PHOSPHORUS AND TRACE ELEMENT STATUS OF ANOESTRUS AND REPEAT BREEDER CROSSBRED COWS

By
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THESIS

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DECLARATION

I hereby declare that the thesis entitled **"Phosphorus and Trace element status of Anoestrus and Repeat breeder crossbred cows"** is a bonafide record of research work done by me during the course of research and that the thesis has not previously formed the basis for the award to me of any degree, diploma, associateship, fellowship or other similar title, of any other University or Society.

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CERTIFICATE

Certified that this thesis, entitled "**Phosphorus and Trace element status of Anoestrus and Repeat breeder crossbred cows**" is a record of research work done independently by **Sri. Joy George**, under my guidance and supervision and that it has not previously formed the basis for the award of any degree, diploma, fellowship or associateship to him.

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Introduction

INTRODUCTION

The state of Kerala as per 1987 census has a total of 34.24 lakhs cattle of which 17.01 lakhs are breedable. Out of these 8.89 lakhs are crossbreds. The cattle in Kerala are mainly reared by the poorer section of the society to augment their meagre income. This probably accounts for the poor feeding standard.

The production capacity of cattle depends to a great extent on reproductive efficiency as measured by its ability to conceive and deliver one viable full term calf each year throughout her reproductive life span. Disturbances in reproduction, of which anoestrum and repeat breeding form a sizable proportion, can markedly reduce reproductive efficiency.

Normal functioning of the reproductive organs may be limited by nutritional deficiencies during critical periods of growth, puberty, gestation and lactation. Peak lactation is all the more critical in this context on account of greater production requirement, overlapping of peak lactation and optimal period for rebreeding and higher requirement for growth in primiparous animals. Even borderline nutrient deficiencies may be manifested as impaired fertility before other clinical symptoms are apparent.

Phosphorus being part of energy rich phosphate bonds and trace elements being part of metalloenzymes are absolutely essential for the metabolic activity of the living tissues, including those of endocrine glands which regulate reproductive function and fertility of animals. Therefore, there should be sufficient levels of phosphorus and trace elements in the diet of animals.

Feeding of mineral mixture not being a regular practice the main source of phosphorus and trace elements in the feed is the concentrate and the greens.

Both qualitative and quantitative deficiencies in the feed and fodder would therefore lead to deficiency of phosphorus and trace elements. The large scale practice of manuring of soil with chemical fertilizers, heavy rainfall and resultant leaching of soil and the changing water management practices in the state upsets the ratio of inter-related microminerals in the soil. This would interfere with the assimilation of trace elements by the fodder plants eventhough there is no deficiency in the soil. There is an added possibility of leaching of the soil especially in the hilly terrains during heavy monsoons. All these factors can lead to a reduced trace element availability from the green fodders resulting in a possible trace element deficiency in cattle.

Eventhough there are many reports on the role of phosphorus and trace elements in the fertility of cattle from all over the world, there are only a few reports from within the country. Barring a few isolated reports on phosphorus and copper status of crossbred cows in the state no scientific and systematic study has so far been undertaken in this important area. Therefore a study on the phosphorus, copper, zinc, manganese and cobalt levels in the serum of crossbred cows of normal and impaired fertility is proposed to be carried out.

Review of Literature

REVIEW OF LITERATURE

Among the various infertility conditions in crossbred cattle anoestrum and repeat breeding occupy important places.

Pillai (1980) recorded an incidence of 28.3 per cent anoestrum in cows of which only 17.4 per cent was true anoestrum. The corresponding figures in heifers were reported to be 28.9 per cent and 22.4 per cent. In contrast Kumar *et al*. (1986) recorded an incidence of 19 per cent anoestrum among indigenous cattle. A much lower incidence of 5.4 per cent anoestrum has been reported by Rehumathulla *et al*. (1986). Based on a clinical survey Chetty and Rao (1987) reported that out of 1463 crossbred cattle examined 341 were anoestrus and 134 were repeat breeders. A field survey on the incidence of anoestrum among the crossbred cattle of Kerala recorded a very high incidence of 30 per cent (Iyer *et al*., 1992). The incidence of repeat breeding among crossbred cattle in District Livestock farm, Mannuthy was reported to be 18.58 per cent (Nambothiripad and Raja, 1972).

There are various reports on the levels of phosphorus and trace elements in the soil, feeds and fodder, body tissues and blood serum and their inter-relationship with body functions.

Swenson et al. (1962) studied the influence of trace minerals such as iron, copper, cobalt, zinc and manganese in increasing the mean red blood cell volume and haemoglobin. Feeding of lucerne hay in required quantities was also found to increase the above values. Eventhough excess dietary calcium with a calcium:phosphorus ratio of 10:1 did neither alter the haemogram nor induce changes in blood serum calcium phosphorus and magnesium, excess calcium added to a non-leguminous basal ration without trace minerals inhibited oestrus cycle and ovulation. In contrast excess calcium added to a leguminous basal ration did not inhibit normal oestrus cycle. Dyer and Rojas (1965) reported that suboptimal intakes of manganese or ingestion of compound antagonistic to its utilization resulted in deformed calves. Their study has further proved that manganese requirement of cows is in the range of 16-20 ppm as against the recommendation of 6-10 ppm by NRC.

Patel et al. (1966) studied the serum levels of phosphorus, calcium and mangnesium in calves and adult animals and the effect of season on the level of these elements. The serum levels of phosphorus, calcium and magnesium (mg%) were recorded to be 8.9, 10.2 and 2.8 respectively in calves aged 2-15 months as against 6.6, 9.6 and 3.41 respectively in adult cows. The calcium and phosphorus levels were found to be highest in summer and lowest in monsoon in all the age groups. Wilson (1966) studied the inter-relationship of calcium, phosphorus and manganese in the causation of functional infertility and the beneficial effect of supplementation of dicalcium phosphate and manganese sulphate. Based on this study he inferred that there was a link between calcium oxide, phosphate and manganese in the aetiology of one type of functional infertility. He further suggested that calcium oxide and phosphate contents in the ration act as conditioning factors in the production of manganese deficiency.

Ford (1972) reported that the extremely variable climate of Great Britain caused marked variation in the quantity and quality of herbage. On highly fertile soils these variations were rarely detrimental, but on soils which had slight mineral deficiencies and imbalance, such variation may be critical. Fluctuatio[illegible] the level of calcium and phosphorus lead to phosphorus deficiency and also the availability of certain trace elements thus affecting the fertility of grazing animals.

Smith (1973) associated outbreaks of post parturient haemaglobinuria with depressed oestrus activity in dairy herds of New Zealand with extremely low liver and blood copper levels. He further drew evidence that application of fertilizers containing molybdenum and/or lime to soil resulted in copper deficiency in the fodder and resultant post parturient haemaglobinuria. The condition was corrected by top dressing of soil with copper sulphate and parenteral administration of copper preparations.

Ulsen (1973) estimated the levels of zinc in the serum of cattle and reported a normal value of 140 µg/ml. He further stated a serum level of 515 µg/ml in case of zinc poisoning. Serum zinc concentration was recorded to be highly variable in dairy cattle with normal values ranging from 85-175 µg/100 ml with a mean of 117 $\pm$ 39 µg/100 ml. These values registered higher variability in hypothermal stress, ketosis and mastitis (Wegner et al., 1973).

Campbell et al. (1974) experimentally produced hypo cuprosis in eight yearling cattle by daily dosing of 30 mg iron per kg body weight as ferric hydroxide for a continuous period of seven months. These animals were grazed in pasture adequate in copper but low in molybdenum. Eventhough there was no effect of this treatment on haemoglobin, PCV, liver zinc, weight gain and general health, there was considerable reduction in liver copper to 7 ppm and blood copper to 0.33 mg/l.

The zinc levels in the blood, plasma, serum and body tissues of slaughtered cattle both in summer and winter were estimated by Decowski (1974). The zinc levels in the blood, plasma and serum (mg/100 ml) and in the liver, spleen, pancreas and kidney (mg/100 g) in summer were respectively 0.24, 0.125, 0.126, 3.42, 2.32, 2.61, and 1.9. The corresponding values in winter were respectively 0.20, 0.125, 0.09, 2.85, 2.02, 2.25 and 1.9.

Schwarz and Kirchgessner (1975) carried out studies on zinc depletion and repletion of lactating dairy cows fed a semi-synthetic diet. They observed that the serum zinc showed extraordinarily high dynamics as was indicated by a very large and rapid response to zinc depletion and repletion. They further stated that the dietary requirement of zinc for the highest zinc concentration in serum was about 110 ppm and serum analysis was well suited to test dietary zinc supply and zinc deficiency in diary cows.

Pandiya et al. (1977) studied the pattern of serum inorganic phosphate levels in growing cattle and reported a fall in concentration from 7.03 mg% at 0-6 months age to 5.5 mg% at 6-12 months, thereafter the level was found to rise to 6.6 mg% at 1-1½ years before registering a fall to 4.92 mg% at 3-3½ years.

Kiatoko et al. (1978) evaluated the mineral status of 12 beef and 9 dairy cattle farms in Costa Rica by estimating the concentration of phosphorus, copper, cobalt, manganese, iron, zinc and magnesium in liver, plasma, bone ash and forages. Based on this they have identified border line to deficient cattle farms.

Parda (1981) investigated zinc, copper and molybdenum levels in blood, serum and in the lucerne pasture near a molybdenite processing factory. Eventhough lucerne was found contaminated with molybdenum the copper and zinc concentration in the fodder were found to be normal. In contrast the molybdenum concentration of serum was high while that of copper was normal. Zinc deficiency was diagnosed based on a reduction in alkaline phosphatase activity and lowered zinc level in blood serum.

