EFFECTS OF *MORINGA OLEIFERA* LEAVES SUPPLEMENTATION ON GROWTH, CERTAIN BLOOD METABOLITES AND HORMONES IN MURRAH BUFFALO HEIFERS

THESIS SUBMITTED TO THE
ICAR-NATIONAL DAIRY RESEARCH INSTITUTE, KARNAL
(DEEMED UNIVERSITY)
IN PARTIAL FULFILMENT OF THE REQUIREMENTS
FOR THE AWARD OF THE DEGREE OF

MASTER OF VETERINARY SCIENCE

IN

ANIMAL PHYSIOLOGY

BY

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B.V.Sc. & A.H.

ANIMAL PHYSIOLOGY DIVISION
ICAR-NATIONAL DAIRY RESEARCH INSTITUTE
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This is to certify that the thesis entitled " **EFFECTS OF *MORINGA OLEIFERA* LEAVES SUPPLEMENTATION ON GROWTH, CERTAIN BLOOD METABOLITES AND HORMONES IN MURRAH BUFFALO HEIFERS**" submitted by **NATASHA SUNIL KOVE** towards the partial fulfilment of the award of the degree of **MASTERS IN VETERINARY SCIENCE IN ANIMAL PHYSIOLOGY** of the **ICAR- NATIONAL DAIRY RESEARCH INSTITUTE (DEEMED UNIVERSITY)**, Kamal. Haryana, India, is a bonafide research work carried out by her under my supervision and no part of the thesis has been submitted for any other degree or diploma.

Dated:

Place: NDRI Karnal

Dr A.K. Roy

MAJOR ADVISOR

Dedicated to

Beloved Family

& Respected Guide

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Date: (Natasha Sunil Kove)

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Abbreviations

LRC	Livestock Research Centre
Kg	Kilogram
g/d	Gram Per Day
BW	Body Weight
$W^{0.75}$	Metabolic Body Weight
CH_4	Methane
CO_2	Carbon Dioxide
FAO	Food Agricultural Organisation
MO	*Moringa oleifera*
DM	Dry Matter
NFE	Nitrogen Free Extract
DCP	Digestible Crude Protein
CP	Crude Protein
TDN	Total Digestible Nutrient
ADG	Average Daily Gain
ADF	Acid Detergent Fibre
NDF	Neutral Detergent Fibre
ADL	Acid Detergent Lignin
HC	Hemicellulose
OM	Organic Matter
EE	Ether Extract
DMI	Dry Matter Intake
TMR	Total Mixed Ration
CSC	Cottonseed Cake
MOLM	*Moringa oleifera* leaf meal
FML	Fresh *Moringa oleifera* leaf

GNH	Groundnut Hay
MSH	*Medicago sativa*
SSC	Sunflower Seed Cake
AHD	Alfa Hay Diet
%	Percent
<	Less than
>	More than
/	Per
Kg	Kilogram
g	Gram
g/kg	Gram Per kilogram
g/dl	Gram Per deciliter
g/ml	Gram Per mililiter
g/kg $W^{0.75}$	Gram per kg metabolic body weight
mg	Miligram
mg/kg	Miligram per kilogram
mg/L	Miligram per liter
mg/dl	Miligram per deciliter
mg/ml	Miligram per mililiter
ml	Mililiter
L	Liter
µl	Microliter
µm	Micromole
ml/g	Mililiter per gram
N	Normal
mm	Millimole
µ mol /l	Micromole per liter

IU/l	International unit per liter
IU /ml	International unit per milliter
meq /l	Mili equivalent per liter
ng /µl	Nanogram per microliter
et al	Et alli / alia
CIRG	Central Institute For Research on Goats
CRD	Complete Randomized Design
CH	Clover hay
LL	Leucaena leucocephala
LBW	Live body weight
CFM	Concenterate feed mixture
ALT	Alanine amino transferase
AST	Aspartate amino transferase
SGPT	Serum glutamic pyruvic transaminase
SGOT	Serum glutamic oxaloacetate transaminase
GH	Growth hormone
IGF	Insulin like growth factor
PCV	Packed cell volume
°C	Degree centigrade
e.g	For example
etc.	And so forth
i.e	That is
@	At the rate of
PH	Negative logarithm of hydrogen ion
Fig.	Figure
h	Hour
min	Minute

sec	Second
C	Control
T	Treatment
M25	*Moringa oleifera* leaves 25% Replacement Level
M50	*Moringa oleifera* leaves 50 % Replacement Level
Hb	Haemoglobin
AOAC	Association of Official Analytical Chemist
ARC	Agricultural Research Council
NRC	National Research Council
ICAR	Indian Council Of Agricultural Research
DW	Dry weight
%BW	Percent body weight
NDRI	National Dairy Research Institute
FRAP	Ferric reducing antioxidant power
ELISA	Enzyme Linked Immunosorbant Assay
OD	Optical density
T-CHO	Total carbohydrates
NFC	Non Fibrous Carbohydrates
EDTA	Ethylene diamine tetraacetic acid
NEFA	Non Esterified Fatty Acid
T_3	Triiodothyronine
T_4	Thyroxine
TAC	Total antioxidant capacity
IAEC	Institutional Animal Ethics Committee
HRP	Horseradish Peroxidase
SA-HRP	Streptavidin conjugated horseradish peroxidase

Abstract

The present study was conducted to evaluate the effect of supplementations of 20% dried *Moringa oleifera* leaves in the diet of growing Murrah buffalo heifers. The experiment was conducted at Livestock Research Center, National Dairy Research Institute, Karnal, Haryana. The experiment was conducted on 14 growing Murrah buffalo heifers aged 13 to 20 months with an initial body weight of 169 kg and 170 kg respectively. The animals were divided into two groups on the basis of their body-weights and age. The control group was fed with the diet recommended by ICAR 2013 guidelines. The treatment groups was supplemented with 20% of DMI (400 g) of dried *Moringa oleifera* leaves in addition to the control diet. Body-weight and dry matter intake was recorded every fortnight. Blood samples were collected at monthly intervals and stored for the analysis of hormones and biochemical attributes. The body-weight and metabolic body-weights were higher (P<0.05) at the end of six months in the treatment group than control group. The DMI was higher in treatment group as compared to control group. The mean (ng/ml) values of T_3 and T_4 were higher (P<0.05) in the treatment than the control group. The mean cortisol (ng/ml) values in the plasma of treatment group was lower (P<0.05) than that of control group. The plasma levels of growth hormone, glucose level and estradiol-17beta showed no difference (P>0.05) between the control and treatment groups. The plasma levels of insulin like growth factor in treatment group was higher (P<0.05) than that of the control group. The total antioxidant capacity of buffalo heifers plasma in the treatment group was higher (P<0.05) in the treatment group as compared to the control group. The digestibility of DM, OM, CP, ADF, NDF were higher (P<0.05) in the treatment group than the control group. There was no difference (P>0.05) in the digestibility of ether extract. Ultrasound examination revealed the number of small (<3 mm), medium (3-8mm) and total number of follicles which were higher (P<0.05) in the treatment group than control group. There was no difference (P>0.05) in the large follicles (>8mm) of control and treatment groups. The supplementation of dried leaves of *Moringa oleifera* @ 20% of the total DMI in addition to the control diet improved body weight, feed intake, growth rate of Murrah buffalo heifers. It also improved the biochemical attributes, metabolic hormone levels and digestibility of nutrients.

सारांश

वर्तमान अध्ययन यौवनावस्था पूर्व मुर्रा भैंस के आहार में मोरिंगा ओलीफेरा अर्थात सहजन पेड़ के सूखे पत्तों के 20% पूरक का प्रभाव देखने हेतु आयोजित किया गया था। यह शोध-कार्य पशुधन अनुसंधान केंद्र, राष्ट्रीय डेयरी अनुसंधान संस्थान, करनाल, हरियाणा में किया गया। तेरह से बीस महीने की आयु वर्ग की 14 मुर्रा भैंस जो क्रमशः 169 किलोग्राम और 170 किलोग्राम के प्रारंभिक दैहिक भार के सामान थी, का चयन परीक्षण हेतु किया गया। इन पशुओं को उनके दैहिक भार और आयु के आधार पर दो समूहों में बांटा गया। नियंत्रण समूह को आईसीएआर-2013 के दिशानिर्देशों द्वारा अनुशंसित आहार खिलाया गया जबकि उपचार समूह को नियंत्रण आहार के अतिरिक्त मोरिंगा ओलीफेरा के सूखे पत्ते जो शुष्क पदार्थ ग्राह्यता के 20% के समान थे, खिलाए गए। प्रत्येक पखवाड़े पर दैहिक भार और शुष्क पदार्थ ग्राह्यता रिकॉर्ड की गई। हार्मोन एवं जैव-रासायनिक विशेषताओं के विश्लेषण हेतु मासिक अंतराल पर रक्त के नमूने एकत्र किए गए। छः माह के अंत पर नियंत्रण समूह की तुलना में उपचार समूह का दैहिक एवं उपचय दैहिक भार अधिक (पी <0.05) पाया गया। उपचार समूह की शुष्क पदार्थ ग्राह्यता नियंत्रण समूह की तुलना में अधिक थी। नियंत्रण समूह की तुलना में उपचार समूह का टी-3 और टी-4 का माध्य (नैनोग्राम / मिलीलीटर) मान अधिक (पी <0.05) था। उपचार समूह के प्लाज्मा में औसत कोर्टिसोल (नैनोग्राम / मिलीलीटर) स्तर नियंत्रण समूह की तुलना में कम (पी <0.05) था। वृद्धि अर्थात प्लाज्मा ग्रोथ-हार्मोन, ग्लूकोज और एस्ट्राडियोल 17-बीटा के स्तर दोनों ही समूहों में लगभग एक जैसे ही (पी>0.05) पाए गए। उपचार समूह में इन्सुलिन जैसे वृद्धि कारक अर्थात आई.जी.एफ.1 का प्लाज्मा स्तर नियंत्रण समूह की तुलना में अधिक (पी <0.05) था। उपचार समूह में प्लाज्मा की कुल एंटीऑक्सीडेंट क्षमता नियंत्रण समूह की तुलना में, उपचार समूह में कहीं अधिक (पी<0.05) थी। नियंत्रण समूह की तुलना में उपचार समूह का शुष्क पदार्थ, कार्बनिक पदार्थ, रुक्ष प्रोटीन, एसिड डिटर्जेंट रेशा एवं न्यूट्रल डिटर्जेंट रेशे की पाचन क्षमता अधिक (पी <0.05) थी। ईथर-अर्क की पाचनशक्ति में कोई विशेष अंतर (पी >0.05) नहीं था। अल्ट्रासाउंड परीक्षण में छोटे (<3 मिमी), मध्यम (3-8 मिमी) और डिम्बों की कुल संख्या का आकलन किया गया जो नियंत्रण समूह की तुलना में उपचार समूह में अधिक (पी <0.05) था। नियंत्रण और उपचार समूहों के बड़े डिम्ब (>8 मिमी) में कोई विशेष अंतर (पी>0.05) नहीं था। नियंत्रण आहार के अतिरिक्त उपचार समूह को मोरिंगा ओलीफेरा के सूखे पत्तों को शुष्क पदार्थ ग्राह्यता के 20% के बराबर खिलाने से दैहिक भार, शुष्क पदार्थ ग्राह्यता एवं भैंस की वृद्धि दर में सुधार पाया गया। इस पूरक को देने से जैव रासायनिक विशेषताओं, उपचय हार्मोन स्तर तथा पोषक तत्वों की पाचनशीलता में भी सुधार हुआ।

Chapter - 1

Introduction

Introduction

Buffaloes play a major role in India's economy for agricultural producers as well as and landless farmers owning over 67% of the dairy animals. Amongst some of the different breeds of buffaloes, Murrah breed is recognized for its milk productivity and improved adaptability and mainly reared in India and many parts of the world for upgrading the non-descript breeds of buffaloes. Farmers in India prefer to rear buffaloes due to its excellent characteristics such as better feed conversion capacity, disease tolerance and proportion of milk fat than that of cows.

Buffalo's low output is mostly due to its inferior genetic potential, insufficient nutrition and unscientific feeding techniques. The most popular techniques of determining an animal's nutritional status are their body weights and body condition score. Buffaloes have a higher feed conversion efficiency as compared to cattle, which allows them to remain productive in pasture-based systems in comparison to cattle (Sabia *et al.*, 2014). The main disadvantages leading to reduced productivity in this species are the late maturity, silent heat combined with poor estrus detection, inconsistent estrous cycle, seasonal patterns in breeding, anoestrus, poor conception rate, large postpartum duration, and repeat breeding.

Because of India's growing population, there is a growing difference between the demand and availability of capital for agriculture (Hoffmann *et al.*, 2003). Agriculture increased by 2-4.5 percent (Economic survey 2017-18), but it cannot meet the demand alone. In the coming decades, livestock products will play a critical role in alleviating the hunger of our country's expanding human population while playing a significant economic role for the progress of India. However, the main constraint for increasing livestock production is to meet the demand of feed capital. Common crops available from agriculture become scarce, creating a difficult situation with crop availability to feed livestock, particularly during the summer season (Anaeto *et al.*, 2009). In general, farmers feed their animals with crop residues and low-quality hay (Manaye *et al.*, 2009). These crop residues, however, are low in essential nutrients and have low digestibility and palatability. All these factors contribute to slow development, delayed sexual maturation, poor reproduction rate, and decreased milk production (Schiene and Ibrahim, 1989). The availability of nutritious feed containing a high amount of soluble carbohydrates and a low amount of fibre such as lignocelluloses is restricted locally. As a result, one of the main concerns for livestock farmers is the lack of alternative feed options in place of concentrates (Asaolu *et al*., 2012; Babeker and Abdalbagi, 2015, Sultana *et al*., 2015). So, there is a need to investigate alternative and cost-effective feed sources that contain useful components for animal diets and are readily available (Mendieta-Araica *et al*., 2011a; Alsersy *et al*., 2015: Salem *et al*., 2015). These factors have recently gained widespread attention as a possible alternative as main source of feed.

Recognizing the ability of tree leaves to create significant volumes of high protein biomass has resulted in the creation of animal husbandry methods that combine the utilisation of tree foliage with local bulky feed resources (Singh and Makkar, 2014). The feeding value of low-quality roughages and grasses can be considerably improved by incorporating tree leaf directly into pastures and fences. *Moringa oleifera* (MO) is gaining popularity due to its various industrial and feeding applications. *Moringa oleifera* Lam (syns. Moringapterygosperm, family Moringaceae) is one of the most fascinating trees, which emerged in the Himalaya region as a local tree and is present scattered almost worldwide. This evergreen tree in areas that are tropical and subtropical. It is one of the fodder species that is most commonly used. *Moringa oleifera* Lam is a multi-purpose, non-leguminous tree with abundant crude protein in the plants. The plant contains large quantities of vitamins A, B and C in the trees with a strong amino acid content. *Moringa oleifera* leaves and green pods are used by human beings as foods and provide ruminants a healthy alternative source of protein. Very high production of *Moringa oleifera* can be obtained up to 100 tonnes of Dry matter if reared under intensive farming. *Moringa oleifera*, also known as the drumstick horseradish tree and sahjan in our local language, is a member of the Moringaceae family (Debela and Tolera. 2013). It is high in essential nutrients, an easily obtainable source of energy and proteins, and has a variety of nutraceutical applications.

Moringa oleifera includes phytochemicals, secondary metabolites, phytosterol, and carbohydrate, as well as anti-cancer agents such as glycoside compounds and glycerol-1-9-octadecanoate (Landrault *et al*. 2001; Iqbal and Bhanger, 2006). *Moringa oleifera* can be used as a feed additive due to the availability of all of these nutrients (Moyo *et al*., 2012).

In most places where *Moringa oleifera* is not native, there has recently been a resurgence of interest in its nutritional qualities (Reyes *et al*., 2006; Oduro *et al*., 2008). This could be because it has nutritional, medicinal, and preventive effects, according to the reports that it boosts animal output (Fahey. 2005). Nutrition plays a critical part in an animal's ability to overcome parasitism and illness (Anwar *et al*., 2007).

Despite the enormous potential of *Moringa oleifera* leaves as a high-quality animal feed, its commercial application has been limited to small farm owners. The following are the reasons for this limited exploration: MO is a fast-growing tree with a thick trunk and branches that pose a problem for commercial machinery-based harvesters (e.g. forage harvester or forage combine). *Moringa oleifera* leaves have a high moisture content (150-200 g/kg DM) and thus became mouldy during direct ensiling, requiring a week of wilting in the field before successful ensiling (Cohen-Zinder *et al*, submitted for publication)

The use of *Moringa oleifera* can be explored in terms of the potential, nutritional values and availability of *Moringa oleifera* leaves. Despite these advantages, no reports of *Moringa oleifera* in feeding studies with Murrah buffalo heifers can be found as such in the literature.

Considering these facts into account, the current study is meant to examine the potential of *Moringa oleifera* leaves as a feed source in growing Murrah buffalo heifers, with the following objectives.

1) **To study the effect of *Moringa oleifera* leaves supplementation on the augmentation of growth in Murrah buffalo heifers**
2) **To investigate the association of blood metabolites and hormones with growth and ovarian status in *Moringa oleifera* leaves supplemented buffaloes**

Chapter -2

Review of Literature

Review of Literature

2.1 Origin and distribution of *Moringa oleifera*

Moringa oleifera is part of the Moringaceae family.Thirteen *Moringa oleifera* species were reported in the family up to the present date. The most frequently grown species is *Moringa oleifera* (Padayachee *et al*., 2012) and comes from northwestern Indian province, namely the southern Himalayan Mountains. A pantropical adaptable tree, with excessive biomass output in various environmental circumstances.The horseradish trees, a drumstick tree, a ben oil tree and a benzoil are various names of *Moringa oleifera*.

2.2 *Moringa oleifera* and its uses

The whole component of *Moringa oleifera* is consumed by humans as food. Several researchers have noted *Moringa oleifera* could be used as a cleaning agent, flower nectar for honey, green leaves for green manure, wood as blue dye, cakes for biogas production and leaves for biogas, as well as the juice extracted from the leaves for foliar nutrient. All portions of *Moringa oleifera* are currently being used either as a medication or for nutrition (both animal and human) (Popoola and Obembe *et al*., 2013).The return of seed oil from *Moringa oleifera* is 30-40% by weight, and Ben is a sweet oil, non-sticky, that is rancidity resistant. It is also a natural alternative source of lipids, digestible proteins and vitamins (ascorbic acid and carotenoids) that most impoverished countries worldwide can utilize for its use of minerals (calcium, iron) (Fahey *et al*., 2001). *Moringa oleifera* leaves have very strong antioxidant activity, they increase host immunity (Yang *et al*., 2006).

Moringa oleifera contains antioxidants that function effectively against the damage produced by free radicals. *Moringa oleifera* leaves contain a high concentration of antioxidants, which have an anti-inflammatory effect in cancer, hypertension, and cardiovascular disease. (Bamishaiye *et al*., 2011; Mensah *et al*., 2012). Β-carotene is another powerful antioxidant found in *Moringa oleifera* leaves. *Moringa oleifera* leaves have a high concentration of antioxidants, including vitamin A. (Ferreira *et al*., 2008; Lopez-Teros *et al*., 2017). *Moringa oleifera* has antibacterial and anticancer properties (Ayoola *et al*., 2008; Davinelli *et al*., 2015).

Because of their redox characteristics, phenolic chemicals present in *Moringa oleifera* have the ability to inactivate lipid free radicals. These phenolic compound capabilities are important in neutralising free radicals, quenching singlet or triplet oxygen, and degrading peroxides (Zheng and Wang, 2001).

Earlier studies discovered that different types of *Moringa oleifera* leaf extracts inhibit 89.7-92.0 percent of linoleic acid peroxidation and scavenge superoxide radicals in different doses in the carotene-linoleic acid system. According to Iqbal and Bhanger. (2006), ambient temperature and soil parameters have a significant impacts on the antioxidant activity of *Moringa oleifera* leaf extracts.

2.3 Nutritional Specifications of *Moringa oleifera* plant

Moringa oleifera-a Potential tree: *Moringa oleifera* is a safe and inexpensive source of protein and micronutrients. *Moringa oleifera* leaf meal has a crude protein content ranging from 17.9 to 26.8 percent (Sultana *et al*., 2015), with approximately 47 percent bypass protein and a sufficient amino acid profile (Nouman *et al*., 2014) The essential basic components of the animal body that is protein, amino acids, fatty acids, minerals, vitamins, calcium, potassium, various phenolics and oxycaroteniodes are present in *Moringa oleifera*. These nutrients are used in osmotic regulation, enzyme activation and growth hormone production, as well as other organic molecules that improve growth, function, and life process maintenance (Anjorin *et al*., 2010)

Over the past years because of its comprehensive nutritional, antioxidant, and medicinal qualities, *Moringa oleifera* has increasingly become the focus of animal husbandry researchers. Due to its rich nutrients and low content of antinutrients, *Moringa oleifera* leaves as animal feed has attracted interest. Leaves are also used as a protein source and were found to boost the growth efficiency of growing lambs, milk yield, and sheep and goat composition. Furthermore, the leaves contain about 200g/kg DM crude protein (CP), making it a low undegradable protein supplementation for ruminants (Kakengi *et al*., 2005) as compared to other shrubs like leucaena leaves (which contain CP ranging between 270 and 350g/kg DM) (Soltan *et al*., 2017)

Many *Moringa oleifera* sections are suggested for use as a complement when minerals or vitamins are deficient are limited or unavailable (FAO, 2014) It tolerates a wide range of soil conditions, but prefers a neutral to slightly acidic (pH 6.3 to 7.0), well-drained sandy or loamy soil (Thurber *et al*., 2009).The mineral content of dried leaves was just as follows: calcium (3.65%), magnesium (0.5%), phoshorus (0.3%), potassium (1.5%), sulphur (0.63%), sodium (0.164%), zinc (13.03 mg/kg), copper (8.25%), magnesium (0.5%), phoshorus

(0.3%), potassium (1.5%), sulphur (0.63%), sodium (0.164%), zinc (13.03 mg/kg), copper (8.25%), manganese (86.8 mg/kg), iron (490 mg/kg) and selenium (363 mg/kg), 17 fatty acids were observed with the highest value is - linolenic acid (44.57 percent), followed by heneicosanoic (14.41 percent), g-linolenic (0.20 percent), palmiteic (0.17 percent), and capric acid (0.07 percent). In the dried leaves, vitamin E had the highest concentration of 77 mg/100 g, followed by beta-carotene, which had 18.5 mg/100 g.

In vitro experiment conducted by Soliva *et al*. (2005) to assess the nutritional content of entire *Moringa oleifera* leaves and extract of its leaves. The OM, CP, NDF, ADF, ADL, and EE of the unextracted *Moringa oleifera* leaves used in the analysis were 899, 321, 167, 133, 23, and 49 g/kg DM, respectively.

Nouala *et al*. (2006) analysed the effect of *Moringa oleifera* leaves as a concentrate feed replacement on in vitro total gas production and nutrient digestibility. On a dry matter basis, the OM, CP, NDF, and ADF of *Moringa oleifera* meal used in the analysis were 89.29, 23.27, 18.74, and 16.07, respectively.

