Effect of replacing protein source in concentrate mixture with dried *Moringa oleifera* leaf powder on the performance of Barbari bucks

THESIS SUBMITTED FOR PARTIAL FULFILLMENT OF THE REQUIREMENT FOR THE DEGREE

OF

MASTER OF VETERINARY SCIENCE

IN

ANIMAL NUTRITION

BY

Anuj Dubey

Enrollment No. V-1820/17

COLLEGE OF VETERINARY SCIENCE & ANIMAL HUSBANDRY

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Vishwavidyalaya Evam Go Anusandhan Sansthan, Mathura – 281001 (UP)

(2019)

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This is to certify that thesis entitled, **"Effect of replacing protein source in concentrate mixture with dried *Moringa oleifera* leaf powder on the performance of Barbari bucks"** submitted by **Dr. Anuj Dubey, Enrollment No. V-1820/17** in partial fulfillment of the requirements for the award of the **Master of Veterinary Science** in **Animal Nutrition** of the **Uttar Pradesh Pandit Deen Dayal Upadhyaya Pashu-Chikitsa Vigyan Vishwavidyalaya Evam Go Anusandhan Sansthan, Mathura (UP), India,** is a bonafide research work carried out by him under my supervision and guidance and no part of the thesis has been submitted for any other degree or diploma.

Dated. 11.7.2019

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HOD

Animal Nutrition

%	:	Percent
@	:	At the rate of
<	:	Less than
>	:	More than
µM	:	Micro mole
^{0}C	:	Degree centigrade
100X	:	Magnification hundred times
10X	:	Magnification ten times
400X	:	Magnification four hundred times
40X	:	Magnification forty times
45X	:	Magnification forty five times
ABTS	:	Azino-Bis- Ethyl-Benzothiazoline Sulphonic-acid
ADF	:	Acid detergent fibre
ADG	:	Average daily gain
ADL	:	Acid detergent lignin
AI	:	Artificial Insemination
AIA	:	Acid insoluble ash
ALP	:	Alkaline phosphatase
ALT	:	Alanine aminotransferase
AOAC	:	Association of official analytical chemists
AR	:	Analytical Grade
ARC	:	Agricultural Research Council
AST	:	Aspartate aminotransferase
ATP	:	Adenosine Triphosphate
AV	:	Artificial Vagina
BHT	:	Butylated Hydroxy Toluene
BW	:	Body weight
Ca	:	Calcium
CAT	:	Catalase
CF	:	Crude fibre

Conc.	:	Concentrate
CP	:	Crude protein
Cu	:	Copper
CuSO4	:	Copper sulphate
DCP	:	Digestible crude protein
DM	:	Dry matter
DMI	:	Dry matter intake
DPPH	:	Diphenyl Picryl Hydrazyl
DUVASU	:	Pandit Deen Dayal Upadhyaya Pashu-Chikitsa Vigyan Vishwavidyalaya Evam Go Anusandhan Sansthan
DW	:	Dry weight/ Distilled water
e.g.	:	For example
EE	:	Ether extract
ELISA	:	Enzyme linked immunosorbant assay
et al	:	*Et alli / alia*
Etc	:	And so forth
FCR	:	Feed conversion ratio
Fe	:	Iron
Fig.	:	Figure
g	:	Gram
g/dl	:	Gram per deciliter
g/ml	:	Gram per milliliter
H_2SO_4	:	Sulphuric acid
Hb	:	Haemoglobin
HCl	:	Hydrochloric acid
$HClO_4$	:	Perchloric acid
HNO_3	:	Nitric acid
HOST	:	Hypo Osmotic Swelling Test
hr (s)	:	Hours
i.e.	:	id est (that is)
ILFC	:	Instructional livestock farm complex
IU	:	International Unit
IU/ L	:	International Unit per liter

kg	:	Kilogram
$kgW^{0.75}$	:	Metabolic body weight
KH_2HPO_4	:	Potassium dihydrogen phosphate
l	:	Litre
LN_2	:	Liquid Nitrogen
LPO	:	Lipid peroxide
MDA	:	Malondialdehyde
meq/l	:	Miliequivalent per litre
mg	:	Milligram
mg/dl	:	Milligram per deciliter
mg/l	:	Milligram per litre
mg/ml	:	Milligram per milliliter
min	:	Minutes
ml	:	Milliliter
Mm	:	Mill mole
MOLP	:	Moringa Oleifera Leaf Powder
mOsm	:	Milliosmole
NDF	:	Neutral detergent fibre
NDS	:	Neutral detergent solution
NFE	:	Nitrogen Free Extract
ng / ml	:	Nanogram per milliliter
NH_3-N	:	Ammonia nitrogen
nm	:	Nanometer
Nmol	:	Nano mole
NRC	:	National research council
NS	:	Non Significant
OD	:	Optical density
OM	:	Organic matter
P	:	Phosphorous
$P\leq 0.01$	:	1% level of significance
$P\leq 0.05$	:	5 % level of significance
PBS	:	Phosphate buffer saline.
PCV	:	Packed cell volume

pH	:	Negative logarithm of Hydrogen ion
ppm	:	Parts per million
RBCs	:	Red blood cells
ROS	:	Reactive Oxygen Species
rpm	:	Revolution per minute.
SE	:	Standard Error
sec (s)	:	Seconds
SEM	:	Standard error of mean
TA	:	Total acid
TBA	:	Thio barbituric acid
TCA	:	Tri-chloral acetic acid
U	:	Unit
U.P	:	Uttar Pradesh
v/v	:	volume by volume
WBC	:	white blood cell

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Date: 11-7-19 **(Anuj Dubey)**

Place: Mathura (U.P.)

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ABSTRACT

The present study was designed to evaluate the effect of replacing protein source in concentrate mixture with dried *Moringa oleifera* leaf powder on growth performance, nutrient utilization, seminal attributes, haematological parameters, blood biochemicals, antioxidant and hormonal parameters of Barbari bucks. For this study, 18 Barbari bucks were selected from the herd maintained at Department of Physiology, DUVASU, Mathura (U.P.). The bucks were divided into 3 groups (Control, T10 and T20) having six animals each on body weight basis. Control group was fed basal diet consisting of concentrate mixture, green berseem and arhar straw where as T10 and T20 groups were fed basal diet (as that of control) along with replacement of soybean meal in concentrate mixture with dried *Moringa oleifera* leaf powder at level 10 and 20% respectively. Nutrient requirement of bucks were fulfilled as per NRC (2007) recommendation of feeding standard. The experimental feeding was intended for 90 days. At the end of experiment, a digestion trial of seven days was conducted to appraise the effect of *Moringa oleifera* supplementation on nutrient utilization. Blood sample were collected at 0, 30, 60 and 90 days interval, respectively. Semen was collected twice a week using artificial vagina from each buck for three weeks, total of six ejaculate from each bucks were collected after 90 days of post feeding of experimental diets. Overall 108 ejaculates were collected. The result revealed no significant ($P>0.05$) difference in the average body weight (kg) and metabolic body weight (kg $W^{0.75}$) between groups. Fortnightly body weight gain (kg) and ADG (g) was found to be significantly higher and comparable in Control and T10 group. Similarly the overall DMI (kg/d, 100 kg BW) was also higher and comparable in Control and T10 group.No significant change in FCR was reported in experimental groups. The supplementation of *Moringa oleifera* had shown no significant ($P>0.05$) difference on nutrient intake and digestibility in treatment groups. Digestible nutrient intake was found similar in the entire experimental groups.CP intake (kg/day), DCP intake (kg/day) and TDN intake (kg/day) remained similar in all experimental groups.DMI (kg/100 kg BW), TDN intake (g/kg $W^{0.75}$) CP and DCP intake (g/kg $W^{0.75}$) remained similar in all experiment groups during digestion trial. Seminal attributes viz. volume of ejaculated semen (ml), semen colour, seminal pH, and spermatozoa concentration (millions/ml) were found similar in all experimental groups. Whereas, mass motility, percent progressive motility, percent live spermatozoa count, HOST reactive spermatozoa percentage and intact acrosomal percentage was found significantly higher ($P< 0.05$) in T10 group and significantly lower ($P<0.05$) in T20 group as compared to Control group. However, total morphological abnormality percentage of spermatozoa in present study was found similar in both Control and *Moringa oleifera* treated groups. The haematological parameters like haemoglobin and PCV was found significantly higher ($P<0.05$) in T20 *Moringa oleifera* supplemented group. The concentrations of plasma glucose, cholesterol, triglycerides, total protein, albumin and globulin did not change significantly ($P> 0.05$) in experimental bucks. Plasma level of enzymes like ALT, AST remain unchanged in Control and treatments group showing no deleterious effect of *Moringa oleifera* supplementation on hepatic metabolism in experimental animal. Overall super oxide dismutase activity (SOD) activity increases significantly ($P<0.05$) in T10 group whereas, plasma lipid peroxidation concentration significantly decreases in T10 groups but there were no significant change in catalase activity in the experimental bucks. Overall plasma cortisol concentration was found significantly lower ($P<0.05$) in T10 *Moringa oleifera* treated group. However, no significant difference in testosterone concentration was found between control and treatment groups. Hence the present results suggested that the soybean meal in concentrate mixture can be effectively replaced with dried *Moringa oleifera* leaf powder at 10 % level without any deleterious effect on blood parameters and hepatic metabolism, with stimulatory effects on their antioxidants status, anti stress and improved seminal attributes of Barbari bucks.

Introduction

CHAPTER-1

INTRODUCTION

Goat production is an important sector of the agro-economy in India. It has served as multipurpose animal that plays a significant role in providing nutrition, supplementary income and livelihood to millions of resource poor farmers and landless labourers of rural India. The rearing of goats is one of the most widely adopted livestock activities in the country. It has the potential to emerge as good source of income and employment for the rural people especially, in the less favoured environment. It possesses several distinct advantages over other livestock because of low capital investment, low input requirements, higher prolificacy, early sexual maturity, wide acceptance of its products and easy marketing. As per 19th livestock census (2012), the goat population in India is 135.17 millions. It contributes 26.40% of the total livestock population of India. The goat sector contributes 14,453 crores to the agriculture economy of the country through meat (6851 crores), milk (4588 crores), skin (648 crores) which account for around 8% of Gross Domestic Product from livestock sector. In addition, it generates about 4% rural employment and about 20 million small and marginal farmers and landless labourers families depend on goat for their livelihood partially or completely (APEDA, 2015-16). In recent years, goat enterprise has also shown promise of its successful commercialization (Kumar et al., 2010).With the increasing consumers concerns on healthier food items, the demand of goat products is increasing owing to their better nutritional profile and health benefits.

Among all farm species, Goats are only widely adapted livestock species that possess inherent capacity to adapt to different physical, nutritional and environmental conditions. They can thrive well on various agro-climatic conditions ranging from arid dry to cold arid to hot humid. They can be efficiently reared in plains, hilly tracts, and sandy zones and at high altitudes on scanty vegetation. Goat farming is prominent in areas with poor irrigation facilities and low agricultural productivity. Goat serves as best alternative source of supplementary income and milk for the resource poor rural farmers, who cannot afford to maintain a cow or a buffalo. Although the goat farming has potential to produce good economic returns, the goat farmers are not able to fetch handsome profit. Most constraining factor in goat production in tropics is

underfeeding, which is mainly assign to limitation of feed in both quantity and quality. In India, basically three feeding system of goats are prevailing *viz*. extensive, semi-intensive and intensive. Extensive system includes migratory, free range, pasture and range grazing. The system is low input based with low level of productivity due to poor nutritional availability. In semi-intensive system, goat are allowed to graze during day time and are supplemented with concentrate mixtures, crop residues, green, dry fodders and tree leaves as per availability. In intensive system, the goats are completely on stalls on cultivated fresh or conserved forages, crop residues and concentrates and are not allowed to graze outside the farm. In conventional extensive system the productivity is severely compromised during the dry season when natural pastures usually dry out and are overgrazed, resulting low content of protein and energy in fodder. It is therefore important to supplement the inadequate available fodder with some amount of concentrate in order to improve intake and digestibility of such poor quality feed resources. However, to untap the full potential of goat husbandry in tropics and for commercial production the rearing of goats should be done under intensive system of production along with adoption of improved technologies and management practices. The demand for feed concentrates, particularly pronounced in developing countries, has been growing steadily over the past decades largely driven by the increasing demand of animal products (Steinfeld and Opio, 2010). However, the high cost and unavailability of the conventional concentrate feed resources has restricted its use under small holder livestock production systems. The rising prices and inadequacy of animal feeds have enforced nutritionists to direct their research to unconventional feed resources, prominently on protein substitutes as it is the most expensive ingredient in animal diet and is always short in supply particularly in developing countries. Thus, searching for alternative unconventional feed sources that may have valuable components of animal diets is indispensable (Melesse, 2012). The use of leguminous multipurpose trees and shrubs has been suggested to be a viable alternative source of proteins, vitamins and minerals for livestock feeding. Among the available forage crops, special focus has been given to the effect of *Moringa oleifera* on livestock growth and production.

Moringa (*Moringa oleifera*) is the most widely cultivated species of a monogeneric family, the Moringaceae (Fahey, 2005). The tree is native of Indian subcontinent and has become naturalized in the tropical and subtropical areas around

the world (Farooq et al., 2012). It is a popular multipurpose legume tree, small, fast growing, evergreen or deciduous tree that usually grows up to 10- 12 m in height. It is drought-resistant tree and can tolerate unfavourable environmental conditions of many developing countries of Asia, Africa, and Latin America. While it grows best in dry sandy or loamy soil that is slightly alkaline (Farooq et al., 2007). It is adaptable to various soil conditions from 4.5 to 8.0 pH, but does not tolerate water logging, freezing or frosts conditions. India is the largest producer of *Moringa oleifera* in the world, with an annual production of 1.1 to 1.3 million tons of tender fruits pods. Average fresh green leaf yield is 6 tons/ha/year (Sagbo, 2006). Andhra Pradesh leads in both area of plantation and production of drumstick, followed by Karnataka and Tamil Nadu. It is well known to have nutritional and pharmacological properties (Soliva et al., 2005). Many parts of *Moringa oleifera* tree were found to have various industrial and medicinal applications thus it is known as " tree of life" or the miracle tree (Soliva et al., 2005; FAO, 2014). In many tropical and subtropical countries, various part of *Moringa oleifera* (leaves, fruit, immature parts and flower) are incorporated into the traditional food of humans (Anhwange et al., 2004). A wide variety of nutritional and medicinal virtue have been attribute to its roots, bark, leaves, flower, fruit and seeds (Anwar and Bhanger, 2003; Kumar et al., 2010).

Among its all parts, the leaves had attracted the attention of ruminant's nutritionists as source of protein, due to optimal balance composition of their amino acid, and high digestible protein content (Babiker et al., 2017). It is considered as alternative to soybean meal and rapeseed meal as protein sources for ruminants (Soliva et al., 2005). Leaves were used as a protein supplement and found to improve the growth performance of growing lamb, milk yield and composition of sheep and goats (Babiker et al., 2017). The phytochemicals analysis have shown that its leaves are particularly rich in Ca, P, K, iron, vitamin A, vitamin D, essential amino acid as well as such known antioxidants like beta carotene, vitamin C and flavonoids (Aslam et al., 2005). The antioxidant effect of *Moringa oleifera* leaf extract and fruit in terms of the presence of polyphenols, tannins, anthocyanin, glycosides and thio carbamates, which remove free radicals, activate antioxidant enzyme and inhibit oxidases (Luqman et al., 2012).

Various phytochemicals with high potency as fertility, cardio tonics, anti cancerous, antianthelmintic, antitubercular, antispasmodic, abortifacient, nantilithic, anti inflammatory and antimicrobial properties are present in *Moringa oleifera* leaves

as well as in other part of *Moringa oleifera* tree (Sholapur and Patil, 2013). These phytochemicals includes saponins, terpenoids, tannins, quercetin, kaempferol, sterols, anthraquinone, glucosinolates, isothiocyanates.

The leaves of *Moringa oleifera* have high nutritive value and have a potential to be used as a protein supplement. They are readily consumed by cattle, sheep, goats, pigs, rabbits, fish and chicken (Moyo et al., 2012). Additionally, the leaves have low or insignificant levels of anti-nutritive factors such as phenols, saponins, trypsin and amylase inhibitors, lectins, cyanogenic glycosides and glucosinolates (Makkar and Becker, 1997; Ben Salem et al., 2004). Literature is available on the use of *Moringa oleifera* leaves in different animal species, indicating improvement in the growth, digestibility, feed intake, seminal attributes, haematology, blood biochemicals, antioxidant and hormonal status. However, research on use of dried *Moringa oleifera* leaf powder as replacement of protein source on the performance of Barbari goat buck is meagre. Taking into account the nutritional profile of *Moringa oleifera* leaf powder, the present study is designed to evaluate the effect of replacing the conventional protein source i.e. soybean meal in concentrate mixture with dried *Moringa oleifera* leaf powder on the performance of Barbari bucks with following objectives:

OBJECTIVES

1) To study the effect of replacing the conventional protein source with dried *Moringa oleifera* leaf powder on feed intake and nutrient utilization of Barbari bucks.
2) To study the effect of incorporating the dried *Moringa oleifera* leaf powder on semen quality of Barbari bucks.
3) To assess the effect of incorporating the dried *Moringa oleifera* leaf powder on haemato-biochemicals, antioxidant and hormonal status of Barbari bucks.

Review of Literature

CHAPTER-2

REVIEW OF LITERATURE

In current scenario, it has become imperative to develop cheap and readily available alternative feeding resources to support livestock growth. Leaf protein sources obtained in leaf vegetables, legume trees, browse plants, fodder trees and shrubs as feed resources to all classes of livestock offer tremendous potentials and are receiving increasing attention worldwide. *Moringa oleifera* is a multi-purpose, legume tree serve as a source of leaf protein concentrate for ruminants. A wide literature is available on its functional and physico-chemical properties, its effect on performances of various livestock species. However, studies are scanty on its effect on performance of bucks. In view of this, present experiment was undertaken to evaluate the effect of replacing the conventional protein source in concentrate mixture with dried *Moringa oleifera* leaf powder on the performance of Barbari bucks. Hence latest research work has been searched upon and efforts have been made to discuss various aspects of it in the present review. Therefore, references pertaining mainly to the effect of feeding of feed intake, nutrient digestibility, rumen fermentation profile, haemato-biochemicals, and antioxidant and semen quality are reviewed and presented.

2.1 *Moringa oleifera*

Moringa oleifera is the only genus in the family *Moringa*ceae and *Moringa oleifera* is the most extensively studied and cultivated species. The taxonomic classification is presented in Table 2.1

Table 2.1 Taxonomic classification of *Moringa oleifera*

Kingdom	**Plantae (Plants)**
Super kingdom	Traceobionta (Vascular plants)
Super division	Spermatophyta (Seed plants)
Division	Magnoliophyta (Flowering plants)
Class	Magnolipsida (Dicotyledonous)
Sub class	Dilleniidae
Order	Capparales
Family	Moringaceae (Horse- radish tree family)
Genus	Moringa
Species	*Moringa oleifera*

Table 2.2 Common name of *Moringa oleifera* in different parts of India

Region	Common Name
Hindi/ Uttar Pradesh	Sahijna,Sojna,Sujana
Bengali	Sajna, Sojna, Sujana,
Gujarat	Suragavo, Midho-saragavo
Orissa	Sanjna, Saijna, Shajna,
Kannada	Nuggekai, Nuggekodu
Konkani/Goa	Moosing, Mosing
Malayalam	Sigru, Moringa, Muringa,
Marathi	Sujna, Shevga, Shivga
Punjab	Sejan
Oriya	Munigha, Sajina
Rajasthan	Lal Sahinjano
Sanskrit	Shobhanjan, Shobanjana,
Tamil	Murungai, Morunga
Telegu	Tella-Munaga, Mulaga,

Moringa oleifera is widespread, multipurpose, perennial evergreen tree of 2.5- 10 m in height (Vlahof et al., 2002). It is native of the western and sub-Himalayan tracts, India, Pakistan, Asia Minor, Africa and Arabia (Somali et al., 1984). It is originated in India, but has become naturalized in many locations worldwide in the tropics and sub-tropic (Mendieta-Araica et al., 2011). It is known by different names in different part of country. Some common names of *Moringa oleifera* in different parts of India are presented in Table 2.2. It is a rapidly growing tree, which is easy to establish and cultivate. It is propagated either by planting stem cuttings or by seeding (Aregheore, 2002). It has a capacity to produce high quantities of fresh biomass up to 120 tons dry matter (DM)/ha/year even at high planting densities. The yield potential of *Moringa oleifera* makes it ideal for forage production. It is drought tolerant and tolerated annual precipitation of 500 to 1500 mm and annual temperatures from 18.7 to 28.5˚C. Although it grows best in sandy soils, it is adapted to a wide range of soil types and conditions, preferring neutral to slightly acidic soil (pH range of 5.0 to 6.5).

2.2 Nutrient composition of *Moringa oleifera*

Moringa oleifera seeds and leaves have almost all essential nutrients in adequate amounts for maintenance and production (Anwar et al., 2005). Dried *Moringa oleifera* leaves may provide macro- and micronutrient to boost the nutritive value of feed. Every part of *Moringa oleifera* is store house of important nutrients. *Moringa oleifera* leaves contain 21.8% CP, 22.8%, acid detergent fibre (ADF), 30.8 % neutral detergent fibre (NDF), 412.0 g/kg of crude fat, 212.2 g/kg of carbohydrates and 44.3g/kg of ash (Sanchez et al., 2006). Nutrient composition and digestibility of morphological parts of *Moringa oleifera* are presented in Table 2.3. The leaves of *Moringa oleifera* are rich in mineral like calcium, potassium, zinc, magnesium, iron, and copper. It provide 7 times more vitamin C than orange, 10 times more calcium than milk, 9 times more protein than yoghurt, 15 time more potassium than banana and 25 time iron than spinach (Rockwood et al., 2013).Vitamins like beta carotene of vitamin A, Vitamin B such as folic acid, pyridoxine and nicotinic acid, Vitamin C, Vitamin D and vitamin E are also present in *Moringa oleifera*.The tree contains high crude protein (CP) in the leaves (251 g/kg DM) and negligible content of tannins(0.89mg GAE/g DM) and other anti-nutritive compounds (Nouala et al., 2006). In raw and extracted *Moringa oleifera* leaves CP are 47% higher than those of common forage and grass consumed by livestock (Soliva et al., 2005). Its leaves contain all of the essential amino acid, which are building blocks of protein. Amino acid composition of different morphological parts of *Moringa oleifera* are presented in Table 2.4.The nutrient compositions of leaves, leaf powder, seeds and pods of *Moringa oleifera* are presented in Table 2.5. The seeds contain high amount of CP, followed by flowers and leaves. Because of adequate amount of protein *Moringa oleifera* can serve as an alternative source of protein to ruminants and non-ruminants livestock.

Table 2.3 Nutrient composition and digestibility of morphological parts of *Moringa oleifera*

Plant parts	DM	Ash	CP	EE	CF	Digestibility
Seeds, g/kg	950.0	34.8	391.7	388.0	48.0	--
Flowers, g/kg	892.5	112.1	314.8	68.0	170.0	--
Pods, g/kg	940.0	97.1	71.2	20.0	490.0	430.7
Leaves, g/kg	930.0	138.9	267.9	64.0	210.0	790.5
Stems, g/kg	940.0	101.1	112.3	32.0	430.0	521.7
Whole plant, g/kg	914.0	123.7	200.0	24.0	270.0	760.9

(Mabruk et al., 2010)

Table 2.4 Amino acid composition of different morphological parts of *Moringa oleifera*

Amino acid contents (per 100g)	Pods	Fresh leaves	Dried leaf powder
Arginine (mg)	360	406.8	1325
Histidine (mg)	110	149.8	613
Lysine (mg)	150	342.4	1325
Tryptophan (mg)	80	107	427
Phenylalanine (mg)	40	310.3	1388
Methionine (mg)	140	117.7	350
Threonine(mg)	390	117.7	1188
Leucine(mg)	650	492.2	1950
Isoleucine (mg)	440	299.6	825
Valine (mg)	540	374.5	1063

(Dhakar et al., 2011)

Table 2.5 Nutrient compositions of leaves, leaf powder, seeds and pods of *Moringa oleifera*

Nutrients	Fresh leaves (per 100g)	Dry leaves (per 100g)	Leaf powder (per 100g)	Seed (per 100g)	Pods (per 100g)
Calories (cal)	92	329	205	-	26
Protein (g)	6.7	29.4	27.1	35.97	2.5
Fat (g)	1.7	5.2	2.3	38.67	0.1
Carbohydrates (g)	12.5	41.2	38.2	8.67	3.7
Fibre (g)	0.9	12.5	19.2	2.87	4.8
Vitamin B1(mg)	0.06	2.02	2.64	0.05	0.05
Vitamin B2 (mg)	0.05	21.3	20.5	0.06	0.07
Vitamin B3 (mg)	0.8	7.6	8.2	0.2	0.2
Vitamin C (mg)	220	15.8	17.3	4.5	120
Vitamin E (mg)	448	10.8	113	751.67	-
Calcium (mg)	440	2185	2003	45	30
Magnesium (mg)	42	448	368	635	24
Phosphorus (mg)	70	252	204	75	110
Potassium (mg)	259	1236	1324	-	259
Copper (mg)	0.07	0.49	0.57	5.20	3.1
Iron (mg)	0.85	25.6	28.2	-	5.3
Sulphur (mg)	-	-	870	0.05	137

(Dhakar et al., 2011)

2.3 Common medicinal uses of different parts morphological parts of *Moringa oleifera*

According to India's ancient tradition of ayurveda, the leaves of the *Moringa oleifera* tree prevent 300 diseases. Number of medicinal properties has been attributed to various parts of this highly valued tree. Almost all the parts of this plant: root, bark, gum, leaf, fruit (pods), flowers, seed and seed oil have been used for various ailments in the indigenous medicine of South Asia, including the treatment of inflammation and infectious diseases along with cardiovascular, gastrointestinal, hematological and hepato renal disorders (The Wealth of India, 1962; Becker, 2003; Singh et al., 2009). Some common medicinal uses of different morphological parts of *Moringa oleifera* are presented in Table 2.6.

Table 2.6 Some common medicinal uses of different parts of *Moringa oleifera*

Plant parts	Medicinal uses	References
Root	Antilithic, rubefacient, vocicent, carminative, antifertility, anti-inflammatory, stimulant in paralytic affections, act as a cardiac/circulatory tonic, used as a laxative, abortifacient, treating rheumatism, articular pains, lower back or kidney pain and constipation.	The Wealth of India (1962), Dahot (1988), Rukmani et al.(1998)
Leaves	Purgative, rubbed on the temples for headaches, used for piles, fevers, sore throat, bronchitis, eye and ear infections, scurvy and catarrhal; leaf juice is believed to control glucose levels, applied to reduce glandular swelling.	Fuglie (2001), The Wealth of India (1962), Dahot (1988)
Stem bark	Rubefacient, vesicant and used to cure eye diseases and for the treatment of delirious patients, prevent enlargement of the spleen and to destroy the tumor and to heal the ulcers, The juice from the root bark is put into ears to relive earaches and also place in a tooth cavity as a pain killer, and has anti-tuberculosis activity.	Bhatnagar et al. (1961), Becker (2003)
Gum	Used for dental caries, and as astringent and rubefacient; Gum, mixed with sesame oil, is used to relive headaches, fevers, intestinal complaints, dysentery, asthma and sometimes used as an abortifacient, and to treat syphilis and rheumatism.	Fuglie (2001)
Flower	High medicinal value as a stimulant, aphrodisiac, abortifacient, cholagogue, used to cure inflammations, muscle diseases, hysteria, tumor, and enlargement of spleen; lower the serum cholesterol, phospholipids,	Nair andSubramanian (1962), Bhattacharya et al. (1982), Dahot (1988), Siddhuraju and Becker (2003),

	triglyceride, VLDL, low-density lipoprotein (LDL), cholesterol to phospholipids ratio and aorta in hypercholesterolemic rabbits and increase the excretion of faecal cholesterol.	Mehta et al. (2003)
Seed	Seed extract exerts its protective effect by decreasing liver lipid peroxides, antihypertensive compounds thiocarbamate and isothiocyanates glycosides have been isolated from the acetate phase of the ethanolic extract of *Moringa oleifera* pods.	Faizi et al. (1998)

2.4 Bioactive constituents in different parts of *Moringa oleifera* plant

Various bioactive constituents are present in *Moringa oleifera* leaves as well as in other part of *Moringa oleifera* tree including saponins, terpenoids, tannins, quercetin, kaempferol, sterols, anthraquinone, glucosinolates, isothiocyanates, glycoside compounds and glycerol-1-9-octadecanoate etc (Eldesoky et al., 2017). It is rich in compounds containing the simple sugar, rhamanose and a fairly unique group of compounds called glucosinolates and isothiocyanates (Fahey et al., 2001; Bennett et al., 2003). The bioactive components present in different parts of *Moringa oleifera* are presented in a Table 2.7.

2.4.1 Leaf

Moringa oleifera leaves have been reported to be a rich source of β-carotene, protein, vitamin C, calcium and potassium and act as a good source of natural antioxidants compounds such as ascorbic acid, flavonoids, phenolics and carotenoids (Becker, 2003). The high concentrations of ascorbic acid, estrogenic substances and β-sitosterol, iron, calcium, phosphorus, copper, vitamins A, B and C, α-tocopherol, riboflavin, nicotinic acid, folic acid, pyridoxine, β-carotene, protein, and in particular essential amino acids such as methionine, cystine, tryptophan and lysine present in *Moringa oleifera* leaves and pods make it a virtually ideal dietary supplement.

2.4.2 Flowers

Flowers contain nine amino acids, sucrose, D-glucose, traces of alkaloids, wax, quercetin and kaempferol, the ash is rich in potassium and calcium (Rukmani et

al., 1998). They have also been reported to contain some flavonoids pigments such as alkaloids, kaemferol, rhamnetin, isoquercitrin and kaempferitrin (Becker, 2003).

2.4.3 Pods

Antihypertensive compounds such as thiocarbamate and isothiocyanates glycosides have been isolated from the acetate phase of the ethanol extract of *Moringa oleifera* pods (Faizi et al., 1998). The cytokines have been shown to be present in the fruit (Nagar et al., 1982).

2.4.4 Seeds

The seed oil mainly consists of groups of sterols such as campesterol, stigmasterol, β-sitosterol, 5-avenasterol and cleroster accompanied by minute amounts of 24-methylenecholesterol, 7-campestanol, stigmastanol and 28-isoavenasterol (Tsaknis J.1998; Anwar et al., 2005). *Moringa oleifera* seed is also a good source of different tocopherol (α-, γ and δ); the concentration of those is reported to be 98.82–134.42, 27.90–93.70, and 48.00–71.16 mg/kg (Anwar et al.,2005).

2.7 Bioactive constituents in different morphological parts of *Moringa oleifera* plant

Moringa part	Bioactive constituents
Leaves	9,12,15-Octadecatrienoic acid, Rhamanose, Pterygospermin, Isothiocyanates, 4-(4'-O-acetyl-a-Lrhamnopyranosyloxy) benzyl Isothiocyanate, Glycosides niazirin, niazirinin, 4-[4'-O-acetyl- α -L- rhamnosyloxy) benzyl] isothiocyanate, niaziminin, vitamins (A, B and E, ascorbic acid), Folates, 2, 6-dihexadecanoate, tetraacetyl-D-xylonic nitrile, phytol and isobenzofuran-1-one 3-acetic acid, flavonol glycosides, quercetin (kaempferol), Amino acids (Arginine, Histidine, Lysine, Tryptophan, Phenylalanine, Methionine Threonine, Leucine, Isoleucine, Valine), Oxalic acid, and minerals (Ca, Mg, Fe Cu, P, S), o-3 and o-6 PUFA

Seeds	Riseofulvin, dechlorogriseofulvin, 8-dihydroramulosin and mullein, Crude protein, Crude fat, carbohydrate, methionine, cysteine, 4-(α-L- rhamnopyranosyloxy) benzylglucosinolate, moringyne, mono-palmitic, di-oleic triglyceride, folates, amino acids (Arginine, Histidine, Lysine, Tryptophan, Phenylanaline, Methionine Threonine, Leucine, Isoleucine, Valine), Oxalic acid, minerals (Fe, Ca, Cu, Mg, P, S), linoleic acid, linolenic acid and oleic acid.
Roots	4- (α- L- rhamnopyranosyloxy) - benzylglucosinolate and benzylglucosinolate, glucotropaeolin, Folates
Flowers	D-mannose, D-glucose, protein, ascorbic acid, polysaccharide, Carotenoids (all-E-luteoxanthin, 13-Z-lutein, all-E-zeaxanthin,15-Z-b- carotene), o-3 and o-6 PUFA
Pods	Nitriles, isothiocyanate, thiocarbanates, 0-[2'-hydroxy-3'-(2''-heptenyloxy)] propylundecanoate, carbamate, methyl-p-hydroxybenzoate and β-sitosterol, Carotenoids (all-E-luteoxanthin, 13-Z -lutein, all-E-zeaxanthin, and 15-Z-b-carotene), Omega-3 and omega-6 polyunsaturated fatty acids
Stem	4-hydroxymellein, vanillin, β-sitosterone, octacosanic acid and β- sitosterol

(Saini et al., 2016)

Makkar and Becker, (1996) investigated nutritional value of whole and ethanol extracted *Moringa oleifera* leaves. They found that crude protein contents of the extracted and unextracted leaves were 43.5% and 25.1%, respectively while the true protein contents of these leaves were 93.8% and 81.3% of the total crude protein and non protein nitrogen contents of 2.7% and 4.7% in extracted and unextracted leaves, respectively. The protein insoluble in acid-detergent fibre (ADIP; protein unavailable to animals) was 13.2% and 9.8% in ADF of the extracted and unextracted leaves respectively (absolute values of 22 g and 11 g ADIP/kg leaves).All essential amino acids including sulphur- containing amino acids were higher than adequate concentration when compared with recommended amino acid pattern of FAO/WHO/UNO. Sarwatt et al. (2002) studied the substitution of sunflower seed-cake (SSC) with *Moringa oleifera* leaves as a supplemental feed to growing goats in

Tanzania. Their study revealed that, chemical composition of *Moringa oleifera* was DM 933 g/kg, CP 253 g/kg, NDF 233 g/kg, Ca 29.1 g/kg and P 3.0 g/kg. Richter et al. (2003) conducted the study to evaluate the nutritional quality of *Moringa oleifera* leaves as an alternative protein source for Nile tilapia. The CP, EE, Ash, NDF and ADF content of *Moringa oleifera* leaf meal as 25%, 10.6%, 8.4%, 15.9% and 12.6% respectively.

