

Effect of dietary supplementation of copper and phosphorus on blood mineral status and biochemical profile in growing crossbred heifers

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ABSTRACT

Crossbred heifers (16) of 12 to 16 months age and an average body weight of 155 kg were divided into 4 groups of animals each in a 2 × 2 factorial completely randomized design. Four respective dietary treatments in the form of mineral (feed) mixture containing copper sulphate to supply copper (Cu) and dicalcium phosphate to supply phosphorus (P) along with the concentrate mixture and *ad lib.* mixed fodder (green oats + oats straw) were provided to all the animals to discern the effect on feed intake, weight gain and blood mineral status and certain serum biological constituents. The heifers in treatment group 1 were fed ration without Cu and P supplements, whereas heifers in treatment groups 2 and 3 were supplemented with Cu and P, respectively, and in treatment group 4 were supplemented with Cu and P both. Feeding trial lasted for 90 days. Blood samples collected at 0, 45 and 90 days of feeding trial were analyzed for Cu and P and certain biochemical constituents in the blood serum. Cu concentration in blood serum was significantly highest in treatment group 2 (Cu supplemented) amongst all the groups at 45 and 90 days. The overall mean Cu concentration in blood serum of heifers was significantly improved due to dietary supplement of Cu. The serum P concentration at 45 and 90 days of feeding trial, differed significantly among the different groups. The overall mean P concentration significantly improved in the heifer due to dietary supplementation of P. The feed intake and weight gain were not significantly influenced due to dietary supplementation of either Cu or P or their interaction; however, heifers supplemented with Cu gained more weight than other groups. The SGPT and SGOT activities were significantly higher in heifers of group 4 (Cu and P supplemented) than other groups. The dietary Cu and P had significant effect in improving the SGPT and SGOT activities. The blood serum total protein, albumin and globulin and blood glucose concentrations were in the normal range and did not differ significantly among the groups and were not influenced due to Cu and/or P supplementation. Serum total cholesterol concentration was higher in heifers of treatment group 2 supplemented with Cu and lowest in the control group but there was no significant effect of Cu or P supplements. Thus Cu and P supplementation is inevitable for ruminant animals in Plain region of Uttarakhand to maintain the blood biochemical constituents and Cu and P status under field as well as under farm condition.

Key words: Blood biochemical profile, Copper, Crossbred heifers, Feed, Phosphorus

Phosphorus (P) has more important functions than any other macro element and is required by rumen microorganism for digestion of cellulose (Burroughs *et al.* 1951), synthesis of microbial protein (Breves and Schroder 1991) and absorption and metabolism of macronutrients in animals. P deficiency causes unthriftiness, inappetence, poor productive performance and impaired fertility (Golf 1998). On the other hand, copper (Cu) is an essential trace element for ruminants which functions as a component of a number of enzyme system. Copper is usually supplemented as sulphate, carbonate

or oxide forms. The essentiality of minerals for growth, health, reproduction as well as normal physiological functions of animal body is well documented by Chew (2000). In plain area of Uttarakhand, cattle and buffaloes are mostly reared on crop residues and dry grasses along with locally available green fodder and small amount of concentrate mixture. Generally, these animals do not receive mineral mixture supplementation and have shown deficiency of P and Cu (Tiwarly *et al.* 2007). In a field study, a significant improvement was observed on the productive and reproductive performance of various categories of cattle due to dietary supplementation of Cu and P (Saxena *et al.* 2008). Keeping in view the present study was carried out in growing crossbred heifers under farm condition to assess the effect of dietary supplementation of Cu and P on feed intake, body

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weight gain and blood Cu and P status and certain biochemical constituents.

MATERIALS AND METHODS

Growing crossbred heifers (16) of 12 to 16 months age with an average body weight of 155 kg, selected from the Instructional Dairy Farm of University were divided into 4 groups of 4 animals each in such a way that each group had equal body weight and age. The 4 groups heifers were provided 4 respective dietary treatments in the form of experimental mineral (feed) mixture containing copper sulphate to supply Cu and dicalcium phosphate to supply P. Treatment 1 (without Cu and P supplements) consisted of maize 54.55% and mustard cake 45.45%, treatment 2 (Cu supplemented) consisted of 54.31% maize, 45.26% mustard cake and 0.43% copper sulphate, treatment 3 (P supplemented) consisted of 30.20% maize, 25.16% mustard cake and 44.64% dicalcium phosphate and treatment 4 (Cu and P supplemented) consisted of 30.07% maize, 25.05% mustard cake, 44.64% dicalcium phosphate and 0.24% copper sulphate.