Towers et al. (1981) proved that experimental hypocuprosis could be produced by adding zinc sulphate to drinking water of Angus cows and calves. The monthly variation in bovine serum copper levels both in beef and dairy cows was correlated with the rainfall (Bain et al., 1986). The higher the rainfall, the lower was the serum copper level.

Bonomi et al. (1988) produced experimental zinc deficiency in Holstein Friesian cows fed optimal amounts of zinc with a calcium:phosphorus ratio of 3.5. The zinc level registered a fall from 98 μg/100 ml to 60 μg/100 ml in a period of 7 months.

There are numerous reports on the role of phosphorus and trace elements in animal reproduction. The levels of phosphorus, calcium and other micronutrients in animals with normal fertility and impaired fertility have been extensively reviewed in the literature. After determining calcium and phosphorus content of 11 pasture plants and whole blood sample from 20 cattle, Hidiroglou et al. (1960) concluded that animals in poor condition tended to have a lower calcium and higher phosphorus content in the blood than healthy cattle. They further observed that phosphorus deficiency was a cause for infertility.

Alderman (1963) investigated the calcium, phosphorus, manganese, copper, cobalt and iodine content of pasture and other fodder plants and their relation to incidence of reproductive disorders and found that deficiency of one or more led to poor reproduction.

Morrow (1969) recorded that the blood phosphorus level of heifers receiving an ad libitum supplement of calcium phosphate in the winter only was 3.9 mg% and they needed an average of 2-8 inseminations per conception. In contrast, in heifers supplemented with calcium phosphate round the year, the blood phosphorus level averaged 6.6 mg% and the number of services per conception was as low as 1.3. Reproductive disorders such as suboestrum, more services per conception, disturbed foetal development and altered sex ratio in favour of males in cattle and goats were attributed to manganese deficiency (Gropel and Anke, 1971).

Petkov and Penkov (1973) estimated the zinc and copper content of the ovaries and corpora lutea and recorded that their ratio varied from 1:2 to 1:3 during different stages of oestrus cycle. It was further observed that the levels of these microminerals in the ovary were directly related to the liver content.

Lorek and Okonski (1974) investigated the influence of phosphorus and manganese content in soil and hay samples from different farms in Poland on conception rate of dairy cows. No significant correlation was found between conception rate and phosphorus content in soil or hay. However, there was a trend of decrease in conception rate as the manganese in soil or hay increased, eventhough the correlation was not statistically significant.

Alswede and Herr (1975) estimated the manganese content of the ovaries and corpora lutea of slaughtered cows and reported that the content was 1.2 ppm in the ovary and

4.5 ppm in the corpus luteum on dry matter basis. They further recorded lower manganese content in the ovaries in disease conditions such as ovarian cyst (0.97 ppm) and genital catarrh (0.87 ppm).

Smith et al. (1975) could reduce the incidence of post partum haemoglobinuria in cows with low copper level by S/C administration of 800 mg of copper glycinate within 48 hours of parturition. Investigating low fertility with high mortality and morbidity in new born calves, Bodai (1976) detected deficiency of phosphorus, copper, zinc, manganese, cobalt and iodine in food stuffs, blood and serum of cattle reared in low lands in Hungary. He further recorded that supplementation of deficient elements in diet improved fertility and reduced the incidence of calf mortality and morbidity.

Eventhough the level of plasma zinc fell significantly either just before or at the onset of parturition, zinc levels covered a wide range and were not particularly low in cows with dystocia (Pryor, 1976).

Dufty et al. (1977) recorded a seasonal change in plasma zinc level but there was little difference in level of the element in cattle sampled at particular stages of oestrus cycle or over a 24 hours period. After conception plasma levels remained relatively constant until late in pregnancy when a decline occurred. A steeper decline occurred during the periparturient period although the trends observed differed between the animals that calved normally and those that developed dystocia.

Blood and plasma samples of 25 regular breeder and 26 repeat breeder cows were analysed for the trace elements; iron, copper, manganese and zinc with a view to determine the possible relationship between mineral content and breeding efficiency (Manickam _et al._, 1977). It was reported that all the four trace elements, registered significantly higher values in both blood and plasma of regular breeders than repeat breeders. There was also a strong negative correlation between iron and zinc content in either blood or plasma and the number of insemination recorded per conception.

While studying the effect of supplementation of copper in early lactating cows, Reddy and Mahadevan (1977) concluded that delayed oestrus in the cows may be due to border line copper deficiency.

Schuh and Brandeis (1977) analysed blood samples from 20 cattle in Austria for vitamin B_{12} content in suspected cases of cobalt deficiency and reported a level of 170 pg/ml (25-280 pg/ml). The affected animals also showed fertility when a decline occurred. A steeper decline occurred during the periparturient period although the trends observed differed between the animals that calved normally and those that developed dystocia.

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Garbacik and Balon (1978) reported that the serum inorganic phosphorus in 50 cows with placental retention as 2.16 to 2.84 mg/100 ml (mean 2.5 mg%) compared to 3.1 to 8.4 mg/100 ml (mean 5.75 mg%) in 50 normally calved cows. Phosphorus deficiency as a cause of prolonged anoestrus in cows was suggested after noting a lower inorganic phosphorus level in serum of 40 cows anoestrus for 6 months (5.71 mg/100 ml) than in 11 normal cows (8.01 mg/100 ml) (Dindorkar and Kohli, 1979).

Murtuza et al. (1979) randomly divided 32 Haryana cattle into 4 groups viz. (a) Heifers, (b) Empty dry cows, (c) Late pregnant cows and (d) Early lactating cows to analyse serum levels of calcium, phosphorus, magnesium, sodium and potassium. Serum calcium significantly differed between early lactating cows and late pregnant cows; early dry cows and late pregnant cows and heifers. Similarly, serum inorganic phosphorus levels differed between groups except between empty dry cows and early lactating cows.

Raising the serum inorganic phosphorus level in 170 infertile cows from 4.5 to more than 5-8 mg/100 ml by adding defluorinated superphosphate at the rate of 2.5 kg/450 litres once weekly resulted in an increased first service pregnancy rate from 36.5 per cent to 63.2 per cent, a decrease in mean calving to conception interval from 109 to 85 days and a drop in culling rate for infertility from 15 per cent to 5 per cent (Scharp, 1979).

Musewe and Gombe (1980) estimated the plasma vitamin B_{12} in both beef and dairy cattle in a well managed and poorly managed farms in the cobalt deficient pasture of Rift valley of Kenya. The levels obtained were compared with the cobalt levels of animals from a well managed farm with cobalt replete pastures outside Rift Valley. They reported that plasma Vit B_{12} levels in well managed farms in Rift Valley (371 $\pm$ 20 pg/ml) were comparable to those for cobalt replete pasture (288 $\pm$ 19 pg/ml). However the cobalt levels of animals in poorly managed farms were significantly lower (156 $\pm$ 13 pg/ml). They further recorded a low conception rate of 30 per cent with high mortality in poorly managed farms as against 67 per cent in well managed farms and suggested that provision of cobalt salt licks and periodical monitoring of plasma Vit B_{12} could be a useful guard against a sub-clinical deficiency.

Homse (1981) induced experimental hypocupraemia in 15 heifers by feeding heptamolybdate and recorded that 10 of the treated heifers had silent oestrus and two were anoestrus.

When heifers were supplemented with copper as copper glycinate injection and zinc, Piper and Spears (1982) observed that copper injected heifers had higher serum copper and ceruloplasmin levels than heifers not receiving copper. Heifers receiving zinc only tended to have lower serum copper and ceruloplasmin. Heifers receiving zinc had more calves (93%) as compared to heifers not receiving zinc (62%). Copper supplementation did not influence calving rates.

Ruskan et al. (1982) could not detect any significant differences in live weight gain, ovarian activity and conception rate in heifers receiving copper supplementation either orally or subcutaneously and those which did not.

Inspite of injection of 400 mg of copper glycinate to cows within 1 week of calving, no significant difference in the average interval between calving to first observed heat, services per conception or first service conception rate was recorded (Whitaker, 1982).

Cates and Christensen (1983) could improve conception rate of cows fed negative forage by daily supplementation of 12 g of calcium and phosphorus and provision of cereal and silage. They further suggested that phosphorus may have been the first limiting nutrient and improvement in conception rate was on account of phosphorus supplementation.

El-Keraby and Abdel-Rahman (1983) studied the effect of supplementation of phosphorus in early lactating cows and reported that the average interval from calving to first post partum oestrus and ovulation could be lowered to 25.7 days.

While studying the incidence of retention of placenta in 1404 calvings in Gir cows and their crosses Shukla et al., (1983) observed that the serum calcium and inorganic phosphorus were significantly lower in crossbred cows which retained placenta than in those which had normal calving. Comparison of the concentration of potassium, magnesium, copper and zinc in the blood of cows which retained placenta and those which did not, failed to reveal any significant difference (Stancioiu and Constantinescu, 1983).

Ullan et al. (1983) estimated the macro and micro minerals in blood plasma of buffaloes during 90 days post partum and reported that the concentration of calcium and phosphorus averaged 8.14 mg% and 5.13 mg% in cycling buffaloes as against 6.89 mg% and 5.3 mg% in anoestrus buffaloes. Eventhough there was significant difference in calcium level between groups, levels of phosphorus zinc, manganese and copper did not register any significant difference.

Brookes et al. (1984) observed low milk yield, unthirftness, osteophagia and infertility in dairy cows which registered low serum inorganic phosphorus, low phosphorus in pasture and a wide calcium-phosphorus ratio in the pasture. Phosphorus supplementation in drinking water restored ovarian cyclic activity.

