INFLUENCE OF SUPPLEMENTATION OF APPROPRIATE MINERAL MIXTURE TO ANESTROUS AND REPEAT BREEDER BUFFALOES IN TRIBAL AREAS OF PANCHMAHAL DISTRICT

A

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By

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INDIA

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DEDICATED

TO

MY

BELOVED PARENTS

ABSTRACT

INFLUENCE OF SUPPLEMENTATION OF APPROPRIATE MINERAL MIXTURE TO ANOESTRUS AND REPEAT BREEDER BUFFALOES IN TRIBAL AREAS OF PANCHMAHAL DISTRICT

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ABSTRACT

Forty buffaloes of post-partum anoestrus and repeat breeder were selected for on-farm trial of 90 days duration at tribal villages viz. Jinjari and Kharod in Panchmahal district. The buffaloes were selected on the basis of history as well as gynecological examination, the postpartum anoestrous (not showing symptoms for more than 100 days after calving) and repeat breeding (not conceiving following three inseminations) animals were randomly allotted to two dietary treatments i.e. T_1 (Ionic mineral mixture) and T_2 (Chelated mineral mixture comprising of Zn in chelate form added extra @ 25% to ionic), following Completely Randomized Design. The buffaloes in control group (T_1) were supplemented ionic mineral mixture with feeding schedule followed by the farmers (concentrate + green roughage + dry roughage) and those in chelated mineral mixture group (T_2) were reared on farm feeding schedule + compound concentrate mixture.

The average DMI (kg/head), DCPI (g/head) and TDNI (kg/head) in T_1 and T_2 groups were 11.46 ± 0.14 and 11.53 ± 0.10; 592.80 ± 17.88 and 567.96 ± 11.22 and 6.40 ± 0.06 and 6.43 ± 0.12, respectively in anoestrus buffaloes. Similarly, in repeat breeder buffaloes, the respective intakes in T_1 and T_2 groups were 11.41 ± 0.10 and 11.47 ± 0.09; 583.82 ± 16.76 and 582.41 ± 5.67 and 6.64 ± 0.08 and 6.60 ± 0.03. The

DMI, DCPI and TDNI in anoestrus and repeat breeder buffaloes were found to be statistically similar and in conformity to ICAR, (1988) standards.

The average intakes of Ca, P and Cu were more than adequate in anoestrus and repeat breeder buffaloes. However, the Zn intake as the per cent of requirement was 66.27±2.37 and 65.58±2.13 in anoestrus buffaloes of ionic and chelated group, and the respective figures in repeat breeder buffaloes was 64.02±1.74 and 66.28±2.37%. The experimental buffaloes were supplemented with appropriate mineral mixture either in ionic or chelated form.

The average blood glucose level (mg/dl) in ionic and chelated group of anoestrus (59.50±0.59 and 61.35±0.77) and repeat breeder (59.68±0.57 and 61.40±0.29) buffaloes. The treatment groups did not differ from each other. The period effect was also not significant.

The ionic and chelated groups did not differ significantly in anoestrus buffaloes for levels (g/dl) of serum total protein (7.14±0.22 and 6.98±0.22), albumin (3.67±0.10 and 3.53±0.14) and globulin (3.48±0.15 and 3.45±0.09). The respective figures for repeat breeder buffaloes were 7.02±0.21 and 7.08±0.20, 3.56±0.12 and 3.58±0.11 and 3.46±0.09 and 3.51±0.09 (g/dl). However, the period effect was found significant ($P<0.01$) in all the variables in anoestrus and repeat breeder buffaloes except for level of globulin in repeat breeder buffaloes. Similarly, the ionic and chelated groups did not differ significantly in anoestrus buffaloes for levels (g/dl) of serum triglycerides (30.40±0.76 and 31.65±0.84), cholesterol (127.98±6.45 and 128.88±7.41), BUN (26.50±1.58 and 24.55±1.57) and Serum creatinine (1.94±0.07 and 1.54±0.03). The respective figures for repeat breeder buffaloes were 32.63±2.54 and 33.00±0.97, 122.00±7.20 and 116.48±5.46, 27.20±1.34 and 25.88±1.42 and 1.93±0.09 and 1.50±0.01 (mg/dl). However, the period effect was found significant ($P<0.01$) in all the

variables in anoestrus and repeat breeder buffaloes except for level of serum triglycerides in anoestrus buffaloes.

In anoestrus buffaloes the ionic and chelated groups did not differ significantly for activity (U/L) of serum AKP (233.13±8.03 and 209.58±9.11), ALT (52.30±1.36 and 54.40±2.26) and AST (172.58±6.07 and 175.13±5.54). The respective figures for repeat breeder buffaloes were 218.83±5.09 and 208.10±9.91, 54.13±1.71 and 56.70±2.17 and 158.10±1.73 and 162.45±2.09 (U/L). However, the period effect was found significant ($P<0.01$) with regard to activities of AKP and ALT both in anoestrus and repeat breeder buffaloes.

The ionic and chelated groups did not differ significantly in anoestrus buffaloes with regard to levels (mg/dl) of serum calcium (8.38±0.61 and 8.64±0.63), Serum phosphorus (5.70±0.30 and 5.93±0.46) and serum magnesium (2.67±0.24 and 2.66±0.24). The respective figures for repeat breeder buffaloes were 8.67±0.58 and 8.76±0.58, 5.76±0.33 and 5.84±0.30 and 2.62±0.22 and 2.63±0.25 (mg/dl). However, the period effect was found significant ($P<0.01$). In both the groups of buffaloes, level of serum Calcium and phosphorus increased by 30th day of supplementation and stabilized thereafter. However, serum magnesium increased by 30th day and slightly declined thereafter.

The levels (mmol/L) in anoestrus buffaloes did not differ significantly for serum sodium (142.13±2.83 and 143.85±1.11) and Serum potassium (8.69±0.37 and 8.48±0.38) in both the groups. The respective figures for repeat breeder buffaloes were 146.63±1.08 and 143.40±2.36 and 9.00±0.48 and 9.43±0.54 (mmol/L). The period effect was found significant ($P<0.01$) with regard to serum potassium level in anoestrus buffaloes.

The ionic and chelated groups did not differ significantly in anoestrus buffaloes with regard to serum zinc level (ppm) which was 1.99±0.27 and 2.25±0.30,

respectively. Similar was the trend in repeat breeder buffaloes and the respective values were 1.95±0.25 and 2.19±0.27. The period effect was found significant ($P<0.01$) and in both groups the level of serum Zn progressively increased up to 90th day.

The average number of days required in ionic and chelated group, for correcting the condition of anoestrus was 63.00±5.81 and 54.33±6.10 and repeat breeding 68.80±5.28 and 51.57±7.26, respectively. Among the animals under study, 50% each of the anoestrus and repeat breeder buffaloes got successfully conceived due to ionic mineral supplementation. Whereas, due to chelate mineral supplementation respectively, 60 and 70% of anoestrus and repeat breeder buffaloes got successfully conceived.

The average total cost (Rs.) including that of feed stuffs and mineral supplement for rearing anoestrus buffaloes worked out as 6637.91±579.34 in ionic group and 5303.40±692.97 in chelated group. In repeat breeder buffaloes the cost worked out as Rs. 6930.51±704.22 in ionic group and 5181.93±740.52 in chelated group, respectively.

It is concluded that anestrous and repeat breeder buffaloes responded favorably to daily supplementation of 30 g appropriate mineral mixture in ionic/inorganic form. But chelated/ organic mineral mixture (comprising of Zn in chelate form added extra @ 25% to ionic) resulted in better response with 25 and 34% lesser cost of rearing in anestrous and repeat breeder buffaloes, respectively.

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CERTIFICATE

This is to certify that the thesis entitled

"Influence of Supplementation of Appropriate Mineral Mixture to Anestrous and Repeat Breeder Buffaloes in Tribal Areas of Panchmahal District"

Submitted by **BUTANI JIGARKUMAR B.** in partial fulfillment of the requirements for the award of the degree of ***"MASTER OF VETERINARY SCIENCE"*** in the subject of **"ANIMAL NUTRITION"** is a record of bonafide research work carried out by him under my guidance and supervision and the thesis has not previously formed the basis for the award of any degree, diploma or other similar title.

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Date: 1/8/2013

CERTIFICATE

This is to certify that I have no objection for providing to any scientist only one copy of any part of this thesis at a time through reprographic process, if necessary for rendering reference service in a library or documentation center.

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DECLARATION

This is to declare that the whole of the research work reported in this thesis for partial fulfillment of the requirement for the degree of "***MASTER OF VETERINARY SCIENCE***" in ***ANIMAL NUTRITION*** by the undersigned is the result of investigations done by me under direct guidance and supervision of ***Dr. SUBHASH PARNERKAR***, Research Scientist and Head, Animal Nutrition Research Department, College of Veterinary Science and Animal Husbandry, Anand Agricultural University, Anand and that no part of the work has been submitted for any other degree so far.

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ABBREVIATIONS

ad lib	*ad libitum*
%	Per cent
<	Less than, not more than
A.No.	Animal number
APMM	Appropriate Mineral Mixture
ASMM	Area Specific Mineral Mixture
AKP	Alkaline phosphatase
ALT	Alanine Aminotransferase
AST	Aspartate Aminotransferase
Avg.	Average
BUN	Blood Urea Nitrogen
BW	Body weight
Ca	Calcium
Ca I	Calcium intake
Ca R	Calcium requirement
CF	Crude Fibre
cm	Centimeter
CP	Crude Protein
CPI	Crude Protein intake
CPR	Crude protein requirement
CV	Coefficient of variation
Cu	Copper
Cu I	Copper intake
CuR	Copper requirement
d	Day
d.f.	Degrees of freedom
DCP	Digestible Crude Protein
dl	Deciliter

DM	Dry Matter
DMI	Dry Matter Intake
EE	Ether Extract
g	Gram
hr	Hour
ICAR	Indian Council of Agricultural Research
IU	International Unit
K	Potassium
kg	Kilogram
L	Litre
mss	Mean Sum of Squares
ME	Metabolizable Energy
mg	Milligram
Mg	Magnesium
ml	Milliliter
mmol	Millimole
Mn	Manganese
Na	Sodium
NFE	Nitrogen Free Extract
NRC	National Research Council
NS	Not significant
OM	Organic matter
P I	Phosphorus intake
PR	Phosphorus requirement
P	Period
Rs.	Rupees
SE	Standard error
SNS	Strategic Nutrient Supplement
T	Treatment
TDMI	Total dry matter intake

TDN	Total digestible nutrients
TDNI	Total digestible nutrients intake
Zn	Zinc
Zn I	Zinc intake
Zn R	Zinc requirement

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source of inspiration for me to proceed ahead in my life. I take this opportunity to dedicate this work to my parents. I fall short of words for the moral support extended by my fiancé ***Ayushi*** *for her kind love, patience, overwhelming support and inspiration.*

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Place: Anand *[Butani Jigarkumar B.]*

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INTRODUCTION

I. INTRODUCTION

In India, the estimated population of buffaloes is 105.34 million (Livestock census, 2007). These animals play an important role in the Indian livestock economy. The buffalo plays an important role in maintaining a sustainable food production system in the developing countries (Nanda and Nakao, 2003). The success of the dairy cattle and buffalo economy lies in proper and optimal reproductive rhythm of each individual cow and 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 calving and lactations during the life span of the animal (Singh *et al.*, 2006).

Anestrous is the most prevalent form of infertility encountered in buffaloes and is the most frustrating and challenging problem. Anestrous due to ovarian dysfunction and silent ovulation and repeat breeding are two major reproductive disorders in buffaloes (Goley and Kadu, 1995).

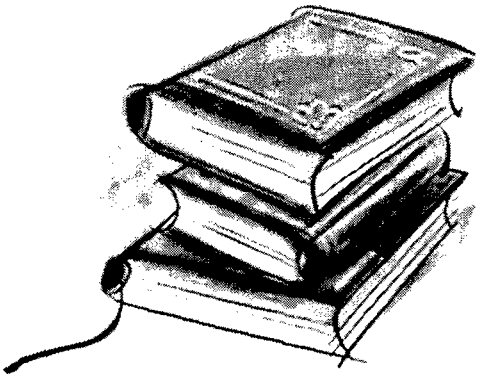
Feeding plays an important role in any livestock development and also for optimum expression of genetic potential of livestock. Though the production and reproduction abilities of animals depend on their genetic potential, it is always prudent to feed them with optimum quantities of different macro as well as micronutrients to exploit their maximum productive and reproductive potentials. Under the tropical conditions of India, the metabolic and/or deficiency diseases in animals are quite common which are mainly due to non availability of balanced diet and also deficiency of specific minerals. Reproductive failure may be induced by deficiencies or imbalances of trace elements (Hidiroglou, 1979). The deficiency of certain minerals like calcium, phosphorus, copper and zinc in dairy cattle has been reported under field conditions (Prasad and Gowda, 2005). Especially, marginal deficiencies are expressed as unthriftiness or sub-normal

growth, fertility or production that are difficult to diagnose and cause significant economic losses. The deficiencies can be corrected by effective mineral supplementation through specifically tailored, high quality, economically feasible mineral supplements that contain only required minerals for correction of disorders.

Beneficial effects of supplementation of deficient minerals either in ionic/inorganic form through area specific mineral mixture (Devasena *et al.,* 2010, Selvaraju *et al.,* 2009, Kumar *et al.,* 2011, Nagalakshmi *et al.,* 2011, Kumar *et al.*, 2012, Mohapatra *et al.,* 2012, Tiwari *et al.,* 2012) and in organic/chelate form (Garg *et al.,* 2008) have been reported on reproductive performance of anoestrus and repeat breeder dairy animals.

Under Tribal Sub Plan (TSP) field study was taken up to demonstrate the benefits of appropriate mineral mixture technology to tribal dairy farmers in Panchmahal district. The department has already evolved appropriate mineral mixture for dairy animals of Panchmahal district which was used for supplementation. In the light of above facts; the present study has been planned in tribal areas in Panchmahal district with the following objectives:

- Survey of feeding practices followed by dairy farmers to work out the nutritional status of anestrous and repeat breeder buffaloes in selected tribal villages of Panchmahal district.
- To study the blood-biochemical status of anestrous and repeat breeder buffaloes in selected tribal villages of Panchmahal district.
- To study the influence of dietary supplementation of appropriate mineral mixture for correcting the condition of anoestrus and repeat breeding in buffaloes of selected tribal villages of Panchmahal district.
- To study the cost: benefit ratio of mineral supplementation.



REVIEW OF LITERATURE

II. REVIEW OF LITERATURE

The dairy sector faces the common constraints of anoestrus, repeat breeding, late maturity, sub-estrus, silent heat in augmenting milk production. Estrous is a physiological reproductive process utilizing minerals, vitamins and micro-nutrients for synthesis of hormones and other bio-molecules (Sharma *et al.*,2003, Kumar *et al.*,1986, Dutta *et al.*,2002 and Beevar, 2006).

Diet adds in endogenous synthesis to manifest normal cycles of cows but inadequate procurement/ improper metabolism disturbs physiological functions, normal metabolism and hormone homeostasis through enzymes and biochemical constituents in blood leading to reproductive failure.

The role of trace minerals in animal production is an area of strong interest for producers, feed manufactures, veterinarians and scientists. Adequate trace 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 ration interfere with the absorption and metabolism of the concerned trace minerals (Olson *et al.,* 1999).

The benefits of supplementation organic/chelated trace minerals in dairy diets have been demonstrated in research and in the field. Traditionally, inorganic forms of trace minerals rapidly dissociation in the rumen and are free to interact with antagonists, resulting in the loss of the trace minerals prior to absorption by the animal (Henry *et al.*, 1992; Ward *et al.*, 1996). Chelated organic trace minerals are bound to organic ligand through coordinate covalent bonds. The bonds between the ligand and the mineral can prevent the minerals from

interacting with antagonists and improve the bioavailability of the mineral (Ward *et al.*, 1996, Bailey *et al.*, 2001). The basic reason for the use of organic forms of trace minerals is the increased bioavailability of organic vs. inorganic sources of the minerals (Spears, 1989; Manspeaker *et al.* 1996).

Keeping above in the view, the present study was undertaken to test the efficiency of varying levels of organic minerals as compared to inorganic trace minerals on reproductive performance of dairy animals to assess the optimum level of supplementation.

2.1. Blood-biochemical status of anoestrus and repeat breeder dairy animals:

2.1.1. Blood biochemical profile:

Serum biochemical constituents, being the true reflections of secretary/excretory functions of different organs, their concentrations vary with metabolic status, physiological state and dietary supplementation (Tamminga, 2006). But cholesterol, the precursor of steroid hormones varies within the cycle, regulates hormonal balance and indicates the reproductive status of the animal.

Vhora *et al.* (1995) estimated the blood serum levels of cholesterol, total proteins in normal cycling and anoestrus cows. In normal cycling cows cholesterol levels (194.94±7.79) were slightly higher than those in anoestrus cows (194.16±16.54), but the difference was non-significant. The level of total proteins (8.62±0.13) was significantly higher in normal cycling cows than in anoestrus cows (6.82±0.40).

Ramakrishna (1996) collected serum samples of 35 anoestrus crossbred Jersey cows and processed for some biochemical constituents and compared with equal number of cycling animals of same age group. Significantly ($P<0.01$) low level of cholesterol and glucose were recorded and also significantly ($P<0.05$) low level of total protein was recorded in anoestrus group as compare to normal cycling animals.

Tandle *et al.* (1997) studied the serum concentration of cholesterol and total proteins in oestrus and anoestrus non-descript cows. In oestrus cows, the cholesterol and total proteins were significantly higher than anoestrus cows.

Chandrahar *et al.* (2003) studied glucose, total protein, albumin, cholesterol and alkaline phosphatase in blood serum of repeat breeder crossbreed cows and their levels were compared with that of normal fertile cows. The repeat breeder cows had significantly ($P<0.01$) lower level of blood glucose while serum albumin and alkaline phosphatase levels were significantly ($P<0.01$) higher level. The differences in total protein and serum cholesterol levels between two groups were non-significant.

Shah *et al.* (2003) studied the blood biochemical profile of 26 suckled post partum Surti buffaloes (18 fertile, 8 infertile by day 90 post partum) at weekly intervals from the day of calving to at least 91 days postpartum and the findings were correlated to reproductive status. The levels of total protein and total cholesterol did not differ significantly between fertile and infertile buffaloes at post partum stages. Only cholesterol increased significantly ($P<0.05$) from the day of calving (109.86±15.46 Vs 125.76±8.14 mg%) to 4-6 weeks postpartum (199.97±13.63 Vs 207.62±20.13 mg%) in both the groups. The cholesterol had significant negative associations with total proteins.

Kumar *et al.* (2007) studied the serum mineral status, vitamins, hormonal and enzymatic profile in buffaloes studied in Gorakhpur, Deoria, Kushinagar and Maharajganj districts of UP having tropical climate. Blood samples (222) were collected randomly from buffaloes of this region. Study revealed that serum enzymes like serum alkaline phosphatase (SAP) was good indicator of such deficiency as their values were significantly lower in deficient buffaloes. While serum alanine amino transferase (ALT) and aspartate amino transferase (AST) values were nonsignificantly lower in these buffaloes.

Tiwari *et al.* (2012a) reported the biochemical profile (blood glucose), macro minerals viz. Ca, Pi, Mg, micro minerals viz. Cu, Co, I, Zn and Mn, hormones, viz. progesterone and insulin in acyclic and cyclic Murrah buffaloes during summer (March to June 2009) in Chhattisgarh. The biochemical profile was estimated in 6 cyclic and 24 acyclic Murrah buffaloes during summer. No significant difference in blood glucose level in cyclic and acyclic buffaloes.

Ali and Shukla (2012) observed the level of blood-biochemical constituents (Hb, TLC, DLC, blood serum glucose, inorganic phosphorus and total serum cholesterol) in anoestrus buffaloes during low breeding season (March to June). The mean levels of blood serum glucose, inorganic phosphorus and cholesterol were significantly ($P<0.01$) higher in normal cycling buffaloes than the anoestrus buffaloes on day 0 and 3 day.

2.2. Mineral profile of anoestrus and repeat breeder buffaloes:

Minerals act at cellular level to regulate synthesis of enzymes, reproductive steroids and nucleic acid metabolism where their imbalance impairs fertility and milk production (Jayanthi *et al.*, 2003). The feeding practices in rural herds / commercial dairy farms either lack proper quality and quantity of minerals or do not maintain bioavailability at required levels for which the heifers exhibit late puberty, anoestrus and repeat breeding (Manston *et al.*, 1975).

2.2.1. Macro-minerals:

Ramakrishna (1996) reported serum samples of 35 anoestrus crossbred Jersey cows of 4.0-6.5 years of age group were processed for some biochemical constituents and are compared with equal number of cycling animals of same age group. Significantly ($P<0.01$) low level of inorganic phosphorus was recorded.

Tandle *et al.* (1997) reported that in oestrus cows the phosphorus levels were significantly higher than anoestrus cows. Higher level of calcium was recorded in oestrus cows but the difference was non significant. The Ca: P ratio was higher (3:1) in anoestrus cows than oestrus cows (2.2:1).

Das *et al.* (2002) reported in repeat breeder cows had significantly ($P<0.05$) lower inorganic phosphorus (4.729 ± 0.150 mg/100ml) than normal cyclic cows where the level was 5.513±0.265 mg/100ml. No significant variation however, was found in Ca between the groups.

Chandrahar *et al.* (2003) reported that repeat breeder cows had significantly ($P<0.01$) lower level of inorganic phosphorus compared to fertile cows while serum calcium was at significantly ($P<0.01$) higher level.

Niazi *et al.* (2003) examined blood mineral level in relation to various stages of oestrous in 35 Friesian and Jersey cows. The experimental animals were divided into following 4 groups. 1. Friesian cows non cyclic, 2. Friesian cows repeat breeder, 3. Jersey cows non cyclic 4. Jersey cows repeat breeder. Blood serum was analysed for Ca, Pi and Mg. The mean values of Ca were 1.96, 2.68, 1.89 and 2.67 millimol/L and of P were 1.46, 1.57, 1.42 and 1.50 millimol/L in four groups respectively. The concentration of Ca and Pi was significantly higher in repeat breeder (2, 4 groups) than in non cyclic cows (1, 3 groups). The Ca and P ratio (Ca:P) was significantly wider/lower 1.34:1,1.33:1 in non cyclic Friesian and Jersey cows than 1.70:1,1.78:1 in repeat breeder Friesian and Jersey cows respectively.

Shah *et al.* (2003) reported that in Surti buffaloes the weekly profile of all macro minerals was higher in fertile than in infertile throughout the postpartum period studied with significant differences in the level of calcium (total and ionized) and Ca:P ratio, which were significantly ($P<0.05$) higher in fertile than the infertile group from 1st to 6th weeks post partum, but not the inorganic phosphorus and none of them differed significantly between different weeks postpartum.

Ceylan *et al.* (2008) estimated the Serum Ca, P and Mg concentration in cows affected with reproductive disorders. The mean concentration of Ca in cows with repeat breeding was found to be significantly ($P<0.01$) higher than that in the control and anoestrus group. The P concentration in cows with repeat breeding and anoestrus was significantly ($P<0.01$) lower than in the control group. The mean Mg concentrations were almost identical for these three groups.

Das *et al.* (2009) reported that the concentrations of Ca and IP varied significantly ($P<0.01$) among the animals with normal ovulation (9.58±0.49 and 5.45±0.15), anovulation (6.48±0.13 and 4.45±0.14) and delayed ovulation (7.36±0.13 and 4.96±0.09). The concentrations of Mg (mg %) was found to be significantly ($P<0.01$) lower in the animals with anovulation (2.64±0.04) compared to the animals with normal (3.12±0.05) and delayed ovulation (2.98±0.06). The results indicated that Ca and IP were probably crucial for the normal ovulatory process and Mg below the optimum level might have caused anovulation in crossbred cattle.

Chaurasia *et al.* (2010) reported that the concentrations of total, free and bound forms of serum sodium, potassium and magnesium were non-significant among the three groups. The total and free forms of serum calcium were significantly ($P<0.01$) lower in anoestrus than in normal cyclic but repeat breeder group was similar to both the groups. Total and free forms of serum phosphorus concentration were significantly ($P<0.01$) higher in normal cyclic as compared to repeat breeder and anoestrus in Murrah buffaloes. Differences in levels of bound forms of serum calcium and phosphorus were nonsignificant among the various groups.

Tiwary *et al.* (2010) conducted the experiment in four villages from two tehsils, namely Roorkee and Laksar of Haridwar district of Uttarakhand. Two villages from each tehsil were indentified from where minimum 10-15 farmers of different categories in each village were selected. Blood samples from cattle and buffaloes of different physiological status viz., lactating, dry, heifer, pregnant and other reproductive health were collected. The blood serum mineral concentration for Ca (10.43±022 mg/dl) and Mg (1.65±0.14 mg/dl) in cattle and buffaloes of different physiological status were found above their respective critical levels, whereas, P (3.79 ± 0.21 mg/dl) concentrations in blood serum of animals were below the respective critical levels. Most of the animals were found to have reproductive problems, which could be attributed to deficiency of different important minerals in this region.

Tiwari *et al.* (2012a) reported that the level of inorganic phosphorus and Mg were significantly lower in acyclic buffaloes than cyclic buffaloes. There was no significant difference in the level of calcium; however, there was significant difference in Ca: P in cyclic and acyclic buffaloes.

Ali and Shukla (2012) observed that the mean levels of inorganic phosphorus was significantly ($P<0.01$) higher in normal cycling buffaloes than the anoestrus buffaloes on day 0 and 3 day.

2.2.2. Micro-minerals:

Kumar and Vadhve (1984) reported low levels of plasma zinc in anoestrus heifers than that in normal cyclic heifers. The concentration of copper was significantly higher during the proestrus phase compared to the oestrus. Anoestrus group of heifers had copper concentration lower than that of proestrus phase but comparable to that in oestrus, metoestrus and dioestrus phases. In heifers of anoestrus group and suboestrus group copper concentration was significantly lower in comparison to proestrus and dioestrus phases.

Pradhan *et al.* (1995) reported that the mean serum copper and zinc in post partum anoestrus cows were found to be 101.31±2.74 mcg percent and 253.87±13.43 mcg percent as compared to 114.20±6.78 mcg percent and 314.00±17.39 mcg percent in normal cycling animals which were found to differ significantly ($P<0.05$).

Vhora *et al.* (1995) reported the respective levels of cobalt and copper (96.67±8.06 and 104.17±3.76)to be significantly higher in normal cycling cows than in anoestrus cows (56.67±6.69 and 73.33±3.35). Higher levels of iron were recorded in normal cycling cows. However the difference between the two groups was non-significant.

Ramakrishna (1996) reported significantly ($P<0.01$) low level of serum iron in anoestrus cross bred Jersey cows compared with equal number of cycling animals of same age group.

Das *et al.* (2002) observed that the repeat breeder cows had significantly ($P<0.05$) lower Cu (0.96 ± 0.017µg/ml) and Zn (1.80 ± 0.033 µg/ml) than normal cyclic cows where the levels were 0.97 ± 0.023 µg/ml and 2.09 ± 0.057 µg/ml, respectively. Higher serum iron level was found in the repeat breeders (3.594 ± 0.043 µg/ml) compared to normal cyclic cows (3.424 ± 0.053 µg/ml). No significant variation however, was found in Mn between the groups.

Shah *et al.* (2003) studied the trace minerals profile of 26 suckled post partum fertile and infertile Surti buffaloes. Plasma iron levels were highest on the day of calving in both the groups, which declined significantly by 1-2 weeks post partum and again, rise to significant level by 11-12 weeks postpartum in fertile group. Zinc, copper and manganese level did not show any specific trend or difference at any stage postpartum in either of the groups, and the values were higher at most intervals in fertile group as compare to infertile group, except copper, which was significantly higher in fertile than infertile group at 7-8 weeks postpartum. Moreover, none of the constituents showed significant variation between different days (0, 7, 14 & 21) of oestrus cycle. Iron was positively correlated with zinc and copper had negative correlation with manganese.

Singh *et al.* (2006) estimated serum concentration of trace minerals in normal cyclic, anoestrus and repeat breeder buffaloes. The serum level of Zn was lower in anoestrus (0.72 ± 0.05 µg/ml) and repeat breeder buffaloes (0.88 ± 0.15 µg/ml) as compare to normal cyclic buffaloes (1.00 ± 0.04 µg/ml). However the difference was not significant. The serum level of Cu was significantly ($P<0.05$) lower in anoestrus (0.59 ± 0.03 µg/ml) and repeat breeder buffaloes (0.62 ±0.03 µg/ml) as compared to normal cyclic buffaloes (0.88 ± 0.03 µg/ml).

Ceylan *et al.* (2008) reported that the serum levels of Zn and Cu in cows with repeat breeding and anoestrus were lower than in the control group, but the variations were not statistically significant even a slight decrease in serum levels of Zn and Cu may induce or predispose animals to repeat breeding and anoestrus.

Patel *et al.* (2010) found that the levels of blood plasma copper, iron, zinc and manganese were significantly ($P<0.05$) higher in normal fertile as compare to repeat breeding Mehsani buffaloes at 0, 10^{th} and 20^{th} days post oestrus.

Tiwari *et al.* (2012a) reported that the micro minerals Zn, Fe, Cu, Co were significantly lower in acyclic buffaloes; however, there was no significant difference in the level of Mn and iodine.

Modi *et al.* (2013) conducted the experiment on 10 normal (Group-I) and 20 repeat breeder (Group-II) Kankrej cows. Blood of each repeat breeding and normally cycling cows were collected aseptically before breeding. Serum was used for the trace minerals estimation. The values observed for copper, iron, zinc and manganese in blood serum of normal and repeat breeding animals were 2.27 ± 0.05 vs. 0.87 ± 0.02, 1.41 ± 0.02 vs. 0.65 ± 0.01, 1.94 ± 0.01 vs. 1.78 ± 0.02 and 0.43 ± 0.02 vs. 0.18 ± 0.01 ppm, respectively. All the values in blood serum were significantly ($P<0.01$) higher in normal than repeat breeder Kankrej cows.