Initially all the experimental heifers were fed concentrate mixture and mixed fodder (green oats + oats straw) as per requirement (Kearl 1982) for 20 days which served as an adaptation period. All the animals were dewormed at the beginning of feeding trial with an anthelmintic. Thereafter animals were switched to respective treatments. All the heifers were housed in a well ventilated shed having concrete floor with individual feeding arrangement and tied with iron chain at such a distance that they had freedom for free movement and prevented them from getting access to the manger of other animals. All the heifers were fed concentrate mixture at 10.00 AM as per requirement (Kearl 1982) and available mixed fodder (green oats+oats straw) was provided *ad lib.* to all the heifers at 9.00 AM daily. The orts were weighed and removed next day in the morning at 8.00 AM and 4.00 PM. The respective experimental feed (mineral) mixtures were supplemented daily as top dressed just after offering the concentrate mixture.

The heifers of treatment group 1 were provided 55 g experimental feed mixture 1 without Cu and P supplement, whereas heifers of treatment group 2 were provided 55 g experimental mineral (feed) mixture 2 containing copper sulphate to supply 60 mg Cu/day. The heifers of treatment group 3 were offered 100 g experimental mineral (feed) mixture 3 containing dicalcium phosphate to supply 5 g P/day; whereas heifers of treatment group 4 were provided 100 g experimental mineral (feed) mixture 4 containing copper sulphate and dicalcium phosphate to supply, respectively, 60 mg Cu and 5 g P/day. The feeding trial lasted for 90 days. Body weights were recorded fortnightly for 2 consecutive days, however, at the start and end of feeding trial the animals were weighed for 3 consecutive days. Blood samples from growing heifers were collected without anticoagulant from jugular vein at 0, 45th and 90th days of experimental feeding

trial before feeding and watering at 8.00 AM and serum was harvested, whereas in another set of tubes containing heparin were used for collection of blood samples for glucose estimation. The serum samples were analyzed for Cu using atomic absorption spectrophotometer P by colorimetric method (Fiske and Subbarow 1925) and blood biochemical parameters, viz. serum total protein (Johnson *et al.* 1999), albumin and globulin (Young 1997), blood glucose and serum total cholesterol (Kaplan and Kavemel 1983) and serum glutamate oxaloacetate transaminase and glutamate pyruvate transaminase activities (Nobert, 1970) using SPAN diagnostic kits. The feeds and fodder samples were also analyzed for proximate principles (AOAC 1995), Cu using AAS and P (Ward and Johnston 1962). The data were analyzed statistically (Snedecor and Cochran 1994) in a 2x2 factorial completely randomized design.

RESULTS AND DISCUSSION

The chemical composition and Cu and P contents in feeds and fodder have been presented in Table 1. The average daily dry matter intake among the different groups of heifers did not differ significantly (Table 2). The dry matter intake per kg metabolic body weight also did not differ significantly amongst the different groups of heifers; however, dry matter intake was higher in groups 3 and 4 supplemented with phosphorus. Similar results were also reported by Mahanta *et al.* (2004). The dry matter intake was not affected due to Cu level in the rations, whereas non-significant increase in dry matter intake was noticed due to increase in P level in the rations. However, Arthington and Spears (2007) reported greater dry matter intake in heifers provided supplemental copper as compared to the control group. There was no significant difference in average body weight gain among different treatment groups. However, there was an improvement in weight gain due to Cu supplementation but the differences were not significant. The P supplementation had no significant effect on weight gain. The interaction effect of Cu and P on weight gain was also non-significant.

The heifers in treatment groups 2 and 4 supplemented with Cu in the ration consumed significantly ($P<0.01$) more quantity of Cu than the heifers of treatment groups 1 and 3 (Table 2). The Cu intake and Cu concentration in the ration fed to heifers of treatment groups 1 and 3 were considerably lower than that recommended by NRC (2001) and ARC (1980) for heifers. Similarly, the heifers in treatment groups 3 and 4 supplemented with P consumed significantly ($P<0.01$) more P than heifers in treatment groups 1 and 2, which were not supplemented with P in the ration. The interaction effect of Cu and P on their intake was non-significant.