Analysis of blood samples from heifers in five Cuban herds fed on different amounts of silage, sugarcane, urea and bagasse revealed no significant relationship of reproductive status with sodium, potassium, magnesium, phosphorus or zinc concentration in blood. However anoestrus heifers had a significantly lower copper concentration in liver than cycling heifers Gonzalez et al. (1984).

Kappel et al. (1984) studied plasma copper and PCV in Holstein cows fed diets averaging 7.5 µg of copper per gram. They observed plasma copper levels within the usual laboratory limits (1.05 ± 0.70 µg/ml) which increased around the time of calving with a maximal level of of 1.13 µg/ml on 11th day post partum. It was further recorded that plasma copper concentration was higher in cows which conceived within 80 days of calving than in those which conceived at 120 days or more. However the relationship between plasma copper level and fertility was not consistent.

A comparative study of blood calcium and phosphorus levels in cycling cows during oestrum and ancestrus cows revealed a mean calcium level of 10.18 mg% which was significantly higher than the level of 9.97 mg% in anoestrus cows (Prasad et al., 1984). They further recorded an inorganic phosphorus level of 7.79 mg% (3.8-12 mg%) at oestrus as against 3.2 to 11.88 mg% in anoestrus cows. The difference was significant.

Gupta et al. (1985) estimated the concentration of zinc and iron in cervico vaginal mucus of 18 fertile and 13 repeat breeder cows and reported no significant difference in the levels of these 2 elements between groups.

Jaskowski and Lachowski (1985) estimated calcium, magnesium, cobalt, zinc, iron and inorganic phosphorus in the blood of 110 cows two weeks before calving, just after calving and thereafter at a frequency of twice a week for a month. Phosphorus and magnesium were low in 5 out of 21 cows which developed placental retention with endometritis while calcium and phosphorus were low in 5 out of 11 cows with delayed uterine involution. Similarly phosphorus, magnesium and copper were low in 4 out of 6 cows which subsequently developed ovarian cyst. They concluded that peurperal disorders were predictable in 20 cows out of a total 55 from the low values of calcium, phosphorus and magnesium and in another 5 cows from low values of trace elements during final month of pregnancy.

Jaskowski (1986) studied the relationship of blood mineral content before and after parturition to fertility in cows and reported that calcium, magnesium, copper and inorganic phosphorus level remained within normal range during the period of 2 weeks prior to calving and at 10, 20 and 30 days post calving. He concluded that no relationship of levels of these elements to subsequent fertility could be traced, except in case of phosphorus values below 1.4 millimol/litre.

Jaskowski *et al*. (1986) found significant correlation in blood levels of calcium, zinc, copper, phosphorus and iron 10 days before calving with the levels of these elements 10 days after calving and then at 3-4 days interval until involution of uterus. They concluded that from the levels prior to calving possible deficiency post calving could be predicted and necessary corrective measures adopted. Serum concentration of phosphorus, calcium, potassium and chlorine were lower in 15 anoestrus and 24 infertile cows and heifers when compared to the levels in 30 normal cycling cows (Kumar *et al*., 1986).

While estimating the blood levels of phosphorus of 52 crossbred heifers on pasture prior to mating Mufarrege *et al*. (1986) reported that there was significantly higher blood phosphorus concentration (3.4 $\pm$ 0.9 mg/dl) in 25 heifers which subsequently conceived than the level of 2.9 $\pm$ 1.3 mg/dl for

25 heifers which did not conceive (P<0.05). They further observed a high significant, correlation between blood phosphorus and body weight at mating.

Parmar _et al_. (1986) estimated levels of iron, manganese, zinc and copper in the blood of normal cyclic and repeat breeding crossbred cows and reported significant difference in the levels of all elements except zinc.

Pedroso _et al_. (1986) estimated the concentration of some biochemical constituents in HF x Zebu and Brown Swiss x Zebu heifers with a view to correlate it with season and herd. They observed that season had significant effect on blood glucose, haemoglobin, inorganic phosphorus, manganese, sodium and potassium (P<0.01), total protein, haematocrit and zinc (P<0.05). Herd significantly affected blood glucose, cholesterol, haemoglobin, inorganic phosphorus, magnesium and potassium concentration. They further observed that the reproductive status affected inorganic phosphorus levels in that, lowest levels were recorded in cycling cow (P<0.01).

Sivaiah _et al_. (1986) studied the serum calcium and inorganic phosphorus levels in 89 crossbred Ongole cows aged 2-6 years grouped as nearing parturition, anoestrus, recently calved and in early pregnancy and found that serum calcium and phosphorus did not differ significantly. Serum calcium ranged from 7.04 ± 2.07 to 8.15 ± 2.46 mg/100 ml while serum inorganic phosphorus ranged from 5.73 ± 0.92 to 7.18 ± 0.9 mg/100 ml. The relatively low Ca:P ratios (1.02 to 1.34) were attributed to the feeding of paddy straw as sole roughage.

Ingraham et al. (1987) attempted correction of subnormal fertility in crossbred cows and heifers with copper and magnesium supplementation. They recorded conception rate to first insemination to be 57 per cent for cows receiving copper and magnesium as against 27 per cent, 38 per cent and 33 per cent for cows receiving copper, magnesium and no supplement respectively, while 92 per cent cows supplemented with copper and magnesium conceived by 210 days of calving only 75 per cent conceived in all the other groups. Plasma magnesium concentration differed among cows grouped according to fertility and concentration was significantly correlated with interval from calving to conception.

A decreased plasma copper level with advancing gestation which increased after calving was observed by Lavin Gonzalez et al. (1987). The levels were lowest in cows returning repeatedly to service and were significantly lower in last 3rd of gestation then 30-90 days after gestation.

O'Gorman et al. (1987) studied the effect of molybdenum induced copper deficiency on reproduction in beef heifers. The mean liver copper levels in beef heifers with hypocupraemia and supplemented with copper was 70 mg/kg DM as against 10 mg/kg DM in heifers with hypocupraemia supplemented with copper and molybdenum. The response of the heifers from both the groups in terms of duration of oestrum, embryo recovery and fertilization rate at the induced cycle with cloprostenol did not differ significantly. However normality of embryos based on microscopic observation was 63 per cent in group I and 16 per cent in group II suggesting that the elevated dietary molybdemum in copper deficient heifers retards the early development of fertilised egg.

Poole et al. (1987) recorded in two groups of beef heifers, one supplemented with molybdemum and the other with copper, a liver copper concentration ranging from 8-11 mg/kg DM and 70-130 mg/kg DM respectively. While the molybdemum treated group gave a pregnancy rate of 41 per cent only the copper treated group gave 71 per cent pregnancy rate.

While investigating reduced fertility in a herd during the interpregnancy period Saba et al. (1987) observed that imbalance in various minerals in the diet was responsible for the condition. Excess calcium in the diet resulted in copper and phosphorus deficiency and slightly depressed zinc levels. High levels of serum calcium, phosphorus and zinc accompanied low levels of copper.

Surendra Singh and Vadnere (1987) induced oestrus in anoestrus cows having deficiency of one or several of the elements such as iodine, copper, calcium, inorganic phosphorus and iron with supplementation for a maximum period of 21 days.

Vukovic et al. (1987) observed 15 per cent of placental retention in Simmental herd. The affected cows had a lower concentration of phosphorus, Vit. A and beta-carotene when compared to the herd average.

Alegria et al. (1988) recorded a pregnancy rate of 61, 85 and 88 per cent for heifers supplemented with common salt, dicalcium phosphate and mineral mixture based on phosphorus, copper and cobalt respectively. Phosphorus supplementation increased live weight gain and pregnancy rates.

After giving a mineral mixture which besides other elements contained cobalt acetate 500 mg, copper sulphate 200 mg, Zinc sulphate 1 g and manganese sulphate 0.1 g at 5 hour intervals for 5 days to 10 cows with acquired utero ovarian hypoplasia caused by deficiencies in feed, Boitor et al. (1988) could detect oestrus in six cows. Gestation was established in five cows 60 days after insemination.

Damir et al. (1988) recorded clinical cases of both zinc and copper deficiencies in a cattle farm in Kordofan region of Sudan after draught. Nearly 55 per cent of the cows sampled had a serum zinc level below 12.24 µmol/litre and most of these cases showed skin lesions of varying severity. Nearly 15 per cent were below 9.18 µmol/litre and all these cases were showing intense skin lesion. Similarly analysis of serum copper level showed that 65 per cent of these cases were below 10.21 µmol/litre. The mean serum calcium, phosphorus and magnesium values were 2.33, 1.74 and 1.09 milli mol/litre respectively. They concluded that both zinc and copper deficiency caused impaired reproductive function.

When Santa Gertrudis cows of Cuba in areas deficient for sodium, potassium, calcium, phosphorus, magnesium, copper, zinc and iron received a mineral mixture supplement, Gonzalez et al. (1988) noted birth rates to increase by 11.8 per cent and abortion rates and calving conception intervals to decrease respectively by 1.3 per cent and 32.8 days.

Lavin Gonzalez et al. (1988) estimated plasma zinc levels of pregnant cows, oestrus cows, cows not coming into oestrus between 30th and 90th day after calving and cows with more than four infertile heats. They observed that plasma zinc decreased as pregnancy advanced and then registered an increase soon after birth. However no significant changes were found in zinc concentration in plasma of cows that repeatedly came into oestrus.

Ropstad *et al*. (1988) observed blood inorganic phosphorus levels to be significantly affecting number of days from calving to first AI and service period in cows with reproductive problem.

