

MANAGEMENTAL MANIPULATIONS FOR SUSTAINABILITY OF BEETAL GOATS UNDER STALL-FED PRODUCTION SYSTEM

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Submitted to the Guru Angad Dev Veterinary and Animal Sciences University in
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in
LIVESTOCK PRODUCTION MANAGEMENT
(Minor Subject: Animal Nutrition)

By

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CERTIFICATE I

This is to certify that the dissertation entitled, “**MANAGEMENTAL MANIPULATIONS FOR SUSTAINABILITY OF BEETAL GOATS UNDER STALL-FED PRODUCTION SYSTEM**” submitted for the degree of Ph.D., in the subject of **LIVESTOCK PRODUCTION MANAGEMENT** (Minor subject: **ANIMAL NUTRITION**) of the Guru Angad Dev Veterinary and Animal Sciences University, Ludhiana is a bonafide research work carried out by **Mandeep Singla (L-2013-V-01-D)** under my supervision and that no part of this dissertation has been submitted for any other degree.

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

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CERTIFICATE II

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ABSTRACT

In order to study the effect of Zn supplementation on sustainability of the Beetal goats, two experiments were conducted on breedable bucks and does under stall-fed production system. Animals under treatment groups were supplemented with either organic (T_{oz}) or inorganic (T_{iz}) Zn formulated ration to get minimum Zn concentration of 50 mg per kg DM against negative control (T_0) on basal diet with no added Zn. DMI and LBW was non-significantly ($P>0.05$) affected by added Zn. Supplementation of Zn helped to maintain better body condition score in does during the physiological reproductive cycle. Feeding of the diet with added Zn numerically increased plasma Zn concentrations. The bucks under T_{oz} and T_{iz} donated 7.24 and 2.63%, respectively less semen volume (ml) over T_0 . However, Sperm concentration ($\times 10^6/ml$), Gross motility (0-5) and Sperm motility (%) were significantly ($P<0.05$) improved by added Zn. Number of live kids born/doe were significantly ($P<0.05$) and numerically ($P>0.05$) higher in T_{oz} and T_{iz} , respectively over T_0 . The percent decrease in PEG of bucks was recorded more in T_{oz} (83%) and T_{iz} (91%) over T_0 (39%). In addition, in does, overall mean PEG were found numerically lower in T_{oz} and T_{iz} over T_0 . Mean OPG in bucks was significantly ($P<0.05$) lower in T_{oz} and T_{iz} , however, in does, mean OPG was significantly ($P<0.05$) and numerically ($P>0.05$) low in T_{oz} and T_{iz} , respectively over T_0 . Cost of adding of organic and inorganic Zn was only Rs 2.67 and 0.17 per kg DMI per month and had not produced any adverse effect on health of animals. It may be concluded that supplementation of added Zn from any source improved the performance and sustainability of Beetal goats under stall-fed production system.

Keywords: Beetal, Goat, Parasitic load, Reproductive performance, Stall-fed, Zinc

Signature of Major Advisor

Signature of Student

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LIST OF ABBREVIATIONS USED

%	:	Percentage
µl	:	microliter
AAS	:	Atomic absorption spectrophotometer
ADF	:	Acid detergent fibre
ALB	:	Albumin
ALP	:	Alkaline phosphatase
ANOVA	:	Analysis of variance
AOAC	:	Association of analytical chemist
BCS	:	Body condition score
BSE	:	Breeding soundness evaluation
CIRG	:	Central Institute of Research on Goats
CP	:	Crude protein
Cu	:	Copper
DLC	:	Differential leukocyte count
DMI	:	Dry matter intake
EE	:	Ether extract
<i>et al</i>	:	et alia (and others)
EU	:	Enzyme Units
g	:	Gram
g/dL	:	Gram per deci liter
GADVASU	:	Guru Angad Dev Veterinary and Animal Sciences University
GLB	:	Globulin
GPX	:	Glutathione peroxidase
HgB	:	Haemoglobin
hrs	:	Hours
i.e.	:	That is
IU	:	International unit
kg	:	Kilogram
LBW	:	Live body weight
LT	:	Lumbar region thickness
mg	:	milligram
ml	:	millilitre
NDF	:	Neutral detergent fibre
NRC	:	National Research Council
OM	:	Organic matter
OPG	:	Oocysts per gram of faeces
PCV	:	Packed cell volume
PEG	:	Parasitic eggs per gram of faeces
ppm	:	Parts per million
RFT	:	Rump fat thickness
SC	:	Scrotal circumference
SE	:	Standard error
SOD	:	Superoxide dismutase
SPSS	:	Statistical package for social sciences
ST	:	sternal region thickness
TDN	:	Total digestible nutrients
TLC	:	Total leucocyte count
Zn	:	Zinc
ZnSO ₄	:	Zinc sulphate

CHAPTER-I

INTRODUCTION

The goat species is an important component of animal genetic resources. Together with sheep, and partly because of their size, both are commonly called “small ruminants”. Goats (*Capra hircus aegarius*) have been associated with mankind since the dawn of agriculture and the domestication of animals. Goats were domesticated as early as 7000-8000 BC, as evidenced by archaeological remains collected in Asia. Since then, it has been involved in development of many aspects of human culture like religion, tradition, folklore, nutrition, livelihood and economics. It is particularly important in tropics and subtropics, where it is used as a major source of meat, milk, fibre, skin and manure in many traditional societies. There is also a tendency to keep goats as a stock of wealth (poor man’s bank) and sell them proportionally less when the demand for funds raises or their number rises.

Goats provide products and services which are important for humans throughout the world. In the developed countries, goats are valued mainly for milk, fibre and meat; while in the developing countries, they are valued mainly for meat, followed by milk, fibre and skin. The socio economic importance of goats is greatest in developing nations, where they fulfil socio economic, cultural and recreational needs. Their small size is especially relevant and relates directly to economic, managerial and biological advantage over other species. The small size of the goat contributes to its popularity. Goats in addition to meeting daily food needs (milk and meat), are easily sold as a source of cash, provide insurance and are valued in religious ceremonies like gifts during marriages. This species is well adapted to Indian subcontinent and to poor and landless marginal farmers. Poor and landless farmers often increase the size of their flocks to provide greater food and economic security (Devendra and Solaiman 2010).

According to the FAO (2016), more than 1006 million goats exist in the world with more than 95% found in the developing countries like India. India with 135 million goats is one of the largest goats owning country in the world and playing a significant role in livelihood and nutritional security as well as providing supplementary income to nearly 70 million farmers of over 5,00,000 remote villages. Goat meat production in the country has increased from 0.47 to 0.59 million tonnes

during the last decade (2002 to 2011) with an annual growth rate of 2.4%. Similarly, goat milk production in the country has also increased from 3.6 to 4.7 million tonnes during the same period with an annual growth rate of 2.6 %. The country stands first in goat milk production and is the second largest in goat meat in the world by sharing 29% & 12% production, respectively. The goat sector contributes 8.4% to the India's livestock GDP (CIRG 2013). The percentage share of goats to national milk yield and meat production is estimated to be 3.54 and 14% in 2014-15 (GoI 2016).

In the coming years, urbanization, increased income and strong preference to goat meat will be some of the major contributing factors for growth in goat meat production. Considering 3% growth in per capita goat meat/mutton consumption, the demand for goat meat by 2050 would be 2.4 million tonnes. To meet out increasing demand of animal products by 2050 it would not be possible just only by increasing their numbers alone, but to a large extent enhancing per animal productivity in a sustainable manner. Since, goat is an important source of meat, milk, fibre, skin and manure especially in the subsistent agriculture in arid and semi-arid zones of Asia and Africa. It can contribute substantially, fulfilling the increasing demand for livestock products in developing and developed countries of the world. Goats are mainly concentrated in ecologically fragile arid and semi arid areas. Hence, it is imperative to address goat production under the present scenario of climate change, which is adversely affecting health, reproduction and production status of animals. It is, therefore, important to reorient our strategies for goat research and development.

Amongst the 23 goat breeds of India, Beetal is one of the prolific breed with an estimated share of 0.53% of total goat population of nation (GoI 2013). The population of purebred animals is gradually declining due to indiscriminate breeding, lack of pure germplasm etc. Moreover, due to shrinkage of pasture land and increasing demand for goat products, goat production is shifting towards intensive or stall-fed production system, characterized by zero grazing for commercial purpose (Shalander Kumar 2007). The advantage of goat rearing under intensive or stall-fed is improved dressing percentage and muscle to bone ratios (Saini *et al* 1988). However, in intensive production system, inputs related to reproductive well-being of goats in turn will be the key for its adaptability by the farmers, breed conservation, improvement and rapid multiplication to meet the market demand. Therefore, supplementation of essential trace minerals like zinc (Zn) and synchronization of

ovulation could be important managemental manipulations. On the other hand, low level of zinc found in the soil and crops of the Punjab and India (Singh 2009) and lack of timely breeding is affecting the reproduction in livestock to great extent.

It is already reported that more than 50% of Indian agricultural soil is Zn deficient (Singh *et al* 2005). Zn deficiency is quite widespread in the Indo-Gangetic plain and other important cereal-growing states like Punjab, Uttar Pradesh, etc. which account for almost three-fourths of the country food grain production. The causes of this severe Zn deficiency are related to the introduction of high-yielding varieties, neglect of application of bulky organic manures, imbalanced use of fertilizer and low Zn uptake and accumulation of Zn which depends upon the pH, soil organic matter, temperature, light intensity, crop species, etc. The total area under Zn deficiency is about 10 Mha in India and approximately 85% of rice–wheat system cropping takes place in the Indo-Gangetic plain which has calcareous soils with high pH and thus low Zn availability. In order to correct the deficiency of soil, use of Zn fertilizers is a common practice which is widely followed in several states such as Punjab and Haryana. However, this approach is neither economical nor environment friendly in the long run, as only 20% of the applied Zn is available for plant uptake, while the remainder gets adsorbed on soil colloids and is, therefore, rendered immobile. As only a small fraction of the applied Zn is utilized by the crop to which it is applied, Zn accumulation in agricultural soils is on the increase, which is an environmental concern (Singh *et al* 2005). Therefore, with regard to livestock nutrition, fortification of Zn in feed could be a potentially important endeavour.

Low Zn intake and insufficient concentrations in body affects immunity both directly and indirectly as it works as an activator of more than 1000 enzymes. Thus, Zn has a great role in body metabolism and immune system function. Furthermore, subclinical parasitism due to lower immunity is also one of the serious constraints under stall-fed conditions and is affecting goat production throughout the globe. Though, sub-clinical parasitic infection may not cause severe mortality, yet at the same time can lead to huge economic losses due to impaired productivity, increased susceptibility to diseases, cost of treatment/prevention etc. Therefore, supplementation of essential trace minerals such as zinc can be an important management manipulation strategy under Indian agro-climatic conditions as

interaction between the parasite and the host leading to the development of clinical infection is affected by many important factors including nutrition.

The influence of Zn on reproductive functions resides on its effect on various hormones (androgens and oestrogens). Spermatogenesis along with functioning of primary and secondary sex organs in males and all phases of reproduction from oestrus to parturition and lactation in females can be adversely affected by Zn deficiency. Forages and grains are the main source of Zn but its content is highly variable resulting in severe deficiencies in farm animals irrespective of sex. Furthermore, the bio-availability of dietary Zn varies between 15-60% and is also influenced by interaction with other trace elements like Calcium, Cadmium, Nickel and Phytic acid which can induce a secondary Zn-deficiency (McDowell 2014). However, the bioavailability of the Zn can be increased through chelation or supplementation of organic source of Zn. Theoretically, the chemical bond formed between metal and ligands should allow organic trace minerals resist many of interactions encountered by inorganic trace mineral sources. Moreover, earlier reports suggested that organic sources of trace minerals had been found to be more bioavailable than inorganic sources (Huang *et al* 2009). Hence, feeding of added organic source of Zn can be an effective tool for goats under stall-fed.

For the economic betterment of goat producers, improvement of semen quality in Beetal bucks and reproductive efficiency in does through supplementation of organic and inorganic zinc along with application of synchronization of oestrus or ovulation could be effective strategy under the agro-climatic conditions of Punjab as well as India. With this state of affairs, improved oestrus synchronization can prove to an important management tool to augment fertility and fecundity in goats. These assisted reproductive techniques can facilitate kidding at predestined time of the year to take maximum advantage of feed, fodder, labour, market, high commodity price, implementation of breeding techniques etc. Earlier work in this area was focussed in large ruminants. However, with increase of interest in goat as “future meat animal” attempts are being made to apply oestrus synchronization programmes in routine management of goats under stall-fed conditions.

Oestrus synchronization in does is achieved either by reducing the length of the luteal phase of the oestrous cycle with prostaglandin F₂ alpha or its analogues (inducing immature luteolysis) or by extending it artificially with exogenous

progesterone or more potent progestagens. Earlier researchers have reported many methodologies for oestrus synchronization. Amongst these, cost effective intravaginal sponges have been the traditional treatment of choice for oestrus synchronization in small ruminants, during the breeding and anoestrus seasons. Moreover, administration of $\text{PGF}_2\alpha$ increases the cost and is not effective in anoestrus goat at any stage for synchronization of oestrus (Goel and Kharche 2009).

The information on presently hypothesized principle to improve goat production under stall-fed rearing conditions is scarce especially in India with specifically reference to agro-climatic conditions of Punjab. Therefore, under the light of above hypothesis, this study was planned with the following objectives:

1. To study the effect of organic and inorganic zinc supplementation on qualitative and quantitative semen attributes of Beetal bucks reared under stall-fed conditions.
2. To analyze the effect of organic and inorganic zinc supplementation and synchronization of ovulation on reproductive efficiency of Beetal does.
3. To compare the economic feasibility of these managerial manipulations under stall-fed production system.

CHAPTER – II

REVIEW OF LITERATURE

Present study was designed to develop and recommend manipulations in management to improve the sustainability of Beetal goats under stall-fed production system. The success of commercial goat farming under intensive system is challenged by many factors such as minerals deficiency and lack of timely breeding programmes. Due to this, Zn supplementation and its effect on reproductive traits, synchronization of oestrus with intra-vaginal progesterone sponges, performance and economic applicability was studied. As the information regarding the hypothesis was scanty, therefore, some of the related findings regarding importance, scope, nature of work and gaps have been reviewed herewith under following sub-headings.

1.1 Factors affecting reproduction in goats

1.1.1 Deficiency of minerals

Clinical and sub-clinical zinc deficiency in livestock is common in all the parts of country. Total and available Zn content in Indian soils varied between 7-2960 mg kg⁻¹ and 0.1-24.6 mg kg⁻¹, respectively with an average deficiency of 12-87%. Zinc deficiency in Indian soils is expected to increase from 42% in 1970 to 63% by 2025 due to continuous depletion of soil fertility. Crops grown in these soils have low Zn content affecting huge population as livestock obtain these essential minerals from feed and fodder, they consume. Continuous intensive cropping of high yielding crop varieties has further aggravated the problem (Singh 2009).

The Zn content of corn fodder at harvest was reported to be 19.9 mg kg⁻¹ DM at farms of GADVASU, Ludhiana (Kumar *et al* 2013) which is below the worldwide mean value of 25-50 mg kg⁻¹ DM in all the pastures (NRC 2007).

Similarly, Chhabra *et al* (2015) conducted a survey to assess the levels of some macro and micro minerals in blood plasma of water buffaloes and forage consumed by these ruminants in the central region of Punjab (India) using apparently healthy animals during two consecutive seasons (summer and winter) of the year. From the results, they found that concentration of zinc in plasma was significantly higher during winters (22.20± 2.53 µmol/l) compared to summers (10.90 ± 0.45 µmol/l). Considering the critical limit of 12.2 µmol/l, the prevalence rate of Zn deficiency among buffaloes was 21.81 and 76.19 percent in winter and summer,

respectively. Analysis of forages collected showed that the variations in the fodder mineral concentrations corresponded to the plasma mineral variations, indicating a direct plant-animal relationship and showing the need of supplementation of the deficient minerals at the place where the livestock were being reared. Mean Zn concentration in fodder falls to lower limits of 7.93 ± 0.61 ppm and rise significantly to 17.39 ± 1.77 ppm during winter. The critical limit for Zn level in fodder was reported to be < 30.0 ppm.

Confirming to the findings at GADVASU, earlier researchers Sharma and Joshi (2005) had also found that fodder zinc (13.46 ± 1.23 ppm) was significantly ($P < 0.01$) deficient against the critical limit of < 30.0 ppm in 10 districts of northern India.

Various investigations are now being focused on zinc supplementation in animals for physiological maximization of animal's growth, reproduction and production as Zn is a co-factor for more than 1000 important enzymes (McDowell 2014).

1.1.2 Semen quality in males

Ray *et al* (1997) during the histological examination of testis of zinc-deficient bucks revealed degenerative changes, including atrophy of the seminiferous tubules, hyperplasia of the germinal epithelium and thickening of the walls of blood vessels affecting spermatogenesis.

1.1.3 Reproductive inefficiency in females

Signs of severe Zn deficiency are rarely seen under natural conditions because most practical diets contain more than 5 mg Zn/kg. However, marginal deficiency, causing reduced fertility is typically observed in sheep and cattle on pastures containing less than 20 mg Zn/kg (White 1993).

Ceylan *et al* (2008) recommended that rations of animals with reproductive disorders should be supplemented with Zn, Cu, and P. Although serum levels of Zn (mg/L) in cows with anoestrus (0.70 ± 0.06) and repeat breeding (0.82 ± 0.12) were lower than in control (0.87 ± 0.03), the variations were not statistically significant. However, even a slight decrease in serum Zn level may lead to reproductive problems.

1.2 Lack of assisted reproductive techniques

It is now advocated that synchronization of ovulation as a valuable management tool to enhance reproductive efficiency in caprine, must not only establish tight synchrony, but also provide an acceptable level of fertility upon natural mating or artificial insemination (Wildeus 2000).

1.3 Zinc supplementation

Zinc requirements of goats are based on estimates, availability and absorbability in diet and other data available on its loss in faeces, urine and milk at the rate of 0.033, 0.012 and 3-8 mg Zn/kg BW, respectively depending on breed, diet and level of production (NRC 2007).

NRC (2007), Meschy (2000) and Tripathi and Karim (2008) advised dietary level of Zn at the rate of 50 mg/kg DM in breeding goats. It is now well established that the requirement is more closely related to the quantity of dry matter intake than to the body weight of the animal (Meschy 2000).

1.3.1 Effect on Feed intake

Puchala *et al* (1999) reported that supplementation of a basal diet containing 22 mg Zn/kg DM with 40 mg/day of Zn had no effect on DMI by Angora goats during a 120-day period.

Malcolm-Callis *et al* (2000) studied the effect of Zn concentration or source in diets of finishing beef steers. They found a linear effect of level of Zn supplementation (20, 100 and 200 mg/kg DM) in DMI by finishing steers consuming a diet with 70 mg Zn/kg DM. Their results indicated that normal dietary Zn concentrations do not influence DMI, but higher concentrations may have a negative influence on diet palatability. Dietary levels of Zn in the current experiment were within normal ranges and would not affect diet palatability.

Mandal *et al* (2007) also did not find any effect on DMI in bulls supplemented with 35 mg Zn/kg DM while consuming a diet containing 32.5 mg Zn/kg DM.

Garg *et al* (2008) indicated that DMI was not affected by adding 20 mg Zn/kg DM to a basal diet with a Zn level of 34 mg/kg DM.

Jia *et al* (2008) reported that supplementation of basal diet containing 22.3 mg Zn/kg DM with 15, 30 or 45 mg Zn/kg DM had no effect on DMI in Liao Ning Cashmere goat wethers during the study of 60 days.

Shinde *et al* (2012) studied the effect of supplementation of copper and zinc in adult Malpura rams of 4-5 years of age. Concentrate feed fed to rams of Group I (G-I) did not contain supplemental Zn and Cu. Inorganic Zn and Cu as sulphate (50% of basal diet) and Cu and Zn methionine (25% of basal diet) was fed to Group II (G-II) and Group III (G-III) rams, respectively. The rams were supplemented with concentrate feed containing Cu and Zn contents of 16.87, 34.67 ppm in group-1; 37.15, 48.56 ppm in group-2 and 26.19, 39.58 ppm in group-3, respectively. During the study, it was found that daily dry matter intake and digestibility along with crude protein, neutral detergent fibre and acid detergent fibre digestibility of feed was not affected by mineral supplementation.

Gupta (2014) elucidated the effect of Zn-sulphate and Zn-lysine in buffalo calves at GADVASU, Ludhiana and found that daily DMI from concentrate and fodder were quantitatively higher in organic Zn supplemented group in comparison to inorganic Zn supplemented group.

1.3.2 Effect on live body weight

Brazle and Stokka (1995) found that newly arrived feed yard calves fed Fourplex (a feed additive from Zinpro Corporation that contains copper lysine, Zn methionine, manganese methionine and cobalt glucoheptonate) did not improved average daily gain.

Abdelrahman *et al* (2003) also observed no significant differences between the group supplemented with Zn oxide or Zn methionine on weight gain or feed conversion in awassi lambs.

Jia *et al* (2008) studied the effects of dietary zinc (Zn) level on the performance, nutrient digestibility and plasma Zn in Cashmere goat wethers (BW= 22.01±0.59 kg). The goats were randomly divided into four groups that were fed a basal diet containing 22.3 mg Zn/kg dry matter (DM) with 0, 15, 30 or 45 mg Zn/kg DM as reagent grade ZnSO₄·7H₂O for a period of 60 days. Both average daily gain (ADG) and feed efficiency were improved ($P < 0.05$) by Zn supplementation and were higher ($P < 0.05$) for the treatment groups supplemented with 30 and 45mg Zn/kg DM compared with 15 mg Zn/kg DM.

Devi *et al* (2012) in case of Assam local male kids, recorded highest body weights in organic zinc supplemented kids compared with inorganic zinc-fed over control.

Shinde *et al* (2012) studied the effect of supplementation of copper and zinc in adult Malpura rams of 4-5 years of age. Concentrate feed fed to rams of Group I (G-I) did not contain supplemental Zn and Cu, inorganic Zn and Cu as sulphate (50% of basal diet) and Cu and Zn methionine (25% of basal diet) was fed to Group II (G-II) and Group III (G-III) rams. The rams were supplemented with concentrate feed containing Cu and Zn contents of 16.87, 34.67 ppm in group-I; 37.15, 48.56 ppm in group-II and 26.19, 39.58 ppm in group-III, respectively. The body weight gain of rams did not differ significantly between the groups.

1.3.3 Effect on Body Condition Score (BCS)

Cope *et al* (2009) supplemented organic and inorganic zinc to HF cows and observed no effect of treatment on DMI, body condition score, body weight, or locomotion score.

Uchida *et al* (2001) reported that feeding combination of these minerals (Zn, Mn, Cu and Co) did not improve BCS of high yielder cows.

1.3.4 Effect on plasma Zn

Jia *et al* (2008) randomly distributed Cashmere goat wethers into four groups that were fed a basal diet containing 22.3 mg Zn/kg dry matter (DM) with 0, 15, 30 or 45 mg Zn/kg DM as reagent grade ZnSO₄·7H₂O for a period of 60 days. During the study it was found that Plasma Zn concentrations were increased ($P < 0.01$) by Zn supplementation and were higher ($P < 0.05$) for the treatment groups supplemented with 30 and 45 mg Zn/kg DM compared with 15 mg Zn/kg DM.

Shinde *et al* (2012) reported that serum concentrations of supplemented Cu and Zn were significantly increased ($P < 0.01$) after feeding the adult Malpura rams with concentrate feed containing Cu and Zn contents of 16.87, 34.67 ppm in group-1; 37.15, 48.56 ppm in group-2 and 26.19, 39.58 ppm in group-3, respectively.

Chhabra *et al* (2013) reported that oral zinc supplementation from organic sources resulted in non-significant increase of Zn plasma levels up to day 60 along with clinical improvement in Zn deficient buffaloes.

Shinde *et al* (2013) observed higher ($P<0.05$) mineral concentrations in blood of rams fed Cu and Zn methionine than Cu and Zn sulphates suggesting higher bioavailability of minerals from organic chelated sources.

Pechova *et al* (2006) supplemented Zn in the chelate form (Bioplex Zn, Alltech Inc., Nicholasville, KY), at the dose of 440 mg Zn per cow per day and found no differences in blood Zn levels of 500 cows in various stages of lactation.

Similarly, Mandal *et al* (2008) studied the effect of Zn supplementation in male crossbred calves kept on basal diet (33 mg Zn/kg diet) without or with added Zn (35 mg/kg feed) from zinc sulphate and zinc propionate. They reported no significant differences in serum zinc and copper concentration between the groups.

1.3.5 Effect on male reproductive attributes (Breeding Soundness)

Kumar *et al* (2006) studied the effect of inorganic and organic sources of Zn supplementation on semen quality in crossbred bulls and reported significantly higher semen volume, sperm concentration (million ml^{-1}), sperm number per ejaculate (million), live sperm (%) and motility in Zn-supplemented groups than the control. However, the group supplemented with organic Zn reflected superiority over other groups.

Shinde *et al* (2012) studied the effect of supplementation of copper and zinc in adult Malpura rams of 4-5 years of age. The rams were supplemented with concentrate feed containing Cu and Zn contents of 16.87, 34.67 ppm in group-1 (no supplemented mineral); 37.15, 48.56 ppm in group-2 (inorganic Zn and Cu as sulphate) and 26.19, 39.58 ppm in group-3 (Cu and Zn methionine), respectively. Semen volume increased from 0.75 in G-I to 0.89 in G-II and 0.78 ml in G-III. Similarly, mass motility on 0-5 scale also increased from 4.03 in G-I to 4.35 in G-II. However, sperm motility decreased from 82.3 in G-I to 76.7 in G-II and 67.6% in G-III. Rapid motile sperm also decreased from 73.7 in G-I to 68.5 in G-II and 56.0% in G-III.

1.3.6 Effect on female reproductive efficiency

Uchida *et al* (2001) reported that feeding of combinations of organic salts of Zn, Mn, Cu and Co to high yielder cows improved conception rate.

Bosseboeuf *et al* (2006) found that daily supplementation of 50 g of Cu, Zn, Mn-amino acid chelates in field dairy cows for a period of 120 days resulted in lower

mean services per conception (1.50 versus 1.90), high conception rate (96% against 87%) and confirmed pregnancies following first services (>36%) as compared to those in inorganic metal supplemented group.

Kundu *et al* (2014) demonstrated that supplementation of basal diet of Teresa goat with 50 to 100 ppm zinc oxide significantly increased the incidence of oestrus (33%), pregnancy rate (12%), kidding rate (5%) and resulted in shorter onset (8 days) of oestrus as compared to the control.

1.3.7 Effect on synchronization of ovulation

The success of synchronization protocol may depend on the supplementation of minerals. In order to test this hypothesis, Sales *et al* (2011) injected crossbred heifers with subcutaneous injections of trace minerals (ITM) prior to embryo transfer. A significant increase in the conception rate (1.58-1.72 times) without any difference in the rate of synchronization (ITM =82.1% versus control=83.1%) was reported in heifers of the ITM group.

1.3.8 Effect of synchronization on goat reproduction

The cost of synchronization can be reduced to a great extent by preparing the indigenous intra-vaginal sponges from naturally available progesterone and are quite successful in induction and synchronization of estrous as developed by CSWRI, Avikanagar for goats (Goel and Kharche 2009).

Kausar *et al* (2009) synchronized oestrus in goats using locally prepared progesterone impregnated intra-vaginal sponges (60 mg medroxyprogesterone acetate (MAP), 17 days) and recorded 100% oestrous response with average time of onset of oestrus after sponge removal (65.4 ± 24.0 hours), duration of oestrus (29.8 ± 6.7 hours), litter size (1.3 ± 0.5) and fertility rate (87.5%).

1.3.9 Effect on serum testosterone

Kumar *et al* (2006) reported in crossbred bulls study that supplementation of inorganic (Zn sulfate) and organic (Zn propionate) sources of Zn @ 70 ppm Zn and 35 ppm Zn for period of 6 months significantly improved serum testosterone concentration (ng/ml) as compared to control. It was earlier reported that Zn stimulates Leydig cells of the testis and enhances the production of testosterone since Zn is an essential component of protein involved in synthesis and secretion of testosterone.

El-Sisy *et al* (2008) revealed in male Baladi goats that supplementation of basal diet with 40 ppm Zn-methionine can result in significant increase of testosterone levels.

1.3.10 Effect on health, morbidity, mortality

Trace minerals, though required in minute quantities (less than 100 mg/kg DM), are essential for maintaining health and immunity. Zinc exerts immune stimulatory effects in various laboratory and farm animals and its deficiency can lead to a variety of pathological abnormalities with impaired performance.