The average values of serum Cu and P profile and certain biochemical constituents in blood serum of crossbred heifers due to dietary supplementation of copper and phosphorus are presented in Table 3. The Cu concentration in blood serum at 0 day of feeding trial in all the groups ranging from 0.39 to

Table 1. Chemical composition and copper and phosphorus content of feedstuffs (DM basis)

Feed stuffs	Proximate constituents								
	Dry	Organic	Crude	Ether	Crude	Nitrogen	Ash (%)	Copper	Phosphorus
Mixed fodder (green oats+ oats straw)	37.61	90.46	9.12	19.97	28.92	49.55	9.54	7.15	0.28
concentrate mixture	86.52	86.94	19.30	3.06	6.46	41.88	13.06	6.78	0.32
Experimental feed mixture 1 (control)	89.36	95.09	25.17	5.61	5.23	40.93	4.91	7.62	0.45
Experimental feed (mineral) mixture 2 (copper supplemented)	88.93	95.21	23.55	5.73	5.09	39.16	4.79	67.34	0.42
Experimental feed (mineral) mixture 3 (phosphorus supplemented)	92.54	84.08	15.26	3.83	4.18	39.20	15.92	6.40	5.54
Experimental feed (mineral) mixture 4 (copper and phosphorus supplemented)	91.08	82.28	14.92	3.75	3.95	40.34	17.72	68.01	5.57

Table 2. Average daily dry matter and copper and phosphorus intakes and weight gain in growing crossbred heifers fed rations supplemented copper and phosphorus

Parameters	Groups/Treatments				Effect of copper supplementation		Effect of phosphorus supplementation	
	1 (Control)	2 (copper supplemented)	3 (phosphorus supplemented)	4 (copper and phosphorus)	Without copper supplementation	With copper supplementation	Without phosphorus supplementation	With phosphorus supplementation
Average body weight (kg)	194.960±15.12	200.94±18.85	194.56±12.15	195.88±11.75	194.63±11.80	198.41±15.16	197.81±16.85	195.22±11.93
Roughage dry intake (kg/d)	4.42±0.72	4.60±0.84	4.98±0.17	4.83±0.14	4.70±0.32	4.72±0.46	4.51±0.77	4.91±0.12
Concentrate dry matter (kg/d)	1.87±0.07	1.91±0.11	1.95±0.06	1.95±0.16	1.91±0.06	1.93±0.08	1.89±0.09	1.95±0.06
Total dry matter intake (kg/d)	6.29±0.79	6.51±0.95	6.93±0.16	6.78±0.18	6.61±0.38	6.65±0.54	6.40±0.86	6.86±0.14
Dry matter intake/W ^{0.75} (g)	119.40±8.97	120.24±10.30	134.24±7.88	130.10±3.81	126.82±0.78	125.17±3.32	119.82±9.61	132.17±5.82
Total copper intake (mg/d)**	26.50 ^c ±4.05	90.66 ^a ±4.98	31.22 ^b ±0.87	99.01 ^a ±1.36	29.77 ^b ±1.02	90.96 ^a ±1.95	59.87±3.00	60.59±0.43
Total phosphorus intake (g/d)**	14.99 ^b ±1.48	14.58 ^c ±1.83	20.27 ^a ±0.37	19.69 ^a ±0.56	17.62±0.79	17.14±1.16	14.78 ^b ±1.63	19.98 ^a ±0.40
Average daily weight gain (g)	552.78±3.46	631.50±4.18	551.86±2.98	567.14±3.24	552.32±2.47	599.32±1.96	592.14±2.04	559.50±1.45

0.50 ppm (Table 3) was below the critical value of 0.65 ppm (Miles and McDowell 1983). At 45th and 90th day of feeding trial, the serum copper concentration increased in all the groups of heifers; however, it was still below the critical level in the heifers of treatment groups 1 and 3 fed ration without

supplementation of Cu, whereas the values of serum Cu concentration improved significantly and were above the critical levels in crossbred heifers of treatment groups 2 and 4 fed ration supplemented with Cu. Thus Cu supplementation in the rations had significant effect on improving the blood