Sanchez et al. (2006) determined the chemical composition of *Moringa oleifera* forage in the proportion of DM 164 g/kg, CP 178 g/kg DM, NDF 506 g/kg DM, ADF 376 g/kg DM and Ash 107.6 g/kg DM. Asaolu et al. (2010) studied the results of chemical composition of *Moringa oleifera* leaves as DM 25%, CP 22.2%, EE 6.68%, NFE 41.3%, Ash 13.2%, NDF 28.0%, ADF 28.9%, K 1.26%, Na 0.28%, Ca 1.97%and P0.13% on DM basis.

Kakengi et al. (2007) studied the possibility of using *M*oringa *oleifera* as a ruminant protein supplement by comparison between nutritive and anti-nutritive value of its different morphological parts with that of conventionally used *Leucaena leucocephala* leaf meal (LL). They found that, crude protein content ranged from 265-308 g/kg DM in *Moringa oleifera* leaves (MOL) and seed cake (MOC) respectively. Between the morphological parts of *Moringa oleifera* the seed cake (MOC) had substantially higher CP content followed by leaves (MOL), leaves and soft twigs (MOLST), soft twigs (MOST) and back (MOB) which exhibited the lowest CP content. More or less the reverse trend was true for the fibre fractions. Between species *Moringa oleifera* cake (MOC) and leaves (MOL) had higher CP values than *Leucaena leucocephala.* It was concluded from this study that the high crude protein content in MOL and MOLST could be well utilized by ruminant animals.

Asaolu et al. (2011) studied the chemical composition of *Moringa oleifera* fodder (MO) that shows DM 95.57%, CP 26.74%, EE 8.06%, CF 11.03%, NDF 26.35%, ADF 40.40%, OM 89.83%, NFE 39.53%, calcium 1.10g/100g, phosphorus 0.43g/100g, sodium 0.20 g/100g, potassium 0.20 g/100g, magnesium 0.20 g/100g, iron 281.00 mg/kg, manganese 80.0 mg/kg, Cu 7.0 mg/kg and Zn 29.0 mg/kg.

Divya et al. (2014) investigated the chemical composition of *Moringa oleifera* leaves and revealed that it contained crude protein (24.01%), crude fibre (9.15%), ash (14.11%), crude fat (2.3%), calcium (1.97%) and phosphorus (0.65%).

Tona et al. (2014) studied the chemical composition of *Moringa oleifera* Leaf Meal that shows DM 90.46%, CP 18.38%, CF 14.04%, EE 14.58%, Ash 8.38%, OM 91.62%, NFE 44.71%, NDF25.68%, ADF 14.78%, ADL 7.11%, hemicelluloses 10.90% and cellulose 6.67%.

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

Asaulo et al. (2010) investigated the performance of grazing West African dwarf goats on *Moringa oleifera* multi nutrient block supplementation. The performance of WAD goats on *Moringa oleifera* multi nutrient block (MMNB) supplementation was assessed relative to cassava peels (CPL) and corn starch residues (CSR) using 4 replicates per treatment. Performance indices were assessed on the basis of supplement intake, experimental animal's weight and haematological changes. The results of this study determined that, only MMNB supplementation resulted in a significant ($P<0.05$) increased in PCV at the end of the study although, all values were within the range considered normal for clinically-healthy WAD goats. Each of the 3 supplements resulted in significant ($P<0.05$) increase in Hb and RBC, although the magnitudes of the increased Hb and RBC were most pronounced with MMNB. Animals on CSR maintained relatively comparable level of WBC at both the commencement and end of the study. However, CPL supplementation resulted in higher ($P<0.05$) WBC value at end of study whereas, MMNB supplementation resulted in corresponding lower ($P<0.05$) values. Hence, adoption of the MMNB feeding technology by small ruminant keeper could be a panacea to the nutritional and health hardship faced by the animals during the usually long dry season.

Divya et al. (2014) evaluated the effect of dietary *Moringa oleifera* leaves powder on growth performance, blood chemistry, meat quality and gut micro flora of broiler chicks. The experiment was conducted using 42 day old broiler chicks. The six dietary treatments included corn-soybean meal based basal diet (control), basal diet supplemented with 20 mg/kg antibiotic; and four levels of *Moringa oleifera* leaves powder (0.5%, 1.0%, 1.5% and 2.0%). Each treatment diet was fed *ad libitum* to seven replicated groups of 10 chicks up to 42 day of age. At the end of feeding trial, seven birds from each group were randomly selected for collection of blood and analysis of serum biochemical profile. The result revealed that, the dietary inclusion of MOL powder in broiler ration significantly ($P<0.05$) decreased the serum total protein, triglycerides, cholesterol, albumin, uric acid and creatinine. Inclusion of

MOL up to 1.5% reduced ALT and AST activities. Additionally, *Moringa oleifera* leaves also had hyper cholesterolemic effect.

Babeker and Abdalbagi, (2015) conducted the study to investigate the effects of feeding different levels of *Moringa oleifera* leaves on haematological, biochemical parameters of Sudan Nudian goats on three different levels of *Moringa oleifera*, group A (0%) as control, group B offered (20%) and group C (50%) fed different levels of *Moringa oleifera* leaves. Thirty yearling females of Nubian goats weighted between 16.00-24.00 kg and their age was nearly 10-12 months were used in this study, the animals were divided according to their live body weight into three groups of ten each. Forage was fed at rate of 1% of live body weight. They were fed for 6 weeks. The erythrocytes indices showed significant variations among the groups except Mean Corpuscular Haemoglobin Concentration (MCHC) in group B, which recorded high significant ($P<0.05$) in all indices of erythrocytes when compared with the other two groups. On other hand leukocytes indices have similar observations for all parameters except total white blood cells count (WBCs) which increased significantly ($P<0.05$) in group B (6.21±0.14) than group C (4.77±0.34) and group A (4 .21±0.09). Glucose decreased a significantly ($P<0.05$) in group B when compared with other two groups, while Total protein and Albumin were significantly ($P<0.05$) higher in group B. Therefore, the study revealed that the *Moringa oleifera* leaf meal could be used to improve livestock system of small ruminants without any adverse effect on blood indices at the 20% diet inclusion level.

Kholif et al. (2015) studied the inclusion of *Moringa oleifera* leaf meal (MLM) in lactating Anglo-Nubian goats. The result showed significant increase ($P < 0.05$) in blood glutamic-pyruvic transaminase and cholesterol concentration while, decrease ($P < 0.05$) urea-N in goats fed MLM at the rate of 15% in diet. Kholif et al. (2016) studied the effect of feeding diets with processed *Moringa oleifera* meal as protein source in lactating Anglo-Nubian goats. They observed that, goats fed *Moringa oleifera* diets had decreased blood serum creatinine ($P=0.003$), triglycerides ($P<0.001$) and cholesterol ($P<0.001$) concentrations. Similarly, GPT concentration increased ($P=0.028$) in goats fed *Moringa oleifera* silage and hay compared with the control diet. In addition, lower blood urea-N and cholesterol ($P< 0.01$) were noted for *Moringa* oleifera silage and hay compared with control diet.

Damor et al. (2017) evaluated the effect of replacing concentrate mixture at three levels on 0, 50, and 100 % with *M*oringa *oleifera* leaves on blood biochemical profile of Mehsana goat kids. The serum total protein, albumin, SGOT and Ca levels were found to be significantly ($P<0.05$) higher in *Moringa oleifera* fed group as compared to control group. The enzyme SGPT concentrations were found to be similar ($P>0.05$) among the treatment groups. It was concluded that *Moringa oleifera* leaves are rich in protein and minerals and replacing the concentrate mixture with *Moringa oleifera* leaves in diet of growing Mehsana goat kids increased the concentrations of blood total protein, albumin, SGOT and calcium while decreased level of blood cholesterol.

Jiwuba et al. (2017) studied the effect of *M*oringa *oleifera* leaf meal (MOLM) on haematology of West African dwarf goats does. Four diets T1, T2, T3 and T4 were formulated containing MOLM at 0%, 5%, 10% and 15%, respectively. Results showed that PCV, RBC, MCV and WBC differed ($P<0.05$) significantly. Packed cell volume (29.50-32.75%) was improved ($P<0.05$) by MOLM supplementation at 15% inclusion level. White blood cell counts for goats in treatment groups were significantly ($P<0.05$) higher and better than the control. Incorporation of 15% MOLM in diets of WAD does enhance their haematological profile. The study concluded that MOLM supplementation level at 15% was recommended for optimum haematological profile for West African Dwarf goats.

Ajugwo et al. (2017) studied the haematinic effect of *Moringa oleifera* in Madonna University Elle Campus Rivers State. A total of 15 rats were divided into three groups. Phenyl hydrazine was used to induce the anaemia in all the groups, the extract was prepared and administered orally using oral gavage. Group A served as control while groups B and C served as test group and were administered with 200 mg per body weight of *Moringa oleifera* leaf extract and 300 mg per body weight of same extract respectively. Blood samples were collected through ocular puncture after 28 days and analyzed for haematological parameter. Results showed that there was significant ($P<0.05$) increase in red blood cell count, haemoglobin count, packed cell volume and white blood cell count.

2.6 Effect of *Moringa oleifera* on antioxidant profile

Moyo et al. (2012) studied polyphenolics content and antioxidant properties of *Moringa oleifera* leaf extracts and enzymatic activity of liver from goats

supplemented with *Moringa oleifera* leaves/sunflower seed cake. The study investigated antioxidant potency of *Moringa oleifera* leaves in different in-vitro systems using standard phyto-chemical methods. The antioxidative effect on the activities of superoxide dismutase (SOD), catalase (CAT), lipid per oxidation (LPO) and reduced glutathione (GSH) were investigated in goats supplemented with *Moringa oleifera* (MOL) or sunflower seed cake (SC).The acetone extract had higher concentrations of total flavonoids (295.01±1.89 QE/g) followed by flavonols (132.74±0.83 QE/g), Phenolic (120.33±0.76 TE/g) and then proanthocyanidins (32.59±0.50 CE/g) than the aqueous extract. The reducing power of both solvent extracts showed strong antioxidant activity in a concentration dependent manner. The acetones extract depicted higher percentage inhibition against DPPH, ABTS and nitric oxide radicals which were comparable with reference standard antioxidants (vitamin C and BHT). MOL increased the antioxidant activity of GSH (186%), SOD (97.8%) and catalase (0.177%). Lipid per oxidation was significantly reduced by MOL. The study suggests that *Moringa oleifera* could be a potential source of compounds with strong antioxidant potential.

Qwele et al. (2012) studied the chemical composition, fatty acid content and antioxidant potential of meat from goats supplemented with *Moringa oleifera* leaves, sunflower cake and grass hay. They determined the chemical composition, fatty acid (FA) content and antioxidant capacity of meat from goats supplemented with *Moringa oleifera* leaves (MOL) or sunflower cake (SC) or grass hay(GH).The meat from goat supplemented with MOL had higher concentrations of total phenolic content (10.62±0.27 mg tannic acid equivalent E/g).The MOL significantly scavenged 2,2-azino-bis-3-ethyl-benzothiazoline-6-sulfonic-acid (ABTS) radical to 93.51±0.19% (93.51±0.19%) and 1,1-diphenyl-2-picrylhydrazyl (DPPH) radical to 58.95± 0.3% than other supplements. The anti-oxidative effect of MOL supplemented meat on catalase (CAT), reduced glutathione (GSH), superoxide dismutase (SOD) and lipid oxidation (LO) was significantly ($P< 0.05$) higher than other meat from goat feed on grass hay or those supplemented with sunflower seed cake. The study indicated that the anti-oxidative potential of MOL may play a role in improving meat quality parameters such as chemical composition, colour and lipid stability.

Ojo and Adetoyl (2017) studied the antioxidant effect of *Moringa oleifera* leaf extract (MOLE). They took twenty four mixed- breed rabbits, having an average

weight of 700g. They were randomly divided into four equal treatments (6 rabbits each) and provide various concentrations of MOLE. Treatment 1 (control) was given 0ml MOLE/kg body weight, treatment 2 (30 ml MOLE/kg body weight), treatment 3 (60 ml MOLE/kg body weight) and treatment 4 (90 ml MOLE/kg body weight). Results showed that *Moringa oleifera* leaf extract at all doses produced significant ($P<0.05$) increase in the serum parameters across the treatments. The total antioxidant capacity (TAC) value of rabbits increased consistently with increased MOLE concentration, while MDA values were not significantly influenced across the treatments. It can be concluded that (MOLE) can be used at 90ml MOLE/kg body weight to reduce lipid per oxidation and enhance oxidative status of rabbits in a semi-humid environment.

2.7 Effect of *Moringa oleifera* on the performance of animal

Sarwatt et al. (2000) studied the substitution of sunflower seed-cake (SSC) with *Moringa oleifera* leaves as a supplemental feed in East African goat. They substituted sunflower seed-cake with *Moringa oleifera* leaves (MOL) at 0%, 25%, 75% and 100% level and all animals were fed with low-quality *Chloris gayana* hay as a basal ration. Their study revealed significant increase in DMI and metabolizable energy intake (MEI) at 75 and 100% MOL supplementary levels along with increase in digestibility of DM (dry matter) and NDF (neutral detergent fibre) as levels of substitution of SSC with MOL increased. The results suggest that MOL could be used as a substitute for sunflower seed-cake with optimum digestibility.

Aregheore (2002) investigated intake and digestibility of *Moringa oleifera*–batiki grass mixtures on Anglo Nubian growing goats. Four dietary treatments were taken i.e. batiki grass alone (M0) as control, and three levels of *Moringa oleifera* leaves at 20% (M20), 50% (M50) and 80% (M80) of the total daily forage allowance. The DMI of the goats on M0, M20, M50 and M80 diets were observed to be 50.6, 50.9, 51.0 and 46.8 g/(W0.75kg), respectively. The DMI was significantly lower ($P < 0.05$) in the goats on M80 diet. The goats on M20 and M50 diets had higher live-weight gain, and higher digestibility of DM, crude protein (CP), neutral detergent fibre (NDF), DM and energy than the goats on M0 and M80. The result suggested that, *Moringa oleifera* at 20% and 50% levels of total daily forage allowance could be used as a cheap protein supplement in batiki grass based diets for goats and improvement in status of digestibility, feed intake and live weight.

Sanchez et al. (2005) evaluated the effect of different levels of foliage of *Moringa oleifera* to Creole dairy cows on intake, digestibility, milk production and consumption. Six *Bos indicus* cows of Creole reyne breed with a mean body weight of 394 ± 24 kg were used in a replicate of 3×3 latin square des ign. The animals were fed with *Brechiara brizantha* hay *ad-lib* with or without supplementation of *Moringa oleifera lam*. *Moringa oleifera lam* is given @ 2-3 kg to the cows. Their study revealed that, the total dry matter intake of cows supplemented with 2 kg DM and 3 kg DM of *Moringa oleifera* to *B. brizantha* hay based diet had significantly ($P < 0.05$) higher intake than the un supplemented cows.

Moyo et al. (2012) studied the effect of supplementing crossbred Xhosa lop-eared goat castrates with *Moringa oleifera* leaves on growth performance, carcass and non-carcass characteristics. A total of 24 castrated goats aged 8 months, with a mean initial weight of 15.1± 2.3 kg, were randomly divided into three diet groups with eight goats in each. The duration of the trial was 60 days. All goats received a basal diet of grass hay (GH) *ad-libitum* and wheat bran (200 g/day each). The MOL and sunflower cake (SC) groups were fed additional 200 g of dried *Moringa oleifera* leaves and 170 g of SC respectively. The third group (GH) did not receive any additional ration. The study showed that, there was significant increase in feed intake ($P<0.05$) in animals fed with *Moringa oleifera* leaves (MOL) and sunflower cake (SC) as compared to animals fed on grass hay (GH). The growth performance of SC and MOL goats were not different. Feeding MOL or SC improved the growth performance of goats in an almost similar way, which indicated that, *Moringa oleifera* could be used as an alternative protein supplement in goats.

Tona et al. (2014) studied the growth performance and nutrient digestibility of West African Dwarf (WAD) goats fed graded levels of *Moringa oleifera* leaf meal. A sixty one days feeding trial was conducted to determine the total feed intake, live weight gain and digestibility coefficients of WAD goats (bucks) fed basal diet of *Panicum maximum* (guinea grass) and concentrate diet containing *Moringa oleifera* leaf meal (MOLM) at 0%, 5%, 10% and 15% levels. Feed intake, live weight gain and digestibility coefficients were determined. The results showed that, the goats fed the concentrate diet with 15% level of inclusion of MOLM had significantly higher ($P<0.05$) rate of growth and digestibility coefficients than those on the 5% and 10% inclusion levels.

Sultana et al. (2015) studied the feeding value of *Moringa oleifera* foliage as replacement of conventional concentrate diet in Bengal goats. The male growing Bengal goats were divided into 5 groups containing 6 goats in each. These five groups were fed at 0%, 25%, 50%, 75% and 100% replacement of concentrate with *Moringa oleifera* foliage. The study revealed significant increase in ADF intake and ADF digestibility with increasing level of *Moringa oleifera* along increase in nitrogen retention ($P<0.05$) in goats fed with 10%, 75% and 50% replacement level of concentrate with *Moringa oleifera*. The highest average daily live weight gain was found in the goats fed with 75% replacement ($P<0.05$) while, the lowest average daily live weight gain was observed in group fed on 0% replacement.

Bebekar and Abdalbagi (2015) studied the effect of different level *Moringa oleifera* of leaves on body weight and feed intake parameters of Sudan Nubian goats on three different levels *viz.* 0%, 20%, and 50% of *Moringa Oleifera* in groups A, B and C respectively. Forage was fed at rate of 1% of the live body weight. Their results revealed significant increase in body weight ($P<0.05$), total gain, ADG and feed conversion ratio ($P<0.05$), feed and water intake ($P<0.05$) at level 20% as compared level 0% and 50%. The study revealed that inclusion of *Moringa oleifera* at 20% of diet improved productive performance and weight gain without any adverse effect on animal health.

Kholif et al. (2015) deliberated *Moringa oleifera* leaf meal as a protein source in lactating Anglo-Nubian goat's diets and its effect on ruminal fermentation. *M*oringa *oleifera* leaf meal inclusion rates were 0% (M0 or control, no MLM, only sesame meal), 10% (M10), 15% (M15) and 20% (M20), replacing sesame meal by 0% (control), 50%, 75% and 100%, respectively. They determined that, goats fed on M15 diet showed increased ($P < 0.05$) ruminal pH, volatile fatty acids and propionate concentrations compared to the control diet. Goats fed on M10 and M20 diets showed increased feed intake ($P<0.05$). The inclusion at the rate of 15% MLM (replacing 75% of sesame meal) in the diet was the most suitable level for lactating goats under the current experiment conditions.

Kholif et al. (2016) studied the effect of feeding diets with processed *Moringa oleifera* meal as protein source in lactating Anglo-Nubian goats. *Moringa oleifera* as fresh foliage, hay or silage replacing 750 g/kg dry matter (DM) of sesame meal were evaluated against a basal diet without *Moringa oleifera* (sesame meal as the sole

protein source) as a control diet. Goats fed *Moringa oleifera* silage or fresh biomass had higher (P<0.05) DM intake (DMI) and digestibility of most nutrients compared with the control diet. In addition, DMI was greater (P<0.05) in goats fed *Moringa oleifera* silage compared with hay. It was concluded that, feeding different forms of *Moringa oleifera* to replace 750 g/kg DM of sesame meal enhanced feed utilization, digestibility dry matter intake in lactating Anglo-Nubian goats. However, the best performance was observed in goats fed *Moringa oleifera* silage. Zinder et al. (2016) determined the effect of feeding lactating cows with ensiled mixture of *Moringa oleifera,* wheat hay and molasses on digestibility and efficiency of milk production. For this, fresh harvested *Moringa oleifera* was mixed with chopped wheat hay and sugar cane molasses at a ratio, of 370:540:90 on DM basis, respectively. This silage was included in the total mixed ration (TMR) of lactating cows at a level of 180 g/kg DM as wheat silage and hay substitute. In this experiment 42 milking cows were divided into 2 groups with 21 cows each group, fed individually, either the MO-TMR or the control ration. The experimental study determined that voluntary DM intake of cows fed the control TMR, tended to be 1.22% higher than that of the MO-TMR fed cows (P = 0.09).

2.8 Effect of *Moringa oleifera* on ruminal fermentation

Dey et al. (2014) investigated the potential of *Moringa oleifera* leaves as supplement to wheat straw and assessed the associative effect on *in vitro* rumen fermentation characteristics in terms of nutritional and environmental impacts. *Moringa oleifera* leaf (ML) and wheat straw (WS) were incubated alone and in mixture (ML: WS; 10:90 and 20: 80) for 24 hours at 39^0C in 100 ml calibrated glass syringes containing buffered rumen fluid. The presence of ML in the mixture exerted positive associative effect on fermentation which resulted in increase in gas production (up to + 2.30%), true degradability of dry matter (up to + 11.47%), true degradability of organic matter (up to + 13.39%) and microbial biomass production (up to (35.60 %) with concomitant reduction in methanogenesis (up to – 18.11) compare with mixture. The mixture (WS- ML) resulted in increased volatile fatty acid production (up to + 5.48%). The present study showed that associative effects of *Moringa oleifera* leaves in improving the fermentation of wheat straw and reducing methane emission and advocate the potentiality of *Moringa oleifera* leaves as a supplement to wheat straw for improving animal performance with improve in rumen fermentation.

Kholif et al. (2015) studied effect of *Moringa oleifera* on ruminal fermentation in lactating Anglo- Nubian goats. The inclusion of *Moringa oleifera* in diet with rate of 0, 10, 15, and 20%. Goat fed with 15% inclusion rate of *Moringa oleifera* showed increased (P< 0.05) ruminal pH volatile fatty acids and propionate concentration to control diet. The result revealed that an inclusion rate of 15% *Moringa oleifera* leaf meal (replacing 75% of sesame meal) in the diet was the most suitable level for lactating goats under the current experiment condition.

2.9 Effect of *Moringa oleifera* on seminal and hormonal attributes

Priyadarshani and Varma (2014) studied the administration of *Moringa oleifera* leaf powder for 21 days. The results revealed significantly increased sperm motility, reduced sperm abnormalities including headless sperm, round head sperm and coiled tail sperm in abundance as well as banana head sperm and amorphous head sperm and significantly increased sperm count.

Raji and Nijidda (2014) studied to evaluate the gonadal and extra gonadal characteristic of Red Skoto goat fed *M. oleifera* leaf supplemented diet. *Moringa olefiera* was supplemented at level 0%, 12.5%, 25%, 37.5% and 50%. The left gonadal plus extra gonadal length recorded significant effect with 25% recording highest value of 9.16 cm. The gonadal sperm reserves were all significant in left and right testes with highest value obtained in 12.5%. The extra gonadal sperm reserve were found to be significant in the caput and the right part of the caudal epididymis and based on these finding, it was observed that supplementation at 50% level of inclusion enhancement in the gonadal and extra- gonadal sperm reserves, motility and pH.

Hairy et al. (2016) studied *Moringa oleifera* extract at level of 60 mg/head as oral administration for 21 days. The *Moringa oleifera* extract had showed significant value in improving the antioxidant status and could serve as supporting treatment in the nutritional management to improve semen production of rabbit buck, and consequently increasing reproductive performance of rabbit does matted by the semen.

Patave et al. (2016) studied the effect of stress on animal in STZ induced diabetic mice using *Moringa oleifera* leaf lam powder and for this swiss albino mice of either sex are randomly divide as i) Normal control (distilled water 0.1 ml/ 10 gm

body weight i.p) ii) Diabetic control (distilled water 0.1 ml/ 10 gm body weight i.p) iii) Diabetic stress (distilled water 0.1 ml/ 10 gm body weight i.p) iv) Diabetic stress + Metformin (120 mg /kg oral) v) Diabetic stress + Diazepam (1mg/ kg i.p.) vi) Diabetic stress + Ethanolic extract of *Moringa oleifera* (100 mg/kg oral) vii) Diabetic stress + Ethanolic extract of *Moringa oleifera* (200 mg/kg oral) and they found in his study that the STZ treated stressed mice have increased Cortisol hormone level when compared with control group. EMO at 100 and 200 mg/kg bought about a significant ($P < 0.05$) and dose dependent decrease in Cortisol hormone level when compared with diabetic stress animal.

Sawsan et al. (2016) studied the ameliorative effect of *Moringa oleifera* leaf extract on male fertility in paroxetine treated rats and for this total forty adult male albino rats were equally allocated into four groups, each of 10 rats. Rats received 0.5 ml distilled water, 400 mg/kg BW of *Moringa oleifera* hydroalcohlic extract, 10 mg/ kg BW of paroxetine simultaneously at same previously mentioned doses. All treatments were administered orally once daily for 60 successive days. Paroxetine treated group showed a significant decrease in serum level of FSH, LH, testosterone, estrogen more ever paroxetine evoked a significant increase in sperm abnormality, testicular DNA fragment and testicular MDA level compared with control group. However the concurrent administration of *Moringa oleifera* extract with paroxetine was ameliorating the aforementioned alternation compared with paroxetine treated group. It can be concluded that *Moringa oleifera* extract reduce reprotoxicity induced by paroxetine in male rats.

Syarifuddin et al. (2017) studied the effect of *Moringa oleifera* leaves supplementation on libido and sperm quality of Bali bulls. Four Bali bulls were kept under individual pens for two periods of eight weeks. During the first period (control), the experimental Bali bulls were fed concentrates (1% of body weight) and rice straw *ad libitum*. During the second period, the experimental Bali bulls were fed similar to the first period with an additional of *Moringa oleifera* leaves 15% of the weight of the concentrate (treatments). Libido and sperm quality were measured twice a week. Plasma samples were taken three times a day (06:00, 14:00 and 22:00h) on the last day of each period. Supplementation of *Moringa oleifera* leaves increased testosterone levels ($P<0.05$) (4.57 vs 4.79, 0.45vs4.78, and 2.35vs 5.63 ng/mL respectively, increased libido ($P<0.05$) (7.20±1.49 vs 3.49±0.40 min), and increased

both the total motility (P<0.05) (63.99±3.37% vs 84.96±3.09%) and the progressive motility (P<0.05) (52.77±1.76% vs 67.03±3.74%) of sperm. Supplementation of *Moringa oleifera* leaves also increased (P<0.05) the velocity and the amplitude and decreased (P<0.05) the linearity of sperm. It can be concluded that the supplementation of *Moringa oleifera* leaves could increase plasma testosterone concentrations, libido, and sperm motility of Bali bulls.

Ajuogu et al. (2019) investigated the influence of supplementing the diet with *Moringa oleifera* Lam. leaf powder on reproductive hormones and semen quality of New Zealand White (NZW) rabbits. Thirty-two NZW rabbits of 50:50 ratio bucks to does, were randomly distributed to four treatment groups (n=4 bucks, n=4 does per group). Graded levels (0, 5, 10 and 15g/kg) of *Moringa oleifera* Lam. leaf powder was incorporated into rabbit growers pellet. The does and bucks were housed separately in hutches and sheltered under the same environmental conditions with free access to their respective treatment diets for a period of 12 weeks. In female rabbits, treatment revealed significantly (P<0.05) dose-dependent reduction in the concentration of serum FSH, LH and estrogen. While in contrast the highest dose of leaf powder significantly (P<0.05) increased progesterone and prolactin concentrations remained unaffected. On the other hand, the concentration of FSH and LH in bucks was significantly (P<0.05) increased in treatment groups compared to the control group. Serum testosterone concentrations were significantly lower in the 5 and 10g/kg treatment groups. Semen volume, sperm count and motility were significantly improved in a dose dependent manner with increasing amounts of *Moringa oleifera* Lam. leaf powder in the diet. We conclude that *Moringa oleifera* Lam. leaf powder supplementation to the diet was more beneficial to male rabbit fertility than the female, where it tended to have a negative impact through the hypothalamic pituitary-gonadal axis. However, with the varying impact of *Moringa oleifera* Lam. leaf powder on the hypothalamicpituitary-gonadal axis of male and female animals, further investigation is necessary to determine the mechanism through which it operates.

Materials and Methods

CHAPTER-3

MATERIALS AND METHODS

The aim of research trial was to evaluate the effect of replaced soybean meal a protein source in concentrate mixture with dried *Moringa oleifera* leaf powder on growth performance, nutrient utilization, semen quality, blood biochemicals, antioxidant and hormonal profile of Barbari bucks. In this chapter, a brief description of material used and experimental techniques adopted during the study has been given in following heads.

3.1 Selection and grouping of experimental animals

The experiment was conducted in the Barbari buck shed at Instructional Livestock Farm Complex (ILFC), DUVASU, Mathura (Uttar Pradesh). A total eighteen Barbari bucks of 1-1.5 year age were selected from the herd maintained at Department of Physiology, DUVASU, Mathura (U.P.). Experimental bucks were randomly assigned into three groups (six bucks in each) on body weight basis.The experiment protocol was in accordance of standard of Institute Animal Ethics committee and the approval of experimentation was also obtained from the committee. The selection and grouping of experimental animals is presented in Table 3.1.

Table 3.1 Selection and grouping of experimental animals

Group	S. No	Animal No.	Body weight(kg)
Control	1	BM017	2.1
	2	BM013	24.5
	3	BM006	22.5
	4	BM014	24.5
	5	BM003	32.5
	6	BM004	43.0
		Mean	**28.0**
T10	1	BM011	24.0
	2	BM018	24.0
	3	BM010	22.0
	4	BM001	30.0
	5	BM002	30.0
	6	BM019	38.0
		Mean	**28.0**

T20	1	BM008	33.5
	2	BM012	25.0
	3	BM015	25.5
	4	BM009	26.0
	5	BM007	28.0
	6	BM0016	30.0
		Mean	**28.0**

3.2 Housing and management of experimental animals

All bucks were housed in a well-ventilated individual sheds having the proper arrangement for feeding and watering. The buck shed was washed daily and thoroughly cleaned to remove faeces and dirt. Deworming of all the animals was done with broad spectrum antibiotic before the start of the experiment.

3.3 Feeding of experimental animal

The bucks were fed basal diet comprising of concentrate mixture, arhar straw and green berseem. The animals of each experimental group were maintained and fed individually on roughage and concentrate based ration to meet out requirement as per NRC (2007) feeding standard. Diets were prepared by taking concentrate and roughage in the ratio of 40:60 respectively. The roughage part composed of arhar straw (36%) and green berseem (24%).Clean and fresh drinking water was offered ad libitum twice to each animal daily. The duration of experiment was of 90 days. *Moringa oleifera* leaves were collected from nearby mature plants and are dried under the shade up to the moisture level of ≤ 12%. The dried leaves were then ground and the material consequently obtained was designated as dried *Moringa oleifera* leaf powder (MOLP). It was then stored in polythene bags in cool and dry place until it is used for feeding and analysis.

Before the start of experiment the animal were given an adaption period of fifteen days. All the three groups were kept on similar feeding regimen, except different level of dried *Moringa oleifera* leaf powder. The soybean meal in concentrate mixture was replaced at 0, 10 and 20% with dried *Moringa oleifera* leaf powder in Control, T10 and T20 group respectively. The animals of three different groups were subjected to the following dietary treatments.

Groups	Diet
Control	Basal diet (concentrate mixture, arhar straw and green berseem)
Treatment 1 (T10)	Basal diet in which 10% of soybean meal in concentrate mixture was replaced with dried *Moringa oleifera* leaf powder.
Treatment 2 (T20)	Basal diets in which 20% of soybean meal in concentrate mixture was replaced with dried *Moringa oleifera* leaf powder.

The animals in Control group were fed on basal diet i.e. arhar straw, concentrate mixture and green berseem without any replacement of soybean meal in concentrate mixture and group T10 was supplemented with basal diet in which 10 % of soybean meal in concentrate mixture was replaced with dried *Moringa oleifera* leaf powder whereas in group T20 experimental bucks were fed on basal diet in which 20 % of soybean meal in concentrate mixture was replaced with dried *Moringa oleifera* leaf powder.

3.4 Ingredient composition of (%) of experimental diet

Diets were prepared by taking concentrate and roughage in the ratio of 40:60 respectively. The roughage part was composed of arhar straw (36%) and green berseem (24%). Concentrate mixture (40%) was composed for Control group having 15.2 parts Maize, 8 parts soybean, 8.4 parts wheat bran, 7.2 parts gram chunni, 1.2 parts mineral mixture, corresponding values for T10 group was 15.2 parts Maize, 7.2 parts soybean, 7.2 parts wheat bran, 8.4 parts gram chunni, 1.2 parts mineral mixture and 0.8 parts dried *Moringa oleifera* leaf powder and corresponding values for T20 group was 15.2 parts Maize, 6.4 parts soybean, 5.6 parts wheat bran, 10.4 parts gram chunni, 1.2 parts mineral mixture and 1.6 parts dried *Moringa oleifera* leaf powder.

Table 3.2 Ingredient composition (%) of experimental diets

Ingredients	Concentrate	Treatment		
		Control	T10	T20
Maize	38	15.2	15.2	15.2
Soybean	20	8	7.2	6.4
Wheat bran	21	8.4	7.2	5.6
Gram chunni	18	7.2	8.4	10.4
Mineral mixture	3	1.2	1.2	1.2
Dried *Moringa oleifera* leaf powder	-	-	0.8	1.6
Green berseem	-	24	24	24
Arhar straw	-	36	36	36

Half of total pre-weighed amount of concentrate mixture, arhar straw and green berseem and was offered to the animals at 09.00 a.m in morning hour and 4.00 pm in evening hour during entire trial period. Clean and fresh drinking water was offered ad libitum to each animal daily.

3.5 Growth performance indices

3.5.1 Body weight and dry matter intake

The animals were weighed before feeding and watering in the morning on two consecutive days at the start of experimental feeding and thereafter at fortnightly interval during experimental period of 90 days. The feeds offered to the animals and residue left were recorded daily to find out the total DMI of the experimental animals. Intake of DM was calculated as the difference between the amount of DM offered and amount of DM left in ort.

3.5.2 Body weight gain

Body weight of experimental animals recorded at start of experiment followed by at fortnightly intervals. The experimental bucks were weighed before feeding and watering. Fortnightly weight gain was calculated by increase in body weight in one fortnight and ADG (g/d) was calculated by dividing the fortnightly weight gain with number of days (15).

3.5.3 Feed to gain ratio or feed conversion ratio (FCR)

Feed-to-gain ratio or FCR was calculated by the amount of DMI (kg) required for unit (per kg) weight gain by animals during the trial period.

