EFFECT OF MINERAL SUPPLEMENTATION IN POST PARTUM ANOESTRUS GRADED MURRAH BUFFALOES

By

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CERTIFICATE

M. V. GOPALA KRISHNA has satisfactorily prosecuted the course of research and that the thesis entitled **“EFFECT OF MINERAL SUPPLEMENTATION IN POST PARTUM ANOESTRUS GRADED MURRAH BUFFALOES”** submitted is the result of original research work and is of sufficiently high standard to warrant its presentation to the examination. I also certify that the thesis or part thereof has not been previously submitted by him for a degree of any university.

Date: 2nd July’ 2012,

Place: Hyderabad.

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CERTIFICATE

This is to certify that the thesis entitled **"EFFECT OF MINERAL SUPPLEMENTATION IN POST PARTUM ANOESTRUS GRADED MURRAH BUFFALOES"** submitted in partial fulfillment of the requirements for the degree of **MASTER OF VETERINARY SCIENCE (Animal Reproduction, Gynaecology And Obstetrics)** of the **Sri Venkateswara Veterinary University**, **Tirupati,** is a record of the bonafide research work carried out by M. V. GOPALA KRISHNA under my guidance and supervision. The subject of the thesis has been approved by the student's Advisory Committee.

No part of the thesis has been submitted for any other degree or diploma. The published part has been fully acknowledged. All assistance and help received during the course of the investigations have been duly acknowledged by the author of the thesis.

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TABLE OF CONTENTS

LIST OF TABLES

I. LIST OF FIGURES

LIST OF PLATES

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(M. V. GOPALA KRISHNA**)**

DECLARATION

I, M. V. GOPALA KRISHNA hereby declare that the thesis entitled **"EFFECT OF MINERAL SUPPLEMENTATION IN POST PARTUM ANOESTRUS GRADED MURRAH BUFFALOES"** submitted to **Sri Venkateswara Veterinary University** for the degree of **MASTER OF VETERINARY SCIENCE in Animal Reproduction, Gynaecology And Obstetrics,** is a result of original research work done by me. It is further declared that the thesis or any part thereof has not been published earlier in any manner.

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ABSTRACT

The present investigation "Effect of mineral supplementation in post partum anoestrus graded Murrah buffaloes" was undertaken in post partum pluriparous true anoestrus buffaloes (above 2 months) after calving, having a body condition score ranging from 3.0 to 4.0 screened clinically for reproductive disorders. 60 animals free from reproductive problems and having completely involuted uterus were selected for the study. Animals were divided into 4 groups comprising 15 in each group and subjected to different mineral supplementation (Group-I: Cyclomin-7 at the dose rate of 2boli/week/animal and Dicalcium phosphate at the dose rate of 30 gm/day/animal, Group-II: Non-chelated minerals at the dose rate of 50 gm/day/animal, Group-III:

Chelated minerals at the dose rate of 50 gm/day/animal, Group-IV: Control group, without any mineral supplementation) protocols for 30 days and were fed with dry fodder, green fodder and concentrates as per the production requirements in intensive feeding system.

During the course of treatment estrus symptoms were monitored with the help of teaser bulls twice or thrice per day and by visual observation 5 to 6 times per day. Animals which were not showing estrus symptoms were examined once in a week per rectally to know the ovarian activity. Animals which showed estrus symptoms have been inseminated twice during late estrus period. After breeding, animals were examined for subsequent estrus cycle and inseminated if required during subsequent cycles. Pregnancy Diagnosis was done per rectally after 50 days of breeding to know the conception status. Blood samples have been collected on zero, 15^{th}, 30^{th} days of treatment for estimation of serum minerals and haematological parameters. Estrus intensity and duration of estrus has been recorded based on the physiological symptoms.

The mean haemoglobin levels on zero, 15^{th} and 30^{th} day in group I were 11.05 ± 0.26, 11.31 ± 0.27 and 11.55 ± 0.27, in group II were 11.01 ± 0.22, 11.32 ± 0.23 and 11.63 ± 0.22, in group III were 11.03 ± 0.18, 11.46 ± 0.26, and 11.89 ± 0.27 and in group IV were 11.01 ± 0.16, 11.03 ± 0.17 and 11.06 ± 0.17 g% respectively. Haemoglobin levels on 15^{th} and 30^{th} day of treatment group III was significantly ($p < 0.05$) higher. In between groups there was no significant difference in haemoglobin levels on zero, 15^{th} day. But on 30^{th} day three treatment groups were having statistically similar concentrations which were significantly ($p < 0.05$) higher than the control group.

The mean Serum calcium levels on zero, 15^{th} and 30^{th} day were 7.20 ± 0.23, 7.69 ± 0.23 and 8.08 ± 0.22 in group I, 7.15 ± 0.21, 7.85 ± 0.12 and 8.63 ± 0.16 in group II, 7.24 ± 0.23, 7.97 ± 0.12 and 8.79 ± 0.07 in group III and 7.31 ± 0.10, 7.34 ±

0.06 and 7.35 ± 0.07 mg/dl in group IV respectively. On 30th day of treatment the serum calcium levels were higher in group III followed by group II, group I and lowest in group IV.

The mean Serum phosphorus levels on zero, 15th and 30th day in group I were 5.46 ± 0.29, 5.79 ± 0.32 and 6.24 ± 0.18 mg/dl, in group II were 5.78 ± 0.18, 5.87 ± 0.28 and 6.30 ± 0.25 mg/dl, in group III were 5.56 ± 0.07, 5.96 ± 0.17 and 6.94 ± 0.05 mg/dl and in group IV were 5.56 ± 0.07, 5.83 ± 0.11 and 5.80 ± 0.10 mg/dl respectively. On 30th day of treatment the serum phosphorus level was significantly ($p < 0.05$) higher in group III followed by group II, I and IV.

The mean Serum iron levels on zero, 15th and 30th day in group I were 2.716 ± 0.112, 3.122 ± 0.084 and 3.180 ± 0.110, in group II were 2.656 ± 0.058, 3.147 ± 0.068 and 3.246 ± 0.140, in group III were 2.689 ± 0.047, 3.174 ± 0.194, and 3.680 ± 0.081 and in group IV were 2.679 ± 0.039, 2.690 ± 0.061 and 2.702 ± 0.060 ppm respectively. Serum iron levels in group I, II were significantly ($p < 0.05$) lower on zero day when compare to 15th and 30th day. In between four groups, on 30th day group III was having significantly ($p < 0.05$) higher Iron levels.

The mean Serum copper levels on zero, 15th and 30th day were 0.047 ± 0.003, 0.097 ± 0.003 and 0.142 ± 0.006 ppm in group I, in group II were 0.049 ± 0.005, 0.127 ± 0.005and 0.163 ± 0.005, in group III were 0.052 ± 0.002, 0.149 ± 0.003 and 0.184 ± 0.005 and in group IV were 0.049 ± 0.002, 0.052 ± 0.002 and 0.054 ± 0.003 ppm respectively. The serum copper levels were significantly ($p < 0.05$) higher on 30th day followed by 15th day and lowest on zero day in all three treatment groups. In between four groups there was significant difference ($p < 0.05$) on 15th and 30th day and group III was having significantly ($p < 0.05$) higher serum copper levels.

The mean serum manganese levels on zero, 15th and 30th day in group I were 0.056 ± 0.003, 0.076 ± 0.003 and 0.151 ± 0.007 ppm, in group II were 0.058 ± 0.002, 0.112 ± 0.002 and 0.165 ± 0.006 ppm, in group III were 0.054 ± 0.002, 0.148 ± 0.002 and 0.182 ± 0.002 ppm and in group IV were 0.056 ± 0.002, 0.056 ± 0.003 and 0.055 ± 0.003 ppm respectively. The serum manganese levels were significantly ($p < 0.05$) different in all three treatment groups on zero, 15th, 30th day. In between four groups there was significant ($p < 0.05$) difference on 15th and 30th day and group III was having higher serum manganese levels.

The mean serum zinc levels on zero, 15th and 30th day in group I were 1.062 ± 0.023, 1.162 ± 0.033 and 2.639 ± 0.061, in group II were 1.089 ± 0.008, 1.248 ± 0.018 and 3.038 ± 0.017, in group III were 1.075 ± 0.010, 1.427 ± 0.039 and 3.767 ± 0.042 and in group IV were 1.032 ± 0.012, 1.050 ± 0.010 and 1.054 ± 0.010 ppm respectively. On 15th and 30th day group III was having significantly ($p < 0.05$) higher serum zinc level followed by II, I and IV.

The percentage of estrus response was 46.66 per cent, 66.66 per cent, 86.66 per cent and 33 per cent; mean duration of estrus was 22.13 ± 0.83, 26.46 ± 0.98, 32.93 ± 0.81 and 15.80 ± 0.65 hours; the intensity of estrus was 10.57 ± 0.69, 12.30 ± 0.76, 13.15 ± 0.72 and 11.4 ± 1.36 points; the interval between treatments to the estrus exhibition 46.67 ± 0.95, 35.20 ± 1.06, 25.0.98 and 70.13 ± 1.79 days and conception rates were 20 per cent, 40 per cent, 73.33 per cent and 13.33 per cent in group I, II, III and IV respectively. Regarding estrus response, duration of estrus and estrus intensity there was significant ($p < 0.05$) difference in between all four groups and group III was having significantly ($p < 0.05$) higher and followed by group II, I and lowest in IV. The interval between treatments to the estrus exhibition there was significant **(**$p < 0.05$**)** difference in between all four groups, group III was having significantly **(**$p < 0.05$**)** lesser interval next higher in group II, I and highest in group IV. The conception rates were significantly ($p < 0.05$) higher in group III and followed by group II and I lowest in IV.

Based on the results of this study come to the concluded that due to the supplementation of chelated mineral mixture, the mean serum mineral levels and bioavailability increased more than non chelated mineral mixture and Di Calcium phosphate plus Cyclomin – 7. Consequently it enhanced the estrus response, duration of estrus and estrus intensity and decreased the interval between the treatment to the estrus exhibition and increased the conception rate in post partum anoestrus graded Murrah buffaloes.

LIST OF ABBREVIATIONS

A.M & P.M	Anti Meridian and Post Meridian
AA	Amino Acids
AAC	Amino Acid Chelate
AAS	Atomic Absorption Spectrophotometer
AATM	Amino Acid Trace Mineral
ad lib.	Adlibidum.
ALP	Alkaline phosphatase
ALT	Alanine transaminase
AMP	Adinine Mono Phosphate
AST	Aspartate Transaminase
BCS	Body Condition Score
Ca	Calcium
Ca: P	Calcium : Phosphorus
CFU	Colony Forming Unit
CL	Corpus Luteum
cm	Centimeter
Co	Cobalt
CR	Conception Rate
Cr	Chromium
CTL	Control
Cu	Copper
DCP	Di Calcium Phosphate
DMI	Dry Matter Intake
EDTA	Ethylene Di amine Tetra Acetic acid
FAO	Food and Agriculture Organization
Fe	Iron
FSH	Follicle Stimulating Hormone
gm/dL	Grams per deciliter
gm/L	Grams per Liter
gm/animal/ day	gram per animal per day
Hb	Haemoglobin
HCl	Hydro Chloric Acid
HF	Holstein-Friesian
IM	Inorganic Metal salts

ING	Inorganic Group
IU	International Unit
L	Liter
LDA	Left Displacement of Abomasum
LH	Luetinizing Hormone
MB	Murrah Buffalo
mcg	Microgram
mg	Milligram
Mg	Megnisium
mL	Milliliter
mm	Millimeter
mmol	Millimole
mmol/l	Millimole per litre
Mn	Manganese
N	Normality
N/10	10 per cent Normalty
NMS	Non-Mineral Supplemented
No	Number
NRC	Nutritional Research Council
ORG	Organic Group
OTM	Organic Trace Minerals
P	Phosphorus
ppm	Parts per million
RP	Retained Placenta
SNF	Solid Not Fat
sp.	species
TEC	Total Erythrocytes Count
U/L	Units per Litre
UMMB	Urea-Molasses Multi-nutrient Block
viz.	Respectively
Vs.	Versus
WCTLZ	West-Central Table Land Zone
Zn	Zinc
µg/kg	Microgram per kilogram
µL	Microliter
µmol	Micromole

CHAPTER I

INTRODUCTION

World buffaloe population is nearly 170 million with 97 percent in Asia, 2 percent in Africa and 0.2 percent in Europe (FAO, 2004). India is possessing 56 per cent of world buffalo population. India has over 97.9 million (census, 2003) buffaloes mostly raised by small farmers owning less than two hectares of land and fewer than five buffaloes. Milk is providing supplementary income to 70 million landless, small and marginal farmers and in over 5, 00,000 remote villages. The dairy industry especially milk production from buffaloes is increasing day by day in rural areas as they get more money from buffalo's milk because of its high fat and solids not fat (SNF) content compared to cows.

Annually buffaloe contributes 72 million tones of milk and three million tones of meat to world food and much of it is from the areas that are prone to nutritional imbalances. The success of the dairy buffalo economy lies in proper and optimal reproductive rhythm of each individual buffalo in the herd within normal physiological range (Dhaliwal, 2005). Any deviation or prolongation in the breeding rhythm results in a progressive economic loss due to widening of the dry period and reduced calvings and lactations during the life span of the animal (Singh *et al*., 2006). Barren or infertile buffaloes cause direct loss in milk production, whereas reduced calf crops hamper the selection efficiency in long term dairy herd improvement (Baghel, 2006).

Reproduction in animals is known to be influenced by environment, nutrition, hormonal status, infections along with genetic traits. For every successful pregnancy and parturition a proper synergism between anabolic and catabolic reactions is essential. Minerals have basic key role in maintenance of metabolism and studies on their nutritional requirements in the body led to the classification of these minerals as major and minor elements. Maintenance of normal levels of various minerals can influence the reproductive performance of ruminants. Reproductive failure may be induced by deficiencies of single or combined trace elements and their imbalance (Hidiroglou, 1979). Mineral deficiencies might be responsible for lowering conception.

Buffaloes are mostly reared on crop residues and dry grasses which are used in combination with locally available green fodder and small amount of concentrate mixture. Generally, the animals do not receive mineral mixture supplementation in the ration therefore, livestock production is often badly influenced by mineral deficiency (Tiwary *et al.,* 2007).

Livestock depend on forages for their mineral requirements, which are deficient in several minerals content as because they grow on a deficient soil (Sharma *et al.,* 2005). In cattle under field conditions reproductive failure is a major problem due to hormonal imbalance, reproductive diseases and deficiency or imbalances of minerals (Prasad and Rao, 1997). Soil mineral content keeps on changing due to pressure on land for maximum crop production, fertilizer application, natural calamities, which may alter their contents in feeds, fodders and their status in animals thereby exhibiting an area specific problem (Underwood and Suttle, 1999).

Mineral content in serum have a great influence in hormonal and vitamin status and marked changes were observed in the hormonal and vitamin status during deficiency of minerals. Mineral deficiency is an area problem (McDowell, 1985). The performance of animal is below than the expectation in spite of good managemental condition practiced to livestock/farm animals. To get rid of deficiency problems an area specific mineral supplement using locally available feed resources is recommended (Sharma *et. al.,* 2002).

Calcium in mineral supplements is generally more available than calcium in forages and common feedstuffs (Hansard *et al.,* 1957). For maintenance of non lactating cattle, the absorbed calcium required is 0.0154 gm/kg body weight (Visek *et al.,* 1953;

Hansard *et al.*, 1957). For lactating animals the maintenance requirement is increased to 0.031 gm/kg live body weight (Martz *et al.*, 1990). The increase in lactating cows reflects the impact increased dry matter intake (DMI) has on intestinal secretion of calcium during digestion. Ca deficiency may be directly or indirectly responsible for infertility, but the disturbed Ca: P ratio has a blocking action on the pituitary gland and consequently on ovarian action (Herrick and John, 1977), prolonging the interval of first ovulation. According to Morrow (1980) at reduced blood calcium level, delayed uterine involution and increase in the incidence of dystocia, retained placenta and prolapsed uterus may occur. Higher Ca: P ratio is associated with infertility in cattle.

Phosphorus is the second most abundant mineral in the animal body after calcium. Phosphorus deficiency is the most common in several developing countries due to the low phosphorus content in soils and plants (McDowell *et al.*, 1986). Phosphorus has more important functions and is required by rumen microorganisms for digestion of cellulose (Burroughs *et al.*, 1951) and synthesis of microbial proteins (Breves and Schroder, 1991). Out of all mineral deficiencies phosphorus deficiency is most widespread and economically important deficiency as far as reproduction is concerned (Underwood, 1981).

On the other hand, copper (Cu) is an essential trace element for ruminants being the component of enzymes and essential for absorption and transport of iron for hemoglobin synthesis. The amount of dietary copper requirement for maintenance, growth and lactation varies with age of the animals. The supplementation of ration with copper and phosphorus as area specific mineral mixture under field condition helped in improving milk production and in amelioration of reproductive problems in cattle and buffaloes (Saxena *et al.*, 2008).

Stanton *et al.* (2000) reported that cows receiving organic trace minerals exhibited higher pregnancy rates with artificial insemination than those receiving inorganic trace minerals. Improved reproductive performance has been reported in dairy cows receiving organic mineral supplements (Manspeaker *et al.,* 1987). Adequate mineral intake and absorption is required for a variety of metabolic functions including immune response to pathogenic challenge, reproduction and growth (Manspeaker *et al.,* 1987; Garg *et al.,* 2009). Dietary imbalances in one or more of the essential minerals are as dangerous as mineral deficiency (Underwood and Suttle, 1999). Trace mineral deficiencies can occur as a primary deficiency when mineral intake is inadequate or as a secondary deficiency when other factors in the diet interfere with the absorption and metabolism of the concerned trace minerals (Olson *et al.,* 1999).

Chelation, which literally means, “bringing together”, refers to a bonding formed between a metal ion (mineral) and a ligand (protein or amino acid chelating agent) carrier. A mineral complex is a mixture consisting of a mineral and an organic compound carrier, such as a protein or polysaccharide; a chelate is a type of complex. Chelates are generated by reacting a mineral salt with amino acid, for example, an enzymatically prepared mixture of amino acids and small peptides, under controlled conditions. The ligand binds the metal at more than one point such that the metal atom becomes part of a ring. Certain amino acids and protein digestion products such as small peptides are ideal ligands because they have at least two functional groups (amino and hydroxyl) that can form a ring structure with the mineral. The resulting mineral-organic complex is a “chelate”. The primary chelated minerals used in animal feed are the trace elements iron, manganese, cobalt, copper, and zinc.

Minerals are supplied to the livestock through mineral mixture in the inorganic form. One of the major disadvantages of using such supplements is that the minerals from such sources are not fully absorbed due to antagonism and anti-nutritional factors present in the diet. However, in recent years, chelated minerals are being supplemented in the ration as the bio-availability of these minerals is more than their inorganic forms

(Spears, 1989). By adhering closely to the recommended dietary levels for each of the trace minerals (NRC, 1989), the production and other physiological problems associated with an impaired mineral metabolism may be avoided (Underwood and Suttle, 1999).

Singal and Lohan (1988) and Shah *et al.* (2003) observed 40 and 46 per cent of estrus with mineral supplementation in anoestrus buffaloes, respectively. Bisla *et al.* (2006) and Kumar (2008) recorded more number of animals exhibited estrus and improved in the conception rate in post partum anoestrum buffaloes with mineral supplementation. Markendeya *et al.* (2010) observed estrus in 40 % of cows and 35 per cent of buffaloes fed with mineral supplements containing feed pellets.

Jyothi Sharma *et al.* (2009) observed estrus in 66.67 per cent of the cattle fed with concentrate feed containing Dicalcium phosphate, copper sulphate and magnesium sulphate. Similarly, with chelate mineral supplementation Campbell *et al.* (1999) and Formigoni *et al.* (1993) noticed early post partum estrus and early first service in cows. Ghadage *et al.* (2010) also observed higher estrus in post partum buffaloes.

Keeping above in the view, the present study was undertaken to test the efficiency of different forms of minerals based on the absorption and bio availability comparing the bolus form of mineral supplementation and non chelated mineral mixture with chelated mineral mixture on reproductive performance in post partum anoestrus graded Murrah buffaloes and also the serum mineral profiles of this experimental buffaloes were estimated before and after treatment to see the changes in serum mineral profiles and effect of mineral supplementation.

At present, the information on blood mineral attributes during the anoestrum and estrum in buffaloes with impaired ovulation is scanty. In view of the above, the present study was conducted to evaluate the effect of different mineral supplements like Dicalcium phosphate plus trace mineral bolus, non chelated mineral mixture and chelated mineral mixture on post partum anestrum in buffaloes with the following objectives.

- To estimate the mineral profile in graded Murrah buffaloes before mineral supplementation and during treatment to observe the change in serum mineral profiles in treatment groups.
- To study the estrus parameters.
- To study the improvement of the reproductive performance in graded Murrah buffaloes after mineral supplementation.
- To evaluate the cost effective mineral supplementation protocol.

CHAPTER II

REVIEW OF LITERATURE

The production potential of buffaloes is constrained by its low reproductive efficiency due to higher age of puberty, poor conception rates, longer service period and calving interval. Reproduction in animals is known to be influenced by environment, nutrition, hormonal status, infections along with genetic traits. For every successful pregnancy and parturition a proper synergism between anabolic and catabolic reactions was essential. Minerals have a basic key role in the maintenance of metabolism and studies on their nutritional requirements in the body led to the classification of these minerals as major and minor elements. Reproductive failure may be induced by deficiencies of single or combined trace elements and or by their imbalance (Hidiroglou, 1979). In general sense all vitamins and essential minerals were required for reproduction because of their cellular roles in metabolism, maintenance and growth. The postpartum female has to make a series of physiological and anatomical readjustments in the uterus and ovaries for restoration of her reproductive potential. The phenomenon of ovarian activity is the key factor in resumption of post partum reproductive function.

Anoestrum is one of the major and most common reproductive disorders in buffaloes, which leads to prolonged intercalving periods, loss of milk as well as calf production and increased maintenance cost of the animal. High incidence of post partum anoestrum is mainly due to ineffective management and malnutrition.

To hasten the post partum reproductive process, various scientists have tried numerous forms of therapeutic protocols such as hormonal treatments, improvement of nutrition, management and climatic conditions etc. In case post partum buffaloes especially in high producing buffaloes there is loss of body condition score and fall in negative energy balance. In this context there extra nutritional supplement could be required for maintenance and also for the optimum further reproduction. Use of different kinds of mineral supplementation in the early postpartum period could be beneficial in improving fertility in buffaloes by decreasing days open, increasing conception rate and reducing the number of services per conception. The relevant literature and the data published for the same have been reviewed and cited below.

2.1 INCIDENCE OF POST PARTUM ANOESTRUM

Four main forms of anestrous i.e. true anoestrum (inactive ovaries and small and medium sized anovulatory follicles), sub-estrus, prolonged luteal activity and ovarian cysts in addition to pregnancy. Differentiation between true anoestrum and sub estrus is particularly important in buffaloes because of their weak estrous signs.

