Effect of Selenium, Zinc, and Copper Supplementation on Blood Metabolic Profile in Male Buffalo (*Bubalus bubalis*) Calves

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Abstract Twenty male buffalo calves (15 months, 200.2 ± 9.75) were divided into four groups of five animals in each and fed diets without (T1) or supplemented with 0.3 ppm selenium (Se)+40 ppm zinc (Zn) (T2), 0.3 ppm Se+40 ppm Zn+10 ppm copper (Cu) (T3), and 40 ppm Zn+10 ppm Cu (T4) for 120 days, during which blood samples were collected on days 0, 40, 80, and 120. Concentrations of glucose, total protein, albumin, globulin, urea, uric acid, and creatinine were similar in all the four groups. The level of different serum enzymes viz. lactate dehydrogenase, alkaline phosphatase, glutamate pyruvate transaminase, and glutamate oxaloacetate transaminase, and hormones viz. T_3, T_4, testosterone and insulin were similar ($P>0.05$) among the four groups but the ratio of T_4/T_3 was reduced ($P<0.05$) in the groups (T2 and T3) where selenium was supplemented at 120th day of supplementation. It was deduced that supplementation of 0.3 ppm Se and/or 10.0 ppm of Cu with 40 ppm Zn had no effect on blood metabolic profile in buffalo calves, except the ratio of T_4 and T_3 hormone which indicates that selenium plays an important role in converting T_4 hormone to T_3 which is more active form of thyroid hormone.

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Introduction

Essentiality of selenium [1], copper [2], and zinc [3], for life is well established, as these elements are essentially required for a number of biochemical functions in living organisms. Selenium-dependent enzymes, notably, the glutathione peroxidase, are components of the physiological antioxidative protection system. Selenium (Se) has also been shown to mediate a number of insulin-like actions both in vivo as well as in vitro [4] and it is also a component of enzyme type I iodothyronine-5′-deiodinase, which converts total thyroxine (T_4) in to total tri-iodothyronine (T_3) which is more active form of the thyroid hormone[5]. Copper is also an essential component of many enzymes (cuproenzymes) and proteins. Studies have shown that copper is required for growth, host defense mechanisms, bone strength, red and white cell maturation, iron transport, and glucose metabolism [6]. Zinc is associated with more than 300 enzymes either as component or as activator [7–9]. In many of the enzymatic reactions, all three, i.e., selenium, copper, and zinc, are essential. For example, superoxide radicals are dismutated into hydrogen peroxide and oxygen by enzyme superoxide dismutase in the presence of copper and zinc as cofactors. Hydrogen peroxide is then reduced to water by the selenium–glutathione peroxidase couple. Efficient removal of these precursors of reactive oxygen species maintains the integrity of membranes, reduces the risk of cancer, and slows

the aging process [10]. However, requirements of these trace elements for buffaloes are yet not specified and their interaction also affects their utilization and till the date levels recommended for cattle [11] are being used. Moreover, recent studies [12] have shown better performance of animals with the higher levels of these elements than their recommended levels by National Research Council. In view of these facts, the present study was conducted to assess the effect of supplementation of Se, copper (Cu), and zinc (Zn) on blood metabolic profile in male buffalo calves.

Materials and Methods

Animals: Selection and Grouping

Twenty healthy male buffalo (*Bubalus bubalis*) calves (15 months, 200.2±9.75 kgs) were taken for this study, vaccinated against common contagious diseases, and dewormed 1 month before the start of experimental feeding. These calves were divided into four groups of five animals each on the basis of their body weight after randomized block design.

Housing and Management

The calves were housed in a well-ventilated, clean and concrete-floored shed, and fed individually. Strict management and hygienic practices were adopted throughout the experimental period. Clean drinking water was provided ad libitum twice a day at about 9 A.M. and 3 P.M.