Singal and Lohan (1988) suggested mineral deficiency as a probable contributing factor to infertility in cattle in Haryana. Serum phosphorus concentration ranging from 1.02 $\pm$ 0.13 to 5.24 $\pm$ 0.70 mg/100 ml and zinc concentration being <9 ppm. When mineral supplements were fed for 5 weeks 40 per cent of previously infertile animals showed oestrus and conceived to matings.

Gaines (1989) interpreted results of blood glucose, urea, haematocrit, haemoglobin, albumin, calcium, phosphorus and magnesium values and their relation with body condition scoring and fertility and concluded that the relationship between nutrition and reproduction is vague, complex and dynamic.

Hahn *et al*. (1989) estimated the serum iron and copper content of 39 high yielding cows which included 24 infertile and 15 healthy controls and recorded a considerably higher iron content averaging 40.59 μ mols/litre in all the cows. However their copper content was low and averaged 10.74 μ mols/litre. Eventhough no significant difference in the mineral levels of healthy and infertile animals was recorded they opined that copper deficiency might lead to infertility.

Prasad et al. (1989) examined 50 crossbred cows with different reproductive disorders and estimated their serum copper, zinc, manganese, cobalt and iron levels besides progesterone and oestradiol. Both high and low level of copper in serum were found to be associated with repeat breeding cases. In all the reproductive conditions studied no significant deviation from physiological range was observed for manganese and cobalt levels in blood serum. Serum iron levels tended to be higher in cycling and longer post partum interval cases. A significant correlation between serum zinc and copper ($r = 0.51$) and between zinc and cobalt ($r = 0.37$) were noticed in different reproductive disorders. They remarked that these elements are likely to play either singly or in combination in maintaining reproductive rhythms.

In cows which conceived within 80 days of calving the concentration of phosphorus, sodium, manganese and zinc were significantly higher than in those which failed to conceive by 5 months post partum (Simeonov et al., 1989). In contrast Forshell et al. (1991) could find no relation between blood calcium, phosphorus and magnesium levels with fertility. Significantly lower serum inorganic phosphorus level was observed in animals in nonfertile heat as compared to values in fertile oestrus (Satishkumar and Sharma, 1991).

Saxena et al. (1991) reported that the plasma levels of calcium, phosphorus and magnesium were not related with age at puberty ($P<0.05$) while plasma levels of copper and zinc were significantly related to age of puberty ($P<0.01$). They concluded that heifers with copper level of 125 mg/dl and zinc level of 230 ug/dl of plasma attain puberty earlier than those having levels lower than this.

Chauhan et al. (1992) studied the seasonal variation in mineral elements of soil, pasture and blood in normal and abnormal reproduction in cows. There was significant fall ($P<0.01$) in soil calcium and phosphorus during rainy season while seasonality had no influence on levels of magnesium, copper, zinc and manganese. The season was found to have no effect on the mineral content of pasture except phosphorus ($P<0.01$). With regards to blood serum there was no significant difference in levels of magnesium copper, zinc and manganese. However there was significant difference in serum calcium and phosphorus levels ($P<0.01$) between groups. Therefore it was concluded that supplementation of ration with calcium and phosphorus is justified to overcome some of the reproductive disorders.

Materials and Methods

MATERIALS AND METHODS

The crossbred cattle brought to the Artificial Insemination Centre, College of Veterinary and Animal Sciences, Mannuthy and those of the University Livestock Farm, Mannuthy, formed the materials for study. The animals were categorised as anoestrus, repeat breeders and normally cycling fertile animals based on breeding history and clinico-gynaecological investigations.

Normal cycling fertile animals (controls)

Heifers and post partum cows which were inseminated on the first observed heat and had conceived with one or two inseminations were taken as cows of normal fertility.

Ancestrus

Post partum cows which did not show any clinical or behavioural signs of heat even 5 months after calving were subjected to detailed clinico-gynaecological examination. Those cows which had small quiscent ovaries without even follicles of prematuration size and/or functional/regressing corpus luteum with flabby genitalia were considered to be suffering from true anoestrum.

Repeat breeders

Cows which showed recurring cycles of normal or nearly normal duration with no clinical signs of any genital diseases and without any gross abnormality of reproductive organs but still did not conceive even with more than two inseminations, using semen from known fertile bulls were taken as repeat breeders.

Collection of serum

Approximately 25 ml blood was collected in a test tube from the jugular vein of each animal and the sample was kept in a slanting position to clot. The clotted blood was kept aside in a refrigerator for 24 hours. The clear serum was collected in 5 ml aliquots and stored in labelled air tight containers at 0°C. Slightly discoloured serum was centrifuged at 1000 rpm for 5 minutes and the clear supernatent was separated and stored in the same manner for the estimation of trace elements.

Inorganic phosphorus content of the serum was estimated soon after collection.

Estimation of minerals

Estimation of inorganic phosphorus

Inorganic phosphorus level in the serum of 17 cows belonging to all the three groups was estimated using phosphorus kits* employing Modified Metol Method.

Procedures

The various reagents and serum were pipetted into clean dry test tubes and labelled in three groups; Blank, Standard and Test as per the following protocol.

	Blank	Standard	Test
Catalyst reagent	1.0 ml	1.0 ml	1.0 ml
Molybdate reagent	1.0 ml	1.0 ml	1.0 ml
Deionized water	1.0 ml	--	--
Standard P solution	--	0.1 ml	--
Serum	--	--	0.1 ml
Metol reagent	1.0 ml	1.0 ml	1.0 ml

They were mixed well and allowed to stand at room temperature for 5 minutes. The absorbance values of Test and Standard were measured against Blank in a Spectrophotometer** at 680 nm within 30 minutes.

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* Stangen Immunodiagnostics

** Spectronic-20 (Bosch & Lomb)

Serum inorganic phosphorus level in mg% was calculated by applying the following formula.

$$\text{Serum inorganic P in mg\%} = \frac{\text{Absorbance of Test}}{\text{Absorbance of Standard}} \times 5$$

Estimation of trace elements

Copper, zinc, manganese and cobalt contents of the serum were estimated by Atomic Absorption Spectrophotometry using a Perkin Elmer 2380 Model Atomic Absorption Spectrophotometer.

Principle of Atomic Absorption Spectrophotometry

Every element has a specific number of electrons which are associated with the atomic nucleus in a unique orbital structure. The electrons occupy orbital position in an orderly and predictable way. The most stable electronic configuration of an atom is the "ground state" in which it has the normal orbital configuration and the lowest energy levels. If light of the right wave length impinges on a free ground state atom, the atom may absorb energy from the light and it enters an excited state in a process known as atomic absorption. The capacity of atom to absorb very specific wave length of light is utilized in atomic absorption spectrophotometry. In Atomic absorption the amount of light at the resonant wave length which is absorbed, as light passes through a cloud of atoms, is measured. As the number of atoms in the light path increases the amount of light absorbed also increases in a predictable way. By measuring the amount of light absorbed a quantitative determination of the amount of analyte element present can be made. The use of special light source and careful selection of wave length allow the specific quantitative determination of individual elements in the presence of others.

Preparation of sample, standard and blank

Copper

Preparation of sample

As per the dilution rate recommended by the manufacturers of the AAS (Perkin Elmer), the serum sample was diluted to 1 in 2 by making up 1 ml of serum to 2 ml using deionized water (1 ml).

Preparation of copper standard

Solution containing 4 ppm copper in 10 per cent aqueous glycerol solution was used as the standard for which 0.4 ml of copper stock standard solution containing 1000 ppm copper was made upto volume with 10 per cent aqueous glycerol in a 100 ml volumetric flask.

Blank

The ten per cent aqueous solution of glycerol in deionized water was used as blank.

Zinc

Preparation of sample

One millilitre of serum was diluted with 4 ml of deionized water.

Preparation of standard

Solution containing 1 ppm Zinc in 5 per cent aqueous glycerol was prepared by making up 0.1 ml of stock standard solution of zinc containing 1000 ppm zinc upto the mark using 5 per cent aqueous glycerol in a 100 ml volumetric flask.

Blank

The five per cent aqueous glycerol solution in deionized water used for the diluting of standard was used as the blank.

Manganese

Preparation of sample

Whole undiluted serum as such was used for manganese estimation.

Preparation of sample

The 2 ppm solution of manganese used as the working standard was obtained by making up 0.2 ml of stock standard solution of manganese containing 1000 ppm of manganese to 100 ml using 20 per cent aqueous glycerol in a 100 ml volumetric flask.

Blank

The twenty per cent aqueous glycerol solution (the one used for preparation of manganese working standard) was used as the blank.

Cobalt

Preparation of sample

Undiluted serum as such was used for the estimation of cobalt.

Preparation of standard

Working standard for cobalt estimation was prepared by making up 0.35 ml of stock standard solution containing 1000 ppm of cobalt in a 100 ml volumetric flask using 20 per cent aqueous glycerol, to yield a 3.5 ppm solution.

Blank

A twenty per cent aqueous solution of glycerol (same as the one used for preparation of working standard) was used as the blank.

Stock standard solutions of elements used to prepare working standards were provided by Perkin Elmer - the manufacturers of the AAS.

The various concentrations of glycerol - 20 per cent, 10 per cent and 5 per cent used as the blank and diluant for the preparation of standards were prepared by pipetting out 40, 20 and 10 ml of glycerol respectively into 200 ml volumetric flasks and making upto mark using deionized water. The various concentrations of glycerol were used to make the working standard attain a viscosity similar to that of diluted serum, viscosity being a factor which governs the amount of solution aspirated into the flame.