2.3. Influence of supplementation of mineral mixture on Blood-biochemical status of anoestrus and repeat breeder dairy animals:

2.3.1. Blood-biochemical profile:

Das *et al.* (2005) selected 12 adult Jersey crossbred heifers for experimental studies. The ages of the heifers were in between 2.5-3 years and the body weights ranged between 185.5 and 252 kg. Selected animals were subjected to gynaecological examination per rectum and conformed they were in anoestrus condition. The heifers were maintained under standard managerial condition and feeding and they were treated with anthelmintic, mineral mixture, vitamin A injections and non-hormonal herbal preparation (*Sajani*, ^{Tm} Sarabhai Zydus) for induction of heat. Blood samples were collected during anoestrus and within 12 h after onset of heat symptoms (oestrus). The results revealed that the serum glucose, total serum protein, serum globulin, serum aspertate transaminase, serum alanine transaminase and serum alkaline phosphatase were significantly ($P<0.01$) higher during oestrus than in anoestrus condition. No significant differences were observed in serum albumin and serum total cholesterol content between oestrus and anoestrus condition.

Behera *et al.* (2012) studied 90 crossbred heifers of normal cycling, anestrus (>2 years), anestrus and repeat breeding (>3 years) category were selected taking 30 heifers in each category. Each category was divided further into before and after treatment group where normal cycling heifers before and after treatment was referred as Gr-I and IA and those of anestrus as Gr-II and IIA and repeat breeding as Gr-III and III-A respectively. The heifers in all the experimental groups except those in Gr-I were orally supplemented with Minfaa @ 30g / heifer / day for 60 days along with ad libitum feed and water under normal farming conditions. The blood was collected on day 0 as before and day 60 as after treatment. The authors reported low levels of metabolic enzymes that was attributed to anoestrous and repeat breeding and Minfa (mineral mixture) supplementation significantly increased ($P<0.05$) the

levels for ASAT, ALAT and ALP in anoestrous and for ALAT and ALP in repeat breeding heifers due to incorporation of metallic minerals in enzyme synthesis/ activity as components/ activators in support of reproductive hormone action to remove the cause of anoestrus and repeat breeding.

Kumar *et al.* (2011) concluded that strategic nutrient supplementation comprising of appropriate mineral mixture significantly ($P<0.05$) improved blood glucose and total serum protein levels in anoestrous lactating buffaloes.

Mohapatra *et al.* (2012) conducted an on-farm trial in which 80 cows having reproductive disorders were allotted to 4 groups with 20 animals — G1 (control); G2, 20 anoestrus heifers supplemented with area specific mineral mixture (ASMM) @ 50 g/day/animal (ANH); G3, 20 anoestrous cows supplemented with ASMM @ 50 g/day/animal (ANC); and G4, 20 repeat breeding cows supplemented with ASMM @ 50 g/day/animal (RBC). The overall serum biochemical values for glucose, total protein, albumin, globulin and cholesterol increased significantly ($P<0.01$) in supplemented group than in un-supplemented group.

Tiwari *et al.* (2012b) conducted to see the effect of dietary supplementation of minerals as area specific mineral mixture in cattle and buffaloes on reproductive performance, milk yield, their profile in blood serum under field conditions in four villages of Nainital district. The calcium, phosphorus, zinc, manganese and copper were supplemented in the form of feed (mineral) mixture which contained ground maize 32.80%, mustard cake 48.69%, di-calcium phosphate 17.85%, copper sulphate 0.12%, manganese sulphate 0.30% and zinc sulphate 0.24%. Fifteen animals (cattle and buffaloes) of different physiological status from each village were selected. Each selected animal was provided 250 g feed mixture daily in order to supply 12 g calcium, 5 g phosphorus, 250 mg zinc, 175 mg manganese and 60 mg copper over and above already present in the ration of animals provided by the farmers for 45 days. The SGPT, SGOT and ALP activities increased in all categories of animals but were within the normal range.

2.4. Influence of supplementation of mineral mixture on Mineral profile of anoestrus and repeat breeder dairy animals:

2.4.1. Macro-minerals:

Lall *et al.* (2004) experimented on twenty one non-pregnant buffaloes heifers (3-4 yrs) showing anoestrus were taken from herd and given high plane of (120%) of nutrition (Kearl, 1982) and mineral mixture (3% including common salt) in their concentrate mixture. Blood samples from these animals were collected before the start of experiment (0 day), then at 30 and 60 days. Serum Calcium content of the sample showed little variation with the dietary treatment and ranged between 7.31 to 7.76 mg/dl. But the serum 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 0, 30 and 60 days, respectively intervals.

Hadiya *et al.* (2010) experimented on twenty freshly calved healthy triple crossbred (HF x J x K) cows and they were divided randomly into four groups each of five animals from the day of calving to observe the effect of supplementation of minerals + proteins-vitamins (Nutri-sacc power pack, Vetcare) and enzymes (Neozyme RU, Biocon India Ltd) on service period and fortnightly plasma profile of macro-micro minerals up to 105 days postpartum. The animals of Group-I (T_0) served as control; of Group-II (T_1) were given nutri-sacc powder (@100 g/d/h; while animals of Group–III (T_2) and Group IV (T_3) received neozyme supplementation @ 750 and 1000 g per ton of concentrate mixture, which was fed @ 1 kg for every 3 kg milk. The pooled plasma calcium, inorganic phosphorus and magnesium concentrations were 10.29±0.11, 4.43±0.10 and 3.40±0.09 mg %, respectively. Significant ($P<0.05$) differences were observed between groups and between periods for all the three traits. Calcium level was significantly higher in enzyme treatment (T_3) as compared to the control group, while phosphorus showed inverse trend. The calcium level was 8.15±0.61 mg % on the day of calving, which increased significantly by day 15 postpartum and again by day 60 postpartum and remained more or less static thereafter. The level of phosphorus and magnesium increased significantly by day 30-45 postpartum.

Effect of supplementation of area specific mineral mixture (ASMM) was studied by Devasena *et al.* (2010) in crossbred cows (40) having different reproductive disorders. At the end of the 90 days, plasma P was improved ($P<0.05$) in anoestrus cows while Ca content increased in anoestrus heifers.

Amrutkar *et al.* (2012) studied the effect of dietary supplementation of limestone powder (LSP) on plasma mineral profile of crossbred calves. Twelve growing male crossbred calves (12–16 months of age) were divided into three groups 4 each on the basis of their body weight. Group I calves (control group I; BW 230.25 kg) were fed oat fodder, wheat straw and concentrate (without LSP) as per requirements. Group II (treatment group II; BW 227.00 kg) and group III (treatment group III; BW 228.52 kg) calves were fed same ration as control group plus 5 and 10% LSP was added in the concentrate mixture of group II and group III calves, respectively. All the groups were fed ration containing 40% concentrate mixture and 60% roughage for a period of 98 days. Plasma concentrations of Ca and P remained similar in the three groups.

Behera *et al.* (2012) orally supplemented Minfa (mineral mixture) @ 30g / heifer / day for 60 days along with *ad libitum* feed and water under normal farming conditions the blood was collected on day 0 as before and day 60 as after treatment . Minfa supplementation significantly increased ($p<0.05$) the serum levels of Na and K in anoestrus heifers.

Mohapatra *et al.* (2012) conducted an on-farm trial in which 80 cows having reproductive disorders were allotted to 4 groups with 20 animals — G1 (control); G2, 20 anoestrus heifers supplemented with area specific mineral mixture (ASMM) @ 50 g/day/animal (ANH); G3, 20 anoestrous cows supplemented with ASMM @ 50 g/day/animal (ANC); and G4, 20 repeat breeding cows supplemented with ASMM @ 50 g/day/animal (RBC). The overall serum mineral levels and biochemical values increased significantly in supplemented group than in un-supplemented group.

Tiwari *et al.* (2012b) reported that the calcium, phosphorus, zinc, manganese and copper were supplemented in the form of feed (mineral) mixture which contained ground maize 32.80%, mustard cake 48.69%, di-calcium phosphate 17.85%, copper sulphate 0.12%, manganese sulphate 0.30% and zinc sulphate 0.24%. Fifteen animals (cattle and buffaloes) of different physiological status from each village were selected. Each selected animal was provided 250 g feed mixture daily in order to supply 12 g calcium, 5 g phosphorus, 250 mg zinc, 175 mg manganese and 60 mg copper over and above already present in the ration of animals provided by the farmers for 45 days. Blood serum calcium and phosphorus concentrations were increased significantly and reached above their critical levels (8.00 mg/dl and 4.00 mg/dl, respectively) in all categories of animals.

2.4.2. Micro-mineral:

The use of chelated Cu, Mn and Zn supplements in controlling repeat breeding and anoestrus conditions was studied at NDRI, Karnal by Prasad *et al.*, 1991. Two sets of experiment were conducted. In experiment No.1. 48 repeaters of NDRI farm which had repeated continuously for 5 times but did not conceive after insemination, were divided into 3 equal treatment groups: in group T1 the ration consisted of normal farm ration as per NRC (1981) with no supplementation of Cu, Mn and Zn: in T2, apart from the ration of T1, Cu, Mn and Zn were supplemented in ionic salt form, whereas in group T3 same level of Cu, Mn and Zn were supplemented in chelate form. It was found that blood Cu, Mn and Zn levels were highest in group T3. Conception in T1, T2 and T3 groups were 43.75, 62.50 and 75%, respectively.

Lall *et al.* (2004) experimented on twenty one non-pregnant buffaloes heifers (3-4 yrs) showing anoestrus were taken from herd and given high plane of (120%) of nutrition and mineral mixture (3% including common salt) in their concentrate mixture. Blood samples from these animals were collected before the start of experiment (0 day), then at 30 and 60

days. The serum Zn content improved significantly (P<0.05) over the period and was 0.58±0.08, 1.08±0.02 and 1.34±0.04 µg/ml respectively. Cu was not influenced with the dietary treatment and ranged between 0.69 to 0.74 µg/ml.

Kinal *et al.* (2005) fed 90 dairy cows in the period from 6 weeks before calving up to 305th day of lactation with diets containing different chemical forms of zinc, manganese and copper supplied in mineral mixtures. The control group received these trace elements as sulphates, but in experimental groups 20 or 30% of daily needs were covered by chelated forms. Cows receiving 30% of needed Zn and Mn (each 315 mg/day) and Cu (63 mg/day) in amino acids-bound forms were characterized by the significant (P≤0.05) increase of both zinc and copper level in blood of cows receiving organically- bound Zn, Mn and Cu.

Garg *et al.* (2008) experimented at Banaskantha district of Gujarat on 35 animals with normal reproductive organs, but suffering from anoestrus were selected. Nine anoestrus animals served as control and remaining twenty-six were given daily 5 g supplement per animal, containing chelated Cu, Zn and Mn and fat-soluble vitamins A, D_3 and E. Minerals (Cu, Zn and Mn) were analyzed in the blood serum before feeding of supplement and at bi-weekly intervals after feeding the supplement. Copper (0.92 ± 0.01 ppm) and zinc (1.02± 0.03 ppm) levels increased significantly ($p < 0.05$) in the blood serum of animals under experimental group, as compared to control Cu (0.50±0.02 ppm) and Zn (0.60±0.03 ppm). However, level of Mn in experimental (0.06±0.01 ppm) and control (0.04±0.01 ppm) groups was similar.

Hadiya *et al.* (2010) experimented on twenty freshly calved healthy triple crossbred (HF x J x K) cows and they were divided randomly into four groups each of five animals from the day of calving to observe the effect of supplementation of minerals + proteins-vitamins (Nutri-sacc power pack, Vetcare) and enzymes (Neozyme RU, Biocon India Ltd) on service period and fortnightly plasma profile of macro-micro minerals up to 105 days

postpartum. The animals of Group-I (T_0) served as control; of Group-II (T_1) were given nutri-sacc powder (@100 g/d/h; while animals of Group–III (T_2) and Group IV (T_3) received neozyme supplementation @ 750 and 1000 g per ton of concentrate mixture, which was fed @ 1 kg for every 3 kg milk. Highly significant differences ($P<0.01$) were observed between periods for plasma zinc, iron, copper and manganese concentrations with pooled values of 1.19±0.03, 1.53±0.04, 0.85±0.02 and 0.09±0.01 ppm, respectively. The zinc and iron levels increased from day 45 postpartum and remained high till day 105 postpartum, while the copper increased significantly by day 15 postpartum. Nutri-saac and/or enzyme supplementation did not influence the plasma trace minerals profile, except for copper.

Effect of supplementation of deficient minerals through area specific mineral mixture (ASMM) was studied by Devasena *et al.* (2010) in Chittoor district of Andhra Pradesh. Crossbred cows (40) having different reproductive disorders were supplemented with ASMM for 90 days. Blood samples were analysed for minerals and hormones (estrogen and progesterone) prior to supplementation and then at the end of the 90 days. Plasma Cu, Zn and Mn content was improved ($P<0.05$) in anoestrus cows. In repeat breeder cows plasma Cu and Zn showed significant ($P<0.05$) increase. Cu contents increased in anoestrus heifers.

Ullah *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% more than NRC recommended Ca, P, Zn and Mn levels. The non-mineral supplemented group (NMS) was kept as control. Progesterone (P4), estradiol (E2), Ca, P, Zn and Mn were analyzed in blood serum of the buffaloes. Blood mineral levels did not differ except for Zn which was higher ($P<0.05$) in MSG in 2 out of 12 samples.

Nagalakshmi *et al.* (2011) conducted the farm trial of 180 days duration in east Godavari district of coastal zone of Andhra Pradesh to test the ASMM along with the concentrate mixture on production and reproductive efficiency in buffaloes. 60 animals selected from various farmers where randomly distributed among three groups. Group-1 animals were fed as per the farmer practise. In group-2 and 3, the concentrate mixture (based on locally available protein supplements) was offered @ 1kg/day to take care of protein deficiency and also offered 30 gm BIS mineral mixture or ASMM (required quantity of deficient minerals), respectively. The serum Cu, Zn and Mn at start, were below the critical levels and increased with ASMM and BIS mineral mixture supplementation.

Amrutkar *et al.* (2012) studied the effect of dietary supplementation of limestone powder (LSP) on plasma mineral profile of crossbred calves. Twelve growing male crossbred calves (12–16 months of age) were divided into three groups 4 each on the basis of their body weight. Group I calves (control group I; BW 230.25 kg) were fed oat fodder, wheat straw and concentrate (without LSP) as per requirements. Group II (treatment group II; BW 227.00 kg) and group III (treatment group III; BW 228.52 kg) calves were fed same ration as control group plus 5 and 10% LSP was added in the concentrate mixture of group II and group III calves, respectively. All the groups were fed ration containing 40% concentrate mixture and 60% roughage for a period of 98 days. DM intake was similar in all groups, thus showing no effect of varying levels of dietary LSP supplementation. Plasma concentrations Mn remained similar in the three groups. However, the overall mean plasma concentration of Cu and Zn was significantly ($P<0.05$) lower in LSP (5 and 10%) supplemented groups. Supplementation of 5 and 10% level of LSP in concentrate mixture showed adverse effect on growth performance and plasma mineral status of Cu and Zn.

Behera *et al.* (2012) reported that oral mineral mixture supplementation @ 30g/ heifer/day for 60 days significantly increased serum levels of Cu, Zn and Mn.

Kumar *et al.* (2011) conducted the study to know the effect of strategic nutrient supplementation for 50 days on blood mineral profile of anoestrus lactating buffaloes in Jabalpur District. They concluded that strategic nutrient supplementation comprising of appropriate mineral mixture improved blood mineral concentrations of Cu, Zn and Co.

Mohapatra *et al.* (2012) conducted an on-farm trial in which 80 cows having reproductive disorders were allotted to 4 groups with 20 animals — G1 (control); G2 20 anoestrus heifers supplemented with ASMM @ 50 g/day/animal (ANH); G3, 20 anoestrous cows supplemented with ASMM @ 50 g/day/animal (ANC); and G4, 20 repeat breeding cows supplemented with ASMM @ 50 g/day/animal (RBC). The results revealed that the mean Zn, Cu, Mn and Fe were higher than critical level. The overall serum mineral levels and biochemical values increased significantly in supplemented group than in un-supplemented group.

Tiwari *et al.* (2012b) reported that calcium, phosphorus, zinc, manganese and copper were found below the critical level in soil, feeds and blood serum of cattle and buffaloes in hill region of Nainital district of Uttarakhand. A study was, therefore conducted to see the effect of dietary supplementation of these minerals as area specific mineral mixture in cattle and buffaloes on reproductive performance, milk yield, their profile in blood serum under field conditions in four villages. The calcium, phosphorus, zinc, manganese and copper were supplemented in the form of feed (mineral) mixture which contained ground maize 32.80%, mustard cake 48.69%, di-calcium phosphate 17.85%, copper sulphate 0.12%, manganese sulphate 0.30% and zinc sulphate 0.24%. Fifteen animals (cattle and buffaloes) of different physiological status from each village were selected. Each selected animal was provided 250 g feed mixture daily in order to supply 12 g calcium, 5 g phosphorus, 250 mg zinc, 175 mg

manganese and 60 mg copper over and above already present in the ration of animals provided by the farmers for 45 days. The serum zinc, manganese and copper concentration also increased but not significantly due to dietary supplementation of these minerals.

2.5. Effect on hormonal profile:

Garg *et al.* (2008) experimented at Banaskantha district of Gujarat on 35 animals with normal reproductive organs, but suffering from anoestrus was selected. Nine anoestrus animals served as control, and remaining twenty-six were given daily 5 g supplement per animal, containing chelated Cu, Zn and Mn and fat-soluble vitamins A, D_3 and E. Minerals (Cu, Zn and Mn) and progesterone were analyzed in the blood serum before feeding of supplement and at bi- weekly intervals after feeding the supplement. In control group, progesterone level did not rise and remained low (0.25-3.81ng/ml) but in experimental group the serum progesterone varied from 0.30-20ng/ml.

Effect of supplementation of deficient minerals (Ca, P, Cu, Zn and Mn) through ASMM was studied by Devasena *et al.* (2010) in Chittoor district of Andhra Pradesh. Crossbred cows (40) having different reproductive disorders were supplemented with ASMM for 90 days. Blood samples were analysed for minerals and hormones (estrogen and progesterone) prior to supplementation and then at the end of the 90 days. Plasma progesterone in anoestrus cows and anoestrus heifers indicated significant ($P<0.05$) increase after ASMM supplementation.

Ullah *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% 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.

Tiwari *et al.* (2012a) reported that the serum progesterone level was significantly higher in cyclic than acyclic buffaloes.

2.6. Influence of supplementation of mineral mixture on reproductive performance of anoestrus and repeat breeder dairy animals:

2.6.1. Ionic/ inorganic mineral mixture:

Pradhan *et al.* (1995) conducted the experiment on 36 post-partum anoestrus cows. Out of 36 post-partum anoestrus cows, 24 cows were supplemented with trace minerals composed of copper sulphate, ferrous sulphate and zinc sulphate and rest 12 cows were left as untreated control. Eighteen out of 24 post-partum anoestrus cows responded to the treatment and exhibited heat within the average period of sixty days.

Lall *et al.* (2000) reported that the supplementation of mineral mixture @ 50 gm/animal/day to about 100 animals (70 buffaloes and 30 adult heifers of more than 3 years of age showing anestrus for more than 5-6 months) showed that more than 70 percent of these animals exhibited heat and conceived within a period of 2-4 weeks of feeding this mineral mixture, indicating the role of mineral supplementation in curing this problem.

Lall *et al.* (2004) experimented on twenty one non-pregnant buffaloes heifers (3-4 yrs) showing anoestrus that were taken from herd and given high plan of (120%) of nutrition (Kearl, 1982) and mineral mixture (3% including common salt) in their concentrate mixture. Eighteen out of twenty one heifers came into heat and became pregnant within first 3 months of the start of this dietary treatment. It is inferred that high plan of nutrition with proper mineral supplementation can bring majority of the anoestrus buffaloes into oestrus.

Das *et al.* (2005) selected 12 adult Jersey crossbred heifers for experimental studies. The ages of the heifers were in between 2.5-3 years and body weights ranged between 185.5-252 kg. Selected animals were subjected to gynaecological examination per rectum and conformed they were in anoestrus condition. Heifers were maintained under standard managerial condition and feeding and they were treated with anthelmintic, mineral mixture, vitamin A injections and non-hormonal herbal preparation (*Sajani*, ^{Tm} Sarabhai Zydus) for induction of heat. Out of 12 animals, 6 animals showed signs of oestrus within 10 days. Non-responded animals were treated in same way, out of which two animals were showed signs of estrus at 14th day of treatment.

Srivastava *et al.* (2008) reported that supplementation of 30-40 g commercial mineral mixture in concentrate for 20 days induced ovulatory oestrus in 93.33% (28/30) anoestrus crossbred heifers. Out of 28 heifers inseminated, 25 (89.28%) conceived with 1 to 5 insemination. First AI conception rate was 32.14%. Maximum conception rate (52.16%) was observed in second AI.

Selvaraju *et al.* (2009) studied that the efficacy of a supplemental ASMM on improving reproductive efficiency in crossbred dairy cattle. Out of the 110 crossbred dairy cattle examined, 73 were had reproductive problems such as postpartum anoestrus and repeat breeding (28% each), followed by delayed puberty (21%), silent heat (10%), infectious endometritis of mild to moderate degree (10%) and cystic ovary (3%). Among the postpartum anoestrus animals, 84.21 and 85.71% exhibited oestrus and conceived, respectively, within 2 months of ASMM supplementation. Among the repeat breeders, which received the ASMM, 78.6% conceived within 2 inseminations. Onset of oestrus occurred in 66.7% of the delayed pubertal animals, which received the ASMM supplementation. Among the silent heat animals, 66.7% conceived within 3 months of the ASMM supplementation.

Effect of supplementation of deficient minerals (Ca, P, Cu, Zn and Mn) through ASMM was studied by Devasena *et al.* (2010) in Chittoor district of Andhra Pradesh. Forty crossbred cows having different reproductive disorders were supplemented with ASMM for 90 days. Among the animals under study, 60% of the anoestrus cows, 62% of repeat breeders cows and 59% of anoestrus heifers responded to supplementation, indicating the beneficial effect of mineral supplementation on their reproductive performance.

Hadiya *et al.* (2010) experimented on twenty freshly calved healthy triple crossbred (HF x J x K) cows and they were divided randomly into four groups each of five animals from the day of calving to observe the effect of supplementation of minerals + proteins-vitamins (Nutri-sacc power pack, Vetcare) and enzymes (Neozyme RU, Biocon India Ltd) on service period and fortnightly plasma profile of macro-micro minerals up to 105 days postpartum. The animals of Group-I (T_0) served as control; of Group-II (T_1) were given nutri-sacc powder (@100 g/d/h; while animals of Group–III (T_2) and Group IV (T_3) received neozyme supplementation @ 750 and 1000 g per ton of concentrate mixture, which was fed @ 1 kg for every 3 kg milk. The supplementation of minerals and enzymes did not influence service period significantly (147±13.69 days).

Kumar *et al.* (2011) planned the study to know the existing rural feeding practices and incidence of reproductive disorders in buffaloes in villages around Jabalpur district of Mahakausal region in Madhya Pradesh. In all 10 villages, 91 farmers where surveyed and they had 176 buffaloes. Among 176 buffaloes surveyed, 65 were found in true oestrus out of which 20 lactating anoestrus buffaloes with normal genitalia (examined per rectally) and without any clinical infections were selected randomly for study. On the basis of macro and micro nutrients level in prevalent feed stuffs and plasma of buffaloes, nutritional deficiencies were calculated and strategic nutritional supplement (SNS) was evolved. Twenty lactating anoestrus buffaloes were divided into 2 groups group-I (Unsupplemented or control or farmer practise) and group-II (supplemented or treatment group) considering their average body

weight, milk yield, parity and stage of lactation (from third to fifth). Buffaloes of supplemented group were fed @ 1kg SNS per day. Supplementation study was conducted for 2 months. During study period, individual animal of both groups were examined for onset of oestrus. After 2 months of supplementation, pregnancy diagnosis of each animal was carried out per rectally to find out the conception rate. Result revealed that, when SNS was supplemented in the ration of anoestrus lactating buffaloes, 70% of the buffaloes exhibited sign of oestrus out of 70% buffaloes who showed sign of oestrus, 60% of them were conceived within a period of 4 months (2 months of supplementation period+2 month of post experimental observation).

Nagalakshmi *et al.* (2011) conducted the farm trial of 180 days duration in East Godavari district of coastal zone of Andhra Pradesh to test the ASMM along with the concentrate mixture on production and reproductive efficiency in buffaloes. Sixty animals selected from various farmers where randomly distributed among three groups. Group-1 animals were fed as per the farmer practise. In group-2 and 3, the concentrate mixture (based on locally available protein supplements) was offered @ 1kg/day to take care of protein deficiency and also offered 30 g BIS mineral mixture or ASMM (required quantity of deficient minerals), respectively. No significant effect of BIS or ASMM supplementation was observed on post partum oestrus and number of insemination/conception, while service period reduced significantly ($P<0.01$) with BIS or ASMM supplementation.

Shukla *et al.* (2011) conducted a study to discern the effect of dietary supplementation of phosphorus and copper in cattle and buffaloes on productive and reproductive performance under field condition in Pauri district of Uttarakhand. Phosphorus (5 g) as di-ammonium phosphate and copper (6.25 mg) as copper propionate as area specific mineral mixture were provided daily to each animal for 60 days over and above the quantity received through feed and fodder provided by the farmers. The incidence of anoestrus and repeat breeding cases reduced from 20.45% to 13.71% and 19.21% to 10.45% respectively.

Behera *et al.* (2012) orally supplemented Minfaa (mineral mixture) @ 30g / heifer / day for 60 days along with ad libitum feed and water under normal farming conditions. The supplementation brought 56.66% of anoestrous heifers into heat and 46.68% repeat breeding heifers into conception.

Kumar *et al.* (2012) studied the efficacy of area specific mineral mixture (ASMM) supplementation on reproductive efficiency in buffaloes in field condition. Out of the 280 buffaloes examined 220 had postpartum anoestrus (40%) and repeat breeding (20%) followed by delayed sexual maturity (20%), silent heat (10.9%) and endometritis of mild to moderate degree (9.09%). Among the postpartum anestrous buffaloes 52.27% and 45.65% exhibited oestrus and conceived, respectively within 3 months of ASMM supplementation. Among the repeat breeders, which received the ASMM, 38.63% conceived within 3 inseminations. Onset of oestrus occurred in 50% of the delayed pubertal animals, which received the ASMM. However, from silent oestrus cases, 37.5% conceived within 3 months of the ASMM supplementation. The present study indicated that supplementation of ASMM improves reproductive performance in buffaloes under rural conditions.

Mohapatra *et al.* (2012) estimated the sample of feeds and fodders and serum of cows for mineral analysis. An on-farm trial was carried out in which 80 cows having reproductive disorders were allotted to 4 groups with 20 animals — G1 (control); G2 20 anoestrus heifers supplemented with ASMM @ 50 g/day/animal (ANH); G3, 20 anoestrous cows supplemented with ASMM @ 50 g/day/animal (ANC); and G4, 20 repeat breeding cows supplemented with ASMM @ 50 g/day/animal (RBC). The percentage conception was 5, 40, 40 and 35 in G1, G2, G3 and G4 groups, respectively. Services per conception were significantly lower in the mineral supplemented group than control. Similarly the post-partum oestrous was significantly lower in G2 and G3 cows compared to control animals. Thus the reproductive performance improved due to supplementation of area specific mineral mixture.

Tiwari *et al.* (2012b) reported calcium, phosphorus, zinc, manganese and copper were found below the critical level in soil, feeds and blood serum of cattle and buffaloes in hill region of Nainital district of Uttarakhand. Each selected animal was provided 250 g feed mixture daily in order to supply 12 g calcium, 5 g phosphorus, 250 mg zinc, 175 mg manganese and 60 mg copper over and above already present in the ration of animals provided by the farmers for 45 days. The incidence of the anoestrus and repeat breeding cases decreased due to dietary supplementation of area specific mineral mixture.

2.6.2. Chelated/organic mineral mixture:

Prasad *et al.* (1991) has carried out an experiment No.II at village level. Accordingly, 4 field centres, mainly Parada, Bagthan, Mayena and Majra in Sirmour district of Himachal Pradesh were used as field camps. Investigations were carried out on 93 anoestrus and 44 repeaters selected in that area. The feeding was the same as carried out by villagers with local feeds and fodders. In addition, supplementation of Cu, Mn, and Zn was adopted in four different groups. The animal distribution in anoestrus was T1, 14; T2, 30; T3, 30 and T4, 19 and in repeat breeders were T1, 6; T2, 15; T3, 15 and T4, 8. While T1 was given no Cu, Mn and Zn supplementation, T2 was given 100% of Cu, Mn and Zn supplementation in ionic salt form as per ISI recommendation, T3 was given chelated Cu, Mn, Zn at the same level as in T2, i.e. 100% where as in T4 the supplementation of chelated Cu, Mn, Zn was at 50% level. The respective, percentage success of conception in anoestrus was 35.71%,73.33%, 86.66% and 63.15% and in repeat breeders were 33.33%, 66.66%, 73.33% and 62.50%. In condition of repeat breeding and anoestrus supplementation Cu, Mn and Zn as a chelate give superior response then their ionic counterpart.

Saker *et al.* (1994) related that copper lysine increased ($P<0.05$) plasma copper compared to control, but controls received only half as much copper as the copper lysine group (43 v 104 ppm).

Manspeaker *et al.* (1996) demonstrated that supplemental metal amino acid chelates (AACs) resulted in improved reproductive performance. Thirty days before expected parturition they supplemented the feed of dairy cows with AACs and IOMs and continued supplementation into lactation until the animals in the study became pregnant with a second calf. Cows receiving the AAC supplement conceived 48 days earlier than heifers receiving the IOM supplement (90 days versus 138 days) ($p<0.05$) and experienced 45% less early embryonic mortality (EEM) ($p<0.05$) (Manspeaker *et al.*, 1996). Other researchers have also reported that AAC supplementation enhanced re-productive performance, but their studies have tended to focus on multiparous cows (Bonomi *et al.*, 1986; Ashmead *et al.*, 2004).

Ballantine *et al.* (2002) had taken three hundred multiparous Holstein cows (150 cows per treatment) that were blocked according to calving date and randomly assigned to a study to determine the effect of trace mineral source on incidence and severity of claw lesions, reproduction and lactation performance of dairy cattle. Treatments were (1) all trace minerals supplied by sulfates (inorganic trace minerals-ITM) or (2) 360 mg Zn from Zn amino acid complex, 200 mg Mn from Mn amino acid complex, 125 mg Cu from Cu amino acid complex and 12 mg Co from Co glucoheptonate per day complex trace minerals (CTM; Availa®; Zinpro Corporation, Eden Prairie, MN) replacing an equivalent amount of Zn, Mn, Cu, and Co from sulfates. Cows received assigned treatments from 21 d prior to calving through 250 d of lactation. The CTM treatment reduced ($P<0.05$) days open by 22 d and tended to increase ($P<0.15$) percentage of cows pregnant by 150 d in milk (54.8% vs 42.7%) and first service conception rate (27.4% vs 18.4%). The CTM treatment decreased days open, and tended to increase first service conception rates and increased percentage of cows pregnant at 150 d in milk.

