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Effect of Mineral Mixture Supplementation on Haemato-biochemical Parameters of Anoestrus Cows in Winter and Summer Season

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ABSTRACT

Background: livestock is mainly reared under a crop livestock integrated farming system and mineral supplementation is not common practice. Out of all nutrients, minerals and vitamins play a crucial role in metabolism, lactation, reproduction and even for microbial fermentation in rumen. Considering the mineral deficiencies in particular zones, the area specific mineral mixtures (ASMM) were formulated for various zones and also found various limiting nutrients in different zones of MP. The current study was planned to find out the effect of mineral mixture supplementation on body weight, body condition score and haemato-biochemical profile of non-descript anoestrus cows.

Methods: A total-forty-eight animal were selected out of the surveyed animals and divided into four groups (n=12) *viz.* T1 (Normal cyclic animals), T2 (Anoestrus Control), T3 (True anoestrus) and T4 (Sub-oestrus). The experiments were carried out in two seasons (summer and winter). All physiological and hemato-biochemical parameters were recorded and analyzed as per standard mentioned protocol.

Result: According to the findings of this experiment weight gain the animals of the true anoestrus and sub-oestrus group's recorded highly significant increases in the winter and summer seasons. The haemoglobin concentration was within the normal range and it was non-significant in all groups except normal cyclic animal (T1) in winter and true anoestrous (T3) in summer after the mineral supplementation. There was no significant difference in the WBC count of all groups except in sub-oestrous control (T2S) and T1 in the winter season. RBC concentration was non-significant in almost all groups. Erythrocytic indices were the normal limits and did not differ significantly in the majority of groups except significant difference showed are MCHC in T3, MCH in T2S, MCV in T1 of winter seasons and were within normal range in all supplemented groups in both. The serum cholesterol concentration showed significantly higher value in the winter and summer season of T3 group. However, in sub-estrous (T4 group) animals, the values in the winter season were significantly higher. We observed significantly higher values of triglycerides in T3 and T4 groups in winter and T3, T4 and T1 groups in summer seasons. The above results indicate the beneficial effects of mineral mixtures in supplement groups. Studies conclude that the supplementation of mineral mixtures can improve reproductive efficiency in non-descript anoestrus cows.

Key words: Anoestrous, Body condition score, Mineral mixture supplementation, Non-descript.

INTRODUCTION

In India, livestock is mainly reared under a crop livestock integrated farming system and mineral supplementation is not common practice. Out of all nutrients, minerals and vitamins play a crucial role in metabolism, lactation, reproduction and even for microbial fermentation in rumen (Bhandari, Garg *et al*., 2014). The majority of animals were maintained on grazing resources with little amount of supplementation in the form of agricultural by-products or cultivated or natural fodder.

Considering the mineral deficiencies in particular zones, the area specific mineral mixtures (ASMM) were formulated for various zones and also found various limiting nutrients in different zones of MP. The limiting nutrients in Vindhya Plateau of Madhya Pradesh are P, Zn and Fe (Baghel *et al*., 2013). By providing area specific mineral mixture *i.e*. ASMM (Ca, P, Cu, Zn and Mn) and iodized salt through concentrate mixture for a period of 30 days to anestrous crossbred heifer, it was found that the rate of expression of estrus and conception was 92.16% and 97.38%, respectively(Puvarajan and Vijayarajan, 2013).

MATERIALS AND METHODS

The present field study was planned to find out the effect of mineral mixture supplementation on body weight, body

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condition score and haemato-biochemical profile of non-descript anoestrus cows in Rewa district of Madhya Pradesh.

A total-forty-eight animal were selected out of the surveyed animals based on two per rectal examination at the 11 day interval and two progesterone level estimates at the 14 day interval. The animals were selected and divided into four groups (n=12) *viz.* T1 (Normal cyclic animals), T2 (Anoestrus Control), T3 (True anoestrus) and T4 (Sub-oestrus) on the basis of body weight, milk yield, parity and stage of lactation. The anoestrus control animals comprise six true anoestrus and six sub-oestrus animals. The experiments were carried out in two seasons; for winter December 2015 to January 2016 (60 days) and for summer March 2016 to April 2016 (60 days) in Rewa district of Madhya Pradesh. These cows were entirely maintained by farmers themselves with close supervision by us. Body weight was determined on the basis of heart girth by using the Shaffer's formula (Shastry *et al*., 1982).

