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Effects of Threonine Level in Low-Protein Diet on Growth Performance and Nutrient Apparent Digestibility in Pre-weaning Sika Deer Fawns: Postprint

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Abstract

This experiment aimed to investigate the effects of threonine levels in low-protein diets on growth performance and nutrient apparent digestibility in pre-weaning sika deer fawns. Twenty-four healthy 3-month-old male sika deer were selected and randomly divided into 4 groups with 6 deer per group. The four groups of fawns were limit-fed four different diets, with dietary lysine and methionine levels at 0.87% and 0.28%, respectively, for all groups. Specifically, the high-protein diet control group (Group I) was fed a high-protein diet with a protein level of 16%, while the low-protein diet experimental groups (Groups II–IV) were fed low-protein diets with a protein level of 14% supplemented with different threonine levels. The dietary threonine levels were 0.54% (Group I), 0.46% (Group II), 0.59% (Group III), and 0.72% (Group IV). The pre-trial period was 15 days, and the formal trial period was 30 days. The results showed: 1) The average daily gain (ADG) of the high-protein diet control group was extremely significantly higher than that of Group II ($P < 0.01$), with no significant differences compared to Groups III and IV ($P > 0.05$). The feed-to-gain ratio (F/G) of the high-protein diet control group was extremely significantly higher than that of Groups III and IV ($P < 0.01$), and extremely significantly lower than that of Group II ($P < 0.01$). 2) The crude protein apparent digestibility of the high-protein diet control group was extremely significantly lower than that of Groups III and IV ($P < 0.01$), and extremely significantly higher than that of Group II ($P < 0.01$). The energy apparent digestibility of the high-protein diet control group was extremely significantly lower than that of Groups III and IV ($P < 0.01$), with no significant difference compared to Group II ($P > 0.05$). The calcium apparent digestibility of Group III was extremely significantly higher than that of Group II ($P < 0.01$), with no significant differences

compared to other groups (P > 0.05). 3) The histidine, aspartic acid, glutamic acid, glycine, and cysteine apparent digestibility of the high-protein diet control group was significantly higher than that of Group II (P < 0.05), with no significant differences in other amino acid apparent digestibility compared to Group II (P > 0.05). The lysine, methionine, threonine, arginine, alanine, and tyrosine apparent digestibility of the high-protein diet control group was extremely significantly lower than that of Group III (P < 0.01), and valine, leucine, phenylalanine, and serine apparent digestibility was significantly lower than that of Group III (P < 0.05). Except for alanine apparent digestibility being extremely significantly lower than that of Group IV (P < 0.01) and lysine, threonine, valine, and alanine apparent digestibility being significantly lower than that of Group IV (P < 0.05), the high-protein diet control group showed no significant differences in other amino acid apparent digestibility compared to Group IV (P > 0.05). It can be concluded that appropriate threonine levels in low-protein diets have a promoting effect on growth performance and nutrient digestion and utilization in pre-weaning sika deer fawns. When dietary lysine and methionine levels are the same, sika deer fawns fed a low-protein diet with 14% protein level and 0.59% threonine level showed superior growth performance and nutrient apparent digestibility compared to those fed a high-protein diet with 16% protein level.

Full Text

Effects of Threonine Level in a Low Protein Diet on Growth Performance and Nutrient Apparent Digestibility of Early Weaning Sika Deer Fawns

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Abstract: This experiment investigated the effects of threonine (Thr) level in a low protein diet on growth performance and nutrient apparent digestibility of early weaning sika deer fawns. Twenty-four healthy 3-month-old male sika deer fawns were randomly divided into 4 groups with 6 fawns per group. All groups received restricted amounts of 4 different diets with lysine (Lys) and methionine (Met) levels held constant at 0.87% and 0.28%, respectively. The high protein diet control group (Group I) received a diet with 16% crude protein, while the low protein diet experimental groups (Groups II–IV) received diets with 14% crude protein supplemented with different Thr levels. Dietary Thr levels were 0.54% (Group I), 0.46% (Group II), 0.59% (Group III), and 0.72% (Group IV). The experiment included a 15-day preliminary period followed by a 30-day formal trial period. The results showed: (1) The average daily gain (ADG) of Group I was extremely significantly higher than that of Group II (P<0.01),

