

Effects of conjugated linoleic acid on growth performance, nutrient digestibility and blood biochemical indexes of male sika deer (*Cervus nippon*)

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ABSTRACT

Context. Conjugated linoleic acid (CLA) is very important for animals and humans. CLA has many important biological functions, such as reducing fat and increasing muscle, antioxidation, improving immunity and so on. CLA requirements for deer have not been established. **Aims.** A single-factor test was conducted to evaluate the effects of CLA supplementation on male sika deer. **Methods.** Sixteen deer were divided in four groups (from G0 to G3) of four animals, each according to their bodyweight. Deer in G0 were fed a basal diet without CLA supplementation. Deer in G1, G2 and G3 were fed diets supplemented with CLA at concentrations of 0.25%, 0.5% and 1.0%. Growth performance, nutrient digestibility and blood biochemical indexes were measured. **Key results.** The results suggested that the average daily gain of deer increased with conjugated linoleic acid supplementation ($P < 0.05$); maximal growth performance was seen in G2. The average daily feed intake showed differences among the treatments ($P < 0.01$). The highest average daily feed intake was observed in Group G2. Feed to gain ratio (F:G) in Groups G1, G2 and G3 was different from that in Group G0 ($P < 0.01$). The digestibility of crude protein and ether extract was increased by conjugated linoleic acid concentrations ($P < 0.05$). The alkaline phosphatase activity showed a significant increase ($P < 0.05$) in Groups G2 and G3, compared with Group G0. There were significant differences in cholesterol between G1 and G2 groups ($P < 0.05$). **Conclusions.** The results indicated that conjugated linoleic acid supplementation to diet plays a positive role in the growth of sika deer. **Implications.** This experiment has shown the effects of dietary supplementation with CLA in sika deer breeding. It has laid a good foundation for the application of CLA supplementation in sika deer industry to promote the healthy development of sika deer breeding industry.

Keywords: biochemical indexes, blood, conjugated linoleic acid, digestibility, growth performance, nutrient, requirement, sika deer.

Introduction

Conjugated linoleic acid (CLA) has a conjugated double bond containing 18 carbons with different positions and spatial configurations. It is a mixture of dienoic acid isomers with various physiological functions. Natural CLA is commonly found in animal products, and ruminant products are the most abundant source of CLA (Kim *et al.* 2016a). The simplest and most effective way to increase the CLA content of animal products is to regulate dietary formulations, such as adding oils and fats to animal diets. The first described bioactive benefit of CLA was reported in 1987; since then, more beneficial activities of CLA have been found, such as having anticancer, anti-obesity and anti-oxidation effects (Kim *et al.* 2016b). As for the mechanism of action of CLA, the results suggest that CLA reduces the lipid accumulation in adipocytes by reducing the pre-differentiation and fat uptake of adipocytes and increasing the decomposition and apoptosis of adipocytes (Kim *et al.* 2016b).

According to the prior research, most of the study of CLA is focused on ruminants such as cattle and sheep. Little work on CLA has been conducted for sika deer. Sika deer are important ruminants and can provide products such as velvet antler. Velvet antler is a very valuable Chinese herbal medicine, and also the main source of income for deer farmers. Antler yield and quality directly affect the income of deer farmers. The effect of CLA on the growth of sika deer has not been reported. The present study was conducted on growing male sika deer to find out the effects of CLA supplementation on growth performance, nutrient digestibility and blood biochemical indexes.

Materials and methods

The experiment was conducted in antler deer (*Cervus nippon*) breeding base at the Institute of Special Animal and Plant Sciences, CAAS (Jilin, China), from 7 July to 22 October 2019.

Animal welfare statement

The animals used in this study and all experimental procedures were approved by the Chinese Academy of Agricultural Sciences Animal Care Committee and by the Institute of Special Animal and Plant Sciences Wild Animal and Plant Subcommittee (Jilin, China). They conformed to the provisions of the National Institutes of Health Guide for the Care and Use of Laboratory Animals (NIH Publications No. 8023, revised 1978). All methods were in accordance with the approved guidelines and regulations.

Experiment design

Sixteen 1-year-old male sika deer were used as experimental animals and the average bodyweight of the animals was 40.25 ± 2.38 kg. Prior to the trial, deer were fed a diet without addition of CLA for 15 days to make the animals accustomed to the experimental diets.