Bosseboeuf *et al.* (2006) conducted the field trial involving 182 multiparous dairy cows from 8 different farms to compare the effects of Cu, Zn, and Mn amino acid chelates (AAC) against Cu, Zn, and Mn inorganic metal salts (IM) on reproductive performance. Each farm had equal numbers of cows in the AAC group and the IM group. Cows in each group

commenced receiving 50 g daily of an isomineral formulation containing AAC or IM following parturition and continuing for the first 120 days of lactation. Mean services per conception were 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% greater in the AAC group compared with the IM group ($P < 0.05$). In the IM group, 87% of the cows ultimately became pregnant compared with 96% in the AAC group ($P < 0.05$). Based on the retail costs of the 2 mineral supplements, the economic return due to improved reproductive performances was approximately 10:1 in favor of the amino acid chelates.

In Banaskantha district of Gujarat, Garg *et al.* (2008) selected 35 animals with normal reproductive organs, but suffering from anoestrus. Nine anoestrus animals served as control, and remaining twenty-six were given daily 5 g supplement per animal, containing chelated Cu, Zn and Mn and fat-soluble vitamins A, D 3 and E. Twenty-four animals in experimental group exhibited estrus, after on an average 27 days of feeding the supplement; however, only two animals exhibited estrus in control group during this period. Animals that showed estrus were subsequently inseminated either through natural service or by artificial insemination. From the present study, the inference could be drawn that the supplementing of trace minerals, in the form of chelates, along with vitamins A, D 3 and E, can help in curing the problems of anoestrus/repeat breeding in dairy animals.

El-Ashry *et al.* (2012) studied the effect of feeding a combination of zinc, manganese and copper methionine chelates of early lactation high producing dairy cows. The experimental treatments include chelated minerals (15 mg Zn as Zn Met, 20 mg Mn as Mn Met, 10 mg Cu as Cu Met). Inorganic mixture contains (15 mg Zn as ZnSO4, 20 mg Mn as MnSO4, 10 mg Cu as CuSO4) in sulphate forms. The experiment was commenced in the dry period of cows, 6 weeks before calving, and after calving the first three months of lactation was taken into consideration. No significant differences were observed among groups concerning the entire blood constituent viz. glucose, total protein, albumin, globulin, urea and activities of AST, ALT. Moreover, they were within the normal average.

MATERIALS AND METHODS

III. MATERIALS AND METHODS

3.1. Location and climate:

An on-farm trial was carried out in tribal areas of Panchmahal district of Gujarat state. The district lies between 22°45′ and 22°75' North latitude and 73°36′ and 73°6' East longitude. The total area is 552049 sq. km. which is distributed in 11 talukas. This district is bordered by Dahod district to the north-east & east, Vadodara district to the south, Kheda district to the west, Sabarkantha district to the north-west and Banswara district of Rajasthan state in the north-east.

The climate of Panchmahal district is characterized by a hot summer and dry in the non-rainy season. It also considered as a drought prone area and average annual temperature ranges from 14°C (min.) to 45°C (max.), having average rainfall 1047 mm, during the year.

3.2. Soil type, crops grown and livestock population:

The soil pattern of Panchmahal district is deep black, shallow black, grey brown and mixed red. The soil texture is black salt or mixed red and brown salt. Main crops of this area are maize, black grams, paddy and wheat. As per the livestock Census (2007), the livestock population (in '000) in Panchmahal district was total Cattle 588 (Indigenous Cattle 537, Crossbreed Cattle 51), Buffalo 615, Goat 446 and Sheep 3.9.

An on-farm trial was conducted to study the effect of supplementation of specific deficient minerals either in ionic or chelate form in of appropriate mineral mixture (AMM) in buffaloes with reproductive disorders.

3.3. Sampling procedure:

For selecting animals, the survey was conducted in Ghoghamba taluka of Panchmahal district. Multistage random sampling technique was used to select the respondents. Jinjari and Kharod villages of Panchmahal district were selected at random,

for identification of farmers for the present study. The help of Government Veterinary officer was available to identify the tribal farmers. Total 40 animals were selected at random. The selected farmers were interviewed and the desired information was collected in the pro-forma developed for the purpose. Information regarding the amount and types of feeds and fodder offered to animals and rate of actual daily feed intake per animal were recorded with a fair degree of precision on a questionnaire from individual farmer using standard sampling procedure and the samples of feeds and fodder were collected for analysis.

3.4. Experimental animals:

Based on the history as well as gynecological examination, the postpartum anoestrous (not showing symptoms for more than 100 days after calving) and repeat breeding (not conceiving following three inseminations) buffaloes were considered for the study. All the animals were free from physiological and anatomical disorders. Each buffalo was examined per rectally to know the status of reproductive organs like cervix, ovary, and uterus. Buffaloes with post partum reproductive problems like persistent corpus luteum, endometritis and other pathological infections were excluded from the mineral supplementation. A minimum of 10 each of anoestrous and repeat breeding buffaloes were selected. The details of experimental buffaloes are given in Table 3.1, 3.2, 3.3 and 3.4.

3.5. Feeding management and plane of nutrition of buffaloes:

The buffaloes received home-made concentrate mixture prepared daily by the farmer using compound concentrate mixture and mixed grain (maize+wheat) bharda. In addition to concentrate mixture, the buffaloes were offered green maize, green bajari, straws of maize and paddy throughout the experimental period. The conventional practice of feeding concentrates in two equal portions at the time of milking in the morning and evening was followed. The roughage portion was offered separately twice or thrice in a day. Clean, fresh and wholesome tap water was made available to all the

Table No. 3.1: Details of experimental anoestrus buffaloes to which ionic mineral mixture distributed

No.	Animal code	Village	Owner name	Address	A/RB	Min.mix. type
1	1.4.1	Jinjari	Rathva Makad Jehara	Leeladhal	A	Ionic
2	1.10.1	Jinjari	Rathva Bhura Ghalubhai	Leeladhal	A	Ionic
3	1.10.2	Jinjari	Rathva Bhura Ghalubhai	Leeladhal	A	Ionic
4	2.3.1	Jinjari	Rathva Tersinh Roopsinh	Surkhed	A	Ionic
5	2.5.1	Jinjari	Rathva Gumal Fulaji	Surkhed	A	Ionic
6	3.2.2	Kharod	Damor Arvind Ratnabhai	Damor	A	Ionic
7	3.6.1	Kharod	Damor Mathur Narsinh	Damor	A	Ionic
8	4.1.1	Kharod	Rathva Thavar Amara	Daudara	A	Ionic
9	4.4.1	Kharod	Rathva Amarsinh Bheema	Daudara	A	Ionic
10	4.6.1	Kharod	Rathva Chhatra Jamsinh	Daudara	A	Ionic

Table No. 3.2: Details of experimental repeat breeder buffaloes to which ionic mineral mixture distributed

No.	Animal code	Village	Owner name	Address	A/RB	Min.mix. type
1	1.1.1	Jinjari	Rathva Bheema Mana	Leeladhal	RB	Ionic
2	1.1.2	Jinjari	Rathva Bheema Mana	Leeladhal	RB	Ionic
3	1.5.1	Jinjari	Rathva Velaji Maka	Leeladhal	RB	Ionic
4	1.8.1	Jinjari	Rathva Seliyas Choher	Leeladhal	RB	Ionic
5	1.8.2	Jinjari	Rathva Seliyas Choher	Leeladhal	RB	Ionic
6	2.4.1	Jinjari	Rathva Devaji Roopsinh	Surkhed	RB	Ionic
7	3.2.1	Kharod	Damor Ratna Punabhai	Damor	RB	Ionic
8	3.2.2	Kharod	Damor Ratna Punabhai	Damor	RB	Ionic
9	3.1.1	Kharod	Damor Arvind Ratnabhai	Damor	RB	Ionic
10	3.6.2	Kharod	Damor Mathur Narsinh	Damor	RB	Ionic

Table No. 3.3: Details of experimental anoestrus buffaloes to which chelated mineral mixture distributed

No.	Animal code	Village	Owner name	Address	A/RB	Min.mix. type
1	1.3.1	Jinjari	Rathva Bharat Bhavsinh	Leeladhal	A	Chelated
2	1.7.2	Jinjari	Rathva Ima Raisinh	Leeladhal	A	Chelated
3	1.9.1	Jinjari	Rathva Nivsinh Chetar	Leeladhal	A	Chelated
4	1.11.1	Jinjari	Rathva Kansinh Mana	Leeladhal	A	Chelated
5	2.2.1	Jinjari	Rathva Shankar Tersinh	Surkhed	A	Chelated
6	3.1.1	Kharod	Damor Natvar Ratnabhai	Damor	A	Chelated
7	3.1.2	Kharod	Damor Natvar Ratnabhai	Damor	A	Chelated
8	3.3.1	Kharod	Damor Soma Narsinh	Damor	A	Chelated
9	3.5.2	Kharod	Rathava Lallu Jaliyabhai	Damor	A	Chelated
10	4.3.1	Kharod	Rathva Ramsinh Gala	Daudara	A	Chelated

Table No. 3.4: Details of experimental repeat breeder buffaloes to which chelated mineral mixture distributed

No.	Animal code	Village	Owner name	Address	A/RB	Min.mix. type
1	1.2.1	Jinjari	Rathva Veghaji Bhaliya	Leeladhal	RB	Chelated
2	1.6.1	Jinjari	Rathva Bhura Galu	Leeladhal	RB	Chelated
3	1.7.1	Jinjari	Rathva Ima Raisinh	Leeladhal	RB	Chelated
4	1.9.2	Jinjari	Rathva Nivsinh Chetar	Leeladhal	RB	Chelated
5	2.1.1	Jinjari	Rathva Dhansinh Soma	Surkhed	RB	Chelated
6	3.3.2	Kharod	Damor Soma Narsinh	Damor	RB	Chelated
7	3.4.1	Kharod	Rathava Manga Jaliyabhai	Damor	RB	Chelated
8	3.5.1	Kharod	Rathava Lallu Jaliyabhai	Damor	RB	Chelated
9	4.2.1	Kharod	Rathva Mansinh Gala	Daudara	RB	Chelated
10	4.5.1	Kharod	Rathva Deepa Raju	Daudara	RB	Chelated

experimental animals in the morning and afternoon. All the buffaloes were housed under hygienic conditions under shed.

Observations regarding the amount and types of feeds and fodder offered to animals, daily feed intakes etc. were recorded with fair degree of precision on a questionnaire developed for the purpose. The amount of DM, DCP and TDN available to dairy buffaloes were calculated from the records of intake of feeds and fodder, using digestibility coefficients/nutritive values given by Sen *et al.* (1978); Ranjhan (1991) and Anonymous (2005). The intake of DCP and TDN of experimental buffaloes was compared with respective requirement for maintenance and milk production given by ICAR (1998). The macro and micro mineral supplied were compared with NRC (2001) standard.

3.6. Experimental treatments:

Forty buffaloes of anoestrus and repeat breeders were selected on the basis of their past history. The trial was conducted up to 90 days. The experimental animals were randomly allotted to two dietary treatments *i.e.* T_1 (ionic mineral mixture) and T_2 (chelated mineral mixture), following Completely Randomized Design.

Group	No.	Diet
T_1	20	Feeding schedule followed by the farmer + appropriate mineral mixture supplementation in ionic form
T_2	20	Feeding schedule followed by the farmer + appropriate mineral mixture supplementation in the form of chelate

The appropriate mineral mixtures were prepared and supplemented to the groups of selected animals daily @ 30 g per buffalo per day packed in plastic sachet until the animal responded or maximum up to 90 days.

3.1 Mixing of minerals

3.2 Preparation of mineral mixture sachets

3.7. Sample preparation and analytical methods:

Composite samples of green fodder, dry fodder, individual concentrate ingredients and concentrate mixtures were collected from the surveyed area. Green samples were dried in oven at 100 ± 5°C for 24 h and subsequently ground (1mm). Ground samples of concentrate and fodder were stored in airtight bags until analysis. The composite samples of green/dry fodder, individual concentrate ingredients and home-made concentrate mixture were collected for analysis of proximate constituents and minerals viz. Ca, P, Mg, Cu, Zn and Mn as per the methods of AOAC (2005).

3.8. Formulation of appropriate mineral mixture:

The appropriate mineral mixture (AMM) was formulated based upon incidence of deficiency prevailing in the animals under investigation. The feed and fodder samples collected were analyzed for macro (Ca, P, and Mg) and micro (Cu, Mn, Zn and Co) minerals. The data on average estimated levels of minerals supplied to selected buffaloes were worked out and compared with their calculated requirements.

The AMM for Panchmahal district (Table 3.5) was developed by the Department under ICAR sponsored All India Coordinated Research Project entitled "***Improvement of feed resources and nutrient utilization in raising animal production***". Ionic mineral salts were used in formulation of ionic mineral mixture. In chelated appropriate mineral mixture the deficient mineral was added extra @ 25 % in ionic mineral mixture.

3.9. Blood sampling:

Blood samples were collected from the jugular vein of the experimental buffaloes in 4 ml sterilized and vacuumized tubes containing sodium fluoride (for plasma) and another 9 ml sterilized and vacuumized tube contained clot activator (for serum) and transported to the laboratory in an ice box at the beginning, mid and 90 days of mineral mixture supplementation. The plasma was separated by centrifugation at 3000

Table 3.5: Appropriate mineral mixture formulation for Panchmahal district

Mineral Element	Requirement (%)	Mineral salt	Quantity (kg)
Calcium	20.000	Dicalcium phosphate	
		Calcite powder	14.554
Phosphorus	12.010	DCP	66.722
Magnesium	5.000	Magnesium oxide	9.259
Sulphur	1.800	Sodium thiosulphate	3.795
Copper	0.100	Copper sulphate	0.400
Zinc	1.140	Zinc sulphate	3.455
Manganese	0.120	Manganese sulphate	0.387
Iron	0.400	Ferrous sulphate	1.333
Cobalt	0.012	Cobalt sulphate	0.060
Iodine	0.026	Potassium iodide	0.034
		Total	**100.00**

3.3 Distribution of mineral mixture to farmer

3.4 Blood collection

rpm for 15 min and stored at –20°C until analyzed for minerals and progesterone levels. Serum also separated and stored in refrigerator until analyzed.

The serum concentration of Total Protein, Albumin, Globulin, Blood Urea Nitrogen, Glucose, Creatinine, Cholesterol, Triglycerides, Sodium (Na), Potassium (K), Calcium (Ca), Magnesium (Mg), Phosphorus (P), Alkaline phosphatase, Alanine Aminotransferases (ALT/SGPT) and Aspartate Aminotransferases (AST/SGOT) was estimated using Semi Automatic Analyzer for clinical chemistry test (Model 3000 EVOLUION) of Coral clinical systems. The commercial diagnostic kits for analysis were procured from CREST Biosystems, Ltd., Goa, India. These all blood-biochemical parameters were analyzed at monthly intervals till the appearance of oestrus and conception in repeat breeders within 3 months of experiment.

3.10. Cost of rearing experimental animal:

The cost of rearing per animal was calculated from the data of feed intake and prevailing procurement price of individual feed ingredients. The average actual price of ingredients purchased and mineral mixtures (Table 3.6) during the experiment was considered for calculating total feed cost during the supplementation period. The daily cost of 30g mineral mixture worked out as Rs. 1.80 for ionic group and Rs. 2.10 for chelated group.

3.11. Statistical analysis:

The data generated during the experiment was analyzed, using Randomized Block Design, as given in Snedecor and Cochran (1994).

Table 3.6: The average actual price of feeds on as such basis (₹/kg)

Ingredients	Price (₹/kg)
Cotton seed cake (CSC)	16.00
Compound concentrate mixture	12.00
Mixed grain bharda	13.00
Green bajari	3.00
Green maize	3.00
Maize straw	4.00
Paddy straw	2.50
Ionic mineral mixture	60.00
Chelated mineral mixture	71.42

RESULTS AND DISCUSSION

IV. RESULTS AND DISCUSSION

4.1: Chemical composition of feeds and fodder:

The chemical composition (% on DM basis) in feeds and fodder used during the experiment is given in Table 4.1. The values were within the normal range.

4.2: Mineral composition of feeds and fodder:

The content of Ca, P, Zn, Cu and Mn in feeds and fodder (% on DM basis) used during the experiment is given in Table 4.2. The values were within the normal range.

4.3: Feed Intake and nutritional status:

4.3.1: DCP and TDN intake (as the % of requirement) of buffaloes:

The average daily TDMI, DCPI and TDNI of anoestrus and repeat breeder buffaloes in ionic and chelated groups are presented in Tables 4.3 and 4.4, respectively. The DCPI and TDNI as per cent of requirement (ICAR, 1998) in the experimental buffaloes in ionic and chelated groups were worked out and are presented in Tables 4.3 and 4.4.

The DMI in anoestrus buffaloes in ionic and chelated groups was 11.46±0.14 and 11.53±0.09 kg/day, respectively. The same was 11.41±0.10 and 11.47±0.09 kg/day in ionic and chelated groups of buffaloes. The DMI did not differ within the groups and was satisfactory.

The average daily DCPI as per cent of requirement of buffaloes was 95.57 ± 1.53 and 92.72 ± 0.85 % in T_1 and T_2 groups, respectively in anoestrus buffaloes. The treatment groups did not differ from each other. Similar trend was observed in repeat breeder buffaloes where it was 93.86 ±1.43 and 93.22 ± 0.64 % in T_1 and T_2 groups, respectively. The intake of DCP and TDN in both the groups of buffaloes was more or less according to ICAR (1998) standard.

Table 4.1: Chemical composition of feeds (% on DM basis)

Particulars	Crude protein	Ether extract	Crude fibre	Ash	Nitrogen free extract	Organic matter
Compound concentrate mixture	17.92±0.52	3.43±0.60	12.02±2.88	6.77±1.01	59.87±3.00	93.23±1.01
Cotton seed cake	21.52±0.91	8.27±1.03	27.12±2.17	5.22±0.48	37.87±2.33	94.78±0.48
Mixed grain bhardo	11.55±1.03	1.54±0.31	7.15±0.77	6.24±0.64	73.52±1.50	93.76±0.64
Green bajari	8.27±0.47	1.59±0.22	27.45±0.34	11.57±0.19	51.12±0.37	88.43±0.19
Green maize	9.70±0.74	3.06±0.64	29.74±0.91	12.81±1.53	44.69±0.91	87.19±1.53
Maize straw	3.63±0.22	1.67±0.37	28.08±3.22	9.13±0.75	57.49±3.29	90.87±0.75
Paddy straw	3.86±0.26	2.49±0.10	31.35±0.74	16.42±0.74	45.69±1.39	83.58±0.74

Table 4.2: Mineral composition of feed stuffs (On DM basis)

Particulars	Ca (%)	P (%)	Cu (ppm)	Zn (ppm)	Mn (ppm)
Compound concentrate mixture	1.46±0.44	1.08±0.65	22.73±3.54	149.76±15.55	81.35±17.98
Cotton seed cake	0.77±0.73	0.81±1.12	12.11±0.44	47.55±3.12	23.44±10.30
Mixed grain bhardo	0.145±1.06	0.475±0.74	8.65±1.20	35.65±1.94	64.75±6.53
Green bajari	0.41±0.64	0.21±0.14	11.99±0.60	44.39±3.66	56.21±13.33
Green maize	0.57±0.29	0.40±0.58	9.34±1.56	17.04±2.45	47.19±7.21
Maize straw	0.33±0.42	0.11±0.64	7.67±1.52	27.35±1.31	52.41±21.71
Paddy straw	0.33±0.26	0.15±0.16	5.71±0.78	37.78±9.21	761.20±192.03

Table 4.3: Average daily DCP and TDN intake (as percent of requirement) of anoestrus buffaloes

A. No.	TDMI (kg/day)		DCPI (g/d)		TDNI (kg/d)		DCPR (g)		TDNR (kg)		DCPI % of R		TDN % of R	
	T_1	T_2	T_1	T_2	T_1	T_2	T_1	T_2	T_1	T_2	T_1	T_2	T_1	T_2
1	11.92	11.5	543.66	573.20	6.12	6.60	563.33	605.27	5.53	5.84	96.51	94.70	110.63	113.05
2	11.23	11.65	685.34	582.50	6.76	6.71	670.34	605.97	6.30	5.84	102.24	96.13	107.16	114.83
3	11.1	11.4	646.80	606.00	6.65	6.52	654.12	658.30	6.19	6.22	98.88	92.05	107.39	104.82
4	12.08	11.9	617.54	507.70	6.29	5.69	624.26	561.86	5.97	5.52	98.92	90.36	105.32	103.01
5	11.95	11.2	640.65	520.80	6.27	6.64	664.90	587.67	6.27	5.71	96.35	88.62	100.01	116.30
6	11.1	11.95	523.50	557.00	6.53	5.85	588.35	604.09	5.71	5.83	88.98	92.21	114.24	100.38
7	11.24	11.75	568.12	604.15	6.32	6.74	608.74	621.30	5.86	5.95	93.33	97.24	107.88	113.30
8	11.87	11.48	622.61	610.74	6.53	6.55	617.72	652.01	5.93	6.17	100.79	93.67	110.13	106.08
9	10.96	11.35	545.93	572.00	6.21	6.62	601.68	626.21	5.81	5.99	90.73	91.34	106.82	110.56
10	11.1	11.08	533.85	545.54	6.37	6.36	599.88	600.52	5.80	5.80	88.99	90.85	109.89	109.66
Av.	**11.46**	**11.53**	**592.80**	**567.96**	**6.40**	**6.43**	**619.33**	**612.32**	**5.94**	**5.89**	**95.57**	**92.72**	**107.95**	**109.20**
±S.E.	**0.14**	**0.10**	**17.88**	**11.22**	**0.06**	**0.12**	**10.96**	**9.08**	**0.08**	**0.07**	**1.53**	**0.85**	**1.19**	**1.70**

		TDMI	DCPI	TDNI	DCPR	TDNR	DCP % of R	TDN % of R
Source	**d.f.**	**m.s.s.**	**m.s.s.**	**m.s.s.**	**m.s.s.**	**m.s.s.**	**m.s.s.**	**m.s.s.**
Treatment	1	0.0252	3084.383	0.230	245.841	0.0125	40.755	7.838
Error	18	0.138	2227.133	2.245	1012.638	0.0529	15.330	21.571
Total	19							

Table 4.4: Average daily DCP and TDN intake (as percent of requirement) of repeat breeder buffaloes

A. No.	TDMI (kg/d)		DCPI (g/d)		TDNI (kg/d)		DCPR (g)		TDNR (kg)		DCPI % of R		TDN % of R	
	T_1	T_2	T_1	T_2	T_1	T_2	T_1	T_2	T_1	T_2	T_1	T_2	T_1	T_2
1	11.05	11.35	561.50	582.35	6.53	6.45	621.30	618.16	5.95	5.93	90.38	94.21	109.73	108.83
2	11.51	11.1	522.58	595.50	6.66	6.44	568.40	626.84	5.57	5.99	91.94	95.00	119.56	107.46
3	11.75	11.4	549.70	570.00	6.79	6.58	603.21	610.78	5.82	5.88	91.13	93.32	116.61	111.99
4	11.67	11.6	632.46	594.85	6.78	6.62	654.12	661.48	6.19	6.24	96.69	89.93	109.62	106.01
5	11.41	11.33	511.28	594.04	6.47	6.46	528.65	645.72	5.29	6.13	96.71	92.00	122.37	105.47
6	11.38	11.45	607.34	568.00	6.69	6.52	673.76	623.95	6.33	5.97	90.14	91.03	105.63	109.27
7	11.36	11.15	655.23	593.50	6.51	6.37	639.06	652.01	6.08	6.17	102.53	91.03	107.14	103.24
8	11.92	11.74	660.26	546.77	7.18	6.80	667.53	584.03	6.28	5.68	98.91	93.62	114.26	119.60
9	10.97	11.65	556.96	608.15	6.34	6.82	621.06	645.41	5.95	6.12	89.68	94.23	106.50	111.35
10	11.11	11.97	580.88	570.96	6.44	6.97	642.02	583.61	6.10	5.68	90.48	97.83	105.52	122.71
Av.	**11.41**	**11.47**	**583.82**	**582.41**	**6.64**	**6.60**	**621.91**	**625.20**	**5.95**	**5.98**	**93.86**	**93.22**	**111.69**	**110.59**
±S.E.	**0.10**	**0.09**	**16.76**	**5.67**	**0.08**	**0.03**	**14.31**	**5.21**	**0.10**	**0.04**	**1.43**	**0.64**	**1.94**	**1.14**

		TDMI	DCPI	TDNI	DCPR	TDNR	DCP % of R	TDN % of R
Source	**d.f.**	**m.s.s.**	**m.s.s.**	**m.s.s.**	**m.s.s.**	**m.s.s.**	**m.s.s.**	**m.s.s.**
Treatment	1	0.0186	9.898	0.00648	54.055	0.00265	2.042	6.061
Error	18	0.0845	1570.901	0.0488	1387.119	0.0714	12.849	37.991
Total	19							

The average daily TDNI as per cent of requirement of buffaloes was 107.95 ± 1.19 and 109.20 ± 1.70 in T_1 and T_2 groups, respectively in anoestrus buffaloes. The treatment group was statistically similar. Similar trend was observed in repeat breeder buffaloes where it was 111.69 ± 1.94 and 110.59 ± 1.14 in T_1 and T_2 groups, respectively. The data indicated that experimental buffaloes of both the groups received adequate quantity of protein and energy to satisfy their requirement.

4.3.2: Ca and P intake (as the % of requirement) of buffaloes:

The average daily intake of Ca and P of buffaloes as per cent of requirement NRC (2001) in ionic and chelated groups were worked out and are presented in Tables 4.5 and 4.6.

The average daily Ca I as per cent of requirement of buffaloes was 161.21 ± 4.90 and 162.43 ± 3.74 % in T_1 and T_2 groups, respectively in anoestrus buffaloes, which was statistically similar. Similar trend was observed in repeat breeder buffaloes where it was 156.33 ±5.49 and 164.47 ± 2.72 % in T_1 and T_2 groups, respectively.

The average daily PI as per cent of requirement of buffaloes was 187.28 ± 2.39 and 182.51 ± 3.54 in T_1 and T_2 groups, respectively in anoestrus buffaloes, which was statistically similar. Similar trend was observed in repeat breeder buffaloes where it was 182.70 ± 5.19 and 184.98 ± 2.16 in T_1 and T_2 groups, respectively. It shows that buffaloes of this experimental group were supplied more than enough quantity of Ca and P compared to their requirement.

4.3.3: Cu and Zn intake (as the % of requirement) of buffaloes:

The average daily Cu I and Zn I of buffaloes as per cent of requirement NRC (2001) in ionic and chelated groups are presented in Tables 4.7 and 4.8.