Brazle and Stokka (1995) reported that feeding of Fourplex (a feed additive from ZinPro corporation containing copper lysine, Zn methionine, manganese methionine and cobalt glucoheptonate) to newly arrived yard calves caused fewer ($P < 0.05$) medication days but did not reduced mortality.

George *et al* (1997) observed that calves fed amino acid trace minerals (AATM) had reduction in incidence and reoccurrence of respiratory diseases. In addition it was also concluded that feeding of elevated organic minerals showed significant improvements in primary and secondary humoral and cell-mediated immunity compared to the feeding of inorganic sources.

However, on the contrary, Spears and Kegley (2002) observed no improvement in cell mediated or humoral immune response in beef cows supplemented with additional Zn from organic and inorganic sources.

Nunnery *et al* (2014) conducted two experiments to evaluate performance, morbidity, and humoral immune response of heifers fed different sources of supplemental Zn. Results from these 2 studies showed no major differences among the sources of supplemental Zn for receiving period morbidity, ADG, DMI, and humoral immune response of beef heifers; however, a lack of supplemental Zn during an extended finishing period tended to negatively affect G:F (feed efficiency in terms of live weight gain observed per unit of dry matter consumed).

1.3.11 Effect on lameness

Cope *et al* (2009) supplemented organic and inorganic zinc to HF cows and observed no effect of treatment on locomotion score. Hoof hardness improved over the duration of the study but there were no differences between treatments.

Uchida *et al* (2001) reported that feeding combination of these minerals (Zn, Mn, Cu and Co) did not improve lameness score of high yielder cows.

1.3.12 Effect on parasitic infestation

Roy *et al* (2013) reported that daily supplementation of Murrah buffalo with 100 g mineral mixture containing 0.75% Zn sulphate salt decreased the parasitic eggs per g of faeces significantly from 183.33 ± 10.54 to 16.67 ± 10.54 in 28 days.

Fausto *et al* (2014) reported that copper and selenium supplementation reduced PEG (parasitic eggs per gram of faeces) and worm burden at the end of the experiment at day 80 in experimentally infected lambs with *Haemonchus contortus*.

1.3.13 Effect on coccidial infection

Rakhshandehroo *et al* (2014) reported significant reductions in serum concentrations of some antioxidant trace elements (zinc, manganese and selenium) and vitamins (vitamin C) in the *Eimeria* infected diseased kids compared with control healthy kids. These observations suggest that *Eimeria* parasites can significantly interfere with the levels of some antioxidant trace elements and vitamins during caprine coccidiosis. Therefore, supplementation of minerals may be an important management strategy to counter the effects of sub-clinical parasitism.

Strnadová *et al* (2011) found that goat kids supplemented with zinc chelate and zinc lactate shed a significantly ($p \leq 0.05$) lower number of oocysts (13.4% and 11.9%, respectively) compared to the number of oocyst shed by control and zinc oxide supplemented groups (25% and 49.7%, respectively). It was further reported that shedding of oocysts was not accompanied by clinical symptoms of coccidiosis in any of the groups. Kids supplemented with zinc chelate showed significantly highest weight gains and blood plasma concentration of zinc ($p \leq 0.05$) as compared to control and inorganic zinc supplemented groups. Organic zinc was recommended to be used as a prophylaxis against coccidiosis in goat kids.

Bun *et al* (2011) during a study on broilers supplemented corn–soybean meal basal diet (29.6 mg of Zn/kg) with methionine hydroxyl analog–Zn chelate at 0, 20, 40, and 60 mg/kg of diet. At 21 d of age, birds were orally gavaged with 1.5×10^4 sporulated *E. tenella* oocysts. Dietary Zn supplementation had no effect on growth performance of either the challenged or non-challenged birds. Activities of Cu–Zn superoxide dismutase and glutathione peroxidase were increased ($P < 0.001$) with

increasing Zn levels in both the challenged and non-challenged groups. It was found that birds fed Zn supplementation excreted significantly fewer oocysts in the excreta than those receiving no Zn supplement ($P < 0.001$).

1.3.14 Effect on haematological profile

Cope *et al* (2009) supplemented organic and inorganic zinc to HF cows and observed no effect of treatment on plasma mineral levels for Zn, Cu, Mo, and Fe in blood. In addition, no effect of treatment on superoxide dismutase activity or blood hematology was also recorded.

Sharma and Joshi (2005) supplemented zinc sulphate enriched mineral mixture to cattle in Zn-deficient districts of north India. Significant improvements were observed in Hgb, WBC, TEC at day 7 of treatment.

1.3.15 Effect on blood biochemical profile and oxidative stress indicators

The primary role of zinc in the body is related to its association with enzymes and proteins both as a part of molecule and as an activator. There are more than thousand proteins associated with zinc. The most thoroughly studied zinc metallo-enzymes are carbonic anhydrase, carboxy peptidase A, alkaline phosphatase, superoxide dismutase, glutathione reductase, glutathione peroxidase, catalase etc. These enzymes act as antioxidant and prevent oxidative stress by neutralizing oxidants produced under stress.

Sharma and Joshi (2005) reported that supplementation of zinc sulphate enriched mineral mixture to cattle in Zn-deficient districts of north India significantly improved serum alkaline phosphatase at day 7 of treatment.

Fadayifar *et al* (2012) observed in lambs that Plasma Zn concentration, serum vitamin A concentration, alkaline phosphatase (ALP) and superoxide dismutase (SOD) activity differed significantly between control and Zn supplemented groups ($P < 0.05$) as Zn (organic and inorganic) supplementation improved these parameters.

Mandal *et al* (2008) supplemented the basal diet (33 mg / kg Zn) of crossbred calves with 35 mg/kg zinc from zinc sulphate and zinc propionate. They observed mean activity of alkaline phosphatase, transaminase and SOD were similar amongst control and supplemented groups.

Bun *et al* (2011) reported in broilers that activities of Cu–Zn superoxide dismutase and glutathione peroxidase were increased ($P < 0.001$) with increasing Zn levels in both the *Eimeria* challenged and non-challenged groups.

Similarly Mostafa *et al* (2012) observed no significant differences amongst the groups of crossbred cows fed with inorganic sulphates and organic sources of Zn, Mn and Cu in the blood constituents (Glucose, Total proteins, Albumin, globulin, urea, AST, ALT).

Shinde *et al* (2012) supplemented the adult Malpura rams with concentrate feed containing Cu and Zn contents of 16.87, 34.67 ppm in group-1; 37.15, 48.56 ppm in group-2 and 26.19, 39.58 ppm in group-3, respectively and found that mean serum glucose, total protein, albumin, globulin, urea and cholesterol concentration were not influenced by mineral supplementation.

Shinde *et al* (2013) reported that supplementation of minerals in the form of Zn and Cu-met or Zn and Cu-sulphate had no significant effect on ALP activity.

Chandrasah *et al* (2015) studied the effect of supplementary inorganic and organic Zn and Cu in Beetal kids. They observed no difference in ALP and SOD, but higher GPx between pre and post challenged period in organic mineral group. Serum BUN and albumin increased after the challenge in organic mineral group. The results suggested that organic mineral supplemented kids had better stress tolerance capability.

1.4 Normal reference values

In order to interpret the data collected correctly, the normal reference values of clinically healthy goats were referred and documented as below:

Normal reference values for buck semen traits as illustrated by Bujarbaruah and Kumaresan (2013)

Parameters	Normal values
Semen volume (ml)	0.5-2
Sperm concentration (x 10 ⁶ /ml)	1500-4000
Total sperms/ejaculate (x 10 ⁶)	1250-5000
Gross motility/ mass motility	4-5
Sperm motility (%)	> 80.00
Live sperm (%)	> 80.00
Semen pH	6.2-6.7

Haematological, Biochemical and oxidative stress indicators (SI units) reference ranges in blood of goats as described by Allen *et al* (2016)

Parameters	Normal range
Hgb ($\times 10$ g/L)	8-12
WBCs ($\times 10^9$ /L)	4-13
Neutrophils (%)	30-48
Lymphocytes (%)	50-70
Monocytes (%)	0-4
Eosinophils (%)	1-8
Basophils (%)	0-1
RBCs ($\times 10^{12}$ /L)	8-18
PCV ($\times 10^{-2}$ L/L)	22-38
Platelets ($\times 10^9$ /L)	300-600
Protein (x 10 g/L)	6.4-7.0
Albumin (x 10 g/L)	2.7-3.9
Globulin (x 10 g/L)	2.7-4.1
ALP (IU/L)	93-387

CHAPTER–III

MATERIALS AND METHODS

3.1 Experimental details

In order to study the effect of Zn supplementation on sustainability of the Beetal goats under stall-fed conditions, the following experiments were conducted during the year 2015-16 as per following details. Various methodologies used during the present study have been explained herewith under different sections given below:-

S. No.	Name of Experiment	Study period	Effective Duration
1.	To study the effect of organic and inorganic zinc supplementation on qualitative and quantitative semen attributes of Beetal bucks reared under stall-fed conditions.	1 st Jun. to 30 th Nov., 2015	6 months
2.	To analyze the effect of organic and inorganic zinc supplementation and synchronization of ovulation on reproductive efficiency of Beetal does.	1 st Jan. to 31 st Aug., 2016	8 months

3.1.1 Location/place of work

Both the experiments were conducted at Goat Research Farm, Department of Livestock Production Management, College of Veterinary Science, Guru Angad Dev Veterinary and Animal Sciences University, Ludhiana 141 004, Punjab, India. The location of work is located at the Latitude of 30°54' North, Longitude of 75°48' East and at the height of 246 meters above the mean sea level. Ludhiana experiences a semi-arid climate with three major seasons i.e. summer (April to June), monsoon (July to September) and winter (December to February). In summer season, the average high temperature ranges from 38-43 °C with low rainfall followed by monsoon season (July to September) that brings more than 700 mm of rainfall and winter months (December to February) remains chilly as the temperature fluctuates between 6-20 °C (Anonymus 2016).

3.1.2 Ethical approval

Since, the present study was a part of dissertation in partial fulfilment of the requirements for the degree of doctor of philosophy in Livestock Production

Management, approval for all the procedures involving animals during the study period had been obtained from the Institutional Animal Ethics Committee (IAEC).

3.1.3 Housing and general management

All the animals were kept under loose housing system through out of the study period. The recommended floor space as per BIS recommendations were provided to each animal under covered as well as open area. Clean potable drinkable water was available to them round the clock. Before the start of experiment, all the animals were de-wormed and vaccinated as per schedule in order to maintain uniform health status amongst them.

3.2 EXPERIMENT I: To study the effect of organic and inorganic zinc supplementation on qualitative and quantitative semen attributes of Beetal bucks reared under stall-fed conditions.

3.2.1 Selection of Animals and Experimental Design:

Before the beginning of trial, testicles of 23 bucks in total (20 Beetal and 3 Beetal cross) with average age of 935.09 ± 86.51 days and weighing 55.10 ± 2.27 kg were examined in May, 2015 with B-mode ultrasound scanner connected to 5.0 MHz linear array transducer. In order to maintain homogeneity, out of these, bucks with extensive fibrosis of testes and crossbred bucks were not considered for the trial. Therefore, in total, 15 donor Beetal bucks having an average age of 832.53 ± 52.91 days and weighing 52.01 ± 1.97 kg were enrolled and randomly distributed into three groups as per following plan.

Treatment	Animals (No.)	Particulars
T ₀	5	Control without Zn supplementation.
T _{oz}	5	Supplementation of organic zinc (Zn chelate of amino acids).
T _{iz}	5	Supplementation of inorganic zinc (Zn sulphate).

3.2.2 Methodology:

The basal diet was prepared containing mineral mixture without any added zinc. Bucks under treatment groups were supplemented with either organic (T_{oz}) or inorganic (T_{iz}) Zn formulated ration. During the study period, the bucks fed on basal diet containing 23.88 ppm Zn/kg DM were supplemented with 30 ppm Zn/kg DM either from Zn chelate of amino acids (T_{oz}) or Zinc sulphate, respectively. Zn chelate

of amino acids and Zinc sulphate contained 15% and 20% Zn, respectively. Therefore, based on Zn content of basal feed, organic zinc and inorganic zinc, the ration was formulated to get minimum Zn intake of 50 mg per kg DM as per the recommendations of NRC (2007), Meschy (2000) and Tripathi and Karim (2008). The bucks in T₀ group were under control on basal diet with no supplemented Zn. Green fodder (Maize/Bajra) was offered *ad.lib* along with concentrate in weighed quantity. The rest of feeding and managerial practices were similar among all the groups throughout the study period of six months from June to November, 2015. Semen samples were collected weekly from 15 donor bucks for 6 months to cover atleast 3 sperm cycles of spermatogenesis in T₀, T_{oz} and T_{iz}, respectively with artificial vagina (AV) connected to graduated collection tube.

3.3 EXPERIMENT II: To analyze the effect of organic and inorganic zinc supplementation and synchronization of ovulation on reproductive efficiency of Beetal does.

3.3.1 Selection of Animals and Experimental Design

Before the start of experiment, all the animals were de-wormed and vaccinated as per schedule in order to maintain uniform health status amongst the does. A total of 30 breed-able Beetal does having an average age of 1343.00 ± 87.59 days and weighing 37.17 ± 1.52 kg were enrolled in one season and randomly distributed into three treatment groups on the basis of average body weight and age. After about one month (adaptation period) of initiation of experiment following supplementation, the does within each group or shed were again arbitrarily divided into sub-groups as per the following plan.

Treat-ment	Animals (No.)	Particulars	Sub- groups (No. of does)
T ₀	10	Control without Zn supplementation	Natural Ovulation (5)
			Synchronization of estrus (5)
T _{oz}	10	Added organic zinc (Zn chelate of amino acid).	Natural Ovulation (5)
			Synchronization of estrus (5)
T _{iz}	10	Added inorganic zinc (Zn-sulphate).	Natural Ovulation (5)
			Synchronization of estrus (5)

3.3.2 Methodology

The basal diet was prepared containing mineral mixture without zinc. Based on Zn content of basal feed, organic and inorganic Zn, the ration was formulated to get minimum Zn intake of 50 mg per kg DM as per the recommendations of NRC (2007), Meschy (2000) and Tripathi and Karim (2008). Therefore, the does fed on basal diet containing 23.66 ppm Zn/kg DM were supplemented with 30 ppm Zn/kg DM either from Zn chelate of amino acids (T_{oz}) or Zinc sulphate (T_{iz}), respectively. The does in T_0 group were under control on basal diet without any supplemented Zn. However, does under treatment groups were supplemented with either organic (T_{oz}) or inorganic Zn (T_{iz}) formulated ration. The does under natural ovulation group were subjected to teaser bucks and bred, accordingly. Progesterone sponges were procured from Division of Animal Physiology and Biochemistry, Central Sheep and Wool Research Institute, Avikanagar-304501, Rajasthan, India. Progesterone releasing Intra-vaginal sponges impregnated with 350 mg natural progesterone was inserted into vagina of 5 does within each group with sterilized and lubricated speculum for a period of 14 days (Fig. 1). Sponges were removed on day 14th and simultaneously an I/M injection of 400 IU PMSG (Folligon® containing PMSG as crystalline powder with concentration of 1000 I.U. per vial) was administered to each doe just after sponge removal (Goel and Kharche 2009). The vagina of each doe was washed thoroughly with normal saline to clear the secretions and to prevent any infection of genitalia.

The does were checked for symptoms of estrus with the use of teaser buck after 24 hours of sponge removal at 6 hours interval. All the synchronized does came into estrus after 48 hours of removal of sponges and were hand mated with Beetal bucks, accordingly.

Blood samples (2 ml) for estimation of variations in plasma progesterone were collected via jugular vein puncture on day 0 (immediately before sponge insertion), 7, 14 and after 48th and 72th hours of sponge removal, in sterilized heparinized vials. Blood samples were centrifuged at 3000 rpm for 20 minutes. Separated plasma was further stored at -20°C until estimation of progesterone. Estrus synchronization was conducted during the month of February, 2016. Green fodder (Maize/Bajra/Berseem) was offered *ad.lib* along with concentrate in weighed quantity. The rest of feeding and managerial practices were similar among all the groups throughout the study period of eight months from January to August, 2016.

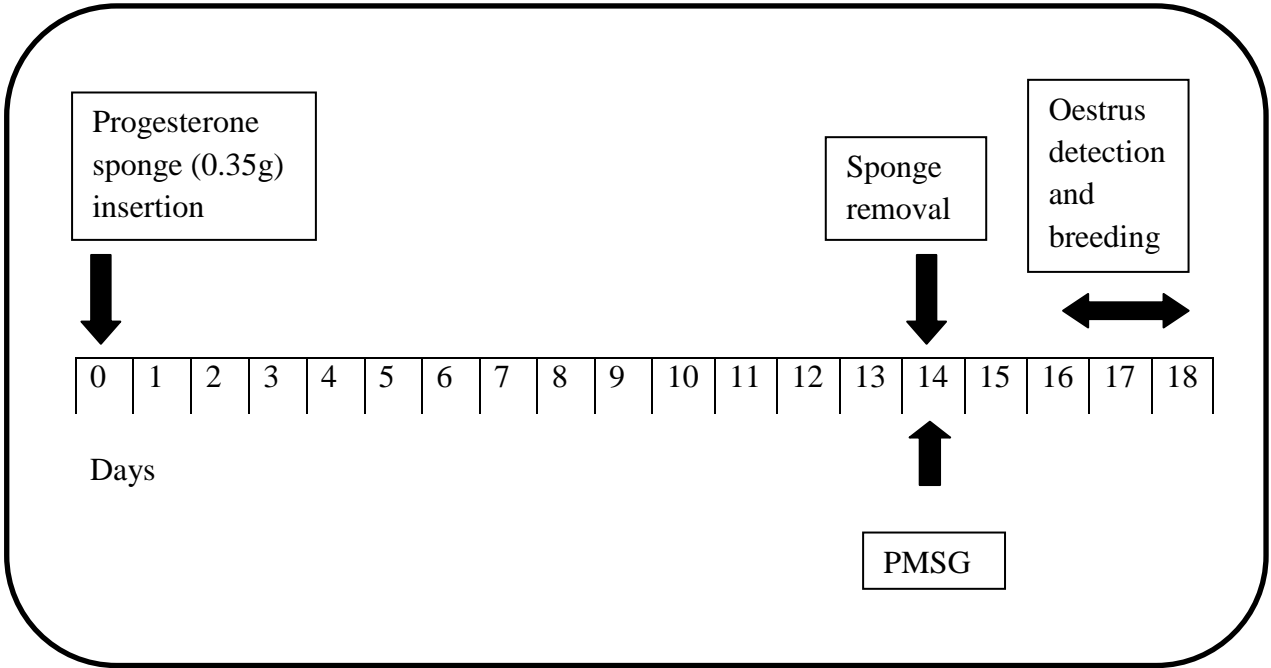


Fig. 1: Estrus synchronization protocol by using Progesterone impregnated Intra-vaginal sponges



Fig. 2: Insertion of intra-vaginal progesterone releasing sponges in does for oestrus synchronization and fixed time breeding.

3.4 Observations recorded

3.4.1 Environmental variables

The data on daily variations in environmental temperature, humidity, sunshine and rainfall were recorded at the observatory of School of Climate Change and Agricultural Meteorology, Punjab Agricultural University, Ludhiana 141004. THI (Temperature Humidity Index) was calculated as measure of thermal comfort as per the formula given by Tucker *et al* (2008).

$$\text{THI} = (1.8T + 32) - [(0.55 - 0.0055 \text{ RH}) (1.8T - 26)]$$

Where T is the air temperature (°C) and RH is the relative humidity (%).

3.4.2 Live body weight (LBW)

The live body weights of the animals were recorded at monthly intervals during period of study under both the experiments. LBW was recorded in the morning hours before offering any feed and water on a 200 kg capacity digital platform weighing machine having 50 g least count.

3.4.3 Body Condition Score (BCS)

The monthly variation in BCS of all bucks and does under study based on a nine point scale i.e. 1-5 scale with increment of 0.5. BCS was assessed by visual and palpation technique. In this methodology, the animal was first observed from the front, side and rear at a distance of 2-3 feet. The body frame of the animal was judged carefully by visualizing various body parts i.e. ribs, hook bone, pin bone, lumbar and sternal region. Thereafter, the animal was palpated at different anatomical locations (ribs, hook bone, pin bones, lumbar and brisket region) which help in assessing fat and muscle reserves in these anatomical locations. After proper assessment of animal's body and its frame, it was assigned its body condition score. The observation chart on the basis of which, BCS was given is as described below (Sharma 2016):

Body Condition Chart for Beetal goats		
BCS	Body Condition	Comment
1.5	Very thin (visible frame)	Wasting in appearance; ribs visible; individual spinal process evident and obvious depressions (ribs, hips) and sunken between pins and hooks; sternum is prominent.
2.0	Slightly thin	Spinous processes (dorsal/transverse) are prominent and sharp; thin flesh covering between hooks and pins; some ribs visible; definite depression between hooks
2.5	Balanced frame	Spinous process smooth; transverse processes have smooth concave curve; hooks and pins are smooth; muscle becoming obvious; sternum can be palpated
3.0	Slightly fleshy (smooth cover)	Spinous process rounded; spinous to transverse processes are smoothly sloped; hooks and pins covered; slight depression between hooks and pins
3.5	Fleshy (frame not visible)	No spinous process noticeable; ribs not visible; hooks and pins rounded with some cover; flat between hooks; difficult to palpate sternum
4.0	Obese	Edge of transverse processes barely noticeable; tail head cavity filled with fat

3.4.3.1 Physical examination of lumbar and sternal regions using Vernier caliper

Manually Vernier caliper with least count of 0.1 was used to record observations before feeding and watering of animals in the morning. The animal was restrained in proper position and plane. In lumbar region, lumbar thickness (LT) measurement was recorded using Vernier caliper with slight pressure at the L₄ position (4th lumbar vertebrae), approximately half distance away from the dorsal midline/ vertebral column. LT of bucks were recorded twice i.e. at initiation and completion of trial. In the sternal region, brisket fold's measurements depicting sternal thickness (ST) were taken near to the 3rd sternbrae position by using Vernier caliper with slight pressure (Sharma 2016).

3.4.4 Daily feed and fodder intake

Daily feed and fodder intake were measured by offering weighed quantity of concentrate feed at 8:00 AM and fodder at 11:30 AM and 3:00 PM to each respective group and taking records of daily residue weight with the help of digital weighing balance having 1 g least count. Consumption of feed and fodder was calculated as per formula given below:

Dry Matter Intake (DMI) = Dry Matter Offered – Dry Matter Residue

The daily intake was also calculated as DMI per kg of metabolic live weight ($LW^{0.75}$) in order to compare the variations between groups as live body weight of goats within group varied significantly.

3.4.5 Proximate Analysis of feed and fodder

The feed and fodder samples collected on monthly basis under both the trials were subjected to proximate analysis for determination of dry matter (DM), Crude Protein (CP), Ether extract (EE) and total Ash as per methodology of AOAC (2000).

3.4.5.1 Dry matter

A known quantity of the sample was taken in a pre-weighed dry aluminium tray, which was dried in hot air oven at 100°C for 24 hours. The dried sample with tray was weighed after being cooled in desiccator and dry matter was calculated as follows:

$$DM (\%) = \frac{\text{Wt. of oven dried sample with tray} - \text{Wt. of empty tray}}{\text{Wt. of the sample before drying}} \times 100$$

The dried samples of experimental diets were then finely grinded in an electric grinder. These grinded samples were further stored in air tight plastic bags for analysis of remaining proximate principles.

3.4.5.2 Crude Protein

Macro-Kjeldahl method was used for determination of nitrogen. A suitable quantity of finely ground material (0.25 g) was weighed and transferred into Kjeldahl flask. The samples were digested with 10 ml conc. H_2SO_4 in presence of 5 g digestion mixture ($CuSO_4:K_2SO_4$; 1:9) till appearance of light blue color. After completion of the digestion, the flask was cooled and then transferred into steam distillation tube. Then 20 ml of 40% NaOH solution was added slowly into distillation flask and ammonia was trapped in 25 ml of 4% boric acid solution with mixed indicator (10 ml/litre) in a conical flask. About 200 ml of distillate was collected. The ammonia borate was titrated later with 0.1 N H_2SO_4 . The reading of blank was also recorded and crude protein percentage of the sample was calculated as follows:

$$N (\%) = \frac{(\text{ml } H_2SO_4 \text{ for sample} - \text{ml } H_2SO_4 \text{ for blank}) \times \text{Strength of acid} \times 0.014}{\text{Wt. of sample}} \times 100$$

The crude protein of feed samples was calculated with the following formula

$$CP (\%) = N (\%) \times 6.25$$

3.4.5.3 Ether Extract

About 2 g of finely ground sample was weighed and quantitatively transferred into a thimble which is made of Whatman filter paper no.1. The sample with thimble was transferred to the extraction beaker of SOCS PLUS[®] having about 100 ml of petroleum ether. This assembly was then fitted in automatic solvent extraction system. The petroleum ether was brought to boiling after increasing the apparatus temperature to 150°C and then kept on boiling for 90 minutes at 90°C. Then again the temperature was raised to 150°C for evaporation of petroleum ether. Three washings were given to thimble for complete ether extraction of oil. After completion of the extraction process, the beaker was dried in the hot air oven at 100°C, cooled in dessicator and final weight of beaker was recorded. The ether extract was calculated as follows:

$$EE (\%) = \frac{\text{Wt. of oil extracted beaker} - \text{Wt. of empty beaker}}{\text{Wt. of the sample}} \times 100$$

3.4.5.4 Total Ash

Finely ground sample of about 2-5 g was charred in a previously weighed bone china crucible. After charring on a hot plate till complete disappearance of smoke, samples were then ignited at 650°C in a Muffle furnace for 3hr. The crucible were taken out, kept in a desiccator till room temperature was attained and weighed. The total ash (TA) content was expressed in percentage as expressed below:

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

3.4.5.5 Organic matter

It was calculated by subtracting the total ash (TA) content from DM of the samples.

$$OM \% = DM \% \text{ of sample} - TA \%$$

3.4.5.6 Estimation of cell wall constituents

Neutral detergent fiber and acid detergent fiber were estimated according to Van Soest *et al* (1991).

3.4.5.6.1 Neutral Detergent Fibre (NDF)

Neutral detergent solution (NDS) was prepared by dissolving 37.22 g disodium salt of EDTA and 13.62 g sodium borate in 700 ml of DW in a beaker. The solution was heated until dissolved. In another beaker 9.58 g disodium hydrogen

orthophosphate was dissolved in 100 ml DW and heated until dissolved. Then add 60 g sodium lauryl sulphate and 20 ml of ethylene glycol or 2-ethoxy ethanol in 850 ml distilled water in another beaker. The contents of all the beakers were mixed and finally the volume was made to two litres.

Finely ground 0.5 g sample was transferred in spoutless beaker and 50 ml of NDS was added to it. The sample with NDS was brought to boiling and refluxed for 60 minutes. The contents were filtered through previously weighed sintered glass crucible and washed with hot water till free from NDS followed by two washings with acetone. The residue was dried at 80°C in a hot air oven for overnight. The difference in initial (empty crucible) and final (crucible+ residue) weight of crucible gave NDF and expressed in percent DM basis as given below:

$$\text{NDF (\%)} = \frac{(\text{Weight of crucible + cell wall constituent}) - \text{weight of empty crucible}}{\text{Wt. of the sample on DM basis}} \times 100$$

3.4.5.6.2 Acid Detergent Fibre (ADF)

One gram sample was transferred in spoutless beaker and 100 ml of acid detergent solution (weight 20 g CTAB and dissolved in one liter of 1N H₂SO₄). The contents were refluxed for 60 minutes, after boiling had started. The contents were filtered through previously weighed sintered glass crucible and washed with hot water till free from acid detergent solution followed by acetone. The residue was dried at 80°C in a forced hot air oven for overnight. The difference in initial (empty crucible) and final (crucible+ residue) weight of crucible gave ADF and expressed in percent DM basis.

$$\text{ADF (\%)} = \frac{(\text{Weight of crucible + ADF}) - \text{weight of empty crucible}}{\text{Wt. of the sample on DM basis}} \times 100$$

3.4.5.6.3 Cellulose and Acid Detergent lignin (ADL)

Sulphuric acid (72%; w/v) was added to the sintered crucibles containing ADF and kept at room temperature for 3 hours. After draining the acid, residue was washed with hot distilled water till it become acid free followed by final two washings of acetone. The crucible was dried in hot air oven overnight at 90°C and then weighed for cellulose calculations as per following formula:

$$\text{Cellulose (\%)} = \frac{(\text{Weight of crucible + ADF}) - \text{weight of crucible after acid treatment}}{\text{Wt. of the sample}} \times 100$$

After calculations of cellulose crucible contents were then ignited in a muffle furnace at 500 °C for 3 hours. ADL was calculated as per the given formula:

$$\text{ADL (\%)} = \frac{(\text{Weight of acid treated crucible}) - (\text{weight of crucible after ignition})}{\text{Wt. of the sample}} \times 100$$

3.4.6 Zinc estimation

The zinc contents of basal feed, fodder and plasma were estimated by Atomic Absorption Spectrophotometer. In case of feed and fodder, 1 g of dried finely ground sample was wet digested using 20.0 ml triple acid (HNO₃: Perchloric acid: H₂SO₄ in the ratio of 9:4:1) and final volume was made to 20.0 ml with double distilled water. However, after thawing the plasma, one ml sample was taken in a conical flask and digested with 5.0 ml triple acid over hot plate and final volume was made to 5.0 ml with double distilled water. These digested samples were further loaded on Atomic Absorption Spectrophotometer (AAS, Perkin Elmer A Analyst 700, USA) for estimation of feed, fodder and plasma zinc level in mg/l (Chhabra *et al* 2015).

