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Moderate Crude Protein Supply and Rumen-Protected Amino Acid Supplementation Enable High-Quality Beef Production and Reduce Nitrogen Excretion in Japanese Black Steers

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Abstract

To reduce the amount of dietary crude protein (CP) and nitrogen excretion, this study aimed to improve the feed for fattening Japanese Black steers. The dietary CP content was 12%-13% on a dry-matter basis in the control diet (CON, n = 4, initial body weight [BW] 304 kg) and 11%-12% in the moderate-protein diet with rumen-protected lysine and methionine during the early and middle fattening stages (LysMet, n = 4, initial BW 297 kg). BW, BW gain, intakes of dry matter and total digestible nutrients, and the gain-to-feed ratio were not affected by dietary CP content. CP intake was significantly lower in LysMet-fed steers than in CON-fed steers ($p < 0.01$); however, there were no differences in the intake of metabolizable lysine or methionine between groups. Total nitrogen excretion was lower in LysMet-fed steers than that in CON-fed steers at the early ($p < 0.10$), middle ($p < 0.05$), and late ($p < 0.10$) fattening stages. Carcass weight and traits were not affected by the dietary CP content. These results indicate that in Japanese Black steers, 11%-12% CP diets supplemented with rumen-protected lysine and methionine reduce nitrogen excretion without affecting productivity.

Discipline: Animal Science
Additional key words: cattle, feces, lysine, methionine, urine

Introduction

Global food insecurity and the environmental impacts of food production represent critical challenges that require technological solutions. The livestock industry also requires efficient production systems that optimize feed utilization and have low environmental impact. The increase in global feed protein demand, such as for soybean meal, has led to supply shortages (Kim et al. 2019). Therefore, the efficient utilization and conservation of feed proteins in livestock systems have become essential priorities to ensure sustainable animal production. Nitrogen is a substance that causes negative environmental loads (Zhang et al. 2015). Nitrous oxide is a nitrogen compound that is a greenhouse gas and ozone-depleting substance (Ravishankara et al. 2009, Montzka et al. 2011). Nitrogen runoff from livestock farming areas is a significant source of nitrate contamination in aquatic ecosystems and contributes to water quality deterioration (Hooda et al. 2000). Therefore, minimizing nitrogen emissions from livestock production is considered a key strategy for safeguarding the environment at both local and global scales.

Optimizing dietary crude protein (CP) levels to the minimum level required to maintain animal performance is essential for conserving feed protein resources and reducing nitrogen emissions from livestock systems (Rotz 2004). Moreover, ureagenesis in the liver contributes to oxygen consumption, accounting for 2.5%-5.0% of the total consumption (Huntington & Archibeque 2000). Jennings et al. (2018) reported that feeding excess CP enhances the maintenance energy requirements of finishing cattle, indicating a decline in energy efficiency. Therefore, optimizing dietary CP is an important technological challenge for improving livestock production efficiency. Modifying dietary

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composition has been demonstrated to mitigate nitrogen excretion, particularly through strategies that couple reduced CP intake with targeted amino acid supplementation (Gerber et al. 2013). Despite the recognized importance of amino acids in animal nutrition, their application in ruminant feed formulations has been less extensively studied than in monogastric species such as swine and poultry. Due to extensive degradation of crystalline amino acids in the rumen, several studies have used abomasal infusion techniques to more precisely evaluate amino acid requirements in ruminants. Studies conducted in growing Holstein steers have indicated that the abomasum infusion of methionine or lysine reduces urinary nitrogen excretion, suggesting improved nitrogen utilization efficiency (Greenwood & Titgemeyer 2000, Löest et al. 2002, Schroeder et al. 2006, Batista et al. 2016). Alternatively, research has focused on the use of rumen-protected amino acids that are chemically or physically treated to resist rumen degradation, thereby reaching the small intestine for absorption. Rumen-protected lysine and methionine supplementation has been associated with increased weight gain in growing cattle (Oke et al. 1986, Klemesrud et al. 2000, Xue et al. 2011). As outlined above, in addition to reducing dietary CP content, supplementation of rumen-protected amino acids has the potential to decrease nitrogen excretion while preserving growth performance in beef cattle.