Body condition scores (BCS) are subjective, visual or physical assessment of the amount of metabolizable energy stored in the fat and muscle in a live animal. The body condition is usually judged through a five-point scale, with one equivalent to an extremely lean cow, while five to a cow having excessive fat reserves (Peters and Ball, 1987).

Blood samples were collected (before and after supplementation study) from selected cattle from different group of Rewa district of Madhya Pradesh. About ten ml blood sample from each animal were collected aseptically from jugular vein in a sterilized plastic tubes containing anti-coagulant heparin solution (0.2 mg/ml of blood) for haematological parameters. One ml of blood sample was treated with sodium fluoride (10 mg/ml) for glucose estimation.

Haemato-biochemical and mineral profiles of animals were studied to identify deficiencies at college of veterinary sciences and AH, Jabalpur (MP). Haematological parameters in blood samples were determined by using Auto blood cell counter (Medonic CA-530 Thor-20). However, DLC was done as per the standard protocol described by Feldman and Jain (2002). The following biochemical parameters were estimated using a serological auto-analyzer. Glucose was estimated by the method of Hutman as described by Pileggi and Szustkiwiecz (1974). Total proteins (albumin and globulin) were estimated by the Biuret method (Tietz, 1986). The amount of albumin in the plasma was estimated after precipitating the albumin with 28 per cent sodium sulphite and quantified by the Biuret method (Tietz, 1986). The globulin content was calculated by subtracting the sum of albumin content from the total protein. Total cholesterol was determined by the method described by Roeschlau *et al*. (1980). Triglycerides were estimated by the Wako method modified by Fossati and Lorenzo (1982) by using commercially available standard kits (Erba).

The animals in different groups were supplemented with a strategic mineral mixture @ 30 gm/day/animal for 60 days. Blood samples were analyzed prior to supplementation and then at the end of 60 days for different haemato-biochemical profiles of animals. This study was carried out in two seasons (summer and winter).

RESULTS AND DISCUSSION

The results of supplementation of mineral mixture on various blood-biochemical and hormonal profile of true anoestrous cows in winter season (December 2015-January 2016) (Table 1) were shows significant ($p<0.05$) increase in body weight of animals (217±8.42) (210.33±9.15), BCS was non-significantly high (2.25±0.13) (2.13.33±9.15), significant increase in total cholesterol (138.91±3.40) (133±3.34), triglycerides was significantly high (11.0±0.96) (9.13±0.87), total protein was non-significantly high (7.05±0.77) (6.69±0.75), albumin was significantly low (0.85±0.27) (1.08±0.30), globulin was significantly high (4.59±0.62) (3.26±0.52), A/G ratio was non-significantly low (1.07±0.30) (1.08±0.30), blood sugar was non-significantly low (67.83±2.38) (71.91±2.45), WBC shows non-significant increase (8.91±0.86) (8.50±0.84), lymphocytes per cent was significantly high (66.37±2.35) (50.19±2.05), monocytes per cent was significantly low (2.30±0.44) (4.25±0.60), RBC was non-significantly low (5.03±0.70) (5.80±0.70), haemoglobin was non-significantly low (8.16±0.83) (8.41±0.84). However, animals showed highly significant decrease in globulin, A/G ratio and the parameters showed non-significant differences of WBC, lymphocytes percent, monocytes percent, RBC numbers, haemoglobin concentration. The majority of parameters in control and normal cyclic animals showed non-significant differences.

The results of before and after supplement of various blood-biochemical profile of true anoestrus cows in summer season (March-April 2016) (Table 2) showed significant increase in triglycerides, RBC and haemoglobin, whereas highly significant differences were seen in total cholesterol, total protein, albumin, blood sugar, whereas non-significant differences were seen in body weight, BCS, globulin, A/G ratio, WBC numbers. Animals belong to sub-oestrus group (T4) showed significant increase in blood sugar and haemoglobin concentrations, whereas parameters that showed significant differences were body weight, triglycerides, total proteins, albumins, RBC numbers. The non-significant differences were found in BCS, total proteins, Globulin, A/G ratio and WBC numbers. The majority of parameters in control and normal cyclic animals show non-significant differences.

The data of average body weight gain showed that the animals of the true anoestrus and sub-oestrus groups had recorded highly significant increases in the winter and summer seasons. However, there was no significant difference in body weight gain amongst different control groups. It may be due to enhanced feed conversion ratio, body condition score, health and immune status and improved metabolic rate after mineral supplementation.

