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Maintenance Crude Protein Requirement of Penned Female Korean Spotted Deer (*Cervus nippon*)

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ABSTRACT: This study was conducted to evaluate the protein requirement for maintenance of 2-year-old female Korean spotted deer. In the course of the experiment, each of three hand-reared female spotted deer was fed three diets that were iso-calorically formulated to contain low (approximately 7%), medium (12%), and high (17%) levels of crude protein (CP). Each of six trials included a 5-day transition, a 10-day preliminary, and a 7-day collection period. Dietary protein levels affected the apparent digestibility of CP ($p<0.05$) but not the apparent digestibility of dry matter, organic matter, or acid detergent fiber. All of the deer showed a positive CP balance on all of the diets. The maintenance CP requirement estimated by regression analysis was 4.17 g/kg metabolic body weight ($W^{0.75}$)·d. The maintenance digestible CP requirement was 1.42 g/kg $W^{0.75}$·d. The metabolic fecal CP was 1.95 g/kg $W^{0.75}$·d. The blood urea nitrogen of spotted deer increased ($p<0.05$) as the dietary protein levels increased. (**Key Words:** Maintenance, Crude Protein Requirement, Blood Urea Nitrogen, Spotted Deer)

INTRODUCTION

Korea has the largest velvet antler market in the world, and deer farming is one of the important animal industries in Korea. Korean spotted deer reared in intensively managed farms are commonly fed oak browse as their main forage, in addition to other conventional forage and concentrates that are usually fed to ruminant livestock (Kwak et al., 1994). Protein requirements can be affected by sex, age, seasons, stage of animal development, body composition, and species. Studies of the protein requirement for maintenance have been conducted on rusa deer (Tomkins and McMeniman, 2006), white-tailed deer (Smith et al., 1975; Holter et al., 1979), elks (Mould and Robbins, 1981), and several other species (Silva et al., 2003; Luo et al., 2004; Kim et al., 2006).

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Crude protein (CP) requirement for the maintenance was 3.13 g/kg $W^{0.75}$·d for goats (Silva Sobrinho, 1989), 2.88 g/kg $W^{0.75}$·d for reindeer (McEwan and Whitehead, 1970), 7.8 and 7.2 g/kg $W^{0.75}$·d for wool and hair lambs, respectively (Silva et al., 2003), 5.83 g/kg $W^{0.75}$·d for beef cows (Susmel et al., 1993), and 5.56 g/kg $W^{0.75}$·d for Korean beef steers (Kim et al., 2006). Digestible CP requirement for the maintenance was 2.82 g/kg $W^{0.75}$·d for goats (NRC, 1981), 2.3 g/kg $W^{0.75}$·d for camels (Farid, 1995), and 1.65 g/kg $W^{0.75}$·d for adult Thai native goats (Cheva-Isarakul et al., 1991). Metabolizable protein requirement for the maintenance of growing goats was 3.07 g/kg $W^{0.75}$·d (Luo et al., 2004).

However, research has not been conducted on the protein requirement for the maintenance of Korean spotted deer in different geographic environments and using intensive feeding systems. Accordingly, this study was conducted to investigate the protein requirements for the maintenance of penned female Korean spotted deer and to provide scientific information on a nutrient feeding system for efficient and profitable production.

MATERIALS AND METHODS

Animals and diets

All animal care protocols were approved by the Konkuk University Institutional Animal Care and Use Committee. Spotted deer (*Cervus nippon*) are particularly difficult to manage for indoor experimentation. For domestication, three experimental deer were hand-reared by a trained feeder for 8 months. The experiment was carried out using three 2-year-old female spotted deer whose mean body weight was 42.7±1.2 kg. During the experiment, the animals were kept in individual metabolism pens (1.4 m×1.8 m×2.0 m) that permitted separate collection of feces and urine. The pens were similar to those designed by Cowan et al. (1969).