3.4.7 Breeding soundness evaluation (BSE)

Various parameters like fibrosis of testis, rump fat thickness, scrotal circumference, testicular volume, internal pelvic area and semen quality were recorded to assess the effect of Zn supplementation on breeding soundness of bucks under study. Observations on variations in fibrosis of testicular tissue, rump fat thickness, scrotal circumference, testicular volume and internal pelvic area were recorded twice i.e. once at beginning and last at completion of trial. The methodology used is explained as below:

3.4.7.1 Ultrasonographic imaging of external genitalia

Ultrasonographic examinations of the testes were done with a B-mode ultrasound scanner connected to 5.0 MHz linear array transducer. The transducer was applied transversally on the external face of the testis halfway between the head and tail of the epididymis (Khushpreet *et al* 2015). Once both testes have been individually assessed, the transducer was then moved longitudinally on both testes.

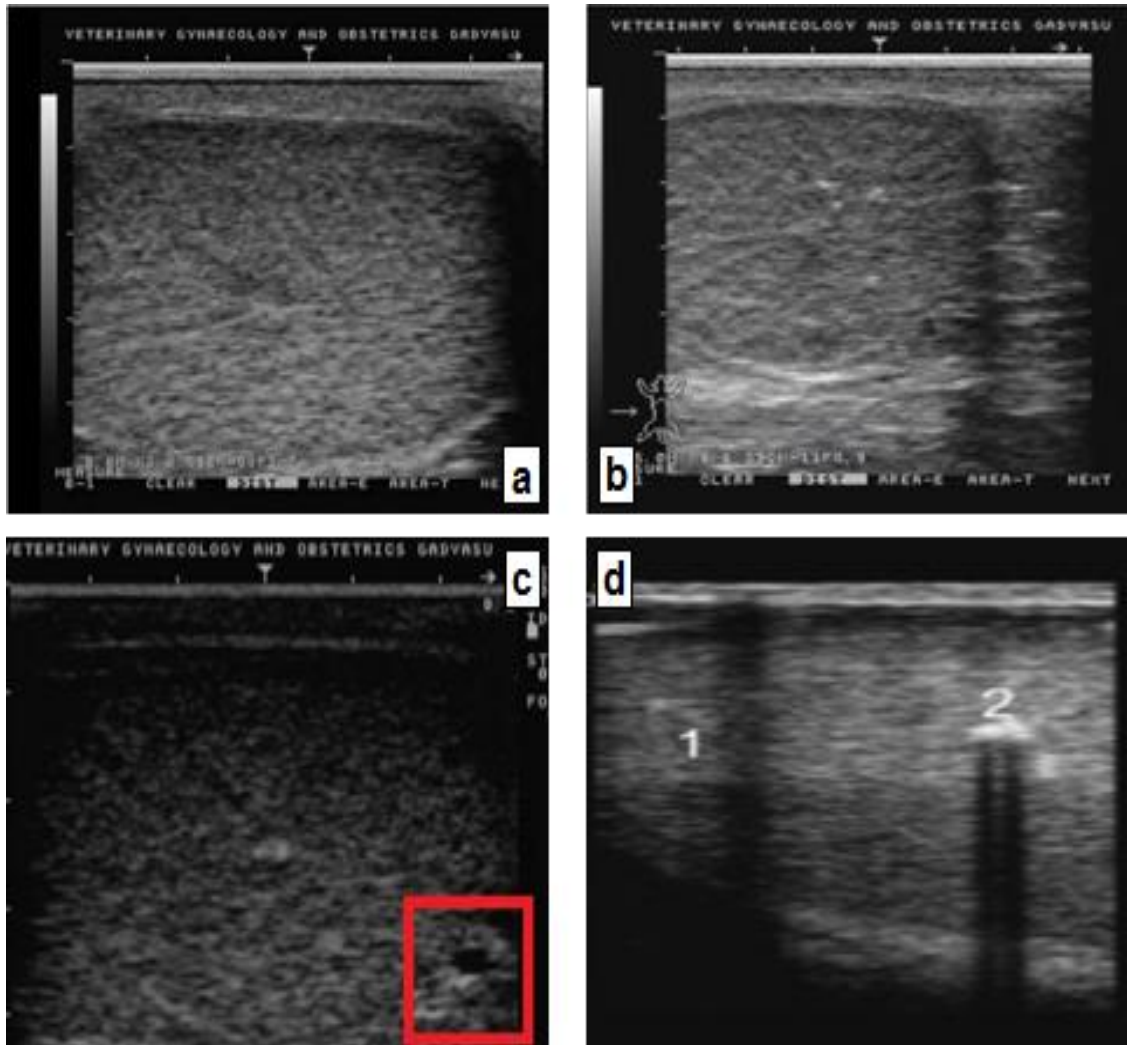


Fig. 3: Ultrasonographic evaluation of testis parenchyma showing (a) normal echo-texture, (b) fibrosis, (c) fluid accumulation and (d) calcification in parenchyma (observations in Fig. (c) and (d) were recorded by Kumar *et al* 2016)

3.4.7.2 Rump fat thickness (RFT)

Ultrasonographic probe of 5 MHz was placed halfway between the hook bone and the pin bone. The distance between the hook and pin bone was measured by measuring tape and the centre point was marked for placing of ultrasonographic probe. The ultrasonographic probe was placed longitudinally and slightly dorsal to the halfway between pin bone and hook bone. The diameter of rump fat thickness was measured in mm (Khushpreet *et al* 2015).

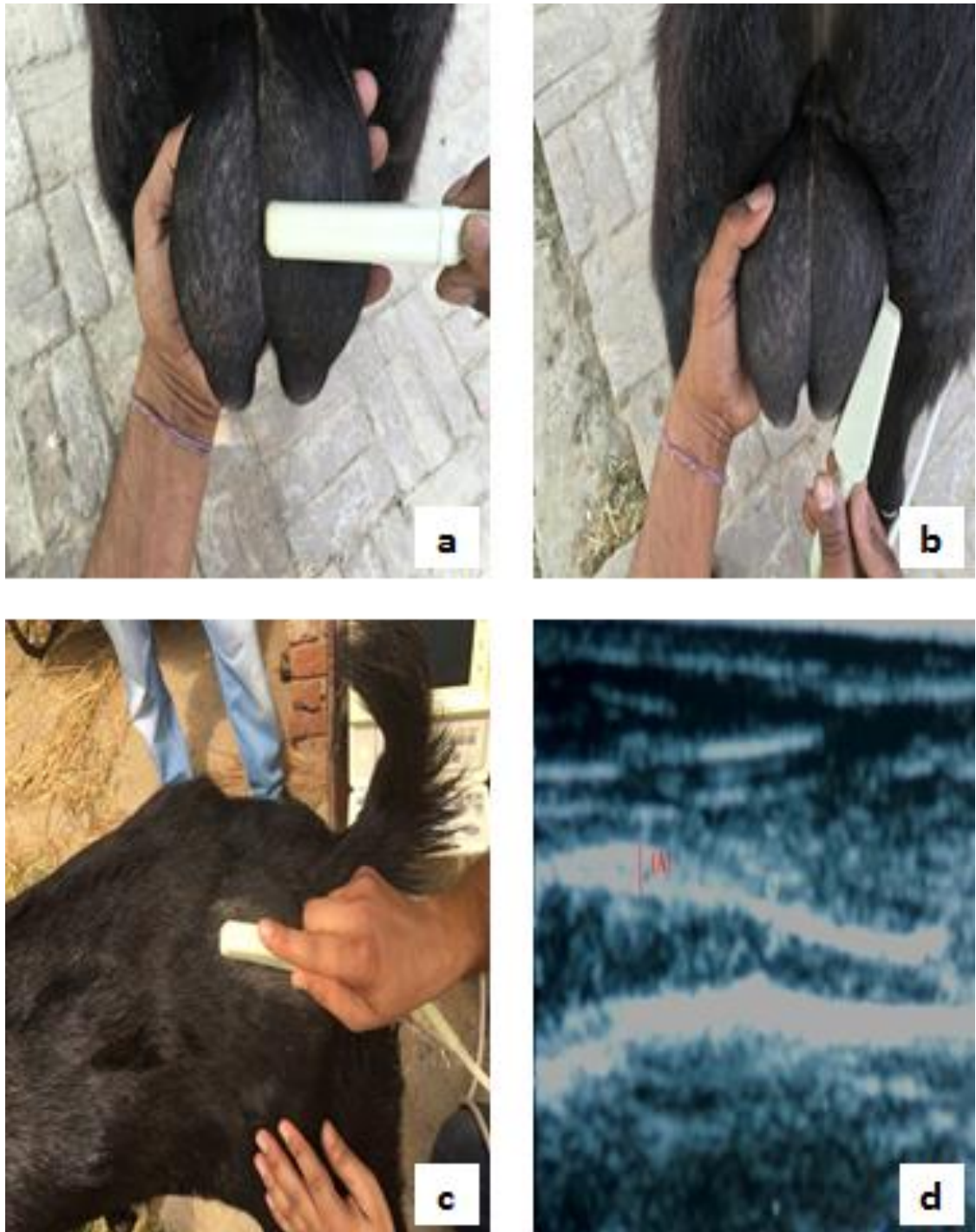


Fig. 4: Transverse (a) and longitudinal (b) placement of probe on testis for ultra-sonographic imaging. Placement of probe for evaluation of rump fat thickness (c). Probe was placed longitudinally and slightly dorsal to the halfway between pin bone and hook bone (c). Thickness of rump fat was measured in mm (d).

3.4.7.3 Scrotal circumference (cm)

Scrotal circumference (SC) was measured by holding the testicles firmly into the lower part of the scrotum so as to minimize the scrotal wrinkles. A looped measuring tape was taken transversely at the greatest diameter of the scrotum and tape was pulled in such a way that it was in close contact with the entire circumference (Singh *et al* 2014). External genitalia were examined by visual assessment and palpation for testicular tone.

3.4.7.4 Testicular volume (cm³)

It was calculated by measuring testicular length and width. Testicular length was measured from the distance between caput and cauda epididymis (dorsal to ventral direction) and its width at the widest testicular point (lateral to medial direction) with the help of Vernier caliper and a scale. Both the right testicular volume (RTV) and the left testicular volume (LTV) were calculated using Harriet's formula:

$$V (\text{cm}^3) = L \times W^2 \times 0.522$$

Where L being the testicle length and W the testicle width and 0.522 the constant (Singh *et al* 2014).

3.4.7.5 Internal pelvic area (cm²)

To measure the internal pelvic area (IPA), distance between hook bones, pin bones and between point of croup to hip joint were measured with the help of a measuring tape. All the measurements were recorded in cm. Then internal pelvic area (IPA) was calculated using Arloing's formula as described by Singh *et al* (2014).

$$\text{Transverse diameter of pelvis outlet (a)} = \frac{\text{Distance between hook bones} + \text{Distance between pin bones}}{4}$$

$$\text{Vertical diameter of pelvis outlet (b)} = \frac{3}{4} \times \text{Distance between point of croup and hip joint}$$

$$\text{Transverse diameter of pelvis inlet} = a \times 1.22$$

$$\text{Vertical diameter of pelvis inlet} = b \times 1.3$$

$$\text{IPA (cm}^2\text{)} = 1.22a \times 1.3b$$

Where 1.22 and 1.3 are constants.

3.4.7.6 Semen evaluation

Semen samples were collected at weekly interval from donor bucks in T₀, T_{oz} and T_{iz}, respectively with artificial vagina (AV) connected to graduated collection tube. Basic semen analysis techniques were adopted to have an idea of effect of Zn

supplementation on semen quality. The ejaculate was examined immediately within shortest possible time to alleviate the effect of environment factors on semen traits as per the guidelines described by Bujarbaruah and Kumaresan (2013). The following parameters were studied to assess the semen quality:

3.4.7.6.1 Volume and colour

Immediately after collection and recording of volume, semen was graded descriptively for colour with unaided eye. The colour of semen was graded descriptively like creamy white, milky white, white etc.

3.4.7.6.2 Gross motility

Gross motility or mass motility based on estimation of vigour of swirls and wave formation in undiluted semen was recorded. An undiluted small drop of freshly collected semen was placed on a clean, pre-warmed grease-free microscopic slide and examined under low power magnification of the microscope. Based on intensity of vigour of waves, ejaculate was graded on 0-5 scale as per the descriptions given below:

Grading	Semen quality	Characteristics
0		No motility
1	Poor motility	Movement of sperm is very weak and oscillatory (motility < 25%)
2	Fair motility	Movement of sperm is vigorous and rapid but no waves or eddies are formed (motility = 20-40 %)
3	Good motility	Movement of sperm is vigorous. Waves and eddies move slowly across the field (motility = 40-60 %)
4	Very good motility	Movement of sperm is rapid, vigorous and progressive. Waves and eddies form and change rapidly across the field (motility = 60-80 %)
5	Excellent motility	Vigorous and progressive sperm movements. Churning of waves and eddies are extremely rapid across the field (motility > 80 %)

Bujarbaruah and Kumaresan (2013)

3.4.7.6.3 Sperm motility

A small drop of semen diluted with buffered saline was placed on clean, pre-warmed grease-free microscopic slide to estimate sperm motility in percentage by wet mount method (Manual motility estimates). A cover-slip was placed onto sample for

avoiding air bubbling and slide was examined under 20X lens of microscope. At least, a minimum of ten widely spaced microscopic fields were examined to estimate the percentage of motile cells as sperm motility. All the motile sperms need not to be fertile but a fertile sperm must always be motile to get the conception. Hence, assessing the sperm motility is the most widely used measure of semen quality.

3.4.7.6.4 Sperm concentration and total sperm count

Sperm concentration per ml was recorded with Accucell photometer (IMV Technologies, Zin^o 1, Est 61300, L'Aigle, France). Total sperm count in one ejaculate was calculated by multiplying sperm concentration per ml with total volume of semen in ml donated by a buck.

3.4.7.6.5 Live percentage of spermatozoa

The percentages of live spermatozoa were estimated by differential staining technique using Eosin-Nigrosin (EN) stain. A small drop of semen and pre-warmed EN stain was mixed thoroughly on a clean, pre-warmed, grease free microscopic slide and left for 30 seconds. A thin smear was prepared from this mixture on a clean microscopic slide and dried rapidly on warming plate. At least 200 spermatozoa were examined and counted under bright field 40X lens of microscope for estimating live (unstained) sperm percentage (Bujarbaruah and Kumaresan 2013).

3.4.7.6.6 pH

Semen pH was tested using digital bench-top Five EasyTM Plus pH meter (Mettler-Toledo AG, 8603 Schwerzenback, Switzerland).

3.4.7.7 Serum testosterone determination by ELISA

To measure changes in testosterone levels during the study period, jugular blood (2 ml) was collected at bimonthly interval from each buck by vein puncture. After this, blood samples were allowed to clot, serum was separated by centrifugation at 3000 rpm for 20 min and stored at -20°C until further analysis. Concentrations of serum testosterone were measured by “DetectX[®] Testosterone Immuneassay kit (Arbor Assays, 1514, Eisenhower place, Ann Arbor, Michigan 48108 USA)”. The sensitivity of this assay was 0.00992 ng/mL of serum. Assay protocol followed is as given below:

- Plate layout sheet was used on the back page to aid in proper sample and standard identification. The number of wells to be used was determined. Zip-loc plate bag was sealed and stored at 4°C.

- 50 μL of samples or standards into wells in the plate was pipeted.
- 75 μL of Assay Buffer into the non-specific binding (NSB) wells was added.
- 50 μL of Assay Buffer into wells was added to act as maximum binding wells (Bo or 0 pg/ml).
- 25 μL of the DetectX[®] Testosterone Conjugate to each well was added using a repeater pipet.
- 25 μL of the DetectX[®] Testosterone Antibody to each well was added, except the NSB wells, using a repeater pipet.
- The sides of the plate were gently tapped to ensure adequate mixing of the reagents. The plate was covered with the plate sealer and shaken at room temperature for 2 hours.
- The plate was aspirated and washed each well 4 times with 300 μL wash buffer. Then, the plate was tapped dry on clean absorbent towels.
- 100 μL of the TMB Substrate was added to each well, using a repeater pipet.
- The plate was incubated at room temperature for 30 minutes without shaking.
- 50 μL of the Stop Solution was added to each well, using a repeater pipet.
- The optical densities generated from each of colorimetric 96 coated wells in a microplate reader were recorded at 450 nm.
- Serum testosterone concentrations were calculated using the online tool from [www. myassays.com/arbora-assays-testosterone-eia-kit.assays](http://www.myassays.com/arbora-assays-testosterone-eia-kit.assays).
- Serum testosterone concentrations were generated in pg/ml, which were converted to ng/ml by multiplying each value with 0.001.

3.4.7.8 Serum estradiol determination by ELISA

The same serum samples collected for estimation of testosterone were used for estimation estradiol concentrations. 17-beta-estradiol (E2) is a C-18 steroid hormone (molecular weight 272.4) produced mainly by the ovary and placenta and in small amounts by adrenals and testes. Concentrations of serum estradiol were measured by “DIA source E2-EASIA Kit (DIA source Immuno Assays S.A., Rue du Bosquet, 2, B-1348 Louvain-la-Neuve, Belgium)”. The sensitivity of this assay was 0.005 ng/mL of serum. Assay protocol followed is as given below:

- Sufficient strips were selected to accommodate calibrators, controls and all test samples.
- The strips were fitted into the holding frame.

- 50 µL of each calibrator, control or sample was dispensed into the appropriate wells with vertical alignment.
- 50 µL of estradiol-HRP conjugate was dispensed into all wells.
- 50 µL of anti-estradiol was added into each well.
- The plate was incubated for 2 hours at room temperature on a horizontal shaker set at 700 ± 100 RPM.
- Then, the plate was washed by:
 - a) Aspirating the liquid from each well;
 - b) Dispensing 0.4 mL of wash solution into each well;
 - c) Aspirating the contents of each well.
 - d) Steps b) and c) were repeated 4 times.
- 200 µL of the freshly prepared substrate solution was dispensed into each well immediately after the washing step.
- The plate was incubated for 30 minutes at room temperature, protecting from direct sunlight, on a horizontal shaker set at 700 ± 100 RPM.
- 50 µL of stop reagent was dispensed into each well.
- The absorbance of each well was read at 450 nm within 1 hour after addition of stop reagent.
- The plate reader's built-in 4PLC (Four Parameter Logarithmic Curve) software available on www.myassays.com was used to calculate estradiol concentration for each sample in pg/ml.
- Serum estradiol concentrations were generated in pg/ml, which were converted to ng/ml by multiplying each value with 0.001.

3.4.8 Reproductive performance of does

The does bred in February and March, 2016 were observed throughout gestation till kidding and fertility, prolificacy and fecundity of does in T_0 , T_{oz} and T_{iz} , respectively was worked out as given below:

3.4.8.1 Fertility

Fertility indicates the number of does kidded. However, breeding group refers to all females exposed to buck.

$$\text{Fertility} = \frac{\text{Number of does kidded}}{\text{Breeding group}} \times 100$$

3.4.8.2 Prolificacy

It was presented by the number of kids born alive divided by number of kidding. Number of kids born alive excluded: abortions, stillbirths, pregnancies terminated and kids died as a result of dystocia.

$$\text{Prolificacy} = \frac{\text{Number of kids born alive}}{\text{Number of does kidded}} \times 100$$

3.4.8.3 Fecundity

Number of kids born alive as above and number of females mated in the breeding group included all the females placed in the breeding treatment that were inseminated or naturally serviced, hence, it excludes goats not coming in estrus in the groups.

$$\text{Fecundity} = \frac{\text{Number of kids born alive}}{\text{Number of does mated}} \times 100$$

3.4.8.4 Visual observation of oestrus response:

Daily observations for behavioural signs of oestrus were conducted at 6 hours interval on 6:00 am, 12:00 noon and 6:00 pm for 30 min. Behavioural signs of oestrus were formally recorded using the previously described weighted scoring method (adapted from Van Eerdenburg *et al* 1996). Each time an animal showed an oestrous sign, the assigned number of points was recorded. A doe was considered to be in oestrus when 100 points were recorded in a 30-minute period (Walker *et al* 2008).

Estrus point scoring scale

Sign of oestrus	Points
Mucous discharge	3
Flehmen/cajoling	3
Restlessness/ swinging of tail	5
Sniffing the vulva of another animal	10
Mounted but not standing	10
Resting with chin on the back of another animal	15
Mounting the rear of other animal (attempt)	35
Mounting head side of other animal (attempt)	45
Standing heat	100

3.4.8.5 Serum progesterone determination by ELISA

To measure changes in progesterone levels during the study period, jugular blood (2 ml) was collected from 15 does at day 0 (immediately before insertion of intravaginal sponges) and then subsequently on day 7, 14 after sponge insertion and 48 and 72 hours after sponge removal and finally after 20th day after mating. The blood samples collected in sterilized heparinized tubes were loaded for centrifugation at 3000 rpm for 20 min and separated plasma was pipetted into plastic vials. These vials were stored at -20°C until further analysis. Concentrations of plasma progesterone were measured by “DetectX[®] Progesterone Immune assay kit (Arbor Assays, 1514, Eisenhower place, Ann Arbor, Michigan 48108 USA)”. The sensitivity of this assay was 0.0479 ng/mL of serum. Following assay protocol was followed while estimating the plasma progesterone levels:

- Plate layout sheet was used on the back page to aid in proper sample and standard identification. The number of wells to be used was determined. ziploc plate bag was sealed and stored at 4°C .
- 50 μL of samples or standards were pipetted into wells in the plate.
- 75 μL of Assay Buffer into the non-specific binding (NSB) wells was pipetted.
- 50 μL of Assay Buffer into wells to act as maximum binding wells (Bo or 0 pg/mL) was pipetted.
- 25 μL of the DetectX[®] Progesterone Conjugate to each well using a repeater pipet was added.
- 25 μL of the DetectX[®] Progesterone Antibody to each well, except the NSB wells, using a repeater pipet.
- The sides of the plate were gently tapped to ensure adequate mixing of the reagents. The plate was covered with the plate sealer and shaken at room temperature for 2 hours.
- The plate was aspirated and each well was washed 4 times with 300 μL wash buffer. The plate was tapped dry on clean absorbent towels.
- 100 μL of the TMB Substrate was added to each well, using a repeater pipet.
- The plate was incubated at room temperature for 30 minutes without shaking.
- 50 μL of the Stop Solution was added to each well, using a repeater pipet.

- The optical densities generated from each of colorimetric 96 coated wells in a microplate reader were recorded at 450 nm.
- Serum progesterone concentrations were calculated using the online tool from [www. myassays.com/arbor-assays-testosterone-eia-kit.assays](http://www.myassays.com/arbor-assays-testosterone-eia-kit.assays).
- Plasma progesterone concentrations were generated in pg/ml, which were converted to ng/ml by dividing each value with 1000.

3.4.9 Health status

Prompt daily observations for any variation in routine behaviour were given to the animals in the morning and evening hours to ascertain their health status. Morbidity, if any, was recorded daily and treated accordingly. Physiological parameters like respiration rate, pulse rate and rectal temperature was recorded randomly. The respiration rate was assessed by the right flank movement for one minute with minimal disturbances to the buck. The pulse rate was counted by placing a stethoscope over the heart between left 4th to 5th rib and heart beats were counted for one minute with minimum disturbances to the animal. Rectal temperature was recorded with clinical thermometer inserted into rectum for 2 minutes in such a manner that bulb of thermometer should touch the mucous membrane of rectum.

3.4.9.1 Fecal parasitic load

Fortnightly variations in faecal parasitic load in terms of parasitic eggs per gram (PEG) and oocyst per gram (OPG) were calculated by using the Modified McMaster Technique (Soulsby 2005). Three gram faeces was weighed and soaked in some quantity of saturated salt solution until they were sufficiently soft. Then 42 ml of saturated salt solution is added and poured through a fine sieve. After thorough shaking sample was withdrawn by means of a wide pipette and run into the McMaster's counting chamber, filling all the spaces. The number of parasitic eggs/oocyst within each ruled area, multiplied by 100, represented the number of PEG/OPG of the faeces of the sample.

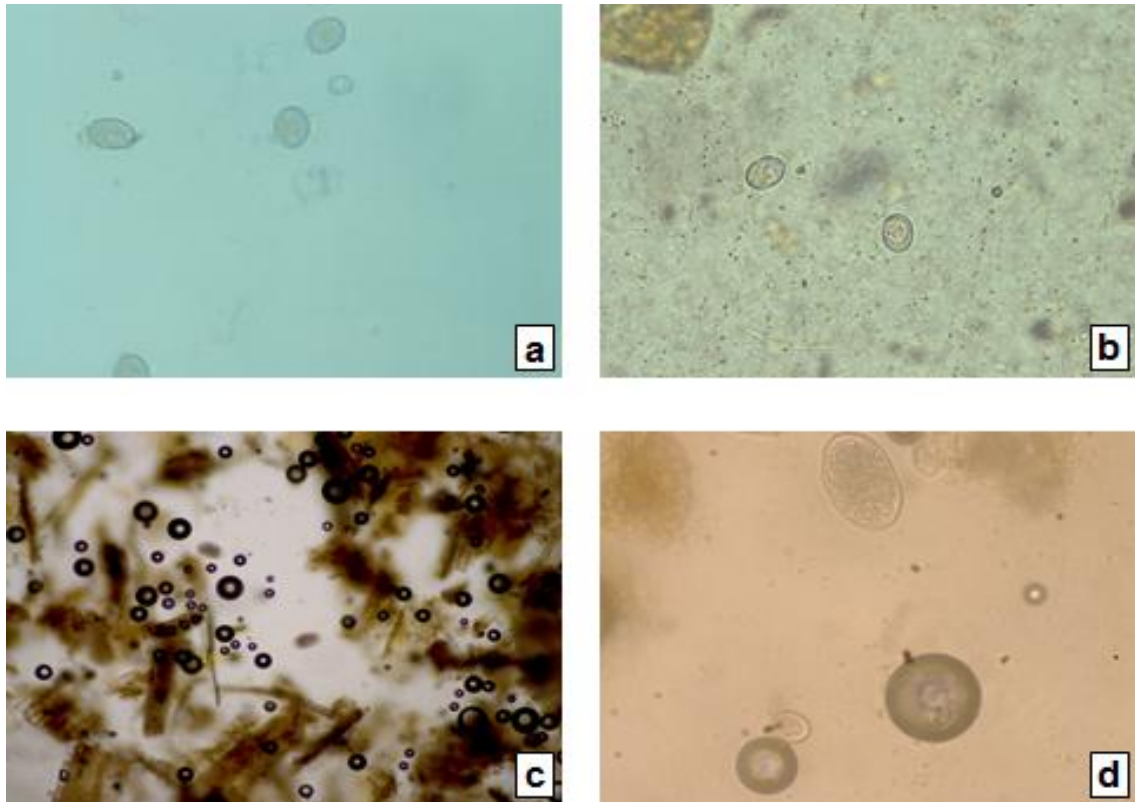


Fig. 5: Examination of coccidial oocysts (a and b) and parasitic eggs (c and d) in faeces of goats under study.

3.4.9.2 Lameness scoring: Individuals were scored for lameness (score 1–3) one morning every month based on gait and posture while walking and standing using methods adapted from Sprecher *et al* (1997) and defined below as described by Walker *et al* 2008. Each individual was scored on visual appraisal during the study.