There was not any significant difference in body condition score in all experimental groups (T3 and T4 groups of both seasons), as body condition score depends on various factors like management, housing energy protein ratio in diet and feeding practices *etc.* A constant body condition score is related with the cow's potential to produce milk while at the

Table 1: Haemato-biochemical profile of different groups in winter season (December 2015- January 2016).

Parameters	Normal cyclic animals (n=12)		Anestrus control animals (n=6)		Anestrus treatment animals (n=12)		Sub-oestrus control animals (n=6)		Sub-oestrus treatment animals (n=12)	
	Day 0	Day 60	Day 0	Day 60	Day 0 pre-treatment	Day 60 post-treatment	Day 0	Day 60	Day 0 pre-treatment	Day 60 Post-treatment
Body weight (kg)	213.41±10.0	216.58±8.95	182.66±10.01	181.66±9.92	210.33±9.15	217.33±8.42**	191.5±5.63	192.16±5.51	208.25±4.17	210.25±4.19**
BCS	2.20±0.17	2.25±0.13	1.83±0.11	1.83±0.11	2.13±0.16	2.25±0.13	1.91±0.08	1.91±0.08	2.29±0.44	2.29±0.44
Total cholesterol (mg/dl)	109.09±9.18	112.07±8.12	94.90±5.21	95.32±8.48	133.91±3.34	138.91±3.40**	117.81±13.96	126.39±11.13	136.23±3.37	142.54±3.45*
Triglycerides (mg/dl)	9.84±0.91	9.85±0.91	6.18±1.02	6.16±1.01	9.13±0.87	11.00±0.96**	5.73±0.98	5.79±0.98	11.90±1.00	12.66±1.03**
Total protein (g/dl)	6.90±0.35	7.46±0.34**	5.61±0.35	5.53±0.43	6.69±0.75	7.05±0.77	6.76±0.32	7.17±0.32**	6.93±0.76	7.81±0.81**
Albumin (g/dl)	3.63±0.18	3.75±0.18*	2.91±0.20	3.06±0.18	1.08±0.30	0.85±0.27*	3.57±0.16	3.75±0.16	3.45±0.54	3.41±0.53
Globulin (g/dl)	3.26±0.19	3.70±0.19**	2.69.±0.22	3.12±0.25*	3.26±0.52	4.59±0.62**	3.17.±0.23	3.42±0.22*	3.47±0.54	1.06±0.61**
A/G ratio	1.12±0.04	1.01±0.04*	1.10±0.09	0.99±0.05	1.08±0.30	1.07±0.30	1.14±0.07	1.11±0.07	1.01±0.29	0.82±0.26**
Blood glucose (mg/dl)	70.83±2.83	71.83±2.89*	50.33±1.26	53.50±1.61**	71.91±2.45	67.83±2.38	62.16±3.41	65.33±3.58	58.41±2.21	59.16±2.22
WBC (× 1000/µl)	10.65±0.41	10.67±0.41*	6.49±0.87	6.67±0.82	8.50±0.84	8.91±0.86	6.91±1.06	7.00±1.06*	9.82±0.90	9.98±0.91
Neutrophils (%)	56.82±3.79	56.65±3.67	47.24±7.41	40.86±9.02	46.68±1.95	28.46±1.54**	47.53±2.81	47.53±2.81	56.27±2.17	43.98±1.91*
Lymphocytes (%)	40.45±1.84	40.37±1.83	48.46±6.47	54.56±8.09	50.19±2.05	66.37±2.35**	51.00±2.92	50.60±0.89**	41.86±1.87	51.30±2.07
Monocytes (%)	2.87±0.89	2.95±0.92	3.4±1.34	2.76±1.18	4.25±2.05	2.3±0.44*	4.85±0.90	4.85±0.89	2.02±0.41	1.47±0.35
RBC (millions/µl)	6.21±0.23	6.25±0.23*	5.53±0.59	5.15±0.54	5.80±0.84	5.03±0.70	5.98±0.35	6.04±0.36	5.69±0.85	5.65±0.69
Hb (g/dl)	9.25±0.26	9.65±0.24*	8.53±0.80	8.00±0.87	8.41±0.84	8.16±0.83	8.4±0.32	8.5±0.36	8.60±0.85	8.81±0.86
MCV (fl)	47.00±0.65	48.16±0.56**	48.00±0.86	46.66±1.20	47.41±1.99	43.83±1.91	48.00±1.21	48.33±1.09	46.50±1.97	49.50±2.03
MCH (pg)	15.41±0.50	15.44±0.50	16.55±1.08	15.73±1.05	14.52±1.10	13.9±1.08	14.20±0.25	14.20±0.31*	15.16±1.12	15.68±1.14
MCHC (g%) or g/dl	32.71±0.89	32.71±0.88	32.71±1.86	32.33±1.96	30.64±1.60	31.71±1.63*	30.35±0.33	30.25±0.39	32.58±1.65	32.57±1.65

