Effects of Threonine Level in Low-Protein Diets on Growth Performance and Nutrient Digestibility of Sika Deer Fawns during the Pre-Overwintering Period: Postprint

Authors: Li Rende, Zhang Tietao, Cui Xuezhe, Si Huazhe, Sun Weili, Zhang Ting, Li Guangyu, Kaiying Wang

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Abstract

This experiment aimed to investigate the effects of threonine (Thr) levels in low-protein diets on growth performance and nutrient digestibility in sika deer fawns during the early overwintering period. Twenty healthy, 6-month-old male sika deer were selected and randomly divided into 4 groups with 5 deer per group. The four groups of deer were limit-fed four different diets. By supplementing rumen-protected lysine, rumen-protected methionine, and rumen-protected threonine, the dietary lysine (Lys) and methionine (Met) levels were identical across all groups at 0.63% and 0.18%, respectively, while Thr levels were 0.55% (Group), 0.47% (Group), 0.53% (Group), and 0.58% (Group). Additionally, Group (control group) received a high-protein diet with a protein level of 15.15%, while the experimental groups (Groups , , and) received low-protein diets with a protein level of 13.46%. The preliminary period was 15 days, and the experimental period was 45 days. The results showed: 1) The final body weight of the control group and Group was significantly higher than that of Group ($P < 0.05$); the average daily gain (ADG) of Group was extremely significantly lower than that of the other groups ($P < 0.01$); the feed-to-gain ratio (F/G) of Group was extremely significantly higher than that of the other groups ($P < 0.01$), while that of Group was extremely significantly lower than that of the control group and Group ($P < 0.01$). 2) The crude fat digestibility of Group was extremely significantly lower than that of Group ($P < 0.01$) and significantly lower than that of Group ($P < 0.05$); the neutral detergent fiber (NDF) digestibility of the control group was significantly lower than that of Group ($P < 0.05$); the energy digestibility of the control group was extremely significantly lower than that of Group ($P < 0.01$); the calcium digestibility of Group was extremely significantly higher than that of the other groups ($P <$

0.01), while that of Group was extremely significantly lower than that of the control group and Group ($P < 0.01$); the phosphorus digestibility of Group was extremely significantly higher than that of Group ($P < 0.01$) and significantly higher than that of the control group and Group ($P < 0.05$). 3) The methionine and threonine digestibility of Group was extremely significantly higher than that of the control group and Group ($P < 0.01$) and significantly higher than that of Group ($P < 0.05$); the aspartic acid digestibility of Group was significantly higher than that of the other groups ($P < 0.05$); the serine digestibility of the control group was extremely significantly lower than that of the other groups ($P < 0.01$); the glutamic acid digestibility of the control group was extremely significantly higher than that of Group and Group ($P < 0.01$); the tyrosine digestibility of the control group was significantly lower than that of Group ($P < 0.05$); the proline digestibility of the control group was extremely significantly lower than that of Group ($P < 0.01$) and significantly lower than that of Group ($P < 0.05$). It can be concluded that under the condition of identical dietary Lys and Met levels, the growth performance and nutrient digestibility of sika deer fawns fed a low-protein diet with a protein level of 13.46% and a Thr level of 0.47% were comparable to those of sika deer fawns fed a high-protein diet with a protein level of 15.15% and a Thr level of 0.55%.

Full Text

Effects of Threonine Level in a Low-Protein Diet on Growth Performance and Nutrient Digestibility of Sika Deer Fawns During Early Winter

LI Rende, ZHANG Tietao, CUI Xuezhe, SI Huazhe, SUN Weili, ZHANG Ting, LI Guangyu, WANG Kaiying*

State Key Laboratory of Special Economic Animal Molecular Biology, Institute of Special Animal and Plant Sciences, Chinese Academy of Agricultural Sciences, Changchun 130112, China

Abstract

This study was conducted to investigate the effects of threonine (Thr) level in a low-protein diet on growth performance and nutrient digestibility of sika deer fawns during early winter. Twenty healthy 6-month-old male sika deer were randomly divided into 4 groups with 5 deer per group. The four groups were fed four different diets on a restricted basis. Through supplementation with rumen-protected lysine (RPL), rumen-protected methionine (RPM), and rumen-protected threonine (RPT), all diets contained identical lysine (Lys) and methionine (Met) levels of 0.63% and 0.18%, respectively, while Thr levels were 0.55% (Group I), 0.47% (Group II), 0.53% (Group III), and 0.58% (Group IV). Additionally, Group I (control) received a high-protein diet with 15.15%