Table 4.5: Average daily Ca and P intake (as percent of requirement) of anoestrus buffaloes

A. No.	Ca I (g/d)		PI (g/d)		Ca R (g/d)		PR (g/d)		Ca I % of R		PI % of R	
	T_1	T_2	T_1	T_2	T_1	T_2	T_1	T_2	T_1	T_2	T_1	T_2
1	65.14	64.63	42.82	45.82	35.40	38.82	21.78	23.90	183.98	166.47	196.59	191.73
2	67.20	68.65	52.89	46.87	44.13	38.88	27.19	23.93	152.28	176.56	194.57	195.83
3	68.70	73.32	51.78	48.80	42.81	43.15	26.37	26.58	160.48	169.91	196.39	183.60
4	71.15	59.39	47.75	39.37	40.37	35.28	24.86	21.70	176.23	168.32	192.09	181.37
5	71.52	54.34	48.48	41.29	43.69	37.39	26.91	23.01	163.70	145.32	180.15	179.43
6	52.32	65.76	40.80	44.34	37.44	38.73	23.04	23.84	139.73	169.79	177.06	185.98
7	66.99	65.18	44.13	44.80	39.11	40.13	24.07	24.71	171.30	162.41	183.33	181.30
8	54.72	59.96	45.72	40.52	39.84	42.64	24.53	26.26	137.35	140.64	186.41	154.32
9	66.76	62.55	44.76	45.49	38.53	40.53	23.72	24.96	173.27	154.31	188.72	182.28
10	59.03	65.57	41.93	44.78	38.38	38.44	23.63	23.66	153.77	170.59	177.46	189.28
Av.	**64.35**	**63.93**	**46.11**	**44.21**	**39.97**	**39.40**	**24.61**	**24.25**	**161.21**	**162.43**	**187.28**	**182.51**
±S.E.	**2.12**	**1.66**	**1.29**	**0.93**	**0.89**	**0.74**	**0.55**	**0.46**	**4.90**	**3.74**	**2.39**	**3.54**

		Ca I	PI	Ca R	PR	Ca I % of R	PI % of R
Source	**d.f.**	**m.s.s.**	**m.s.s.**	**m.s.s.**	**m.s.s.**	**m.s.s.**	**m.s.s.**
Treatment	1	0.874	18.012	1.630	0.630	7.479	113.526
Error	18	36.258	12.636	6.743	2.589	190.262	91.365
Total	19						

Table 4.6: Average daily Ca and P intake (as percent of requirement) of repeat breeder buffaloes

A. No.	Ca I (g/d)		PI (g/d)		Ca R (g/d)		PR (g/d)		Ca I % of R		PI % of R	
	T_1	T_2	T_1	T_2	T_1	T_2	T_1	T_2	T_1	T_2	T_1	T_2
1	56.44	72.36	44.11	47.83	40.13	39.88	24.71	24.55	140.64	181.45	178.53	194.83
2	55.28	64.11	40.66	46.20	35.82	40.58	22.03	24.99	154.35	157.97	184.52	184.89
3	59.96	61.22	43.90	43.67	38.66	39.27	23.79	24.18	155.11	155.87	184.48	180.61
4	67.19	67.37	48.29	46.18	42.81	43.41	26.37	26.74	156.95	155.19	183.16	172.69
5	65.46	72.69	43.86	48.25	32.57	42.12	20.03	25.94	200.95	172.56	219.00	186.01
6	66.95	66.10	48.09	44.27	44.41	40.35	27.36	24.84	150.75	163.81	175.79	178.21
7	56.57	72.10	39.06	48.02	41.58	42.64	25.60	26.26	136.04	169.09	152.56	182.87
8	67.11	63.05	51.95	44.29	43.90	37.09	27.04	22.82	152.86	169.99	192.08	194.03
9	62.50	67.84	43.87	48.45	40.11	42.10	24.70	25.93	155.81	161.14	177.65	186.86
10	66.87	58.40	46.17	43.06	41.82	37.06	25.75	22.80	159.89	157.59	179.26	188.81
Av.	**62.43**	**66.52**	**45.00**	**46.02**	**40.18**	**40.45**	**24.74**	**24.90**	**156.33**	**164.47**	**182.70**	**184.98**
±S.E.	**1.57**	**1.56**	**1.20**	**0.65**	**1.17**	**0.70**	**0.72**	**0.43**	**5.49**	**2.72**	**5.19**	**2.16**

		Ca I	PI	Ca R	PR	Ca I % of R	PI % of R
Source	**d.f.**	**m.s.s.**	**m.s.s.**	**m.s.s.**	**m.s.s.**	**m.s.s.**	**m.s.s.**
Treatment	1	83.681	5.263	0.362	0.139	330.566	25.946
Error	18	24.398	9.294	9.230	3.543	187.638	157.882
Total	19						

Table 4.7: Average daily Cu and Zn intake (as percent of requirement) of anoestrus buffaloes

A. No.	Cu I (mg/d)		Zn I (mg/d)		Cu R (mg/d)		Zn R (mg/d)		CuI % of R		ZnI % of R	
	T_1	T_2	T_1	T_2	T_1	T_2	T_1	T_2	T_1	T_2	T_1	T_2
1	118.74	122.40	574.54	597.13	109.20	115.00	873.60	920.00	108.74	106.43	65.77	64.91
2	129.57	131.71	643.84	638.49	112.30	116.50	898.40	932.00	115.38	113.06	71.67	68.51
3	133.04	135.38	677.93	682.16	111.00	114.00	888.00	912.00	119.85	118.76	76.34	74.80
4	128.03	116.72	632.02	500.06	110.80	119.00	886.40	952.00	115.55	98.09	71.30	52.53
5	129.15	109.91	626.86	535.47	109.50	99.50	876.00	796.00	117.94	110.46	71.56	67.27
6	109.04	129.49	480.47	594.14	111.00	119.50	888.00	956.00	98.24	108.36	54.11	62.15
7	122.88	125.92	584.04	546.52	112.40	117.50	899.20	940.00	109.32	107.17	64.95	58.14
8	110.39	112.26	487.39	596.87	108.70	102.30	869.60	818.40	101.56	109.74	56.05	72.93
9	122.68	122.82	624.15	585.50	109.60	113.50	876.80	908.00	111.94	108.21	71.18	64.48
10	114.50	124.91	530.44	621.31	111.00	110.80	888.00	886.40	103.15	112.74	59.73	70.09
Av.	**121.80**	**123.15**	**586.17**	**589.76**	**110.55**	**112.76**	**884.40**	**902.08**	**110.17**	**109.30**	**66.27**	**65.58**
±S.E.	**2.66**	**2.60**	**21.38**	**16.64**	**0.40**	**2.15**	**3.20**	**17.22**	**2.31**	**1.69**	**2.37**	**2.13**

		CuI	ZnI	Cu R	Zn R	CuI % of R	ZnI % of R
Source	**d.f.**	**m.s.s.**	**m.s.s.**	**m.s.s.**	**m.s.s.**	**m.s.s.**	**m.s.s.**
Treatment	1	9.112	64.692	24.421	1562.912	3.741	2.346
Error	18	69.270	3668.604	23.967	1533.899	41.073	50.829
Total	19						

Table 4.8: Average daily Cu and Zn intake (as percent of requirement) of repeat breeder buffaloes

A. No.	Cu I (mg/d)		Zn I (mg/d)		Cu R (mg/d)		Zn R (mg/d)		CuI % of R		ZnI % of R	
	T_1	T_2	T_1	T_2	T_1	T_2	T_1	T_2	T_1	T_2	T_1	T_2
1	113.93	133.06	532.51	681.46	110.50	113.50	884.00	908.00	103.11	117.23	60.24	75.05
2	112.89	121.71	491.52	583.17	115.10	111.00	920.80	888.00	98.08	109.65	53.38	65.67
3	118.73	119.49	553.46	538.82	117.50	114.00	940.00	912.00	101.05	104.81	58.88	59.08
4	128.51	126.17	600.35	597.15	116.70	116.00	933.60	928.00	110.12	108.76	64.30	64.35
5	124.14	133.92	633.45	680.34	114.10	113.30	912.80	906.40	108.80	118.20	69.40	75.06
6	130.79	125.71	631.08	584.13	113.80	114.50	910.40	916.00	114.93	109.79	69.32	63.77
7	106.99	132.71	496.81	674.48	101.10	111.50	808.80	892.00	105.83	119.03	61.43	75.61
8	136.16	125.98	651.44	590.07	119.20	117.40	953.60	939.20	114.23	107.31	68.31	62.83
9	120.39	132.59	571.27	639.88	109.70	116.50	877.60	932.00	109.75	113.82	65.09	68.66
10	128.10	119.95	620.90	504.65	111.10	119.70	888.80	957.60	115.30	100.21	69.86	52.70
Av.	**122.06**	**127.13**	**578.28**	**607.41**	**112.88**	**114.74**	**903.04**	**917.92**	**108.12**	**110.88**	**64.02**	**66.28**
±S.E.	**2.88**	**1.78**	**18.38**	**19.19**	**1.64**	**0.85**	**13.09**	**6.80**	**1.90**	**1.94**	**1.74**	**2.37**

		CuI	ZnI	Cu R	Zn R	CuI % of R	ZnI % of R
Source	**d.f.**	**m.s.s.**	**m.s.s.**	**m.s.s.**	**m.s.s.**	**m.s.s.**	**m.s.s.**
Treatment	1	128.322	4244.532	17.298	1107.072	38.116	25.470
Error	18	57.465	3531.488	16.996	1087.716	36.840	43.148
Total	19						

The average daily Cu I as per cent of requirement of anoestrus buffaloes was 110.17 ± 2.31 and 109.30 ± 1.69 % in T_1 and T_2 groups, respectively, which was statistically similar. Similar was the trend observed in repeat breeder buffaloes where it was 108.12 ± 1.90 and 110.88 ± 1.94 % in T_1 and T_2 groups, respectively. This indicated that experimental buffaloes received adequate Cu through their ration.

The average daily Zn I as per cent of requirement of buffaloes was 66.27 ± 2.37 and 65.58 ± 2.13 in T_1 and T_2 groups, respectively in anoestrus buffaloes. The treatment groups did not differ statistically. Similar trend was observed in repeat breeder buffaloes where it was 64.02 ± 5.19 and 66.28 ± 2.16 in T_1 and T_2 groups, respectively. It shows that buffaloes of this experimental group had severe deficiency of this mineral, which might have led to anoestrus and repeat breeder condition.

4.4: Biochemical profile of anoestrus and repeat breeder buffaloes:

4.4.1: Blood glucose:

The average blood glucose level (mg/dl) of anoestrus and repeat breeder buffaloes in ionic and chelated groups are presented in Table 4.9.

The average level in ionic and chelated group of anoestrus buffaloes was 59.50±0.59 and 61.35±0.77 (mg/dl), respectively. The treatment groups did not differ from each other. Similar was the finding of Behera *et al.* (2012) who did not find any difference in blood glucose level of anoestrus crossbred heifers supplemented with mineral mixture.

The average blood glucose (mg/dl) at 0, 30th, 60th and 90th days was found to be 57.40 ± 2.48, 59.40 ± 1.26, 62.50 ± 1.15 and 58.70 ± 1.71 in ionic group and 57.60 ± 1.89, 64.30 ± 0.71, 62.30 ± 2.49 and 61.20 ± 1.47 in chelated group of anoestrus buffaloes which was statistically similar. During supplementation period highest concentration of blood glucose was seen at 60th day in ionic group and thereafter it decreased and reached to normal range. In chelated group highest concentration was seen at 30th day and the decreased at 60th and 90th day, respectively.

Table 4.9: Blood glucose level (mg/dl) of anoestrus and repeat breeder buffaloes

Days of supplementation	Ionic	Chelated	Mean
	Anoestrus buffaloes		
0	57.40	57.60	57.50
30	59.40	64.30	61.85
60	62.50	62.30	62.40
90	58.70	61.20	59.95
Mean	**59.50**	**61.35**	
±S. E.	**0.59**	**0.77**	
	Repeat breeder buffaloes		
0	57.40	60.10	58.75
30	60.10	62.10	61.10
60	58.90	61.00	59.95
90	62.30	62.40	62.35
Mean	**59.68**	**61.40**	
±S. E.	**0.57**	**0.29**	

Analysis of variance for anoestrus buffaloes (mean sum of squares)

Source	d.f.	m.s.s.	Test	SEM	P value
P	3	98.11	NS	1.36	0.057
T	1	35.11	NS	0.96	0.335
PT	3	9.94	NS	1.93	0.849
Error	72	37.32			

Analysis of variance for repeat breeder buffaloes (mean sum of squares)

Source	d.f.	m.s.s.	Test	SEM	P value
P	3	46.513	NS	1.283	0.24
T	1	70.313	NS	0.90	0.14
PT	3	1.246	NS	1.81	0.99
Error	72	32.92			

The average level in ionic and chelated group of repeat breeder buffaloes was 59.68±0.57 and 61.40±0.29 (mg/dl), respectively. The treatment groups did not differ from each other. Similar was the finding of Behera *et al.* (2012) who did not find any difference in blood glucose level of repeat breeding crossbred heifers supplemented with mineral mixture and contrary to Mohapatra *et al.* (2012) recorded significantly higher values of blood glucose on account of supplementation of mineral mixture.

The average blood glucose (mg/dl) in repeat breeder buffaloes at 0, 30th, 60th and 90th days was found to be 57.40 ± 1.33, 60.10 ± 1.49, 58.90 ± 0.98 and 62.30 ± 1.45 in ionic group and 60.10 ± 1.69, 62.10 ± 2.35, 61.00 ± 1.50 and 62.40 ± 1.97 in chelated group which was statistically similar. During supplementation the highest blood glucose concentration was seen at 90th day in both ionic and chelated group.

4.4.2: Serum total protein:

The average serum total protein level (g/dl) of anoestrus and repeat breeder buffaloes in ionic and chelated groups are given in Table 4.10 and has been depicted in figures 4.1 and 4.2.

The average total protein level (g/dl) in ionic and chelated group of anoestrus buffaloes was 7.14±0.22 and 6.98±0.22 (mg/dl), respectively. The treatment groups did not differ from each other. Similar to the findings of present study, Behera et al, (2012) reported no elevation in serum protein level of anoestrus cow heifers on account of mineral mixture supplementation for 60 days. But higher total serum protein levels in anoestrous lactating buffaloes (Kumar *et al.*, 2012) and cows (Mohapatra *et al.,* 2012) has been reported due to supplementation of ASMM.

In anoestrus buffaloes the average total protein (g/dl) at 0, 30th, 60th and 90th days was found to be 6.46 ± 0.21, 6.53 ± 0.17, 7.40 ± 0.14 and 8.18 ± 0.05 in ionic group and 6.34 ± 0.29, 6.51 ± 0.18, 6.97 ± 0.28 and 8.10 ± 0.11 in chelated group. The period

Table 4.10: Serum total protein level (g/dl) of anoestrus and repeat breeder buffaloes

Days of supplementation	Ionic	Chelated	Mean
	Anoestrus buffaloes		
0	6.46	6.34	6.40^{a}
30	6.53	6.51	6.52^{a}
60	7.40	6.97	7.19^{b}
90	8.18	8.10	8.14^{c}
Mean	**7.14**	**6.98**	
±S. E.	**0.22**	**0.22**	
	Repeat breeder buffaloes		
0	6.34	6.40	6.37^{a}
30	6.95	6.73	6.84^{b}
60	6.71	7.09	6.90^{b}
90	8.08	8.10	8.09^{c}
Mean	**7.02**	**7.08**	
±S. E.	**0.21**	**0.20**	

Means bearing different superscripts in column differ significantly (P<0.01)

Analysis of variance for anoestrus buffaloes (mean sum of squares)

Source	d.f.	m.s.s.	Test	SEM	P value
P	3	10.44	**	0.15	<0.01
T	1	1.378	NS	0.11	0.098
PT	3	0.094	NS	0.22	0.901
Error	72	0.490			

Analysis of variance for repeat breeder buffaloes (mean sum of squares)

Source	d.f.	m.s.s.	Test	SEM	P value
P	1	5.40	**	0.16	<0.01
T	3	0.27	NS	0.11	0.48
PT	1	0.26	NS	0.23	0.70
Error	72	0.57			

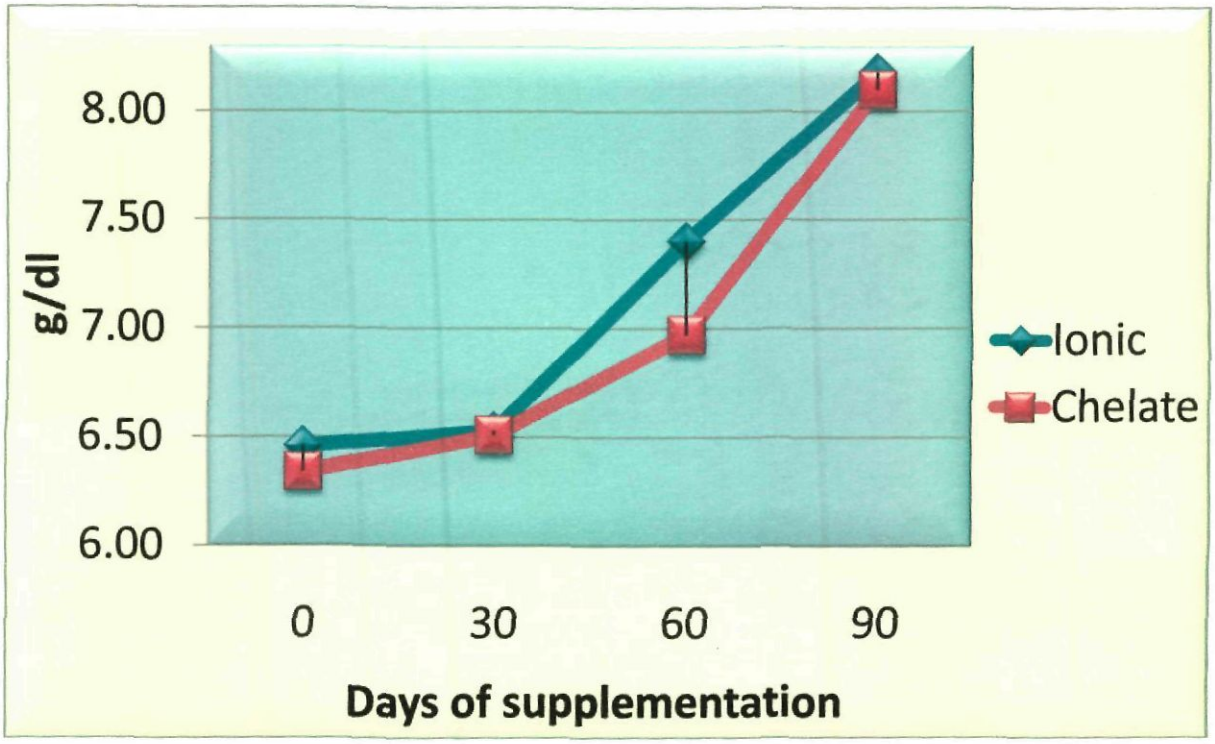


Figure 4.1: Serum total protein level (g/dl) in anoestrus buffaloes

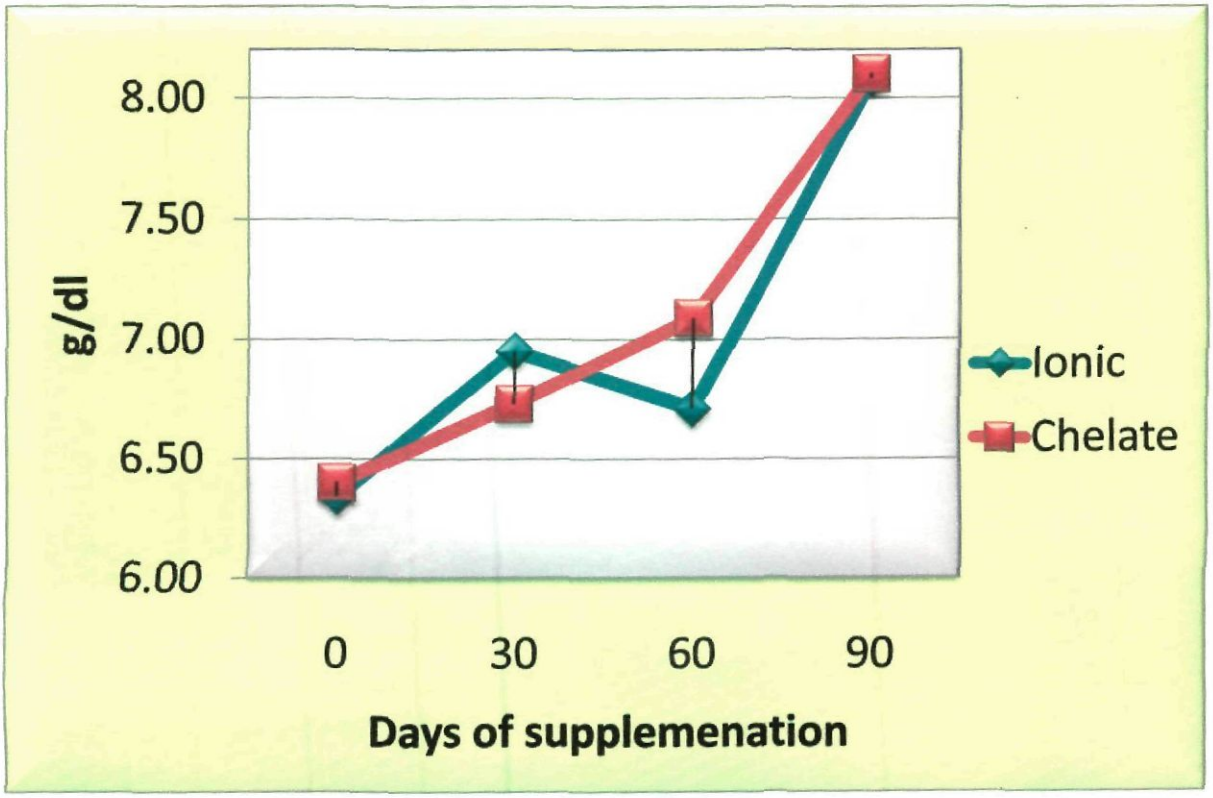


Figure 4.2: Serum total protein level (g/dl) in repeat breeder buffaloes

effect was found significant (P<0.01). During supplementation up to 30 days the serum protein level remained similar to 0 day but increased by 60th days of supplementation and the highest concentration was seen at 90th day both in ionic and chelated groups.

The average total protein level (g/dl) in ionic and chelated group of repeat breeder buffaloes was 7.02±0.21 and 7.08±0.20 (mg/dl), respectively. The treatment groups did not differ from each other. Similarly, Behera et al, (2012) reported no elevation in serum protein level of repeat breeder cow heifers on account of mineral mixture supplementation for 60 days. But Mohapatra *et al.* (2012) reported elevated levels of serum protein in repeat breeding buffaloes.

In repeat breeder buffaloes, the respective average total protein (mg/dl) at 0, 30th, 60th and 90th days was found to be 6.34 ± 0.25, 6.95 ± 0.13,6.71 ± 0.28 and 8.08 ± 0.10 in ionic group and 6.40 ± 0.43, 6.73 ± 0.37,7.09 ± 0.26 and 8.10 ± 0.26 in chelated group in anoestrus buffaloes. There was significant (P<0.01) difference between period effect. During supplementation period highest concentration of total protein was seen at 90th day in ionic and chelated group.

4.4.3: Serum albumin:

The average serum albumin level (g/dl) of anoestrus and repeat breeder buffaloes in ionic and chelated groups are given in Table 4.11 and has been depicted in figures 4.3 and 4.4.

The average level in ionic and chelated group of anestrous buffaloes was 3.67±0.10 and 3.53±0.14 (g/dl), respectively. The treatment groups did not differ from each other. Contrary to this, Mohapatra *et al.* (2012) found albumin levels to increase significantly (P<0.01) in ASMM supplemented group of anoestrus cows than in un-supplemented ones.

Table 4.11: Serum albumin level (g/dl) of anoestrus and repeat breeder buffaloes

Days of supplementation	Ionic	Chelated	Mean
	Anoestrus buffaloes		
0	3.50	3.18	3.34[a]
30	3.50	3.28	3.39[a]
60	3.47	3.37	3.42[a]
90	4.19	4.28	4.24[b]
Mean	**3.67**	**3.53**	
±S. E.	**0.10**	**0.14**	
	Repeat breeder buffaloes		
0	3.28	3.33	3.31[a]
30	3.45	3.27	3.36[a]
60	3.30	3.54	3.42[a]
90	4.22	4.16	4.19[b]
Mean	**3.56**	**3.58**	
±S. E.	**0.12**	**0.11**	

Means bearing different superscripts in column differ significantly (P<0.01)

Analysis of variance for anoestrus buffaloes (mean sum of squares)

Source	d.f.	m.s.s.	Test	SEM	P value
P	3	2.641	**	0.089	<0.01
T	1	0.741	NS	0.063	0.035
PT	3	0.10	NS	0.127	0.589
Error	72	0.16			

Analysis of variance for repeat breeder buffaloes (mean sum of squares)

Source	d.f.	m.s.s.	Test	SEM	P value
P	1	2.169	**	0.079	<0.01
T	3	0.018	NS	0.056	0.706
PT	1	0.12	NS	0.11	0.421
Error	72	0.126			

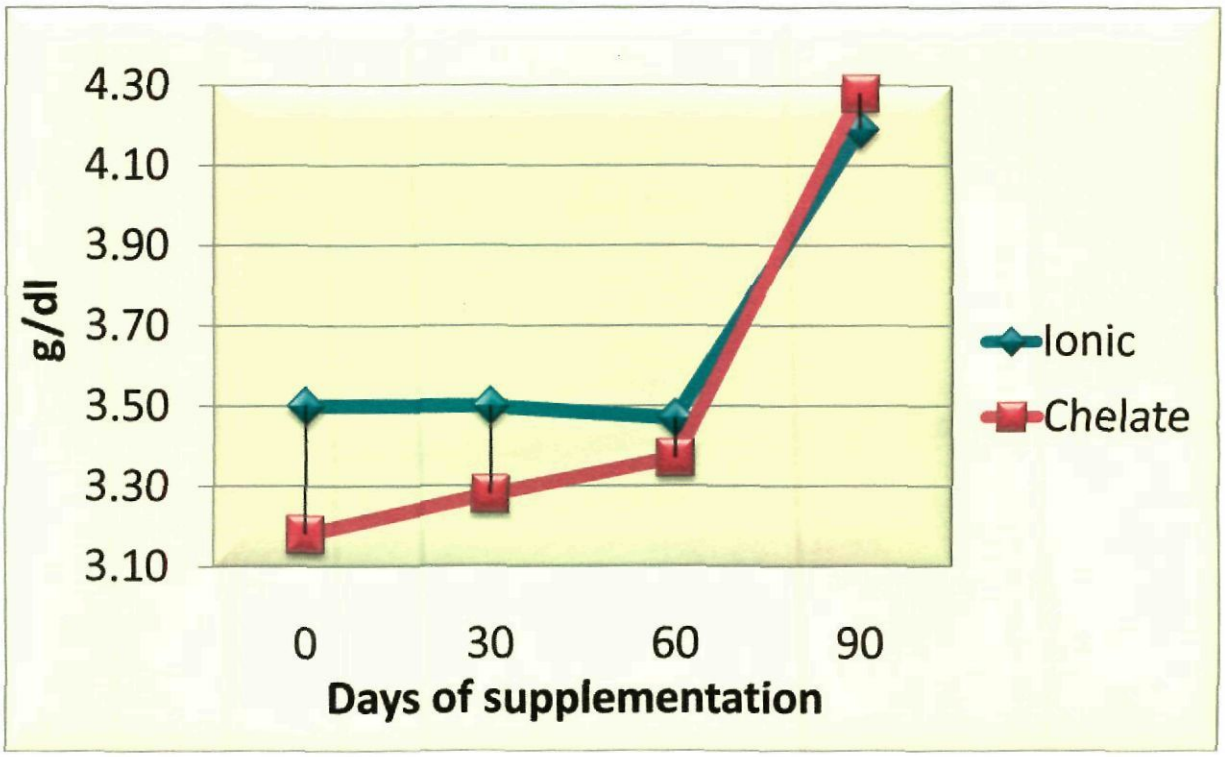


Figure 4.3: Serum albumin level (g/dl) in anoestrus buffaloes

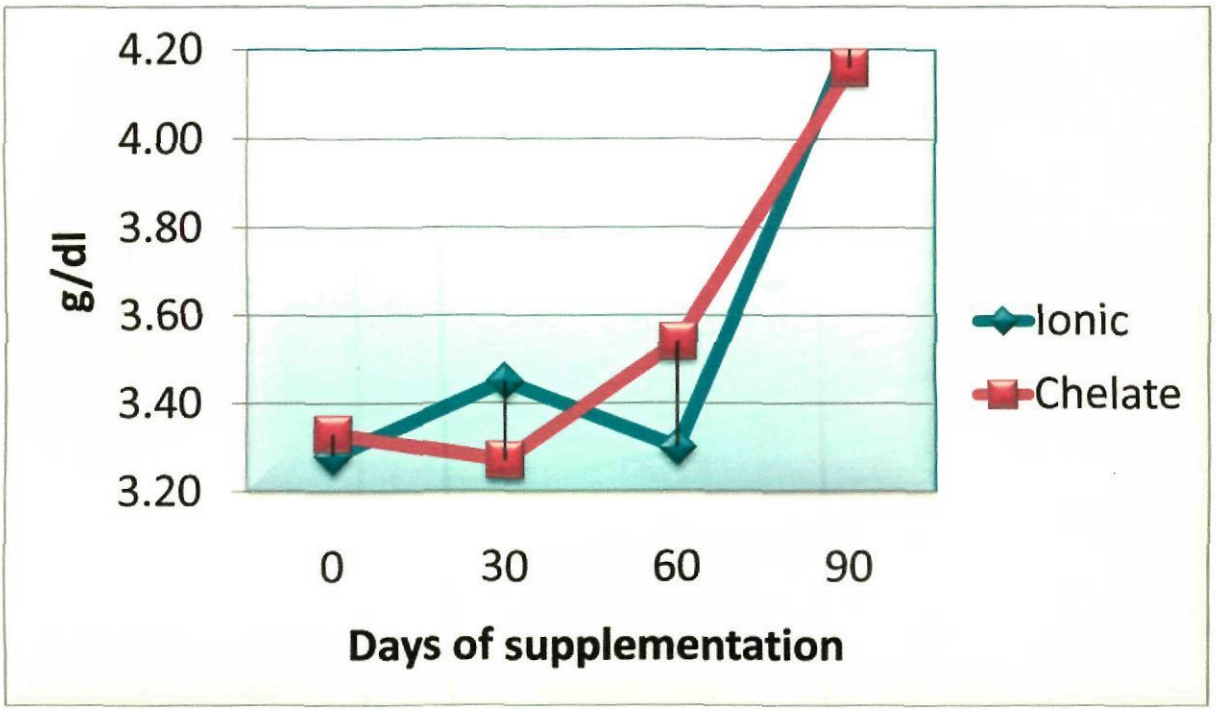


Figure 4.4: Serum albumin level (g/dl) in repeat breeder buffaloes

The respective average serum albumin level (g/dl) at 0 day was 3.50 ± 0.14 in ionic group and 3.18 ± 0.11 in chelated group. However, the period effect was found significant (P<0.01). During supplementation up to 30 days the serum albumin level remained similar to 0 day but increased by 60th days of supplementation and the highest concentration was seen at 90th day both in ionic and chelated groups.

The average level in ionic and chelated group of repeat breeder buffaloes was 3.56±0.12 and 3.58±0.11 (g/dl), respectively. The treatment groups did not differ from each other. Contrary to this, Mohapatra *et al.* (2012) found albumin levels to increase significantly (P<0.01) in ASMM supplemented group of repeat breeder cows than in un-supplemented ones.

The average serum albumin level (g/dl) at 0 day was 3.28 ± 0.12 in ionic group and 3.33 ± 0.11 in chelated group. However, the period effect was found significant (P<0.01). During supplementation up to 30 days the serum albumin level remained similar to 0 day but increased by 60th days of supplementation and the highest concentration was seen at 90th day in both the ionic and chelated groups.

4.4.4: Serum globulin:

The average serum globulin level (g/dl) of anoestrus and repeat breeder buffaloes in ionic and chelated groups are given in Table 4.12 and has been depicted in figures 4.5.

The average level in ionic and chelated group of anoestrus buffaloes was 3.48±0.15 and 3.45±0.09 (g/dl), respectively. The treatment groups did not differ from each other. Contrary to this, Mohapatra *et al.* (2012) reported globulin level to increase significantly (P<0.01) in ASMM supplemented group of anoestrus cows than in un-supplemented ones.