The CP requirement for the maintenance of female spotted deer was based on a nitrogen balance study using yearling white-tailed deer (Holter et al., 1979). Three iso-caloric (64% total digestible nutrients, TDN) diets containing low (7%), medium (12%), and high (17%) levels of CP on a dry matter basis were formulated based on the dietary CP levels of 7% to 22% used in a white-tailed deer study of Holter et al. (1979). Feed ingredients are presented in Table 1; oak leaves provided a main source of forage, corn cobs a fiber source, cracked corn grain an energy source, and soybean meal a protein source. Appropriate levels of minerals and vitamins were added. In chemical composition, CP contents analyzed were 6.9%, 11.7%, and 17.3% for low, medium, and high CP diets, respectively. All the diets contained similar levels of energy, calcium, and phosphorus.

In vivo digestion trials were carried out on each diet, totaling six trials with two replications in a 3×3 Latin square design (three diets, three animals). Diet trial order was randomly selected for each animal. A 15-day diet adjustment period (comprising a 5-day transition period and 10-day preliminary period) was followed by a 7-day collection period. Between trials, animals were maintained on a medium protein (12%) diet. Feed was restricted and offered twice per day (1,400 g/d, wet basis, at 0700 and 1900 h). Animals had free access to water.

Table 1. Ingredient and chemical composition (%, DM basis) of diets with different levels (approximately 7%, 12%, and 17%) of crude protein fed to female spotted deer

	Dietary protein level		
	Low	Medium	High
Ingredient composition			
Corn grain, cracked	39.0	29.0	16.5
Soybean meal	0	9.6	22.1
Oak leaves (*Quercus* spp.)	17.0	30.7	30.8
Corn cobs	42.4	29.5	29.6
Dicalcium phosphate	0.9	0.6	0.4
Limestone	0.5	0.5	0.5
Vitamin-mineral premix[1]	0.2	0.2	0.2
Chemical composition			
Dry matter	89.7	89.7	90.1
Organic matter	93.5	93.1	92.2
Crude protein	6.9	11.7	17.3
Acid detergent fiber	28.5	26.2	28.7
Crude ash	6.5	6.9	7.8
Calcium	0.6	0.6	0.6
Phosphorus	0.4	0.4	0.4
Total digestible nutrients[2]	64.0	64.0	64.0

[1] The premix contained 1,312,500 IU/kg vitamins A, 437,500 IU/kg D_3, 4,900 IU/kg E, 19,950 ppm Fe, 4,200 ppm Cu, 9,800 ppm Mn, 14,000 ppm Zn, and 84 IU/kg Se.

[2] Calculated value based on NRC (1985).

Sampling procedures

Urine was collected in a collection jar containing 15 mL 50% H_2SO_4 (v/v) and 500 mL distilled water. Collected urine was diluted to a constant volume with distilled water. After the urine was weighed, 2% of the urine volume was sampled, refrigerated and bulked for the collection period. Fecal and urine samples, each about 100 g (or mL), were separately pooled and thoroughly mixed. These samples were dried in a forced-air oven at 60°C, ground to pass through a 1-mm screen, and stored for further analysis.

On the last day of each trial, deer were anesthetized with xylazine hydrochloride, which was administered using a blow gun–dart syringe at a dosage of 1.0 to 2.0 mg/kg BW. Blood was obtained in heparinized vacutainers via jugular venipuncture 6 h after feeding, deproteinized, and stored at −20°C for subsequent urea analysis.

Analytical techniques

Daily feed and orts samples were frozen and analyzed for dry matter (DM), organic matter (OM), CP, and acid detergent fiber (ADF) (AOAC, 1990). Daily fecal samples were dried in a 60°C dry oven and analyzed for DM, OM, CP, and ADF as above. The nitrogen content of urine was determined (AOAC, 1990). Calcium and phosphorus of feed samples were analyzed according to the mineral analysis method of Standard Analysis Protocol for Feeds (KMAFF, 2010). Blood urea nitrogen (BUN) was determined using the method of Coulombe and Favreau (1963).