Cu levels. The low serum Cu concentration in crossbred heifers prior to experimental feeding trial might be due to less bioavailability of copper in feeds and fodder as well as decreased gut absorption and increased excretion of Cu (McDowell 1985). The overall average values of serum copper concentration differed significantly ($P<0.01$) amongst the different groups and the values in heifers of treatment groups 2 and 4 were similar and were significantly higher than in heifers of treatment groups 1 and 3. The interaction effect of

Cu and P in increasing the Cu level in blood was also significant ($P<0.01$). Kegley *et al.* (1994) Similar findings reported in growing cattle and Gengelbach *et al.* (1994) in beef cows and calves. The blood serum P concentration in crossbred heifers was also below the normal value of 4-6 mg/dl (NRC 2001) for cattle in all the groups of heifers on 0 day of feeding trial. Similar trend remained throughout the experimental feeding period in heifers of treatment groups 1 and 2 in which no dietary P was supplemented. Whereas there

Table 3. Mean copper and hoshorus profile and biochemical constituents in blo od serum of growing crossbred heifers fed ration supplemented with copper and hoshorus

Parameters	Groups/Treatments				Effect of copper supplementation		Effect of phosphorus supplementation	
	1 (control)	2 (copper supplemented)	3 (phosphorus supplemented)	4 (copper and phosphorus)	without copper supplementation	With copper supplementation	Without phosphorus supplementation	With phosphorus supplementation
Serum copper concentration (ppm)								
0 day	0.39±0.03	0.41±0.05	0.41±0.03	0.50±0.04	0.40±0.02	0.46±0.04	0.40±0.03	0.46±0.02
45 days**	0.44±0.02	0.62a±0.03	0.52b±0.03	0.63a±0.03	0.48b±0.01	0.62a±0.02	0.53±0.02	0.57±0.03
90 days**	0.58±0.04	0.73a±0.02	0.65b±0.02	0.71a±0.02	0.62b±0.01	0.72a±0.02	0.65±0.03	0.68±0.01
Mean**	0.47±0.01	0.59a±0.03	0.53b±0.02	0.61a±0.02	0.50b±0.06	0.60a±0.08	0.53±0.07	0.57±0.07
Serum phosphorus concentration (mg/dl)								
0 day	3.45±0.12	3.52±0.03	3.47±0.12	3.29±0.10	3.46±0.06	3.41±0.05	3.49±0.06	3.38±0.05
45 days**	3.82b±0.12	3.60c±0.07	4.05a±0.02	4.11a±0.02	3.93±0.07	3.85±0.04	3.71b±0.07	4.08a±0.01
90 days**	3.86b±0.13	3.69c±0.09	5.10a±0.23	5.12a±0.06	4.48±0.06	4.40±0.06	3.77b±0.03	5.11a±0.12
Mean**	3.71b±0.10	3.60c±0.05	4.21a±0.07	4.17a±0.05	3.96±0.29	3.89±0.29	3.66b±0.09	4.19a±0.50
SGPT activity (IU/litre)								
0 day	12.25±0.85	14.00±0.71	12.25±0.25	13.50±1.04	12.25±0.47	13.75±0.60	13.12±0.55	12.88±0.52
45 days***	13.50±1.20	16.25b±0.63	17.50a±1.20	20.75a±1.38	15.50b±0.54	18.50a±0.89	14.88b±0.75	19.12a±0.85
90 days***	14.25±0.25	20.25b±0.63	19.75b±1.75	27.25a±1.50	17.38b±0.80	23.75a±0.88	17.25b±0.25	23.88a±0.85
Mean***	13.33±0.68	16.83b±0.29	16.75b±0.94	20.50a±0.29	15.04b±1.50	18.67a±2.89	15.08b±1.19	18.63a±3.19
SGOT activity (IU/litre)								
0 day	56.00±0.91	55.00±1.08	56.50±0.97	55.25±1.11	56.25±0.52	55.12±0.55	55.50±0.71	55.88±0.83
45 days***	59.25±0.48	63.75ab±1.15	64.75a±1.80	68.00a±1.08	62.00b±0.89	65.88a±0.38	61.50b±0.54	66.38a±0.66
90 days***	61.00±1.08	70.00b±0.82	71.50b±0.96	78.50a±1.71	66.25b±0.60	74.25a±0.75	65.50b±0.84	75.00a±1.08