3.6 Digestibility trial

To compare the efficiency of nutrient utilisation in Barbari bucks, a digestion trial for a period of 6 days was conducted at the end of the study. Bucks were weighed before start and at the end of digestion trial to find out body weight gain. Weighed amount of feeds and fodders was offered during digestion trial. Representative samples of the feed offered and residue left were collected and analysed for chemical composition. Faeces voided during 24:00 hours were collected and measured daily for 6 days. About $1/10^{th}$ of thoroughly mixed total faecal matter (as such basis) was taken for chemical analysis. Additionally, for N estimation, approximately $1/30^{th}$ of total faecal sample was collected daily for 6 days and stored in plastic containers having 25% sulphuric acid solution. Dried dung samples were grounded to pass through 1mm sieve size and analyzed for proximate principles and fibre constituents (NDF and ADF) as per standard techniques. The digestibility coefficient of nutrients was calculated from the nutrient intake and nutrient outgo in feces during digestion trial:

$$\text{Digestibility (\%)} = \frac{\text{(Nutrient intake – Nutrient outgo in faeces)}}{\text{Nutrient intake}} \times 100$$

3.7 Chemical analysis

The representative samples of feeds and fodders offered and residue left and faeces were ground and analyzed for DM, OM, CP, EE, CF and total ash (AOAC, 2000). Fibre fraction of feed, fodder and faecal sample were analyzed by using detergent method of fibre estimation (Van Soest et al., 1991).

3.7.1 Dry matter (DM)

DM content in samples of feedstuffs and faeces was analyzed by using AOAC, 2000) method. 100 g sample was taken in a pre-weighed moisture cup or tray and it was placed in a hot air oven at 100 ± 5°C for 24:00 hours. The loss in moisture content after drying was estimated and DM was calculated as follows:

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

3.7.2 Organic matter (OM)

OM content of sample was determined by subtracting the total ash content from DM content of respective sample.

$$OM\ (\%) = DM\ \% - Total\ ash\ (\%)$$

3.7.3 Crude protein (CP)

Apparatus: Kjeldahl flasks, digester, Kjeldahl distillation apparatus, 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 to 1000 ml), concentrated H_2SO_4 (98% purity and specific gravity 1.84), 4% boric acid indicator solution (40 g boric acid dissolved to 1 L and added with 10 ml 0.2% bromocresol green and 20 ml 0.1% methyl red indicators) and N/10 H_2SO_4 solution.

Procedure

A known quantity of sample (about 0.5-1 g) was taken in digestion tube and digested with 20-30 ml concentrated H_2SO_4 and 2-3 g of digestion mixture till the solution became colorless. After digestion, the contents were cooled and volume was made to 250 ml. 10 ml of aliquot was distilled in Kjeldahl distillation apparatus (KELPLUS Nitrogen Analyzer, Chennai) after adding 10-15 ml of 40% NaOH solution. About 60-75 ml of distillate (light green colour) was collected into an Erlenmeyer flask containing 10 ml of 4% boric acid indicator solution. The distillate was then titrated against N/10 H_2SO_4 solution and the end point was recorded when colour changed to slight pinkish. Volume of N/10 H_2SO_4 solution used in titration was recorded.

Calculation

$$N\ (\%) = \frac{0.0014 \times 0.1 \times \text{Volume of N/10 } H_2SO_4 \text{ used} \times \text{Volume made (ml)}}{\text{Aliquot taken (ml)} \times \text{sample taken (g)}} \times 100$$

The CP (%) of sample was calculated by multiplying the N content with factor 6.25 (protein contains 16% nitrogen).

3.7.4 Ether extract (EE)

EE content in representative samples was analyzed by using AOAC (2000).

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

Reagent: Petroleum ether (40-60°C).

Procedure: A known quantity of grounded sample (about 3g) was taken in a cellulose thimble and extracted for 8 hours with petroleum ether (40-60°C) in Soxhlet extraction apparatus attached to a pre weighed oil flask. The oil flask was removed after evaporating the excess of ether and then it was dried overnight in a hot air oven (100 ± 5°C). The flask was cooled in desiccator and weighed to a constant value. The EE was estimated as given below:

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

3.7.5 Total Ash (TA)

TA content in samples of feedstuffs and faeces was analyzed by using AOAC (2000) method (942.05). A known quantity of sample (about 2.5-3 g) was taken in pre-weighed silica crucible. After charring the sample on heater (till the smoke disappeared), the crucible was kept in muffle furnace for ignition at 550-600°C for 2-3 hours. Then the crucible was removed on cooling and kept in desiccator and weighed again to find out weight of ash. The ash content was calculated as given below

$$\text{TA (\%)} = \frac{\text{Weight of crucible + ash after drying – Weight of crucible}}{\text{Weight of sample taken}} \times 100$$

3.7.6 Crude fiber (CF)

Apparatus: Reflux assembly, suction apparatus, muslin cloth, Erlenmeyer's flask, hot air oven, desiccator, weighing balance.

Reagents: 1.25% H_2SO_4 and 1.25% NaOH

Procedure: A moisture and fat free sample of known quantity (after ether extraction) was taken in spoutless beaker of 1 L capacity previously marked to 200 ml. Then, 25

ml of 10% sulphuric acid (w/v) was added and volume was made up to 200 ml with water from the sides of the beaker to have 1.25 % H_2SO_4 solution in the beaker. The contents of beaker were refluxed for 30 minutes and then filtered through muslin cloth using Buchner funnel with the help of vacuum pump. After repeated hot water washings, the residue left on muslin cloth was transferred to the same spout less beaker with smooth steel spatula, followed by little washing of muslin cloth. 25 ml of 10% sodium hydroxide (w/v) was added and volume was made to 200 ml with water to have 1.25% alkali solution. Contents were refluxed for 30 min and filtered through the muslin cloth and washed with hot water. The residue left on muslin cloth was transferred to a clean silica crucible with the help of steel spatula. The contents were dried in hot air oven at 100 ± 5°C and weighed. Silica crucible containing dried residue was kept in a muffle furnace at 550°C for 2 hours for ashing and weighed after cooling. The CF content of sample was estimated as follows:

$$\text{CF (\%)} = \frac{\text{Weight of dried residue} - \text{Weight of ash}}{\text{Weight of sample taken}} \times 100$$

3.7.7 Nitrogen free extracts (NFE)

The NFE of sample was estimated by using following equation:

$$\text{NFE (\%)} = 100 - (\text{CP\%} + \text{EE\%} + \text{CF\%} + \text{\% TA})$$

3.7.8 Estimation of cell wall constituents

The fractions of cell wall (fibre fraction) such as neutral detergent fibre (NDF), acid detergent fibre (ADF) and acid detergent lignin (ADL) were estimated as per methodology described by Van Soest et al., (1991).

3.7.9 Neutral detergent fibre (NDF)

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

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

Table. 3.3 Composition of neutral detergent solution (NDS)

Chemical	Amount
Sodium lauryl sulphate	30 g
Disodium ethylene diamino tetra acetate (EDTA)	18.61 g
Sodium borate decahydrate	6.81 g
Disodium hydrogen phosphate (anhydrous)	4.56 g
Triethyleneglycol	10 ml
Distilled water	990 ml

Solution preparation

EDTA and sodium borate decahydrate were put together in a large beaker with some distilled water and heated on hot plate untill dissolved. Similarly sodium lauryl sulphate was dissolved in distilled water and triethylene glycol was added to it. The solution of sodium lauryl sulphate and tri ethylene glycol was added to the previous solution. Disodium hydrogen phosphate was taken in another beaker and some amount of distilled water was added and the contents were heated untill dissolved. Then, it was added to solution containing other ingredients and volume was made up to one litre with distilled water.

Procedure

A known quantity of ground sample (1.0 g) was taken in a spoutless beaker and to this add 100 ml NDS. The contents were heated to boil and refluxed for 60 min. The contents were filtered through a pre-weighed Gooch crucible (G1 porosity) under vacuum. The contents were given 3-4 washings with hot distilled water and a final washing of acetone. The crucibles were dried to a constant weight at 100º C and weighed. Cell wall contents or NDF was calculated as follows:

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

3.7.10 Acid detergent fibre (ADF)

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

Reagents: Acid detergent solution (ADS), acetone, hot boiling water, 1N H_2SO_4 (Taken 26.63 ml of conc. H_2SO_4 and dissolved in 1litre of distilled water. This yield 1N H_2SO_4 solution).

Preparation of acid detergent solution (ADS): 20 g Cetyl trimethyl ammonium bromide (CTAB) was dissolved in one litre of 1 N H_2SO_4 (Taken 26.63 ml of conc. H_2SO_4 and dissolved in 1litre of distilled water. This yield 1N H_2SO_4 solution)

Procedure

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

$$\text{ADF (\%)} = \frac{\text{(Weight of crucible with residue – Weight of empty crucible}}{\text{Weight of sample taken}} \times 100$$

3.7.11 Hemicellulose (HC)

Hemicellulose content was calculated as the difference between NDF and ADF.

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

3.7.12 Acid detergent lignin (ADL)

Apparatus and reagents: Same as that of ADF estimation, and 72% sulphuric acid (measured 665ml of concentrated H_2SO_4 and slowly poured into 300 ml of distilled water and immersed the beaker in cold water. After the solution was cool down, made the final volume to 1 litre with distilled water this yield 72% sulphuric acid solution).

Procedure

ADF was used as preparatory step for ADL estimation. Prepared ADF crucible placed on glass tray. One end of glass tray kept 2cm higher so that the acid drained away from crucibles. Content of the crucible covered with cooled (15^0C) 72% sulphuric acid (w/v) and stirred with a glass rod to a smooth paste. Crucible filled about halfway with acid and stir. Glass rod remained in crucibles; refilled with 72% sulphuric acid (w/v) and stirred at hourly intervals as acid drained away. Crucibles

dried at 105^0 C for 8 hours or overnight and cooled in desiccator for at least 1 hour and weighed. Crucible ignited in a muffle furnace at 500^0C for 2 hours. While still hot, crucible placed in desiccator, cooled to constant temperature and weighed. The acid detergent lignin was calculated as follows:

$$\text{ADL (\%)} = \frac{\text{(Weight of crucible with dry residue – Weight of crucible with ash}}{\text{Weight of sample taken}} \times 100$$

3.7.13 Estimation of Calcium (Ca) by titration method Talpatra method (Talpatra et al., 1940)

Apparatus & reagents: Beaker 250ml, Bunsen burner, conical flasks, filtration stand, glass rod, burette stand, test tubes, wash bottle, graduate pipette, Whatman filter paper no. 42, glass funnel and test tube rack, Saturated solution of Ammonium oxalate, methyl red indicator. 5% ammonium hydroxide, concentrated H_2SO_4 and N/10 $KMnO_4$.

Procedure

10 ml aliquot was taken from the stock ash solution into 250 ml beaker and was diluted by adding 100 ml distilled water and to this 10 ml saturated solution of Ammonium oxalate and 2-3 drops of 0.2% alcoholic solution of methyl red indicator was added. The solution was mixed thoroughly with glass rod and then solution was turn into red colour as the ash has been extracted with HCl in the preparation of the acid soluble ash. The solution which was acidic in nature was neutralized by adding diluted 5% ammonium hydroxide drop by drop to just reached the pH range in the alkaline medium. The content of the beaker was boiled for 10-15 minutes and then cooled. The precipitate of calcium oxalate was formed. The precipitate was washed with hot distilled water and filtered through filter paper no. 42 to made it chloride free. The filtrate was tested with 1% silver nitrate solution to detect presence of chloride. The real technique of filtration was keeping maximum quantity of calcium oxalate in the original beaker then it was poured on filter paper during filtration. The filter paper was transferred to original beaker in such a way that outer surface of filter paper was sticked to inner wall of beaker. After that the precipitate was dissolved with jet of distilled water. After that 10 ml of concentrated H_2SO_4 added to the original beaker. After that beaker was heated till the content was started to boiling.

Finally the solution (when it was hot) was titrated against N/10 $KMnO_4$ solution. The pink colour was appeared by running $KMnO_4$ solution and it was remained at least for 15 seconds. The required volume of N/10 $KMnO_4$ for titration was recorded.

Calculation

$$\text{Ca (\%)} = \frac{\text{ml of N/10 } KMnO_4 \text{ required} \times 0.002 \times \text{A.F.}}{\text{Weight of sample}} \times 100$$

1ml of N/ 10 $KMnO_4$ = 0.002g of Ca

Where A.F is aliquot factor

A.F = volume of aliquot prepared / volume of aliquot taken.

3.7.14 Estimation of phosphorus (P) by Volumetric method.

Apparatus & reagents: Beaker 250ml, Bunsen burner, conical flasks, filtration stand, glass rod, burette stand, test tubes, wash bottle, graduate pipette, Whatsman filter paper no. 42, glass funnel and test tube rack.

Method

10 ml aliquot was taken into 250 ml beaker and the volume was made 100 ml by adding distilled water. 10 ml of 20% ammonium molybdate and 10 ml of conc. HNO_3 was taken in two different test tube and both of the reagents was poured in a common stream in the beaker containing aliquot and the content of beaker was stirred vigorously with glass rod till canary yellow colour precipitate was formed. The beaker was allowed to stand for overnight. After that the supernatant fluid was transferred to the filter paper with the help of conical flask and funnel. When all the supernatant liquid was transferred to funnel, wait for complete filtration. The next step was washing of the precipitate.

Washing of precipitate

3% of KNO_3 was used to wash the acid from the precipitate. 10 ml pipette was used to transfer 10ml of 3% KNO_3 on the filter paper containing the precipitate and it was mixed well and allowed to settle. The supernatant fluid was transferred to filtering funnel. By this time another 10 ml of 3% KNO_3 was transferred to the beaker and it was mixed well and allowed it to settle. When the previous filtration was

completed, the supernatant fluid was transferred from beaker to the whatsman filter paper 42. The washing and filtration was continued till the filtrate was acid free.

Dissolving the precipitate

When the filtrate was free from acid, gently taken the filter paper and made it into a pulp. Now the side of beaker was washed with distilled water. The entire content was now yellow. Known amount of 0.1 N NaOH was added till yellow colour was disappeared and the content was become colourless except for the white filter paper residue. Solution was stirred well and a drop or 2 of phenolphthalein indicator was added. The pink colour was appeared indicating excess of alkali.

Back titration

The amount of excess alkali added was found out by back titration with 0.1 N HCl (8.6 ml HCl was taken and it was diluted with distilled water up to 1 litre). From the total amount of 0.1 N NaOH added, the quantity of acid required for titration was deducted to arrived at the actual quantity of alkali was required for dissolving the precipitate. The end point of titration was pink to colourless. The initial and final reading was recorded.

Calculation

$$P\ (\%) = \frac{0.0001347\ N/10 \times A.F.}{\text{Weight of sample}} \times 100$$

1ml of 0.1N NaOH = 0.0001347 g of P

Where A.F is aliquot factor

A.F = volume of aliquot prepared / volume of aliquot taken.

3.8. Calculation of nutrient digestibility coefficient

The nutrients (Dry matter, organic matter, crude protein, ether extract, NDF and ADF) digestibility coefficient was calculated from the nutrient intake and nutrient out go in faeces during digestion trial as follows:

$$\text{Digestibility}\ (\%) = \frac{(\text{Nutrient intake} - \text{Nutrient outgo in faeces})}{\text{Nutrient intake}} \times 100$$

3.9 Semen collection

Semen was collected twice a week using artificial vagina (length= 20 cm and diameter = 4.5 cm).The artificial vagina was prepared by filling with water at a temperature of 42- 45 ^{0}C and air through a valve to maintain optimum pressure. The temperature of the water varied according to season of collection to maintain optimal warmth in the artificial vagina. Just before collection the inner lining was lubricated with non-spermicidal lubricant followed by checking of temperature and pressure. A non-estrus doe was used for mounting of bucks and semen was collected into graduated tubes attached to one end of artificial vagina.

3.9.1 Semen Samples

Semen of Barbari bucks were collected with the use of sterilized artificial vagina and evaluated for various parameters.

3.9.2 Transportation of semen samples

Immediately after semen collection, samples were transported to Semen Analytical Laboratory, Department of Animal Physiology, DUVASU Mathura for evaluation of various parameters.

3.9.3 Volume

The volume of semen was directly measured in milliliter (ml) from the graduated semen collection tube.

3.9.4 Colour

Semen sample were also observed for colour and consistency by direct visualization with naked eyes and any abnormalities in colour or consistency were treated as abnormal and the sample were discarded.

3.9.5 Mass motility

It was assessed by placing a small drop of semen of uniform size and thickness over a clean dry glass slide. The semen drop was examined under low power objective (10X) of microscope on a thermostatically controlled warm stage at 37°C. Nazir (1988) reported that motility was rated according to the vigour wave motion on grade scale of 0 to 5 as given below.

Table 3.4 Mass motility scores

S. No.	Observation	Mass motility score (0-5 scale)
1	No motility	0
2	No wave but sperm movement evident	+1
3	Slow wave formation	+2
4	Relatively more wave formation with swirls	+3
5	Wave with swirls and eddies	+4
6	Wave with very rapid swirls and eddies	+5

3.9.6 Progressive motility

The progressive motility of the spermatozoa was observed under high power phase objective (40 X) on a thermostatically controlled stage maintained at 37°C. A small drop of diluted semen was put on a clean grease free slide and was covered with a cover slip. The slide was examined to observe vigorously motile spermatozoa exhibiting progressive path. The progressive motility of spermatozoa was then calculated as below

$$\text{Progressive motility (\%)} = \frac{\text{No of progressively motile spermatozoa}}{\text{Total number of spermatozoa observed}} \times 100$$

3.9.7 Sperm concentration

Concentration of spermatozoa in the semen sample was estimated by haemocytometer (improved Neubauers chamber) method. The diluting fluid used was mixture of 2.9 g of sodium citrate and 0.05 g of eosin Y9 (water soluble) in 100 ml of formalized distilled water.

Steps for examining the sample

1) Semen was sucked into RBC pipette up to 0.5 mark.
2) The outside of pipette was cleaned with cotton to wiped off any semen sample sticking outside the tip of pipette. Then the diluting fluid was sucked up to 101 mark.
3) After filling the pipette, it was held horizontally between palm of hands and rolled for 2-3 min to ensure through mixing.

4) Haemocytometer was well cleaned, dried and placed on level surface, a clean dry cover slip was placed over haemocytometer.
5) The diluting pipette was shaken gently and discarding the first few drops of the diluted semen, a small drop was used to charge the haemocytometer through capillary action.
6) The haemocytometer was then left undisturbed for ten minutes to ensure the setting of spermatozoa.
7) The total number of spermatozoa was counted in five secondary square, namely top right, top left, bottom right, bottom left and central square of central primary square.
8) Each secondary square contains sixteen tertiary square, so total no. of spermatozoa was counted in eighty tertiary square. The average of the count on both side of haemocytometer chamber was taken for calculating the sperm density and total no. of spermatozoa per ml of neat semen. The calculation is given below.

Area of 1 tertiary square	$= 1/20 \times 1/20\ mm^2$
Volume of 1 tertiary square	$= 1/4000\ mm^3$
Number of spermatozoa in 80 tertiary squares	$= n$
Volume of 80 tertiary squares	$= 1/4000 \times 80\ mm^3$
(Where $80/4000\ mm^3$ volume has n number of spermatozoa)	
Therefore,	$= n/80 \times 4000$
	$= n \times 50$
Dilution factor	$= 200$
Total number of spermatozoa (undiluted)	$= n \times 50 \times 200$
$1\ mm^3$ neat semen has	$= n \times 10000$
Spermatozoa concentration (per ml)	$= n \times 10000 \times 1000$
	$= 10n \times 10^6$

3.9.8 Differential staining for live and dead spermatozoa

Dead spermatozoa could be differentiated by their ability to get stained by Eosin dye. The live spermatozoa, which were alive at the time of staining, remain colourless since they were impermeable to Eosin stain. Nigrosin provided a blue-black background (Hancock, 1952).

Preparation of Stain

Eosin-Nigrosin stain was prepared by the method described by Hancock (1951). The composition of the stain was as follows (Table given below)

The mixture was kept overnight and on the next day filtered through quality filters paper (Whatman filter paper no. 40) and stored in a dark and sealed glass bottle. Before use, the staining solution was brought to room temperature. Fresh stain was prepared every 15 days to prevent artifacts.

Table 3.5 Composition of Eosin-Nigrosin stain

Nigrosin	10.0 g
Eosin-Y (water soluble)	1.67 g
Sodium citrate dehydrate	2.9 g
Distilled water	100 ml

Ingredient of eosin–nigrosin stain were accurately weighed on an electronic balance and triturated using a glass pestle and mortar, the final volume of the solution was made up to 100 ml in a volumetric flask by adding double distilled water. The stain was kept in an incubator at 37^0 C for two days and then the supernatant was carefully decanted and stored in a refrigerator in a tight stopper glass bottle.

At the time of estimation, one drop of neat semen was mixed with 5- 6 drops of stain in a glass tube at 30^0 C in a water bath and incubated for 5-10 second. Smears were made on a clean, grease free slide and dried in air. At least 200 spermatozoa were counted and identified as live or dead under the oil immersion objective (100X). The stained spermatozoa (eosinophilic) obtained a pinkish colour and were categorized as dead and the unstained ones against a dark background of Nigrosin were counted as live. The percentage of live spermatozoa was calculated as follows:

$$\text{Per cent live spermatozoa} = \frac{\text{Live spermatozoa counted}}{\text{Total spermatozoa counted}} \times 100$$

3.9.9 Morphological Abnormalities

Live and dead count was also used for enumerating abnormalities. The classification suggested by (Lasley, 1951) was used for the study. The slides were observed under oil immersion in Phase Contrast Microscope. These studies were broadly classified in three groups as per abnormalities listed below.

Table 3.6 Sperm abnormality categories

S. No.	Head Abnormalities	Mid Piece Abnormalities	Tail Abnormalities
1.	Micro Head	Abaxial Attachment	Simple bent tail
2.	Macro head	Beaded	Coiled Tail
3.	Giant head	Proximal protoplasmic Droplet	Loose tail
4.	Loose head	Distal protoplasmic droplet	Tail stump
5.	Detached head	Corn screw mid piece	Double tail
6.	Pear shaped head (pyriform)	Bent Mid piece	Corkscrew tail
7.	Bent head	-	-
8.	Knobbed acrosome detect	-	-

About 200 spermatozoa were counted in different fields and total abnormal spermatozoa were calculated as follows:

$$\text{Total Abnormalities (\%)} = \frac{\text{Total Number of abnormal spermatozoa}}{\text{Total number of spermatozoa counted}} \times 100$$

3.9.10 Hypo-osmotic swelling test (HOST)

To analyze the functional integrity of plasma membrane hypo-osmotic swelling test was performed according to the technique described by Jeyendran et al. (1984). The protocol was modified in reference to incubation time (1 hour) and osmalality required of goat semen. Hypo-osmotic solution of 150 mOsm/l was prepared as follows.

Table 3.7 Composition of Hypo-osmotic solutions of 150 mOsm/l

Ingredients	Amounts
Sodium citrate	0.735 g
Fructose	1.351 g
Millipore/ double distilled water upto	100 ml
Osmolality	150 mOsm/l

Procedure

One ml of HOST solution, having an osmotic strength of 150 mOsm/l was mixed with 0.1 ml of semen and incubated at 37^0C for one hour. Following incubation, a drop of well-mixed solution was taken on clean glass slide and covered with a cover slip. Sperm tail curling was recorded as an effect of swelling due to influx of water. A total of about 200 spermatozoa were counted in different fields under 40X phase objective under phase contrast microscope. The total proportion of swollen spermatozoa was calculated by dividing the number of reacted cells by the total spermatozoa counted in the same area and multiplying the figure by 100. These spermatozoa were classified in four different classes according to presence of following swelling pattern (Takahashi et al., 1990).

A. No swelling, no membrane reaction
B. Swelling of the tip of the tail
C. Different type of hair pin like swelling or swelling of mid-piece
D. Complete tail coiling

Spermatozoa showing B, C, D type of pattern was considered to be HOST positive.

$$\text{HOST positive Spermatozoa (\%)} = \frac{\text{Number of curled tail spermatozoa}}{\text{Total number of spermatozoa counted}} \times 100$$

3.9.11 Acrosome integrity

Acrosome play a vital role in the fertilization of ovum, hence percentage of intact acrosome is a good indicator in analyzing the fertilization potential of sperm. Acrosome integrity was judged by Giemsa staining technique as per the methodology described by (Watson, 1975).

Sorenson Phosphate buffer:

Solution A

Sodium phosphate dibasic	11.876g
Distilled water	1000ml

Solution B

Potassium phosphate	9.08g
Distilled water	1000ml

0.1 M Sorenson phosphate buffer was prepared by adding, 33 ml of solution A and 17 ml of solution B.

Fixative

5% Formalin solution was used for fixation of spermatozoa.

Working solution

Giemsa stain	3 ml
0.1 M Sorenson phosphate buffer	2ml
Distilled stain	35ml

Staining procedure

A thin smear of diluted semen drop was made on clean, grease free slide. Smear was dried and kept in 5% formaldehyde solution for 30 minute at 37^0C. The slides were washed in double distilled water and are air-dried.

In a staining jar, 3 ml of Giemsa solution was added drop by drop in 2ml Sorenson phosphate buffer solution and 35 ml of double distilled water was added and the resulting mixture was mixed thoroughly. Then the slides were kept in the staining jar solution for 4 hours at 37^0C in the incubator. The stained slides were washed with distilled water, air dried and 200 spermatozoa per slide were examined under high power (100X) of phase contrast microscope. This method stained the acrosome dark purple.

$$\text{Acrosomal Integrity (\%)} = \frac{\text{Number of acrosome stained spermatozoa}}{\text{Total number of spermatozoa counted}} \times 100$$

3.10 Estimation of blood metabolites

Blood samples were collected from all the bucks before the start of experimental feeding and thereafter, at monthly intervals (i.e. day 0, 30, 60, 90). About 9 ml of blood was collected aseptically from the jugular vein of each experimental animal in heparinized vacutainer (BD Franklin, USA). Immediately after the collection, the vials were gently rolled in between palms for proper mixing of the anticoagulant, kept in the icebox and were brought to the laboratory for analyzing various hematological and biochemical parameters.

Hematological parameters haemoglobin (Hb) concentration, packed cell volume (PCV) was estimated in whole blood. Antioxidant enzymes and lipid peroxidation were estimated in RBC hemolysate. The blood biochemicals and hormone estimation were done in blood plasma. For plasma, the blood samples were centrifuged at 3,000 rpm for 15 min, and plasma was collected into cryo vials and stored at -20^0C until further analysis were performed. Collected plasma samples were analyzed for blood biochemical with the help of automated biochemical analyzer in diagnostic lab. Instrument was calibrated according to span diagnostic kit supplied by Span Diagnosis Ltd for the estimation of glucose, total cholesterol, triglycerides, total protein, albumin, globulin, ALT, AST.

3.10.1 Haematological attributes

3.10.1.1 Haemoglobin (Hb) concentration

Haemoglobin concentration was estimated in the fresh blood with the help of MS4Se auto haemoanalyser by Nexus medical solution 002296745-A.

3.10.1.2 Packed cell volume (PCV) or haematocrit (HCT)

PCV was estimated in the fresh blood with the help of MS4Se auto haemoanalyser by Nexus medical solution 002296745-A.

3.10.2 Plasma attributes

3.10.2.1 Plasma glucose

Plasma glucose was estimated by "GOD-POD, End point assay test kit" supplied by Span Diagnosis Ltd. Glucose oxidase (GOD) oxidizes glucose to gluconic acid and Hydrogen Peroxide. In presence of enzyme Peroxidase, released Hydrogen Peroxide is coupled with phenol and 4-Aminoantipyrine (4-AAP) to form coloured Quinoneimine dye. Absorbance of coloured dye is measured at 505 nm and is directly proportional to glucose concentration in the sample.

Procedure

20 µl of plasma aliquot was pipetted in 10×75 mm tubes in duplicate, to which 1500 µl of working glucose reagent was added. Blank (20 µl distilled water) and standard (20 µl from standard 50 mg/dl) were pipetted in duplicate to which 1500 µl of working glucose reagent was added. The contents were mixed well and incubated at 37 °C for 10 min. 1500 µl of purified water was added to each tube. The contents

were mixed well. UV-spectrophotometer was blanked with reagent blank and the absorbance of standard and test sample was measured.

Calculation: Plasma glucose levels were calculated as per formula and expressed in mg/100ml:

$$\text{Glucose (mg/100 ml)} = \frac{\text{Absorbance of test}}{\text{Absorbance of standard}} \times 100$$

3.10.2.2 Plasma cholesterol

Plasma cholesterol was estimated in plasma samples by "CHOD-PAP, End point assay test kit" supplied by Span Diagnosis Ltd. The principle of assay was cholesterol esters are hydrolyzed by cholesterol esterase to give free cholesterol and fatty acids. In subsequent reaction, cholesterol oxidase, oxidizes the 3-OH group of free cholesterol to liberate cholest-4-en-3-one and hydrogen peroxide. In presence of peroxidase, hydrogen peroxide couples with 4-aminoantipyrine and phenol to produce red quinoneimine dye. Absorbance of coloured dye was measured at 505 nm and was proportional to amount of total cholesterol concentration in the sample.

Procedure

10 µl of plasma aliquots were pipetted in 10×75 mm tubes in duplicate, to which 1000 µl of working cholesterol reagent was added. Blank (10 µl distilled water) and standard (10 µl from standard 200 mg/dl) was pipetted in duplicate, to which 1000 µl of working cholesterol reagent was added. The content were mixed well and incubated at 37ºC temperature for 10 minutes. UV-spectrophotometer was blanked with reagent blank and the absorbance of standard and test sample was measured at 505 nm.

Calculation: The cholesterol concentration was calculated as per the formula and expressed in mg/100ml:

$$\text{Cholesterol (mg/100 ml)} = \frac{\text{Absorbance of test}}{\text{Absorbance of standard}} \times 200$$

3.10.2.3 Plasma triglyceride/Triacyglycerol

Plasma triglycerides were estimated in plasma samples by "End point assay test kit" supplied by Span Diagnosis Ltd. The principle of assay was triglycerides are

hydrolyzed by lipoprotein lipase (LPL) to give glycerol and free fatty acids. In subsequent reaction, glycerol 3-PO_4 oxidase, oxidizes the 3-PO_4 group of free glycerol to liberate dihydroxy acetone phosphate and hydrogen peroxide. In presence of peroxidase, hydrogen peroxide couples with 4-aminoantipyrine and 4-chlorophenol to produce red quinoneimine dye. Absorbance of coloured dye was measured at 505 nm and is proportional to amount of total triglycerides concentration in the sample.

Procedure

10 µl of plasma aliquots were pipetted in 10×75 mm tubes in duplicate, to which 1000 µl of working triglycerides reagent was added. Blank (10 µl distilled water) and standard (10 µl from standard 200 mg/dl) was pipetted in duplicate, to which 1000 µl of working triglycerides reagent was added. The content were mixed well and incubated at 37ºC temperature for 10 minutes. UV-spectrophotometer was blanked with reagent blank and the absorbance of standard and test sample was measured at 505 nm.

Calculation The triglyceride concentration was calculated as per the formula and expressed in mg/100ml

$$\text{Triglyceride (mg/100 ml)} = \frac{\text{Absorbance of test}}{\text{Absorbance of standard}} \times 200$$

3.10.2.4 Plasma total protein

Total protein was estimated in blood plasma samples by using “Modified Biuret, End point assay test kit” supplied by Span Diagnosis Ltd. The principal of the assay is that the peptide bonds of proteins react with cupric ions in alkaline solution to form a colored chelate; the absorbance was measured at 578 nm. The absorbance of final color is proportional to the concentration of total protein in the sample.

Procedure

10 µl of plasma aliquot was pipetted in 10×75 mm tubes in duplicate, to which 1000 µl of working Biuret reagent was added. Blank (10 µl distilled water) and standard (10 µl from protein standard 6.5 g/dl) was pipetted in duplicates, to which 1000 µl of working Biuret reagent was added. The content were mixed well and incubated at room temperature for 1 minute. UV-spectrophotometer was blanked with

reagent blank and the absorbance of standard and test sample was measured at 578 nm.

Calculation Total protein concentration was calculated as per formula and expressed in g/100ml

$$\text{Total protein (g/100 ml)} = \frac{\text{Absorbance of test}}{\text{Absorbance of standard}} \times 6.5$$

3.10.2.5 Plasma albumin

Albumin was estimated in blood plasma samples by using "Bromocresol green, End point assay test kit" supplied by Span Diagnosis Ltd. It is based on the principal that albumin binds with anionic dye Bromocresol green (BCG) to form green color complex, which is measured at 630 nm. Kit reagent were prepared and stored as per the instruction provided with the assay kit.

Procedure

10 µl of plasma aliquots were pipetted in 10×75 mm tubes in duplicate, to which1000 µl of working albumin reagent was added. Blank (10 µl distilled water) and standard (10µl from standard 4g/dl) was pipetted in duplicates, to which 1000 µl of working albumin reagent was added. The content were mixed well and incubated at room temperature for 1 minute. UV-spectrophotometer was blanked with reagent blank and the absorbance of standard and test sample was measured at 630 nm.

Calculation The albumin concentration was calculated as per the formula and expressed in g/100ml:

$$\text{Albumin (g/100 ml)} = \frac{\text{Absorbance of test}}{\text{Absorbance of standard}} \times 4$$

3.10.2.6 Plasma globulin

It was determined by subtracting the albumin content from total protein content:

Plasma globulin (g/100ml) = Total protein concentration – albumin concentration

3.10.2.7Aspartate aminotransferase (AST) activity

AST in plasma of bucks was determined by UV modified, kinetic assay AST test kit (Span diagnostic ltd. Surat, India).

Assay Principle

AST catalyses the transamination of L-aspartate and α- ketoglutamate and oxaloacetate. In subsequent reaction, malate dehydrogenase reduces oxaloacetate to malate with simultaneous oxidation of reduced nicotinamide adenine dinucleotide (NADH) to nicotinamide adenine dinucleotide (NAD). The rate of oxidation of NADH is measured kinetically by monitoring the decrease in absorbance at 340 nm and is directly proportional to AST activity in the sample. Lactate dehydrogenase was added to enzyme system to prevent endogenous pyruvate interference, which is normally present in the plasma.

Reagent	Composition	Concentration
1 (Buffer)	Tris buffer (pH 7.8)	80 mmol/l
	L- Aspartate	240 mmol/l
	MDH	≥ 600 U/l
	LD	≥ 600 U/l
2 (Substrate)	α- Ketoglutarate	12 mmol/l
	NADH	0.18 mmol/l

Procedure

Working AST reagent was prepared, as mentioned in test kit protocol, by mixing reagent 1 and reagent 2 in proportion of 1:4. 100 µl of plasma was mixed well with 1000 µl of working AST reagent and read absorbance at 340 nm in biochemical analyzer. Blank the analyzer with purified water. Read absorbance after 60 seconds. The reading was repeated after every 30 seconds i.e. up to 120 seconds. The mean absorbance changed per minute (Δ A/minute) was determined.