Feeds and Feeding

Buffalo calves in all the four groups were offered concentrate mixture (20% ground maize grain, 27% soybean meal, 50% wheat bran, 2% mineral mixture, and 1% common salt) and ad libitum wheat straw to meet nutrient requirements for 500 g/day body weight gain [13]. The amount of concentrate mixture offered to calves was regularly revised every fortnight based on their body weight. All the calves were provided with about 2.5 kg of available (maize/oats/berseem) green fodder, twice a week to meet vitamin A requirements. Feeding schedule was similar in all the four groups, except for mineral supplementation, which was no mineral supplementation in group T1 (control), 0.3 ppm Se+40 ppm Zn in group T2, 0.3 ppm Se+40 ppm Zn+10 ppm Cu in group T3, and 40 ppm Zn+10 ppm Cu in group T4. Supplementation of Se, Zn, and Cu was done as aqueous solutions of sodium selenite, zinc sulfate, and cupric sulfate, respectively, which were mixed daily in the concentrate mixture of each animal. The quantity of mineral solution was revised every week according to total dry matter intake of the animals. Experimental feeding was done for a period of 120 days.

Collection of Blood Samples and Separation of Serum

Blood samples from buffalo calves were collected initially (0 day) and subsequently at 40 days interval through jugular venipuncture, observing all aseptic precautions in the morning (before watering and feeding), into clean and dry test tubes and kept in slanting position for 45 min, followed by centrifugation at 700×*g* for 15 min to separate out serum. Serum samples were stored in 2 mL plastic vials at −20°C for further analysis.

Serum Biochemical Constituents and Enzyme Activity

Serum samples were analyzed for glucose [14], total protein (TP) and albumin [15], urea [16], creatinine [17], uric acid [18], alkaline phosphatase (ALP) [19], glutamate oxaloacetate transaminase (SGOT), and glutamate pyruvate transaminase (SGPT) [20], and lactate dehydrogenase (LDH) [21]. Concentration of globulin was calculated by subtracting serum albumin from TP.

Serum Hormone Levels

Serum hormone profile of calves was determined by radioimmunoassay (RIA) kits using a gamma counter (Model COBRA II, Packard, USA). The RIA kits used for the estimation of T_3, T_4 were from Immunotech, Radiova Czech Republic; testosterone from Immunotech, France; and insulin from Medicorp, Canada.

Statistical Analysis

All the data generated in the experiment were analyzed statistically using analysis of variance [22] and Duncan's multiple range test was used to compare the means [23].

Results and Discussion

Chemical Composition of Feeds

The chemical composition of the concentrate mixture (CM), wheat straw (WS) and complete basal diet (calculated on the basis of proportion of CM and WS consumed during entire experimental period) is presented in Table 1. The contents of crude protein, ether extract, total carbohydrates, neutral detergent fiber, and acid detergent fiber contents were comparable to the levels recommended for growing buffalo calves for 500 g daily gain [13]. The Se (0.28 ppm), Zn (31.60 ppm), and Cu (11.47 ppm) contents

Table 1 Chemical composition of feeds offered to animals (percent DM basis)

Attributes	Concentrate mixture	Wheat straw	Complete diet[a]
Organic matter	91.74	93.34	92.64
Crude protein	21.99	2.80	11.19
Ether extract	3.26	2.57	2.87
Total ash	8.26	6.66	7.36
Acid insoluble ash	1.68	4.50	3.26
Total carbohydrates	66.49	87.98	78.58
Neutral detergent fiber	39.03	84.52	64.62
Acid detergent fiber	12.72	54.56	36.26
Cellulose	10.37	47.47	31.24
Hemicellulose	26.31	29.96	28.36
Acid detergent lignin	2.35	7.10	5.02
Calcium	1. 41	0.46	0.59
Phosphorus	0.82	0.06	0.40
Sulfur	0.21	0.20	0.20
Zinc (ppm)	47.97	18.87	31.60
Copper (ppm)	15.95	7.98	11.47
Selenium (ppm)	0.34	0.23	0.28
Iron (ppm)	303.69	256.34	277.06
Manganese (ppm)	43.97	33.71	38.20
Cobalt (ppm)	3.25	4.28	3.83
Molybdenum (ppm)	1.18	1.05	1.11

[a] Calculated on the basis of proportion of concentrate mixture and wheat straw consumed during entire feeding trial

in the diet were also comparable to the levels recommended for cattle calves [11].

