical Chemistry (IFCC).

Procedure

To 100 µl of plasma, 1 ml reagent was added and mixed at room temperature. Concentration was noted by using Erba (Mannheim) Chem 5X (Transasia) at 405 nm.

3.8 Assessment of cell mediated immune response (Abbas et al., 2014)

In vivo delayed type of hypersensitivity reaction against phytohemagglutinin-P (PHA-P) was adopted to study the cell-mediated immune (CMI) response. 150 µg of PHA-P in 200 µl of phosphate buffer saline solution (pH 7.4) was injected intra-dermally at the marked area on neck region. To avoid inflammatory reaction due to abrasion, the skin area of neck region was cleaned and shaved prior to 24 h. The skin thickness was measured by a digital vernier caliper at 0 h which represented the basal value. After intradermal injection of PHA-P, the skin thickness was measured up to 96 h at 24 h interval.

3.9 Economic evaluation

Cost of feeding CSM in experimental groups was calculated by taking into account dry matter intake, cost of concentrate, cost of fodder and cost of CSM. Net profit per kg body weight gain was calculated for all groups.

3.10 STATISTICAL ANALYSIS

The data were analyzed using (SPSS, 2012) version 21 by simple ANOVA, as stated by (Snedecor and Cochran, 1994). Differences in means were tested by Tukey’s b.

CHAPTER IV

RESULTS AND DISCUSSION

The findings of the study "Effect of cottonseed meal on nutrient utilization and blood biochemical profile of male buffalo calves" obtained is discussed under the following heads.

4.1 *In vitro evaluation*

- 4.1.1 Chemical composition of CSM and conventional oil cakes
- 4.1.2 Chemical composition of concentrate mixtures containing graded levels of CSM
- 4.1.3 Chemical composition of TMRs containing graded levels of CSM
- 4.1.4 *In vitro* evaluation of CSM and conventional oil cakes
- 4.1.5 *In vitro* evaluation of concentrate mixtures containing graded levels of CSM
- 4.1.6 *In vitro* evaluation of TMRs containing graded levels of CSM

4.2 *In vivo* evaluation

- 4.2.1 Effect of dietary level of CSM on fortnightly body weight (kg) in male buffalo calves
- 4.2.2 Effect of dietary level of CSM on nutrient intake in male buffalo calves
- 4.2.3 Effect of dietary level of CSM on digestibility of nutrients in male buffalo calves
- 4.2.4 Effect of dietary level of CSM on nitrogen balance in male buffalo calves
- 4.2.5 Effect of dietary level of CSM on blood profile in male buffalo calves
- 4.2.6 Effect of dietary level of CSM on cell mediated immune response in growing calves
- 4.2.7 Effect of dietary level of CSM on performance of buffalo calves
- 4.2.8 Economics of feeding

4.1 *In vitro* evaluation

4.1.1. Chemical composition of CSM and conventional oil cakes

The chemical composition of ingredients used in the experiment is given in Table 4.1. The organic matter (OM) content in cottonseed meal (CSM), soybean meal

(SBM) and groundnut cake (GNC) was 92.5%, 89.35% and 93.06%, respectively. The OM content in mustard cake (MC) was 93.46% which was higher and OM present in deoiled mustard cake was 88.08% and it was lower than other oil seed cakes evaluated. Wanapat et al. (2013) reported that CSM contained 90.6 % OM which was almost similar to the present study. The CP content of CSM was 47.85% while CP content of SBM was 47.76%. The CP content of GNC, MC and DMC was 43.64%, 39.59% and 42.01%, respectively. The CP of CSM in the current study was slightly higher than that reported by Fadel and Ashmawy (2015) (45.05% CP) and Sun et al. (2013) (46.2% CP).

Table 4.1. Chemical composition of ingredients used in the experiment, % DM basis

Parameter	CSM	SBM	GNC	MC	DMC
OM	92.50	89.35	93.06	93.46	88.08
CP	47.85	47.76	43.64	39.59	42.01
EE	1.71	1.36	0.91	8.34	1.25
Total ash	7.50	10.65	6.94	6.53	11.91
NDF	29.00	30.73	30.06	21.40	21.80
ADF	17.40	20.43	22.36	19.03	21.13
Hemicellulose	11.60	10.30	7.70	2.37	0.67
ADL	6.30	4.13	7.93	6.26	7.06
ADICP	6.27	7.04	5.89	6.67	5.13
NDICP	9.31	16.53	12.54	7.79	5.51
TCHO	42.94	40.23	48.51	45.54	44.83

OM- Organic matter, CP- Crude protein, EE- Ether extract, NDF- Neutral detergent fibre, ADF- Acid detergent fibre, ADL- Acid detergent lignin, TCHO- Total carbohydrates, ADICP- Acid detergent insoluble crude protein, NDICP- Neutral detergent insoluble crude protein

The ether extract (EE) of CSM and SBM was 1.71% and 1.36%, respectively. The EE content of MC (8.34%) was higher and GNC (0.91%) was lower than other oilseed cakes evaluated. EE content of DMC was 1.25%. The EE content of CSM in the present study was similar to that reported by Silva et al. (2009). The total ash content of CSM, SBM, GNC, MC and DMC was 7.50%, 10.65%, 6.94%, 6.53% and 11.91%, respectively. The total ash content in the present study was similar to that reported by Silva et al. (2009). The NDF content of CSM (29.00%) was almost similar to SBM (30.73%) and NDF content of GNC, MC and DMC was 30.06%, 21.40% and 21.80%, respectively. Silva et al. (2009) and Wanapat et al. (2013) reported 28.21% and 32.3% NDF in cottonseed meal which is almost similar to that reported in the present study. The ADF content in CSM, SBM, GNC, MC and DMC was 17.4%, 20.43%, 22.36%, 19.03% and 21.13%, respectively. Wanapat et al. (2013) reported 20.7% ADF which was slightly higher than that reported in the present study whereas Silva et al. (2009) reported 11.41% ADF which was lower than that in the current study.

The hemicellulose content of CSM, SBM, GNC, MC and DMC was 11.60%, 10.30%, 7.70%, 2.37% and 0.67%, respectively. ADL content in CSM, SBM, GNC, MC and DMC was 6.3%, 4.13%, 7.93%, 6.26% and 7.06%, respectively. The ADICP and NDICP content in CSM was 6.27% and 9.31%, respectively. The ADICP and NDICP in SBM was 7.04% and 16.53%, respectively. The ADICP and NDICP in CSM was lower than SBM. The carbohydrate content was 42.94%, 40.23%, 48.51%, 45.54% and 44.83% in CSM, SBM, GNC, MC and DMC, respectively.

4.1.2. Chemical composition of concentrate mixtures containing graded levels of CSM

The chemical composition of various concentrates with graded level of cotton seed meal is given in Table 4.2. The OM of concentrates was 91.66% in concentrate 1, 91.32% in concentrate 2, 91.53% in concentrate 3, 92% in concentrate 4 and 91.98% in concentrate 5. All the concentrates mixtures were iso-nitrogenous as the CP content of concentrate mixtures varied from 20.07% to 20.95% (Table 4.2). The ether extract content in concentrate mixtures varied from 5.26% to 5.64%. The total ash content was 8.33% in concentrate 1, 8.67% in concentrate 2, 8.46% in concentrate 3, 7.92% in concentrate 4 and 8.06% in concentrate 5. The NDF content in concentrate mixtures varied from 30.46% to 33.40%. ADF content in concentrate 1 was 15.16% and then from concentrate 2 to 5 it varied from 13.36% to 15.23%. The hemicellulose content varied from 16.24% to 19.04% in the concentrate mixture evaluated. The ADL content varied from 4.36% to 4.80%.

Table 4.2. Chemical composition of concentrate mixtures containing graded levels of CSM, % DM basis

Parameter	CONC 1 (0% CSM)	CONC 2 (25% CSM)	CONC 3 (50% CSM)	CONC 4 (75% CSM)	CONC 5 (100% CSM)
OM	91.66	91.32	91.53	92.00	91.98
CP	20.07	20.31	20.95	20.52	20.86
EE	5.36	5.26	5.50	5.36	5.64
Total ash	8.33	8.67	8.46	7.92	8.06
NDF	31.40	32.33	33.40	30.46	30.73
ADF	15.16	15.23	14.36	13.93	13.36
Hemicellulose	16.24	17.10	19.04	16.53	17.37
ADL	4.36	4.50	4.73	4.80	4.63
ADICP	7.79	7.22	7.79	7.98	7.41
NDICP	9.31	9.12	9.69	9.70	9.88
TCHO	66.24	65.76	65.09	66.20	65.44

OM- Organic matter, CP- Crude protein, EE- Ether extract, NDF- Neutral detergent fibre, ADF- Acid detergent fibre, ADL- Acid detergent lignin, TCHO- Total carbohydrates, ADICP- Acid detergent insoluble crude protein, NDICP- Neutral detergent insoluble crude protein

The ADICP of the concentrate mixtures ranged from 7.22% to 7.98%. The NDICP in concentrate mixtures varied from 9.12% to 9.88%. The Total carbohydrate content in concentrates ranged between 65.09% to 66.24% indicating almost similar carbohydrate content in the concentrate mixtures.

4.1.3. Chemical composition of TMRs containing graded levels of CSM

The chemical composition of TMRs is given in Table 4.3. The OM content of TMRs varied from 90.71% to 91.40%. The OM content increased with increasing level of cotton seed meal in TMRs. The CP content of TMRs varied from 15.07% to 15.83%. All the TMRs prepared were iso-nitrogenous. The ether extract content was almost similar and ranged from 3.09% to 3.30% in TMRs. The total ash content varied between 8.60% to 9.28% in TMRs.

The NDF content varied from 56.40% to 59.30% in TMRs. The ADF content varied between 32.90% to 34.13% in TMRs. The hemicellulose content varied from 22.57% to 27.23%. The ADL content of TMRs was in range of 5.06% to 8.33%. The

ADICP content varied from 5.89% to 6.84% in TMRs and NDICP content in TMRs ranged from 8.65% to 9.12%. The total carbohydrate content in TMRs ranged between 72.07% to 72.56%.

Table 4.3. Chemical composition of TMRs containing graded levels of CSM, % DM basis

Parameter	TMR 1	TMR 2	TMR 3	TMR 4	TMR 5
OM	90.71	90.86	90.86	91.23	91.40
CP	15.07	15.71	15.47	15.55	15.83
EE	3.29	3.09	3.30	3.13	3.21
Total ash	9.28	9.13	9.13	8.76	8.60
NDF	59.30	59.13	57.30	56.40	56.60
ADF	32.90	31.90	34.13	32.26	34.03
Hemicellulose	26.40	27.23	23.17	24.14	22.57
ADL	8.33	5.43	6.30	7.50	5.06
ADICP	6.84	6.27	6.65	5.89	6.27
NDICP	8.94	8.65	9.12	8.74	8.65
TCHO	72.36	72.07	72.10	72.56	72.36

OM- Organic matter, CP- Crude protein, EE- Ether extract, NDF- Neutral detergent fibre, ADF- Acid detergent fibre, ADL- Acid detergent lignin, TCHO- Total carbohydrates, ADICP- Acid detergent insoluble crude protein, NDICP- Neutral detergent insoluble crude protein

4.1.4. *In vitro* evaluation of CSM and conventional oil cakes

The net gas production (NGP, ml/g DM/24h) in CSM (160.48) was lower ($P<0.05$) than SBM (198.04) and MC (199.55), however, it was similar to that of GNC (173.78) and DMC (164.95) (Table 4.4). DeVore (2018) also reported lowest ($P<0.05$) *in vitro* gas production in CSM when compared to soyhulls, corn gluten feed (CGF) and wheat middling's. The partitioning factor (PF, mg/ml) was higher ($P<0.05$) in CSM (4.91) than SBM (3.60) and MC (3.91). The PF of various oilseed cakes tested in the present study varied in the following order as CSM>DMC>GNC>MC>SBM. The OM digestibility (%) was higher ($P<0.05$) in CSM (85.09) and MC (83.88) than the other oilseed cakes tested. The OM digestibility (%) of SBM (79.83) was statistically similar to that of GNC (77.80) and DMC (81.76). The OM digestibility of CSM in the present study was higher than that reported by Tripathi et al. (2014).

Table 4.4. ***In vitro* gas production and digestibility of CSM and conventional oil cakes (24 h)**

Parameter	CSM	SBM	GNC	MC	DMC	SEM
NGP, ml/g DM/24h	160.48^{a}	198.04^{b}	173.78^{a}	199.55^{b}	164.95^{a}	4.59
PF, mg/ml	4.91^{c}	3.60^{a}	4.19^{abc}	3.91^{ab}	4.52^{bc}	0.16
OMD, %	85.09^{c}	79.83^{ab}	77.80^{a}	83.88^{c}	81.76^{b}	0.89
NDFD, %	52.44^{c}	41.35^{b}	31.27^{a}	29.59^{a}	26.29^{a}	3.23
MMP, mg	159.44^{c}	98.61^{a}	125.23^{ab}	123.24^{ab}	134.44^{b}	6.76
EMMP, %	53.73^{c}	36.92^{a}	45.81^{bc}	41.91^{ab}	49.56^{bc}	2.03
DMD, %	87.14^{c}	85.31^{b}	81.72^{a}	85.60^{b}	86.72^{c}	0.64
SCFA, mmole	0.71^{a}	0.88^{b}	0.77^{a}	0.88^{b}	0.73^{a}	0.02
ME, MJ/kg DM	9.89^{b}	10.73^{c}	9.82^{b}	11.96^{d}	9.08^{a}	0.27
NH_3-N, mg/dl	70.00^{ab}	64.50^{a}	78.50^{c}	71.00^{b}	72.00^{b}	1.55
Fer CO_2, mmoles	51.11^{b}	49.62^{a}	49.36^{a}	49.77^{a}	49.04^{a}	0.25
Fer CH_4, mmoles	30.64^{b}	31.35^{c}	29.73^{a}	30.55^{b}	31.27^{c}	0.20

NGP- Net gas production, PF- Partitioning factor, D- Digestibility, OM- Organic matter, NDF- Neutral detergent fibre, MMP- Microbial mass production, EMMP- Efficiency of microbial mass production, DM- Dry matter, SCFA- Short chain fatty acids, NH_3-N- Ammoniacal nitrogen, Fer CO2- Fermentable carbon dioxide, Fer CH_4- Fermentable methane, Means bearing different superscripts in a row differ significantly (P<0.05)

The NDF digestibility (%) of CSM (52.44) was higher (P<0.05) than SBM (41.35) as well as other oilseed cakes tested. The microbial mass production (MMP, mg) was highest (P<0.05) in CSM (159.44) followed by DMC (134.44), GNC (125.23), MC (123.24) and lowest (P<0.05) in SBM (98.61). The efficiency of microbial mass production (EMMP, %) was higher (P<0.05) in CSM (53.73) and lower (P<0.05) in SBM (36.92) than the other oil seed cakes tested. The EMMP was similar (P<0.05) in GNC (45.81) and DMC (49.56).

The DM digestibility (%) was higher (P<0.05) in CSM (87.14) and DMC (86.72) than SBM (85.31). The DM digestibility (%) in SBM (85.31) was similar to that of MC (85.60) and was lowest (P<0.05) in GNC (81.72). The DM digestibility of CSM (72.64%) in the present study was higher than that reported by Devore (2018).

The short chain fatty acid (SCFA, mmole) production in CSM (0.71) was lower (P<0.05) than SBM (0.88), however, it was similar to that of GNC (0.77) and

DMC (0.73). The SCFA production in SBM was similar to that of MC. The SCFA production of CSM in the present study was higher than SCFA production in cottonseed cake (0.62 mmole) reported by Raji et al. (2017). The metabolizable energy (ME, MJ/kg DM) in CSM (9.89) was higher (P<0.05) than DMC (9.08) but it was lower (P<0.05) than SBM (10.73) and MC (11.96). However, the ME content of CSM (9.89 MJ/kg DM) was similar to that of GNC (9.82 MJ/kg DM). The results of the present study are in accordance with those of Khanum et al. (2007) who reported 9.74 MJ/kg DM ME in CSM.

The ammonia nitrogen (mg/dl) was lowest (P<0.05) in SBM (64.50) and highest (P<0.05) in GNC (78.50) among the oilseed cakes tested with intermediate values for CSM (70.0), MC (71.0) and DMC (72.0). The fermentable CO_2 in CSM (51.11) was higher (P<0.05) than other oilseed cakes tested viz., SBM (49.62), GNC (49.36), MC (49.77) and DMC (49.04). The fermentable CH_4 (mmol) in CSM (30.64) was lower (P<0.05) than SBM (31.35) and DMC (31.27), however, it was higher (P<0.05) than GNC (29.73). The fermentable CH_4 (mmole) in SBM was similar to that in DMC.

The total volatile fatty acid (TVFA) production of ingredients used in the experiment is presented in Table 4.5. The acetic acid (mM/dl) production in CSM (4.04) was lower (P<0.05) than SBM (4.44), MC (4.37) and DMC (4.62), however, it was similar to GNC (3.93). The propionic acid, isobutyric acid and isovaleric acid production varied non-significantly among the protein sources evaluated. The butyric acid (mM/dl) production in CSM (0.69) was higher (P<0.05) than SBM (0.60), GNC (0.58) and DMC (0.60), however, it was similar to that in MC (0.67).

The results of the present study are in agreement with those of Lamba et al. (2014) who reported almost similar values for acetic acid (4.64mM/dl), propionic acid (2.51 mM/dl) and butyric acid (0.58mM/dl) in cottonseed cake. The valeric acid (mM/dl) in CSM (0.15) was lower (P<0.05) than other protein sources evaluated. The TVFA (mM/dl) production in CSM (6.63) was higher (P<0.05) than GNC (6.45) but lower (P<0.05) than SBM (6.97), MC (7.11) and DMC (7.25). The acetate: propionate ratio in SBM was higher (P<0.05) than CSM, GNC and MC, however, it was similar to DMC. TVFA production in CSM in the present study was slightly lower than that reported by Lamba et al. (2014) (CSC: 7.89 mM/dl).