Calculation

$$\text{AST activity (IU/I)} = \Delta A/\text{minute} \times K$$

Where; Δ A/minute is change in absorbance per minute and K is kinetic factor

3.10.2.8 Alanine aminotransferase (ALT) activity

ALT in plasma of calves was determined by UV modified, kinetic assay ALT (GPT) test kit (Span diagnostic ltd. Surat, India)

Assay principle

ALT catalyses the transamination of L-alanine and α- ketoglutarate to form pyruvate and L-glutamate. In subsequent reaction, lactate dehydrogenase reduces pyruvate to lactate with simultaneous oxidation of reduced NADH to NAD. The rate of oxidation of NADH is measured kinetically by monitoring the decrease in absorbance at 340 nm and is directly proportional to ALT activity in the sample. Lactate dehydrogenase rapidly and completely reduces endogenous sample pyruvate during the initial incubation period, so that it does not interfere with the assay.

Reagent	Composition	Concentration
1 (Buffer)	Tris buffer (pH 7.5)	100 mmol/l
	L- Alanine	500 mmol/l
	LD	≥ 1200 U/l
2 (Substrate)	α- Ketoglutarate	15 mmol/l
	NADH	0.18 mmol/l

Procedure

Working ALT reagent was prepared, as mentioned in test kit protocol, by mixing reagent 1 and reagent 2 in proportion of 1:4. 100 µl of plasma was mixed well with 1000 µl of working ALT reagent and read absorbance at 340 nm in biochemical analyzer. The analyzer was blanked with purified water. the absorbance was read after 60 seconds. The reading was repeated after every 30 seconds i.e. upto 120 seconds. The mean absorbance changed per minute (Δ A/minute) was determined.

Calculation

$$\text{ALT activity (IU/l)} = \Delta A/\text{minute} \times K$$

Where; Δ A/minute is change in absorbance per minute and K is kinetic factor

3.10.3 Assessment of antioxidant status

Antioxidant enzymes (superoxide dismutase (SOD), Catalase (CAT) and Lipid peroxidation (LPO). The procedure employed for the estimation of each enzyme is discussed below

3.10.3.1 Blood lysate preparation

Blood was taken in 2 ml micro-centrifuge tube and centrifuged at 3000 rpm in refrigerated centrifuge for 15 minute. Plasma was taken off and the buffy coat was discarded and the compact RBC pellet was used for the preparation of the lysate. The pellets were washed thrice with normal saline (0.9% NaCl) solution and stored at -20^0C for further analysis.

1) For SOD estimation, washed RBC pellets were first diluted to 33% with phosphate buffer saline [(PBS) (NaCl, 8g + KCl, 0.2 g +KH_2PO_4, 0.2g +Na_2HPO_4, 0.94g dissolved in about 800ml distilled water and final volume was made upto 1L)] and again diluted 1:10 with PBS and was stored at 4^0C till analysis.
2) The stock solution of hemolysate for catalase estimation, was prepared by taking 0.1 ml of washed pellets and adding 0.9 ml of chilled distilled water.

3.10.3.2 Superoxide Dismutase (SOD) activity

SOD was estimated as per the method described by Madesh and Balasubramanian (1997). It involved generation of super oxide by pyrogallol autoxidation and the inhibition of super oxide dependent reduction of the tetrazolium dye MTT (3- (4-5 dimethyl 2-xl) 2, 5 diphenyltetrazolium bromide) to its formazan which was measured at 570 nm. The reaction was terminated by the addition of dimethyl sulfoxide (DMSO), which helps to solubilize the formazane formed. The colour evolved was stable for many hours and was expressed as SOD units (one unit of SOD is the amount (mg) of hemoglobin required to inhibit the MTT reduction by 50%).

Chemical used and reagents made

1) 100 µM pyragallol (Sisco Research Laboratories Pvt. Ltd, India): 6.3 mg of pyragallol was dissolved in the 5 ml of distilled water. One ml from this solution was added to 100 ml of distilled water.

2) 1.25 mM MTT (Sigma-Aldrich, Missouri, USA): 2.58 mg MTT was dissolved in 5 ml of distilled water.
3) Phosphate buffer saline (PBS): PBS was prepared as described elsewhere for separation of erythrocytes.
4) DMSO (Dimethyl sulfoxide)

Reagents	Sample	Control	Blank (duplicate)
PBS	0.65ml	0.65 ml	0.65 ml
MTT	30 µl	30 µl	30 µl
Hemolysate	10µl	--	--
Pyragallol	75 µl	75 µl	75 µl
Incubated for 5 min at room temperature			
DMSO	0.75 ml	0.75ml	0.75ml
Hemolysate	--	10µl	--

The absorbance of sample was read at 570 nm against blank.

Calculation

$$\text{SOD (U)} = \frac{\text{mg of haemoglobin} \times 50 \times \text{Dilution factor}}{\text{Y \%}} \times 100$$

$$\text{(Y \%) Inhibition of MTT reduction by SOD protein (\%)} = \frac{\text{(OD of test)}}{\text{(OD of control)}} \times 100$$

The Haemoglobin was calculated as discussed earlier (section 3.10.1.1)

3.10.3.3 Catalase

The enzyme activity was estimated by spectrophotometer (Specord 200) by following the method of Aebi (1984).

Reagents

1) Phosphate buffer (50 mM; pH 7.0), prepared by mixing two solutions as below:

Solution A: 8.90 g of $NaHPO_4.2H_2O$ dissolved in DW and volume made to 1 litre.

Solution B: 6.81 g of KH_2PO_4 dissolved in DW and volume made to 1 litre.

To solution A, solution B was added until pH reached to 7.0.

2) H_2O_2 (30mM): Diluted 0.34 ml of 30% H_20_2 with phosphate buffer to 100 ml.

Procedure

Took 100 µl of RBC pellet in 1.5 ml eppendorf tube and to it 900µl of cold distilled water was added for lysis. From this 1 ml dilution (100µl pellet + 900 µl cold water for lysis) 100 µl was taken in a fresh clean test tube and 4.9 ml PBS was added to it. The reaction mixture (total volume of 3.0 ml) contained 2.0 ml of RBC lysate in appropriate dilution with phosphate buffer (50mM, pH 7.0) and 1 ml of H_2O_2 (30mM) at 250C against a blank containing one ml phosphate buffer instead of substrate (H_2O_2) and 2 ml appropriately diluted RBC lysate. The reaction was started by addition of H_2O_2. The decomposition of H_2O_2 was shown by decrease in absorbance at 240 nm using double beam UV/visible spectrophotometer. The initial absorbance was approximately A= 0.500 and the decrease in absorbance was followed for 1 minute. The difference in absorbance per unit time was the measure of catalase activity. The enzyme activity was calculated using an extinction coefficient of 0.0394 litres $mM^{-1}mm^{-1}$ and expressed as µ moles of H_2O_2 consumed /min/g Hb in blood.

$$\text{Catalase} = \frac{(5 \text{ second O.D} - 65 \text{ Second O.D}) \times \text{Dilution factor} \times 3}{\text{Hb g \%} \times 0.0394 \times 1000} \times 100$$

0.0394= extinction coefficient

3.10.3.4 Lipid peroxidation (LPO)

TBARS is the measure of lipid peroxidation. The extent of lipid peroxidation was evaluated in terms of malondialdehyde (MDA) production, which was determined by the method of Shafiq-U-Rehman (1984). One ml packed erythrocyte (33%) was taken to which 1ml of 10%TCA was added and thoroughly mixed, vortex and centrifuged at 2000 rpm of 10 min. To one ml of supernatant liquid, an equal amount of 0.67% TBA was added and kept in boiling water bath for 10 min. The reaction mixture was cooled under running tap water and diluted with one ml distilled water. Absorbance was recorded ad 535 nm. Calculation was done by using the major extinction coefficient of MDA-TBA complex at 535 nm, i.e. 1.56×10^{8}M/cm. The amount of lipid peroxidation was expressed as nM MDA formed /ml packed RBCs.

LPO (nM MDA/ml packed RBCs = (OD/EC) × (Total volume of the reaction mixture/Amount of sample taken) $\times 10^9 \times DF \times 2$ (Incubation time)

Where, EC = 1.56×10^8 M/cm molar extinction coefficient.

3.10.4 Assessment of hormonal status

3.10.4.1 Assay for plasma Cortisol

Cortisol was determined in the plasma of Barbari bucks by "Goat Cortisol ELISA Test kit"

(Catalog No. E0021Go) by Bioassay Technology Laboratory from yangpu District Shanghai, China.

Assay Principle

This kit was an Enzyme-Linked Immunosorbent Assay (ELISA). COR was added to the wells pre- coated with COR monoclonal antibody. After incubation a biotin-conjugated anti goat COR antibody was added and binded to goat COR. After incubation unbound biotin conjugated anti goat COR antibody was washed away during a washing step. Streptavidin-HRP was added and binded to the biotin-conjugated anti- goat COR 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 goat COR. The reaction was terminated by addition of acidic stop solution and absorbance was measured at 450 nm.

Reagent required

Components	Quantity
Standard Solution(64ng/ml)	0.5ml x1
Pre-coated ELISA Plate	12 * 8 well strips x1
Standard Diluent	3ml x1
Streptavidin-HRP	6ml x1
Stop Solution	6ml x1
Substrate Solution A	6ml x1
Substrate Solution B	6ml x1
Wash Buffer Concentrate (30x)	20ml x1
Biotin- Conjugate Anti goat COR Antibody	1ml x1

Reagent Preparation

Standard Preparation

120µl of the standard (64ng/ml) was reconstituted with 120µl of standard diluents was to generate 32ng/ml standard stock solution. The standard was allowed to sit for 15 min with gentle agitation prior to made dilutions. Duplicate standard was prepared by serially diluted the standard stock solution (32ng/ml) 1:2 with standard diluent to produced 16ng/ml, 8ng/ml, 4ng/ml and 2ng/ml solutions. Standard diluent was served as the zero standard (0 mg/ml. Dilution of standard solutions suggested are as followed:

32ng/ml	Standard No.5	120µl Original Standard + 120µl Standard Diluent
16ng/ml	Standard No.4	120µl Standard No.5 + 120µl Standard Diluent
8ng/ml	Standard No.3	120µl Standard No.4 + 120µl Standard Diluent
4ng/ml	Standard No.2	120µl Standard No.3 + 120µl Standard Diluent
2ng/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
64ng/ml	32ng/ml	16ng/ml	8ng/ml	4ng/ml	2ng/ml

Wash Buffer

20 ml of Wash Buffer Concentrate 30X was diluted into deionized or distilled water to yield 500 ml of 1X Wash Buffer.

Assay Procedure

The reagents were brought to room temperature before use. The assay was performed at room temperature. 50µl standard was added to standard well and 40µl sample was added to sample wells and also 10µl anti-COR antibody was added to sample wells then 50µl streptavidin-HRP was added to sample wells and standard wells (Not blank control well). It was mixed thoroughly and the plate was covered with a sealer and was incubated for 60 minutes at 37°C. The sealer was removed and the plate was washed 5 times with wash buffer. The wells were soaked with at least 0.35 ml wash buffer for 30 seconds to 1 minute for each wash. The plate was blotted on paper towels.50µl substrate solution A was added to each well after that 50µl

substrate solution B was also added to each well. The plate was covered with a new sealer and was incubated for 10 minutes at 37°C in the dark. After that 50µl stop solution was added to each well, then blue color was changed into yellow colour immediately. The optical density (OD value) of each well was determined immediately with the help of microplate reader at 450 nm.

Calculation of Result

A standard curve was constructed by plotted the average OD for each standard on the vertical (Y) axis against the concentration on the Horizontal (X) axis and a best fit line curve was drawn through the points on the graph. and the best fit line was determined by regression analysis method.

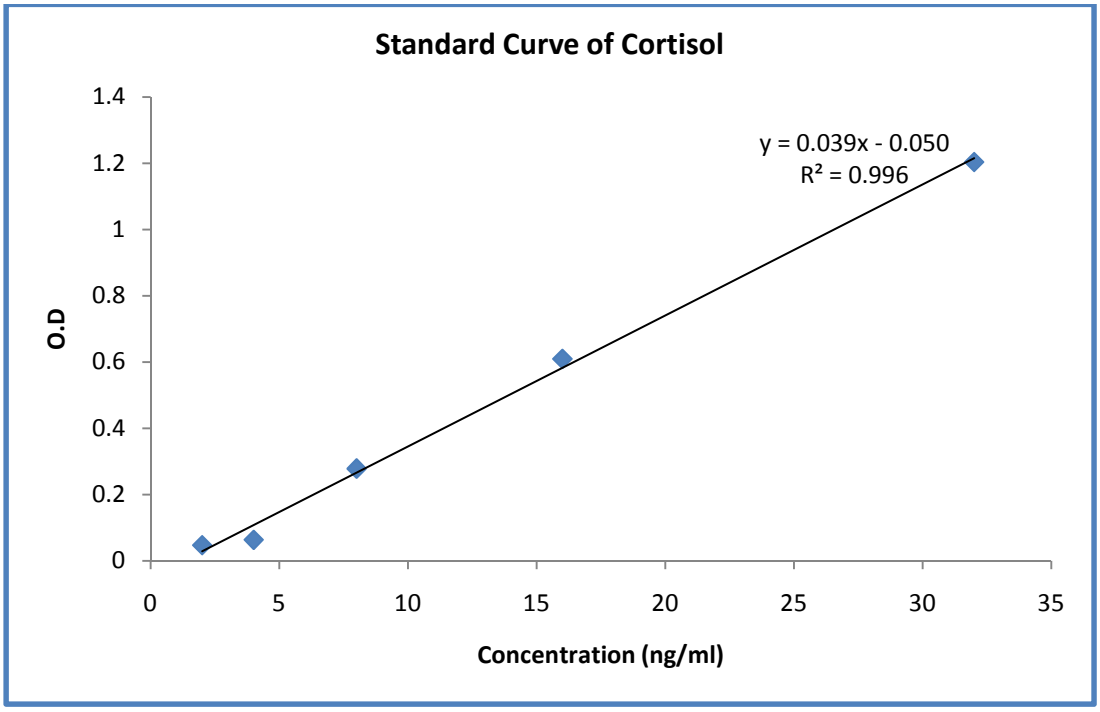


Fig. 3.1 Standard curve of Cortisol

3.10.4.2 Assay for plasma Testosterone

Testosterone was determined in the plasma of Barbari bucks by "Goat Testosterone ELISA Test kit" (Catalog No. E0026Go) by Bioassay Technology Laboratory from yangpu District Shanghai, China.

Assay Principle

This kit is an Enzyme-Linked Immunosorbent Assay (ELISA).The plate had been pre- coated with Goat T antibody. T present in the sample was added and binds

to antibodies coated on the wells. And then biotinylated Goat T antibody was added and binded to T in the sample. Streptavidin- HRP was added and bind to the biotinylated T antibody. After incubation unbound Strepytavidin-HRP was washed away during a washing step. Substrate solution was then added and colour developed in proportion to the amount of goat T. The reaction was terminated by addition of acidic stop solution and absorbance was measured at 450 nm.

Reagent required

Components	Quantity
Standard Solution (32ng/ml)	0.5ml x1
Pre-coated ELISA Plate	12 * 8 well strips x1
Standard Diluent	3ml x1
Streptavidin-HRP	6ml x1
Stop Solution	6ml x1
Substrate Solution A	6ml x1
Substrate Solution B	6ml x1
Wash Buffer Concentrate (30x)	20ml x1
Biotin- Conjugate Anti goat COR Antibody	1ml x1

Reagent Preparation

Standard Preparation

120µl of the standard was reconstituted (32ng/ml) with 120µl of standard diluent to generate a 16ng/ml standard stock solution. The standard was allowed to sit for 15 min with gentle agitation prior to making dilutions. Prepared duplicate standard was prepared by serially diluting the standard stock solution (16ng/ml) 1:2 with standard diluent was to produce 8ng/ml, 4ng/ml, 2ng/ml and 1ng/ml solutions. Standard diluent was served as the zero standard (0 ng/ml). Dilution of standard solutions suggested are as followed:

16ng/ml	Standard No.5	120µl Original Standard + 120µl Standard Diluent
8ng/ml	Standard No.4	120µl Standard No.5 + 120µl Standard Diluent
4ng/ml	Standard No.3	120µl Standard No.4 + 120µl Standard Diluent
2ng/ml	Standard No.2	120µl Standard No.3 + 120µl Standard Diluent
1ng/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
32ng/ml	16ng/ml	8ng/ml	4ng/ml	2ng/ml	1ng/ml

Wash Buffer

20ml of Wash Buffer Concentrate 25X was diluted into deionized or distilled water to yield 500 ml of 1X Wash Buffer.

The reagents were brought to room temperature before use. The assay was performed at room temperature. 50µl standard was added to standard well and 40µl sample was added to sample wells and also 10µl anti-COR antibody was added to sample wells after that 50µl streptavidin-HRP was added to sample wells and standard wells (Not blank control well). It was mixed thoroughly and the plate was covered with a sealer and was incubated for 60 minutes at 37°C. The sealer was removed and the plate was washed 5 times with wash buffer. The wells were soaked with at least 0.35 ml wash buffer for 30 seconds to 1 minute for each wash. The plate was blotted on paper towels. After that 50µl substrate solution A was added to each well also 50µl substrate solution B was added to each well. The plate was covered with a new sealer and was incubated for 10 minutes at 37°C in the dark. After that 50µl stop solution was added to each well, then blue color was changed into yellow colour immediately. The optical density (OD value) of each well was determined immediately with the help of microplate reader at 450 nm.

Calculation of Result

A standard curve was constructed by plotted the average OD for each standard on the vertical (Y) axis against the concentration on the Horizontal (X) axis and a best fit line curve was drawn through the points on the graph and the best fit line was determined by regression analysis method.

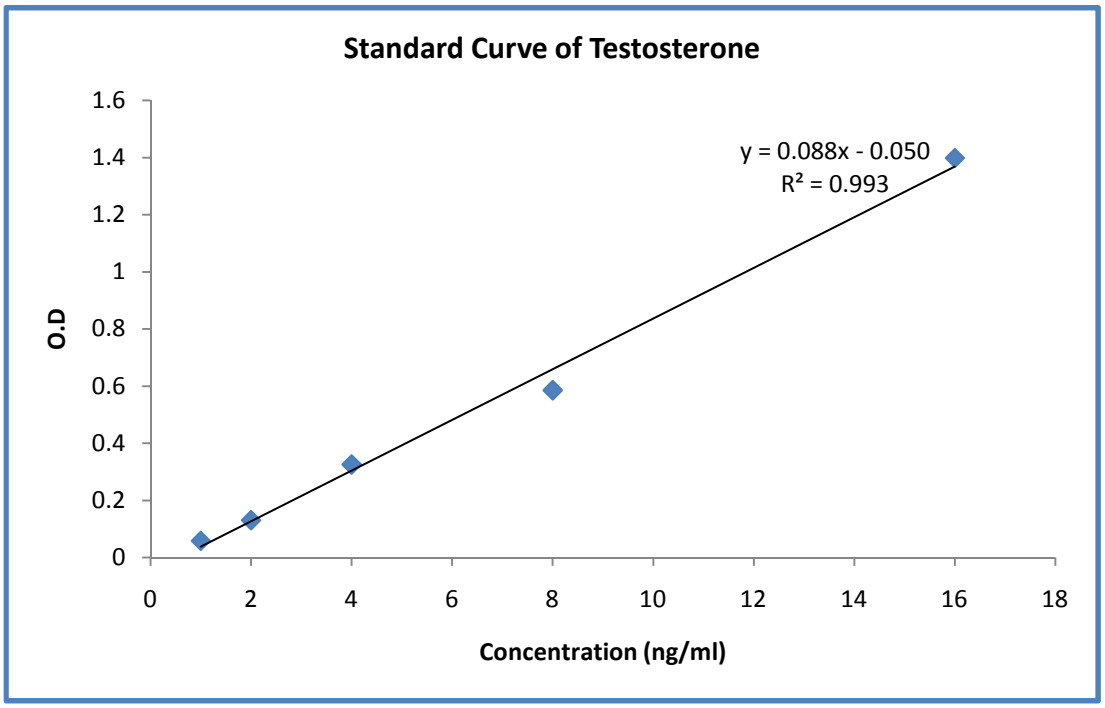
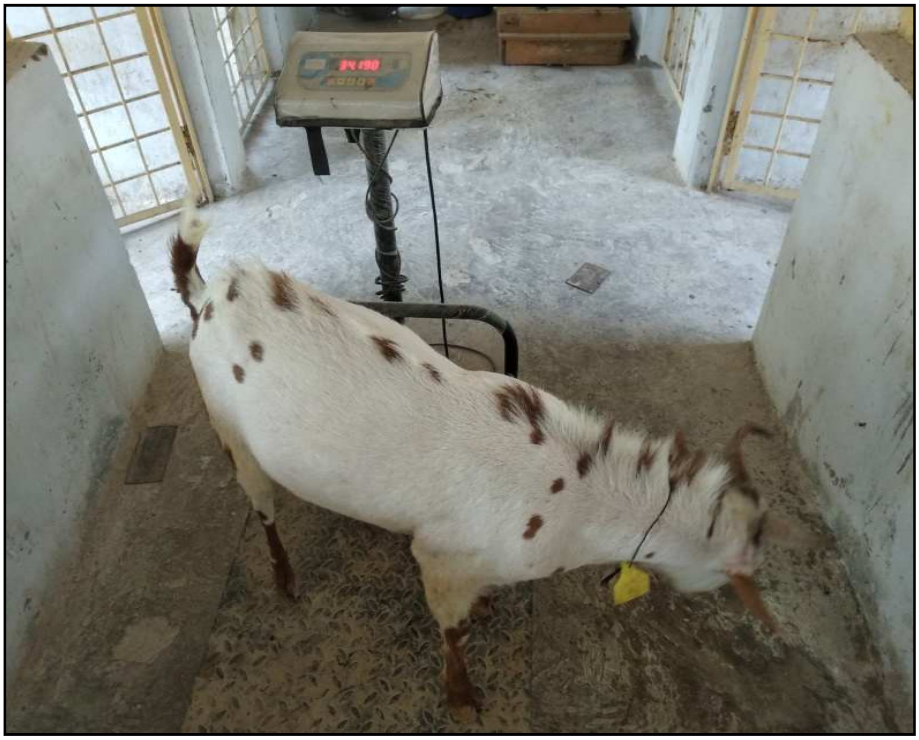


Fig. 3.2 Standard curve of Testosterone

3.10.5 Statistical

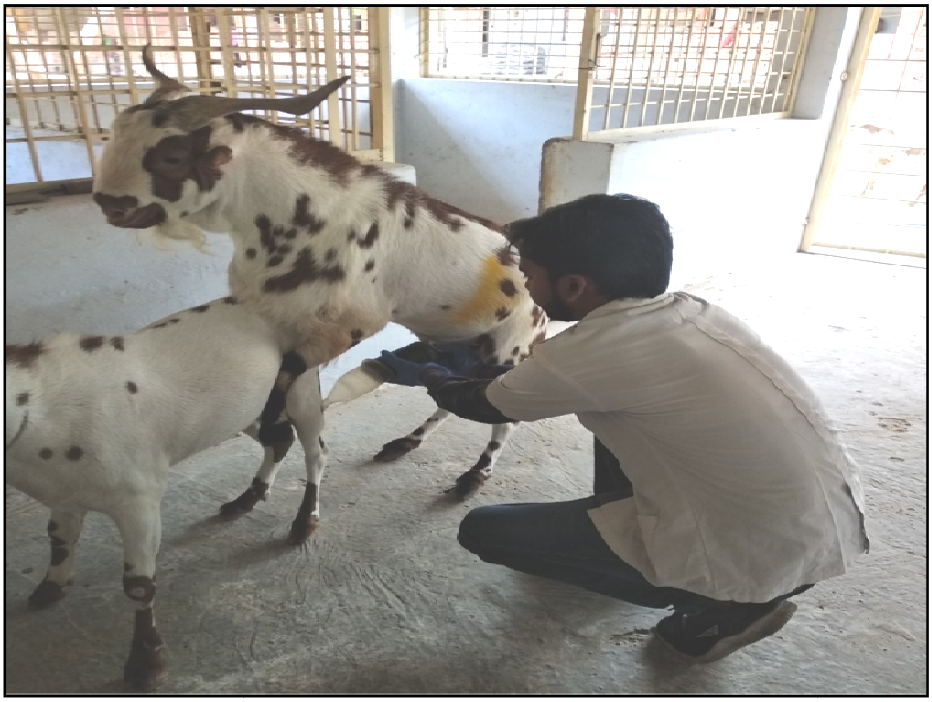
The data was analyzed using the general linear model (GLM) procedure of Statistical Package for the Social Sciences (SPSS Inc., Chicago, IL, USA) as a randomize block design with animal as the experimental unit. The pair-wise comparison of means was carried out by using "Tukey's honest significant difference (HSD) test". Significance was determined at $P<0.05$.

Pic 3.1 Shed of Buck



Pic 3.2 Taking body weight of buck

Pic 3.3 Digestion trial

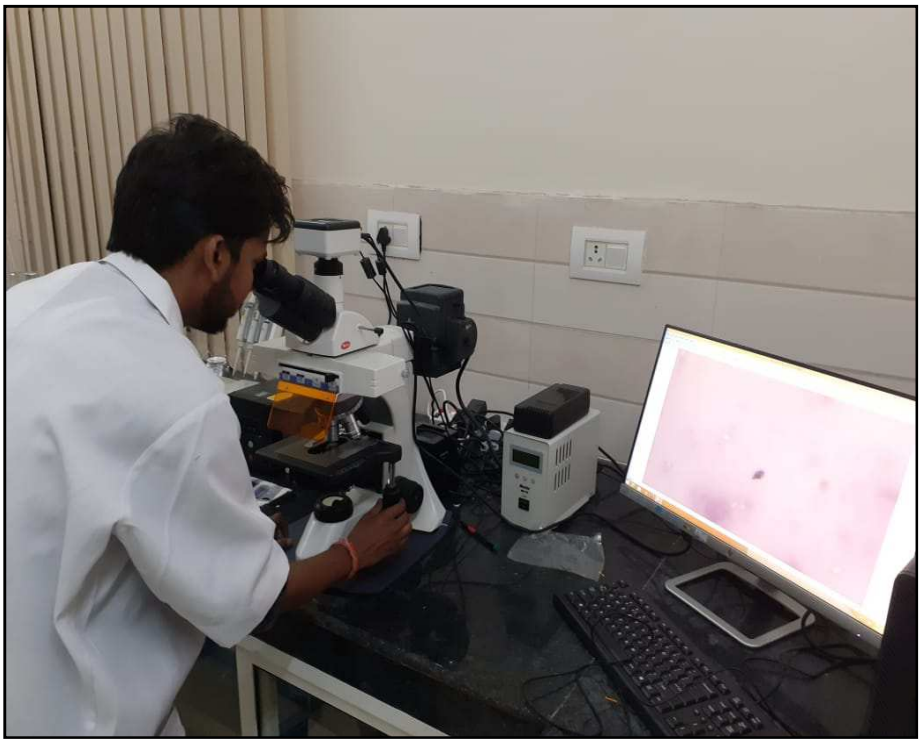


Pic 3.4 Semen collection by using Artificial Vagina

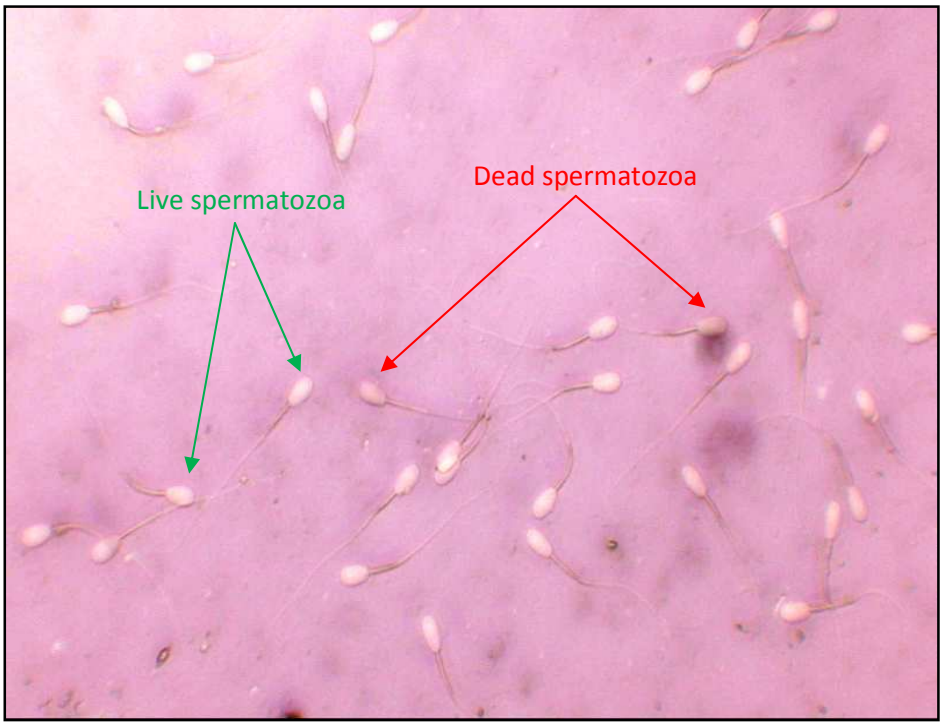
Pic 3.5 Feeding of Buck

Pic 3.6 Estimation of crude protein

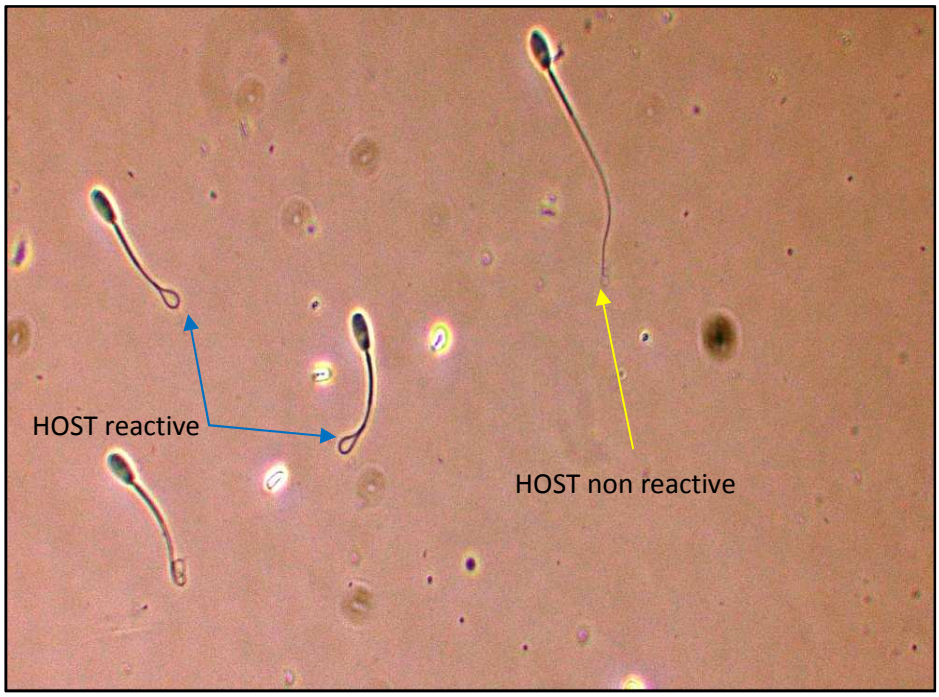
Pic 3.7 Estimation of ether extract



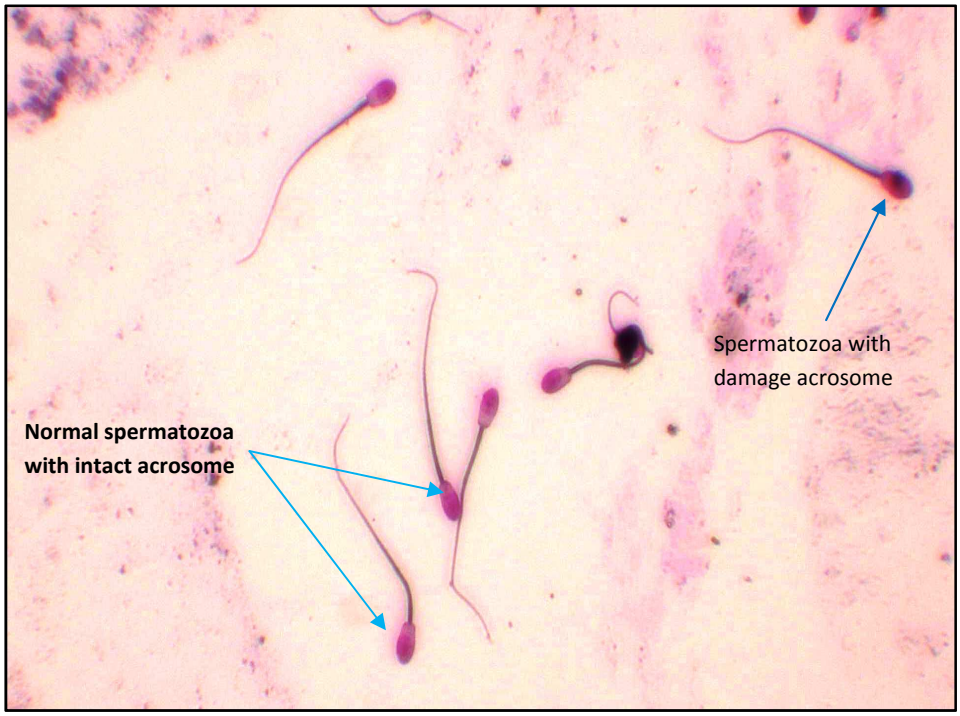
Pic 3.8 Evaluation of semen sample



Pic 3.9 Photograph showing live and dead spermatozoa (Eosin- Nigrosin stain, magnification 100x)



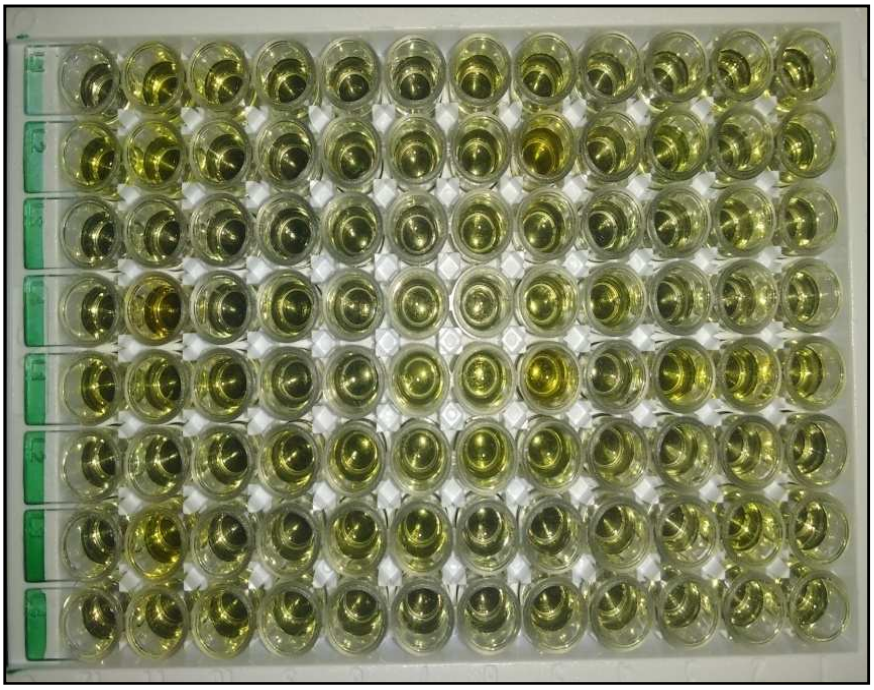
Pic 3.10 Photograph showing hypo osmotic swelling test (HOST) reactive and non reactive spermatozoa (magnification 100x)



Pic 3.11 Photograph showing Acrosomal integrity of Barbari buck spermatozoa (by using giemsa staining method)

Pic 3.12 Biochemical examination

Pic 3.13 Haematological examination



Pic 3.14 ELISA plate

Pic 3.15 ELISA reader

Pic 3.16 Hormone estimation by ELISA kit method

Results

CHAPTER- 4

RESULTS

The objective of the study was to evaluate the effect of replacing protein source in concentrate mixture with dried *Moringa oleifera* leaf powder on the animal growth performance, nutrient utilization, seminal attributes, blood biochemicals, antioxidant status, and hormonal status of Barbari bucks. In this experiment, soybean meal which is used as protein source in concentrate mixture was replaced with 10% and 20% by dried *Moringa oleifera* leaf powder and supplemented to Barbari bucks on dry matter basis. The observations on chemical composition of experimental diets, growth parameters, nutrient utilization, seminal attributes, blood biochemicals, antioxidant status and hormonal status are presented in this chapter.