Preliminary analyses with replication as a factor in the model indicated no differences between first and second replication; therefore, the data were pooled across replicates.

After quantifying CP intake and retention in the experimental period, the variable retained CP in animal bodies was expressed as a function of CP intake, in g/kg $W^{0.75}$·d, through a linear regression model (retained CP = a +b*CP intake). This regression model was used to assess

Table 2. Daily dry matter intake and apparent nutrient digestibility of diets with different levels (approximately 7%, 12%, and 17%) of crude protein fed to female spotted deer

Item	Dietary protein level			
	Low	Medium	High	SE
Daily dry matter intake (kg)	1.0	1.1	1.1	0.09
Apparent digestibility (%)				
Dry matter	65.9	61.7	65.7	1.23
Organic matter	67.4	63.7	67.9	1.11
Crude protein	42.6[a]	58.3[b]	68.7[c]	3.43
Acid detergent fiber	45.5	33.9	41.1	4.40

[a,b,c] Means with different superscripts within the same row differ ($p<0.05$).

Table 3. Crude protein balance of female spotted deer fed diets with different levels (approximately 7%, 12%, and 17%) of crude protein

Item	Dietary protein level			
	Low	Medium	High	SE
Intake (g/d)	76.2[a]	146.9[b]	217.8[c]	2.35
Excretion (g/d)				
Fecal	43.9[a]	61.5[b]	68.3[b]	5.15
Urinary	29.1[a]	43.5[a]	75.6[b]	9.49
Total	73.0[a]	105.0[b]	143.9[c]	8.68
Absorption (g/d)	32.3[a]	85.4[b]	149.5[c]	5.45
Retention (g/d)	3.2[a]	41.9[b]	73.9[c]	9.36
% intake	4.1[a]	28.5[b]	33.9[c]	6.90
% absorbed	9.6[a]	48.9[b]	49.4[b]	14.60

[a, b, c] Means with different letters within the same row differ ($p<0.05$).

CP requirement for maintenance. The *x*-axis intercept was considered to be the maintenance CP requirement. The *b* coefficient represents CP utilization efficiency (Silva, 1996). Digestible CP requirement for maintenance was also estimated in the same manner. Metabolic fecal CP was estimated by regressing digestible CP (g/kg $W^{0.75}$·d) on CP intake and extrapolating the regression line to Y = 0 (Smith et al., 1975; Holter et al., 1979).

Statistical analysis

A randomized complete block ANOVA was used to compare CP balance data (CP intake, urinary CP, fecal CP, and retained CP) with apparent digestibility characteristics among treatments for each trial (Statistix7, 2000). Treatment means were compared using Tukey's test, when necessary. A statistical program (Statistix7, 2000) was used to perform regression analysis and create equations and graphs.

RESULTS

Apparent digestibility of nutrients

The apparent digestibility of nutrients is presented in Table 2. Dietary CP levels did not affect the apparent digestibility of DM, OM, or ADF in treated diets. However, the apparent digestibility of CP increased linearly as dietary CP increased.

Protein utilization and requirement for maintenance

Crude protein balance in spotted deer is presented in Table 3. The dietary CP intake linearly increased ($p<0.05$) as dietary CP increased. The linear increase in CP intake resulted in a linear increase of CP in total fecal and urinary excretion, absorption, and retention ($p<0.05$). Retained CP was 4% to 34% per daily CP intake and 10% to 49% per daily absorbed CP for all treatments. For the high CP diet, the excess CP absorbed in the body was lost mainly through the urine.

The regression equation of retained CP according to CP intake to estimate the CP requirement for maintenance is shown in Figure 1. The estimated regression equation was retained CP = −1.9924+0.4787×CP intake (r^2 = 0.82). The maintenance CP requirement for female spotted deer was calculated to be 4.17 g/kg $W^{0.75}$·d.