Blood Biochemical Parameters

Results showed that supplementation of Se (0.3 ppm) and/or Cu (10 ppm) with Zn (40 ppm) had no effect on different blood biochemical parameters in the buffalo calves as their values were similar ($P>0.05$) among the four groups throughout the experimental period (Table 2). In contrast to our observations, a declined plasma glucose level was reported in buffalo calves supplemented with Se [24]; but the level of Se supplementation in that experiment [24] was very high (8.54 ppm) compared to 0.3 ppm level used by us. However, similar to our findings, kids supplemented with 10 ppm Cu in their diet had no effect on blood glucose levels [25]. Similarly, no effect on plasma protein concentration has been reported with the supplementation of 0.1 ppm Se in steers [26], 8.54 ppm Se in buffalo calves [24], and 10 ppm of Cu in kids [25]. Similarly in one of our experiment [27], we also did not observed any effect on serum glucose and total protein level in buffalo calves supplemented with either Se (0.3 ppm) and Cu (10 ppm) alone on in combination.

However, the concentration of serum globulin was increased and albumin and A/G ratio were found to be reduced in our previous experiment when Se (0.3 ppm) either alone or with 10 ppm Cu was supplemented in the

diet of buffalo calves [27]. But in most of the reports, supplementation of Se [24, 26] in buffalo calves and steers, respectively, or Cu [25] in kids had no effect on either serum albumin and globulin levels or their ratios.

Results also revealed no significant effect of supplementation of Se and/or Cu with Zn on serum urea, creatinine, and uric acid, as values of these constituents were comparable ($P>0.05$) among the four groups throughout the experimental period (Table 2). In contrast to our results, steers supplemented with 0.1 ppm of Se [26] had higher levels of urea and creatinine in plasma; but the basal diet used in their experiment had very low levels of Se (0.015 ppm), which might be the reason for an increase in the levels of plasma urea and creatinine on 0.1 ppm of Se supplementation in that experiment. However, our findings are supported by an experiment conducted on buffalo calves where supplementation of as high as 8.54 ppm selenium had no effect on serum creatinine levels [24], similarly we also did not find any effect on either serum urea, creatinine, or uric acid levels when supplemented 0.3 ppm Se either alone or in combination with 10 ppm Cu in buffalo calves [27].

Serum Enzyme Profile

Supplementation of Se (0.3 ppm) and/or Cu (10 ppm) with Zn (40 ppm) had no effect on the activity of different blood enzymes, i.e., ALP, SGPT, SGOT, and LDH in buffalo

Table 2 Mean concentration of different blood biochemical parameters in different groups