Conditions used for the operation of AAS

The AAS was set for operation as per the recommendations of the instrument manufacturers (Perkin Elmer). The standard conditions for atomic absorption of various elements studied are furnished below.

Item	Copper	Zinc	Manganese	Cobalt
Wave length (nm)	324.8	213.9	279.5	279.5
Slit SBW (nm)	0.7	0.7	0.2	0.2
Flame gases*	A-AC	A-AC	A-AC	A-AC
Lamp - Hollow cathode	Copper	Zinc	Manganese	Cobalt
Lamp current	15 m A (25 m A)	15 m A (20 m A)	28 m A (30 m A)	30 m A (40 m A)
Time (seconds)*	0.2	0.2	0.2	0.2
Average*	5	5	5	5

* Reading taken at every 0.2 seconds and 5 such readings averaged

Working of the instrument

After setting the instrument to the required specifications it was standardized using a reagent blank and the recommended working standard solution for each element. The instrument was then set to register a reading every 0.2 seconds and to display the average of 5 such readings. The working samples were then aspirated into the flame and concentrations in ppm displayed directly were noted. Between samples deionized water was aspirated to clean up the nebulizer system. Concentration of each element in the original serum sample was then computed using necessary dilution factors.

The data obtained was statistically analysed (Snedecor and Cochran, 1967) to correlate with the fertility status of the animal.

Results

RESULTS

The serum levels of phosphorus, copper, zinc, manganese and cobalt in normal fertile, anoestrus and repeat breeder cows are presented in Tables 1 to 5 and Fig.1 to 5.

Phosphorus

The serum inorganic phosphorus level in normal fertile group was 7.526 ± 0.5304 mg % which was significantly higher than that for anoestrus group ($P<0.05$) which registered a level of 6.082 ± 0.3337 mg %. Eventhough the repeat breeder group too registered a lower value of 6.345 ± 0.4474 mg % the difference was not statistically significant.

Copper

The copper concentration in serum of anoestrus group was 0.509 ± 0.0591 ppm and that in repeat breeder group was 0.542 ± 0.0415 ppm. Both these were significantly lower ($P<0.01$) than the serum copper level of normal fertile group which registered a value of 0.733 ± 0.0511 ppm.

Zinc

Zinc levels in serum of anoestrus group was 1.028 ± 0.0984 ppm and that of repeat breeder group was 1.017 ± 0.0654 ppm. Both values were lower than that for normal breeding group which registered a value of 1.337 ± 0.1555 ppm. But the difference was statistically insignificant.

Manganese

The serum manganese level of 0.0339 ± 0.0052 ppm registered for the anoestrus group was lower than that of repeat breeders which registered a value of 0.0429 ± 0.0033 ppm which in turn was lower than the values for control which was 0.0553 ± 0.0095 ppm. However the difference was not statistically significant.

Cobalt

Eventhough the concentration of cobalt obtained for different groups were not significantly different highest value was registered for repeat breeders (0.0795 ± 0.0111 ppm) followed by the control (0.0702 ± 0.0100 ppm) and the lowest in anoestrus group 0.0641 ± 0.0052 ppm.

The result could be summarized as follows:

Statistically significant lower values existed between normal fertile, anoestrus and repeat breeding groups for serum copper levels. The significance was limited to that between normal and anoestrus in case of phosphorus eventhough repeat breeder group too registered a lower value. There was no significant difference in the serum values registered of zinc, manganese and cobalt between any of these groups. The repeat breeder group registered a higher value than that for control with regard to serum cobalt level.

Table 1. Serum phosphorus levels in fertile, anoestrus and repeat breeder cows (mg%)

No.	Fertile	Anoestrus	Repeat breeders
1.	8.86	5.68	4.55
2.	11.30	4.77	5.80
3.	10.90	4.77	4.33
4.	7.72	7.27	4.32
5.	6.59	7.95	4.32
6.	7.04	7.73	4.55
7.	4.54	5.68	5.45
8.	7.04	8.86	6.82
9.	9.77	3.64	6.82
10.	6.81	6.82	7.73
11.	8.86	5.68	5.45
12.	6.81	5.68	6.82
13.	10.00	4.77	5.00
14.	4.66	5.00	8.18
15.	4.33	6.82	10.00
16.	7.72	6.82	8.63
17	5.00	5.45	9.09
Mean ± SE	7.526 0.5304	6.082 0.3337	6.345 0.4474

Table 2. Serum copper levels in fertile, anoestrus and repeat breeder cows (ppm)

No.	Fertile	Anoestrus	Repeat breeders
1.	0.70	0.51	0.78
2.	0.45	0.42	0.20
3.	0.95	0.60	0.50
4.	0.50	0.35	0.30
5.	0.75	0.30	0.70
6.	1.20	0.35	0.60
7.	0.65	0.45	0.70
8.	0.80	0.20	0.45
9.	0.95	0.40	0.55
10.	0.80	0.30	0.40
11.	0.80	0.96	0.70
12.	0.50	0.70	0.45
13.	0.60	0.66	0.55
14.	0.65	0.96	0.60
15.	0.70	0.48	0.65
Mean ± SE	0.733 0.0511	0.509 0.0591	0.542 0.0415

Table 3. Serum zinc levels in fertile, anoestrus and repeat breeder cows (ppm)

No.	Fertile	Anoestrus	Repeat breeders
1.	1.75	0.96	0.72
2.	1.65	0.66	1.00
3.	1.15	1.68	0.60
4.	1.30	1.02	1.15
5.	1.25	1.02	0.90
6.	1.05	0.54	0.95
7.	0.95	1.65	0.95
8.	1.60	0.54	1.00
9.	1.45	1.05	0.90
10.	2.95	0.95	0.90
11.	1.05	1.15	1.45
12.	1.05	1.25	1.00
13.	0.70	0.90	1.35
Mean ± SE	1.337 0.1555	1.028 0.0984	1.017 0.0654

Table 4. Serum manganese levels in fertile, anoestrus and repeat breeder cows (ppm)

No.	Fertile	Anoestrus	Repeat breeders
1.	0.09	0.011	0.027
2.	0.05	0.026	0.058
3.	0.04	0.019	0.035
4.	0.04	0.080	0.060
5.	0.05	0.060	0.070
6.	0.04	0.020	0.020
7.	0.03	0.020	0.040
8.	0.04	0.060	0.050
9.	0.03	0.030	0.030
10.	0.03	0.040	0.050
11.	0.03	0.050	0.040
12.	0.04	0.070	0.060
13.	0.03	0.018	0.050
14.	0.19	0.018	0.030
15.	0.06	0.018	0.040
16.	0.08	0.015	0.040
17	0.07	0.024	0.030
Mean ± SE	0.0553 0.0095	0.0339 0.0052	0.0429 0.0033

Table 5. Serum cobalt levels in fertile, anoestrus and repeat breeder cows (ppm)

No.	Fertile	Anoestrus	Repeat breeders
1.	0.040	0.046	0.036
2.	0.050	0.065	0.066
3.	0.040	0.093	0.180
4.	0.50	0.070	0.070
5.	0.060	0.040	0.090
6.	0.090	0.070	0.070
7.	0.090	0.030	0.090
8.	0.060	0.070	0.120
9.	0.050	0.070	0.090
10.	0.110	0.050	0.040
11.	0.100	0.074	0.020
12.	0.030	0.110	0.110
13.	0.030	0.050	0.130
14.	0.075	0.060	0.040
15.	0.178	0.060	0.040
Mean ± SE	0.0702 0.0100	0.0641 0.0052	0.0795 0.0111