Table 4.5. ***In vitro*** **VFA production (mM/dl) in CSM and conventional oil cakes (24 h)**

Parameter	CSM	SBM	GNC	MC	DMC	SEM
Acetate	4.04^{a}	4.44^{b}	3.93^{a}	4.37^{b}	4.62^{c}	0.09
Propionate	1.33	1.34	1.36	1.39	1.37	0.01
Isobutyrate	0.16	0.12	0.13	0.15	0.14	0.01
Butyrate	0.69^{b}	0.60^{a}	0.58^{a}	0.67^{b}	0.60^{a}	0.01
Isovalerate	0.26	0.28	0.24	0.26	0.27	0.01
Valerate	0.15^{a}	0.19^{b}	0.20^{b}	0.27^{c}	0.24^{c}	0.01
TVFA	6.63^{b}	6.97^{c}	6.45^{a}	7.11^{cd}	7.25^{d}	0.10
A:P	3.03^{ab}	3.30^{c}	2.89^{a}	3.15^{b}	3.36^{c}	0.06
Relative Proportion, %						
Acetate	60.90^{a}	63.71^{b}	60.97^{a}	61.41^{a}	63.69^{b}	0.44
Propionate	20.09^{b}	19.29^{ab}	21.13^{c}	19.53^{ab}	18.93^{a}	0.26
Isobutyrate	2.36	1.67	1.97	2.18	1.98	0.12
Butyrate	10.42^{d}	8.63^{ab}	9.06^{bc}	9.46^{c}	8.31^{a}	0.25
Isovalerate	3.98	4.05	3.74	3.67	3.75	0.07
Valerate	2.25^{a}	2.66^{a}	3.13^{b}	3.76^{d}	3.34^{bc}	0.18

TVFA- Total volatile fatty acids, A:P- acetate: propionate, Means bearing different superscripts in a row differ significantly (P<0.05)

The relative proportion (%) of acetic acid in SBM (63.71) was higher (P<0.05) than CSM (60.90), GNC (60.97) and MC (61.41), however, it was similar to that in DMC (63.69). The relative proportion (%) of propionic acid in CSM (20.09) was similar to SBM (19.29), MC (19.53). The relative proportion of propionic acid was highest (P<0.05) in GNC and lowest (P<0.05) in DMC among the protein sources evaluated. The relative proportion of isobutyric acid and isovaleric acid was similar among the protein sources evaluated. The relative proportion (%) of butyric acid was highest (P<0.05) in CSM (10.42) followed by MC (9.46), GNC (9.06), SBM (8.63) and DMC (8.31). The relative proportion of valeric acid was similar in CSM and SBM and was lower (P<0.05) than other protein sources evaluated.

Table 4.6. Hydrogen balance of CSM and conventional oil cakes (24h)

Parameter	CSM	SBM	GNC	MC	DMC	SEM
H- recovery, %	78.16^{c}	75.12^{b}	79.57^{d}	74.73^{b}	73.22^{a}	0.79
H consumed via CH_4	5.03^{b}	4.97^{ab}	4.79^{a}	5.13^{b}	5.04^{b}	0.04
FE, %	74.70^{c}	73.96^{a}	74.97^{c}	74.38^{b}	73.81^{a}	0.15
VFA UI	3.76^{bc}	3.81^{c}	3.39^{a}	3.61^{b}	3.76^{bc}	0.05

FE- Fermentation efficiency, H- Hydrogen, VFA UI- Volatile fatty acids utilization index, Means bearing different superscripts in a row differ significantly (P<0.05)

The H- recovery (%) in CSM (78.16) was higher (P<0.05) than SBM (75.12), MC (74.73) and DMC (73.22), however, it was lower (P<0.05) than GNC (79.57) (Table 4.6). The hydrogen consumed via methane was lowest (P<0.05) in GNC (4.79). The hydrogen consumed via methane in CSM was similar to that in SBM, MC and DMC.

The fermentation efficiency (%) in CSM (74.70) and GNC (74.97) was higher (P<0.05) than SBM (73.96), MC (74.38) and DMC (73.81). The VFA utilization index (VFA UI) was highest (P<0.05) in SBM (3.81) and lowest (P<0.05) in GNC (3.39). The VFA UI in CSM (3.76) was similar to DMC (3.76). The results conclusively revealed that cottonseed meal could be used as a potential source of nutrients for livestock feeding.

4.1.5. *In vitro* evaluation of concentrate mixtures containing graded levels of CSM

The net gas production (NGP, ml/g DM/24h) was 196.19, 194.50, 196.94, 195.27 and 192.97 in concentrate mixtures 1, 2, 3, 4 and 5, respectively (Table 4.7). No significant difference was observed in NGP among the concentrate mixtures. The results are in agreement with those of Abdalla et al. (2012) who reported no significant difference in gas production on replacing cottonseed meal with soybean meal. The PF was 3.80, 3.78, 3.75, 3.83, 3.86 mg/ml in concentrate mixtures 1, 2, 3, 4 and 5, respectively and varied non-significantly. The partitioning factor (PF) is the ratio of organic matter degraded (mg) *in vitro* to the volume of gas (ml) produced. A higher partitioning factor (PF) means proportionally more of degraded matter is incorporated into microbial mass i.e., the efficiency of microbial protein synthesis is higher. The PF of ruminant diets should be in the range of 2.71- 4.4 (Blummel et al., 1997).

Table 4.7. ***In vitro* utilization of nutrients of concentrate mixtures containing graded levels of CSM (24 h)**

Parameter	CONC 1	CONC 2	CONC 3	CONC 4	CONC 5	SEM
NGP, ml/g DM/24h	196.19	194.50	196.94	195.27	192.97	1.07
PF, mg/ml	3.80	3.78	3.75	3.83	3.86	0.04
OMD, %	79.85	80.07	80.68	81.64	81.22	0.33
NDFD, %	41.17	43.69	47.05	44.49	43.80	0.94
MMP, mg	110.60	109.11	109.71	115.98	114.89	1.85
EMMP, %	40.16	39.95	39.51	40.76	41.19	0.56
DMD, %	81.79	82.06	82.32	83.09	82.73	0.28
SCFA, mmole	0.87	0.86	0.87	0.86	0.85	0.00
ME, MJ/kg DM	9.41	9.33	9.53	9.45	9.46	0.04
NH_3-N, mg/dl	34.00^{b}	31.00^{ab}	30.50^{ab}	30.00^{ab}	27.00^{a}	0.79
Fer CO_2, mmoles	46.86	48.17	48.07	47.70	47.41	0.36
Fer CH_4, mmoles	24.30	25.11	25.27	26.32	25.98	0.36

NGP- Net gas production, PF- Partitioning factor, D- Digestibility, OM- Organic matter, NDF- Neutral detergent fibre, MMP- Microbial mass production, EMMP- Efficiency of microbial mass production, DM- Dry matter, SCFA- Short chain fatty acids, NH_3-N- Ammoniacal nitrogen, Fer CO2- Fermentable carbon dioxide, Fer CH_4- Fermentable methane, Means bearing different superscripts in a row differ significantly ($P<0.05$)

The PF in the present study was within the suggested range. The OM digestibility (%) in concentrate mixture 1, 2, 3, 4 and 5 was 79.85, 80.07, 80.68, 81.64 and 81.22, respectively. There was no significant difference in OM digestibility among the concentrate mixtures evaluated. Abdalla et al. (2012) also reported no significant difference in organic matter true digestibility (OMTD) on replacing cottonseed meal with soybean meal in the *in vitro* study. The NDF digestibility varied non-significantly among the concentrate mixtures. Microbial mass production (MMP) in concentrate mixtures 1, 2, 3, 4 and 5 was 110.60, 109.11, 109.71, 115.98, 114.89 mg and efficiency of microbial mass production (EMMP) was 40.16, 39.95, 39.51, 40.76 and 41.19% in concentrate mixtures 1, 2, 3, 4 and 5, respectively (Table 4.7). The MMP and EMMP results varied non significantly among the concentrate mixtures.

The DM digestibility (%) was similar in the concentrate mixtures showing no significant difference. DM digestibility % of concentrate 1, 2, 3, 4 and 5 was 81.79, 82.06, 82.32, 83.09 and 82.73%, respectively. Nasser (2009) also reported no significant difference in true degradability of dry matter (TDMD) when jojoba meal was added to a concentrate diet at 6%, 9% and 18% levels, substituting for cottonseed meal.

The short chain fatty acids (SCFA, mmole) production was similar among all the concentrate mixtures and varied from 0.85 to 0.87 (Table 4.7). The metabolizable energy (ME) was 9.41, 9.33, 9.53, 9.45 and 9.46 MJ/kg DM in concentrate mixtures 1 to 5, respectively. There was no significant difference in ME value among the concentrate mixtures. The ammonia nitrogen (NH_3-N, mg/dl) was highest ($P<0.05$) in concentrate mixture 1 (34.00) and lowest ($P<0.05$) in concentrate mixture 5 (27.00) (Table 4.7). Ammonia- N showed a declining trend with increasing level of CSM in concentrate mixtures. Concentrate mixtures 2 (31.00 mg/dl), 3 (30.50 mg/dl) and 4 (30.00 mg/dl) had similar NH_3-N concentration. However, Nasser (2009) reported no significant difference in NH_3-N on adding jojoba meal to a concentrate diet at 6%, 9% and 18% levels, substituting for cottonseed meal. The fermentable carbon dioxide (Fer CO_2, mmol) level in the concentrate mixtures containing graded levels was similar to that of control concentrate (conc 1). Concentrate mixture 1, 2, 3, 4 and 5 produced 46.86, 48.17, 48.07, 47.70 and 47.41 mmole fermentable CO_2, respectively. The fermentable methane (Fer CH_4, mmol) in concentrate mixtures varied non significantly and concentrate 1, 2, 3, 4 and 5 produced 24.30, 25.11, 25.27, 26.32 and 25.98 mmol fermentable CH_4, respectively.

The acetic acid content (mM/dl) in concentrate mixture 1 (2.70), concentrate mixture 2 (2.64), concentrate mixture 3 (2.73), concentrate mixture 4 (2.83) and concentrate mixture 5 (2.81) were similar (Table 4.8). The propionic acid content (mM/dl) in concentrate mixture varied non significantly and concentrate 1, 2, 3, 4 and 5 had 1.51, 1.44, 1.47, 1.40 and 1.42 mM/dl propionic acid, respectively. There was non-significant difference in isobutyric acid content among the concentrate mixtures (Table 4.8). Concentrate mixture 1, 2, 3, 4 and 5 had 0.06, 0.06, 0.05, 0.06, 0.05 mM/dl isobutyric acid, respectively. The butyric acid concentration (mM/dl) in concentrate mixture 1 (0.27), concentrate mixture 2 (0.31), concentrate mixture 3 (0.30), concentrate mixture 4 (0.28) and concentrate mixture 5 (0.26) varied non significantly.

The total volatile fatty acid production (TVFA, mM/dl) was similar ($P>0.05$) in concentrate mixture 1 (4.54), concentrate mixture 2 (4.45), concentrate mixture 3 (4.55), concentrate mixture 4 (4.56) and concentrate mixture 5 (4.55) and differed non significantly. However, Nasser (2009) reported significant increase ($P<0.05$) in *in vitro* TVFA concentration when jojoba meal was added at L2 (9%) and L3 (18%) levels substituting cottonseed meal. The acetate: propionate (A: P) was similar among the concentrate mixtures evaluated. Concentrate mixture 1, 2, 3, 4 and 5 had 1.79, 1.84, 1.86, 2.02 and 1.98 mM/dl A:P ratio, respectively. Nasser (2009) also reported similar results where no significant difference was observed in A:P ratio in concentrate mixture having graded levels of jojoba meal replacing cottonseed meal.

Table 4.8. *In vitro* volatile fatty acids production (mM/dl) in concentrate mixtures containing graded levels of CSM (24 h)

Parameter	CONC 1	CONC 2	CONC 3	CONC 4	CONC 5	SEM
Acetate	2.70	2.64	2.73	2.83	2.81	0.031
Propionate	1.51	1.44	1.47	1.40	1.42	0.022
Isobutyrate	0.06	0.06	0.05	0.06	0.05	0.003
Butyrate	0.27	0.31	0.30	0.28	0.26	0.014
Isovalerate	0.00	0.00	0.00	0.00	0.00	0.000
Valerate	0.00	0.00	0.00	0.00	0.00	0.000
TVFA	4.54	4.45	4.55	4.56	4.55	0.036
A:P	1.79	1.84	1.86	2.02	1.98	0.040
Relative proportion, %						
Acetate	59.38	59.40	60.03	62.00	61.80	0.436
Propionate	33.33	32.24	32.29	30.74	31.27	0.467
Isobutyrate	1.40	1.42	1.03	1.25	1.14	0.069
Butyrate	5.89	6.94	6.65	6.01	5.79	0.284
Isovalerate	0.00	0.00	0.00	0.00	0.00	0.000
Valerate	0.00	0.00	0.00	0.00	0.00	0.000

TVFA- Total volatile fatty acids, A:P- acetate: propionate, Means bearing different superscripts in a row differ significantly ($P<0.05$)

The relative proportion (%) of acetic acid in concentrate mixture 1 (59.38), concentrate mixture 2 (59.40), concentrate mixture 3 (60.03), concentrate mixture 4 (62.00) and concentrate mixture 5 (61.80) was similar (Table 4.8). The relative proportion (%) of propionic acid was 33.33, 32.24, 32.29, 30.74 and 31.27 in concentrate mixture 1, 2, 3, 4 and 5, respectively.

The relative proportion (%) of isobutyric acid in concentrate mixture 1, 2, 3, 4 and 5 was 1.40, 1.42, 1.03, 1.25 and 1.14, respectively. The relative proportion of butyrate in concentrate mixture 1 (5.89%), concentrate mixture 2 (6.94%), concentrate mixture 3 (6.65%), concentrate mixture 4 (6.01%) and concentrate mixture 5 (5.79%) was similar among the concentrate mixtures evaluated.

The hydrogen recovery (%) was 99.05, 100.16, 99.01, 98.88 and 98.89 in concentrate mixture 1, 2, 3, 4 and 5, respectively (Table 4.9). The hydrogen consumed via CH_4 was similar among concentrates. Concentrate mixture 1, 2, 3, 4 and 5 had 3.81, 3.83, 3.92, 3.84 and 3.82 hydrogen consumed via CH_4, respectively. The fermentation efficiency (%) in concentrate 1 (78.98), concentrate 2 (78.64), concentrate 3 (78.56), concentrate 4 (77.80) and concentrate 5 (78.00) was similar.

Table 4.9. Hydrogen balance of nutrients of concentrate mixtures containing graded levels of CSM (24 h)

Parameter	CONC 1	CONC 2	CONC 3	CONC 4	CONC 5	SEM
H- recovery, %	99.05	100.16	99.01	98.88	98.89	0.45
H- consumed via CH_4	3.81	3.83	3.92	3.84	3.82	0.05
FE, %	78.98	78.64	78.56	77.80	78.00	0.20
VFA UI	2.14	2.28	2.27	2.41	2.36	0.05

FE- fermentation efficiency, H- Hydrogen, VFA UI- volatile fatty acids utilization index, Means bearing different superscripts in a row differ significantly (P<0.05)

The volatile fatty acids utilization index (VFA UI) was 2.14, 2.28, 2.27, 2.41 and 2.36 in concentrate mixtures 1, 2, 3, 4 and 5, respectively. No significant difference was observed in VFA utilization index among the concentrate mixtures. VFA utilization index is non-glucogenic to glucogenic VFA ratio (NGGR). The data conclusively revealed that soybean meal could be replaced by CSM up to 100% without affecting the nutrient digestibility and microbial biomass production. The NH_3-N showed a declining trend with increasing level of CSM in the concentrate mixtures.

4.1.6. *In vitro* evaluation of TMRs containing graded levels of CSM

The net gas production (NGP, ml/g DM/24h) in TMR 1, 2, 3, 4 and 5 was 187.17, 185.95, 190.23, 181.99 and 185.76, respectively (Table 4.10). There was no significant difference in NGP among the TMRs. The partitioning factor (PF, mg/ml) in TMR 1, 2, 3, 4 and 5 was 2.93, 3.13, 2.99, 3.05 and 2.97, respectively. No significant difference was seen in PF in the present study. The OM digestibility (%) in the TMRs containing graded levels of CSM was similar to that of control TMR (TMR

1). The NDF digestibility was also similar among the TMRs. The microbial mass production (MMP, mg) in TMR 1, 2, 3, 4 and 5 was 51.15, 64.05, 55.21, 58.72 and 55.04, respectively. The efficiency of microbial mass production (EMMP, %) in TMR 1, 2, 3, 4 and 5 was 24.95, 29.66, 26.33, 27.86 and 25.99%, respectively.

Table 4.10. Effect of level of CSM on the *in vitro gas* production and digestibility of nutrients in the TMRs (24 h)

Parameter	**TMR1**	**TMR2**	**TMR3**	**TMR4**	**TMR5**	**SEM**
NGP, ml/g DM/24h	187.17	185.95	190.23	181.99	185.76	1.42
PF, mg/ml	2.93	3.13	2.99	3.05	2.97	0.03
OMD, %	60.13	63.41	61.46	61.19	61.78	0.44
NDFD, %	39.00	43.78	38.88	37.21	38.28	0.87
MMP, mg	51.15	64.05	55.21	58.72	55.04	1.96
EMMP, %	24.95	29.66	26.33	27.86	25.99	0.77
DMD, %	65.29	67.69	66.31	66.05	66.53	0.32
SCFA, mmole	0.82	0.81	0.83	0.81	0.84	0.01
ME, MJ/kg DM	8.17	8.16	8.32	8.06	8.23	0.04
NH_3-N, mg/dl	30.00	29.50	27.00	27.00	24.50	0.73
Fer CO_2, mmoles	50.89^{ab}	48.47^{a}	50.61^{ab}	51.77^{b}	50.95^{ab}	0.41
Fer CH_4, mmoles	33.15^{c}	28.29^{a}	29.53^{b}	29.26^{b}	29.12^{b}	0.57

NGP- Net gas production, PF- Partitioning factor, D- Digestibility, OM- Organic matter, NDF- Neutral detergent fibre, MMP- Microbial mass production, EMMP- Efficiency of microbial mass production, DM- Dry matter, SCFA- Short chain fatty acids, NH_3-N- Ammoniacal nitrogen, Fer CO_2- Fermentable carbon dioxide, Fer CH_4- Fermentable methane, roughage to concentrate ratio in TMRs was 60:40 on dry matter basis, all TMRs contained bajra fodder and wheat straw in the ratio of 1:1, Means bearing different superscripts in a row differ significantly (P<0.05)

The MMP and EMMP varied non-significantly among the TMRs tested. The DM digestibility of TMRs ranged from 65.29% to 67.69% with non-significant difference among TMRs tested. The short chain fatty acids (SCFA) varied from 0.81 to 0.84 mmole and differed non-significantly among the TMRs. The metabolizable energy (ME) ranged from 8.06 to 8.32 MJ/kg DM. No significant difference was observed among TMRs. The ammonia nitrogen in TMR 1, TMR 2, TMR 3, TMR 4 and TMR 5 was 30.00, 29.50, 27.00, 27.00 and 24.50 mg/dl, respectively. There was non-significant decrease in NH_3-N from TMR 1 to TMR 5. The fermentable CO_2 (mmol) was lowest (P<0.05) in TMR 2 (48.47) and highest (P<0.05) in TMR 4 (51.77) with intermediate values in TMR 1 (50.89), TMR 3 (50.61), TMR 5 (50.95).