Day	T1	T2	T3	T4	SEM±
Glucose (mg/dl)					
0	30.77±2.89	26.51±3.40	29.70±4.27	30.63±1.14	1.49
40	27.29±2.30	29.82±2.52	30.38±2.51	31.29±1.12	1.06
80	31.21±2.00	28.35±1.76	31.36±2.28	33.09±3.18	1.15
120	31.38±5.41	29.86±3.34	27.48±3.37	30.32±3.43	1.86
Mean[a]	29.96	29.34	29.74	31.57	
Total protein (g/dl)					
0	6.34±0.07	6.18±0.04	6.19±0.19	6.47±0.11	0.060
40	6.28±0.09	6.18±0.11	6.32±0.20	6.31±0.17	0.069
80	6.50±0.12	7.05±0.10	6.79±0.24	6.62±0.12	0.085
120	6.70±0.16	6.63±0.17	6.62±0.16	6.59±0.20	0.080
Mean[a]	6.49	6.62	6.58	6.51	
Albumin (g/dl)					
0	3.79±0.08	3.84±0.03	3.79±0.13	3.93±0.06	0.039
40	3.82±0.08	3.89±0.07	3.89±0.12	3.93±0.14	0.051
80	3.25±0.10	3.54±0.03	3.50±0.15	3.47±0.08	0.053
120	3.40±0.09	3.44±0.06	3.36±0.11	3.41±0.08	0.040
Mean[a]	3.49	3.62	3.58	3.60	
Globulin (g/dl)					
0	2.55±0.09	2.33±0.05	2.40±0.11	2.54±0.10	0.048
40	2.46±0.05	2.29±0.16	2.42±0.13	2.38±0.24	0.075
80	3.25±0.12	3.51±0.10	3.29±0.13	3.15±0.13	0.063
120	3.30±0.14	3.19±0.18	3.25±0.13	3.18±0.17	0.071
Mean[a]	3.00	3.00	2.99	2.90	
Albumin/globulin					
0	1.50±0.08	1.65±0.04	1.59±0.08	1.56±0.07	0.035
40	1.56±0.05	1.74±0.14	1.62±0.09	1.73±0.21	0.064
80	1.01±0.05	1.01±0.03	1.07±0.05	1.11±0.06	0.025
120	1.04±0.05	1.09±0.07	1.04±0.07	1.08±0.06	0.028
Mean[a]	1.20	1.28	1.24	1.31	
Urea (mg/dl)					
0	29.75±1.49	30.30±1.78	30.16±1.98	29.58±1.37	0.77
40	27.00±0.78	24.25±0.93	23.15±0.93	26.94±2.11	0.71
80	17.24±1.14	17.66±1.06	16.94±0.51	16.92±1.07	0.45
120	19.26±1.86	19.91±1.35	17.15±0.45	17.24±1.45	0.69
Mean[a]	21.17	20.61	19.08	20.37	
Creatinine (mg/dl)					
0	1.28±0.10	1.53±0.10	1.46±0.12	1.42±0.06	0.051
40	1.16±0.12	1.37±0.11	1.18±0.05	1.13±0.08	0.048
80	1.37±0.08	1.41±0.11	1.39±0.05	1.37±0.08	0.039
120	1.18±0.09	1.22±0.08	1.17±0.06	1.20±0.06	0.035
Mean[a]	1.24	1.33	1.25	1.23	
Uric acid (g/dl)					
0	1.11±0.06	1.12±0.06	1.25±0.08	1.21±0.03	0.032
40	1.21±0.08	1.28±0.15	1.18±0.06	1.34±0.08	0.047
80	1.42±0.03	1.47±0.09	1.40±0.07	1.41±0.06	0.047
120	1.43±0.09	1.42±0.11	1.37±0.05	1.41±0.08	0.040
Mean[a]	1.35	1.39	1.32	1.42	

[a] Value is the mean of observations made on days 40, 80, and 120

Table 3 Blood enzyme profile of buffalo calves in different groups

Day	T1	T2	T3	T4	SEM±
ALP (KA units)					
0	20.23±2.05	14.30±3.08	10.23±2.10	17.96±4.61	1.68
40	21.03±1.56	14.70±4.79	11.67±1.84	19.10±3.39	1.68
80	25.19±1.70	19.77±4.69	14.90±2.74	22.85±4.41	1.87
120	24.70±1.80	17.50±4.99	14.42±3.55	23.63±4.97	2.07
Mean[a]	23.64	17.32	13.66	21.86	
SGPT (units/ml)					
0	27.47±0.79	29.78±1.70	29.19±1.38	30.24±1.28	0.65
40	29.45±0.95	31.49±1.43	32.74±1.50	33.47±1.78	0.75
80	30.70±1.42	31.62±0.95	34.39±1.10	32.55±1.53	0.66
120	30.17±0.32	34.39±1.55	34.65±1.26	32.74±2.08	0.77
Mean[a]	30.11	32.50	33.93	32.92	
SGOT (units/ml)					
0	68.64±0.90	68.40±3.10	65.56±2.96	67.78±1.74	1.12
40	62.96±1.42	61.60±1.88	61.36±1.33	63.09±2.50	0.86
80	51.76±2.87	52.85±1.04	54.79±2.22	54.91±2.07	1.03
120	47.64±2.65	53.33±2.18	51.03±3.02	50.30±2.32	1.23
Mean[a]	54.12	55.93	55.73	56.10	
LDH (IU/L)					
0	439±26.96	448±44.93	448±21.35	454±49.75	17.33
40	477±45.52	442±40.13	429±22.04	463±30.16	16.90
80	450±25.99	459±23.34	444±60.86	445±11.36	16.36
120	461±35.99	426±35.02	429±22.04	469±42.90	16.56
Mean[a]	463	442	434	459	

[a] Value is the mean of observations made on days 40, 80, and 120

calves, as their values were comparable ($P>0.05$) in the four groups throughout the experimental period (Table 3). As these enzymes are the indicator of muscular or liver damages and the observations of the present experiment suggested that the level of mineral supplementations were within the safe limits in the diet of buffalo calves.