4.1 Chemical composition (%DM basis) of dietary feed ingredient fed to Barbari buck

The chemical composition of experimental diets and dietary components (on dry matter basis) is presented in Table 4.1. The proximate principle i.e. dry matter, organic matter, ether extract, crude protein, total ash, crude fibre, nitrogen free extract in concentrate mixture were found to be 91, 91.3,2.96, 18.9, 8.72, 12.8 and 55.5% respectively. The corresponding values for arhar straw were 90, 86.1, 2.10, 10.5, 13.9, 28.6 and 41.4% respectively. In case of green berseem the values were 10, 87.8, 3.43, 16.3, 12.2, 19.0 and 49.0% respectively. The values for dried *Moringa oleifera* leaf powder were 91.1, 91.6, 5.45, 24.78, 8.34, 14.32 and 47.11% respectively.

Table 4.1 Chemical composition (%DM basis) of dietary feed ingredient fed to Barbari bucks.

Sr. No	Item	Concentrate	Arhar straw	Green berseem	*Moringa oleifera*	Control TMR	T10 TMR	T20 TMR
1	DM	91.00	90.00	10.00	91.11	71.32	71.50	71.40
2	OM	91.30	86.10	87.80	91.66	88.55	88.91	88.90
3	EE	2.96	2.10	3.43	5.45	11.45	11.05	11.06
4	CP	18.90	10.50	16.30	24.78	14.89	14.89	14.89
5	ASH	8.72	13.90	12.20	8.34	11.45	11.41	11.42
6	CF	12.80	28.60	19.00	14.32	20.19	21.09	20.46
7	NFE	55.50	41.40	49.07	47.11	48.31	48.15	48.69
8	NDF	51.10	62.90	49.87	33.46	52.10	52.32	52.71
9	ADF	13.10	38.70	38.64	26.92	28.45	28.96	28.87
10	ADL	1.50	3.60	2.90	2.80	2.49	2.55	2.63
11	Cellulose	11.70	35.20	35.80	23.20	25.20	26.40	26.40
12	Hemicellulose	38.10	24.30	11.30	18.00	24.60	23.30	23.70
13	Calcium	0.90	0.60	1.60	1.20	0.96	0.97	0.98
14	Phosphorus	0.40	0.41	0.70	0.80	0.49	0.51	0.53

The percentage of cell wall constituent as per Van Soest fraction i.e. NDF, ADF, ADL, cellulose and hemicelluloses in concentrate mixture was 51.1, 13.1, 1.50, 11.7 and 38.1 % respectively. The corresponding values in arhar straw were 62.9, 38.7, 3.60, 35.2 and 24.3% respectively. The values in green berseem were 49.87, 38.6, 2.9, 35.8, and 11.3% respectively. In dried *Moringa oleifera* leaf powder the values were 33.46, 26.92, 2.8, 23.2, 18% respectively. The percentage of calcium and phosphorus in concentrate mixture were 0.9 and 0.4 % respectively. In arhar straw wit was 0.6 and 0.41% respectively. The corresponding values in green berseem were 1.60 and 0.70 % respectively. In dried *Moringa oleifera* leaf powder the values were found to be 1.2 and 0.8 % respectively. The nutrient content in total mixed ration was according to the value of feed ingredients.

4.2 Different growth parameter in buck fed ration replacing soybean meal with dried *Moringa oleifera* leaf powder (MOLP)

4.2.1 Body weight (BW) and Metabolic body weight ($W^{0.75}$)

Average BW of Barbari bucks of all three groups measured at fortnight intervals during the three months experimental period has been given in Table 4.2. Initially, average BW of Barbari bucks were 28.00, 28.00 and 28.00 kg in Control, T10 and in T20 groups respectively. Final body weights of corresponding groups were 34.40, 34.35 and 33.60 kg, respectively. The average BW of all the fortnights was not significantly different ($P>0.05$) between groups. The overall BW (kg) was found similar in all the experimental groups. Average metabolic body weight of Barbari bucks of all three groups measured at fortnight intervals during the three months experimental period has been given in Table 4.3. Initially, average Metabolic body weights ($W^{0.75}$) of Barbari bucks were 12.09, 12.12 and 12.15 kg in Control, T10 and T20 groups respectively. Final Metabolic body weights of corresponding groups were 14.14, 14.15 and 13.92 kg, respectively. The averages values of all the fortnights were not significantly different ($P>0.05$) within the groups.

Table 4.2 Body weight (kg) in buck fed ration replacing soybean meal with dried *Moringa oleifera* leaf powder (MOLP)

Days	Treatment			SEM	P value
	Control	T10	T20		
0	28.00	28.00	28.00	2.53	0.99
15	28.98	28.86	28.80	2.52	0.99
30	29.96	29.90	29.58	2.52	0.99
45	31.10	30.95	30.56	2.51	0.98
60	32.23	32.18	31.53	2.47	0.97
75	33.38	33.20	32.68	2.44	0.97
90	34.40	34.35	33.60	2.40	0.96
Total	31.15	31.06	30.68	0.93	0.93

Table 4.3 Metabolic body weight ($W^{0.75)}$ in buck fed ration replacing soybean meal with dried *Moringa oleifera* leaf powder (MOLP)

Days	Treatment			SEM	P value
	Control	T10	T20		
0	12.09	12.12	12.15	0.81	0.99
15	12.41	12.41	12.41	0.80	0.99
30	12.73	13.09	12.66	0.79	0.99
45	13.09	13.08	12.98	0.78	0.99
60	13.46	13.47	13.29	0.76	0.98
75	13.82	13.79	13.65	0.75	0.98
90	14.14	14.15	13.92	0.73	0.97
Total	13.10	13.15	13.00	0.96	0.96

4.2.2 Dry matter intake (DMI)

Dry matter intake (kg/d) in Control, T10 and T20 groups during different fortnights of experimental feeding has been presented in Table 4.4. At fortnightly interval DMI of Control, T10 and T20 ranged from 0.91 to 1.19, 0.90 to 1.15 and 0.86 to 1.00 kg/d respectively. Overall DMI (kg/d) in present study was found significantly higher ($P<0.05$) and comparable in T10 and Control than T20 group. The percent DMI (kg/100 kg BW) in experimental animals in Control, T10 and T20 groups during

different fortnights of experimental feeding has been presented in Table 4.5. The percent DMI was significantly higher (P<0.05) and comparable in T10 and Control than T20 group in second, third, fourth, fifth and sixth fortnight. Overall percentage DMI (kg/100 kg BW) in present study was also found significantly higher (P<0.05) in T10 and Control group as compared to T20 group.

Table 4.4 Dry matter intake (kg/day) in buck fed ration replacing soybean meal with dried *Moringa oleifera* leaf powder (MOLP)

Days	Treatment			SEM	P value
	Control	T10	T20		
15	0.91	0.90	0.86	0.10	0.93
30	1.02	1.00	0.88	0.10	0.60
45	1.06	1.03	0.93	0.10	0.66
60	1.09	1.09	0.97	0.10	0.62
75	1.15	1.11	0.99	0.10	0.51
90	1.19	1.15	1.00	0.09	0.37
Total	1.07^{b}	1.04^{b}	0.94^{a}	0.02	0.02

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

Table 4.5 Dry matter intake (kg/100kg BW) in buck fed ration replacing soybean meal with dried *Moringa oleifera* leaf powder (MOLP)

Days	Treatment			SEM	P value
	Control	T10	T20		
15	3.20	3.20	3.05	0.08	0.38
30	3.49^{b}	3.46^{b}	3.04^{a}	0.07	0.01
45	3.51^{b}	3.45^{b}	3.12^{a}	0.07	0.03
60	3.52^{b}	3.50^{b}	3.12^{a}	0.08	0.04
75	3.54^{b}	3.42^{b}	3.05^{a}	0.10	0.01
90	3.50^{b}	3.42^{b}	2.99^{a}	0.08	0.01
Total	3.46^{b}	3.41^{b}	3.06^{a}	0.03	0.01

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

4.2.3 Body weight gain

The fortnightly BW gain of Barbari bucks of Control, T10 and T20 groups have been presented in Table 4.6. Fortnightly body weight gain (kg) ranged from 0.98 to 1.15, 0.87 to 1.23 and 0.80 to 1.15kg in Control, T10 and T20 group respectively. At second and fourth fortnight, T10 and Control group animals showed significantly higher ($P<0.05$) fortnightly body weight gain as compared to T20 group. The overall fortnightly BW gain (kg) in present study was found significantly higher ($P<0.05$) and comparable in T10 and Control group as compared to T20 group. Average daily gain (g/d) of Barbari bucks of all the three groups measured at fortnightly intervals is presented in Table 4.7. At first fortnight the average daily gain were 65.56, 57.78 and 53.33 g/d in Control, T10 and T20 groups, respectively. The overall average daily gain (g/d) was found significantly higher ($P<0.05$) and comparable in T10 and Control group as compared to T20 group.

Table 4.6 Fortnightly body weight gain (kg) in buck fed ration replacing soybean meal with dried *Moringa oleifera* leaf powder (MOLP)

Days	Treatment			SEM	P value
	Control	T10	T20		
15	0.98	0.87	0.80	0.07	0.21
30	0.98^{b}	1.04^{b}	0.78^{a}	0.07	0.04
45	1.13	1.04	0.98	0.07	0.35
60	1.13^{b}	1.23^{b}	0.97^{a}	0.07	0.04
75	1.15	1.02	1.15	0.11	0.61
90	1.02	1.15	0.92	0.11	0.34
Total	1.07^{b}	1.06^{b}	0.92^{a}	0.37	0.02

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

Table 4.7 Average daily gain (g) in buck fed ration replacing soybean meal with dried *Moringa oleifera* leaf powder (MOLP)

Days	Treatment			SEM	P value
	Control	T10	T20		
15	65.56	57.78	53.33	4.67	0.21
30	65.55	69.33	52.22	4.66	0.05
45	75.55	69.56	65.55	4.73	0.35
60	75.55	82.22	64.44	4.66	0.05
75	76.67	67.78	76.66	7.16	0.61
90	67.78	76.67	61.11	7.21	0.34
Total	71.11^{b}	70.55^{b}	62.2^{a}	2.44	0.02

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

4.2.4 Feed conversion ratio (FCR)

The feed conversion ratio of experimental animals at different fortnight is given in Table 4.8. The FCR ranged from 14.20 to 19.40, 13.51 to 21.75 and 13.10 to 18.08 in Control, T10 and T20 groups respectively. The averages values of FCR of all the fortnights were not significantly different ($P>0.05$) within the groups. The overall FCR was found similar in all the experimental groups.

Table 4.8 Feed conversion ratio (FCR) in buck fed ration replacing soybean meal with dried *Moringa oleifera* leaf powder (MOLP)

Days	Treatment			SEM	P value
	Control	T10	T20		
15	14.20	16.67	16.55	2.22	0.68
30	16.18	14.63	18.02	2.33	0.60
45	14.37	15.22	14.70	1.84	0.95
60	15.96	13.51	15.00	2.34	0.76
75	15.77	21.75	13.10	4.64	0.42
90	19.40	15.95	17.15	2.94	0.71
Total	15.98	16.29	15.76	1.14	0.95

4.2.5. Nutrient intake and digestibility in buck fed ration replacing soybean meal with dried *Moringa oleifera* leaf powder (MOLP)

The data pertaining to digestibility coefficient of Control and treatments group have been presented in Table 4.9. The DM digestibility was 64.40, 64.03 and 66.70 % in Control, T10 and T20 groups, respectively. Whereas, the OM digestibility was 66.54, 65.83 and 68.74 % in Control, T10 and T20 groups, respectively. The statistical analysis of data on DM and OM digestibility coefficient revealed that there were no significant difference (P>0.05) between the groups. The CP digestibility coefficient in Control, T10 and T20 groups were 70.88, 69.33 and 70.01%, respectively. The EE digestibility coefficient in Control, T10 and T20 groups were 80.72, 79.31 and 80.04% respectively. The statistical analysis of data on CP and EE digestibility coefficient revealed that there were no significant different (P>0.05) between the groups. The CF digestibility coefficient in Control, T10 and T20 groups were 57.60, 55.59 and 56.40% respectively. The NFE digestibility coefficient in Control, T10 and T20 groups were 77.28, 77.93 and 77.69 % respectively. The digestibility coefficient of CF and NFE were also found similar in all Control and treatments group. The NDF digestibility coefficient in Control, T10 and T20 groups were 58.59, 56.89 and 56.19 % respectively. The ADF digestibility coefficient in Control, T10 and T20 groups were 53.25, 50.49 and 51.10 % respectively. Both NDF and ADF digestibility coefficient were found similar in Control and treatments groups. Digestible DM, OM, EE, NFE, CF, NDF, ADF and TDN intake were found similar in all the three groups (Table 4.10). Total body weight and body weight gain was found similar in all the experimental groups during digestion trial.

Table 4.9 Nutrient intake and digestibility in buck fed ration replacing soybean meal with dried *Moringa oleifera* leaf powder (MOLP)

Attributes	Treatment			SEM	P value
	Control	T10	T20		
Initial wt(kg)	30.41	29.86	29.76	2.62	0.98
Final wt (kg)	30.94	30.39	30.27	2.62	0.98
Wt gain(kg)	0.53	0.53	0.51	0.26	0.86
DM intake kg/day	0.98	0.91	0.97	0.10	0.88
CP intake kg/day	0.16	0.15	0.17	0.02	0.73
DCP intake kg/day	0.12	0.12	0.12	0.01	0.72
DCP intake g/kg $W^{0.75}$	8.93	8.09	9.30	0.60	0.36
TDN intake kg/day	0.69	0.62	0.65	0.08	0.84
TDN intake g/kg $W^{0.75}$	51.76	47.18	50.65	3.14	0.57
Digestibility coefficient %					
DM digestibility	64.40	64.03	66.70	0.63	0.18
OM digestibility	66.54	65.83	68.74	0.59	0.11
CP digestibility	70.88	69.33	70.01	0.78	0.74
CF digestibility	57.6	55.59	56.40	0.93	0.70
EE digestibility	80.72	79.31	80.04	0.79	0.78
NFE digestibility	77.28	77.93	77.69	0.59	0.91
NDF digestibility	58.59	56.89	56.19	0.80	0.48
ADF digestibility	53.25	50.49	51.10	1.21	0.65

Table 4.10 Digestible nutrient intake in buck fed ration replacing soybean meal with dried *Moringa oleifera* leaf powder (MOLP)

Attributes	Treatment			SEM	P value
	Control	T10	T20		
DM	0.73	0.65	0.68	0.09	0.83
OM	0.65	0.59	0.62	0.08	0.86
CP	0.12	0.11	0.12	0.01	0.69
CF	0.12	0.10	0.11	0.02	0.78
EE	0.02	0.02	0.02	0.03	0.65
CF	0.12	0.10	0.11	0.02	0.78
NFE	0.39	0.36	0.38	0.04	0.88
NDF	0.38	0.32	0.35	0.05	0.66
ADF	0.14	0.12	0.12	0.02	0.72
TDN	0.69	0.62	0.65	0.08	0.84

4.3 Seminal attributes

4.3.1 Ejaculated semen volume (ml)

Ejaculated semen Volume (ml) in Control, T10 and T20 groups during different collection interval of experimental feeding has been presented in Table 4.11. At different collection interval volume of ejaculated semen (ml) of Control, T10 and T20 ranged from 0.55 to 0.66, 0.55 to 0.70 and 0.51 to 0.66 ml respectively. Overall ejaculated volume of semen (ml) in present study was found similar in all experimental groups.

Table 4.11 Ejaculated Semen volume (ml) in buck fed ration replacing soybean meal with dried *Moringa oleifera* leaf powder (MOLP)

Interval	Treatment			SEM	P value
	Control	T10	T20		
1st collection	0.55	0.55	0.51	0.05	0.88
2nd collection	0.58	0.56	0.55	0.05	0.90
3rd collection	0.60	0.58	0.56	0.04	0.84
4th collection	0.65	0.63	0.61	0.04	0.87
5th collection	0.66	0.68	0.66	0.04	0.93
6th collection	0.66	0.70	0.65	0.04	0.68
Total	0.62	0.62	0.60	0.02	0.66

4.3.2 Colour of semen

Colour of semen in Control, T10 and T20 groups during different collection interval of experimental feeding has been presented in Table 4.12. At different collection interval colour of semen is Creamy white colour.

Table 4.12 Colour of semen in buck fed ration replacing soybean meal with dried *Moringa oleifera* leaf powder (MOLP)

Intervals	Treatment		
	Control	T10	T20
1st collection	Creamy white colour	Creamy white colour	Creamy white colour
2nd collection	Creamy white colour	Creamy white colour	Creamy white colour
3rd collection	Creamy white colour	Creamy white colour	Creamy white colour
4th collection	Creamy white colour	Creamy white colour	Creamy white colour
5th collection	Creamy white colour	Creamy white colour	Creamy white colour
6th collection	Creamy white colour	Creamy white colour	Creamy white colour

4.3.3 Seminal pH

Seminal pH in Control, T10 and T20 groups during different collection interval of experimental feeding has been presented in Table 4.13. At different collection interval mean of seminal pH of all Control, T10 and T20 groups was 6.8. Overall seminal pH in present study was found similar in all experimental groups.

Table 4.13 Seminal pH in buck fed ration replacing soybean meal with dried *Moringa oleifera* leaf powder (MOLP)

Intervals	Treatment			SEM	P value
	Control	T10	T20		
1st collection	6.7	6.8	6.8	0.01	0.39
2nd collection	6.7	6.8	6.8	0.01	0.39
3rd collection	6.8	6.8	6.7	0.01	0.39
4th collection	6.8	6.7	6.8	0.01	0.39
5th collection	6.7	6.8	6.8	0.01	0.39
6th collection	6.8	6.7	6.8	0.01	0.39
Total	6.8	6.8	6.8	0.04	0.59

4.3.4 Concentration of spermatozoa (millions/ml)

Concentration of spermatozoa (millions/ml) in Control, T10 and T20 groups during different collection interval of experimental feeding has been presented in Table 4.14. At different collection interval concentration (millions/ml) of Control, T10 and T20 ranged from 3716 to 3816, 3766 to 3850 and 3733 to 3816 million respectively. Overall concentration of spermatozoa in present study was found similar in all experimental groups.

Table 4.14 Concentration of spermatozoa (millions/ml) in buck fed ration replacing soybean meal with dried *Moringa oleifera* leaf powder (MOLP)

Intervals	Treatment			SEM	P value
	Control	T10	T20		
1st collection	3716	3800	3766	21.5	0.05
2nd collection	3783	3766	3750	25.1	0.65
3rd collection	3800	3800	3766	32.2	0.70
4th collection	3800	3800	3733	32.2	0.27
5th collection	3816	3850	3816	28.2	0.63
6th collection	3800	3833	3783	30.1	0.50
Total	3786	3808	3775	11.9	0.14

4.3.5 Mass motility (0-5 scales)

Mass motility of sperms in Control, T10 and T20 groups during different collection interval of experimental feeding has been presented in Table 4.15. At different collection interval mass motility of Control, T10 and T20 ranged from 3.66 to 4.00, 4.25 to 3.97 and 3.66 to 4.00 respectively. Overall mass motility of sperm was found significantly higher (P<0.05) in treatment T10 as compared to Control and T20 group.

Table 4.15 Mass motility in buck fed ration replacing soybean meal with dried *Moringa oleifera* leaf powder (MOLP)

Intervals	Treatment			SEM	P value
	Control	T10	T20		
1st collection	3.66	4.08	3.66	0.16	0.15
2nd collection	3.83^{ab}	4.25^{b}	3.75^{a}	0.13	0.04
3rd collection	4.00	3.97	3.75	0.15	0.51
4th collection	3.91	4.08	3.91	0.08	0.18
5th collection	3.91	4.25	4.00	0.11	0.17
6th collection	4.00	4.16	3.97	0.13	0.41
Total	3.88^{a}	4.12^{b}	3.80^{a}	0.12	0.01

Means with different superscript in row differ significantly (P<0.05)

4.3.6 Percent progressive motility of spermatozoa

Percent progressive motility of spermatozoa in Control, T10 and T20 groups during different collection interval of experimental feeding have been presented in Table 4.16. At different collection interval percent progressive motility of spermatozoa of Control, T10 and T20 ranged from 82.50 to 85.83, 85.33 to 87.33 and 80.07 to 85.33% respectively. In fourth collection percent progressive motility of spermatozoa count was significantly lower (P<0.05) in T20 group as compared to Control and T10 group. Overall percent progressive motility of spermatozoa count was found significantly higher (P<0.05) in T10 group animals and significantly lower (P<0.05) in T20 group.

Table 4.16 Percent progressive motility of spermatozoa in buck fed ration replacing soybean meal with dried *Moringa oleifera* leaf powder (MOLP)

Intervals	Treatment			SEM	P value
	Control	T10	T20		
1st collection	82.83^{ab}	86.50^{b}	80.07^{a}	1.50	0.03
2nd collection	84.00^{ab}	85.50^{b}	80.83^{a}	1.20	0.04
3rd collection	82.50^{ab}	87.33^{b}	81.66^{a}	1.41	0.02
4th collection	84.01^{b}	85.83^{b}	80.33^{a}	0.99	0.03
5th collection	83.60	85.33	83.16	0.99	0.29
6th collection	85.83	87.16	85.33	1.10	0.49
Total	83.80^{b}	86.27^{c}	81.88^{a}	0.51	0.01

Means with different superscript in row differ significantly (P<0.05)

4.3.7 Percent live spermatozoa

Percent live spermatozoa in Control, T10 and T20 groups during different collection interval of experimental feeding have been presented in Table 4.17. At different collection interval percent live spermatozoa of Control, T10 and T20 ranged from 87.83 to 90.83, 91.66 to 92.83 and 85.83 to 90.66% respectively. Overall percent live spermatozoa count was found significantly higher (P<0.05) in T10 as compared to Control and T20 group.

Table 4.17 Percent live spermatozoa in buck fed ration replacing soybean meal with dried *Moringa oleifera* leaf powder (MOLP)

Intervals	Treatment			SEM	P value
	Control	T10	T20		
1st collection	87.83^{ab}	92.33^{b}	83.66^{a}	1.72	0.01
2nd collection	89.16^{ab}	91.66^{b}	85.83^{a}	1.22	0.01
3rd collection	88.50	91.66	86.50	1.52	0.08
4th collection	90.83^{ab}	91.66^{b}	87.66^{a}	0.99	0.03
5th collection	90.33	91.83	88.83	1.05	0.16
6th collection	90.66	92.83	90.66	1.08	0.29
Total	89.55^{b}	92.00^{c}	87.19^{a}	0.55	0.01

Means with different superscript in row differ significantly (P<0.05)

4.3.8 HOST reactive spermatozoa (%)

HOST reactive spermatozoa (%) in Control, T10 and T20 groups during different collection interval of experimental feeding have been presented in Table 4.18. At different collection interval HOST reactive spermatozoa (%) of Control, T10 and T20 ranged from 80.16 to 84.00, 84.16 to 86.16 and 78.33 to 81.50 % respectively. In second and fourth collection percent HOST reactive spermatozoa count was significantly (P<0.05) higher in T10 group and significantly lower (P<0.05) in T20group. In sixth collection, percent HOST reactive spermatozoa count was found significantly higher (P<0.05) in T10 as compared to Control and T20 group. Overall percent HOST reactive spermatozoa count was found significantly higher (P<0.05) in T10 group as compared to Control and T20 group.

Table 4.18 HOST reactive spermatozoa percentage in buck fed ration replacing soybean meal with dried *Moringa oleifera* leaf powder (MOLP)

Intervals	Treatment			SEM	P value
	Control	T10	T20		
1st collection	80.16	84.16	79.00	1.47	0.06
2nd collection	82.00^{b}	84.50^{c}	79.33^{a}	0.72	0.01
3rd collection	81.00	84.50	80.80	1.18	0.07
4th collection	82.66^{b}	86.00^{c}	78.33^{a}	0.73	0.01
5th collection	84.00^{ab}	86.16^{b}	81.50^{a}	0.97	0.01
6th collection	83.00^{a}	85.83^{b}	81.00^{a}	0.69	0.01
Total	82.13^{b}	85.19^{c}	80.00^{a}	0.42	0.01

Means with different superscript in row differ significantly (P<0.05)

4.3.9 Intact acrosomal percentage

Intact acrosomal percentage in Control, T10 and T20 groups during different collection interval of experimental feeding have been presented in Table 4.19. At different collection interval intact acrosomal percentage of Control, T10 and T20 ranged from 79.00 to 84.83, 82.00 to 86.19 and 77.50 to 82.00 % respectively. In fifth collection intact acrosomal percentage was found significantly lower in (P<0.05) in T20 group and in sixth collection intact acrosomal percentage was significantly higher (P<0.05) in T10 group as compared to Control and T20 group. Overall intact acrosomal percentage was found significantly higher (P<0.05) in T10 as compared to Control and T20 group bucks.

Table 4.19 Intact Acrosomal percentages in buck fed ration replacing soybean meal with dried *Moringa oleifera* leaf powder (MOLP)

Intervals	Treatment			SEM	P value
	Control	T10	T20		
1st collection	79.00	82.00	79.50	1.43	0.20
2nd collection	79.83	83.33	79.00	1.22	0.05
3rd collection	81.50^{ab}	85.00^{b}	77.50^{a}	1.22	0.002
4th collection	82.33^{ab}	85.66^{b}	78.83^{a}	1.05	0.001
5th collection	84.83^{b}	84.83^{b}	81.00^{a}	0.65	0.001
6th collection	81.66^{a}	86.19^{b}	82.00^{a}	0.75	0.001
Total	81.52^{b}	84.58^{c}	79.63^{a}	0.49	0.001

Means with different superscript in row differ significantly (P<0.05)

4.3.10 Total morphological abnormality percentage

Total morphological abnormality percentage in Control, T10 and T20 groups during different collection interval of experimental feeding has been presented in Table 4.20. At different collection interval total morphological abnormality percentage of Control, T10 and T20 ranged from 3.08 to 3.50, 3.00 to 3.16 % and 3.16 to 3.66 % respectively. Overall total morphological abnormality percentage of spermatozoa in present study was found similar in all experimental groups.

Table 4.20 Total morphological abnormality percentage in buck fed ration replacing soybean meal with dried *Moringa oleifera* leaf powder (MOLP)

Intervals	Treatment			SEM	P value
	Control	T10	T20		
1st collection	3.33	3.08	3.33	0.09	0.15
2nd collection	3.50	3.08	3.66	0.19	0.13
3rd collection	3.16	3.01	3.41	0.13	0.11
4th collection	3.08	3.00	3.25	0.13	0.42
5th collection	3.08	3.16	3.16	0.09	0.79
6th collection	3.16	3.00	3.16	0.11	0.50
Total	3.22	3.05	3.36	0.06	0.06

4.4 Haematological parameters

4.4.1 Blood haemoglobin concentration

Blood haemoglobin concentration (g/dl) of experimental animals at monthly interval is presented in Table 4.21. The overall concentration of haemoglobin at end of experiment was found to be 6.88, 7.13 and 7.55g/dl in Control, T10 and T20 groups, respectively. In last fortnight the haemoglobin concentration (g/dl) in T20 groups was found significantly higher (P<0.05) than Control and T10 group. Overall haemoglobin concentration (g/dl) was found significantly higher (P<0.05) in T20 group than Control and T10 group bucks.

Table 4.21 Blood Haemoglobin concentration (g/dl) in buck fed ration replacing soybean meal with dried *Moringa oleifera* leaf powder (MOLP)

Days	Treatment			SEM	P value
	Control	T10	T20		
0	6.57	6.95	7.28	0.37	0.06
30	6.78	7.10	7.31	0.22	0.26
60	6.91	7.18	7.66	0.25	0.14
90	7.26^{a}	7.28^{a}	7.98^{b}	0.16	0.02
Total	6.88^{a}	7.13^{a}	7.55^{b}	0.13	0.01

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

4.4.2 Packed cell volume (%)

The Packed cell volume (%) of experimental animals at monthly interval is presented in Table 4.22. The overall PCV percentage was found to be 19.76, 19.87 and 22.08% in Control, T10 and T20 groups respectively. Overall packed cell volume (%) was found significantly higher (P<0.05) in T20 group than Control and T10 group bucks.

Table 4.22 Packed cell volume (%) in buck fed ration replacing soybean meal with dried *Moringa oleifera* leaf powder (MOLP)

Days	Treatment			SEM	P value
	Control	T10	T20		
0	17.73	16.63	19.86	0.99	0.97
30	20.91	18.78	20.20	0.71	0.13
60	18.31	19.61	21.36	0.85	0.07
90	22.10	24.45	26.60	1.31	0.08
Total	19.76^{a}	19.87^{a}	22.08^{b}	0.70	0.04

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

4.5 Blood biochemical parameters

4.5.1 Plasma glucose

The plasma glucose concentration (mg/dl) of experimental bucks at monthly interval is presented in Table 4.23. The value ranged from 45.08 to 69.77 mg/dl in Control, 52.45 to 66.71 mg/dl in T10 and 46.97 to 71.20 mg/dl in T20 groups. The overall glucose concentration (mg/dl) of treatments group was found similar with Control group for whole trial period.

Table 4.23 Plasma glucose concentration (mg/dl) in buck fed ration replacing soybean meal with dried *Moringa oleifera* leaf powder (MOLP)

Day	Treatment			SEM	P value
	Control	T10	T20		
0	63.48	65.29	64.51	3.17	0.98
30	69.77	66.71	71.20	2.04	0.68
60	59.39	59.86	66.62	3.10	0.60
90	45.08	52.45	46.97	2.19	0.38
Total	59.43	61.08	62.33	1.60	0.77

4.5.2 Plasma cholesterol

The plasma cholesterol concentration (mg/dl) of experimental animals at monthly interval is presented in Table 4.24. The values ranged from 78.10 to 85.27mg/dl in Control, 80.71 to 100.14 mg/dl in T10 and 73.47 to 90.90 mg/dl in T20

groups. Overall cholesterol concentration of treatments group was found similar with Control group bucks.

Table 4.24 Plasma cholesterol concentration (mg/dl) in buck fed ration replacing soybean meal with dried *Moringa oleifera* leaf powder (MOLP)

Days	Treatment			SEM	P value
	Control	T10	T20		
0	81.91	83.54	73.47	2.95	0.35
30	85.27	100.14	90.90	4.991	0.50
60	78.10	83.12	82.51	2.594	0.71
90	82.29	80.71	86.37	2.504	0.66
Total	81.89	86.88	83.31	1.765	0.50

4.5.3 Plasma triglycerides

The plasma triglyceride concentration (mg/dl) of experimental animals at monthly interval is presented in Table 4.25. The values ranged from 25.79 to 31.87 mg/dl in Control, 27.90 to 34.35 mg/dl in T10 and 28.18 to 35.11 mg/dl in T20 groups. Overall plasma triglyceride concentration of treatments group was found similar with Control group bucks.

Table 4.25 Plasma triglycerides concentration (mg/dl) in buck fed ration replacing soybean meal with dried *Moringa oleifera* leaf powder (MOLP)

Days	Treatment			SEM	P value
	Control	T10	T20		
0	25.79	27.90	28.18	1.50	0.80
30	29.04	31.37	28.44	1.47	0.72
60	31.87	29.47	34.60	1.46	0.38
90	30.59	34.35	35.11	1.55	0.47
Total	29.32	30.77	31.58	0.78	0.49

4.5.4 Plasma total protein

The plasma total protein concentration (g/dl) of experimental animals at monthly interval is presented in Table 4.26. The values ranged from 6.93 to 7.86 g/dl

in Control, 6.97 to 7.97 g/dl in T10 and 6.29 to 7.14 g/dl in T20 groups. Overall plasma total protein concentration of treatments group was found similar with Control group bucks.

Table 4.26 Plasma total protein concentration (g/dl) in buck fed ration replacing soybean meal with dried *Moringa oleifera* leaf powder (MOLP)

Days	Treatment			SEM	P value
	Control	T10	T20		
0	7.37	7.11	6.29	0.29	0.31
30	7.10	7.08	6.85	0.29	0.93
60	6.93	6.97	6.69	0.29	0.93
90	7.86	7.97	7.14	0.22	0.25
Total	7.31	7.28	6.74	0.14	0.17

4.5.5 Plasma albumin

The plasma albumin concentration (g/dl) of experimental animals at monthly interval is presented in Table 4.27. The values ranged from 3.36 to 3.87g/dl in Control, 3.45 to 3.64 g/dl in T10 and 3.25 to 3.69 g/dl in T20 groups. Overall plasma albumin concentration of treatments group was found similar with Control group bucks.