The fermentable CH_4 (mmol) was lowest (P<0.05) in TMR 2 (28.29) and highest (P<0.05) in TMR 1 (33.15) and similar (P<0.05) in TMR 3 (29.53), TMR 4 (29.26) and TMR 5 (29.12).

The effect of different levels of cotton seed meal in TMRs on individual and total volatile fatty acids (TVFAs) is presented in Table 4.11. The acetic acid (mM/dl) production was highest (P<0.05) in TMR 1 (4.34) and lowest (P<0.05) in TMR 4 (3.39). The acetic acid production (mM/dl) was similar (P<0.05) in TMR 3 (3.61) and TMR 5 (3.57).

Table 4.11. Effect of level of CSM on *in vitro* VFA production (mM/dl) in TMRs (24 h)

Parameter	TMR 1	TMR 2	TMR 3	TMR 4	TMR 5	SEM
Acetate	4.34^{d}	3.76^{c}	3.61^{b}	3.39^{a}	3.57^{b}	0.11
Propionate	1.21^{a}	1.50^{e}	1.38^{c}	1.32^{b}	1.43^{d}	0.03
Isobutyrate	0.06^{a}	0.16^{b}	0.07^{a}	0.12^{b}	0.04^{a}	0.01
Butyrate	0.54	0.50	0.55	0.64	0.56	0.02
Isovalerate	0.30^{b}	0.29^{b}	0.27^{ab}	0.29^{b}	0.26^{a}	0.01
Valerate	0.00	0.00	0.00	0.00	0.00	0.00
TVFA	6.45^{c}	6.21^{b}	5.88^{a}	5.76^{a}	5.86^{a}	0.09
A:P	3.58^{c}	2.51^{a}	2.62^{b}	2.57^{ab}	2.49^{a}	0.14
Relative Proportion, %						
Acetate	67.38^{c}	60.55^{b}	61.40^{b}	58.97^{a}	60.89^{b}	0.97
Propionate	18.82^{a}	24.15^{c}	23.43^{b}	22.95^{b}	24.47^{c}	0.68
Isobutyrate	0.89^{a}	2.50^{b}	1.22^{a}	2.01^{b}	0.62^{a}	0.24
Butyrate	8.33^{a}	8.11^{a}	9.37^{ab}	11.03^{b}	9.59^{ab}	0.38
Isovalerate	4.59	4.70	4.58	5.04	4.42	0.08
Valerate	0.89^{a}	2.50^{b}	1.22^{a}	2.01^{b}	0.62^{a}	0.24

TVFA- Total volatile fatty acids, A:P- Acetate: propionate, Means bearing different superscripts in a row differ significantly (P<0.05)

The propionic acid (mM/dl) production was higher (P<0.05) in TMR 2 (1.50) and lower (P<0.05) in TMR 1 (1.21) than other TMRs. The propionic acid production (mM/dl) in TMR 3, TMR 4 and TMR 5 was 1.38, 1.32 and 1.43, respectively. The butyric acid (mM/dl) production in TMR 1 (0.54), TMR 2 (0.50), TMR 3 (0.55), TMR 4 (0.64) and TMR 5 (0.56) varied non significantly. The isovaleric acid (mM/dl) production was lowest (P<0.05) in TMR 5 (0.26). The total VFA (mM/dl) production varied significantly among the TMRs and was highest (P<0.05) in TMR 1 (6.45) (Table 4.11). The TVFA production in TMR 3, TMR 4 and TMR 5 was similar. The acetate: propionate ratio was higher (P<0.05) in TMR 1 (3.58) and lower (P<0.05) in TMR 2 (2.51) and TMR 5 (2.49) as compared to other TMRs evaluated. The A: P ratio in TMR 3 (2.62) and TMR 4 (2.57) varied non significantly.

The relative proportion (%) of acetic acid was highest (P<0.05) in TMR 1 (67.38) and was lowest (P<0.05) in TMR 4 (58.97). It was similar (P<0.05) in TMR 2 (60.55%), TMR 3 (61.40%) and TMR 5 (60.89%). The relative proportion (%) of propionic acid was highest (P<0.05) in TMR 2 (24.15) and TMR 5 (24.47) and lowest (P<0.05) in TMR 1(18.82). The relative proportion (%) of propionic acid was similar (P<0.05) in TMR 3 (23.43) and TMR 4 (22.95). The relative proportion (%) of isobutyric acid was higher (P<0.05) in TMR 2 (2.50) and TMR 4 (2.01) than TMR 1 (0.89), TMR 3 (1.22) and TMR 5 (0.62). The relative proportion (%) of butyric acid was highest (P<0.05) in TMR 4 (11.03) and was lowest (P<0.05) in TMR 1 (8.33) and TMR 2 (8.11). It was similar in TMR 3 (9.37%) and TMR 5 (9.59%). The relative proportion (%) of isovaleric acid in TMR 1, 2, 3, 4 and 5 was 4.59, 4.70, 4.58, 5.04 and 4.42, respectively. The relative proportion (%) of valeric acid was lower (P<0.05) in TMR 1 (0.89), TMR 3 (1.22) and TMR 5 (0.62) than TMR 2 (2.50) and TMR 4 (2.01).

The H- recovery (%) was lowest (P<0.05) in TMR 1 (78.18) and highest (P<0.05) in TMR 3 (84.55), TMR 4 (85.82) and TMR 5 (85.00) followed by TMR 2 (82.08) (Table 4.12). The hydrogen consumed via methane was similar in TMR1 (4.67), TMR 2 (4.80), TMR 3 (4.73), TMR 4 (4.72) and TMR 5 (4.85). The fermentation efficiency (%) was highest (P<0.05) in TMR 5 (76.10) and was lowest (P<0.05) in TMR 1 (73.36) (Table 4.12).

Table 4.12. Effect of level of CSM on hydrogen balance in TMRs (24h)

Parameter	TMR 1	TMR 2	TMR 3	TMR 4	TMR 5	SEM
H- recovery, %	78.18[a]	82.08[b]	84.55[c]	85.82[c]	85.00[c]	0.93
H consumed via CH_4	4.67	4.80	4.73	4.72	4.85	0.04
FE, %	73.36[a]	75.99[bc]	75.69[b]	75.93[bc]	76.10[c]	0.35
VFA UI	4.31[c]	2.98[a]	3.30[b]	3.33[b]	3.22[b]	0.15

FE- Fermentation efficiency, H- Hydrogen, VFA UI- Volatile fatty acids utilization index, Means bearing different superscripts in a row differ significantly (P<0.05)

The VFA utilization index (VFA UI) was lowest (P<0.05) in TMR 2 (2.98) and highest (P<0.05) in TMR 1 (4.31). It was similar (P<0.05) in TMR 3 (3.30), TMR 4 (3.33) and TMR 5 (3.22). The low VFA UI in TMR 2 corroborates well with high molar proportion of propionate in TMR 2. VFA utilization index or the non-glucogenic to glucogenic VFA ratio (NGGR) is associated with effects on methane production, milk composition and energy balance (Morvay et al., 2011). Glucogenic propionate contributes to energy deposition in body tissues, where non-glucogenic acetate and butyrate are sources for long chain fatty acid synthesis. Too high VFA UI indicates a high loss of energy in the form of gases (Orskov, 1975). The low value of VFA UI indicates the best utilization of VFAs.

The data conclusively revealed that soybean meal could be replaced by CSM upto 100% without affecting the nutrient digestibility and microbial mass production. The fermentation efficiency was higher (P<0.05) in TMRs containing graded levels of CSM than control TMR. On the basis of digestibility of nutrients of TMRs observed in the *in vitro* study, two levels of CSM (75 and 100% replacement of soybean meal in concentrate) were selected for feeding to buffalo calves in the *in vivo* study.

4.2. *In vivo* evaluation

4.2.1. Effect of dietary level of CSM on fortnightly body weight (kg) in buffalo calves

The mean body weight (kg) of buffalo calves at the start of the experiment was 149.29, 150.08 and 150.25 in control (0% CSM), T1 (75% CSM) and T2 (100% CSM) groups, respectively (Table 4.13 and Fig.8). Gradual increase in mean BW was observed each fortnight during the experimental period of 120 days. The overall mean average body weight of buffalo calves during experimental period was higher (P<0.05) in T2 (203.44 kg) than control group (197.44 kg). The BW of calves of T1 (200.52 kg) group was similar to that of T2 group (203.44 kg).

The overall mean metabolic body weight (kg) of buffalo calves was lowest (P<0.05) in control group (52.00 kg), and similar in T1 (53.14 kg) and T2 (53.68 kg) groups. Nomeary et al. (2021) also reported maximum (P<0.05) daily BW gain in lambs fed CSM diet compared to lambs fed black cumin seed meal (BCSM), sesame seed meal (SSM) and soybean meal (SBM) diets. However, Tripathi et al. (2014) reported no significant difference in final BW, total BW gain and ADG of lambs fed groundnut meal, insect protected cottonseed meal or conventional cottonseed meals.

Solomon et al. (2008) reported that BW of goats supplemented with the medium (300 g) and high level (400 g) of CSM were higher (P<0.01) than low (200 g) level of supplementation. However, Ojewola et al. (2006) observed no significant difference (P>0.05) in birds mean daily weight gain in which cottonseed meal was substituted for soybean meal at 0, 25, 50, 75 and 100%.

Table 4.13. Effect of dietary level of CSM on fortnightly average body weight (kg $W^{0.75}$) in buffalo calves

Fortnight	Average body weight (kg)				Average metabolic BW (kg)			
	C	T_1	T_2	SEM	C	T_1	T2	SEM
0*	149.29	150.08	150.25	2.38	42.20	42.84	42.87	0.51
1	164.58	163.88	165.00	2.77	45.38	45.76	45.98	0.58
2	172.90	175.55	177.98	2.79	47.14	48.19	48.68	0.58
3	190.20	193.98	195.19	3.09	50.62	51.95	52.16	0.63
4	201.67	206.29	206.25	3.20	52.92	54.41	54.36	0.64
5	213.00	217.17	222.08	3.25	55.19	56.55	57.47	0.64
6	222.25	225.25	229.75	3.28	56.99	58.13	58.96	0.64
7	228.00	231.00	239.17	3.33	58.12	59.23	60.76	0.65
8	238.25	244.83	248.83	3.41	60.10	61.87	62.58	0.66
Overall mean	197.44^{a}	200.52^{ab}	203.44^{b}	1.27	52.00^{a}	53.14^{b}	53.68^{b}	0.26

0 represents weight taken at start of trial*

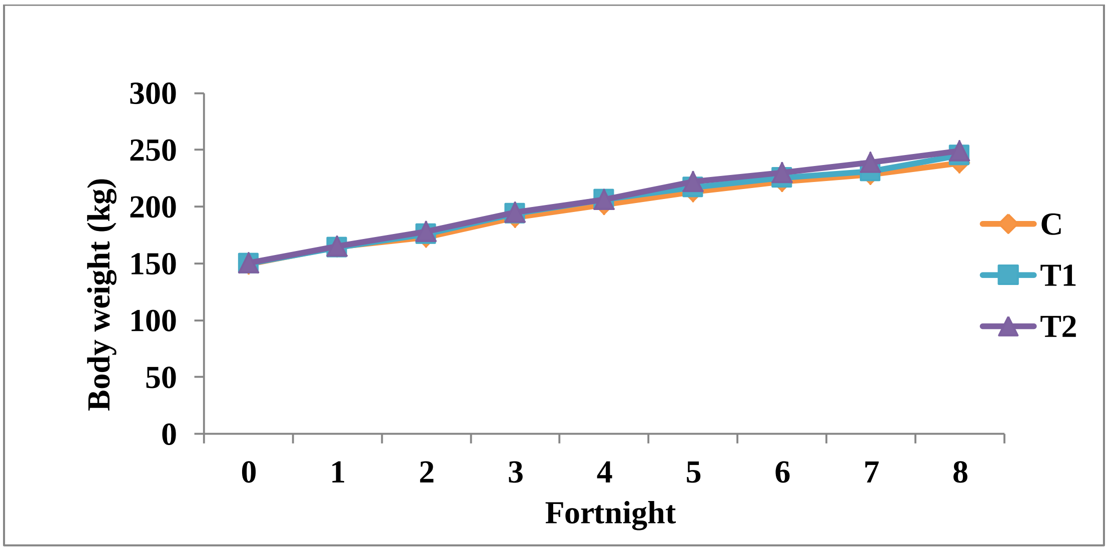

Fig. 8. Effect of dietary level of CSM on fortnightly average body weight (kg) in buffalo calves

Table 4.14. Effect of dietary level of CSM on fortnightly DM intake in buffalo calves

Fortnight	DMI (kg/animal/day)				DMI (kg/100 kg body weight)				DMI (g/kg $W^{0.75}$)			
	C	T1	T2	SEM	C	T1	T2	SEM	C	T1	T2	SEM
0*	3.76	3.99	3.92	0.06	2.54	2.66	2.60	0.02	87.62	92.98	91.07	0.64
1	3.89	4.22	4.18	0.07	2.40	2.58	2.53	0.02	84.84	92.12	90.57	0.70
2	4.55	4.74	4.74	0.08	2.63	2.70	2.66	0.01	94.34	98.18	97.17	0.69
3	4.70	4.84	4.79	0.09	2.47	2.50	2.45	0.02	90.58	93.09	91.36	0.80
4	4.84	5.10	4.88	0.10	2.35	2.47	2.36	0.02	88.07	93.72	89.35	1.03
5	4.76	5.00	4.92	0.09	2.21	2.30	2.20	0.02	83.91	88.39	85.09	0.87
6	5.14	5.31	5.21	0.10	2.27	2.36	2.26	0.02	87.15	91.32	87.89	0.87
7	5.74	5.82	5.89	0.10	2.48	2.51	2.46	0.02	95.79	98.07	96.54	0.87
8	5.92	6.02	6.14	0.10	2.44	2.46	2.47	0.01	95.50	97.13	97.87	0.74
Overall mean	4.80^{a}	5.00^{b}	4.95^{ab}	0.03	2.42^{a}	2.51^{b}	2.44^{a}	0.01	89.70^{a}	93.88^{c}	91.87^{b}	0.28

0 represents mean values of the adaptation period*

Means bearing different superscripts in a row differ significantly (P<0.05)

Table 4.15. Effect of dietary level of CSM on fortnightly OM intake in buffalo calves

Fortnight	OMI (kg/animal/day)				OMI (kg/100 kg body weight)				OMI (g/kg $W^{0.75}$)			
	C	T1	T2	SEM	C	T1	T2	SEM	C	T1	T2	SEM
0*	3.35	3.54	3.48	0.06	2.26	2.36	2.31	0.14	77.92	82.47	80.89	0.55
1	3.47	3.76	3.73	0.06	2.14	2.29	2.26	0.15	75.38	81.98	80.78	0.59
2	4.09	4.25	4.25	0.07	2.36	2.42	2.39	0.13	84.67	87.98	87.15	0.62
3	4.23	4.35	4.30	0.08	2.22	2.24	2.20	0.16	81.51	83.68	82.19	0.70
4	4.37	4.61	4.42	0.09	2.12	2.24	2.13	0.20	79.51	84.65	80.83	0.90
5	4.31	4.54	4.47	0.08	2.00	2.09	2.00	0.17	75.95	80.22	77.22	0.77
6	4.65	4.82	4.74	0.09	2.05	2.14	2.06	0.16	78.84	82.90	79.98	0.77
7	5.18	5.28	5.34	0.09	2.24	2.28	2.23	0.15	86.49	88.90	87.57	0.77
8	5.36	5.46	5.56	0.09	2.21	2.23	2.24	0.11	86.37	88.04	88.68	0.65
Overall mean	4.33^{a}	4.50^{b}	4.47^{b}	0.03	2.18^{a}	2.25^{b}	2.20^{a}	0.06	80.72^{a}	84.52^{c}	82.80^{b}	0.25

0 represents mean values of the adaptation period*

Means bearing different superscripts in a row differ significantly ($P<0.05$)

Table 4.16. Effect of dietary level of CSM on fortnightly CP intake in buffalo calves

Fortnight	CPI (kg/animal/day)				CPI (kg/100 kg body weight)				CPI (g/kg $W^{0.75}$)			
	C	**T1**	**T2**	**SEM**	**C**	**T1**	**T2**	**SEM**	**C**	**T1**	**T2**	**SEM**
0*	0.57	0.60	0.60	0.01	0.387	0.400	0.401	0.002	13.31	13.97	14.01	0.09
1	0.58	0.62	0.63	0.01	0.358	0.378	0.381	0.002	12.60	13.52	13.63	0.09
2	0.64	0.68	0.69	0.01	0.372	0.389	0.386	0.002	13.34	14.16	14.08	0.10
3	0.66	0.71	0.70	0.01	0.346	0.366	0.356	0.003	12.70	13.63	13.29	0.12
4	0.77	0.81	0.78	0.02	0.374	0.392	0.379	0.003	14.00	14.85	14.35	0.15
5	0.75	0.78	0.78	0.01	0.350	0.361	0.349	0.003	13.27	13.84	13.47	0.12
6	0.77	0.83	0.83	0.01	0.342	0.367	0.360	0.002	13.13	14.19	14.00	0.12
7	0.87	0.92	0.94	0.02	0.378	0.397	0.394	0.002	14.59	15.46	15.47	0.12
8	0.93	0.95	0.98	0.02	0.386	0.389	0.396	0.002	15.07	15.37	15.69	0.10
Overall mean	0.73[a]	0.77[b]	0.77[b]	0.01	0.366[a]	0.382[c]	0.378[b]	0.001	13.56[a]	14.33[b]	14.22[b]	0.04

0 represents mean values of the adaptation period*

Means bearing different superscripts in a row differ significantly (P<0.05)

Table 4.17. Effect of dietary level of CSM on fortnightly EE intake in buffalo calves

