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Effects of dietary zinc supplementation on nutrient digestibility, haematological biochemical parameters and production performance in male sika deer (*Cervus nippon*)

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Abstract. The purpose of the present study was to investigate the effects of dietary zinc (Zn) supplementation on antler growth, haematological biochemical parameters and nutrient digestibility in farmed male sika deer. Twenty-five 2-year-old growing male sika deer were randomly divided into five Groups (A, B, C, D and E; 5/group). Animals in Groups A, B, C, Dand E received 0, 5, 15, 40, 100 mg Zn/kg, respectively. Group A, without supplementation acted as a control. The treatment diets were the same basal diet supplemented with 5, 15, 40 and 100 mg Zn/kg (provided as Zn methionine), respectively. The results showed that the digestibility of dry matter (DM) and crude protein in Groups D and E were greater than that in Groups A, B and C ($P < 0.05$). Digestibility of neutral detergent fibre in Group E was higher than in the control group ($P < 0.05$). Plasma Zn concentrations were increased by Zn supplementation and were higher ($P < 0.01$) for the treatment groups supplemented with 15, 40 and 100 mg Zn/kg DM than for the control and 5 mg/kg Zn groups ($P < 0.01$). Faecal Zn content in Groups D and E was higher than that in Group A ($P < 0.01$). The content of albumin in plasma from Group E was greater than in the control and Groups B, C and D ($P < 0.05$). The concentrations of testosterone in plasma from Groups C, D and E were decreased ($P < 0.05$) compared with the control. The days between antler initiation and harvesting of deer in Groups D and E were decreased ($P < 0.05$). Average daily gain of fresh antler and dry antler of deer in Groups D and E was significantly higher than that in the control group ($P < 0.05$). In conclusion, a control diet containing 58.6 mg Zn/kg was inadequate for achieving optimal productivity for sika deer. The recommended Zn supplementation is from 76.7 to 99.0 mg/kg for 2-year-old male sika deer.

Additional keywords: antler, apparent digestibility, trace minerals, Zn.

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Introduction

Trace minerals are essential in ruminant diets as they participate in biochemical processes required for normal growth and development. Trace minerals have been traditionally added to ruminant diets as inorganic salts such as oxides and sulfates. In recent years, there have been considerable interests in feeding animals organic trace minerals that increase the bioavailability of the minerals above that of soluble inorganic forms (Rojas *et al.* 1995; Socha and Johnson 1998; Miles and Henry 2006). Zinc (Zn) is an essential trace mineral that plays a role in multiple enzyme systems (Underwood and Suttle 1999) and, therefore, deficiencies would cause varied symptoms (Robbins 1993). In ruminants, deficiency of the Zn mineral could affect growth and reproduction, impair immune function, alter nervous system function, and cause poor pelage growth (Minson 1990).

Anecdotal reports from private deer farmers suggest that high dietary concentrations of Zn may increase antler size; this is particularly true for white-tailed deer (*Odocoileus virginianus*) and elk (*Cervus elaphus*) (Bartoskewitz *et al.* 2007). In addition, holding deer in captivity may increase their exposure and susceptibility to disease because of the close interaction among animals and stress of confinement. Zn can affect immune function (Galyean *et al.* 1999; Fraker *et al.* 2000; Spears 2000). Thus, high concentrations of dietary Zn may allow growth of deer with larger bodies and antlers, as well as increased disease resistance (Bartoskewitz *et al.* 2007).

Minimum requirements for sheep and cattle range from 20 to 30 mg/kg for Zn (National Research Council 1985; Minson 1990). Zn requirement for deer has not been established. Our objective for the present study was to evaluate the effects of dietary Zn on antler growth, blood biochemical parameters and nutrient digestibility for farmed male sika deer.

Materials and methods

The experiment was carried out at the experimental deer farm of institute of special animal and plant sciences, Chinese Academy

of Agricultural Sciences in north-east of China, in the period from 15 May to 23 August in 2008.