Luktuke and Roy (1964) reported that the incidence of silent and anovulatory first post partum estrus was 14.6 per cent and 6.5 per cent in Murrah buffaloes respectively. Kodagli (1968) recorded a higher incidence of anoestrum (43.85 per cent) than luteal persistency (12.27 per cent) and silent ovulations (8.76 per cent) in Jaffarabadi buffaloes. Rao and Murthy (1971) reported that anoestrum condition was 46.76 and 41.22 per cent in cows and buffaloes respectively, in village herds of Andhra Pradesh. Bansal (1976) recorded the incidence of reproductive disorders in buffaloes ranging from 6.20 to 12.50 per cent, of which anoestrum was found to be 56.82 to 70.57 per cent of total disorders. Luktuke and Sharma (1978) observed an incidence of 56 per cent true anoestrus in buffalo heifers compared to 32.82 per cent in buffaloes. Rao and Sreemannarayana (1982) indicated overall incidence of quiescent ovaries as 56.36 per cent, while its incidence was 79.07 per cent in heifers and 42.59 per cent in cows. Shrivastava and Kharche (1986) reported that the incidence of normal and abnormal cycling was 29.47 and 69.73 per cent respectively in buffaloes where as in anoestrum was recorded to be 30.47 per cent. Kumar and Agarwal (1986) indicated that the incidence of post partum anoestrum in cows showed great variation from as low as 3 per cent and as high as 41.40 per cent.

Banerjee *et al.* (1992) reported that the incidence of true anoestrum, sub-functional ovaries, and missed estrus, heat during examinations, pregnancy and persistent corpus luteum was 33.47, 22.82, 36.08, 6.08, 0.86 and 0.65 per cent respectively in graded Murrah buffaloes. Rao *et al.* (1993) and Kumar and Kumar (1993) reported that the incidence of anoestrus in field conditions has been reported between 30 to 70 per cent in breedable buffaloe populations. Sreemannarayana and Narasimha Rao (1997) observed that the incidence of smooth ovaries, post-service anoestrus, postpartum anoestrus, silent ovulation, anovular heat and cystic ovarian degeneration were 26.1, 15.1, 10.5, 6.8, 1.4 and 0.5 per cent respectively in buffaloes under village management. Singh *et al.* (1998) indicated that 58.8 per cent lameness affected cattle were found to have prevalent reproductive disorders of which 23.97 per cent accounted for anoestrus in Punjab. Tomar *et al.* (2002) indicated that the incidence of anoestrum in buffaloes was as low as 55.99 per cent to as high as 66.73 per cent in autumn and summer respectively. Vivek Kunj *et al.* (2002) recorded month wise and season wise post-partum anoestrum in buffaloes 34.18 per cent and stated that the higher per cent observed in autumn with 38.75 per cent followed by summer (37.73), winter (29.41) and spring (28.30). Sathesh Kumar and Punniamurthy (2003) reported that the incidence of anoestrum due to smooth ovaries was higher in buffaloe heifers. Pandit (2004) indicated that the incidence of anoestrum was 25.23 per cent in buffaloes. Naidu and Baburao (2004) reported that true anoestrum was 30.76 per cent in post-partum buffaloes. Kasthuri (2006) recorded the incidence of reproductive disorders like anoestrum, unobserved estrus, and infantile genitalia, anovulatory heat, delayed ovulation, repeat breeder, cervicitis, endometritis, cystic overies and miscellaneous cases recorded 43.08, 3.41, 10.22, 1.48, 0.53, 4.38, 3.90, 10.88, 3.88 and 0.58 per cent respectively in cross bred cows. Reddy (2007), Prahalad (2009) indicated that the post-partum anoestrus was found to be 18.45 and 18.65 per cent respectively in farm conditions.

2.2 EFFECT OF BODY CONDITION SCORE (BCS) IN POST PARTUM ANOESTRUM

Generally used body condition scoring (BCS) system in dairy cattle is a 5-point scale, with 1 corresponding to an extremely thin cow, and 5 to a cow with excessive fat deposits (Wildman *et al.,* 1982). It has been demonstrated that the body condition score (BCS) recorded one month after calving had the largest correlation with calving interval, particularly in first-lactation cows (Price *et al.,* 2000). Similarly, high-producing cows with low BCS five weeks or more after calving can be expected to have delayed estrus resumption. Cows with poor BCS had fewer normal oocytes than cows with higher scores, whereas follicle numbers were higher in cows with BCS of 3 to 5 (Dominguez, 1995).

Body condition score 1. Cows will have deep cavity around tail head. Bones of pelvis and short ribs are sharp and easily felt. No fatty tissue in pelvic or loin area. Deep depression in loin.

Body condition score 2. Cows will have shallow cavity around tail head with some fatty tissue lining it and covering pin bones. Pelvis easily felt. Ends of short ribs feel rounded and upper surfaces can be felt with slight pressure. Depression visible in loin area.

Body condition score 3. Cows will have no cavity around tail head and fatty tissue easily felt over whole area. Pelvis can be felt with slight pressure. Thick layer of tissue covering the top of short ribs which can still be felt with pressure. Slight depression in loin area.

Body condition score 4. Cows will have folds of fatty tissue are seen around tailhead with patches of fat covering pin bones. Pelvis can be felt with firm pressure. Short ribs can no longer be felt. No depression in loin area.

Body condition score 5. Cows will have tail head is buried in thick layer of fatty tissue. Pelvic bones cannot be felt even with firm pressure. Short ribs covered with thick layer of fatty tissue.

Richards *et al.* (1989) reported that anovulation occurred in mature Hereford cows when they had lost 24 per cent of their body weight and 36 per cent of their BCS in 26 weeks. Ryan *et al.* (1994) observed that Body Condition Score of cows at calving influenced the number of medium and large (≥ 10 mm) follicles at 5 to 17 days of postpartum. Obese cows (BCS 8) had more medium follicles at 5 days of postpartum than cows with BCS of 3 (very thin), 4 (thin), or 6 (optimal), and BCS 8 cows had more large follicles at 9 days of postpartum than cows with BCS of 3 or 4 at 17 days of post partum, very thin cows had fewer medium and large follicles than cows with BCS of 4, 6, or 8.

Anitha *et al.* (2010) developed a new BCS system for Murrah buffaloes. The skeletal check points were identified by studying the anatomical features and amount of fat reserves in slaughtered animals. The scores were assigned from 1 to 5 based on the amount of fat reserves in slaughtered animals. A score of 1 represents least and 5 represents most amount of fat. The skeletal check points identified were ordered based on the amount of carcass fat reserves and scores assigned to prepare a preliminary BCS chart on a 1 to 5 scale at 0.25 increments. The BCS chart was further modified by eliminating the skeletal check points at which the fat reserves were less evident on palpation in most of the buffaloes and a new BCS chart on a 1 to 5 scale at 0.5

increments examining eight skeletal check points was developed. The new BCS system developed was tested for precision in 10 buffaloes for each point of the 1-5 scale by ultrasonographic measurements of body fat reserves. Ultrasonographic measurements showed that as the BCS increased, the amount of fat reserves also increased ($p < 0.01$), indicating that the BCS adequately reflected the amount of actual fat reserves. BCS was significantly correlated ($r = 0.860$) with the carcass fat reserves as well as the ultrasonographic fat reserves ($r = 0.854$).

2.3 INTERACTION OF MINERALS IN SOIL, PLANT AND ANIMAL

Williams (1977) and Fletcher and Doyle *et al.* (1978) stated that trace minerals of natural feeds is determined primarily by the mineral availability from the soil and secondly by the actual mineral composition of soil. Mineral composition of the soils is influenced by origin of parent rock (Fletcher and Doyle, 1978), glaciation, leaching, surface, erosion, evaporation, salinization, application of pesticides to soil, fertilizers, trace elements, manures and sludges, aerial fallout from industry and transportation (Frank *et al.,* 1976; Horvath and Reid, 1980 and Williams, 1977). Horvath and Reid (1980) reported that mineral uptake by crops and pastures influenced by soil factors like acidity, moisture or drainage conditions, temperature and seasonal effect.

The availability of mineral in soil depends upon their effective concentration in soil solution (Hockstca, 1973) which is influenced by pH, moisture, organic matter, leaching, and presence of other elements and microbial activity of soil (Burk, 1978 and Williams, 1977). Alkaline soils lead to an increased biological availability of trace elements selenium and molybdenum (Burk, 1978 and Williams, 1977). With decreasing soil pH, mineral is less available but the uptake of cationic metal copper improves (Burk, 1978 and Williams, 1977). Copper besides some trace minerals form insoluble complexes with the organic matter in the soil (Williams, 1977). Soil leaching erosion and long term cropping lead to a depletion of biologically active trace elements and some elements in the soil from complexes with the minerals (Smart *et al.*, 198l), e.g. sulphate, which will inhibit the uptake of selenate and selenite from the soil (Burk, 1978).

Chauhan and Nderingo (1997) reported that sixteen representative samples each of soil and pasture were collected during the rainy and dry seasons in Tanzania. Blood samples were collected from 42 cows during different phases of normal reproduction. Ranges of the mineral contents of the soils were: Ca 0.62-0.92 per cent, P 1.25-1.96 per cent, Mg 1.83-1.88 per cent, Cu 11.68-13.93 ppm (mg/kg), Zn 5.34 ppm, and Mn 40.64-41.14 ppm. The pasture content of Ca, P, Mg, Cu, Zn and Mn were 0.34-0.36 per cent, 0.25-0.32 per cent, 0.21-0.27 per cent, 4.68-4.85 ppm, 13.39-13.56 ppm, 39.30-41.51 ppm, respectively. The mineral content in the blood serum of cycling, pregnant, and early and late post partum cows was Ca 9.64-1.75 mg/100 ml, P 3.90-5.27 mg/100 ml, Mg 2.53-2.94 mg/100 ml, Cu 0.64-0.75 ppm (mg/litre), Zn 0.57-0.74 ppm and Mn 0.28-0.35 ppm, respectively.

Lall *et al*. (2000) observed in survey on the feeding practices of buffaloes in a village in Hissar district, India revealed that more than 80 percent of the lactating buffaloes were in short supply of essential minerals like calcium, phosphorus, zinc and manganese as indicated by the analysis of samples of feeds and fodders collected from the village. These rations were deficient in calcium and phosphorus to the extent of 30-40 per cent, zinc (Zn), manganese (Mn) and copper (Cu) to the extent of 50 percent. Low contents of Zn (18-42 ppm), Mn (12-18 ppm) and Cu (3-10 ppm) in all of the feeds (wheat, bajra, cottonseed cake and wheat straw) is leading to a problem of long anoestrus periods in 70 per cent of buffaloes. Crop management and climatic conditions also influences the eventual trace mineral level in feeds (Sharma *et al.*, 2003). Fertilization and/or heavy rainfall can result in lush pasture growth and the dilution of some trace minerals (Burk, 1978). In one study nitrogen fertilizer increased the copper content of the forage (Mudd, 1970). Stage of plant maturity and method of forage handling influence the availability of trace elements to animals (Horvath and Reid, 1980). As the plant matures there is a gradual decline in the trace mineral content particularly copper and zinc (Gladstine and Loneragan, 1967).

Rajiv *et al.* (2005) correlated calcium, phosphorus and magnesium status of soil, fodder and crossbred cattle in sub-mountainous zone of Shiwalik hills involving Ropar, Hoshiarpur and Gurdaspur districts of Punjab. He analyzed 84 blood samples from crossbred cattle of different age groups along with 31 fodder samples *(Sorghum* spp., *Pennisatum typhoides* and *Zeumays. L* and *Trifolium alexandrium L*) and 20 soil samples were collected for estimation of calcium, phosphorus and magnesium levels. The average values of Ca, P and Mg in plasma samples of crossbred cattle were 2.22 ± 0.03 mmol/l, 1.87 ± 0.04 mmol/l and 0.97 ± 0.02 mmol/l, respectively. All the sampled fodders were adequate in Ca and Mg contents, whereas, 36.84 per cent of non-leguminous fodders were deficient in phosphorus.

Sharma *et al.* (2006) conducted a survey in 2 phases in certain parts of Northern India to record the prevalence of mineral deficiency in bovine. Soil samples (547), fodder samples (609) and serum samples (638) were collected from the districts of Nainital, Almora, Bageshwar, U.S. Nagar, Pilibhit, Bareilly, Badaun and Rampur. The prevalence of soil Ca, P and Mg deficiency was 21.35 per cent, 23.30 per cent and 28.64 per cent, respectively, while that of fodder Ca, P and Mg deficiency was 13.88 per cent, 16.55 per cent and 19.72 per cent, respectively. The overall prevalence of serum Ca, P and Mg deficiency was 27.15 per cent, 27.61 per cent and 23.13 per cent, respectively. The correlation of coefficient of Ca, P and Mg in soil, fodder and serum was highly significant and in most of the cases the values were above 0.7. The highest deficiency was observed in plain region, followed by Tarai (foothills of Himalayas) region and then hilly region. Regarding haemato biochemical profile significant decrease in Hb, TEC was observed while the values of TLC were slightly higher in deficient buffaloes. The values of serum enzymes, viz. AST, ALT and Ca were lower, whereas that of SAP was higher. The values of thyroid hormones (T3 and T4) and vitamins (A and E) were significantly lower in deficient animals.

Saraswathi (2009) reported that the macro and micro mineral contents in soils of Pithoragarh district were higher than their respective critical levels except Calcium. 12.5 per cent of soil samples were deficient with Cu. The feed ingredients were found low in Ca, P, Mg and Cu while blood serum of cattle and buffaloes was low in Ca, Cu and Mn contents. Concentrate samples were found optimum for all minerals, but Ca (0.156 per cent) and Cu (7.1 ppm) contents were below their critical levels. The roughages had 0.175 per cent of Ca and 6.78 ppm of Cu was below critical levels, while P, Mg, Zn, Fe, Co and Mn contents were above the critical levels. The blood serum P (5.29 mg/dl), Mg (4.22 mg/dl), Zn (1.06 ppm) and Fe (2.76 ppm) concentrations were above the critical levels, whereas serum Ca (4.33 mg/dl), Cu (0.34 ppm) and Mn (0.18 ppm) concentrations were below their respective critical levels. Present investigation indicated the critical deficiency of Ca and Cu and slight deficiency of Mn in the animals of this region. There is a need of dietary supplementation of these minerals as area-specific mineral mixture for improving the productive and reproductive efficiency of animals of this region.

Das *et al.* (2011) reported the mineral status in soil, fodder and serum from four districts of Tripura state. There is a significant deficiency of P, Zn, and Cu in the soil, fodder, serum (cattle) of Tripura state. The levels of Hb (8.58 ± 0.45, 9.16 ± .38, 9.25 ± 0.42, 8.69 ±0.40 g %), PCV (20.30 ± 1.73, 24.64 ± 1.65, 25 ± 1.70, 22.77 ± 1.55%), TEC (5.62 ± 0.17, 5.38 ± 0.28, 5.43 ± 0.25, 5.11 ± 0.19 × 10^6/µl) in the region of west, south, north and Dhalai districts of Tripura state respectively. A mineral mixture was prepared according to the deficiency obtained and a therapeutic trial was conducted on some cattle in local and Govt. dairy farm for 90 days. Significant improvement was observed in haemato biochemical status of the deficient animals.

Singh *et al.* (2011) reported that sample of feeds and fodders and serum samples of cows in 8 villages, 2 from each block and 2 blocks from each of the 2 districts namely Bargarh and Jharsuguda of West-central table land zone (WCTLZ) of Odisha were collected and analyzed for macro and micro mineral content. Among the fodders, paddy straw was deficient in calcium (Ca), phosphorus (P), copper (Cu) and manganese (Mn). Deficiency of P was observed in most of the fodders. Most of the concentrate contained higher level of the analyzed minerals. The average serum Ca, P, zinc (Zn), Cu, Mn and iron (Fe) content of cows in WCTLZ were found to be 6.81 ± 0.14 mg/dl, 3.67 ± 0.09 mg/dl, 0.90 ± 0.02 ppm, 0.68 ± 0.01 ppm, 0.26 ± 0.004 ppm and 2.38 ± 0.07 ppm, respectively. The percentage of animals deficient in serum Ca, P, Zn, Cu and Mn were observed to be 75.8, 72.5, 13.3, 29.1, 9.1 and 5.8 per cent, respectively. The serum mineral content of the animals of WCTLZ was highly deficient in Ca and P. Deficiency of Zn, Cu, Mn and Fe was also noticed in some animals.

Kumar *et al.* (2011) reported that micro minerals have a great impact on animal's reproductive physiology and its imbalance causes various problems leading to lowered reproductive efficiency and resultant monetary loss to the dairy industry. Adequate micro minerals supplementation is required as most of the roughages, greens, concentrates and even most of commercial feeds available to Indian market are deficient in trace mineral elements. Correcting an imbalance in mineral levels can solve a nagging problem by improving reproductive performance and health with little additional cost. As terrain and agro climatic area of India is quite diverse, so one therapeutic treatment may not be suitable for other regions. Hence there is a need to map of the various nutrient statuses in soil, fodder and animal, so that accordingly an area specific mineral may be supplemented.

2.4 STUDIES ON THE ESTRUS CYCLE

2.4.1 Estrus Induction

Bhalla *et al.,* (1967) reported that the average interval of post partum estrus was 185.0 ± 13.65 and 96.55 ± 6.38 days in buffaloes and sahiwal cows respectively. Rao *et al*. (1973), Paragoanker and Kaikini (1974) and Sheikh and Mohmed (1976) reported that the first postpartum estrus was 125.73 days in Surti buffaloes, 55.10 days in Nagapuri buffaloes and 147.37 ± 12.61 days in Egyptian buffaloes, respectively. Singh *et al*. (1979), Jainudeen *et al*. (1983), Swain and Bhatnagar (1983) and Usmani *et al.* (1985) reported that the first postpartum estrus was 76.96 ± 4.62 days in buffaloes, 88 ± 26 days in Suckled swamp buffaloes, 87.38 ± 3.66 days in Murrah buffaloes and 56.40 ± 3.90 days in Nili-Ravi buffaloes respectively.

Jainudeen (1986) and Barkawi *et al*. (1986) reported that an average of 75 days (range 35-185 days) for first post partum estrus in River buffaloes and 90 days (range 40-275 days) for postpartum Swamp buffaloes and 40.4 ± 3.8 days in Egyptian buffaloes. Shrivastava and Kharche (1986) reported that 33.50 per cent of buffaloes exhibited postpartum estrus within 120 days of calving.

Suthar and Kavani (1992) reported that an average first postpartum estrus was 56.72 ± 3.58 days in Mehasana buffaloes. Tiwari and Pathak (1995) recorded that the onset of estrus after postpartum was 57.7 ± 5.61 and 28.3 ± 2.32 days for Suckled and non-suckled groups of buffaloes and Shah (1999) recorded 96.75 ± 14.39 days in post partum estrus Surti buffaloes.

Chowdary (2003) reported that the onset of postpartum estrus was 137 days in Murrah Buffaloes. Khasatiya *et al.* (2004) reported that the onset of postpartum estrus was 125 and 134.80 days in Surti buffaloes. Khasatiya *et al.* (2005) recorded that the average interval between parturition and first onset of estrus as 69.00 ± 7.36 and 141.82 ± 21.32 days in fertile and infertile Surti buffaloes, respectively.

2.4.2 Duration of Estrus

Pradyumna Rao *et al.* (1982) recorded the mean duration of estrus was 24.1±0.69 hours (ranged between 15 and 36 hours) in Murrah buffaloes. Satishkumar (1986) noticed that the duration of estrus was ranged between 20 and 36 hours in non-descript buffaloes. Danell (1987) reported that the average duration of estrus appears to be slightly longer in River buffalo (23.8 ± 6.2 hours) than in Swamp buffaloes (19.9 ± 4.4 hours).

Lamothe-Zavaleta *et al.* (1991) recoded the duration of estrus in Zebu cattle was shorter with increasing environmental temperature (>27^{0}C). Figueiredo *et al.* (1997) reported that the estrus length of 13.6±1.0 hours in natural estrus of Nellore cows in Brazil.

2.4.3 Intensity of Estrus

Intensity of estrus was assessed by the symptoms exhibited by the buffaloes during estrus. Estrus symptoms are less obvious in buffaloes then cows. Agarwal and Purbey (1983) reported that the intensity of estrus in buffaloes under rural condition was observed to be 15-38 per cent in pronounced state while 45.19 and 39.43 per cent found in normal and weak respectively. Rao and Kodgali (1983) and Singh *et al.* (1984) observed that the prominent estrus symptoms of bellowing (68.8 and 58.7 per cent), mucus discharge (83.7 and 79.3 per cent), congestion of vulva (86.8 and 66.3 per cent) and frequent urination (83.7 and 79.3 per cent) in buffaloes, respectively.

Banerjee *et al.* (1989) observed that the symptoms of swollen vulva, mucus discharge and frequent urination in 88.0, 68.0 and 75.5 per cent of buffaloes, respectively and also noticed that the temporary engorgement of teats in 81.00 per cent of buffaloes prior to estrus. Kaikini (1992) recorded that the incidence of silent estrus was 20-40 per cent in buffaloes. Gordon (1996) reported that the acceptance of male by female was the most prominent symptoms of estrus in buffaloes. Srivastava *et al.* (1998) observed that the estrus symptom of bellowing in 35.92 per cent buffaloes during estrus. Kumaresan *et al.* (2001) recorded that the typical fern pattern in mucus discharge during estrus in 57.0 per cent of buffalo.

2.4.4 Conception Rate

Chauhan and Singh (1979) and Singh *et al.* (1979) reported that the overall conception rate was 88.50 and 88.70 per cent in post partum buffaloes respectively where as Agarwal and Purbey (1983) recorded that the percentage of conception was 36.36 per cent in early heat and 52.77 per cent and 28.66 per cent in mid and late heat in postpartum buffaloes, respectively. Shanker *et al.* (1983) observed that the conception rates as 40, 40, 13.3 and 6.7 per cent at first, second, third and fourth inseminations respectively in postpartum cows.

Singh and Singh (1989) reported that the average conception rate in cows through AI under field conditions 37.5 per cent and also reported that 38.1 per cent conception rate in local breeds as against 37.0 per cent in crossbred cows. Bagal and Kadu (1989) reported that 50 per cent conception rate in postpartum crossbred cows.

2.5 SERUM MINERAL PROFILES

Several researchers have conducted studies to access the variations in blood serum inorganic phosphorus, calcium values in healthy buffaloe having normal regular estrus cycles and those in anoestrum. Assessment of macro and micro nutrients by screening blood profile is a satisfactory index for diagnosing deficiencies and also the nutritional and related reproductive status and abilities in buffaloes.