Fortnight	EEI (kg/animal/day)				EEI (kg/100 kg body weight)				EEI (g/kg $W^{0.75}$)			
	C	T1	T2	SEM	C	T1	T2	SEM	C	T1	T2	SEM
0*	0.12	0.13	0.13	0.002	0.082	0.088	0.085	0.0005	2.83	3.08	2.99	0.02
1	0.12	0.14	0.13	0.002	0.076	0.083	0.081	0.0004	2.66	2.96	2.89	0.02
2	0.14	0.14	0.15	0.002	0.081	0.082	0.084	0.0005	2.90	2.98	3.08	0.02
3	0.15	0.15	0.15	0.003	0.076	0.076	0.079	0.0006	2.80	2.84	2.94	0.02
4	0.16	0.19	0.16	0.003	0.080	0.090	0.079	0.0007	2.98	3.41	2.98	0.03
5	0.16	0.19	0.16	0.003	0.075	0.087	0.072	0.0006	2.83	3.34	2.78	0.03
6	0.16	0.19	0.18	0.003	0.072	0.084	0.078	0.0005	2.78	3.24	3.05	0.03
7	0.18	0.21	0.20	0.003	0.079	0.089	0.085	0.0005	3.05	3.48	3.33	0.03
8	0.19	0.20	0.21	0.003	0.080	0.083	0.084	0.0003	3.12	3.26	3.33	0.02
Overall mean	0.15^{a}	0.17^{c}	0.16^{b}	0.001	0.078^{a}	0.085^{c}	0.081^{b}	0.0002	2.88^{a}	3.18^{c}	3.04^{b}	0.01

0 represents mean values of the adaptation period*

Means bearing different superscripts in a row differ significantly (P<0.05)

Table 4.18. Effect of dietary level of CSM on fortnightly NDF intake in buffalo calves

Fortnight	NDFI (kg/animal/day)				NDFI (kg/100 kg body weight)				NDFI (g/kg $W^{0.75}$)			
	C	T1	T2	SEM	C	T1	T2	SEM	C	T1	T2	SEM
0*	1.96	2.04	1.99	0.03	1.32	1.37	1.32	0.010	45.52	47.71	46.25	0.37
1	2.05	2.21	2.16	0.04	1.27	1.35	1.31	0.011	44.59	48.21	46.80	0.41
2	2.44	2.52	2.50	0.04	1.41	1.43	1.40	0.009	50.52	52.18	51.16	0.39
3	2.50	2.54	2.48	0.05	1.32	1.31	1.27	0.011	48.30	48.95	47.40	0.47
4	2.44	2.53	2.36	0.05	1.18	1.23	1.14	0.015	44.13	46.41	43.22	0.62
5	2.41	2.47	2.40	0.05	1.12	1.14	1.08	0.012	42.40	43.67	41.53	0.52
6	2.66	2.75	2.63	0.05	1.17	1.22	1.14	0.011	44.88	47.19	44.36	0.53
7	2.96	3.00	2.98	0.06	1.27	1.29	1.24	0.011	49.23	50.49	48.86	0.52
8	3.06	3.05	3.07	0.05	1.26	1.24	1.23	0.008	49.26	49.08	48.93	0.42
Overall mean	2.49	2.56	2.50	0.02	1.26[b]	1.29[c]	1.24[a]	0.004	46.53[a]	48.21[b]	46.50[a]	0.17

0 represents mean values of the adaptation period*

Means bearing different superscripts in a row differ significantly (P<0.05)

Table 4.19. Effect of dietary level of CSM on fortnightly ADF intake in buffalo calves

Fortnight	ADFI (kg/animal/day)				ADFI (kg/100 kg body weight)				ADFI (g/kg $W^{0.75}$)			
	C	T1	T2	SEM	C	T1	T2	SEM	C	T1	T2	SEM
0*	1.15	1.21	1.20	0.02	0.78	0.81	0.80	0.006	26.71	28.20	27.92	0.23
1	1.20	1.30	1.30	0.02	0.74	0.79	0.79	0.007	26.05	28.41	28.18	0.27
2	1.46	1.52	1.51	0.03	0.84	0.87	0.85	0.006	30.24	31.54	31.02	0.25
3	1.50	1.53	1.49	0.03	0.79	0.79	0.76	0.008	28.87	29.52	28.36	0.31
4	1.51	1.58	1.47	0.03	0.73	0.77	0.71	0.009	27.40	29.12	26.98	0.40
5	1.51	1.57	1.51	0.03	0.70	0.72	0.68	0.008	26.57	27.66	26.15	0.34
6	1.67	1.73	1.61	0.03	0.73	0.77	0.70	0.008	28.17	29.71	27.14	0.34
7	1.86	1.90	1.84	0.04	0.80	0.82	0.77	0.007	31.06	31.95	30.11	0.33
8	1.8	1.88	1.88	0.03	0.77	0.77	0.76	0.005	29.96	30.27	30.04	0.28
Overall mean	1.52	1.58	1.53	0.01	0.76^{a}	0.79^{b}	0.76^{a}	0.003	28.32^{a}	29.59^{b}	28.43^{a}	0.11

0 represents mean values of the adaptation period*

Means bearing different superscripts in a row differ significantly (P<0.05)

Table 4.20. Effect of dietary level of CSM on fortnightly cellulose (CL) intake in buffalo calves

Fortnight	CLI (kg/animal/day)				CLI (kg/100 kg body weight)				CLI (g/kg $W^{0.75}$)			
	C	T1	T2	SEM	C	T1	T2	SEM	C	T1	T2	SEM
0*	0.90	0.92	0.90	0.02	0.61	0.62	0.59	0.005	20.86	21.51	20.82	0.17
1	0.94	1.00	0.97	0.02	0.58	0.61	0.59	0.005	20.43	21.78	21.10	0.19
2	1.14	1.17	1.16	0.02	0.66	0.67	0.65	0.004	23.72	24.27	23.74	0.18
3	1.19	1.20	1.17	0.02	0.62	0.62	0.60	0.005	22.89	23.02	22.25	0.21
4	1.17	1.18	1.13	0.02	0.56	0.57	0.54	0.006	21.17	21.75	20.64	0.27
5	1.16	1.16	1.15	0.02	0.54	0.54	0.51	0.006	20.39	20.54	19.88	0.24
6	1.28	1.33	1.28	0.02	0.57	0.59	0.56	0.005	21.69	22.77	21.62	0.23
7	1.41	1.45	1.44	0.03	0.61	0.62	0.60	0.005	23.65	24.36	23.71	0.22
8	1.45	1.46	1.47	0.02	0.60	0.59	0.59	0.003	23.40	23.53	23.47	0.19
Overall mean	1.18	1.21	1.18	0.01	0.59^{b}	0.60^{b}	0.58^{a}	0.002	22.01^{a}	22.61^{b}	21.91^{a}	0.08

0 represents mean values of the adaptation period*

Means bearing different superscripts in a row differ significantly (P<0.05)

Table 4.21. Effect of dietary level of CSM on fortnightly hemicellulose (HC) intake in buffalo calves

Fortnight	HCI (kg/animal/day)				HCI (kg/100 kg body weight)				HCI (g/kg $W^{0.75}$)			
	C	**T1**	**T2**	**SEM**	**C**	**T1**	**T2**	**SEM**	**C**	**T1**	**T2**	**SEM**
0*	0.81	0.84	0.79	0.01	0.55	0.56	0.52	0.004	18.82	19.51	18.33	0.15
1	0.86	0.91	0.86	0.02	0.53	0.55	0.52	0.004	18.54	19.80	18.62	0.15
2	0.98	1.00	0.98	0.02	0.57	0.57	0.55	0.003	20.28	20.64	20.14	0.14
3	1.01	1.01	1.00	0.02	0.53	0.52	0.51	0.004	19.42	19.43	19.04	0.17
4	0.92	0.94	0.89	0.02	0.45	0.46	0.43	0.006	16.73	17.30	16.24	0.24
5	0.90	0.91	0.89	0.02	0.42	0.42	0.40	0.004	15.83	16.01	15.37	0.18
6	1.00	1.02	1.02	0.02	0.43	0.45	0.44	0.004	16.71	17.48	17.22	0.20
7	1.10	1.10	1.14	0.02	0.47	0.48	0.48	0.004	18.17	18.54	18.75	0.20
8	1.20	1.17	1.19	0.02	0.49	0.48	0.48	0.003	19.30	18.81	18.90	0.16
Overall mean	0.97	0.99	0.97	0.01	0.49[b]	0.50[b]	0.48[a]	0.002	18.20[a]	18.62[b]	18.07[a]	0.07

0 represents mean values of the adaptation period*

Means bearing different superscripts in a row differ significantly ($P<0.05$)

4.2.2. Effect of dietary level of CSM on nutrient intake in buffalo calves

The overall mean DM intake (kg/animal/d) was higher (P<0.05) in T1 (5.00) group than control group (4.80). However, DMI in T2 (4.95 kg/animal/d) group was similar to that in T1 group (5.0 kg/animal/d) (Table 4.14 and Fig. 9). The DMI as %BW in the current study was highest (P<0.05) in T1 (2.51) group. The DMI (g/kg $W^{0.75}$) was highest (P<0.05) in T1 (93.88) group followed by T2 (91.87) group and lowest (P<0.05) in C (89.70) group. However, Nomeary et al. (2021) reported no significant difference in DM intake among sheep groups fed black cumin seed meal, cottonseed meal and sesame seed meal replacing soybean meal in the ration. Kannan et al. (2013) also reported non-significant difference in DM intake of lambs fed 40 % raw CSM, 40 % raw CSM supplemented with vitamin E and 40 % CSM with 1.5 % calcium hydroxide replacing soybean meal. However, our results are similar to that of Solomon et al. (2008) who reported significant difference (P<0.01) in DM intake of goats supplemented with the high level of CSM than those on the control treatment.

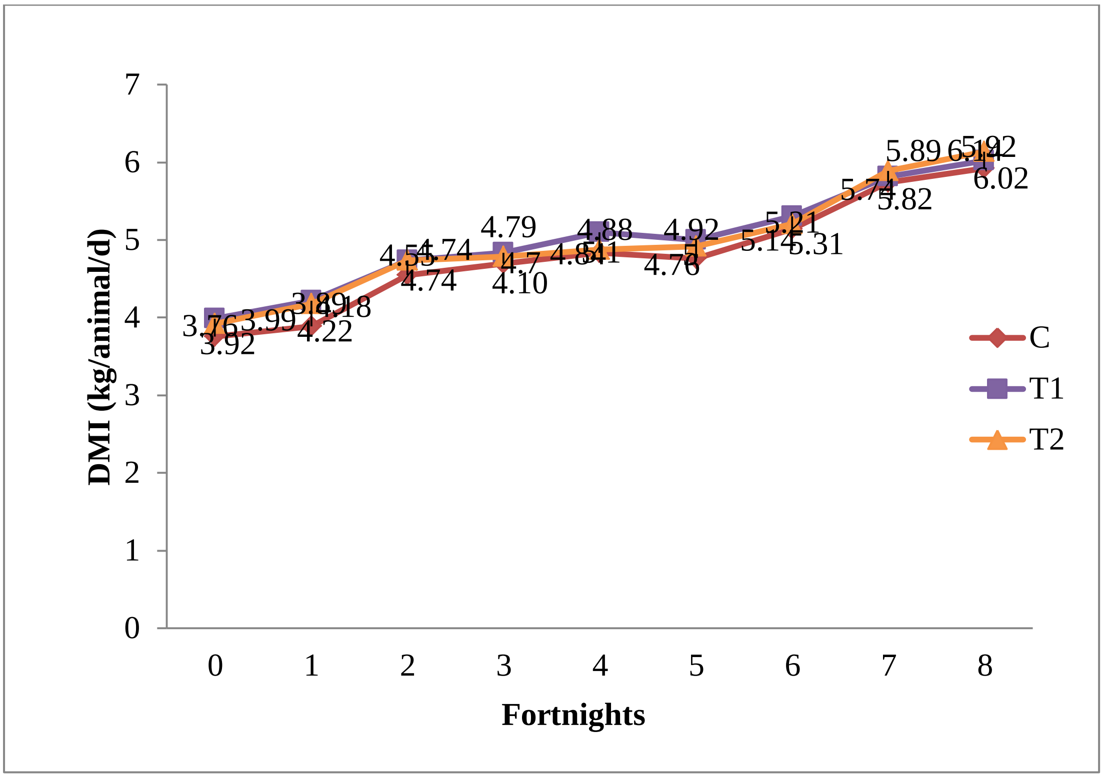

Fig. 9. Effect of dietary level of CSM on fortnightly DMI (kg/animal/d) in buffalo calves

The overall mean OM intake (kg/animal/d) was higher (P<0.05) in T1 (4.50) and T2 (4.47) groups than C (4.33) group (Table 4.15 and Fig. 10). The OMI (% BW) was higher (P<0.05) in T1 (2.25) group than C (2.18) and T2 (2.20) groups. The OM intake (g/kg $W^{0.75}$) was lowest (P<0.05) in C (80.72) group and highest (P<0.05) in T1 (84.52) group followed by T2 (82.80) group. Our results are contrary to those of Tripathi et al. (2012) who reported no significant difference in lambs fed Bt-CSM and C-CSM replacing groundnut meal.

The overall mean CP intake (kg/animal/d) was higher (P<0.05) in T1 (0.77) and T2 (0.77) groups than control (0.73) group (Table 4.16). The lower mean CP intake in control group may be due to slightly lower DM intake in this group. The CP intake (% BW) was highest (P<0.05) in T1 (0.382) group followed by T2 (0.378) group and was lowest (P<0.05) in C (0.366) group. The CPI (g/kg $W^{0.75}$) was similar in both T1 (14.33) and T2 (14.22) groups and was higher (P<0.05) than C (13.56) group (Table 4.16). However, Kannan et al. (2013) reported no significant difference in CP intake of lambs fed raw CSM, raw CSM supplemented with vit E and CSM treated with 1.5% $Ca(OH)_2$ replacing soybean meal. Tripathi et al. (2012) also reported similar CPI among lambs fed Bt-CSM and CSM as a replacement of groundnut meal. However, our results are in tune with those of Solomon et al. (2008) who reported higher (P<0.01) CPI (g/day) in CSM supplemented goats compared to control group fed hay.

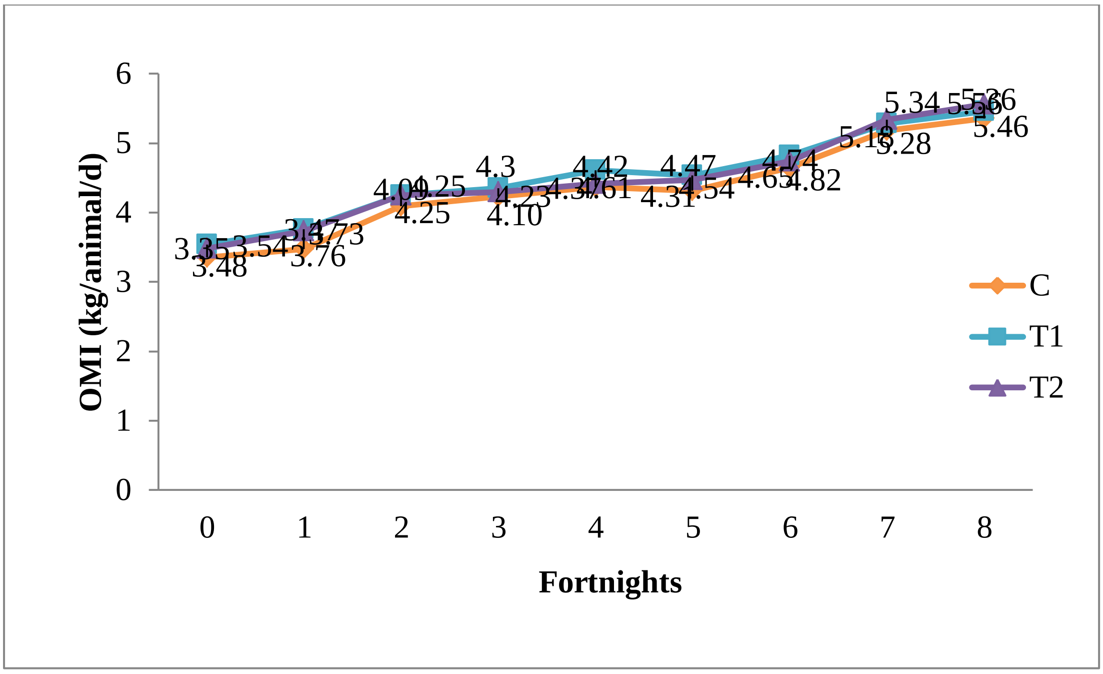

Fig. 10. Effect of dietary level of CSM on fortnightly OMI (kg/animal/d) in male buffalo calves

The overall mean EE intake (kg/animal/day, % BW and g/kg $BW^{0.75}$) during the whole experimental period was highest (P<0.05) in T1 group and lowest (P<0.05) in control group (Table 4.17). Our results are contrary to those of Silva et al. (2009) who reported no significant difference in EE intake of lactating cows fed cottonseed meal replacing 0, 25, 50, 75 and 100% of soybean meal in the concentrate. The overall mean NDF intake (kg/animal/d) of C, T1 and T2 groups was 2.49, 2.56 and 2.50, respectively and was similar among the groups (Table 4.18). The NDF intake (% BW) was highest (P<0.05) in T1 (1.29) group followed by C (1.26) and lowest (P<0.05) in T2 (1.24) group. NDF intake (g/kg $W^{0.75}$) was highest (P<0.05) in T1 (48.21) group and similar in C (46.53) and T2 (46.50) groups. Tripathi et al. (2012) also reported similar NDFI (g/day) among groups fed Bt-CSM and CSM which is similar to present study. Our results are contrary to those of Solomon et al. (2008) who reported higher (P<0.01) NDFI (g/day) in CSM supplemented goats compared to control group.

The overall mean ADF intake (kg/animal/d) in C, T1 and T2 groups was 1.52, 1.58 and 1.53 kg/animal/d, respectively and the values were similar among the groups (Table 4.19). The ADF intake (% BW and g/kg $W^{0.75}$) was highest (P<0.05) in T1 group compared to other groups. Our results are similar to those of Tripathi et al. (2012) who reported non-significant difference in ADFI among lambs fed Bt-CSM and CSM replacing groundnut meal.

The overall mean cellulose intake (kg/animal/d) was similar among C (1.18), T1 (1.21) and T2 (1.18) groups (Table 4.20). The CL intake (% BW) was higher (P<0.05) in C (0.59) and T1(0.60) groups than T2 (0.58) group. The CL intake (g/kg $W^{0.75}$) was highest (P<0.05) in T1 (22.61) group. Tripathi et al. (2012) reported no significant difference in cellulose intake among groups fed Bt-CSM and CSM replacing groundnut meal.