The Japanese Black, one of the four Wagyu breeds, is the predominant breed in Japan's beef cattle industry, accounting for the largest proportion of the national herd owing to its superior meat quality, particularly its intramuscular fat deposition (Yamada 2022). Characteristically, the fattening phase for Japanese Black cattle is extended relative to the typical fattening durations observed in international beef production systems (Ogino et al. 2004), reflecting specific management practices aimed at enhancing meat quality. Therefore, it is necessary to optimize feeding strategies that combine lower CP diets with supplemental amino acids for fattening Japanese Black cattle. Kamiya et al. (2026) reported the first experiment in which Japanese Black steers were fed an 11% CP diet supplemented with rumen-protected lysine and methionine, which resulted in reduced weight gain during the early fattening stages. These findings suggest that diets with very low CP levels may be insufficient to meet the nutritional demands during the early fattening stage and should be reconsidered for optimization. Specifically, it is suggested that a diet with a higher CP level and reduced supplementation of rumen-protected amino acids may be more suitable for the early fattening stage than the lower-CP diet formulated by Kamiya et al. (2026). The objective of this study was to assess the effects of dietary protein optimization combined with supplementation of rumen-protected lysine and methionine on feed intake, growth performance, carcass traits, and nitrogen excretion in Japanese Black steers during the fattening period.

Materials and methods

1. Cattle and feeding management

Eight Japanese Black steers (average age, 10 months) were assigned to either a control group (CON, n = 4) or a rumen-protected lysine and methionine group (LysMet, n = 4). Steers in the LysMet group were fed a moderate-protein diet supplemented with rumen-protected lysine (AjiPro-L, Ajinomoto Co., Inc., Tokyo, Japan) and methionine (Mepron, Evonik Nutrition and Care GmbH, Hanau, Germany) during the early and middle fattening periods. Initially, the control and lower CP diets were formulated without the rumen-protected amino acids. The NASEM (2016) model was subsequently employed to estimate the amounts of metabolizable lysine and metabolizable methionine in both diets. To compensate for the reduction in these amino acids in the lower CP diet, rumen-protected lysine and methionine were supplemented, resulting in the formulation of the LysMet diet, which was designed to provide metabolizable lysine and methionine levels equivalent to those in the CON diet. In the late fattening period, no protein deficiency was anticipated even with the low CP diet; therefore, the steers were fed a diet without rumen-protected amino acids.

The chemical compositions and ingredients of the experimental diets are listed in Table 1. The steers were housed in barns and fed hay and concentrate mixtures individually (Table 2). The steers were fed hay once daily (in the morning) and a concentrate mixture twice daily (in the morning and evening), with water and mineral blocks available ad libitum. The hay and concentrate mixture was collected and weighed each day before morning feeding. The body weight (BW) of each steer was measured once a week before the morning feeding.

On average, steers were kept from 10 to 28 months of age, covering three stages of fattening: early (9-15 months), middle (16-22 months), and late (23-28 months). Steers were slaughtered at an average age of 28 months. This animal study was approved by the Animal Care and Use Committee of the National Agriculture and Food Research Organization (NARO), Japan (approval no. R4-R35-NILGS) and was conducted according to the NARO Implementation Regulations on

Animal Experiments.

2. Feed analysis

The dry matter (DM) content of the diets was determined by forced-air drying at 105°C for 16 hr (Barbosa et al. 2015). The CP, acid detergent fiber (ADF), neutral detergent fiber (NDF), ether extract, crude ash, and starch contents of each diet were chemically analyzed. The CP content was determined by the macro-Kjeldahl method (AOAC 1990) using an automatic analyzer (KjeltecTM 8400, FOSS, Hilleroed, Denmark). The ADF and NDF contents were determined by the detergent method (Van Soest et al. 1991) using a raw fiber extractor (FIWE 6, VELP Scientifica, Usmate Velate, Italy). Ether extract content was measured using a solvent extractor (SER148, VELP Scientifica, Usmate Velate, Italy) with

