



Effect of supplementation of area specific mineral mixture on reproductive performance of the cows

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Received: 22 October 2011; Accepted: 6 June 2012

ABSTRACT

Sample of feeds and fodders and serum of cows were collected 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 anoestrous 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). In cows, the mean serum Ca and P levels were lower while the mean Zn, Cu, Mn and Fe were higher than critical level. At the end of experimental feeding, the body weights, metabolic body weights, dry matter intake per kg metabolic body weight, DCP and TDN intake of the animals did not differ significantly. The overall serum mineral levels and biochemical values increased significantly in supplemented group than in un-supplemented group. 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.

Key words: Cows, Mineral supplementation, Reproductive disorder

Reproductive efficiency in dairy cows plays a pivotal role in profitable dairy farming. Improving reproductive efficiency of cows 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 (Judson and McFarlane 1998). 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 (Akhtar *et al.* 2009). Trace elements like copper (Cu), cobalt (Co), zinc (Zn), iron (Fe), selenium (Se), iodine (I), manganese (Mn) and certain macro-elements like Ca, Na, P are very essential for normal livestock growth. 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 (Shinde *et al.*

2009, Prasad *et al.* 2007). Among the infertility problems occurring in cattle, an oestrous and repeat breeding are the most common. On mineral supplementation, improvement in reproductive status was reported by many workers (Newar *et al.* 1998, Hussain *et al.* 2004). The present study was undertaken to find the effect of supplementation of area specific mineral mixture on the reproductive performance of cows.

MATERIALS AND METHODS

An on-farm trial was carried out in the villages namely Baniapada and Gopalpur of Gandia block of Dhenkanal district of Odisha, which is under mid-central table land zone. On the basis of survey work conducted, 80 animals were selected from the 2 villages. All the animals were free from physiological and anatomical disorders. Each animal was examined per rectally to know the status of reproductive organs like cervix, ovary, and uterus. Animals were then allotted to four groups with 20 animals each on the basis of their breeding status. The experimental treatment of the animals was: G₁ comprising 5 crossbred anoestrous heifers, 7 crossbred anoestrous cows and 8 repeat breeding crossbred cows. They were maintained under normal feeding and animal husbandry practices of farmers (control group); G₂,

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20 crossbred Jersey anoestrous heifers supplemented with area specific mineral mixture (ASMM) @ 50 g/day/animal (ANH); G₃, 20 crossbred Jersey an oestrous cows supplemented with ASMM @ 50 g/day/animal (ANC); and G₄, 20 repeat breeding crossbred Jersey cows supplemented with ASMM @ 50 g/day/animal (RBC). The body weight of the cows was recorded at the onset of the experiment and monthly intervals during the experiment using Johnson's formula (1940). Taking the average body weight of the cows to be 400 kg and dry matter intake 6 kg/animal/day the minerals intake were calculated for major minerals, viz. Ca and P, and minor minerals, viz. Zn, Cu and Mn. Out of this analysis the deficient minerals like Ca, P, Zn, Cu and Mn were incorporated as per the requirement of the experimental animals (ICAR 1998) for preparation of ASMM. Though iodine and cobalt were not estimated in the experimental animals, but they were added in the prepared mineral mixture as per ICAR recommendation (1998). Blood samples were collected at the onset of experiment and subsequently at monthly intervals and serum samples were harvested for further analysis in the laboratory. The serum samples were stored in deep freeze at a temperature of -20 °C in properly capped and labeled glass vials for analysis (Oser 1971) of macro- and micro- minerals and blood metabolites, viz. glucose, total protein, albumin, globulin and cholesterol. Samples of soil from the different grazing and cultivated fodder land were collected from each of the identified survey area. The procedure suggested by Jackson (1973) was adopted for collection of soil samples. The soil Ca was analyzed as per Jackson (1973). The soil P was analyzed as per Olsen *et al.* (1954). The soil samples were extracted as per Lindsay and Norvell (1978) for micro-mineral study. Representative samples of pastures grass, agricultural byproducts, feed ingredients, straws and compounded feed were collected either from the grazing land of the village or from the farmers. This prepared mineral extract was used for analysis of Ca and P (AOAC 1995). The mineral extract thus prepared was used for micro-mineral analysis (AOAC 1995) using atomic absorption spectrophotometer. The concentration of Ca in

serum was estimated by using the kit. Data retrieved from the experiment were subjected to statistical analysis wherever required. The statistical analysis of the data was done according to Snedecor and Cochran (1994).