crude protein, while the experimental groups (Groups II, III, and IV) received low-protein diets with 13.46% crude protein. The experiment consisted of a 15-day preliminary period followed by a 45-day formal trial period. The results showed: (1) The final body weight of the control group and Group II was significantly higher than that of Group IV ($P<0.05$). The average daily gain (ADG) of Group IV was extremely significantly lower than that of the other groups ($P<0.01$). The feed-to-gain ratio (F/G) of Group IV was extremely significantly higher than that of the other groups ($P<0.01$), while that of Group II was extremely significantly lower than that of the control group and Group III ($P<0.01$). (2) The ether extract (EE) digestibility of Group IV was extremely significantly lower than that of Group II ($P<0.01$) and significantly lower than that of Group III ($P<0.05$). The neutral detergent fiber (NDF) digestibility of the control group was significantly lower than that of Group II ($P<0.05$). The energy digestibility of the control group was extremely significantly lower than that of Group II ($P<0.01$). The calcium (Ca) digestibility of Group II was extremely significantly higher than that of the other groups ($P<0.01$), while that of Group IV was extremely significantly lower than that of the control group and Group III ($P<0.01$). The phosphorus (P) digestibility of Group II was extremely significantly higher than that of Group IV ($P<0.01$) and significantly higher than that of the control group and Group III ($P<0.05$). (3) The digestibility of methionine and threonine in Group II was extremely significantly higher than that in the control group and Group IV ($P<0.01$) and significantly higher than that in Group III ($P<0.05$). The digestibility of aspartic acid (Asp) in Group II was significantly higher than that in the other groups ($P<0.05$). The digestibility of serine (Ser) in the control group was extremely significantly lower than that in the other groups ($P<0.01$). The digestibility of glutamic acid (Glu) in the control group was extremely significantly higher than that in Groups II and III ($P<0.01$). The digestibility of tyrosine (Tyr) in the control group was significantly lower than that in Group II ($P<0.05$). The digestibility of proline (Pro) in the control group was extremely significantly lower than that in Group II ($P<0.01$) and significantly lower than that in Group III ($P<0.05$). In conclusion, under conditions of identical dietary Lys and Met levels, sika deer fawns fed a low-protein diet containing 13.46% crude protein and 0.47% Thr exhibited comparable growth performance and nutrient digestibility to those fed a high-protein diet containing 15.15% crude protein and 0.55% Thr.

Keywords: sika deer fawns; threonine; growth performance; nutrient digestibility; low-protein diet

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Author Introduction: LI Rende (1991-), male, from Shangqiu, Henan, master' s student, engaged in research on nutrition and feeding of special economic animals. E-mail: 1432846608@qq.com

Corresponding Author: WANG Kaiying, associate researcher, master' s supervisor, E-mail: tcswky@126.com

Introduction

In China, sika deer farming has a long history, and deer products have extremely high medicinal and economic value. As feeding costs continue to rise, people increasingly recognize the importance of improving feed utilization and reducing feed costs. Reducing dietary protein level is an effective way to solve the problem of high feed costs, and adding limiting amino acids to low-protein diets can reduce the negative impacts of insufficient protein on feed utilization and production performance [1-3]. Numerous studies have shown that adding rumen-protected amino acids to low-protein diets for ruminants can effectively overcome the disadvantage of crystal amino acids being degraded by rumen microorganisms, allowing them to be directly utilized by the body and improving feed utilization [4-5]. Threonine plays important roles in the growth and development of young animals, including improving growth performance, feed conversion efficiency, and immunity. However, research on rumen-protected threonine in deer fawns is still blank and urgently needs to be studied and explored [6-10]. This experiment investigated the effects of adding rumen-protected threonine to low-protein diets on growth performance and nutrient digestibility of sika deer fawns during winter, aiming to screen for the appropriate supplementation level of rumen-protected threonine in low-protein diets and provide data support for amino acid nutrition research in sika deer.

1.1 Experimental Animals and Design

Twenty healthy 6-month-old male sika deer fawns with an initial body weight of (38.55$±$1.44) kg and no significant differences in body weight and body size indices (P>0.05) were randomly divided into 4 groups with 5 deer per group. The dietary threonine levels in this experiment were determined based on the Lys:Thr ratio of 100:77 reported by Hill et al. [7] and Wang Jianhong et al. [11], as well as the threonine level in the high-protein diet. The four groups were fed four different diets, where Group I (control) received a high-protein diet with 15.15% crude protein, and the experimental groups (Groups II, III, and IV) received low-protein diets with 13.46% crude protein supplemented with different levels of rumen-protected threonine. The threonine levels in the diets for Groups I, II, III, and IV were 0.55%, 0.47%, 0.53%, and 0.58%, respectively. By supplementing rumen-protected lysine and rumen-protected methionine, all four diets contained identical lysine and methionine levels of 0.63% and 0.18%, respectively. The Lys:Thr ratios for Groups I, II, III, and IV were 100.00:86.50, 100.00:74.24, 100.00:83.48, and 100.00:92.87, respectively. The total experimental period was 60 days, including a 15-day preliminary period and a 45-day formal trial period.