Lameness scoring scale

Lameness score	Description	While standing	While walking	Gait
1	Non-lame	Level back posture	Level back posture	Normal
2	Moderately lame	Level back posture	Arched back	Normal to short-striding
3	Severely lame	Arched posture	Arched back	Takes one step at a time; reluctant to bear weight on one or more limbs/feet

3.4.9.3 Haematological attributes

Blood samples (2 ml) were collected aseptically from jugular vein at monthly interval in disposable vials containing different types of anticoagulants for different blood parameters. EDTA coated vials were used for haematological profile such as haemoglobin (Hgb), WBC count, RBC count, differential leukocyte count (DLC) and Packed cell volume (PCV). Heparinized vials were used for oxidative stress indicating enzyme like Superoxide Dismutase (SOD), Glutathione peroxidase (GPx) and catalase. Plain vials for blood biochemical parameters like Total protein, Albumin, Alkaline Phosphatase (ALP). Blood samples with and anti-coagulant were centrifuged at 3000 rpm for 10 minutes to separate plasma and serum, respectively. These plasma and serum samples were further stored at -20°C till further used for estimation of various parameters. Samples were thawed properly before any estimation.

3.4.9.3.1 Haemoglobin (Hgb) as per Sahli's method

Exactly 100 micro litre of N HCL was added to 20 microlitre of the sample and shaken 5 times gently. The solution was transferred to the graduated tube of Sahli's haemoglobin meter and allowed to stand for 5 minutes, so that Hgb gets converted into acid haematin. The colour of solution in the graduated tube was compared with that of reference strip on either side of haemoglobin meter. If the colour of the graduated tube was darker, drop by drop either 0.1 N HCL or distilled water was added using dropping pippete and mixed with glass rod, until the colour matched with the reference strips. The reading was noted on graduated tube and presented in SI unit of X 10 g/L.

3.4.9.2 Packed cell volume as per haematocrit centrifugation technique

Anti-coagulant was mixed with capillary blood sample. Capillary tube was filled with two-thirds to three-quarters full with well-mixed, oxalated venous blood sample. One end of the tube was sealed with plasticine (sealing wax), and tubes were placed in the micro haematocrit centrifuge. The samples were centrifuged for 5 minutes at a speed of 10,000 rpm. This separated RBCs from the plasma and left a band of buffy coat at the interface consisting of WBCs and platelets. The tube was taken and placed in the holder of micro-haematocrit scale in such a way that the bottom margin of red cells layer was against 0 mark of the scale. Similarly, capillary tube was adjusted so that top margin of plasma layer coincides with the slanting line of 100 mark, the sliding line was adjusted so that it cut between the red cell layer and buffy coat, reading was noted. This denoted the packed cell volume.

3.4.9.3 WBC count as per Neubauer's counting chamber method (Benjamin 1985).

3.4.9.4 Differential leukocyte count (Jain 1986)

For DLC, a drop of blood was placed on a clean glass slide and a thin smear was prepared. These were air dried, fixed in a methanol for 2 minutes and stained with Wright Giemsa stain (composition: Wright powder 300 mg, Giemsa powder 30 mg and methanol 100 ml) after diluting in ratio 1:10 with phosphate buffer (pH 6.8) for 30 minutes. Slides were washed under a jet of buffer from the wash bottle to remove the extra stain. Stained slides were air dried and examined under oil immersion to count 100 WBCs per slide.

3.4.9.5 RBC counting

20 microlitre of a sample was aspirated into a tube and 4 ml of diluent added and mixed gently 5 times. The coverslip was placed on the Neubauer chamber and the slide placed in the chamber for RBC count. After 1-2 minutes, the RBC settled and then RBCs were counted in five small squares (one in each corner and one in centre) of the central secondary square. Platelets were counted using automated haematology analyser (ADVIVA 2120 Haematology System, Siemens).

3.4.9.6 Serum biochemical parameters

VITROS DT60 II chemistry system (Ortho-Clinical Diagnostics, Johnson and Johnson Company, New Brunswick, NJ, USA) was used to determine the serum activities of alkaline phosphatase (ALP), total proteins and albumin with the help of in-vitro Erba[®] Mannheim diagnostic kit.

3.4.9.7 Assay of Alkaline Phosphatase (ALP)

ALP hydrolyses p-Nitrophenyl Phosphate (PNPP) into p- Nitrophenyl and Phosphate. At alkaline pH of buffered medium p- Nitrophenyl is yellow. The reagent also contains a metal ion buffer system to ensure that optimal concentrations of Zinc and Magnesium are maintained. The metal ion buffer can also chelate other potentially inhibitory ions which may be present. The reaction is monitored by measuring the rate of increase in absorbance at 405 or 415 nm which is proportional to the activity of ALP in the serum.

3.4.9.8 Assay of Total Protein (TP)

Total protein estimation was done with biuret method. Peptide bonds of proteins react with copper II ions to form a blue violet coloured complex in an

alkaline medium (biuret reaction). Each copper ion form complex with 5 or 6 peptide bonds. The intensity of the colour is proportional to the number of peptide bonds and the colour was read at 546 nm (530-570 nm).

3.4.9.9 Assay of Albumin (ALB)

Albumin binds with Bromo Cresol Green (BCG) at pH 4.2 causing a shift in absorbance of the yellow BCG dye. The blue-green colour formed is proportional to the concentration of albumin, when measured photo-metrically between 540–630 nm with maximum absorbance at 625 nm.

3.4.9.10 Assay of Globulin (GLB)

Total protein includes albumin and globulin. So to get the value of globulin, estimated value of albumin was subtracted from value of total protein.

3.4.9.11 Assay of Superoxide dismutase (SOD)

The activity of superoxide dismutase (SOD) in erythrocyte lysate was determined by the method of Marklund and Marklund (1974).

Reagents:

Pyrogallol, 0.6 mM: Dissolve 76 mg of pyrogallol in 100 ml of water, store in brown bottle, always prepare this solution fresh every hour.

EDTA, 6 mM: Dissolve 223 mg EDTA disodium salt in 100 ml distilled water.

Tris-HCL buffer 100 mM: Dissolve 1.21 g Tris in 80 ml of distilled water. Adjust pH to 8.2 with 10 mM HCL and make volume to 100 ml.

Procedure:

	Control	Test	Blank
Tris buffer	1.5 ml	1.5 ml	1.5 ml
EDTA	0.5 ml	0.5 ml	0.5 ml
Pyrogallol	1 ml	1 ml	-
Enzyme preparation (10% lysate)	-	20µl	-

Read absorbance at 420 nm in every 30 seconds upto 4 minutes. The unit of enzyme activity is defined as the amount of enzyme causing 50% inhibition of auto oxidation of pyrogallol observed in control.

3.4.8.12 Assay of Glutathione Peroxidase (GPx)

The activity of glutathione peroxidase (GPx) in erythrocyte lysate was assessed by the method of Hafeman *et al* (1974).

Reagents

Glutathione, 20 mM,: 9.2 mg GSH dissolved in 15 ml of distilled water.

Sodium phosphate buffer, 0.4 M, pH 7, containing 0.4 mM EDTA.

Sodium azide, 0.01M: 65 mg sodium azide in 100 ml of distilled water.

Hydrogen peroxide, 1.2 mM: 0.012 ml 30% H₂O₂ in 100 ml of distilled water.

Disodium hydrogen phosphate (Na₂HPO₄), 0.4 M: 7.12 g Na₂HPO₄ in 100 ml distilled water.

M-phosphoric acid precipitation solution: 1.67 g m-phosphoric acid. 0.2 EDTA and 30 g NaCl in 100ml of distilled water.

DTNB reagent: 40 mg 5-5' -dithiobis (2-nitrobenzoic acid) in 100 ml of 1% trisodium citrate solution.

Test procedure:

S. No.	Test	Control
1	0.1 ml of erythrocyte lysate/tissue homogenate	0.1 ml of erythrocyte lysate/tissue homogenate
2	1 ml of GSH (Reduced)	1 ml of GSH (Reduced)
3	1ml of phosphate buffer	1ml of phosphate buffer
4	0.5 ml of sodium azide	0.5 ml of sodium azide
5	Volume was made to 4ml with distilled water	Volume was made to 4ml with distilled water
6	Incubate for 5 minutes	Incubate for 5 minutes
7	1 ml of H ₂ O ₂ (pre-warmed to 37°C)	1 ml of H ₂ O ₂ (pre-warmed to 37°C)
8	Mix well and remove 1 ml after 1 minute in a separate centrifuge tube	Mix well and remove 1 ml after 1 minute in a separate centrifuge tube
9	Add 4 ml m-phosphoric acid precipitation solution	Add 4 ml m-phosphoric acid precipitation solution
10	Centrifuge at 3000 rpm for 15 minutes	Centrifuge at 3000 rpm for 15 minutes
11	Take 2 ml of supernant	Take 2 ml of supernant
12	Add 2 ml of Na ₂ HPO ₄	Add 2 ml of Na ₂ HPO ₄
13	Add 1 ml of DTNB reagent (at the time of reading)	Add 1 ml of DTNB reagent (at the time of reading)
14	Read absorbance at 412 nm within two minutes after mixing	Read absorbance at 412 nm within two minutes after mixing

Activity of GPx = $10 \log Co/C$

Where,

Co = concentration of GSH at zero time

C = concentration of GSH after one min incubation.

The activity was expressed as enzyme unit per mg of HgB.

3.4.9.13 Assay of Catalase

The activity of Catalase in erythrocyte lysate was determined according to the method described by Aebi (1983).

Reagents

Phosphate buffer, 50 mM, pH 7.0

Hydrogen peroxide, mM: 0.34 ml of 30% H₂O₂ was diluted with buffer. The optical density of diluted H₂O₂ at 240 nm should be around 1.5. Prepared Buffered H₂O₂ solution was prepared fresh every time.

Test procedure: Take 2 ml of phosphate buffer in quartz cuvette, add 20 µl of erythrocyte lysate/tissue homogenate and mixed well. The reaction was started by the addition of 1ml of 30 m M H₂O₂ and the decrease in absorbance was recorded at every 10 sec interval for 1 min at 240 nm in a U.V. spectrophotometer.

2 ml of phosphate buffer was taken to set zero base value.

The results were expressed as µmol H₂O₂ decomposed per min per mg Hb using 36 as molar extinction coefficient of H₂O₂.

3.4.10 Statistical analysis

The statistical significance of the mean differences within control and treated groups was analysed by ANOVA, with Duncan's multiple range test (Snedecor and Cochran 1994). The software used was IBM's Statistical Package for Social Sciences (SPSS - 22.0). Numerical data are represented as mean ± SE and differences were considered to be significant at P<0.05.

CHAPTER-IV

RESULTS AND DISCUSSION

The results and discussion for both the experiments are presented in this chapter in the same order as observations are listed in the materials and methods.

4.1 EXPERIMENT I: To study the effect of organic and inorganic zinc supplementation on qualitative and quantitative semen attributes of Beetal bucks reared under stall-fed conditions.

4.1.1 Meteorological attributes

The results pertaining to the monthly variations in environmental parameters during the study period of 6 months from June to November, 2015 are presented in Table 1. The bucks reared under loose housing system were exposed to external environment of the open area. The mean daily air temperature, relative humidity, sunshine, rainfall and THI as per outside weather during the study period were recorded as $27.62 \pm 0.33^{\circ}\text{C}$, $67.63 \pm 0.92 \%$, 6.80 ± 0.29 hours, 2.96 ± 0.80 mm and 77.42 ± 0.48 , respectively. The bucks reared under hot-humid climate of the study period experienced a high THI, particularly during the first four months of study. Though, goats can tolerate high THI, but associated with poor growth performance as well as adverse effects on physiological characteristics of the goats. Therefore, management strategies needed to improve goat production by minimizing stress in order to attain optimal animal comfort (Popoola *et al* 2014).

4.1.2 Daily feed fodder intake and proximate chemical analysis

The data on daily feed, fodder intake, their proximate chemical analysis and ingredients composition of concentrate feed fed during study period is presented in Table 2 to 4. The bucks sorted under different groups were reared together without any individual feeding. Therefore, concentrate and fodder consumption by bucks collected under different treatment groups was recorded and is presented. The bucks fed inorganic Zn consumed significantly ($P < 0.05$) higher fresh fodder over control group.

Table 1. Daily variations of environmental variables in the specified months during the study period in bucks i.e. Jun. to Nov., 2015

		Jun.	Jul.	Aug.	Sept.	Oct.	Nov.	Mean
Air Temp. (°C)	Max.	37.55 ± 0.57 ^a	33.48 ± 0.46 ^b	33.34 ± 0.34 ^b	33.21 ± 0.48 ^b	31.33 ± 0.38 ^c	26.93 ± 0.25 ^d	32.64 ± 0.29
	Min.	26.03 ± 0.44 ^a	27.17 ± 0.34 ^a	26.46 ± 0.21 ^a	24.20 ± 0.43 ^b	18.98 ± 0.52 ^c	12.58 ± 0.44 ^d	22.60 ± 0.42
	Mean	31.79 ± 0.42 ^a	30.33 ± 0.36 ^b	29.90 ± 0.22 ^b	28.71 ± 0.35 ^c	25.15 ± 0.41 ^d	19.76 ± 0.28 ^e	27.62 ± 0.33
RH (%)	Mor.	60.90 ± 2.35 ^a	81.58 ± 1.40 ^b	86.35 ± 1.01 ^c	87.47 ± 0.96 ^{cd}	90.06 ± 1.1 ^{cd}	91.17 ± 1.03 ^d	82.97 ± 0.94
	Eve.	38.27 ± 2.99 ^a	66.61 ± 2.65 ^b	67.16 ± 1.68 ^b	58.17 ± 2.84 ^c	45.35 ± 1.50 ^d	37.43 ± 1.91 ^a	52.29 ± 1.31
	Mean	49.58 ± 2.43 ^a	74.10 ± 1.79 ^b	76.76 ± 1.08 ^b	72.82 ± 1.61 ^b	67.71 ± 0.87 ^c	64.30 ± 1.08 ^c	67.63 ± 0.92
THI	Mean	80.38 ± 0.42 ^a	82.38 ± 0.40 ^b	82.22 ± 0.26 ^b	79.75 ± 0.48 ^a	73.90 ± 0.65 ^c	65.69 ± 0.41 ^d	77.42 ± 0.48
Wind speed (km/h)	Mean	6.18 ± 0.48 ^a	6.57 ± 0.55 ^a	4.12 ± 0.33 ^b	4.97 ± 0.66 ^{ab}	2.65 ± 0.31 ^c	1.86 ± 0.25 ^c	4.39 ± 0.22
Sunshine (hrs.)	Mean	8.64 ± 0.77 ^a	5.85 ± 0.77 ^b	6.11 ± 0.71 ^{bc}	8.00 ± 0.65 ^{ac}	7.56 ± 0.50 ^{abc}	4.65 ± 0.64 ^b	6.80 ± 0.29
Rainfall (mm)	Mean	0.60 ± 0.31 ^a	8.26 ± 3.73 ^b	5.34 ± 2.35 ^{ab}	2.85 ± 1.36 ^{ab}	0.52 ± 0.36 ^a	0.00 ± 0.00 ^a	2.96 ± 0.80
Rainfall (mm)	Total	17.9	256.1	165.6	85.4	16	0	541
Rainy days	Total	6	11	7	5	2	0	31
Evaporation (mm)	Mean	8.54 ± 0.54 ^a	5.46 ± 0.40 ^b	4.20 ± 0.21 ^{cd}	4.52 ± 0.25 ^c	3.52 ± 0.14 ^d	2.42 ± 0.13 ^e	4.76 ± 0.19
Evaporation (mm)	Total	256.1	152.9	130.1	135.5	109.1	72.5	856.2

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

Table 2. Daily DM consumption (kg/day) by the bucks sorted under different groups

Parameters	T ₀	T _{oz}	T _{iz}
Fresh fodders intake	24.47 ± 0.15 ^a	24.62 ± 0.14 ^{ab}	25.00 ± 0.15 ^b
DMI from fodder	8.65 ± 0.07	8.71 ± 0.07	8.84 ± 0.07
DMI from conc.	2.26	2.26	2.26
Total DMI	10.91 ± 0.07	10.97 ± 0.07	11.10 ± 0.07
Total DMI/kg metabolic live weight (LW ^{0.75} , g/d)	171.62 ± 4.37	170.77 ± 4.08	174.35 ± 3.41

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

Table 3. Chemical composition (%) of offered fodder and concentrate (on DM basis) during the study period

	Fodder	Concentrate
DM	35.36	90.50
OM	88.68	92.53
Ash	11.32	7.47
CP	9.71	16.94
EE	1.64	2.31
NDF	66.76	29.14
ADF	43.46	12.85
Cellulose	30.52	6.54
Lignin	8.64	2.14

Table 4. Composition of concentrate feed (per 100 kg)

Ingredients	Proportion (%)
Maize	47
De-oiled rice bran	30
Soybean meal	20
Mineral mixture	2
Salt	1

Though, bucks in organic Zn group also consumed higher fresh fodder than in control, but the variations between them are not statistically significant. DMI from fodder and total DMI were recorded highest in T_{iz} followed by T_{oz} over T₀. However, the differences between groups were not significant (P<0.05). No variation was recorded in DMI from concentrate between any of groups under study.

The trend recorded in feed and fodder consumption revealed that supplementation of organic and inorganic Zn had not produced any adverse effects on appetite, ruminal micro-flora and digestibility of nutrients (DM, CP, ADF, and NDF). The result in DMI was in line with the finding Jia *et al* (2008) that supplementation of basal diet containing 22.3 mg Zn/kg DM with 15, 30 or 45 mg Zn/kg DM had no effect on DMI in Liao Ning Cashmere goat wethers during the study of 60 days. In the same way, in accordance with the finding of this study, Puchala *et al* (1999) reported that supplementation of a basal diet containing 22 mg Zn/kg DM with 40 mg/day of Zn had no effect on DMI by Angora goats during a 120-day period. Similarly, Garg *et al* (2008) indicated that DMI was not affected by adding 20 mg Zn/kg DM to a basal diet with a Zn level of 34 mg/kg DM. Mandal *et al* (2007) also did not find any effect on DMI in bulls supplemented with 35 mg Zn/kg DM while consuming a diet containing 32.5mg Zn/kg DM. However, Malcolm-Callis *et al* (2000) found a linear effect of level of Zn supplementation (20, 100 and 200 mg/kg DM) in DMI by finishing steers consuming a diet with 70 mg Zn/kg DM. These results indicate that normal dietary Zn concentrations do not influence DMI, but higher concentrations may have a negative influence on diet palatability (Malcolm-Callis *et al* 2000). Dietary levels of Zn in the current experiment were within normal ranges and did not affect diet palatability.

4.1.3 Live body weight (LBW)

The data recorded on monthly variations in live body weight (kg) of Beetal bucks is presented in Table 5 and Fig. 2. The initial LBW (kg) of bucks sorted by groups were 53.20 ± 4.11 , 52.04 ± 3.92 and 50.80 ± 2.80 for T₀, T_{oz} and T_{iz}, respectively. On completion of trial at 180th day, the LBW (kg) of bucks was 49.62 ± 3.71 , 52.64 ± 3.54 and 51.84 ± 2.29 for T₀, T_{oz} and T_{iz}, respectively. Variations in LBW recorded a decline between day 0 and 60 amongst the three groups under study. After 60th day of recording, LBW had registered a continuous increasing pattern in T_{oz} and T_{iz}, respectively. Whereas, a zig-zag observations were recorded in bucks grouped under T₀ group.

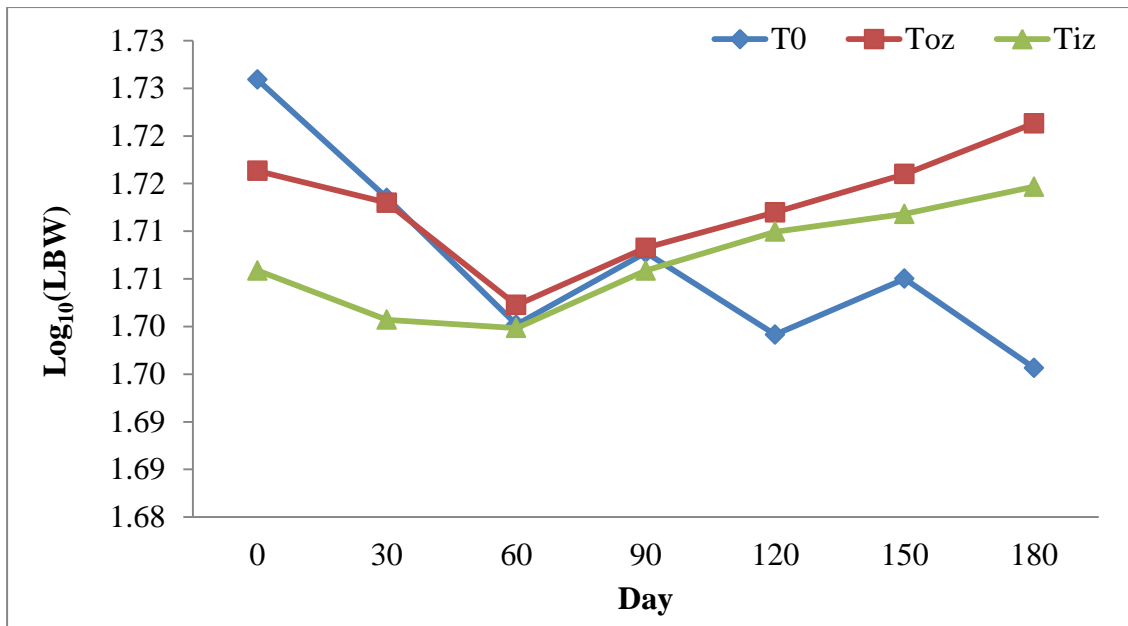


Fig. 6: Monthly variations in live body weights (kg) of Beetal bucks sorted by groups

Table 5. Monthly variations in LBWs (kg) of Beetal bucks sorted by groups

Day	T ₀	T _{0z}	T _{iz}
0	53.20 ± 4.11	52.04 ± 3.92	50.80 ± 2.80
30	51.70 ± 4.33	51.64 ± 3.40	50.20 ± 1.91
60	50.14 ± 3.99	50.38 ± 3.29	50.10 ± 2.35
90	51.02 ± 3.72	51.08 ± 3.27	50.80 ± 2.34
120	50.02 ± 4.07	51.52 ± 3.11	51.28 ± 2.44
150	50.70 ± 3.16	52.00 ± 3.53	51.50 ± 2.14
180	49.62 ± 3.71	52.64 ± 3.54	51.84 ± 2.29
Mean	50.91 ± 1.35	51.61 ± 1.22	50.93 ± 0.81

The bucks under treatment groups T_{0z} and T_{iz} showed percent gain of 1.2 and 2.0, respectively against control where loss in average LBW was 6.7 percent over the study period of 180 days (Table 5). The overall LBW (kg) was highest in T_{0z} (51.61 ± 1.22) followed by T_{iz} (50.93 ± 0.81) which was higher than the control (50.91 ± 1.35). Similarly, in case of Assam local male kids, highest body weights were reported in organic zinc supplemented kids compared with inorganic zinc-fed over control (Devi *et al* 2012). However, the differences between and within groups during period of 180 days were statistically non-significant. Statistically non-significant variations in LBW

could be attributed to the fact that no clinical symptom of any deficiency disease was observed in any buck under study. Similar non-significant variations were reported in live body weight of adult Malpura rams supplemented with inorganic and organic Cu and Zn minerals against control with no added mineral (Shinde *et al* 2012). However, Jia *et al* (2008) reported in Liao Ning Cashmere goat wethers on basal diet containing 22.3mg Zn/kg DM that during the study of 60 days, both average daily gain (ADG) and feed efficiency were improved ($P < 0.05$) by Zn supplementation and were higher ($P < 0.05$) for the treatment groups supplemented with 30 and 45 mg Zn/kg DM compared with 15 mg Zn/kg DM and control.

4.1.4 Body condition scoring (BCS) and lumbar thickness (LT) of bucks

Data recorded on BCS and lumbar thickness of bucks under study is presented in Table 6. Results revealed that BCS had declined in control group (T_0), whereas, bucks under supplementation of organic (T_{oz}) and inorganic (T_{iz}) Zn maintained same BCS. Mean BCS was also found lowest in T_0 followed by T_{oz} and T_{iz} . Contrary to the findings in BCS, lumbar thickness had increased in bucks with no supplemented Zn (T_0). However, bucks supplemented with organic (T_{iz}) and inorganic (T_{iz}) Zn had demonstrated decrease in LT. Mean LT was found highest in T_0 over T_{oz} and T_{iz} . However, the variations recorded in BCS and LT between groups were statistically non-significant.

Table 6. Body condition score and lumbar muscle thickness of Beetal bucks grouped under different groups

Particulars	T_0	T_{oz}	T_{iz}
BCS at day 0	2.35 ± 0.10	2.25 ± 0.11	2.30 ± 0.18
BCS at day 180	2.15 ± 0.13	2.25 ± 0.16	2.30 ± 0.22
Mean BCS	2.22 ± 0.04	2.26 ± 0.05	2.30 ± 0.07
LT at day 0	2.28 ± 0.20	2.18 ± 0.17	2.44 ± 0.26
LT at day 180	2.39 ± 0.18	2.02 ± 0.12	2.01 ± 0.25
Mean LT	2.33 ± 0.13	2.10 ± 0.10	2.22 ± 0.19

4.1.5 Plasma zinc

Effects of dietary Zn supplementation on plasma Zn concentration of Beetal bucks are shown in Table 7. Bimonthly variations in plasma zinc illustrated in Table 7 demonstrated that initial Zn level value was lower in plasma of bucks under T_{oz} (0.51

± 0.18) and T_{iz} (0.51 ± 0.13) over T_0 (0.73 ± 0.11). The plasma Zn level of bucks showed increasing trend towards 120th day in T_0 , T_{oz} and T_{iz} , respectively. However, as the experiment progressed towards completion on 180th day, plasma Zn levels showed decline in control. On the contrary, plasma Zn values maintained raising trend in T_{oz} and T_{iz} , respectively. At the time of completion of trial on 180th day, plasma Zn was highest in T_{iz} (1.23 ± 0.07) followed by T_{oz} (1.17 ± 0.08) over T_0 (1.05 ± 0.06). Overall mean plasma zinc values were recorded lowest for bucks enrolled under T_{oz} (0.85 ± 0.09) over T_0 (0.98 ± 0.05) and T_{iz} (0.95 ± 0.08), which was due to low plasma Zn levels at the initial stages of experiment. However, the variations recorded in plasma Zn between groups during period of 180 days were statistically non-significant. Likewise, non-significant variation in serum Zn and Cu were also reported by Mandal *et al* (2008) in male calves. Similarly, Puchala *et al* (1999) also reported that supplementation of the diet with Zn-Methionine and ZnO increased ($P < 0.03$) plasma Zn concentration (0.92 and 0.87 versus 0.72 mg/l for control). When Zn is adequate, supplemental Zn would not be expected to increase plasma Zn concentration due to homeostatic mechanisms (Jia *et al* 2008). Increase in plasma Zn of bucks under treatment groups T_{oz} and T_{iz} in response to supplemental Zn indicate that the animals were deficient in Zn intake fed on basal diet. However, variations in T_0 could not be explained. In concurrence with the findings, Shinde *et al* (2012) reported that serum concentrations of supplemented Cu and Zn were significantly increased ($P < 0.01$) after feeding the adult Malpura rams with concentrate feed containing Cu and Zn contents of 16.87, 34.67 ppm in group-1; 37.15, 48.56 ppm in group-2 and 26.19, 39.58 ppm in group-3, respectively.

Table 7. Bimonthly variations in plasma zinc (mg/L) of Beetal bucks sorted by groups

Day	T_0	T_{oz}	T_{iz}
0	0.73 ± 0.11	0.51 ± 0.18	0.51 ± 0.13
60	0.93 ± 0.08	0.72 ± 0.19	0.93 ± 0.08
120	1.22 ± 0.04	0.99 ± 0.10	1.14 ± 0.07
180	1.05 ± 0.06	1.17 ± 0.08	1.23 ± 0.07
Mean	0.98 ± 0.05	0.85 ± 0.09	0.95 ± 0.08

4.1.6 Breeding soundness evaluation (BSE)

Various parameters recorded to study the effect of Zn supplementation on breeding soundness of bucks are presented in Table 8. In addition, quantitative and qualitative semen attributes like ejaculate volume (ml), sperm concentration (millions/ml), total sperm number per ejaculate (millions), gross motility, sperm motility (%), live sperm (%) and semen pH were recorded at weekly interval and are presented in Table 9.

4.1.6.1 Visual and physical appraisal

It revealed that all the bucks under study were having two large, well developed, functional and equal sized testicles in a single scrotum. Testicles did not exhibit sizable splits in the scrotum and were not of overly pendulous shape. Textures of testes were smooth and free from any inflammations and hardened spots. No lameness was reported in bucks during the study period. No variations were recorded during the study period.