with no significant differences compared to Groups III and IV (P>0.05). The feed/gain ratio (F/G) of Group I was extremely significantly lower than that of Groups III and IV (P<0.01) and extremely significantly higher than that of Group II (P<0.01). (2) The crude protein apparent digestibility of Group I was extremely significantly lower than that of Groups III and IV (P<0.01) and extremely significantly higher than that of Group II (P<0.01). The energy apparent digestibility of Group I was extremely significantly lower than that of Groups III and IV (P<0.01), with no significant difference compared to Group II (P>0.05). The calcium apparent digestibility of Group III was extremely significantly higher than that of Group II (P<0.01), with no significant differences compared to other groups (P>0.05). (3) The apparent digestibility of histidine, aspartic acid, glutamic acid, glycine, and cysteine in Group I was significantly higher than that in Group II (P<0.05), with no significant differences for other amino acids (P>0.05). The apparent digestibility of lysine, methionine, threonine, arginine, alanine, and tyrosine in Group I was extremely significantly lower than that in Group III (P<0.01), while valine, leucine, phenylalanine, and serine were significantly lower (P<0.05). Compared to Group IV, Group I showed extremely significantly lower alanine digestibility (P<0.01) and significantly lower lysine, threonine, valine, and alanine digestibility (P<0.05), with no significant differences for other amino acids (P>0.05). These results indicate that appropriate Thr levels in low protein diets can promote growth performance and nutrient utilization in early weaning sika deer fawns. Under the same dietary Lys and Met levels, fawns fed a 14% protein diet with 0.59% Thr demonstrated superior growth performance and nutrient apparent digestibility compared to those fed a 16% protein diet.

Keywords: sika deer; threonine; growth performance; low protein diet

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Sika deer has been the primary deer species farmed in China due to the significant health benefits of deer products in strengthening the body and delaying aging. However, like other livestock producers, deer farmers have recently focused on increasing dietary protein levels to maximize product yield while neglecting nutritional balance. This has resulted in low nutrient utilization efficiency and substantially increased emissions of greenhouse gases such as methane and carbon dioxide, as well as malodorous gases and wastewater, raising production costs and causing environmental pollution. While reducing dietary protein levels can effectively decrease nitrogen in feces and ammonia emissions, it directly impairs animal performance [1-3]. Supplementing low protein diets with limiting amino acids to create amino acid-balanced formulations can improve animal performance while reducing environmental pollution [4-6]. This technology is well-established in pigs and poultry but remains poorly studied in sika deer, particularly in fawns. Existing research has focused only on methionine (Met) and lysine (Lys) [1-3], while threonine (Thr), which directly affects nutrient digestibility, immunity, and growth performance in young animals, remains unstudied and warrants urgent investigation. This experiment investigated the

effects of Thr supplementation in low protein diets on growth performance and nutrient utilization in early weaning sika deer fawns to determine optimal dietary Thr levels and provide data support for amino acid nutrition research in sika deer.

1.1 Experimental Animals and Design

Twenty-four healthy, disease-free, 3-month-old male sika deer fawns with an average body weight of (21.44$±$3.00) kg and no significant differences in body measurements (P>0.05) were randomly divided into 4 groups of 6 fawns each. All groups received restricted feeding of 4 different diets. The high protein diet control group (Group I) received a diet with 16% crude protein, while the low protein diet experimental groups (Groups II–IV) received diets with 14% crude protein supplemented with different Thr levels. Dietary Thr levels were 0.54% (Group I), 0.46% (Group II), 0.59% (Group III), and 0.72% (Group IV). All diets were supplemented with Lys and Met to maintain levels of 0.87% and 0.28%, respectively. The Lys:Thr ratios were 100.0:62.5, 100.0:52.6, 100.0:68.3, and 100.0:83.0 for Groups I, II, III, and IV, respectively. The total experimental period was 45 days, including a 15-day preliminary period and a 30-day formal trial period.

1.2 Experimental Diets and Management

To ensure consistent concentrate-to-forage ratios and corresponding crude protein levels across groups, different formulations were used. Diets were prepared using dried distillers grains with solubles (DDGS), corn, soybean meal, corn germ meal, alfalfa meal, Chinese wildrye meal, wheat bran, molasses, salt, premix, and other ingredients to create a 16% protein diet and 14% protein diets with varying Thr levels. The high protein diet was supplemented with Lys and Met, while the low protein diets maintained the same Lys and Met levels with Thr added according to the experimental design. After thorough mixing, ingredients were processed into total mixed ration (TMR) pellets with a diameter of 0.4 cm and length of 1.2–1.5 cm. The diet composition and nutrient levels are shown in Table 1 , and amino acid contents are presented 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 August 14 to September 28, 2016. Animals were fed twice daily at 07:30 and 15:00 with quantitative rations and had free access to water.

1.3 Fecal Sample Collection and Analysis

During the final 4 days of the formal trial period, fresh feces were collected continuously each day. Between 09:00–10:00 daily, approximately 100 g of fresh feces were collected from 6 fixed points in each deer pen. Following the acid insoluble ash (AIA) method requirements, impurities were carefully removed. Fecal samples were dried in a 65°C oven, ground, and passed through a 0.425

mm sieve. Dry matter (DM), organic matter (OM), crude protein (CP), ether extract (EE), neutral detergent fiber (NDF), acid detergent fiber (ADF), calcium (Ca), and phosphorus (P) contents were determined according to *Feed Analysis and Feed Quality Detection Technology* (2nd edition) [7]. Amino acid contents were measured using a Hitachi L-8900 automatic amino acid analyzer. Nutrient apparent digestibility was determined using the 2 mol/L AIA method.