1.2 Experimental Diets and Management

To ensure that each group had the same concentrate-to-forage ratio and corresponding crude protein content, different formulas were used for each group' s diet. According to Diao Qiyu et al. [12], the rumen environment of calves gradually stabilizes after 4 months of age, with pH, ammonia nitrogen (NH3-N), and volatile fatty acid content fluctuating within a certain range. Therefore, this experiment used rumen-protected amino acids. High-protein diets (15.15% crude protein) and low-protein diets (13.46% crude protein) were formulated by mixing dried distillers grains with solubles (DDGS), corn, soybean meal, corn germ meal, alfalfa hay powder, wheat bran, salt, premix, and other ingredients in different proportions. The low-protein diets were additionally supplemented with different levels of rumen-protected threonine, rumen-protected lysine, and rumen-protected methionine (using high-stability coating technology with a rumen bypass rate above 90% and intestinal release rate above 95%) to achieve the designed levels of threonine, lysine, and methionine. The composition and nutrient levels of each group' s diet are shown in Table 1 , and the amino acid contents are shown in Table 2 .

The experiment was conducted at the Deer Antler Experimental Base of the Institute of Special Animal and Plant Sciences, Chinese Academy of Agricultural Sciences, from November 12, 2016, to January 11, 2017. Deer were fed twice daily (08:30 and 15:00) with quantitative feeding and free access to water.

Table 1 Composition and Nutrient Levels of Diets in Different Groups (Air-Dry Basis)

Table 2 Amino Acid Contents of Diets in Different Groups (Air-Dry Basis)

1.3 Fecal Sample Collection and Index Determination

During the last 4 days of the formal trial period, feces were collected continuously each day. Fresh fecal samples were collected daily from 09:00–10:00 at 5 fixed points in each deer pen, with approximately 100 g collected per point. Following the acid-insoluble ash method requirements, impurities were removed as much as possible. The collected fecal samples were dried in a 65°C oven, ground, and passed through a 0.425 mm sieve. The crude protein, ether extract, neutral detergent fiber, acid detergent fiber, calcium, and phosphorus contents in diet and fecal samples were determined according to *Feed Analysis and Feed Quality Detection Technology* [13]. Amino acid contents were determined using a Hitachi L8900 automatic amino acid analyzer. Nutrient digestibility was calculated using the 2 mol/L hydrochloric acid-insoluble ash method with reference to the calculation formula in literature [14], as follows:

Nutrient digestibility (%) = 100 -100 × [dietary acid-insoluble ash content (%) / fecal acid-insoluble ash content (%)] × [fecal nutrient content (%) / dietary nutrient content (%)]

Energy apparent digestibility (%) = 100 -100 × [dietary AIA content (%) /

fecal AIA content (%)] × [fecal gross energy (MJ/kg) / dietary gross energy (MJ/kg)]

1.4 Body Weight and Body Size Index Determination

On the morning of day 60, experimental deer were fasted and anesthetized, then weighed using a Shanghai Yingzhan electronic scale (capacity 150 kg, precision 0.01 kg) to record body weight and calculate average daily gain (ADG). Daily feed intake was recorded to calculate average daily feed intake (ADFI) and feed-to-gain ratio (F/G). Body size indices (body height, body length, chest circumference) were measured according to methods described in *Cattle Production Science* [15].

1.5 Statistical Methods

Data were statistically analyzed using the ANOVA procedure of SAS 9.3 software, and Duncan's multiple comparison method was used to analyze significant differences between groups. $P<0.05$ was considered significant, and $P<0.01$ was considered extremely significant. Data are expressed as mean ± standard deviation.

2.1 Effects of Threonine Level in Low-Protein Diet on Growth Performance of Sika Deer Fawns

As shown in Table 3 , the final body weight of the control group and Group II was significantly higher than that of Group IV ($P<0.05$), with no significant difference from Group III ($P>0.05$). The average daily gain (ADG) of Group IV was extremely significantly lower than that of the other groups ($P<0.01$), with no significant differences among the other groups ($P>0.05$). The feed-to-gain ratio (F/G) of Group IV was extremely significantly higher than that of the other groups ($P<0.01$), while that of Group II was extremely significantly lower than that of the control group and Group III ($P<0.01$).