Consistent to our finding in Holstein Friesian calves [28], no effect was observed on ALP activity when supplemented Zn (30 or 60 ppm) with Cu (20 ppm) also. Similarly in ewes [29], no effect on ALP activity was observed due to supplementation of Zn with Cu (90 and 20 ppm, respectively) also. Similar observations were made in the study on buffalo calves [27] where no effect of Se (0.3 ppm) and/or Cu (10 ppm) supplementation on ALP activity was observed.

Contrary to our observations, reduced serum ALP activity was observed in steers supplemented with 0.1 ppm of Se [26], but it was probably due to a very low level of Se (0.015 ppm) in their basal diet. However, our basal diet had about 0.28 ppm Se; and probably therefore, we did not observe any change in the ALP activity due to further supplementation of Se. Our results are supported by an experiment in Holstein Friesian calves, where ALP activity remained unaltered on supplementation of Cu at 10 and 20 mg/kg of diet [28].

Contrary to our results, buffalo calves supplemented with 8.54 ppm of Se in their diet [24] and adult goats supplemented with 0.15–0.30 mg Se per kilogram body weight [29] showed increased SGOT and SGPT activity. But in both these experiments, the level of Se supplementation was too high compared to our experiment (0.3 ppm of the diet). Similarly, significant increase in SGOT activity has been reported in cows and heifers on Cu supplementation [30], but probably, it was also due to very high levels (80 ppm) of Cu supplementation, which might have been toxic to the animals. However, our results are supported by a study on male Holstein calves, where no effect was observed on SGOT and LDH activity on supplementation of 1 and 10 ppm of Se and Cu, respectively [31]. Similarly, supplementation of 10 ppm Cu in the diet had no effect on SGOT activity in male cattle calves [32]. The results of our previous study [27] also supports that supplementation of either 0.3 ppm Se or 10 ppm Cu either alone or in combination did not have any effect on the serum levels of enzyme SGOT, SGPT, and LDH.

Serum Hormone Profile

Supplementation of 0.3 ppm of Se and/or 10 ppm of Cu with 40 ppm Zn had no effect on insulin, testosterone, T_3

and T_4 levels as their values were comparable ($P>0.05$) among the four groups throughout the experimental period (Table 4). However, there was an overall increasing tendency in the testosterone levels with increasing post-feeding period in all the groups, which may be associated with physiological changes that accompany male sexual maturation [33]. Similar to the present findings, Angus steers supplemented with 10 and 20 ppm Cu also did not show any effect on serum insulin values [34]. In buffalo calves [27], also no difference on the insulin level was observed when supplemented with 0.3 ppm Se with 10 ppm Cu.

Se supplementation has been reported to increase T_3 and decrease T_4 concentrations [26, 35]; but it was probably due to very low levels of Se being 0.015 [26] and 0.02–0.03 ppm [35] in the basal diets in these experiments. On the contrary, basal diet in our experiment had much higher levels of Se (0.28 ppm) and probably for that reason; we did not observe any significant effect of Se

supplementation on T_3 and T_4 hormone levels as such in the buffalo calves. Similar to our observations, sheep (0.11 ppm Se in the basal diet) supplemented with 0.2 ppm of Se did not show any effect on thyroid (plasma T_3 and T_4 levels) metabolism [34]. In buffalo calves [27] also, no effect on thyroid metabolism was observed by supplementation of Se (0.3 ppm) with Cu (10 ppm). Goats [36] also did not show any effect on thyroid hormone levels when even a high level (1 ppm) of selenium was supplemented in their diet. Though, statistically there was no difference in values of T_4 and T_3 among different treatment groups, the ratio of T_4/T_3 was lower ($P<0.05$) in groups supplemented with Se (T2 and T3), as compared to the groups where Se was not supplemented (T_1 and T_4). Thus, supplementation of Se had an effect on T_4/T_3 ratio in the buffalo calves. The results were also supported by the previous findings where in two different experiments conducted on Holstein Friesian calves [28] and reported