Table 4.12: Serum globulin level (g/dl) of anoestrus and repeat breeder buffaloes

Days of supplementation	Ionic	Chelated	Mean
	Anoestrus buffaloes		
0	2.96	3.16	3.06[a]
30	3.03	3.23	3.13[a]
60	3.93	3.60	3.77[b]
90	3.99	3.82	3.91[b]
Mean	**3.48**	**3.45**	
±S. E.	**0.15**	**0.09**	
	Repeat breeder buffaloes		
0	3.06	3.07	3.07
30	3.50	3.46	3.48
60	3.41	3.54	3.48
90	3.86	3.94	3.90
Mean	**3.46**	**3.51**	
±S. E.	**0.09**	**0.09**	

Means bearing different superscripts in column differ significantly (P<0.01)

Analysis of variance for anoestrus buffaloes (mean sum of squares)

Source	d.f.	m.s.s.	Test	SEM	P value
P	3	4.124	**	0.163	<0.01
T	1	0.015	NS	0.115	0.867
PT	3	0.238	NS	0.231	0.720
Error	72	0.53			

Analysis of variance for repeat breeder buffaloes (mean sum of squares)

Source	d.f.	m.s.s.	Test	SEM	P value
P	1	0.878	NS	0.154	0.140
T	3	0.435	NS	0.10	0.342
PT	1	0.067	NS	0.218	0.934
Error	72	0.475			

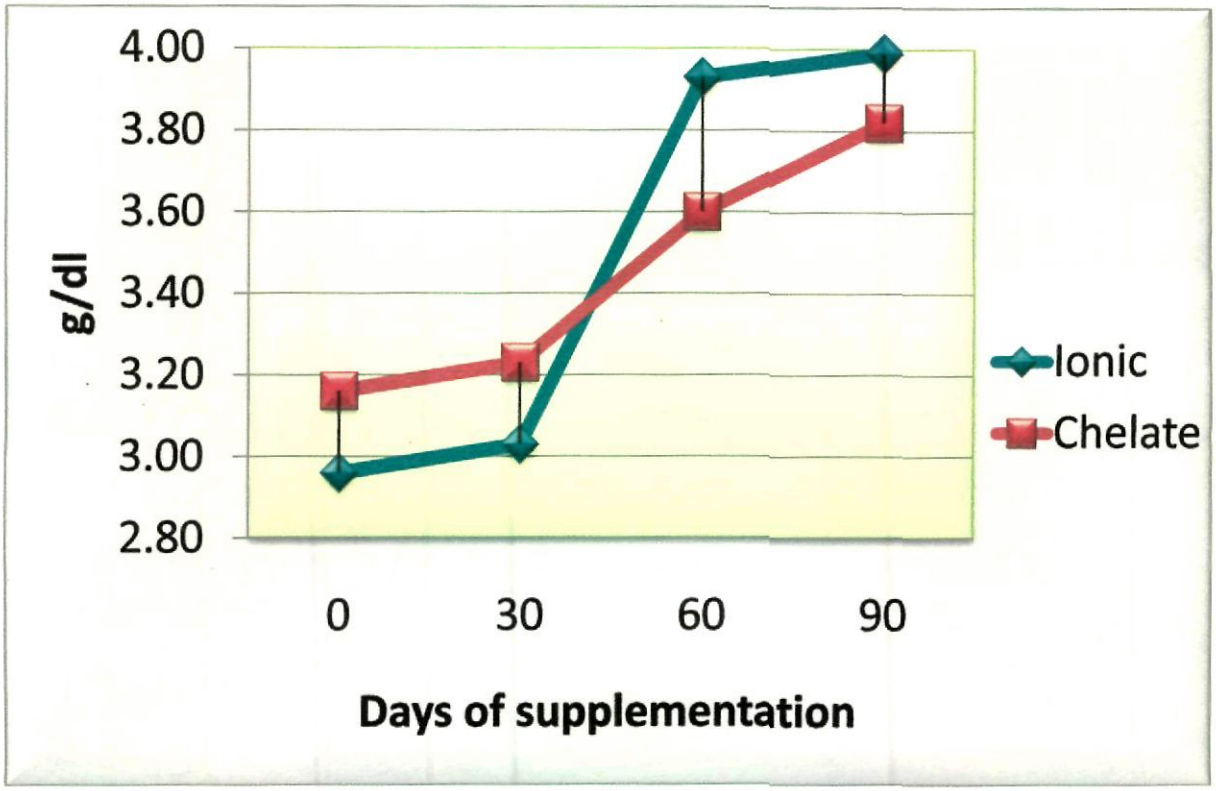


Figure 4.5: Serum globulin level (g/dl) in anoestrus buffaloes

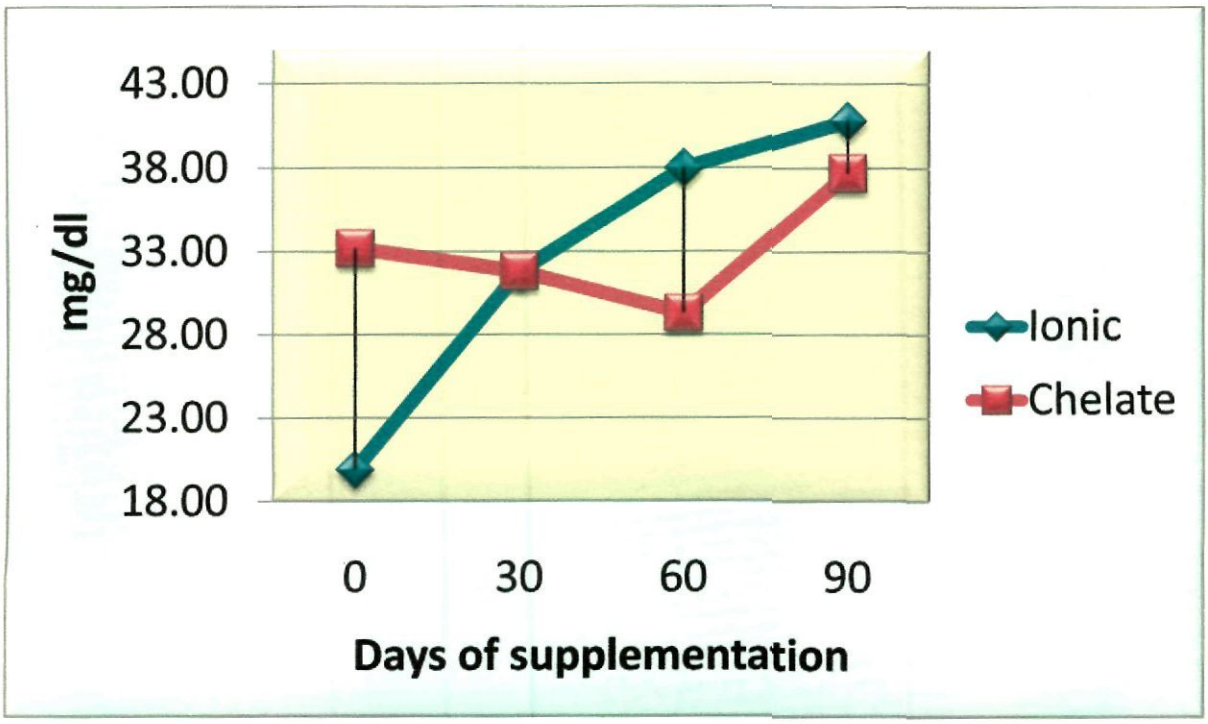


Figure 4.6: Serum TG level (mg/dl) in repeat breeder buffaloes

The period effect was found significant (P<0.01). The levels of serum globulin (g/dl) in ionic and chelated group was lower at the beginning (2.96±0.15 and 3.16±0.24), and it increased at 30th day (3.03±0.20 and 3.23±0.24) and at 60th day (3.93±0.13 and 3.60±0.18) and was the highest at 90th day (3.99±0.07 and 3.82±0.16) in ionic and chelated group.

In repeat breeder buffaloes, the average level in ionic and chelated group was 3.46±0.09 and 3.51±0.09 (g/dl), respectively. The treatment groups did not differ from each other. Contrary to this, Mohapatra *et al.* (2012) reported serum globulin level to increase significantly (P<0.01) in ASMM supplemented group of repeat breeder cows than in un-supplemented ones.

The period effect was not statistically different. The respective level of serum globulin in ionic and chelated group at 0, 30, 60 and 90th days was 3.06±0.27 and 3.07±0.24, 3.50±0.11 and 3.46±0.26, 3.41±0.15 and 3.54±0.24 and 3.86±0.12 and 3.94±0.11 (g/dl).

4.4.5: Serum Triglycerides:

The average serum triglycerides level (mg/dl) of anoestrus and repeat breeder buffaloes in ionic and chelated groups are given in Table 4.13 and has been depicted in figures 4.6.

The average level in ionic and chelated group of anoestrus buffaloes was found as 30.40±0.76 and 31.65±0.84 (mg/dl), respectively. The treatment groups did not differ from each other. The level of serum triglycerides (mg/dl) at 0, 30th, 60th and 90th day was 29.30±3.96, 27.00±1.60, 32.70±3.33, and 32.60±1.85 in ionic group and 27.30±2.05, 31.80±2.69, 33.20±2.29, and 34.30±1.68 in chelated group, respectively. The period effect was also not found statistically different.

Table 4.13: Serum triglycerides level (mg/dl) of anoestrus and repeat breeder buffaloes

Days of supplementation	Ionic	Chelated	Mean
	Anoestrus buffaloes		
0	29.30	27.30	28.30
30	27.00	31.80	29.40
60	32.70	33.20	32.95
90	32.60	34.30	33.45
Mean	**30.40**	**31.65**	
±S. E.	**0.76**	**0.84**	
	Repeat breeder buffaloes		
0	19.90	33.20	26.55^{a}
30	31.80	31.80	31.80^{a}
60	38.00	29.30	33.65^{a}
90	40.80	37.70	39.25^{b}
Mean	**32.63**	**33.00**	
±S. E.	**2.54**	**0.97**	

Means bearing different superscripts in column differ significantly (P<0.01)

Analysis of variance for anoestrus buffaloes (mean sum of squares)

Source	d.f.	m.s.s.	Test	SEM	P value
P	1	3067.23	NS	3.361	0.06
T	3	99.01	NS	2.37	0.51
PT	1	485.13	NS	4.75	0.546
Error	72	16271.30			

Analysis of variance for repeat breeder buffaloes (mean sum of squares)

Source	d.f.	m.s.s.	Test	SEM	P value
P	1	1022.41	**	3.36	0.01
T	3	99.01	NS	2.37	0.56
PT	1	161.71	NS	4.75	0.85
Error	72	225.99			

In repeat breeder buffaloes, the average level in ionic and chelated group was 32.63±2.54 and 33.00±0.97 (mg/dl), respectively. The treatment groups did not differ from each other.

The period effect was found significant (P<0.01). The level of serum triglycerides (mg/dl) at 0, 30th, 60th and 90th day was 19.90±1.75, 31.80±2.69, 38.00±2.70 and 40.80±5.37 in ionic group and 33.20±5.28, 31.80±2.69, 29.30±1.37 and 37.70±3.28 in chelated group, respectively.

4.4.6: Serum cholesterol:

The average serum cholesterol level (mg/dl) of anoestrus and repeat breeder buffaloes in ionic and chelated groups are given in Table 4.14 and has been depicted in figures 4.7 and 4.8.

The average level in ionic and chelated group of anoestrus buffaloes was 127.98±6.45 and 128.88±7.41 (mg/dl), respectively. The treatment groups did not differ from each other. Behera *et al.* (2012) also did not find any elevation in serum cholesterol level in anoestrus crossbred heifers. Contrary to this, Mohapatra *et al.* (2012) found cholesterol level was increased significantly (P<0.01) in ASMM supplemented group of anoestrus cows than in un-supplemented group.

The period effect was found significant (P<0.01). The average cholesterol concentration (mg/dl) on 0, 30th, 60th and 90th day was 94.90±5.44, 128.30±5.69, 148.40±8.75 and 140.30±3.47 in ionic group and 98.40±6.06, 164.30±7.25, 127.20±6.64 and 125.60±2.82 in chelated group.

In repeat breeder buffaloes, the average level in ionic and chelated group was 122.00±7.20 and 116.48±5.46 (mg/dl), respectively. The treatment groups did not differ from each other. Contrary to this, Mohapatra *et al.* (2012) in repeat breeder cows and Behera *et al.* (2012) in repeat breeder crossbred heifers found cholesterol level to

Table 4.14: Serum cholesterol level (mg/dl) of anoestrus and repeat breeder buffaloes

Days of supplementation	Ionic	Chelated	Mean
	Anoestrus buffaloes		
0	94.90	98.40	96.65^{a}
30	128.30	164.30	146.30^{b}
60	148.40	127.20	137.80^{b}
90	140.30	125.60	132.95^{b}
Mean	**127.98**	**128.88**	
±S. E.	**6.45**	**7.41**	
	Repeat breeder buffaloes		
0	85.10	92.30	88.70^{a}
30	128.30	109.00	118.65^{b}
60	147.40	137.30	142.35^{b}
90	127.20	127.30	127.25^{b}
Mean	**122.00**	**116.48**	
±S. E.	**7.20**	**5.46**	

Means bearing different superscripts in column differ significantly (P<0.01)

Analysis of variance for anoestrus buffaloes (mean sum of squares)

Source	d.f.	m.s.s.	Test	SEM	P value
P	1	9482.04	**	4.48	<0.01
T	3	0.61	NS	3.17	0.96
PT	1	2486.21	**	6.34	<0.01
Error	72	402.05			

Analysis of variance for repeat breeder buffaloes (mean sum of squares)

Source	d.f.	m.s.s.	Test	SEM	P value
P	1	17279.10	**	4.87	<0.01
T	3	540.80	NS	3.44	0.29
PT	1	710.63	NS	6.89	0.22
Error	72	475.42			

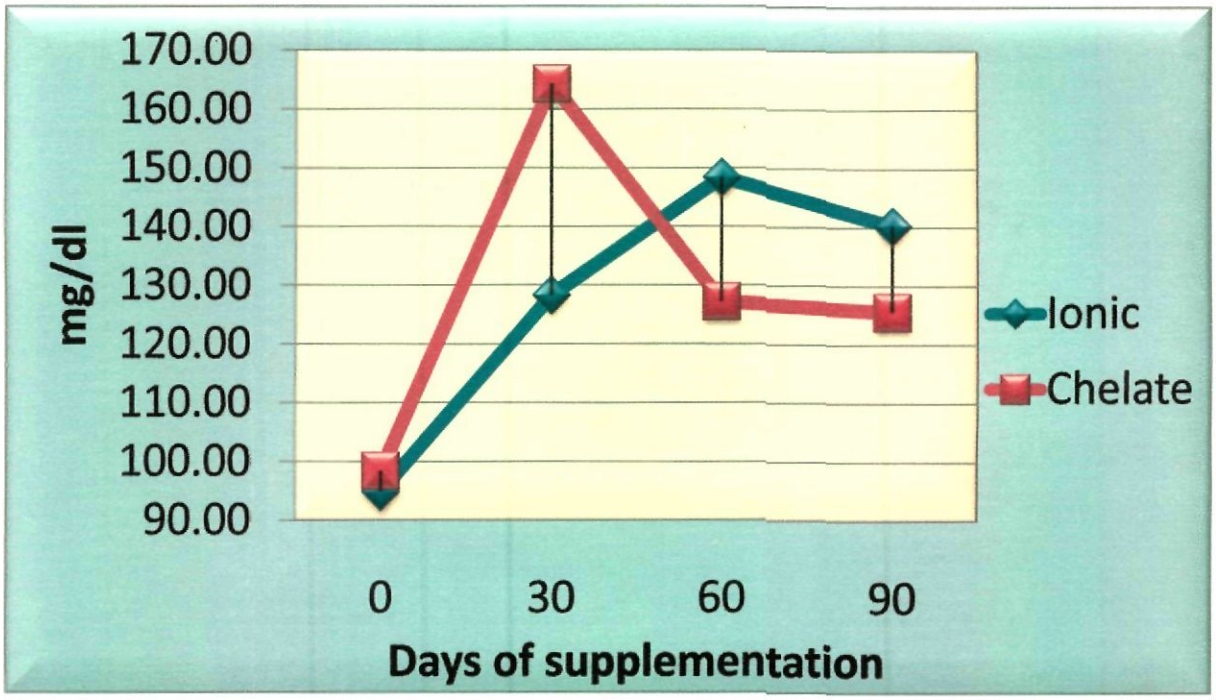


Figure 4.7: Serum cholesterol level (mg/dl) in anoestrus buffaloes

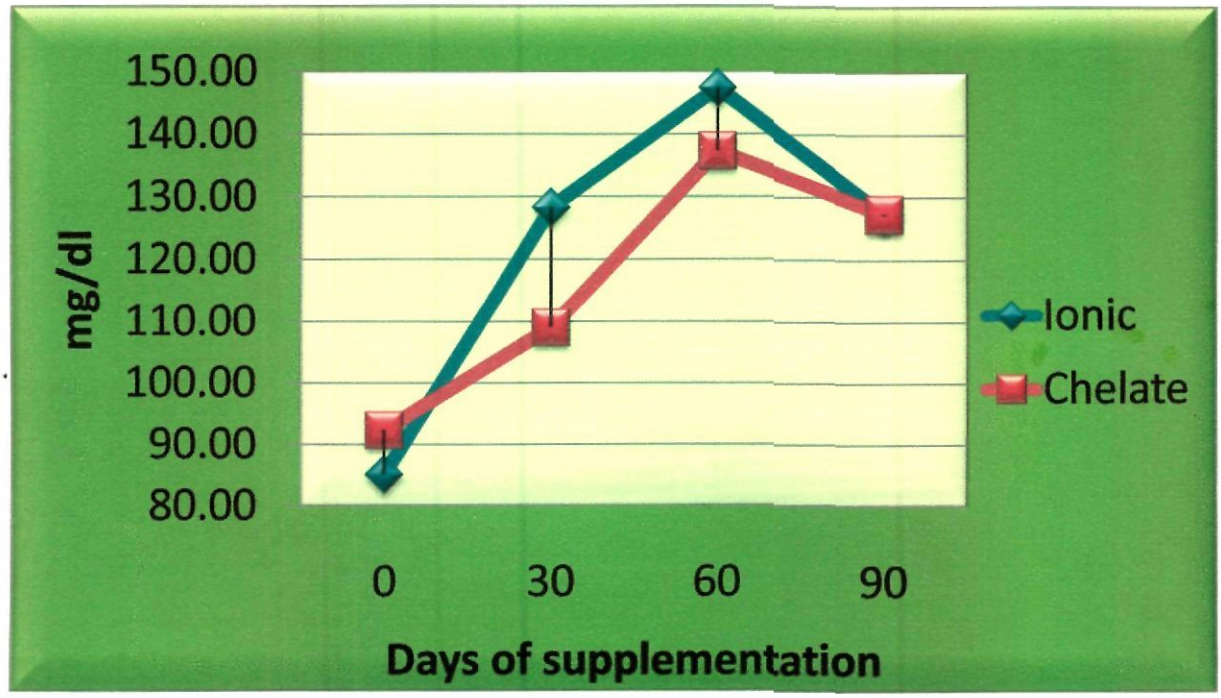


Figure 4.8: Serum cholesterol level (mg/dl) in repeat breeder buffaloes

increase significantly in mineral mixture supplemented groups of than in un-supplemented group.

In the present study, the period effect was found significant (P<0.01). The average level of cholesterol (mg/dl) at 0, 30th, 60th and 90th day was 85.10±5.55, 128.30±8.15, 147.40±7.58 and 127.20±6.64 in ionic group and 92.30±1.94, 109.00±5.00, 137.30±7.09, 127.30±6.64 in chelated group.

4.4.7: Blood urea nitrogen:

The average BUN level (mg/dl) of anoestrus and repeat breeder buffaloes in ionic and chelated groups are given in Table 4.15 and has been depicted in figures 4.9 and 4.10.

The average BUN level in ionic and chelated group of anoestrus buffaloes was 26.50±1.58 and 24.55±1.57 (mg/dl), respectively. The treatment groups did not differ from each other. However, Behera *et al*, (2012) reported elevated levels of BUN in anoestrus crossbred heifers.

The period effect was found significant (P<0.01). The average BUN concentration (mg/dl) during 0, 30th, 60th and 90th day was 33.30±2.75, 29.10±3.48, 22.90±1.75 and 20.70±1.41 in ionic group and 31.80±1.58, 25.80±1.29, 22.30±1.97 and 18.30±2.44 in chelated group.

In repeat breeder buffaloes, the average BUN level in ionic and chelated group was 27.20±1.34 and 25.88±1.42 (mg/dl), respectively. The treatment groups did not differ from each other. Similarly, Behera *et al*, (2012) reported no effect of mineral supplementation on levels of BUN.

The period effect was found significant (P<0.01). The level of BUN (mg/dl) at 0, 30th, 60th and 90th day in ionic group was 34.30± 2.46, 25.80± 1.29, 25.70± 2.84 and 23.00± 2.08 and 32.00± 2.16, 26.40± 2.34, 25.80± 1.29, 19.30± 1.52 in chelated group.

Table 4.15: Blood urea nitrogen level (mg/dl) of anoestrus and repeat breeder buffaloes

Days of supplementation	Ionic	Chelated	Mean
	Anoestrus buffaloes		
0	33.30	31.80	32.55^{b}
30	29.10	25.80	27.45^{b}
60	22.90	22.30	22.60^{a}
90	20.70	18.30	19.50^{a}
Mean	**26.50**	**24.55**	
±S. E.	**1.58**	**1.57**	
	Repeat breeder buffaloes		
0	34.30	32.00	33.15^{b}
30	25.80	26.40	26.10^{a}
60	25.70	25.80	25.75^{a}
90	23.00	19.30	21.15^{a}
Mean	**27.20**	**25.88**	
±S. E.	**1.34**	**1.42**	

Means bearing different superscripts in column differ significantly ($P<0.01$)

Analysis of variance for anoestrus buffaloes (mean sum of squares)

Source	d.f.	m.s.s.	Test	SEM	P value
P	3	622.81	**	1.668	<0.01
T	1	61.25	NS	1.180	0.298
PT	3	5.08	NS	2.360	0.965
Error	72	55.67			

Analysis of variance for repeat breeder buffaloes (mean sum of squares)

Source	d.f.	m.s.s.	Test	SEM	P value
P	1	535.046	**	1.72	<0.01
T	3	21.01	NS	1.21	0.55
PT	1	30.37	NS	2.43	0.67
Error	72	59.15			

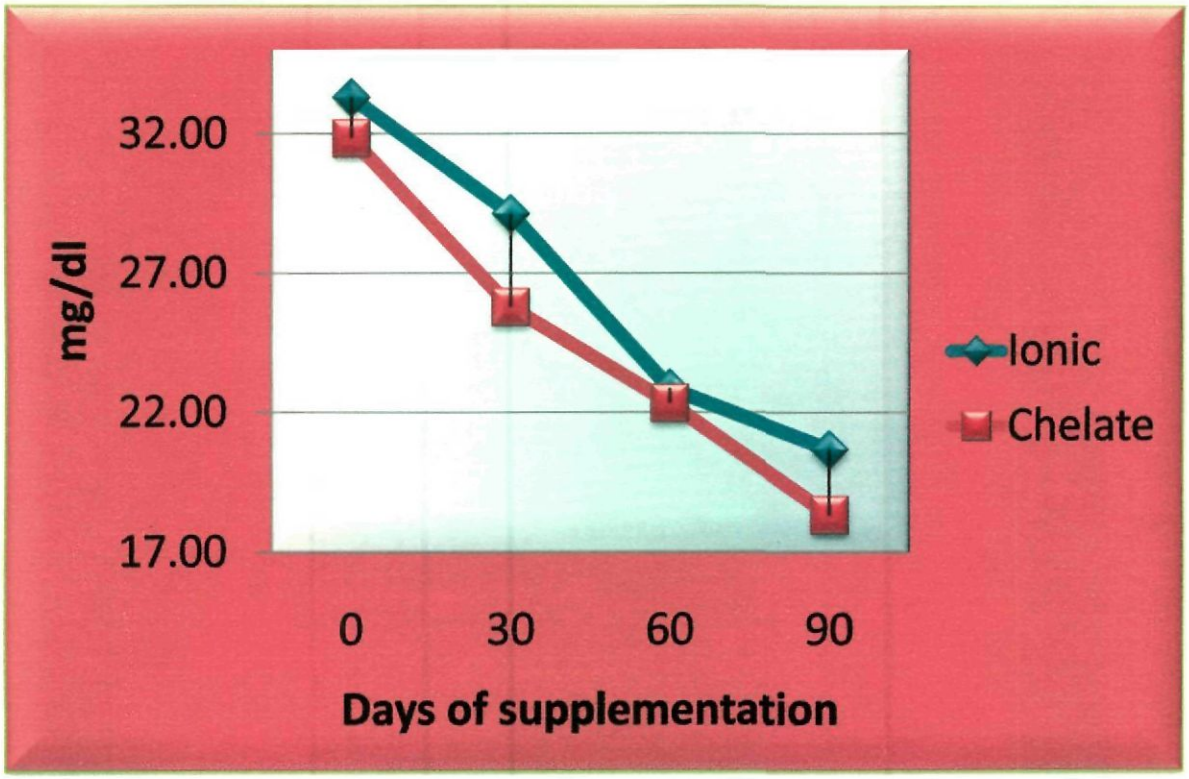


Figure 4.9: Serum BUN level (mg/dl) in anoestrus buffaloes

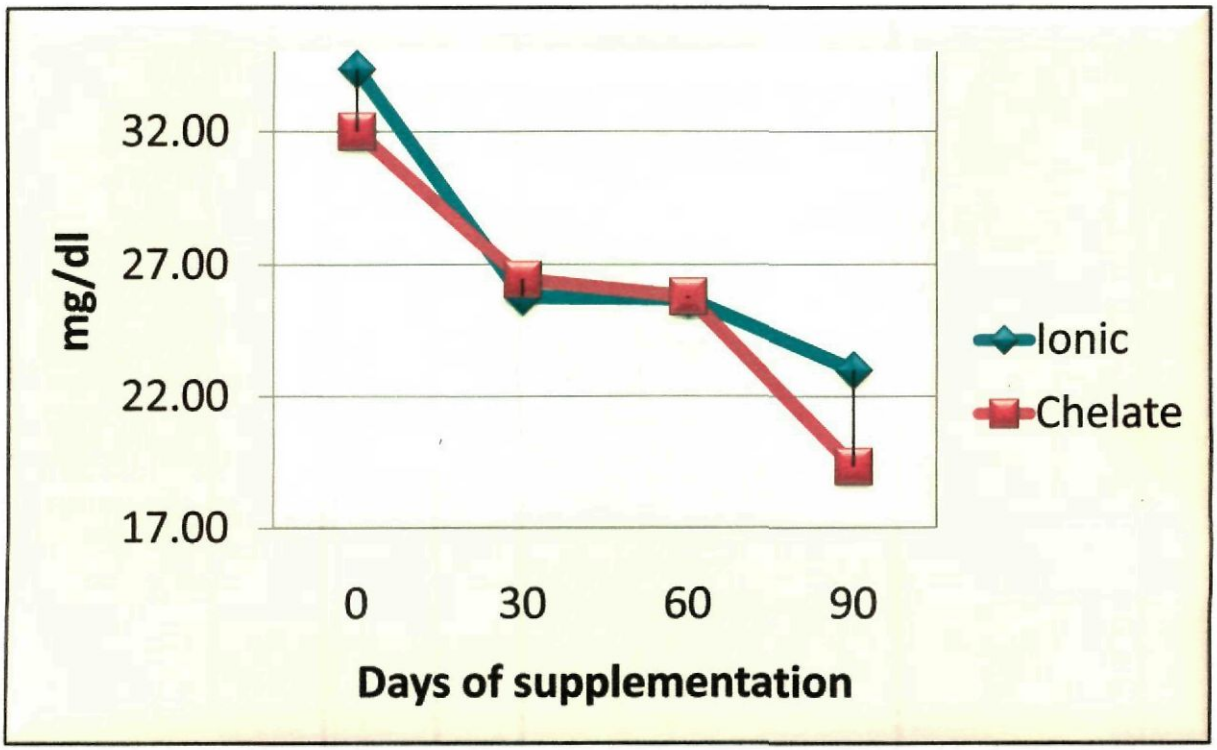


Figure 4.10: Serum BUN level (mg/dl) in repeat breeder buffaloes

4.4.8: Serum creatinine:

The average serum creatinine level (mg/dl) of anoestrus and repeat breeder buffaloes in ionic and chelated groups are given in Table 4.16 and has been depicted in figures 4.10 and 4.11.

The average serum creatinine level in ionic and chelated group of anoestrus buffaloes was 1.94±0.07 and 1.54±0.03 (mg/dl), respectively. The treatment groups did not differ from each other. However, the period effect was found significant ($P<0.01$). The average serum creatinine concentration (mg/dl) during 0, 30th, 60th and 90th day was 2.27±0.10, 2.00±0.15, 1.80±0.07 and 1.68±0.08 in ionic group and 1.58±0.10, 1.68±0.07, 1.43±0.16 and 1.48±0.08 in chelated group.

The average serum creatinine level in ionic and chelated group of repeat breeder buffaloes was 1.93±0.09 and 1.50±0.01 (mg/dl), respectively. The treatment groups did not differ from each other. But the period effect was found significant ($P<0.01$). The average concentration of serum creatinine (mg/dl) at 0, 30th, 60th and 90th day in ionic group was 2.38±0.06, 1.83±0.08, 1.81±0.16 and 1.68±0.11 and 1.54±0.05, 1.53±0.09, 1.50±0.12, 1.43±0.16 in chelated group.

4.4.9: Serum Alkaline phosphatase (AKP) activity:

The average serum AKP activity (U/L) of anoestrus and repeat breeder buffaloes in ionic and chelated groups are given in Table 4.17 and has been depicted in figures 4.13 and 4.14.

The average activity in ionic and chelated group of anoestrus buffaloes was 233.13±8.03 and 209.58±9.11 (U/L), respectively. The treatment groups did not differ from each other. Contrary to this, Behera *et al.* (2012) found Minfa (mineral mixture) supplementation to significantly increase ($P<0.05$) the activity of AKP in anoestrous heifers.