2.5.1 Serum Calcium

Dabas *et al.* (1987) reported that the average blood serum calcium levels were 9.5 ± 0.2 mg/100ml in anoestrous buffalo against 11.20 ± 0.15 mg/100ml in normal cyclic buffaloes.

Umesh *et al.* (1995) studied 40 pluriparous buffaloes consisting of 20 each of anoestrus and normal cyclic animals and reported that the serum calcium level was 10.432 ± 0.240 and 9.769 ± 0.230 mg/100ml in follicular phase and luteal phase and 9.798 ± 0.244 mg/100ml in anoestrus buffaloes respectively.

Newer *et al.* (1999) observed that the serum calcium in buffaloes during anoestrus, estrus and diestrus periods were 9.20 ±0.08, 10.76 ± 0.17 and 10.30 ± 0.21 mg % respectively. Pant (2000) reported that the serum calcium levels in anoestrus buffalo heifers, normal cycling heifers, anoestrus buffaloes and normal cycling buffaloes were observed to be 7.53 ± 0.39, 11.27 ± 0.70, 7.53 ± 0.39 and 11.51 ± 0.37 mg %, respectively. Paul *et al.* (2000) analyzed blood serum of anoestrus heifers, anoestrus lactating buffalo and cyclic buffalo for calcium and found to be 12.15 ± 0.37, 9.97 ± 0.80, 11.20 ± 0.3 mg/100 ml, respectively.

Shah *et al.* (2003) studied blood serum mineral profile from calving to conception in fertile and in infertile post-partum buffaloes and found that the overall levels of calcium in fertile buffaloe were found to be significantly higher than the infertile group during 1-2, 3-4 and 5-6 wks post partum. Khasatiya *et al.* (2005) reported that the serum calcium levels were 10.29 ± 0.18 and 11.32 ± 0.31 mg % in true anoestrus and sub-estrus Surti buffaloes, respectively.

2.5.2 Serum Phosphorus

Dabas *et al.* (1987) stated that the levels of serum inorganic phosphorus in post-partum anoestrus buffaloes was found to be 4.0 ± 0.3 mg/100ml against 6.2 ± 0.25 mg/100ml normal cyclic buffaloes and found that the level was found to be significantly lower in anoestrus buffaloes compared to normal cycling animals.

Umesh *et al.* (1995) analyzed buffalo blood serum for phosphorus and found to be 5.369 ± 0.207, 3.261 ± 0.171 mg/100 ml in follicular and luteal phase respectively against 2.030 ± 0.099 mg/100 ml in post partum anoestrus buffaloe and observed significant ($p < 0.01$) difference in the level of inorganic phosphorus. Jani *et al*. (1995) reported mean serum inorganic phosphorus was 8.50 ± 0.58 mg % in normal cyclic buffaloes.

Khattab *et al.* (1995) collected blood samples from 26 postpartum buffaloes and reported that serum phosphorus levels were 6.45 and 5.01 mg % in regular cyclic and in inactive ovaries, respectively. Chaurasia (1999) reported that the total and free form of serum phosphorus concentrations were significantly higher in normal cyclic (5.39 ± 0.14 and 5.29 ± 0.14 mg %) when compared to anoestrus (4.06 ± 0.20 and 3.96 ± 0.20 mg %) in Murrah buffaloes.

Lall *et al.* (2000) observed in survey on the feeding practices of buffaloes in a village in Hisar district, India revealed that more than 80 per cent of the lactating buffaloes were in short supply of essential minerals like calcium, phosphorus, zinc and manganese as indicated by the analysis of samples of feeds and fodders collected from the village. These rations were deficient in calcium and phosphorus to the extent of 30-40 per cent and zinc (Zn), manganese (Mn) and copper (Cu) to the extent of 50 percent. Low contents of Zn (18-42 ppm), Mn (12-18 ppm) and Cu (3-10 ppm) in all of the feeds (wheat, bajra, cottonseed cake and wheat straw) is leading to a problem of long anoestrus periods in 70 per cent of buffaloes. Pant (2000) reported that the serum inorganic phosphorus values in true anoestrus buffaloe heifers (3.73 ± 0.12 mg %) and cows (3.57 ± 0.09 mg %) were significantly lower than the corresponding values of normal cyclic buffalo heifers (5.25 ± 0.10 mg %) and cows (5.45 ± 0.12 mg %) respectively. Paul *et al*. (2000) reported that serum inorganic phosphorus levels were 7.13 ± 1.5 and 6.96 ± 0.26 mg % in normally conceived and anoestrus Nili-Ravi buffaloes, respectively.

Shah *et al.* (2003) analyzed calcium and phosphorus levels and stated that the overall inorganic phosphorus level of fertile group was significantly higher than infertile group. He also compared phosphorus from calving to conception in fertile and in infertile buffaloe and stated that there was no significant variation observed in phosphorus level of fertile and infertile estrus cycle.

Singh *et al.* (2004) estimated the serum calcium and inorganic phosphorus levels in 28 anoestrus buffaloes and 35 cyclic buffaloes. The levels of serum calcium and phosphorus were 8.33 ± 0.15 and 5.50 ± 0.22 in anoestrus and 10.45 ± 0.16 and

6.81 ± 0.11 mg/dl in cyclic buffaloes, respectively. Khasatiya *et al*. (2005) reported that serum inorganic phosphorus levels in true anoestrus and sub-estrus Surti buffaloes were 4.79 ± 0.16 and 5.93 ± 0.17 mg per cent, respectively.

2.6 ROLE OF MINERALS IN FEMALE REPRODUCTION

Animals require minerals, which act as structural components of organs and tissues, as cofactors or activators in enzyme and hormone systems, as constituents of body fluids and tissues, and as regulators of cell replication and differentiation. Deficiencies of certain mineral elements cause reproductive disorders as minerals play an important role in health and reproduction of the livestock. Infertility and reproductive disorders has emerged as an important problem in our livestock population.

Acharya (1960) stated that lack of phosphorus and calcium upset the proper functioning of the reproductive organs in cattle. Trace elements also related to fertility. Wagner (1962) reported that most common cause for anoestrus was probably with nutritional deficiency of cobalt and phosphorus in New York.

Doisy (1974) reported that the manganese has a role in steroid synthesis and inhibition of cholesterol and cholesterol precursor synthesis, manganese deficiency may be involved in inhibiting sex hormones and steroid synthesis and it also had a role in luteal tissue metabolism. Porwal *et al.* (1976) showed that deficiencies of certain serum macro- and micro-elements may cause reproductive disorders.

Desai *et al.* (1982) reported that the higher serum copper levels indicated higher oestrogenic (higher copper levels during follicular phage) and lower FSH and LH activity in the serum. Veldhuis and Klase (1982) observed that calcium dependent mechanisms are involved in steroid biosynthesis in the testes, adrenal glands and ovaries. Sharma *et al.* (1983) reported that serum calcium and phosphorus levels were deficient in the anoestrus cows and were statistically significant.

Maas (1987) reported that the reproductive performance of cattle may be compromised if Zn, Cu, or Mn status is in the marginal to deficient range. Common Cu deficiency symptoms in cattle include delayed or suppressed estrus, decreased conception, infertility and embryo death. Inadequate Zn levels have been associated with decreased fertility, abnormal estrus, abortion, and altered myometrial contractibility with prolonged labour. Manganese deficiency in cows results in lower of CR (Conception Rate), delayed estrus in post partum females and young prepuberal heifers, infertility, abortion, immature ovaries and dystocia.

Olson *et al.* (1999) reported that the combination of Cu, Co, Mn, and Zn in an organic or inorganic form fed at higher than nutrient recommendations for 2-year-old cows from calving to breeding would affect pregnancy rate, calving date, calf performance, and cow liver and serum mineral concentrations. Cows were allotted to one of three treatments after calving, 1) nosupplemental minerals (CTL), 2) organic minerals (ORG), or 3) inorganic minerals (ING). Minerals were fed to all cows at the same quantity for both organic and inorganic treatments: Cu (125 mg), Co (25 mg), Mn (200 mg), and Zn (360 mg). Cows were individually fed mineral-protein supplement with grass hay from calving (February-March) to before breeding (May, 15). Hay intakes were calculated using chromium oxide boluses to determine faecal output. Faecal excretion of minerals was calculated following trace element analysis of faeces. Liver biopsies were obtained before calving, after calving (start of supplementation), at the end of supplementation and in midsummer. Over 2 years, more cows did not become pregnant ($p < 0.01$) in ORG (11/ 78) and ING (11/78) treatments than in CTL (0/80) treatments. Cows in the ORG group conceived later ($p < 0.01$) than cows in the ING or CTL groups in 1994. In 1995, there was no difference ($p > 0.10$) in day of conception among groups. Liver Zn and Mn concentrations were not different ($p > 0.10$) and Cu concentrations increased ($p < 0.01$) for the ORG and ING groups. Cows in the ORG and ING groups had higher ($p < 0.01$) concentrations of Cu, Mn, and Zn in the faeces than the CTL cows. Trace elements in the faeces did not differ for ORG and ING groups. Results indicate that combinations of Cu, Co, Mn, and Zn fed at higher levels than are required reduced reproductive performance.

Hypocalcemic cows at parturition had 4.8 times greater risk of developing left displacement of abomasum (LDA) than normocalcemic cows (Massey *et al.,* 1993). Cobalt, Cu, Mn, and Zn have important roles in maintaining fertility, health and production of dairy cattle (Miller *et al.,* 1988 and NRC, 2001) and cows experiencing LDA had delayed postpartum first service (Raizman and Santos, 2002). Whiteford and Sheldon (2005) reported that cows experiencing hypocalcaemia had increased incidence and severity of uterine diseases and delayed return of ovarian cyclic activity. Brunob *et al*. (2010) reported that the deleterious effect of hypocalcaemia on subsequent fertility.

Wilde (2006) reported that infertility in dairy cattle is a complex, multi-factorial problem that cannot be evaluated in isolation of other diseases and disorders. Clearly there is a role for the prevention of problems in the peri-parturient period, in particular hypocalcaemia, mastitis, lameness and retained placenta (RP), that all have a negative impact on the subsequent fertility of the cow. Minerals, trace elements and vitamins play a vital role in the prevention of these disorders. Macro minerals are involved in the acid base status of the dairy cow and influence calcium metabolism. The use of anionic salts in combination with adequate calcium and magnesium supplementation may help to improve dry matter intakes and reduce negative energy balance in the post-calving period as well as prevent hypocalcaemia. Vitamin E and zinc are effective in prevention of mastitis that occurs predominantly in the first weeks of lactation, through enhanced antioxidant function and keratinisation of the teat canal. Lameness in dairy cattle also occurs mainly in lactation though most of the original insults to the hoof can occur prior to calving. Zinc and biotin are implicated in improving keratinisation of the hoof and prevention of this disease.

Pankaj Kumar *et al.* (2007) reported that micro mineral deficiency also affected the level of serum oestrogen and progesterone during various stage of physiology, thus resulted in reduced fertility, repeat breeding, poor conception, abortion and increase in age for sexual maturity of heifers.

Juchem (2010) evaluated the effect of feeding protected fat as Ca salts and observed increased conception rates in cows fed calcium salts of linoleic and monoenoic C18 trans fatty acids (unsaturated fatty acids) compared with cows fed calcium salts of palm oil (highly saturated fatty acid). This improvement in fertility was attributed to higher fertilization rates (87.2 per cent versus 73.3 per cent), and the greater proportion of embryos graded as 1^{st} and 2^{nd} (73.5 per cent versus 51.5 per cent), for saturated and unsaturated fatty acid diets, respectively (Cerri *et al.*, 2009).

2.7 EFFECT OF MINERAL SUPPLEMETATION ON SERUM MINERALS PROFILE

Henry *et al.* (1992) and Ward *et al.* (1996) reported that the traditional inorganic forms of trace minerals rapidly dissociate in the rumen and are free to interact with antagonists, resulting in the loss of the trace minerals prior to absorption by the animal. Ward *et al.* (1996) and Bailey *et al.* (2001) reported that Chelated organic trace minerals are bound to organic ligands through coordinate covalent bonds. The bonds between the ligand and the mineral can prevent the mineral from interacting with antagonists and improve the bioavailability of the mineral.

Spears (1996) suggested that organic minerals have an increased bioavailability, resulting in an increased absorption in the gastrointestinal tract and also lead to an improvement in performance or health or may reduce the level of mineral supplementation required in the diet.

Tambe (1996) reported that the mean concentration of the serum mineral levels like copper were 64.34 ± 2.35, 63.88 ± 4.23, 63.48 ± 2.72 µg% , iron were 325.6 ± 27.14, 345.92 ± 24.49, 399 ± 24.29 µg%, manganese were 39.62 ± 1.93, 49.42 ± 3.6 and 42.38 ± 3.2 µg% and for zinc were 1.24 ± 0.211, 1.73 ± 0.294 and 0.991 ± 0.116 mg% and calcium were 10.81 ± 0.51, 10.78 ± 0.38, 11.07 ± 0.37 mg%, respectively in follicular, luteal and anestrous phases of the reproduction in post partum anoestrus cows. There is no significant difference in the mean serum levels of all four minerals in follicular, luteal and anestrous phase of reproduction ($p < 0.05$).

Lall *et al.* (2004) reported that twenty one non-pregnant buffaloe heifers (3-4 years of age) showing anoestrus were taken from the institute's herd and given high plane (120 per cent) of nutrition and mineral mixture (3 per cent including common salt) in their concentrate diet mix. Blood samples from these animals were collected before the start of the experiment (0 day), then at 30 and 60 days; and the serum samples were analysed for calcium (Ca), phosphorus (P), zinc (Zn), copper (Cu). Calcium content of the samples showed little variation with the dietary treatment and ranged between 7.31-7.76 mg/dl. Phosphorus content improved significantly ($p < 0.05$) over the period and the levels were 4.65 ± 0.33, 5.35 ± 0.23 and 5.26 ± 0.17 mg/dl, at zero, 30^{th} and 60^{th} day intervals, respectively. Zn content also improved significantly ($p < 0.05$) over the period and the levels were 0.58 ± 0.08, 1.08 ± 0.02 and 1.34 ± 0.04 µg/ml, respectively. Cu was not influenced by the dietary treatment and ranged between 0.69-0.74 µg/ml.

Jyoti Sharma *et al.* (2009) reported that calcium, copper and manganese concentration in blood serum of all categories of animals also increased significantly and reached above the critical levels in animals due to dietary supplementation of calcium, copper and manganese.

Krys (2009) fed organic forms of microelements (Se, Zn, Cu, Mn) for four to five weeks before parturition and 1 week after parturition and observed slight increase of serum selenium and copper levels 60 μg/1 and 9 μg / l, respectively over the initial levels one week after parturition.

Chaurasia *et al.* (2010) recorded the low levels of free form of serum calcium, phosphorus in anoestrus buffaloes, when compare to the normal cyclic and repeat breeding Murrah buffaloes, there was no significant difference of bound form of calcium and phosphorus was found between the three groups.

Ghadage *et al.* (2010) studied the efficacy of chelated and conventional mineral supplementation on apparently healthy lactating buffaloes. 60 lactating Marathwadi buffaloes were divided into groups A, B and C and were supplemented with chelated mineral mixture, conventional mineral mixture and non-mineral mixture, respectively. Both mineral mixtures were administered at 30 gm orally once daily for 90 days. There were significant increases in Ca, P and Mg of groups A and B. The copper, iron and zinc levels were significantly higher in chelated mineral mixture supplemented animals than conventional mineral mixture supplemented buffaloes.

Hayat (2010) reported that serum calcium level were decreased after two weeks of post partum where as serum zinc levels were increased throughout the trail in zinc-methionine treated group when compared with that of control group.

Tiwary *et al.* (2010) collected blood samples from cattle and buffaloes of different physiological status viz., lactating, dry, heifer, pregnant and other reproductive health were collected. Minerals such as Ca, Mg, Zn, Fe, Cu and Mn were estimated and serum mineral concentration for Ca (10.43 ± 0.22 mg/dl), Mg (1.65 ± 0.14 mg/dl), Zn (1.54 ± 0.11 ppm), Fe (1.97 ± 0.19 ppm) and Mn (0.54 ± 0.02 ppm) in cattle and buffaloes of different physiological status were found above their respective critical levels. Whereas, P (3.79 ± 0.21 mg/dl) and Cu (0.46 ± 0.07 ppm) concentrations in blood serum of animals were below the respective critical levels. The average serum Ca, Mg, Zn and Fe differ significantly ($p < 0.05$) between the Roorkee and Laksar tehsils of Haridwar district of Uttarakhand. There was significantly ($p < 0.05$) higher serum Ca, Mg, Zn and Fe levels in animals of Laksar tehsil (10.82 ± 0.31 mg/dl, 1.88 ± 0.16 mg/dl, 1.92 ± 0.14 ppm and 2.44 ± 0.29 ppm, respectively) than in animals of Roorkee tehsil (10.03 ± 0.25 mg/dl, 1.42 ± 0.22 mg/dl, 1.16 ± 0.06 ppm and 1.49 ± 0.14 ppm, respectively).

Ullah *et al.* (2010) reported that blood mineral levels did not differ except for Zn, which was higher ($p < 0.05$) in mineral supplemented group in 2 out of 12 samples and concluded that mineral supplementation did not affect the post partum ovarian activity in buffaloes, in different seasons. Ashmead (1970), Wedekind *et al.* (1992) and Nockels *et al.,* (1993) reported that specific amino acid complexes of trace minerals are more bioavailable and are better retained in the body than inorganic sources of trace minerals.

2.8 EFFECT OF MINERAL TREATMENT ON POST PARTUM ANOESTRUM IN BUFFALOES

2.8.1 Mineral Bolus and Di Calcium Phosphate

2.8.1.1 *Estrus Induction*

Biswas *et al.* (2005) observed in 16 postpartum anoestrus Murrah buffaloes were equally divided into two groups which were fed a control diet (Group I) and a diet containing Complemin Forte mineral mixture at 50 g orally plus a tablet containing cobalt, iron and copper orally for 30 days (Group II). Blood samples were analysed before and after treatments and compared between groups. Serum calcium and phosphorus levels were significantly higher after mineral supplementation. Except iron, other minerals like manganese, copper and zinc concentrations were higher after supplementation. No buffalo in the control group came into oestrus, while only one buffalo in Group II came in to estrus after 21 days of supplementation and conceived by artificial insemination. It was indicating that the supplementation improves the serum mineral profile but does not influence the reproductive status of buffaloes.

Mavi *et al.* (2005) reported that in extreme summer (June), 26 true anoestrus buffalo heifers including two dry buffaloes aged 28 to 66 months and weighing 256 to 628 kg were divided into two groups. Eighteen heifers were supplemented for 20 days with 2 boluses of a mineral supplement, each having composition as: iron 200 mg, iodine 20 mg, cobalt 80 mg, manganese 80 mg, copper 100 mg, zinc 40 mg, selenium 0.5 mg and *Asparagus racemorus* 50 mg. Eight animals including 2 dry ones were kept as control. The treatment improved reproductive efficiency. Eight heifer in the treatment group (44 per cent) exhibited oestrus within 60 days of supplementation and 4 (22 per cent) conceived. In the control group, only 1 (12.5 per cent) exhibited oestrus and none conceived in the same period.

2.8.1.2 *Interval between Treatment and Onset of Estrus*

Rekhis *et al.* (2002) observed the effects of supplementation of di-calcium-phosphate in the form of blocks in late pregnancy (2 months before calving), on reproduction parameters of dairy cattle in smallholder farms. The experiment covered 63 animals in 20 smallholder farms, divided into control and supplemented groups. Results showed that mineral supplementation had a significant effect on calf weight, milk fat content and reproduction parameters. Inter-calving interval was lower by 38 days ($p < 0.05$) in the mineral supplemented cows compared to the control group. The body condition score of the cows were higher in the supplemented group than in the control group but the effect was not significant ($p > 0.05$).

2.8.1.3 *Conception Rate*

Biswas *et al.* (2005) observed in 16 postpartum, anoestrus Murrah buffaloes were equally divided into two groups which were fed a control diet (Group I) and a diet containing Complemin Forte mineral mixture at 50 gm orally plus a tablet containing cobalt, iron and copper orally for 30 days (Group II). No buffaloe in the control group conceived, while only one buffalo in group II conceived by artificial insemination.

2.8.2 Non chelated Mineral Mixture

Singal and Lohan (1988) estimated the serum phosphorus, zinc levels in infertile buffaloes and phosphorus levels were ranging from 1.02 ± 0.13 to 5.24 ± 0.70 mg/ 100 ml and zinc levels were less than 9 ppm, after 5 weeks of mineral supplementation for these infertile buffaloes, 40 per cent of them were exhibited the estrus and conceived after mating. Samanta (1995) observed in spontaneous cases of anaemia were recorded in grazing cattle. The anaemic cattle with loss of milk production (group II) and anoestrus (group III) showed decreased levels of Hb, PCV, blood glucose, total protein, serum P, Fe, Cu and Mn compared to non-anaemic controls (group I). Supplementation of minerals to animals of both groups (group II and III) improved body condition, increased milk yields and improved reproductive performance.

Sharma *et al.* (2002) prepared three types of mineral mixture according to deficiency obtained and fed to three groups of deficient animals. Observations were recorded on 0, 30th, 60th and 75th day. In group A animals normal mineral mixture was provided, where as in group B and C 10 per cent and 25 per cent more of Ca, P, Mg were provided, respectively. There was an increase in body weight, milk yield, haemoglobin concentration, and total erythrocyte count. Alanine aminotransferase, Aspartate amino transferase in group C animals. There was a decrease in heart rate, respiratory rate and alkaline phosphatase in group C animal after mineral supplement.

Lehmkuhler (2010) reported that with the mineral supplementation at appropriate levels optimized reproductive success and fertility of both the male and female.

Singh *et al.* (2010) studied the effect of supplementary feeding of Urea-Molasses Multi-nutrient Block (UMMB) enriched with area specific mineral mixture on productive traits of buffaloes, significant increase in the total plasma protein and albumin level were observed with no significant effect on Hb, PCV, glucose, BUN, calcium, phosphorous, magnesium, copper, zinc, iron, plasma inorganic iodine (PII) and manganese and closely monitored buffaloes revealed an average increase of 46.66 per cent in fodder intake, 51.70 per cent in milk yield and 13.88 per cent in milk fat following UMMB supplementation.

Singh (2011) observed that supplementation of deficient minerals, viz. Ca, P, Zn and Cu in the diet of cattle under existing feeding practices in West-Central Table Land Zone (WCTLZ) of Odisha is imperative for better health and productivity.