Animals and experiment design

Twenty-five 2-year-old growing male sika deer with an average bodyweight of 55.35 ± 3.15 kg were selected before antler plates drop off from the pedicles and the deer were still in the velvet-antler stage. Animals were randomly divided into five groups (A, B, C, D and E; five/group) at the same level of nutrients, except Zn concentration. Group A, without any supplementation, acted as a control. Concentrated pellets were used as a control diet (Table 1). Nutrient content was measured using standard procedures of AOAC (2000). The control diet contained 58.55 mg Zn/kg DM. The treatment diets were the same as the control diet, except that they contained 5.0 mg Zn/kg, 15.0 mg Zn/kg, 60.0 mg Zn/kg, 100.0 mg Zn/kg (from Zn methionine), respectively, added by the manufacturer during the pelleting process. Levels of Zn supplementation are shown in Table 2.

Management and measurement

Animals were kept under uniform managerial condition by housing them in 10 m × 20 m pens. Ample clean and fresh drinking water was available to the animals at all times. All training and testing of the animals was performed by the same person. The experiment was preceded by 1-week adjustment period, during which the animals were accustomed to the experimental feed. Digestion trial was carried out from 20 to 24 June.

Table 1. Composition and nutritive concentrations of control diet
Measured nutrient concentrations are on a DM basis

Parameter	Concentration
Composition	
Corn flour (%)	15
Soybean meal (%)	15
Puffed rice bran (%)	2
Lucerne (%)	20
Corn germ meal (%)	8
Corn stalk (%)	25
Distillers dried grains with soluble (%)	13.5
Salt (%)	0.5
Additives (without zinc)[A] (%)	1
Total (%)	100
Measured nutrient concentration	
Metabolisable energy (MJ/kg)	12.49
Organic matter (%)	93.64
Crude protein (%)	17.50
Neutral detergent fibre (%)	42.71
Ether extract (%)	3.16
Calcium (%)	1.35
Phosphorus (%)	1.14
Zinc (mg/kg)	58.55

[A]Contained the following per kg of premix: Mg,76 mg; Cu,36 m g; Mn,43 mg; Fe,53 mg; Se, 31 mg; vitamin A, 2 484 IU; vitamin D_3,496.8 IU; vitamin E, 0.828 IU; vitamin K_3, 0. 23 mg; vitamin B_1, 10.092 mg; vitamin B_2 0.69 mg; vitamin B_{12},1.38 m g; folic acid 0.023 mg; nicotinic acid 1.62 mg; calcium pantothenate 1.15 mg; $CaHPO_4$ 5.17 g; $CaCO_3$ 4.57 g.

Sample collection and treatment

Collection of faeces

The faeces free of sand and other debris were carefully collected by part faecal collection method everywhere in the pen at 0530 hours every day during the trial period, and then sprayed with dilute sulfuric acid to avoid the loss of nitrogen. For chemical analysis, faecal material was dried at 60°C and ground to pass a 1-mm screen in a Wiley mill and preserved in airtight bottles. Feeds and refusals were processed similarly before chemical analysis.

Collection of blood

Blood samples were collected 30 days after the start of the treatments through jugular venipuncture in the morning (before watering and feeding). Deer were anesthetised with xylazine hydrochloride (Qing dao Hanhe Animal and Plant Medicine Co., Qing dao, Shandong Province), which was administered by blow-gun-dart syringe at a dosage of 0.5~3.0 mg/kg of body mass. Deer were administered an intraveneous injection of tolazoline hydrochloride (Sigma Chemical Co., St Louis, MO, USA) for recovery from the xylazine hydrochloride. Each deer also received 2 mL penicillin after sampling as a prophylactic. The blood was collected into tubes with heparin sodium to prevent the blood from clotting. After centrifuging for 10 min at 3000 r.p.m., plasma was collected and stored in 2-mL plastic vials at −20°C for further analysis.

Collection of antler

Velvet antlers were removed by our professional technician under the guidance of the institutional velveting regime. Antlers were removed at the same time. The procedures for this were as follows: lignocaine analgesia consisted of administration of 2% lignocaine hydrochloride at a high dose (1.2 mL/cm pedicle circumference) in a ring around the base of the pedicle, followed by a 4-min wait, testing of analgesia, and antler removal as with compression. Fresh antler yield, the days between antler initiation and harvesting were measured at the same time. Antlers were stored at −80°C and then processed by free drier. Dry antler yield was measured after being processed. The whole dry antlers were ground to pass a 1-mm screen in a Wiley mill for further analysis. One antler of each group was selected to analyse Zn content.