Mean***	58.75±0.76	63.93b±0.77	64.25b±0.37	67.25a±0.13	61.50b±2.90	65.08a±5.54	60.83b±2.96	65.75a±5.53
Serum total protein (g/dl)								
0 day	6.34±0.19	6.90±0.43	7.39±0.38	6.51±0.36	6.87±0.17	6.71±0.15	6.62±0.29	6.95±0.32
45 days	6.12±0.20	6.49±0.13	6.13±0.13	6.09±0.30	6.12±0.14	6.29±0.20	6.30±0.07	6.11±0.11
90 days	6.66±0.11	6.30±0.23	6.55±0.12	6.66±0.37	6.31±0.03	6.48±0.26	6.18±0.08	6.61±0.21
Mean	6.18±0.08	6.57±0.24	6.69±0.16	6.42±0.19	6.43±0.22	6.49±0.12	6.37±0.13	6.56±0.25
Serum albumin (g/dl)								
0 day	3.58±0.25	3.51±0.09	3.66±0.19	3.78±0.11	3.62±0.08	3.64±0.09	3.54±0.12	3.72±0.11
45 days	2.97±0.17	3.18±0.06	3.13±0.19	3.20±0.12	3.05±0.14	3.19±0.12	3.08±0.12	3.17±0.06
90 days	3.34±0.09	3.29±0.08	3.46±0.04	3.29±0.10	3.40±0.04	3.29±0.06	3.32±0.06	3.37±0.06
Mean	3.30±0.12	3.33±0.03	3.42±0.13	3.42±0.06	3.36±0.16	3.37±0.14	3.31±0.13	3.42±0.16
Serum globulin (g/dl)								
0 day	2.77±0.20	3.39±0.37	3.73±0.49	2.76±0.28	3.25±0.17	3.08±0.11	3.08±0.24	3.25±0.35
45 days	3.15±0.04	3.31±0.09	3.00±0.09	2.89±0.26	3.07±0.04	3.10±0.21	3.23±0.07	2.94±0.15
90 days	2.72±0.18	3.01±0.25	3.09±0.16	3.38±0.31	2.91±0.06	3.19±0.25	2.87±0.03	3.23±0.20
Mean	2.88±0.08	3.24±0.23	3.27±0.23	3.01±0.14	3.08±0.10	3.12±0.04	3.06±0.11	3.14±0.10
Blood glucose (mg/dl)								
0 day	59.57±0.70	70.21±3.91	73.14±4.74	61.31±4.18	66.36±1.46	65.76±3.70	64.89±3.18	67.22±3.37
45 days	76.60±4.30	76.00±1.22	74.50±6.28	65.75±2.87	75.25±3.89	70.88±1.21	76.00±2.11	70.13±4.56
90 days***	48.50b±2.70	50.61b±3.70	66.73a±2.78	60.27ab±3.64	57.66±0.68	55.44±3.20	49.60b±2.46	63.51a±2.95
Mean	61.38±2.18	65.61±1.98	71.46±3.61	62.45±3.08	66.42±5.08	64.03±4.54	63.50±7.66	66.95±1.92
Serum total cholesterol (mg/dl)								
0 day	63.13±9.97	60.59±2.17	61.19±2.82	61.83±7.25	62.17±6.12	61.21±2.97	61.87±5.60	61.51±3.52
45 days	66.45±7.47	81.30±4.09	69.32±7.99	70.50±4.97	67.88±7.24	75.90±3.24	73.88±8.98	69.91±2.56
90 days	82.08±6.79	97.22±2.57	78.98±5.92	93.33±6.21	80.53±7.96	95.27±3.40	89.65±4.38	86.75±4.51
Mean	70.56±7.63	79.70±1.08	69.83±5.14	75.22±3.86	70.19±5.43	77.46±9.87	75.13±8.05	72.72±7.24

was significant ($P < 0.01$) improvement in blood P concentration in heifers of treatment groups 3 (P supplemented) and 4 (Cu and P supplemented) and P level in the blood serum of these groups reached above the critical level (< 4.00 mg) at 45 and 90 days of feeding trial. The values of P concentration in blood serum at 45 and 90 days were significantly ($P < 0.01$) different amongst the different treatment groups and were higher in heifers of treatment groups 3 and 4 than in treatment groups 1 and 2. The overall mean P concentration in blood serum of heifers was significantly ($P < 0.01$) higher in treatment groups 3 and 4 than in treatment groups 1 and 2 (Table 3). Thus P supplementation had significant ($P < 0.01$) effect in improving P level in blood. The result of the present study corroborated with the findings of Wu and Satter (2000), Gimbi *et al.* (2004), Lopez *et al.* (2004) and Tallam *et al.* (2005), who also reported improvement in the serum P levels in dairy cattle due to dietary P supplementation.