Table 1. Chemical composition and ingredients of experimental diets

	Concentrate mixture for the early stage		Concentrate mixture for the middle stage		Concentrate mixture for the late stage		Timothy hay
	CON	LysMet	CON	LysMet	CON	LysMet	
Dry matter, % of FM	87.8	87.7	87.9	87.8	87.6	87.5	89.3
Chemical composition, % of DM							
TDN[a]	83.5	83.7	83.8	84.0	84.9	85.1	54.1
CP	14.9	13.5	13.3	12.1	13.0	11.7	6.5
ADFom	7.1	6.9	6.8	6.6	6.5	6.3	41.3
aNDFom	22.3	22.2	20.8	20.7	20.0	20.0	65.8
Ether extract	2.9	3.0	3.0	3.1	3.0	3.1	1.3
Crude ash	3.8	3.6	3.7	3.5	3.3	3.1	6.1
Starch	45.1	47.7	48.1	50.1	49.8	52.0	1.3
Ingredients, % of FM							
Corn	28.3	31.9	32.3	35.1	40.4	43.5	-
Barley	37.4	37.4	37.3	37.3	32.9	32.9	-
Rice	0.9	0.9	0.8	0.8	0.7	0.7	-
Wheat bran	17.0	17.0	17.0	17.0	15.0	15.0	-
Soybean meal	8.3	4.6	4.5	1.6	3.8	0.7	-
Other by-products[b]	6.9	6.9	6.9	6.9	6.1	6.1	-
Supplement[c]	1.2	1.2	1.2	1.2	1.0	1.0	-
Rumen-protected lysine	-	0.09	-	0.07	-	-	-
Rumen-protected methionine	-	0.01	-	0.01	-	-	-

[a] The TDN of the concentrate mixture was estimated according to Standard Tables of Feed Composition in Japan (NARO 2010). The TDN of Timothy hay was calculated using the following formula: TDN(%DM) = 87.09 − 0.752 × ADF(%DM).
[b] Barley bran with hull and polish, corn gluten feed, and rapeseed meal.
[c] Cane molasses, minerals, vitamins, and feed additives. ADFom: acid detergent fiber on organic matter basis; aNDFom: amylase-treated neutral detergent fiber on organic matter basis; CON: control; CP: crude protein; DM: dry matter; FM: fresh matter; LysMet: moderate-protein diet with rumen-protected lysine and methionine; TDN: total digestible nutrients

Table 2. Feeding program used for fattening the Japanese Black steers

	Age (months)		
	10-15	16-22	23-28
Concentrate mixture for the early stage of fattening	5.5-8.0		
Concentrate mixture for the middle stage of fattening		8.5-9.4	
Concentrate mixture for the late stage of fattening			9.4-10.0
Timothy hay	3.0	2.5	2.5

The data are in kgFM/day. FM: fresh matter diethyl ether (AOAC 1990). Crude ash content was determined by heating the sample in a muffle furnace at 600°C for 2 hr (AOAC 1990). Total starch content was determined by using the amyloglucosidase/α-amylase method (Total starch assay kit, Megazyme, Ltd., Wicklow, Ireland). The total digestible nutrients (TDN) content of the concentrate mixture was estimated based on the feed ingredient values in the Standard Tables of Feed Composition in Japan (NARO 2010). The TDN content of Timothy hay was estimated using the following formula (Otsuki 2001):

$$\mathrm{TDNhay} = 87.09 - 0.752 \times \mathrm{ADFhay}$$

where TDNhay is the TDN content (% of DM) and ADFhay is the ADF content (% of DM) of Timothy hay.

The intake of metabolizable lysine and methionine was estimated by calculating the amount of absorbed amino acids supplied by ruminally undegradable protein (RUP) and ruminal bacteria (NASEM 2016). The metabolizable intake was estimated for each amino acid as follows:

$$\mathrm{Metabolizable}_{AA} = \mathrm{FeedRUP}_{AA} \times 0.8 + \mathrm{MicrobialCP}_{AA} \times 0.8 \times 0.8$$

$$\mathrm{FeedRUP}_{AA} = \mathrm{DMI} \times (\mathrm{FeedCP}/100) \times (\mathrm{FeedRUP}/100) \times (\mathrm{Feed}_{AA}/100)$$

$$\mathrm{MicrobialCP}_{AA} = (42.73 + \mathrm{TDNI} \times 0.087) \times (\mathrm{Bacteria}_{AA}/100)$$

where $\mathrm{Metabolizable}_{AA}$ is the metabolizable amino acid intake (g/day), $\mathrm{FeedRUP}_{AA}$ is the amino acid intake derived from the RUP (g/day), $\mathrm{MicrobialCP}_{AA}$ is the amino acid intake derived from the microbial CP (g/day), FeedCP is the CP content of feed (% of DM), FeedRUP is the RUP content in the CP (% of CP), Feed_{AA} is the composition of the amino acid in feed (% of CP), DMI is the DM intake (gDM/day), TDNI is the TDN intake (g/day), and $\mathrm{Bacteria}_{AA}$ is the composition of the amino acid in the ruminal bacteria (% of microbial CP), with lysine at 7.9% and methionine at 2.6%.