4.1.6.2 Ultrasonography of external genitalia

Ultrasonographic imaging was conducted to evaluate the external genitalia. Ultrasonographic imagings of the testes with a B-mode ultrasound scanner connected to 5.0 MHz linear array transducer revealed that homogenous pattern of echo texture throughout the testis parenchyma tissue with no visible lesions was found in 9 out of 15 bucks. Two bucks each with mild fibrosis in one or both the testicles were allocated in T₀, T_{oz} and T_{iz}, respectively. Bucks with fibrosis showed hyperechoic areas scattered in the testicular parenchyma, acoustic shadowing, showing testicular degenerations with mineralization. The percentages of testicles diagnosed with mild fibrosis were 20, 30 and 30 in T₀, T_{oz} and T_{iz}, respectively. The overall prevalence of mild fibrosis in individual testicle was 26.67 % diagnosed in 40% of total bucks. No variation was recorded in texture of testicular parenchyma during the study period of 180 days. Here, it is important to mention that appearance of fibrosis in any buck was not associated with any clinical symptoms resembling orchitis, fever, lethargy, off-fed etc. Fibrotic lesions were reported common in the testes of bulls raised under intensive rearing conditions in western Canada as well as in the more extensive rearing conditions of Argentina (Barth *et al* 2008). The causes of appearance of fibrotic lesions in teste's parenchyma were unknown and speculative. Some of earlier workers had also failed to establish the cause of fibrosis in testicles and viewed that

developmental changes of the testes might be involved in the etiology of testis fibrotic lesions (Barth *et al* 2008).

4.1.6.3 Rump fat thickness (RFT)

Mean RFT (mm) was 2.58 ± 0.10 , 2.10 ± 0.25 and 2.52 ± 0.17 in T_0 , T_{oz} and T_{iz} , respectively. The RFT (mm) was highest ($P>0.05$) bucks enrolled under group with no Zn supplementation. Supplementation of organic Zn resulted in lowest accumulation of rump fat in T_{oz} followed by T_{iz} over the control group. However, the variations were statistically non-significant. Recording of lower RFT could be an important observation for selection and judging of bucks under field conditions of traditional farming where no authenticated documented records of age and body weight are available. Moreover, it was well documented selection with low BFT was expected to improve fertility at the farm. Likewise, Khushpreet *et al.* (2015) recorded significantly ($P<0.05$) higher RFT (mm) in poor libido buffalo bulls (7.29 ± 0.93) over high libido buffalo bulls (5.29 ± 0.62). Advancement of age, LBW and increased nutrition may had increased rump fat accumulation in bucks, though no overfeeding was practiced at the farm.

4.1.6.3 Scrotal circumference (SC)

Scrotal circumference in cm (Mean \pm SE) was recorded higher in bucks supplemented with organic and inorganic Zn (T_{oz} and T_{iz} , respectively) over the bucks with no additional Zn supplementation in T_0 (Table 8). However, the variations amongst groups were statistically non-significant. Likewise, non-significantly higher scrotal circumference of 35.6 ± 1.23 cm was reported in high libido buffalo bull over value of 34.55 ± 0.50 in low libido buffalo bull, respectively (Khushpreet *et al.*, 2015).

4.1.6.4 Right and left testicular volume (RTV and LTV)

Data on testicular volume revealed non-significant variations for both RTV and LTV in the bucks. Here, an important observation was recorded that both RTV and LTV had declined over the study period of 6 months in all the three groups under trial. This decrease of testicular size could be attributed to significant decline in air temperature from 31.79 ± 0.42 in June to 19.76 ± 0.28 in November, 2015. It was also documented earlier that continuous production of semen may also lead to shrinkage of testicles.

4.1.6.5 Internal pelvic area (IPA)

The internal pelvic areas recorded in bucks were 142.03 ± 1.76 , 137.68 ± 5.52 and 142.74 ± 3.75 in T_0 , T_{oz} and T_{iz} , respectively. The variations in IPA were statistically non-significant. Pelvic area is an important desirable trait affecting fertility in male animals. Many workers had authored that bulls with smaller pelvic areas were classified as substandard potential breeders. They further had reported a positive correlation of the pelvic area with fertility ($r=0.45$). Clearly, IPA involved a complex interplay of body development, age and other physical cues (Singh *et al* 2014).

Usually, the biometry parameters like testicular volume, IPA etc. depends upon age, testicular abnormalities like hypoplasia, orchitis or testicular degeneration. However, in present study, the bucks were of similar age group, thus the variation was not due to age. Also, the physical and ultrasonographic examination failed to reveal any testicular pathological conditions. Thus, non-significant variations were recorded amongst three groups.

Table 8. Various parameters of BSE of Beetal bucks sorted under different groups

Parameter	T_0	T_{oz}	T_{iz}
Testicles (%) with mild fibrosis	20	30	30
RFT (mm) at day 0	2.58 ± 0.15	2.12 ± 0.41	2.52 ± 0.28
RFT (mm) at day 180	2.58 ± 0.14	2.08 ± 0.33	2.52 ± 0.22
Mean RFT (mm)	2.58 ± 0.10	2.10 ± 0.25	2.52 ± 0.17
SC (cm) at day 0	25.40 ± 0.80	26.16 ± 0.51	26.16 ± 1.31
SC (cm) at day 180	25.38 ± 0.87	26.12 ± 0.51	26.16 ± 1.27
Mean SC (cm)	25.39 ± 0.56	26.14 ± 0.34	26.16 ± 0.86
RTV (cm ³) at day 0	111.63 ± 8.58	111.22 ± 5.32	113.77 ± 2.26
RTV (cm ³) at day 180	107.80 ± 6.97	107.57 ± 4.93	110.08 ± 1.84
Mean RTV (cm ³)	109.72 ± 5.25	109.40 ± 3.47	111.93 ± 1.50
LTV (cm ³) at day 0	112.23 ± 6.09	114.98 ± 6.07	115.61 ± 1.84
LTV (cm ³) at day 180	106.63 ± 4.30	110.38 ± 5.97	111.93 ± 2.26
Mean LTV (cm ³) at day 0	109.43 ± 3.64	112.68 ± 4.09	113.77 ± 1.50
IPA (cm ²) at day 0	142.03 ± 2.64	137.68 ± 8.28	142.74 ± 5.63
IPA (cm ²) at day 180	142.03 ± 2.64	137.68 ± 8.28	142.74 ± 5.63
Mean IPA (cm ²)	142.03 ± 1.76	137.68 ± 5.52	142.74 ± 3.75

4.1.6.6 Semen evaluation

As per data depicted in Table 9, it was found that bucks enrolled under control (T_0) ejaculated significantly ($P < 0.05$) higher volume of semen over bucks supplemented with organic Zn (T_{oz}) and non-significantly higher semen volume over bucks supplemented with inorganic Zn (T_{iz}). The variations between treatment groups T_{oz} and T_{iz} were statistically non-significant. The bucks under T_{oz} and T_{iz} donated 7.24 and 2.63 percent less volume of semen over T_0 , respectively. The semen donated by all the bucks was creamy white indicating no attributing effect fibrosis of testes on semen colour. On the contrary, Shinde *et al* (2012) reported that supplementation of inorganic and organic Cu and Zn to Malpura rams non-significantly increased semen volume from 0.75 in control to 0.89 in rams supplemented Cu and Zn sulphate and 0.78 ml in rams supplemented with Cu and Zn methionine.

The mean concentration of sperms ($\times 10^6/\text{ml}$) in semen ejaculated was 2356.53 ± 46.94 , 2586.10 ± 29.43 and 2703.07 ± 59.05 in T_0 , T_{oz} and T_{iz} , respectively. Interestingly, an opposite trend to semen volume was observed in sperm concentration. The bucks supplemented with organic or inorganic Zn ejaculated semen with significantly ($P < 0.05$) higher sperm concentration ($\times 10^6/\text{ml}$) over bucks supplemented with no additional Zn. The variations in sperm concentration were statistically not significant amongst T_{oz} and T_{iz} groups.

Total sperms donated per ejaculate ($\times 10^6$) were significantly ($P < 0.05$) higher T_{iz} over T_0 . The bucks under T_{oz} had also generated higher ($P > 0.05$) total sperms per ejaculate over T_0 . However, the variations between treatment groups were statistically non-significant. Bucks under T_{oz} and T_{iz} generated 5.63 % and 11.96 % more total sperms over bucks in T_0 , respectively.

Data analysed for semen attributes depicted that significant variations were found in Gross motility and sperm motility amongst the groups (Table 9). From the findings, it was revealed that supplementation of organic and inorganic Zn lead to significant ($P < 0.05$) improvement in gross motility and sperm motility. However, the variations between treatment groups T_{oz} and T_{iz} in gross motility and sperm motility (%) were statistically non-significant. Similarly, Shinde *et al* (2012) reported that supplementation of inorganic and organic Cu and Zn to Malpura rams improved the mass motility from 4.03 in control to 4.35 in supplemented inorganic group. However,

sperm motility decreased in rams from 82.3% in control to 76.7% in rams fed inorganic minerals and 67.6% in rams fed organic minerals.

The value of percent live sperm was higher than the desirable level of 80% in all the three groups under study. The differences amongst the three groups regarding live sperm (%) were statistically non-significant.

Mean semen pH was found 6.60 ± 0.02 , 6.46 ± 0.02 and 6.50 ± 0.02 in T_0 , T_{oz} and T_{iz} , respectively. Mean semen pH was recorded within physiological limits of 6.2-6.7 (Bujarbaruah and Kumaresan 2013). However, significantly ($P < 0.05$) higher pH was recorded in the semen of bucks in control (T_0) over bucks in treatment groups (T_{oz} and T_{iz} , respectively). The semen pH seemed to be the least variable parameter to evaluate the breeding soundness. It was earlier documented that semen pH remained within normal limits of 6.68 ± 0.04 , 6.7 ± 0.10 and 6.8 in bucks suffering from acute, subacute and chronic orchitis, respectively (Njenga *et al* 1999).

Table 9. Semen quantitative and qualitative traits of Beetal bucks sorted under different treatment groups on the basis of zinc supplementation

Parameters	T_0	T_{oz}	T_{iz}
Semen volume (ml)	1.52 ± 0.03^a	1.41 ± 0.03^b	1.48 ± 0.03^{ab}
Sperm concentration ($\times 10^6$ /ml)	2356.53 ± 46.94^a	2586.10 ± 29.43^b	2703.07 ± 59.05^b
Total sperms/ejaculate ($\times 10^6$)	3480.88 ± 71.35^a	3676.87 ± 91.57^{ab}	3897.15 ± 90.32^b
Gross motility/mass motility	4.06 ± 0.06^a	4.40 ± 0.05^b	4.36 ± 0.05^b
Sperm motility (%)	80.52 ± 0.72^a	83.04 ± 0.50^b	82.35 ± 0.48^b
Live sperm (%)	82.82 ± 0.70	83.46 ± 0.63	82.92 ± 0.71
Semen pH	6.60 ± 0.02^a	6.46 ± 0.02^b	6.50 ± 0.02^b

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

Mean values of semen quantitative and qualitative characteristics at the start of the experiment were statistically non-significant ($P > 0.05$) in all the bucks sorted under different groups. However, there were statistically significant differences among the bucks of different groups after 6 months of zinc supplementation except for live sperm (%). Similarly, Kumar *et al* (2006) studied the effect of inorganic (Zn

sulfate) and organic (Zn propionate) sources of Zn supplementation on semen quality in crossbred bulls and reported significantly higher semen volume, sperm concentration (million ml⁻¹), sperm number per ejaculate (million), live sperm (%) and motility in Zn-supplemented groups than the control. However, the group supplemented with organic Zn reflected superiority over other groups. Therefore, it may be concluded that Zn supplementation either in the inorganic or organic form in the diet of Beetal bucks improved the qualitative and quantitative attributes of semen. Here it is also important to mention that all the semen quality parameters recorded in bucks under T₀, T_{oz} and T_{iz}, respectively varied within normal physiological limits as described by Bujarbaruah and Kumaresan (2013).

4.1.6.7 Serum testosterone

The variations in serum testosterone of bucks grouped under T₀, T_{oz} and T_{iz} are presented in Table 10. Mean testosterone levels (ng/ml) were recorded highest in T_{oz} followed by T_{iz} over T₀, respectively (Table 10). It seemed that Zn supplementation had led to improved serum testosterone values in treatment group compared with control with no supplementation of Zn. It is earlier established that increase in serum testosterone is useful in induction of an intense sexual behaviour and improvement of semen quality supporting improvement of semen quality of Beetal bucks under treatment groups in this study. Similarly, in agreement with findings of this study, Kumar *et al* (2006) reported in crossbred bulls study that supplementation of inorganic (Zn sulfate) and organic (Zn propionate) sources of Zn @ 70 ppm Zn and 35 ppm Zn for period of 6 months significantly improved serum testosterone concentration (ng/ml) as compared to control. In accordance with the findings of this study, El-Sisy *et al* (2008) revealed in male Baladi goats that supplementation of basal diet with 40 ppm Zn-methionine can result in significant increase of testosterone levels. In addition, it was also observed that serum testosterone level had shown elevated values at completion of trial at 180th day over the initial values at 0th day in all the groups. This increase of serum testosterone could be due to weekly sampling of semen. However, the variations recorded between the groups were statistically non-significant, which could be due to wide individual variations within the groups.

Table 10. Bimonthly variations (Mean±SE) in serum testosterone (ng/ml) of Beetal bucks

Day	T ₀	T _{oz}	T _{iz}
0	2.99± 1.69	3.73± 0.89	3.39± 0.69
60	3.89 ± 1.86	4.52 ± 1.00	4.23 ± 1.23
120	3.01 ± 1.49	6.05 ± 2.86	5.33 ± 0.82
180	3.84 ± 1.63	5.16 ± 2.25	4.35 ± 1.18
Mean	3.43 ± 0.77	4.87 ± 0.91	4.33 ± 0.49

4.1.6.8 Testosterone to estradiol ratio

Data depicting testosterone to estradiol ratio of Beetal bucks in T₀, T_{oz} and T_{iz}, respectively is presented in Table 11. Exactly opposite to the findings for testosterone, the estradiol concentration in serum was lowest in T_{oz} followed by T_{iz} over the control group. Similarly, Testosterone to estradiol ratio was highest in T_{oz} followed by T_{iz} and lowest in T₀ group. However, the variations recorded were statistically non-signification.

Testosterone and estrogen are no longer considered male only and female only hormones. Both hormones are important in both sexes. Testosterone to estradiol ratio plays an important role in exhibition of libido, breeding soundness and semen quality. High levels of estradiol can affect libido and breeding soundness as testosterone and estradiol have been found to be negatively correlated to each other. The ratios of testosterone to estrogen are more important than their individual values in regulating libido and breeding soundness. In the present study testosterone to estrogen ratio was higher in bucks supplemented with Zn over the control. Leydig cells produce testosterone, which is converted to estradiol by aromatization in sertoli cells, adipose tissues and hypothalamic pre-optic area. Increased aromatization of testosterone to estradiol causes decreased testosterone to estrogen ratio. Hence, higher estradiol level might be associated with poor libido and poor breeding performance in breeding male animals (Singh *et al* 2015).

Table 11: Mean value of serum testosterone, estradiol (ng/ml) and testosterone to estradiol ration in Beetal bucks sorted by supplementation of different sources of Zn

Particulars	T ₀	T _{oz}	T _{iz}
Testosterone	3.43 ± 0.77	4.87 ± 0.91	4.33 ± 0.49
Estradiol	0.07 ± 0.01	0.04 ± 0.01	0.06 ± 0.01
Testosterone to estradiol ratio	105.43 ± 33.63	188.79 ± 59.71	116.11 ± 31.65

4.1.7 Health status

The bucks under study were examined daily in the morning and evening for any variation in routine behaviour. In addition to this, the physiological responses of the animals were also recorded randomly. The physiological responses in terms of rectal temperature, respiration rate and pulse rate were within normal physiological limits. Hooves of bucks were examined and trimmed regularly as per routine management practice. Therefore, lameness score was recorded as 1 in all the bucks during study. No variation in lameness score was recorded in bucks under different groups during the study period of 6 months. No case of lameness, morbidity or mortality was observed during the study period indicating no harmful effect of Zn supplementation on bucks.

It is well established that gastrointestinal parasitic infections under stall-fed are one of the serious constraints affecting goat production throughout the globe. Moreover, deficiency of zinc affects immunity both directly and indirectly, making the animal susceptible to subclinical parasitism. This subclinical parasitism may not cause heavy morbidity or mortality, but may lead to huge economic losses due to production performance losses. Therefore, the effect of organic and inorganic zinc supplementation on the faecal parasitic load of Beetal bucks under stall feeding rearing system were also studied and is presented under the following subheads.

4.1.7.1 Parasitic eggs shed per gram of faeces (PEG)

Fortnightly variations in number of parasitic eggs shed per gram (PEG) of faeces shown in Table 12 and Fig. 7. The data recorded depicted a declining trend in all the naturally infected groups under study period. The percent decline was reported more in treatment groups supplemented with organic and inorganic zinc over the

control with no zinc supplementation i.e. 39%, 83% and 91% in T₀, T_{oz} and T_{iz}, respectively. Overall mean number of parasitic eggs shed in 1 g of faeces in Beetal bucks sorted by groups were found significantly lowest in T_{iz} over T_{oz} and T₀ (Table 12). The high mean value in T_{oz} group was due to high PEG during initial stages of trial period over control (T₀) and T_{iz}, respectively. Similarly, It was documented by Scott and Koski (2000) using a zinc deficient nematode infected mouse model that parasites are better able to survive in the zinc deficient hosts than in well nourished. Therefore, earlier, many researchers had shown that use of minerals can boost immune response to cope up with subclinical naturally infested parasitism. On the contrary to the findings of this study, Schafer *et al* (2015) reported that administration of Zn and Cu did not reduced PEG in experimentally infected lambs with *H. contortus* along with enhancing the immunity. Mixed infections of *Strongyle* with *Amphistomes*, *Schistosoma* and *Trichuris* were observed in bucks. Similarly, Singla (1995) observed mixed subclinical parasitic gastrointestinal infection of *Strogyles* with *Fasciola*, *Amphistomes* and coccidia in 89.90% faecal samples of sheep and goat population at Ludhiana, Punjab. Here it is important to report that subclinical parasitism was not accompanied by clinical symptoms of diarrhea in any group.

4.1.7.2 Oocysts shed per gram of faeces (OPG)

The variations in OPG of Beetal bucks in T₀, T_{oz} and T_{iz}, respectively are presented in Table 13 and Fig. 8. Mean numbers of oocysts shed per gram of faeces of naturally coccidia infected bucks of individual groups during the whole experiment were transformed logarithmically and are presented in Fig. 8. During the study period of 120 days, a total of 45 samples each were collected in T₀, T_{oz} and T_{iz}, respectively. *Eimeria* oocysts were recovered in 86.7, 71.1 and 86.7 percent of faecal samples of Beetal bucks sorted by groups in T₀, T_{oz} and T_{iz}, respectively. No oocyst was recovered in 6, 13 and 6 out of each 45 faecal samples in T₀, T_{oz} and T_{iz}, respectively. Maximum OPG count in a specimen recorded was 12500, 4500 and 3200 in T₀, T_{oz} and T_{iz}, respectively.

Table 12. Number of parasitic eggs shed in 1 g (PEG) of faeces in Beetal bucks sorted by supplementation of different sources of Zn

Day	T ₀	T _{oz}	T _{iz}
0	660.00 ± 208.81	1680.00 ± 381.31	900.00 ± 430.12
15	1020.00 ± 233.24 ^{ab}	1820.00 ± 257.68 ^a	800.00 ± 311.45 ^b
30	1120.00 ± 395.47	1380.00 ± 263.44	540.00 ± 199.00
45	400.00 ± 104.88	500.00 ± 309.84	380.00 ± 120.00
60	1200.00 ± 418.33	780.00 ± 355.53	520.00 ± 146.29
75	1060.00 ± 338.53	460.00 ± 297.66	340.00 ± 124.90
90	120.00 ± 58.31	440.00 ± 390.64	260.00 ± 188.68
105	320.00 ± 320.00	640.00 ± 450.11	240.00 ± 150.33
120	400.00 ± 170.29	280.00 ± 255.73	80.00 ± 58.31
Mean	700.00 ± 101.50 ^a	886.67 ± 130.72 ^a	451.11 ± 76.38 ^b

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

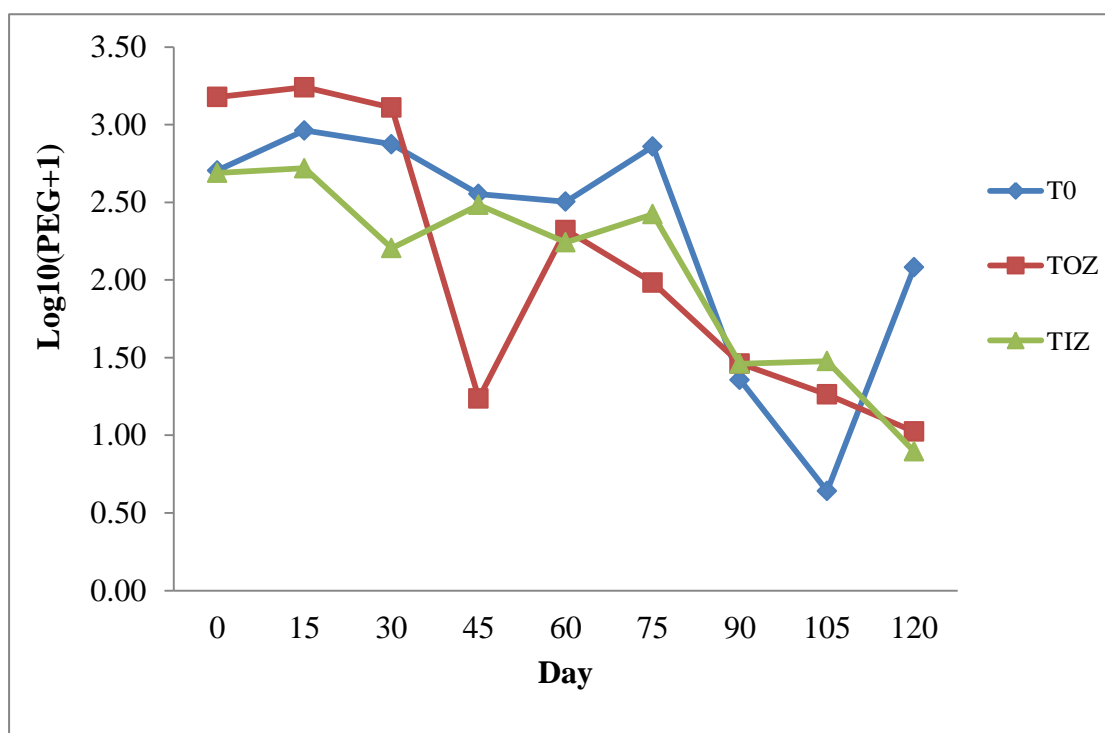


Fig. 7: Fortnightly mean number of PEG in faeces of Beetal bucks sorted by groups.

Results indicated that Zn supplementation (organic or inorganic) kept the highest OPG towards lower count in T_{oz} and T_{iz} compared with T_0 . Irrespective of groups, a total of 81.5 percent samples having variable intensity of OPG (100-12500) were found positive for *Eimeria* oocysts. High positive specimen percentages were consistent with findings elsewhere (Singla 1995 and Faizal and Rajapakse 2001).

The mean OPG was reported low during the first month of study and then it has shown increasing trend towards the completion of study period (Table 13 and Fig. 8). The percent increase in shedding of oocysts per gram of faeces during the study period of 120 days were reported to be 1046, 710 and 367 for T_0 , T_1 and T_2 , respectively. The overall mean OPG during the study period reported (1908.89 ± 388.83 , 817.78 ± 158.95 and 864.44 ± 120.02 in T_0 , T_{oz} and T_{iz} , respectively) was higher than 190.03 in Jakhrana goats under semi-arid regions of India (Sharma *et al* 2009) and lower than 2667 in cross-bred goats (Jamnapari, Kottukachchiya and Boer) in dry areas of Sri Lanka (Faizal and Rajapakse 2001).

Bucks in groups T_{oz} and T_{iz} that received organic and inorganic Zn, respectively shed significantly less oocysts ($P \leq 0.05$) compared to the control (Fig. 8). The variations between T_{oz} and T_{iz} were statistically non-significant. Bucks under control (T_0) with no Zn supplementation shed the highest mean oocysts per gram of faeces (53.1%). On the contrary, bucks from chelated Zn group (T_{oz}) shed the least number of oocysts (22.8%) followed by bucks from T_{iz} group (24.1%). Contrary to the findings of this study, Strnadová *et al* 2011 reported that goat kids fed inorganic Zn shed the most oocysts (49.7%), whereas in concurrence, kids from Zn lactate and chelate groups shed the least number of oocysts (11.9 and 13.4%, respectively) compared to control (25.0%). Confirming the finding of this study, many researchers had studied the influence of Zn supplementation on the performance, antioxidant status, and immune responses of broilers birds challenged with *Eimeria* and reported that birds fed Zn supplementation excreted fewer oocysts ($P < 0.001$) in the excreta than those receiving no Zn supplement (Bun *et al* 2011). Here it is important to report that shedding of oocysts was not accompanied by clinical symptoms of coccidiosis (diarrhea) in any group. In the same way, goat kids receiving inorganic and organic zinc shed oocysts without any clinical manifestation of disease (Strnadová *et al* 2011).

Some authors had considered coccidiosis to be clinical when more than 100,000 *Eimeria* spp. oocysts are detected in 1 g of faeces (Scott 2007). However, some are of view that a high OPG more than $10^5/g$ is not always suggestive of clinical infection, though in conjunction with clinical symptoms can be a good diagnostic tool (Soulsby 2005). It was also reported that some Saanen or crossbreed kids showed sub-clinical signs even when more than 5×10^5 number of oocysts were found in 1 g of their faeces (Koudela and Boková 1998). Here, it did not viewed unreasonable to conclude that the *Eimeria* oocysts encountered in bucks of about 29 months of age reared under intensive stall-fed production system in the semi-arid zone were mainly sub-clinical. Presence of these sub-clinically *Eimeria* infected goats with no clinical signs can be potential carriers and may facilitate in precipitating the disease in the susceptible immune-compromised groups (Woji *et al* 1994). Moreover, sub-clinical parasitism can reduce the performance (Rahman 1994 and Faizal *et al* 1999) of bucks under stall-fed.

The prevalence and the severity of infection vary considerably depending on the local environmental conditions such as humidity, temperature, rainfall, vegetation and management practices (Swarnkar *et al* 2010 and Radfar *et al* 2011). An opposite trend was reported between fortnightly environment temperature, relative humidity, THI and OPG during the study period. This could be attributed to that declining fortnightly environment temperature (29.79 ± 0.35 to 18.85 ± 0.42 °C), relative humidity (78.6 ± 1.52 to 61.53 ± 1.37 %) and THI (82.31 ± 3.90 to 64.23 ± 5.76) had created favourable conditions for propagation of infective coccidial larvae on pasture. However, earlier studies indicated that lowering of air temperature, humidity and rainfall reduced the shedding of oocysts in faeces (Sharma *et al* 2009). However, increasing trend recorded in this study may be due to housing of bucks, sorted by groups, together. As the temperature started decreasing, more and more bucks may come close and sit together in close extremity to each other during cooler parts of day, resulting in spread of coccidiosis and rise of OPG. Confirming to findings of this study, highest OPG in clinically healthy goats, at the Raeini Goats Research Center of Iran, with fall of air temperature during winter was recorded because the flock was being kept more compact (Radfar *et al* 2011). However, no seasonal fluctuation was observed in the prevalence of the coccidia in Saanen or crossbreed milch goats kept in the Czech Republic (Koudela and Boková 1998).