The mean serum glutamate-pyruvate transaminase (SGPT) activity was significantly ($P < 0.01$) highest in heifers of treatment group 4 fed ration supplemented with both Cu and P (20.00 IU/litre) followed by treatment group 2 fed ration supplemented with Cu (16.83 IU/litre) and group 3 fed ration supplemented with P only (16.75 IU/litre) and lowest in heifers of treatment group 1 fed ration without dietary Cu and P supplementation (13.33 IU/litre). The SGPT activity in treatment group 1 was below the normal range of 14–38 IU/litre (Sastry *et al.* 1999). In fact SGPT activity at 0 day of feeding trial was below the critical level in all groups of heifers (Table 3). The SGPT activity was considerably improved on 45th and 90th day of feeding trial in Cu and P supplemented groups. The overall mean SGPT activity significantly ($P < 0.01$) increased due to Cu and P supplementation and also the interaction effect of Cu and P was significant ($P < 0.01$) and values were within the normal range. However, Sharma (2007) reported higher SGPT activity (20.20 to 29.90 IU/litre) in crossbred heifers fed complete ration or conventional ration than that obtained in the present study.

The mean values of serum glutamate-oxaloacetate transaminase (SGOT) activity at 0 day were below the normal range of 60–118 IU/litre (Sastry *et al.* 1999) in all groups of crossbred heifers. After dietary supplementation of Cu or P or Cu and P both in the ration of heifers of treatment groups 2, 3 and 4, respectively, SGOT activity increased significantly ($P < 0.01$) on 45th and 90th day of feeding trial in comparison to the control group (treatment group 1) wherein the SGOT activity remained below the critical level throughout the feeding trial of 90 days. The heifers in treatment group 4 had higher SGOT activity (67.25 IU/litre) and values were within the normal range due to dietary supplementation of Cu and P both. The SGOT activity at 45th and 90th day of feeding trial differed significantly ($P < 0.01$) amongst the different groups of crossbred heifers. The overall mean SGOT activity was significantly ($P < 0.01$) higher in heifers of group 4; however,

the values were above the critical level in heifers of treatment groups 2, 3 and 4 supplemented with either Cu or P or both. The supplementation of Cu and /or P and their interaction had significant ($P < 0.01$) positive effect on SGOT activity (Table 3).

The values of total protein, albumin and globulin concentration in blood serum did not differ significantly amongst the different groups of heifers at 0, 45 and 90 days of the feeding trial (Table 3). The overall mean values of serum total protein, albumin and globulin concentration were in the normal range and were not affected due to dietary Cu and/or P supplementation. Similar values for serum total protein, albumin and globulin were also reported by Divya (2005) and Sharma (2007) in crossbred heifers. Mondal *et al.* (2004) also did not find any effect of Cu supplementation on serum total protein, albumin and globulin concentration in black Bengal kids.

The values of blood glucose concentration at 90 days of feeding trial differed significantly ($P < 0.01$) amongst the different treatment groups of heifers. The overall mean serum glucose concentration was higher in heifers of group 3 supplemented with P as compared to other groups. However, it does not seem to be due to supplementation of P or Cu. Tripathi *et al.* (2001) also recorded similar observation in crossbred calves. Similarly, the serum total cholesterol level was not influenced significantly due to dietary supplementation of Cu and P or due to their interaction in crossbred heifers. Similar findings were not reported by Engle *et al.* (2001) in Holstein cows fed varying levels of copper in the diet. It was concluded that dietary Cu and P supplementation is necessary in plain region of Uttarakhand for ruminant animals for improving the feed intake and to maintain the profile of biochemical constituents as well as and Cu and P status in blood in field as well as farm conditions under existing feeding practices for improving the reproductive and productive performance.

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