Table 4.27 Plasma albumin concentration (g/dl) in buck fed ration replacing soybean meal with dried *Moringa oleifera* leaf powder (MOLP)

Days	Treatment			SEM	P value
	Control	T10	T20		
0	3.87	3.45	3.62	0.13	0.44
30	3.48	3.64	3.32	0.09	0.42
60	3.36	3.53	3.25	0.09	0.54
90	3.61	3.45	3.69	0.08	0.52
Total	3.58	3.45	3.47	0.53	0.56

4.5.6 Plasma globulin

The plasma globulin concentration (g/dl) of experimental animals at monthly interval is presented in Table 4.28. The values ranged from 3.50 to 4.25g/dl in Control, 3.44 to 4.49 g/dl in T10 and 3.44 to 3.67 g/dl in T20 groups. Overall plasma

globulin concentration of treatments group was found similar with Control group bucks.

Table 4.28 Plasma globulin concentration (g/dl) in buck fed ration replacing soybean meal with dried *Moringa oleifera* leaf powder (MOLP)

Days	Treatment			SEM	P value
	Control	T10	T20		
0	3.50	3.66	3.67	0.28	0.32
30	3.62	3.44	3.54	0.31	0.97
60	3.57	3.44	3.44	0.29	0.98
90	4.25	4.79	3.44	0.23	0.57
Total	3.73	3.83	3.52	0.14	0.23

4.5.7 Plasma ALT activity

The plasma ALT activity (IU/L) of experimental animals at monthly interval is presented in Table 4.29. Mean plasma ALT activity at the beginning of experiment were 14.50, 16.40 and 13.26 IU/L in Control, T10 and T20 groups, respectively and corresponding values at the end of experiment were 19.92, 16.87 and 20.27 IU/L respectively. Overall plasma ALT activity of treatments group was found similar with Control group for whole trial period.

Table 4.29 Plasma ALT activity (IU/L) in buck fed ration replacing soybean meal with dried *Moringa oleifera* leaf powder (MOLP)

Days	Treatments			SEM	P value
	Control	T10	T20		
0	14.50	16.40	13.26	1.05	0.50
30	17.99	16.44	13.31	1.03	0.17
60	18.51	19.17	19.42	1.15	0.95
90	19.92	16.87	20.27	0.85	0.21
Total	17.73	17.22	16.56	0.55	0.69

4.5.8 Plasma AST activity

The plasma AST activity (IU/L) of experimental animals at monthly interval is presented in Table 4.30. Mean plasma AST concentration at the beginning of

experiment was 54.33, 62.72 and 53.68 IU/L in Control, T10 and T20 groups, respectively and corresponding values at the end of experiment were 50.74,55.08 and 54.95 IU/L respectively. Overall plasma AST activity of treatments group was found similar with Control group for whole trial period.

Table 4.30 Plasma AST activity (IU/L) in buck fed ration replacing soybean meal with dried *Moringa oleifera* leaf powder (MOLP)

Days	Treatment			SEM	P value
	Control	T10	T20		
0	54.33	62.72	53.68	2.58	0.30
30	65.04	62.47	57.08	2.44	0.42
60	51.37	53.18	52.42	1.62	0.91
90	50.74	55.08	54.95	2.11	0.66
Total	55.37	54.52	54.53	0.16	0.37

4.6 Antioxidant parameters

4.6.1 Super oxide dismutase (SOD) activity

The plasma SOD activity (U/mgHb) of experimental animals at monthly interval is presented in Table 4.31. Mean plasma SOD activity at the beginning of experiment were 39.70, 35.91 and 38.70 U/mgHb in Control, T10 and T20 groups, respectively and corresponding values at the end of experiment were 44.57, 53.55 and 49.22U/mg/Hb, respectively. In third and fourth fortnight the SOD activity U/mgHb in T10 groups was found significantly higher ($P<0.05$) than Control and T20 group. Overall plasma SOD activity of T10 group was found significantly higher ($P<0.05$) than Control group bucks.

Table 4.31 SOD activity (U/mgHb) in buck fed ration replacing soybean meal with dried *Moringa oleifera* leaf powder (MOLP)

Month	Treatment			SEM	P value
	Control	T10	T20		
0	39.70	35.91	38.70	1.95	0.38
30	42.17	46.93	41.09	2.50	0.25
60	43.19^{a}	53.93^{b}	44.21^{ab}	2.84	0.03
90	44.57^{a}	53.55^{b}	49.22^{ab}	1.88	0.02
Total	42.41^{a}	47.57^{b}	43.30^{ab}	1.49	0.04

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

4.6.2 Catalase activity

The Catalase activity (μmoles of H_2O_2 consumes /min/gHb) of experimental animals at monthly interval is presented in Table 4.32. Mean plasma Catalase activity (μmoles of H_2O_2 consumes /min/gHb) at the beginning of experiment were, 61.81, 54.32 and 57.08 μmoles of H_2O_2 consumes /min/gHb in Control, T10 and T20 groups, respectively and corresponding values at the end of experiment were 59.21, 56.07 and 52.32μmoles of H_2O_2 consumes /min/gHb respectively. Overall plasma catalase activity of treatments group was found similar with Control group bucks for whole trial period.

Table 4.32 Catalase activity (μmoles of H_2O_2 consumes /min/gHb) in buck fed ration replacing soybean meal with dried *Moringa oleifera* leaf powder (MOLP)

Days	Treatment			SEM	P value
	Control	T1	T2		
0	61.81	54.32	57.08	4.29	0.48
30	61.57	52.67	56.98	3.57	0.24
60	54.77	57.85	51.81	6.54	0.81
90	59.21	56.07	52.32	2.78	0.25
Total	59.34	55.23	52.32	5.55	0.25

4.6.3 Lipid peroxidation (LPO) activity

Plasma lipid peroxidation (nM MDA/ml packed RBCs) value of experimental animals at monthly interval is presented in Table 4.33. Mean plasma lipid peroxidation (nM MDA/ml packed RBCs) activity at the beginning of experiment were 7.22, 7.21 and 7.16 nM MDA/ml packed RBCs in Control, T10 and T20 groups, respectively and corresponding values at the end of experiment were 8.15, 5.37 and 7.33nM MDA/ml packed RBCs respectively. Lipid peroxidation value in last fortnight of experimental period was significantly lower ($P<0.05$) in T10 groups as compared to Control. Overall lipid peroxidation value was found significantly lower ($P<0.05$) in T10 group bucks as compared to Control group bucks.

Table 4.33 Lipid peroxidation (nM MDA/ml packed RBCs) level in buck fed ration replacing soybean meal with dried *Moringa oleifera* leaf powder (MOLP)

Month	Treatment			SEM	P value
	Control	T10	T20		
0	7.22	7.21	7.16	0.81	0.99
30	7.32	6.66	7.67	0.67	0.57
60	7.93	6.18	6.87	0.59	0.15
90	8.15^{b}	5.37^{a}	7.33^{ab}	0.69	0.03
Total	7.65^{b}	6.35^{a}	7.26^{ab}	0.34	0.02

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

4.7. Hormonal attributes

4.7.1 Plasma Cortisol

The plasma Cortisol concentration (ng/ml) of experimental animals at monthly interval is presented in Table 4.34. The value ranged from 22.14 to 34.42 ng/ml in Control, 18.43 to 21.81ng/ml in T10 groups and 21.51 to 28.89 ng/ml in T20 groups. In last fortnight the Cortisol level was significantly lower in T10 group than Control group. Overall plasma Cortisol concentration was found significantly lower (P<0.05) in T10 group bucks as compared to Control group bucks.

Table 4.34 Plasma Cortisol (ng/ml) level in buck fed ration replacing soybean meal with dried *Moringa oleifera* leaf powder (MOLP)

Month	Treatment			SEM	P value
	Control	T1	T2		
0	22.14	21.40	21.51	2.75	0.98
30	25.55	21.81	24.68	3.37	0.72
60	28.34	21.52	28.42	3.71	0.35
90	34.42^{b}	18.43^{a}	28.89^{b}	2.13	0.01
Total	27.62^{b}	20.79^{a}	25.87^{ab}	1.57	0.009

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

4.7.2 Plasma Testosterone

The plasma Testosterone concentration (ng/ml) of experimental animals at monthly interval is presented in Table 4.35. The value ranged from 1.36 to 2.29 ng/ml

in Control, 1.53 to 1.99 ng/ml in T10 groups and 1.70 to 2.15 ng/ml in T20 groups. Overall plasma Testosterone concentration of treatments group bucks was found similar with Control group bucks.

Table 4.35 Plasma Testosterone (ng/ml) level in buck fed ration replacing soybean meal with dried *Moringa oleifera* leaf powder (MOLP)

Month	Treatment			SEM	P value
	Control	T1	T2		
0	1.81	1.83	1.74	0.21	0.94
30	1.36	1.53	1.70	0.15	0.32
60	2.29	1.87	2.15	0.25	0.51
90	1.98	1.99	1.77	0.32	0.86
Total	1.86	1.81	1.84	0.12	0.95

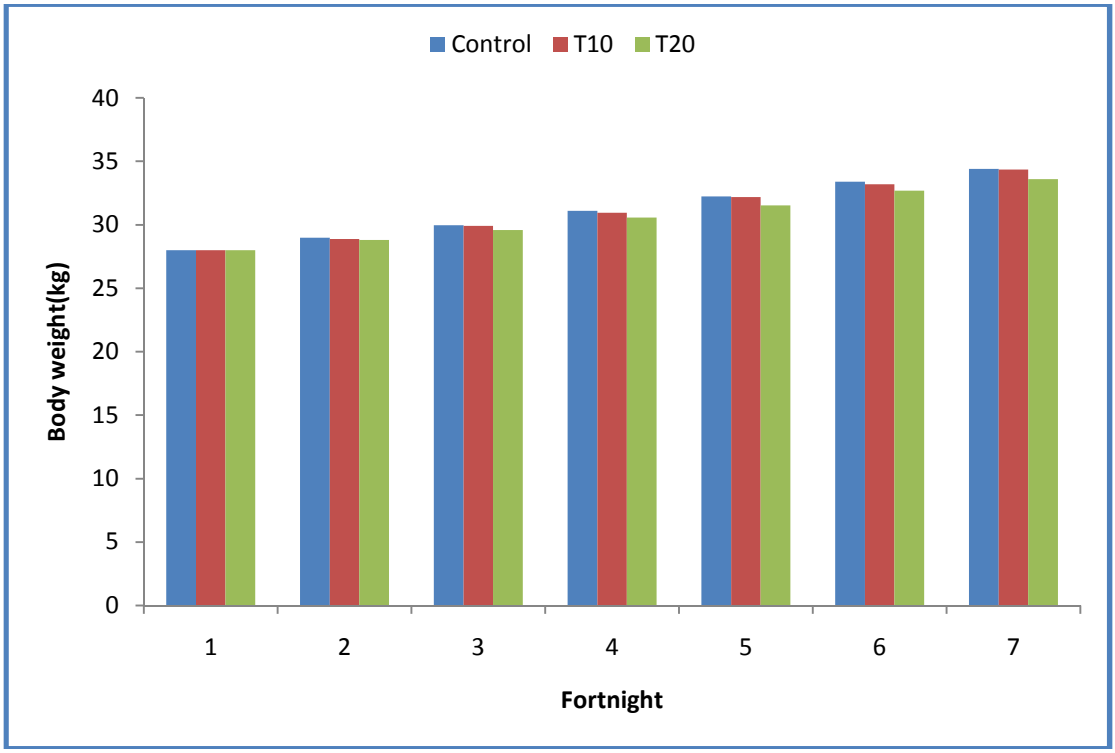


Fig. 4.1 Fortnightly body weight of experimental bucks

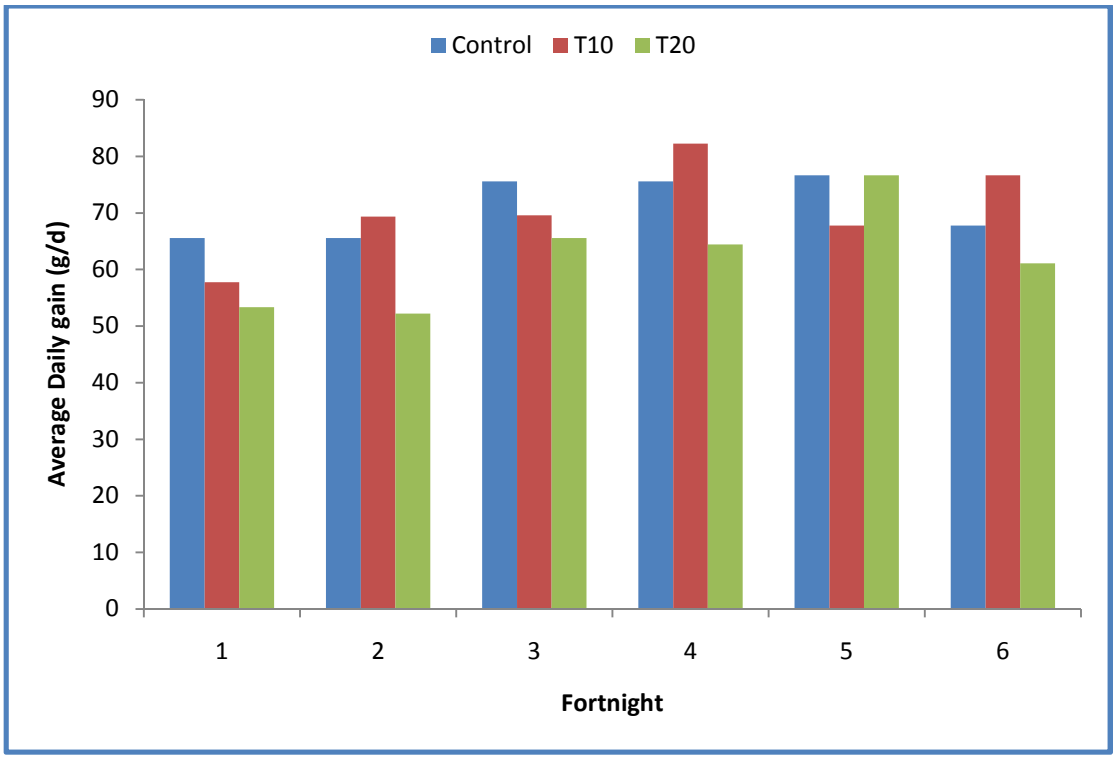


Fig. 4.2 Average daily gain of experimental bucks

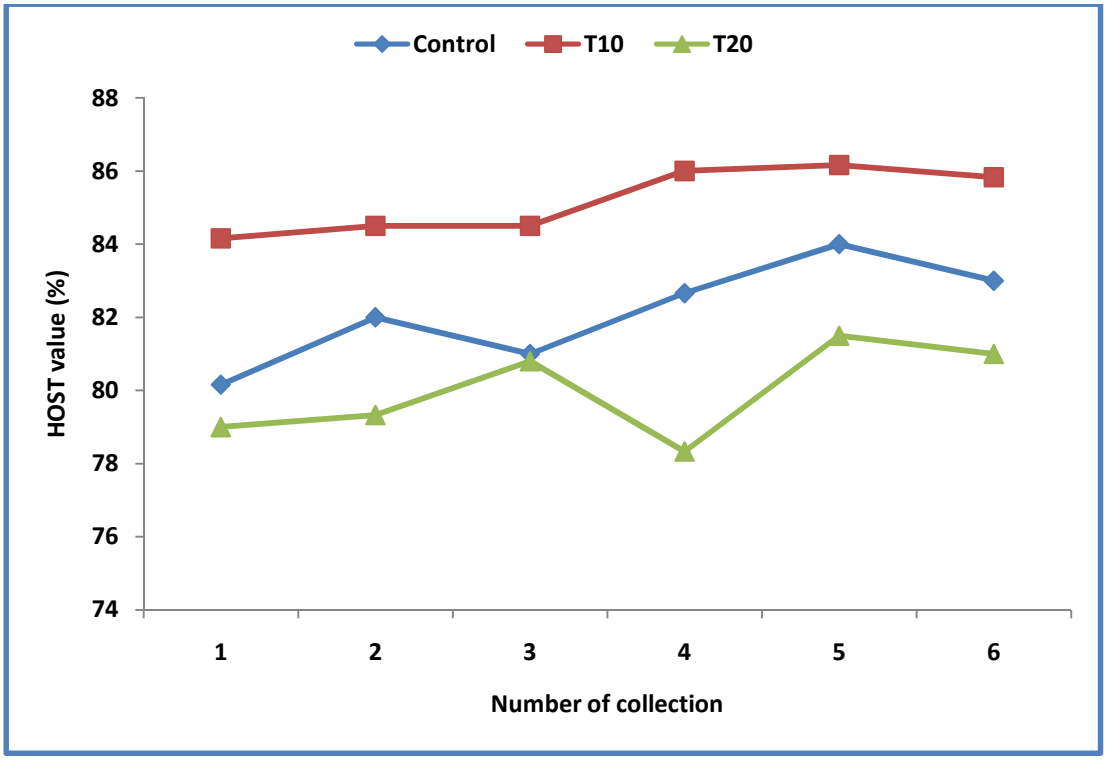


Fig. 4.3 Hypo Osmotic Swelling Test (HOST) percentage of experimental bucks

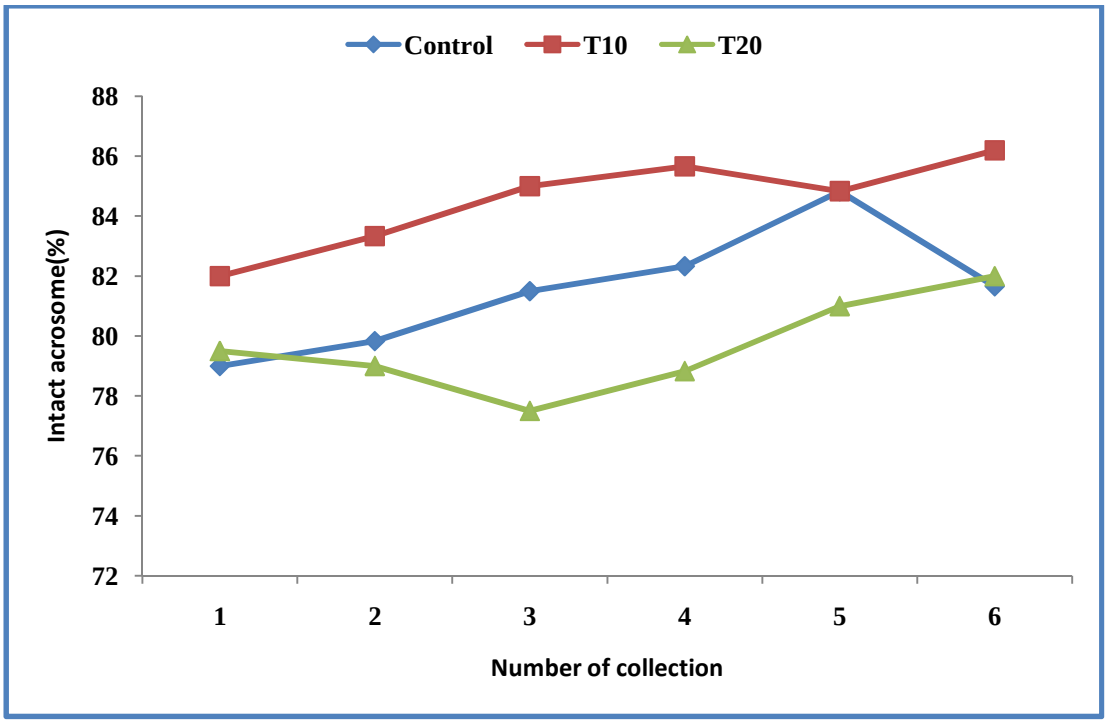


Fig. 4.4 Intact Acrosomal percentage of experimental bucks

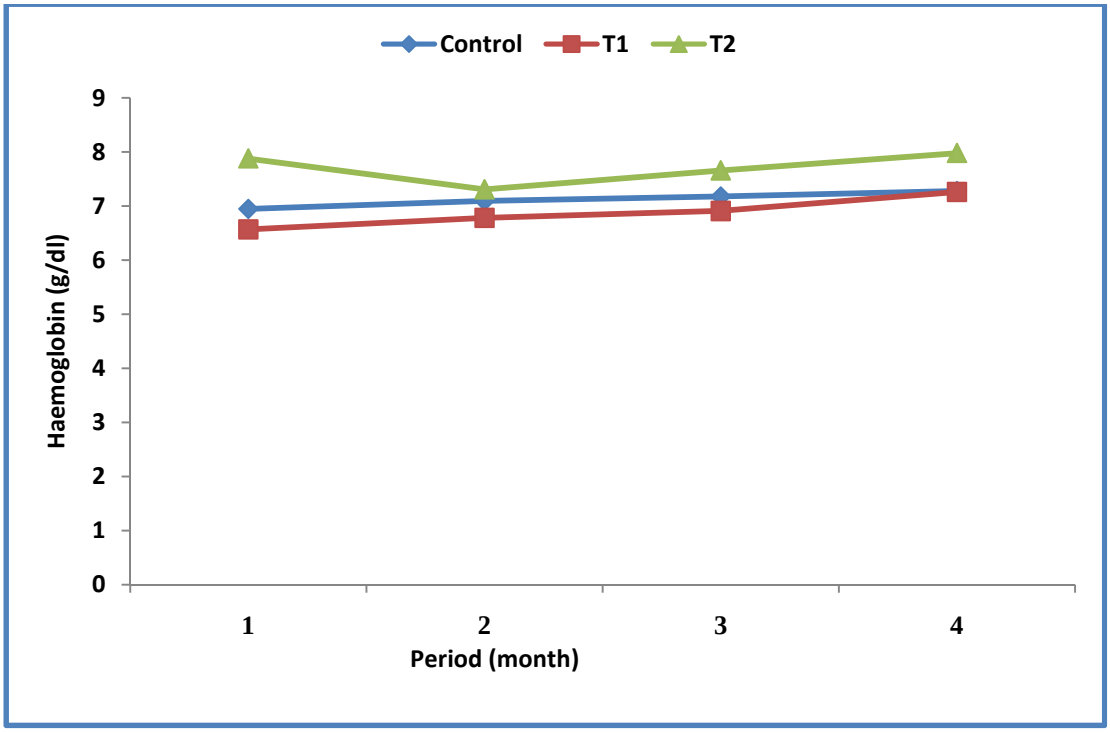


Fig. 4.5. Haemoglobin concentration (g/dl) of experimental bucks

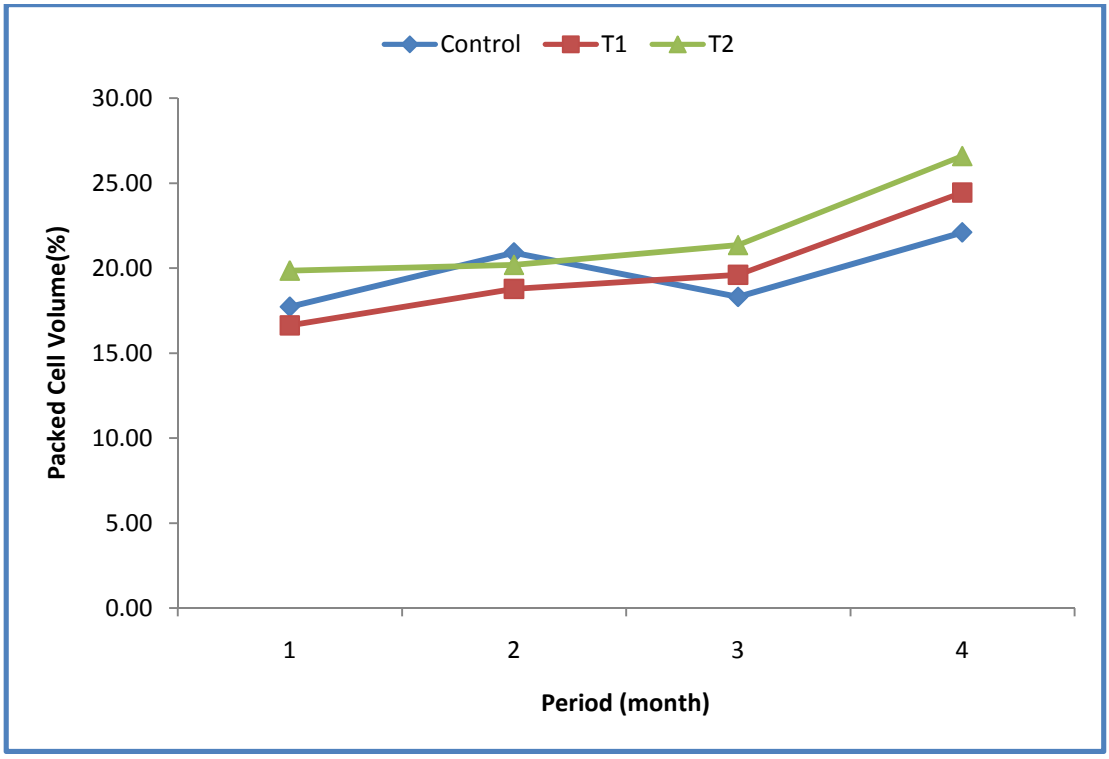


Fig. 4.6. Packed Cell Volume (%) of experimental bucks

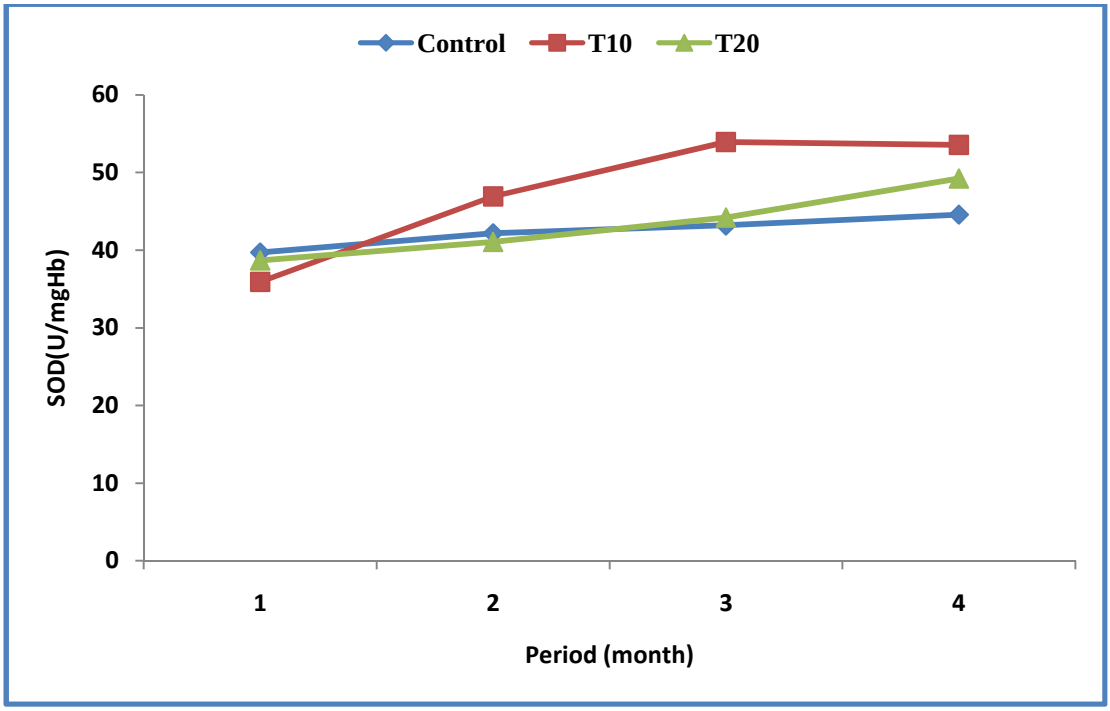


Fig. 4.7. Superoxide dismutase (U/mgHb) of experimental bucks

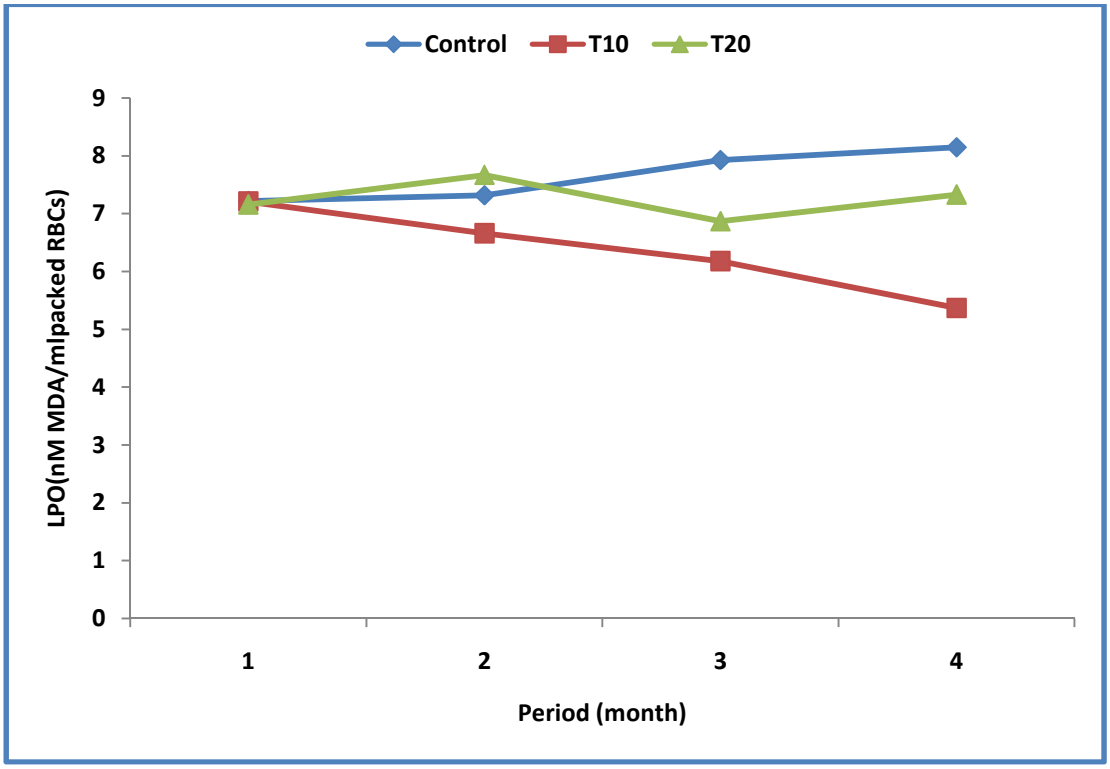


Fig. 4.8. Lipid peroxiation (LPO) (nM MDA / ml) of experimental bucks

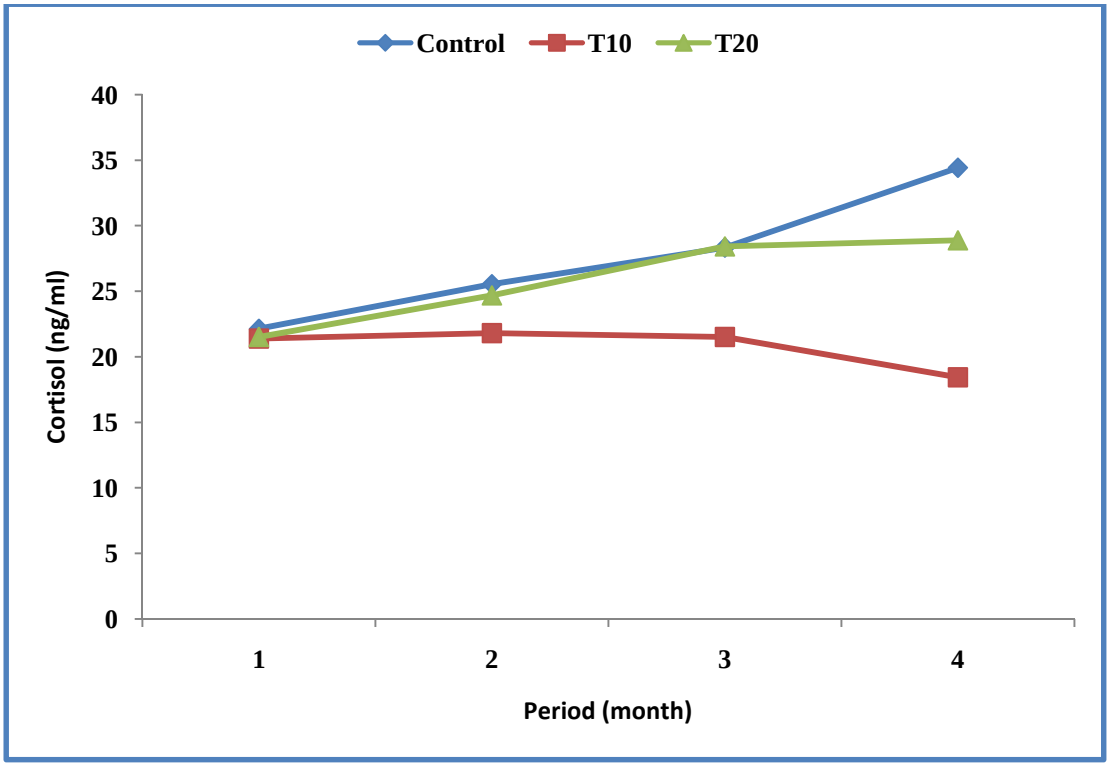


Fig. 4.9. Plasma Cortisol (ng/ml) of experimental bucks

Discussion

CHAPTER-5

DISCUSSION

The results obtained on replacing soybean meal in concentrate mixture with dried *Moringa oleifera* leaf powder on growth performance, nutrient utilization, seminal attributes, haematological parameters, blood biochemicals, antioxidant and hormonal parameters of Barbari bucks are discussed in following chapter.

5.1 Chemical composition of experimental diet

The chemical composition (% DM basis) of experimental diet offered to Barbari bucks during the experimental period was estimated. The ratio of concentrate and roughage in experimental diet was 40:60. Out of total 60% contribution of roughage, green fodder (berseem) contributed 24% and arhar straw contributed 36% dry matter. The values of proximate composition and fibre fractions of arhar straw, green berseem, concentrate mixture and dried *Moringa oleifera* leaf powder obtained fell within earlier reported ranges. (Asaolu et al., 2010; Divya et al., 2014; Singh et al., 2017; Damor et al., 2017; Kholif et al., 2018). The crude protein content of *Moringa oleifera* leaves used in the study was comparable with the values 25.95, 22.60, 29.70, 23.24, 29.14 and 26.30% obtained by Manh et al., 2005; Sánchez et al., 2005; Fadiyimu et al., 2010; Jiwuba et al., 2016; Oyedele et al., 2016 and Damor et al., 2017 respectively, but higher than the values 19.30, 19.50, 18.26 and 20.56% reported by Aregheore (2002); Kakengi et al., 2003; Sultana et al., 2015 and Ali, 2017 respectively. The variations in nutritive value of *Moringa oleifera* could be due to the age of harvest, soil type and fertility, proportion of leaf and stem and agro ecological zone where trees are growing. The high CP content of *Moringa oleifera* serve it as a good protein source that can conveniently substitute the conventional protein sources like soybean and rapeseed meals for ruminants, and they are able to improve the microbial protein synthesis in the rumen (Soliva et al., 2005).