The regression equation of retained CP according to

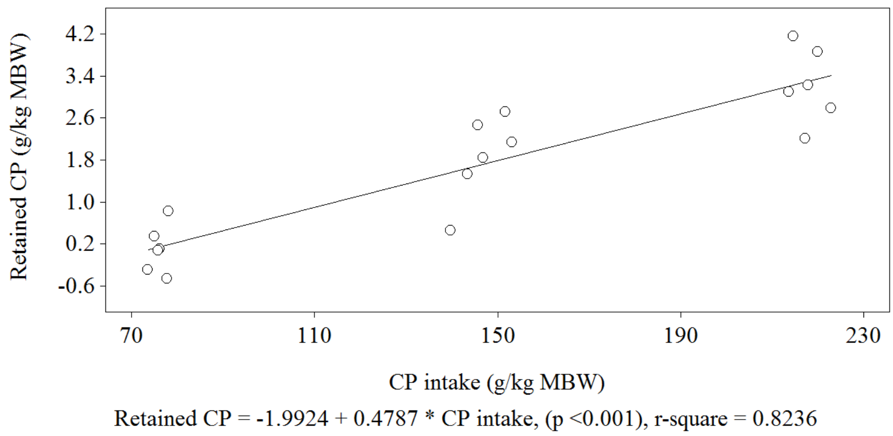

Figure 1. Regression equation of daily retained crude protein (CP) according to daily CP intake by female spotted deer (MBW = $BW^{0.75}$) (MBW, metabolic body weight; BW, body weight).

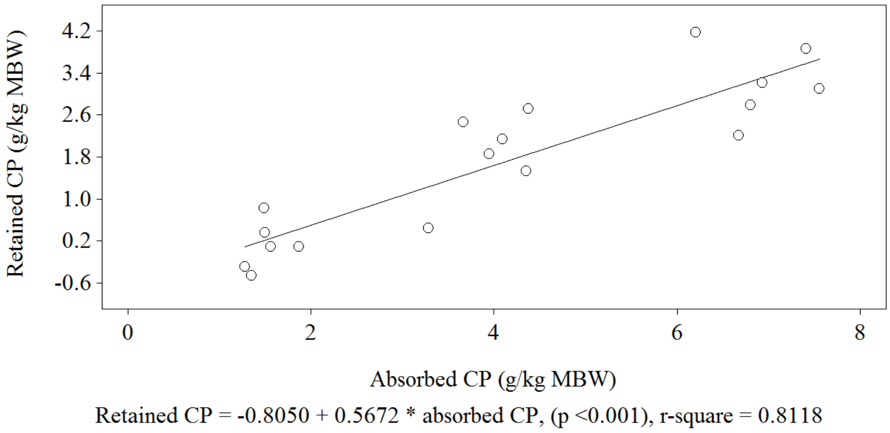

Figure 2. Regression equation of daily retained crude protein (CP) according to daily absorbed CP by female spotted deer (MBW = $BW^{0.75}$) (MBW, metabolic body weight; BW, Body weight).

absorbed CP to estimate absorbed CP requirement for maintenance is shown in Figure 2. The estimated regression equation was retained CP = –0.8050+0.5672×absorbed CP (r^2 = 0.81). The absorbed or digestible CP requirement for the maintenance of female spotted deer was calculated to be 1.42 g/kg $W^{0.75}$·d.

The regression equation of absorbed CP according to CP intake to estimate metabolic fecal CP is shown in Figure 3. The estimated regression equation was absorbed CP = –1.9467+0.8273×CP intake (r^2 = 0.97). The metabolic fecal CP level was calculated to be 1.95 g/kg $W^{0.75}$·d by extrapolating the line to zero CP intake. Regression analysis of absorbed or digestible CP on CP intake yielded a highly significant linear relationship (r^2 = 0.97, $p<0.001$).

Blood urea nitrogen

Blood urea nitrogen of female spotted deer is presented in Table 4. Blood urea nitrogen is a good index for dietary CP intake; it increased significantly ($p<0.05$) as the level of dietary CP increased. Blood urea nitrogen was highly correlated with CP intake (r^2 = 0.92) and absorbed CP (r^2 = 0.91), but not as strongly correlated with urinary CP (r^2 = 0.73).