3. Blood sampling and analysis

Blood samples were collected monthly from the jugular vein into heparinized tubes before the morning feeding from 10 (pre-experiment) to 27 months of age. The plasma was separated by centrifugation at 1,760 g for 20 min at 4°C and stored at −30°C until analysis. Plasma urea nitrogen concentrations were determined using an automated biochemical analyzer (Dri-Chem 3500V, FUJIFILM Co., Tokyo, Japan) based on an enzymatic method using urease.

4. Nitrogen excretion

Nitrogen excretion was determined at 12 months of age for the early stage, 19 months for the middle stage, and 25 months for the late stage of fattening. Spot fresh urine and fecal grab samples were collected from each steer during the morning and evening for three consecutive days, following Baldwin et al. (1983). After collection, the fecal samples were stored at 4°C whereas 80 mL of the urine per sample was stored at −30°C. The samples from each steer were mixed prior to analysis.

The nitrogen content of each sample was determined using the macro-Kjeldahl method for the measurement of dietary CP content. Urinary creatinine concentration was determined using the Jaffe method (LabAssay Creatinine, FUJIFILM Wako Pure Chemical Co., Ltd., Osaka, Japan). Daily urinary excretion of creatinine was defined as 26 mg/kg BW (Whittet 2004), and urine volume was estimated from the BW and urinary creatinine concentration (Chizzotti et al. 2008). Thus, urinary nitrogen excretion was estimated as follows:

$$\mathrm{UNE} = 26 \times \mathrm{BW}/\mathrm{Cr} \times (\mathrm{UN}/100) \times 1000$$

where UNE is urinary nitrogen excretion (g/day), BW is the body weight (kg), Cr is the urinary creatinine concentration (mg/L), and UN is the urinary nitrogen concentration (% FM).

Fecal weight was estimated from the acid-insoluble ash (AIA) concentrations of the feed and fecal samples, measured using the 4 N hydrochloric acid method (Terada et al. 1979, Sales & Janssens 2003). Thus, fecal nitrogen excretion was estimated as follows:

$$\mathrm{FNE} = \mathrm{AI}/(\mathrm{FA}/100) \times (\mathrm{FN}/100)$$

where FNE is fecal nitrogen excretion (g/day), AI is the AIA intake (g), FA is the fecal AIA concentration (% DM), and FN is the fecal nitrogen concentration (% DM).

5. Carcass weight and traits

Each carcass was sectioned between the sixth and seventh ribs one day after slaughter. The rib eye area, rib thickness, and subcutaneous fat thickness at the incision plane between the sixth and seventh ribs were measured according to the Japan Meat Grading Association guidelines. The yield score was calculated using the following formula:

$$\text{Yield score} = 67.37 + 0.13 \times \mathrm{REA} + 0.667 \times \mathrm{RT} - 0.025 \times \mathrm{CHCW} - 0.896 \times \mathrm{SF} + 2.049$$

where REA is the rib eye area (cm^2), RT is the rib thickness (cm), CHCW is the cold half-carcass weight (kg), and SF is the subcutaneous fat thickness (cm). The rib eye area was measured as the area enclosed by the fascial line of the longissimus thoracis muscle. Rib thickness was measured from the thoracic side of the pleura to the dorsal side of the latissimus dorsi muscle, approximately at the midpoint of the total rib length. Subcutaneous fat thickness was measured as the length from the dorsal side of the latissimus dorsi muscle to the carcass surface on a line drawn perpendicular to the carcass surface from the side edge of the iliocostalis muscle.

Marbling, meat color, fat color, meat brightness, meat firmness, meat texture, and fat brightness and quality at the incision plane between the sixth and seventh ribs were measured according to the Japan Meat Grading Association guidelines. The beef marbling standard (BMS) numbers ranged from 1 to 12; the higher the number, the more the marbling. The beef color standard (BCS) numbers ranged from 1 to 7, with a higher number indicating darker meat colors. The beef fat color standard (BFS) numbers ranged from 1 to 5, with a high number of yellow fats. Meat brightness, meat firmness, meat texture, and fat brightness and quality were rated on a scale of 1 to 5, with the higher values indicating better quality. Two to three days after slaughter, half of the carcass was dissected and weighed for lean meat, fat, bones, and other qualities. The lean meat included intramuscular fat.