The overall mean hemicellulose intake (HCI) (kg/animal/day) varied non significantly among the groups (C, T1, T2) (Table 4.21). HCI (% BW) in T1 (0.50) group was higher (P<0.05) than T2 (0.48) group, however, it was similar to that in C (0.49) group. The HCI (g/kg $W^{0.75}$) was higher in T1 (18.62) group than C and T2 groups. Our results are similar with those of Tripathi et al. (2012) who reported no significant difference in hemicellulose intake in lambs fed conventional and Bt cottonseed meal replacing groundnut oil meal.

4.2.3. Effect of dietary level of CSM on digestibility of nutrients in buffalo calves

The values of various proximate principles and fiber fractions in concentrate mixtures with graded levels of CSM (0%, 75% and 100% replacing SBM), green fodder (berseem, oats and mustard mixed) and wheat straw fed to buffalo calves during metabolic trial are given in Table 4.22. The DM was 91.0, 91.0 and 93.0 in concentrate mixtures fed to C, T1 and T2 groups, respectively. The DM in green fodder and wheat straw was 17.30 and 86.80%, respectively. OM (%) in concentrate mixtures fed to control, T1 and T2 groups was 90.45, 90.85 and 90.55%, respectively. Green fodder and wheat straw fed during metabolic trial had 88.53 and 92.40% OM, respectively.

Table 4.22. Chemical composition of feedstuffs offered during metabolic trial, %DM basis

Parameter	C (0% CSM)	T1 (75% CSM)	T2 (100% CSM)	Green fodder	Wheat straw
DM	91.00	91.00	93.00	17.30	86.80
OM	90.45	90.85	90.55	88.53	92.40
CP	20.07	20.52	20.86	16.50	4.40
EE	5.36	5.36	5.64	3.35	1.14
Total ash	9.55	9.15	9.45	11.47	7.60
NDF	31.40	30.46	30.73	48.30	84.90
ADF	16.40	16.05	14.75	31.90	58.65
Cellulose	9.40	8.10	7.70	20.60	46.90
Hemicellulose	15.00	14.41	15.98	16.40	26.25
TCHO	65.02	64.97	64.05	68.68	86.86

DM- Dry matter, OM- Organic matter, CP- Crude protein, EE- Ether extract, NDF- Neutral detergent fibre, ADF- Acid detergent fibre, TCHO- Total carbohydrates

The CP content was 20.07%, 20.52% and 20.86% in concentrate mixtures fed to control, T1 and T2 groups, respectively, indicating that concentrate mixtures were isonitrogenous (Table 4.22). The CP in green fodder and wheat straw was 16.50% and 4.40%, respectively. The EE content in control concentrate was 5.36% and in concentrates fed to T1 and T2 groups was 5.36% and 5.64%, respectively. The EE content in green fodder and wheat straw was 3.35% and 1.14%, respectively. Total ash content ranged from 9.15% (T1 group) to 9.55% (C group). Green fodder and wheat straw fed during metabolic trial contained 11.47% and 7.60 %, total ash, respectively. NDF content was 31.40%, 30.46% and 30.73% in control, T1 and T2 concentrate mixtures, respectively. NDF content in green fodder and wheat straw was 48.30% and 84.90%, respectively. ADF content was 16.40%, 16.05% and 14.75% in control, T1, and T2 concentrate mixtures, respectively. Green fodder (berseem, oats, mustard mixed) and wheat straw had 31.90% and 58.65% ADF content. Cellulose content in control, T1 and T2 concentrates was 9.40%, 8.10% and 7.70%, respectively. Green fodder and wheat straw had 20.60% and 46.90% cellulose, respectively.

The DM intake (kg/d) during metabolic trial was 5.40, 5.02 and 5.12 in control (0% CSM), T1 (75% CSM) and T2 (100% CSM) groups, respectively and was similar among the groups (Table 4.23). All the groups had similar DMI indicating that CSM inclusion in the diet of buffalo calves had no adverse effect on palatability of the diet. However, Tripathi et al. (2014) reported higher DM intake in lambs fed Bt-CSM based diet replacing groundnut oil meal.

The DM digestibility (%) was similar ($P>0.05$) among the groups. The DM digestibility in C group was 63.38% which was similar to that of T1 (61.24%) and T2 (63.17%) groups (Table 4.23). The results are in agreement with those of Fadel and Ashmawy (2015) who reported no significant difference in DM digestibility of goats fed cottonseed meal untreated and CSM treated with tannin. The OM digestibility in C, T1 and T2 groups was 66.64%, 64.80% and 66.42%, respectively. There was no significant ($P>0.05$) difference in OM digestibility among the groups. Similarly, Fadel and Ashmawy (2015) also reported no significant difference in OM digestibility (%) in goats fed CSM untreated and CSM treated with tannin. Silva et al. (2009) also reported that lactating cows that were fed with cottonseed meal at 0, 25, 50, 75 and 100% replacing soybean meal had shown no significant difference in OM digestibility.

The CP digestibility in the present study was similar in C (75.43%), T1 (76.29%) and T2 (76.06%) groups fed 0%, 75% and 100% CSM replacing SBM, respectively (Table 4.23). Tripathi et al. (2014) also reported non-significant difference in CP digestibility in lambs fed Bt-CSM and CSM replacing groundnut oil meal. Similarly, Silva et al. (2009) also reported no significant difference in CP digestibility in cows fed with cottonseed meal replacing soybean meal. However, our results are contrary to those of Nomeary et al. (2021) who reported significant difference in CP digestibility (%) which was higher ($P<0.05$) in lambs fed cottonseed meal than those fed black cumin seed meal and sesame seed meal based diets.

Table 4.23. Effect of dietary level of CSM on nutrient digestibility (%) in buffalo calves

Parameter	C (0% CSM)	T1 (75% CSM)	T2 (100% CSM)	SEM
DM intake, kg/d	5.40	5.02	5.12	0.15
% Nutrient digestibility				
DM	63.38	61.24	63.17	0.83
OM	66.64	64.80	66.42	0.76
CP	75.43	76.29	76.06	0.55
NDF	53.45	51.80	51.94	1.05
ADF	49.34	46.48	48.53	1.19
Cellulose	60.50	56.06	57.72	1.02

DM- Dry matter, OM- Organic matter, CP- Crude protein, NDF- Neutral detergent fibre, ADF-Acid detergent fibre

The NDF digestibility in C, T1 and T2 groups was 53.45%, 51.80% and 51.94%, respectively (Table 4.23). No significant difference was observed among groups. The results are in accordance with those of Tripathi et al. (2014) who reported similar NDF digestibility in lambs fed Bt-CSM and CSM replacing groundnut oil meal. Silva et al. (2009) also reported no significant difference in NDF digestibility in cows which were fed cottonseed meal at graded levels replacing soybean meal. The ADF digestibility was 49.34%, 46.48% and 48.53% in C, T1 and T2 groups, respectively (Table 4.23). No significant difference ($P>0.05$) was observed in ADF digestibility among the groups. Solomon et al. (2008) also reported no significant difference in apparent digestibility of ADF among control and CSM supplemented goats. However, Tripathi et al. (2012) reported significantly higher ($P<0.05$) ADF digestibility in lambs fed CSM based diet than Bt cottonseed meal diet and control (groundnut oil meal) diet.

The cellulose digestibility in C, T1 and T2 groups was 60.50%, 56.06% and 57.72%, respectively (Table 4.23). No significant effect (P>0.05) was seen on cellulose digestibility in buffalo calves fed graded levels of CSM. However, Tripathi et al. (2012) reported significantly higher (P<0.05) cellulose digestibility in CSM and Bt-CSM supplemented lambs replacing groundnut meal.

4.2.4 Effect of dietary level of CSM on nitrogen balance in male buffalo calves

The total nitrogen intake (g/d) in C, T1 and T2 groups was 126.06, 121.75 and 124.87 g/d, respectively and varied non significantly among the groups indicating no effect of CSM inclusion on N intake (Table 4.24 and Fig. 11). Tripathi et al. (2012) also reported similar N intake (g/d) among the lambs fed CSM, Bt-CSM and control (groundnut oil meal) groups. Lorena-Rezende et al. (2012) also observed no significant difference in N intake in barrows fed cottonseed meal based diets with or without enzymes and conventional corn-soybean meal based diet.

Table 4.24. Effect of dietary level of CSM on nitrogen balance (g/d) in buffalo calves

Parameter	C	T1	T2	SEM
Total nitrogen intake	126.06	121.75	124.87	3.50
Urinary N	29.18	32.91	29.36	1.00
Faecal N	30.27	28.74	29.76	0.86
Total N outgo	59.45	61.64	59.12	1.43
N balance	66.61	60.10	65.74	2.73

The mean urinary N (g/d) excretion in T1 (32.91) and T2 (29.36) groups was similar to control (29.18) group (Table 4.24 and Fig 11). Li et al. (2012) also reported no significant difference in N excretion from urine of pigs fed cottonseed meal diets and control (corn and soybean meal) diet. Tripathi et al. (2012) also reported similar urinary N excretion among the lambs fed CSM, Bt-CSM and control (groundnut oil meal) diet. The mean faecal N output was similar in C, T1 and T2 groups. The values of faecal N in C, T1 and T2 groups were 30.27, 28.74 and 29.76 g/d, respectively. Khan et al. (2000) also reported similar nitrogen excretion through faeces in lambs fed cottonseed meal based rations (40% untreated CSM, formaldehyde treated mechanical and solvent extracted CSM). Tripathi et al. (2012) also reported similar faecal N excretion among the lambs fed CSM, Bt-CSM and control (groundnut oil meal) groups.

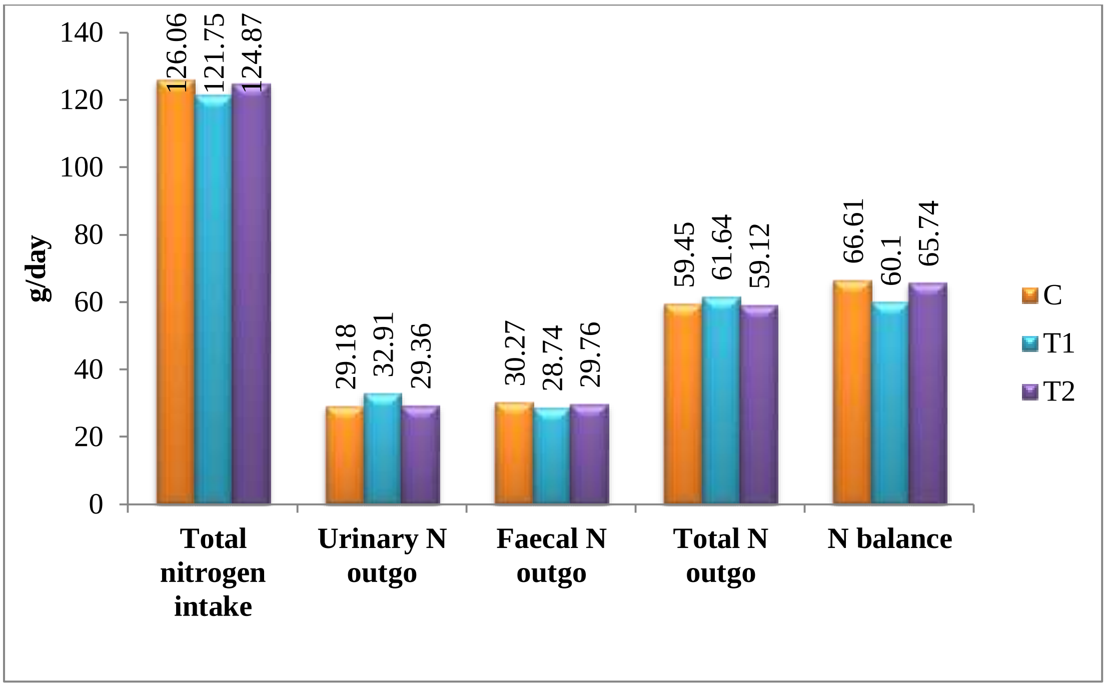

Fig. 11. Effect of dietary level of CSM on nitrogen balance (g/d) in buffalo calves

The mean total N outgo in T1 and T2 groups was similar to C group (Table 4.24 and Fig. 11). The N balance in C, T1 and T2 groups was 66.61, 60.10 and 65.74g/d, respectively. However, Lorena-Rezende et al. (2012) reported that the diets containing cottonseed meal (30% replacement with control diet with or without enzymes) resulted in higher ($P<0.01$) amount of retained nitrogen compared to control (corn and soybean meal) diet in barrows. Tripathi et al. (2012) reported no significant difference in N retention among lambs fed CSM and Bt-CSM diets replacing groundnut oil meal.

4.2.5 Effect of dietary level of CSM on blood profile in male buffalo calves

The blood glucose (mg/dl) levels in the present study were 71.33, 69.07 and 70.00 mg/dl in C, T1 and T2 groups, respectively (Table 4.25). The values were within normal physiological range. Kannan et al. (2013) also reported non-significant difference in blood glucose (mg/dl) in lambs fed 40 % raw CSM (RCSM), 40 % raw CSM supplemented with vitamin E (ERCSM), and 40 % CSM with 1.5 % calcium hydroxide (CaCSM) replacing soybean meal. The results are also in agreement with those of Saijpaul et al. (2006) who reported no significant difference in blood glucose in lactating crossbred cows fed water soaked whole linted cottonseed (WSWLCS) replacing equal quantity of control concentrate mixture. The values of BUN in the current study were 27.09, 28.89 and 26.85mg/dl in C, T1 and T2 groups, respectively. All the values were within normal physiological range. He et al. (2015) also reported no significant difference in BUN (mg/dl) in layers fed CSM at 25%, 50%, 75% and 100% replacing soybean meal in the diets. Kannan et al. (2013) also reported no significant difference in BUN in lambs fed 40% raw CSM, 40% vit E supplemented raw CSM and 40% calcium hydroxide treated CSM replacing soybean meal.

Table 4.25. Effect of dietary level of CSM on blood profile in buffalo calves

Parameter	C	T1	T2	SEM
Glucose, mg/dl	71.33	69.07	70.00	0.16
BUN, mg/dl	27.09	28.89	26.85	1.15
Total protein, g/dl	6.61	6.79	6.20	0.16
Albumin, g/dl	3.53	3.51	3.42	0.05
Triglycerides, mg/dl	25.31	29.12	27.75	1.30
Cholesterol, mg/dl	86.90	83.26	81.40	1.89
AST, U/L	98.94	101.51	96.11	1.05
ALT, U/L	29.12	28.28	29.87	1.32
GGT, U/L	10.97	12.90	13.20	0.85

The total protein and albumin in the present study were 6.61, 6.79 and 6.20 g/dl and 3.53, 3.51 and 3.42g/dl in C, T1 and T2 groups, respectively and were similar among the groups. The results of the present study are in accordance with those of Thirumalaisamy et al. (2016) who reported similar total protein and albumin in broiler chicks fed CSM at 2% and 4% diets replacing soybean meal. Similarly, He et al. (2015) also reported no significant difference in total protein and albumin in layers fed CSM at 25%, 50%, 75%, 100% replacing soybean meal in the diet. Kannan et al. (2013) also reported no significant difference in serum albumin (g/dl) in lambs fed 40% raw CSM, 40% vit E supplemented raw CSM and 40% calcium hydroxide treated CSM replacing soybean meal.

The values of triglycerides and cholesterol were similar among the groups and ranged from 25.31 to 29.12 mg/dl and 81.40 to 86.90 mg/dl, respectively (Table 4.25). The results of the present study are in accordance with those of Thirumalaisamy et al. (2016) who reported similar cholesterol and triglycerides in broiler chicks fed CSM at 2% and 4% diets replacing soybean meal. Similarly, Saijpaul et al. (2006) also reported no significant difference in cholesterol in lactating crossbred cows fed water soaked whole linted cottonseed (WSWLCS) replacing equal quantity of control concentrate mixture.

The values of AST, ALT and GGT were 98.94, 101.51, 96.11 U/l; 29.12, 28.28, 29.87 U/l and 10.97, 12.90, 13.20 U/l in C, T1and T2 groups, respectively (Table 4.25). AST, ALT and GGT had no significant difference among the groups. Our results are in accordance with those of He et al. (2015) who reported no significant difference in AST and ALT in layers fed CSM at 25%, 50%, 75%, 100% replacing soybean meal. Similarly, Kannan et al. (2013) also reported no significant difference in AST and ALT in lambs fed 40% raw CSM, 40% vit E supplemented raw CSM, 40% calcium hydroxide treated CSM replacing soybean meal.

4.2.6. Effect of dietary level of CSM on cell mediated immune response in buffalo calves

The cell mediated immune (CMI) response of the buffalo calves after administration of phyto hemagglutinin-P (PHA-P) is shown in Table 4.26. Treatment groups T1 and T2 exhibited similar CMI response to control group.

Table 4.26. Effect of dietary level of CSM on *in vivo* delayed type of hypersensitivity response (DTH response) to phyto haemagglutinin-P (PHA-P) in buffalo calves

Hours post - inoculation	C	T1	T2	SEM
0	100	100	100	0.00
24	148.24	147.80	128.81	4.93
48	127.48	120.72	114.70	4.82
72	115.57	110.27	106.14	3.33
96	103.35	100.82	100.72	0.72

The CMI response was higher after 24 h of antigen administration in all the groups as compared to response after 48 h. Decrease in skin thickness i.e., CMI response after 48h, 72h and 96h was almost similar in all the groups. Our results are contrary to those of Nagalakshmi et al. (2001) who reported that cooked, $Ca(OH)_2$ and iron treated raw CSM showed better cell mediated immunity (CMI) than raw CSM where the diets included 40% of either raw, 45 min cooked, 1% calcium hydroxide or iron treated CSM replacing deoiled groundnut cake in lambs. The similar immune response in CSM fed groups to that of control group in the present study could be due to similar nutrient utilization which ultimately affects the cellular integrity and immune response of the animals.

4.2.7. Effect of dietary level of CSM on performance of buffalo calves

The initial body weight (kg) of buffalo calves in C, T1 and T2 groups was 149.29, 150.08 and 150.25 kg at the beginning of the experiment and the final body weight at the end of the experiment was 238.25, 244.83 and 248.83 kg, respectively (Table 4.27). The gain in body weight during the experimental period of 120 days was 88.96, 94.75 and 98.58 kg/animal in control, T1 and T2 groups, respectively (Table 4.27 and Fig. 12). No significant difference was observed among the groups, indicating that CSM was not having any adverse effect on body weight of buffalo calves. The average daily gain in T2 group was numerically higher than other groups (Table 4.27 and Fig. 13). The FCR in T1 (6.61) group was similar to control (6.61) group. However, FCR in T2 (6.38) group was marginally better than C and T1 groups.