5.2 Growth parameter and nutrient utilization in buck fed ration replacing soybean meal with dried *Moringa oleifera* leaf powder (MOLP)

5.2.1 Growth indices

The results revealed no significant ($P>0.05$) difference in the average body weight and metabolic body weights (kg $W^{0.75}$) between groups. However, the overall

fortnightly body weight gain (kg) and average daily gain (g) was found to be significantly higher and comparable in Control and T10 group. Similarly, the overall DMI (kg/d, kg/100 kg BW) were also found to be significantly higher and comparable in Control and T10 group. No significant change in overall FCR was observed in the experimental groups. In accordance with the present study, Sarwatt et al. (2002) observed no significant difference in body weight and metabolic body weight of East African goats fed with 0, 25, 75 and 100% level of *Moringa oleifera* substituting sunflower seed cake in the concentrate mixture. Kholif et al. (2018) also observed no significant difference in body weight in lactating Nubian goats supplemented with different doses of *Moringa oleifera* extract. Despite that, findings reported by Tono et al. (2014) are in difference of opinion with the present observations as they recorded better gain in body weight of goats fed on diets with inclusion of *Moringa oleifera*. The observed differences in the above study are attributed to variation in the types of basal diet, protein source substituted and level of replacement with *Moringa oleifera*. Similar to our results, Tono et al. (2014) also reported significantly higher daily dry matter intake and daily weight gain in the West African Dwarf (WAD) goats fed the concentrate diet with 15% level of inclusion of *Moringa oleifera* leaf meal. Sultana et al. (2015) found significantly higher average daily gain and dry matter intake of Bengal goats fed on sole *Moringa oleifera* foliage diet. Similarly, Bebekar and Abdalbagi (2015) observed higher dry matter intake and average daily gain in Sudan Nubian goats fed *Moringa oleifera* leaves at 20% level. However, the observations made in present study contradict with the findings of Asaulo et al. (2010) who reported no significant difference in DM intake in WAD goats fed on equal but separate combinations of *Moringa oleifera* with *Leucaena* and *Gliricidia* fodders. The results differ with present findings as they evaluated the effect of *Leucaena* and *Gliricidia* fodder relative to sole *Moringa oleifera* fodder. Similarly, Aregheore (2002) reported no significant difference in dry matter intake among the treatments groups on incorporation of *Moringa oleifera* foliage as forage in Anglo-Nubian goat ration at 0%, 20%, 50% and 100% levels. In the present study the mean ADG (g/d) fortnightly BW gain (kg) and DMI (kg/d, kg/100 kg BW) were significantly lower ($P<0.05$) in T20 group as compared to Control and T10 group. The findings of present study suggest that on substituting the soybean meal with higher level (20%) of *Moringa oleifera* leaf powder the performance of Barbari bucks was depressed. Similar to our observations Ritcher et al. (2003) also reported that the

performance of the fish fed with higher levels of *Moringa oleifera* (20% and 30% of protein base) was inferior to those of fish fed with the Control diet and the diet containing 10% *Moringa oleifera* leaf meal. The average daily weight gains are also in accordance with the reports of Argeherore (2002) who reported that the ADG of the goats offered the diet with 80% inclusion of *Moringa oleifera* leaf meal was lower than those of the goats on 20% and 50% diets, but similar to those of Control, in batiki grass based diets for goats. 20% substitution of alfalfa meal by *Moringa oleifera* leaves would result in better ADG and feed conversion ratio (FCR), probably regarded the better protein quality and amino acids content of *Moringa oleifera* leaves. However, diets that contained *Moringa oleifera* leaves 30% substitution issued in lower ADG and FCR than *Moringa oleifera* 20% substitution group (Sultana et al., 2015). Adverse effect of feeding high levels of *Moringa oleifera* leaves might be associated with the high content of several phytochemical compounds (phenols, alkaloids, coumarins and tannins) which are existing in *Moringa oleifera* leaves naturally (El-Badawi et al., 2014). The depression of growth performance and feed intake at higher levels of inclusions could likely be attributed to several factors, among which the presence of anti nutrients (saponins, phenols, etc) and the levels of cell wall constituents (NDF and ADF) in *Moringa oleifera* leaf will have been important. A further possible reason for the reduced performance at higher level of substitution may be due to the combination of anti nutrients with fibre that have caused the significant decrease in performance (Francis et al., 2001). It could be envisaged from the findings of present study that the dietary treatment had no effect on the feed conversion ratio in experimental groups of bucks. The findings are consistent to those of Argeherore (2002) who reported no change in feed efficiency with *Moringa oleifera* supplementation in goats .Likewise Sultana et al. (2015) observed that FCR was not affected in the Black Bengal goats fed on napier grass mixed with different levels of *Moringa oleifera* foliage .However, study by Murro et al. (2003) resulted in poorer feed conversion at higher (66.6 and 100%) rate of replacement of cottonseed cake (CSC) with *Moringa oleifera* leaf meal supplementation in growing sheep fed basal diet of Rhodes grass hay. The discrepancy in results may be attributed to higher level of substitution of *Moringa oleifera* with cotton seed cake.

5.2.2 Nutrient utilization

The present findings showed no significant (P>0.05) difference on nutrient intake and nutrient digestibility in barbari bucks fed ration replacing soybean meal with dried *Moringa oleifera* leaf powder (MOLP). The overall DM intake (kg/d), CP intake and TDN intake were found to be similar in all the experimental groups. In the present study replacement of soybean meal with *Moringa oleifera* leaf powder at 10 and 20% level did not adversely affect the digestibility of DM, CP, EE, NDF and ADF. The results indicated that *Moringa oleifera* leaves had similar digestibility with soybean meal on the most nutrient profiles and had an almost identical pattern of all the essential amino acids (Foidl et al., 2001). Conversely, Sultana et al. (2015) observed that the CP and ADF digestibility increased linearly with increasing level of *Moringa oleifera* foliage in the Napier grass based diet in goats. Fadiyimu et al. (2010) observed higher CP digestibility in sheep on sole *Moringa oleifera* foliage diet. A similar observation was reported by Arigbede et al. (2005) who found that the digestibility coefficients of DM, OM, CP, NDF, ADF and ADL was higher in diets containing graded levels of combinations of Grewia pubescens and Panicum maximum. Apparent digestibility coefficients (%) of nutrients were also reported higher in WAD goats fed graded levels of inclusions of *Moringa oleifera* leaf meal (MOL) (Tona et al. (2013). This could be attributed to the differences in the type of basal diet fed, the enhance digestibility is probably because *Moringa oleifera* fodder consists of more degradable components especially crude protein than Napier and Panicum that substituted *Moringa oleifera* in above studies. The activities of fibrolytic bacteria in the rumen is also elevated probably as a result of the increased availability of essential nutrients especially protein, energy and minerals in balanced proportions to enhance microbial growth and multiplication that led to increase in nutrient digestibility.

5.3 Seminal attributes in buck fed ration replacing soybean meal with dried *Moringa oleifera* leaf powder (MOLP)

In the present study no significant differences (P<0.05) were observed on substituting the soybean meal in concentrate mixture with different levels of *Moringa oleifera* leaf powder on the semen pH, colour, volume, spermatozoa concentration and morphological abnormality in spermatozoa. However, significantly (P<0.05) higher values were noticed in mass motility, progressive motility, livability, acrosomal

integrity and HOST reactive spermatozoa in T10 group as compared to Control and T20 group. The observation reported is consonance with the findings of Syarifuddin et al. (2017) reported that supplementation of *Moringa oleifera* leaves in Bali bull increased both the total motility and the progressive motility of sperm. Similarly supplementation of 4% and 8% *Moringa oleifera* leaves to buffalo bulls improved mass motility, progressive motility, livability, acrosomal integrity, HOST reactive spermatozoa count (Wafa et al., 2017). *Moringa oleifera* leaves significantly increased percentage of motility, live percentage, membrane integrity in rabbit buck semen as an extract (Khalifa et al., 2016) or as meal (Oyedemi et al., 2008). Priyadarshani and Verma (2014) studied the effect of administration of *Moringa oleifera* leaf powder for 21 days and the result revealed significantly increase sperm motility and reduces abnormality percentage. The result may suggest the pronounced effect of dried *Moringa oleifera* leaf powder as an antioxidant on improving most sperm characteristics, including sperm motility, live percentage, membrane integrity and may be attributed to prevention of excessive generation of free radicals. The observed positive influence of the leaf powder on buck's reproductive function may be related to the highly digestible nutritional and amino acids profiles of *Moringa oleifera* leaves (Rubanza et al., 2005; Leone et al., 2015). It is rich in carotenoids, potassium, calcium, vitamins (particularly C and E), and iron (Jongrungruangchok et al., 2010; Moyo et al., 2011; Yameogo et al., 2011). It is also an established rich source of omega-6 and polyunsaturated fatty acids (PUFAs) (Saini et al., 2014). The phyto-compounds present in the *Moringa oleifera* have the potential to improve semen quality characteristics. Purdy et al. (2004) demonstrated that flavonoids present in *Moringa oleifera* leaves causes an increase in sperm motility. It is evident that *Moringa oleifera* leaf powder could be used to enhance bucks reproductive function clearly manifested through the improvement of the semen quality characteristics.

5.4 Haematological parameters

The haematological parameters reported in present study viz. haemoglobin concentration (g/dl) and packed cell volume percentage was found significantly higher ($P<0.05$) in T20 group than Control. The values of the reported haematological parameters were found to be in the normal physiological ranges (Merck, 2011; Asaolu, 2011).The present results are in agreement with the finding of Asaulo et al. (2011) who investigated the performance of grazing WAD goats on *Moringa oleifera*

multi nutrient block (MMNB) supplementation relative to cassava peels and corn starch residue and result revealed that only MMNB supplementation resulted in significant increase in haemoglobin and PCV. Likewise, Jiwuba et al. (2017) studied the effect of *Moringa oleifera* leaf meal (MOLM) on haematology of WAD goat fed diets containing MOLM at 0%, 5%, 10% and 15% levels and found that haemoglobin and PCV was improved by MOLM supplementation at 15% inclusion level. Similarly, Ajugwo et al. (2017) studied the haematinic effect of *Moringa oleifera* on rats and observed that supplementation of *Moringa oleifera* leaf extract resulted significant increase in haemoglobin count and packed cell volume in treatment group as compared to Control but in normal reference ranges. The increase of haemoglobin and packed cell volume in this study may be due to high content of iron in *Moringa oleifera* leaves and vitamin C that could help the absorption of iron in the body. Hematocrit or PCV value is proportional to the hemoglobin concentration.

5.5 Blood biochemicals parameters

With regard to plasma biochemical parameters, in buck fed ration replacing soybean meal with dried *Moringa oleifera* leaf powder (MOLP) the overall plasma glucose concentration (mg/dl) of treatments group was found similar with Control for whole experimental period. The mean plasma glucose values in all three groups were in normal reference range (More, 2006). The present findings are in agreement with Damor et al. (2017) who reported non-significant differences in serum glucose values in Mehsana goat kids fed with *Moringa oleifera* leaves replacing concentrate mixture at 0, 50 and 100% levels. The results are not in tuned with the observations made by Bebikar and Abdalbagi. (2015) who reported significant decrease in serum glucose values in goats fed *Moringa oleifera* leaves at 20% in goat ration. However, the levels were within the variation range (50-75mg/dl) indicated for healthy goats (Zubcic, 2001; Dhanotiya, 2004) and the observed depressed serum glucose in treatment group B is not due to *Moringa oleifera* leaf meal intoxication, but that the dietary energy was sufficiently utilized for growth and the animals were not surviving at the expense of body tissues (Ologhobo et al., 1992). Although some studies also indicate that the presence of flavonoid in *Moringa oleifera* inhibit a-amylase activity to regulate the amount of glucose in the blood (Bebekar and Abdalbagi, 2015), but the present results were not in accordance with this.

The plasma triglyceride and cholesterol concentration of all experimental groups was found similar and the observed reports are not in agreement with the studies of Kholif et al. (2015), Babiker et al. (2016), and Zhang et al. (2017) who reported that feeding does diets containing *Moringa oleifera* decreased serum cholesterol and triglycerides concentrations. Lowered blood cholesterol and triglyceride concentrations observed with oral supplementation of *Moringa oleifera* extract could be a result of a functional effect of the phenolic acids in the extract (Babiker et al., 2016). Saxena et al. (2013) showed that phytochemicals in the medicinal plants or their phytogenic extracts could decrease the synthesis and absorption of cholesterol and triglycerides. Moreover, Jain et al. (2010) observed that *Moringa oleifera* inhibits endogenous cholesterol biosynthesis by reducing the activity of HMG-CoA reductase. However, the variation in result may be due to that in these experiments phytogenic extracts of *Moringa oleifera* leaves were supplemented. Furthermore, it has been proposed that *Moringa oleifera* leaves extracts contain a large number of phenolic compounds and some of the secondary metabolites identified have cholesterol-lowering effects. But, the mechanism by which *Moringa oleifera* extract decreased cholesterol and triglycerides is not clear.

The observations on protein metabolism showed no significant effect of dietary treatment on the plasma total protein, albumin and globulin concentration in experimental groups. The results suggest minimal protein catabolism and normal kidney function in present study. The observed levels of plasma total protein in all the groups were in normal clinical range as cited by Kaneko et al. (1997). Ali (2017) documented similar findings and reported no difference in serum total protein, albumin and globulin levels in the goats fed *Moringa oleifera* leaves. Conversely, Bebekar and Abdalbagi (2015) reported significantly higher level of serum total protein, albumin and globulin in goat fed *Moringa oleifera* leaves at different levels in the ration. Divya et al. (2014) reported significant decrease in total protein level in broiler chicken; fed *Moringa oleifera* leaves at1.5% level.

Reported values of GOT and GPT concentrations were within the normal physiological range (Kaneko et al., 1997) suggesting that inclusion of *Moringa oleifera* leaf meal in the diet of goat did not alter liver function. The present findings are in agreement with report of Damor et al. (2017) who observed non-significant difference in SGPT values in Mehsana goat kids fed on *Moringa oleifera* leaves by

replacing concentrate mixture at the rate of 0%, 50%and 100%. Decreased GOT and GPT concentrations are important indicators of normal or enhanced liver function suggesting the absence of pathological lesions in the liver (Stanek et al., 1992). Kholif et al. (2015, 2016) observed greater GPT concentration with feeding *Moringa oleifera* leaf meal. This might be due to that in their experiments, does were fed diets containing 150 and 200 g *Moringa oleifera* leaf meal/kg DM of diet, which means that greater amounts of secondary metabolites were delivered to does compared with the present study. Moreover, Rivera et al. (2005) showed that secondary metabolites at high doses for a considerable time of consumption can cause anemia, damage to the liver and kidney, and sometimes death. Zhang et al. (2017) reported that feeding *Moringa oleifera* silage did not affect serum concentrations of GOT, GPT, glucose, total protein, and albumin, revealing no effects on hepatic metabolism and immune response of lactating cows. Results from present study showed that dose of supplementation of *Moringa oleifera* in bucks did not impair the liver functions. Thus, replacing soybean meal with dried *Moringa oleifera* leaf powder at 10 and 20% levels in concentrate mixture fed to Barbari bucks did not negatively affect blood parameters and hepatic metabolism in this study.

5.6 Antioxidant parameters

In the present study, plasma SOD activity of the bucks fed T10 was significantly higher ($P<0.05$) than those of bucks fed T20 and Control diet. However, the catalase activity of treatments group was found similar with Control for whole experimental period. SOD has been reported as one of the most important antioxidant defense enzymes that scavenge superoxide anion in order to lessen toxic effect caused by this radical (Curtis et al., 1972; Liyana et al., 2006). The present study revealed the high percentage inhibition of superoxide anion in the bucks supplemented with T10 diet in comparison to Control and T20 group diets. The obtained result are as per the findings of Moyo et al. (2012) who observed increased SOD activities in goats supplemented with *Moringa oleifera* leaf. Qwele et al. (2012) also studied the antioxidant potential of meat from goat supplemented with *Moringa oleifera* leaves and reported that the meat from goat fed with *Moringa oleifera* had higher SOD activities than goat fed with grass hay or with sunflower seed cake. Catalase is another antioxidant enzyme widely distributed in the animal tissues (Oyedemi et al., 2008). The enzyme is reported to protect the system from highly reactive hydroxyl

radicals through hydrogen peroxide decomposition (Chance et al., 1952). Reduction of this enzyme activity may promote the cellular damage caused by the assimilation of superoxide and hydrogen peroxide. However, in this study catalase activity was not significantly affected on supplementation of *Moringa oleifera* leaves in bucks. Obtained result were not in consistent with the findings of Moyo et al. (2012) who reported increase catalase activity in goat fed *Moringa oleifera* in comparison with grass hay and sun flower seed cake. The present observation implies an efficient protective mechanism of *Moringa oleifera* leaves against superoxide anion radical relative to the high concentration of phenolics and flavonoids contents (Robak & Gryglewski, 1988). The phenolic compounds present in *Moringa oleifera* leaf extracts are good electron donors and could terminate the radical chain reaction by converting free radicals to stable product. However, the effect of dosage factors affecting the antioxidant levels of *Moringa oleifera* leaves requires further analysis. The overall lipid peroxidation value was found significantly lower ($P<0.05$) in T10 as compared to Control group. The decrease LPO level in present study is in accordance with the Moyo et al. (2012) who reported decrease LPO levels in goat fed with *Moringa Oleifera* as comparison to goat fed with grass hay or with sun flower seed cake. Serum malondialdehyde (MDA) concentrations in both male and female mice were significantly decreased by dietary *Moringa oleifera* supplementation (Zeng et al., 2009). Lowering erythrocytic LPO values in T10 group highlights the anti-peroxidative potential of the *Moringa oleifera*. A significant elevation of the activity of SOD and reduction of LPO was observed in bucks fed *Moringa oleifera* leaf powder at 10% replacement with soybean meal as compared with the Control and T20 group. Overall, the data indicates that *Moringa oleifera* was effective in raising the antioxidant status of the bucks. It was reported that *Moringa oleifera* leaves have high amount of phenols and potent antioxidant properties, and it could prevent morphological changes and oxidative damage in human and animals effectively by increasing the activities of antioxidant enzymes (Sreelather and Padma, 2009; Verma et al., 2009; Osman et al., 2012).

5.7 Hormonal attributes

The Cortisol levels was significantly ($P<0.05$) lower in T10 group when compared with that of Control and T20 values, showing positive effect of dietary treatment at 10% level of substitution. The decrease Cortisol level in accordance with

the reports of Patave et al. (2016) who reported decreased Cortisol level in STZ induced diabetic mice treated with ethanolic *Moringa oleifera* extract at the dose rate of 100 and 200 mg/kg. Similarly, Elabd et al. (2019) studied that by dietary supplementation of *Moringa oleifera* leaf meal to Nile tilapia (Oreochromisniloticus) fish the Cortisol level was significantly decrease in *Moringa oleifera* supplemented group throughout the experiment. Thus decrease in Cortisol level is indicative of lowered stress level that might be due to antioxidant properties of *Moringa oleifera* (Chumark et al. 2008). In present study the plasma testosterone of treatment groups was found similar with Control group. In accordance with our results, Sawsan et al. (2016) revealed no significant change in plasma testesterone level in rats administered *Moringa oleifera*. *Moringa oleifera* had no significant effects on the serum testosterone concentration in male mice (Zeng et al., 2009). Similar results have been reported by Cajuday and Pocsidio (2010) who fed male mice with a hexane extract of *Moringa oleifera*. However, the present reports are in disagreement with Syarifuddin et al. (2017) who reported significantly higher level of testosterone, when fed *Moringa oleifera* leaves at different levels in the ration of Bali bulls. Administration of *Moringa oleifera* leaf powder at 50 mg per kg body weight orally for 100 days improved the plasma testosterone levels (Akunna et al., 2012). It is studied that *Moringa oleifera* extract attenuates the reduction in the interstitial cells of Leydig and serum testosterone levels induced by stress exposure. Moreover in our study, it was suggested that the antioxidant effect of the *Moringa oleifera* alleviates oxidative stress-related toxicity that resulted in preventing the depression in plasma testosterone levels and enhance seminal characteristics. It has been reported that the elevation of glucocorticoid levels induced by stress can also inhibit the synthesis of testosterone in interstitial cells of Leydig (Gao et al., 1996). However, the stress level in present study was lowered which was evident with the lowered levels of Cortisol in treatment groups.

Summary and Conclusions

CHAPTER- 6

SUMMARY AND CONCLUSIONS

The aim of the experiment was to investigate the effect of replacing protein source in concentrate mixture with dried *Moringa oleifera* leaf powder on growth performance, nutrient utilization, seminal attributes, haematological parameters, blood biochemicals, antioxidant and hormonal parameters of Barbari bucks.

For this study, 18 Barbari bucks were selected from the herd maintained at Department of Physiology, DUVASU, Mathura (U.P.). The bucks were divided into 3 groups (Control, T10 and T20) having six animals each on body weight basis. Control group was fed basal diet consisting of concentrate mixture, green berseem and arhar straw where as T10 and T20 groups were fed basal diet (as that of control) along with replacement of soybean meal in concentrate mixture with dried *Moringa oleifera* leaf powder at level 10 and 20% respectively. Nutrient requirement of bucks were fulfilled as per NRC (2007) recommendations of feeding standard. The experimental feeding was for 90 days. During the experimental period DMI, body weight of animal was monitored fortnightly. At the end of experimental period, a digestion trial of seven days was conducted to assess the effect of replacing protein source in concentrate mixture with dried *Moringa oleifera* leaf powder on growth performance and nutrient utilization. Blood samples were collected at 0, 30, 60 and 90 days interval, respectively for haematological, biochemical, antioxidant and for hormonal assay. Haematological parameters were estimated in whole blood. The blood biochemicals and hormonal estimation were done in blood plasma at monthly interval. The antioxidant enzymes were estimated in RBC haemolysate. Semen was collected twice a week using artificial vagina from each buck for three weeks, total of six ejaculates from each buck was collected after 90 days of post feeding of experimental diets. Overall 108 ejaculates were collected and evaluated for different seminal attributes.

6.1 Growth performance and nutrient utilization in buck fed ration replacing soybean meal with dried *Moringa oleifera* leaf powder (MOLP)

The average initial body weight of Control, T10 and T20 groups were 28.0, 28.0 and 28.0 kg respectively. Final body weight of corresponding groups was 34.40, 34.35 and 33.60 kg, respectively. The average BW and metabolic BW of all fortnights were similar (P>0.05) between the groups. At fortnightly interval DMI of Control, T10 and T20 ranged from 0.91 to 1.19, 0.90 to 1.15 and 0.86 to 1.00 kg/d respectively. Overall DMI (kg/d, kg/100 kg BW) was found significantly (P<0.05) higher and comparable in Control and T10 group. Fortnightly body weight gain (kg) ranged from 0.98 to 1.15, 0.87 to 1.23 and 0.80 to 1.15kg in Control, T10 and T20 group respectively. Overall fortnightly BW gain (kg) was found significantly higher (P<0.05) and comparable in Control and T10 group. At first fortnight the average daily gain were 65.56, 57.78 and 53.33 g/d in Control, T10 and T20 groups, respectively. The Overall average daily gain (g/d) was also found significantly higher (P<0.05) and comparable in Control and T10 group. The FCR ranged from 14.20 to 19.40, 13.51 to 21.75 and 13.10 to 18.08 in Control, T10 and T20 groups respectively. No significant change in overall FCR was reported in the experimental groups.

Buck fed ration replaced with soybean meal at 10 and 20 % with dried *Moringa oleifera* leaf powder showed no significant (P>0.05) difference on nutrient digestibility. DM, OM, CP, EE, CF, NFE, ADF digestibility remained unaffected on dried *Moringa oleifera* supplementation. Dry matter intake (kg/day), CP intake (kg/day), DCP intake (kg/day, g/kg $W^{0.75}$) and TDN intake (kg/day, g/kg $W^{0.75}$) remained similar in all experimental groups during the digestion trial.

6.2 Seminal attributes in buck fed ration replacing soybean meal with dried *Moringa oleifera* leaf powder (MOLP)

The mean of seminal parameters viz semen colour, seminal pH, volume of ejaculated semen (ml) and spermatozoa concentration (millions/ml) were not affected by *Moringa oleifera* supplementation. Whereas, the average mass motility of Control, T10 and T20 ranged from 3.66 to 4.00, 4.25 to 3.97 and 3.66 to 4.00 respectively showed significantly higher (P<0.05) in T10 as compared to Control and T20 group. Likewise, percent progressive motility of spermatozoa of Control, T10 and T20 ranged from 82.50 to 85.83, 85.33 to 87.33 and 80.07 to 85.33% respectively and found

significantly higher (P<0.05) in T10 and significantly lower (P<0.05) in T20 as compared to Control group. Similarly, at different collection intervals percent live spermatozoa of Control, T10 and T20 ranged from 87.83 to 90.83, 91.66 to 92.83 and 85.83 to 90.66% respectively. And overall percent live spermatozoa count was found significantly higher (P<0.05) in T10 as compared to Control group however, the significantly lower values were obtained in T20 group. HOST reactive spermatozoa (%) of Control, T10 and T20 ranged from 80.16 to 84.00, 84.16 to 86.16 and 78.33 to 81.50 % respectively. Overall percent HOST reactive spermatozoa count was found significantly higher (P<0.05) in T10 as compared to Control and T20 group. Intact acrosomal percentage of Control, T10 and T20 ranged from 79.00 to 84.83, 82.00 to 86.19 and 77.50 to 82.00 % respectively. Intact acrosomal percentage was found significantly higher (P<0.05) in T10 and significantly lower (P<0.05) in T20 as compared to Control group. The total morphological abnormality percentage of spermatozoa in present study was found similar in both Control and *Moringa oleifera* treated groups.

6.3 Haematological and blood biochemicals parameters in buck fed ration replacing soybean meal with dried *Moringa oleifera* leaf powder (MOLP)

The overall concentration of haemoglobin at end of experiment was found to be 6.88, 7.13 and 7.55g/dl in Control, T10 and T20 groups, respectively and was found significantly higher (P<0.05) in T20 *Moringa oleifera* supplemented group than Control group. The overall concentration of PCV was found to be 19.76, 19.87 and 22.08% in Control, T10 and T20 groups respectively and was found significantly higher (P<0.05) in T20 *Moringa oleifera* supplemented group than Control group. The plasma glucose, cholesterol, triglyceride value did not change significantly (P>0.05) in the treatment group. Plasma total protein, albumin and globulin concentrations were also non significant in Control and *Moringa oleifera* treated groups. Plasma levels of enzymes like ALT, AST remain unchanged in Control and treatments group. Results from present study showed that dose of supplementation of *Moringa oleifera* in bucks did not impair the liver functions. Thus, replacing soybean meal with dried *Moringa oleifera* leaf powder at 10 and 20% levels in concentrate mixture fed to Barbari bucks did not negatively affect blood parameters and hepatic metabolism in this study.

6.4 Antioxidant status in buck fed ration replacing soybean meal with dried *Moringa oleifera* leaf powder (MOLP)

In the present study, plasma SOD activity of the bucks fed T10 diet was significantly higher ($P < 0.05$) than those of bucks fed T20 and Control diet. Mean plasma SOD activity at the beginning of experiment were 39.70, 35.91 and 38.70 U/mgHb in Control, T10 and T20 groups, respectively and corresponding values at the end of experiment were 44.57, 53.55 and 49.22 U/mg Hb respectively. Overall SOD activity increased significantly ($P<0.05$) in T10 group where as catalase activity had no significant difference between *Moringa oleifera* treated and Control groups. Whereas, mean plasma lipid peroxidation (nM MDA/ml packed RBCs) activity at the beginning of experiment were 7.22, 7.21 and 7.16 nM MDA/ml packed RBCs in Control, T10 and T20 groups, respectively and corresponding values at the end of experiment were 8.15, 5.37 and 7.33 nM MDA/ml packed RBCs respectively. Plasma lipid peroxidation concentration of T10 groups was found significantly lower as compared to Control group. A significant elevation of the activity of SOD and reduction of LPO was observed in bucks fed *Moringa oleifera* leaf powder at 10% replacement with soybean meal as compared with the Control and T20 group. Overall, the data indicates that *Moringa oleifera* was effective in raising the antioxidant status of the bucks.

6.5 Hormonal status in buck fed ration replacing soybean meal with dried *Moringa oleifera* leaf powder (MOLP)

The plasma Cortisol concentration (ng/ml) of experimental animals at monthly interval ranged from 22.14 to 34.42 ng/ml in Control, 18.43 to 21.81ng/ml in T10 groups and 21.51 to 28.89 ng/ml in T20 groups. Overall plasma Cortisol concentration was found significantly lower ($P<0.05$) in T10 *Moringa oleifera* treated group as compared to Control group. The plasma testosterone concentration of treatment groups was found similar to that of Control group. No significant difference in testosterone concentration was found between Control and treatment groups. In present study, it was suggested that the antioxidant effect of the *Moringa oleifera* alleviates oxidative stress that resulted in preventing the depression in plasma testosterone levels and improved seminal attributes. The elevated level of glucocorticoids during stress inhibits the synthesis of testosterone in interstitial cells of

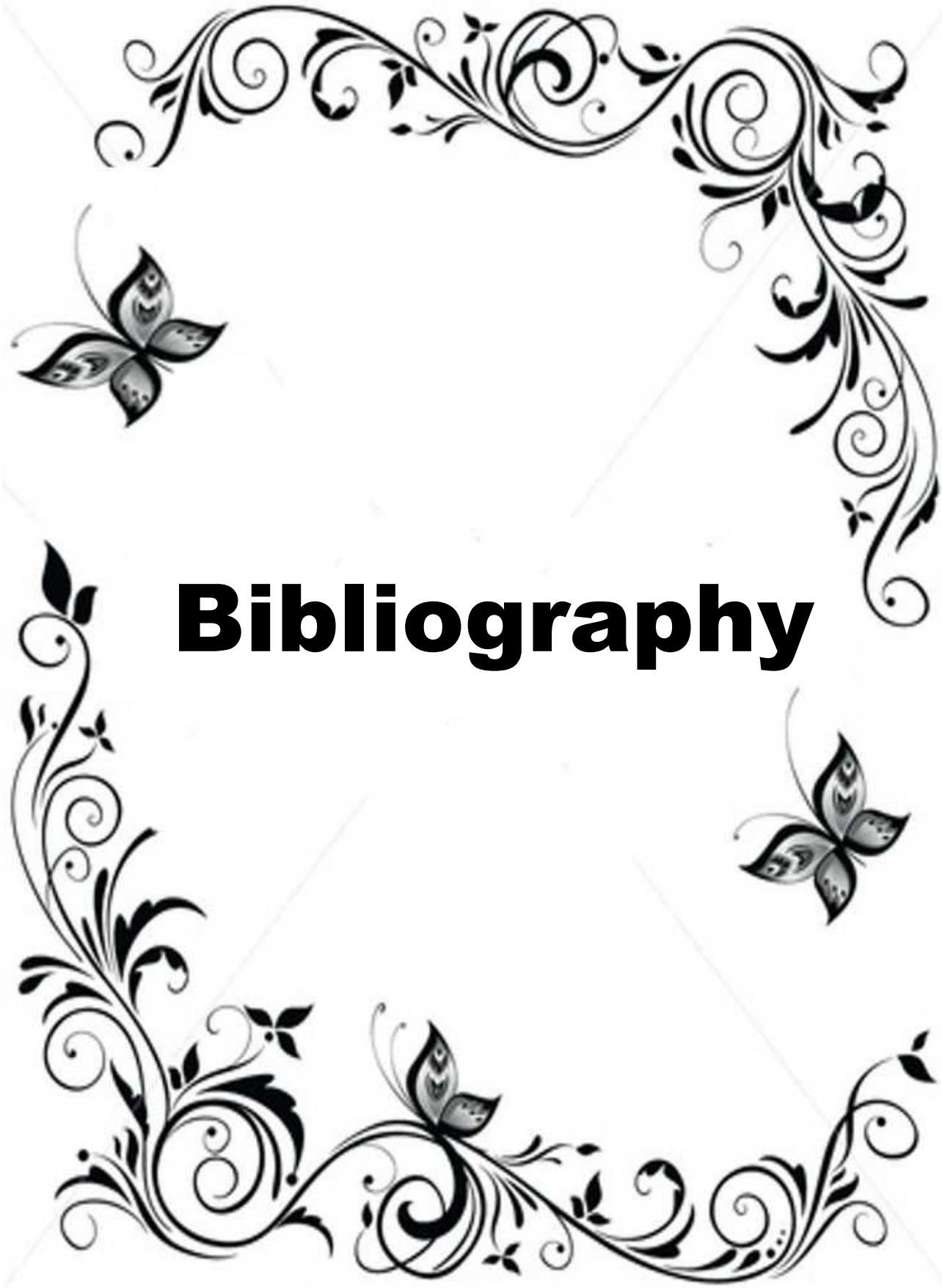
Leydig. However, the stress level in present study was lowered which was evident with the lowered levels of Cortisol in treatment groups.

6.6 Conclusions

The effect of replacing soybean meal in concentrate mixture with dried *Moringa oleifera* leaf powder on the performance of Barbari bucks may be concluded as:

1) Replacing the soybean meal in concentrate mixture with dried *Moringa oleifera* leaf powder at 10% level showed significant ($P< 0.05$) increase in DMI, fortnightly weight gain and daily gain of Barbari bucks.
2) Inclusion of dried *Moringa oleifera* leaf powder showed no significant ($P>0.05$) difference on nutrient intake and digestibility.
3) Semen analysis showed significant increase in mass motility, progressive motility, HOST and acrosomal integrity in T10 group as compared to control and T20 group.
4) Haemoglobin and PCV values were found significantly higher in *Moringa oleifera s*upplemented groups. However, the concentration of all other blood biochemicals studied remained unaltered.
5) Incorporation of dried *Moringa oleifera* leaf powder at 10% showed improvement in antioxidant profile with significant ($P< 0.05$) increase in SOD activity and decreased LPO values.
6) The assay of Cortisol was lower ($P< 0.05$) in *Moringa oleifera* supplemented group. However, the testosterone concentration remained unchanged.

Hence the present results suggested that the soybean meal in concentrate mixture can be effectively replaced with dried *Moringa oleifera* leaf powder at 10 % level without any deleterious effect on blood parameters and hepatic metabolism, with stimulatory effects on their antioxidants status, anti stress and improved seminal attributes of Barbari bucks.



Bibliography

19th Livestock Census. 2012. Department of Animal Husbandry and Dairying, Government of India.