2.8.2.1 *Estrus Induction*

Singal *et al.* (1988) were reported after 5 weeks of mineral supplementation in infertile buffaloes, 40 per cent of them were exhibited the estrus. Baruah *et al.* (2000) reported in thirty post partum swamp buffaloes in their second to fourth lactation, not exhibiting estrus for more than six months and above were grouped into three groups, group A and B were allowed to graze and were given 60 gm and 100 gm mineral mixture, Group C were allowed to graze but without supplements. Soil samples from where the animals were grazed were also analyzed for their mineral contents. Results showed that in group A and B majority (n=8) of the animals exhibited estrus within 120-132 days of supplementation. In the case of non-supplemented group, only 2 out of 10 animals exhibited estrus within 168-178 days. Shah *et al.* (2003) reported that 14 out of the 30 (46.66 per cent) anoestrus buffaloes treated with mineral supplementation for a period of 30 days.

Lall *et al.* (2004) reported that twenty one non-pregnant buffalo heifers (3-4 years of age) showing anoestrus were taken from the institute's herd and given high plane (120 per cent) of nutrition and mineral mixture (3 per cent including common salt) in their concentrate diet mix. Eighteen out of 21 heifers came into heat and became pregnant within the first 3 months from the starting of dietary mineral supplementation.

Bisla *et al.* (2006) observed that buffaloes either at an organized farm or reared by marginal farmers are affected by a multitudes of parasites and nutritional deficiencies resulting in heavy economic losses. A total of 1000 buffaloes maintained by individual farmers in two adopted villages of Krishi Vigyan Kendra, Panipat, received different anti-parasitic treatments and 60 gm of mineral mixture/day/animal for 30 days during October to February months from the years 2001 to 2004. Following the treatment, 235/1000 buffaloes exhibited estrus and conceived. Kumar (2008) reported that 45 dairy buffaloes were divided into three groups. All the three groups were dry buffaloes of around 8 month pregnant which were supplemented without mineral mixture, with mineral mixture and with fortified mineral mixture respectively. The highest reproductive performance like post partum estrus occurrence, conception rate was observed in fortified mineral mixture supplemented group than the mineral mixture supplemented and control group.

Jyoti Sharma *et al.* (2009) examined the effect of strategic dietary supplementation of calcium, copper and manganese on reproduction and lactation performance of cattle in Pithoragarh district (Hill region) of Uttarakhand, India. The calcium, copper and manganese were supplemented in the form of concentrate feed which containing maize (34 per cent), deoiled mustard cake (16.5 per cent), deoiled soyabean meal (33.5 per cent), dicalcium phosphate (15.32 per cent), copper sulfate

(0.16 per cent) and manganese sulfate (0.52 per cent). The experimental animals were provided with 250 gm concentrate feed containing mineral mixture daily so as to supply 11.3 gm calcium, 96 mg copper and 320 mg manganese for 60 days over and above the quantity received by the animals through feeds and fodder provided by the farmers. Dietary supplementation of Ca, Cu and P made 66.67 per cent improvement in anoestrous animals and 50 per cent improvement in repeat breeding cases.

Markandeya *et al.* (2010) evaluated the efficacy of special feed pellets for optimum reproductive performance in post partum dairy animals. 20 Holstein-Friesian (HF) cows and 20 Murrah buffaloes were administered with 100 gm special feed pellets (200 gm Ca, 100 gm P and 40 000 IU vitamin D3/per kg pellets manufactured by Indian Immunological Limited, Hyderabad) daily for 15 days, forming the HFI and MBI groups. Similarly other 20 HF cows and 20 Murrah buffaloes were administered with market preparation of 100 ml liquid mineral supplement (1650 mg Ca, 850 mg P, 8000 IU vitamin D3, 100 micro g vitamin B12 and 40 000 mg carbohydrates per 100 ml liquid) daily for 15 days, serving as groups HF2 and MB2. Estrus was induced in 8 (40.00 per cent) cows and 7(35.00 per cent) buffaloes after treatment of special feed supplementation and 5 (25.00 per cent) cows and 2 (10.0 per cent) buffaloes after liquid mineral supplementation.

Saghar (2003) collected data from Nili-Ravi lactating buffaloes stationed at the Livestock Experiment Station, Bahadurnagar, Okara, Punjab, Pakistan was analysed to study the effect of supplementation of mineral mixture on onset of oestrus and observed 33 per cent buffaloes showed estrus in Group A (control) and 67 per cent in Group B (mineral mixture supplement), respectively.

Singh *et al.* (2010) studied the effect of supplementary feeding of Urea-Molasses Multi-nutrient Block (UMMB) enriched with area specific mineral mixture reproductive traits of buffaloes, none of the anoestrus control buffaloes came in heat during the study period however, after 30-45 days of completion of trial 72.72 per cent (9/11) buffaloes came to heat.

Ulla *et al.* (2010) investigated the effect of mineral supplementation on ovarian activity of post partum buffaloes in different season. Ten buffaloes were divided in two groups of five buffaloes each. The mineral supplemented group (MSG) was offered minerals 10 per cent more than NRC recommended Ca, P, Zn and Mn levels. The non-mineral supplemented group (NMS) was kept as control. Progesterone (P4), Oestradiol (E2), Ca, P, Zn and Mn were analyzed in blood serum of the buffaloes. The first post partum rise in P4 in NMS and MSG was observed on day 91.0 ± 29.7 and 54.8 ± 16.0 ($p > 0.05$), respectively. The first post partum rise in E2 in NMS and MSG was on day 24.0 ± 4.43 and 30.6 ± 6.33 ($p > 0.05$), respectively. Post partum ovarian activity resumed in 80 per cent in both groups. The ovarian activity lasted for 86.0 ± 23.8 and 132.0 ± 23.6 days and then the animals underwent anoestrus period for 174.0 ± 18.3 and 111.0 ± 38.6 days ($p > 0.05$) in MSG and NMS, respectively.

2.8.2.2 ***Duration of Estrus***

Chaudhry (1991) observed in three groups of Nili-Ravi buffaloes (10 per group), aged 10-14 months, and were fed *ad lib.* on green fodder plus a concentrate and a mineral supplement (group 1), *ad lib.*, Fodder plus concentrate (group 2), or *ad lib.* fodder alone (group 3). Concentrate feeding (1 per cent of body weight per day) was continued until attainment of sexual maturity. For the 3 groups duration of oestrus 19.8 ± 2.5, 19.1 ± 2.0 and 18.5 ±1.51 hours, respectively.

2.8.2.3 ***Interval between Treatment and Onset of Estrus***

Lall *et al.* (2000) reported supplementation with a mineral mixture at 50 gm/animal/day to 70 buffaloes and 30 adult heifers more than 3 years of age showing anoestrus for more than 5-6 months showed that more than 70 percent of these animals showed estrus and conceived within a period of 2-4 weeks of mineral supplementation in mineral deficiency.

2.8.2.4 ***Conception Rate***

Singal *et al.* (1988) reported that infertility in cattle and buffaloes in 2 villages in Haryana and observed that mineral deficiency was contributory factor to the infertility, after 5 weeks of mineral supplementation for these infertile buffaloes, 40 per cent were conceived after mating.

Black and French (2004) reported that the effect on the fertility of three commercial dairy herds of three types of copper and selenium-containing mineral supplements was investigated. As the cows on each farm were dried off they were allocated to one of three treatment groups, and treated with either subcutaneous injections of copper and selenium, or two matrix intraruminal trace element boluses, or two glass intraruminal trace element boluses. When the data from the 406 cows on the three farms were combined, there was a significant difference between the conception rates of the three groups ($p < 0.001$). The conception rate in cows treated with the glass boluses were 1.8 times greater than those treated by injection ($p < 0.001$), and 1.5 times greater than those treated with matrix boluses ($p = 0.002$).

Mavi *et al.* (2005) reported that 4 out of eight heifers were conceived in mineral supplemented group 4 conceived where as in the control group none were conceived. Ahola (2004) also found higher conception rate in a 60 days breeding season in beef cows supplemented with Zn, Cu and Mn compared to cows not supplemented.

Singh *et al.* (2010) studied the effect of supplementary feeding of Urea-Molasses Multi-nutrient Block (UMMB) enriched with area specific mineral mixture supplementation none of anoestrus buffaloes came in heat during the study period however, after 30-45 days of completion of trial 72.72 per cent (9/11) of the buffaloes came in heat and conceived to first insemination.

2.8.3 Chelated Minerals

Biological availability is the ability of the element or ion under consideration to support some physiological process (Peeler, 1972). It is well documented that when trace elements are complexed to organic molecules, such as amino acids (AA) their absorption into the animal's body and biological availability is increased compared to inorganic sources (Ashmead, 1970; Wedekind *et al.,* 1992; Nockels *et al.,* 1993).

The word chelation refers to a complex in which a metal atom is bound to two or more ligands (Rubin and Princiotto, 1963). The metal complex or chelate is stable in the digestive tract and protected from forming complexes with other dietary compounds that would otherwise inhibit its absorption (Spears, 1996). Metal Amino Acid (AA) chelates can be absorbed intact from the intestine which reduces the competition between the metal ions for absorption sites. The absorption of AA chelates from the intestine may stimulate certain physiological responses or enter target tissues at higher levels (Ashmead, 1970). In the animal, trace minerals occur and function as organic complexes or chelates and not as free inorganic ions (Spears, 1996). Therefore, inorganic mineral sources must be converted to organic forms for use by the animal (Ashmead, 1993). The trace minerals that occur naturally in feeds exist primarily as organic chelates or complexes (Spears, 1996). The differences in bioavailability between complexed and inorganic sources of trace minerals are confounded by level of antagonists (Wedekind *et al.,* 1992) and stress (Nockels *et al.,* 1993), with antagonists and stress having a greater negative impact on the bioavailability of inorganic trace mineral sources than amino acid complex sources. As a result, chelated trace minerals have traditionally been fed to cows experiencing stress from disease, health, reproduction or the environment with the goal of increasing the bioavailability of minerals to the animal to support performance and production. Increased display of oestrus and conception rates have been observed when trace minerals were added to deficient diets (Kropp, 1993). Even when dietary levels were adequate in trace mineral content, the supplementation of diets with Amino Acid Trace Mineral (AATM) have resulted in improved reproductive performance in beef cattle (Kropp, 1993) and dairy heifers (Manspeaker and Robl, 1993; Ashmead *et al.,* 2004). Toni *et al.* (2007) and Siciliano-Jones *et al*. (2008) observed minimal effects of replacing inorganic minerals with Amino Acid Treated Mineral (AATM) in dairy cow diets on fertility, but herd culling rate was decreased with feeding AATM. Wilde (2006) reported that organic forms of zinc are retained better than inorganic sources and may provide greater benefit in disease prevention. Retained placenta can be reduced by prevention of hypocalcaemia and also adequate selenium status of the dairy cow. Selenium yeast is known to have higher retention in tissues and may play an important role in ensuring sufficient selenium is available to the cow for reduction of disease.

Boga and Filik (2011) observed that organisms needed trace minerals to be able to conduct their life functions and be optimally and efficiently productive. Trace minerals have inorganic and organic forms. These are given to farm animals and included in the premix in inorganic structure melting form as chloride or sulfate, and non-melting form as oxide or carbonate. Trace mineral mixtures in these forms can begin antagonistic effects inside the premix, and their digestibility decreases significantly. In recent years, by using high technology, the trace minerals have been produced in the forms of capsule or chelate. These forms inside the premix have been saved from the antagonistic effects and have shown very high digestibility values. The higher bioavailability of organic minerals has positive effects on growing, immunity functions, amendment of metabolism, carcass quality and prevention of vitamin loss in vitamin-mineral complexes. It is well known that trace minerals has also environmental effects due to the representatives of environmental protectors. Supplementing the feeds with Cr, Cu, Mn, Se, and Zn causes increase of these minerals in faeces and prevent using higher organic complexes which may be a potential option.

2.8.3.1 ***Estrus Induction***

Di Costanzo *et al.* (1986) observed that supplemented a low manganese diet, with a chelated form of Mn, decreased the number of services required to achieve conception. Manspeaker *et al.* (1987) reported that in Holstein heifers from the University of Maryland dairy herd 2 groups receiving a formulation containing Mg, Fe, Mn, Cu, and Zn as Amino Acid Chelate (AAC) exhibited improved fertility ($p < 0.05$) compared with herd mates receiving the same minerals as inorganic metal salts (IM). The AAC group had 75 per cent more mature ovarian follicles with vaginal secretions indicating estrus ($p < 0.05$).

Floyd *et al.* (1995) reported that no improvement in reproductive performance of beef cows with AATM supplementation. Similarly, Olson *et al.* (1999) noticed that AATM supplementation 60 days prior to breeding had a reduction in reproductive performance compared to cows with inorganic or no supplementation. However, Olson *et al.* (1999) reported that animals fed with the AATM at twice the recommended levels and they believe that this may have caused a mineral imbalance or subclinical toxicosis that lead to the decrease in reproductive performance.

Campbell *et al.* (1999) observed that cows given an additional 359 mg Zn, 199 mg Mn, 125 mg Cu, and 26 mg Co in the form of AATM sources had reduced days to first estrus and also noted that cows given additional AATM that had less incidence of retained placenta had fewer days to first estrus, days to first luteal activity and days to first corpus luteum than control cows that had a retained placenta. Formigoni *et al.* (1993) observed a similar response in days open, cows fed AATM had similar days to first service as those fed inorganic minerals.

Ahola (2004) observed that supplemented beef cows with AATM, inorganic minerals and no supplementation, over a two-year period and observed in estrus 77 per cent, 65 per cent and 58 per cent of cows in three groups, respectively.

Bosseboeuf *et al.* (2006) reported that the mean number of days from calving to first estrus in AAC group was 78 days when comparing with inorganic mineral group 72 days ($p < 0.05$) and it was found significantly different. For those cows that did not conceive on first and second services, the mean number of days from calving to last estrus (3 or more services) was the same for both groups (AAC = 96 days versus IM = 99 days). The difference in the number of days from calving to first estrus seemed to be greater in the spring/summer than in the fall/winter for both groups, but the differences between seasons were not significant. Nocek *et al.* (2006) were recorded when in AATM fed with addition of inorganic minerals or in substitution for inorganic sources received fewer days to first estrus.

Ghadage *et al.* (2010) studied the efficacy of chelated and conventional mineral supplementation on apparently healthy lactating buffaloes. 60 lactating Marathwadi buffaloes were divided into groups A, B and C and were supplemented with chelated mineral mixture, conventional mineral mixture and without mineral mixture, respectively. Both mineral mixtures were administered at 30 gm orally once daily for 90 days. Results revealed that there was significant improvement in appetite and water intake in the chelated mineral mixture supplemented group than the conventional mineral mixture group. There were 35, 25 and 10 per cent buffaloes from groups A, B and C which showed first postpartum estrus, respectively.

Hackbart *et al.* (2010) reported that treatment response of in-organic and organic mineral supplementation in post partum anoestrus cows for the number of days required for first ovulation was 23.05 ± 1.96 and 25.14 ± 1.86 days respectively.

2.8.3.2 *Interval between Treatment and Onset of Estrus*

Bonomi *et al.* (1986) indicated that mean first estrus occurred in the University of Parma dairy herd 16.8 days sooner when AAC (amino acid chelates) were supplemented compared with cows receiving IM (inorganic metal salts).

Kropp (1990) reported the number of days from calving to first estrus may be less (19 days) in cows receiving AAC (amino acid chelates) supplementation when comparing to the control group of cows.

Tambe (1996) was reported that the mean time required from beginning of treatment to first cyclic oestrus in 40 days of post-partum cows was 32, 24 ± 6.65 and 21 ± 8.98 days in control (Group I), Chelated mineral mixture group (group II) and Inorganic mineral mixture group (Group III), respectively, where as in above 100 days of post partum cows was 28.66 ± 0.04, 29 ± 6.35 and 36 ± 12.9 days in Group I, II & III, respectively.

Uchida *et al.* (2001), Ballantine *et al.* (2002), Kellogg *et al.* (2003) and Bosseboeuf *et al.* (2006) were recorded when in AATM fed with addition of inorganic minerals or in substitution for inorganic sources reduced days to conception.

2.8.3.3 ***Conception Rate***

Manspeaker *et al.* (1987) reported that the mean first conception occurred 45 days earlier in the AAC (Amino Acid Chelates) group 90 days versus IM 135 days ($p < 0.05$). Maas (1987) reported feeding of complexed trace minerals (minerals bound to another molecule, usually amino acids, to improve absorption) to dairy cows enhances reproductive performance early in the breeding season and in similar experiment resulted pregnancy 10 days earlier in first calf heifers and 17 to 35 per cent improvement in conception rate with artificial insemination.

Ahola *et al.* (2003) reported that over a two-year period, crossbred, multiparous beef cows (n = 164/year) were used to determine the effect of trace mineral supplementation from 90 days prior to parturition through 120 days post parturition on cow performance. Overall 60 days pregnancy rate tended ($p = 0.10$) to be higher for supplemented cows relative to controls.

Ballantine *et al.* (2002), Ferguson *et al.* (2004), Bosseboeuf *et al.* (2006) and Nocek *et al.* (2006) were noticed higher pregnancy rate when in AATM fed with addition of inorganic minerals or in substitution for inorganic sources.

Bosseboeuf *et al.* (2006) reported that mean services per conception was less in the AAC group compared with the IM group (1.50 versus 1.90) ($p < 0.001$). Confirmed pregnancies following first services was 36 per cent greater in the AAC group compared with the IM group ($p < 0.05$). In the IM group, 87 per cent of the cows ultimately became pregnant compared with 96 per cent in the AAC.

Hayat (2010) reported that deit supplemented with chelated zinc decreased ($p < 0.05$) the number of days to conception (43.2 per cent Vs 54.4 per cent) when compare with the non - supplemented control group and conception rate was (80 per cent vs 60 per cent) respectively, in Baladi does.

Formigoni (2011) reported that during the first 150 days of post partum, organic trace minerals (OTM) increased milk fat content by 4.4 per cent ($p < 0.05$) while milk yield, protein content and somatic cell count were not influenced by treatment. Because more cows from the OTM group became pregnant between 150 and 240 days after calving.

CHAPTER III

MATERIALS AND METHODS

The present work was carried out at Dairy Experimental station, College of Veterinary Science, Rajendranagar, Hyderabad and commercial dairy farms surrounding the College of Veterinary Science, Rajendranagar locating in Rangareddy district.

The animals selected for this study were maintained under good housing, feeding and health with well managemental practices and all the buffaloes were graded Murrah buffaloes. The present experiment was carried out on 60 graded Murrah buffaloes.

3.1 SELECTION OF BUFFALOES

All the buffaloes selected were post-partum pluriparous true anoestrus buffaloes 60 days after calving, having a body condition score from 3.0 to 4.0. Genitalia of these buffaloes were examined per rectally twice at an interval of 8 days and confirmed their non cyclicity before they were selected for experimental trial. All the selected buffaloes were free from pathological conditions like endometritis and ovarian disorders. Finally

60 graded Murrah buffaloes were selected for the experimental trail. All these 60 selected post partum anestrus graded Murrah buffaloes belonged to different farms were randomly distributed in to following four groups with 15 animals in each group. After grouping all the experimental animals were treated with albidol* bolus (containing albendazole 125 mg in each gram, 5mg/kg body weight orally) and experiment was started 8-10 days after deworming.

*** Concept Pharmaceuticals Limited, 167 Cst Road, Kalina, Santacruz (East), Mumbai, Maharashtra - 400098 (India).**

3.2 EXPERIMENTAL DESIGN

These experiment buffaloes were randomly distributed in to four groups, each group comprised of 15 animals which contained three treatments and one control group. The treatment groups were given three different types of minerals as mentioned below.

Group - I: Buffaloes were administered with Cyclomin-7* at the dose rate of 2 boli to each animal weekly once for a period of 30 days and Dicalcium Phosphate** at the dose rate of 30 grams every day to each animal for 30 days.

Composition of Each Cyclomin -7 bolus:

Copper	0.700 gm
Cobalt	0.056 mg
Iodine	0.14gm
Iron	1.4 gm
Manganese	0.56gm
Selenium	0.004gm
Zinc	0.28 gm

Composition of Di Calcium Phosphate is calcium 26 per cent and phosphorus 16 per cent.

Group – II: Buffaloes were administered with Non-chelated mineral mixture*** at the rate of 50 grams per day to each animal for 30 days.

***Pfizer Animal Health India Ltd., Pfizer Centre, Patel Estate, Off S.V Road, Jogeswari (W), Mumbai – 400 102, Maharastra, India.**

****Sri Krishna Minerals, Vanasthali puram, Hydertabad, 500 030, AP, India.**

*****Ultramin. NEOSPARK Drugs and chemicals Private Limited, Unit-II, Plot No: 64/B, Phase-I, IDA, Jeedimetla, Hyderabad-500055, Andhra Pradesh, India.**

Composition of non-chelated mineral mixture in each Kg is as follows

Calcium	24%
Phosphorus	12 %
Sodium	5.9 mg
Magnesium	6000mg
Potassium	100 mg
Sulphur	0.922 %
Copper	1200 mg
Cobalt	150 mg
Iron	5000 mg
Iodine	325 mg
Zinc	9600 mg
Selenium	10 mg
Manganese	1500 mg
DL – Methionine	1.929 gm
L – Lysine	4.40 gm

Group – III: Buffaloes were administered with Chelated mineral mixture* at the dose rate of 50 grams per day to each animal for 30 days.

Composition of chelated mineral mixture in each Kg is as follows

Vitamin A	7, 00,000 IU
Vitamin D_3	70,000 IU
Vitamin E	250 mg
DL – Methionine	1.929 gm
L – Lysine	4.40 gm
Copper	1200 mg
Cobalt	150 mg
Magnesium	6000mg
Iron	5000 mg
Zinc	9600 mg
Iodine	325 mg
Selenium	10 mg
Manganese	1500 mg
Calcium	25.5 %
Phosphorus	12.75 %
Sulphur	0.72 %
Sodium	5.9 mg
Potassium	100 mg
Lactobacillus sp.	1.5 X10^{11} CFU
Sacchoromyces cerevisiae.	30,000 Million CFU

***Chelated Ultramin Forte. NEOSPARK Drugs and chemicals Private Limited, Unit-II, Plot No: 64/B, Phase-I, IDA, Jeedimetla, Hyderabad-500055, Andhra Pradesh, India.**

Group – IV: Buffaloes were not fed with any mineral supplementation and fed with dry fodder, green fodder and concentrates as per the production requirements in intensive feeding system and kept as Control Group.

3.3 COLLECTION OF THE BLOOD SAMPLE

Blood samples were collected in different intervals from jugular venepuncture on 0^{th} day (on the day of starting of experiment or treatment), 15^{th} day and 30^{th} day from starting of the experiment in all the buffaloes of four treatment groups from each animal about 4 ml and 10 ml of blood in 4 ml BD vacutainers containing EDTA as anticoagulant (BD Franklin Lakes NJ USA) and BD vacutainers without anticoagulant (BD Franklin Lakes NJ USA) for haemotological study and serum mineral estimation respectively. Haemoglobin was estimated from the blood samples collected in BD vacutainers containing of EDTA as anticoagulant (BD Franklin Lakes NJ USA) on same day of blood collection. Serum was separated from the blood samples collected in BD vacutainers without anticoagulant (BD Franklin Lakes NJ USA) within 12 hours of blood collection by centrifugation at 3000 rpm/min for 10 minutes. Serum was collected in serum collection tubes and stored at -20 ^{0}C until the estimation of serum mineral profile.