Table 13. Number of oocysts in 1 g of faeces in Beetal bucks of different groups

Day	T ₀	T _{oz}	T _{iz}
0	480.00 ± 165.53	200.00 ± 200.0	360.00 ± 215.87
15	120.00 ± 58.31	100.00 ± 44.72	480.00 ± 174.36
30	200.00 ± 154.92	500.00 ± 428.95	440.00 ± 150.33
45	900.00 ± 167.33	1160.00 ± 838.21	880.00 ± 195.96
60	1420.00 ± 476.86	800.00 ± 380.79	760.00 ± 376.30
75	1900.00 ± 613.19	780.00 ± 436.35	980.00 ± 451.00
90	1960.00 ± 672.76	1260.00 ± 574.98	1320.00 ± 519.04
105	4700.00 ± 1985.95 ^a	940.00 ± 386.78 ^b	880.00 ± 318.43 ^b
120	5500.00 ± 1535.58 ^a	1620.00 ± 514.20 ^b	1680.00 ± 449.89 ^b
Mean	1908.89 ± 388.83 ^a	817.78 ± 158.95 ^b	864.44 ± 120.02 ^b

Mean values bearing different superscripts within a row differ significantly (P<0.05)

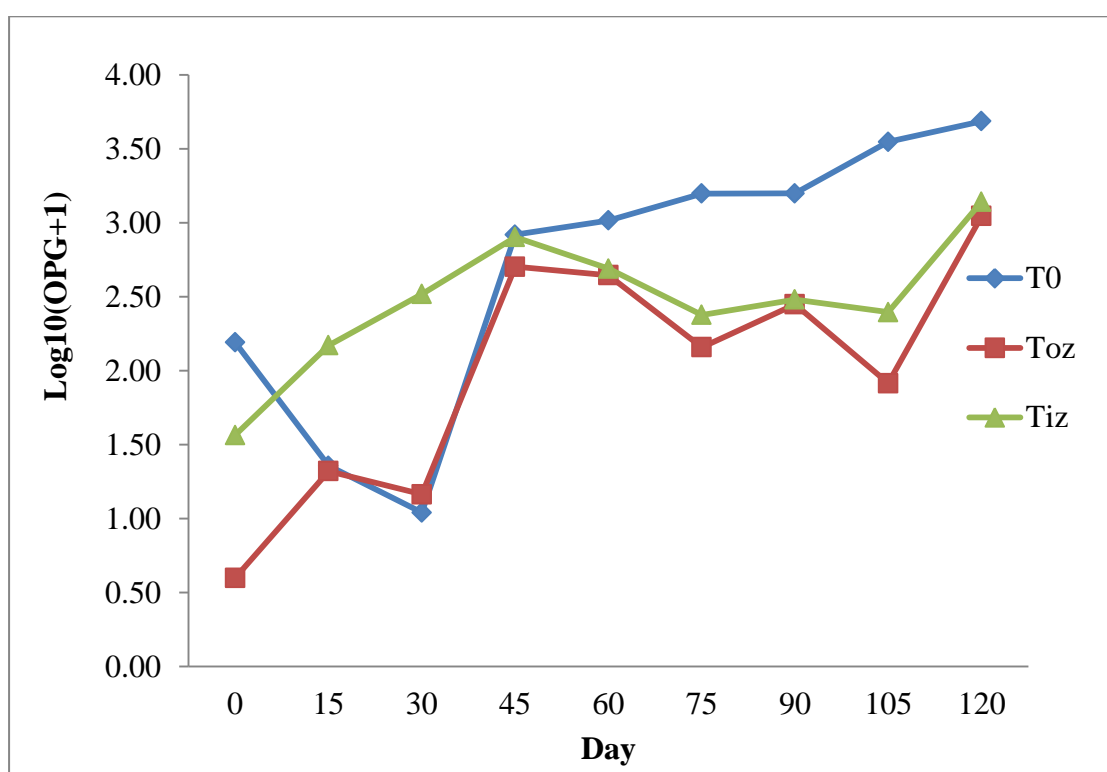


Fig. 8: Fortnightly mean number of OPG in faeces of Beetal bucks sorted by groups.

4.1.7.3 Blood Profile

4.1.7.3.1 Haematological profile

The blood haematological picture of bucks supplemented with different sources of Zn is presented in Table 14. There were no differences in HgB, RBCs, PCV, DLC and platelet count in the blood of bucks sorted under different groups. The variations were within normal physiological limits. The WBCs ($\times 10^9/L$) were reported higher ($P < 0.05$) in T_0 over T_{oz} and T_{iz} group. However, the mean WBC count was higher than normal recommended range in all the groups under study. Similarly, earlier researchers at the same farm had also reported higher WBC count and neutrophils (%) in blood of goats under stall-fed conditions (Chandrasah 2011) without any clinical symptom. The variations in blood parameters during the study period were also statistically non-significant. All the bucks were reared, starting from their birth, under similar standard management practices at the goat farm. Hence, similarity was observed in haematological parameters of bucks in T_0 , T_{oz} and T_{iz} , respectively without any statistically significant variations. Thus, indicating that supplementation of Zn had not produced any adverse effect on health of bucks under study. Similarly, Gupta (2014) observed no effect of organic (Zn-lysine) and inorganic Zn ($ZnSO_4$) containing mineral mixture on haematological profile in buffalo calves.

4.1.7.3.2 Biochemical profile and oxidative stress indicators

The biochemical profile and oxidative stress indicators of bucks supplemented with organic and inorganic Zn are presented in Table 14. Results indicated that the variations in total serum protein, albumin, globulin, SOD, GPx and catalase were statistically non-significant and within the normal physiological limits indicative of apparently healthy condition of animals throughout the study period in all the groups. Similarly, Gupta (2014) observed no effect of organic (Zn-lysine) and inorganic Zn ($ZnSO_4$) containing mineral mixture on blood biochemical profile in buffalo calves.

Table 14. Haematological, Biochemical and oxidative stress indicators (SI units) in blood of bucks under different groups

Parameters	T ₀	T _{oz}	T _{iz}
Hgb (× 10 g/L)	8.38 ± 0.14	8.36 ± 0.12	8.50 ± 0.22
WBCs (× 10 ⁹ /L)	17.55 ± 0.63 ^a	14.14 ± 0.50 ^b	15.47 ± 0.43 ^b
Neutrophils (%)	53.13 ± 1.51	49.07 ± 1.94	51.67 ± 1.45
Lymphocytes (%)	59.37 ± 1.46	57.30 ± 0.96	58.20 ± 1.07
Monocytes (%)	0	0	0
Eosinophils (%)	0.10	0.13	0.07
Basophils (%)	0	0	0
RBCs (× 10 ¹² /L)	16.15 ± 0.50	15.67 ± 0.39	14.99 ± 0.45
PCV (× 10 ⁻² L/L)	26.27 ± 0.62	26.43 ± 0.68	25.82 ± 0.72
Platelets (× 10 ⁹ /L)	382.97 ± 28.40	323.83 ± 28.54	366.63 ± 40.40
Protein (x 10 g/L)	6.86 ± 0.05 ^a	6.84 ± 0.07 ^a	6.66 ± 0.06 ^b
Albumin (x 10 g/L)	3.17 ± 0.06	3.26 ± 0.05	3.12 ± 0.09
Globulin (x 10 g/L)	3.69 ± 0.08	3.58 ± 0.10	3.55 ± 0.09
ALP (IU/L)	179.26 ± 21.46	201.81 ± 18.41	144.20 ± 18.75
SOD (EU/mg HgB)	2.52 ± 0.06	2.47 ± 0.05	2.46 ± 0.06
GPX (EU/mg HgB)	6.18 ± 0.07	6.14 ± 0.07	6.17 ± 0.07
Catalase (µmole H ₂ O ₂ decomposed/ min/ mg Hgb)	2407.03 ± 39.69	2287.02 ± 69.13	2350.61 ± 51.96

Mean values bearing different superscripts within a row differ significantly (P<0.05)

4.1.8 Economics of supplementing Zn to ration of bucks

Economic viability of adding organic and inorganic Zn to bucks sorted under different groups was assessed and presented in Table 15. One common basal feed containing 2% mineral mixture and 1% salt without any added Zn was formulated and prepared for all the bucks under study. For example a lot of 3 quintal basal feed was prepared as per standard practices. This basal feed was further divided into three equal parts of 100 kg each for feeding of 5 bucks for 40 days in T₀, T_{oz} and T_{iz}, respectively. Fresh Zn powder requirement was calculated to supplement Zn @ 30 mg/kg DM. Then Zn powder from organic and inorganic sources was added to basal feed for feeding in T_{oz} and T_{iz}, respectively. Economical calculations (Table 15) revealed that

monthly supplementation of organic and inorganic Zn to each buck costed only Rs. 6.00 and 0.39 in T_{oz} and T_{iz}, respectively over the control group. However, added Zn maintained healthy live body weight, improved semen quality and reduced parasitic load. Thus, it is economically viable to supplement Zn to bucks from various sources under stall-fed conditions.

Table 15. Economics of supplementing Zn to bucks sorted under different groups

Parameters	T ₀	T _{oz}	T _{iz}
Daily DMI from fodder by a group of 5 bucks	8.65	8.71	8.84
Daily DMI from concentrate by a group of 5 bucks	2.26	2.26	2.26
Daily total DMI by a group of 5 bucks	10.91	10.98	11.10
Daily total DMI per buck	2.18	2.20	2.22
Daily Zn requirement @ 30 mg/Kg DMI (mg)	No added Zn	66.00	66.6
Zn Percentage in source powder	-	15	20
Quantity of source powder containing daily required Zn in mg (on dry matter basis)	-	440.00	333.00
Fresh source powder (containing 10% moisture) added to meet daily requirement of a buck (mg)	-	488.89	370.00
Daily fresh source powder added per buck (g)	-	0.49	0.37
Purchase cost of source Zn powder per kg (Rs)	-	400	35
Daily cost of source Zn powder supplementation (Rs)	-	0.20	0.013
Monthly cost of source Zn powder supplementation (Rs)	-	6.00	0.39
Monthly cost of adding Zn per kg DMI (Rs)	-	2.67	0.17

4.2 EXPERIMENT II: To analyze the effect of organic and inorganic zinc supplementation and synchronization of ovulation on reproductive efficiency of Beetal does.

4.2.1 Meteorological attributes

The results pertaining to the monthly variations in environmental parameters during the study period of 8 months from Jan. to Aug., 2016 are presented in Table 16. The does reared under loose housing system were exposed to external environment of the open area. The mean daily air temperature, relative humidity, sunshine, rainfall and THI as per outside weather during the study period were recorded as 25.50 ± 0.50 °C, 62.73 ± 1.05 %, 7.02 ± 0.26 hours, 2.38 ± 0.57 mm and 73.18 ± 0.69 , respectively. The does under study experienced a high THI, particularly during the last five months of study. Though, goats can tolerate high THI, but associated with poor growth performance as well as adverse effects on physiological characteristics of the goats. Therefore, management strategies needed to improve goat production by minimizing stress in order to attain optimal animal comfort (Popoola *et al* 2014).

4.2.2 Daily feed fodder intake and proximate chemical analysis

The data on daily feed, fodder intake, their proximate chemical analysis and ingredients composition of concentrate feed fed during study period is presented in Table 17-19. The does sorted under different groups were reared together without any individual feeding. Therefore, concentrate and fodder consumption by does enrolled and placed under different treatment groups was recorded and is presented. The differences between groups in feed and fodder intake were statistically non-significant ($P < 0.05$). However, does fed added organic Zn consumed highest kg of fresh fodder, DM from fodder and total DM from feed and fodder followed by does fed Inorganic Zn over the does in control with no added Zn, indicating that supplementation of organic and inorganic Zn had not produced any adverse effects on appetite, ruminal micro-flora and digestibility of nutrients (DM, CP, ADF, and NDF). Similar results reported by earlier workers are explained in Experiment I. In agreement with findings of this study, it was reported in buffalo calves that daily DMI from fodder were quantitatively higher in the organic Zn supplemented group in comparison to inorganic Zn supplemented group (Gupta 2014).

Table 16. Daily variations of environmental variables in the specified months during the study period in does i.e. Jan. to Aug., 2016

		Jan.	Feb.	Mar.	Apr.	May	Jun.	Jul.	Aug.	Mean
Air Temp. (°C)	Max.	17.22 ±0.83 ^a	23.04 ±0.47 ^b	28.00 ±0.57 ^c	36.57 ±0.56 ^d	39.62 ±0.56 ^e	38.85 ±0.52 ^e	33.63 ±0.50 ^f	33.31 ±0.38 ^f	31.29 ±0.52
	Min.	7.44 ±0.39 ^a	8.99 ±0.58 ^b	14.59 ±0.32 ^c	19.59 ±0.44 ^d	24.63 ±0.53 ^e	28.49 ±0.25 ^f	27.34 ±0.38 ^g	26.10 ±0.25 ^h	19.70 ±0.52
	Mean	12.33 ±0.50 ^a	16.02 ±0.46 ^b	21.30 ±0.36 ^c	28.08 ±0.41 ^d	32.13 ±0.45 ^e	33.67 ±0.28 ^f	30.48 ±0.39 ^g	29.70 ±0.26 ^g	25.50 ±0.50
RH (%)	Mor.	94.81 ±0.56 ^a	91.55 ±1.35 ^{ab}	88.35 ±1.15 ^b	62.00 ±2.08 ^c	57.26 ±2.13 ^d	64.57 ±1.84 ^c	84.61 ±1.09 ^b	84.58 ±0.98 ^b	78.48 ±1.03
	Eve.	63.97 ±2.95 ^a	45.62 ±1.55 ^b	43.32 ±1.95 ^b	21.67 ±1.45 ^c	26.87 ±1.74 ^c	42.27 ±3.47 ^b	66.29 ±2.35 ^a	64.81 ±1.89 ^a	46.98 ±1.30
	Mean	79.39 ±1.48 ^a	68.59 ±1.09 ^b	65.84 ±1.28 ^b	41.83 ±1.53 ^c	42.07 ±1.66 ^c	53.42 ±2.46 ^d	75.45 ±1.43 ^{ae}	74.69 ±1.17 ^e	62.73 ±1.05
THI	Mean	54.47 ±0.85 ^a	60.36 ±0.69 ^b	67.92 ±0.46 ^c	74.63 ±0.48 ^d	79.57 ±0.49 ^e	83.60 ±0.34 ^f	82.84 ±0.46 ^{fg}	81.58 ±0.30 ^g	73.18 ±0.69
Wind speed (km/h)	Mean	2.30 ±0.25 ^a	3.07 ±0.26 ^{ab}	3.66 ±0.40 ^b	3.80 ±0.35 ^b	6.02 ±0.62 ^c	6.44 ±0.54 ^c	3.93 ±0.46 ^b	2.94 ±0.41 ^{ab}	4.02 ±0.17
Sunshine (hrs.)	Mean	2.35 ±0.54 ^a	7.73 ±0.48 ^b	8.20 ±0.61 ^{bc}	9.73 ±0.57 ^c	9.08 ±0.68 ^{bc}	7.77 ±0.63 ^b	5.82 ±0.72 ^d	5.66 ±0.72 ^d	7.02 ±0.26
Rainfall (mm)	Mean	0.63 ±0.52 ^a	0.30 ±0.26 ^a	1.33 ±0.58 ^a	0.10 ±0.06 ^a	0.81 ±0.41 ^a	2.87 ±2.02 ^a	9.85 ±3.47 ^b	3.02 ±1.21 ^a	2.38 ±0.57
Rainy days	Total	3	3	8	3	8	6	13	10	6.75
Evaporation (mm)	Mean	1.05 ±0.08 ^a	2.50 ±0.11 ^b	4.01 ±0.20 ^c	8.35 ±0.32 ^d	9.89 ±0.35 ^e	9.32 ±0.49 ^e	4.88 ±0.25 ^c	4.20 ±0.24 ^c	5.51 ±0.22
Evaporation (mm)	Total	32.60	72.50	124.20	250.40	124.20	270.30	146.30	130.20	143.84

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

Table 17. Daily DM consumption (kg/day) by the does sorted under different groups

Parameters	T ₀	T _{oz}	T _{iz}
Fresh fodders intake	80.24 ± 3.43	80.81 ± 3.41	80.51 ± 3.42
DMI from fodder	14.45 ± 0.23	14.59 ± 0.25	14.52 ± 0.24
DMI from concentrate	4.54	4.54	4.54
Total DMI	19.00 ± 0.23	19.14 ± 0.25	19.06 ± 0.24
Total DMI/kg metabolic live weight (LW ^{0.75} , g/d)	211.59 ± 6.85	207.92 ± 7.40	204.01 ± 6.57

Table 18. Chemical composition (%) of offered fodder and concentrate to does (on DM basis) under study

	Fodder	Concentrate
DM	20.61	90.82
OM	88.64	94.01
Ash	11.36	5.99
CP	14.31	15.43
EE	1.73	2.57
NDF	66.28	27.30
ADF	40.62	14.39
Cellulose	30.29	7.62
Lignin	6.38	1.95

Table 19. Composition of concentrate feed (per 100 kg)

Ingredients	Proportion (%)
Maize	47
De-oiled rice bran	30
Soybean meal	20
Mineral mixture	2
Salt	1

4.2.3 Live body weight (LBW)

The data recorded on monthly variations in live body weight (kg) of Beetal does is presented in Table 20 and Fig. 9. The monthly variations in LBW depicted an increasing trend in all the groups, which may be due to advancing of pregnancies in all the does. However, the differences between groups during period were statistically non-significant. Statistically non-significant variations in LBW could be attributed to the fact that no clinical symptom of any disease was observed in any doe under study. Likewise, Gupta (2014) revealed that supplementation of organic and inorganic Zn in ration caused similar variations in LBW of male and female buffalo calves.

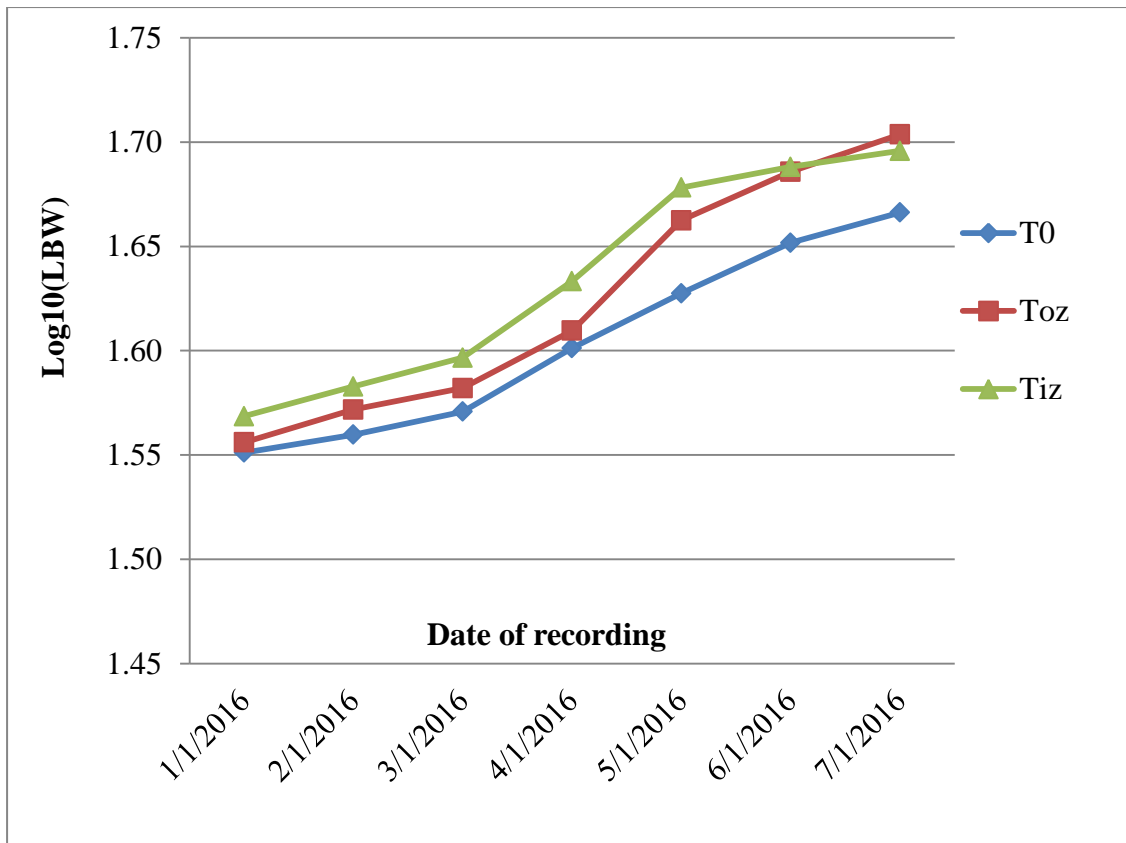


Fig. 9: Monthly variations in live body weights (kg) of Beetal does sorted by groups

Table 20. Monthly variations in live body weights (kg) of Beetal does sorted by groups

Date	T ₀	T _{oz}	T _{iz}
01/01/2016	37.35 ± 3.73	36.74 ± 2.30	37.43 ± 1.78
01/02/2016	37.74 ± 3.43	38.11 ± 2.43	38.68 ± 1.85
01/03/2016	38.60 ± 3.39	39.04 ± 2.52	39.93 ± 1.90
01/04/2016	41.05 ± 3.19	41.54 ± 2.62	43.47 ± 2.11
01/05/2016	43.49 ± 3.21	46.77 ± 2.71	48.21 ± 2.43
01/06/2016	45.89 ± 3.22	49.54 ± 3.20	49.37 ± 2.64
01/07/2016	47.41 ± 3.30	51.63 ± 3.39	50.46 ± 3.10
Mean	41.65 ± 1.29	43.34 ± 1.20	43.94 ± 1.03

4.2.4 Body condition score (BCS), Lumbar thickness (LT) and Sternal Thickness (ST) of does

Data recorded on variations in BCS, LT and ST of does distributed under different groups on the basis of Zn supplementation is presented in Table 21. Results revealed that BCS of does keep on increasing after conception till kidding and then declined during post kidding period in all the groups under study. However, treatment groups supplemented with added Zn maintained significantly ($P < 0.05$) higher mean BCS over control group towards marginally positive side. The mean BCS was highest in T_{oz} followed by T_{iz} , though the variations between T_{oz} and T_{iz} were not significant. Therefore, it is concluded that supplementation of Zn helped to maintain better condition during the physiological reproductive cycle. Moreover, it is also earlier documented that healthier conditioned does have longer and better reproductive activity than lesser conditioned does (Sharma 2016). In consonance with the findings of this study, it was also earlier reported in Beetal Does that BCS tended to increase during pregnancy and decrease during early lactation period (Sharma 2016). This could be attributed to negative energy balance due to milk production, kidding performance and lower DMI during early lactation.

BCS recording using visual and palpation technique was correlated with physical measurements of lumbar and sternal regions as thickness of these regions gives indication of the condition and body reserves of the animal. Similar trend was observed for variations in LT between groups. Mean LT was recorded highest in T_{iz} followed by T_{oz} over T_0 group. Though, the variations were statistically non-significant between groups. Almost likewise, sternal thicknesses increased after conception till fourth month of gestation and thereafter keep on declining till post kidding recording of observation. The variations were statistically non-significant between groups. Mean ST was recorded significantly ($P < 0.05$) higher in treatment groups T_{oz} and T_{iz} over the control group T_0 . The variations between treatment groups were not significant. It is documented earlier in Beetal goats that BCS had better correlation with physical measurements using Vernier caliper than ultrasonographic measurements in lumbar and sternal regions. This information can be very useful for an unskilled farmer for estimating the condition of his animal (Sharma 2016).

Table 21. Body condition score, lumbar and sternal region thickness (cm) of Beetal does sorted by groups

Particulars	T ₀	T _{oz}	T _{iz}
BCS at day 0	2.55 ± 0.14	2.85 ± 0.10	2.70 ± 0.11
BCS at time of breeding	2.55 ± 0.14	2.85 ± 0.10	2.70 ± 0.11
BCS after one month of breeding	2.55 ± 0.14	2.85 ± 0.10	2.70 ± 0.11
BCS after two month of breeding	2.65 ± 0.15	2.88 ± 0.11	2.78 ± 0.11
BCS after three month of breeding	2.68 ± 0.15	2.95 ± 0.09	2.95 ± 0.12
BCS after four month of breeding	2.83 ± 0.11	2.95 ± 0.09	2.95 ± 0.11
BCS before kidding	2.83 ± 0.13	3.03 ± 0.09	3.03 ± 0.21
BCS after a week of kidding	2.28 ± 0.18	2.19 ± 0.12	2.28 ± 0.15
Mean BCS	2.62 ± 0.05 ^a	2.83 ± 0.04 ^b	2.76 ± 0.05 ^b
LT at day 0	2.28 ± 0.19	2.35 ± 0.16	2.41 ± 0.16
LT at time of breeding	2.28 ± 0.19	2.35 ± 0.16	2.41 ± 0.16
LT after one month of breeding	2.28 ± 0.19	2.35 ± 0.16	2.41 ± 0.16
LT after two month of breeding	2.34 ± 0.17	2.46 ± 0.18	2.41 ± 0.16
LT after three month of breeding	2.38 ± 0.22	2.79 ± 0.20	2.64 ± 0.18
LT after four month of breeding	2.41 ± 0.19	2.86 ± 0.21	3.05 ± 0.26
LT before kidding	2.27 ± 0.22	2.35 ± 0.21	2.57 ± 0.24
LT after a week of kidding	1.76 ± 0.27	1.48 ± 0.13	1.64 ± 0.17
Mean LT	2.25 ± 0.07	2.40 ± 0.07	2.45 ± 0.08
ST at day 0	2.37 ± 0.16	2.64 ± 0.12	2.67 ± 0.19
ST at time of breeding	2.37 ± 0.16	2.64 ± 0.12	2.67 ± 0.19
ST after one month of breeding	2.37 ± 0.16	2.64 ± 0.12	2.67 ± 0.19
ST after two month of breeding	2.42 ± 0.15	2.64 ± 0.12	2.67 ± 0.19
ST after three month of breeding	2.59 ± 0.14	2.84 ± 0.13	2.75 ± 0.20
ST after four month of breeding	2.70 ± 0.13	2.89 ± 0.12	3.00 ± 0.19
ST before kidding	2.57 ± 0.08	2.63 ± 0.23	2.77 ± 0.19
ST after a week of kidding	1.76 ± 0.20	1.89 ± 0.17	2.09 ± 0.19
Mean ST	2.40 ± 0.06 ^a	2.62 ± 0.06 ^b	2.67 ± 0.07 ^b

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

4.2.5 Plasma zinc

Effects of dietary Zn supplementation on plasma Zn concentration of Beetal does are shown in Table 22. Variations in plasma level demonstrated that supplementation of added Zn for the first two months resulted in rise of plasma Zn by 20.0 % and 13.04 % in T_{oz} and T_{iz} against the control, where the rise was 5.56% only. Under optimum conditions of Zn intake, supplementation of added Zn is not supposed to increase the plasma Zn due to homeostatic mechanisms (Jia *et al* 2008). Increase in plasma Zn of does under T_{oz} and T_{iz} in response to supplemental Zn indicate that the animals were deficient in Zn intake fed on basal diet. However, marginal variations in T₀ could not be explained. However, Pechova *et al* (2006) found no effect of chelated Zn supplementation on blood Zn levels. Similarly, Mandal *et al* (2008) reported non-significant variations in serum zinc and copper levels after supplementation of organic and inorganic sources. However, in concurrence with findings of this study, Chhabra *et al* (2013) reported that oral zinc supplementation from organic sources resulted in non-significant increase of Zn plasma levels up to day 60 along with clinical improvement in Zn deficient buffaloes. Thereafter, it had decreased in all the groups owing to growing foetus, lactation after kidding and reducing fodder Zn concentration. Supplementation of organic Zn resulted in significantly (P<0.05) improved mean plasma Zn level over the control. However, feeding of added inorganic Zn numerically improved the mean plasma Zn values. Similarly, Shinde *et al* (2013) observed higher (P<0.05) mineral concentrations in blood of sheep fed Cu and Zn methionine than Cu and Zn sulphates suggesting higher bioavailability of minerals from organic chelated sources.

Table 22. Bimonthly variations in plasma zinc (mg/L) of Beetal does sorted by groups

Day	T ₀	T _{oz}	T _{iz}
Day 0	1.26 ± 0.14	1.95 ± 0.48	1.38 ± 0.14
Day 60	1.33 ± 0.15	2.34 ± 0.79	1.56 ± 0.13
Day 120	1.28 ± 0.09	1.74 ± 0.24	1.49 ± 0.14
After a week of kidding	1.09 ± 0.13	1.40 ± 0.27	1.14 ± 0.13
Mean	1.24 ± 0.06 ^a	1.86 ± 0.24 ^b	1.39 ± 0.07 ^{ab}

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

4.2.6 Reproductive performance of does

The effect of supplementation of Zn and synchronization of ovulation with intra-vaginal progesterone sponges on reproductive performance indicators are presented in Table 23. Supplementing with different sources of an iso-mineral formula during study period resulted in several desirable differences between groups. Therefore, Zn supplementation had a positive effect on reproductive performance traits of Beetal does compared with does receiving only basal diet. The conception rate did not differ amongst does sorted under different groups. However, Uchida *et al* (2001) reported that feeding combination of these minerals (Zn, Mn, Cu and Co) improved conception rate in high yielding dairy cows.

The mean number of days from start of buck parading to breeding was comparable amongst does, indicating the strong effect of buck on induction of oestrous. All the artificially synchronized does irrespective of group, responded to progesterone sponges and exhibited standing oestrous at fixed time after removal of sponges. In total, all the 30 does become pregnant following one service representing 100 percent conception rate in T_0 , T_{oz} and T_{iz} , respectively supporting the fact that goats are highly prolific specie compared with other large ruminants (cow, buffalo) and male effect is more pronounced in the onset of breeding season and synchronizing the does. Therefore, it is concluded that addition supplementation and synchronization protocol did not affected days to first service after start of parading, conception and fertility rate. Similarly, inorganic and organic forms of Zn, Mn, Cu, and Se trace mineral supplementation to Holstein cows did not affect days to first service, days to first estrus, services per conception and conception rate (Karkoodi *et al* 2012). However, it was reported in Teresa goat that supplementation of basal diet with 50 and 100 ppm zinc oxide significantly increased the incidence of oestrus (33%), pregnancy rate (12%), kidding rate (5%) and resulted in shorter onset (8 days) of oestrus as compared to the control (Kundu *et al* 2014).