Dry matter content and average daily gain of antlers were calculated by the following formulae:

$$\text{DM content}\,(\%) = (\text{dry antler yield}/\text{fresh antler yield}) \times 100;$$

$$\text{Average daily gain of fresh antler}\,(\text{g/day}) = \text{fresh antler yield}/\text{the days between antler initiation and harvesting;}$$

$$\text{Average daily gain of dry antler}\,(\text{g/day}) = \text{dry antler yield}/\text{the days between antler initiation and harvesting.}$$

Chemical analysis

The content of crude protein (CP), neutral detergent fibre (NDF), phosphorus (P) and calcium (Ca) were determined in our laboratory by using standard procedures of AOAC (2000).

Table 2. Level of zinc (Zn) supplementation in different treatment groups

Group	Zn (mg/kg DM)	Total Zn (mg/kg DM)
A	0	58.6
B	5.0	63.3
C	15.0	76.7
D	40.0	99.0
E	100.0	163.0

The digestibilities of DM, CP, NDF, P and Ca were calculated by the acid-insoluble ash method (2 N HCL) (Van Keulen and Young 1977). Zn concentrations in plasma, faeces, antlers and diets were measured by atomic absorption spectroscopy (VARIAN, Palo Alto, CA, USA). Blood biochemical parameters such as alkaline phosphatase (ALP), alanine transaminase (ALT), total protein (TP), albumin (ALB) and testosterone (T) were measured by diagnostic kits. ALP, TP, ALB and ALT (Nanjing Jiancheng Bioengineering Institute, Nanjing, China) were measure using colourimetric (biuret, bromocresol green and disodium phenyl phosphate) methodsand velocity (lactate dehydrogenase). Plasma testosterone concentrations were determined by time-resolved fluoroimmunoassay, using a commercially available DELFIA testosterone kit (Wallac Oy, Turku, Finland), according to the manufacturer's instructions.

Statistical analyses

The data are presented as means and s.e.m. ANOVA and comparison of significance were performed by Duncan's multiple-range test in SAS (SAS Institute Inc. 2008). Trends were considered significant if probability values of $P < 0.05$ were obtained.

Results

Nutrient digestibility

Effects of diet Zn on the digestibility of nutrients in antler growing period is shown in Table 3. The digestibility of DM in Groups D and E was greater than that in Groups A and B ($P < 0.05$). The digestibility of crude protein in Groups D and E was greater than that in Groups A and B ($P < 0.05$). The digestibility of NDF in Group E was higher than that in the control ($P < 0.05$).

Zn concentration in plasma, faeces and antler

Zinc concentration in plasma, faeces and antler is shown in Table 4. With the increasing Zn supplementation, Zn concentration in plasma, faeces and antler increased. Plasma Zn concentrations increased and were higher ($P < 0.01$) for the treatment groups supplemented with 15, 40 and 100 mg Zn/kg DM than those of the control and 5 mg Zn/kg DM groups ($P < 0.01$). Faecal Zn concentration in Groups D and E was higher than that in Group A ($P < 0.01$). Zn concentration in antler was increased for treatment groups compared with the control group.

Hematological biochemical parameters

Effects of dietary Zn on blood biochemical parameters is shown in Table 5. As shown in Table 5, the activity of alanine transaminase (ALT) in Group E was greater than that in the control and Group B

Table 3. Effect of diet zinc supplementation on the digestibility of nutrients in antler growing period (%)

Means followed by different letters within a row differ significantly (at $P = 0.05$); $n = 5$ per treatment

Item	Treatment group					s.e.m.	*P*-value
	A	B	C	D	E		
Dry matter	56.9b	56.6b	59.4ab	63.2a	67.1a	4.00	0.034
Crude protein	68.1c	68.2c	71.9bc	72.9ab	76.8a	2.68	0.002
Nneutral detergent fibre	48.1b	49.5b	51.4ab	57.9ab	59.9a	5.30	0.024
Calcium	46.6	49.1	51.9	55.0	56.9	5.61	0.062
Phosphorus	59.3	57.7	59.8	57.6	58.9	5.31	0.095

($P < 0.05$). The concentration of albumin (ALB) in Group E was greater than that in the control and Groups B, C and D groups ($P < 0.05$). The concentration of testosterone (T) was decreased by Zn supplementation. The concentration of testosterone in Groups C, D and E was lower than that in the control and Group B ($P < 0.05$). The activity of alkaline phosphatase (ALP) and the concentration of total protein were not affected by supplementation level of dietary Zn.