3.4 HAEMATOLOGICAL PARAMETERS

Blood samples were examined for estimation of haemoglobin. Estimations were carried out on the day of collection.

3.4.1 Haemoglobin (Hb)

The estimation of Haemoglobin was conducted using acid- haematin method as described by Coles (1986). The results were expressed in grams per deciliter (gm/dL) of blood. The detailed procedure is furnished in Appendix – ‘A’.

3.5 SERUM MINERAL ESTIMATION

The serum samples were analyzed for estimation of the Calcium, Phosphorus, Iron, Copper, Manganese and Zinc levels by prescribed methods.

AAS AND ANALYZER

3.5.1 Estimation of Serum Calcium

The calcium level in serum was estimated by the a-cresolpthalein complexone method described in the kit* the values were expressed in mg%. The detailed procedure is furnished in Appendix – 'B'

3.5.2 Estimation of Serum Phosphorus

The phosphorus level in serum was estimated by modified metol method as described in the kit** supplied and the values were expressed in mg%. The detailed procedure is furnished in Appendix – ‘C’

3.5.3 Estimation of Serum Micro minerals

The Tri-Acid Digestion (AOAC-1999 Official method of analysis 18th ed, Association of Official Analytical chemicals, Washington, D.C.) method is the most frequently used procedure for serum micro minerals like Iron, Copper, Manganese and Zinc estimation by Atomic absorption Spectrophotometer*** (AAS). All the serum samples were subjected to the Tri – acid digestion method and estimated the levels of Iron, Copper, Manganese and Zinc by using AAS and reading were recorded and calculated the values by using dilution factor and expressed the values in ppm. The detailed procedure is furnished in Appendix – ‘D’

3.6 OBSERVATION OF ESTRUS SYMPTOMS

All the four groups of treated animals were observed for the estrus parameters like estrus response, interval between treatment and onset of estrus, estrus intensity and duration of estrus during the period of study.

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*Clacium Kit. Asapen Laboratories Pvt Ltd, S-82, G.T, Karnal Road, India, Area, Delhi-3.
**Phosphorus Kit - DIZYME DIAGNISTICS PVT LTD. Plot No.44, Road No- 7, IDA, MALLAPUR, HYDERABAD-76, AP, INDIA.
*** ATOMIC ABSORPTION SPECTROPHOTOMETER SL 173 - ELICO Limited - #1

3.6.1 Estrus Detection

All the four treatment group animals were kept under observation for a period of 60 days from staring day of the treatment for estrus symptoms. The farm attendants in the farms were advised about the estrus symptoms and heat detection and requested to monitor the animals at least 5 times a day particularly in the early hours of the day for estrus signs like bellowing, edema and congestion of vulva, mucus discharge, micturition and all other behavioural signs. Detection of estrus in all the buffaloes were carried out on the basis of the behavioural heat symptoms coupled with using teaser bull twice daily (once in the morning and once in the evening). Confirmation of estrus was done with the rectal examination.

3.6.2 Estrus Response

The per cent of estrus was calculated as number of buffaloes exhibited estrus divided by the number of animals treated in each group and multiplied by 100.

3.6.3 Interval between Treatment and Onset of Estrus

Interval between treatment and onset of estrus was calculated in days from the beginning of the treatment to the time of first appearance of the behavioural estrus in four treatment groups of buffaloes

3.6.4 Estrus intensity and Score Card

The intensity of estrus was assessed in post partum buffaloes based on the visual and rectal examination of the external and internal genetalia for exhibited estrus symptoms and certain physiological changes in the reproductive organs as per the score card designed by Rao and Rao (1981). The intensity of heat was classified as weak, normal and intense estrus. The intensity of oestrus was graded as weak estrus, normal estrus, and intense estrus by scoring less than 10, 10-15, and more than 15 points, respectively as per the modified score card (Table1).

Exhibited symptoms during estrus	Points allotted
Bellowing	2
Excitement (or) restlessness	2
Estrual mucus discharge	3
Edema, congestion and wetness of vulva	3
Raising of tail in response to placing of palm on the rump region	2
Micturation	2

Cervical relaxation	2
Uterine tonicity	3
Graffian follicle on the ovary	3
Temporary engorgement of teats	1
Decreased milk yield	1
Decreased feed intake	1
TOTAL	**25**

Score card for assessment of intensity of estrus in anoestrus buffaloes.

GRADING

Weak estrus : < 10 Points

Normal estrus : 10-15 Points

Intense estrus : >15 Points

3.6.5 Duration of Estrus (hours)

Duration of behavioural estrus was estimated in hours from the time of first appearance of estrus to the time of detection of last estrus sign.

3.7 BREEDING OF THE ESTRUS BUFFALOES

Estrus exhibited animals were inseminated twice with 8 hours interval by following A.M & P.M rule 12 hours after the exhibiting of the estrus symptoms by using good quality Murrah buffalo bull frozen semen.

3.8 PREGNANCY DIAGNOSIS

Inseminated buffaloes were examined per rectally for pregnancy diagnosis after 50 days of post insemination and recorded the result.

3.9 CONCEPTION RATE

The conception rates were calculated and recorded on number of animals inseminated and found positive for pregnancy by palpation per rectum on day 50 of post insemination. The efficacies of various mineral mixture supplements in four treatment groups of post partum buffaloes were analyzed.

3.10 STATISTICAL ANALYSIS

The results of the study were recorded and statistically analyzed as per the methods suggested by Snedecor and Cochran (1994). The soft ware used for statistical analysis was SPSS 1999 SPSS® User's Guide Release 10.0.1. SPSS Inc., USA, (16.0 version).

CHAPTER-IV

RESULTS

4.1 STUDY OF HAEMOTOLOGICAL PARAMETERS

The mean haemoglobin levels in mineral supplemented post partum anoestrus buffaloes at the end of the mineral supplementation were presented in Table No 1.

4.1.1 Haemoglobin Concentration

The mean haemoglobin levels on zero, 15^{th} and 30^{th} day in group I were 11.05 ± 0.26, 11.31 ± 0.27 and 11.55 ± 0.26, in group II were 11.01 ± 0.22, 11.32 ± 0.23 and 11.63 ± 0.22, in group III were 11.03 ± 0.18, 11.46 ± 0.26, and 11.89 ± 0.27 and in group IV were 11.01 ± 0.16, 11.03 ± 0.17 and 11.06 ± 0.17 gm % respectively.

Haemoglobin levels in group I, II and IV there was no significant difference between zero, 15^{th} and 30^{th} day of treatment, but in group III levels were statistically similar on zero & 15^{th} day and 15^{th} and 30^{th} day of treatment.

There was no significant difference in haemoglobin levels among all four groups on zero, 15th day. But on 30th day three treatment groups were having statistically similar concentrations which were significantly higher than the control group.

4.2 STUDY OF SERUM MINERAL PROFILES

In the present study serum mineral levels were estimated on starting day of mineral supplementation, 15th day and 30th day in post partum anoestrum buffaloes. To study the change in the serum mineral levels before and after supplementation of the different kinds

HAEMOGLOBIN TABLE

HAEMOGLOBIN GRAFF

of mineral supplements to the post partum anoestrus graded Murrah buffaloes, serum calcium, phosphorus, iron, copper, manganese and zinc levels were estimated.

4.2.1 Serum Calcium levels

The mean Serum calcium levels in different mineral supplementation groups of post partum anestrus buffaloes on zero, 15th and 30th day were 7.20 ± 0.23, 7.69 ± 0.22 and 8.08 ± 0.22 in group I (DCP + Cyclomin - 7), 7.15 ± 0.21, 7.85 ± 0.12 and 8.63 ± 0.16 in group II (non – chelated mineral mixture), 7.24 ± 0.23, 7.98 ± 0.12 and 8.79 ±

0.07 in group III (chelated mineral mixture) and 7.31 ± 0.10, 7.34 ± 0.06 and 7.35 ± 0.07 mg/dl in group IV (control group), respectively (Table 2).

The statistical analysis revealed that in group I, II and III there was significant ($p < 0.05$) difference in serum calcium levels on zero, 15th and 30th day. The levels were significantly ($p < 0.05$) higher on 30th day when compared with other two days. In group IV there was no significant difference ($p < 0.05$) (before treatment, during treatment and after treatment) in between the days of treatment.

The statistical analysis revealed that in between all four groups on zero day there was no significant difference ($p < 0.05$), on 15th day there was no significant ($p < 0.05$) difference in three treatment groups (I,II and III), but these were differ significantly ($p < 0.05$) with the control group (IV). On 30th day of treatment the serum calcium levels were higher in group III (chelated) followed by group II (non - chelated), group I (DCP + Cyclomin - 7) and lowest in group IV (control group), but statistically group II and III were having similar levels of calcium and these two groups were having significant ($p < 0.05$) difference with group I and IV.

CALCIUM TABLE

CALCIUM GRAFF

4.2.2 Serum Phosphorus levels

The mean Serum phosphorus levels in different mineral supplementation in post partum anoestrus buffaloes on zero, 15th and 30th day in group I were 5.46 ± 0.29, 5.79 ± 0.32 and 6.24 ± 0.18 mg/dl, in group II were 5.78 ± 0.18, 5.87 ± 0.28 and 6.30 ± 0.25 mg/dl, in group III were 5.56 ± 0.07, 5.96 ± 0.17 and 6.94 ± 0.05 mg/dl and in group IV were 5.56 ± 0.7, 5.83 ± 0.11 and 5.80 ± 0.10 mg/dl, respectively (Table No. 3).

The statistical analysis revealed that in group I and II there was no significant difference ($p < 0.05$) in serum phosphorus levels on zero, 15th and 30th day, but in group III the levels were significantly ($p < 0.05$) higher on 30th day followed by 15th day and zero day. In group IV the levels were significantly higher ($p < 0.05$) on 15th and 30th day when compared with zero day.

The statistical analysis revealed that in between groups on zero day there was no significant difference ($p < 0.05$), on 15th day between three treatment groups (I, II and III) the phosphorus levels were differ significantly ($p < 0.05$) with control group (IV), on 30th day of treatment the serum phosphorus levels were significantly higher in group III followed by group II, group I and IV were having statistically similar lowest levels.

4.2.3 Serum Iron Levels

The mean Serum iron levels in different mineral supplementation in post partum anoestrus buffaloes on zero, 15th and 30th day in group I were 2.716 ± 0.112, 3.122 ± 0.084 and 3.180 ± 0.110, in group II were 2.656 ± 0.058, 3.147 ± 0.068 and 3.246 ± 0.140, in group III were 2.689 ± 0.047, 3.174 ± 0.194, and 3.680 ± 0.081 and in group IV were 2.679 ± 0.039, 2.690 ± 0.061 and 2.702 ± 0.060 ppm, respectively (Table 4).

PHOSPHORUS TABLE

PHOSPHORUS GRAFF

IRON TABLE

IRON GRAFF

The statistical analysis revealed that the serum iron levels in group I, II were significantly ($p < 0.05$) lower on zero day when compare to 15^{th} and 30^{th} day, in between 15^{th} and 30^{th} day there was no significant difference ($p < 0.05$); In group III levels were differ significantly ($p < 0.05$) on zero, 15^{th} and 30^{th} day, levels on 30^{th} day were significantly higher, when compared with other two days. In group IV there was no significant difference ($p < 0.05$) on zero, 15^{th} and 30^{th} days of treatment.

The statistical analysis revealed that in between four groups, the serum iron levels on zero day there was no significant difference ($p < 0.05$). On 15^{th} day there was no significant difference in three treatment groups (I, II and III) but they were differ significantly ($p < 0.05$) with control (group IV). On 30^{th} day there was significant difference ($p < 0.05$) between treatment and control group, among treatment groups I and II were having similar levels and group III was significantly ($p < 0.05$) higher Iron levels than all other groups (Table 4)

4.2.4 Serum Copper levels

The mean Serum copper levels in different mineral supplementation in post partum anoestrus buffaloes on zero, 15th and 30th day were 0.047 ± 0.003, 0.097 ± 0.003 and 0.142 ± 0.006 ppm in group I, in group II were 0.049 ± 0.005, 0.127 ± 0.005and 0.163 ± 0.005, in group III were 0.052 ± 0.002, 0.149 ± 0.003 and 0.184 ± 0.005 and in group IV were 0.049 ± 0.002, 0.052 ± 0.002 and 0.054 ± 0.003 ppm (Table 5), respectively.

The statistical analysis revealed that the serum copper levels were significantly ($p < 0.05$) higher on 30th day followed by 15th day and lowest on zero day in all three treatment groups. In group IV there was no significant difference ($p < 0.05$) in between zero, 15th and 30th day of treatment.

COPPER TABLE

COPPER GRAFF

Statistical analysis revealed that in between four groups there was no significant ($p < 0.05$) difference on zero day, but significant difference ($p < 0.05$) was observed on 15^{th} and 30^{th} day in all four groups Group III was having significantly ($p < 0.05$) higher and group IV was having lowest serum copper levels (Table 5).

4.2.5 Serum Manganese levels

The mean Serum manganese levels in different mineral supplementation in post partum anoestrus buffaloes on zero, 15th and 30th day in group I were 0.056 ± 0.003, 0.076 ± 0.003 and 0.151 ± 0.007, in group II were 0.058 ± 0.002, 0.112 ± 0.002 and 0.165 ± 0.006, in group III were 0.541 ± 0.002, 0.148 ± 0.002 and 0.182 ± 0.002 and in group IV were 0.055 ± 0.002, 0.056 ± 0.003 and 0.055 ± 0.003 ppm, respectively (Table. 6).

The statistical analysis revealed that the serum manganese levels were significantly ($p < 0.05$) different in all three treatment groups on zero, 15th, 30th day. There was no significant difference ($p < 0.05$) observed in group IV in between zero, 15th and 30th day of treatment.

The statistical analysis revealed that in between four groups on zero day there was no significant difference ($p < 0.05$). On 15th and 30th day all four groups differed significantly ($p < 0.05$), the control group was having lower and group III was having higher level of serum manganese levels.

4.2.6 Serum Zinc levels

The mean Serum zinc levels in different mineral supplementation in post partum anestrus buffaloes on zero, 15th and 30th day in group I were 1.062 ± 0.023, 1.162 ± 0.033

MANGANESE TABLE

MANGANESE GRAFF

and 2.639 ± 0.061, in group II were 1.089 ± 0.008, 1.248 ± 0.018 and 3.038 ± 0.017, in group III were 1.075 ± 0.010, 1.427 ± 0.039 and 3.767 ± 0.042 and in group IV were 1.032 ± 0.012, 1.050 ± 0.010 and 1.054 ± 0.010 ppm, respectively (Table 7).

The statistical analysis revealed that the serum zinc concentrations on zero day in group I significantly ($p < 0.05$) differ from 15th and 30th day, there was no significant difference ($p < 0.05$) between 15th and 30th day of treatment, in group II and III there was significant difference ($p < 0.05$) between zero, 15th and 30th day, but there was no significant difference ($p < 0.05$) in group IV.

The statistical analysis revealed that in between four groups on zero day similar zinc levels were observed in between group I, II, III and in between group I, III and IV. On 15th and 30th day significant difference ($p < 0.05$) was observed in all four groups, control group was having lower and group IV was having higher level of zinc.

4.3 STUDY OF ESTRUS PATTERN

4.3.1 Estrus Response

The estrus response (Table 8) in mineral supplemented post partum graded Murrah buffaloes were 7, 10, 13 and 5 of animals out of 15 animals in group I, II, III and IV respectively. The percentage of animals exhibited estrus were 46.66 per cent, 66.66 per cent, 86.66 per cent and 33 per cent in group I, II, III and IV respectively. Regarding estrus response there was significant ($p < 0.05$) difference in between four groups. Group III was having significantly higher estrus response followed by group II and I, where as group IV was having significantly ($p < 0.05$) lowest in estrus response.

ZINC TABLE

ZINC GRAFF

4.3.2 Duration of Estrus

The mean duration of estrus in group I, II, III and IV were 22.13 ± 0.83, 26.46 ± 0.98, 32.93 ± 0.81 and 15.80 ± 0.65 hours, respectively (Table 9). Statistically there was significant difference ($p < 0.05$) in between all four groups. Among the groups, group III was having significantly ($p < 0.05$) higher duration of estrus when compared to all other three groups and group IV was having significantly ($p < 0.05$) lesser duration of estrus.

4.3.3 Mean Intensity of Estrus

Intensity of estrus was assessed by the symptoms exhibited by the buffaloes during estrus. The estrus signs were scored in 1 to 25 scale to know the intensity of the estrus of an individual animal. The mean intensity of estrus score in different treated groups were 10.57 ± 0.69, 12.30 ± 0.76, 13.15 ± 0.72 and 11.4 ± 1.36, in group I, II, III and IV, respectively. Statistically there was no significant difference ($p < 0.05$) among four groups, however group III was having higher estrus intensity score followed by group II, IV and I (Table 9).

4.3.4 Interval between Treatment to the Onset of the Estrus

The mean interval from starting of the treatment to the exhibition of the estrus (Table No. 9) were 46.67 ± 0.95, 35.20 ± 1.06, 25.0.98 and 70.13 ±1.79 days in group I, II, III and IV, respectively. The ranges of the interval between treatments to the estrus exhibition in four groups were 44-49, 33-37, 24- 28, and 66 – 74 days in group I, II, III and IV, respectively. Statistically there was significant ($p < 0.05$) difference among all four groups for the interval between treatment to estrus exhibition, group III was having significantly ($p < 0.05$) lesser interval followed by group II, I higher and the group IV was having significantly ($p < 0.05$) highest interval between treatment to the estrus exhibition.

ESTRUS RESPONSE

TABLE NO 9

TABLE 10

4.3.5 Conception Rate

Conception rate was calculated based on the number of animals treated. The pregnancy diagnosis was done after 50 days of breeding and number of animals conceived was 3, 6, 11 and 2 in group I, II, III and IV, respectively. The conception rates were 20 per cent, 40 per cent, 73.33 per cent and 13.33 per cent in group I, II, III and IV, respectively. Statistically significantly higher in group III and followed by group II and I. Group IV was having significantly ($p < 0.05$) lowest conception rate (Table no.8).

CHAPTER V
DISCUSSION

The results of haemoglobin level, serum mineral profile, estrus pattern and conception rates in postpartum anoestrus graded Murrah buffaloes of different treatment groups of mineral supplementation are discussed below. The response of mineral supplementation group wise viz. in Group-I (Cyclomin-7 at the dose rate of 2boli/week/animal and Dicalcium phosphate at the dose rate of 30 gm/day/animal), Group-II (Non-chelated minerals at the dose rate of 50 gm/day/animal), Group-III (Chelated minerals at the dose rate of 50 gm/day/animal) and Group-IV (Control group, without any mineral supplementation) were discussed.

5.1 Haemoglobin Levels

The mean haemoglobin levels in postpartum anoestrus graded Murrah buffaloes during the period of study were presented in Table No. 1. There was no significant difference in haemoglobin levels during the course of treatment in group I, II, and IV, but in group III haemoglobin levels were statistically similar on 0 day and 15th day, 15th and 30th day. In between groups no significant difference was observed in haemoglobin levels on zero day and 15th day. But on 30th day three treatment groups were having statistically similar concentrations which were significantly higher than the control group.

In this study, haemoglobin levels were improved with the mineral supplementation and more improvement was observed in the chelated mineral supplementation. Anand *et al.* (2004) recorded slightly higher haemoglobin levels (13.29 ± 0.29 gm %) in post partum buffaloes. Sharad *et al.* (2010) reported 12.20 ± 0.23 and 13.60 ± 0.51 gm% haemoglobin in anoestrum and cyclic buffaloes. The improvement of the haemoglobin levels of the present study were in agreement with the findings of Ghadage *et al.* (2010), who recorded more improvement of haemoglobin levels in chelated mineral supplementation, slight improvement in non- chelated mineral supplementation in lactating buffaloes. Sharma *et al.* (2002) reported significant increase in haemoglobin which is in corroboration with the present findings. Improvement in haematological status of cattle after supplementation of zinc and copper has been reported by Sharma and Joshi (2005). Mehta *et al.* (2010) also observed similar improvement in haemoglobin concentration with mineral supplementation in kids. Ramakrishna (1997) reported that haemoglobin concentration influence tissue oxygenation of the reproductive tract, which in turn affect the cyclicity. In this study also the improvement of haemoglobin levels in treatment groups helped for the better estrus response.

5.2 STUDY OF SERUM MINERAL PROFILES

5.2.1 Serum Calcium levels

The mean serum calcium levels in four treatment groups on zero, 15th and 30th day were presented in Table No. 2. In three treatment groups there was significant improvement of calcium levels from zero to 30th day, where as in control group no significant improvement observed. In between the groups, group III (chelated mineral supplemented group) had significantly ($p < 0.05$) higher calcium levels on 30th day followed by group II and I and lowest levels were observed in group IV (control).

In the present study the mean serum calcium levels on zero day ranging from 7.15 ± 0.21 to 7.31 ± 0.10 mg/dl were below the critical value (< 8.00 mg/dl) as reported by Miles and McDowell (1983). Low levels of serum calcium might be due to the low content of the calcium in locally available feedstuffs.

Statistically more improvement in calcium levels were observed in group II and III when compared to group I and IV. Lall *et al.* (2004) recorded serum calcium levels (7.31-7.76 mg/dl) in non chelated mineral mixture supplementation non-pregnant buffalo heifers. Pant (2000) reported low calcium levels (7.53 ± 0.39 mg %) in anoestrus and high calcium levels (11.51 ± 0.37 mg %) in normal cycling buffaloes. Similarly, Dhoble *et al.* (1987) reported that serum calcium concentrations ranging from 7.62 ± 0.68 to 10.75 ± 0.45 mg /dl in buffaloes. Singh *et al*. (2004) reported serum calcium levels as 8.33 ± 0.15 mg /dl in anoestrus buffaloes.

Jyoti Sharma *et al.* (2009) and Biswas *et al.* (2005) observed significant improvement in calcium levels with mineral supplementation in anoestrus buffaloes. Ghadage *et al.* (2010) reported significant increase in calcium levels with the chelated mineral supplementation in lactating buffaloes, which was in agreement with the higher calcium levels in the chelated mineral supplementation group of the present study. Calcium deficiency is responsible for infertility and disturbed Ca: P ratio had blocking action on the pituitary gland and consequently on ovarian action (Herrick and John, 1977), prolonging the interval of first ovulation. Calcium in mineral supplements is generally more available than calcium in forages and common feedstuffs (Hansard *et al.*, 1957). Morrow (1980) reported that reduced blood calcium level causes delayed uterine involution and increase in the incidence of dystocia, retained placenta and prolapsed uterus. Higher Ca: P ratio is associated with infertility in cattle.