Fig.1 SERUM INORGANIC PHOSPHORUS LEVELS(mg%)IN FERTILE, ANOESTRUS AND REPEAT BREEDER COWS

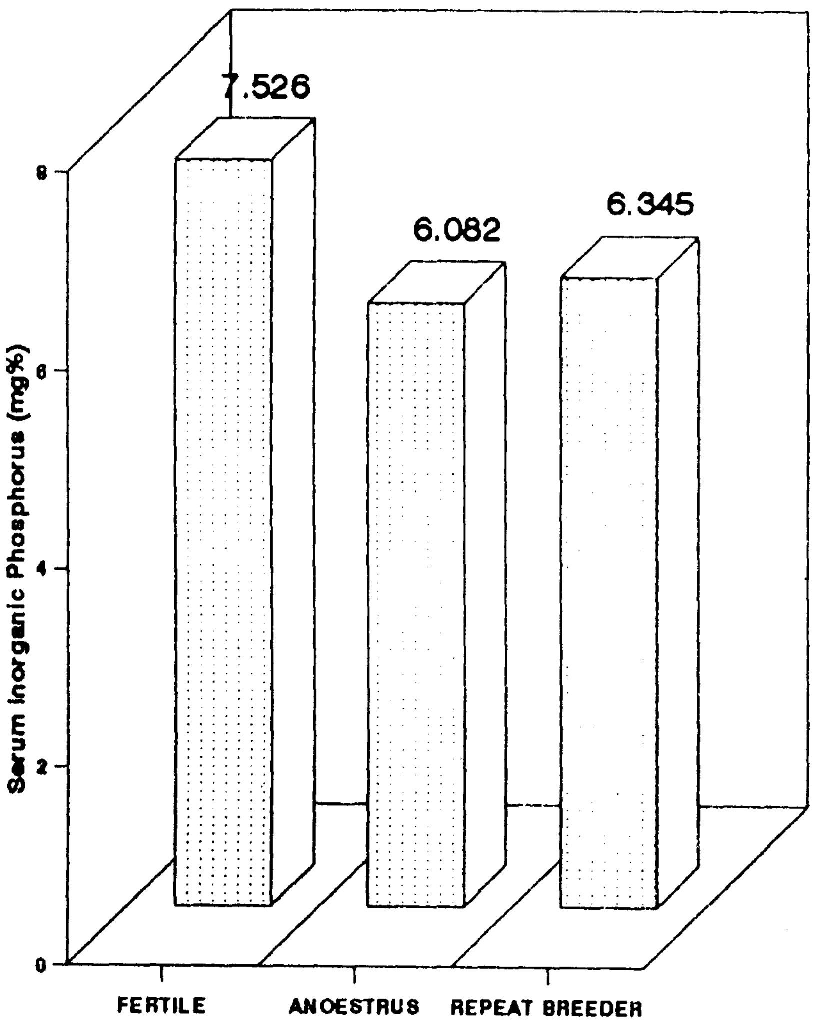


Fig.2 SERUM COPPER LEVELS(ppm) IN FERTILE, ANOESTRUS AND REPEAT BREEDER COWS

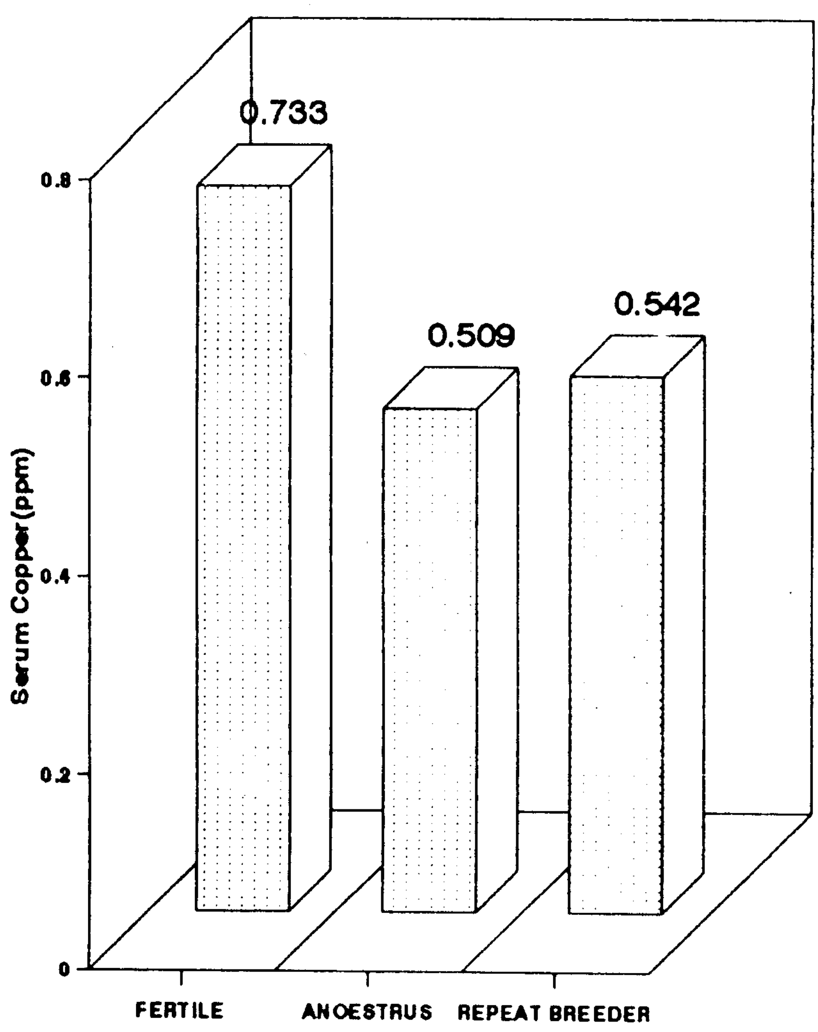


Fig.3 SERUM ZINC LEVELS(ppm) IN FERTILE, ANOESTRUS AND REPEAT BREEDER COWS

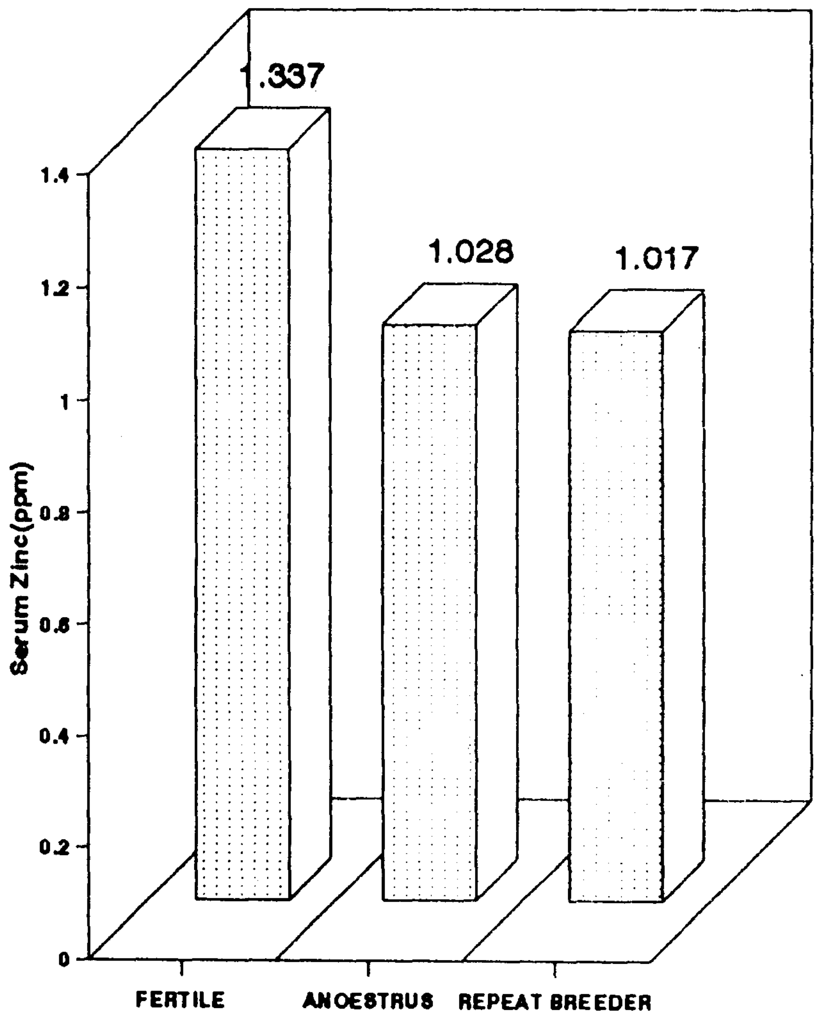


FIG.4 SERUM MANGANESE LEVELS(ppm) IN FERTILE, ANOESTRUS AND REPEAT BREEDER COWS

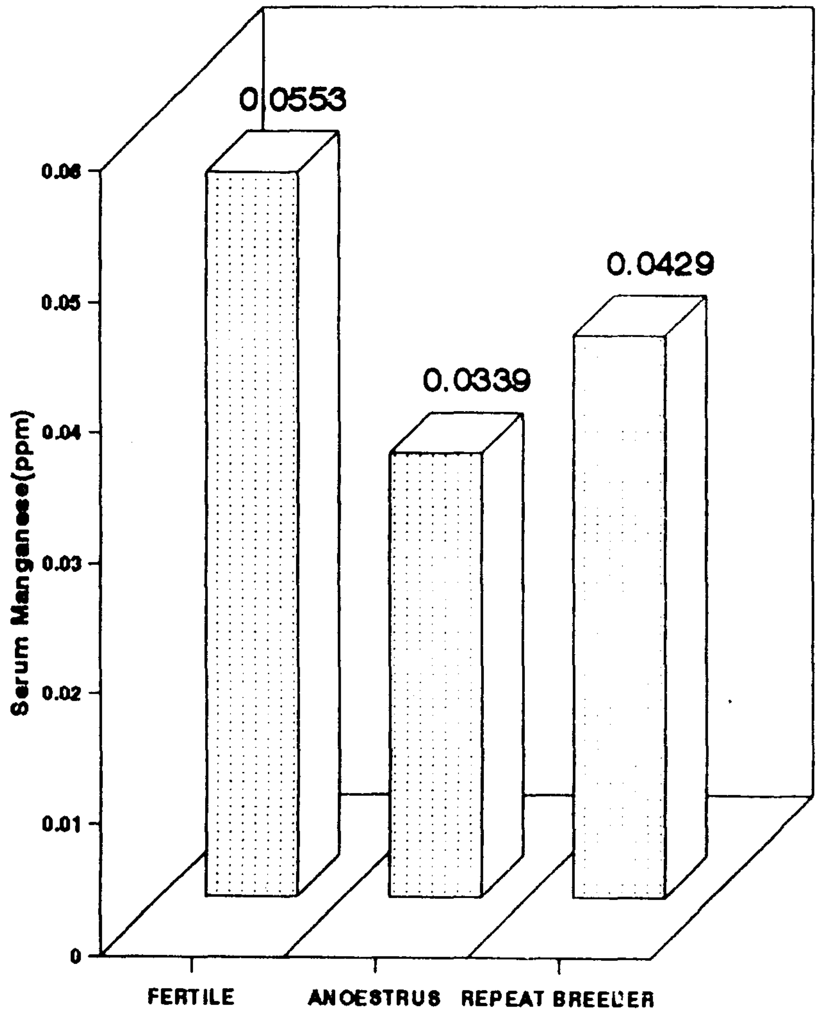
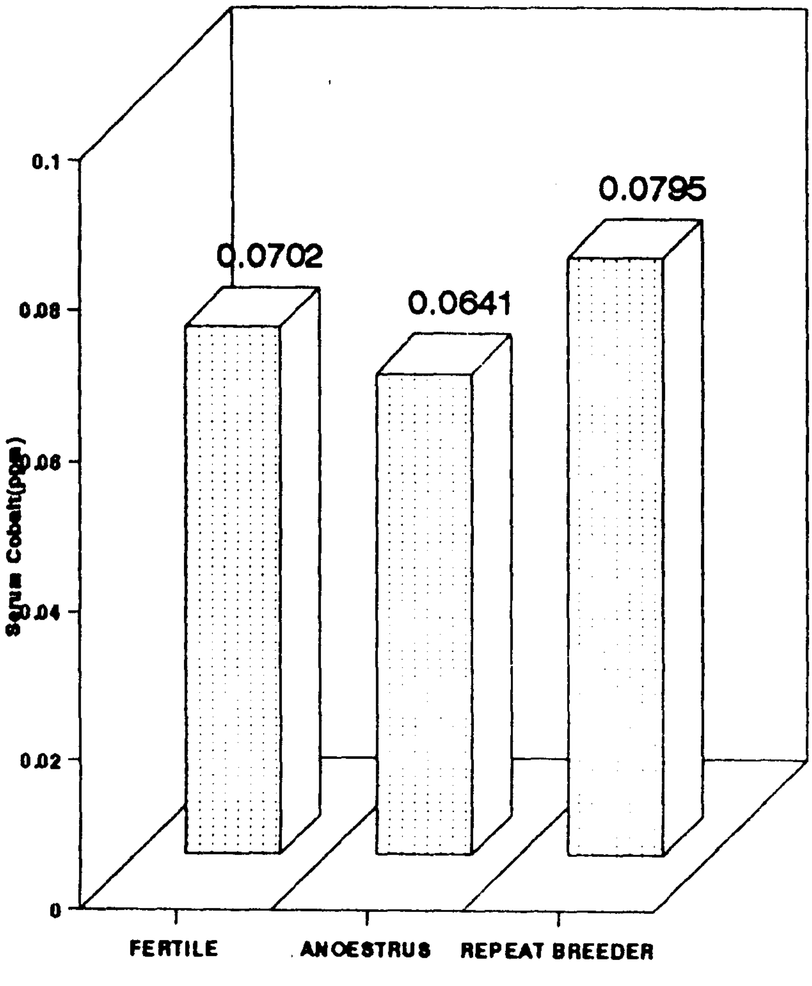