Nutrient apparent digestibility was calculated using the formula from Reference [8]:

Nutrient apparent digestibility (%) = 100 -100 × [AIA content in diet (%) / AIA content in feces (%)] × [Nutrient content in feces (%) / Nutrient content in diet (%)].

1.4 Body Weight and Body Measurement Indices

On day 45 of the experiment, fawns were anesthetized before morning feeding and weighed using a Shanghai Yingzhan electronic scale (capacity 150 kg, precision 0.01 kg) after fasting (precision 0.01 kg). Body weight was recorded to calculate average daily gain (ADG). Daily feed intake was recorded to calculate average daily feed intake (ADFI) and feed/gain ratio (F/G). Body measurements (body height, body length, chest circumference) were determined according to methods described in *Cattle Production Science* [9].

1.5 Statistical Methods

Data were analyzed using the ANOVA procedure in SAS 9.3 software. Duncan' s multiple comparison test was used to analyze significant differences among groups. Differences were considered significant at $P<0.05$ and extremely significant at $P<0.01$.

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

As shown in Table 3 , Group II exhibited the lowest values for all indices except F/G. Its final body weight was significantly lower than other groups ($P<0.05$), ADG was extremely significantly lower ($P<0.01$), and F/G was extremely significantly higher ($P<0.01$). The ADFI of the high protein diet control group was extremely significantly lower than all low protein diet groups ($P<0.01$), while its F/G was extremely significantly lower than Group II but extremely significantly higher than Groups III and IV ($P<0.01$). Groups III and IV showed no significant differences from the high protein control group in any indices except for extremely significantly higher ADFI ($P<0.01$) and extremely significantly lower F/G ($P<0.01$). No significant differences were observed between Groups III and IV for any growth performance indices ($P>0.05$).

2.2 Effects of Threonine Level in Low Protein Diet on Nutrient Apparent Digestibility of Fawns

As shown in Table 4 , in the low protein diet groups, apparent digestibility of all nutrients except EE, NDF, and energy showed an initial increase followed by a decrease as Thr levels increased. Groups III and IV showed extremely significantly higher CP and energy apparent digestibility compared to both the high protein control group and Group II ($P<0.01$). Group III exhibited extremely significantly higher Ca apparent digestibility than Group II ($P<0.01$). Group II showed significantly lower NDF and Ca apparent digestibility compared to other groups ($P<0.05$). No significant differences were observed among groups for EE, ADF, or P apparent digestibility ($P>0.05$). The high protein control group showed extremely significantly higher CP apparent digestibility ($P<0.01$) and significantly higher NDF apparent digestibility ($P<0.05$) than Group II, with no significant differences for other nutrients ($P>0.05$), though values were numerically higher.

2.3 Effects of Threonine Level in Low Protein Diet on Amino Acid Apparent Digestibility of Fawns

As shown in Table 5 , in the low protein diet groups, apparent digestibility of all amino acids initially increased then decreased as Thr levels increased. Group II showed the lowest apparent digestibility for all amino acids, while Group III showed the highest. Group III exhibited extremely significantly higher apparent digestibility of lysine, methionine, threonine, arginine (Arg), alanine (Ala), and tyrosine (Tyr) compared to the high protein control group ($P<0.01$), and significantly higher valine (Val), leucine (Leu), phenylalanine (Phe), and serine (Ser) digestibility ($P<0.05$). Group III also showed extremely significantly higher apparent digestibility of all amino acids except Val compared to Group II ($P<0.01$), with Val digestibility significantly higher ($P<0.05$). Compared to the high protein control group, Group IV showed extremely significantly higher Ala digestibility ($P<0.01$) and significantly higher Lys, Thr, Val, and Ala digestibility ($P<0.05$), with no significant differences for other amino acids ($P>0.05$). The high protein control group exhibited significantly higher apparent digestibility of histidine (His), aspartic acid (Asp), glutamic acid (Glu), glycine (Gly), and cysteine (Cys) compared to Group II ($P<0.05$), with no significant differences for other amino acids ($P>0.05$).