Calcium requirement changes depending on the animal age and production status. Non lactating, pregnant cows require calcium at a level of 0.18 percent of total dry matter intake, while the requirement for lactating cows is 0.27 percent of total dry matter intake. Growing and finishing cattle require 0.31 percent calcium for optimal growth. The maximum tolerable level of calcium is not known (Hale Chad and Olson, 2001). Calcium plays an important role in the gonadotropic regulation of ovarian steroidogenesis (Carnegie and Tsang, 1984) and regulation of the membrane potential of oocytes. It is also suggested that calcium is involved in the disruption of cumulus cell cohesiveness by regulating the number of gap junctions between the cells (Peracchia, 1978), which contributes to the process of ovulation. Moreover, a disturbed calcium-phosphorus ratio has a blocking action on the pituitary gland and consequently on the ovarian function (Herrick and John, 1977).

5.2.2 Serum Phosphorus levels

The mean serum phosphorus levels of four groups of post partum anoestrus buffaloes were presented in Table No. 3. Increase in serum phosphorus levels were observed in all four groups from zero day to 30th day of treatment. In group III (chelated mineral) significantly ($p < 0.05$) higher improvement of serum phosphorus levels were observed.

The serum phosphorus levels on zero day was similar in all four groups, whereas on 15th and 30th day serum phosphorus levels were higher in three treatment groups compared to control group of buffaloes . Serum phosphorus levels were highest on 30th day in group III (chelated mineral supplemented group).

The mean serum phosphorus levels on zero day of present study were slightly higher (5.06 ± 0.13 mg/dl) than the observations of the Singh *et al.* (2011) in buffaloes, Singh *et al.* (2004) 5.50 ± 0.22 mg/dl in anoestrus buffaloes. Similarly, Khasatiya *et al.*

(2005) also noticed lower levels of serum phosphorus in true anoestrus (4.79 ± 0.16 mg %) and sub-estrus (5.93 ± 0.17 mg %) in Surti buffaloes.

Phosphorus is essential for transfer of biological energy, particularly through ATP, and deficiency of it may arrest the phenomenon of fertilization and this in turn may cause early embryonic death resulting in the repeat breeder and anoestrus conditions of animals. Phosphorus has more important functions and is required by rumen microorganisms for digestion of cellulose (Burroughs *et al.*, 1951) and synthesis of microbial proteins (Breves and Schroder, 1991).

Phosphorus is the second most abundant mineral in the animal body after calcium. Phosphorus deficiency is the most common in several developing countries due to the low phosphorus content in soils and plants (McDowell *et al.,* 1986). Out of all mineral deficiencies phosphorus deficiency is most widespread and economically important deficiency as far as reproduction is concerned (Underwood, 1981). Phosphorus is critically deficient for most classes of productive ewes throughout the year and being most deficient along with calcium during the period of early lactation. Read *et al.* (1986) showed that the sub normal fertility associated with depressed or irregular estrus and decreased conception in cattle. Scharp (1979) reported that improved fertility in dairy cows, having subnormal serum inorganic phosphorus with the supplementation of defluorinated super phosphate in drinking water. Increased phosphorus levels with the mineral supplementation during the treatment period were useful for the better estrus exhibition and conception rate in the treatment group of buffaloes, these findings were in agreement with the above authors.

5.2.3 Serum Iron Levels

The mean serum iron levels were presented in Table No. 4. Statistically mean iron levels were improved significantly ($p < 0.05$) from zero to 15th day in all three treatment groups, which from 15th to 30th day a significant ($p < 0.05$) improvement was observed in group III (chelated mineral), but there was no significant ($p < 0.05$) difference in control group from zero to 15th and 30th day.

Among three treatment groups, there was no significant ($p < 0.05$) difference in serum iron levels from zero and 15 day, but they differ significantly ($p < 0.05$) with control group. On 30th day significant ($p < 0.05$) improvement was observed in all three treatment groups, when compared with control group. Group III (chelated mineral) was having significantly ($p < 0.05$) higher iron levels than all other groups on 30th day, which is attributed to the supplementation of chelated form of the mineral mixture in this group.

The higher levels of serum iron in chelated mineral supplementation group of this study were in agreement with the findings of the Ghadage *et al.* (2010) in buffaloes. Tiwari *et al.* (2010) reported slightly lower serum iron levels (1.97 ± 0.19 ppm) in dairy cattle of different physiological periods. Tambe (1996) reported significantly ($p < 0.05$) higher levels of serum iron in follicular, luteal and anestrus phases of the reproduction in post partum anoestrus cows, the levels were 325.6 ± 27.14, 345.92 ± 24.49, 399 ± 24.29 µg per cent respectively, which were higher than the present study. Kumar *et al.* (2007) reported the lower levels of serum iron in different physiological status (ranging from 1.570 to 2.4 ppm) which were lower than the present study.

The iron levels of the present study were higher than the critical levels observed by McDowell (1987) and Tiwary *et al*. (2010) who reported 0.89 and 1ppm, respectively. In present study improvement was observed in serum iron levels with the mineral supplementation even though the initial levels were above the critical value. Iron deficiency was observed seldomly in dairy cattle, because most of diets were having the adequate levels. Iron deficiency can be observed in dairy cattle when there is high parasitic infestation or disease exists that cause chronic blood loss.

The improvement of iron levels with mineral supplementation in the present study helped for the slight increase in haemoglobin levels. More than half of the iron in the animal body is found as a constituent of haemoglobin. A small amount is found in myoglobin and certain enzymes which play a part in oxygen utilization

High dietary iron levels affects the absorption of the dietary copper and manganese in dairy cattle, therefore the iron dietary supplementation should not be too high, otherwise it affects the absorption of the other minerals and affects the reproduction. In the present study, the serum iron levels were less than the higher levels (400 to 500 µg/100 ml) (Kincaid *et al.*, 1999).

5.2.4 Serum Copper Levels

The mean serum copper levels of four groups of post partum anoestrum buffaloes were presented in Table No.5. In all three treatment groups a significant ($p < 0.05$) improvement of serum copper were observed with the supplementation of the mineral from zero to 30th day, where as in control group no significant ($p < 0.05$) improvement was observed during the experiment period. In between groups also there was no significant ($p < 0.05$) difference on zero day, but significant ($p < 0.05$) difference was observed on 15th and 30th day and the group III (chelated mineral) was having significantly ($p < 0.05$) higher levels of serum copper when compared with group I and II. The higher copper levels in group III (chelated mineral supplementation group) was attributed to the supplementation of the chelated mineral mixture. The critical levels of plasma copper levels reported by Kincaid *et al.* (1999) were less than 0.2 µg/ml in clinically deficient and 0.2 to 0.52 µg/ml in deficient cattle, Singh *et al.* (2011) 0.65 ppm and 0.52 µg/ml (NRC, 2001). The serum copper levels in the present study were less than the critical values as described by the previous authors, this may be due to the antagonistic effect of the other trace elements like iron, molybdenum and sulphur. Iron levels of the present study were slightly higher, so it may be the one of the factors for decreasing absorption of the dietary copper. The copper deficiency causes reproductive inefficiency characterised by depressed estrus (Underwood, 1981).

The study revealed that the serum copper level is less than the critical value even after 30 days of mineral supplementation in all the treatment groups. The levels in the control group were much lower than the critical level, so the quantity of copper supplementation may further increased and the iron supplementation levels should be decreased in the diet. The study of molybdenum and sulphur levels has to be conducted to know the antagonistic effect of these elements, to arrive at the exact conclusion for the cause of copper levels less than the critical values. The feed stuffs may be deficient in copper, which was inadequate to meet the dietary requirement of animals leading to low copper concentration in blood serum. Similar observations have also been reported by Jumba *et al*. (1995) who observed that a low level of copper in feeds reflected lower serum copper levels in animals.

Copper is required in the body for the production of red blood cells, as it is essential for absorption and transport of iron which is necessary for haemoglobin synthesis (Tuormaa, 2000). Copper is necessary for production of melanin pigment and interaction of copper and estrogen are also observed (Hidiroglou, 1979). Copper deficiency is associated with high molybdenum levels as crops grown on 'tert soils' (having high organic matter) have high Mo and low Cu. Mo and Cu interactions further lower the available Copper absorption.

The important sign of copper deficiency related to reproduction in cattle is decline in fertility. Changes in steroidal metabolism may lead to alter reproductive behaviour (Hidiroglou, 1979). Low fertility associated with delayed or depressed estrus has been reported in cattle grazed on copper deficient pastures (Kreplin and Yaremcio, 1992). The normal body requirement of copper in dairy cattle is 10 ppm (Puls, 1994). Copper treatment has been found to improve conception rate (Hunter, 1977).

5.2.5 Serum Manganese Levels

The mean serum manganese levels in different groups of post partum anestrum buffaloes were presented in Table No.6. Significant ($p < 0.05$) improvement of serum manganese levels were observed in all three treatment groups from zero to 30th day but there was no change in the control group. In between the groups there was a significant ($p < 0.05$) difference in all four groups on 15th and 30th day, the mean serum mineral manganese levels in group III (chelated mineral) were recorded highest on 15th and 30th day of treatment.

Singh (2011) recorded critical level of serum manganese which was 0.2 ppm and ranging from 0.23 ± 0.008 to 0.31 ± 0.01 ppm in different zones of Odisha. Garg *et al.* (2011) reported the total manganese requirement per day was 560 mg in diet for Murrah buffaloes having milk yield of 10 litres per day and also reported most of the feed ingredients consisted of sufficient quantity of the manganese (more than 63 ppm). In contrast to this in the present study serum manganese levels were less than the critical value i.e. 0.2 ppm. It indicates that the feed ingredients of this region are very much deficient of manganese levels. Similarly, Lall *et al.* (2000) reported the manganese deficiency of the feed ingredients, which were containing 12-18 ppm. Saraswathi *et al.* (2009) also reported that the serum manganese levels were less than the critical value (0.18 ± 0.09 ppm, it is 87 per cent deficient), which is in agreement with the present study. Singh (2011) reported higher manganese levels in serum (0.26 ± 0.004 ppm) than the present study.

In the present study in group III, serum manganese levels were higher. It might be due to the chelated trace minerals, which were bound to organic ligands through covalent bonds. The bonds between the ligand and the mineral can prevent the mineral from interacting with the antagonist and improve the bioavailability of the mineral (Ward *et al.*, 1996; Bailey *et al.*, 2001).

Most of the manganese in the body is found in skeleton, liver and hair. Manganese accumulates in the liver and is directly proportion to the dietary manganese provided in the precise index of manganese status. High concentrations of the dietary calcium, potassium or phosphorus increase excretion of manganese in the faeces, presumably by reducing absorption of manganese (Lassiter, 1972; Hartmans, 1974). Excessive dietary iron depresses retention of manganese in calves (Ho *et al*., 1984).

The National Research Council's nutrient requirement of dairy cattle (1989) suggested that 40 mg of manganese/kg dietary dry matter should be adequate for all classes of cattle, though a requirement was not actually determined. Heifers and cows that are fed with low manganese diet are slower to exhibit estrus and more likely to have silent heats and lower conception rate than the cows fed with sufficient manganese in their diet (Bentley and Philips, 1951). It revealed that the supplementation of the manganese in the diet was very much important for exhibiting estrus and for high conception rate.

Deficiency of manganese may be associated with suppression of estrus, silent estrus, irregular estrus cycle, cystic ovary, poor follicular developments with delayed ovulation, and increase in embryonic mortality and reduced conception rate (Kreplin and Yaremcio, 1992; Corrah, 1996). Even manganese deficient goats were observed to exhibit no apparent sign of estrus despite normal ovulation (Groppel and Anke, 1971). Manganese supplementation has proven to be effective in shortening the postpartum anoestrus and increasing conception rates in dairy cows (Krolak, 1968). Manganese has a role in steroid synthesis (Keen and Zidenberg-cheer, 1990) and it is associated with impaired reproductive functions such as anoestrus, delayed ovulation and repeat breeding (Corrah, 1996). Delayed ovulation has been induced experimentally in dairy cows by withholding Mn over a long period (Rojas *et al.*, 1965).

5.2.6 Serum Zinc Levels

The mean serum zinc levels in different mineral supplemented groups of postpartum anoestrus buffaloes were present in Table No. 7. In all three treatment groups significant ($p < 0.05$) increase in serum zinc levels were observed from zero day to 30th day. In group II and III the serum zinc levels were significantly ($p < 0.05$) higher than the other groups on 30th day. In control group there was no significant ($p < 0.05$) difference in the serum zinc levels on different days during the course of treatment. In between groups, the group III was having significantly ($p < 0.05$) higher serum zinc levels on 15th and 30th day of treatment and control group had the significantly ($p < 0.05$) lowest serum zinc levels on zero, 15th and 30th day. The higher levels of serum zinc levels in chelated mineral supplementation group were due to the chelation effect of the trace minerals.

Singh *et al.* (2011) reported the critical level of zinc in serum was 0.6 ppm and Singh *et al.* (2011) reported the average serum zinc levels of dairy cattle in different parts of Orissa ranging from 0.64 ± 0.002 to 0.74 ± 0.01 ppm and Saraswathi (2009) reported the serum mean zinc levels as 1.06 ± 0.17 ppm in Uttarakhand, which was lower than the present study. Garg *et al.* (2011) reported the daily requirement of zinc in feed was 11.20 mg for high yielding Murrah buffaloes in Panjab. Hambridge *et al.* (1986), Goff and Stable (1990) were reported the serum zinc concentration normally between 0.7 and 1.3 µg/ml, concentration of zinc in serum below 0.4 µg/ml are considered deficient.

Tiwary *et al.* (2010) reported the mean serum zinc levels in cattle and buffaloes were higher than the initial zinc levels on the zero day of the present study. Zinc supplementation through the organic source had a higher retention and tissue concentration (Lardy *et al.*, 1992; Cao *et al.*, 2000). The higher serum zinc levels in chelated mineral supplementation (group III) were in accordance with the findings of the Hayat (2010) and Ghadage *et al.* (2010). Ashmead (1970), Wedekind *et al.* (1992)

and Nockles *et al.* (1993) it may be due to the specific amino acid complexes of trace minerals are more bioavailable and had better retention in the body than inorganic source of trace minerals.

Lower vitamin A can be correlated with zinc deficiency. Zinc is required for mobilization of vitamin A from liver stores and vitamin A deficiency has resulted for grazing cattle due to shortage of zinc (Guerin, 1981). Since tropical forage is low in zinc (McDowell, 1985), conditioned vitamin A deficiencies may be resulting even though liver vitamin A values indicate adequate concentration of this vitamin.

Zinc is a component of many metallo enzymes which affects the metabolism of carbohydrates, protein and nucleic acids. Zinc regulates thyroid hormone binding and inositol phosphate synthesis. Zinc deficiency alters the prostaglandin synthesis which in turn affects the luteal function.

Zinc is a component of thiomosin hormone produce by thymic cells that regulate the cell mediated immunity (Graham, 1991). The higher serum zinc levels of the present study might be due to higher availability of zinc from feed and fodder of the farms in this study. The effect on prostaglandin synthesis suggests that zinc deficiency have profound effect on reproductive cycle and pregnancy. Delayed puberty and lower conception rates, failure of implantation and reduction of litter size are also found in association with the zinc deficiency in feed (Kreplin and Yaremcio, 1992). Zinc has a significant role in repair and maintenance of endometrium following parturition and early return of post partum estrus (Green *et al.*, 1998).

5.3 ESTRUS PATTERTNS

5.3.1 Estrus Response

Effect of different groups of mineral supplementation in postpartum anoestrum graded Murrah buffaloes on estrus induction were presented in the Table No.8. Among the four groups, the chelated mineral (group III) group buffaloes exhibited highest estrus response (86.66 per cent) followed by group II (66.66 per cent) and group I (46.66 per cent) and significantly ($p < 0.05$) lowest estrus response was observed in group IV (control) group (33.33 66 per cent). Similar type of improvement in estrus exhibition was recorded by Biswas *et al.* (2005) in post partum anoestrus Murrah buffaloes with the supplementation in combination with mineral mixture and trace mineral tablet. Mavi and Bahga, (2005) also observed that 65 per cent estrus response with trace mineral bolus supplementation.

5.3.2 Duration of Estrus

The mean duration of estrus in group I, II, III and IV were 22.13 ± 0.83, 26.46 ± 0.98, 32.93 ± 0.81 and 15.80 ± 0.65 hours, respectively (Table 9). The duration of estrus was statistically differed among all four groups. Among groups, group III was having significantly ($p < 0.05$) higher duration of estrus when compared to all other three groups and group IV was having significantly ($p < 0.05$) lesser duration of estrus.

Pradyumna Rao *et al.* (1982) recorded the mean duration of estrus was 24.1±0.69 hours in Murrah buffaloes. Satishkumar (1986) observed 20 and 36 hours in non-descript buffaloes. Danell (1987) recorded 23.8 ± 6.2 hrs in River buffalo, 19.9 ± 4.4 hours in Swamp buffaloes, which were slightly lesser than the observations of the present study. Figueiredo *et al.* (1997) reported that the estrus length was 13.6 ± 1.0 hours in natural estrus of Nellore cows in Brazil. Smilarly, Chaudary *et al.* (1991) also reported 18.5 ± 1.51 to 19.8 ± 2.5 hours in Neli-Ravi buffaloes and observed longer duration of estrus (19.8 ± 2.5 hours) in mineral supplemented group when compared with non-mineral supplemented group. Similar trends were observed in mineral supplemented groups of the present study.

In the present study longer duration of the estrus observed in chelated mineral supplemented group (group III). It might be due to relatively high level of serum trace minerals like copper, zinc, manganese and iron. These minerals effect the estrus symptoms exhibition, so in other groups these minerals were at lower levels, hence may exhibit lesser duration of estrus.

5.3.3 Intensity of Estrus

The mean estrus intensity scores of different treatment groups of post partum anoestrus buffaloes of present study were presented in Table No. 9. Statistically, there was no significant ($p < 0.05$) difference among the four groups based on the estrus intensity scores, but higher estrus intensity score was observed in group III followed by II, IV and I. Based on estrus intensity scale in the present study three treatment groups were having normal (10-15 points) estrus intensity and in control group it was weak (< 10 points) estrus intensity. The present findings are in agreement with estrus intensity classification of Rao and Rao (1981).

The better estrus intensity score is attributed to the higher serum trace elements in mineral supplementation groups compared with the control group. Rao and Kodagali (1983) observed that the prominent estrus symptoms of bellowing (68.8 and 58.7 per cent) in buffaloes. The findings of the present study were in agreement with the above authors. Different authors recorded estrus symptoms like mucus discharge (68.0 per cent) and frequent urination (75.5 per cent) by Banerjee *et al.* (1989), the acceptance of male by female (Gordon, 1996), bellowing (35.92 per cent) by Srivastava *et al.* (1998) in buffaloes.

In buffaloes silent estrus was a major problem, exhibition of the estrus symptoms were influenced by the environment, managemental and nutritional factors.

In nutrition, dietary mineral levels influence the estrus symptoms. So in the present study supplementation of the chelated mineral mixture, non–chelated and Dicalcium phosphate plus trace mineral bolus supplementation might have been helped to overcome the silent heat problem in treatment buffaloes.

5.3.4 Interval between Treatment and Onset of Estrus

In the present study, the interval between treatment and onset of estrus in different mineral supplemented groups in post partum anoestrus buffaloes were presented in Table No. 9. Group III was having significantly ($p < 0.05$) lesser interval than group II followed by group I. The group IV was having significantly ($p < 0.05$) highest interval between treatments to the estrus exhibition. On the other hand, Baruah *et al.* (2000) reported 120-132 days in mineral supplemented group and 168-178 days in non mineral supplemented group in post partum swamp buffaloes from the day of calving. Similarly, Swenson *et al.* (1998) reported a shorter postpartum interval for cows supplemented with organic sources of copper, zinc, manganese, and cobalt when compared with sulfate forms of the trace minerals and un-supplemented controls. Boland *et al.* (1996) found that cows receiving organic trace minerals (copper, zinc and manganese proteinates and selenium yeast) had a non-significant reduction in days to emergence of the first dominant follicle (7.8 vs. 9.3 days). The calcium soaps of fatty acids may be used in relatively small amounts in beef cow diets on range to improve reproductive efficiency during the postpartum period. Espinoza *et al.* (1995) reported that feeding of megalac resulted in more cows cycling at 30 to 90 days of post partum.

5.3.5 Conception Rate

In the present study, the percentages of conception rate in different mineral supplemented groups in post partum anoestrus buffaloes were presented in Table No. 8.

The conception rates were significantly ($p < 0.05$) higher in group III followed by group II and I and lowest in group IV. The observations in the present study were corroborating with the findings of by Bhat *et al.* (2009), who reported 71.4 per cent of conception rate in post partum anoestrus cows with mineral supplementation when compared to 33.3 per cent in control group. Similarly, Singh *et al.* (2006) reported that the conception rates in mineral supplemented anoestrus buffalo heifers and buffaloes were 63.63 per cent and 71.92 per cent respectively which were higher than the present study. Ahola *et al.* (2004) recorded 77 per cent conception rate in organic mineral supplementation group, 65 per cent in inorganic mineral supplementation group and 58 per cent in control group of cows. Similarly, Stanton *et al.* (2000) and Kropp (1990) reported higher pregnancy rate in cows receiving organic copper, zinc and manganese than the inorganic forms which was in agreement with the conception rate of the present study. Bisla *et al.* (2006) reported lower conception rate 23.5 per cent in buffaloes after mineral supplementation. This variation could be due to the feeding practices, level of mineral supplementation, breeding practices and other managemental factors etc.

Fallon *et al.* (1993) showed that superovulated, cross-bred heifers receiving organic Cu, Zn, and Mn supplementation displayed a significant (8.5 per cent) increase in fertilization rate and 36 per cent increase in the numbers of fertilized embryos. Britt (1996) suggested that supplementation of organic trace minerals in diets of superovulated cows resulted in increase in the number of transferable embryos per flush and a marked increase in the number of Grade I embryos collected. These observations coupled with positive observations in the field suggested that the use of organic trace minerals in dairy diets might play a beneficial role in the improvement of reproductive performance.

CHAPTER VI
SUMMARY

The present investigation was carried out at Dairy Experimental Station, College of Veterinary Science, Rajendranagar, Hyderabad and commercial dairy farms in Rangareddy district in postpartum pluriparous true anoestrus Murrah buffaloes having a body condition score ranging from 3.0 to 4.0 screened clinically for reproductive disorders. A total of 60 buffaloes free from reproductive problems and having completely involuted uterus were selected for the study.

Animals selected for the experiment were divided into 4 groups comprising 15 in each group and were subjected to different mineral supplementation (Group-I: Cyclomin-7 at the dose rate of 2 boli/week/animal and Dicalcium phosphate at the dose rate of 30gm/day/animal, Group-II: Non-chelated mineral mixture at the dose rate of 50gm/day/animal, Group-III: Chelated mineral mixture at the dose rate of 50gm/day/animal/30days, Group-IV: Control group, without any mineral supplementation) and fed with dry fodder, green fodder and concentrates as per the production requirements in intensive feeding system.