FIG.5 SERUM COBALT LEVELS(ppm) IN FERTILE, ANOESTRUS AND REPEAT BREEDER COWS



Discussion

DISCUSSION

Reproduction in animals is known to be influenced by genetic traits, nutrition, hormonal status, and environment. For every successful pregnancy and parturition a proper synergism between anabolic and catabolic reactions is essential and hence minerals have a key role in maintenance of reproductive efficiency. Various indepth studies have been undertaken to elucidate the nature and extent of involvement of minerals and trace elements in the body metabolism and reproduction which yielded conflicting results reflecting the complex nature of their involvement in body function.

The present investigation undertaken to assess the role of phosphorus, copper, zinc, manganese and cobalt in reproduction of crossbred cattle of Kerala too yielded results confirming and contradicting previous findings.

Phosphorus

Serum inorganic phosphorus level of normal fertile animals (control group) was found to be 7.526 $\pm$ 0.5304 mg% which was significantly higher ($P<0.05$) than the values recorded for anoestrus group (6.082 $\pm$ 0.3337 mg%) suggesting that hypophosphataemia might lead to anoestrum. The repeat breeder group registered a lower value of 6.345 $\pm$ 0.4474 mg% but it was not statistically significant.

The level of inorganic phosphorus in serum markedly influenced oestrous cycle and conception rate in bovines with low levels of phosphorus tending to reduce conception rate (Morrow, 1969; Mufarrege et al., 1986; Gonzales et al., 1988; Simeonov et al., 1989). It was further observed that supplementation of phosphorus improved conception rate of the affected animals (Morrow, 1969; Cates and Christensen, 1983; Mufarrege et al., 1986; Alegria et al., 1988; Gonzales et al., 1988; Simeonov et al., 1989).

Anoestrum has been found to be associated with lower levels of serum inorganic phosphorus (Dindorker and Kohli, 1979; Prasad et al., 1984; Kumar et al., 1986). Initiation or re-establishment of normal cyclic activity and increased fertility have been noticed following phosphorus supplementation (Brooks et al., 1984; Surender Singh and Vadnere, 1987; Singal and Lohan, 1988).

Repeat breeders too were found to register a lower serum inorganic phosphorus level (Satishkumar and Sharma, 1991). However in the present study there was no significant difference in the serum phosphorus values of fertile and repeat breeder cows.

There are a few reports contradicting the above findings about the role of phosphorus in reproductive function. Serum inorganic phosphorus was not found to influence the onset of puberty and fertility of cows (Forshell et al., 1991; Saxena et al., 1991).

Phosphorus deficiency affects most cell types as phosphorus is an integral component of nucleic acids, nucleotides, phospholipids and some proteins. It is required for transfer and utilization of energy and normal phospholipid metabolism. It is also an integral part of large number of enzymes. The involvement of phosphorus in the phospholipid and CAMP synthesis may be a key to its effect on reproduction. The role of calcium and phospholipid dependent proteinkinase may be crucial in mediating hormone action (Hurley and Doane, 1989). Phosphorus deficiency could occur on account of imbalance in Ca:P ratio in the diet, deficiency of phosphorus in diet and also a gross imbalance between phosphorus assimilated and that secreted through milk. There will be gradual reduction in phosphorus mobilisation from the skeletal system leading to fall in serum phosphorus level. Thus the availability of phosphorus for reproductive function becomes meagre or scanty leading to anoestrum and impaired fertility. Anoestrum in the present study could be attributed to low serum phosphorus levels since serum inorganic phosphorus in anoestrus cow was significantly lower than that of fertile cows. Eventhough serum inorganic phosphorus level was lower in repeat breeders also, when compared to fertile animals, it was not statistically significant and hence the role of phosphorus in repeat breeding could not be established with certainty.

Copper

The mean serum copper level for control group (0.733 ± 0.0511 ppm) was significantly higher ($P<0.01$) than those of anoestrus group (0.509 ± 0.0591 ppm) and repeat breeders (0.542 ± 0.0415 ppm). This finding is in agreement with earlier findings (Manickam et al., 1977; Prasad et al., 1989).

Supplementation of copper to anoestrus animals has been found to induce heat (Surendra Singh and Vadnere, 1987). Animals repeatedly coming into service had low levels of copper in blood (Parmar et al., 1986) and in plasma (Lavin Gonzalez et al., 1987). Both high and low levels of copper in serum has been observed in repeat breeders (Prasad et al., 1989).

While there are many reports suggestive of the important role played by copper in reproduction there are a few others which contradict this. The relationship between plasma copper level and fertility has been reported to be inconsistent (Kappel *et al.*, 1984). Chauhan *et al.* (1992) also could not detect any significant difference in blood copper levels in animals exhibiting normal and abnormal reproduction.

Copper related reproductive disorders may be due to actual deficiency or interference with copper utilization (Allcroft and Parker, 1949). Availability of copper is reduced by an excess of other minerals such as sulphur, iron, calcium, zinc and molybdenum in the soil and fodder. Molybdenum is thought to reduce capacity of liver to store copper (Blakemore and Venn, 1950). High levels of serum calcium, phosphorus and zinc have been reported to be accompanied by low levels of copper (Saba *et al.*, 1987).

Copper as an integral component of metalloenzyme play a significant role in metabolic functions including those of endocrine organs. Copper is also reported to modulate the PGE_2 receptor binding, thus regulating the release of LH releasing hormone (Barnea *et al.*, 1985).

Since liver acts as a storage organ for copper, serum copper levels register subnormal values only after depletion of liver storage. In the present study low serum copper levels in anoestrus and repeat breeder cows clearly indicate hypocuprosis and resultant interference in reproductive function.

Zinc

The mean serum level zinc for anoestrus group was 1.028 ± 0.0984 ppm as against 1.337 ± 0.1555 ppm for control group, the difference being statistically insignificant. Similarly the serum zinc levels in repeat breeder (1.017 ± 0.065 ppm) also did not vary significantly from those of fertile and anoestrus cows.

Prasad et al. (1989) also did not get any significant difference in serum zinc levels between fertile and anoestrus cows. However he observed that serum zinc levels was higher in repeat breeders when compared to normal and anoestrus animals. Low serum zinc levels were reported in anoestrus cows (Dufty, 1977, Kumar et al., 1984) and in repeat breeders (Manickam et al., 1977).

Cows which failed to conceive within five months post partum were found to have a lower zinc level than those which conceived within 80 days of calving. Heifers with plasma zinc levels of 230 ug/dl were observed to attain puberty faster than those having levels lower than this (Saxena, 1991). Supplementation studies have shown that zinc was essential for reproduction. Zinc, when supplemented along with other elements to cattle improved the fertility (Bodai, 1976; Boiter *et al.*, 1988; Gonzalez *et al.*, 1988).