Table 4 Serum hormone levels of buffalo calves in different groups

Day	T1	T2	T3	T4	SEM±
Insulin (pmol/L)					
0	13.15±3.15	22.13±1.63	30.84±4.49	25.97±3.76	2.16
40	58.72±14.18	93.06±24.72	77.77±11.84	60.61±12.17	8.26
80	72.77±5.12	69.69±13.68	62.64±4.66	63.32±6.99	3.99
120	53.74±8.71	64.76±26.87	54.75±9.25	51.47±10.70	7.34
Mean[a]	61.74	78.84	65.05	58.47	
T_3 (nmol/L)					
0	1.75±0.05	1.50±0.07	1.64±0.09	1.80±0.08	0.048
40	1.74±0.08	1.83±0.15	1.72±0.09	2.02±0.12	0.058
80	1.62±0.09	1.64±0.07	1.54±0.09	2.00±0.20	0.069
120	1.68±0.10	1.89±0.11	1.73±0.05	1.84±0.09	0.047
Mean[a]	1.68	1.79	1.66	1.95	
T_4 (nmol/L)					
0	38.77±2.68	35.87±3.09	39.17±3.40	46.31±3.10	1.66
40	47.27±2.31	44.83±4.16	39.51±2.26	54.15±7.11	2.36
80	34.27±5.49	36.21±4.32	36.05±4.17	49.88±4.92	2.61
120	50.49±3.57	47.39±4.27	42.88±2.96	53.83±0.87	1.73
Mean[a]	44.01	42.81	39.48	52.62	
T_4/T_3					
0	22.13±1.16	24.19±2.45	23.80±1.55	25.68±0.86	0.797
40	27.30±1.43	24.49±0.72	22.97±0.93	26.87±3.06	0.914
80	21.85±3.97	21.90±2.14	23.35±2.50	25.00±1.05	1.242
120*	30.09±1.53[b]	24.94±1.07[a]	24.79±1.69[a]	29.57±1.92[b]	0.924
Mean[a]	26.41	23.78	23.70	27.15	
Testosterone (ng/ml)					
0	0.257±0.079	0.153±0.031	0.205±0.050	0.093±0.011	0.0267
40	0.242±0.064	0.214±0.048	0.194±0.061	0.167±0.046	0.0263
80	0.512±0.356	0.294±0.064	0.210±0.135	0.216±0.014	0.0930
120	0.636±0.371	0.398±0.079	0.383±0.082	0.251±0.063	0.0957
Mean[a]	0.463	0.302	0.262	0.211	

[a] Value is the mean of observations made on days 40, 80, and 120

*$P<0.05$, means bearing different superscripts in a row differ significantly

decreased T_4/T_3 ratio ($P<0.01$) by intraruminal Se pellet infusion. Similarly, in Frisian steers [26] also lower plasma T_4/T_3 ratio was observed by supplementation of 1 ppm Se.

As Se is a component of enzyme Type I Iodothyronine-5′-deiodinase, which converts T_4 in to T_3 [5] and a lower T_4/T_3 ratio is indicative of better thyroid status of the animals because T_3 is more active form of the thyroid hormone as compared to T_4. Thus the findings of present experiment suggested that supplementation of Se at 0.3 ppm along with either Zn or Zn+Cu had a beneficial effect on thyroid status of animals.

The serum testosterone concentration among different groups remained similar ($P>0.05$) during the entire experimental period. Similar to our previous work in buffalo calves [27] supplementation of Se (0.3 ppm) and/or Cu (10 ppm) had no effect on serum testosterone levels.

On the basis of these results, it is deduced that supplementation of 0.3 ppm Se and/or 10 ppm of Cu with 40 ppm Zn in the basal diet of buffaloes having 0.28 ppm Se, 11.47 ppm Cu and 31.60 ppm Zn had no effect on their blood metabolic profile, except the ratio of T_4 and T_3 hormone which indicates that selenium plays an important role in converting T_4 hormone to T_3 which is more active form of thyroid hormone.

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