Then the deer were assigned to four treatments (four deer/treatment) in such a way that the average bodyweight was similar among the treatments ($P > 0.05$; Wu *et al.* 2015). The experimental deer were fed the following diets: (1) basal diet (containing 0% CLA, G0); (2) basal diet and 0.25% CLA (G1); (3) basal diet and 0.5% CLA (G2); and (4) basal diet and 1% CLA (G3). CLA was purchased from Xi'an tianguangyuan biological Co. Ltd., with a concentration of 99%, and the main component of Trans10, Cis12-CLA. The dietary composition and nutritional content of basal diets are shown in Table 1.

Management

The experimental deer were managed in a unified group. Adequate and clean water was available to deer at all times.

Table 1. Composition and nutritive levels of control diet.

Parameter	Concentration
Composition (%)	
Corn flour	22
Soybean meal	12
Lucerne	50
Distillers dried grains with soluble (DDGS)	4
Corn germ meal	5.5
Molasses	5
NaCl	0.5
Additives ^A	1
Total	100
Measured nutrient concentration (dry matter)	
Gross energy (GE, MJ/kg)	14.03
Crude protein (CP, %)	15.80
Neutral detergent fibre (NDF, %)	41.54
Ether extract (EE, %)	3.31
Acid detergent fibre (ADF, %)	16.16
Ca (%)	0.76
P (%)	0.50

^AContaining the following per kilogram of premix: Mg, 76 mg; Cu, 36 mg; Zn, 43 mg; Fe, 53 mg; vitamin A, 2484 IU; vitamin D₃, 496.8 IU; vitamin K₃, 0.23 mg; vitamin B₁, 10.092 mg; vitamin B₂, 0.69 mg; vitamin B₁₂, 1.38 mg; folic acid, 0.023 mg; nicotinic acid, 1.62 mg; calcium pantothenate, 1.15 mg; CaHPO₄, 5.17 g; CaCO₃, 4.57 g.

The experimental deer were fed total mixed rations (TMR) twice a day, at 6 am and 4 pm. The digestion trial took place from 18 to 24 August.

Growth trial

The growth trial lasted for 70 days, from 9 July to 18 September. During this period, feed supply and feed residue were recorded every day to calculate average daily feed intake (ADFI) and feed efficiency (Bao *et al.* 2020). Deer were weighed every 2 weeks and their weight was recorded to calculate the average daily gain (ADG). Feed to gain ratio (F:G) = ADFI / ADG.

Collection of blood and faeces

Blood was taken intravenously on 30 days of the trial in the morning (the deer must not be fed or drunk). To prevent blood clotting, blood was collected in tubes containing heparin. Plasma was collected after centrifuging for 10 min at 3000g at 4°C and stored in 1.5 mL plastic vials at -20°C for further analysis (Bao *et al.* 2020).

On the 40th day of the growth period, 16 animals were selected from each treatment and placed in a metabolic cage to separate urine and faeces to determine the digestibility of

nutrients (Bao et al. 2020). The digestion experiment lasted for 6 days; faeces and feed residue were collected every day. The feed samples were further analysed. The faeces of deer were collected every day. After weighing, 10% of faeces was reserved for further analysis. The faecal material was dried at 65°C and ground to pass through a 1 mm sieve. Prior to chemical analysis, feed and refusals were treated similarly (Bao et al. 2017).

Chemical analysis

The concentrations of crude protein (CP), dry matter (DM), ether extracts (EE), neutral detergent fiber (NDF), acid detergent fiber (ADF), calcium (Ca) and phosphorus (P) were determined according to the methods of AOAC (Association of Official Analytical Chemists) (2005). Blood biochemical parameters such as total protein (TP), albumin (ALB), glutamic-pyruvic transaminase (ALT), glutamic-oxalacetic transaminase (AST), alkaline phosphatase (ALP), triglyceride (TG), low-density lipoprotein (LDL), high-density lipoprotein (HDL), cholesterol (CHO), urea nitrogen (BUN) and glucose (GLU) were measured by diagnostic kits (Nanjing Jiancheng Bioengineering Institute). The total protein content was determined by Coomassie brilliant blue method. The albumin content was determined by bromocresol green method. The glutamic-pyruvic transaminase activity was detected by Wright's method. Glutamic-oxaloacetic transaminase activity was detected by colorimetry. Alkaline phosphatase activity was detected by visible light colorimetry. The concentration of triglyceride was determined by single reagent GPO-PAP method. Low-density lipoprotein and high-density lipoprotein were determined by double-reagent direct method. The concentration of cholesterol was determined by single-reagent GPO-PAP method. Urea nitrogen was determined by urease method. The concentration of glucose in the serum was determined by hexokinase method. All operations were performed strictly in accordance with the requirements of the kit.