Asaolu *et al*. (2010) investigated the effects of *Moringa oleifera* and bamboo leaves on ground nut hay utilisation by West African goats. The proximate analysis of *Moringa oleifera* leaves used in the study revealed that they contained 25 percent DM, 22 percent CP, 6.68 percent EE, 11 percent CF, 41.3 percent NFE, 13.2 percent Ash, 28 percent NDF, 28.9 percent ADF, potassium 1.26 percent, sodium 0.28 percent, calcium 1.97 percent and phosphorus 0.13 percent.

Adegun *et al*. (2013) conducted a study to evaluate the growth performance of West African rams fed *Moringa oleifera* as a protein supplement to *Panicum maximum*. *Moringa oleifera* leaf meal (MLM) contained DM 88.6 percent, OM 78.2 percent, CP 22.1 percent, ADF 31.8 percent, NDF 36.2 percent, and total ash 2 percent, according to the study.

Moyo *et al*. (2014) studied the effect of *Moringa oleifera* Lam supplementation in goat diets. The DM, CP, ADF, NDF, acid detergent cellulose, phosphorus, and calcium content of *Moringa oleifera* leaf meal (MOL) were 88.93, 23.76, 2.06, 34.77, 17.15, 7.93, 0.64, and 2.78 percent, respectively.

Kholif *et al*. (2018) examine the impact of *Moringa oleifera* as a replacement for berseem clover in goat diets. The chemical composition of the *Moringa oleifera* used was measured, and it revealed that it contains DM- 296 g/kg DM, OM-888 g/kg DM, NSC 265 g/kg DM, CP 211 g/kg DM, EE 49 g/kg DM, NDF 362 g/kg, ADF 289 g/kg DM, ADL 63 g/kg DM, cellulose 226 g/kg DM, and hemicellulose 73 g/kg DM, Total phenols were 45 g/kg DM, tannins were 26 g/kg DM, and saponins were 11.2 g/kg DM.

Meel *et al*. (2018a) investigated the growth performance of Sirohi goat kids fed varying amounts of *Moringa oleifera* leaves. The researchers determined the approximate composition of *Moringa oleifera* leaves used in the study. This includes the following: the dry matter; organic matter; crude protein; ether extract; crude fibre; nitrogen-free extract;and total ash levels in *Moringa oleifera* leaves were 85.69, 90.24, 23.31, 4.7, 9.26, 52.97, and 9.76 percent, respectively.

Khalel *et al*. (2014) had compared and noted their impact on cattle's performance with the nutritional value of *Moringa oleifera* and *Trifolium alexandrinium*. The chemical composition of leaves of *Moringa oleifera* in the study indicated that 76.42% Moisture contained 18.12% CP, 22.73% CF, 2.11% EE, 46.90% NFE, 10,14% Ash, 3.69% Calcium and 0.33% Phosphorus.

A study was conducted by Jiwuba *et al*. (2016) to investigate feed and body weight increase in West African goats fed moringa leaf food. The next study of *Moringa oleifera* found that it has 87.90% dry matter, 23.24% crude protein, 15.16% crude fibre, 4.15% ether extract, NFE 39.14%, ash 6.21%, and 4.03% kcal/g gross energy.

2.4 .Properties of Phytochemicals in *Moringa oleifera* with uses

2.4.1 Tannins and Saponins

Moringa oleifera leaves are high in saponin and tannins, which are water soluble phenolic compounds that cause alkaloids, gelatin, and other proteins to precipitate. Tannin concentrations in dry leaves range from 13.2 to 20.6 tannin/kg (Richter *et al*., 2003). Tannins are anti-cancer, anti-atherosclerotic, anti-inflammatory, and anti-hepatotoxic (Adedapo *et al*., 2015). Saponin, on the other hand, has anti-cancer properties (Tian *et al*., 2013).Saponin is a naturally occurring compound composed of an isoprenoidal-derived aglycone moiety covalently linked to one or more sugar moieties (Augustin *et al*., 2011).

2.4.2 Alkaloids, glucosinolates and isothiocyonates

Moringa oleifera leaves have yielded a variety of alkaloids, including -L-rhamnopyranosyl vincosamide, phenylacetonitrile, pyrrolemarumine, 40hydroxyphenylethanamide-, and -L-rhamnopyranoside glucopyranosyl derivative (Panda *et al*., 2013, Sahakitpichan *et al*., 2011).

2.4.3 Glucosinolates

Glucosinolates, 4-0-(a-L-rhamnopyranosyloxy)-benzylglucosinolate or glucomoringin, are found in almost every part of *Moringa oleifera*(stem, leaves, flowers, pods, and seeds), whereas roots are high in benzyl glucosinolate (glucotropaeolin) (Amaglo *et al*., 2010). Glucosinolates and isothiocyanates are both materials possess beneficial to health (Dinkova-Kostova and Kostov, 2012).

2.4.4 Polyphenol

Moringa oleifera contains a variety of antinutrients that have a minor negative impact on its beneficial actions. It contains anti-cancer substances such as glycerol-1 9-octadecanoate, isothiocyanates, glucosinolates, and a small amount of glycoside compounds, as well as plant secondary metabolites such as polyphenols, tannins, terpenoids, anthraquinones, alkaloids, terpenoids, sterols, and some types of soluble carbohydrate (Makkar and Becker, 1997).

2.5 Effect of *Moringa oleifera* on Body weight and Metabolic Body weight

Choudhary *et al*. (2018) assessed the effects of *Moringa oleifera* leaves on lactating Bengal goat growth performance. Goats were fed rations containing 100% concentrate modure (T1), 70% concentrate mixture+30% *Moringa oleifera* leaves (T2), and 50% concentrate mixture-50% *Moringa oleifera* leaves (T3), respectively. In the experiment, the T3 group grew the fastest, followed by the T2 and T1 group.

Babiker *et al*. (2017) investigated the effect of *Moringa oleifera* leaves diet (MOD) on lamb and kid growth performance when leaves were used as a replacement for Alfa hay diet (AHD). The inclusion of *Moringa oleifera* leaves in the diet of lambs resulted in a significant increase in the animals' total body weight. The inclusion of *Moringa oleifera* leaves in the diet had no effect on weight gain in goat kids.

Ahmad *et al*. (2017) investigated the effect of dried *Moringa oleifera* leaves on calves' growth performance. Calves were classified into 5 groups and fed rations containing 0, 5, 10, 15, and 20% of DMOL by replacing equal proportions of calf starters, the study found that calves fed diets supplemented with MO had significantly higher total body weight gain and average daily gain.

Damor *et al*. (2017a) investigated the influence of *Moringa oleifera* leaves on Mehsana goat kids' growth performance. In the survey of 18 goat kids, T1-100% concentrate combination was divided into three experimental treatments: T2-50% concentrate mixture +50% *Moringa oleifera* leaves and T3-100% *Moringa oleifera* leaves. T3 had the highest ($P<0.001$) changes in overall body weight and average daily body weight gain among goat kids, followed by T2 and T1.

Aharwal *et al*. (2018) investigated the impact of *Moringa oleifera* leaves on buffalo calves' growth results. Twelve calves were split into two categories. The control group received calf starter along with milk, while the M10 group received *Moringa oleifera* leaves in place of 10% calf starter. In the report, the final average body weight of the calves in each category increased by 8.30 percent. During the pre-weaning phase, there were no substantial differences in ADG ($P< 0.05$). Statistical analysis revealed that there were significant differences in ADG among the groups ($P<0.05$) during the post-weaning phase.

Paguia *et al*. (2014) investigated and evaluated the use of *Moringa oleifera* as broiler chicken feed. The effects of *Moringa oleifera* leaf powder and leaf meal on broiler chicken diets were investigated in three experiments. In the experiment, 150 day-old Cobbs broilers were randomly assigned to one of five nutritional treatments using a Complete Randomized Design (CRD).Each treatment involves 30 birds. T1-Basal diets, T2-0.20 percent MOLM, T3-0.30 percent MOLM, T4-0.40 percent MOLM, and T5- 0.50 percent MOLM are among the therapies. In terms of ADG, feed intake, FCR, final weight, and income over feed cost, broiler performance was not significantly better than the control.

Sultana *et al*. (2015b) investigated the effect of *Moringa oleifera* foliage on goat growth and nutrient utilisation. Goats in groups T1 to T5 were fed diets containing 100 percent, 75 percent, 50 percent, 25 percent, and 0 percent *Moringa oleifera* foliage, respectively. The study found that as the amount of *Moringa oleifera* foliage in the diet increased, the weight gain of the experimental goats increased. The average daily gain in goats fed the T1 diet was significantly higher than in goats fed other diets.

Adegun and aye. (2013) examined into the growth and economy in West African Dwarf Rams given *Moringa oleifera* and cotton seed cake as protein supplements to *Panicum maximum*. The objective of this research was to assess the protein substitution values of

Moringa oleifera leaf meal and cotton seed cake in concentrates supplied as a supplement to Panicum maximum for West African Dwarf (WAD) ram for 84 days. In Diet 1, 2, 3, 4, 5, *Moringa oleifera* leaf meal (MLM) was replaced with cotton seed cake (CSC) at 0, 25, 50, 75, and 100%, respectively. The highest CP (21.68g/100gm) was of Diet 1 (0 percent MLM), while 19.84, 19.47, 17.85 and 15.75 g/100gm were other values of diet 3, 2, 4 and 5. The dietary level of MLM ($P<0.05$) increased, however there were no variations to ram weight when intake decreased. Every animal had an average weight gain of 47.25g per day. The FCR rate was 6.9 less in diet 5, while diets 1 and 3 were 7.9 and 7.8. Diet 5 showed an increase in the cost effectiveness of MLM in diets. This means that CSC as a protein source can be fully substituted by MLM in the concentrate mix offered to western African dwarf sheep in addition to the *Panicum maximum* diet. Reduced production costs will benefit poor farmers' resources.

2.6 Effect of *Moringa oleifera* on Feed Intake

The effect of *Moringa oleifera* leaf meal on the performance of Murrah buffalo calves was studied by (Aharwal *et al*., 2018). 18 Murrah buffalo calves of body weight equivalent to either sex were identified on the 5th day after birth and uniformly divided into different groups (Control, M5 and M15) with six calves for every group. The control group was raised including milk and on the calf starter/concentrate mixture. *Moringa oleifera* leaf meal @ 5% and 15% was substituted with the milk calf starter concentrate mixture for the M5 and M15 groups. Results showed that the intake of dry matter, average daily intake of dry matter (percent BW), crude protein, and protein quality differed significantly amongst these groups.

Adegun *et al*. (2013) investigated the effects of *Moringa oleifera* and cotton seed cake on feed intake in rams. Rams were classified into five groups and fed diets containing 0 %, 25%, 50%, 75%, and 100% *Moringa oleifera* leaves as a replacement for cotton seed cake. According to the study, feed intake differed significantly between groups, with the highest feed intake recorded in the group supplemented with 0% *Moringa oleifera* leaves.

Khalel *et al*. (2014) conducted a study in cows to compare the nutritional value of *Moringa oleifera*(drumstick) and *Trifolium alexandrinum* (berseem) as supplements. The cows were divided into three groups and fed 60 percent concentrate with 40 percent berseem R1, 40 percent *Moringa oleifera*(R2) and 20 percent berseem + 20 percent *Moringa oleifera* (R3) respectively. The dry matter intake did not significantly differ among the groups in the study, but the dry matter intake of the goats fed 75% and 50% *Moringa oleifera* foliage was significantly greater than that of the goats fed 100%.

In a study, Sultana *et al*. (2015) evaluated *Moringa oleifera* foliage as goat feed. Growing male goats were divided into five treatment groups for the study: T1 (100 percent *Moringa oleifera* foliage); T2 (75 percent *Moringa oleifera* foliage+25 percent napier grass); T3 (50 percent *Moringa oleifera* foliage +50 percent napier grass); T4 (25 percent *Moringa oleifera* foliage+75 percent napier grass); and T5 (100 percent napier grass). The study's findings revealed that the T1 diet had significantly higher DM intake than the T2, T3 and T4 or T5 diet.

Jiwuba *et al*. (2016) investigated the feed intake of goats fed *Moringa oleifera* leaf meal. Experimental goats were fed diets containing *Moringa oleifera* leaf meal at 0%, 5%, 10%, and 15% concentrations. The study found that goats fed 15% *Moringa oleifera* leaf meal had the highest average daily feed intake.

Roy *et al*. (2016) investigated the effects of *Moringa oleifera* on growing bull performance. The bulls were classified into three categories based on their dietary treatment. The control group was fed maize silage, while the two treatment groups were fed *Moringa oleifera* foliages and Australian Sweet Jumbo silage, respectively. The daily DM intake of bulls fed *Moringa oleifera* foliage was significantly higher ($p<0.01$) than that of bulls fed maize or Australian sweet Jumbo, according to the study.

Kholif *et al*. (2016) conducted a study in which fresh *Moringa oleifera* leaves, *Moringa oleifera* hay, and *Moringa oleifera* silage were integrated into the diets of goats. When compared to a control diet or *Moringa oleifera* hay, goats fed *Moringa oleifera* silage or fresh leaves had higher DM intake (DMI).

2.7 Effect of *Moringa oleifera* on Nutrient Digestibility

Asaolu *et al*. (2011) examined the effects of *Moringa oleifera* and bamboo leaves on ground nut hay uptake by West African goats. The goats were divided into three groups and fed 50 percent *Moringa oleifera* leaves addition 50 percent GNH, 50 percent Bamboo leaves plus 50 percent GNH, and 100 percent GNH. The study's findings revealed that goats supplemented with *Moringa oleifera* leaves had considerably higher dry matter crude protein and NFE digestibility values. The nitrogen intake, nitrogen excreted, and nitrogen balance of the *Moringa oleifera* supplemented group all increased significantly.

Fadiyimu *et al*. (2010) studied nutritional digestibility and nitrogen balance in rams fed *Moringa oleifera* leaf diets. In the study, five treatment groups with four rams each were examined. At 0, rams were fed meals containing *Moringa oleifera* leaves. By replacing equal proportions of Panicum maximum at 25, 50, 75, and 100 percent levels. The digestibility of DM, OM, and CF declined linearly as the amount of *Moringa oleifera* in the diet increased, although the digestibility of CP and nitrogen balance values increased.

Khalel *et al*. (2014) investigated nutritional digestibility and nitrogen balance in cows fed diets containing *Moringa oleifera* and *Trifolium alexandrinum* (berseem). Cows were grouped into different dietary regimens, each of which consisted of a 60 percent concentrate feed mixture and a 40 percent berseem (R1), 40 percent *Moringa oleifera*(R2) and 20 percent berseem plus 20 percent *Moringa oleifera* (R3).The results showed that *Moringa oleifera* supplemented groups had considerably greater digestibility values for all nutrients. The R2 group had the highest values, followed by the R1 and R3 groups

Tona *et al*. (2014) investigated the effect of *Moringa oleifera* leaf on goat nutritional digestibility. Goats were fed *Moringa oleifera* leaf meal at 0%, 5%, 10% and 15% levels, respectively. It was discovered that goats fed a diet containing 15% *Moringa oleifera* leaf had significantly greater digestibility coefficients than other levels.

Kholif *et al*. (2015) examine the influence of using *Moringa oleifera* leaf meal (MLM) as a protein source in the meals of lactating goats. Animals were separated into four dietary groups, with *Moringa oleifera* leaf meal supplemented at 0, 10, 15, and 20% levels, replacing 0, 50, 75, and 100% of sesame meal, respectively. In the study dry matter, organic matter, and fibre digestibility were increased in animals fed the M15 diet.

Azzaz *et al*. (2016) investigated the influence of *Moringa oleifera* supplements on the diets of lactating ewes. Three groups of ewes were formed. The ewes in the first group were fed 30% complete feed and 70% berseem and served as controls. In addition to the control diet, the ewes in the second group were supplemented with *Moringa oleifera* dried leaves at a rate of 15 gm per Kg DM. The study examined the nutrient digestibility of experimental ewes, which demonstrated that the digestibility of DM, OM, CP, and NFE increased significantly when *Moringa oleifera* leaves were supplemented in the diet compared to control and Echinacea purpura supplemented diets. However, the digestibility of CF and EE showed no significant variations among the groups. A similar pattern was noticed in the DCP and TDN values. The *Moringa oleifera* supplemented group had the highest DCP and TDN levels, and the difference between groups was significant.

Richter *et al*. (2003) determined the CP, EE, and ash levels of *Moringa oleifera* leaf meal to be 25.6%, 10.6%, and 8.4%, respectively. There were also significantly high quantities of NDF 15.9% and ADF 12.6% in *Moringa oleifera* leaves. Crude protein, acid detergent fibre, and neutral detergent fibre, as well as crude fat, carbs, and ash, were found to be beneficial in increasing animal output. Furthermore, low-quality livestock fodders or rations can be enhanced by supplementing with *Moringa oleifera* leaves, which increases the dry matter intake and digestibility of the fodder by cattle.

Akinyemi *et al.* (2010) have evaluated the effect of the inclusion level of the sheep fed *Moringa oleifera* as *Panicum maximum* supplements on nutrients intakes, digestibility, nitrogen balance and haematological parameters in the West African Dwarf rams (WAD). Twenty WAD rams with an initial average weight of 16.1 kg were undertaken for this investigation. Five treatments 1 - 5 were allocated with inclusion levels of 0, 25, 50, 75 and 100%, respectively. The following results have been discovered: (DM) Dry Matter, Organic Matter (OM), Crude Protein (CP) and Nitrogen-free extract (NFE) digestibility were greatest at 100 percent *Moringa oleifera* inclusion, while CF and ether extract (EE) digestibility were greatest at 25 percent inclusion, yielding equivalent DM and CP digestibility with 100 percent inclusion.

2.8 Effect of *Moringa oleifera* on Haemato-biochemical attributes

Meel *et al*. (2018) performed the study on *Moringa oleifera* feeding on the hemato-biochemical profile of the young sirohi goat kids. They took 40 Sirohi goat kids divided on the basis with similar age and identical conformity. Group T1 was provided with 60% of methi straw and 40% of concentrate, and in groups T2, T3, T4 and T5, the concentrate was replaced by *Moringa oleifera* in percentage of 25%,50%,75%.The result showed as T5 (6.84±0.06 g/dl) and (3.03±0.07g/dl) serum total protein and serum albumin were significantly increased ($P<0.05$). Improved serum globulin levels (3.86±0.09 g/dl in T5 and 3.69±0.11 g/dl in T1) and decreased serum glucose levels (58.27±1.06 mg/dl in T5) by *Moringa oleifera* leaves. Concentrate feed when substituted with 100 percent *Moringa oleifera* leaves, the biochemical profile showed improvement in sirohi goat kid's diet.

The experiment carried out by Babiker *et al*. (2017) on 20 dairy Aardi goats. They had two groups with 10 animals, each in a duplicated 2-2 crossover design. They were divided into two groups. The inclusion rate for *Moringa oleifera* Hay was 25% of the total mixed ration (TMR) when a 40 percent TMR alfalfa hay was replaced by the TMR. In comparison to the diet for Alfalfa hay, *Moringa oleifera* hay had serum with more antioxidants (TAC) and vitamin C. The serum of goats fed *Moringa oleifera* hay contained lower levels of cholesterol and glucose.

Khalel *et al*. (2014) investigated the effect of feeding different amounts of *Moringa oleifera* on multiparous cross-bred Friesian cows. They divided fifteen cross-bred Friesian cows into three groups of five animals each: control (60 percent concentrate and 40 percent berscem), second (60 percent concentrate and 40 percent berseem replaced with 40 percent *Moringa oleifera*), and third (60 percent concentrate and 20 percent berseem replaced with 20 percent Moringa).They discovered that feeding *Moringa oleifera* rations was related with greater (P<0.05) blood glucose concentrations and enzymatic antioxidant activity (GSH-PX, CAT, and SOD), as well as lower (P<0.05) blood cholesterol and urea contents than animals fed all berseem diets (R1).

Babiker *et al*. (2017) conducted study on the feeding value of *Moringa oleifera* leaves diet (MOD) as a partial substitution for alfalfa hay diet (AHD) in ewes and goats. In a 6-week trial, twenty animals from each group were involved. Each animal group was separated into two groups of ten animals each, and the animals were distributed in a replicated 2×2 crossover arrangement. The total antioxidant capacity (TAC) and vitamin C levels in milk and serum of goats and ewes fed MOD were higher than those in AHD-fed goats and ewes. Lower levels of glucose was seen in the serum of goats and ewes given MOD.

An experiment was conducted by Oparinde, D. P., & Atiba, A. S. (2014) on Wistar rats in order to assess plasma malondialdehyde, total antioxidant status, and red blood cell glutathione peroxidase. A total of seventy healthy Wistar rats were used in the study. Over the course of 4 weeks, 40 rats were fed a food fortified with *Moringa oleifera* leaf and 30 rats were fed standard diet. The mean serum TAS in rats feeding *Moringa oleifera* was reported to be significantly higher (P<0.01) compared in rats fed a conventional diet.

2.9 Effect of *Moringa oleifera* on Hormones and Reproduction

The research assessed the effect of aqueous leaf extracts of *Moringa oleifera* on follicle-stimulating hormones and serum cholesterol in Wistar rats (Nwamarah *et al*., 2015) .Mean concentrations of follicle stimulating hormone (FSH) in U/ml of male and female Wistar rats administered with graded doses of aqueous leaf extract *Moringa oleifera* were observed. 1 percent was administered to male rats in group A aqueous leaves extract of *Moringa oleifera* with a percentage increase of 38.52 %, it had the highest concentration of FSH (3.92 U/ml) followed by the rats in group C that were given 10% of *Moringa oleifera* (3.50 U/ml) (23.67 percent). Group B rats were fed 5% *Moringa oleifera* leaf extract showed low concentration of FSH (2.23 U/ml) with a 21.20% decrease. In contrast with the control group, there was a major statistical difference ($P<0.05$) between the mean FSH concentration of the groups.

In adult Swiss rats, the function of *Moringa oleifera* aqueous leaf extract in regulating thyroid hormone status has been studied by (Tahiliani *et al*., 2000) 175mg/kg BW on day 1 for 10 days extract of *Moringa oleifera* were given to both males and females. After administration of extract, serum T3 and hepatic LPO reduced and serum T4 increase observed in female rats. As there was no significant change in males it suggest that *Moringa oleifera* has less effect in males than in females. There were no significant change in other parameters.

To evaluate the possible role of *Moringa oleifera* on sow productivity and ghrelin-growth hormone (GH)/insulin-like growth factor-1 (IGF-1) metabolic pathway, two experiments were performed by (Gonzales *et al*., 2019). 15% *Moringa oleifera* leaf meal (MoLM) was included in diets of four sexually mature gilts to determine plasma ghrelin and IGF levels. There were no statistically different levels of plasma ghrelin and IGF-1 between the control and experimental classes. The level of IGF-1 in pigs fed with MoLM seemed to be regulated down. The observations demonstrated the ability of 15 percent MoLM to increase sow productivity via the metabolic pathway of ghrelin-GH/IGF-1.

