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Effect of zinc-sulphate treated soybean-meal feeding on nutrient utilization and blood metabolic profile in male Murrah buffalo calves

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ABSTRACT

An experiment was conducted on 15 male buffalo calves (18-months-old, 263.0±7.8 kg mean body weight), divided into 3 groups of 5 calves in each, to elucidate the effect of feeding zinc-sulphate treated soybean-meal on nutrient utilization, nitrogen balance and blood metabolic profile. All the buffalo calves were fed on wheat straw and concentrate mixture to meet their nutrient requirement. Concentrate mixture was having 25% untreated, 1% and 2% zinc sulphate treated soybean meal in group 1, 2 and 3, respectively. This feeding practice lasted for 60 days. A metabolism trial was conducted to know the digestibility of nutrients and nitrogen balance at the end of feeding trial. Blood was collected to estimate the blood biochemical profile from all the animals after 60 days of experimental feeding. Results revealed no significant difference in the digestibility of proximate principles and fiber fractions and nitrogen balance. Results revealed non-significant difference in serum concentration of total protein, albumin, globulin, urea, calcium and phosphorus, whereas the concentration of serum cholesterol and blood glucose decreased and serum zinc level increased significantly in group 2 and 3 in comparison to control group. The activity of alkaline phosphatase was significantly higher in calves fed concentrate mixture having zinc sulphate treated soybean meal than the control group calves. There was no significant difference in plasma concentration of T_3, whereas levels of plasma T_4, testosterone and insulin were significantly higher in groups 2 and 3 as compared to group 1. It may be concluded that addition of zinc sulphate treated soybean meal in concentrate mixture of buffalo calves decreased blood glucose and serum cholesterol concentration and increased the levels of serum alkaline phosphatase, zinc, and plasma hormones like insulin, testosterone and thyroxine, though there was no significant effect on weight gain, digestibility of nutrients and nitrogen balance.

Key words: Buffalo calves, Blood hormone, Nutrient utilization, Soybean meal, Zinc sulphate

Britton and Klopfenstein (1986) reported improved efficiency of nitrogen utilization in calves fed diets supplemented with zinc treated soybean meal. Similarly, Froetschel *et al*. (1990) reported increased flow of feed amino acids in steers fed diets having high concentrations of zinc sulphate. Similarly, Jadhav *et al.* (2008) reported improved growth performance and feed conversion efficiency in buffalo calves given high levels of zinc as zinc sulphate. No information is available on the effect of feeding high levels of zinc through zinc treated soybean meal in buffalo calves on their growth performance and changes in blood metabolites. Therefore, the present work was conducted to study the effect of feeding zinc-sulphate treated soybean-meal on nutrient utilization, nitrogen balance, blood metabolic profile and some plasma hormones in male buffalo calves

MATERIALS AND METHODS

Treatment of soybean meal: Zinc sulphate (1 kg and 2 kg) was dissolved in 50 liters of tap water; to each solution 100 kg of soybean meal was added and mixed well and kept for 3 h before drying the same in the sun on a polythene sheet. Dried cake was collected and stored in polythene bags for feeding to animals.

Animals, management and feeding: This study was conducted on 15 male Murrah buffalo (*Bubalus bubalis*) calves (18 months old; 263.0±7.8 kg mean body weight), divided randomly in to 3 groups of 5 animals in each on the basis of their body weights, following completely randomized

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block design. All the calves were fed on wheat straw and concentrate mixture in the ratio of 1: 1 to meet their nutrients requirement as per Kearl (1982). Concentrate mixture comprised maize grain 30%, soybean meal 25%, wheat bran 42%, mineral mixture 2% and common salt 1%. Soybean meal in the concentrate mixture of calves of group 1, 2 and 3 was untreated, 1 and 2% zinc sulphate treated, respectively. Clean drinking water was offered to the calves at 9.00 and 15.00 h daily. The animals were weighed at the start and after every 15 days to assess the change in body weight.