The prolificacy (%), fecundity (%), litter size/doe, number of kids born were significantly ($P < 0.05$) higher in does supplemented with organic Zn followed by does with inorganic Zn than the control group (Table-23). Litter size/ doe at birth, number of live kids born were improved in groups fed basal diet containing either organic or inorganic Zn by 56.25 and 23.61%; 38.46 and 23.08% over control diet containing no added Zn, respectively. Does with multiple births were 4, 7 and 6 in T_0 , T_{oz} and T_{iz} , respectively.

Table 23. Reproductive performance of does under different groups supplemented with different sources of Zn

Particulars		T ₀	T _{oz}	T _{iz}	Mean
DFS (days)		10.80 ± 3.46	12.40 ± 4.43	10.40 ± 2.48	11.2 ± 1.91
Conception (%)	SYN	100	100	100	100
	NOV	100	100	100	100
	Mean	100	100	100	100
Fertility (%)	SYN	80	80	80	80.00
	NOV	100	80	100	93.33
	Mean	90	80	90	86.67
Fertility (%) after excluding mortality and abortion	SYN	100	100	100	100
	NOV	100	100	100	100
	Mean	100	100	100	100
Prolificacy (%)	SYN	150.00	275.00	175.00	200.00
	NOV	140.00	175.00	180.00	164.29
	Mean	144.44	225.00	177.78	180.77
Fecundity (%)	SYN	120	220	140	160.00
	NOV	140	140	180	153.33
	Mean	130	180	160	156.67
Number of live kids born	SYN	6	11	7	24
	NOV	7	7	9	23
	Total	13	18	16	47
Number of does kidded	SYN	4	4	4	12
	NOV	5	4	5	14
	Total	9	8	9	26
Number of kids /doe kidded	SYN	1.50±0.29 ^a	2.75±0.25 ^{bx}	1.75±0.48 ^a	2.00±0.25
	NOV	1.40±0.25	1.75±0.25 ^y	1.80±0.20	1.64±0.13
	Mean	1.44 ± 0.18 ^a	2.25 ± 0.25 ^b	1.78 ± 0.22 ^{ab}	1.81 ± 0.14
Number of kids born/doe under treatment	SYN	1.2	2.2	1.4	1.6
	NOV	1.4	1.4	1.8	1.53
	Mean	1.3	1.8	1.6	1.57

Particulars		T ₀	T _{oz}	T _{iz}	Mean
Number of does yielding twins	SYN	2	1	1	4
	NOV	2	3	4	9
	Total	4	4	5	13
Number of does yielding triplets	SYN	0	3	1	4
	NOV	0	0	0	0
	Total	0	3	1	4
Gestation period (days)	SYN	151.75±1.84	148.00±1.87	150.75±3.20	150.17±1.34
	NOV	146.80±1.66	144.50±1.55	145.00±2.17	145.50±1.03
	Mean	149.00±1.44	146.25±1.31	147.56±2.01	147.65±0.94
Male : female kid born	SYN	1:5	7:4	2:5	10:14
	NOV	4:3	5:2	6:3	15:8
	Mean	5:8	12:6	8:8	25:22
Average kid's birth weight	SYN	2.96±0.22	2.50±0.14	2.97±0.11	2.75±0.17
	NOV	2.75±0.21	2.41±0.34	2.72±0.31	2.63±0.10
	Mean	2.85 ± 0.15	2.46 ± 0.15	2.83 ± 0.18	2.69 ± 0.10
% loss of LBW within a week of kidding	SYN	7.06±1.74	4.64±2.17	6.16±1.77	5.96±1.04
	NOV	3.55±1.07	6.82±2.63	6.65±1.27	5.59±0.97
	Mean	5.11 ± 1.10	5.73 ± 1.63	6.43 ± 0.99	5.76 ± 0.69

DFS: Days to first service after start of buck parading (excluding artificially synchronised does as synchronised does were bred at fixed time after removal of PRID)

SYN: Does synchronized, NOV: Does subjected to natural ovulation

^{ab}Mean values bearing different superscripts within a row differ significantly (P<0.05)

^{xy}Mean values bearing different superscripts within a column differ significantly (P<0.05)

The average birth weight did not statistically differed in groups. However, average birth weight was numerically lowest in T_{oz} compared with T₀ and T_{iz}, respectively. Likewise in Teresa goat, supplementation of basal diet with 50 and 100 ppm zinc oxide had resulted in improved reproductive performance, was reported (Kundu *et al* 2014). In the same way, Habeeb *et al* (2013) observed that supplementation of doe's diet with 30 and 80 ppm Zn improved litter size at birth, number of kids per doe and litter weight at birth along with increase in number of

does kidding triple and twins. Synchronization of does numerically improved prolificacy and fecundity. The synchronized does supplemented with organic zinc yielded significantly ($P < 0.05$) higher number of kids per doe over the other groups. Similarly, Kusina *et al* (1999) observed in does that different methods were effective in synchronizing oestrus with no variation in fertility, prolificacy and fecundity.

The variations in percent loss of LBW after a week of kidding were statistically non-significant. However, percent loss in LBW was numerically higher in treatment group, which can be correlated with higher prolificacy (%) and fecundity (%) over the control group. On the contrary, Chandrahas *et al* (2014) reported that the post kidding live body weight loss of Beetal dams fed organic Zn-lysine and Cu-lysine were 6.26% lower than dam's supplemented with sulphate salt of Zn and copper. However, the variations were non-significant in the findings of Chandrahas *et al* (2014) and our study.

The variations in oestrous response of does to Zn supplementation is presented in Table 24. The variations recorded in oestrous behaviour between groups were statistically non-significant. However, supplementation of organic and inorganic Zn resulted in numerical increase of standing heat duration over the does with no added Zn. It was also observed that synchronization protocol with progesterone sponges had also increased duration and intensity of oestrus in does against non-synchronized does. It was also experienced that it was difficult to detect the oestrous with conspicuous signs in does without the presence of male. All the synchronized does started revealing various signs of estrus after 36 hours of removal of intra-vaginal sponges without standing heat. However, standing heat was exhibited after 48 hours of sponge removal.

Table 24. Variations in estrous response of does sorted under different groups supplemented with different sources of Zn

Particulars	T ₀	T _{oz}	T _{iz}
Duration of standing estrous (hrs.)	8.40 ± 0.98	9.60 ± 0.98	9.60 ± 0.98
Duration of standing estrous in artificially synchronized does	10.80 ± 1.20	12.00 ± 0.00	10.80 ± 1.20
Duration of standing estrous in non-synchronized does	6.00 ± 0.00	7.20 ± 1.20	8.40 ± 1.47

4.2.7 Effect of Zn supplementation on synchronization of does

The success of synchronization protocols for fixed time breeding or timed embryo transfer depends on various factors such as breed, body condition scores and nutrition (including trace mineral status). In the present study, does were supplemented with organic and inorganic Zn, against control with no added Zn, for one month prior to start of synchronization with intra-vaginal progesterone sponges. Out of 5 does each assigned for synchronization in T₀, T_{oz} and T_{iz}, respectively, all the goats responded to protocol (Table 25) indicating that 100 % of treated does had been successfully synchronized irrespective of group. Further, no statistical difference between plasma progesterone levels of successfully synchronized does in T₀, T_{oz} and T_{iz}, respectively was observed (Table 25). Therefore, the hypothesis that added Zn supplementation would increase the number of synchronized does with progesterone releasing intra-vaginal device protocol was not confirmed. In accordance with the findings of this study, subcutaneous injections of trace minerals (ITM-100 mg zinc, 100 mg manganese, 50 mg copper and 25 mg selenium) to crossbred heifers 17 days prior to embryo transfer, did not increase the number of heifers successfully synchronized compared to non-injected controls (ITM =82.1% (308/375) versus control=83.1% (375/451); P=0.76, Sales *et al* 2011).

Table 25. Variations in plasma progesterone (Mean ± SE) values of does sorted under different groups

	Day 0	Day 7	Day 14	48 hrs	72 hrs	Day 20 after mating
T ₀	1.52 ±0.44	5.27 ±0.30	3.80 ±0.21	0.28 ±0.02	0.40 ±0.06	4.29 ±0.33
T _{oz}	2.19 ±0.17	4.56 ±0.25	3.42 ±0.10	0.23 ±0.01	0.36 ±0.02	4.50 ±0.31
T _{iz}	2.33 ±0.33	5.10 ±0.29	3.19 ±0.14	0.20 ±0.03	0.37 ±0.04	3.97 ±0.51

4.2.8 Health status of does

The does under study were examined daily in the morning and evening for any variation in routine behaviour. In addition to this, the physiological responses of the animals were also recorded randomly. The physiological responses in terms of rectal temperature, respiration rate and pulse rate were within normal physiological limits. One goat under control group aborted after completing 124 days of gestation. In addition, two goats in T_{oz} and one goat in T_{iz} died in the fifth month of gestation. Post

findings revealed no abnormality and enteritis. Except these, no other case of severe lameness, morbidity or mortality was observed during the study period indicating no harmful effect of Zn supplementation on does. It was earlier reported that supplementation of amino acid trace minerals (AATM) had a role reduction in incidence and reoccurrence of diseases along with significant improvement in primary and secondary humoral, and cell-mediated immunity (George *et al* 1997). However, on the contrary, Spears and Kegley (2002) observed no improvement in cell mediated or humoral immune response in beef cows supplemented with additional Zn from organic and inorganic sources.

Hooves of does were examined and trimmed regularly as per routine management practice. Therefore, overall mean lameness score was recorded as 1.09 ± 0.03 , 1.10 ± 0.04 and 1.09 ± 0.03 for the does in T₀, T_{oz}, and T_{iz}, respectively indicating no effect of added Zn supplementation on the lameness of does during gestation period. Similarly, Karkoodi *et al* (2012) found that Sources of trace mineral supplements did not affect hoof status and thus lameness score index did not significantly differ among the animals (1.71 ± 0.29 , 1.50 ± 0.27 , and 1.57 ± 0.37 for control, 25%, and 50% organic supplement groups, respectively; P=0.88). Similar non-significant effect of mineral supplementation was also reported by Cope *et al* (2009) and Uchida *et al* (2001).

The effect of organic and inorganic zinc supplementation on the faecal parasitic load of Beetal does under stall feeding rearing system were also studied and is presented under the following subheads.

4.2.8.1 Parasitic eggs shed per gram of faeces (PEG)

Fortnightly variations in number of parasitic eggs shed per gram (PEG) of faeces shown in Table 26 and Fig. 6. The data recorded revealed a declining trend in shedding of parasitic eggs in faeces naturally infected does grouped under T₀, T_{oz} and T_{iz}, respectively. The percent change between maximum and minimum PEG of does was recorded to be 48.84 (day 60 and 105), 47.89 (day 60 and 120) and 58.11 (day 30 and 120) in T₀, T_{oz} and T_{iz}, respectively. Overall mean number of parasitic eggs shed in 1 g of faeces were found numerically (P>0.05) lower in Beetal does supplemented with added inorganic or organic Zn over the does with no added Zn. However, variations in PEG between groups were statistically non-significant. Likewise,

Schafer *et al* (2015) observed non-significant variations in PEG after administration of Zn and Cu in lambs infected with *H. contortus*.

Table 26. Number of parasitic eggs shed in 1 g (PEG) of faeces in Beetal does sorted by supplementation of different sources of Zn

Day	T ₀	T _{oz}	T _{iz}
0	750.00 ± 140.83	816.67 ± 175.91	1066.67 ± 287.13
15	1133.33 ± 252.54	1016.67 ± 212.00	816.67 ± 268.85
30	1333.33 ± 420.85	1166.67 ± 274.06	1233.33 ± 299.63
45	1216.67 ± 363.70	1150.00 ± 256.58	1033.33 ± 312.69
60	1433.33 ± 440.96	1183.33 ± 329.06	1166.67 ± 320.07
75	1050.00 ± 323.27	1083.33 ± 338.05	966.67 ± 252.54
90	966.67 ± 272.85	750.00 ± 166.83	883.33 ± 305.96
105	733.33 ± 166.67	683.33 ± 132.71	716.67 ± 183.33
120	750.00 ± 138.44	616.67 ± 147.01	516.67 ± 132.71
Mean	1040.74 ± 98.36	940.74 ± 78.44	933.33 ± 87.80

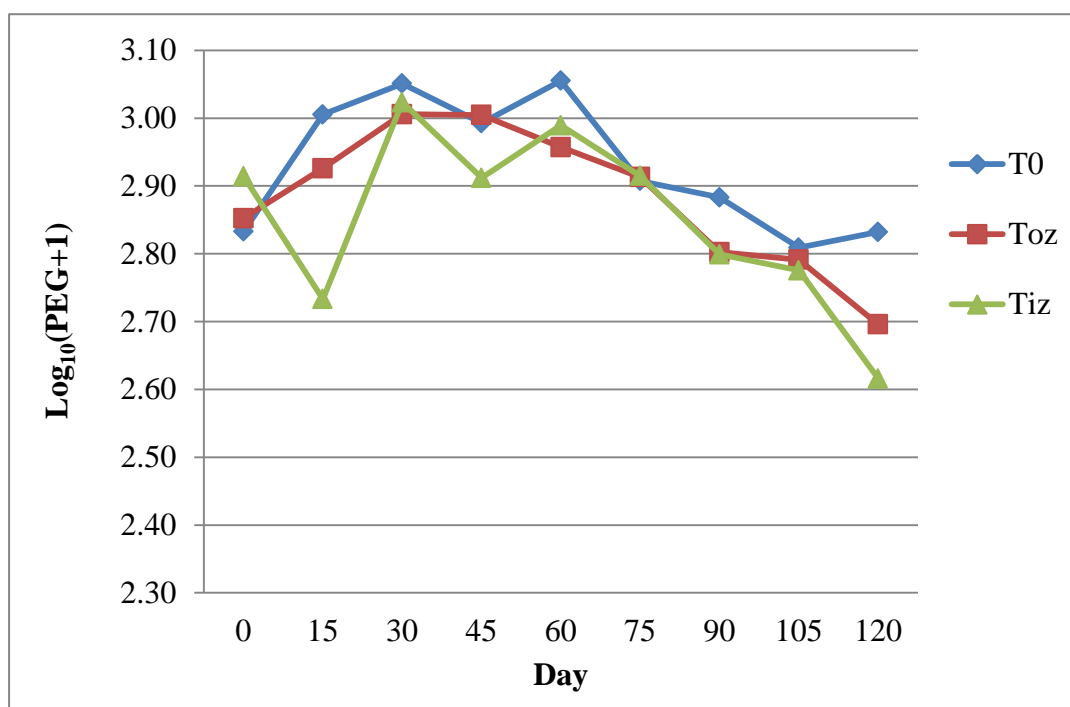


Fig. 10: Fortnightly mean number of PEG in faeces of Beetal does sorted by groups

Similarly, Roy *et al* (2013) reported that daily supplementation of Murrah buffalo with 100 g mineral mixture containing 0.75% Zn sulphate salt decreased the parasitic eggs per g of faeces significantly from 183.33 ± 10.54 to 16.67 ± 10.54 in 28 days. Mixed infections of *Strongyle* with *Amphistomes*, *Schistosoma* and *Trichuris* were observed in does. Similarly, mixed subclinical parasitic infection was reported earlier in sheep and goat population of Ludhiana (Singla 1995). It was also observed that shedding of parasitic eggs in faeces were not associated with any clinical symptom i.e. diarrhoea etc.

4.2.8.2 Oocysts shed per gram of faeces (OPG):

The variations in OPG of Beetal does in T_0 , T_{oz} and T_{iz} , respectively are presented in Table 27 and Fig. 11. Mean numbers of oocysts shed per gram of faeces of naturally coccidia infected does of individual groups during the whole experiment were transformed logarithmically and are presented in Fig. 11. *Eimeria* oocysts were recovered in 85.19, 75.93 and 83.33 percent of faecal samples of Beetal does examined in T_0 , T_{oz} and T_{iz} , respectively. Maximum OPG count in a specimen recorded was 1800, 2100 and 1900 in T_0 , T_{oz} and T_{iz} , respectively. Irrespective of groups, a total of 81.48 percent samples having variable intensity of OPG (100-2100) were found positive for *Eimeria* oocysts. High positive specimen percentages were consistent with findings elsewhere (Singla 1995 and Faizal and Rajapakse 2001).

The mean OPG in groups T_0 , T_{oz} and T_{iz} , respectively was recorded towards higher side during first and second month of study; however, it started decreasing towards lowest side in the third and fourth month of study. The percent decline between maximum and minimum mean OPG was 94.74 (day 15 and 120), 98.18 (day 30 and 120) and 96.05 (day 45 and 120) in T_0 , T_{oz} and T_{iz} , respectively. The overall OPG was significantly ($P < 0.05$) lowest in does supplemented with organic Zn over the does supplemented with in inorganic Zn and no added source of Zn. Does under control (T_0) with no added Zn supplementation shed the highest mean oocysts per gram of faeces (40.50%). On the contrary, does from chelated Zn group (T_{oz}) shed the least number of oocysts (25.33 %) followed by does from inorganic Zn (T_{iz}) group (34.17 %). Here it is important to report that shedding of oocysts was not accompanied by clinical symptoms of coccidiosis (diarrhoea) in any group.

Table 27. Number of oocysts in 1 g of faeces in Beetal does of different groups

Day	T ₀	T _{oz}	T _{iz}
0	766.67 ± 192.64	783.33 ± 235.82	400.00 ± 131.66
15	1266.67 ± 160.56	900.00 ± 284.02	816.67 ± 110.81
30	1250.00 ± 125.83	916.67 ± 174.01	1100.00 ± 255.60
45	1000.00 ± 136.63 ^a	383.33 ± 183.33 ^b	1266.67 ± 108.53 ^a
60	1050.00 ± 210.95	616.67 ± 205.62	950.00 ± 152.21
75	900.00 ± 180.74 ^a	366.67 ± 102.20 ^b	800.00 ± 73.03 ^a
90	316.67 ± 90.98	183.33 ± 74.91	216.67 ± 83.33
105	100.00 ± 51.64	33.33 ± 21.08	66.67 ± 33.33
120	66.67 ± 42.16	16.67 ± 16.67	50.00 ± 34.16
Mean	746.30 ± 75.32 ^a	466.67 ± 69.39 ^b	629.63 ± 70.82 ^a

Mean values bearing different superscripts within a row differ significantly (P<0.05)

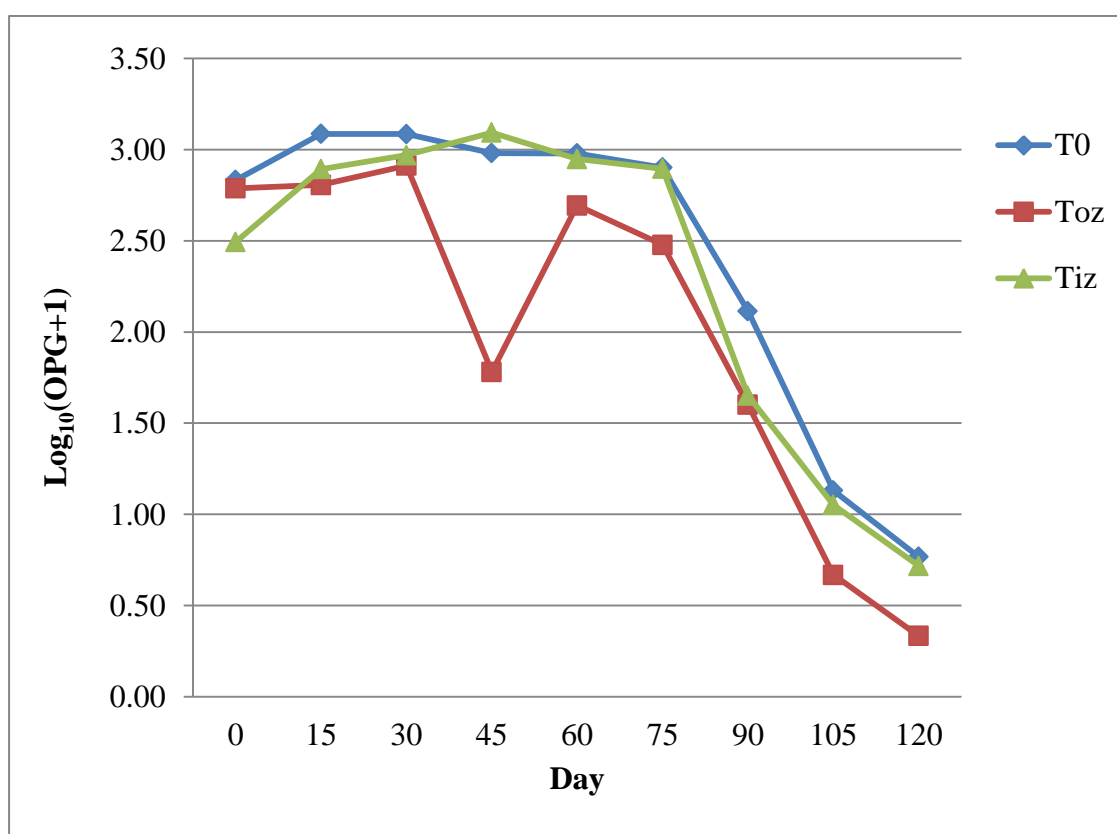


Fig. 11: Fortnightly mean number of OPG in faeces of Beetal does under different groups.

Therefore, it may be concluded from the findings that the health of does was improved by reducing the subclinical *Eimeria* infestation significantly through supplementation of organic Zn and numerically through added inorganic Zn.

4.2.8.3 Blood Profile

4.2.8.3.1 Haematological profile

Blood haematological picture of does supplemented with different sources of Zn is presented in Table 28. The variations in HgB, PCV and platelets were statistically non-significant. The WBC count was recorded significantly higher in T₀ and T_{iz} over T_{oz}. The blood RBCs were significantly higher in T₀ and T_{oz} over T_{iz} group. The reason for these results in WBC and RBC could not be explained. Moreover, the variations were within physiological limits. The overall WBC and neutrophil count was found towards higher side without any clinical manifestation in does in all the groups under study, which is in consonance with the findings of earlier workers at the same farm (Chandrasahs 2011). Non-significant variation in haematological parameters of does during the study period indicated that supplementation of Zn had not produced any adverse effect on health of does under study. Likewise, feeding of different sources of Zn had produced non-significant variations in haematological profile of calves (Gupta 2014). As all the does at farm right from their birth were reared under similar standard management practices. Hence, there was similarity observed in haematological profile of does in T₀, T_{oz} and T_{iz}, respectively. Similar observations were recorded by Cope *et al* (2009) in HF cows. However, Sharma and Joshi (2005) recorded significant (P<0.01) increase in blood HgB (7.47 ± 0.38 to 8.22 ± 0.42 g/dl), TEC (4.02 ± 0.42 to $4.52 \pm 0.41 \times 10^6/\mu\text{l}$) after 7 days and TLC (5.84 ± 0.49 to $6.56 \pm 0.29 \times 10^3/\mu\text{l}$) after 14 days of supplementing Zinc sulphate enriched mineral mixture to Zn deficient lactating cows. In addition the values of HgB, TEC after 21 days and TLC reached the standard after 28 days of feeding Zn-sulphate, while, no improvement was observed in untreated cows.

4.2.8.3.2 Biochemical profile and oxidative stress indicators

The biochemical profile and oxidative stress indicators of does supplemented with organic and inorganic Zn are presented in Table 27. Results indicated that the variations in total serum protein, albumin and globulin were statistically non-significant and within the normal physiological limits indicative of apparently healthy condition of animals throughout the study period in all the groups.

Table 28. Haematological, Biochemical and oxidative stress indicators (SI units) in blood of does supplemented with different sources of Zn

Parameters	T ₀	T _{oz}	T _{iz}
Hgb (× 10 g/L)	8.06 ± 0.19	8.47 ± 0.18	8.57 ± 0.17
WBCs (× 10 ⁹ /L)	14.40 ± 0.40 ^a	12.80 ± 0.41 ^b	14.40 ± 0.40 ^a
Neutrophils (%)	44.14 ± 2.25	42.31 ± 2.14	42.69 ± 2.24
Lymphocytes (%)	56.52 ± 2.30	55.55 ± 2.13	55.29 ± 1.98
Monocytes (%)	0	0	0
Eosinophils (%)	0	0.05	0.10
Basophils (%)	0	0	0
RBCs (× 10 ¹² /L)	14.56 ± 0.31 ^a	14.31 ± 0.42 ^a	13.29 ± 0.43 ^b
PCV (× 10 ⁻² L/L)	25.55 ± 0.51	25.74 ± 0.72	25.27 ± 0.61
Platelets (× 10 ⁹ /L)	327.07 ± 12.01	339.24 ± 14.06	331.93 ± 16.90
Protein (x 10 g/L)	7.88 ± 0.12	7.52 ± 0.15	7.68 ± 0.11
Albumin (x 10 g/L)	3.25 ± 0.04	3.18 ± 0.05	3.14 ± 0.08
Globulin (x 10 g/L)	4.63 ± 0.11	4.34 ± 0.16	4.55 ± 0.12
ALP (IU/L)	231.51 ± 24.78	211.74 ± 12.45	181.23 ± 11.92
SOD (EU/mg HgB)	2.54 ± 0.06	2.47 ± 0.06	2.52 ± 0.06
GPX (EU/mg HgB)	6.76 ± 0.06	6.59 ± 0.06	6.63 ± 0.07
Catalase (µmole H ₂ O ₂ decomposed/ min/ mg Hgb)	2721.35 ± 71.71	2666.56 ± 69.99	2699.87 ± 73.24

Mean values bearing different superscripts within a row differ significantly (P<0.05)

Similar observations were reported in calves (Gupta 2014). Likewise Shinde *et al* (2012) found that feeding of adult rams with concentrate feed containing Cu and Zn contents of 16.87, 34.67 ppm in group-1; 37.15, 48.56 ppm in group-2 and 26.19, 39.58 ppm in group-3, respectively did not influenced mean serum glucose, total protein, albumin, globulin, urea and cholesterol concentration. Non-significant variations were reported in blood ALP of does sorted under different groups. Variations in ALP of does supplemented with Zn against control were within physiological limits. However, Sharma and Joshi (2005) recorded significant (P<0.01) increase in blood ALP (1.02 ± 0.12 to 2.34 ± 0.35 RE units/dl), after 7 days and reached close to standard values after 14 days of feeding Zn sulphate enriched mineral mixture to Zn deficient lactating cows against the un-supplemented control, where, no improvement was observed.

4.2.9 Economics of supplementing Zn to ration of does:

Economic viability of supplementing organic and inorganic zinc to does sorted under different groups was calculated and presented in Table 29. One common basal feed containing 2% mineral mixture and 1% salt without any added Zn was formulated and prepared for all the does under study. For example a lot of 12 quintal basal feed was prepared as per standard practices. This basal feed was further divided into three equal parts of 400 kg each for feeding of 10 does for 80 days in T₀, T_{oz} and T_{iz}, respectively. Fresh Zn powder requirement was calculated to supplement added Zn @ 30 mg/kg DM. Then Zn powder from organic and inorganic sources was added to basal feed for feeding in T_{oz} and T_{iz}, respectively. Economical calculations revealed that monthly supplementation of organic and inorganic Zn to each doe costed only Rs. 5.10 and 0.30 in T_{oz} and T_{iz}, respectively over the control group. However, added Zn maintained healthy live body weight with better condition, improved reproductive performance and reduced parasitic load. Thus, it is economically viable to supplement Zn to does from various sources under stall-fed conditions.