In the study conducted by (Adeyemi, 2014), sixty rabbits were subjected to four diets with supplementation MoLM in a 24-week trial: 0 (control), 2.5, 5.0 and 7.5 percent with 10 bucks and 5 bucks in every treatment group.The hormonal assay outcomes showed a rise in testosterone and oestrogen levels, when the dietary levels of MoLM was increased. In male rabbits, serum testosterone levels did not change significantly between treatments, but there was lower level in rabbits fed with control diet than to the ones on the MoLM diet. Similar but higher levels of serum oestrogen were observed in rabbits with 2.5 and 7.5 percent of MoLM diet.

The main objective of this study by Abdel-Raheem *et al*. (2021) was to evaluate how feeding *Moringa oleifera* leaf meal (MLM) as a substitute for soybean meal affected nutritional digestibility, rumen fermentation, rumen enzyme activity, blood metabolites, growth-related hormones, and specific growth rate in buffalo calves. The experimental diets had no statistically significant difference on serum growth hormone concentrations (P> 0.05). Plasma IGF 1 levels were considerably higher in calves supplemented with 15% MLM compared to calves in the M0 and M20 treatment groups.

Under heat stress conditions, the experiment was carried out by (Hassan *et al*., 2016) to investigate the productive performance, carcass characteristics, and certain blood parameters of broiler chicks feeding corn-soybean meal diets with three levels of *Moringa oleifera* leaves meal (MOLM). Two hundred and eighty-one day old chicks were randomised to one of four treatments at random. As a control, the first treatment provided a commercial basal diet, whereas the other treatments 2, 3, and 4 provided a commercial basal diet supplemented with MOLM (0.1, 0.2 and 0.3 percent, respectively). Increased MOLM levels resulted in significant (p<0.05) increases in thyroid hormones (T3 and T4), whereas the ratio of T3/T4 was unaffected by the rise in MOLM level.

The objective of current experiment by (El Badawi *et al*., 2014) is to observe different levels of *Moringa oleifera* leaves (ML) supplementation influence the haematological profile, blood biochemical components, and histopathological modifications in growing rabbits. Thirty-six white male New Zealand (NZW) rabbits about 4-5 weeks with an initial average body weight of 566.5 g were assigned randomly to one of four treatment groups (9 rabbits each) and fed ad libitum for 56 days on a basal diet supplemented with 0, 0.15, 0.30, and 0.45 percent ML. At the end of the experiments, three rabbits from each group were slaughtered for blood and histological investigations. Triiodothyronine (T_3) and thyroxine (T4) levels were considerably (P<0.05) higher with ML treatments compared with control, with the greatest levels recorded with 0.30 percent ML, while a significant reduction was observed with 0.45 percent ML treatment.

The research by (Khalid *et al*., 2020) evaluated the effects of *Moringa oleifera* leaf powder (MOLP) supplementation .Young New Zealand White rabbits (males) aged 32 weeks (n = 21, mean body weight 3318±171 g) were raised on commercially pelleted diet for four weeks and separated into three groups: control (CON, 25°C), HS (35±1°C), and HS (35±1°C) with MOLP (HSM) supplemented orally (200 mg/kg body weight). The HS group had higher serum cortisol levels than the CON group (P<0.05), but MOLP had lower cortisol levels than the HS group (P<0.05). After going through the review, following gaps in knowledge have been observed.

Gaps in Knowledge

1. A limited information is available on the effect of *Moringa oleifera* leaves feeding on the performance of Murrah buffalo heifers.
2. There is a scarcity of literature on the usage of *Moringa oleifera* to improve reproductive physiology of buffaloes.

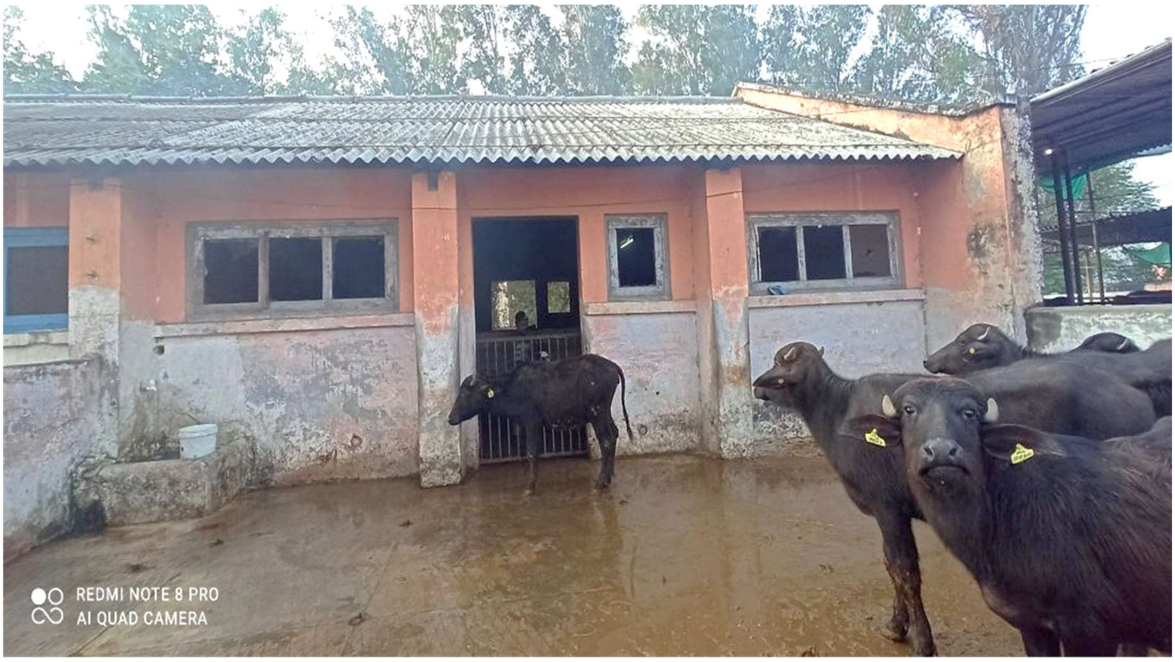


Photo- 1. Housing of animals in well ventilated Shed

Photo- 2. Feeding of Chaffed green oats, Wheat straw and concentrate to control and experimental animals

Photo- 3. Green roughage

Photo- 4. Dried *Moringa oleifera* leaves

Photo- 5. Feeding of dried *Moringa oleifera* leaves with concenterate in tub

Photo- 6. Individual feeding of Dried *Moringa oleifera* leaves

Photo- 7. Digestion trial in Murrah buffalo heifers

Photo- 8. Separation of plasma after centrifugation of blood

Photo- 9. Analysis of Plasma samples for Hormones using ELISA kits

Chapter -3

Materials and methods

Materials and Methods

3.1 In Vivo Study

The general procedure used to study the effect of *Moringa oleifera* leaves as a feed source in growing heifers.

3.1.1 Location of Study

The present study was conducted at Livestock Research Centre (LRC), National Dairy Research Institute, Karnal, Haryana, India, located at 29°41′N 76°59′E/29.68°N 76.98°E. The location has an elevation of 250 metres (748 feet) from sea level. Minimum and maximum ambient temperature range from near 0°C in winter to 45°C in summer with diurnal variation in the order of 15-20°C along with an annual rainfall of 700 mm.

3.1.2 Ethical approval

The experiment was approved by the Institutional Animal Ethics Committee (IAEC) of the Indian Council of Agricultural Research Institute (ICAR) - National Dairy Research Institute (NDRI) on 27-10-2020 under approval no. M-20/AG&B/2018/387-393 constituted as per Article 13 of CPCSEA rules, laid down by the Government of India. All ethical guidelines were followed during the experiment. Animals were fed recommendations of ICAR, 2013 guidelines.

3.1.3 Selection of experimental animals

The study was conducted from December 2020 to May 2021 on 14 growing Murrah Buffalo heifers of age 11-13 months at the time of selection. These animals were divided into two groups of 7 animals each on basis of body weight and age of animals. The two groups as control were fed with diet as per ICAR guidelines and the treatment group was fed with control diet of roughages, concentrates and along with it supplementation of *Moringa oleifera* leaves at 20% of the dry matter intake.

3.1.4 Housing and Management of animals

All experimental animals were provided with normal feed and fodder as per the availability with the Livestock Research Centre. The concentrate mixture was given as provided by LRC with a CP of 20%. The animals in the treatment group were fed a control diet along with it were supplemented with 400 g of *Moringa oleifera* dried leaves to individual animals. Fresh and clean water was provided free choice to each buffalo. Animals were fed with roughage, concentrate and *Moringa oleifera* dried leaves in the morning and roughage and concentrate in the evening. Animals were kept in a well-ventilated shed with a proper supply of water and electricity.

3.1.5 Feeding of experimental animals

The experimental diets were balanced for energy and protein. All the animals were fed as per recommendations of ICAR (2013) feeding standards to meet their nutrient requirement. Green oats fodder was supplied twice daily, wheat straw once daily and concentrate mixture was fed in proportions. Fresh and clean tap water for drinking was made available *ad libitum* atleast twice daily

A. Control group (C)

Animals in the control group were fed chopped oats fodder, threshed wheat straw and concentrate mixture.

B. Treatment group (T)

Animals in the treatment group were fed 20 % of DMI of dried *Moringa oleifera* leaves in addition to the feed as given in the control group of chopped oat fodder, wheat straw and concentrate mixture.

3.1.6 Measurement of Bodyweight and dry matter intake

3.1.6.1 Body Weight

Every fortnightly animals were weighed on digital platform balance in the morning before feeding. Body-weight records were maintained during the experiment. The total body-weight gain (kg) of each group was calculated at the end of the experiment.

3.1.6.2 Dry Matter Intake

Growing Murrah buffalo heifers were fed measured quantity of experimental feed and during the feeding trial and left over feed was the measured the next day to obtain daily dry matter intake of animals. Dry matter intake of experimental feeds in terms of kg/100 kg BW was calculated from records of dry matter consumption and body weights of Murrah buffalo heifers.

3.1.7 Blood sampling and analysis

Blood Samples were collected early in the morning. It was collected on 0, 30, 60, 90, 120 and 150, 180 days. Blood was drawn aseptically from the jugular vein of each animal in a sterile EDTA vacutainer (Vacutte®, K_3EDTA, Greiner bio-one (9ml) GMDH, Austria). Immediately after collection blood was transported to the lab in an icebox and then centrifuged at 2500 rpm for 25 minutes to separate plasma. Plasma obtained was decanted in clean, dried plastic Eppendorf vials and stored at -20˚C for subsequent analysis of various plasma metabolites.

Analysis of Hormones and Biochemical attributes:

3.1.7.1 Bovine plasma growth hormone

Growth Hormone was determined in the plasma of animals by Bovine ELISA kit (Catalog No.**E0013Bo**) from Bioassay Technology Laboratory, Shanghai, China.

Principle of assay

The Enzyme-Linked Immunosorbent Assay (ELISA) plate has been pre-coated with Bovine GH antibody. GH present in the sample was added that binds to antibodies coated on the wells. The biotinylated Bovine GH Antibody was added that binds to GH in the sample. Then Streptavidin-HRP was added that binds to the Biotinylated GH antibody. After incubation unbound Streptavidin-HRP was washed away during a washing step. Substrate solution was then added and colour developed in proportion to the amount of Bovine GH. The reaction was terminated by addition of the acidic stop solution and absorbance was measured at 450 nm.

Materials provided in the kit

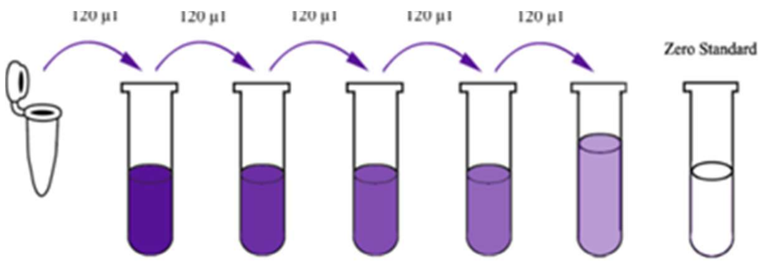
Standard Solution (12.8ng/ml)-0.5ml x1, Pre-coated ELISA Plate-12 * 8 well strips x1, Standard Diluent-3ml x1, Streptavidin-HRP-6ml x1, Stop Solution-6mlx1, Substrate Solution A -6ml x1, Substrate Solution B-6ml x1, Wash Buffer Concentrate (25x)-20ml x1, Biotinylated Bovine GH Antibody 1ml x1, User Instruction-1 , Plate Sealer -2 pics, Zipper bag-1 pc

Reagent Preparation

1. All reagents were brought to room temperature before use.
2. **Standard** 120µl of the standard (12.8ng/ml) was reconstituted with 120µl of standard diluent to generate a 6.4ng/ml standard stock solution. The standard was allowed to sit for 15 min with gentle agitation prior to making dilutions. Other standards were prepared by serially diluting the standard stock solution 6.4ng/ml 1:2 with standard diluent to produce

3.2ng/ml, 1.6ng/ml, 0.8ng/ml and 0.4ng/ml solutions. Standard diluent serves as the zero standard (0 ng/ml). The remaining solution was frozen at -20°C for use within one month. Dilutions of standard solutions suggested were as follows:

6.4ng/ml	Standard No.5	120µl Original Standard + 120µl Standard Diluent
3.2ng/ml	Standard No.4	120µl Standard No.5 + 120µl Standard Diluent
1.6ng/ml	Standard No.3	120µl Standard No.4 + 120µl Standard Diluent
0.8ng/ml	Standard No.2	120µl Standard No.3 + 120µl Standard Diluent
0.4ng/ml	Standard No.1	120µl Standard No.2 + 120µl Standard Diluent



Standard Concentration	Standard No.5	Standard No.4	Standard No.3	Standard No.2	Standard No.1
12.8ng/ml	6.4ng/ml	3.2ng/ml	1.6ng/ml	0.8ng/ml	0.4ng/ml

Procedure

1. 20ml of Wash Buffer Concentrate 25x was diluted with deionized or distilled water to yield 500ml of 1x Wash Buffer. The solution was mixed gently until the crystals have been completely dissolved.
2. The no of strips required for the assay was determined. The no. of strips needed was determined by that of samples to be tested by that of standards. The strips were inserted in the frames for use.
3. 50µl standard was added to the standard well.

4. 40µl sample to sample wells was added and then 10µl anti- GH antibody was added to the sample wells, followed by the addition of 50µl streptavidin- HRP to sample wells and standard wells. The plate was mixed and the plate was covered with a sealer.
5. The plate was incubated at 37°C for 60 minutes.
6. The sealer was removed and the plate was washed 5 times with wash buffer. The wells were soaked with at least 0.35ml wash buffer for 30 seconds-1 minutes for each washing.
7. 50 µl substrate solution A was added to each well and 50 µl substrate solution B was added to each well. The plate was then covered with a new sealer and then incubated for 10 minutes at 37◦C in the dark.
8. 50 µl Stop solution was added to each well, the blue colour changed to yellow colour immediately.
9. The optical density (OD) of each well was determined immediately using a microplate reader set at 450 nm within 30 minutes after the addition of stop solution.

Calculation of Result

Standard curve was constructed by plotting the average OD for each standard on the vertical (Y) axis against the concentration on the horizontal (X) axis with computer-based curve-fitting software and concentrate was determined by using regression analysis.

3.1.7.2 Bovine plasma estradiol

Estradiol was determined in the plasma of animals by Bovine ELISA kit (Catalog No. **E0004Bo**) from Bioassay Technology Laboratory, Shanghai, China.

Principle of assay

The Enzyme-Linked Immunosorbent Assay (ELISA) plate has been pre-coated with Bovine Estradiol antibody. Estradiol present in the sample was added that binds to antibodies coated on the wells. The biotinylated Bovine Estradiol Antibody was added that binds to Estradiol in the sample. Then Streptavidin-HRP was added that binds to the Biotinylated Estradiol antibody. After incubation unbound Streptavidin-HRP was washed away during a washing step. Substrate solution was then added and colour developed in proportion to the amount of Bovine Estradiol. The reaction was terminated by addition of the acidic stop solution and absorbance was measured at 450 nm.

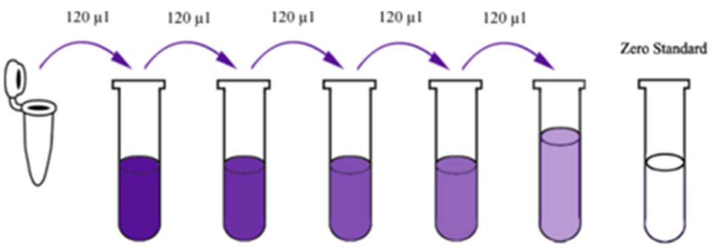
Materials provided in kit

Standard Solution (12.8ng/ml)-0.5ml x1, Pre-coated ELISA Plate-12 * 8 well strips x1, Standard Diluent-3ml x1, Streptavidin-HRP-6ml x1, Stop Solution-6mlx1, Substrate Solution A -6ml x1, Substrate Solution B-6ml x1, Wash Buffer Concentrate (25x)-20ml x1, Biotinylated Bovine estradiol Antibody 1ml x1, User Instruction-1 , Plate Sealer -2 pics, Zipper bag-1 pc

Reagent Preparation

1. All reagents were brought to room temperature before use.
2. **Standard** 120µl of the standard (1600pg/ml) was reconstituted with 120µl of standard diluent to generate 800pg/ml standard stock solution. The standard was allowed to sit for 15 min with gentle agitation prior to making dilutions. Other standard were prepared by serially diluting the standard stock solution 800pg/ml 1:2 with standard diluent to produce 400pg/ml, 200pg/ml, 100pg/ml and 50pg/ml solutions. Standard diluent serves as the zero standard (0 ng/ml). Remaining solution was frozen at -20°C for use within one month. Dilution of standard solutions suggested were as follows:

800pg/ml	Standard No.5	120µl Original Standard +120µl Standard Diluent
400pg/ml	Standard No.4	120µl Standard No.5 + 120µl Standard Diluent
200pg/ml	Standard No.3	120µl Standard No.4 + 120µl Standard Diluent
100pg/ml	Standard No.2	120µl Standard No.3 + 120µl Standard Diluent
50pg/ml	Standard No.1	120µl Standard No.2 + 120µl Standard Diluent



Standard Concentration	Standard No.5	Standard No.4	Standard No.3	Standard No.2	Standard No.1
1600pg/ml	800pg/ml	400pg/ml	200pg/ml	100pg/ml	50pg/ml

Procedure

1. 20ml of Wash Buffer Concentrate 25x was diluted with deionized or distilled water to yield 500ml of 1x Wash Buffer. The solution was mixed gently until the crystals have been completely dissolved.
2. The no of strips required for the assay was determined. The no. of strips needed was determined by that of samples to be tested by that of standards. The strips were inserted in the frames for use.
3. 50µl standard was added to standard well.
4. 40µl sample to sample wells was added and then 10µl anti-estradiol antibody was added to the sample wells, followed by the addition of 50µl streptavidin- HRP to sample wells and standard wells. The plate was mixed and the plate was covered with a sealer.
5. The plate was incubated at 37°C for 60 minutes.
6. The sealer was removed and the plate was washed 5 times with wash buffer. The wells were soaked with at least 0.35ml wash buffer for 30 seconds- 1 minutes for each washing.
7. 50 µl substrate solution A was added to each well and 50 µl substrate solution B was added to each well. The plate was then covered with a new sealer and then incubated for 10 minutes at 37◦C in the dark.
8. 50 µl Stop solution was added to each well, the blue colour changed to yellow colour immediately.
9. The optical density (OD) of each well was determined immediately using a microplate reader set at 450 nm within 30 minutes after the addition of stop solution

Calculation of Result

Standard curve was constructed by plotting the average OD for each standard on the vertical (Y) axis against the concentration on the horizontal (X) axis with computer-based curve-fitting software and concentrate was determined by using regression analysis.

3.1.7.3 Bovine plasma insulin-like growth factors 1(IGF-1)

IGF-1 was determined in plasma of animals by Bovine IGF-1 ELISA kit (Catalog No. **E0016Bo**) from Bioassay Technology Laboratory, Shanghai, China.

Principle of assay

The Enzyme-Linked Immunosorbent Assay (ELISA) plate has been pre-coated with Bovine IGF-1 antibody. IGF-1 present in the sample was added that binds to antibodies coated on the wells. And then biotinylated Bovine IGF-1 Antibody was added that binds to IGF-1 in the sample. Then Streptavidin-HRP was added that binds to the Biotinylated IGF-1 antibody. After incubation unbound Streptavidin-HRP was washed away during a washing step. Substrate solution was then added and color developed in proportion to the amount of Bovine IGF-1. The reaction was terminated by addition of acidic stop solution and absorbance was measured at 450 nm.

Materials provided in kit

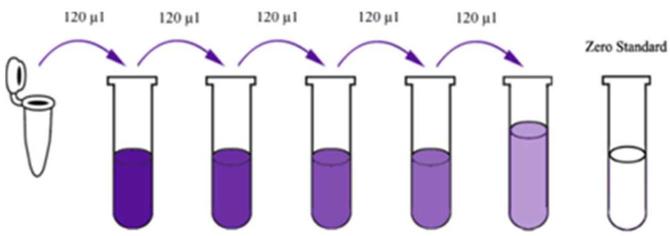
Standard Solution (12.8ng/ml)-0.5ml x1, Pre-coated ELISA Plate-12 * 8 well strips x1, Standard Diluent-3ml x1, Streptavidin-HRP-6ml x1, Stop Solution-6mlx1, Substrate Solution A -6ml x1, Substrate Solution B-6ml x1, Wash Buffer Concentrate (25x)-20ml x1, Biotinylated Bovine IGF Antibody 1ml x1, User Instruction-1 , Plate Sealer -2 pics, Zipper bag-1 pc

Reagent Preparation

1. All reagents were brought to room temperature before use.
2. **Standard** 120µl of the standard (480ng/ml) was reconstituted with 120µl of standard diluent to generate a 240ng/ml standard stock solution. The standard was allowed to sit for 15 min with gentle agitation prior to making dilutions. Other standard were prepared

by serially diluting the standard stock solution 240ng/ml 1:2 with standard diluent to produce 120ng/ml, 60ng/ml, 30ng/ml and 15ng/ml solutions. Standard diluent serves as the zero standard (0 ng/ml). Remaining solution was frozen at -20°C for use within one month. Dilution of standard solutions suggested were as follows:

240ng/ml	Standard No.5	120µl Original Standard + 120µl Standard Diluent
120ng/ml	Standard No.4	120µl Standard No.5 + 120µl Standard Diluent
60ng/ml	Standard No.3	120µl Standard No.4 + 120µl Standard Diluent
30ng/ml	Standard No.2	120µl Standard No.3 + 120µl Standard Diluent
15ng/ml	Standard No.1	120µl Standard No.2 + 120µl Standard Diluent



Standard Concentration	Standard No.5	Standard No.4	Standard No.3	Standard No.2	Standard No.1
480ng/ml	240ng/ml	120ng/ml	60ng/ml	30ng/ml	15ng/ml

Procedure

1. 20ml of Wash Buffer Concentrate 25x was diluted with deionized or distilled water to yield 500ml of 1x Wash Buffer. The solution was mixed gently until the crystals have been completely dissolved.
2. The no of strips required for the assay was determined. The no. of strips needed was determined by that of samples to be tested by that of standards. The strips were inserted in the frames for use.
3. 50µl standard was added to standard well.