Metabolism trial: After 60 days of experimental feeding, a metabolism trial of 6 days was conducted to determine the nutrient digestibility and balance of nitrogen. For this purpose, calves were shifted to metabolic cages, having arrangements for separate collection of faeces and urine. During collection period feed offered, and leftover were quantified and samples of feeds offered and residue, if any, were taken in separate polythene bags for each animal daily for chemical analysis. During metabolism trial, total faeces voided by individual animal during 24 h was collected into pre-weighed bucket, and weighed at a fixed time (9.30 h) daily. Suitable aliquots of faeces were taken for DM estimation, which were pooled and kept in polythene bags for further analysis. For the estimation of nitrogen, a suitable aliquot was taken daily into a glass bottle containing 20% sulphuric acid. Urine excreted in 24 h, collected in plastic bottles was also measured daily at fixed time (9.00 h). Suitable aliquots of urine were taken daily and pooled into Kjeldahl flask containing 40 ml commercial sulphuric acid for nitrogen estimation. For chemical analysis, faecal material was dried at 60°C and ground to pass through a 1 mm screen in a mill and preserved in airtight bottles. Feeds were also processed similarly for chemical analysis.

Collection of blood: Blood samples were collected from all the calves at the end of experimental feeding from jugular vein in clean dry test tube with or without anticoagulant; serum and plasma were separated and stored at –20°C for further analysis.

Chemical analysis: Proximate composition of feeds and fodders was determined using methods recommended by AOAC (2000) and cell wall constituents as per Van Soest *et al*. (1991). Serum total protein and albumin were determined by the modified Biuret and Doumas method (Doumas *et al.* 1971). Diacetyl monooxime method was followed to estimate the concentration of serum urea (Wybenga *et al*. 1971). The serum alkaline phosphatase activity was estimated as per Kind and King (1954) and serum cholesterol as per Wybenga *et al.* (1970). Calcium and inorganic phosphorus in the blood serum were estimated colorimetrically using the diagnostic kits. Estimation of triiodothyronine (T_3), thyroxin (T_4), insulin and testosterone in blood plasma samples was done by radioimmunoassay technique using an automatic gamma counter. T_3, T_4 testosterone, and insulin were estimated using commerical kit.

Statistical analysis: A completely randomized design was used to determine the dietary effect. One-way analysis of variance (ANOVA) was carried out on the experimental data using treatments as independent variable (Snedecor and Cochran 1989). Duncan's multiple range test was used to know the significance of difference between means (Steel and Torrie 1980).

RESULTS AND DISCUSSION

The crude protein content of the 3 concentrate mixtures was alike (Table 1), indicating that treatment of soybean-meal with zinc sulphate had no effect on its protein content. Zinc level as envisaged was higher in concentrate mixtures 2 and 3 as compared to concentrate mixture 1. Change in body weight and DM intake (g/d^{-1} and g/kg $w^{0.75}$) were similar in 3 groups (Table 2), indicating that feeding of zinc sulphate treated soybean meal, vis-à-vis higher level of Zn in the diet of calves of group 2 and 3 did not have any significant effect on their body weight gain and DM intake. Similarly, earlier researchers have also reported that Zn concentration in cattle and sheep diets did not influence the body weight gain (Mandal *et al*. 2007, Garg *et al*. 2008). These workers did not find any beneficial effect of zinc supplementation on body weight gain to a basal diet containing more than 20ppm Zn. Similarly, Khan (1978) did not observe any effect of supplemental zinc on DM intake in growing calves, when their dietary Zn was increased from 26.02 to 85.67ppm. Results revealed no significant effect of treatments on the digestibility of proximate principles and fibre fractions (Table 2). Contrary to our observations, Kegley and Spears (1994) reported that supplemental Zn tended to increase DM digestibility in lambs. Salma Ahmad *et al*. (2003) reported that apparent digestibility of DM ($P<0.05$), CP ($P<0.05$) and OM ($P<0.05$) were significantly greater in dairy goats supplemented with zinc methionine in their diet as compared to control. However, Mandal *et al*. (2007) did

Table 1. Chemical composition (%) of feeds on dry matter basis

Attribute	Concentrate mixture 1*	Concentrate mixture 2*	Concentrate mixture 3*	Wheat straw
Organic matter	91.74	91.16	91.04	92.46
Crude protein	17.5	17.6	17.4	4.14
Ether extract	2.63	2.84	2.35	1.41
Neutral detergent fibre	38.8	38.0	35.8	83.0
Acid detergent fibre	18.7	18.6	18.1	60.5
Hemicelluloses	20.1	19.4	17.7	22.5
Cellulose	13.6	13.9	13.4	51.05
Zn, ppm	48.97	85.17	163.32	10.62

Concentrate mixture 1*: having 25 parts untreated soybean meal; concentrate mixture 2*: having 25 parts zinc-sulphate (1%) treated soybean-meal; concentrate mixture 3*: having 25 parts zinc-sulphate (2%) treated soybean-meal.