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

As shown in Table 3, significant differences in F/G, ADG, and ADFI among groups after the experiment indicate that Thr level in low protein diets affects fawn growth performance, and appropriate dietary Thr levels are essential for rapid growth. Early weaning fawns have underdeveloped rumens but are undergoing rapid body development, making their nutrient utilization more similar to monogastric animals. Research shows that threonine is an indispensable amino

acid for animal growth, and its deficiency can reduce feed efficiency and retard growth [10-12]. Scheideler [13] and Sklan et al. [14] both found that low protein diets inhibit growth in young animals, consistent with Group II results in this study. In the low protein diet groups, growth performance initially increased then decreased with rising Thr levels, indicating that fawn performance improves with Thr level within the appropriate range, consistent with findings by Liu Weidong et al. [15], Zhang Changming et al. [16], Feng Weixian [17], and Zhang Chun et al. [18].

Çifici et al. [19] found interactive effects between dietary protein and Thr levels, primarily affecting feed conversion efficiency, weight gain, and feed intake. Zhang Weibing et al. [20] and Lou Can et al. [21] demonstrated that feed intake and nutrient digestion/absorption reflect not only changes in digestive organ structure and function but also effectively indicate animal health, performance, and nutrient utilization efficiency. Fawns enter a rapid growth phase after weaning, and as feed intake increases, digestive organs also develop rapidly, manifested by increases in rumen microbial populations, gastrointestinal volume and surface area, and developmental changes in digestive enzymes. These changes inevitably affect fawn digestibility of dietary nutrients. In summary, low protein diets with appropriate Thr levels can promote animal growth, improve feed conversion efficiency, and enhance growth performance.

3.2 Effects of Threonine Level in Low Protein Diet on Nutrient Apparent Digestibility of Fawns

Appropriate dietary protein levels and balanced amino acids are required for optimal animal growth and production performance [22], while excessive or insufficient protein levels cause feed waste and impair growth [14,23-24]. The 16% protein level in the high protein diet in this study is similar to the optimal level reported by Yu Liwei [25]. Reducing dietary protein from 16% to 14% without additional Thr supplementation resulted in the lowest CP apparent digestibility, consistent with results from Lee et al. [3] and Russell et al. [26]. As Thr levels increased, CP apparent digestibility increased, then decreased at excessive Thr levels, possibly because excess Thr affects absorption of other amino acids. As protein building blocks, amino acids directly affect protein digestion and absorption. The Lys:Thr ratio of 100.0:68.3 in Group III' s diet is consistent with Wang et al. [27]. The intestine can utilize two-thirds of dietary Thr, with absorbed Thr ultimately used for body protein synthesis [19,28-33].

Group II showed the lowest nutrient apparent digestibility, likely because Thr deficiency disrupted amino acid balance and affected rumen microbial activity, ultimately reducing intestinal digestion capacity. While amino acid excess rarely damages the body under natural conditions due to metabolic conversion pathways, excessive synthetic amino acids can have negative effects. Air pollution from livestock mainly originates from organic matter in feces and urine, which produces malodorous gases and greenhouse effects when fermented [34]. In this study, CP, Ca, ADF, and P apparent digestibility in low protein diet groups

showed an initial increase then decrease with rising Thr levels, indicating that appropriate Thr levels can promote organic matter digestion and utilization, reduce organic matter content in feces and urine, and achieve energy conservation and environmental protection.

3.3 Effects of Threonine Level in Low Protein Diet on Amino Acid Apparent Digestibility of Fawns

Amino acids have complex effects on animal organisms, and amino acid utilization efficiency increases with improved amino acid balance. Only balanced amino acids achieve maximum utilization efficiency. Early weaning fawns grow rapidly with underdeveloped rumens, making their nutrient utilization more similar to monogastric animals. Threonine is an essential amino acid indispensable for young animal growth; its deficiency causes growth retardation, reduced feed efficiency, and even impaired immunity. With widespread application of Met and Lys in animal production, Thr has become a major limiting factor affecting animal performance. Current Thr research focuses primarily on monogastric animals, with limited studies on amino acid apparent digestibility in ruminants, possibly due to diverse rumen microorganisms.

Tang Maoyan et al. [35] found that growing pigs' ADG increased with dietary Thr level within a certain range at the same Lys level, otherwise decreasing. Zhou Yanwen [36] found that Hepu geese' s metabolic rates of Met and Lys initially increased then decreased with rising dietary Thr levels, consistent with this study's results. The high protein control group's lower amino acid apparent digestibility compared to Group III may be due to the lower Thr level in the high protein diet disrupting amino acid balance, or because the supplemental Thr in Group III was free amino acid that could be directly utilized by rumen microbes and rumen wall, thereby improving Thr absorption. Therefore, further in-depth research on Thr' s amino acid nutritional effects in fawns is essential.

This study demonstrates that supplementing a 14% protein diet with appropriate Thr levels can effectively improve apparent digestibility of CP, NDF, and various amino acids in sika deer fawns, achieving growth performance comparable to or better than a 16% protein diet, thereby reducing resource waste and promoting healthy development of the sika deer industry. Under the conditions of this experiment, the optimal Thr level in a 14% protein diet is 0.59%.

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