6. Statistical analyses

Plasma urea nitrogen concentration was evaluated using a linear mixed model with treatment, period, and the interaction of treatment and period as fixed effects and cattle as a random effect (R ver. 4.2.0). Differences in each measurement between groups were evaluated using analysis of variance (ANOVA: R ver. 4.2.0). Probability (p) values < 0.05 were considered significant and those < 0.10 were considered a trend.

Results

1. Feeding performance

The effects of moderate-protein diets supplemented with rumen-protected lysine and methionine on feeding performance are presented in Table 3. No effect of diet was observed on BW, BW gain, DM and TDN intake, feed efficiency, or metabolizable lysine and methionine intake.

Starch intake during the early fattening period or entire fattening period was significantly higher ($p < 0.01$) or tended to be higher ($p < 0.10$) in the LysMet group than in the CON group. The LysMet group had reduced protein content in the concentrate diet; therefore, CP intake was lower than that in the CON group during the early ($p < 0.01$), middle ($p < 0.05$), late ($p < 0.10$), and entire ($p < 0.01$) fattening stages. The dietary CP content of the CON group was 12.9% DM, 12.3% DM, and 12.2% of the DM during the early, middle, and late stages of fattening, respectively. The dietary CP contents in the LysMet group were 11.9% DM, 11.2% DM, and 11.1% of the DM during the early, middle, and late stages of fattening, respectively.

2. Nitrogen excretion and plasma urea nitrogen concentrations

Table 4 shows the effects of moderate-protein diets supplemented with rumen-protected lysine and methionine on nitrogen excretion. Urinary nitrogen excretion was significantly lower ($p < 0.01$) in the LysMet group than in the CON group at all three stages. Total nitrogen excretion was lower in the LysMet group than in the CON group during the early ($p < 0.10$), middle ($p < 0.05$), and late ($p < 0.10$) stages. However, the effects of treatment, period, and their interaction on plasma urea nitrogen concentration were not significant (Fig. 1).

3. Carcass weight and traits

The effects of moderate-protein diets supplemented with rumen-protected lysine and methionine are summarized in Table 5. There were no significant between-group differences in carcass weight or in the weights of lean meat, fat, or bone in half of the carcasses. Moderate-protein diets supplemented with rumen-protected lysine and methionine had no significant effects on the rib eye area, rib thickness, or subcutaneous fat thickness at the incision plane between the sixth and seventh ribs. Dietary CP content had no effects on the BMS number, BCS number, BFS number, meat brightness, meat firmness, meat texture, or fat brightness and quality.

Discussion

During the early fattening stage, the dietary CP content of the LysMet group in the present study was approximately 12%, positioned between 11% reported for the low-CP group in a previous experiment (Kamiya et al. 2026) and the 13% observed in the CON group. The intakes of metabolizable lysine and methionine in the present study were similar between the LysMet and CON groups, likely due to the supplementation of rumen-protected lysine and methionine in the LysMet diet. Although a previous study reported reduced weight gain during the early fattening stage when 11% CP diets

Table 3. Body weight and feed intake of Japanese Black steers fed conventional or moderate crude protein diets supplemented with rumen-protected lysine and methionine