4. 40µl sample to sample wells was added and then 10µl anti-IGF 1 antibody was added to the sample wells, followed by the addition of 50µl streptavidin- HRP to sample wells and standard wells. The plate was mixed and the plate was covered with a sealer.
5. The plate was incubated at 37°C for 60 minutes.
6. The sealer was removed and the plate was washed 5 times with wash buffer. The wells were soaked with at least 0.35ml wash buffer for 30 seconds- 1 minutes for each washing.
7. 50 µl substrate solution A was added to each well and 50 µl substrate solution B was added to each well. The plate was then covered with a new sealer and then incubated for 10 minutes at 37◦C in the dark.
8. 50 µl Stop solution was added to each well, the blue colour changed to yellow colour immediately.
9. The optical density (OD) of each well was determined immediately using a microplate reader set at 450 nm within 30 minutes after the addition of stop solution

Calculation of Result

Standard curve was constructed by plotting the average OD for each standard on the vertical (Y) axis against the concentration on the horizontal (X) axis with computer-based curve-fitting software and concentrate was determined by using regression analysis.

3.1.7.4 Bovine plasma thyroxine (T_4)

Thyroxine was determined in plasma of animals by Bovine T_4 ELISA Kit (Catalog No. **E0216Bo**) from Bioassay Technology Laboratory, Shanghai, China.

Principle of assay

The Enzyme-Linked Immunosorbent Assay (ELISA) plate has been pre-coated with Bovine T_4 antibody. T_4 present in the sample was added that binds to antibodies coated on the wells. And then biotinylated Bovine T_4 Antibody was added that binds to T_4 in the sample. Then Streptavidin-HRP was added that binds to the Biotinylated T_4 antibody. After incubation unbound Streptavidin-HRP was washed away during a washing step. Substrate solution was then added and color developed in proportion to the amount of Bovine T_4. The reaction was terminated by addition of acidic stop solution and absorbance was measured at 450 nm.

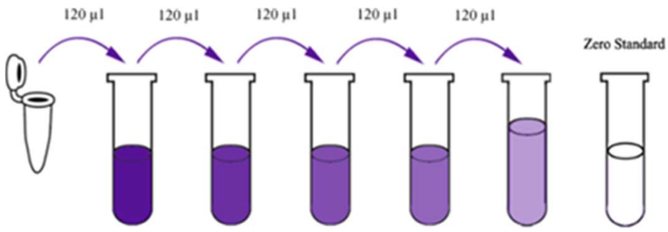
Materials provided in kit

Standard Solution (12.8ng/ml)-0.5ml x1, Pre-coated ELISA Plate-12 * 8 well strips x1, Standard Diluent-3ml x1, Streptavidin-HRP-6ml x1, Stop Solution-6mlx1, Substrate Solution A -6ml x1, Substrate Solution B-6ml x1, Wash Buffer Concentrate (25x)-20ml x1, Biotinylated Bovine T_4 Antibody 1ml x1, User Instruction-1 , Plate Sealer -2 pics, Zipper bag-1 pc

Reagent Preparation

1. All reagents were brought to room temperature before use.
2. **Standard** 120μl of the standard (640ng/ml) was reconstituted with 120μl of standard diluent to generate a 320ng/ml standard stock solution. The standard was allowed to sit for 15 min with gentle agitation prior to making dilutions. Other standard were prepared by serially diluting the standard stock solution 320ng/ml 1:2 with standard diluent to produce 160ng/ml, 80ng/ml, 40ng/ml and 20ng/ml solutions. Standard diluent serves as the zero standard (0 ng/ml). Remaining solution was frozen at -20°C for use within one month. Dilution of standard solutions suggested were as follows:

320ng/ml	Standard No.5	120μl Original Standard + 120μl Standard Diluent
160ng/ml	Standard No.4	120μl Standard No.5 + 120μl Standard Diluent
80ng/ml	Standard No.3	120μl Standard No.4 + 120μl Standard Diluent
40ng/ml	Standard No.2	120μl Standard No.3 + 120μl Standard Diluent
20ng/ml	Standard No.1	120μl Standard No.2 + 120μl Standard Diluent



Standard Concentration	Standard No.5	Standard No.4	Standard No.3	Standard No.2	Standard No.1
640ng/ml	320ng/ml	160ng/ml	80ng/ml	40ng/ml	20ng/ml

Procedure

1. 20ml of Wash Buffer Concentrate 25x was diluted with deionized or distilled water to yield 500ml of 1x Wash Buffer. The solution was mixed gently until the crystals have been completely dissolved.
2. The no of strips required for the assay was determined. The no. of strips needed was determined by that of samples to be tested by that of standards. The strips were inserted in the frames for use.
3. 50µl standard was added to standard well.
4. 40µl sample to sample wells was added and then 10µl anti- T4 antibody was added to the sample wells, followed by the addition of 50µl streptavidin- HRP to sample wells and standard wells. The plate was mixed and the plate was covered with a sealer.
5. The plate was incubated at 37°C for 60 minutes.
6. The sealer was removed and the plate was washed 5 times with wash buffer. The wells were soaked with at least 0.35ml wash buffer for 30 seconds- 1 minutes for each washing.
7. 50 µl substrate solution A was added to each well and 50 µl substrate solution B was added to each well. The plate was then covered with a new sealer and then incubated for 10 minutes at 37◦C in the dark.
8. 50 µl Stop solution was added to each well, the blue colour changed to yellow colour immediately.
9. The optical density (OD) of each well was determined immediately using a microplate reader set at 450 nm within 30 minutes after the addition of stop solution

Calculation of Result

Standard curve was constructed by plotting the average OD for each standard on the vertical (Y) axis against the concentration on the horizontal (X) axis with computer-based curve-fitting software and concentrate was determined by using regression analysis.

3.1.7.5 Bovine plasma triiodothyronine (T_3)

Tri-iodothyronine was determined in plasma of animals by Bovine T_3, ELISA Kit (Catalog No. **E0215Bo**) procured from Bioassay Technology Laboratory, Shanghai,China.

Principle of assay

The Enzyme-Linked Immunosorbent Assay (ELISA) plate has been pre-coated with Bovine T_3 antibody. T_3 present in the sample was added that binds to antibodies coated on the wells. And then biotinylated Bovine T_3 Antibody was added that binds to T_3 in the sample. Then Streptavidin-HRP was added that binds to the Biotinylated T_3 antibody. After incubation unbound Streptavidin-HRP was washed away during a washing step. Substrate solution was then added and color developed in proportion to the amount of Bovine T_3. The reaction was terminated by addition of acidic stop solution and absorbance was measured at 450 nm.

Materials provided in kit

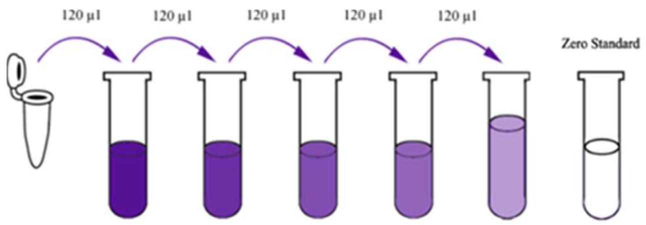
Standard Solution (12.8ng/ml)-0.5ml x1, Pre-coated ELISA Plate-12 * 8 well strips x1, Standard Diluent-3ml x1, Streptavidin-HRP-6ml x1, Stop Solution-6mlx1, Substrate Solution A -6ml x1, Substrate Solution B-6ml x1, Wash Buffer Concentrate (25x)-20ml x1, Biotinylated Bovine T_3 Antibody 1ml x1, User Instruction-1 , Plate Sealer -2 pics, Zipper bag-1 pc

Reagent Preparation

1. All reagents were brought to room temperature before use.
2. **Standard** 120µl of the standard (9.6ng/ml) was reconstituted with 120µl of standard diluent to generate a 4.8ng/ml standard stock solution. The standard was allowed to sit for 15 min with gentle agitation prior to making dilutions. Other standard were prepared by serially diluting the standard stock solution 4.8ng/ml 1:2 with standard diluent to produce

2.4ng/ml, 1.2ng/ml, 0.6ng/ml and 0.3ng/ml solutions. Standard diluent serves as the zero standard (0 ng/ml). Remaining solution was frozen at -20°C for use within one month. Dilution of standard solutions suggested were as follows:

4.8ng/ml	Standard No.5	120μl Original Standard + 120μl Standard Diluent
2.4ng/ml	Standard No.4	120μl Standard No.5 + 120μl Standard Diluent
1.2ng/ml	Standard No.3	120μl Standard No.4 + 120μl Standard Diluent
0.6ng/ml	Standard No.2	120μl Standard No.3 + 120μl Standard Diluent
0.3ng/ml	Standard No.1	120μl Standard No.2 + 120μl Standard Diluent



Standard Concentration	Standard No.5	Standard No.4	Standard No.3	Standard No.2	Standard No.1
9.6ng/ml	4.8ng/ml	2.4ng/ml	1.2ng/ml	0.6ng/ml	0.3ng/ml

Procedure

1. 20ml of Wash Buffer Concentrate 25x was diluted with deionized or distilled water to yield 500ml of 1x Wash Buffer. The solution was mixed gently until the crystals have been completely dissolved.
2. The no of strips required for the assay was determined. The no. of strips needed was determined by that of samples to be tested by that of standards. The strips were inserted in the frames for use.
3. 50μl standard was added to standard well.
4. 40μl sample to sample wells was added and then 10μl anti- T3 antibody was added to the sample wells, followed by the addition of 50μl streptavidin- HRP to sample wells and standard wells. The plate was mixed and the plate was covered with a sealer.
5. The plate was incubated at 37°C for 60 minutes.

6. The sealer was removed and the plate was washed 5 times with wash buffer. The wells were soaked with at least 0.35ml wash buffer for 30 seconds- 1 minutes for each washing.
7. 50 µl substrate solution A was added to each well and 50 µl substrate solution B was added to each well. The plate was then covered with a new sealer and then incubated for 10 minutes at 37◦C in the dark.
8. 50 µl Stop solution was added to each well, the blue colour changed to yellow colour immediately.
9. The optical density (OD) of each well was determined immediately using a microplate reader set at 450 nm within 30 minutes after the addition of stop solution

Calculation of Result

Standard curve was constructed by plotting the average OD for each standard on the vertical (Y) axis against the concentration on the horizontal (X) axis with computer-based curve-fitting software and concentrate was determined by using regression analysis.

3.1.7.6 Bovine plasma cortisol (COR)

Cortisol was determined in plasma of animals by Bovine Cortisol ELISA kit (Catalog No. **E0110Bo**) from Bioassay Technology Laboratory, Shanghai, China.

Principle of assay

The Enzyme-Linked Immunosorbent Assay (ELISA) plate has been pre-coated with Bovine COR antibody. COR present in the sample was added that binds to antibodies coated on the wells. And then biotinylated Bovine COR Antibody was added that binds to COR in the sample. Then Streptavidin-HRP was added that binds to the Biotinylated COR antibody. After incubation unbound Streptavidin-HRP was washed away during a washing step. Substrate solution was then added and color developed in proportion to the amount of Bovine COR. The reaction was terminated by addition of acidic stop solution and absorbance measured at 450 nm.

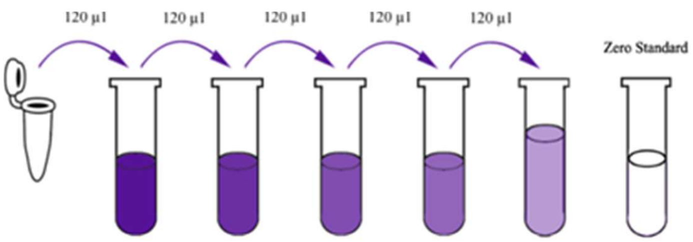
Materials provided in kit

Standard Solution (12.8ng/ml)-0.5ml x1, Pre-coated ELISA Plate-12 * 8 well strips x1, Standard Diluent-3ml x1, Streptavidin-HRP-6ml x1, Stop Solution-6mlx1, Substrate Solution A -6ml x1, Substrate Solution B-6ml x1, Wash Buffer Concentrate (25x)-20ml x1, Biotinylated Bovine COR Antibody 1ml x1, User Instruction-1 , Plate Sealer -2 pics, Zipper bag-1 pc

Reagent Preparation

1. All reagents were brought to room temperature before use.
2. **Standard** 120µl of the standard (240ng/ml) was reconstituted with 120µl of standard diluent to generate a 120ng/ml standard stock solution. The standard was allowed to sit for 15 min with gentle agitation prior to making dilutions. Other standard were prepared by serially diluting the standard stock solution 120ng/ml 1:2 with standard diluent to produce 60ng/ml, 30ng/ml, 15ng/ml and 7.5ng/ml solutions. Standard diluent serves as the zero standard (0 ng/ml). Remaining solution was frozen at -20°C for use within one month. Dilution of standard solutions suggested were as follows:

120ng/ml	Standard No.5	120µl Original Standard + 120µl Standard Diluent
60ng/ml	Standard No.4	120µl Standard No.5 + 120µl Standard Diluent
30ng/ml	Standard No.3	120µl Standard No.4 + 120µl Standard Diluent
15ng/ml	Standard No.2	120µl Standard No.3 + 120µl Standard Diluent
7.5ng/ml	Standard No.1	120µl Standard No.2 + 120µl Standard Diluent



Standard Concentration	Standard No.5	Standard No.4	Standard No.3	Standard No.2	Standard No.1
240ng/ml	120ng/ml	60ng/ml	30ng/ml	15ng/ml	7.5ng/ml

Procedure

1. 20ml of Wash Buffer Concentrate 25x was diluted with deionized or distilled water to yield 500ml of 1x Wash Buffer. The solution was mixed gently until the crystals have been completely dissolved.
2. The no. of strips required for the assay was determined. The no. of strips needed was determined by that of samples to be tested by that of standards. The strips were inserted in the frames for use.
3. 50µl standard was added to standard well.
4. 40µl sample to sample wells was added and then 10µl anti- COR antibody was added to the sample wells, followed by the addition of 50µl streptavidin- HRP to sample wells and standard wells. The plate was mixed and the plate was covered with a sealer.
5. The plate was incubated at 37°C for 60 minutes.
6. The sealer was removed and the plate was washed 5 times with wash buffer. The wells were soaked with at least 0.35ml wash buffer for 30 seconds- 1 minutes for each washing.
7. 50 µl substrate solution A was added to each well and 50 µl substrate solution B was added to each well. The plate was then covered with a new sealer and then incubated for 10 minutes at 37◦C in the dark.
8. 50 µl Stop solution was added to each well, the blue colour changed to yellow colour immediately.
9. The optical density (OD) of each well was determined immediately using a microplate reader set at 450 nm within 30 minutes after the addition of stop solution

Calculation of Result

Standard curve was constructed by plotting the average OD for each standard on the vertical (Y) axis against the concentration on the horizontal (X) axis with computer-based curve-fitting software and concentrate was determined by using regression analysis.

3.1.7.7 Plasma total antioxidant capacity (TAC)

Cortisol was determined in plasma of animals by Bovine Cortisol ELISA kit (Catalog No. **E0384Bo**) from Bioassay Technology Laboratory, Shanghai, China.

Principle of assay

The Enzyme-Linked Immunosorbent Assay (ELISA) plate has been pre-coated with Bovine T-AOC antibody. T-AOC present in the sample was added that binds to antibodies coated on the wells. And then biotinylated Bovine T-AOC Antibody was added that binds to T-AOC in the sample. Then Streptavidin-HRP was added that binds to the Biotinylated T-AOC antibody. After incubation unbound Streptavidin-HRP was washed away during a washing step. Substrate solution was then added and color developed in proportion to the amount of Bovine T-AOC. The reaction was terminated by addition of acidic stop solution and absorbance was measured at 450 nm.

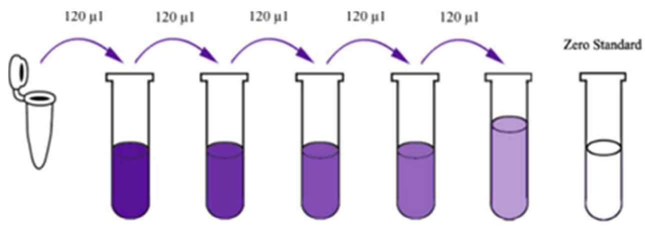
Materials provided in kit

Standard Solution (12.8ng/ml)-0.5ml x1, Pre-coated ELISA Plate-12 * 8 well strips x1, Standard Diluent-3ml x1, Streptavidin-HRP-6ml x1, Stop Solution-6mlx1, Substrate Solution A -6ml x1, Substrate Solution B-6ml x1, Wash Buffer Concentrate (25x)-20ml x1, Biotinylated Bovine TAC Antibody 1ml x1, User Instruction-1 , Plate Sealer -2 pics, Zipper bag-1 pc

Reagent Preparation

1. All reagents were brought to room temperature before use.
2. **Standard** 120µl of the standard (48U/ml)) was reconstituted with 120µl of standard diluent to generate a 24U/ml standard stock solution. The standard was allowed to sit for 15 min with gentle agitation prior to making dilutions. Other standard were prepared by serially diluting the standard stock solution 24U/ml 1:2 with standard diluent to produce 12U/ml, 6U/ml, 3U/ml and 1.5U/ml solutions. Standard diluent serves as the zero standard (0 ng/ml). Remaining solution was frozen at -20°C for use within one month. Dilution of standard solutions suggested were as follows:

24U/ml	Standard No.5	120µl Original Standard + 120µl Standard Diluent
12U/ml	Standard No.4	120µl Standard No.5 + 120µl Standard Diluent
6U/ml	Standard No.3	120µl Standard No.4 + 120µl Standard Diluent
3U/ml	Standard No.2	120µl Standard No.3 + 120µl Standard Diluent
1.5U/ml	Standard No.1	120µl Standard No.2 + 120µl Standard Diluent



Standard Concentration	Standard No.5	Standard No.4	Standard No.3	Standard No.2	Standard No.1
48U/ml	24U/ml	12U/ml	6U/ml	3U/ml	1.5U/ml

Procedure

1. 20ml of Wash Buffer Concentrate 25x was diluted with deionized or distilled water to yield 500ml of 1x Wash Buffer. The solution was mixed gently until the crystals have been completely dissolved.
2. The no of strips required for the assay was determined. The no. of strips needed was determined by that of samples to be tested by that of standards. The strips were inserted in the frames for use.
3. 50µl standard was added to standard well.
4. 40µl sample to sample wells was added and then 10µl anti- TAC antibody was added to the sample wells, followed by the addition of 50µl streptavidin- HRP to sample wells and standard wells. The plate was mixed and the plate was covered with a sealer.
5. The plate was incubated at 37°C for 60 minutes.

6. The sealer was removed and the plate was washed 5 times with wash buffer. The wells were soaked with at least 0.35ml wash buffer for 30 seconds- 1 minutes for each washing.
7. 50 µl substrate solution A was added to each well and 50 µl substrate solution B was added to each well. The plate was then covered with a new sealer and then incubated for 10 minutes at 37◦C in the dark.
8. 50 µl Stop solution was added to each well, the blue colour changed to yellow colour immediately.
9. The optical density (OD) of each well was determined immediately using a microplate reader set at 450 nm within 30 minutes after the addition of stop solution

Calculation of Result

Standard curve was constructed by plotting the average OD for each standard on the vertical (Y) axis against the concentration on the horizontal (X) axis with computer-based curve-fitting software and concentrate was determined by using regression analysis.

3.1.7.8 Estimation of plasma glucose

Plasma glucose was estimated using commercial diagnostic kit purchased (Recombigen Laboratories Pvt. Ltd., New Delhi)

Principle

Glucose oxidase (GOD) oxidized glucose to gluconic acid and hydrogen peroxide in presence of enzyme peroxidase. Released hydrogen peroxide wass coupled with phenol and 4-aminoantipyrine (4-AAP) to form colored dye. Intensity of colored dye was measured at 505 nm.

Reagents

(I) L1: Glucose Reagent

(II) S: Glucose Standard (100 mg/dl)

Procedure

(I) Labelled 3 clean dry test tubes as were taken for blank (B), standard (S) and test serum (T). Pipetting into each test tube was done as shown below:

Addition Sequence	B (ml)	S (ml)	T (ml)
Glucose reagent (L1)	1.0	1.0	1.0
Distilled water	0.01	-	-
Glucose Standard (S)	-	0.01	-
Serum sample	-	-	0.01

(II) Mixed well and incubated at 37ºC for 10 min. Absorbance of standard and unknown sample was measured at 505 nm against the Blank using UV-Spectrophotometer (__505___) within 20 min.

Concentration was calculated as per formula and expressed as mg/dl.

$$\textit{Serum glucose (mg/dl)} = \frac{\textit{Absorbance of test}}{\textit{Absorbance of standard}} X\ 100$$

3.2 Chemical Evaluation of Feedstuff

3.2.1 Sample collection and Preparation

The *Moringa oleifera* leaves were collected from the trees in the campus. These leaves were dried under sun for removal of moisture from leaves. Dried *Moringa oleifera leaves* were also bought from Sashi Neutraceuticals, Alwar, Rajasthan for research purpose under funds provided by NICRA. The chemical composition of dried *Moringa oleifera* leaves samples was determined using AOAC standard procedures (2005).

3.2.2 Chemical Composition

3.2.2.1 Dry matter (DM)

Apparatus: Aluminum tray, hot air oven, desiccator, weighing balance, tong, spatula

Procedure

100 g of sample was taken in a pre-weighed aluminum tray, placed in hot air oven at 100 ± 5 °C for 24 h and weighed again. The loss in moisture content after drying was estimated and the dry matter was calculated as follows

$$\text{Moisture (\%)} = \frac{\text{Weight of sample after drying}}{\text{Weight of fresh sample}} \times 100$$

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

After drying the samples were ground to pass through 1 mm sieve, which was used in further analysis

3.2.2.2 Organic matter (OM)

OM was determined by subtracting the total ash content from 100, i e.

OM (%) = 100- total ash (%)

3.2.2.3 Crude protein (CP)

Total nitrogen was estimated by Kjeldahl method

Apparatus: Kjeldahl flasks, digester, Kjeldahl distillation apparatus (KELPLUS Nitrogen Analyzer), Erlenmeyer flasks titration assembly

Reagents

Digestion mixture (Na_2SO_4 and $CuSO_4$ in the ratio of 9:1), 40% NaOH solution (400 g NaOH pellets dissolved in distilled water and volume made upto 1000 ml). concentrated H_2SO_4 (98% purity and specific gravity of 1.84), 2% boric acid solution (20 boric acid dissolved in distilled water and volume made up to 1L and added with 10 ml of 0.2 bromocresol green and 20 ml 0.1% methyl red indicators), N/100 H_2SO_4 solution.

Procedure

• **Digestion**: One g sample with 2-3 g of digestion mixture was taken in Kjeldahl flask and digested with 20 ml concentrated H_2SO_4, till the solution became colourless. After digestion, the contents were cooled and volume was made to 100 ml.