Table 4.16: Serum creatinine level (mg/dl) of anoestrus and repeat breeder buffaloes

Days of supplementation	Ionic	Chelated	Mean
	Anoestrus buffaloes		
0	2.27	1.58	1.93^{b}
30	2.00	1.68	1.84^{a}
60	1.80	1.43	1.62^{a}
90	1.68	1.48	1.58^{a}
Mean	**1.94**	**1.54**	
±S. E.	**0.07**	**0.03**	
	Repeat breeder buffaloes		
0	2.38	1.54	1.96^{b}
30	1.83	1.53	1.68^{a}
60	1.81	1.50	1.66^{a}
90	1.68	1.43	1.56^{a}
Mean	**1.93**	**1.50**	
±S. E.	**0.09**	**0.01**	

Means bearing different superscripts in column differ significantly (P<0.01)

Analysis of variance for anoestrus buffaloes (mean sum of squares)

Source	d.f.	m.s.s.	Test	SEM	P value
P	3	2.49	**	0.080	<0.01
T	1	0.25	NS	0.057	0.167
PT	3	0.030	NS	0.114	0.870
Error	72	0.130			

Analysis of variance for repeat breeder buffaloes (mean sum of squares)

Source	d.f.	m.s.s.	Test	SEM	P value
P	1	3.63	**	0.082	<0.01
T	3	0.021	NS	0.058	0.69
PT	1	0.060	NS	0.11	0.72
Error	72	0.13			

Table 4.17: Serum AKP activity (U/L) in anoestrus and repeat breeder buffaloes

Days of supplementation	Ionic	Chelated	Mean
	Anoestrus buffaloes		
0	217.40	159.80	188.60[a]
30	221.10	223.30	222.20[a]
60	217.00	228.20	222.60[a]
90	277.00	227.00	252.00[b]
Mean	**233.13**	**209.58**	
±S. E.	**8.03**	**9.11**	
	Repeat breeder buffaloes		
0	193.20	203.90	190.60[a]
30	221.20	160.00	198.55[a]
60	223.30	223.50	223.40[a]
90	237.60	245.00	241.30[b]
Mean	**218.83**	**208.10**	
±S. E.	**5.09**	**9.91**	

Means bearing different superscripts in column differ significantly (P<0.01)

Analysis of variance for anoestrus buffaloes (mean sum of squares)

Source	d.f.	m.s.s.	Test	SEM	P value
P	1	45461.23	**	19.97	<0.01
T	3	12650.45	NS	14.12	0.212
PT	1	13789.21	NS	28.2	0.169
Error	72	7982.04			

Analysis of variance for repeat breeder buffaloes (mean sum of squares)

Source	d.f.	m.s.s.	Test	SEM	P value
P	1	37540.56	**	17.40	<0.01
T	3	21978.45	NS	12.30	0.061
PT	1	7323.01	NS	24.61	0.313
Error	72	6058.75			

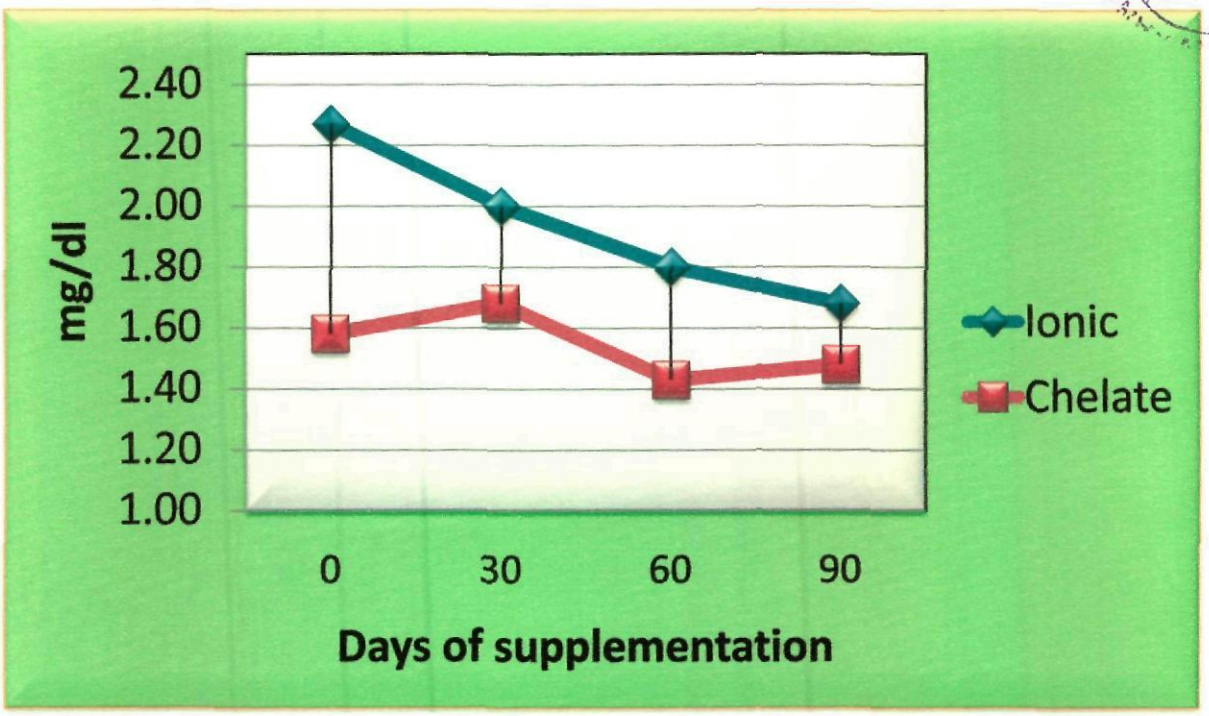


Figure 4.11: Serum creatinine level (mg/dl) in anoestrus buffaloes

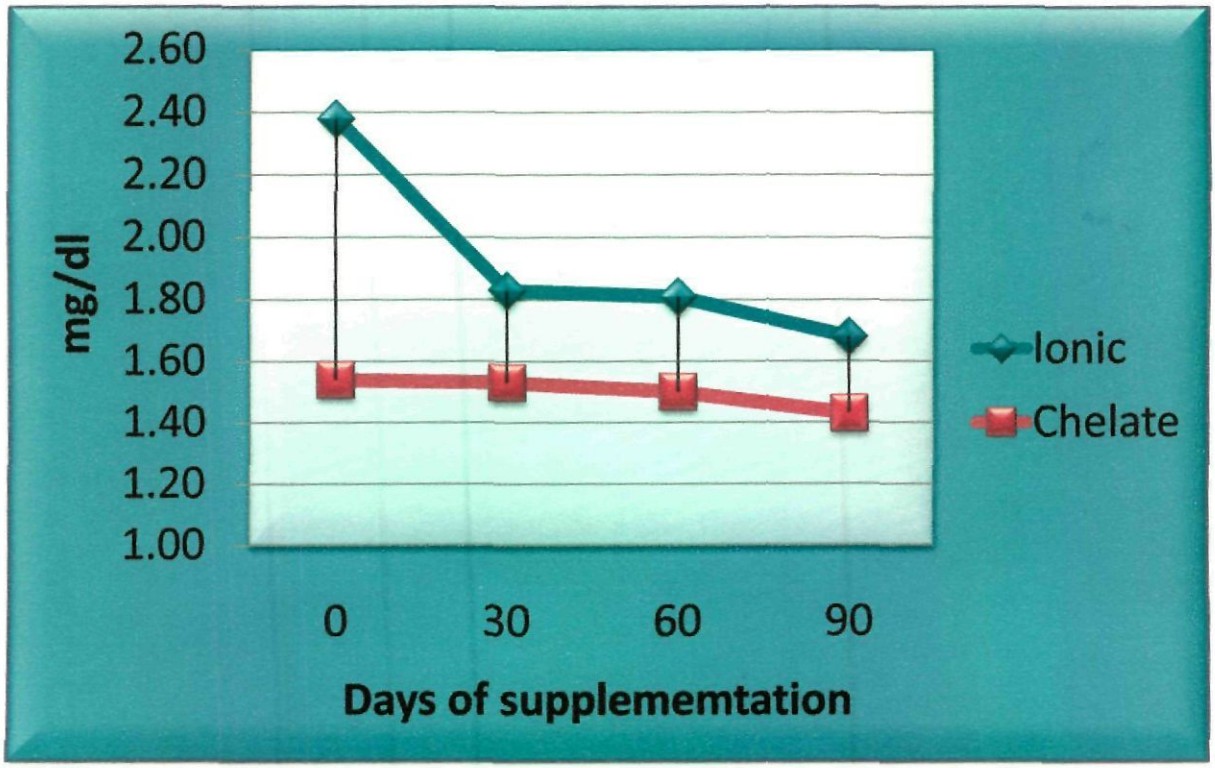


Figure 4.12: Serum creatinine level (mg/dl) in repeat breeder buffaloes

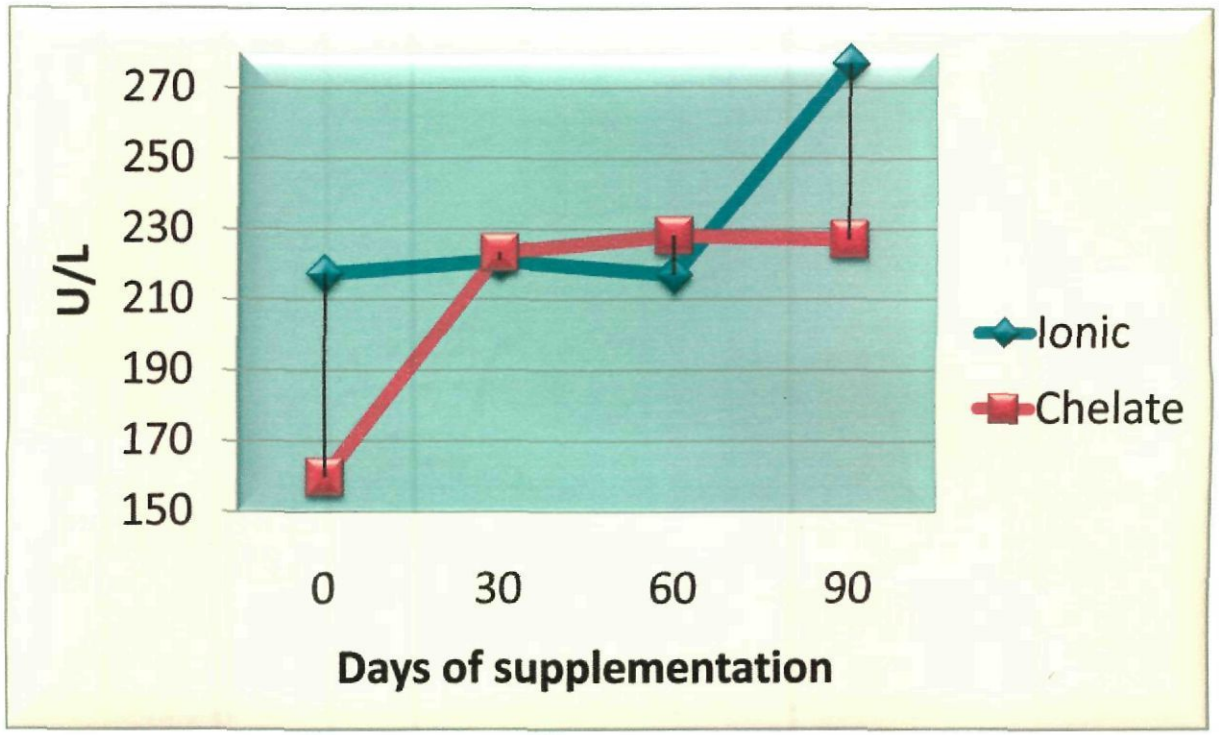


Figure 4.13: Serum AKP activity (U/L) in anoestrus buffaloes

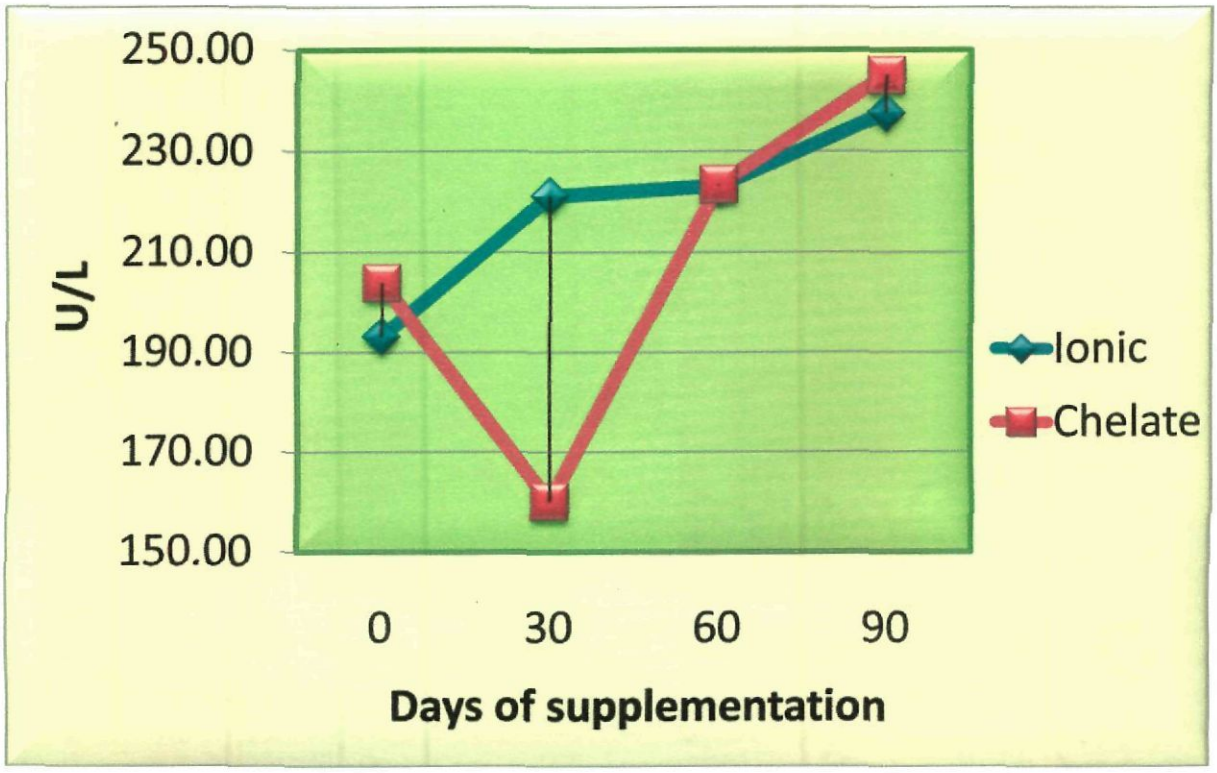


Figure 4.14: Serum AKP activity (U/L) in repeat breeder buffaloes

The period effect was found significant (P<0.01). The average serum AKP activity (U/L) during 0, 30th, 60th and 90th day was 217.40±20.66, 221.10±18.15, 217.00±15.10 and 277.00±23.38 in ionic group and 159.80±18.96, 223.30±25.17, 228.20±18.30 and 227.00±18.60 in chelated group.

In repeat breeder buffaloes, the average activity in ionic and chelated group was 218.83±5.09 and 208.10±9.91 (U/L), respectively. The treatment groups did not differ from each other. Contrary to this, Behera *et al.* (2012) found Minfa (mineral mixture) supplementation to significantly increase (P<0.05) the activity of AKP in repeat breeder heifers.

The period effect was found significant (P<0.01). The average serum AKP activity (U/L) at 0, 30th, 60th and 90th day was 193.20± 13.78, 221.20± 22.15, 223.30± 25.17 and 237.60± 30.49 in ionic group and 245.00± 32.58, 160.00± 20.80, 203.90± 17.45, 223.50± 25.17 in chelated group.

4.4.10: Serum Alanine Aminotransferase (ALT) activity:

The average serum ALT activity (U/L) of anoestrus and repeat breeder buffaloes in ionic and chelated groups are given in Table 4.18 and has been depicted in figures 4.15 and 4.16.

The average activity in ionic and chelated group of anoestrus buffaloes was 52.30±1.36 and 54.40±2.26 (U/L), respectively. The treatment groups did not differ from each other. Contrary to this, Behara *et al.* (2012) found Minfa (mineral mixture) supplementation to significantly increase (P<0.05) the activity of ALT in anoestrous heifers.

Table 4.18: Serum ALT activity (U/L) in anoestrus and repeat breeder buffaloes

Days of supplementation	Ionic	Chelated	Mean
	Anoestrus buffaloes		
0	47.30	48.20	47.75[a]
30	50.60	49.80	50.20[a]
60	52.20	53.20	52.70[a]
90	59.10	66.40	62.75[b]
Mean	**52.30**	**54.40**	
±S. E.	**1.36**	**2.26**	
	Repeat breeder buffaloes		
0	49.80	49.80	49.80[a]
30	51.50	53.60	52.55[a]
60	51.80	55.30	53.55[a]
90	63.40	68.10	65.75[b]
Mean	**54.13**	**56.70**	
±S. E.	**1.71**	**2.17**	

Means bearing different superscripts in column differ significantly (P<0.01)

Analysis of variance for anoestrus buffaloes (mean sum of squares)

Source	d.f.	m.s.s.	Test	SEM	P value
P	3	782.53	**	2.13	<0.01
T	1	336.20	NS	1.50	0.059
PT	3	49.93	NS	3.01	0.651
Error	72	91.13			

Analysis of variance for repeat breeder buffaloes (mean sum of squares)

Source	d.f.	m.s.s.	Test	SEM	P value
P	1	625.87	**	2.29	<0.01
T	3	143.112	NS	1.62	0.247
PT	1	43.37	NS	3.24	0.744
Error	72	105.046			

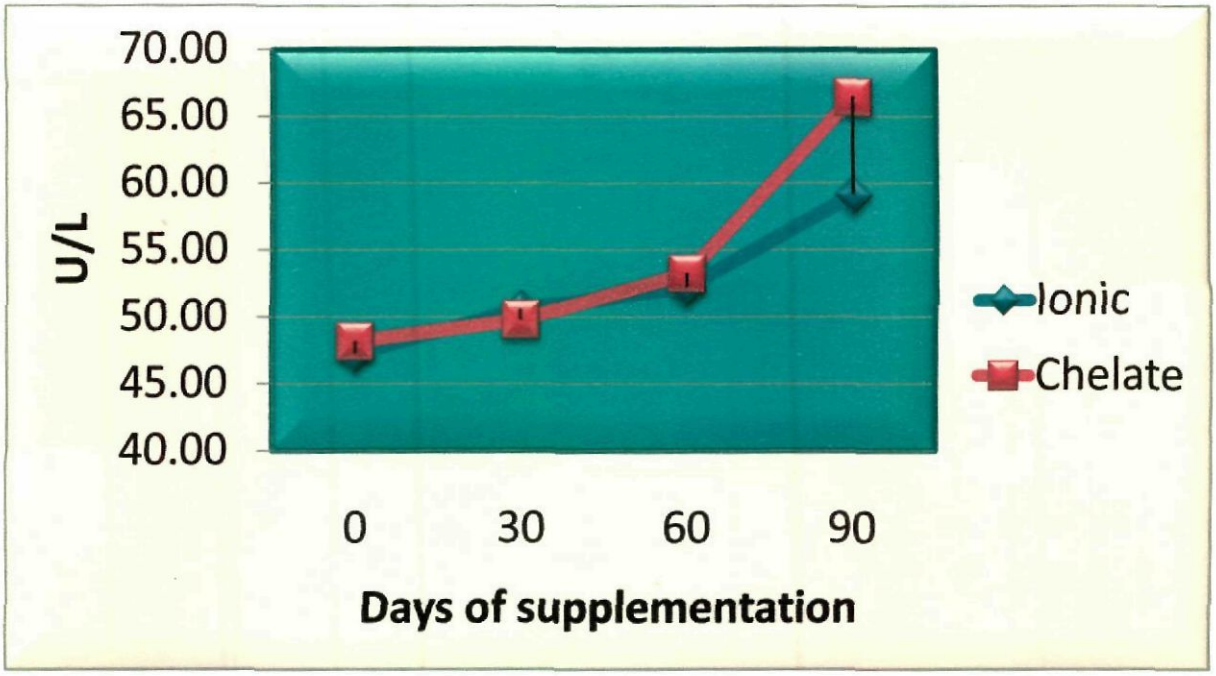


Figure 4.15: Serum ALT activity (U/L) in anoestrus buffaloes

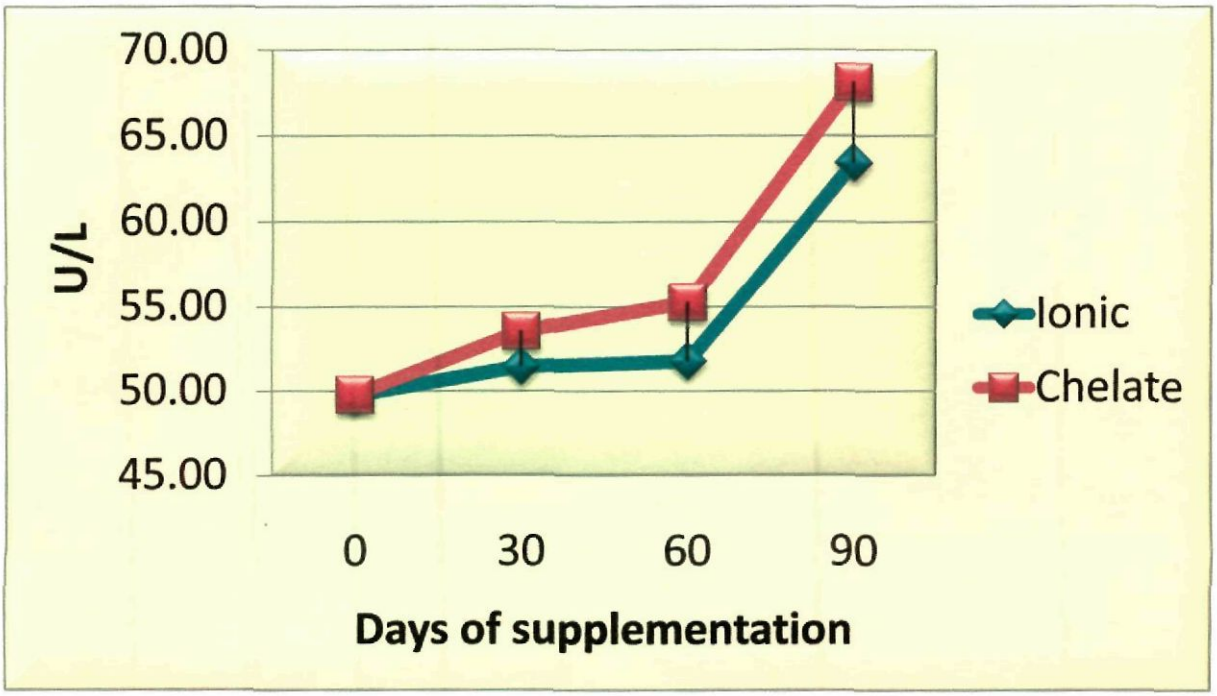


Figure 4.16: Serum ALT activity (U/L) in repeat breeder buffaloes

The period effect was found significant (P<0.01).The average serum ALT activity (U/L) during 0, 30th, 60th and 90th day was 47.30±2.29, 50.60±1.86, 52.20±2.61 and 59.10±3.36 in ionic group and 48.20±3.91, 49.80±3.13, 53.20±1.78 and 66.40±3.46 in chelated group. The activity was increased as period effect was increase, respectively.

In repeat breeder buffaloes, the average activity in ionic and chelated group was 54.13±1.71 and 56.70±2.17 (U/L), respectively. The treatment groups did not differ from each other. Contrary to this, Behara *et al.* (2012) found Minfa (mineral mixture) supplementation to significantly increase (P<0.05) the activity of ALT in repeat breeder heifers.

The period effect was found significant (P<0.01). The average serum ALT activity (U/L) at 0, 30th, 60th and 90th day was 49.80± 3.13, 51.50± 2.16, 51.80± 1.96 and 63.40± 3.73 in ionic group and 49.80± 3.13, 53.60± 2.64, 55.30± 2.45, 68.10± 3.24 in chelated group.

4.4.11: Serum Aspartate Aminotransferase (AST) activity:

The average serum AST (U/L) of anoestrus and repeat breeder buffaloes in ionic and chelated groups are given in Table 4.19.

The average serum AST activity in ionic and chelated group of anoestrus buffaloes was 172.58±6.07 and 175.13±5.54 (U/L), respectively. The treatment groups did not differ from each other. Contrary with Behara *et al.* (2012) found Minfa (mineral mixture) supplementation to significantly (P<0.05) increase the activity of AST in anoestrous heifers.

The period effect was also found statistically similar. The average serum AST activity (U/L) during 0, 30th, 60th and 90th day was 156.30± 5.45, 163.60± 7.50,165.10± 2.04 and 205.30± 8.59 in ionic group and 161.60± 6.54, 155.80± 8.72, 183.50± 21.37 and 199.60±13.82 in chelated group.

Table 4.19: Serum AST activity (U/L) in anoestrus and repeat breeder buffaloes

Days of supplementation	Ionic	Chelated	Mean
	Anoestrus buffaloes		
0	156.30	161.60	158.95
30	163.60	155.80	159.70
60	165.10	199.60	182.35
90	205.30	183.50	194.40
Mean	**172.58**	**175.13**	
±S. E.	**6.07**	**5.54**	
	Repeat breeder buffaloes		
0	150.30	158.30	154.30
30	155.80	155.80	155.80
60	164.30	162.60	163.45
90	162.00	173.10	167.55
Mean	**158.10**	**162.45**	
±S. E.	**1.73**	**2.09**	

Analysis of variance for anoestrus buffaloes (mean sum of squares)

Source	d.f.	m.s.s.	Test	SEM	P value
P	3	2534.58	NS	9.79	0.27
T	1	510.05	NS	6.92	0.60
PT	3	4442.05	NS	13.85	0.08
Error	72	1918.66			

Analysis of variance for repeat breeder buffaloes (mean sum of squares)

Source	d.f.	m.s.s.	Test	SEM	P value
P	1	671.81	NS	6.72	0.53
T	3	369.80	NS	4.75	0.52
PT	1	35.70	NS	9.51	0.98
Error	72	904.65			

In repeat breeder buffaloes, the average serum AST activity in ionic and chelated group was 158.10±1.73 and 162.45±2.09 (U/L), respectively. The treatment groups did not differ from each other. Contrary with Behara *et al.* (2012) found Minfa (mineral mixture) supplementation to significantly increase ($P<0.05$) the activity of AST in repeat breeder heifers.

The period effect was also found statistically similar. The average serum AST activity (U/L) at 0, 30th, 60th and 90th day was 150.30± 6.95, 155.80± 8.72, 164.30± 5.98 and 162.00± 13.04 in ionic group and 158.30± 7.69,155.80± 8.72, 162.60± 7.56 and 173.40± 10.81 in chelated group.

4.5.: Mineral profile of anoestrus and repeat breeder buffaloes:

4.5.1.: Macro mineral:

4.5.1.1.: Serum Calcium Level:

The average serum Calcium level (mg/dl) of anoestrus and repeat breeder buffaloes in ionic and chelated groups are given in Table 4.20 and has been depicted in figures 4.17 and 4.18.

The average serum Calcium level in ionic and chelated group of anoestrus buffaloes was 8.38±0.61 and 8.64±0.63 (mg/dl), respectively. The treatment groups did not differ from each other. Similar findings of significantly increased serum Ca level due to supplementation of mineral mixture were reported by Devasena *et al.* (2010) in anoestrus heifers and Mohapatra *et al.* (2012) in anoestrus cows. Whereas, Lall *et al.* (2004) reported non-significant effect of mineral mixture supplementation on Ca level in anoestrus buffalo heifer.

Table 4.20: Serum calcium level (mg/dl) of anoestrus and repeat breeder buffaloes

Days of supplementation	Ionic	Chelated	Mean
	Anoestrus buffaloes		
0	5.09	5.29	5.19^{a}
30	9.22	10.47	9.85^{b}
60	9.84	9.42	9.63^{b}
90	9.37	9.36	9.37^{b}
Mean	**8.38**	**8.64**	
±S. E.	**0.61**	**0.63**	
	Repeat breeder buffaloes		
0	5.53	5.60	5.57^{a}
30	10.06	10.09	10.08^{b}
60	9.78	9.93	9.86^{b}
90	9.32	9.42	9.37^{b}
Mean	**8.67**	**8.76**	
±S. E.	**0.58**	**0.58**	

Means bearing different superscripts in column differ significantly (P<0.01)

Analysis of variance for anoestrus buffaloes (mean sum of squares)

Source	d.f.	m.s.s.	Test	SEM	P value
P	1	104.37	**	0.33	<0.01
T	3	2.77	NS	0.23	0.265
PT	1	3.19	NS	0.46	0.235
Error	72	2.20			

Analysis of variance for repeat breeder buffaloes (mean sum of squares)

Source	d.f.	m.s.s.	Test	SEM	P value
P	1	116.032	**	0.335	<0.01
T	3	0.00012	NS	0.237	0.99
PT	1	0.0891	NS	0.473	0.98
Error	72	2.239			

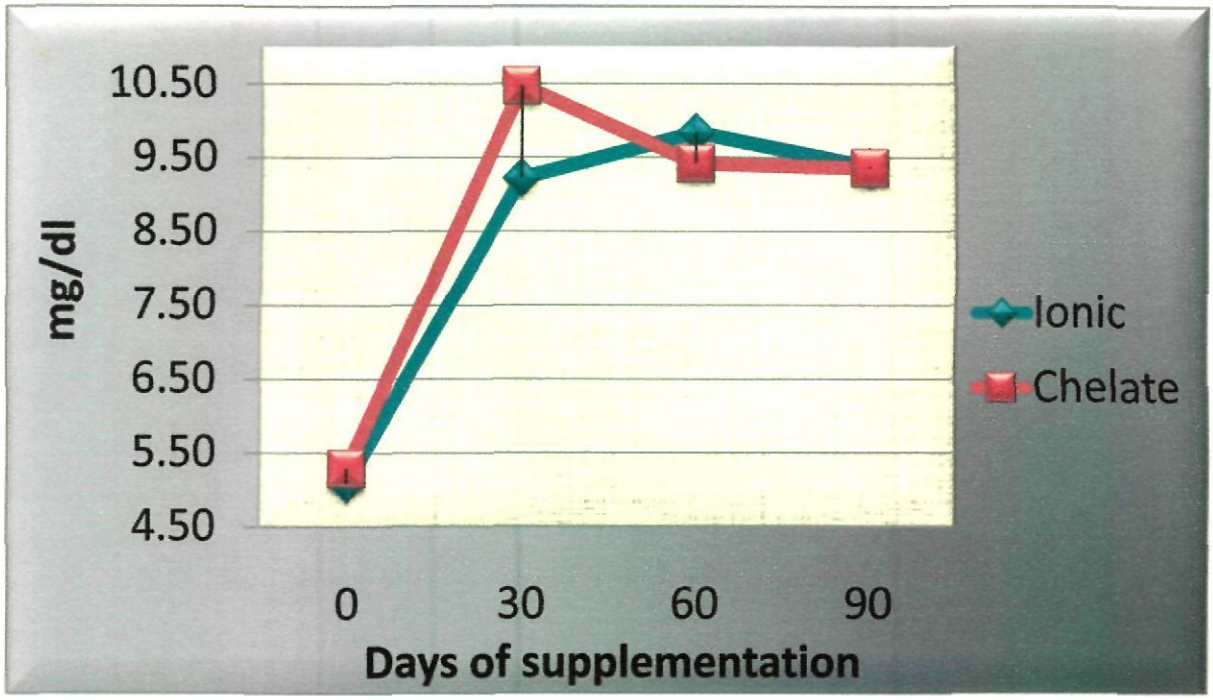


Figure 4.17: Serum Ca level (mg/dl) in anoestrus buffaloes

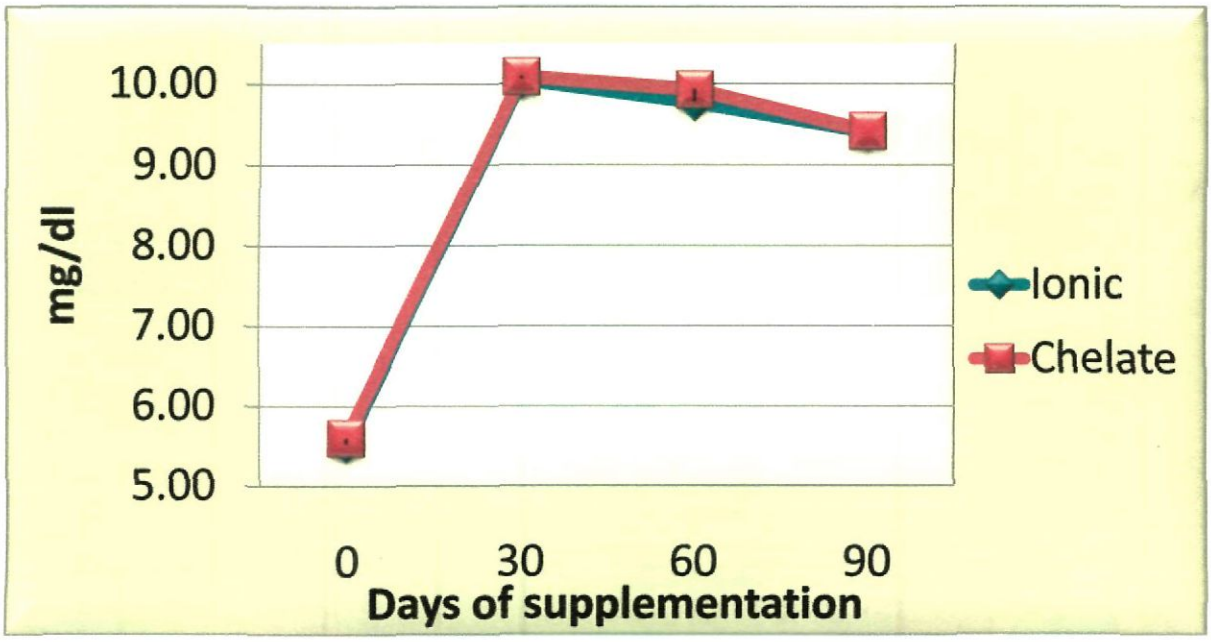


Figure 4.18: Serum Ca level (mg/dl) in repeat breeder buffaloes

The period effect was found significant (P<0.01). The level (mg/dl) during 0, 30th, 60th and 90th day was 5.09±0.41, 9.22±0.40, 9.84±0.60 and 9.37±0.34 in ionic group and 5.29±0.44, 10.47±0.29, 9.42±0.53 and 9.36±0.41 in chelated group. In both groups the level of Calcium increased by 30th day and stabilized thereafter.