		CON	LysMet	p-value
Body weight, kg	Initial (early stage)	301 ± 19	297 ± 34	0.85
	Initial (middle stage)	478 ± 15	476 ± 39	0.92
	Initial (late stage)	670 ± 29	667 ± 52	0.93
	Final	773 ± 13	774 ± 60	0.98
Body weight gain, kg/day	Early stage	1.01 ± 0.11	1.02 ± 0.10	0.91
	Middle stage	0.91 ± 0.07	0.91 ± 0.09	0.98
	Late stage	0.71 ± 0.18	0.73 ± 0.07	0.83
	Entire	0.89 ± 0.04	0.90 ± 0.04	0.79
DM intake, kg/day	Early stage	7.8 ± 0.2	7.8 ± 0.1	0.67
	Middle stage	8.6 ± 0.3	8.7 ± 0.3	0.66
	Late stage	8.6 ± 0.2	8.7 ± 0.9	0.82
	Entire	8.3 ± 0.2	8.4 ± 0.3	0.77
Gain-to-feed ratio	Early stage	0.129 ± 0.013	0.131 ± 0.011	0.82
	Middle stage	0.106 ± 0.005	0.105 ± 0.008	0.74
	Late stage	0.082 ± 0.020	0.084 ± 0.003	0.89
	Entire	0.106 ± 0.004	0.107 ± 0.006	0.93
TDN intake, kg/ day	Early stage	6.0 ± 0.1	6.0 ± 0.1	0.77
	Middle stage	6.8 ± 0.2	6.9 ± 0.3	0.69
	Late stage	7.0 ± 0.2	7.1 ± 0.6	0.79
	Entire	6.6 ± 0.1	6.6 ± 0.2	0.72
Starch intake, kg/ day	Early stage	2.7 ± 0.0	2.8 ± 0.0	< 0.01
	Middle stage	3.6 ± 0.1	3.7 ± 0.1	0.13
	Late stage	3.8 ± 0.2	4.0 ± 0.3	0.33
	Entire	3.3 ± 0.1	3.5 ± 0.1	< 0.10
CP intake, kg/day	Early stage	1.01 ± 0.02	0.92 ± 0.02	< 0.01
	Middle stage	1.06 ± 0.04	0.97 ± 0.04	< 0.05
	Late stage	1.06 ± 0.05	0.97 ± 0.08	< 0.10
	Entire	1.04 ± 0.02	0.95 ± 0.03	< 0.01
Dietary CP content, %[a]	Early stage	12.9 ± 0.1	11.9 ± 0.1	< 0.01
	Middle stage	12.3 ± 0.1	11.2 ± 0.1	< 0.01
	Late stage	12.2 ± 0.4	11.1 ± 0.3	< 0.01
	Entire	12.5 ± 0.1	11.4 ± 0.1	< 0.01
Metabolizable lysine intake, g/day	Early stage	38.6 ± 0.6	39.3 ± 0.5	0.13
	Middle stage	43.1 ± 1.4	44.2 ± 1.6	0.34
	Late stage	43.7 ± 1.6	43.2 ± 3.5	0.84
	Entire	41.7 ± 0.7	42.3 ± 1.3	0.49
Metabolizable methionine intake, g/day	Early stage	12.4 ± 0.2	12.7 ± 0.2	0.11
	Middle stage	14.2 ± 0.5	14.5 ± 0.5	0.33
	Late stage	14.6 ± 0.6	14.7 ± 1.2	0.86
	Entire	13.7 ± 0.2	14.0 ± 0.4	0.35

Values are mean ± SD. [a] This value represents the percentage of CP intake relative to the dry matter intake. CON: control; CP: crude protein; DM: dry matter; LysMet: moderate-protein diet with rumen-protected lysine and methionine; TDN: total digestible nutrients were used (Kamiya et al. 2026), the diet of the LysMet group used in the present study supported satisfactory growth performance. These results suggest that a moderate CP diet combined with rumen-protected amino acids does not adversely affect the growth performance of fattened Japanese Black steers. Other studies in Angus, Simmental, or Limousin-sired crossbred, or Holstein steers have also reported increases in BW gain in beef cattle when low-CP diets were supplemented with a combination of rumen-protected lysine and methionine (Oke et al. 1986, Veira et al. 1991, Hussein & Berger 1995), or with rumen-protected lysine alone (Klemesrud et al. 2000, Xue et al. 2011). These findings suggest that supplementation with rumen-protected lysine and methionine can effectively compensate for reduced CP content, thereby maintaining amino acid availability at levels similar to that achieved with conventional CP diets.

The abomasum infusion of methionine leads to decreased urinary nitrogen excretion and increased nitrogen retention in growing Holstein steers (Greenwood & Titgemeyer 2000, Löest et al. 2002, Schroeder et al. 2006). Batista et al. (2016) demonstrated that low-CP diets supplemented with rumen-protected lysine reduced urinary nitrogen excretion and enhanced nitrogen retention in growing Holstein steers. In the present study, urinary nitrogen excretion was consistently lower in the LysMet group that received a moderate CP diet combined with rumen-protected amino acids than in the conventional CP diets. These results support those of previous studies (Kamiya et al. 2020, 2021, 2026), indicating that dietary CP reduction is an effective strategy for mitigating nitrogen loss in intensive beef production systems in Japan. In the nitrogen excretion measurement trial, the LysMet group exhibited a progressive reduction in nitrogen intake relative to the CON group, with decreases of 13, 12, and 14 g per day observed during the early, middle, and late fattening stages, respectively. This reduction was accompanied by a corresponding decrease in urinary nitrogen excretion, amounting to 13, 17, and 23 g per day at the same stages, respectively. The present study demonstrated that approximately a 10% reduction in dietary nitrogen intake led to approximately a 20% decrease in urinary nitrogen excretion, suggesting a disproportionately high reduction efficiency. According to the estimation guidelines provided by the Greenhouse Gas Inventory Office of