• **Distillation**: An aliquot of 10 ml was distilled in Kjeldahl distillation apparatus (KELPLUS Nitrogen Analyzer) after adding 20 ml of 40% NaOH solution. About 75 ml of distillate (light green colour) was collected into an Erlenmeyer conical flask containing 10 ml of 2% boric acid with mixed indicator.

• **Titration**: The distillate was then titrated against standard N/100 H_2SO_4 and the end point was recorded when colour changed to slight pinkish. Volume of N/100 H_2SO_4 solution used in titration was measured and recorded.

3.2.2.4 Ether extract (EE)

Apparatus: Soxhlet's extraction apparatus, oil flask, thimble, hot air oven, desiccator, weighing balance

Reagent: Petroleum ether (40-60^{o}C)

Procedure

About 3 g of accurately weighed sample was taken into a thimble. The thimble was placed in the Soxhlet's extraction apparatus to which pre weighed oil flask was attached and extraction was done for 8 to 10 hours with petroleum ether (BP 40-60^{o}C) The oil flask was removed and after evaporating the excess of ether, it was dried overnight in a hot air oven (temp.80+-5^{o}C).

Calculation:

$$EE (\%) = \frac{\text{(Weight of oil flask with ether extract-weight of oil flask)}}{\text{Weight of sample}} \times 100$$

3.2.2.5 Total ash

Apparatus: Silica crucible, electric plate heater, muffle furnace, desiccator und weighing balance

Procedure

3 g sample was taken in a pre-weighed silica crucible. After charring the sumple on heater (till the smoke disappeared), the crucible was kept in muffle furnace for ignition at 550^{o}C for 2-3h. The crucible was removed on cooling and kept in a desiccator and weighed. The ash content was calculated as given below:

$$\text{Total ash (\%)} = \frac{\text{Weight of crucible + ash after cooling - weight of crucible}}{\text{Weight of sample}} \times 100$$

3.2.3 Estimation of Cell wall constituents

The cell wall constituents such as neutral detergent fibre (NDF), acid detergent fibre (ADF) were estimated as per Van Soest *et al*. (1991).

3.2.3.1 Neutral detergent fibre

Apparatus and glassware: Spout less beaker, sintered crucible, vacuum pump, hot air oven, muffle furnace, weighing balance and desiccator.

Reagents: Neutral detergent solution (NDS), amylase solution, acetone, hot boiling water

Procedure

Approximately 1 g sample was taken in spoutless beaker of 1 L capacity. To this, 100 mL NDS and 0.5 g of sodium sulphite were added. The contents of spoutless beaker were refluxed for half an hour. Thirty minutes after onset of boiling, beaker was removed and 2 mL of enzyme solution was added. One hour after initial boiling, the contents of beaker were filtered through pre-weighed 50 mL sintered glass crucible (G-1) using oil free vacuum pump. The contents were washed repeatedly with hot boiling water and then with acetone to remove all salts. The sintered crucible containing residue was dried hot air oven (100 + 5°C) overnight, cooled and weighed to a constant value.

$$\text{NDF}(\%) = \frac{\text{(Weight of crucible with residue - Weight of crucible with residual ash)}}{\text{Weight of sample taken}} \text{x100}$$

3.2.3.2 Acid detergent fiber (ADF)

Procedure

Approximately 1 g of sample was taken in a spoutless beaker of 1 L capacity. To this, 100 mL ADS was added and the contents were refluxed for exactly 1 hour. After refluxing, the residue was filtered through pre-weighed sintered glass crucible G-1 using vacuum pump and washed with hot water 2-3 times followed by acetone to remove the salts. The sintered glass crucible containing residue was dried in hot air oven (100+5°C) and weighed again. The ADF was calculated as follows:

$$\text{ADF (\%)} = \frac{\text{Weight of crucible + ADF) - Weight of crucible}}{\text{Weight of sample (DM basis)}} \text{ x 100}$$

3.3 Digestibility trial

A digestibility trial was conducted for 5 days towards the end of the experiment to evaluate nutrient utilization in animals. Samples of feed, faeces were collected during the experimental period.

3.3.1 Sampling, processing and storage of samples

During the 5-day collection period, an accurate account of feed offered, residues left, faeces voided, during a 24-h feeding cycle was kept. Representative samples of feed offered, residue, faeces were collected at 24 h intervals. The total amount of faeces voided was recorded, after thorough mixing, an aliquot (1/200) was taken for the estimation of dry matter (DM) in duplicate. Another aliquot (1/1000) was composited in a separate container under acidic conditions (mixed with 25% sulphuric acid) to prevent losses of N in the form of ammonia from the samples. Samples of feed, refusals and faeces were dried in a hot-air oven at 100 °C for 24 h to determine the DM content. Each animal's refusals and faeces were pooled across the 5-day trial period. Dried samples of feed, residuals and faeces were ground to pass through a 1-mms screen in a grinding mill and stored in air-tight plastic containers for further laboratory analysis.

3.3.2 Analytical procedures

Samples of feed, faeces were analyzed for proximate constituents as per AOAC (2005). The total crude protein of feed, faeces were determined by standard Kjeldahl's method. Determination of ether extract in feed and faces was done with help of Soxhlet's apparatus. For determination of dry matter, crude fibre, NFE and total ash standard procedures were followed. Digestibility of CP, CF, EE and NFE was estimated by the records of feed intake and faeces voided during the metabolic trial and proximate analysis of feed and faeces samples.

3.4 Ultrasonography

Murrah buffalo heifers were subjected to ultrasound examination towards the end of the experiment to observe the ovarian status of the control and treatment group fed with dried *Moringa oleifera* leaves. The age and body weight of each animal was recorded. A transrectal ultrasound examination was performed. Ovaries of animals were examined for the number of follicles and the size of the largest follicle. The follicles were measured and categorized into three sizes based on their diameter: small (≤3 mm), medium (3 -8 mm) and large (>8 mm).

3.4.1 Observation of number of follicles

The number of follicles present on ovaries of the control and treatment group was examined to compare the effect of *Moringa oleifera* leaves on the follicular growth and age of puberty in growing Murrah buffalo heifers.

3.4.2 Observation of Size of follicles

The size of the largest follicle was determined by calculating the diameter of follicles present on the ovaries of the control and treatment groups. The size of follicles were measured in the USG machine in Livestock Research Centre.

3.5 Statistical Analysis

Data are presented as Mean±SE. Statistical analysis was carried out by Repeated measures Analysis of Variance (ANOVA), one way ANOVA using SPSS (2007) Software version 16.0 for Windows. Continuous variables have been tested by Levene's test for equality of variance. Figures were developed by Graphpad Prism 8.0 (USA). The effects ($P<0.05$) were considered statistically significant whereas effects ($P>0.05$) were considered non-significant.

Chapter-4

Results and Discussions

Results and Discussion

Heifers are the potential population of a dairy farm. Balanced diet, improved management, and a low disease occurrence can all contribute to lower the age of first calving (Heinrichs *et al.*, 2005). Herbal plants are employed in animal feeds as hunger stimulants, digestive stimulants, physiological activity stimulants, prevention and therapy of specific pathological disorders and antioxidants. (Mohamed *et al.*, 2016a; Mohamed *et al.*, 2016b; Abdellatief *et al.*, 2017; Emam *et al.*, 2018; Farouk *et al.*, 2020; Gad *et al.*, 2021) The experiment was carried out to investigate the influence of *Moringa oleifera* on association of blood metabolites and hormones with growth and ovarian status of Murrah buffalo heifers. Results of the study "Effects of *Moringa oleifera* leaves supplementation on Growth, certain blood metabolites and hormones in Murrah buffalo heifers" are presented below.

4.1 Chemical Composition of feedstuff

The Proximate composition of dried *Moringa oleifera* leaves, wheat straw, oats and concentrate provided to the animal during the experiment of 180 days is given in Table no (1) The DM (90%), OM(89.2%), CP(21.35%), EE(4.09%), TA (10.57%), CF(12.8%), NFE (57.5%), NDF(22.9%) and ADF(16.56%) was present in dried *Moringa oleifera* leaves. The feedstuff had composition as follows DM (90.6%), OM(84.5%), CP(3.18%), EE(1.57%), TA (11.5%), CF(40.54%), NFE (41.86%),NDF(82.9%) and ADF(53.1%) in wheat straw, DM (91%), OM(89.14%), CP(19.38%), EE(4.33%), TA (10.86%), CF(28.55%), NFE (40.37%),NDF(24.34%) and ADF(13.12%) in concentrate and DM (19.5%), OM(90.75%), CP(12.95%), EE(2.51%), TA (9.25%), CF(24.23%), NFE (53.15%),NDF(64.52%) and ADF(42.13%) in green fodder(oats). The CP in *Moringa oleifera* leaves (21.35%) were higher than the concentrate (19.38%) and therefore can be a supplement to concentrate for a higher intake of protein.

The results in the study are in line with Meel *et al.* (2018) where the dry matter, organic matter, crude protein, ether extract, crude fibre, nitrogen-free extract and total ash levels in *Moringa oleifera* leaves were 85.69, 90.24, 23.31, 4.7, 9.26, 52.97 and 9.76 per cent respectively. The CP per cent in *Moringa oleifera* leaves is comparable with the earlier study as 211 g/kg DM by Kholif *et al.* (2018), 22.1 % by Adegun *et al.* (2013), 200 g/kg DM CP by Kakengi *et al.* (2005) and 22 % Asaolu *et al.* (2010). 18.38 % CP by Tona *et al.* (2014), 18.23% by Sultana *et al.* (2015b), 18.12% by Khalel *et al.*(2014) and 18.62% DM CP by Roy *et al.*(2016) reported the values which are lower than the values observed in the study and the values in the study were lower in comparison to the values (25.95 and 22.6%) as was obtained by Manh *et al*. (2005) and Sánchez *et al*. (2006b). The EE per cent in *Moringa oleifera* leaves is comparable with the earlier study as 49 g/kg DM by Kholif *et al*. (2018), 4.15% by Jiwuba *et al*. (2016), 4.7 % by Meel *et al*. (2018a). The NDF per cent in *Moringa oleifera* leaves is comparable with the earlier study as 219 g/kg DM by Makkar *et al*. (1996), 233 g/kg DM by Sarwatt *et al.* (2002), 212 g/kg DM by Soliva *et al*. (2005). The ADF percent in *Moringa oleifera* leaves is comparable with the earlier study as 163 g/kg DM by Makkar *et al*. (1997), 16.07 % by Nouala *et al*. (2006). Moringa leaf meal has a crude protein content ranging from 17.9 to 26.8 percent (Sultana *et al*., 2015), with approximately 47 percent bypass protein and a sufficient amino acid profile (Nouman *et al*., 2014). *Moringa oleifera* is a safe and inexpensive source of protein and micronutrients. Increased consumption, digestibility and body weight gain were observed when tree leaves were used as a low-quality supplement**.**

4.2. Body weights (Kg) and Metabolic body weights $W^{0.75}$ (Kg) in Murrah buffalo heifers supplemented with rations containing dried *Moringa oleifera* leaves in addition to concentrate mixture

The mean values (±SEM) of the body weight (kg) in the control and treatment groups on the basis of feeding of dried *Moringa oleifera* leaves are presented in table (2) Fig (1). The initial body weight of the animals in control group was 169.83±2.10 and in treatment group was 170.60±1.32. The body weight at the end of experiment were 292.06±2.95and 304.11±2.70 in control and treatment group respectively. The body weight gain in treatment group was significantly higher (P<0.05) than the control group. The overall mean values of body weight (kg) was observed in treatment (236.88±12.21) than in control group (232.58±11.95) at a significant level of (P<0.05). Metabolic body weight (kg) was calculated in control and treatment group and are presented in table number (3) and Fig (2).The mean values of metabolic body weight in initial day in control and treatment were 47.04±0.44 and 47.08±0.50. The metabolic body weight (kg) at the end of experiment were 70.64±0.54 and 72.41±0.53 in control and treatment group respectively. The overall mean values of metabolic body weight (kg) was observed in treatment (60.18±2.35) than in control group (59.38±2.31) at a significant level of (P<0.05). Metabolic body weight in treatment group was significantly higher (P<0.05) than the control group. The Murrah buffalo heifers grew at a faster rate in treatment than in control group. Statistical data analysis showed variation among the groups and period. The change in body weight during the period is because of the growing age of heifers.

The increase in body weight of treatment group is due to better nutrient utilization of heifers on *Moringa oleifera* diet when compared to diet of control group. These findings were in accordance with earlier studies by Babeker and Bdalbagi, (2015); Melesse *et al*. (2015) and Sultana *et al*. (2015) who found considerably enhanced body weight changes in goat kids that were fed Moringa leaves. The considerable rise in overall body weights observed in Moringa leaf fed groups could be attributed. Enhancing body weight can be due to the abundant nutrient content of DMOL (Fahey *et al*., 2001). Mahmoud, (2013) carried out study on growing lambs and observed R2 group (with highest level of *Moringa oleifera* stems (18.75% on DM basis) had highest daily gain and total Body weight (20.14 kg). Abdel-Raheem *et al*. (2021) found that final body weight, total and daily weight gain increased ($P< 0.001$) for calves on M15 and M20 than in control group. According to Aregheore, (2002), substituting 20 % and 50% batiki grass for fresh *Moringa oleifera* increased weight gain and nutrient digestibility (DM, OM, CP, NDF) in developing goats. The increased body weight of the goats in this study could be attributed to the fact that *Moringa oleifera* is high in amino acids, vitamins and minerals, particularly iron (Subadra *et al*., 1997; Faye, 2011).

4.3. Dry Matter Intake (% Body Wt) in Murrah buffalo heifers supplemented with rations containing dried *Moringa oleifera* leaves in addition to concentrate mixture

Dry matter intake (DMI) is a crucial indicator of energy intake and performance for the utilization of ruminant feed. Table (4), Fig (3) show the Dry Matter Intake (%BW) in Murrah buffalo heifers fed with rations containing supplementation of dried *Moringa oleifera* leaves. Initial DMI %BW was 2.28±0.11 in control and 2.28±0.02 in treatment group. The DMI %BW at the end of experiment was 2.43±0.13 in control and 2.69±0.02 in treatment group. The overall mean values of dry matter intake (%BW) was observed in treatment (2.61±0.04) than in control group (2.37±0.01) at a significant level of ($P<0.05$). The finding in the result shows the comparison of DMI % body weight in control and treatment group. The overall mean values of DMI % BW was significantly higher ($P<0.05$) in treatment group than in control group. The result of study is accordance with earlier works of Jiwuba *et al*. (2016) who investigated the feed intake of goats fed *Moringa oleifera* leaf meal. The study found that goats fed 15% *Moringa oleifera* leaf meal had the highest average daily feed intake.

Reyes *et al*. (2006) have reported that the DMI of *Moringa oleifera* has enhanced by the increased availability and enhancement of the microbial activity of important nutrients in rumen microorganisms. This could be due to the fact that the addition of concentrates mixture would provide nutrients that were lacking or insufficient in the control group, it could also be due to larger concentrate to roughage ratios supplemented to the animals in the control and T1 groups (Yusuf *et al*., 2018) fed *Moringa oleifera* leaf meal (MOLM) to three groups of goats at DM levels of 0, 50 and 100 g/kg. According to the findings, the inclusion of Moringa leaves in the supplements had no effect on goat growth results. In a study by (Khalel *et al*., 2014) the dry matter intake did not significantly differ among the groups in the study, but the dry matter intake of the goats fed 75% and 50% Moringa foliage was significantly greater than that of the goats fed 100%.

4.4 Plasma growth hormone (ng/ml) in Murrah buffalo heifers supplemented with rations containing dried *Moringa oleifera* leaves in addition to concentrate mixture

The result of mean (±SE) values of plasma growth hormone (ng/ml) in control and treatment groups are given in the table (5) Fig (4).The mean values of GH in control group was (6.57±0.31) and in treatment group was (6.58±0.41) initially. The mean values of plasma growth hormone (GH) of the control and treatment groups of Murrah buffalo heifers at the end of experiment were 4.55±0.42 and 5.21±0.60 (ng/ml) respectively. The overall mean values of GH in control and treatment group was 4.81±0.34 and 5.16±0.33 respectively. Both the groups were growing heifers and both gain body weight at increasing pace. The GH hormone levels in control and treatment was not significantly different ($P<0.05$) but levels of hormone were higher in treatment group.

These results are in consistent with findings of Abdel-Raheem *et al*. (2021) conducted a study to investigate inclusion of *Moringa oleifera* leaves on diet of buffalo calves where the experimental diets had no statistically significant ($P<0.05$) effect on serum growth hormone levels. Most elements of postnatal somatic growth require growth hormone (GH). Throughout its role in postnatal growth and metabolism, pituitary GH is released in almost all species in a short-term, very episodic, pulsatile manner.

Table (1). Chemical Composition of feedstuff

Parameters	**Dried *Moringa oleifera* leaves**	**Wheat Straw**	**Concentrate**	**Green fodder (Oats)**
DM (%)	**90**	**90.6**	**91**	**19.5**
OM	**89.2**	**84.5**	**89.14**	**90.75**
CP	**21.35**	**3.18**	**19.38**	**12.95**
EE	**4.09**	**1.57**	**4.33**	**2.51**
TA	**10.57**	**11.5**	**10.86**	**9.25**
CF	**12.8**	**40.54**	**28.55**	**24.23**
NFE	**57.5**	**41.86**	**40.37**	**53.15**
NDF	**22.9**	**82.9**	**24.34**	**64.52**
ADF	**16.56**	**53.1**	**13.12**	**42.13**

DM %: Dry Matter percent; OM: Organic Matter; CP: Crude Protein; EE: Ether Extract; TA: Total Ash; AIA: Acid Insoluble Ash; CF: Crude Fibre; NFE: Nitrogen Free Extract; NDF: Neutral Detergent Fibre; ADF: Acid Detergent Fibre

Table (2).Mean (±SE) of Body weights (Kg) of experimental Murrah buffalo heifers

Fortnight	Control	Treatment	P value
0	$169.83^{a}\pm2.10$	$170.60^{a}\pm1.32$	<0.05
1	$179.70^{Bb}\pm2.93$	$187.71^{Ab}\pm1.86$	
2	$185.87^{b}\pm2.67$	$190.10^{b}\pm1.27$	
3	$199.57^{c}\pm2.50$	$208.50^{c}\pm2.41$	
4	$211.07^{d}\pm1.93$	$210.10^{c}\pm1.96$	
5	$243.29^{e}\pm3.29$	$237.97^{d}\pm3.32$	
6	$265.51^{g}\pm3.93$	$258.74^{e}\pm1.66$	
7	$257.81^{fg}\pm3.77$	$263.10^{ef}\pm3.37$	
8	$249.21^{ef}\pm2.72$	$262.64^{ef}\pm3.14$	
9	$260.17^{g}\pm3.26$	$269.43^{f}\pm4.05$	
10	$276.80^{h}\pm3.90$	$281.00^{g}\pm1.71$	
11	$292.06^{i}\pm2.95$	$304.11^{h}\pm2.70$	
Overall mean	$232.58^{y}\pm11.95$	$236.88^{x}\pm12.21$	

The values are mean ±SE of eleven fortnight observations on seven animals in each group.

Means in columns with different superscript (a,b,c,d,e,f,g,h,i) differ significantly ($P\leq0.05$).

Overall means ± SE in rows with superscript (x,y) differ significantly

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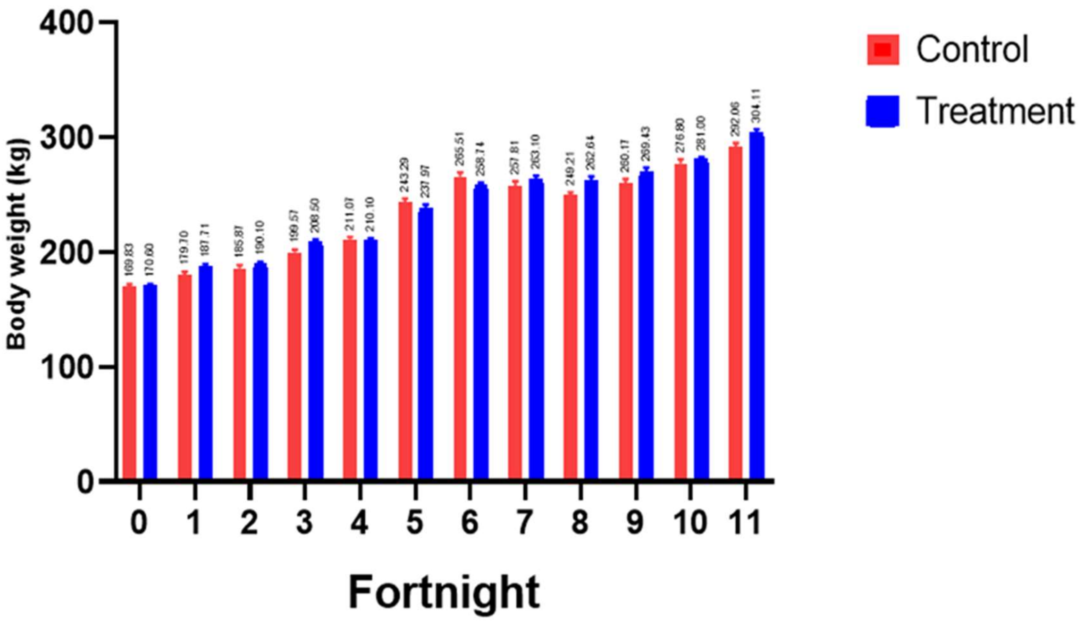


Fig(1) Mean body weights of Murrah buffalo heifers in two groups

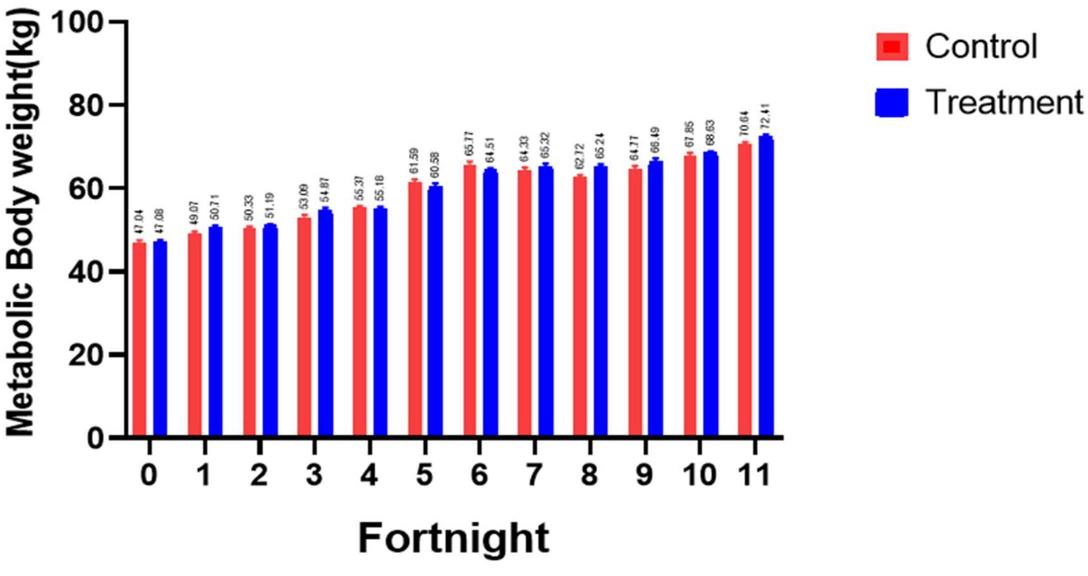


Fig (2) Mean Metabolic body weights $W^{0.75}$ in two groups of Murrah buffalo heifers