In repeat breeder buffaloes, the average serum Calcium level in ionic and chelated group was 8.67±0.58 and 8.76±0.58 (mg/dl), respectively. The treatment groups did not differ from each other. Similar findings were reported by Mohapatra *et al.* (2012) in repeat breeder cows supplemented with mineral mixture.

The period effect was found significant (P<0.01). The serum Calcium level (mg/dl) at 0, 30th, 60th and 90th day was 5.53±0.45, 10.06±0.41, 9.78±0.50 and 9.32±0.53 in ionic group and 5.60±0.39, 10.09±0.41, 9.93±0.50 and 9.42±0.53 in chelated group. In both groups the level of Calcium increased by 30th day and stabilized thereafter.

4.5.1.2.: Serum Phosphorus Level:

The average serum Phosphorus level (mg/dl) of anoestrus and repeat breeder buffaloes in ionic and chelated groups are given in Table 4.21 and has been depicted in figures 4.19 and 4.20.

The average level in ionic and chelated group of anoestrus buffaloes was 5.70±0.30 and 5.93±0.46 (mg/dl), respectively. The treatment groups did not differ from each other. The results of this study are corroborated by the findings of Lall *et al*, (2004) in anoestrus buffalo heifers, Devasena *et al.* (2010) in anoestrus heifers and Mohapatra *et al.* (2012) in anoestrus cows.

The period effect was found significant (P<0.01).The serum Phosphorus level (mg/dl) during 0, 30th, 60th and 90th day was 4.16±0.39, 5.74±0.22, 6.51±0.45 and 6.39±0.30 in ionic group and 3.40±0.22, 6.68±0.24, 6.80±0.49 and 6.82±0.19 in chelated group. In both groups the level of Phosphorus increased by 30th day and stabilized thereafter.

Table 4.21: Serum phosphorus level (mg/dl) of anoestrus and repeat breeder buffaloes

Days of supplementation	Ionic	Chelated	Mean
	Anoestrus buffaloes		
0	4.16	3.40	3.78^{a}
30	5.74	6.68	6.21^{b}
60	6.51	6.80	6.66^{b}
90	6.39	6.82	6.61^{b}
Mean	**5.70**	**5.93**	
±S. E.	**0.30**	**0.46**	
	Repeat breeder buffaloes		
0	4.03	4.27	4.15^{a}
30	5.93	6.10	6.02^{b}
60	6.80	6.19	6.50^{b}
90	6.27	6.80	6.54^{b}
Mean	**5.76**	**5.84**	
±S. E.	**0.33**	**0.30**	

Means bearing different superscripts in column differ significantly (P<0.01)

Analysis of variance for anoestrus buffaloes (mean sum of squares

Source	d.f.	m.s.s.	Test	SEM	P value
P	1	37.111	**	0.239	<0.01
T	3	1.40	NS	0.16	0.22
PT	1	1.693	NS	0.33	0.27
Error	72	1.14			

Analysis of variance for repeat breeder buffaloes (mean sum of squares)

Source	d.f.	m.s.s.	Test	SEM	P value
P	1	25.379	**	0.22	<0.01
T	3	1.15	NS	0.15	0.28
PT	1	0.16	NS	0.31	0.92
Error	72	1.008			

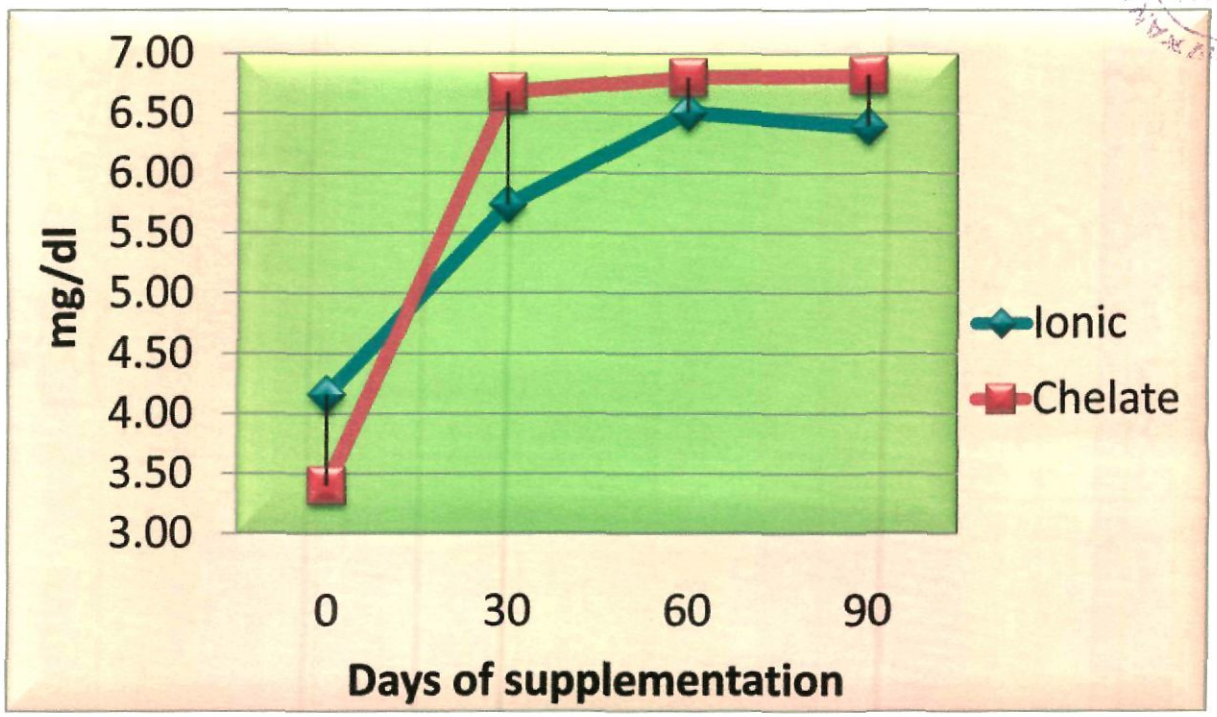


Figure 4.19: Serum inorganic P level (mg/dl) in anoestrus buffaloes

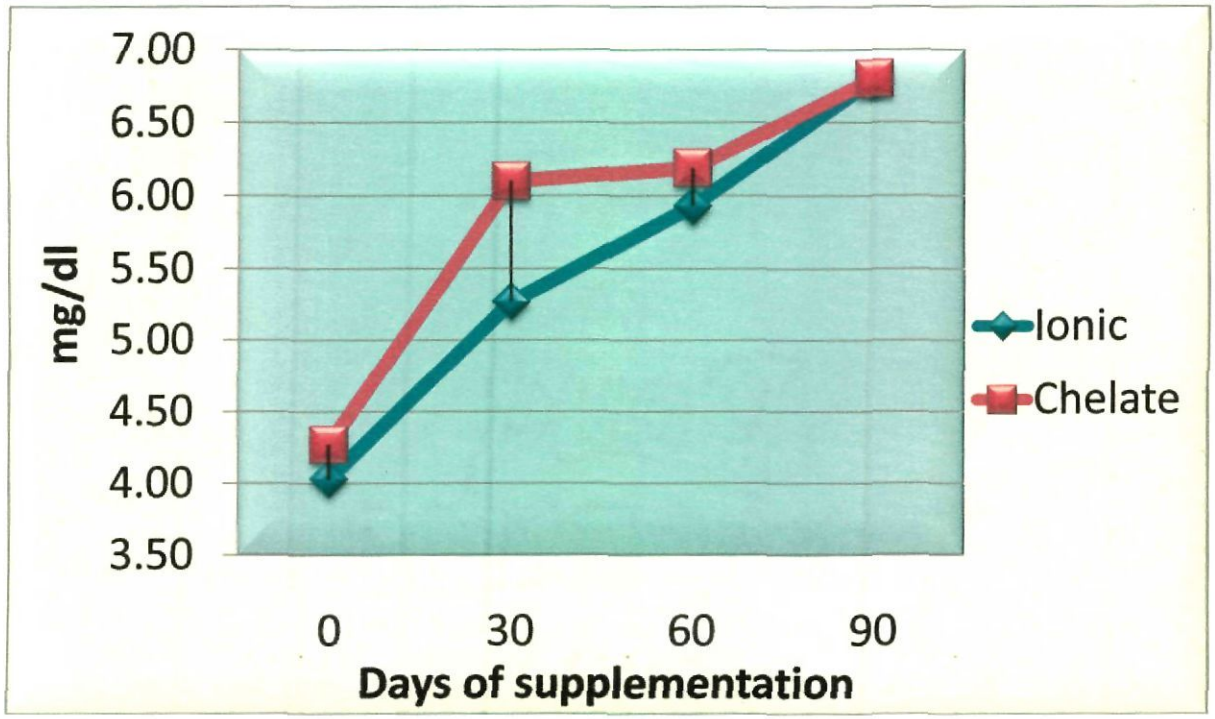


Figure 4.20: Serum inorganic P level (mg/dl) in repeat breeder buffaloes

In repeat breeder buffaloes, the average level in ionic and chelated group was 5.76±0.33 and 5.84±0.30 (mg/dl), respectively. The treatment groups did not differ from each other. However, Mohapatra *et al.* (2012) observed the serum Phosphorus level to elevate significantly (P<0.01) in repeat breeding cows due to supplementation of mineral mixture.

The period effect was found significant (P<0.01). The serum Phosphorus level (mg/dl) at 0, 30th, 60th and 90th day was 4.03±0.35, 5.93±0.22, 6.80±0.26 and 6.27±0.49 in ionic group and 4.27±0.33, 6.10±0.15, 6.19±0.31, 6.80±0.49 in chelated group. In both groups the level of Phosphorus to elevate up to 90th day.

4.5.1.3.: Serum Magnesium Level:

The average serum Magnesium level (mg/dl) of anoestrus and repeat breeder buffaloes in ionic and chelated groups are given in Table 4.22 and has been depicted in figures 4.21 and 4.22.

The average level in ionic and chelated group of anoestrus buffaloes was 2.67±0.24 and 2.66±0.24 (mg/dl), respectively. The treatment groups did not differ from each other. The period effect was found significant (P<0.01).The serum Magnesium level (mg/dl) during 0, 30th, 60th and 90th day was 1.39±0.09, 3.40±0.15, 2.99±0.19 and 2.90±0.19 in ionic group and 1.38±0.08, 3.45±0.15, 2.99±0.25 and 2.82±0.12 in chelated group. In both groups the level of Magnesium increased by 30th day and slightly declined thereafter. Similar were the findings of Behera *et al.* (2012) who reported Minfa (mineral mixture) supplementation for 60 days to significantly (P<0.05) increase the serum levels of Mg in anoestrus crossbred heifers.

In repeat breeder buffaloes, the average level in ionic and chelated group was 2.62±0.22 and 2.63±0.25 (mg/dl), respectively. The treatment groups did not differ from each other. The period effect was found significant (P<0.01). The serum Magnesium

Table 4.22: Serum magnesium level (mg/dl) of anoestrus and repeat breeder buffaloes

Days of supplementation	Ionic	Chelated	Mean
	Anoestrus buffaloes		
0	1.39	1.38	1.39^{a}
30	3.40	3.45	3.43^{b}
60	2.99	2.99	2.99^{b}
90	2.90	2.82	2.86^{b}
Mean	**2.67**	**2.66**	
±S. E.	**0.24**	**0.24**	
	Repeat breeder buffaloes		
0	1.43	1.28	1.36^{a}
30	3.03	3.29	3.16^{b}
60	3.01	2.94	2.98^{b}
90	2.99	3.01	3.00^{b}
Mean	**2.62**	**2.63**	
±S. E.	**0.22**	**0.25**	

Means bearing different superscripts in column differ significantly (P<0.01)

Analysis of variance for anoestrus buffaloes (mean sum of squares)

Source	d.f.	m.s.s.	Test	SEM	P value
P	1	18.34	**	0.135	<0.01
T	3	0.024	NS	0.095	0.79
PT	1	0.104	NS	0.19	0.83
Error	72				

Analysis of variance for repeat breeder buffaloes (mean sum of squares)

Source	d.f.	m.s.s.	Test	SEM	P value
P	1	13.72	**	0.107	<0.01
T	3	1.137	NS	0.0754	1.00
PT	1	0.129	NS	0.151	0.63
Error	72	0.227			

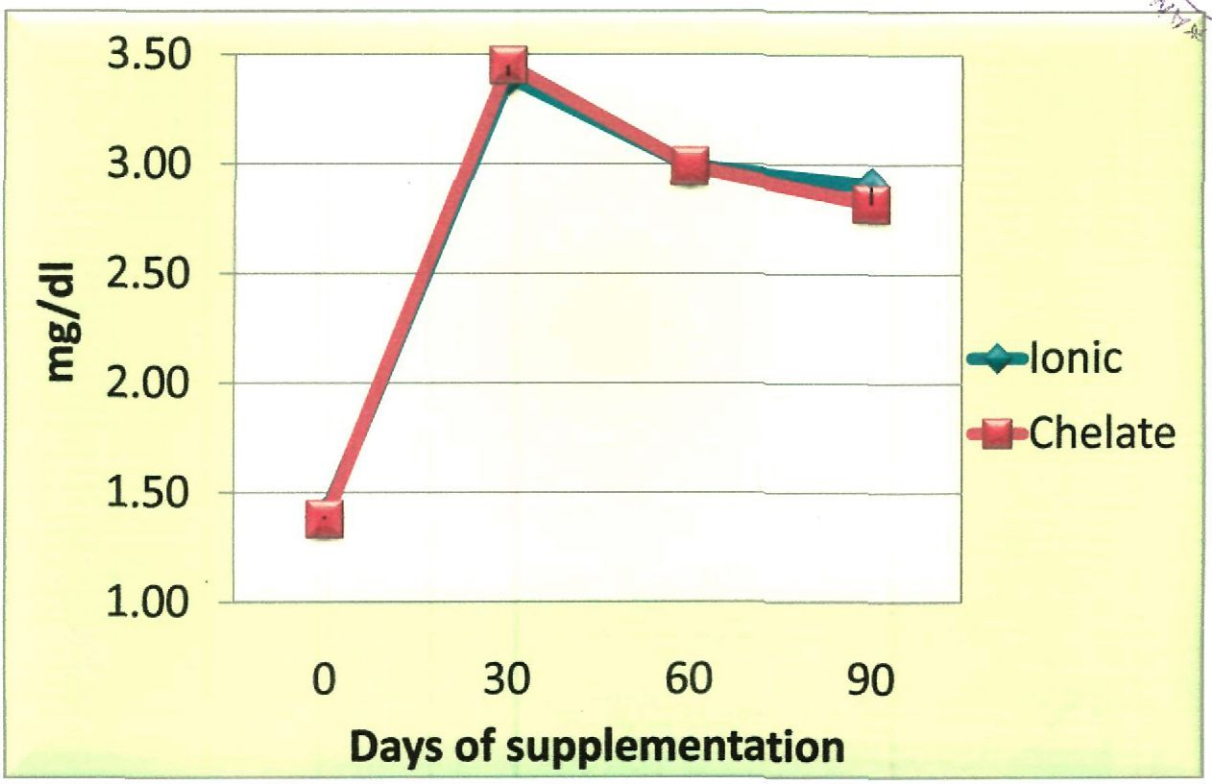


Figure 4.21: Serum Mg level (mg/dl) in anoestrus buffaloes

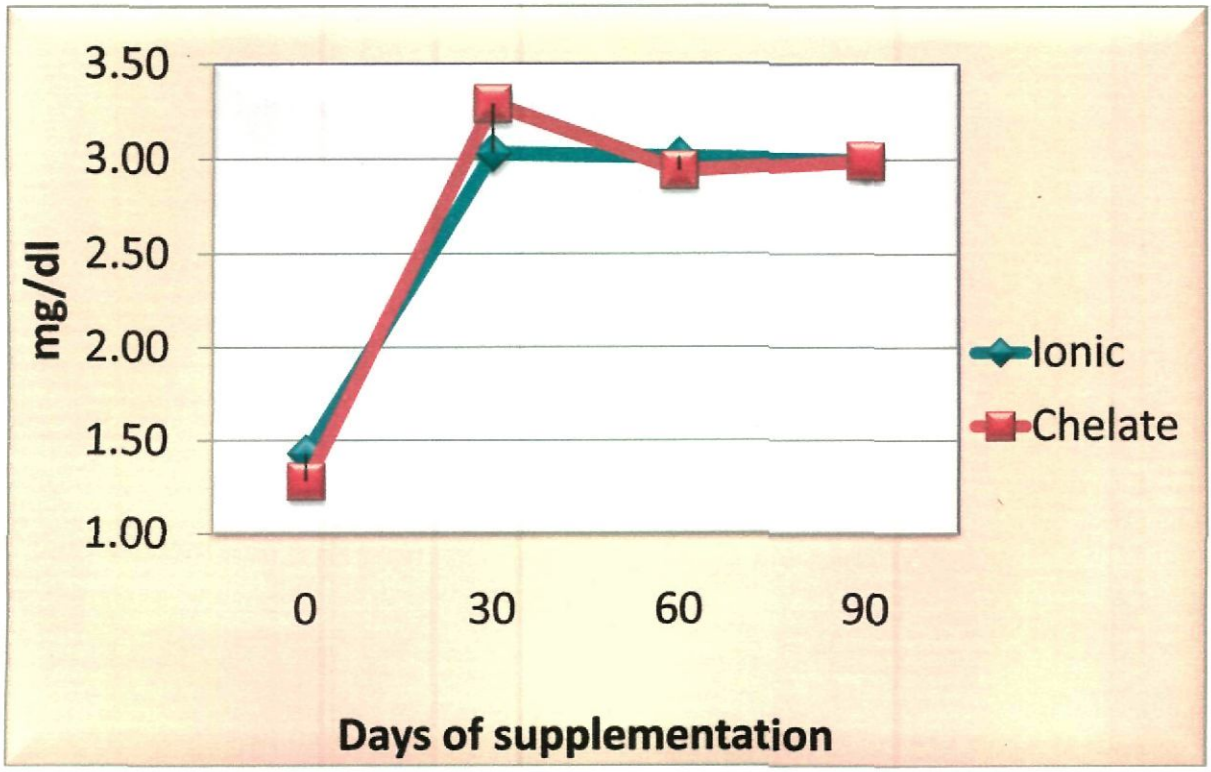


Figure 4.22: Serum Mg level (mg/dl) in repeat breeder buffaloes

level (mg/dl) at 0, 30th, 60th and 90th day was 1.43±0.08, 3.03±0.18, 3.01±0.11 and 2.99±0.25 in ionic group and 1.28±0.07, 3.29±0.19, 2.94±0.12 and 3.01±0.25 in chelated group. In both groups the level of Magnesium increased by 30th day and slightly declined thereafter. Similar were the findings of Behera *et al.* (2012) who reported Minfa (mineral mixture) supplementation for 60 days to significantly ($P<0.05$) increase the serum levels of Mg in repeat breeder crossbred heifers.

4.5.1.4.: Serum Sodium Level:

The average serum Sodium level (mmol/l) of anoestrus and repeat breeder buffaloes in ionic and chelated groups are given in Table 4.23.

The average level in ionic and chelated group of anoestrus buffaloes was 142.13±2.83 and 143.85±1.11 (mg/dl), respectively. The treatment groups did not differ from each other. The period effect was also found statistically similar. The average serum Sodium level (mmol/l) during 0, 30th, 60th and 90th day in anoestrus buffaloes was 155.20±3.52, 129.90±6.10, 142.10±4.61 and 141.30±2.83 in ionic group and 142.20±4.01, 141.50±8.84, 149.90±5.64 and 141.80±3.02 in chelated group. Similar were the findings of Behera *et al.* (2012) who reported that Minfa (mineral mixture) supplementation for 60 days significantly increased ($p<0.05$) the serum levels of Na in anoestrus crossbred heifers.

The average level in ionic and chelated group of repeat breeder buffaloes was 146.63±1.08 and 143.40±2.36 (mg/dl), respectively. The treatment groups did not differ from each other. Also the period effect was statistically similar. In repeat breeder buffaloes the average serum Sodium level (mmol/l) at 0, 30th, 60th and 90th day was 148.00±4.76, 140.90±6.13, 147.70±4.00 and 149.90±5.64 in ionic group and 144.70±3.45, 130.90±1.92, 148.10±4.83 and 149.90±5.64 in chelated group. Similar were the findings of Behera *et al.* (2012) who reported that Minfa (mineral mixture) supplementation for 60 days significantly increased ($p<0.05$) the serum levels of Na in repeat breeder crossbred heifers.

Table 4.23: Serum sodium level (mmol/L) of anoestrus and repeat breeder buffaloes

Days of supplementation	Ionic	Chelated	Mean
	Anoestrus buffaloes		
0	155.20	142.20	148.70
30	129.90	141.50	135.70
60	142.10	149.90	146.00
90	141.30	141.80	141.55
Mean	**142.13**	**143.85**	
±S. E.	**2.83**	**1.11**	
	Repeat breeder buffaloes		
0	148.00	144.70	146.35
30	140.90	130.90	135.90
60	147.70	148.10	147.90
90	149.90	149.90	149.90
Mean	**146.63**	**143.40**	
±S. E.	**1.08**	**2.36**	

Analysis of variance for anoestrus buffaloes (mean sum of squares)

Source	d.f.	m.s.s.	Test	SEM	P value
P	1	79.83	NS	3.22	0.764
T	3	125.0	NS	2.27	0.440
PT	1	46.30	NS	4.55	0.880
Error	72	207.34			

Analysis of variance for repeat breeder buffaloes (mean sum of squares)

Source	d.f.	m.s.s.	Test	SEM	P value
P	1	253.81	NS	3.77	0.45
T	3	19.012	NS	2.66	0.79
PT	1	26.21	NS	5.33	0.96
Error	72	284.88			

4.5.1.5.: Serum Potassium Level:

The average serum potassium level (mmol/l) of anoestrus and repeat breeder buffaloes in ionic and chelated groups are given in Table 4.24 and has been depicted in figures 4.23 and 4.24.

The average level in ionic and chelated group of anoestrus buffaloes was 8.69±0.37 and 8.48±0.38 (mmol/l), respectively. The treatment groups did not differ from each other. The period effect was found significant ($P<0.01$). The serum potassium level (mmol/l) during 0, 30th, 60th and 90th day was 7.06±0.33, 8.29±0.88, 9.21±0.58 and 10.20±0.42 in ionic group and 6.77±0.40, 8.32±0.78, 8.74±0.50 and 10.10±0.38 in chelated group. In both groups the level of serum K progressively increased up to 90th day. This result is corroborated with the findings of Behera *et al.* (2012) who reported Minfa (mineral mixture) supplementation to significantly ($P<0.05$) increase the serum levels of K in anoestrus heifers.

In repeat breeder buffaloes, the average level in ionic and chelated group was 9.00±0.48 and 9.43±0.54 (mmol/l), respectively. The treatment groups did not differ from each other. In both the groups the period effect was also found to be statistically similar. The serum potassium level (mmol/l) at 0, 30th, 60th and 90th day was 7.50±0.37, 8.30±0.78, 8.70±0.59 and 11.50±0.64 in ionic group and 7.40±0.33, 8.40±0.78, 10.00±0.89 and 11.90±0.49 in chelated group.

4.5.2.: Micro mineral:

4.5.2.1. Serum Zinc level:

The average serum zinc level (ppm) of anoestrus and repeat breeder buffaloes in ionic and chelated groups are given in Table 4.25 and has been depicted in figures 4.25 and 4.26.