Statistical analyses

Data are represented in the form of means \pm s.d. Multiple comparison and significance analysis were performed by

Duncan's method in SAS (SAS Institute Inc., Cary, NC, USA, 2008). It was considered to be significant if $P < 0.05$.

Results

Growth performance

As shown in Table 2, bodyweight (BW) showed no difference after dietary treatment in this study ($P > 0.05$). But the average daily gain (ADG) of deer increased with the CLA supplementation in diet ($P < 0.05$); maximal growth performance was seen in the G2 group. The average daily feed intake (ADFI) showed significant differences among the treatments ($P < 0.01$). The highest ADFI was observed in Group G2. At the same time, feed to gain ratio (F:G) in Groups G1, G2 and G3 showed significant differences from that of Group G0 ($P < 0.01$).

Nutrient digestibility

Effect of dietary CLA concentration on apparent digestibility of nutrients is shown in Table 3. There were no differences in apparent digestibility of DM, ADF, NDF, Ca and P among the four treatments ($P > 0.05$). Apparent CP and EE digestibilities were increased by CLA supplementation ($P < 0.05$).

Blood biochemical indexes

Data regarding plasma biochemical indexes are shown in Table 4. No significant ($P > 0.05$) differences were observed among different CLAs with respect to total protein, albumin, ALT, AST, GLU, TG, LDL, HDL and BUN activity. While the ALP concentration showed a significant ($P < 0.05$) increase in G2 and G3 compared with that in Group G0. It can also be noted that there was a significant difference in CHO between Groups G1 and G2 ($P < 0.05$).

Discussion

Conjugated linoleic acid has important biological functions and is essential for animal growth. CLA has been widely

Table 2. Effects of supplementation of CLA on growth performance of deer.

Item	G0	G1	G2	G3	P-value
Initial BW (kg)	56.63 \pm 8.19	56.00 \pm 5.21	52.63 \pm 5.11	56.88 \pm 8.77	0.8139
Final BW (kg)	62.63 \pm 7.34	65.63 \pm 4.17	63.63 \pm 3.20	66.00 \pm 7.45	0.8225
ADG (g/day)	120.00 \pm 26.25b	160.42 \pm 18.48a	183.33 \pm 34.02a	152.08 \pm 43.76a	0.0198
ADFI (g/day)	1685.12 \pm 0.083c	1785.15 \pm 0.065ab	1793.23 \pm 0.063a	1765.34 \pm 0.1008b	0.010
F:G	16.81 \pm 2.57A	11.53 \pm 1.56B	10.62 \pm 1.85B	12.16 \pm 2.73B	0.0045

Note: values in the same row followed by different upper-case letters (A, B) are significantly different at $P = 0.01$, and those followed by different lower-case letters (a, b) are significantly different at $P = 0.05$.

Table 3. Effects of supplementation of CLA on nutrients digestibility of deer.

Item	G0	G1	G2	G3	P-value
DM	54.32 ± 3.18	55.25 ± 2.60	58.66 ± 3.08	63.18 ± 3.69	0.4852
CP	62.65 ± 3.52b	62.71 ± 2.62b	69.21 ± 2.08a	63.32 ± 4.21a	0.0153
EE	47.43 ± 2.11b	49.26 ± 3.12b	59.81 ± 3.05a	55.26 ± 3.15a	0.0225
ADF	44.16 ± 3.51	44.66 ± 2.16	45.88 ± 1.56	46.03 ± 2.36	0.2561
NDF	50.02 ± 3.15	52.32 ± 2.18	54.05 ± 2.38	54.64 ± 3.52	0.1387
Ca	48.26 ± 1.57	48.33 ± 2.18	49.33 ± 1.55	49.25 ± 2.18	0.1252
P	54.11 ± 2.88	53.26 ± 3.45	54.66 ± 3.78	53.25 ± 2.87	0.2053

Note: values in the same row followed by different letters (a, b) differ significantly at $P = 0.05$.