Table 4. Blood urea nitrogen of female spotted deer fed diets with different levels (approximately 7%, 12%, and 17%) of crude protein

Item	Dietary protein level			
	Low	Medium	High	SE
Blood urea nitrogen (mg/100 mL)	11.6[a]	28.1[a,b]	40.8[b]	3.37

[a,b] Means with different superscripts within the same row differ ($p<0.05$).

DISCUSSION

Apparent digestibility of nutrients

Dietary CP levels did not affect the apparent digestibility of DM or OM, and deer showed a similar digestion pattern between treatments. In contrast, apparent DM digestibility was greater for the high-protein diet fed to rusa deer (Tomkins and McMeniman, 2006). Organic matter digestibility was within the normal range of 58% to 75%

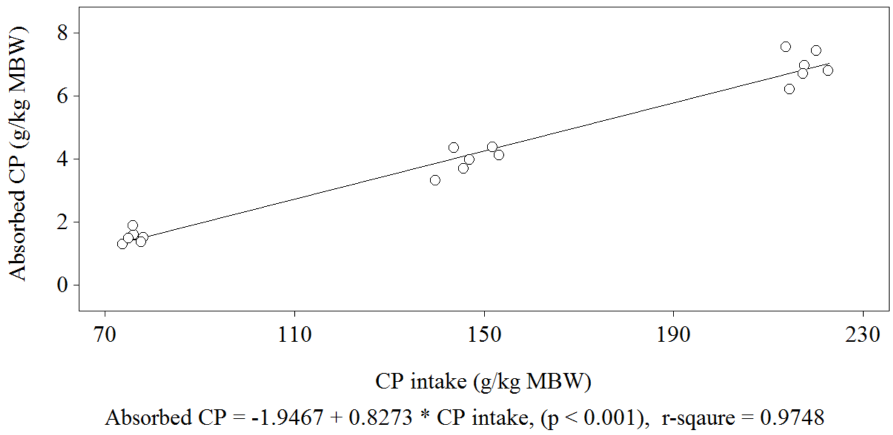

Figure 3. Regression equation of daily absorbed crude protein (CP) according to daily CP intake by female spotted deer (MBW = $BW^{0.75}$) (MBW, metabolic body weight; BW, Body weight).

reported by Das et al. (2010), Kim et al. (1996), and Garg (1996). Apparent digestibility of CP increased significantly as dietary CP level increased up to 17%. Similar findings have been reported in studies using white-tailed deer (Holter et al., 1979), Indian spotted deer (Das et al., 2010), elks (Mould and Robbins, 1981), and beef steers (Kim et al., 2006). The relatively low CP digestibilities in low and medium CP diets could be attributed to the protein-to-energy ratio being low for efficient protein digestion, or to a non-optimal amino acid pattern as explained by Smith et al. (1975). Crude protein digestibility was within the normal range of 42% to 67% for penned Korean spotted deer, as reported by Kim et al. (1996).

No effect of the dietary levels of CP on digestibility of ADF in the present study has also been reported earlier in white-tailed deer (Smith et al., 1975). Further, the digestibility of neutral detergent fiber (NDF) was not affected by dietary protein level for rusa deer (Tomkins and McMeniman, 2006). In contrast, the apparent digestibility of ADF was affected by dietary CP levels for white-tailed deer (Holter et al., 1979) and elks (Mould and Robbins, 1981). These different findings may be due to differences in dietary conditions and experimental environments.

Protein utilization and requirement for maintenance

The increased CP intake, digestion, urinary excretion, and retention in the present study were also observed in rusa deer when they were fed a higher protein diet (Tomkins and McMeniman, 2006). Under a high CP regimen, the excess CP absorbed in the body was lost mainly through the urine among growing beef cattle (Griffiths, 1984) and sheep (Wanapat et al., 1982). Among red deer, urine excretion was generally considered a major route of N loss when dietary CP was supplied in excess of requirements (Freudenberger et al., 1994).