Production performance of male sika deer

Effects of dietary Zn on production performance are shown in Table 6. Fresh antler mass, dry antler mass and antler drying matter rate were not affected by dietary Zn concentration ($P > 0.05$). The days between antler initiation and harvesting in Groups D and E decreased compared with the control group ($P < 0.05$). Average daily gain of fresh antler and dry antler in Groups D and E was significantly higher than that in the control ($P < 0.05$).

Discussion

Nutrient digestibility

The results of nutrient digestibility were in disagreement with Mandal *et al.* (2007) who indicated that DM, CP and NDF digestibility were not affected by dietary Zn in bulls fed a basal diet (32.5 mg Zn/kg DM) supplemented with 35 mg Zn/kg DM. In most studies, Zn supplementation ranging from 20 to 135 mg/kg DM does not influence digestibility of DM and CP (Bedi 1976; Khan 1978; Kumar *et al.* 2002), or NDF (Salama *et al.* 2003) in ruminants. Salama *et al.* (2003) reported that apparent digestibility of DM and CP was higher in dairy goats supplemented with Zn methionine (1 g/day) in their diet than in non-supplemented goats. A likely reason for the effects of Zn supplementation on digestibility may be that Zn requirements of ruminal microbes were not met by the basal diet. The results may be associated with special rumen system (methanogen composition and diversity) of domestic sika deer as compared with other ruminants such as domestic cows, sheep, yak and camels. It appears that the processed diet in the present experiment containing 99.0 and 163.0 mg Zn/kg DM was sufficient.

Zn content in plasma, faeces and antler

Plasma Zn concentrations can reflect the metabolism of Zn and utilisation of Zn in the body. The normal plasma Zn range in

Table 4. Zinc (Zn) concentration in plasma, faeces and antler

One antler from each group was selected for analysis of Zn concentration. Variance and comparison of significance was not performed. Means followed by different letters within a row differ significantly (at $P = 0.05$); $n = 5$ per treatment

Item	Treatment group					s.e.m.	*P*-value
	A	B	C	D	E		
Plasma Zn (μg/mL)	0.4b	0.4b	0.5a	0.5a	0.5a	0.03	0.003
Faeces Zn (mg/kg)	171.1d	173.1d	201.7d	275.5b	416.6a	10.84	<0.001
Antler Zn (mg/kg)	22.7	24.0	24.5	24.7	25.2		

Table 5. Effect of dietary zinc supplementation on blood biochemical parameters

Means followed by different letters within a row differ significantly (at $P = 0.05$); $n = 5$ per treatment

Item	Treatment group					s.e.m.	*P*-value
	A	B	C	D	E		
Alkaline phosphatase (U/L)	27.4	30.1	32.7	32.2	35.8	4.12	0.792
Alanine transaminase (U/L)	52.8b	54.1b	63.4ab	57.7ab	73.4a	5.84	0.025
Total protein (g/L)	71.7	72.7	73.0	76.1	74.0	3.97	0.832
Albumin (g/L)	25.4b	26.8b	27.5b	27.4b	31.0a	2.27	0.032
Testosterone (ng/mL)	0.9a	0.8a	0.5c	0.7bc	0.6bc	0.12	0.022

Table 6. Effect of dietary zinc supplementation on production performance of sika deer

Means followed by different letters within a row differ significantly (at $P = 0.05$); $n = 5$ per treatment