Table 29. Economics of supplementing Zn to does sorted under different groups

Parameters	T ₀	T _{oz}	T _{iz}
Daily DMI from fodder by a group of 10 does	14.45	14.59	14.52
Daily DMI from concentrate by a group of 10 does	4.54	4.54	4.54
Daily total DMI by a group of 10 does	19.00	19.14	19.06
Daily total DMI per doe	1.90	1.91	1.91
Daily Zn requirement @ 30 mg/Kg DMI (mg)	no added Zn	57.3	57.3
Zn Percentage in source powder	-	15	20
Quantity of source powder containing daily required Zn in mg (on dry matter basis)	-	382	286.5
Fresh source powder (containing 10% moisture) added to meet daily requirement of a buck (mg)	-	424.44	318.33
Daily fresh source powder added per buck (g)	-	0.42	0.32
Purchase cost of source Zn powder per kg (Rs)	-	400	35
Daily cost of source Zn powder supplementation (Rs)	-	0.17	0.01
Monthly cost of source Zn powder supplementation (Rs)	-	5.1	0.3
Monthly cost of adding Zn per kg DMI (Rs)	-	2.67	0.17

CHAPTER – V

SUMMARY

In, order to improve the sustainability of Beetal goat under stall-fed, two trials were conducted in the year 2015-16 viz. i) To study the effect of organic and inorganic zinc supplementation on qualitative and quantitative semen attributes of Beetal bucks reared under stall-fed conditions and ii) To analyze the effect of organic and inorganic zinc supplementation and synchronization of ovulation on reproductive efficiency of Beetal does. Both the experiments were conducted at Goat Research Farm, Department of Livestock Production Management, College of Veterinary Science, Guru Angad Dev Veterinary and Animal Sciences University, Ludhiana 141 004, Punjab, India after the ethical approval of IAEC. A total of fifteen donor bucks and thirty breedable does were selected and distributed randomly into three groups. Different observations on meteorological attributes, Dry matter intake, live body weight, body condition score, plasma zinc, breeding soundness evaluation in bucks, reproductive attributes in does, health status, blood profile and economic feasibility of Zn adding were recorded. All the animals were kept under similar management conditions except supplementation of added organic and inorganic Zn in the treatment groups.

The mean daily air temperature, relative humidity, sunshine, rainfall and THI during the study period on bucks were recorded as $27.62 \pm 0.33^{\circ}\text{C}$, $67.63 \pm 0.92\%$, 6.80 ± 0.29 hours, 2.96 ± 0.80 mm and 77.42 ± 0.48 , respectively. Supplementation of inorganic Zn significantly ($P < 0.05$) improved the fresh fodder intake. However, added Zn in organic and inorganic form improved numerically the DMI from fodder and total DMI compared with control with no added Zn. No variation was recorded in concentrate consumption by bucks. Added Zn from different sources helped the bucks to maintain the live body weight and body condition score. The plasma Zn of bucks responded to supplemental Zn indicating that basal diet was deficient to meet body's Zn requirement. Moreover, at completion of trial, treatment groups showed higher plasma Zn over the control.

Ultra-sonographic imaging of buck's testes revealed no variation in texture of testicular parenchyma during the study period of 180 days. In addition, Non-significant variations were recorded in rump fat thickness (mm), scrotal

circumference (cm), right and left testicular volume (cm³) and internal pelvic area (cm²). Interestingly, opposite to the trend observed in semen volume, the bucks with supplemented Zn produced significantly (P<0.05) higher sperm concentration (x 10⁶/ml) over bucks with no additional mineral. Bucks fed organic and inorganic Zn generated 5.63 % and 11.96 % more total sperms over bucks in control, respectively. Moreover, it was revealed that supplementation of organic and inorganic Zn lead to significant (P<0.05) improvement in gross motility and sperm motility. Variations in semen pH and live sperm percentage were within acceptable physiological limits. Mean testosterone levels (ng/ml) and testosterone to estradiol ratio were recorded highest in organic group followed by inorganic over control, respectively.

During the study period of 180 days, no case of lameness, morbidity or mortality was observed in bucks indicating no harmful effect of Zn supplementation. The percent decline in shedding of parasitic eggs in 1 g faeces was recorded more in bucks supplemented with organic (83%) and inorganic (91%) zinc over the control (39%) with no zinc supplementation. Examination of faecal samples also revealed that Zn supplementation (organic or inorganic) kept the shedding of oocysts towards lower count compared with control. Variations in haematological, biochemical and oxidative stress indicators in blood of bucks supplemented with different sources of Zn were within physiological limits indicating no adverse effect of mineral supplementation on health of bucks. Economical calculations revealed that monthly supplementation of organic and inorganic Zn @ 30 mg/kg DM to each buck costed only Rs. 5.85 and 0.38, respectively over the control group under stall-fed conditions.

The mean daily air temperature, relative humidity, sunshine, rainfall and THI as per outside weather during the study period were recorded as 25.50 ± 0.50 °C, 62.73 ± 1.05 %, 7.02 ± 0.26 hours, 2.38 ± 0.57 mm and 73.18 ± 0.69, respectively. The differences between groups in feed and fodder intake by does were statistically non-significant, indicating no adverse effects on appetite, ruminal micro-flora and digestibility of nutrients. Supplementation of organic and inorganic Zn in ration caused similar variations in live body weight of does during pregnancy period. However, treatment groups supplemented with added Zn maintained significantly (P<0.05) higher mean BCS and sternal region thickness indicating better condition over control group. Supplementation of added mineral had improved mean plasma Zn

significantly ($P < 0.05$) and numerically in organic and inorganic group, respectively over the control.

Zn supplementation had produced positive effect on reproductive performance traits of Beetal does compared with does receiving only basal diet. The prolificacy (%), fecundity (%), litter size/doe, number of live kids born were significantly ($P < 0.05$) higher in does supplemented with organic Zn followed by does with inorganic Zn than the control group. Number of multiple births were higher in treatment groups. Added Zn supplementation did not increase the number of synchronized does with progesterone releasing intra-vaginal device protocol along with non-significant and similar variations in plasma progesterone.

One goat under control group aborted during late gestation. In addition, two goats under organic group and one goat under inorganic died in the fifth month of gestation. Post findings revealed no abnormality and enteritis. Except these, no other case of severe lameness, morbidity or mortality was observed during the study period indicating no harmful effect of Zn supplementation on does. Overall mean number of parasitic eggs shed in 1 g of faeces were found numerically ($P > 0.05$) lower in Beetal does supplemented with added inorganic or organic Zn over the does with no added mineral. Does under control with no added Zn shed the highest mean oocysts per gram of faeces (40.50%). On the contrary, does from chelated Zn group shed the least number of oocysts (25.33 %) followed by does from inorganic Zn group (34.17 %). Variations in haematological, biochemical and oxidative stress indicators in blood of does were within physiological limits indicating no adverse effect of mineral supplementation on health of does. Economical calculations revealed that monthly supplementation of organic and inorganic Zn to each doe @ 30 mg/kg DM costed only Rs. 4.64 and 0.33 in T_{oz} and T_{iz} , respectively over the control group.

It was observed during the study on bucks that supplementation of organic and inorganic Zn lead to harvest 5.63 % and 11.96 % more total sperms over control, respectively along with significant ($P < 0.05$) improvement in sperm concentration ($\times 10^6/ml$), gross motility and sperm motility. Does supplemented with organic and inorganic Zn produced Prolificacy (%) of 225.00 and 177.78, respectively which was higher 144.44 in control. Adding of Zn also kept PEG and OPG towards lower side. It was also found from the results of study that adding zinc had not produced any adverse effects on DMI, LBW, BCS and health of goats under study. Thus, it may be

concluded that supplementation of added Zn from any source improved the performance and sustainability of Beetal goat under stall-fed production system. However, the group supplemented with organic Zn reflected superiority over other groups. Financial designs revealed that monthly adding of organic and inorganic Zn costed only Rs 2.67 and 0.17 per kg DMI in bucks and does. However, further studies are needed to assess and develop its sustainable, economical and field applicable design to harvest maximum productivity in commercial goat farming under stall-fed for a prolonged period of time.

REFERENCES

- Abdelrahman M M, Numan A M, Rayyan A L, Faisal, Awawdeh T and Alazzeah A Y. 2003. The effect of dietary levels of zinc methionine on the performance of growing awasi lambs. *Pakistan Journal of Biological Science* **6**: 979-983.
- Aebi H E. 1983. *Catalase* In: Bergmeyer H O (ed). *Methods of Enzymatic Analysis* Vol III Pp: 273-386. Academic press, New York.
- Allen D G, Constable P D, Dart A, Davies P R, Quesenberry K E, Reeves P T and Sharma J M (ed). 2016. *The Merck Veterinary Manual*. 11th Edn. Pp 3176-318. Merck and Co., Inc. Kenilworth, NJ, USA.
- Anonymus. 2016. Ludhiana yearly weather summary. Retrieved on May 14, 2016 from <http://worldweatheronline.com/ludhiana-weather-averages/punjab/> in.aspx.
- AOAC. 2000. *Official Methods of Analysis of AOAC International*. 17th edn. AOAC International, Maryland.
- Barth A D, Alisio L, Avilés M, Arteaga A A, Campbell J R and Hendrick S H. 2008. Fibrotic lesions in the testis of bulls and relationship to semen quality. *Animal Reproduction Science* **106(3)**: 274-288.
- Benjamin M M. 1985. *Outline of Veterinary Clinical Pathology*. 3rd Edn. Kalyani Publishers, New Delhi.
- Bosseboeuf Y, Bourdonnais A, Ashmead H D and Ashmead S D. 2006. The effect of copper, zinc and manganese amino acid chelates on dairy cow reproduction on eight farms: A field trial. *International Journal of Applied Research in Veterinary Medicine* **4(4)**: 313-19.
- Brazle F K and Stokka G. 1995. Effect of four trace mineral elements on gain and health of newly arrived calves. *The Professional Animal Scientist* **12**: 50-55.
- Bujarbaruah K M and Kumaresan A. 2013. Semen biology and artificial insemination. In: *Handbook of Animal Husbandry*. Indian Council of Agricultural Research, New Delhi. Pp: 446-505.
- Bun S D, Guo Y M, Guo F C, Ji F J and Cao H. 2011. Influence of organic zinc supplementation on the antioxidant status and immune responses of broilers challenged with *Eimeria tenella*. *Poultry Science* **90(6)**: 1220-1226.
- Ceylan A, Serin I, Aksit H and Seyrek. 2008. Concentrations of some elements in dairy cows with reproductive disorders. *Bulletin of the Veterinary Institute in Pulawy* **52**:109-12.

- Chandrasah, Saijpaul S and Saini A L. 2014. Supplementation of prepared organic copper and zinc on performance of Beetal does and kids. In: Proceedings of national seminar on “New dimensional approaches for livestock productivity and profitability enhancement under era of climate change” and XXI annual convention of Indian Society of Animal Production and Management, January 28-30, 2014 at Anand-388001.
- Chandrasah, Saijpaul S, Sandhu K and Saini A L. 2015. Stress appraisal by organic-inorganic zinc and copper supplementation in Beetal kids. *The Indian Journal of Animal Sciences* **85(8)**: 908-12.
- Chandrasah. 2011. ‘Studies on some managerial interventions for sustainability of Beetal goat under stall-fed conditions.’ Ph.D. Thesis, Guru Angad dev Veterinary and Animal Sciences University, Ludhiana.
- Chhabra S, Randhawa S N S and Bhardwaj S D. 2015. Macro and micro mineral profile in forage and blood plasma of water buffaloes with respect to seasonal variation. *Buffalo Bulletin* **34(1)**: 45-50.
- Chhabra S, Randhawa S S and Bhardwaj S D. 2013. Effect of oral zinc supplementation on plasma zinc status in zinc deficient buffalo. *Indian Journal of Veterinary Medicine* **33(2)**: 125-127.
- CIRG. 2013. Vision 2050-Central Institute for Research on Goats. Mathura. Retrieved on May 14, 2016 from www.cirg.res.in.
- Cope C M, Mackenzie A M, Wilde D and Sinclair L A. 2009. Effects of level and form of dietary zinc on dairy cow performance and health. *Journal of dairy science* **92(5)**: 2128-2135.
- Devendra C and Solaiman S G. 2010. Perspectives on goats and global production. In: Solaiman S G (Eds). *Goat Science and Production*, Wiley-Blackwell, pp 3-20.
- Devi J, Goswami J, Sarmah B C and Sarma K. 2012. Effect of zinc supplementation on growth rate of Assam local male kids. *Indian Journal of Animal Research* **46(1)**: 95-97.
- El-Sisy G A, Abdel-Razek A M A, Younis A A, Ghallab A M and Abdou M S S. 2008. Effect of dietary zinc or selenium supplementation on some reproductive hormone levels in male Baladi Goats. *Global Veterinaria* **2(2)**: 46-50.
- FAO. 2016. Statistical Databases. Food and Agriculture Organization of the United Nations. Retrieved on May 18, 2016 from <http://faostat3.fao.org>.
- Fadayifar A, Aliarabi H, Tabatabaei M M, Zamani P, Bahari A, Malecki, M and Dezfoulian A H. 2012. Improvement in lamb performance on barley based diet supplemented with zinc. *Livestock Science* **144(3)**: 285-289.

- Faizal A C M and Rajapakse R P V J. 2001. Prevalence of *coccidia* and gastrointestinal nematode infections in cross bred goats in the dry areas of Sri Lanka. *Small Ruminant Research* **40**: 233-238.
- Faizal A C M, Rajapakse R P V J, Jayasinghe S R and Rupasinghe V. 1999. Prevalence of *Eimeria* Spp. and gastrointestinal nematodes versus weight gains in treated goats raised in the dry areas of Sri Lanka. *Small Ruminant Research* **34(1)**: 21-25.
- Fausto G C, Pivoto F L, Costa M M, dos Anjos Lopes S T, França R T, Molento M B, Minervino A H H, Da-Rocha J B T and do Rêgo Leal M L. 2014. Protein profile of lambs experimentally infected with *Haemonchus contortus* and supplemented with selenium and copper. *Parasites and Vectors* **7(1)**: 1-6.
- Garg A K, Mudgal V and Dass R S. 2008. Effect of organic zinc supplementation on growth, nutrient utilization and mineral profile in lambs. *Animal Feed Science Technology* **144**: 82-96.
- George M H, Nockels C F, Stanton T L, Johnson B, Cole N A and Brown M A. 1997. Effect of source and amount of zinc, copper, manganese, and cobalt fed to stressed heifers on feedlot performance and immune function. *The Professional Animal Scientist* **13(2)**: 84-89.
- Goel A K and Kharche S D. 2009. Advances in reproductive techniques in goats-A review. *Indian Journal of Small Ruminants* **15(1)**: 1-34.
- GoI (Government of India). 2013. *Estimated Livestock Population-Breed Wise 2013*. Ministry of Agriculture and Farmers Welfare, Department of Animal Husbandry, Dairying and Fisheries, Krishi Bhawan, New Delhi.
- GoI (Government of India). 2016. *Annual Report 2015-16*. Ministry of Agriculture and Farmers Welfare, Department of Animal Husbandry, Dairying and Fisheries, Krishi Bhawan, New Delhi.
- Gupta P. 2014. 'Bioavailability of zinc from inorganic and organic sources in calves' M.V.Sc. Thesis, Guru Angad Dev Veterinary and Animal Sciences University, Ludhiana.
- Habeeb A A M, EL-Tarabany A A and Gad A E. 2013. Effect of zinc levels in diet of goats on reproductive efficiency, hormonal levels, milk yield and growth aspects of their kids. *Global Veterinaria* **10(5)**: 556-564.
- Hafeman D G, Sunde R A and Hoekstra W G. 1974. Effect of dietary selenium on erythrocyte and liver glutathione peroxidase in the rat. *The Journal of Nutrition* **104(5)**: 580-587.
- Huang Y L, Lu L, Luo X J and Liu B. 2009. An optimal dietary zinc level of broiler chicks fed a corn-soybean meal diet. *Poultry Science* **86**: 2582-89.

- Jain N C. 1986. *Schalm's Veterinary Hematology*. 4th edn. Lea and Febiger, 600 Washington square, Philadelphia, USA.
- Jia W, Jia Z, Zhang W, Wang R, Zhang S and Zhu X. 2008. Effects of dietary zinc on performance, nutrient digestibility and plasma zinc status in Cashmere goats. *Small Ruminant Research* **80(1)**: 68-72.
- Karkoodi K, Chamani M, Beheshti M, Mirghaffari S S and Azarfar A. 2012. Effect of organic zinc, manganese, copper, and selenium chelates on colostrum production and reproductive and lameness indices in adequately supplemented Holstein cows. *Biological Trace Element Research* **146(1)**: 42-46.
- Kausar R, Khanum S A, Hussain M and Shah M S. 2009. Estrus synchronization with medroxyprogesterone acetate impregnated sponges in goats (*Capra hircus*). *Pakistan Veterinary Journal* **29(1)**: 16-18.
- Khushpreet S, Ajeet K, Honparke M and Dadarwal D. 2015. Ultrasonographic approaches for breeding soundness evaluation of high and low libido buffalo bulls. *Indian Journal of Animal Sciences* **85(5)**: 13–15.
- Koudela B and Boková A. 1998. Coccidiosis in goats in the Czech Republic. *Veterinary Parasitology* **76(4)**: 261-267.
- Kumar A, Singh K, Honparkhe M, Kaur S, Ghuman S and Brar P. 2016. Physical confirmation and ultrasonographic approaches for breeding soundness evaluation of high and low libido crossbred bulls. *The Indian Journal of Animal Reproduction* **37(1)**: 19-22.
- Kumar B, Ram H, Dhaliwal S S and Singh S T. 2013. Productivity and quality of fodder corn (*Zea mays* L.) under soil and foliar zinc application. *Proceedings of 27th International Plant Nutrition Conference*. pp. 752-53. Istanbul.
- Kumar N, Verma R P, Singh L P, Varshney V P and Dass R S. 2006. Effect of different levels and sources of zinc supplementation on quantitative and qualitative semen attributes and serum testosterone level in crossbred cattle (*Bos indicus* X *Bos taurus* bulls). *Reproduction Nutrition Development* **46**: 663-75.
- Kundu M S, De A K, Jeyakumar S, Sunder J, Kundu A and Sujatha T. 2014. Effect of zinc supplementation on reproductive performance of Teressa goat. *Veterinary World* **7(6)**: 380-383.
- Kusina N T, Tarwirei F, Hamudikuwanda H, Agumba G, and Mukwena J. 2000. A comparison of the effects of progesterone sponges and ear implants, PGF₂alpha, and their combination on efficacy of estrus synchronization and fertility of Mashona goat does. *Theriogenology* **53(8)**: 1567-1580.

- Malcolm-Callis K J, Duff G C, Gunter S A, Kegley E B and Vermeire D A. 2000. Effects of supplemental zinc concentration and source on performance, carcass characteristics and serum values in finishing beef steers. *Journal of Animal Science* **78**: 2801–2808.
- Mandal G P, Dass R S, Garg A K, Varshney V P and Mondal A B. 2008. Effect of zinc supplementation from inorganic and organic sources on growth and blood biochemical profile in crossbred calves. *Journal of Animal and Feed Sciences* **17(2)**: 147-156.
- Mandal G P, Dass R S, Isore D P, Garg A K and Ram G C. 2007. Effect of zinc supplementation from two sources on growth, nutrient utilization and immune response in male crossbred cattle (*Bos indicus* × *Bos taurus*) bulls. *Animal Feed Science Technology* **138**: 1–12.
- Marklund S and Marklund G. 1974. Involvement of the superoxide anion radical in the autoxidation of pyrogallol and a convenient assay for superoxide dismutase. *European Journal of Biochemistry* **47**: 469-474.
- McDowell L R. 2014. *Minerals in Animal and Human Nutrition*. 2nd Edn. (Indian print). Reed Elsevier India Pvt. Ltd., New Delhi.
- Meschy F. 2000. Recent progress in the assessment of mineral requirements of goats. *Livestock Production Science* **64**: 9-14.
- Mostafa E A G, Hassan A A M and Soliman S M. 2012. Effect of feeding a combination of zinc, manganese and copper methionine chelates of early lactation high producing dairy cow. *Food and Nutrition Sciences* **3(8)**: 1084-1091.
- Njenga M J, Munyua S J M, Mutiga E R, Gathuma J M, Kang'ethe E K, Bwangamoi O, Mugeru G M and Mitaru B N. 1999. Semen characteristics of goats with subacute, acute and chronic besnoitiosis: research communication. *Journal of the South African Veterinary Association* **70(1)**: 18-20.
- NRC. 2007. *Nutrient requirements of small ruminants: sheep, goats, cervids and new world camelids*. National Academic Press, Washington DC.
- Nunnery G A, Vasconcelos J T, Parsons C H, Salyer G B, Defoor P J, Valdez F R and Galyean M L. 2007. Effects of source of supplemental zinc on performance and humoral immunity in beef heifers. *Journal of Animal Science* **85(9)**: 2304-2313.
- Pechova A, Pavlata L and Lokajova E. 2006. Zinc supplementation and somatic cell count in milk of dairy cows. *Acta Veterinaria Brno* **75(3)**: 355-361.
- Popoola M A, Bolarinwa M O, Yahaya M O and Adebisi G L. 2014. Thermal comfort effects on physiological adaptation and growth performance of West

- African dwarf goats raised in Nigeria. *European Scientific Journal* **3**: 275-281.
- Puchala R, Sahlu T and Davis J J. 1999. Effects of zinc-methionine on performance of Angora goats. *Small Ruminant Research* **33**: 1–8.
- Radfar M H, Sakhaee E, Shamsaddini Bafti M and Haj Mohammadi H. 2011. Study on gastrointestinal parasitic infections of Raeini goats. *Iranian Journal of Veterinary Research* **12(1)**: 76-80.
- Rahman W A. 1994. Effect of sub-clinical *Eimeria* species infections in tropical goats subsequently challenged with caprine *Haemonchus contortus*. *Veterinary Research* **134**: 235-237.
- Rakhshandehroo E, Razavi S M and Nazifi S. 2014. Experimental caprine coccidiosis: the pattern of changes in antioxidant micronutrients and vitamins. *Veterinary Science Development* **4(2)**: 46-50.
- Ray S K, Roychoudhury R, Bandopadhyay S K and Basu S. 1997. Studies on “zinc deficiency syndrome” in Black Bengal goats (*Capra hircus*) fed with fodder (*Andropogon gayanus*) grown on soil treated with an excess of calcium and phosphorus fertilizer. *Veterinary Research Communications* **21(8)**: 541-546.
- Roy D, Jaiswal A K, Kumar V and Shankar D. 2013. Effect of supplementing mineral mixture on gastro intestinal parasitic load in Murrah buffaloes. *Indian Veterinary Journal* **90(2)**: 112-113.
- Saini A L, Khan B U and Singh K. 1988. Growth performance of goats under three systems of feeding management. *Indian Journal of Animal Sciences* **58**: 604-609.
- Sales J N S, Pereira R V V, Bicalho R C and Baruselli P S. 2011. Effect of injectable copper, selenium, zinc and manganese on the pregnancy rate of crossbred heifers (*Bos indicus* × *Bos taurus*) synchronized for timed embryo transfer. *Livestock Science* **142**: 59-62.
- Schafer A S, Leal M L R, Molento M B, Aires, A R, Duarte M M M F, Carvalho F B, Tonin A A, Schmidt L, Flores E M M, França R T and Grando T H. 2015. Immune response of lambs experimentally infected with *Haemonchus contortus* and parenterally treated with a combination of zinc and copper. *Small Ruminant Research* **123(1)**: 183-188.
- Scott M E and Koski K G. 2000. Zinc deficiency impairs immune responses against parasitic nematode infections at intestinal and systemic sites. *The Journal of Nutrition* **130(5)**: 1412S-1420S.
- Scott P R. 2007. *Sheep Medicine*. Manson Publishing, London, 336 p.

- Shalander Kumar. 2007. Commercial Goat Farming in India: An Emerging Agri-Business Opportunity. *Agricultural Economics Research Review* **20**: 503-520.
- Sharma A. 2016. 'Effect of body condition score on performance of Beetal goat.' M.V.Sc. Thesis, Guru Angad Dev Veterinary and Animal Sciences University, Ludhiana.
- Sharma D K, Agrawal N, Mandal A, Nigam P and Bhushan S. 2009. Coccidia and gastrointestinal nematode infections in semi-intensively managed Jakhrana goats of semi-arid region of India. *Tropical and Subtropical Agroecosystems* **11**: 135-139.
- Sharma M C and Joshi C. 2005. Therapeutic efficacy of zinc sulphate used in clustered model treatment in alleviating zinc deficiency in cattle and its effect on hormones, vitamins and production parameters. *Veterinary Research Communications* **29(7)**: 609-628.
- Shinde A K, Sankhyan S K, Kumar D and Regar R K. 2012. Effects of supplementation of copper and zinc on nutrient intake, utilization, blood profile, wool yield and semen quality of Malpura rams. *Indian Journal of Small Ruminants* **18(2)**: 191-197.
- Shinde A K, Sankhyan S K, Ramkesh M and Kumar R R. 2013. Effect of feed supplementation with copper and zinc salts on growth, wool yield, nutrient utilization, blood constituents and mineral profile of Malpura lambs. *Agricultural Science Research Journal* **3**: 284-291.
- Singh A K, Brar P S and Cheema R S. 2014. Relationships among frozen-thawed semen fertility, physical parameters, certain routine sperm characteristics and testosterone in breeding Murrah buffalo (*Bubalus bubalis*) bulls. *Veterinary World* **7(9)**: 644-651.
- Singh B, Natesan S K A, Singh B K and Usha K. 2005. Improving zinc efficiency of cereals under zinc deficiency. *Current Science India* **88(1)**: 36-44.
- Singh K, Kumar A and Honparkhe M. 2015. Endocrine status of serum testosterone, estradiol, prolactin and thyroid hormones in good and poor libido breeding buffalo bulls. *Indian Veterinary Journal* **92(12)**: 39-40.
- Singh M V. 2009. Micronutrient nutritional problems in soils of India and improvement for human and animal health. *Indian Journal of Fertilisers* **5(4)**: 11-26.
- Singla L D. 1995. A note on sub-clinical gastro-intestinal parasitism in sheep and goats in Ludhiana and Faridkot districts of Punjab. *Indian Veterinary Medical Journal* **19**: 61-62.
- Snedecor G W and Cochran W G. 1994. *Statistical Methods*. 8th Edn. Oxford & IBH Publishing Co., Calcutta, India.

- Soulsby E J L. 2005. *Helminths Arthropods and Protozoa of Domesticated Animals*. pp. 707–719, ELBS-7, Bailliere Tindal, London.
- Spears J W and Kegley E B. 2002. Effect of zinc source (zinc oxide vs zinc proteinate) and level on performance, carcass characteristics, and immune response of growing and finishing steers. *Journal of Animal Science* **80(10)**: 2747-2752.
- Sprecher D J, Hostetler D E and Kaneene J B. 1997. A lameness scoring system that uses posture and gait to predict dairy cattle reproductive performance. *Theriogenology* **47(6)**: 1179-1187.
- SPSS. Statistical Package for Social Sciences (IBM) Version 22.0. Chicago, IL, United States of America.
- Strnadová, P, Svobodová V, Pavlata L, Mišurová Ľ and Dvořák R. 2011. Effect of inorganic and organic zinc supplementation on coccidial infections in goat kids. *Acta Veterinaria Brno* **80(2)**: 131-137.
- Swarnkar C P, Singh D and Solanki V K. 2010. Prevalence of gastrointestinal parasites of sheep in semi-arid Rajasthan: a field study. *Indian Journal of Small Ruminants* **16(2)**: 221-227.
- Tripathi M K and Karim S A. 2008. Mineral requirement of small ruminants with special reference to their role in rumen fermentation-A review. *Indian Journal of Small Ruminants* **14(1)**: 1-47.
- Tucker C B, Rogers A R and Schutz K E. 2008. Effect of solar radiation on dairy cattle behaviour, use of shade and body temperature in a pasture-based system. *Applied Animal Behaviour Science* **109**: 141–154.
- Uchida K, Mandebvu P, Ballard C S, Sniffen C J and Carter M P. 2001. Effect of feeding a combination of zinc, manganese and copper amino acid complexes, and cobalt glucoheptonate on performance of early lactation high producing dairy cows. *Animal Feed Science and Technology* **93(3)**: 193-203.
- Van Eerdenburg F J, Loeffler H S and Van Vliet J H. 1996. Detection of oestrus in dairy cows: a new approach to an old problem. *The Veterinary Quarterly* **18(2)**: 52–54.
- Van Soest P J, Robertson J B and Lewis B A. 1991. Methods for dietary fiber, neutral detergent fiber and non-starch polysaccharides in relation to animal nutrition. *Journal of Dairy Science* **74**: 3583-3597.
- Walker S L, Smith R F, Jones D N, Routly J E and Dobson H. 2008. Chronic stress, hormone profiles and estrus intensity in dairy cattle. *Hormones and Behaviour* **53(3)**: 493-501.

- White C L. 1993. The zinc requirements of grazing ruminants. *Proceedings of the International Symposium on Zinc in Soils and Plants*. Pp. 197-206. Australia.
- Wildeus S. 2000. Current concepts in synchronization of estrus: Sheep and goats. *Journal of Animal Science* **77**:1-14.
- Woji A Y, Little D A and Ikwuegbu O A. 1994. Prevalence of coccidial infections in the west African dwarf goat in the sub humid zone of Nigeria. *Tropical Animal Health and Production* **26**: 1-6.

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