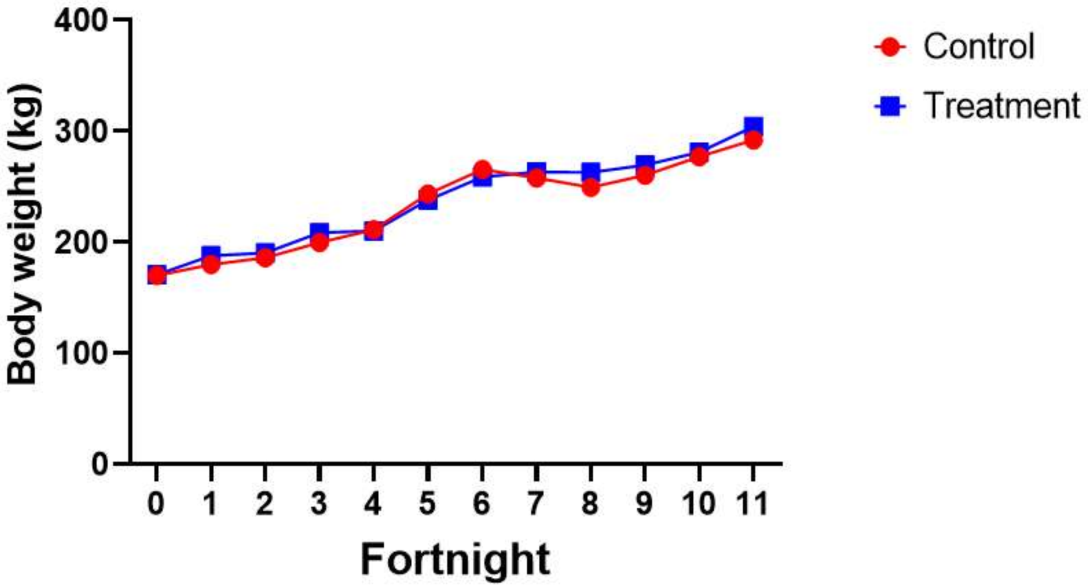


Fig (1) Mean body-weights in two groups of Murrah buffalo heifers

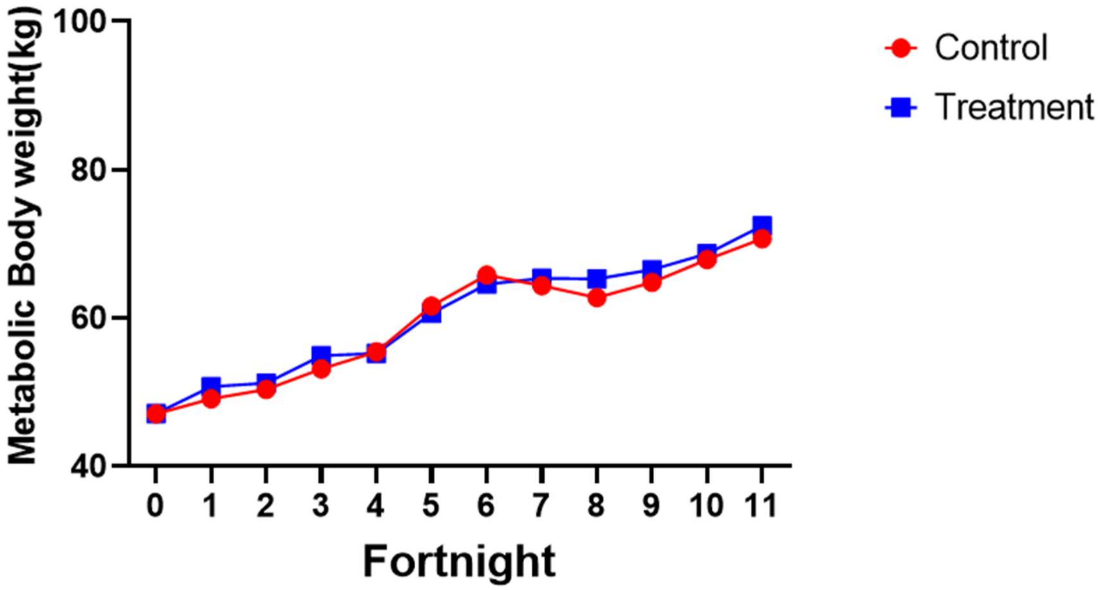


Fig (2). Mean Metabolic body weights $W^{0.75}$ in two groups of Murrah buffalo heifers

Table (3) Mean (±SE) of Metabolic body weights $W^{0.75}$ (Kg) in experimental Murrah buffalo heifers

Fortnight	Control	Treatment	P value
0	47.04^{a}±0.44	47.08^{a}±0.50	<0.05
1	49.07^{Bb}±0.60	50.71^{Ab}±0.38	
2	50.33^{b}±0.54	51.19^{b}±0.26	
3	53.09^{c}±0.50	54.87^{c}±0.47	
4	55.37^{d}±0.38	55.18^{c}±0.39	
5	61.59^{e}±0.63	60.58^{d}±0.63	
6	65.77^{gh}±0.73	64.51^{e}±0.31	
7	64.33^{fg}±0.71	65.32^{ef}±0.63	
8	62.72^{ef}±0.51	65.24^{ef}±0.59	
9	64.77^{g}±0.61	66.49^{f}±0.75	
10	67.85^{h}±0.72	68.63^{g}±0.31	
11	70.64^{i}±0.54	72.41^{h}±0.53	
Overall mean	59.38^{y}±2.31	60.18^{x}±2.35	

The values are mean ±SE of eleven fortnight observations on seven animals in each group.

Means in columns with different superscript (a,b,c,d,e,f,g,h,i) differ significantly ($P \leq 0.05$).

Means in rows with different superscript (A,B) differ significantly ($P \leq 0.05$).

Overall means ± SE in rows with superscript (x,y) differ significantl

According to Joakimsen and Blom, (1976) and Keller *et al*. (1979) that GH level improves when the animals reach advanced phases of pubertal growth, our experimental buffaloes may be entering the peripubertal stage There is compelling evidence that GH plays numerous functions in pubertal gonadotrophin secretion and response, folliculogenesis and steroidogenesis in cattle (Hall *et al*. 1994; Yelich *et al*.1996), sheep (Shirley *et al*., 2001), humans (Darendeliler *et al*., 1990, Hull KL and Harvey S, 2001) and monkeys (Wilson *et al*., 1989) However, there is no evidence released on the role of GH during pubertal development in buffalo heifers. In vivo GH treatment improved the number of big follicles in GH-deficient dwarfed rats and the number of corpora lutea in GH transgenic rats (Ozawa *et al*., 1996), (Danilovich *et al*., 2000)

4.5 Plasma estradiol (pg/ml) in Murrah buffalo heifers fed with rations containing supplementation of dried *Moringa oleifera* leaves in addition to concentrate mixture

Table (6) Fig (5) depicts the mean values (pg/ml) of plasma estradiol in Murrah buffalo heifers at monthly intervals. The overall mean values (pg/ml) of plasma estradiol levels in the control and treatment groups of Murrah heifers were 5.33±0.09 and 5.65±0.09 respectively. The mean value of plasma estradiol was 5.42±0.74 and 5.38±1.01 (pg/ml) on the zero day in control and treatment groups. At the end of the experiment, mean plasma estradiol levels were 5.55±1.02 and 6.01±1.07 (pg/ml) in control and treatment groups. In Murrah buffalo heifers, plasma estradiol levels did not differ significantly ($P<0.05$) between treatment and controls. The treatment group had numerically greater mean plasma estradiol levels ($P<0.05$) than the control group.

These findings are similar to the earlier work of the study conducted by (Adeyemi, 2014), sixty rabbits were subjected to four diets with supplementation MoLM in a 24-week trial: 0 (control), 2.5, 5.0 and 7.5 percent with 10 bucks and 5 bucks in every treatment group. The hormonal assay outcomes showed a insignificant ($P<0.05$) rise in testosterone and oestrogen levels, when the dietary levels of MoLM was increased. Higher levels of serum oestrogen were observed in rabbits with 2.5 and 7.5 percent of MoLM diet. (Zeng *et al*., 2018) stated that in female mice, *Moringa oleifera* leaf had no effect on serum estradiol levels($P<0.05$).

Table (4) Mean (±SE) of dry matter intake (%BW) in experimental Murrah buffalo heifers

Fortnight	Control	Treatment	P value
0	2.28^{B}±0.11	2.28^{Aa}±0.02	<0.05
1	2.36^{B}±0.11	2.40^{Ab}±0.02	
2	2.36^{B}±0.12	2.53^{Ac}±0.01	
3	2.42^{B}±0.10	2.54^{Ac}±0.02	
4	2.36^{B}±0.11	2.61^{Ad}±0.02	
5	2.40^{B}±0.10	2.67^{Adef}±0.03	
6	2.44^{B}±0.11	2.65^{Ade}±0.02	
7	2.34^{B}±0.10	2.71^{Aef}±0.04	
8	2.31^{B}±0.11	2.74^{Af}±0.02	
9	2.41^{B}±0.17	2.71^{Aef}±0.04	
10	2.37^{B}±0.11	2.74^{Af}±0.02	
11	2.43^{B}±0.13	2.69^{Aef}±0.02	
Mean± SE	2.37^{y}±0.01	2.61^{x}±0.04	

The values are mean ±SE of eleven fortnight observations on seven animals in each group.

The means in columns with different superscript (a,b,c,d,e,f) differ significantly (P≤0.05).

The means in rows with different superscript (A,B) differ significantly (P≤0.05).

Overall means ± SE in rows with superscript (x,y) differ significantly.

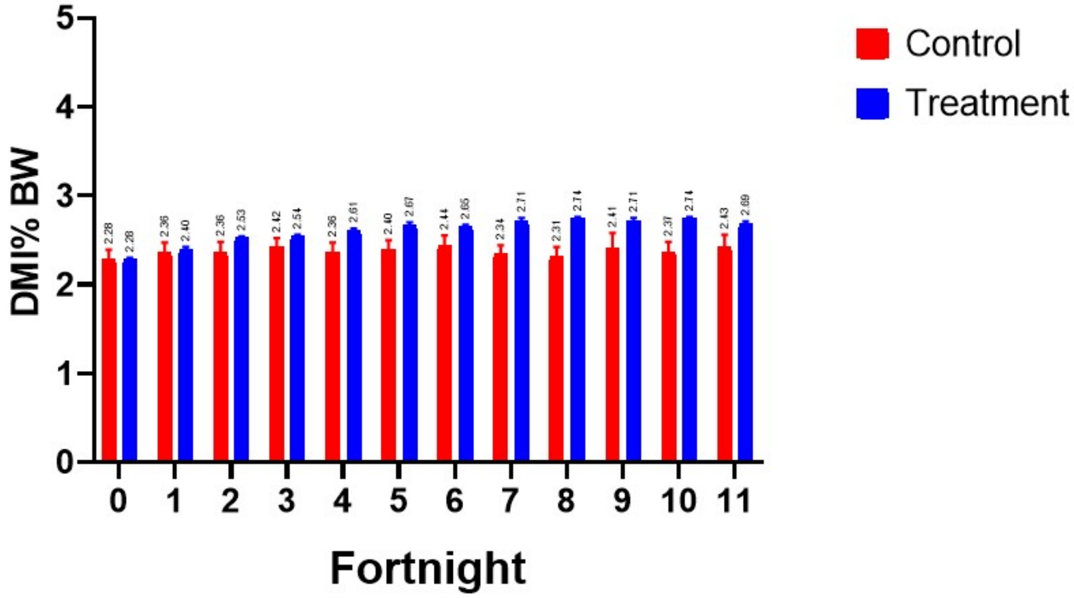
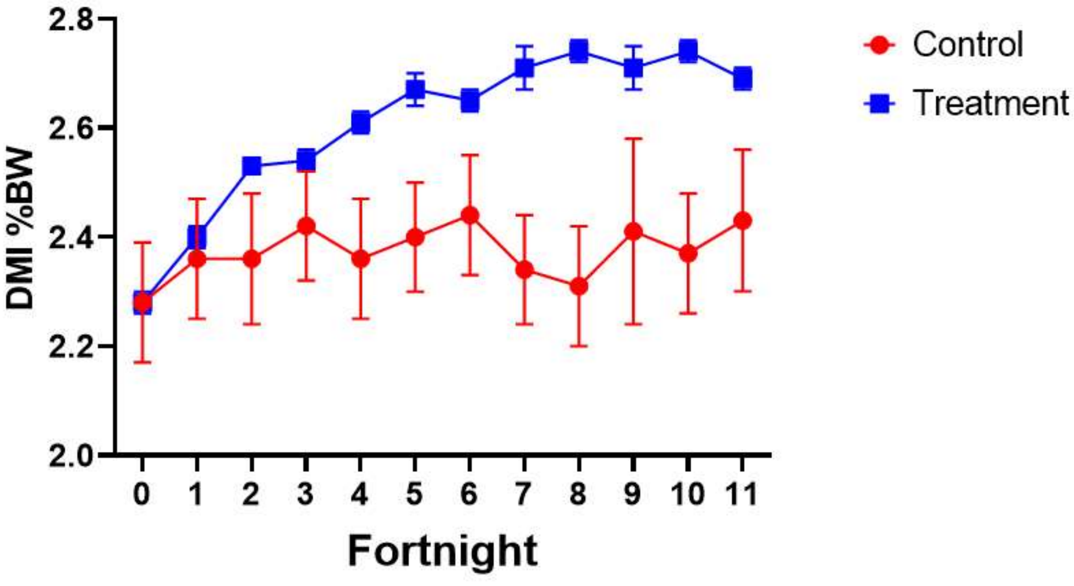


Fig.(3) Mean of dry matter intake (%BW) in two groups of Murrah buffalo heifers



Fig(3). Mean of dry matter intake (%BW) in two groups of Murrah buffalo heifers

Mehrotra *et al*. (2003 and 2004) found increased steroidogenic activity, predominantly oestradiol, in the ovaries of M.koenigii-supplemented rats. Curry leaves (M. koenigii) are an excellent source of minerals, particularly calcium, phosphorus, iron and zinc (Gopalan *et al*., 1996; Shanthala and Prakash, 2005) which are the minerals also present in *Moringa oleifera* leaves. Phytoestrogens influence steroidogenesis by boosting ovulation rate and reducing ovarian follicle atresia (Suttner *et al*., 1998), as well as by causing a preponed FSH surge by plant active principles, resulting in an increased follicle population (Mehrotra *et al*., 2004).

4.6 **Plasma insulin like growth factor (ng/ml) in Murrah buffalo heifers fed with rations containing supplementation of dried *Moringa oleifera* leaves in addition to concentrate mixture**

Table (7) Fig (6) shows the mean values (ng/ml) of plasma insulin like growth factor (IGF) in Murrah buffalo heifers during monthly intervals. The overall mean values (ng/ml) plasma insulin like growth factor (IGF) levels in the control and treatment groups of Murrah heifers were 119.54±2.71 and 123.43±3.25 respectively. On the zero day (before the experiment started), the plasma mean value was 106.6±4.21 and 107.93±4.31 (ng/ml) in the control and treatment groups of Murrah heifers. At the end of the experiment, the mean plasma insulin like growth factor (IGF) levels in the control and treatment groups of Murrah buffalo heifers were 126.75±3.08 and 130.86±3.78 respectively (ng/ml).Plasma IGF levels differed significantly ($P<0.05$) between treatments and controls in Murrah buffalo heifers. The treatment group had higher mean plasma insulin like growth factor (IGF) levels ($P<0.05$) than the control group.

These findings are congruent with those of Abdel-Raheem *et al*. (2021) where plasma IGF 1 levels were considerably higher in calves supplemented with 15% MLM compared to calves in the M0 and M20 treatment groups. The study is in contrary to experiment performed by (Gonzales *et al*., 2019), 15% *Moringa oleifera* leaf meal (MoLM) was included in diets of four sexually mature gilts to determine plasma ghrelin and IGF levels. There were no statistically different levels of plasma ghrelin and IGF-1 between the control and experimental classes. The level of IGF-1 in pigs fed with MoLM seemed to be regulated down. In growing buffalo calves, dietary supplementation with 15% MLM increased rumen fermentation, growth rates, blood metabolites, plasma IGF-I and reduced ammonia and methane. The study found a positive link with the concentrations of the IGF-1 plasma in growing steers (Röpke *et al*. 1994, Davis and Simmens, 1997), which show a high level of nutrition, high average daily increase and weight. Richards *et al*. (1991) discovered that when feed intake reduced due to negative energy balance, circulation concentrations of IGF-1 and LH dropped. IGF-1 has a vital function in the development and maturing of oocytes (Pawshe *et al*., 1998). Heifers provided the HE diet produced more IGF-1 localised within follicles, or their follicular fluid contained higher concentrations of IGF-1 binding proteins. Both would have resulted in a higher proportion of systemically generated IGF-1 being preserved.

4.7 Plasma thyroid hormones (ng/ml) in Murrah buffalo heifers fed with rations containing supplementation of dried *Moringa oleifera* leaves in addition to concentrate mixture

The mean values (ng/ml) of plasma thyroxine (T_4) of Murrah buffalo during monthly interval are shown in table (8) Fig (7). The overall mean (ng/ml) values of plasma thyroxine in the control and treatment groups of Murrah heifers were 37.91±1.4, 42.07±0.64 respectively. The plasma mean value on the zero day (before to the start of the experiment) was 43.77±2.74 and 44.29±1.65 (ng/ml) in control and treatment groups. The mean values of plasma thyroxine of the control and treatment groups of Murrah buffalo heifers at the end of experiment were 34.84±2.85 and 39.93±2.11 (ng/ml), respectively. Plasma thyroxine levels in Murrah buffalo heifers differed significantly ($P<0.05$) between control and treatment. The mean plasma thyroxine levels in the treatment group were higher ($P<0.05$) than in the control group. The mean values (ng/ml) of Murrah buffalo plasma triiodothyronine (T_3) throughout monthly intervals are provided in Table (9) Fig (8). The overall mean (ng/ml) plasma triiodothyronine(T_3) levels in the control and treatment groups of Murrah heifers were 1.68±0.09 and 1.88±0.06 respectively. The plasma mean value on the zero day (before the study started) was 2.14±0.28 in control and 2.14±0.05 (ng/ml) in treatment group. At the end of the experiment, the mean plasma triiodothyronine (T_3) levels in the control and treatment groups of Murrah buffalo heifers were 1.47±0.15 and 1.70±0.07 (ng/ml) respectively. Plasma triiodothyronine levels in Murrah buffalo heifers differed significantly ($P<0.05$) between treatment and control groups. The treatment group had greater ($P<0.05$) mean plasma triiodothyronine (T_3) levels than the control group.

These reported similar with those of (El Badawi *et al*., 2014) where triiodothyronine (T_3) and thyroxine (T_4) levels were considerably ($P<0.05$) higher with ML treatments compared with control, with the greatest levels recorded with 0.30 percent ML, while a significant reduction was observed with 0.45 percent ML treatment. The aqueous leaf extract of *Moringa oleifera* was tested for its ability to improve thyroid function in a rat model. T3 and T4 levels were significantly higher (P<0.05) and TSH levels were significantly lower (P<0.05) at high doses when compared to the control group (Wazida *et al*., 2013) The study is in contrast to the study of adult Swiss rats, the increase in *Moringa oleifera* may cause a decrease in T_3 and T_4 levels due to a decrease in thyroid gland secretion as a result of the action on the thyroid gland. (Tahiliani P. Kar A, 2000) said that 10 days supplementing of 175 and 350 mg/kg of Moringa leaf extract in rat's diet, female rats reported to have a reduction in circulating T_3 by 30%, with an increase in T_4 by 15%, but male rats did not encounter a decrease in circulating T_3 by 30%.

After administration of extract, serum T3 and hepatic LPO reduced. Thyroid hormones are responsible, either directly or indirectly, on qualities which are regulated by Moringa leaves. The increased T_3 levels in the MOLM groups could be attributed to the high concentration of antioxidants, particularly vitamin C, which can counteract the negative effects of heat stress (Hassan *et al*., 2016).

4.8 Plasma cortisol (ng/ml) in Murrah buffalo heifers fed with rations containing supplementation of dried *Moringa oleifera* leaves in addition to concentrate mixture

The mean values (ng/ml) of plasma cortisol of Murrah buffalo during monthly interval are shown in table (10) Fig (9). The overall mean (ng/ml) values of plasma cortisol in the control and treatment groups of Murrah heifers were 6.67±0.20, 6.06±0.13 respectively. The mean value of plasma cortisol levels on the zero day in the control and treatment groups was 6.47±0.23 and 6.48±0.38 (ng/ml). The mean values of plasma cortisol of the control and treatment groups of Murrah buffalo heifers at the end of experiment were 7.49±0.25 and 6.11±0.49 (ng/ml), respectively. There was significant difference (P<0.05) in plasma cortisol levels in Murrah buffalo heifers between control and treatment group. The mean plasma cortisol levels in the treatment group were lower (P<0.05) than in the control group.

These results are consistent with the findings provided by Gbadamosi *et al*. (2016) where the results demonstrated that supplementing the fish with *Moringa oleifera* leaf extract in the meals significantly lowered cortisol and glucose levels generated by stressors (P<0.05). Study by (Abdel-Latif *et al*., 2018) aimed to investigate *Moringa oleifera* extract (MOE), vitamin C and sodium bicarbonate (NaHCO3) affected heat stress (HS)-induced changes in rabbits.

Table (5) Mean (±SE) of plasma growth hormone (ng/ml) in Murrah buffalo heifers

Days	Control	Treatment	P value
0	$6.57^{c}\pm0.31$	$6.58^{c}\pm0.41$	0.057
30	$5.45^{bc}\pm0.61$	$5.97^{bc}\pm0.46$	
60	$4.00^{a}\pm0.27$	$4.12^{a}\pm0.19$	
90	$4.32^{ab}\pm0.49$	$4.28^{a}\pm0.12$	
120	$4.35^{ab}\pm0.18$	$4.91^{ab}\pm0.56$	
150	$4.47^{Bab}\pm0.30$	$5.09^{Aab}\pm0.58$	
180	$4.55^{Bab}\pm0.42$	$5.21^{Aab}\pm0.60$	
Mean ± SE	4.81±0.34	5.16±0.33	

The values are mean ±SE of seven observations on seven animals in each group.
Means in columns with different superscript (a,b,c) differ significantly (P≤0.05).
Means in rows with different superscript (A, B) differ significantly (P≤0.05).