Table 4.27. Effect of dietary level of CSM on changes in body weight (kg) of buffalo calves

Parameter	**C**	**T1**	**T2**	**SEM**
Initial body weight	149.29	150.08	150.25	9.93
Final body weight	238.25	244.83	248.83	13.76
BW gain (120 days)	88.96	94.75	98.58	4.54
Average daily gain, g	741.32	789.58	821.53	37.82
FCR	6.61	6.61	6.38	0.22

The results of the present study are in accordance with those of Tripathi et al. (2014) who reported no significant difference in final BW, total BW gain and ADG among control, CSM and Bt-CSM fed lambs for 120 days. Ojewola et al. (2006) also reported no significant difference in birds mean daily weight gain and feed to gain ratio in which cottonseed meal was substituted for soybean meal at 0, 25, 50, 75 and 100%.

However, Nomeary et al. (2021) reported highest (P<0.05) daily BW gain in lambs fed CSM diet compared to lambs fed black cumin seed meal, sesame seed meal and soybean meal diets, respectively. Hassanabadi et al. (2009) also reported improved (P<0.05) weight gain and FCR in lysine supplemented CSM with 5, 10, 15, 20 % levels in broiler chicks as compared to control group fed corn and soybean meal based diet.

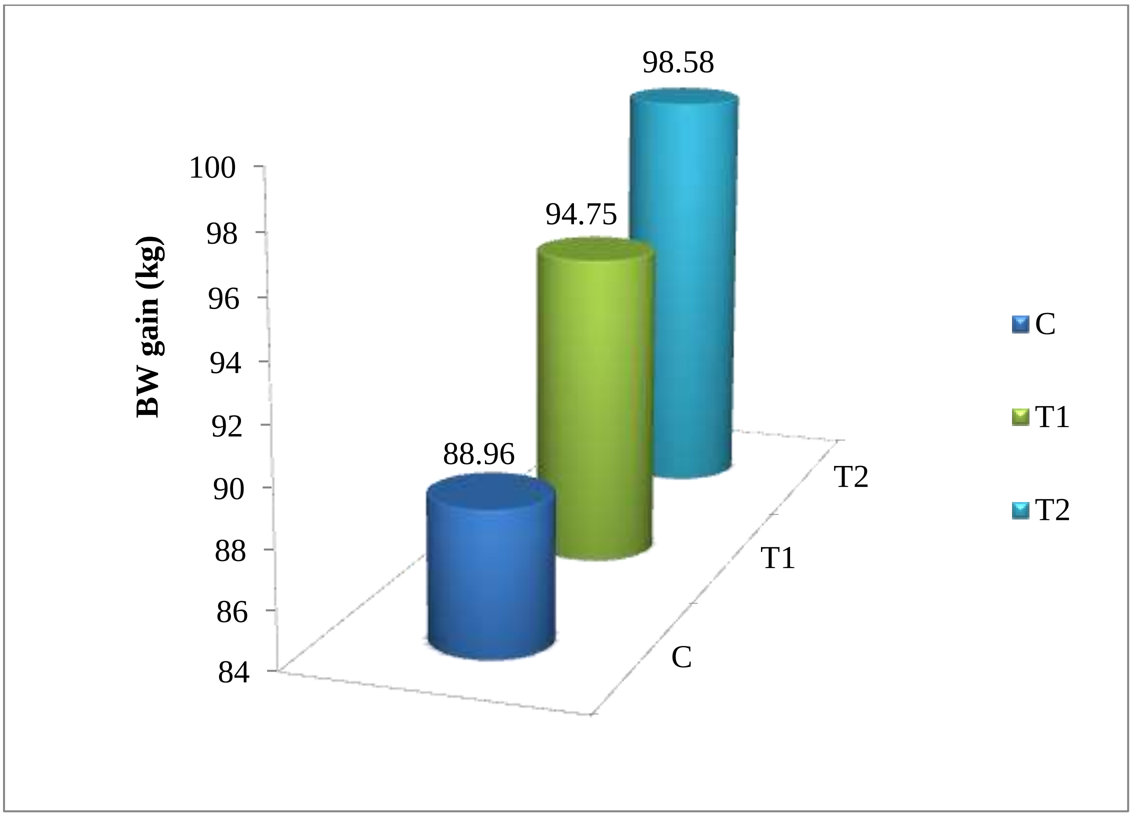

Fig. 12. Effect of dietary level of CSM on body weight gain (kg) in 120 days in buffalo calves

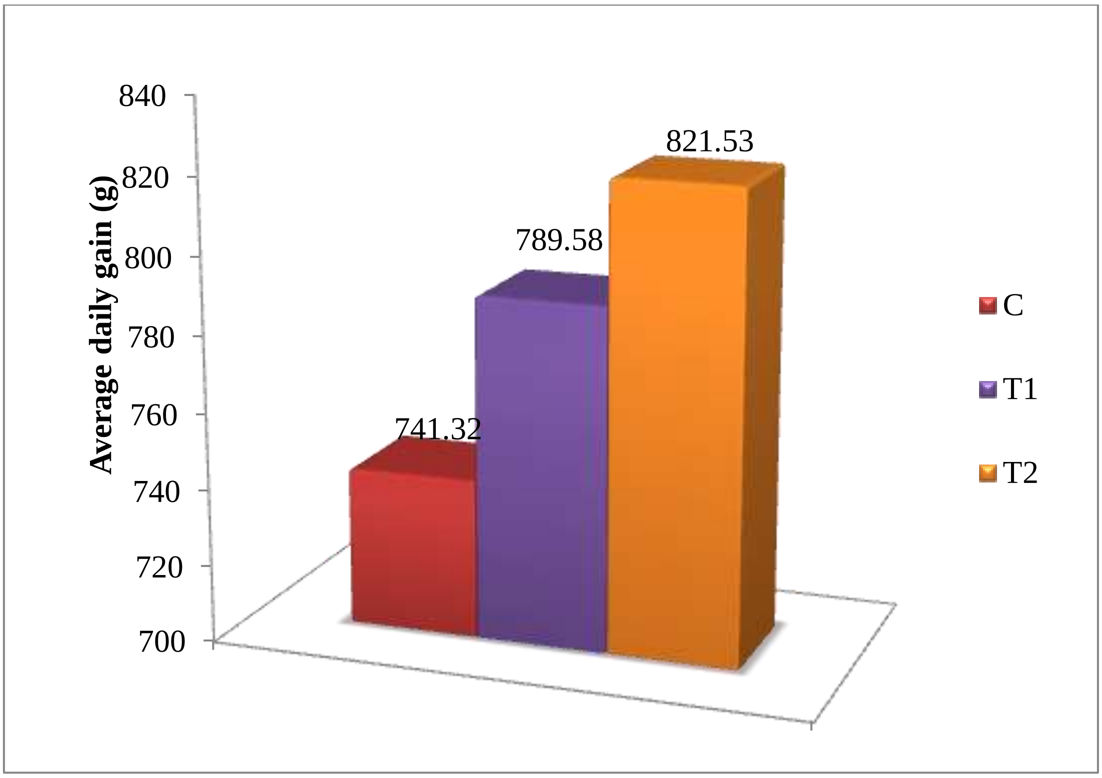

Fig. 13. Effect of dietary level of CSM on average daily gain (g) in buffalo calves

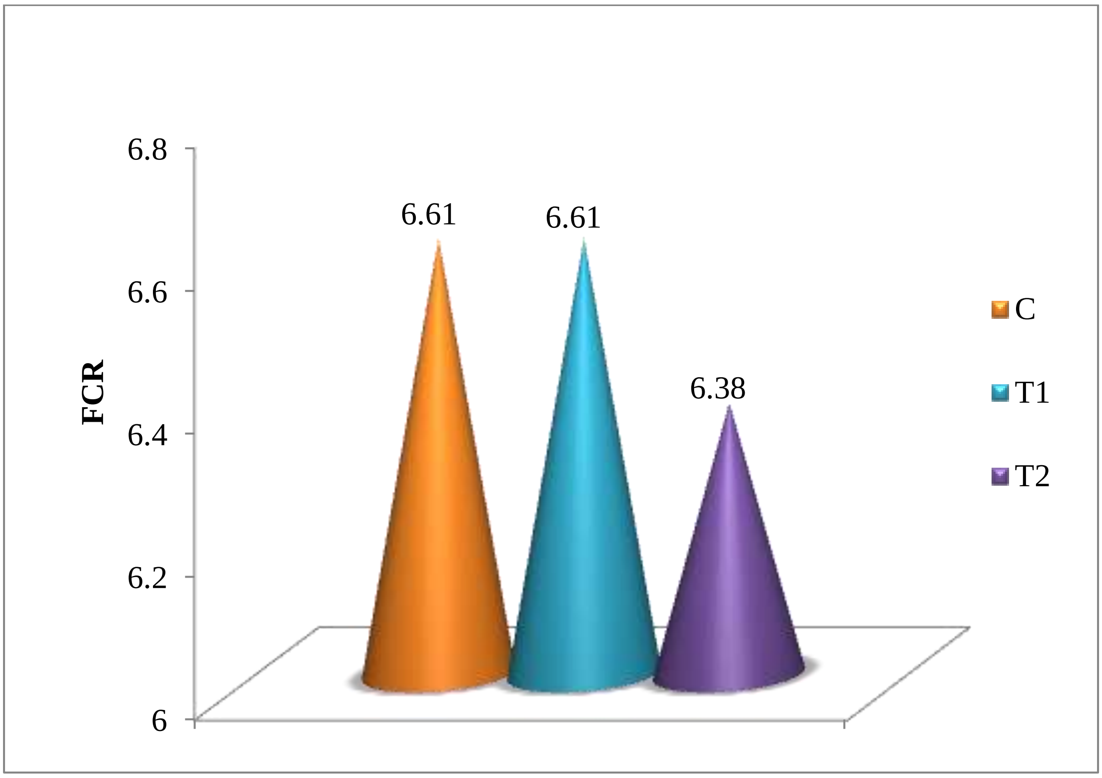

Fig. 14. Effect of dietary level of CSM on feed conversion ratio (FCR) in buffalo calves

4.2.8. Comparative cost of feeding

The economics of feeding cottonseed meal (CSM) replacing soybean meal (SBM) at graded levels in the diet of buffalo calves during 120 days is given in Table 4.28.

Table 4.28. Comparative cost of feeding graded levels of CSM as a replacement of soybean meal to buffalo calves over 120 days experimental period

Parameter	**C**	**T1**	**T2**
Amount of fresh feed given, kg			
Concentrate mixture	1191.20	1207.13	1222.25
Green fodder	6253.06	6338.62	6412.24
Wheat straw	912.60	924.78	936.14
Feed cost (Rs/kg)			
Concentrate mixture	22.77	21.11	20.55
Green fodder	2.50	2.50	2.50
Wheat straw	4.50	4.50	4.50
Total feed cost for 120 days (Rs)	46860.59	45487.59	45365.40
Total BW gain in 120 days/ group (kg)	355.84	379.00	394.33
Feed cost (Rs/kg BW gain)	131.69	120.02	115.04

At the time of experiment, the cost of CSM and SBM was Rs. 3524/Q and Rs. 5000/Q, respectively. The cost of concentrate mixtures fed to buffalo calves was worked out to be Rs. 22.77/kg, Rs. 21.11/kg and Rs. 20.55/kg for control, T1 and T2 groups, respectively. Replacement of SBM by CSM reduced the cost of concentrate mixtures fed to T1 and T2 groups by 7.29% and 9.75%, respectively as compared to concentrate mixture fed to control group. CSM inclusion at 75% and 100% levels replacing SBM resulted in net saving of Rs. 11.67 and 16.65 per kg BW gain in T1 and T2 groups, respectively. Thus, based on the results of the present study, it could be concluded that feeding of CSM to male buffalo calves was cost effective.

The results obtained in the present study are in tune with those of Nomeary et al. (2021) who reported that CSM had higher values of revenue and relative efficiency i.e., it was more economical in lambs compared to black cumin seed meal and sesame seed meal replacing soybean meal.

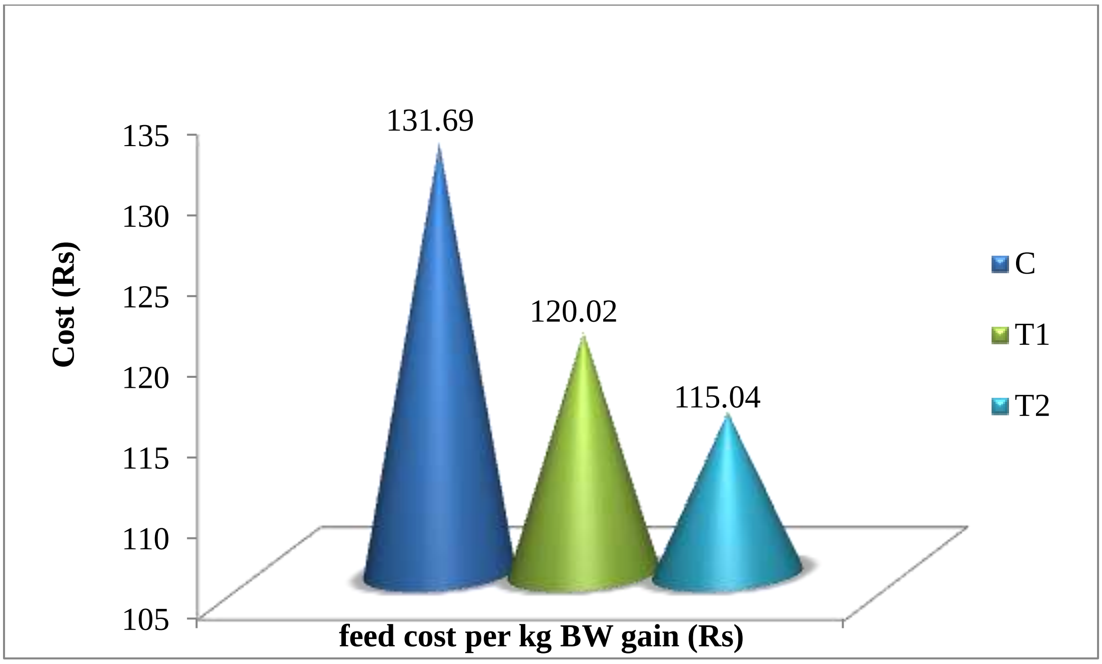

Fig. 15. Effect of dietary level of CSM on feed cost per kg BW gain in buffalo calves

Attanayaka et al. (2016) also reported that least cost of production per bird was observed in the diet containing 15% CSM when SBM was replaced at the levels of 0%, 5%, 10% and 15% CSM. They concluded that CSM could be used to replace SBM up to 10% of the diet safely and would be significant in commercial perspective especially when the price of SBM is unusually high. Fadel and Ashmawy (2015) reported that the protected linseed meal and cotton seed meal (treated with quebracho tannin) at 2% resulted in better economics in goats when compared to untreated linseed and cottonseed meal. Ojewola et al. (2006) observed least cost when soybean meal was excluded completely and replaced by CSM in broilers. Khan et al. (2002) reported that economic efficiency was better on CSM based diets with and without lysine and methionine compared to control diet in 14-21 d old crossbred calves.

CHAPTER V

SUMMARY AND CONCLUSIONS

The present study was performed to evaluate the effect of feeding cottonseed meal (CSM) as an alternate protein source in the diet of male buffalo calves. The SBM (0, 25, 50, 75 and 100%) was replaced by CSM in graded levels in the concentrate mixtures. Firstly, proximate composition of ingredients (CSM and conventional oil cakes), concentrate mixtures and TMRs was analyzed. *In vitro* evaluation of CSM and conventional oil cakes, five concentrate mixtures and TMRs containing graded levels of CSM (0, 25, 50, 75 and 100%) replacing SBM on N-basis was done. *In vivo* experiment was conducted on male buffalo calves to study the effect of CSM inclusion at different levels on fortnightly body weight, nutrient intake, digestibility of nutrients, nitrogen balance, blood parameters, cell mediated immune response and performance. The results obtained during the course of this study have been summarized as below:

Chemical composition of CSM and conventional oil cakes

The organic matter (OM) content in cottonseed meal (CSM), soybean meal (SBM) and groundnut cake (GNC) was 92.5%, 89.35% and 93.06%, respectively. The OM content in mustard cake (MC) was 93.46% which was higher and OM present in deoiled mustard cake was 88.08% and it was lower than other oil seed cakes evaluated. The CP content of CSM was 47.85% while CP content of SBM was 47.76%. The CP content of GNC, MC and DMC was 43.64%, 39.59% and 42.01%, respectively. The ether extract (EE) of CSM and SBM was 1.71% and 1.36%, respectively. The EE content of MC (8.34%) was higher and GNC (0.91%) was lower than other oilseed cakes evaluated. EE content of DMC was 1.25%. The total ash content of CSM, SBM, GNC, MC and DMC was 7.50%, 10.65%, 6.94%, 6.53% and 11.91%, respectively. The NDF content of CSM (29.00%) was almost similar to SBM (30.73%) and NDF content of GNC, MC and DMC was 30.06%, 21.40% and 21.80%, respectively The ADF content in CSM, SBM, GNC, MC and DMC was 17.4%, 20.43%, 22.36%, 19.03% and 21.13%, respectively. The hemicellulose content of CSM, SBM, GNC, MC and DMC was 11.60%, 10.30%, 7.70%, 2.37% and 0.67%, respectively. ADL content in CSM, SBM, GNC, MC and DMC was 6.3%, 4.13%, 7.93%, 6.26% and 7.06%, respectively. The ADICP and NDICP content in CSM was

6.27% and 9.31%, respectively. The ADICP and NDICP in SBM was 7.04% and 16.53%, respectively. The ADICP and NDICP in CSM was lower than SBM. The carbohydrate content was 42.94%, 40.23%, 48.51%, 45.54% and 44.83%, in CSM, SBM, GNC, MC and DMC, respectively.