Table 4.24: Serum potassium level (mmol/L) of anoestrus and repeat breeder buffaloes

Days of supplementation	Ionic	Chelated	Mean
	Anoestrus buffaloes		
0	7.06	6.77	6.92[a]
30	8.29	8.32	8.31[a]
60	9.21	8.74	8.98[a]
90	10.2	10.1	10.15[b]
Mean	**8.69**	**8.48**	
±S. E.	**0.37**	**0.38**	
	Repeat breeder buffaloes		
0	7.50	7.40	7.45
30	8.30	8.40	8.35
60	8.70	10.00	9.35
90	11.50	11.90	11.70
Mean	**9.00**	**9.43**	
±S. E.	**0.48**	**0.54**	

Means bearing different superscripts in column differ significantly (P<0.01)

Analysis of variance for anoestrus buffaloes (mean sum of squares)

Source	d.f.	m.s.s.	Test	SEM	P value
P	1	250.27	**	0.40	<0.01
T	3	0.045	NS	0.28	0.90
PT	1	0.71	NS	0.56	0.88
Error	72	3.22			

Analysis of variance for repeat breeder buffaloes (mean sum of squares)

Source	d.f.	m.s.s.	Test	SEM	P value
P	1	56.19	NS	0.383	0.06
T	3	5.356	NS	0.271	0.18
PT	1	0.69	NS	0.541	0.87
Error	72	2.93			

Table 4.25: Serum zinc level (ppm) of anoestrus and repeat breeder buffaloes

Days of supplementation	Ionic	Chelated	Mean
	Anoestrus buffaloes		
0	0.74	0.86	0.80^{a}
30	2.24	2.43	2.34^{b}
60	2.38	2.73	2.56^{b}
90	2.60	2.97	2.79^{b}
Mean	**1.99**	**2.25**	
±S. E.	**0.27**	**0.30**	
	Repeat breeder buffaloes		
0	0.82	0.96	0.89^{a}
30	1.98	2.31	2.15^{b}
60	2.42	2.69	2.56^{b}
90	2.61	2.80	2.71^{b}
Mean	**1.95**	**2.19**	
±S. E.	**0.25**	**0.27**	

Means bearing different superscripts in column differ significantly ($P<0.01$)

Analysis of variance for anoestrus buffaloes (mean sum of squares)

Source	d.f.	m.s.s.	Test	SEM	P value
P	1	16.104	**	0.188	<0.01
T	3	1.309	NS	0.133	0.177
PT	1	0.0741	NS	0.266	0.957
Error	72				

Analysis of variance for repeat breeder buffaloes (mean sum of squares)

Source	d.f.	m.s.s.	Test	SEM	P value
P	1	14.311	**	0.160	<0.01
T	3	1.551	NS	0.113	0.085
PT	1	0.0499	NS	0.226	0.961
Error	72				

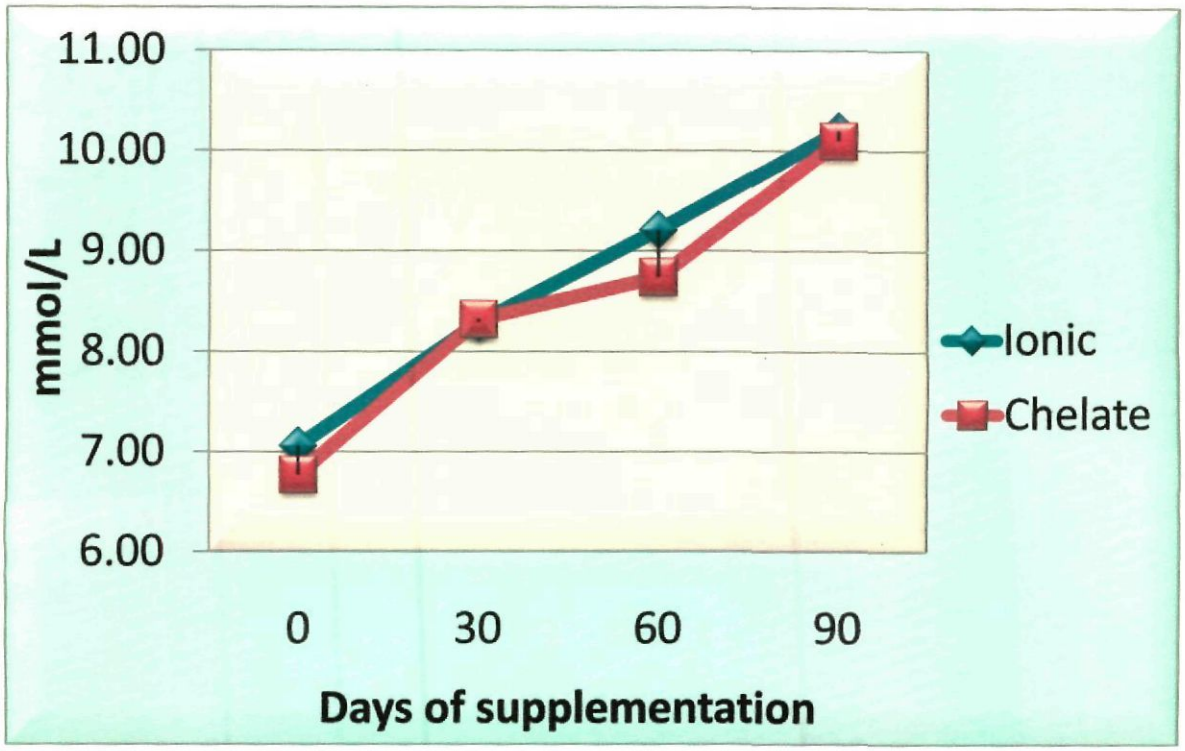


Figure 4.23: Serum K level (mmol/L) in anestrous buffaloes

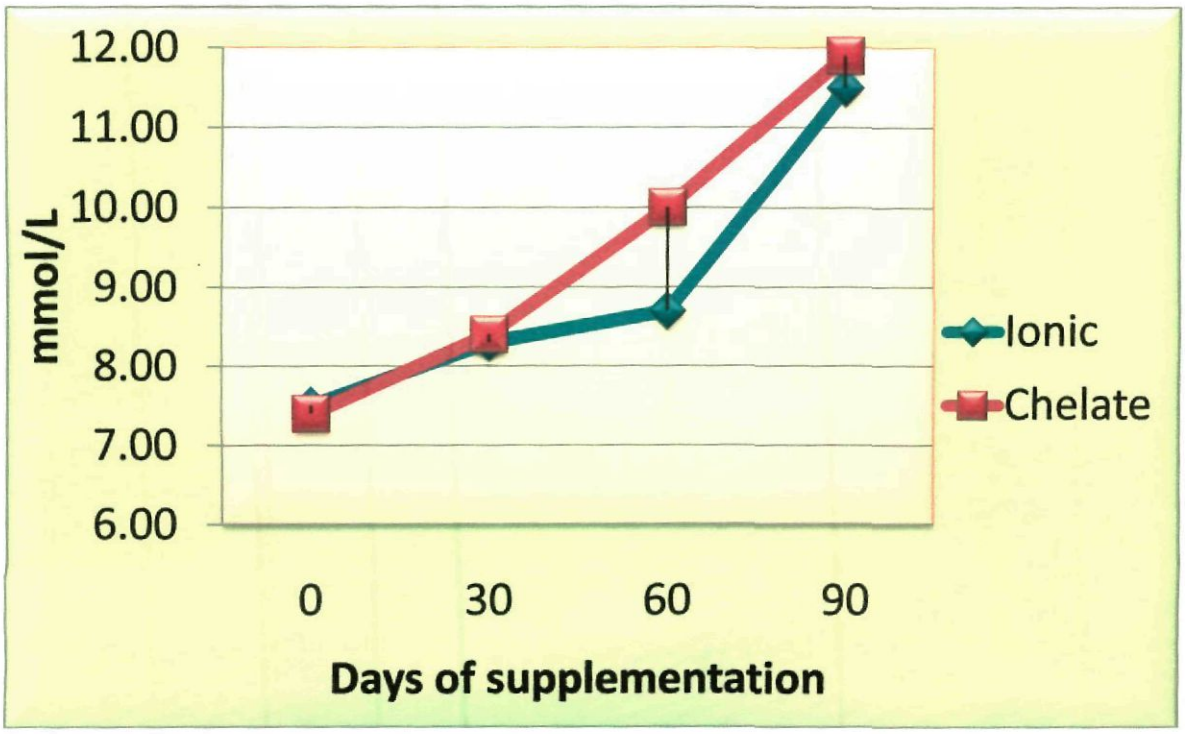


Figure 4.24: Serum K level (mmol/L) in repeat breeder buffaloes

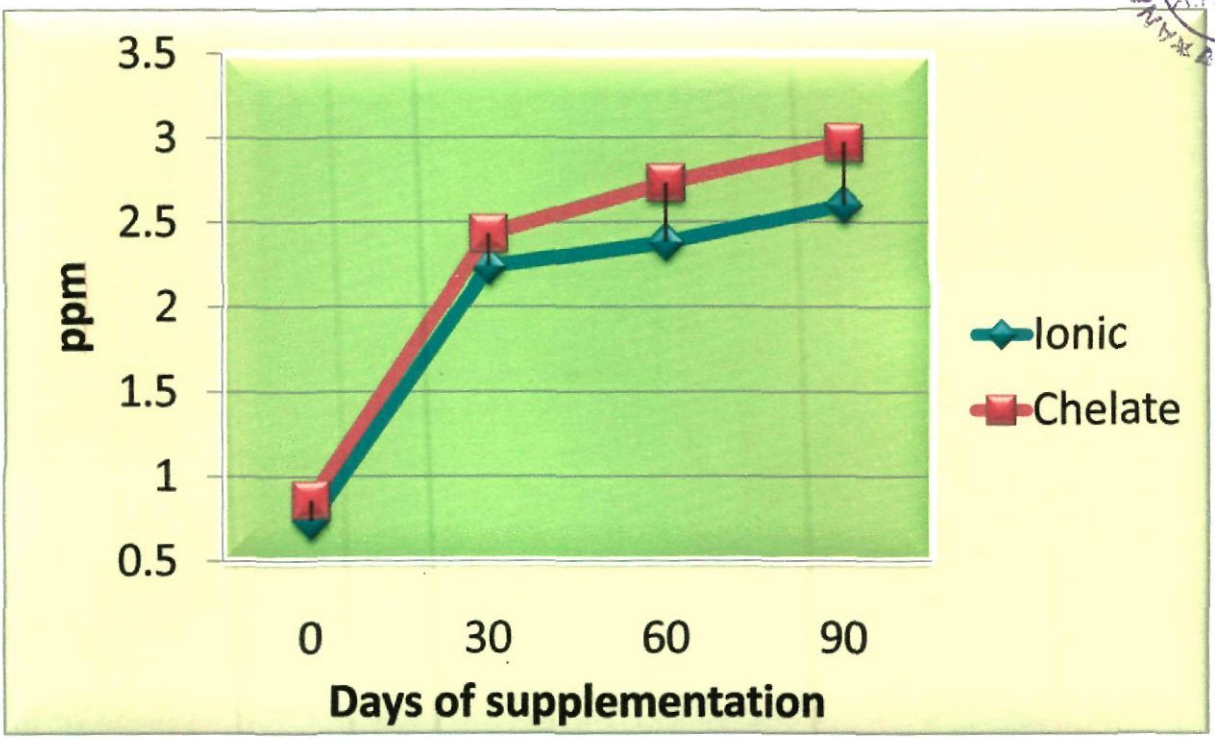


Figure 4.25: Serum Zn level (ppm) in anestrous buffaloes

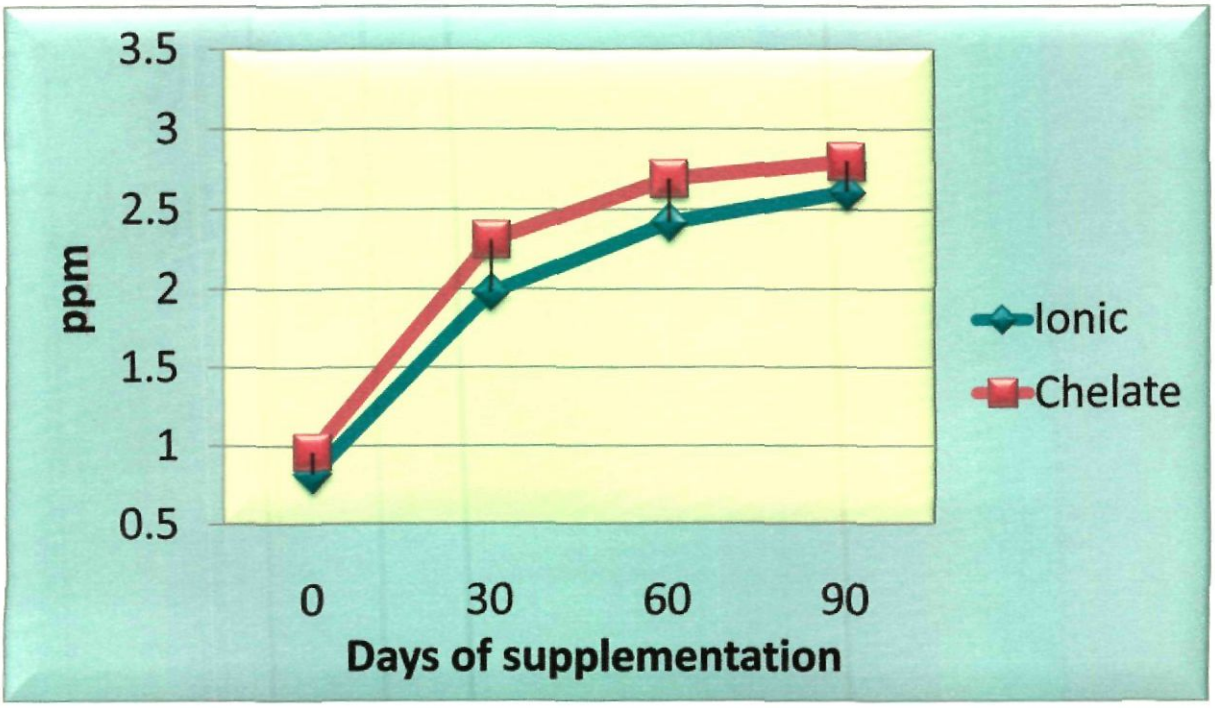


Figure 4.26: Serum Zn level (ppm) in repeat breeder buffaloes

The average level in ionic and chelated group of anoestrus buffaloes was 1.99±0.27 and 2.25±0.30 (ppm), respectively. The treatment groups did not differ from each other. The period effect was found significant (P<0.01). The average serum zinc level (ppm) during 0, 30th, 60th and 90th day was 0.74±0.10, 2.24±0.31, 2.38±0.53 and 2.60±0.17 in ionic group and 0.86±0.07, 2.43±0.22, 2.73±0.29 and 2.97±0.12 in chelated group. In both groups the level of serum Zn progressively increased up to 90th day. Due to presence of Zn in chelate form in the mineral mixture, the overall mean value was higher than in ionic mineral mixture group. The supplementation of mineral mixture elevated the level of Zn in serum as observed by Lall *et al.* (2004) in anoestrus buffalo heifers, Garg *et al.* (2008) in anoestrus animals, Devasena *et al.* (2010) in anoestrus cows and heifers, Behra *et al.* (2012) in anoestrus heifers, Kumar *et al.* (2012) in anoestrus lactating buffaloes and Mohapatra *et al.* (2012) in anoestrus cows.

In repeat breeder buffaloes, the average level in ionic and chelated group was 1.95±0.25 and 2.19±0.27 (ppm), respectively. The treatment groups did not differ from each other. The period effect was found significant (P<0.01). In the average serum zinc level (ppm) at 0, 30th, 60th and 90th day was 0.82±0.13, 1.98±0.19, 2.42±0.33 and 2.61±0.17 in ionic group and 0.96±0.12, 2.31±0.29, 2.69±0.32, 2.80±0.34 in chelated group. In both groups the level of serum Zn progressively increased up to 90th day. These findings are corroborated with that of Devasena *et al.* (2010) in repeat breeding cows, Behra *et al.* (2012) in repeat breeding heifers, Mohapatra *et al.* (2012) in repeat breeding cows.

4.7: Reproduction status of anoestrus and repeat breeder buffaloes:

The list and days of appropriate mineral mixture supplementation required and % of conception rates of successful anoestrus and repeat breeder buffaloes are given in Tables 4.26 and 4.27.

Table 4.26: List and days of supplementation required by successful anoestrus and repeat breeder buffaloes.

Anoestrus buffaloes					
Ionic mineral mixture			Chelated mineral mixture		
Animal No.	**Animal Code**	**No. of days of mineral supplementation**	**Animal No.**	**Animal Code**	**No. of days of mineral supplementation**
1.	1.10.1	74	1.	1.3.1	44
2.	2.5.1	41	2.	1.11.1	70
3.	3.3.2	63	3.	2.2.1	32
4.	4.1.1	50	4.	3.1.1	67
5.	4.6.1	87	5.	3.4.1	36
			6.	4.3.1	77
Mean		**63.00**	**Mean**		**54.33**
±SE		**5.81**	**±SE**		**6.10**
Repeat breeder buffaloes					
Ionic mineral mixture			Chelated mineral mixture		
Animal No.	**Animal Code**	**No. of days of mineral supplementation**	**Animal No.**	**Animal Code**	**No. of days of mineral supplementation**
1.	1.1.1	43	1.	1.3.1	72
2.	1.8.2	75	2.	1.7.2	32
3.	3.2.1	70	3.	1.9.1	22
4.	3.2.2	89	4.	2.2.1	39
5.	3.3.1	67	5.	3.1.1	85
			6.	3.1.2	46
			7.	3.6.2	65
Mean		**68.80**	**Mean**		**51.57**
±SE		**5.28**	**±SE**		**7.26**

Table 4.27: Effect of appropriate mineral mixture supplementation on % of conception rates in anoestrus and repeat breeding buffaloes.

Type of mineral mixture	Ionic	Chelated
	Anoestrus buffaloes	
No. of buffaloes under study	**10**	**10**
No. of anoestrus buffaloes coming into oestrus within 90 days of treatment	**5**	**6**
% success of treatment	**50**	**60**
	Repeat breeder buffaloes	
No. of buffaloes under study	**10**	**10**
No. of repeat breeder buffaloes coming into oestrus within 90 days of treatment	**5**	**7**
% success of treatment	**50**	**70**

The numbers of days of mineral mixture supplementation required for correcting the condition of anoestrus was 63.00±5.81 and 54.33±6.10 in ionic and chelated group, respectively. Thus, chelated group required 15.96% less number of days compared to ionic group. The repeat breeder buffaloes required 68.80±5.28 in ionic group and 51.57±7.26 days in chelated group, respectively. Accordingly, chelated group required 33.41% less number of days compared to ionic group.

In case of anoestrus buffaloes 50% in ionic group and 60% in chelated group successfully conceived within the supplementation period of 90 days. Whereas, in repeat breeder buffaloes 60% in ionic group and 70% in chelated group successfully conceived on account of mineral mixture supplementation period of 90 days.

The success rate of conception of the anestrous buffaloes on account of supplementation of ionic mineral mixture was variable in different studies. Accordingly, 70 % (Lall *et al.*, 2000), 90% (Lall *et al.,* 2004), 60% (Kumar *et al.,* 2011) and 45.65% (Kumar *et al.*, 2012) of anoestrus buffaloes were reported to successfully conceive. The anoestrus cattle responded better than buffaloes on account of supplementation of ionic mineral mixture as evident from the figures of 75% (Pradhan *et al.*, 1995) and 66% (Das *et al.*, 2005) in cows, 93.33 % in crossbred heifers (Srivastava *et al.*, 2008) and 85.71% cows (Selvaraju *et al.*, 2009).

Prasad *et al.* (1991) reported 73.33% of anoestrus buffaloes to successfully conceive when Cu, Mn and Zn were supplemented in ionic form. The respective success rate was 86.66% and 63.15% when the same minerals were supplied in chelate form @ of 100 and 50% of requirement.

The success rate of conception of the repeat breeder buffaloes on account of supplementation of ionic mineral mixture was reported as 38.63% by Kumar *et al.* (2012) in different studies. Similar studies with crossbred cows the success rate was

variable. Accordingly, 78.6% (Selvaraju *et al.*, 2009), 46.68% (Behera *et al.*, 2012) and 35 % (Mohapatra *et al.*, 2012) repeat breeding animals successfully conceived.

Prasad *et al.* (1991) reported 66.66% of repeat breeder buffaloes to successfully conceive when Cu, Mn and Zn were supplemented in ionic form. The respective success rate was 73.33% and 62.50% when the same minerals were supplied in chelate form @ of 100 and 50% of requirement.

4.8: Cost: benefit ratio:

The cost of rearing successful anoestrus and repeat breeder buffaloes in the present investigation is presented in Tables 4.28 and 4.29.

The average total cost (Rs.) including that of feed stuffs and mineral supplement for rearing anoestrus buffaloes worked out as 6637.91±579.34 in ionic group and 5303.40±692.97 in chelated group. The ionic group recorded 25.16% higher cost of rearing than the chelated group. In case of repeat breeder buffaloes the cost worked out as Rs. 6930.51±704.22 in ionic group and 5181.93±740.52 in chelated group, respectively. The ionic group recorded 33.74% higher cost of rearing than the chelated group. These findings could be attributed to less numbers of days of mineral mixture supplementation required for correcting the condition of anoestrus and repeat breeding in chelate group.

Table 4.28: Cost of rearing successful anoestrus buffaloes

Animal No.	Animal Code	No. of days of mineral supplementation	Cost involved (Rs.)		
			Feed	Mineral supplement	Total
Ionic Mineral Mixture Group					
1.	1.10.1	74	8110.40	133.94	8244.34
2.	2.5.1	41	4079.50	74.21	4153.71
3.	3.3.2	63	6489.00	114.03	6603.03
4.	4.1.1	50	5545.00	90.50	5635.5
5.	4.6.1	87	8395.50	157.47	8552.97
Mean		**63.00**	**6523.88**	**114.03**	**6637.91**
±SE		**5.81**	**569.09**	**10.52**	**579.34**
Chelated Mineral Mixture Group					
1.	1.3.1	44	4268.00	92.40	4360.40
2.	1.11.1	70	7105.00	147.00	7252.00
3.	2.2.1	32	2912.00	67.20	2979.20
4.	3.1.1	67	7035.00	140.70	7175.70
5.	3.4.1	36	2685.60	75.60	2761.20
6.	4.3.1	77	7130.20	161.70	7291.90
Mean		**54.33**	**5189.30**	**114.10**	**5303.40**
±SE		**6.10**	**680.40**	**12.81**	**692.97**

Table 4.29: Cost of rearing successful repeat breeder buffaloes

Animal No.	Animal Code	No. of days of mineral supplementation	Cost involved (Rs.)		
			Feed	Mineral supplement	Total
Ionic Mineral Mixture Group					
1.	1.1.1	43	4386.00	77.83	4463.83
2.	1.8.2	75	6540.00	135.75	6675.75
3.	3.2.1	70	6174.00	126.70	6300.70
4.	3.2.2	89	10404.10	161.09	10565.19
5.	3.3.1	67	6525.80	121.27	6647.07
Mean		**68.80**	**6805.98**	**124.53**	**6930.51**
±SE		**5.28**	**695.55**	**9.56**	**704.22**
Chelated Mineral Mixture Group					
1.	1.3.1	72	6624.00	151.20	6775.20
2.	1.7.2	32	3200.00	67.20	3267.20
3.	1.9.1	22	2288.00	46.20	2334.20
4.	2.2.1	39	3669.90	81.90	3751.80
5.	3.1.1	85	8542.50	178.50	8721.00
6.	3.1.2	46	4301.00	96.60	4397.60
7.	3.6.2	65	6890.00	136.50	7026.50
Mean		**51.57**	**5073.63**	**108.30**	**5181.93**
±SE		**7.26**	**725.41**	**15.24**	**740.52**

SUMMARY AND CONCLUSIONS

V. SUMMARY AND CONCLUSIONS

Reproductive efficiency in dairy animals plays a pivotal role in profitable dairy farming. Improving reproductive efficiency of dairy animals can be difficult as it is influenced by many factors including plane of nutrition, rate of weight gain, level of stress and level of milk production. Apart from energy and protein, minerals are the main limiting factor for livestock production. Minerals are the essential nutrients bearing a significant role in the animal reproduction, because their excess or deficiency produces detrimental effect on the performance of livestock. Among the infertility problems occurring in dairy animals, anoestrous and repeat breeding are the most common.

Mineral deficiencies or imbalances in animals are an area specific problem and are caused to a greater extent, by available feed resources and feeding practices followed by the farmers. The deficiency of certain minerals like calcium, phosphorus, copper and zinc in dairy cattle has been reported under field conditions. On mineral supplementation, improvement in reproductive status was reported by many workers. The present study was undertaken to find the effect of supplementation of appropriate mineral mixture for Panchmahal district formulated by Animal Nutrition Research Department, AAU, Anand on correcting the condition of anoestrus and repeat breeding in buffaloes. Two mineral mixtures were formulated: one in ionic/inorganic form and the other as chelated appropriate mineral mixture comprising of deficient micro-mineral Zinc that was added extra @ 25 % in chelate form to ionic mineral mixture. The present study was planned with following objectives:

- Survey of feeding practices followed by dairy farmers to work out the nutritional status of anestrous and repeat breeder buffaloes in selected tribal villages of Panchmahal district.
- To study the blood-biochemical and hormonal status of anestrous and repeat breeder buffaloes in selected tribal villages of Panchmahal district.
- To study the influence of dietary incorporation of appropriate mineral mixture for correcting the condition of anoestrus and repeat breeding in buffaloes of selected tribal villages of Panchmahal district.
- To study the cost: benefit ratio of mineral supplementation.

Forty buffaloes of post partum anoestrus and repeat breeder were selected for on-farm trial of 90 days duration at tribal villages viz. Jinjari and Kharod in Panchmahal district. The buffaloes were selected on the basis of history as well as gynecological examination, the postpartum anoestrous (not showing symptoms for more than 100 days after calving) and repeat breeding (not conceiving following three inseminations) animals were randomly allotted to two dietary treatments i.e. T_1 (Ionic mineral mixture) and T_2 (Chelated mineral mixture), following Completely Randomized Design. The buffaloes in control group (T_1) were supplemented ionic mineral mixture with feeding schedule followed by the farmers (concentrate + green roughage + dry roughage) and those in T_2 were supplemented with chelated mineral mixture group + farm feeding schedule as in T_1.

The average DMI (kg/head), DCPI (g/head) and TDNI (kg/head) in T_1 and T_2 groups were 11.46 ± 0.14 and 11.53 ± 0.10; 592.80 ± 17.88 and 567.96 ± 11.22 and 6.40 ± 0.06 and 6.43 ± 0.12, respectively in anoestrus buffaloes. Similarly, in repeat breeder buffaloes, the respective intakes in T_1 and T_2 groups were 11.41 ± 0.10 and 11.47 ± 0.09; 583.82 ± 16.76 and 582.41 ± 5.67 and 6.64 ± 0.08 and 6.60 ± 0.03. The DMI, DCPI and TDNI in anoestrus and repeat breeder buffaloes were found to be statistically similar and in conformity to ICAR, (1988) standards.

The average intakes of Ca, P and Cu were more than adequate in anoestrus and repeat breeder buffaloes. However, the Zn intake as the per cent of requirement was 66.27±2.37 and 65.58±2.13 in anoestrus buffaloes of ionic and chelated group, and the respective figures in repeat breeder buffaloes was 64.02±1.74 and 66.28±2.37%. The experimental buffaloes were supplemented with appropriate mineral mixture either in ionic or chelated form.

The average blood glucose level in ionic and chelated group of anoestrus buffaloes was 59.50±0.59 and 61.35±0.77 and the respective level in repeat breeder buffaloes was 59.68±0.57 and 61.40±0.29 mg/dl. The treatment groups did not differ from each other in both anoestrus and repeat breeder buffaloes. The period effect was also not significant.

The ionic and chelated groups did not differ significantly in anoestrus buffaloes for levels (g/dl) of serum total protein (7.14±0.22 and 6.98±0.22), albumin (3.67±0.10 and 3.53±0.14) and globulin (3.48±0.15 and 3.45±0.09). The respective figures for repeat breeder buffaloes were 7.02±0.21 and 7.08±0.20, 3.56±0.12 and 3.58±0.11 and 3.46±0.09 and 3.51±0.09 (g/dl). However, the period effect was found significant ($P<0.01$) in all the variables in anoestrus and repeat breeder buffaloes except for level of globulin in repeat breeder buffaloes.

The ionic and chelated groups did not differ significantly in anoestrus buffaloes for levels (g/dl) of serum triglycerides (30.40±0.76 and 31.65±0.84), cholesterol (127.98±6.45 and 128.88±7.41), BUN (26.50±1.58 and 24.55±1.57) and Serum creatinine (1.94±0.07 and 1.54±0.03). The respective figures for repeat breeder buffaloes were 32.63±2.54 and 33.00±0.97, 122.00±7.20 and 116.48±5.46, 27.20±1.34 and 25.88±1.42 and 1.93±0.09 and 1.50±0.01 (mg/dl). However, the period effect was found significant ($P<0.01$) in all the variables in anoestrus and repeat breeder buffaloes except for level of serum triglycerides in anoestrus buffaloes.

In anoestrus buffaloes the ionic and chelated groups did not differ significantly for activity (U/L) of serum AKP (233.13±8.03 and 209.58±9.11), ALT (52.30±1.36 and 54.40±2.26) and AST (172.58±6.07 and 175.13±5.54). The respective figures for repeat breeder buffaloes were 218.83±5.09 and 208.10±9.91, 54.13±1.71 and 56.70±2.17 and 158.10±1.73 and 162.45±2.09 (U/L). However, the period effect was found significant ($P<0.01$) with regard to activities of AKP and ALT both in anoestrus and repeat breeder buffaloes.

The ionic and chelated groups did not differ significantly in anoestrus buffaloes with regard to levels (mg/dl) of serum calcium (8.38±0.61 and 8.64±0.63), Serum phosphorus (5.70±0.30 and 5.93±0.46) and serum magnesium (2.67±0.24 and 2.66±0.24). The respective figures for repeat breeder buffaloes were 8.67±0.58 and 8.76±0.58, 5.76±0.33 and 5.84±0.30 and 2.62±0.22 and 2.63±0.25 (mg/dl). However, the period effect was found significant ($P<0.01$). In both the groups of buffaloes, level of serum Calcium and phosphorus increased by 30th day of supplementation and stabilized thereafter. However, serum magnesium increased by 30th day and slightly declined thereafter.

The levels (mmol/L) in anoestrus buffaloes did not differ significantly for serum sodium (142.13±2.83 and 143.85±1.11) and Serum potassium (8.69±0.37 and 8.48±0.38) in both the groups. The respective figures for repeat breeder buffaloes were 146.63±1.08 and 143.40±2.36 and 9.00±0.48 and 9.43±0.54 (mmol/L). The period effect was found significant ($P<0.01$) with regard to serum potassium level in anoestrus buffaloes.

The ionic and chelated groups did not differ significantly ($P<0.01$) in anoestrus buffaloes with regard to serum zinc level (ppm) which was 1.99±0.27 and 2.25±0.30, respectively. Similar was the trend in repeat breeder buffaloes and the respective values were 1.95±0.25 and 2.19±0.27. The period effect was found significant ($P<0.01$) and in both groups the level of serum Zn progressively increased up to 90th day.

The average number of days required in ionic and chelated group, for correcting the condition of anoestrus was 63.00±5.81 and 54.33±6.10 and repeat breeding 68.80±5.28 and 51.57±7.26, respectively. Among the animals under study, 50% each of the anoestrus and repeat breeder buffaloes got successfully conceived due to ionic mineral supplementation. Whereas, due to chelate mineral supplementation respectively, 60 and 70% of anoestrus and repeat breeder buffaloes got successfully conceived.

The average total cost (Rs.) including that of feed stuffs and mineral supplement for rearing anoestrus buffaloes worked out as 6637.91±579.34 in ionic group and 5303.40±692.97 in chelated group. The ionic group recorded 25.16% higher cost of rearing than the chelated group. In case of repeat breeder buffaloes the cost worked out as Rs. 6930.51±704.22 in ionic group and 5181.93±740.52 in chelated group, respectively. The ionic group recorded 33.74% higher cost of rearing than the chelated group.

Conclusions:

1) The daily intake of DM, protein and energy was adequate both in anestrous and repeat breeding buffaloes selected for the on-farm trial.
2) The intakes of Ca, P, Cu and Mn were more than adequate in both the anestrous and repeat breeding buffaloes selected for the on-farm trial. However, the intake of Zn worked out as 65.58-66.27% in anestrous and 64.02-66.28% in repeat breeders buffaloes of their actual requirement.
3) The blood glucose level of both the anestrous and repeat breeder buffaloes was not influenced by supplementation of mineral mixture. The level of serum total protein, albumin and globulin was not different in anestrous and repeat breeder buffaloes supplemented either with ionic or chelated mineral mixture but the level significantly ($P<0.01$) increased after 30 days of mineral mixture supplementation in both anestrous and repeat breeder buffaloes.

4) The ionic and chelated groups did not differ significantly in both anoestrus and repeat breeder buffaloes for serum levels of triglycerides, cholesterol, BUN and creatinine. However, the period effect was found significant ($P<0.01$) in all the variables except for level of serum triglycerides in anoestrus buffaloes. The activities of enzymes AST, ALT and AKP followed an increasing trend as the days of supplementation increased. The serum levels of Ca, P, Mg, Na, K and Zn significantly increased on account of mineral supplementation. However, the treatment groups did not differ from each other.

5) The conception rates in anoestrus buffaloes that were supplemented daily with 30 g ionic and chelated (comprising of Zn in chelate form added extra @ 25% to ionic) mineral mixture was 50 and 60%, respectively and the respective figure was 50 and 70% in case of repeat breeder buffaloes. The number of days of mineral supplementation required in successful anoestrus buffaloes in ionic and chelated group was 63.00±5.81, 54.33±6.10 and the same for repeat breeder buffaloes was 68.80±5.28, 51.57±7.26, respectively. Therefore, chelated mineral mixture resulted in 25 and 34% lesser cost of rearing in anestrous and repeat breeder buffaloes, respectively.

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