Table 2. Change in body weight, digestibility (%) of nutrients and nitrogen balance in buffalo calves

Attributes	Group			SEM
	1	2	3	
Change in body weight				
Initial body weight, kg	263.6	262.8	264.0	7.8
Final body weight, kg	292.0	292.2	291.6	5.3
Gain in weight, kg	28.6	29.2	27.8	3.4
Weight gain, g/ d	493.1	503.5	463.3	34.0
DM intake, g/d	6750	6610	6620	45.9
DM intake,g/100 kg	2560	2530	2510	14.5
DM intake, g/kg $w^{0.75}$	103.0	101.6	101.0	0.59
Digestibility of nutrients (%)				
Dry matter	56.2	55.9	53.6	0.65
Organic matter	59.0	58.9	57.0	0.62
Crude protein	64.6	65.3	62.9	0.94
Ether extract	67.2	68.1	65.0	1.15
Neutral detergent fiber	52.2	50.9	49.0	0.71
Acid detergent fiber	46.7	45.1	45.5	0.78
Hemicelluloses	63.3	62.1	65.2	1.73
Cellulose	57.0	52.6	52.0	0.66
Nitrogen balance				
N intake (g)	104.2	103.7	104.7	2.68
Fecal-N (g)	37.0	35.8	38.8	1.35
Urinary -N (g)	28.7	32.9	32.2	1.91
Total N excreted	65.7	68.6	71.0	2.97
Nitrogen balance (g)	38.4	35.0	33.7	1.71
N Absorption (g/d)	67.1	67.9	65.9	1.96
% Absorbed retained	57.7	51.0	51.9	2.32
% Intake retained	37.5	33.4	33.6	1.81

Table 3. Blood biochemical profile and hormonal status in buffalo calves

Attributes	Group			SEM
	1	2	3	
Glucose, mg/ dl*	40.55 [a]	35.02 [b]	31.34 [c]	1.76
Total protein, g /dl	6.69	7.14	6.30	0.39
Albumin, g/ dl	3.57	3.60	3.74	0.10
Globulin, g/dl	3.11	3.55	2.56	0.40
Urea, mg/ dl	23.89	25.06	23.13	0.60
Cholesterol, mg/ dl*	169.70[a]	156.36[b]	141.01[c]	5.42
Calcium, mg/ dl	8.61	8.35	7.38	0.24
Phosphorus, mg/ dl	6.62	7.54	5.92	0.36
Zinc, mg/ L**	0.75	0.95	1.25	0.25
Alkaline phosphatse,KA Unit**	7.29[a]	13.08[b]	19.05[c]	2.27
Triiodothyronine, nmol/ L	1.91	1.97	1.96	0.09
Thyroxin, nmol/ L**	45.57 [a]	55.00 [b]	55.63 [b]	3.00
Testosterone, ng /ml**	0.115 [a]	0.254 [b]	0.351[c]	0.045
Insulin, pmol /L*	51.64[a]	56.36[b]	65.63[c]	5.78

*Means bearing different alphabets in rows differ significantly (P< 0.05); **means bearing different alphabets in rows differ significantly (P< 0.01).

not find any difference in the digestibility of CP, EE, NDF, ADF, cellulose and hemicelluloses in crossbred calves supplemented with 0, 35 or 70 ppm zinc in the basal diet containing 32.5 ppm zinc.

Results of present study revealed no significant effect of treatment of soybean meal with zinc sulphate vis-à-vis higher levels of zinc feeding on nitrogen intake, its excretion through feces and urine, and nitrogen balance (Table 2). Similarly, Khan (1978) and Mandal *et al*. (2007) in cattle calves and Kumar *et al*. (2002) in goat kids did not find any significant effect of Zn supplementation on nitrogen retention. Contrary to these, Kinal *et al*. (1996) observed improved nitrogen balance in dairy cows with the increasing concentration of dietary zinc from 40 to 70 ppm.