Zinc being an integral part of over 200 proteins and enzymes has been attributed several important biological role. Some of these enzymes may be of particular importance in their function on reproductive tissue, but little is known about how zinc deficiency influences manifestations of zinc dependent functions. A role in reproduction may involve zinc as an essential component or activator of enzymes involved in steroidogenesis. Zinc may act indirectly through the pituitary to influence gonadotrophic hormone or directly through complexing with specific ligands in the gonads and prostate gland (Apagar, 1985). Sterility due to zinc deficiency has been attributed to defects in Prostaglandin metabolism. But it is quite evident that zinc deficiency in ruminants as in other species causes a more pronounced impairment of fertility in the male than in female (Hidiroglou, 1979). However, from the present study no definite conclusion on the role of zinc in anoestrum and repeat breeding could be drawn.

Manganese

The serum manganese level in control group (0.0553 ± 0.0095 ppm) did not vary significantly from those of anoestrus (0.0339 ± 0.0052 ppm) and repeat breeder (0.0429 ± 0.0033 ppm) group. Similar observation has been made by Prasad *et al.* (1989) who did not find any significant difference in the serum content of Mn between normal (0.026 $\pm$ 0.007 ppm) repeat breeder (0.019 $\pm$ 0.009 ppm) and anoestrus (0.015 $\pm$ 0.009 ppm) cows. But significantly lower manganese values have been reported by other workers (Manickam *et al.*, 1977; Parmer *et al.*, 1986; Simeonov *et al.*, 1989).

Eventhough manganese deficiency is rare in ruminants, dietary, deficiency and reduced tissue concentration can lead to impaired reproductive function characterised by anoestrum, suboestrum, anovulation, delayed ovulation and reduced fertility (Wilson, 1966). Reproductive disorders such as suboestrus, more services per conception, disturbed foetal development and altered sex ratio in favour of males in cattle and goats have been attributed to manganese deficiency (Groppel and Anke, 1971).

Negative effects of higher levels of manganese on reproduction have also been reported. A decrease in conception rate though not statistically significant was associated with a higher manganese content in soil or hay (Lorek and Okaniski, 1974).

Many of the gross effects of manganese deficiency can be explained in term of the effect of manganese on mucopolysaccharide synthesis (Underwood, 1977). Deficiency also affects several manganese metalloenzymes including hydroxylase, kinase, decarboxylases and transferases, manganese also plays an active role in redox process, tissue respiration, bone formation, growth, reproduction, blood formation and endocrine function (Georgievskii, 1981).

Although manganese is well known as an activator of enzyme systems in many metabolic pathways and as constituent of certain metalloenzymes, it also has been implicated explicitly in synthesis of steroids and hence gonadal hormones. Gonadotropins like HCG may influence manganese transport and modify its availability to different tissues or organs. No definite conclusion on the role of manganese in anoestrum and repeat breeding condition of crossbred cows can be drawn from the results obtained in this study.

Cobalt

The serum cobalt levels were 0.0702 ± 0.0100 ppm in fertile cows as against (0.0641 ± 0.0052 ppm for anoestrus and 0.0795 ± 0.0111 ppm for repeat breeders. Similar trend was observed by Prasad et al. (1989) who reported much lower cobalt values for all the three groups.

Chauhan et al. (1991) has reported the cobalt level in serum to range from 0.04 to 0.63 ppm in animals showing abnormal reproduction.

Cobalt as an integral part of Vitamin B_{12} play an important role in body function including reproductive function. Cobalt is necessary for microbial synthesis of cobalamin and deficiency will lead to impaired synthesis of this vitamin. Selective uptake of free cobalt versus cobalamin by cellular organelles suggests that the metabolic effect of cobalt may not be limited to its requirement for cobalamin synthesis (Georgievskii, 1981). Cobalt deficiency is associated with anaemia and general unthriftness which might indirectly lead to delayed onset of puberty, anoestrum and infertility, supplementation of cobalt help to alleviate higher incidence of suboestrum, anoestrum and poor conception rate (Hidiroglou, 1979).

Since no significant difference in the serum cobalt levels was observed in anoestrus and repeat breeder cows when compared to fertile group, no definite conclusion could be drawn about the role of this element in these two infertility conditions.

Summary

SUMMARY

The objective of the study was to evaluate the levels of phosphorus, copper, zinc, manganese and cobalt in fertile cows and in those which were anoestrus and repeat breeders. The data generated would throw light on the role of these minerals and trace elements in lowering the fertility of crossbred cows in this area.

Serum samples were collected form seventeen fertile, anoestrus and repeat breeder cows each and analysed for serum phosphorus, copper, zinc, manganese and cobalt. Heifers and post partum cows which conceived on the first or second insemination were considered as normal cycling fertile animals (control). Post partum cows which did not come into heat even five months after calving and which had small quiescent ovaries were considered to be anoestrus . Cows which had recurring cycles with no clinical signs of genital abnormality but still did not conceive even with more than two inseminations were considered as repeat breeders.

Inorganic phosphorus was estimated soon after collection of serum by employing modified metol method using phosphorus kits. Serum copper, zinc, manganese and cobalt were estimated by Atomic Absorption Spectrophotometry by using

Perkin Elmer 2380 Atomic Absorption Spectrophotometer. The data so generated were assembled and analysed by standard statistical procedure.

The serum inorganic phosphorus level in normal cycling fertile cows was 7.526 ± 0.5304 mg per cent as against 6.082 ± 0.3337 mg per cent and 6.345 ± 0.4474 mg per cent respectively for anoestrus and repeat breeder cows. There was significant difference ($P<0.05$) in the serum phosphorus level, only between normal cycling fertile and anoestrus animals.

The serum copper in normal cycling fertile cows registered a value of 0.733 ± 0.0511 ppm which was significantly higher ($P<0.01$) than these recorded for anoestrus cows (0.509 ± 0.0591 ppm) and repeat breeders (0.542 ± 0.0415 ppm).

There was no significant difference in the serum zinc levels of fertile, anoestrus and repeat breeder cows. While the fertile cows recorded 1.337 ± 0.1555 ppm, the corresponding values for anoestrus and repeat breeder cows were 1.028 ± 0.0984 ppm and 1.017 ± 0.0654 ppm respectively.

The manganese levels in the serum of control anoestrus and repeat breeders cows were respectively 0.0553 ± 0.0095 ppm, 0.0339 ± 0.0052 ppm and 0.0429 ± 0.0033 ppm.

However there was no significant difference in values between the three groups.

Eventhough the concentration of cobalt in the serum for the three groups were not significantly different the highest value was obtained for repeat breeders (0.0795 $\pm$ 0.0111 ppm) followed by the control (0.0702 $\pm$ 0.0100 ppm) and anoestrus group (0.0641 $\pm$ 0.0052 ppm).

From the results of the study it can be inferred that there is significantly lower serum phosphorus value in anoestrus cows. Similarly the serum copper value in both anoestrus and repeat breeder cows were significantly lower than that in normal cycling fertile animals. With respect to other trace elements no significant difference in the values was obtained between fertile cows and cows with lowered fertility which were either anoestrus or repeat breeder. It can be concluded that trace element deficiency, with possible exception of copper is not prevalent in the crossbred cows in the area of study.

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PHOSPHORUS AND TRACE ELEMENT STATUS OF ANOESTRUS AND REPEAT BREEDER CROSSBRED COWS

By

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ABSTRACT OF A THESIS

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ABSTRACT

The role of phosphorus, copper, zinc, manganese and cobalt in reproductive function was evaluated on the basis of serum values of these elements in fertile, anoestrus and repeat breeder cows.

Serum samples drawn from seventeen cows each of fertile, anoestrus and repeat breeder cows were analysed for serum inorganic phosphorus and trace elements namely copper, zinc, manganese and cobalt. Cows and heifers which conceived with one or two inseminations were considered as fertile animals. Anoestrus and repeat breeder cows were selected based on the breeding history and clinico-gynaecological examination. Serum inorganic phosphorus was estimated by modified metol method using kits. The levels of serum inorganic phosphorus was 7.526 ± 0.5304 mg% in fertile cows as against 6.082 ± 0.3337 mg% for anoestrus cows and 6.345 ± 0.4474 mg% in repeat breeder cows. The level was significantly lower ($P<0.05$) in anoestrus cows than in fertile cows. It can be surmised that hypophosphataemia might be the cause for anoestrum. Though inorganic phosphorus level in serum was lower in repeat breeders than in fertile cows it was not statistically significant. Hence the effect of hypophosphataemia in repeat breeding could not be established with certainty.

Serum copper, zinc, manganese and cobalt were estimated by Atomic Absorption Spectrophotometry by Perkin Elmer-2380 Atomic Absorption Spectrophotometer. Among these trace elements the serum level of copper only was found to significantly vary among fertile, anoestrus and repeat breeder cows. The serum copper in fertile cows registered a value of 0.733 ± 0.0511 ppm which was significantly higher ($P<0.01$) than those recorded for anoestrus (0.509 ± 0.0591 ppm) and repeat breeder cows (0.542 ± 0.0415 ppm). Since liver is a storage organ for copper the serum levels of copper will drop only after depletion of liver storage. It is therefore reasonable to assume that hypocuprosis as evidenced by lower serum values might have contributed to anoestrum and repeat breeding.

The serum zinc, manganese and cobalt levels in fertile cows were respectively 1.337 ± 0.1555 ppm, 0.0553 ± 0.0095 ppm and 0.0702 ± 0.0100 ppm. The corresponding values for anoestrus cows were 1.028 ± 0.0984; 0.0339 ± 0.0052 ppm and 0.0641 ± 0.0052 ppm. These values did not vary significantly from those of fertile cows. Similarly the corresponding values for repeat breeders were recorded to be 1.017 ± 0.0654 ppm, 0.0429 ± 0.0033 ppm and 0.0795 ± 0111 ppm which did not differ significantly from values of fertile cows. Therefore the role of zinc, manganese and cobalt in anoestrum and repeat breeding could not be established.