Table 4. Effects of supplementation of CLA on serum biochemical indexes in deer.

Item	G0	G1	G2	G3	P-value
TP (g/L)	66.76 ± 6.22	65.26 ± 8.83	68.865 ± 5.899	69.828 ± 4.57	0.760
ALB (g/L)	25.085 ± 3.692	23.257 ± 4.677	26.962 ± 3.88	24.89 ± 2.228	0.591
ALT (U/L)	54.498 ± 6.41	51.092 ± 8.23	52.575 ± 6.72	52.975 ± 6.286	0.973
AST (U/L)	61.255 ± 11.90	64.773 ± 14.875	78.777 ± 9.566	69.032 ± 13.179	0.427
ALP (U/L)	79.808 ± 22.75a	89.473 ± 25.49ab	126.69 ± 30.86b	217.345 ± 63.73a	0.043
TG (mmol/L)	0.460 ± 0.067	0.5175 ± 0.071	0.2475 ± 0.029	1.160 ± 0.074	0.249
LDL (mmol/L)	0.185 ± 0.060	0.2075 ± 0.039	0.195 ± 0.033	0.150 ± 0.014	0.256
CHO (mmol/L)	1.700 ± 0.322ab	2.082 ± 0.504a	1.890 ± 0.249ab	1.465 ± 0.196b	0.043
HDL (mmol/L)	1.335 ± 0.118	1.567 ± 0.368	1.333 ± 0.188	1.215 ± 0.165	0.228
BUN (mmol/L)	9.530 ± 1.024	10.043 ± 1.945	7.925 ± 0.499	7.555 ± 0.754	0.034
GLU (mmol/L)	5.645 ± 0.779	5.298 ± 0.489	5.777 ± 0.468	5.622 ± 0.640	0.974

Note: values in the same row without different letters (a, b) differ significantly at $P < 0.05$.

studied in fat metabolism and adipogenesis in cell culture and animal models (Shen *et al.* 2018). Benchaar *et al.* (2012) and Kholif *et al.* (2016) found that feed supplemented with linseed oil did not affect the feed intake of cows and goats respectively. Hoffmann *et al.* (2016) fed full-fat rapeseed or rapeseed oil (DM 2.2%) to dairy cows, and found that the DM intake of the experimental group increased significantly. Prieto-Manrique. (2018) added 20 g/kg and 40 g/kg sunflower oil to the diet, and found that it reduced the feed intake of dairy cows, which is similar to the results of the present experiment. This may be because of the unsaturated fatty acids in sunflower oil, especially long-chain fatty acids, account for 60% of the total fatty acids. The number of protozoa in rumen is significantly reduced and the number of fibrinolytic bacteria is significantly increased if the diet is rich in C18:2 fatty acids. When the number of protozoa is reduced, the utilisation efficiency of feed can be increased, and unnecessary energy cycle consumption can be reduced, which may affect the intake of animals (Ivan *et al.* 2004). The fat-containing long-chain polyunsaturated fatty acids will increase cholecystokinin and reduce rumen motility, and eventually affect food intake (Agazzi *et al.* 2010). In this experiment, the pre-test

time was 15 days, and the amount of CLA increased day by day, which had little effect on the palatability of deer. In the normal test period of 90 days, the amount of CLA added to each group was less than 2%, which may be because the level of oil added was lower than the safe level for rumen microbiota activity (Gomaa *et al.* 2018), which was not enough to inhibit the intake. The results of the effect of oil supplementation on ruminant intake are different, which may be due to the different amount of oil added and the different effect on rumen environment.