RESULTS AND DISCUSSION

Mineral status of soil, feeds, fodders and cows: The mineral status of the soil, feed and fodder, and blood serum of the cows in Mid-central table land zone of Odisha are presented in Table 1. The average soil Ca concentration (0.15%) of the analyzed samples of the surveyed area was marginally above the reported critical level whereas the average P concentration in the soil was below the reported critical level (Jackson 1973). The lower P level as observed in the present study of the soil of the surveyed areas could be attributed to low pH of the soil (Singh *et al.* 2011) and the ionic acidity of soil might be induced the formation of complex with iron and thereby lowered the availability of inorganic P. The average Zn content of the soil of the analyzed samples of the surveyed area was higher than the critical levels as suggested by Rojas *et al.* (1993). The reported mineral concentration of the soil is in agreement with the values reported by Singh *et al.* (2011). Wheat bran and maize contained marginally higher P than that of critical level. Garg *et al.* (2008) and Singh *et al.* (2011) reported similar range of P in maize and wheat bran as observed in the present study. The Zn concentration of paddy straw, maize, wheat bran and *mung chuni*, was marginally higher than the critical level (Prabowo *et al.* 1990). The reason for higher Zn content in all the feed ingredients might be transfer of Zn from plant segment to seeds, was quite substantial (Garg *et al.* 2002). The Cu content of paddy straw, maize and wheat bran was marginally higher, while *mung chuni* and mixed grass contained higher level of Cu than critical level (8 ppm).

The average serum mineral levels of the surveyed cows were deficient in Ca (69.1%) and P (83.3%). Though, the average levels of serum Zn, Cu and Mn were above the critical level, still 29.1%, 44% and 17%, respectively of surveyed cows contained lower levels. Lower serum ca levels

Table 1. Mineral status of the surveyed area of mid-central table land zone

Attributes		Ca	P	Zn (ppm)	Cu (ppm)	Mn (ppm)	Fe (ppm)
Soil	Mineral status	0.15 *±0.005	25.11\$±0.73	0.73±0.02	2.83±0.05	53.20±1.07	63.70±.12
	CL	0.1*	45–130\$	0.6	0.3	5.0	2.5
Feed and fodder	Paddy straw	0.23 *±0.77	0.04 *±0.003	33.06±1.02	9.47±0.45	25.26±0.77	138.42±2.18
	Maize	0.27 *±0.01	0.40 *±0.01	49.46±1.87	8.98±0.48	27.68±1.70	146.94±1.96
	Wheatbran	0.20 *±0.01	0.25 *±0.02	48.63±1.37	7.53±0.55	46.43±1.78	151.09±2.66
	<i>Mung chunni</i>	0.36 *±0.02	0.32 *±0.02	48.97±3.04	15.24±1.42	42.83±2.86	209.10±9.01
	Mixed grass	0.47 *±0.03	0.33 *±0.01	36.13±2.41	11.46±0.86	34.17±2.78	244.26±11.02
	CL	0.3*	0.25*	40	8	30	50
Serum	Mineral status	6.55#±0.13	3.09#±0.08	0.88±0.02	0.75±0.01	0.29±0.01	2.17±0.06
	CL	8.0#	4.5#	0.6	0.65	0.2	1.0

*Percentage, # mg/dl, \$ ppm, †McDowell (1985), CL- Critical level†.

(Singh *et al.* 2011), P level (Sharma and Joshi 2004 and Singh *et al.* 2011), Zn (Sharma and Joshi 2004), Cu (Singh *et al.* 2011) and Mn (Singh *et al.*, 2011) were reported by different workers.

Effect on serum mineral status: The overall mean serum mineral content of experimental cows are presented in Table 2. Before experimental feeding period, the average serum Ca levels of all the groups were below the critical level (Mc Dowell 1985). On supplementation of mineral mixture the average serum Ca level of all the treated groups were above critical level. The overall serum Ca levels of G₁, G₂, G₃ and G₄ differed significantly ($P<0.01$), with higher Ca level in supplemented than un-supplemented group. This corroborated with the findings of Samanta *et al.* (2005). The Ca level in the serum might be one of the reasons of lower fertility status of the experimental animals as Ca dependant mechanisms are involved in steroid biosynthesis in testes, adrenal gland ovaries and (Bodesta *et al.* 1980). Also gonadotropin releasing hormone stimulation of LH released from pituitary cells involves a Ca dependant mechanism (Naor and Catt 1981) and a Ca deficiency would impede LH release resulting in anoestrous condition. In addition to this, reduced blood Ca level may delay uterine involution and increased incidence of dystocia, retained placenta and prolapse of uterus (Hurley and Doane 1981). The overall serum P concentration of all the treated groups were significantly ($P<0.05$) higher than the control group. The present findings corroborated with the observations of Das *et al.* (2002) and Samanta *et al.* (2005). The significant lower level of serum P might be one of the reasons of these reproductive disorders as the P deficiency is most prevalent cause affecting reproduction (Salisbury and Vandermark 1961). Anoestrus, sub estrus, irregular and delayed sexual maturity are reported to be the consequence of P deficiency. Hypophosphotemia adversely affects the cell functions, as P is an integral component of nucleic acid, nucleotides, phospholipids and some protein. P is essential for transfer and utilization of energy, phospholipid metabolism and large number co-enzyme activation (Hurley and Doane 1989). The Ca and P concentration in the soil of Dhenkanal was deficient