Item	Treatment group					s.e.m.	*P*-value
	A	B	C	D	E		
Fresh antler yield (g)	643.8	661.3	668.8	671.3	703.7	37.21	0.531
Dry antler yield (g)	197.5	213.8	218.3	216.3	227.5	25.28	0.242
Dry matter content (%)	30.7	32.7	32.8	31.2	32.4	1.31	0.103
The days between antler initiation and harvesting (days)	47.5a	50.5a	43.8ab	41.5b	42.5b	4.12	0.038
Average daily gain of fresh antler (g/day)	11.2c	13.1bc	14.8ab	16.2a	16.9a	1.84	0.022
Average daily gain of dry antler (g/day)	3.6b	4.3b	4.5ab	5.2a	5.5a	0.70	0.024

ruminants is 0.9–1.5 mg/L (ARC 1980). Results of the present study indicated that control animals may have been deficient in Zn because the plasma Zn concentration was below 0.9 mg/L. Similar to our results, Chhabra and Arora (1985) found that plasma Zn concentrations were higher in goats fed a basal diet (15 mg Zn/kg DM) supplemented with 65 mg Zn/kg DM, than in the control goats. When Zn is adequate, supplement Zn would not be expected to increase plasma Zn concentration due to homeostatic mechanisms (Lu 2004). Therefore, since blood Zn concentration has been served as a popular Zn index in the clinical field, the increased Zn concentration might be a useful parameter to evaluate Zn status.

The apparent absorption of inorganic Zn for adult animals is between 5% and 10%, and more than 50% of Zn in feed is not absorbed and it is excreted along with faeces into the soil and cannot be degraded, which would pollute the environment. Moreover, using organic Zn can enhance the utilisation rate of Zn and decrease the environmental pollution at the same time. Zn methionine has been studied extensively and has been reported to have equal, or higher, bioavailability than Zn sulfate (Kellog 1990; Wedekind *et al.* 1992). Zn faecal excretion from deer of Groups D and E was greater for the treatment diet than the control ($P < 0.01$). The results would mean that 98.97 mg Zn/kg DM was sufficient for sika deer. More Zn would be excessive and excreted if diet Zn was ~160.0 mg Zn/kg DM.

Hematological biochemical parameters

Although in our study, some of the biochemical parameters were slightly elevated ($P > 0.05$) in Zn-supplement group, there were no differences between treatments in concentrations of ALP and T in blood plasma. Liver is the main organ for the synthesis and storage of ALT and ALB. Liver may be damaged when the activity of ALT increases or decreases (Bao *et al.* 2010). Activity of alanine transaminase in Group E was higher than that in the control and Group B ($P < 0.05$). The content of albumin in Group E was higher than that in the control and Groups B, C and D ($P < 0.05$). These results indicated that when diet Zn content is 162.95 mg/kg DM, it would not be beneficial to sika deer. In our study, the concentration of testosterone in Groups C, D and E was lower than that in the control and Group B ($P < 0.05$). The concentration of testosterone was decreased by Zn supplementation. Velvet antler ossification process depends on androgen. The body of sika deer controls testosterone synthesis by adjusting the activity of follicle-stimulating hormone and luteinising hormone. Testosterone reaches a minimum level

when antler is growing fast (Li *et al.* 2003; Bubenik *et al.* 2005). Suitable Zn supplementation as Zn methionine was from 15 to 40 mg/kg (total Zn content 76.7–99.0 mg/kg DM) according to haematological biochemical parameters.

Production performance

Antlers are bony appendages developed from outgrowths of the frontal bone of the skull, referred to as pedicles, in most species of the deer family (Li *et al*. 2009). In our study, the number of days between antler initiation and harvestings in Groups D and E was lower than that in the control ($P < 0.05$). Average daily gain of fresh antler and dry antler in Groups D and E was significantly higher than that in the control ($P < 0.05$). These results indicated that Zn supplementation could raise average daily gain of antler. Suitable Zn supplementation was from 15 to 40 mg/kg (total Zn content 76.7–99.0 mg/kg DM).

Conclusions

The intensity of deer management is increasing as more animals are intended to be held in captivity for commercial and recreational purposes. Safe and effective feeding practices are needed to ensure animal health, wellbeing and productivity. Our results suggested that diet with different levels Zn supplementation could influence digestibility of nutrient, Zn concentration in plasma, faeces and antler, haematological biochemical parameters and antler mass. Suitable level of Zn supplementation was found to be from 15 to 40 mg/kg (total Zn content 76.7–99.0 mg/kg DM) in antler-growing period for 2-year-old sika deer.

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