Table (6)Mean (±SE) of plasma estradiol (pg/ml) in Murrah buffalo heifers

Days	Control	Treatment	P value
0	5.42±0.74	5.38±1.01	0.760
30	5.59±0.84	5.71±1.37	
60	5.28±1.30	5.48±1.11	
90	5.10±1.06	5.36±1.02	
120	4.98±1.25	5.83±1.04	
150	$5.4^{B}\pm0.49$	$5.79^{A}\pm1.21$	
180	5.55±1.02	6.01±1.07	
Mean ± SE	5.33±0.09	5.65±0.09	

The values are mean ±SE of seven observations on seven animals in each group..

The means ± SE in rows with superscript (A, B) differ significantly.

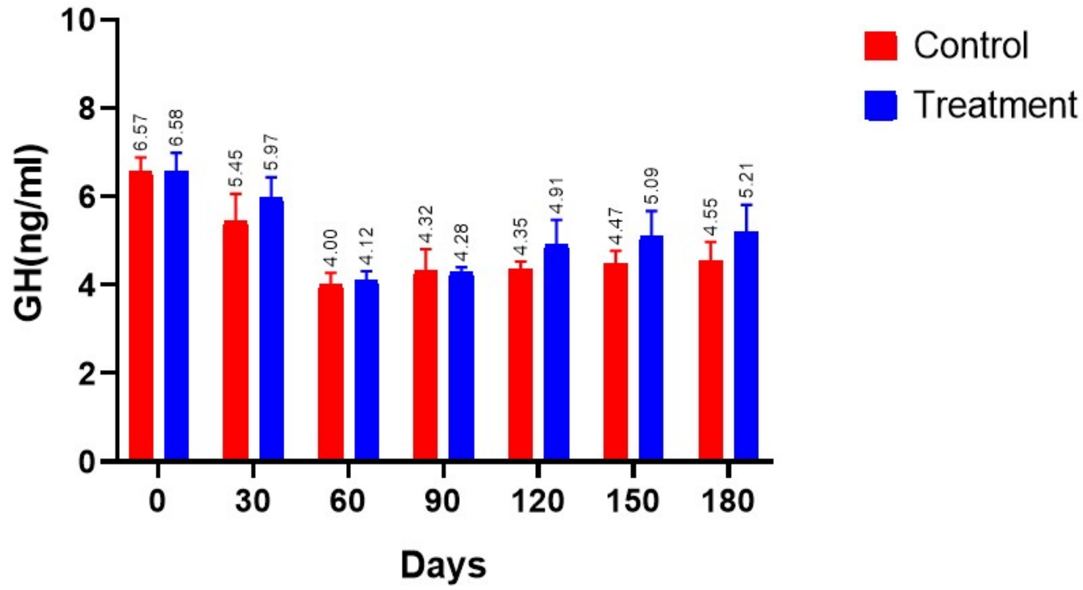


Fig (4) Mean plasma GH (ng/ml) in two groups of Murrah buffalo heifers

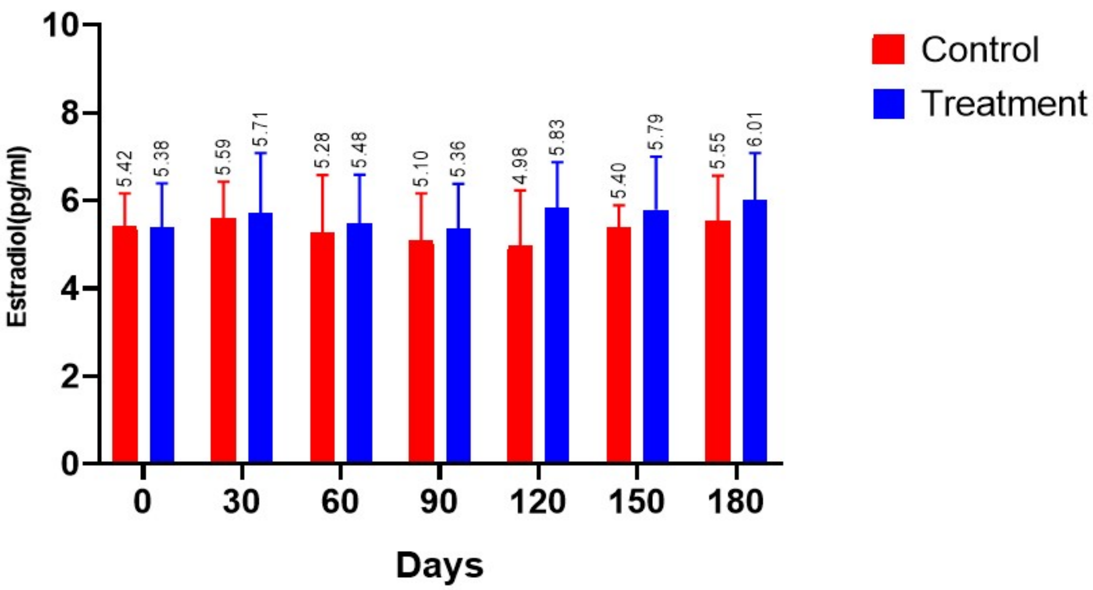


Fig (5) Mean plasma estradiol (pg/ml) in two groups of Murrah buffalo heifers

The research by (Khalid *et al*., 2020) evaluated the effects of *Moringa oleifera* leaf powder (MOLP) supplementation on rabbit productivity and intestinal health during prolonged heat stress (HS). The HS group had higher serum cortisol levels than the CON group (P<0.05), but MOLP had lower cortisol levels than the HS group (P<0.05). Somagond, Y. M (2019) observed cortisol levels were significantly lower (P<0.05) in the antioxidant (astaxanthin) supplemented group compared to the control and prill fat supplemented groups. As *Moringa oleifera* is high in antioxidant properties it is assumed to lower down cortisol levels in plasma just like asthaxanthin (antioxidant) supplementation. In Tharparkar and Karan Fries heifers, Kumar (2018) showed positive effects of a summer astaxanthin supplementation which contributed to a reduction in the level of cortisol in the treatment group. Banerjee *et al*. (2016) discovered a positive association between cortisol and physiological responses and a negative correlation between cortisol and thyroid hormones.

The anti-stress qualities of Shativari led in the period of expression to non-significant Gholap, S. and Kar, A. (2004) noticed a concurrent drop in serum cortisol in treated animals with extracts of *I. racemosa, B. diffusa* and *O.sanctum*. The reduction in cortisol concentration caused by plant extracts could be attributable to a suppression of adrenocorticotrophin (ACTH) release in pituitary glands or a direct action at the adrenal cortex level.

4.9 Plasma total antioxidant capacity (U/ml) in Murrah buffalo heifers fed with rations containing supplementation of dried *Moringa oleifera* leaves in addition to concentrate

The mean values (U/ml) of plasma total antioxidant capacity (TAC) of Murrah buffalo during monthly interval are shown in table (11) Fig (10). The overall mean (U/ml) values of plasma total antioxidant capacity (TAC) in the control and treatment groups of Murrah heifers were 3.55±0.08 and 3.92±0.11 respectively. The mean value of plasma total antioxidant capacity (TAC) on the zero day in the control and treatment groups was 3.61±0.42 and 3.59±0.29 (U/ml). The mean value of TAC at the end of the experiment was 3.56±0.15 and 4.28±0.22 (U/ml) in control and treatment group respectively. Plasma total antioxidant capacity (TAC) levels in Murrah buffalo heifers differed significantly (P<0.05) between control and treatment. The mean plasma total antioxidant capacity (TAC) levels in the treatment group were higher (P<0.05) than in the control group.

These results are consistent with the findings provided by the experiment carried out by Babiker *et al*. (2017) on 20 dairy Aardi goats. In comparison to the diet for Alfalfa hay, *Moringa oleifera* hay fed had serum with more antioxidants (TAC) and vitamin C. They found that goats given a *Moringa oleifera* leaf diet had significantly higher TAC ($P<0.01$) and vitamin C ($P<0.05$) levels in their serum than animals on Alfa hay diet. Oparinde, D. P. and Atiba, A. S. (2014) shown the reduced TAS in test rats compared to control rats could imply that *Moringa oleifera* is providing antioxidants. Because of its high amount of phenolic compounds and isothiocyanate, MOL showed excellent antioxidant activities (Tumer *et al*., 2015; Verma *et al*., 2009). *Moringa oleifera* has strong antioxidant properties against free radicals, which helps to avoid oxidative damage to key macromolecules and offers additional antioxidant protection (Sreelatha and Padma, 2009). The high phenolic and natural antioxidants such as the vitamin C, E, A in *Moringa oleifera* may result in improved antioxidant activity in the serum, and this factor develops the animals' health for increasing the use and absorption of minerals, protein, vitamins and other essential elements (Saxena *et al*., 2013).

4.10 Plasma glucose (mg/dl) in Murrah buffalo heifers fed with rations containing supplementation of dried *Moringa oleifera* leaves in addition to concentrate mixture

The mean values (mg/dl) of Murrah buffalo plasma glucose throughout monthly intervals are provided in table (12) Fig (11). The overall mean (mg/dl) plasma glucose levels in the control and treatment groups of Murrah heifers were 68.91±0.50 and 67.05±0.82, respectively. The plasma mean value on the zero day (before the study started) was 70.38±1.99 in control and 70.28±1.25 (mg/dl) in treatment group. At the end of the experiment, the mean plasma glucose levels in the control and treatment groups of Murrah buffalo heifers were 69.21±1.36 and 68.20±2.32, respectively. There was no significant differences ($P<0.05$) in plasma glucose levels of Murrah buffalo heifers between treatments and controls. The treatment group had similar ($P<0.05$) mean plasma glucose levels than the control group.

These reported similar with those of Jelali *et al*. (2014) that showed MLM diets declined in total serum proteins ($P<0.05$), but have no effect on the levels of serum glucose ($P>0.05$). Glucose is the primary metabolic fuel required by essential organs, foetal growth and milk production (Leblanc, 2010).

Table (7) Mean (±SE) of plasma IGF-1 (ng/ml) in Murrah buffalo heifers

Days	Control	Treatment	P value
0	106.6^{a}±4.21	107.93^{a}±4.31	<0.05
30	117.43^{bc}±2.59	118.06^{ab}±3.39	
60	114.9^{ab}±3.18	120.04^{bc}±3.1	
90	121.11^{bc}±2.73	128.68^{bcd}±3.39	
120	124.37^{bc}±3.11	126.78^{bcd}±3.24	
150	125.65^{c}±3.9	131.69^{d}±4.08	
180	126.75^{c}±3.08	130.86^{cd}±3.78	
Mean ± SE	119.54^{y}±2.71	123.43^{x}±3.25	

The values are mean ±SE of seven observations on seven animals in each group.
Means in columns with different superscript (a,b,c,d) differ significantly (P≤0.05).
Overall means ± SE in rows with superscript (x,y) differ significantly.

Table (8) Mean (±SE) of plasma thyroxine (ng/ml) in Murrah buffalo heifers

Days	Control	Treatment	P value
0	43.77^{c}±2.74	44.29^{a}±1.65	<0.05
30	40.58^{bc}±1.65	42.53^{a}±1.10	
60	39.20^{abc}±1.12	43.54^{a}±2.00	
90	36.27^{abc}±2.28	39.93^{a}±2.11	
120	32.74^{a}±2.92	42.66^{a}±4.44	
150	37.96^{abc}±2.75	41.59^{a}±3.02	
180	34.84^{ab}±2.85	39.93^{a}±2.11	
Mean ± SE	37.91^{y}±1.4	42.07^{x}±0.64	

The values are mean ±SE of seven observations on seven animals in each group.
Means in columns with different superscript (a,b,c) differ significantly (P≤0.05).
Overall means ± SE in rows with superscript (x,y) differ significantly

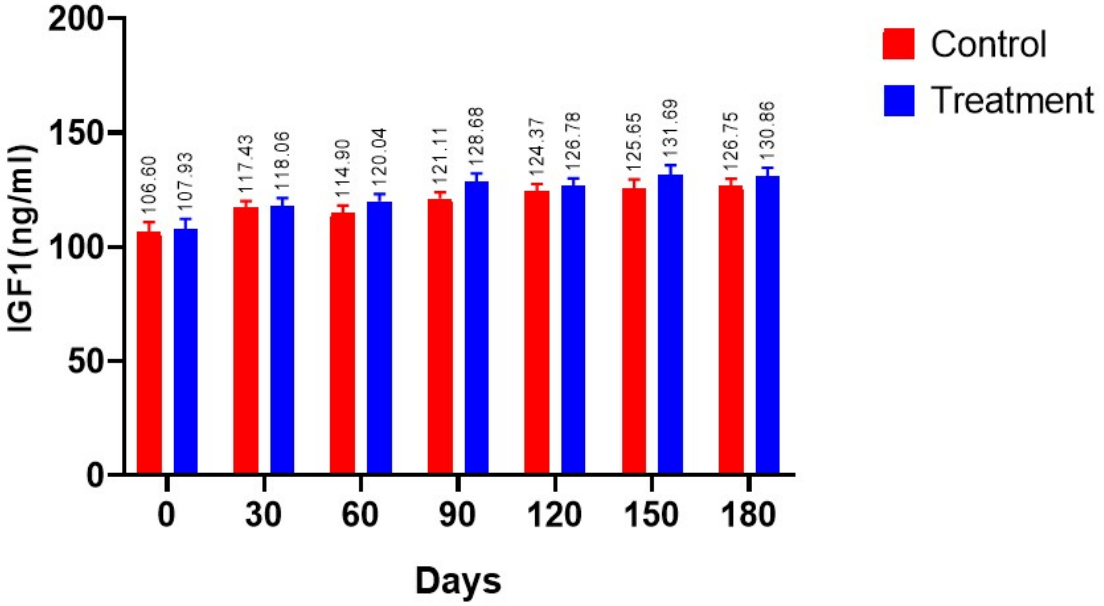


Fig (6) Mean plasma IGF-1 (ng/ml) in two groups of Murrah buffalo heifers

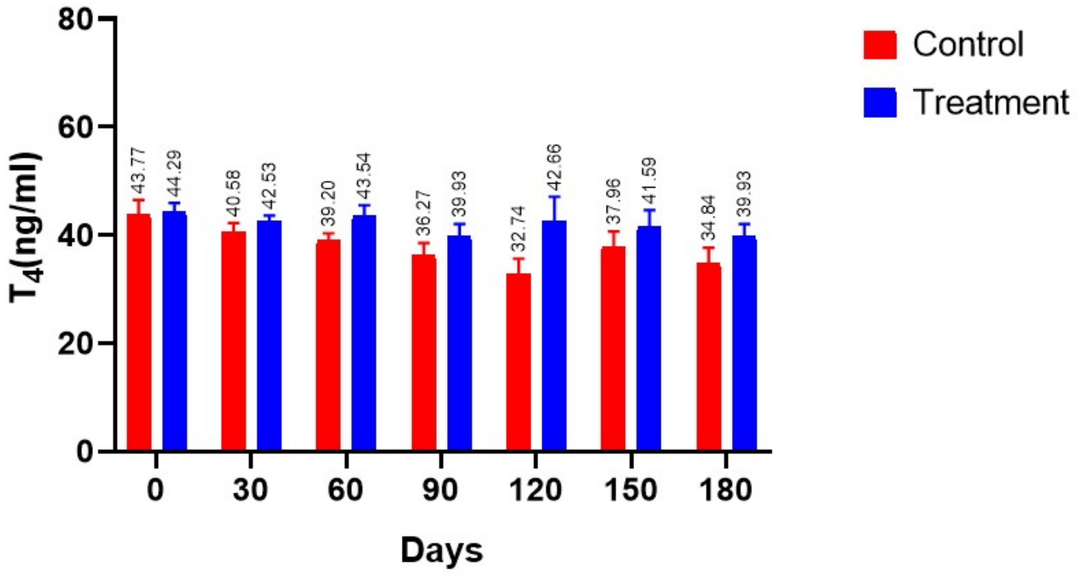


Fig (7) Mean plasma thyroxine (ng/ml) in two groups of Murrah buffalo heifers

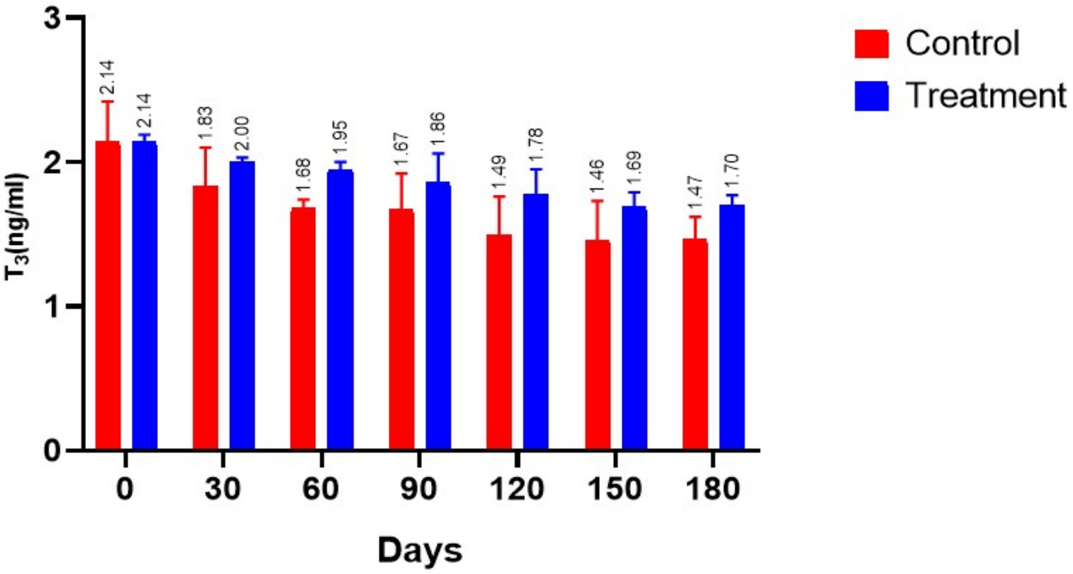


Fig (8) Mean plasma T_3 (ng/ml) in two groups of Murrah buffalo heifers

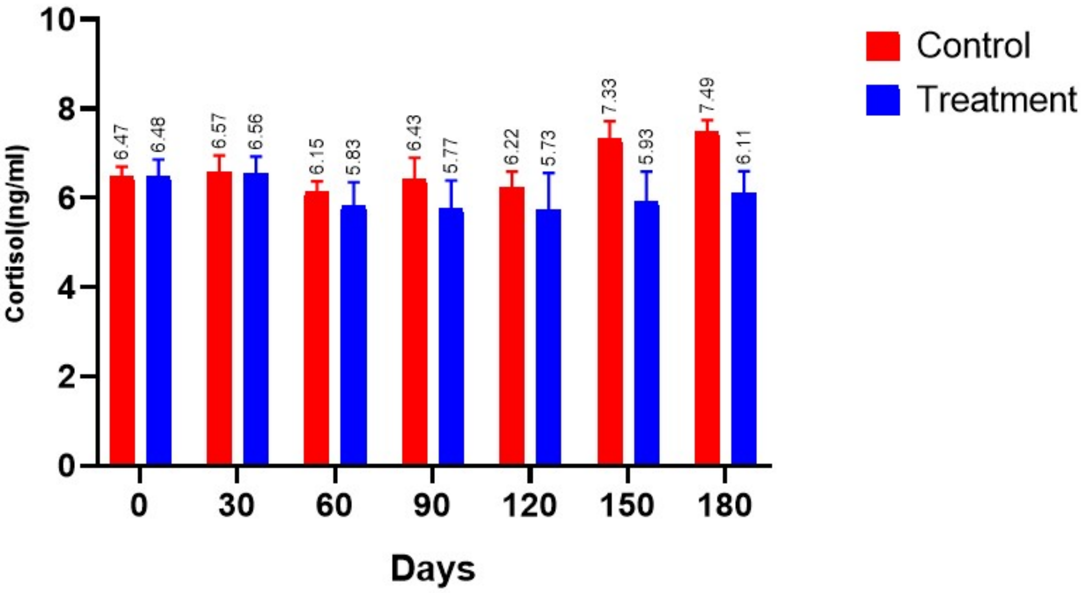


Fig (9). Mean plasma cortisol (ng/ml) in two groups of Murrah buffalo heifers

Glucose levels were considerably (P<0.05) lower with 0.15 and 0.30 percent ML, but significantly higher with *Moringa oleifera* up to 0.45 percent compared to control. The lower glucose could be attributed to an increase in glucose utilisation. However, with a 0.45 percent ML diet, this trend was greatly reduced. *Moringa oleifera* leaves supplemented at low amounts (0.15 to 0.30 percent) appear to improve insulin production, while greater levels may have an unfavourable effect (0.45 percent).It appears reasonable to conclude that the observed decrease in serum glucose in treatment group is not due to *Moringa oleifera* leaf meal intoxication, but rather that the dietary energy was used efficiently for growth and the animals were not living at the expense of bodily tissues (Ologhobo *et al*., 1992). The low glucose level detected suggests that it is good for human diabetic consumption, as the presence of flavonoid also fits with the results of (Farooq *et al*., 2012) who said that the *Moringa oleifera* plant is one of the highly prospective antidiabetic plants most likely due to the ability of its components and some flavonoids to block amylase activity, which regulates the amount of glucose in the blood.

4.11 Apparent digestibility of nutrient (%) in Murrah buffalo heifers fed with rations containing supplementation of dried *Moringa oleifera* leaves in addition to concentrate

The results of the study are presented in the table (13), Fig (12) .The apparent digestibility of DM (61.27±1.88), OM (61.76±0.86), CP (66.91±0.92), EE (75.27±0.67), NDF (61.97±2.23) and ADF (62.62±2.75) was observed in control group. The apparent digestibility of DM (69.78±0.79), OM (71.52±0.69), CP (75.46±0.77), EE (74.54±1.16), NDF (69.52±1.79) and ADF (70.74±1.19) was observed in treatment group. Statistical analysis showed significant difference in digestibility of DM, OM, CP, NDF, ADF (P<0.05) .There was no significant difference in EE among the control and treatment group. This finding is in line with earlier study by (Imran *et al*.,2016) showed that the apparent digestibility coefficients of DM,OM,CP,NDF,ADF of buffaloes supplemented *Moringa oleifera* hay were significantly (P<0.05) higher than those of buffaloes on feeding *Medicago sativa*. Reyes *et al*. (2006) discovered similar results, describing the apparent digestibility coefficients for DM, OM, CP, NDF and ADF improved in dietary supplementation with *Moringa oleifera*.

This result is in accordance to study by Mahmoud *et al*. (2013), Abdel-Raheem *et al*. (2021), Kholif *et al*.(2015), Azzaz *et al*. (2016) where the EE values did not show significant (P<0.05) increase in treatment group as compared to control group. The digestibility of organic matter (OM), dry matter and neutral detergent fibre (NDF) in Nubian goats was improved (P<0.01) by *Moringa oleifera* extract (Kholif *et al*., 2018, 2019).