Chemical composition of concentrate mixtures

The OM of concentrates was 91.66% in concentrate 1, 91.32% in concentrate 2, 91.53% in concentrate 3, 92% in concentrate 4 and 91.98% in concentrate 5. All the concentrates mixtures were iso-nitrogenous as the CP content of concentrate mixtures varied from 20.07% to 20.95%. The ether extract content in concentrate mixtures varied from 5.26% to 5.64%. The total ash content was 8.33% in concentrate 1, 8.67% in concentrate 2, 8.46% in concentrate 3, 7.92% in concentrate 4 and 8.06% in concentrate 5. The NDF content in concentrate mixtures varied from 30.46% to 33.40%. ADF content in concentrate 1 was 15.16% and then from concentrate 2 to 5 it varied from 13.36% to 15.23%. The hemicellulose content varied from 16.24% to 19.04% in the concentrate mixture evaluated. The ADL content varied from 4.36% to 4.80%. The ADICP of the concentrate mixtures ranged from 7.22% to 7.98%. The NDICP in concentrate mixtures varied from 9.12% to 9.88%. The Total carbohydrate content in concentrates ranged between 65.09% to 66.24% indicating almost similar carbohydrate content in the concentrate mixtures.

Chemical composition of total mixed rations (TMRs)

The OM content of TMRs varied from 90.71% to 91.40%. The OM content increased with increasing level of cotton seed meal in TMRs. The CP content of TMRs varied from 15.07% to 15.83%. All the TMRs prepared were iso-nitrogenous. The ether extract content was almost similar and ranged from 3.09% to 3.30% in TMRs. The total ash content varied between 8.60% to 9.28% in TMRs. The NDF content varied from 56.40% to 59.30% in TMRs. The ADF content varied between 32.90% to 34.13% in TMRs. The hemicellulose content varied from 22.57% to 27.23%. The ADL content of TMRs was in range of 5.06% to 8.33%. The ADICP content varied from 5.89% to 6.84% in TMRs and NDICP content in TMRs ranged from 8.65% to 9.12%. The total carbohydrate content in TMRs ranged between 72.07% to 72.56%.

***In vitro* evaluation of CSM and conventional oil cakes**

The net gas production (NGP, ml/g DM/24h) in CSM (160.48) was lower (P<0.05) than SBM (198.04) and MC (199.55), however, it was similar to that of GNC (173.78) and DMC (164.95). The PF of various oilseed cakes tested in the present study varied in the following order as CSM>DMC>GNC>MC>SBM. The OM digestibility (%) was higher (P<0.05) in CSM (85.09) and MC (83.88) than the other oilseed cakes tested. The NDF digestibility (%) of CSM (52.44) was higher (P<0.05) than SBM (41.35) as well as other oilseed cakes tested. The microbial mass production (MMP, mg) was highest (P<0.05) in CSM (159.44) followed by DMC (134.44), GNC (125.23), MC (123.24) and lowest (P<0.05) in SBM (98.61). The efficiency of microbial mass production (EMMP, %) was higher (P<0.05) in CSM (53.73) and lower (P<0.05) in SBM (36.92) than the other oil seed cakes tested. The DM digestibility (%) was higher (P<0.05) in CSM (87.14) and DMC (86.72) than SBM (85.31). The short chain fatty acid (SCFA, mmole) production in CSM (0.71) was lower (P<0.05) than SBM (0.88), however, it was similar to that of GNC (0.77) and DMC (0.73 The metabolizable energy (ME, MJ/kg DM) in CSM (9.89) was higher (P<0.05) than DMC (9.08) but it was lower (P<0.05) than SBM (10.73) and MC (11.96). The ammonia nitrogen (mg/dl) was lowest (P<0.05) in SBM (64.50) and highest (P<0.05) in GNC (78.50) among the oilseed cakes tested with intermediate values for CSM (70.0), MC (71.0) and DMC (72.0). The fermentable CO_2 in CSM (51.11) was higher (P<0.05) than other oilseed cakes tested viz., SBM (49.62), GNC (49.36), MC (49.77) and DMC (49.04). The fermentable CH_4 (mmol) in CSM (30.64) was lower (P<0.05) than SBM (31.35) and DMC (31.27). The acetic acid (mM/dl) production in CSM (4.04) was lower (P<0.05) than SBM (4.44), MC (4.37) and DMC (4.62). The propionic acid, isobutyric acid and isovaleric acid production varied non-significantly among the protein sources evaluated. The butyric acid (mM/dl) production in CSM (0.69) was higher (P<0.05) than SBM (0.60), GNC (0.58) and DMC (0.60), however, it was similar to that in MC (0.67). The valeric acid (mM/dl) in CSM (0.15) was lower (P<0.05) than other protein sources evaluated. The TVFA (mM/dl) production in CSM (6.63) was higher (P<0.05) than GNC (6.45) but lower (P<0.05) than SBM (6.97), MC (7.11) and DMC (7.25). The acetate: propionate ratio in SBM was higher (P<0.05) than CSM, GNC and MC, however, it was similar to DMC. The relative proportion (%) of acetic acid in SBM (63.71) was higher

(P<0.05) than CSM (60.90), GNC (60.97) and MC (61.41). The relative proportion (%) of propionic acid in CSM (20.09) was similar to SBM (19.29), MC (19.53). The relative proportion of isobutyric acid and isovaleric acid was similar among the protein sources evaluated. The relative proportion (%) of butyric acid was highest (P<0.05) in CSM (10.42) followed by MC (9.46), GNC (9.06), SBM (8.63) and DMC (8.31). The relative proportion of valeric acid was similar in CSM and SBM and was lower (P<0.05) than other protein sources evaluated.

The H- recovery (%) in CSM (78.16) was higher (P<0.05) than SBM (75.12), MC (74.73) and DMC (73.22), however, it was lower (P<0.05) than GNC (79.57). The hydrogen consumed via methane was lowest (P<0.05) in GNC (4.79). The hydrogen consumed via methane in CSM was similar to that in SBM, MC and DMC. The fermentation efficiency (%) in CSM (74.70) and GNC (74.97) was higher (P<0.05) than SBM (73.96), MC (74.38) and DMC (73.81). The VFA utilization index (VFA UI) was highest (P<0.05) in SBM (3.81) and lowest (P<0.05) in GNC (3.39). The VFA UI in CSM (3.76) was similar to DMC (3.76).

***In vitro* evaluation of concentrate mixtures containing graded levels of CSM**

The net gas production (NGP, ml/g DM/24h) was 196.19, 194.50, 196.94, 195.27 and 192.97 in concentrate mixtures 1, 2, 3, 4 and 5, respectively. No significant difference was observed in NGP among the concentrate mixtures. The PF was 3.80, 3.78, 3.75, 3.83, 3.86 mg/ml in concentrate mixtures 1, 2, 3, 4 and 5, respectively and varied non-significantly. The OM digestibility (%) in concentrate mixture 1, 2, 3, 4 and 5 was 79.85, 80.07, 80.68, 81.64 and 81.22, respectively. There was no significant difference in OM digestibility, NDF digestibility, DM digestibility among the concentrate mixtures. The MMP and EMMP results varied non significantly among the concentrate mixtures. The short chain fatty acids (SCFA, mmole) production was similar among all the concentrate mixtures and varied from 0.85 to 0.87. There was no significant difference in ME value among the concentrate mixtures.

Ammonia-N showed a declining trend with increasing level of CSM in concentrate mixtures. The fermentable carbon dioxide (Fer CO_2, mmol) level in the concentrate mixtures containing graded levels was similar to that of control concentrate (conc 1). The fermentable methane (Fer CH_4, mmol) in concentrate mixtures varied non significantly and concentrate 1, 2, 3, 4 and 5 produced 24.30, 25.11, 25.27, 26.32 and 25.98 mmol fermentable CH_4, respectively. The acetic acid, propionic acid, isobutyric acid, butyric acid content varied non significantly. The total volatile fatty acid production (TVFA, mM/dl) was similar ($P>0.05$) in concentrate mixture 1 (4.54), concentrate mixture 2 (4.45), concentrate mixture 3 (4.55), concentrate mixture 4 (4.56) and concentrate mixture 5 (4.55) and differed non significantly. Concentrate mixture 1, 2, 3, 4 and 5 had 1.79, 1.84, 1.86, 2.02 and 1.98 A:P ratio, respectively. The relative proportion of acetic acid, propionic acid, isobutyric acid, butyrate was similar among the concentrate mixtures evaluated.

The hydrogen recovery (%) was 99.05, 100.16, 99.01, 98.88 and 98.89 in concentrate mixture 1, 2, 3, 4 and 5, respectively. The hydrogen consumed via CH_4 was similar among concentrates. The fermentation efficiency (%) in concentrate 1 (78.98), concentrate 2 (78.64), concentrate 3 (78.56), concentrate 4 (77.80) and concentrate 5 (78.00) was similar. No significant difference was observed in VFA utilization index among the concentrate mixtures.

***In vitro* evaluation of TMRs containing graded levels of CSM**

There was no significant difference in NGP among the TMRs. The partitioning factor (PF, mg/ml) in TMR 1, 2, 3, 4 and 5 was 2.93, 3.13, 2.99, 3.05 and 2.97, respectively. No significant difference was seen in PF in the present study. The OM digestibility (%) in the TMRs containing graded levels of CSM was similar to that of control TMR (TMR 1). The NDF digestibility was also similar among the TMRs. The MMP and EMMP varied non-significantly among the TMRs tested. The DM digestibility of TMRs ranged from 65.29 to 67.69% with non-significant difference among TMRs tested. The short chain fatty acids (SCFA) varied from 0.81 to 0.84 mmole and differed non-significantly among the TMRs. The metabolizable energy (ME) ranged from 8.06 to 8.32 MJ/kg DM. No significant difference was observed among TMRs. There was non-significant decrease in NH_3-N from TMR 1 to TMR 5.

The fermentable CO_2 (mmol) was lowest ($P<0.05$) in TMR 2 (48.47) and highest ($P<0.05$) in TMR 4 (51.77) with intermediate values in TMR 1 (50.89), TMR 3 (50.61), TMR 5 (50.95). The fermentable CH_4 (mmol) was lowest ($P<0.05$) in TMR 2 (28.29) and highest ($P<0.05$) in TMR 1 (33.15) and similar ($P<0.05$) in TMR 3

(29.53), TMR 4 (29.26) and TMR 5 (29.12). The acetic acid (mM/dl) production was higher (P<0.05) in TMR 1 (4.34) and lowest (P<0.05) in TMR 4 (3.39). The propionic acid (mM/dl) production was higher (P<0.05) in TMR 2 (1.50) and lower (P<0.05) in TMR 1 (1.21) than other TMRs. The butyric acid (mM/dl) production in TMR 1 (0.54), TMR 2 (0.50), TMR 3 (0.55), TMR 4 (0.64) and TMR 5 (0.56) varied non significantly. The isovaleric acid (mM/dl) production was lowest (P<0.05) in TMR 5 (0.26). The total VFA (mM/dl) production varied significantly among the TMRs and was highest (P<0.05) in TMR 1 (6.45). The TVFA production in TMR 3, TMR 4 and TMR 5 was similar. The acetate: propionate ratio was higher (P<0.05) in TMR 1 (3.58) and lower (P<0.05) in TMR 2 (2.51) and TMR 5 (2.49) as compared to other TMRs evaluated. The relative proportion (%) of acetic acid was highest (P<0.05) in TMR 1 (67.38) and was lowest (P<0.05) in TMR 4 (58.97). The relative proportion (%) of propionic acid was highest (P<0.05) in TMR 2 (24.15) and TMR 5 (24.47) and lowest (P<0.05) in TMR 1 (18.82). The relative proportion (%) of propionic acid was similar (P<0.05) in TMR 3 (23.43) and TMR 4 (22.95). The relative proportion (%) of isobutyric acid was higher (P<0.05) in TMR 2 (2.50) and TMR 4 (2.01) than TMR 1 (0.89), TMR 3 (1.22) and TMR 5 (0.62). The relative proportion (%) of butyric acid was highest (P<0.05) in TMR 4 (11.03) and was lowest (P<0.05) in TMR 1 (8.33) and TMR 2 (8.11). The relative proportion (%) of isovaleric acid in TMR 1, 2, 3, 4 and 5 was 4.59, 4.70, 4.58, 5.04 and 4.42, respectively. The relative proportion (%) of valeric acid was lower (P<0.05) in TMR 1 (0.89), TMR 3 (1.22) and TMR 5 (0.62) than TMR 2 (2.50) and TMR 4 (2.01).

The H- recovery (%) was lowest (P<0.05) in TMR 1 (78.18) and highest (P<0.05) in TMR 3 (84.55), TMR 4 (85.82) and TMR 5 (85.00) followed by TMR 2 (82.08). The hydrogen consumed via methane was similar in TMR1 (4.67), TMR 2 (4.80), TMR 3 (4.73), TMR 4 (4.72) and TMR 5 (4.85). The fermentation efficiency (%) was highest (P<0.05) in TMR 5 (76.10) and was lowest (P<0.05) in TMR 1 (73.36). The VFA utilization index (VFA UI) was lowest (P<0.05) in TMR 2 (2.98) and highest (P<0.05) in TMR 1 (4.31).

Effect of graded levels of CSM on nutrient intake and digestibility in buffalo calves

The DMI, OMI, CPI, EEI, NDFI, ADFI (kg/100 kg BW) in the 120 day experimental period was higher (P<0.05) in T1 group than C and T2 groups. CLI and

HCI (kg/100 kg BW) of T1 group was similar to C group and was higher ($P<0.05$) than T2 group.

The DM intake (kg/d) during metabolic trial was similar among the control and treatment groups fed CSM. The DM, OM, CP, NDF, ADF, cellulose digestibility showed no significant difference among C, T1 and T2 groups.

Effect of graded levels of CSM on nitrogen balance in buffalo calves

The total nitrogen intake, faecal N, urinary N, N outgo and N balance varied non significantly among C, T1 and T2 groups.

Effect of dietary levels of CSM on blood profile

Blood parameters like glucose, BUN, total protein, albumin, triglyceride, cholesterol, AST, ALT and GGT showed no significant difference with inclusion of CSM in T1 (% 11.25 CSM) and T2 (15 % CSM) groups replacing SBM in the concentrate mixtures as compared to control (0% CSM) group.

Effect of dietary level of CSM on cell mediated immune response in buffalo calves

Treatment groups T1 and T2 exhibited similar CMI response to control groups. The CMI response was higher after 24 h of antigen administration in all the groups as compared to response after 48 h. Decrease in skin thickness i.e., CMI response after 48h, 72h and 96h was almost similar in all the groups.

Effect of dietary levels of CSM on performance of buffalo calves

The initial body weight (kg) of buffalo calves in C, T1 and T2 groups was 149.29, 150.08 and 150.25 kg at the beginning of the experiment and the final body weight at the end of the experiment was 238.25, 244.83 and 248.83 kg, respectively. The gain in body weight during the experimental period of 120 days was 88.96, 94.75 and 98.58 kg/animal in control, T1 and T2 groups, respectively. The average daily gain in T2 group was numerically higher than other groups. The FCR in T1 (6.61) group was similar to control (6.61) group. However, FCR in T2 (6.38) group was marginally better than C and T1 groups.

Economics of feeding

Replacement of SBM by CSM reduced the cost of concentrate mixtures fed to T1 and T2 groups by 7.29% and 9.75%, respectively as compared to concentrate mixture fed to control group. CSM inclusion at 75% and 100% levels replacing SBM resulted in net saving of Rs. 11.67 and 16.65 per kg BW gain in T1 and T2 groups, respectively. Thus, based on the results of the present study, it could be concluded that feeding of CSM to male buffalo calves was cost effective.

CONCLUSIONS

- CSM had similar CP, EE and NDF to SBM. NDICP in CSM was lower than SBM.
- *In vitro* study of concentrates and TMRs revealed that soybean meal could be replaced by CSM upto 100% without affecting the nutrient digestibility (OM, NDF and DM), and microbial mass production.
- Digestibility of nutrients and nitrogen balance was comparable in all the groups, indicating CSM did not have any effect on nutrient digestibility and nitrogen retention.
- CSM inclusion in the diet of buffalo calves did not have any adverse effect on blood profile, cell mediated immune response, ADG and FCR in male buffalo calves.
- Inclusion of CSM at 75% and 100% levels replacing SBM resulted in net saving of Rs. 11.67 and 16.65 per kg body weight gain respectively, in buffalo calves.

Therefore, from the present study, it was concluded that CSM could economically replace soybean meal upto 100 % in the concentrate mixture of buffalo calves on w/w basis without any adverse effect on palatability, digestibility of nutrients, nitrogen balance and health of animals.

REFERENCES

Abdalla, A. L., Louvandini, H., Sallam, S. M. A. H., da Bueno, I. C. S., Tsai, S. M., & de Figueira, A. V. O. (2012). In vitro evaluation, in vivo quantification, and microbial diversity studies of nutritional strategies for reducing enteric methane production. *Tropical Animal Health and Production*, 44(5), 953–964. https://doi.org/10.1007/s11250-011-9992-0.

Ash, M. S. (1992). Animal Feeds Compendium, AER-656. US Department of Agriculture, Economic.

Adeyemo, G. O., & Longe, O. G. (2007). Effects of graded levels of cottonseed cake on performance, haematological and carcass characteristics of broilers fed from day old to 8 weeks of age. *African Journal of Biotechnology,* 6(8), 1064–1071. https://doi.org/10.4314/ajb.v6i8.57103.

Attanayaka, P., Pathirana, A., Priyankarage, N., Silva, S., & Nayananjalie, W. (2016). Effect of Substitution of Soybean Meal with Cottonseed Meal on the Performances of Broiler Chicken. *International Journal of Livestock Research,* 6(3), 24. https://doi.org/10.5455/ijlr.20160306102626.

Azman, M. A., & Yilmaz, M. (2005). The growth performance of broiler chicks fed with diets containing cottonseed meal supplemented with lysine. *Revue de Medecine Veterinaire,* 156(2), 104–106.

Abbas, A. K., Lichtman, A. H., & Pillai, S. (2014). Cellular and molecular immunology E-book. Elsevier Health Sciences.

AOAC. (2007). Official Methods of Analysis, 18th edition. Association of Official Analytical Chemists, Arlington, Virginia, USA.

Blummel, B. Y. M., Gab, H. S., & Becker, K. (1997). The relationship between in vitro gas production, in vitro microbial biomass yield and 15N incorporation and its implications for the prediction of voluntary feed intake of roughages. *British Journal of Nutrition*, 77(6), 911-921.

Barros, L. V. De, Paulino, M. F., Campos, S. De, Filho, V., Detmann, E., Gomes, F., Egidio, É., Valente, L., Antonio, S., & Martins, L. S. (2011). Replacement of soybean meal by cottonseed meal 38 % in multiple supplements for grazing beef heifers. *Revista Brasileira de Zootecnia*, 40(4), 852–859.