Conjugated linoleic acid can participate in the metabolism of fat and other substances, and promote the digestion and absorption of nutrients through the body's metabolism. CLA can realise nutrient redistribution and promote animal production performance. At present, dietary CLA supplementation has been reported to be improve, inhibit or not affect the animal growth performance. The reasons for these differences may be related to animal species, dietary nutrient level, or different isomer and dosage of CLA. Jiang *et al.* (2014) reported that 1% CLA supplementation to broiler diet had no significant effect on the growth performance of broilers. Du and Ahn (2002) and Zhang *et al.* (2008) also obtained similar results in the trials on broilers. Zhang *et al.* (2008)

pointed out that adding hydrogenated palm oil to the diet significantly improved the ADG and feed conversion rate of Xiza beef cattle. Ferreira *et al.* (2014) reported that the mixture of fish oil and soybean oil did not affect the ADG of male lambs. Zhang *et al.* (2021) found that the supplementation of CLA could increase the total weight gain and ADG of Tan sheep during lactation. In the present experiment, the ADG of the experimental group was 3.49%, 17.44%, 10.47% higher than that of Group G0, and the F:G of the experimental group was 9.74%, 21.85%, 21.59% lower than that of G0 group, which indicated that the addition of CLA showed a trend of increasing the daily weight of cows, reducing the ratio of consumption to weight, and had a positive effect on improving the feed utilisation rate, and the effect in the group with 1.0% CLA was greater. Guo *et al.* (2019) found that adding different levels of CLA in the diet had no significant effect on the final weight, ADG, average DM intake and the ratio of feed consumption to weight gain of the cows. 1.0% CLA supplementation showed a trend of promoting the growth of the cows. This is similar to the results of our study.

Concentration of TP and ALB in serum is an important index to evaluate the liver function. Serum transaminase is a sensitive index of hepatocyte injury. The main function of the liver is to metabolise fat, protein and carbohydrate. Under normal circumstances, the activities of ALT and AST are stable. Liver and heart may be damaged if the activities of ALT and AST increase or decrease. In this study, the addition of CLA had no significant effect on TP, ALB, AST and ALT concentrations in the serum. The ALT concentration in Groups G1, G2 and G3 was lower than that in Group G0. However, they were all in the normal range, indicating that the addition of CLA would reduce ALT concentration and have no adverse effect on the liver.

ALP participates in the metabolism of fat, and an increase or decrease in the activity of ALP will affect growth rate and production performance of animals. An increase in the activity of ALP in the serum could help improve daily gain (Wu *et al.* 1999). ALP in the serum of growing animals mainly comes from bone. In normal conditions, the intensity of osteogenesis correlates positively to the activity of ALP. At the same time, ALP participates in the metabolism of phosphonium and calcium and is closely related to the deposition of phosphonium and calcium (Xing *et al.* 2008).

TG, CHO, LDL and HDL are common indexes used to reflect the metabolism level of blood lipid in the animal body. TG can reflect the development of fatty tissue and the ability of fat deposition in the animal body; LDL transports CHO from the liver to the whole body, and HDL transports CHO from various tissues back to the liver for metabolism. Serum TG is mainly synthesised in liver and adipose tissue, and can also be absorbed and synthesised from food through intestinal mucosa. Zhao (2014) found that no effect on TG concentration was observed by adding 2.4% fish oil, sunflower oil and the mixed oil to the diet of Bamei mutton sheep, but the

total concentration of CHO, HDL and LDL in the blood of sheep was higher than that in the G0 group after adding sunflower oil.

The concentration of BUN can reflect protein catabolism and renal function. GLU concentration in the serum is an important index of energy balance of the animal body. A change in the GLU concentration can reflect a physiological state of the body, which is generally positively related to the growth rate, to a certain extent. Roy *et al.* (2013) found that the serum GLU concentration in goats was not affected by oil supplement. However, some studies have reported an increase in plasma GLU concentration in goats after oil supplementation (Li *et al.* 2012). No significant difference in BUN and GLU concentrations was observed in the present experiment. With the increase of CLA, the GLU concentration in the serum increased at first, and then decreased, and the GLU concentration in the 0.5% group was the highest, which was consistent with the trend of ADG in this experiment. It indicated that the addition of CLA promoted the growth of deer and increased the utilisation rate of energy *in vivo*.

Conclusion

CLA supplementation promotes the ADG, ADFI and feed utilisation of deer significantly according to our current results. At the same time, a number of serum biochemical indexes were improved because of CLA supplementation.

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Data availability. The data that support this study are available in the article.

Conflicts of interest. This paper has not been published elsewhere in whole or in part. All authors have read the paper and have agreed to submit it in its current form for consideration for publication in the Journal. There are no ethical/legal conflicts involved in the article.

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