*****Significant ($p<0.05$); **Highly significant ($p<0.01$); NS- non-significant.

Table 2: Haemato-biochemical profile of control and treatment groups in summer season (March-April 2016).

Parameters	Normal cyclic animals (n=12)		Anestrus control animals (n=6)		Anestrus treatment animals (n=12)		Sub-oestrus control animals (n=6)		Sub-oestrus treatment animals (n=12)	
	Day 0	Day 60	Day 0	Day 60	Day 0 pre-treatment	Day 60 post-treatment	Day 0	Day 60	Day 0 pre-treatment	Day 60 post-treatment
Body weight (kg)	212.5±10.15	213.41±9.77	182.5±9.67	183.33±9.87	213.67±9.88	213.75±9.37	191.16±5.20	191.83±5.45	207.91±15.67	209.33±15.69**
BCS	2.29±0.19	2.25±0.19	1.83±0.11	1.83±0.11	2.33±0.17	2.37±0.16	1.83±0.11	1.91±0.08	2.30±0.27	2.33±0.26
Total cholesterol (mg/dl)	108.35±7.91	110.96±7.85**	94.13±7.03	95.51±6.93**	133.60±15.07	136.22±14.80**	129.39±11.33	128.30±11.29**	138.75±11.97	140.74±11.75
Triglycerides (mg/dl)	9.00±0.55	9.37±0.59*	6.46±0.61	6.45±0.62	9.60±0.66	10.94±0.48*	6.80±0.40	6.76±0.41	10.26±0.56	11.03±0.55**
Total protein (g/dl)	8.03±0.55	7.62±0.17**	7.38±0.21	6.57±0.18**	7.54±0.39	6.62±0.40**	7.19±0.23	6.70±0.23**	8.39±0.31	7.54±0.23**
Albumin (g/dl)	3.70±0.21	3.83±0.23	3.05±0.15	2.98±0.12	3.49±0.24	2.96±0.25**	3.66±0.30	3.35±0.22	4.13±0.25	3.73±0.26**
Globulin (g/dl)	3.70±0.16	3.78±0.17	3.58±0.16	0.84±0.05**	3.74±0.21	3.65±0.18	3.34±0.13	3.35±0.14	3.81±0.10	3.80±0.10
A/G ratio	1.06±0.12	1.06±0.11	0.84±0.05	0.84±0.05	0.81±0.05	0.80±0.05	1.01±0.08	1.00±0.08	1.00±0.09	0.99±0.09
Blood glucose (mg/dl)	70.25±2.78	70.75±2.96	54.33±1.36	55.16±1.14	64.83±1.77	67.58±1.48**	67±3.64	69±3.70	60.08±2.52	62.08±2.12*
WBC (× 1000/µl)	10.46±0.45	10.51±0.38	7.08±0.81	7.28±1.73	8.19±0.57	7.99±0.54	7.40±1.27	7.48±1.21	9.56±0.47	9.89±0.35
Neutrophils (%)	56.70±3.69	56.62±4.41	40.86±9.02	40.95±9.10	25.04±2.14	25.20±2.09	47.53±3.68	45.63±4.45	43.48±6.43	44.98±5.82
Lymphocytes (%)	40.21±2.94	41.46±3.81	54.73±8.16	54.90±8.04	64.59±2.66	70.25±2.08	50.11±2.99	50.16±4.07	52.39±4.99	54.54±5.47
Monocytes (%)	2.48±0.88	2.50±0.88	2.76±1.18	2.71±1.23	2.30±0.41	2.29±0.42	4.85±1.19	5.03±1.30	4.47±0.29	1.45±0.29
RBC (millions/µl)	6.63±0.19	6.75±0.12	5.15±0.40	5.40±0.36	6.16±0.15	6.87±0.25*	6.30±0.29	6.39±0.32	5.97±0.28	6.46±0.24**
Hb (g/dl)	9.78±0.21	9.81±0.21	7.81±0.77	8.08±0.71	8.25±0.28	8.81±0.28*	8.76±0.28	8.83±0.24	8.78±0.33	9.11±0.28*
MCV (fl)	47.25±0.60	48.16±0.56	46.66±1.20	47.33±1.12	44.08±0.98	43.83±1.25	48.33±1.09	49.16±1.40	49.58±1.03	49.5±1.05
MCH (pg)	15.40±0.50	15.44±0.50	15.63±1.03	15.73±1.05	13.99±0.34	13.90±0.36	14.2±0.25	14.25±0.30	15.62±0.55	15.68±0.55
MCHC (g%) or g/dl	32.73±0.85	32.71±0.88	32.33±1.96	32.18±1.83	31.69±0.29	31.72±0.32	30.35±0.33	30.28±0.31	32.53±1.11	32.57±1.11