Bo, Y. K., Yang, H. J. J., Wang, W. X., Liu, H., Wang, G. Q., & Yu, X. (2012). Metabolisable energy, in situ rumen degradation and in vitro fermentation characteristics of linted cottonseed hulls, delinted cottonseed hulls and cottonseed linter residue. *Asian-Australasian Journal of Animal Sciences,* 25(2), 240–247. https://doi.org/10.5713/ajas.2011.11304.

Brien, R. D., Jones, L. A., King, C. C., Wakelyn, P. J., & Wan, P. J. (2005). Cottonseed Oil: Chapter 5. Bailey's Industrial Oil and Fat Products, Sixth Edition, John Wiley & Sons, Inc.

Baran, M., & Zitnan, R. (2002). Effect of monensin sodium on fermentation efficiency in sheep rumen (short communication). *Archives Animal Breeding*, 45(2), 181–185. https://doi.org/10.5194/aab-45-181-2002.

Bucolo, G., & David, H. (1973). Quantitative determination of serum triglycerides by the use of enzymes. *Clinical Chemistry*, 19(5), 476–482. https://doi.org/ 10.1093/clinchem/19.5.476.

Church, D. C. (1991). Livestock feeds and feeding (No. Ed. 3). Prentice Hall.

Cheng, Z. J., & Hardy, R. W. (2002). Apparent digestibility coefficients and nutritional value of cottonseed meal for rainbow trout (Oncorhynchus mykiss). Aquaculture, 212(1–4), 361–372. https://doi.org/10.1016/S0044-8486(02) 00260-0.

Crampton, E. W., & Maynard, L. A. (1938). The Relation of Cellulose and Lignin Content to the Nutritive Value of Animal Feeds. *The Journal of Nutrition*, 15(4), 383–395. https://doi.org/10.1093/jn/15.4.383.

Cottyn, B. G., & Boucque, C. V. (1968). Rapid method for the gas-chromatographic determination of volatile fatty acids in rumen fluid. *Journal of Agricultural and Food Chemistry*, 16(1), 105-107.

Dhanda, O. P. (2004). Developments in water buffalo in Asia and Oceania. Proc. of the Seventh World Buffalo Congress, Manila, Philippines. pp. 17-28.

Devore, D. W. (2018). In-Vitro Digestibility and Gas Production of Wheat Middlings , Solvent Extracted Cottonseed Meal , Soyhulls , and Corn Gluten Feed and the Effects of Monensin on In-Vitro Digestibility and Gas Production. *MSU Graduate Theses*, 3309. https://bearworks.missouristate.edu/theses/3309.

Doumas, B. T., Ard Watson, W., & Biggs, H. G. (1971). Albumin standards and the measurement of serum albumin with bromcresol green. *Clinica Chimica Acta*, 31(1), 87–96. https://doi.org/10.1016/0009-8981(71)90365-2.

Demeyer, D. I. (1991). Quantitative aspects of microbial metabolism in the rumen and hindgut. *Rumen microbial metabolism and ruminant digestion*, (Jouany, J. P edition) published in association with I.N.R.A. Paris. pp. 217-237.

Fadel, M., & Ashmawy, T. (2015). Influence of Protected Linseed Meal and Cotton Seed Meal By Tannins on Zaraibi Dairy Goats and Their Offspring Performance. *Journal of Animal and Poultry Production*, 6(4), 219–234. https://doi.org/10.21608/jappmu.2016.52750.

France, J., Dhanoa, M. S., Theodorou, M. K., Lister, S. J., Davies, D. R., & Isac, D. (1993). A model to interpret gas accumulation profiles associated with in vitro degradation of ruminant feeds. *Journal of Theoretical Biology*, 163(1), 99–111. https://doi.org/10.1006/jtbi.1993.1109.

Food and Agriculture Organization. (2018). http://www.fao.org/faostat.

Ghanbari, F., Ghoorchi, T., Shawrang, P., Mansouri, H., & Torbati-Nejad, N. M. (2012). Comparison of electron beam and gamma ray irradiations effects on ruminal crude protein and amino acid degradation kinetics, and in vitro digestibility of cottonseed meal. *Radiation Physics and Chemistry*, 81(6), 672–678. https://doi.org/10.1016/j.radphyschem.2012.02.014.

He, T., Zhang, H. J., Wang, J., Wu, S. G., Yue, H. Y., & Qi, G. H. (2015). Application of low-gossypol cottonseed meal in laying hens' diet. *Poultry Science*, 94(10), 2456–2463. https://doi.org/10.3382/ps/pev247.

Hassanabadi, A., Heidariniya, A., & Shahir, M. H. (2009). Histological effects of cottonseed meal with and without ferrous sulfate and lysine in male broiler rations. *Journal of Animal and Veterinary Advances*, 8(8), 1499–1502.

Henry, M. H., Pesti, G. M., Bakalli, R., Lee, J., Toledo, R. T., Eitenmiller, R. R., & Phillips, R. D. (2001). The performance of broiler chicks fed diets containing extruded cottonseed meal supplemented with lysine. *Poultry Science*, 80(6), 762–768. https://doi.org/10.1093/ps/80.6.762.

Hinze, L. L., Horn, P. J., Kothari, N., Dever, J. K., Frelichowski, J., Chapman, K. D., & Percy, R. G. (2015). Nondestructive measurements of cottonseed nutritional trait diversity in the U.S. national cotton germplasm collection. *Crop Science*, 55(2), 770–782. https://doi.org/10.2135/cropsci2014.04.0318.

Henry, R. J., Cannon, D. C., & Winkelman, J. W. (1974). Clinical Chemistry Principles and Techniques, 11th Edn. pp 1629. Happer and Row Publishers, New York.

ICAR. (2013). Nutrient requirements of cattle and buffalo. Indian Council of Agricultural Research, New Delhi.

Khan, A. G., Azim, A., & Mirza, I. H. (2002). Response of feeding amino acids supplemented cottonseed meal on growth performance and digestibility of early weaned cow calves. *Asian-Australasian Journal of Animal Sciences*, 15(2), 184–187. https://doi.org/10.5713/ajas.2002.184.

Khan, A. G., Azim, A., Nadeem, M. A., & Ayaz, M. (2000). The Effect of Formaldehyde Treatment of Solvent and Mechanical Extracted Cottonseed Meal on the Performance, Digestibility and Nitrogen Balance in Lambs. *Asian-Australasian Journal of Animal Sciences*, 13(6), 785–790. https://doi.org/10.5713/ajas.2000.785.

Khanum, S. A., Yaqoob, T., Sadaf, S., Hussain, M., Jabbar, M. A., Hussain, H. N., Kausar, R., & Rehman, S. (2007). Nutritional Evaluation of Various Feedstuffs for Livestock. *Pakistan Vetereinary Jounal*, 27(3), 129–133.

Kannan, A., Sastry, V. R. B., Agrawal, D. K., & Kumar, A. (2013). Effect of feeding of calcium hydroxide-treated or vitamin E-supplemented cottonseed meal on plasma gossypol levels, blood parameters, and performance of Bikaneri lambs. *Tropical Animal Health and Production*, 45(6), 1289–1295. https://doi.org/10.1007/s11250-013-0359-6.

Lamba, J. S., Hundal, J. S., Wadhwa, M., & Bakshi, M. P. S. (2014). In-vitro methane production potential and in-sacco degradability of conventional and non-conventional protein supplements. *Indian Journal Animal Science*, 84(5), 539-43.

Lorena-Rezende, I. M. B., Dutra, W. M., de Rezende, F. M., Palhares, L. O., Ludke, M. D. C. M. M., & Rabello, C. B. V. (2012). Digestibilidade do farelo de algodão com ou sem adição das enzimas protease e fitase na alimentação de suínos. *Acta Scientiarum - Animal Sciences*, 34(3), 259–265. https://doi.org/10.4025/actascianimsci.v34i3.12360.

Licitra, G., Hernandez, T. M., & Van Soest, P. J. (1996). Feedbunk management evaluation techniques. *Animal Feed Science Technology*, 57, 347–358.

Li, J. T., Li, D. F., Zang, J. J., Yang, W. J., Zhang, W. J., & Zhang, L. Y. (2012). Evaluation of energy digestibility and prediction of digestible and metabolizable energy from chemical composition of different cottonseed meal sources fed to growing pigs. Asian-Australasian *Journal of Animal Sciences*, 25(10), 1430–1438. https://doi.org/10.5713/ajas.2012.12201.

Mcqueen, R. E., & Nicholson, J. W. G. (1979). Modification of the Neutral-Detergent Fiber Procedure for Cereals and Vegetables by Using α-Amylase. *Journal of AOAC International*, 62(3), 676–680. https://doi.org/10.1093/jaoac/62.3.676.

Menke, K. H., Raab, L., Salewski, A., Steingass, H., Fritz, D., & Schneider, W. (1979). The estimation of the digestibility and metabolizable energy content of ruminant feedingstuffs from the gas production when they are incubated with rumen liquor in vitro. *The Journal of Agricultural Science*, 93(1), 217–222. https://doi.org/10.1017/S0021859600086305.

Morvay, Y., Bannink, A., France, J., Kebreab, E., & Dijkstra, J. (2011). Evaluation of models to predict the stoichiometry of volatile fatty acid profiles in rumen fluid of lactating Holstein cows. *Journal of dairy science*, 94(6), 3063-3080.

Matondi, G. H. M., Masama, E., Mpofu, I. D. T., & Muronzi, F. F. (2007). Effect of feeding graded levels of cottonseed meal on goat erythrocyte membrane osmotic fragility. *Livestock Research for Rural Development*, 19(11).

Menke, K. H., & Steingass, H. (1988). Estimation of the energetic feed value obtained from chemical analysis and in vitro gas production using rumen fluid. *Animal research and development*, 28, 7-55.

National livestock census. (2019). 20th Livestock census. All India reports. Ministry of Agriculture, Department of Animal Husbandry, Dairying and Fisheries, Krishi Bhawan, New Delhi.

Nagalakshmi, D., Sastry, V. R. B., & Kesava Rao, V. (2002). Influence of feeding processed cottonseed meal on meat and wool production of lambs. *Asian-Australasian Journal of Animal Sciences*, 15(1), 26–33. https://doi.org/10.5713/ajas.2002.26.

Nasser, M. E. A. W. (2009). Effect of partial or complete replacement of cottonseed meal by jojoba meal on gas production, rumen fermentation and produced amylase and carboxymethyle cellulase activity, in vitro. *Livestock Research for Rural Development*, 21(5), 2009.

Nagalakshmi, D., Sastry, V. R. B., Agrawal, D. K., & Katiyar, R. C. (2001). Haematological and immunological response in lambs fed on raw and variously processed cottonseed meal. *Asian-Australasian Journal of Animal Sciences*, 14(1), 21-29.

Nomeary, Y. A. A., Abd El-Rahman, H. H. H., Shoukry, M. M., Abedo, A. A., Salman, F. M., & Mohamed, M. I. (2021). Effect of different dietary protein sources on digestibility and growth performance parameters in lambs. *Bulletin of the National Research Centre*, 45(1), 1–11. https://doi.org/10.1186/s42269-021-00486-1.

Orskov, E. R. (1975). Manipulation of rumen fermentation for maximum food utilization. *World Review of Nutrition and Dietetics,* 22, 152–182. https://doi.org/10.1159/000397977.

Ojewola, G. S., Ukachukwu, S. N., & Okulonye, E. I. (2006). Cottonseed meal as substitute for soyabean meal in broiler ration. *International Journal of Poultry Science,* 5(4), 360–364. https://doi.org/10.3923/ijps.2006.360.364.

Oguz, F. K., Oguz, M. N., Hatipoglu, S., & Gulay, M. S. (2006). The Effects of Iron Sulphate Supplementation to Diets Containing Cottonseed Meal on Performance and Haematological Parameters of Broilers. *Erciyes Üniversitesi Veteriner Fakültesi Dergisi*, 3(1), 9–14.

Pattanaik, A. K., Sastry, V. R. B., Singh, D. K., Goswami, T. K., & Mohanty, D. N. (2003). Effect of gossypol from cottonseed meal diets on some clinico-biochemical parameters and humoral immune response of crossbred calves fed barley or sorghum. *Asian-Australalasian Journal of Animal Sciences,* 16(9), 1291–1296. https://doi.org/10.5713/ajas.2003.1291.

Raji, M. O., Atanda, R. A., Adeyemo, G. O., Muraina, T. O., Adebisi, I. A. & Akinosun, A. A. (2017). evaluation of chemical composition and in vitro gas production of cottonseed and its by-products. *Journal of animal Production Research*, 29(2), 215-221.

Roeschlau, P., Bernt, E., & Gruber, W. (1974). Enzymatic determination of total cholesterol in serum, *Clinical Chemistry Clinical Biochemistry,* 12(9), 403-407.

Salas, C., Ekmay, R., England, J., Cerrate, S., & Coon, C. N. (2013). The TMEn, proximate analysis, amino acid content and amino acid digestibility of glandless and commercial cottonseed meal for broilers. *International Journal of Poultry Science*, 12(4), 212-216.

Saijpaul, S., Grewal, R., Ahuja, C., & Naik, P. (2006). Effect of supplementation of whole linted cottonseed on the performance and blood biochemical profile of crossbred cows in summer. *Animal Nutrition and Feed Technology,* 6(1), 95–102.

Silva, F. M. da, Ferreira, M. de A., Guim, A., Pessoa, R. A. S., Gomes, L. H. dos S., & Oliveira, J. C. V. de. (2009). Replacement of soybean meal by cottonseed meal in diets based on spineless cactus for lactating cows. *Revista Brasileira de Zootecnia,* 38(10), 1995–2000. https://doi.org/10.1590/s1516-3598200900 1000020.

Solomon, M., Melaku, S., & Tolera, A. (2008). Supplementation of cottonseed meal on feed intake, digestibility, live weight and carcass parameters of Sidama goats. Livestock Science, 119(1–3), 137–144. https://doi.org/10.1016/j. livsci.2008.03.011.

Snedecor, G. W. & Cochran, W, G. (1994). Statistical methods, 11th Edn. pp 267. The Iowa State University Press, Ames, IA.

SPSS. (2012). Statisical package for windows. Chicago, IL, USA.

Sniffen, C. J., O'connor, J. D., Van Soest, P. J., Fox, D. G., & Russell, J. B. (1992). A net carbohydrate and protein system for evaluating cattle diets: II. Carbohydrate and protein availability. *Journal of animal science,* 70(11), 3562-3577.

Sun, H., Tang, J. wu, Yao, X. hong, Wu, Y. fei, Wang, X., & Feng, J. (2013). Effects of dietary inclusion of fermented cottonseed meal on growth, cecal microbial population, small intestinal morphology, and digestive enzyme activity of broilers. *Tropical Animal Health and Production,* 45(4), 987–993. https://doi.org/10.1007/s11250-012-0322-y.

Thirumalaisamy, G., Purushothaman, M. R., Vasantha Kumar, P., & Selvaraj, P. (2016). Effect of feeding cottonseed meal on some hematological and serum biochemical parameters in broiler birds. *Veterinary World,* 9(7), 723–727. https://doi.org/10.14202/vetworld.2016.723-727.

Thirumalaisamy, G., Purushothaman, M. R., Vasantha Kumar, P., & Selvaraj, P. (2015). Nutritive and feeding value of cottonseed meal for broilers. M.V.Sc. thesis. Tamil Nadu Veterinary and Animal Sciences University, Namakkal - 637 002.

Tripathi, M. K., Mondai, D., Raghuvansi, S. K. S., & Karim, S. A. (2012). Effect of Bt-cottonseed meal feeding on intake, growth, nutrient utilization, serum cholesterol, immunological status, organ weight and slaughtering performance of growing lambs. *Animal Nutrition and Feed Technology,* 12(2), 165–178.

Tripathi, M. K., Raghuvansi, S. K. S., Mondal. D., & Karim, S. A. (2014). Effect of Bt-cottonseed meal feeding on performance, fermentation, ciliates population and microbial hydrolytic enzymes in lamb. *African Journal of Biotechnology,* 13(3), 509–522. https://doi.org/10.5897/ajb2013.13111.

Tang, J. W., Sun, H., Yao, X. H., Wu, Y. F., Wang, X., & Feng, J. (2012). Effects of replacement of soybean meal by fermented cottonseed meal on growth performance, serum biochemical parameters and immune function of yellow-feathered broilers. *Asian-Australasian Journal of Animal Sciences,* 25(3), 393–400. https://doi.org/10.5713/ajas.2011.11381.

Trinder, P. (1969). Determination of glucose in blood using glucose oxidase with an alternative oxygen acceptor. *Annals of clinical Biochemistry,* 6(1), 24-27.

Talke, H., & Schubert, G. E. (1965). Enzymatic determination of urea using the coupled urease-GLDH enzyme system. Mediators of Inflammation, 43, 174-176.

Van Soest, P. J., Robertson, J. B., & Lewis, B. A. (1991). Methods for Dietary Fiber, Neutral Detergent Fiber, and Nonstarch Polysaccharides in Relation to Animal Nutrition. *Journal of Dairy Science*, 74(10), 3583–3597. https://doi.org/10.3168/jds.S0022-0302(91)78551-2.

Van Soest, P. J., & Robertson, J. B. (1988). A laboratory manual for animal science. pp 612. Cornell university, USA.

Wanapat, M., Pilajun, R., Polyorach, S., Cherdthong, A., Khejornsart, P., & Rowlinson, P. (2013). Effect of carbohydrate source and cottonseed meal level in the concentrate on feed intake, nutrient digestibility, rumen fermentation and microbial protein synthesis in swamp buffaloes. *Asian-Australasian Journal of Animal Sciences*, 26(7), 952–960. https://doi.org/10.5713/ajas.2013.13032.

Worrell, M. A., Undersander, D. J., Thompson, C. E., & Bridges Jr, W. C. (1990). Effects of time of season and cottonseed meal and lasalocid supplementation on steers grazing rye pastures. *Journal of animal science,* 68(4), 1151-1157.

Zhou, J. Z., Zhang, H., Gao, L., Wang, L., & Qian, H. F. (2015). Influence of pH and ionic strength on heat-induced formation and rheological properties of cottonseed protein gels. *Food and Bioproducts Processing,* 96, 27–34. https://doi.org/10.1016/j.fbp.2015.06.004.

Zotte, A., Brand, T. S., Hoffman, L. C., Schoon, K., Cullere, M., & Swart, R. (2013). Effect of cottonseed oilcake inclusion on ostrich growth performance and meat chemical composition. *Meat Science*, 93(2), 194–200.

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