The feeding of MLM to buffalo calves significantly improved (P<0.05) the digestibility of dry matter (DM), organic matter (OM). MLM protein degradability in the rumen may also affect intake, as higher availability of undegradable protein might boost feed intake (M'hamed *et al*., 2001; Kholif *et al*., 2014). Tona *et al*. (2014) discovered that goats provided a concentrate diet containing 15% *Moringa oleifera* leaf meal exhibited significantly higher rate of digestibility coefficients compared to goats fed a diet containing 5% or 10% inclusion level. The results obtained are in contrary to Sultana *et al*. (2015a) where *Moringa oleifera* foliage did not show any significant (P<0.05) difference in digestibility of nutrients between groups. The high dry matter and ash contents of MOD may enhance the levels of cellulolytic rumen microflora, resulting in rapid breakdown of the diet and passing of digesta in the rumen. MLM may play a function in limiting ammonia output in bovine digestive tracts by lowering ruminal protein breakdown and deamination and decreasing rumen ammonia (Bhatta *et al*., 2012; Jelali and Salem, 2014).*Moringa oleifera* leaves contain more degradable compounds, particularly crude protein and could be used as a supplementary to ruminant diets (Fayomi *et al*., 2014). According to Nuhu, (2010), feeding *Moringa oleifera* dry leaves improved CP digestibility in rabbits.

4.12 Effect of feeding of dried *Moringa oleifera* leaves on ultrasonographic attributes

The ovarian follicular features of Murrah buffalo heifers in both groups are provided in table (14), Fig (13). In the present study the Mean ±SE in number of follicles (<3mm) of control group and treatment group were 2.5±0.29 and 3.5±0.26respectively. The number of follicles (3-8mm) showed a Mean ±SE of 1.50±0.30 in control group and 2.50±0.27 in treatment group. The total number of follicles showed a Mean ±SE of 4.50±0.25 in control group and 6.75±0.29in treatment group. There was significant difference (P>0.05) in BW as a result of supplementation. The number of small (<3 mm), medium (3-8mm) and total no of follicles were significantly higher (P<0.05) in treatment group than in control group.

Table (9) Mean (±SE) of plasma triodothyronine (ng/ml) in Murrah buffalo heifers

Days	Control	Treatment	P value
0	$2.14^{a}\pm0.28$	$2.14^{b}\pm0.05$	<0.05
30	$1.83^{Ba}\pm0.27$	$2.00^{Aab}\pm0.03$	
60	$1.68^{a}\pm0.06$	$1.95^{ab}\pm0.05$	
90	$1.67^{a}\pm0.25$	$1.86^{ab}\pm0.20$	
120	$1.49^{a}\pm0.27$	$1.78^{a}\pm0.17$	
150	$1.46^{Ba}\pm0.27$	$1.69^{Aa}\pm0.1$	
180	$1.47^{a}\pm0.15$	$1.70^{a}\pm0.07$	
Mean ± SE	$1.68^{y}\pm0.09$	$1.88^{x}\pm0.06$	

The values are mean ±SE of seven observations on seven animals in each group
The means ± SE in rows with superscript (A,B) differ significantly.
The means ± SE in columns with superscript (a,b) differ significantly
Overall means ± SE in rows with superscript (x,y) differ significantly.

Table (10) Mean (±SE) of plasma cortisol (ng/ml) in Murrah buffalo heifers

Days	Control	Treatment	P value
0	$6.47^{ab}\pm0.23$	$6.48^{a}\pm0.38$	<0.05
30	$6.57^{ab}\pm0.38$	$6.55^{a}\pm0.37$	
60	$6.15^{a}\pm0.22$	$5.83^{a}\pm0.52$	
90	$6.43^{ab}\pm0.47$	$5.77^{a}\pm0.62$	
120	$6.22^{a}\pm0.37$	$5.73^{a}\pm0.83$	
150	$7.33^{b}\pm0.39$	$5.93^{a}\pm0.66$	
180	$7.49^{b}\pm0.25$	$6.11^{a}\pm0.49$	
Mean ± SE	$6.67^{x}\pm0.2$	$6.06^{y}\pm0.13$	

The values are mean ±SE of seven observations on seven animals in each group.
The means in columns with different superscript (a,b) differ significantly (P≤0.05).
Overall means ± SE in rows with superscript (x,y) differ significantly.

There was no significant difference in large follicles (>8mm) in control and treatment group (P<0.05).The results are in agreement with the study by (Saki *et al*., 2014) the weight of the ovary and the quantity of small follicles were favourably affected by supplementation of herbal feed additive. Mehrotra *et al*. (2004) discovered that when ethanolic extracts of M. koenigii (1000 mg/kg) were used to study the effect of M. koenigii on follicular development in rats, the mean number of follicles was significantly higher in the treatment group comparison to the control group where Murraya appears to have a beneficial effect on follicle population. They proposed that M. koenigii stimulated FSH secretion, resulting in the early recruitment of follicles with a greater number of FSH receptors, resulting in an increased larger follicle population. Aloe vera has an angiogenic and FSH-like action, according to Kosif and Aktas, (2009). On histological sections of the ovaries of female Wistar albino rats treated with Aloe vera, they noticed vascular increase and hyperemic ovary with increased follicle counts. Bushmich *et al*. (1980), who discovered that monensin-fed gonadotrophin-treated pubertal heifers had more follicles and corpora luteas than controls. Moseley *et al*. (1982) discovered that in heifers fed monensin, the age at puberty decreased and this difference was not related to higher average daily gain or body weight. In heifers, a slower growth rate is related with a later onset of puberty and hence a later age at first calving (Bhatti *et al*., 2007; Maquivar *et al*., 2006). Puberty occurs at an average age of 37 and 34 months in buffalo and cow heifers, respectively (Bashir, 2006; Rehman, 2006).

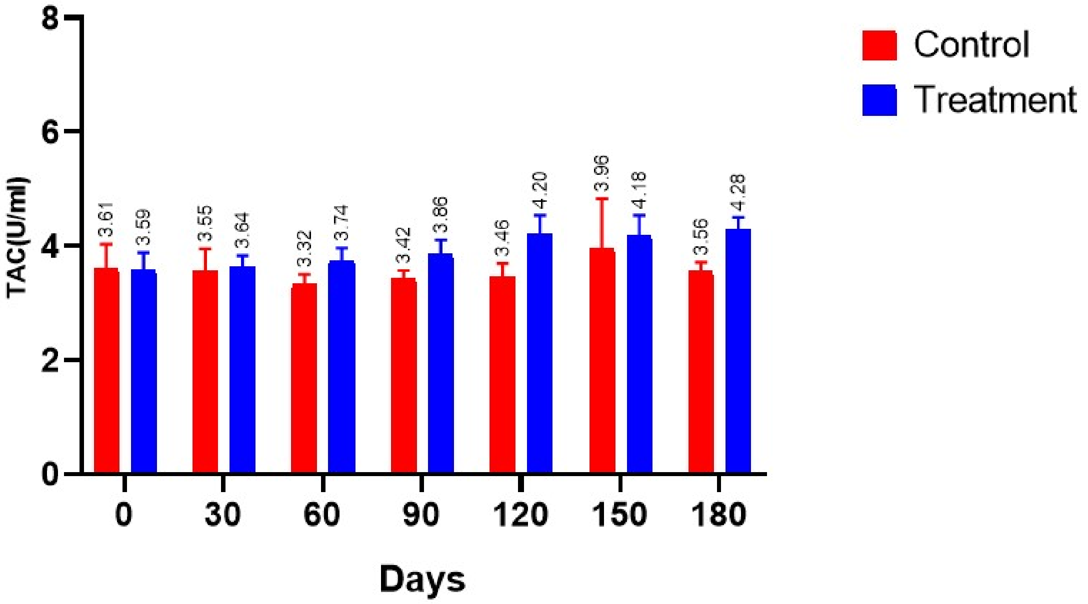


Fig (10). Mean plasma total antioxidant capacity (U/ml) in Murrah buffalo heifers

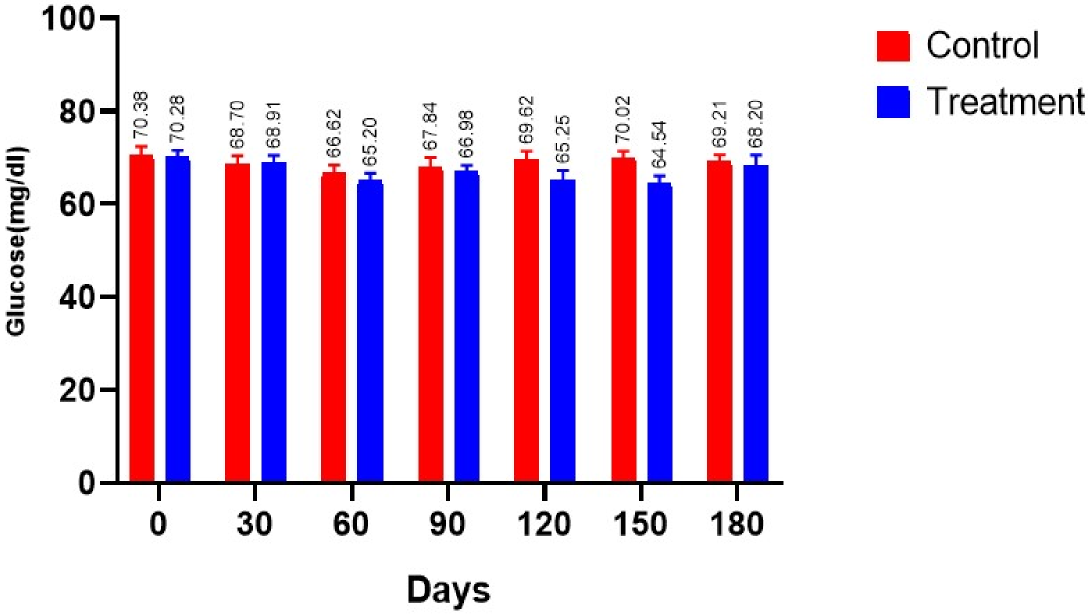


Fig (11) Mean plasma glucose (mg/dl) in two groups of Murrah buffalo heifers

Table (11) Mean (±SE) of plasma total antioxidant capacity (U/ml) in buffalo heifers

Days	Control	Treatment	P value
0	3.61±0.42	3.59±0.29	<0.05
30	3.55±0.4	3.64±0.19	
60	3.32±0.18	3.74±0.22	
90	3.42±0.15	3.86±0.24	
120	3.46±0.23	4.20±0.34	
150	3.96±0.87	4.18±0.36	
180	3.56±0.15	4.28±0.22	
Mean ± SE	3.55^{y}±0.08	3.92^{x}±0.11	

The values are mean ±SE of seven observations on seven animals in each group.
Overall means ± SE in rows with superscript (x,y) differ significantly.

Table (12) Mean (±SE) of plasma glucose (mg/dl) in Murrah buffalo heifers

Days	Control	Treatment	P value
0	70.38^{a}±1.99	70.28^{b}±1.25	0.098
30	68.70^{a}±1.64	68.91^{ab}±1.52	
60	66.62^{a}±1.79	65.20^{ab}±1.42	
90	67.84^{a}±2.17	66.98^{ab}±1.33	
120	69.62^{a}±1.74	65.25^{ab}±1.95	
150	70.02^{a}±1.32	64.54^{a}±1.52	
180	69.21^{a}±1.36	68.20^{ab}±2.32	
Mean ± SE	68.91±0.50	67.05±0.82	

The values are mean ±SE of seven observations on seven animals in each group.
Means in columns with different superscript (a,b) differ significantly ($P \leq 0.05$).

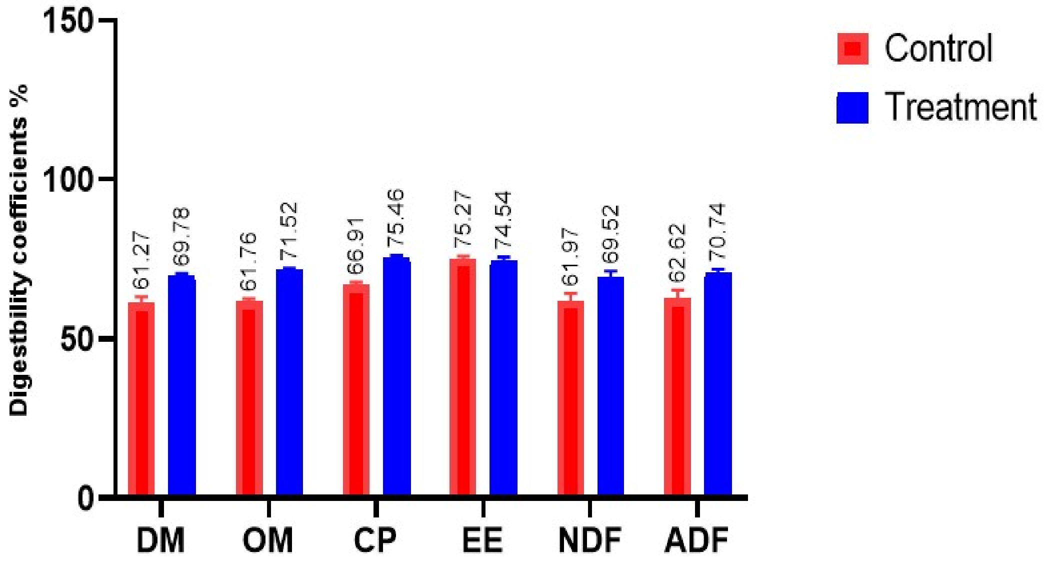


Fig (12). Mean apparent digestibility of nutrients (%) in Murrah buffalo heifers

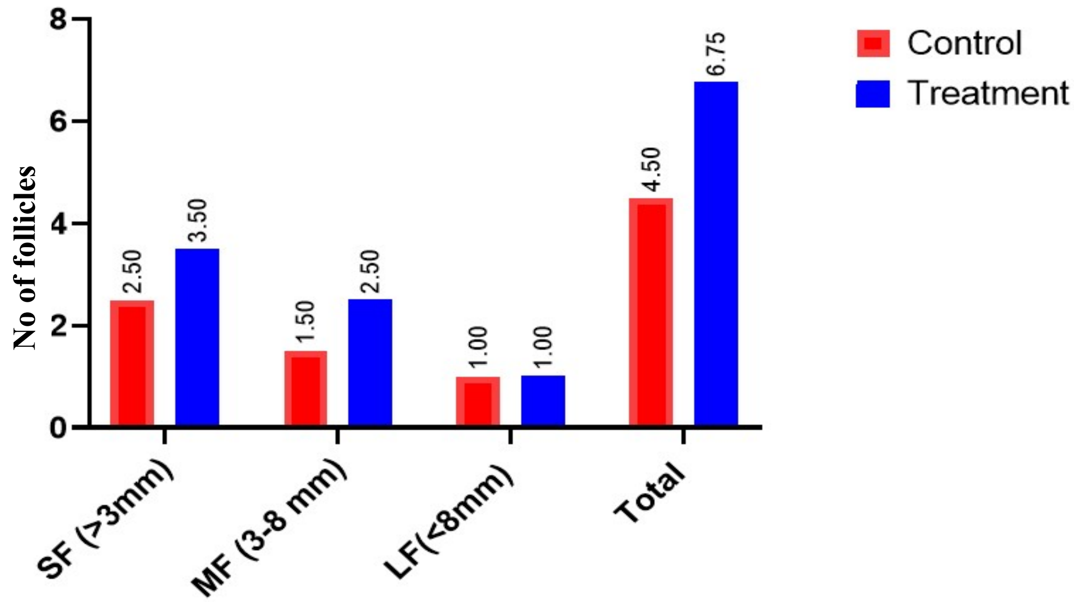


Fig (13) Mean ovarian follicles in two groups of Murrah buffalo heifers

Table (13) Mean (±SE) of apparent digestibility of nutrients (%) in Murrah buffalo heifers

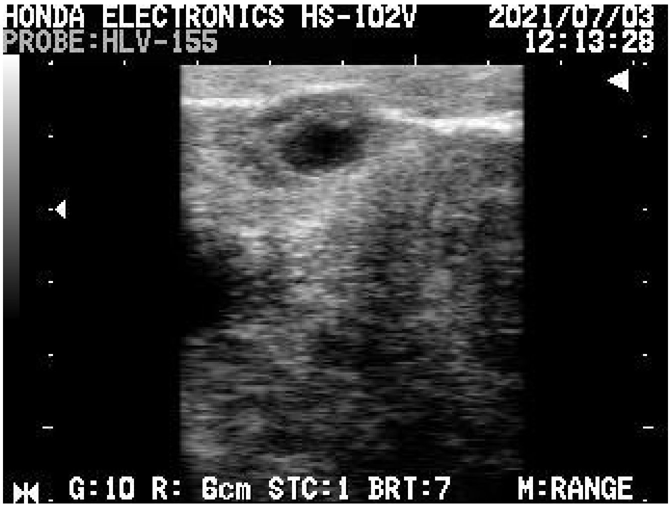
Apparent digestibility coefficients (%)	Control	Treatment
DM	61.27^{b}±1.88	69.78^{a}±0.79
OM	61.76^{b}±0.86	71.52^{a}±0.69
CP	66.91^{b}±0.92	75.46^{a}±0.77
EE	75.27±0.67	74.54±1.16
NDF	61.97^{b}±2.23	69.52^{a}±1.79
ADF	62.62^{b}±2.75	70.74^{a}±1.19

The values are mean ±SE of seven observations on seven animals in each group.
Means in rows with different superscript (a,b) differ significantly (P≤0.05).

Table (14) Mean (±SE) of ovarian follicles in experimental Murrah buffalo heifers

Attributes	Control	Treatment
No of small follicles(<3mm)	2.5^{b}±0.29	3.5^{a}±0.26
No of medium follicles(3-8mm)	1.50^{b}±0.30	2.50^{a}±0.27
No of large follicles(>8mm)	1.00±0	1.00±0
Total no of follicles	4.50^{b}±0.25	6.75^{a}±0.29
Diameter of largest follicle	8.5	11.8

The values are mean ±SE of observations on control and treatment animals
The Means in rows with different superscript (a,b) differ significantly (P≤0.05).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2021/07/03
PROBE:HLV-155
12:13:28
G:10 R: 6cm STC:1 BRT:7
M:RANGE


HONDA ELECTRONICS HS-102V
2021/07/03
PROBE:HLV-155
12:14:04
G:10 R: 6cm STC:1 BRT:7
M:RANGE


HONDA ELECTRONICS HS-102V
2021/07/03
PROBE:HLV-155
12:18:56
G:10 R: 6cm STC:1 BRT:7
M:RANGE


HONDA ELECTRONICS HS-102V
2021/07/03
PROBE:HLV-155
12:21:17
G:10 R: 6cm STC:1 BRT:7
M:FOCUS


HONDA ELECTRONICS HS-102V
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G:10 R: 6cm STC:1 BRT:7
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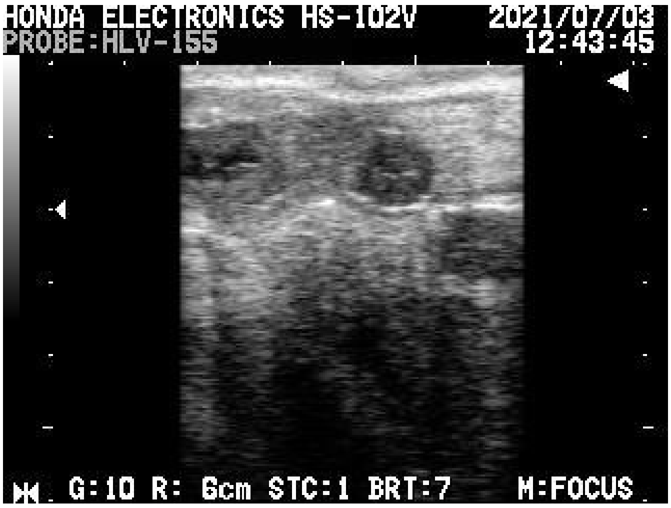
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Chapter-5

Summary and Conclusions

Summary

The present experiment was performed to evaluate **"Effects of *Moringa oleifera* leaves supplementation on growth, certain blood metabolites and hormones in Murrah buffalo heifers" with the following objectives.**

1. To study the effect of *Moringa oleifera* leaves supplementation on the augmentation of growth in Murrah buffalo heifers.

2. To investigate the association of blood metabolites and hormones with growth and ovarian status in *Moringa oleifera* leaves supplemented buffaloes

The study was conducted at Livestock Research Centre (LRC), National Dairy Research Institute, Karnal with effect from December 2020 to May 2021 on 14 growing Murrah Buffalo heifers aged 11-13 months at the time of selection. These animals were divided into two groups of 7 animals each on basis of body weight and age of animals. The two groups as Control was fed with diet as per ICAR guidelines and the Treatment group was fed with control diet of roughages, concentrates and along with it supplementation of *Moringa oleifera* leaves at 20% of the dry matter intake.

Every fortnightly animals were weighed on digital platform balance in morning before feeding. Body weight records were maintained during experiment. Total body weight gain (kg) and Dry matter intake of experimental feeds in terms of kg/100 kg BW were calculated from records of dry matter consumption and body weights of Murrah buffalo heifers.

Blood Samples were collected early in the morning. It was collected on 0, 30, 60, 90, 120 and 150, 180 days. Blood drawn aseptically from jugular vein of each animal in sterile EDTA vacutainer (Vacutte®, K3EDTA, Greiner bio-one (9ml) GMDH, Austria). Immediately after collection blood was transported to lab in ice box and then centrifuged at 2500 rpm for 25 minutes to separate plasma. Plasma obtained was decanted in clean, dried plastic eppendorf vials and stored at -20˚C for subsequent analysis of various plasma metabolites.

1. The overall mean values of Body weight, Metabolic body weight, DMI % BW was significantly higher ($P<0.05$) in treatment group than in control group.
2. The overall means of levels of plasma IGF (ng/ml), plasma thyroxin (ng/ml), plasma triiodothyronine (ng/ml), plasma total antioxidant capacity (U/ml) differed significantly ($P<0.05$) between treatments and controls in Murrah buffalo heifers. Plasma IGF (ng/ml), Plasma thyroxin (ng/ml), Plasma triiodothyronine (ng/ml) were higher ($P<0.05$) in the treatment group than in the control group.
3. The mean values of GH hormone (ng/ml), Plasma estradiol (pg/ml), Plasma glucose (mg/dl) in control and treatment was ($P>0.05$) but levels of hormone were higher for GH and Estradiol and similar for Glucose in treatment groups.
4. There was significant difference ($P<0.05$) in the overall means of Plasma Cortisol (ng/ml) levels in Murrah buffalo heifers between control and treatment group. The mean plasma cortisol levels in the treatment group were lower ($P<0.05$) than that of the control group.
5. Significant difference ($P<0.05$) was observed in the apparent digestibility (%) of DM, OM, CP, NDF, ADF in treatment group than in control group. No significant difference ($P<0.05$) was observed in digestibility of EE.
6. The number of small (<3 mm), medium (3-8mm) and total no of follicles were significantly higher ($P<0.05$) in treatment group than in control group. There was no significant difference in large follicles (>8mm) in control and treatment group ($P>0.05$). The largest follicle was observed in treatment group.

Conclusion

The following conclusions were drawn from the present study.

1. The feeding of dried *Moringa oleifera* leaves for 6 months improved the body-weights, feed intake, digestibility of nutrients and growth rate of Murrah buffalo heifers.
2. The ovarian follicular development was also improved in the *Moringa oleifera* leaves supplemented Murrah buffalo heifers.

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