Table 3 Effects of Threonine Level in a Low-Protein Diet on Growth Performance of Sika Deer Fawns

In the same row, values with different small letter superscripts indicate significant difference (P<0.05), different capital letter superscripts indicate extremely significant difference (P<0.01), and no letters indicate no significant difference (P>0.05). The same applies below.

2.2 Effects of Threonine Level in Low-Protein Diet on Energy and Nutrient Digestibility of Sika Deer Fawns

As shown in Table 4 , under low-protein dietary conditions, both energy and nutrient digestibility showed a gradual decreasing trend with increasing threonine levels. The energy digestibility of the control group was extremely significantly lower than that of Group II ($P<0.01$), with no significant differences among

other groups (P>0.05). The ether extract (EE) digestibility of Group IV was extremely significantly lower than that of Group II (P<0.01) and significantly lower than that of Group III (P<0.05), with no significant difference from the control group (P>0.05). The neutral detergent fiber (NDF) digestibility of the control group was significantly lower than that of Group II (P<0.05), with no significant differences from other groups (P>0.05). The calcium (Ca) digestibility of Group II was extremely significantly higher than that of the other groups (P<0.01), while that of Group IV was extremely significantly lower than that of the control group and Group III (P<0.01), with no significant difference between the control group and Group III (P>0.05). The phosphorus (P) digestibility of Group II was extremely significantly higher than that of Group IV (P<0.01) and significantly higher than that of the control group and Group III (P<0.05).

Table 4 Effects of Threonine Level in a Low-Protein Diet on Energy and Nutrient Digestibility of Sika Deer Fawns

2.3 Effects of Threonine Level in Low-Protein Diet on Amino Acid Digestibility of Sika Deer Fawns

As shown in Table 5 , the digestibility of methionine and threonine in Group II was extremely significantly higher than that in the control group and Group IV (P<0.01) and significantly higher than that in Group III (P<0.05). The digestibility of aspartic acid (Asp) in Group II was significantly higher than that in the other groups (P<0.05), with no significant differences among the other groups (P>0.05). The digestibility of serine (Ser) in the control group was extremely significantly lower than that in the other groups (P<0.01), with no significant differences among the other groups (P>0.05). The digestibility of glutamic acid (Glu) in the control group was extremely significantly higher than that in Groups II and III (P<0.01), with no significant difference from Group IV (P>0.05). The digestibility of tyrosine (Tyr) in the control group was significantly lower than that in Group II (P<0.05), with no significant differences among the other groups (P>0.05). The digestibility of proline (Pro) in the control group was extremely significantly lower than that in Group II (P<0.01) and significantly lower than that in Group III (P<0.05), with no significant difference from Group IV (P>0.05).

Table 5 Effects of Threonine Level in a Low-Protein Diet on Amino Acid Digestibility of Sika Deer Fawns

3.1 Effects of Threonine Level in Low-Protein Diet on Growth Performance of Sika Deer Fawns

The experimental results showed that threonine level in low-protein diets had extremely significant effects on average daily gain and feed-to-gain ratio, and significant effects on final body weight of sika deer fawns during early winter. Yang Yufen et al. [16] found that supplementing appropriate levels of lysine, methionine, and threonine in low-protein diets for growing pigs could achieve

growth performance comparable to feeding high-protein diets. Yin Xihan [17] in Holstein dairy cattle and Yang Kui [18] in fattening cattle both found that supplementing appropriate levels of rumen-protected lysine and rumen-protected methionine in low-protein diets could achieve growth performance comparable to high-protein diets while saving feed costs. Yun Qiang et al. [19] reported similar findings in calf experiments. Socha et al. [20] and Broderick et al. [21] found that supplementing amino acids in dairy cow diets could improve protein digestibility and feed conversion efficiency. Kidd et al. [22] reported that adding threonine to broiler diets and reducing dietary protein level by 1.0%~1.5% did not significantly affect weight gain or feed conversion ratio. Zhang Yanlei [23] in rabbits and Qu Zhengxiang [24] in broilers found that average daily gain increased with increasing threonine level before reaching the optimal requirement, but decreased when threonine level exceeded the requirement. Tang Maoyan et al. [25] found that average daily gain of growing pigs showed a trend of first increasing then decreasing with increasing dietary threonine level at the same lysine level. Chee et al. [26], Maroufyan et al. [27], and Habte-Tsion et al. [28] all found that appropriate threonine levels could promote intestinal mucin secretion and nutrient absorption, thereby improving production performance and protein deposition. Under low-protein dietary conditions, average daily gain decreased with increasing threonine level, while feed-to-gain ratio increased, possibly because excess threonine caused amino acid imbalance in the body, increasing energy consumption through deamination, or because excess threonine interfered with the absorption and utilization of other amino acids. Therefore, during early winter, when dietary protein level is 13.46%, the appropriate threonine level is 0.47%, at which fawns show optimal growth performance comparable to the control group fed a high-protein diet with 15.15% crude protein and 0.55% threonine.