During the course of treatment estrus symptoms were monitored with help of teaser bulls twice or thrice per day and by visual observation 5 to 6 times per day. Animals which were not showing estrus symptoms were examined once in a week per rectally to know the ovarian activity. Animals which showed estrus symptoms have been inseminated twice during late estrus period. After breeding, animals were examined for subsequent estrus cycle and inseminated if required during subsequent cycles. Pregnancy Diagnosis is done per rectally after 50 days of breeding to know the conception status. Blood samples have been collected on zero, 15th and 30th day of treatment for estimation of serum minerals and haematological parameters. Estrus intensity and duration of estrus has been recorded based on the physiological symptoms.

The mean haemoglobin levels on 15th and 30th day of treatment group III was significantly ($p < 0.05$) higher. In between groups there was no significant ($p < 0.05$) difference in haemoglobin levels on zero, 15th day. But on 30th day three treatment groups were having statistically similar concentrations which were significantly ($p < 0.05$) higher than the control group. Basing on the present study results the dietary mineral supplementation increased in haemoglobin concentration over a period of 30 days, moreover in chelated mineral supplemented group the haemoglobin concentration significantly ($p < 0.05$) higher due to the bioavailability of minerals like iron and copper which are necessary for haemoglobin formation.

The mean serum calcium levels and phosphorous levels on 30th day of treatment were significantly ($p < 0.05$) higher in group III followed by group II, group I and lowest in group IV. It was concluded that the dietary mineral supplementation increased the serum calcium and phosphorus level in a period of 30 days which intern improved the reproductive performance. Calcium plays an important role in the gonadotropic regulation of ovarian steroidogenesis and regulation of the membrane potential of oocytes. It is also suggested that calcium is involved in the disruption of cumulus cell cohesiveness by regulating the number of gap junctions between the cells, which contributes to the process of ovulation. Moreover, a disturbed calcium-phosphorus ratio has a blocking action on the pituitary gland and consequently on the ovarian function. Increased phosphorus levels with the mineral supplementation during the treatment period were useful for the better estrus exhibition and conception rate in the treatment group of buffaloes.

The mean serum iron levels in group I, II were significantly ($p < 0.05$) lower on zero day when compare to 15^{th} and 30^{th} day. In between four groups, on 30^{th} day group III was having significantly ($p < 0.05$) higher iron levels. The improvement of iron levels with mineral supplementation in the present study helped for the slight improvement in haemoglobin levels. More than half of the iron in the animal body is found as a constituent of haemoglobin. A small amount is found in myoglobin and certain enzymes which play a part in oxygen utilization and increased the ovarian activity which in turn improved the reproductive performance.

The mean serum copper level was significantly ($p < 0.05$) higher on 30^{th} day followed by 15^{th} day and lowest on zero day in all three treatment groups. In between four groups there was significant difference ($p < 0.05$) on 15^{th} and 30^{th} day and group III was having significantly ($p < 0.05$) higher serum copper levels. The higher copper level in present study was due to the chelated mineral supplementation and more bioavailability of the copper will bring changes in the steroidal metabolism which led to altered reproductive behaviour and performance.

The mean serum manganese level was significantly ($p < 0.05$) different in all three treatment groups on zero, 15^{th} and 30^{th} day. In between four groups there was significant ($p < 0.05$) difference on 15^{th} and 30^{th} day and group III was having higher serum manganese levels. In the present study, in group III serum manganese levels were higher. It was due to the chelated trace minerals, which were bound to organic ligands through covalent bonds. The bonds between the ligand and the mineral prevent the mineral from interacting with the antagonist and improve the bioavailability of the mineral. It revealed that the supplementation of the manganese in the diet was very much important for exhibiting estrus and for high conception rate.

The mean serum zinc level on 15^{th} and 30^{th} day group III was having significantly ($p < 0.05$) higher serum zinc level followed by II, I and IV. Higher serum zinc levels in group III was due to chelated mineral supplementation. Zinc regulates thyroid hormone binding and inositol phosphate synthesis and alters the prostaglandin synthesis which in turn affects the luteal function. The increased levels of serum zinc was increased the luteal function and increased the estrus response, duration of estrus and conception rate.

The serum mineral profiles of buffaloes in this experiment were lesser in calcium, copper and manganese levels when compared to the critical values and zinc and iron were above the critical values. Fodder crops of these dairy farms are mostly irrigated with sewage water of Hyderabad town flows in the Musi River. So it may responsible for higher in zinc and iron levels in the sorghum. This indicates in mineral supplementation calcium, phosphorus, manganese and copper levels be more and zinc and iron levels will be lesser for optimum reproductive performance.

Higher increase of serum mineral levels calcium, phosphorus, iron, copper, manganese and zinc were observed in group III chelated mineral group when compared to other two treatment groups and control group from zero day to 30th day of treatment, it indicating that the chelated minerals were having better bioavailability and not interfering with antagonistic action. So due to this effect in chelated mineral group increase in serum minerals were higher the estrus response, duration of estrus and estrus intensity were significantly ($p < 0.05$) different in between all four groups and group III was having significantly ($p < 0.05$) higher estrus response, duration of estrus and estrus intensity followed by group II, I and lowest in IV. The interval between treatment to the estrus exhibition there was significant ($p < 0.05$) difference in between all four groups, group III was having significantly ($p < 0.05$) lower interval and higher in group II, I with highest in group IV. The conception rates were significantly ($p < 0.05$) higher in group III and followed by group II and I lowest in IV.

It was concluded that in the present study the different kind of mineral supplementation in post partum anoestrus graded Murrah buffaloes have positive effect on haematological parameters and increased in serum mineral concentrations, which in turn increased the reproductive performance by increased in estrus response, duration of estrus and estrus intensity. The number of animals came to heat was higher in mineral supplemented groups when compared with the control group (non mineral supplemented group) and among mineral supplemented groups the estrus response was higher in chelated mineral supplemented group when compared with that of non chelated mineral supplementation and also with Di Calcium Phosphate plus Cyclomin – 7 which was due to serum mineral bioavailability and increased in serum mineral levels. Increased serum mineral levels enhanced the estrus response, duration of estrus and estrus intensity and decreased the interval between the treatment to the estrus exhibition and increased the conception rate.

It was concluded that use of chelated mineral is better option in postpartum buffaloes for better absorption of minerals in stress full condition from the gut of the animal when compared to the than non chelated minerals. It will reduce the service period and increase the conception rate.

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APPENDIX 'A'

ESTIMATION OF HAEMOGLOBIN (Hb)

Method: Acid – haematin method

Principle:

Hb is converted to acid – haematin on reaction with dilute hydrochloric acid. The resulting brownish yellow mixture is matched with a standard in a comparator.

Materials required:

1. Sahli's Haemometer
2. N/10 hydrochloric acid (HCl)

Procedure:

0.1 N HCl was added to whole blood and mixture was allowed to stand until haematin was developed. Colour of the blood and acid mixture was compared with standard in comparator.

APPENDIX 'B'

ESTIMATION OF SERUM CALCIUM

The calcium level in serum was estimated by the a-cresolpthalein complexone method described in the kit. The values were expressed in mg%.

Reagents:

Diagnostic reagent kit supplied by M/S. SPAN Diagnostics Limited was used for determination of serum calcium, which contained the following:

1) **OCPC r**eagent 2) **AMP** buffer 3) Calcium standard 10 mg%

Preparation of working reagents:

Equal volumes of **OCPC** reagent and **AMP** buffer were mixed as per daily requirement. Calcium Standard was ready to use reagent.

Procedure:

The test tubes labelled Blank (B), Standard (S) and Test (T) were taken and **1000 µl** of the working calcium Reagent was added to all the 3 tubes. To the test tube marked (S) **20 µl** of calcium standard was added and the test tube marked (T) **20 µl** of serum was added, mixed well and allowed to stand at room temperature for 5 minutes. Then the absorbance of Standard (S) and Test (T) were measured against Blank (B) on a spectrophotometer at 578 nm within 30 minutes.

Calculations:

$$\text{Serum Calcium (in mg \%)} = \frac{\text{Absorbance of Test}}{\text{Absorbance of Standard}} \times 10$$

APPENDIX 'C'

ESTIMATION OF PHOSPHORUS

The phosphorus level in serum was estimated by modified metol method as described in the kit supplied and the values were expressed in mg%.

Reagents:

Phosphorus kit (modified metol method) supplied by M/S Qualigens Diagnostics, was used for estimation of serum phosphorus, which contained the following reagents:

1) Catalyst Reagent 2) Molybdate Reagent 3) Metol Reagent 4) Standard (5 mg %)

Procedure:

Three test tubes labelled Blank (B), Standard (S) and Test (T) were taken and **1 ml** of catalyst reagent (1) was added to each of them. **1 ml** of Molybdate was added to each test tube. To the tube labelled (B) deionized water **0.1 ml** was added, and **0.1 ml** of Standard (4) was added to the test tube labelled (S). To the test tube labelled (T) **0.1 ml** of serum was added and **1 ml** of metol reagent (3) was added to each test tube.

All the contents of three test tubes were mixed, and allowed them to standard at room temperature for 5 minutes. Then the absorbance of the Test (T) and Standard (S) were measured against Blank (B) on a spectrophotometer at 680 nm, within 30 minutes.

Calculations:

$$\text{Serum Phosphorus (in mg \%)} = \frac{\text{Absorbance of Test}}{\text{Absorbance of Standard}} \times 5$$

APPENDIX 'D'

SERUM MINERAL ESTIMATION

The Tri- acid digestion method is the most frequently used procedure for serum minerals like Cu, Zn, Fe, Mn estimations by Atomic absorption Spectrophotometer.

Reagents:

1. Tri-acid Mixture: - 9:3:1 Nitric acid, Perchloric acid, Sulphuric acid.

Example- 90ml- nitric acid (concentrated)

30ml- perchloric acid (concentrated)

10ml- sulphuric acid. (concentrated)

Method:

Take 1ml serum in 100 ml conical flask and added 10ml of Tri-acid mixture. It gives pale yellow color. Digest the sample by using hot plate at 60°c until colorless or 1ml sample remains in conical flask. Cool the sample and makeup volume to 25 ml by using double distilled water. Read the sample by using AAS for minerals.

Calculation:

ppm (mg/1000ml) = Reading x 25 (25ml Dilution).

Precautions:-

1. Serum should always be clear and should not interfere with any porphyrins (red color).
2. Blood sample interfere with the Fe readings.
3. Conical flasks should always be cleaned by using double distilled water and dry them.
4. After reading, store the digested sample at 10°c or freeze.

5. AAS-Standards Range for serum minerals (ppm):

Copper (Cu):	0.5, 1.0, 1.5, 2.0, 2.5, and 3.0
Zinc (Zn):	0.5, 1.0, 1.5, 2.0, 2.5, and 3.0
Iron (Fe):	0.5, 1.0, 1.5, 2.0, 2.5, and 3.0
Manganese (Mn):	0.5, 1.0, 1.5, 2.0, 2.5, and 3.0

Table 1: Haemoglobin (g%) levels in post partum anoestrus graded Murrah buffaloes of different mineral supplementation groups.

S. No	Name of the Group	Days of sample collection		
		0 day	15^{th} day	30^{th} day
1	GROUP I	11.05 ± 0.26	11.31 ± 0.27	11.55^{AB} ± 0.26
2	GROUP II	11.01 ± 0.22	11.32 ± 0.23	11.63^{AB} ± 0.22
3	GROUP III	11.03^{a} ± 0.18	11.46^{ab} ± 0.26	11.89^{Bb} ± 0.27
4	GROUP IV	11.01 ± 0.16	11.03 ± 0.17	11.06^{A} ± 0.17

[a, b, c, ...] differ significantly ($p < 0.05$) within a row.
[A, B, C, D...] differ significantly ($p < 0.05$) within a column.

Table 2: Serum Calcium levels (mg/dl) in post partum anoestrus graded Murrah buffaloes of different mineral supplementation groups.

S. No	Name of the Group	Days of sample collection		
		0 day	15th day	30th day
1	GROUP I	$7.20^{a} \pm 0.23$	$7.69^{ABb} \pm 0.23$	$8.08^{Bc} \pm 0.22$
2	GROUP II	$7.15^{a} \pm 0.21$	$7.85^{Bb} \pm 0.12$	$8.63^{Cc} \pm 0.16$
3	GROUP III	$7.24^{a} \pm 0.23$	$7.97^{Bb} \pm 0.12$	$8.79^{Cc} \pm 0.07$
4	GROUP IV	7.31 ± 0.10	$7.34^{A} \pm 0.06$	$7.35^{A} \pm 0.07$

a, b, c, ...differ significantly ($p < 0.05$) within a row.
A, B, C, D.... differ significantly ($p < 0.05$) within a column.

Table 3: Serum Phosphorus levels (mg/dl) in post partum anoestrus graded Murrah buffaloes of different mineral supplementation groups.

S. No	Name of the Group	Days of sample collection		
		0 day	15th day	30th day
1	GROUP I	5.46 ± 0.29	5.79 ± 0.32	6.24^{AB} ± 0.18
2	GROUP II	5.78 ± 0.18	5.87 ± 0.28	6.30^{B} ± 0.25
3	GROUP III	5.56^{a} ± 0.07	5.96^{b} ± 0.17	6.94^{Cc} ± 0.05
4	GROUP IV	5.56^{a} ± 0.07	5.83^{b} ± 0.11	5.80^{Aab} ± 0.10

a, b, c, ...differ significantly ($p < 0.05$) within a row.
A, B, C, D... differ significantly ($p < 0.05$) within a column.

Table 4 : Serum Iron levels (ppm) in post partum anoestrus graded Murrah buffaloes of different mineral supplementation groups.

S. No	Name of the Group	Days of sample collection		
		0 day	**15th day**	**30th day**
1	GROUP I	2.716[a] ± 0.112	3.122[Bb] ± 0.084	3.180[Bb] ± 0.110
2	GROUP II	2.656[a] ± 0.058	3.147[Bb] ± 0.068	3.246[Bb] ± 0.140
3	GROUP III	2.689[a] ± 0.047	3.174[Bb] ± 0.194	3.680[Cc] ± 0.081
4	GROUP IV	2.679 ± 0.039	2.690[A] ± 0.061	2.702[A] ± 0.060

a, b, c, ...differ significantly ($p < 0.05$) within a row.

A, B, C, D... differ significantly ($p < 0.05$) within a column.

Table 5: Serum Copper

concentration (ppm) in post partum anoestrus graded Murrah buffaloes with mineral supplementation in different groups.

S. No	Name of the Group	Days of sample collection		
		0 day	15^{th} day	30^{th} day
1	GROUP I	$0.047^{a} \pm 0.003$	$0.097^{Bb} \pm 0.003$	$0.142^{Bc} \pm 0.006$
2	GROUP II	$0.049^{a} \pm 0.005$	$0.127^{Cb} \pm 0.005$	$0.163^{Cc} \pm 0.005$
3	GROUP III	$0.052^{a} \pm 0.002$	$0.149^{Db} \pm 0.003$	$0.184^{Dc} \pm 0.005$
4	GROUP IV	0.049 ± 0.002	$0.052^{A} \pm 0.002$	$0.054^{A} \pm 0.003$

$^{a, b, c, ...}$differ significantly ($p < 0.05$) within a row.
$^{A, B, C, D...}$ differ significantly ($p < 0.05$) within a column.

Table 6: Serum Manganese levels (ppm) in post partum anoestrus graded Murrah buffaloes of different mineral supplementation groups.

S. No	Name of the Group	Days of sample collection		
		0 day	15^{th} day	30^{th} day
1	GROUP I	$0.056^{a} \pm 0.003$	$0.076^{Bb} \pm 0.003$	$0.151^{Bc} \pm 0.007$
2	GROUP II	$0.058^{a} \pm 0.002$	$0.112^{Cb} \pm 0.002$	$0.165^{Cc} \pm 0.006$
3	GROUP III	$0.054^{a} \pm 0.002$	$0.148^{Db} \pm 0.002$	$0.182^{Dc} \pm 0.002$
4	GROUP IV	0.056 ± 0.002	$0.056^{A} \pm 0.003$	$0.055^{A} \pm 0.003$

a, b, c, …differ significantly ($p < 0.05$) within a row.

A, B, C, D... differ significantly ($p < 0.05$) within a column.

Table 7: Serum Zinc levels (ppm) in post partum anoestrus graded Murrah buffaloes of different mineral supplementation groups.

S. No	Name of the Group	Days of sample collection		
		0 day	15th day	30th day
1	GROUP I	$1.062^{ABa} \pm 0.023$	$1.162^{Bb} \pm 0.033$	$2.639^{Bb} \pm 0.061$
2	GROUP II	$1.089^{Ba} \pm 0.008$	$1.248^{Cb} \pm 0.018$	$3.038^{Cc} \pm 0.017$
3	GROUP III	$1.075^{ABa} \pm 0.010$	$1.427^{Db} \pm 0.039$	$3.767^{Dc} \pm 0.042$
4	GROUP IV	$1.032^{A} \pm 0.012$	$1.050^{A} \pm 0.010$	$1.054^{A} \pm 0.010$

a, b, c, ...differ significantly ($p < 0.05$) within a row.

A, B, C, D... differ significantly ($p < 0.05$) within a column.

Table No 8: Details of the Estrus response, Conception rate in mineral supplemented post partum anoestrus graded Murrah buffaloes.

S. No.	Name of the group	No. of animals treated	No. of Animals Exhibited Estrus	No. of Animals found Positive	Per cent of Estrus response	Conception rate on treated animals (%)	Conception rate on Estrus exhibited animals (%)
1	GROUP I	15	7	3	(7/15) 46.66 %	(3/15) 20 %	(3/7) 42.85 %
2	GROUP II	15	10	6	(10/15) 66.66 %	(6/15) 40 %	(6/10) 60 %
3	GROUP III	15	13	11	(13/15) 86.66 %	(11/15) 73.33 %	(11/13) 84.61 %
4	GROUP IV	15	5	2	(5/15) 33.33 %	(2/15) 13.33 %	(2/5) 40 %

*Figures indicating in the parenthesis were number of animals exhibited estrus, conceived in respective groups.

Table No 9 : Details of Estrus patterns in mineral supplemented post partum anoestrus graded Murrah buffaloes.

S. No	Name of the group	No. of animals in the group	No. Of animals exhibited Estrus	No. Days to estrus (Mean ± S.E., Days)	Duration of Estrus (Mean ± S.E., Hrs)	Intensity of Estrus (Mean ± S.E, Points)
1	GROUP I	15	7	46.67[b] ± 0.95	22.13[b] ± 0.83	10.57[b] ± 0.69
2	GROUP II	15	10	35.20[c] ± 1.06	26.46[c] ± 0.98	12.30[c] ± 0.76
3	GROUP III	15	13	25.93[d] ± 0.98	32.93[d] ± 0.81	13.15[d] ± 0.72
4	GROUP IV	15	5	70.13[a] ± 1.79	15.80[a] ± 0.65	9.40[a] ± 1.36

a,b,c,d… differ significantly ($p < 0.05$) within a column.

Table No.10: The Details of estrus pattern in mineral supplemented post partum anoestrus graded Murrah buffaloes.

S. No	Name of the group	Significance of Estrus			Significance of Conception rate (based on treated animals)			Significance of Conception rate (based on estrus animals)		
		No. of animals treated	No .of animals exhibited estrus	Chi square test	No. of animals treated	No. of Animals found positive	Chi square test	No. of animals came to heat	No. of animals found positive	Chi square test
1	GROUP I	15	7	10.08 ** Chi Square Value	15	3	14.06 ** Chi Square Value	7	3	4.98 ** Chi Square Value
2	GROUP II	15	10		15	6		10	6	
3	GROUP III	15	13		15	11		13	11	
4	GROUP IV	15	5		15	2		5	2	

** Among the groups significant ($p < 0.01$) level.

Fig. 2: Serum Calcium levels (mg/dl) in post partum anoestrus graded Murrah buffaloes of different mineral supplementation groups.

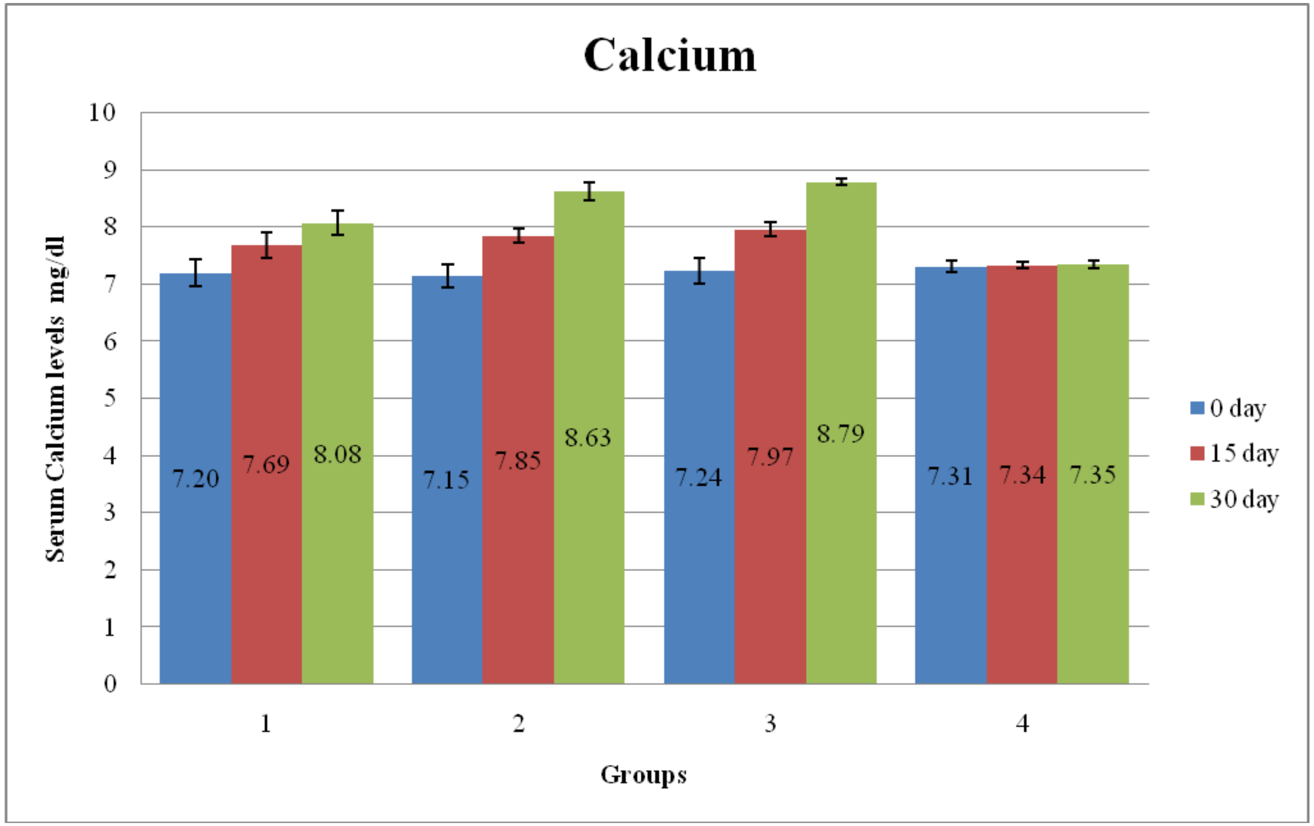

Fig. 3: Serum Phosphorus levels (mg/dl) in post partum anoestrus graded Murrah buffaloes of different mineral supplementation groups.