Blood biochemical parameters and plasma hormones levels in different groups of animals are presented in Table 3. Glucose concentration (mg/dl) was 40.55, 35.02 and 31.34 in 3 groups, respectively, indicating a statistical significantly (P<0.01) lower values in zinc-sulphate treated soybean-meal fed calves as compared to untreated soybean meal fed calves. Similarly, Tobia *et al*. (1998) reported that Zn supplementation improved glucose tolerance and decreased serum glucose levels rats. However, no information is available in the literature on the effect of high level of zinc supplementation on serum glucose levels in ruminants. Concentrations of total protein, albumin and globulin (g/dl) were statistically (P>0.05) similar in 3 groups, without showing any effect of feeding zinc-sulphate treated soybean-meal. Urea concentration in blood serum (mg/dl) was 23.89, 25.06 and 23.13 in 3 groups, respectively, and statistically alike. Similar were the findings of earlier workers in cattle and buffalo calves given different levels of zinc in their diet. (Jadhav 2005, Mandal *et al*. 2008). Similarly, Huerta *et al*. (2002) also did not find any change in plasma proteins and blood urea-N concentrations due to zinc supplementation. Cholesterol (mg/dl) was significantly higher (P<0.01) in control group (169.70) as compared to group 2 (156.36) and group 3 (141.01). Similarly, Dean *et al*. (1991) reported that high level of Zn decreased the level of total cholesterol and high density lipoprotein (HDL) cholesterol in broiler chicks. Concentrations of calcium and phosphorus (mg/100 ml) in blood serum of buffalo calves were statistically (P>0.05) comparable in 3 groups, without showing any effect of treatments on these parameters.

Blood alkaline phosphatase has been used as an indicator of animal Zn status. The activity of alkaline phosphatase (KA Units) was significantly lower (P<0.01) in group 1 as compared to groups 2 and 3. Similar higher activity of alkaline phosphatase was reported in lambs supplemented with 100 mg Zn/day (Saraswat and Arora 1972). Spears 1989) observed higher plasma activity of alkaline phosphatase in zinc supplemented heifers than non-supplemented control. Contrary to this, Hatfield *et al*. (2002) in goats and Mandal *et al*. (2008) in crossbred calves did not find any effect of Zn supplementation on alkaline phosphatase activity. This may be due to low levels of zinc supplementation in their experiment as compared to present study. The concentration of T_3 was alike in 3 groups without showing any significant effect of zinc supplementation. Similarly, Mandal *et al*. (2008) also did not find any significant difference in T_3 concentration of crossbred calves supplemented with zinc from inorganic and organic sources. In the present study, the concentration of T_4 was significantly ($P < 0.01$) higher in groups 2 and group 3 than control group, but Mandal *et al*. (2008) did not find any significant difference in T_4 level in crossbred calves supplemented with 35ppm zinc. Contrary to above, Kececi and Keskin (2002) in lambs observed reduced concentration of serum T_3 and T_4 on supplementation of 290mgZn/day. These variations observed in concentration of T_3 and T_4 in different studies may be due to different species of animals and different levels of zinc supplementation. Testosterone is the male sex hormone and responsible for secondary sexual characters in male. Zinc is known to influence the testosterone synthesis by virtue of its role in stimulation of Ledig cells of testis, and also as a structural component of proteins, responsible in the synthesis, secretion and transport of testosterone. Its concentration (ng/ml) in blood plasma was significantly ($P<0.01$) higher in groups 2 and 3 as compared to group 1, indicating that higher levels of zinc from zinc sulphate treated cake in group 2 and 3 increased the serum levels of zinc, which in turn enhanced the synthesis and secretion of testosterone in these group. Similar higher testosterone levels were reported by Kumar *et al*. (2006) in crossbred calves and Imam *et al*. (2009) in buffalo bulls on Zn supplementation in their diet. Contrary to these, Mandal *et al*. (2008) did not find any effect of zinc supplementation on testosterone level in crossbred calves. Zinc is also associated with insulin in the pancreas, as it participates in the synthesis and storage of insulin in the ß-cells of pancreas (Quarterman and Florence 1972). Results revealed that plasma concentration of insulin (pm/l) was significantly higher in group 3 as compared to group 2 and group 1. Contrary to above, Engle *et al*. (1997) in heifers, Mandal *et al*. (2008) in crossbred calves and Jadhav (2005) in buffalo calves did not find any significant effect of zinc supplementation on their plasma insulin level. Kirchgessner *et al*. (1978) working on cattle observed that Zn was associated with insulin release from the pancreas and that pancreatic Zn concentrations were markedly reduced during dietary Zn deficiencies, resulting in reduced concentration of serum insulin and hyperglycemia in animals. This may be due to lower level of Zn supplementation in the diet of their experimental animals as compared to present study.

It may be concluded that addition of zinc-sulphate treated soybean meal in concentrate mixture of buffalo calves decreased blood glucose and serum cholesterol concentration and increased the levels of serum alkaline phosphatase, zinc and plasma hormones like insulin, testosterone and thyroxin, though there was no significant effect on weight gain, digestibility of nutrients and nitrogen balance.

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