Adegun MK and Aye PA. 2013. Growth performance and economic analysis of West African Dwarf Rams fed *Moringa oleifera* and cotton seed cake as protein supplements to *Panicum maximum*. American Journal of Food and Nutrition, 3(2):58-63.

Aebi H. 1984. Catalase in vitro. Methods in Enzymology, 105:121-126.

Agricultural and Processed Food Products Export Development Authority. 2015-16.

Ajugwo AO, Mounbegna PE, Kemajou TS, Ofakansi VC. 2017. Effects of *Moringa oleifera* leaves extract on haematological parameters of phenylhydrazine anaemia induced Wistar rats. International Journal of Public health and Safety, 2: 139.

Ajuogu PK, Mgbere OO, Bilas DS, McFarlane JR. 2019. Hormonal change, semen quality and variance in reproductive activity outcomes of post pubertal rabbits fed *Moringa oleifera* Lam. Leaf Powder. Journal of Ethanopharmacology, 233: 80-86.

Akhouri S, Prasad A, Ganguly S. 2014. World Journal of Pharmacy and Pharmaceutical Sciences, 3(7): 523- 525.

Akunna GG, Ogunmodede OS, Sallu CL, Ogunlade B, Bello AJ, Salawu EO 2012. Ameliorative Effect of *Moringa oleifera* (drumstick) Leaf extract on chromium- induced testicular toxicity in rat testis. World Journal of Life Science and Medical Research, 2:6-20.

Ali SB. 2017. Growth performance of goat fed *Moringa oleifera* leaf meal incorporated in concentrate mixture. MVSc Thesis submitted to Maharashtra Animal and Fisheries Sciences University, Nagpur.

Anhwange BA, Ajibola VO, Oniye. 2004. Amino acid composition of seed of *Moringa oleifera*, *Detarium microcarpum* and *Bauhinia monandra*. Journal of Chemical Education, 7:9-13.

Anwar F, Bhanger MI. 2003. Analytical characterization of *Moringa oleifera* seed oil grown in temperate regions of Pakistan. Journal of Agricultural and Food Chemistry, 51:6558–6563.

Anwar F, Latif S, Ashraf M, Gilani AH. 2005. *Moringa oleifera* plants with medicinal uses. Phytotherapy Research, 21(1):17-25.

AOAC. 2000. Association of Official Analytical Chemist. Official Method of Analysis.17th edition, Washington DC.

Aregheore EM. 2002. Intake and digestibility of *Moringa oleifera* - batiki grass mixture by growing goats. The Official Journal of the International Goat Association, 46(1):23-28.

Arigbede OM, Olanite JA, Bamikole MA. 2005. Intake, performance and digestibility of West African Dwarf goats supplemented with graded level of Grewia pubescens and Panicum maxicum. Nigerian Journal of Animal Production, 32(2):293-300.

Arti R, Verma M, Vijayakumar, Chandra S. 2009. In vitro and in vivo antioxidant properties of different fractions of *Moringa oleifera* leaves. Food and Chemical Toxicology, 47(9):2196-2201.

Asaolu VA, Akinlade JA Aderinola OA, Okewoye AT, Atalade JA. 2011. Performance of grazing West African Dwarf Goats on *Moringa* multi nutrient block supplementation. Asian Journal of Animal Sciences, 6(6):263-277.

Asaolu VA, Binuomote RT, Akinlade JA, Oyelami OS, Kolapo KO. 2010. Utilization of *Moringa oleifera* fodder combination with *Leucaena leucocephala* (LEU) and *Gliricidia sepium* (GLI) fodders by West African Dwarf goats (WAD). International Journal of Agricultural Research, 6(8):607-619.

Aslam V, Binuomote R, Akinlade J. 2005. Intake and growth performance of West African dwarf goats fed *Moringa oleifera*, *Gliricidia sepium* and *Leucaena leucocephala* dried leaves as supplements to cassava peels. Journal of Biology Agriculture and Health, 2:76–88.

Aye PA, Adegun MK. 2013. Chemical composition and some functional properties of *Moringa*. Leucaena and Gliricidia leaf meals. Agriculture and Biology Journal of North America, 4(1):71-77.

Babeker EE, Abdalbagi YM. 2015. Effect of feeding different levels of *Moringa oleifera* leaves on performance, haematological, biochemical and some physiological parameters of Sudan Nubian goats. Journal of Animal and Feed Research, 5:50–61.

Babiker EE, Al Juhaimi F, Ghafoor K, Mohammad HE, Abdoun KA. 2016. Effect of partial replacement of alfa alfa hay with *Moringa* species leaves on milk yield and composition of Najdi ewes. Tropical Animal Health Production, 48(7):1427-1433.

Babiker EE, Juhaimi FA, Ghafoor K, Abdoun KA. 2017. Comparative study on feeding value of *Moringa* leaves as a partial replacement for alfalfa hay in ewes and goats. Livestock Science, 195:21–26.

Becker M. 2003. Studies on utilization of *Moringa oleifera* leaves as animal feed. Institute for Animal Production in Tropic and Sub Tropic, 480:15.

Ben Salem H, Nefzaoui A, Makkar HPS.2004. Towards better utilization of non-conventional feed sources by sheep and goats in some African and Asian countries. International Center for Advanced Mediterranean Agronomic Studies 59:177-187.

Bennet RN, Mellon FA, Foidl N, Pratt J, Dupont MS, Perkins L, Kroon PA. 2003. Profiling glucosinolates and phenolics in vegetative and reproductive tissues of the multi- purpose trees *Moringa oleifera* and *Moringa Stenoptela.* Journal of Agriculture and Food Chemistry, 51:3546-3553.

Bergmeyer HU. 1983. Method of enzymatic analysis volume 1, 2nd edition VCH Weinheim.

Bhatnagar SS, Santapau H, Desai JDH, Yellore S, Rao TNS. 1961. Biological activity of Indian medicinal plants. Part 1. Antibacterial, anti tubercular and antifungal action. Indian Journal of Medical Research, 49:799-805.

Bhattacharya SB, Das AK, Banerji N. 1982. Chemical investigation on the gum exudates from Sajna *(Moringa oleifera*). Carbohydrate Research, 102:253-262.

Cajuday LA, Pocsidio GL. 2010. Effect of *Moringa oleifera* Lam. (Moringaceae) on the reproduction male mice (*Mus musculus*). Journal of Medicinal Plant Research, 4(12):1115-1121.

Chance B, Green stein DS, Roughton FJ. 1952. The mechanism of catalase action. Archieve of Biochemistry and Biophysics, 37(2):301- 321.

Choudhary RK, Roy A, Roy PS, Singh KM, Kumar P. Effect of replacing Concentrate mixture with *Moringa* leaves (*Moringa oleifera*) on performance of lactating Bengal goats. International Journal of Current Microbiology and Applied Sciences, 7:2895-2900.

Chukwunomso PD, Ahamefule FO, Okechukwu 0S. 2017. Feed intake, body weight and haematology of West African Dwarf goats fed dietary level of *Moringa oleifera* leaf meal. Agricultra, 13(1):71-77.

Chumark P, Khunawat P, Sanvarinda Y, Phornchirasilp S, Morales NP, Phivthong-ngam L, Ratnachamnong P, Srisawat S, Pongra peeporn KS. 2008. The in vitro and ex vivo antioxidant properties, hypolipidaemic and antiatherosclerotic activities of water extract of *Moringa oleifera* lam. Leaves. Journal of Ethnopharmacology, 116:439-446.

Curtis SJ, Mortiz M, Snodgrass PJ. 1972. Serum enzymes derived from liver cell fractions. The response to carbon tetrachloride intoxication in rats. Gastroenterology, 62(1):84-92.

D'souza J, Kulkarni AR. 1993. Comparative studies on nutritive values of tender foliage of seedlings and mature plants of *Moringa oleifera*. Journal of Economic and Taxonomic Botany, 17:479–485.

Dahot MU.1988. Vitamin content of flower and seed of *Moringa oleifera*. Journal of Biochemistry, 21:21-24.

Damor SV, Pawar MM, Ankuya KJ, Gami YM, Srivastava AK, Chauhan HD, Chaudhary KR. 2017. Effect of feeding different levels of *Moringa* (*Moringa oleifera*) leaves on growth performance of Mehsana goat kids. Trends in Bioscience, 10(18):3190-3193.

Damor SV, Pawar MM, Gami YM *et al.* 2013. Effect of replacing concentrate mixture with *Moringa oleifera* leaves on blood biochemical and mineral profile of Mehsana goat kids. Life Science, 89:28-35.

Dewangan G, Koley KM, Vadlamudi VP, Mishra A, Poddar A, Hirppurkar SD.2010. Antibacterial activity of *Moringa Oleifera* (drumstick) root bark. Journal of Chemical and Pharmaceutical Research, 2:424–428.

Dey A, Paul SS, Pandey P, Rathore R. 2014. Potential of *Moringa oleifera* leaves in modulating invitro methanogenesis and fermentation of wheat straw in buffalo. Indian Journal of Animal Sciences, 84:533- 538.

Dhakar RC, Maurya SD, Pooniya BK, Bairwa N, Gupta M. 2011. *Moringa*: the herbal gold to combat malnutrition. Chronicles of Young Scientist, 2:119.

Dhanotiya. 2004. Text book of Veterinary Biochemistry, College of veterinary Science and animal husbandry; India, 448.

Divya, Mandal AB, Biswas A, Yadav AS, Biswas AK. 2014. Effect of dietary *Moringa oleifera* leaves on growth performance, blood chemistry, meat quality and gut micro flora of broiler chicks. Animal Nutrition and Feed Technology, 14:349-357.

El- Badawi AY, Omer HAA, Abedo AA, Yacout MHM. 2014. Response of growing New Zealand White rabbit to ration supplemented with different level of *Moringa* dry leaves. Global Veterinaria, 14:400-408.

Elabd H, Soror E, Asley EL. 2019. Dietary supplementation of *Moringa* leaf meal for Nile Tilapia *Oreochromis niloticus*: Effect on growth and stress indices. Egyptian Journal of Aquatic Research, https://doi.org/10.1016/j.ejar.2019.05. 009.

Eldesoky NI, Hashem NM, Elkomy A, Aboelezz ZR. 2017. Physiological response and semen quality of rabbit buck supplemented with *Moringa oleifera* leaves ethanolic extract during summer season. Animal 11: 1549- 1557.

Fadiyimu AA, Julias AA, Fajemisin AN. 2010. Digestibility, nitrogen balance, and haematological profile of West African Dwarf sheep fed dietary level of *Moringa oleifera* as supplement to *Panicum maxicum*. Journal of Animal Science, 6(10): 634-643.

Fahey J, Zalcmann A, Talalay P. 2001. The chemical diversity and distribution of glucosinolates and isothiocyanates among plants. Phytochemistry 2001, 56, 5–51.

Fahey JW. 2005. *Moringa oleifera*: A review of the medical evidence for its nutritional, therapeutic and prophylactic properties. Trees for Life Journal, 1:5.

Faizi S, Siddiqui B, Saleem R, Saddiqui S, Shaheen F, Gilani AH.1998. Hypotensive constituent from the pods of *Moringa oleifera*. Planta Medicine, 64:225-228.

Farooq A, Sajid L, Muhammad A, Anwarul HG. 2007. *Moringa oleifera*: A food plant with multiple medicinal uses. Phytotherapy Research, 21: 17-25.

Farooq F, Rai M, Tiwari A, Khan AA, Farooq S. 2012. Medicinal properties of *Moringa oleifera*. An overview of promising healer. Journal of Medicinal Plant Research, 6(27):4368- 4374.

Foidl N, Makkar HPS, Becker K. 2001. The Potential of *Moringa oleifera* for agricultural and industrial uses. In: Proceeding of the international Workshop, What Development potential of *Moringa* Products, Tanzania, pp 47-67.

Food and Agriculture Organization of the United Nations (FAO). 2014.

Francis G, Makkar HPS, Becker K. 2001. Anti nutritional factor present in plant derived alternate fish feed ingredient and their effect in fish. Fish Aquaculture, 199(3-4):197-227.

Fuglie LJ. 2001. Combating malnutrition with *Moringa* the miracle tree: The multiple attributes of *Moringa*, Phytotherapy Research, pp 117.

Gao HB, Shan LX, Monder C, Hardy MP. 1996. Suppression of endogenous corticosterone level in vivo increases the steroidogenics capacity of purified rat leydig cell invitro. Endocrinology, 143(1):130-138.

Hairy E, Shamiah M, Ghodaia AE. 2016. Influence of oral whole extract from *Moringa oleifera* on semen characteristic of rabbits. Journal of Animal and Poultry Production, 7(6):217-224.

Hancock JL. 1951. A staining techniques for the study of temperature shock in semen. Nature (London), 169:323-326.

Hankok. 1952. The morphology of bull spermatozoa. Journal of Experimental Biology. 29(3):445-453.

Harairy E, Shamiah M, Ghodaia AE. 2016. Influence of oral whole extract from *Moringa oleifera* on semen characteristic of rabbits. Journal of Animal and Poultry Production, 7(6):217-224.

Hoffmann EM, Muetzel S, Becker K. Effects of *Moringa oleifera* seed extract on rumen fermentation in vitro. Archives of Animal Nutrition, 57(1):65-81.

Jain PG, Patil SD, Haswani NG, Girase MV, Surana SJ. 2010. Hypolipidemic activity of *Moringa oleifera* lam, Moringacae, on high fat diet induced hyperlipidemia in albino rats. Revista Brasileira de Farmacognosia, 20:969-973.

Jayendran RS, Vandervent HH, Perez Peleae ZM, Crabo BG and Zanevald LJD. 1984. Development of an assay to assess the functional integrity of human sperm membrane and its relationship to their semen characteristics. Journal of Reproduction and Fertility, 70:219-225.

Jiwuba PC, Ahamefule FO, Ogbuewu IP, Ikwunze K. 2017. Blood chemistry and haematology of West African dwarf goat fed *Moringa oleifera* leaf meal (MOLM) in their diets. Comparative Clinical Pathology, 26:621-624.

Jiwuba PC, Ahamefule FO, Okechukwu OS, Ikwunze K. 2016. Feed intake, body weight changes and haematology of West African dwarf goats fed dietary levels of *Moringa oleifera* leaf meal. Agricultura, 13(1-2):71-77.

Jongrungruangchok S, Bunrathep S, Songsak T. 2010. Nutrient and mineral content of eleven different sample of *Moringa oleifera* cultivated in Thailand. Journal of Health Research, 24:123-137.

Kakengi AMV, Kaijage JT, Sarwatt SV, Mutayoba MN, Fujihara. 2007. Effect of *Moringa oleifera* leaf meal as substitute for sun flower seed meal on performance of laying hens. Livestock Resources for Rural Development, 19:12.

Kakenji AMV, Shen MN, Servert SV, Fujihara T. 2003. Can *Moringa oleifera* be used as protein supplement to ruminant. Asian Australian Journal of Animal Science, 18: 42-47.

Kaneko JJ, Harvey JW, Bruss ML.1997 Clinical biochemistry of domestic animals. Harcourt Brace and Company Asia Academic Press, USA, 5:890-893.

Khalifa WH, Ibrahim FM, Aida I, Makawy EL, Hafiza A, Wagbdy KB, Nagwa A. 2016. Safety and fertility enhancing role of *Moringa oleifera* leaves aqueous extract in New Zealand rabbit buck. International Journal of Pharmacy, 6(1): 156-168.

Kholif AE, Gouda G.A, Anele UY, Galyean ML. 2018. Extract of *Moringa oleifera* leaves improves feed utilization of lactating Nubian goats. Small Ruminant Research, 158:69-75.

Kholif AE, Gouda GA, Morsy TA, Salem AZ, Lopez S, Kholif AM. 2015. *Moringa oleifera* leaf meal as a protein source in lactating goat's diets: feed intake, digestibility, ruminal fermentation, milk yield and composition, and its fatty acids profile. Small Ruminant Research, 129:129-137.

Kholif AE, Morsy TA, Gouda GA, Anele UY, Galyean ML. 2016. Effect of feeding diets with processed *Moringa oleifera* meal as protein source in lactating Anglo-Nubian goats. Animal Feed Science and Technology, 217:5-55.

Kumar S, Rama Rao CA, Kareemulla B, Venkateswarlu. 2010. Role of goats in livelihood security of rural poor in the less favoured environments. Indian Journal of Agriculture Economy, 65(4):761-783.

Kumar SP, Mishra D, Ghosh D, Panda CS. 2009. Medicinal uses and pharmacological properties of *Moringa oleifera*. International Journal of Phytomedicine, 2(3): 210-216.

Lasley JF. 1951. Spermatozoa motility as a measure of semen quality. Journal of Animal Science, 10:211-218.

Leone A, Spada A, Battezzati A. 2015. Cultivation, genetic, ethnopharmacology, phytochemistry and pharmacology of *Moringa oleifera* leaves: an overview. International Journal of Molecular Science, 16:12791-12835.

Liyana – Pathirana C, Dexter J, Shahidi F. 2006. Antioxidant properties of wheat pearling . Journal of Agriculture and Food chemistry, 54: 6177- 6184

Luqman S, Srivastava S, Kumar S, Maurya AK, Chanda D. 2012. Experimental assessment of *Moringa oleifera* leaf and fruit for its antistress, anti oxidant and

scavenging potential using in vitro and in vivo assays. Hindawi, http://dx.doi.org/10.1155/2012/519084.

Mabruk AA, Talib HN, Mohamed MA, Alawad AH. 2010. A note on the potential use of *Moringa oleifera* tree as animal feed. Journal of Veterinary Medicine and Animal Production, 1(2):184-188.

Madesh M, Balasubramanian KA. 1997. A microtiter plate assay for superoxide using MTT reduction method. Indian Journal of Biochemistry and Biophysics, 34, 535-539.

Makkar HPE, Becker K. 1996. Nutritional value and nutritional components of whole and extracted *Moringa oleifera* leaves. Animal Feed Science and Technology, 63:211-228.

Manh LH, Dung N, Xuan VT 2005. Introduction and evaluation of *Moringa oleifera* for biomass production and as feed for the goats in the Mekong Delta. Livestock Resource Rural Development. Available at http// www.lrrd.org/ lrrd17/9/manh17104.htm

Mehta K, Balaraman R, Amin AH, Bafna PA, Gulati OD. 2003. Effect of fruit of *Moringa oleifera* on the lipid profile of normal and hypercholesterolaemic rabbit. Journal of Ethanopharmacology, 86:191-195.

Melesse A. 2012. The feeding value of deseeded pods from *Moringa stenoptela* and *Moringa oleifera* as evaluated by chemical analyses and in vitro gas production. Journal of Animal and Feed Science, 21:527-550.

Mendieta-Araica B, Sporndly R,Sanchez N, Sporndly E. 2011. *Moringa* (*Moringa oleifera*) leaf meal as a source of protein in locally produce concentrate for dairy cows fed low proteins diets in tropical areas. Livestock Science, 137(1/3):10-17.

Merk, 2011. Merk sharp and Dohme Corporation, white house station, NJ,USA.

More T. 2006. Animal Clinical Biochemistry. 1st Edition, Kalyani Publishers, Ludhina, pp 317-318.

Mortan JF.1990. The horseradish tree, *Moringa pterygosperma* (Moringaceae) - A boon to arid lands. Economic Botany, 45(3):318-333.

Moyo B, Masika PJ and Muchenje V. 2014. Effects of feeding *Moringa* leaf meal on the physico-chemical characteristic and sensory properties of goat meat. South African Journal of Animal Science, 44(1):64-70.

Moyo B, Masika PJ, Muchenje V. 2011. Effect of supplementing crossbred Xhosa lop-eared goat castrates with *Moringa oleifera* leaves on growth performance, carcass and non-carcass characteristics. Tropical Animal Health Production, 44:801–809.

Moyo BS, Oyedemi PJ, Masika V, Muchenje. 2012. Polyphenolics content and anti oxidant properties of *Moringa oleifera* leaf extracts and enzymatic activity of liver from goats supplemented with *Moringa oleifera* leaves / sun flower seed cake. Meat Science, 91:441-447.

Murro J, MuhikambeleV, Sarwatt S. 2003. *Moringa oleifera* leaf meal can replace cotton seed cake in concentrate mix feed with Rhodes grass (*Choloris gayana*) hay for growing sheep. Livestock Research for Rural Development, 15(11):234-236.

Nagar PK, Indira R, Sircar PK. 1982. Cytokinins in developing fruits of *Moringa Pterigosperma* Gaertn. Physiologia Plantarum, 55(1):45-50.

Nair AGR, Subramanian. 1962. Pigments of the flowers of *Moringa pterygosperma*. Current Science 31: 55-56.

National Research Council. 2007. Nutrient requirement of goat. National Academy Press, Washington DC, USA.

Nazir M. 1988. Semen evaluation and sperm morphology. Monograph on reproductive pattern of riverine buffaloes and recommendations to improve their reproductive performance at small farmer level. Pakistan Agricultural Research Council, Islamabad, Pakistan.

Nouala FS, Akinbamijo OO, Adewumi A, Hoffman E, Muetze S, Becker K.2006. The influence of *Moringa oleifera* leaves as substitute to conventional concentrate on the in vitro gas production and digestibility of ground nut hay. Livestock Research for Rural Development, 18:12-16.

Obembe AO, Urom SE, Ofutet EO, Ikpi DE, Okpo-Ene AI. 2015. The effect of aqueous seed extracts of *Moringa Oleifera* on sperm count, motility and morphology in male albino wistar rats. Der Pharmacia Lettre, 7(3):129-133.

Ohkawa H, Ohishi N, Yagi K. 1979. Assay for lipid peroxides in animal tissues by thiobarbituric acid reaction. Analytical Biochemistry, 95(2):351-358.

Ojo OA, Adetoyl SA. 2017. Effect of *Moringa oleifera* leaf extract on the haematological and serum biochemistry of rabbit reared in a semi humid environment. African Journal of Biotechnology, 16(25):1386-1390.

Ologhobo AD, Apata DF, Oyegide A, Akinpelu RO. 1992. Toxicity of raw lima beans (*Phaseolus lunatus*) and lima bean fraction for growing chicks. British Poultry Science, 34:505-522.

Osman HM, Shayoub ME, Babiker EM. 2012. The effect of *Moringa oleifera* leaves on blood parameters and body weights of Albino rats and rabbits. Jordan Journal of Biological Sciences, 5:147-150.

Oyedele OJ, Asalou VO, Odeyinka SM. 2016. Nutrient digestibility and growth performance of West African Dwarf (WAD) goats fed foliage combination of *Moringa Oleifera* and gliricidia sepium with equal proportion of low- cost concentrate. Journal of Natural Sciences Research, 18:2224-3186.

Oyedemi MO, Oluwatoyin O, Leigh O, Pidesiji T, Fisayo A. 2008. The spermiogram of male wistar rats treated with aqueous leaf extract of vernonia amygdalina. Folia Veterinarias, 52(2):98-103.

Patave Tarannum R, Unehemant D, Quadri Neha A. 2016. Effect of stress on Anxiety in streptozocin induced diabetic mice using *Moringa oleifera* Lam pod. International Journal of Pharmacist and Pharmaceutical Research 6(2):80-94.

Priyadarshani NM, Varma MC. 2014. Effect of *Moringa oleifera* leaf powder on sperm count, histology of testis and epididymis of hyperglycemicmice *Mus musculus*. American International Journal of Applied and Natural Science, 7:07-13.

Purdy PH, Ericsson SA, Dodson RE, Sternes KL. 2004. Effect of the flavonoids, silibinin and catechin, on the motility of extended cooled caprine sperm. Small Ruminant Research, 55(1):239-243.

Qwele K, Hugo A, Oyedemi B. 2012. Chemical composition, fatty acid content and antioxidant potential of meat from goats supplemented with Moringa (*Moringa oleifera*) leaves, sunflower cake and grass hay. Journal of Meat Science, 93(3):455-462.

Raji AY, Nijidda. 2014. Gonadal and extra- gonadal sperm reserves of the Red Skoto goats fed *Moringa oleifera* supplemented diets. International Journal of Agriculture and Biosciences, 3(2):61-64.

Ramchandran C, Peter KV, Gopalkrishnan PK. 1980. Drumstick (*Moringa oleifera*): A multipurpose Indian vegetable. Economical Botany, 34:276-283.

Richter N, Siddhuraju P, Becker K. 2003. Evaluation of nutritional quality of *Moringa* (*Moringa oleifera* Lam.) leaves as an alternative protein sources for Nile tilapia. Aquaculture, 217(1/4):599-611.

Rivera S, Nemeth E, Gabayan V, Lopez MA, Farshidi D, Ganz T. 2005. Synthetic hepcidin causes rapid dose dependent hypoferramia and is concentrated in ferroportin- containing organs. Blood, 106:2196-2199.

Robak J, Gryglewski RJ. 1988. Flavonoid are scavenger of superoxide anions. Biochemistry and Pharmacology, 37(5):837- 841.

Rockwood JL, Anderson BG, Casmatta DA.2013.Potential uses of *Moringa oleifera* and an examination of antibiotic efficacy conferred by *Moringa oleifera* seed and leaf extract using crude extraction technique available to underserved indigenous population. International Journal of Phytotherapy, 3:61-71.

Rubanza CDK, Shem MN, Otsyina ER, Bakengesa SS, Ichinohem T, Fujihara T. 2005. Polyphenolic and tannin effect on in vitro digestibility of selected Acacia species leaves. Animal Feed Science Technology, 119(1-2):129-142.

Rukmani K, Kavinami S, Anandan R, Jaykar B. 1998. Effect of *Moringa oleifera* Lam on paracetamol induced hepatotoxicity. Indian Journal of Pharmaceutical Sciences, 60:33-35.

Saini RK, Shetty NP, Giridhar P. 2014. Carotenoid content in vegetative and reproductive part of commercially grown *Moringa oleifera* Lam cultivator from india by LC-APCI-MS. European Food Research Technology, 238: 971-978.

Saini RK, Shetty NP, Giridhar P. 2016. GC- FID/MS analysis of fatty acid in Indian cultivars of *Moringa oleifera*: potential source of PUFA. Journal of the American Oil Chemists Society, 91:1029-1034.

Sanchez- Machado DI, Nunez-Gastelum JA, Reyes Moreno C, Ramirez-Wong B, Lopenz-Cervantes J. 2010. Nutritional quality of edible parts of *Moringa oleifera*. Food Analytical Methods, 3:175-180.

Sanchez NR, Sporndly E, Ledin. 2005. Effect of different level of foliage of *Moringa oleifera* to creole dairy cows on intake, digestibility, milk production and composition. Livestock Science, 2810:8.

Sarwatt SV, Kapange SS, Kakengi AMV. 2002. Substituting sunflower seed cake with *Moringa oleifera* leaves as a supplemental goat feed in Tanzania. Agro Forestry Systems, 56(3):241-247.

Sawsan M, Khairy MA, Fadil HA, Azza MA.2016. Ameliorative effect of *Moringa oleifera* extract on Male fertility in Paroxetime treated rats. Zagazig Veterinary Journal 44(3):244-250.

Saxena M, Saxena J, Nema R, Singh D, Gupta A. 2013. Phytochemistry of medicinal plant. Journal of Pharmacognosy and Phytochemistry, 1(4) 82-168.

Shafiq-U-Rehman. 1984. Lead-induced regional lipid peroxidation in brain. Toxicology Letters, 21:333- 337.

Sharifudin SA, Fakurazi S, Hidayat MT, Hairuszah, Moklas MA, Arulselvan P. 2013. Therapeutic potential of *Moringa oleifera* extracts against acetaminophen-induced hepatotoxicity in rats. Pharmaceutical Biology, 51(3):279-288.

Sholapur HPN, Patil BM. 2013. Pharmacognostic and phytochemical investigation on the bark of *Moringa oleifera* Lam. Indian Journal of Natural Products and Resources, 4(1):96-101.

Siddhuraju P, Becker K.2003. Antioxidant properties of various solvent extracts of total phenolic constituents from three different agro-climatic origins of drumstick tree (*Moringa oleifera* Lam.). Journal of Agriculture and Food Chemistry, 15:2144–2155.

Singh BN, Singh BR, Singh RL, Prakash D, Dhakarey R Upadhya G. 2009. Oxidative DNA damage protective activity, antioxidant and anti quorum sensing

potentials of *Moringa oleifera.* Food and Chemical Toxicology, 47:1109-1116.

Singh C, Chauhan PA, Parmar VN, Thakur D. 2017. Nutritional evaluation of total mixed ration comprising of pigeon pea (*Cajanus cajan*) straw in cattle. Journal of Entomology and Zoology studies, 5(6): 2605-2610.

Sogbo KA. (2006). *Moringa* leaf farming systems: conditions for profitability and sustainability. Retrieved, pp 11-19.

Soliva CR, Kreuzer M, Foidl N, Foidl G, Mach- Muller A, Hess HD. 2005. Feeding value of whole and extracted *Moringa oleifera* leaves for ruminants and their effects on ruminal fermentation in vitro. Animal Feed Science and Technology, 118(1):47-62.

Somali MA, Bajnedi MA, Alfhaimani SS. 1984. Chemical composition and characteristic of *Moringa* peregrine seeds and seed oil. Journal of America, 61:85-86.

Sreelathar S, Padma PR. 2009. Antioxidant activity and total phenolic content of *Moringa oleifera* leaves in two stages of maturity. Plant Foods Human Nutrition Journal, 64:303–311.

Stanek M, Florek S, Rydzik W, Rusiecka L. 1992. Effect of energy feed in diet for sheep on nutrient digestibility and rumen digestion process. Acta Acadamey of Agriculture Technology, 37:3-11.

Steinfold H, Opio C. 2010. Responces on human nutrition issues. Livestock in a Changing Landscape. Proceedings of the National Academy of Science, 1: 373.

Sudha P, Asdaq SM, Dhamingi SS, Chandrakala GK. 2010. Immune-modulatory activity of methanolic leaf extract of *Moringa oleifera* in animals. Indian Journal of Physiology and Pharmacology, 54(2):133-140.

Sultana N, Alimon AR, Haque KS, Sazili AQ, Yaakub H, Hossain, SMJ. (2014). The effect of cutting interval on yield and nutrient composition of different plant fraction of *Moringa oleifera* tree. Journal of Food Agriculture and Environment, 12(2):599-604.

Sultana N, Alimon AR, Huque KS et al. 2016. The feeding value of Moringa (*Moringa oleifera*) foliage as replacement to conventional concentrate diet in Bengal goats. Advance in Animal and Veterinary Sciences, 3(3):164–173.

Sultana N, Alimon AR, Huque KS, Baba M and Hossain J. 2015.Evaluation of Moringa foliage (*Moringa oleifera*) as goat Feed. Iranian Journal of Applied Animal Science, 5(4) 865:871.

Syarifuddin NA, Toleng AL, Rahardja, Yusuf M. 2017. Improving libido and sperm quality of Bali bulls by supplementation of *Moringa oleifera* Leaves. Media Peternakan Journal, 40(2):88- 93.

Takahasi K, Vehida A, Kitao M. 1990. Hypo-osmotic swelling test of sperm. Archieves of Andrology, 25:225-242.

Talpatra SK, Ray SC, Sen KC. 1940. Estimation of phosphorus, chlorine, calcium, magnesium, sodium and potassium in foodstuffs. Indian Journal of Veterinary Science and Animal Husbandry, 10:243-246.

The Wealth of India (A Dictionary of Indian Raw Materials and Industrial Products). 1962. Council of Scientific and Industrial Research: New Delhi, 4:25–429.

Tona GO, Asaolu VO, Amao OA, Akingbade A 2013). Evaluation of chemical composition and in-vitro fermentation parameter of *Moringa oleifera* leaf meal based diets as feed for ruminants in Nigeria. Agricultural Journal, 8(4): 165-169.

Tona GO, Ogunbosoye, Bakare. 2014. Growth performance and nutrient digestibility of West African Dwarf goats fed graded levels of *Moringa oleifera* leaf meal. International Journal of Current Microbiology and Applied Science, 3(8):99-106.

Tsaknis J.1998.Characterisation of Moringa peregrine Arabia seed oil. Grasses Aceites, 49:170-176.

Van Soest PJ, Robertson JB, Lewis BA (1991). Methods for dietary fiber, neutral detergent fiber and non-starch polysaccharides in relation to animal nutrition. Journal of Dairy Science, 74(10):3583-3597.

Verma AR, Vijay Kumar M, Mathela CS, Rao CV. 2009. In vitro and in vivo antioxidant properties of different fractions of *Moringa oleifera* leaves. Food and Chemical Toxicology, 47:2196-2201.

Vlahof G, Chepkwony PK, Ndalut PK. 2002. Characterization of triglycerols of *Moringa oleifera* seed oil: An oleic vaccenic acid oil. Journal of Agricultural and Food Chemistry, 50:970-975.

Wafa WM, El-Nagar HA, Gabr AA, Rezk, MM. 2017. Impact of dietary *Moringa oleifera* leaves supplementation on semen characteristic, oxidative stress, physiological response and blood parameters of heat stressed buffalo bulls. Journal of Animal and Poultry production 8(9):367-379.

Wang L, Chen X, Wu A. 2016. Mini review on antimicrobial activity and bioactive compounds of *Moringa oleifera*. Medicinal Chemistry, 6(9):578-582.

Watson PF. 1975. Use of Giemsa stain to detect changes in acrosomes of frozen ram spermatozoa. Veterinary Record, 97:12-15.

Yameogo CW, Bengaly MD, Savadogo A, Nikiema PA, Traore SA. 2011. Determination of chemical composition and nutritional value of *Moringa oleifera* leaves. Pakistan Journal of Nutrition, 10(3):264:268.

Yusuf AO, Mlambo V, Iposu SO. 2017. A nutritional and economic evaluation of *Moringa oleifera* leaf meal as a dietary supplementation in West African Dwarf goats. South African Journal of Science, 4:1.

Zeng B, Luo J, Wang P, Yang L, Chen T, Sun J, Xie M, Li M, Zhang H, Zhang Y 2009. The beneficial effects of *Moringa oleifera* leaf on reproductive performance in mice. Food Science Nutrition, 7:738-746.

Zhang J, Zhou Y, Li S, Zhang P, Zhou T, Xu DP, Li HB. 2017. Effect and mechanism of fruit and vegetables juices on cardiovascular disease. International Journal of Molecular Science, pp 18.

Zubcic D. 2001. Some biochemical parameter in the blood of grazing german improved fawn goats from Istria, Crotia. Veterinary Archive, 71:237-244.

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UNDERTAKING OF COPY RIGHT

I, **Dr. Anuj Dubey,** Enrolment No. **V-1820/17** undertake that I give copy right to the DUVASU, Mathura of my thesis entitled **"Effect of replacing protein source in concentrate mixture with dried *Moringa oleifera* leaf powder on the performance of Barbari bucks''**

I also undertake that patent, if any, arising out of research work conducted during the programme shall be filed by me only with due permission of the competent authority of DUVASU, Mathura (UP).

Signature of Student