*Significant ($p<0.05$); **Highly significant ($p<0.01$); NS- non-significant.

same time maintaining its energy balance. BCS correlated positively with fat and protein and negatively with milk yield.

The serum cholesterol concentration showed significantly higher value in the winter and summer season of True anoestrous (T3 group). However, in the sub-oestrous (T4 group) animals, the values in the winter season were significantly higher, but in summer they were not significant. In the control groups T1 and T2 (T2TA and T2S) groups, in the winter season, it was non- significant, but in the summer season it was significant. Similar results were reported by Engle *et al*. (2001). This may have ramifications related to the improvement in reproductive performance of dairy cattle. The result is contradictory. In another study, the serum cholesterol level was not influenced significantly due to dietary supplementation of minerals among the different groups (Saxena *et al*., 2010). The above results indicate beneficial effects of mineral mixtures in supplement groups.

Blood haemoglobin is an indicator of erythrocytic normal level and general well being of animals. The haemoglobin concentration was within the normal range and it was non-significant in all groups except normal cyclic animal (T1) in winter and true anoestrous (T3) in summer after mineral supplementation. There was not any significant difference in the WBC count of all groups except significant in sub-oestrous control (T2s) and normal cyclic animal (T1) of winter season. RBC concentration was statistically non significant in all groups except in group T1 winter and T3 summer but the values were in the normal range.

Erythrocytic indices in present study were in normal limits and did not differ significantly in majority of groups except significant difference showed by MCHC in T3 , MCH in T2S, MCV in T1 of winter seasons and were within normal range in all supplemented groups *i.e.* T3 and T4 of both seasons may be due to mineral mixture supplementation. These values are helpful in classification of different types of anaemia.

Blood glucose concentration was statistically ($p<0.05$) similar in all the groups except significant in T_{2TA} and T_1 winter and T3 summer. An increased or decreased level of blood glucose level is an indicator of stress to the animals. However, in present study, analogous glucose level indicates normal physiological condition of all the experimental animals throughout the experimental period.

In the present study, serum total proteins was differ significantly ($p<0.01$) among different dietary treatments. However, non-significant increase was recorded in T_3 and T_{2TA} groups in winter season. Findings of present study were in close agreement to the findings of Amle *et al*. (2014). Higher serum total protein might have been associated with infectious process *i.e.* mastitis, metritis or had improved because of dietary intake of nutrients.

The mean plasma albumin was statistically similar in all groups except significant increase in T3 and T1 of winter and T3 and T4 of summer seasons. Marginally lower values of albumin have been reported by other research workers (Singh *et al*., 2002 and Sharma, 2004).The total plasma globulin concentration differed significantly ($p<0.01$) among different dietary treatments due to higher total plasma protein concentration in the animals. The mean plasma A: G ratio did not differ significantly ($p<0.05$) among different dietary treatments except it is significant in T1 and T4 of winter season.

CONCLUSION

There is significant increase in majority of hemato-biochemical, mineral and hormonal parameters of animals supplemented with mineral mixture in winter and summer season. Improvement in animal performance is indicative of beneficial effects of mineral mixture supplementation under field condition. It can be concluded that, supplementation of mineral mixture can improve reproductive efficiency in non-descript anoestrus cows.

Conflict of interest: None.

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