Table 2. Average serum mineral concentration of experimental animals supplemented with or without area specific mineral mixture

Parameters	Group 1 (control)	Group 2 (ANH)	Group 3 (ANC)	Group 4 (RBC)
Ca (mg/dl)	6.35 ^a ±0.04	7.70 ^b ±0.08	7.94 ^c ±0.08	8.11 ^d ±0.08
P (mg/dl)	3.24 ^a ±0.06	3.94 ^b ±0.07	4.00 ^b ±0.07	4.02 ^b ±0.07
Zn (PPM)	0.87 ^a ±0.01	1.73 ^b ±0.04	1.77 ^b ±0.05	1.88 ^c ±0.05
Cu (PPM)	0.73 ^a ±0.01	1.02 ^b ±0.02	1.66 ^c ±0.02	1.22 ^d ±0.02
Mn (PPM)	0.32 ^a ±0.005	0.45 ^b ±0.01	0.50 ^c ±0.01	0.54 ^d ±0.01

Values bearing different superscripts in rows varied significantly ($P<0.01$).

in Ca and P and this could lead to low level of P in the feed ingredients and roughages.

The serum Zn and Mn concentrations of cows in all the groups before supplementation were nearly same and the average values were above their respective critical levels. Probably, deficiency of Zn and Mn might not be the exclusive cause of reproductive disorder in the present study as Zn plays an important role in male fertility rather than female fertility (Hidiroglou 1979). In spite of higher average serum Mn level, low serum Mn level was observed in 8.3% of cows of this study. The present observation of significantly ($P<0.05$) higher level of Zn and Mn in supplemented groups than un-supplemented animals corroborated with the findings of Samanta *et al.* (2005). Tambe *et al.* (1998) also reported increase in serum Mn concentration due to mineral supplementation. In contrast to this no significant increase in serum Mn level was observed in supplemented group than un-supplemented group by Upadhyaya (2004). The serum Cu level before the experimental period of all the groups of animals were almost similar, whereas on supplementation of mineral mixture, the serum Cu level increased significantly ($P<0.05$) in supplemented group than un-supplemented group and also there was significant ($P<0.05$) differences observed among all the supplemented groups. Considering the higher percentage of animals deficient in Cu (44%) in the initial period of survey of this study implied that lower Cu level in the serum might be one of the reasons for the incidence of reproductive disorder as deficiency of Cu has an effect on physiological function in general and reproductive function in particular and might be associated with delayed or depressed oestrous (Hidiroglou 1979). Cu is an integral part of enzyme component and the catalyst involved in steroidogenesis and prostaglandin synthesis and its deficiency lead to low fertility, delayed/depressed oestrus, abortion/foetal resorption (Smith and Akinbamijo 2000). The present observations of significantly higher level of serum Cu in

Table 3. Overall blood metabolic status of experimental animals supplemented with or without area specific mineral mixture

Parameters	Group 1 (control)	Group 2 (ANH)	Group 3 (ANC)	Group 4 (RBC)
Glucose (mg/dl)	35.15 ^a ±0.45	40.88 ^b ±0.48	42.14 ^c ±0.61	43.80 ^d ±0.66
Total protein (g/dl)	4.87 ^a ±0.06	6.22 ^b ±0.09	6.36 ^b ±0.09	6.60 ^c ±0.11
Albumin (g/dl)	2.54 ^a ±0.03	3.01 ^b ±0.04	3.21 ^c ±0.04	3.21 ^c ±0.04
Globulin (g/dl)	2.33 ^a ±0.07	3.21 ^{bc} ±0.07	3.14 ^b ±0.08	3.39 ^c ±0.09
Cholesterol (mg/dl)	94.64 ^a ±0.7	131.66 ^b ±2.90	135.10 ^b ±2.46	134.95 ^b ±2.47

Values bearing different superscripts in rows varied significantly ($P<0.01$).