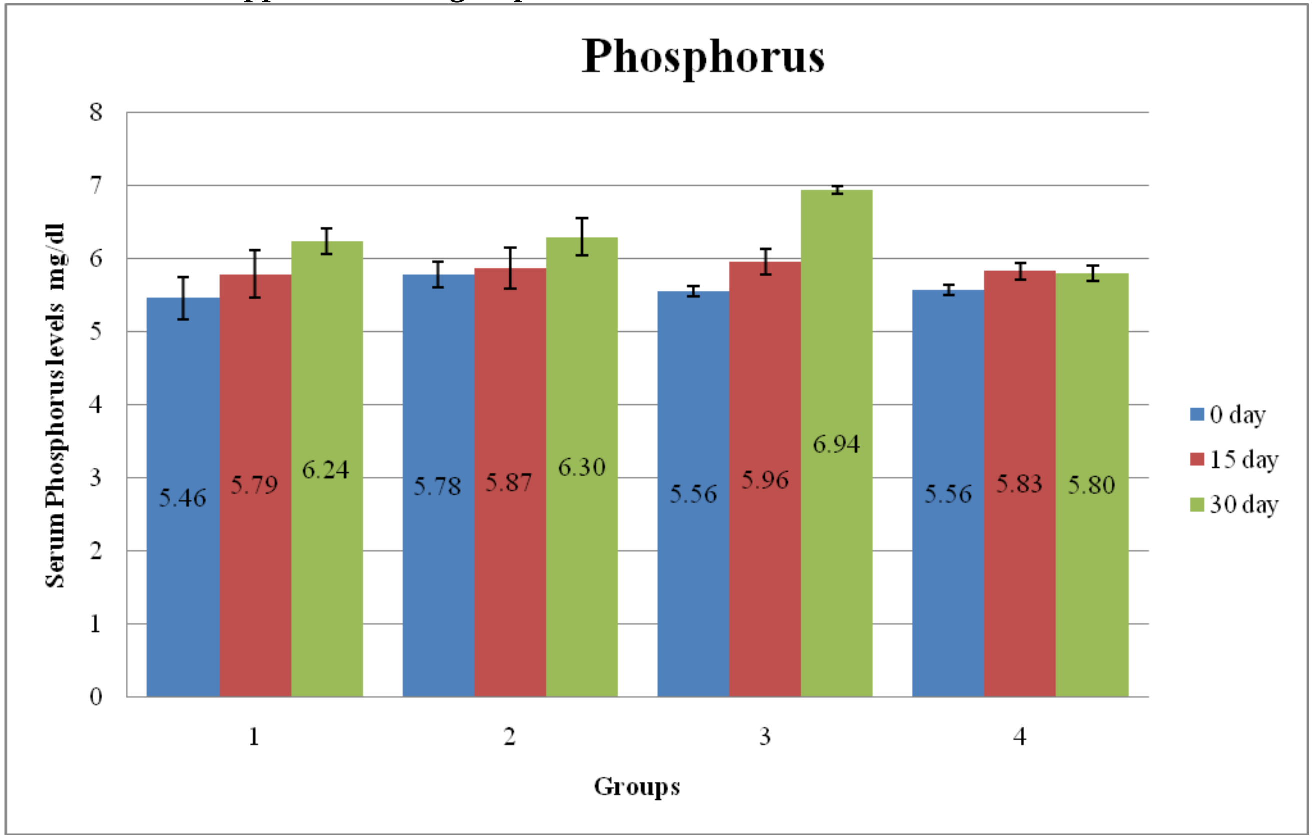

Fig. 4: Serum Iron levels (ppm) in post partum anoestrus graded Murrah buffaloes of different

mineral supplementation groups.

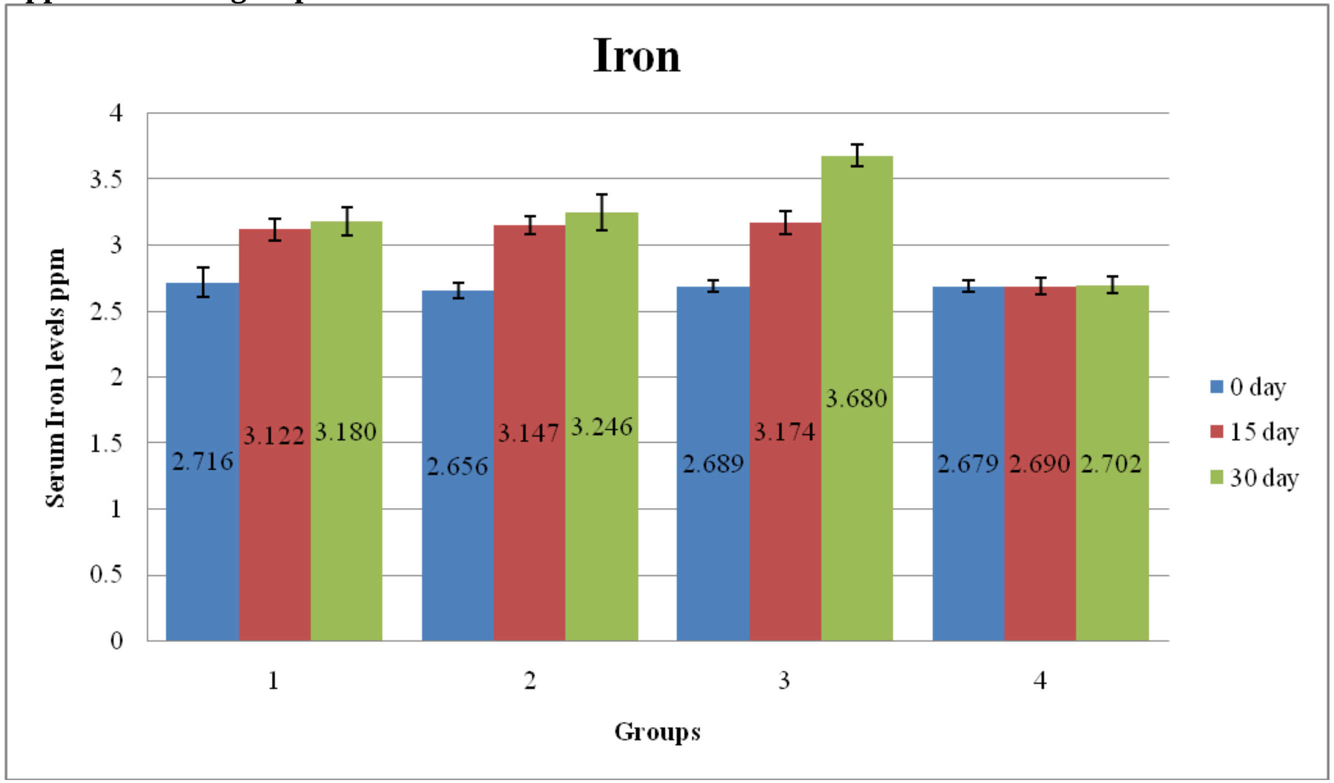

Fig. 5: Serum Copper levels (ppm) in post partum anoestrus graded Murrah buffaloes of different mineral supplementation groups.

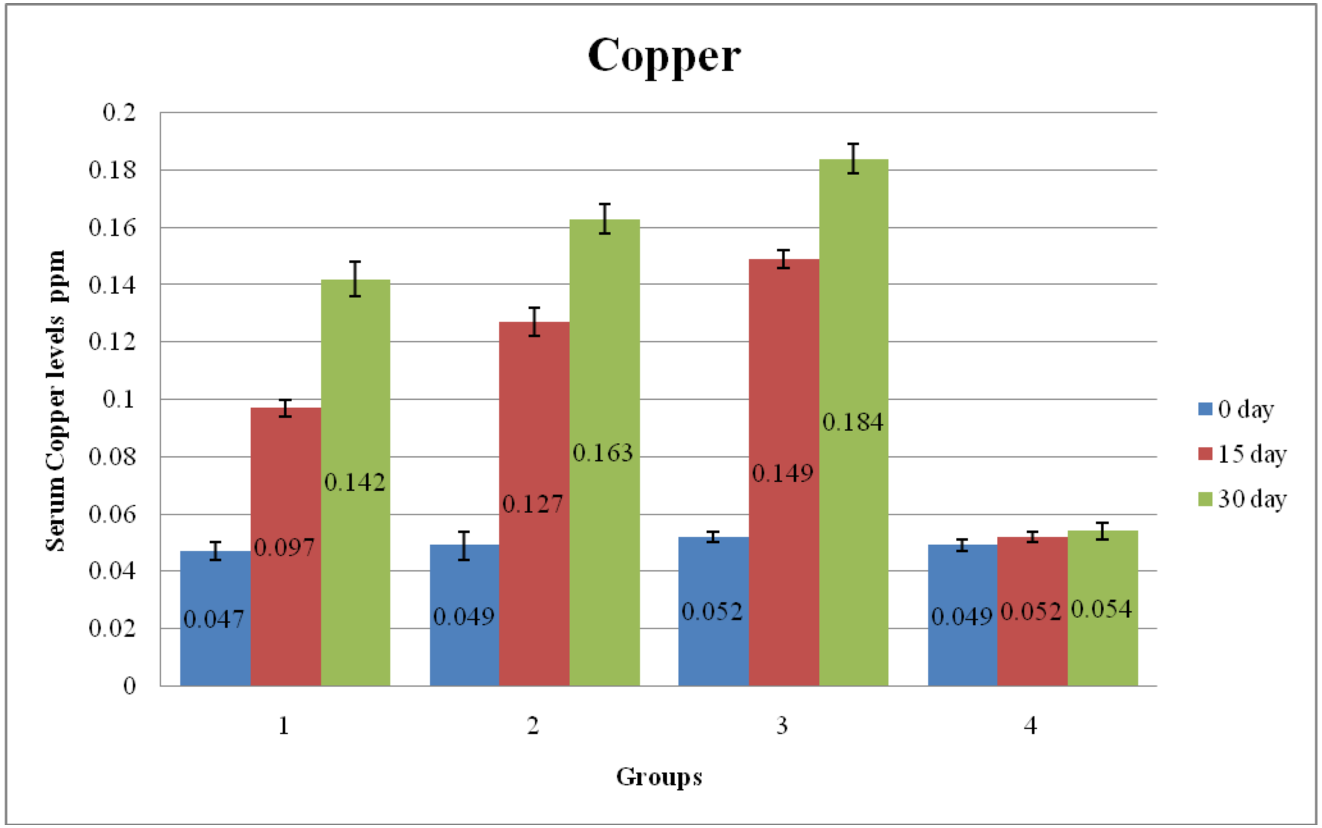

Fig. 6: Serum Manganese levels (ppm) in post partum anoestrus graded Murrah buffaloes of different mineral supplementation groups.

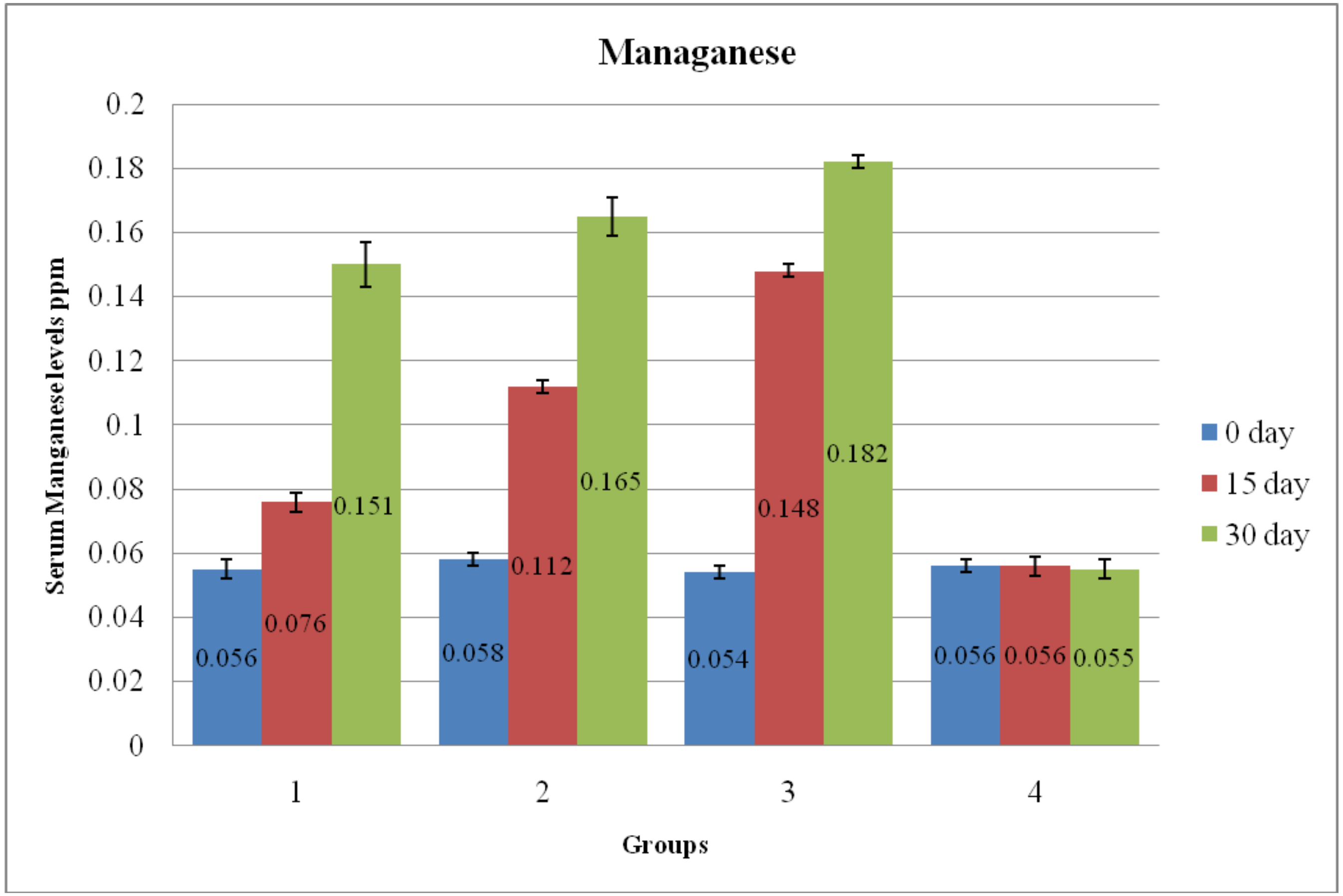

Fig. 7: Serum Zinc levels (ppm) in post partum anoestrus graded Murrah buffaloes of different mineral supplementation groups.

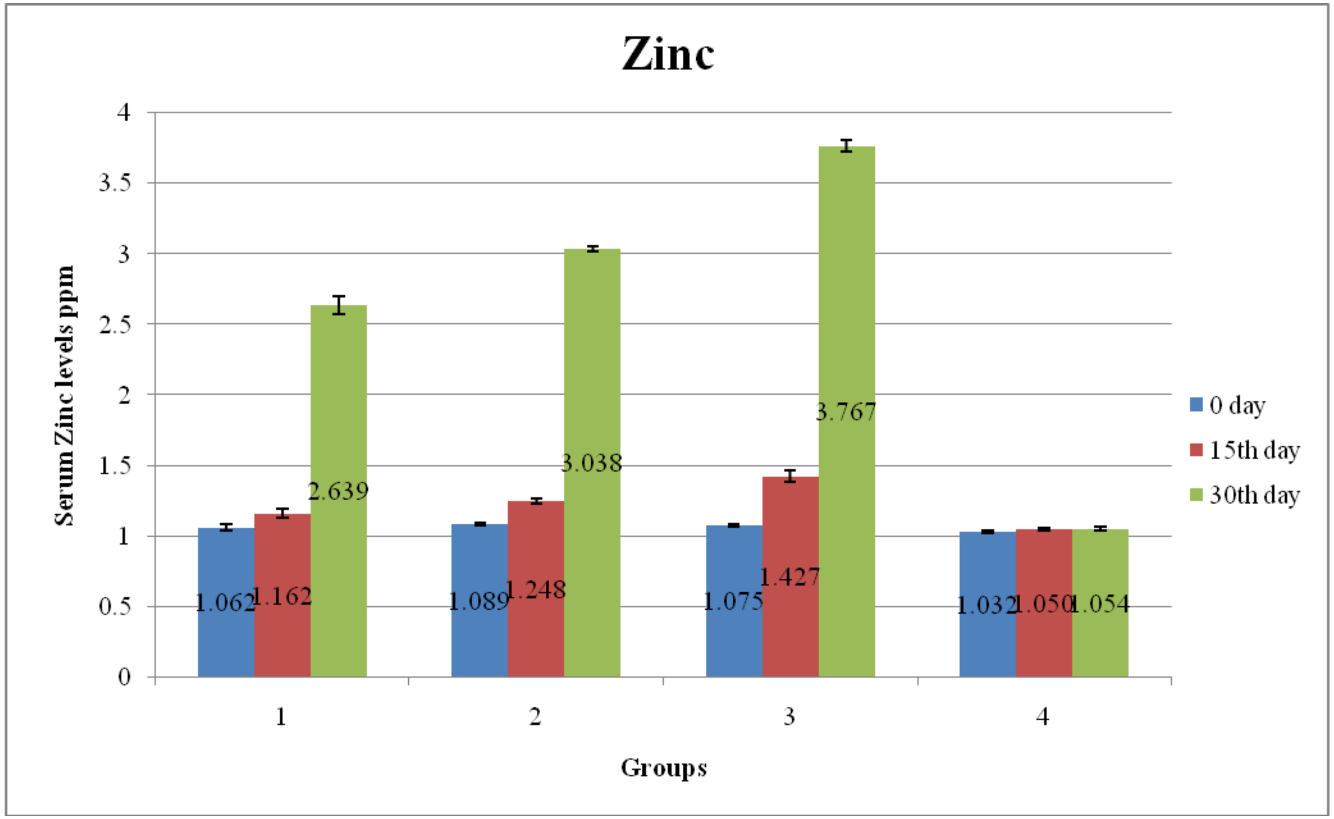

Fig. 1: Haemoglobin levels (g %) in post partum anoestrus graded Murrah buffaloes of different mineral supplementation groups.

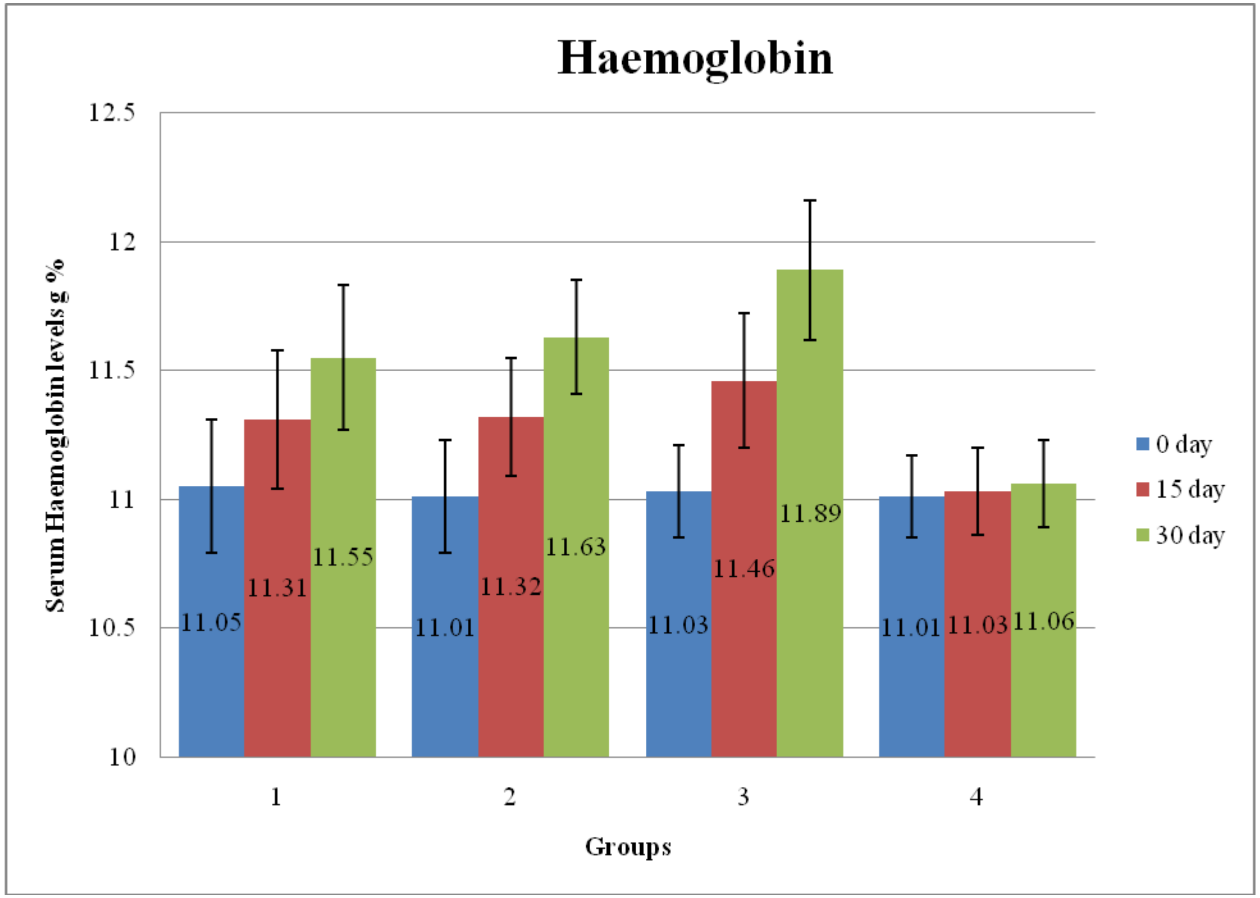
Haemoglobin
Serum Haemoglobin levels g %
12.5
12
11.5
11
10.5
10
11.05
11.31
11.55
11.01
11.32
11.63
11.03
11.46
11.89
11.01
11.03
11.06
1
2
3
4
Groups
0 day
15 day
30 day

PLATE - I

A. Atomic Absorption Spectrometer

B. Chemistry Analyzer