3.2 Effects of Threonine Level in Low-Protein Diet on Energy and Nutrient Digestibility of Sika Deer Fawns

Appropriate dietary protein level and balanced amino acids are required for animals to achieve optimal growth performance and nutrient digestibility [29], while levels that are too high or too low cause feed waste and affect animal growth [30-32]. In this experiment, the control group received a high-protein diet with 15.15% crude protein, which is similar to the 15.09% optimal protein level for sika deer during winter reported by Wang Xin et al. [33]. The ether extract digestibility of Group II was extremely significantly higher than that of Group IV, showing a decreasing trend with increasing threonine level, indicating that excessive threonine reduces fat digestion and utilization in fawns. This contradicts the conclusion of Westermeier et al. [34] that threonine promotes fat decomposition and utilization, possibly because threonine levels in Groups III and IV of this experiment were already excessive, affecting nutrient digestibility and thus reducing ether extract digestibility. The energy, calcium, and phosphorus digestibility of the control group were extremely significantly or significantly lower than those of Group II, and the calcium and phosphorus

digestibility of Groups II, III, and IV all showed decreasing trends with increasing threonine level, indicating that reducing protein level can improve dietary energy, calcium, and phosphorus digestibility, while excessive threonine reduces energy and threonine digestibility, increases calcium and phosphorus excretion, and intensifies environmental pollution. The NDF digestibility of Group II was significantly higher than that of the control group, with no significant differences from other groups, indicating that reducing dietary protein level can improve NDF digestibility but this effect is independent of dietary threonine level. Therefore, appropriate dietary threonine level can promote the digestion and utilization of ether extract, energy, calcium, and phosphorus, reduce calcium and phosphorus excretion, and alleviate environmental pressure. In summary, the appropriate threonine level in low-protein diets for fawns during early winter is 0.47%.

3.3 Effects of Threonine Level in Low-Protein Diet on Amino Acid Digestibility of Sika Deer Fawns

Amino acids are the basic structural units of protein and play important roles in protein metabolism. The experimental results showed that the digestibility of methionine, threonine, serine, glutamic acid, and proline in Group II was extremely significantly higher than that in the control group, and the digestibility of aspartic acid and tyrosine was significantly higher than that in the control group, indicating that reducing dietary protein level can improve the digestibility of methionine, threonine, serine, glutamic acid, proline, aspartic acid, and tyrosine. This contradicts the findings of Dong Zhiyan [35] in growing pigs. The closer the amino acid composition of dietary protein is to that of animal body protein, the higher its biological value, and when an essential amino acid is completely lacking, its biological value is zero [36]. Combined with the results of this experiment, the amino acid composition of Group II diet was relatively balanced, resulting in higher amino acid digestibility. Adding limiting amino acids to diets can reduce protein usage. Chen Aodong et al. [37] found that adding rumen-protected amino acids to diets could improve metabolic performance and feed conversion efficiency in dairy cows. Zhou Yanwen [38] found that the metabolic rate of methionine in Hepu geese first increased then decreased with increasing dietary threonine level. In this experiment, the digestibility of methionine and threonine gradually decreased with increasing dietary threonine level, indicating that threonine was excessive in the control group and Groups III and IV. Threonine is the only amino acid that does not undergo deamination or transamination for catabolism and can be converted to serine and glycine through threonine dehydrogenase, threonine dehydratase, and threonine aldolase catalysis. Glutamic acid has some threonine-sparing effect [39]. Therefore, dietary levels of serine, glycine, and glutamic acid are closely related to threonine metabolism. In this experiment, the digestibility of serine, glycine, glutamic acid, and aspartic acid all decreased with increasing dietary threonine level, further indicating that threonine was excessive in the control group and Groups III and IV. In summary, the dietary threonine level of 0.47% maximized

amino acid digestibility and utilization in fawns during early winter.

4 Conclusion

Under the conditions of this experiment, a low-protein diet (13.46% crude protein) with a threonine level of 0.47% provided optimal feed utilization, nutrient digestibility, and amino acid digestibility for sika deer fawns during early winter, and produced growth performance comparable to that achieved with a high-protein diet (15.15% crude protein with 0.55% threonine).

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Note: Figure translations are in progress. See original paper for figures.