Table 4. Reproductive performances of experimental animals

Type of animals	Total no. of animals	No. of animals came to heat	No. of experimental days to come to first heat	Services per conception	No. of animals conceived	Post partum oestrous period	Percentage of rate of success of pregnancy
Control	20	2	120 ^a ±19	3 ^a	1	140 ^a	5
ANH	20	9	51.88 ^b ±4.64	1.37 ^b ±0.18	8	—	40
ANC	20	14	71.35 ^c ±6.02	1.50 ^b ±0.18	8	126.12 ^b ±4.98	40
RBC	20	12	66.83 ^c ±4.34	1.57 ^b ±0.20	7	119.57 ^b ±5.71	35

Values bearing different superscripts in rows varied significantly ($P<0.01$).

supplemented conceived animals than un-supplemented animals corroborated with the findings of Upadhyay (2004).

Effect on serum biochemical status: The overall metabolic status of the experimental cows is presented in Table 3. The mean serum glucose level of all the groups before the experimental supplementation period was in the same range and below the normal level as reported by Kaneco *et al.* (1997). Energy nutrition has a strong influence on pregnancy rate in lactating cows as well as heifers (Randel 1990). Mc Clure (1965) observed that variations in blood glucose were clearly linked to estrus cyclicity and fertility. High blood glucose level increased the progesterone production directly by increasing LH pulse and mean concentration of LH (Richards *et al.* 1989) and indirectly by increasing blood insulin level which stimulated progesterone secretion from leuteal cells (Mc Ardle and Holtfort 1989). On mineral supplementation, there was significant increase in the serum glucose level in the entire supplemented group than un-supplemented group. The higher glucose level in all the supplemented groups might be due to the fact that minerals are either the cofactor and/or activator of many enzymatic systems associated with the metabolism of nutrients. The serum protein, albumin and globulin levels of all the experimental groups were almost similar on the 0 month of the experimental period. Protein has a certain role for expression of oestrous signs in cows (Patil and Deshpande 1979). On mineral supplementation, the average serum protein, albumin and globulin levels of supplemented groups were higher than the un-supplemented group. These findings corroborated with that of Samanta *et al.* (2005) and they observed significant improvement of protein level on mineral supplementation. The role of mineral for improvement of serum protein level is mostly limited to the better availability of P in supplemented than un-supplemented group, as P plays an significant role in amino acid and protein synthesis (Underwood and Suttle 1999). On mineral supplementation, there was significant ($P<0.05$) higher average serum cholesterol level in supplemented groups than un-supplemented group. Similar findings were reported by Sharma *et al.* (1984). Presence of higher serum cholesterol in supplemented groups corroborated with the findings of

Samanta *et al.* (2005).

Effect on reproductive status: The reproductive performances of experimental animals are presented in Table 4. During the experimental period the pregnancy rate of the control, ANH, ANC and RBC animals were 5, 40, 40 and 35%, respectively. There was significant ($P<0.01$) reduction in number of experimental days required for the animals to come to heat between the unsupplemented and supplemented groups. Among the supplemented groups, ANH group responded at earlier days of mineral supplementation on the onset of oestrous than that of ANC and RBC group. Similarly, the services per conception was significantly ($P<0.01$) higher in unsupplemented group than that of supplemented groups. The post partum oestrous period was also significantly ($P<0.01$) lower in supplemented groups than the un-supplemented group. The present findings of conception/pregnancy rate on supplementation of mineral mixture to anoestrus cows are in agreement with the findings of many workers (Samanta *et al.* 1995, Vhora and Dindokar 1997, Lall *et al.* 2000, and Upadhyay 2004). The pregnancy percentage ranged from 66.66 to 90% by different workers on mineral supplementation, which was observed to be higher than the pregnancy percentage observed in the present study. This might be due to difference in mineral availability to the animals under study as it depends on agro-climatic condition and cropping pattern of the region. In addition to the deficiency of minerals, the principal deficiency influencing reproduction is general nutrition due to feed shortage and poor quality feed (Randel 1990). Diets that are low either in protein or energy lowers the reproductive performance in animals (Randel 1990). However, the improvement in reproductive performance due to mineral supplementation when compared to the performance of unsupplemented group was very clear.

ACKNOWLEDGEMENT

Authors are highly obliged to the All India Coordinated Research Project on "Improvement of feed resources and nutrient utilization in raising animal production" of ICAR, New Delhi, for providing all financial support to carry out this research work.

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