Intake and fecal, urinary, and absorbed CP in the present balance study were within the normal range, similar to those of female Korean spotted deer fed medium and high CP diets reported by Kim et al. (1996). However, that study obtained a value of 6 to 25 g for retained CP, lower than the value found in our study, possibly due to more indigestible roughage feeding.

The CP requirement of female spotted deer for maintenance in the present study was higher than the 2.88 $g/W^{0.75}\cdot d$ for reindeer (McEwan and Whitehead, 1970) and 3.13 $g/kg\ W^{0.75}\cdot d$ for goats (Silva Sobrinho, 1989), and lower than the 7.8 and 7.2 $g/W^{0.75}\cdot d$ for wool and hair lambs (Silva et al., 2003), 5.56 $g/W^{0.75}\cdot d$ for Korean beef steers (Kim et al., 2006), and 5.83 $g/W^{0.75}\cdot d$ for beef cows (Susmel et al., 1993).

The recommended digestible CP (DCP) allowance for maintenance of camels was 2.3 g $DCP/kg\ W^{0.75}\cdot d$ (Farid, 1995). The mean protein requirement for maintenance of adult Thai native goats was 1.65 g $DCP/kg\ W^{0.75}\cdot d$ (Cheva-Isarakul et al., 1991). These values are a little higher than the value of 1.42 g $DCP/kg\ W^{0.75}\cdot d$ obtained in the present study.

The fecal metabolic CP (1.95 $g/kg\ W^{0.75}\cdot d$) in the present study was lower than the value found for white-tailed deer (2.25 $g/kg\ W^{0.75}\cdot d$) (Smith et al., 1975) and higher than the value obtained for goats (1.43 $g/kg\ W^{0.75}\cdot d$) (Silva Sobrinho, 1989).

Blood urea nitrogen

The increased BUN correlated with increased levels of CP in the diet in the present study was also observed in studies using deer (Seal et al., 1978) and calves (Bunting et al., 1989). In the present study, the concentrations (21 to 41 mg/100 mL) of BUN in the medium and high CP diets were similar to the concentrations of 31 to 38 mg/100 mL found in Indian spotted deer that were fed similar levels of CP, as reported by Das et al. (2010). The concentration of BUN was greater when rusa deer were fed a high-protein (15.8% CP) diet than when they received a low-protein (10.2% CP) diet. Values ranged from 12 to 25 mg/100 mL. The data showed that BUN can be used as an indicator of diet quality in spotted deer, as suggested also by Das et al. (2010). Blood urea nitrogen was highly correlated with CP intake (r^2 = 0.92). The positive linear relationships between CP intake and BUN have previously been reported among red deer, white-tailed deer, and sheep (Maloiy et al., 1970; Robbins et al., 1974). The weaker correlation (r^2 = 0.73) with urinary CP observed in the present study could be explained from the fact that up to 62% of the BUN was lost through urine and 47% was recycled through secondary urea pools (Tomkins and McMeniman, 2006).

In general, the dietary CP requirement of female spotted deer for maintenance in the present study was higher than that of reindeer (McEwan and Whitehead, 1970) and goats (Silva Sobrinho, 1989) and lower than that of wool and hair lambs (Silva et al., 2003), Korean beef steers (Kim et al., 2006), and beef cattle (Susmel et al., 1993).

CONCLUSION

The CP requirement for the maintenance of Korean female spotted deer farmed under intensive management conditions was 4.17 $g/kg\ W^{0.75}\cdot d$. The digestible CP requirement for maintenance was 1.42 $g/kg\ W^{0.75}\cdot d$. The metabolic fecal CP level was 1.95 $g/kg\ W^{0.75}\cdot d$. These results could be used as basic data for the scientific diet formulation of spotted deer.

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