Table 4. Nitrogen excretion of Japanese Black steers fed conventional or moderate crude protein diets supplemented with rumen-protected lysine and methionine

	CON	LysMet	*p*-value
During the early fattening stage, g/day			
Nitrogen intake	161 ± 4	148 ± 4	< 0.01
Fecal nitrogen excretion	49 ± 9	50 ± 2	0.89
Urinary nitrogen excretion	78 ± 1	65 ± 3	< 0.01
Total nitrogen excretion	127 ± 10	115 ± 3	< 0.10
Nitrogen retention	35 ± 8	33 ± 5	0.71
During the middle fattening stage, g/day			
Nitrogen intake	170 ± 10	158 ± 6	< 0.10
Fecal nitrogen excretion	54 ± 6	52 ± 6	0.64
Urinary nitrogen excretion	96 ± 5	79 ± 6	< 0.01
Total nitrogen excretion	150 ± 11	131 ± 8	< 0.05
Nitrogen retention	21 ± 4	27 ± 5	0.11
During the late fattening stage, g/day			
Nitrogen intake	171 ± 24	157 ± 18	0.39
Fecal nitrogen excretion	66 ± 11	58 ± 11	0.37
Urinary nitrogen excretion	110 ± 13	87 ± 16	< 0.10
Total nitrogen excretion	176 ± 21	146 ± 22	< 0.10
Nitrogen retention	–5 ± 10	11 ± 9	< 0.05

Values are mean ± SD. Nitrogen excretion was measured at 12, 19, and 25 months of age during the early, middle, and late fattening stages, respectively. CON: control; LysMet: moderate-protein diet with rumen-protected lysine and methionine

Japan (GIO 2020), nitrous oxide emissions originating from manure management are directly proportional to the amount of nitrogen excreted via urinary and fecal pathways. In the present study, the reduction in total nitrogen excretion, including both the urinary and fecal components, was 12, 19, and 30 g per day during the early, middle, and late fattening stages, respectively. The decrease in total nitrogen excretion was equivalent to or approximately double the reduction in nitrogen intake, suggesting an improved metabolic efficiency under the applied dietary conditions. Nitrogen compounds have been identified as contributors to multiple environmental loads, including greenhouse gas emissions (Montzka et al. 2011), stratospheric ozone depletion (Ravishankara et al. 2009), and nitrate pollution in water systems (Hooda et al. 2000). Therefore, reducing nitrogen excretion in beef production systems in Japan is expected to mitigate a broad range of environmental effects.

The diets used in the present study demonstrated no statistically significant effects on either carcass weight or lean tissue mass. Additionally, the rib eye area, rib thickness, and yield score did not differ significantly between the groups, suggesting no detrimental effects on carcass yield traits in Japanese Black steers. Dietary treatments did not significantly affect the marbling score, meat color, fat color, or other quality-related parameters, suggesting that differences in diet composition within the tested range did not compromise the visual quality of beef. Glucose serves as a key substrate for de novo fatty acid synthesis in the intramuscular adipose tissue of fattening cattle (Smith et al. 2018). Park et al. (2018) demonstrated that high-starch diets during the early fattening stage enhanced intramuscular fat deposition, likely owing to increased glucose availability and subsequent lipogenic activity in muscle-associated adipocytes. In the present study, partial substitution of soybean meal with corn in the concentrate mixture for the LysMet group led to increased dietary starch content relative to the CON group. Nevertheless, the extent of starch intake elevation was limited and did not result in measurable enhancement of intramuscular fat deposition. The meat quality observed in the present study was consistent with the typical characteristics of Japanese Black steers. These findings indicate that the feed composition used in the present study was sufficient to support the production of high-quality beef.

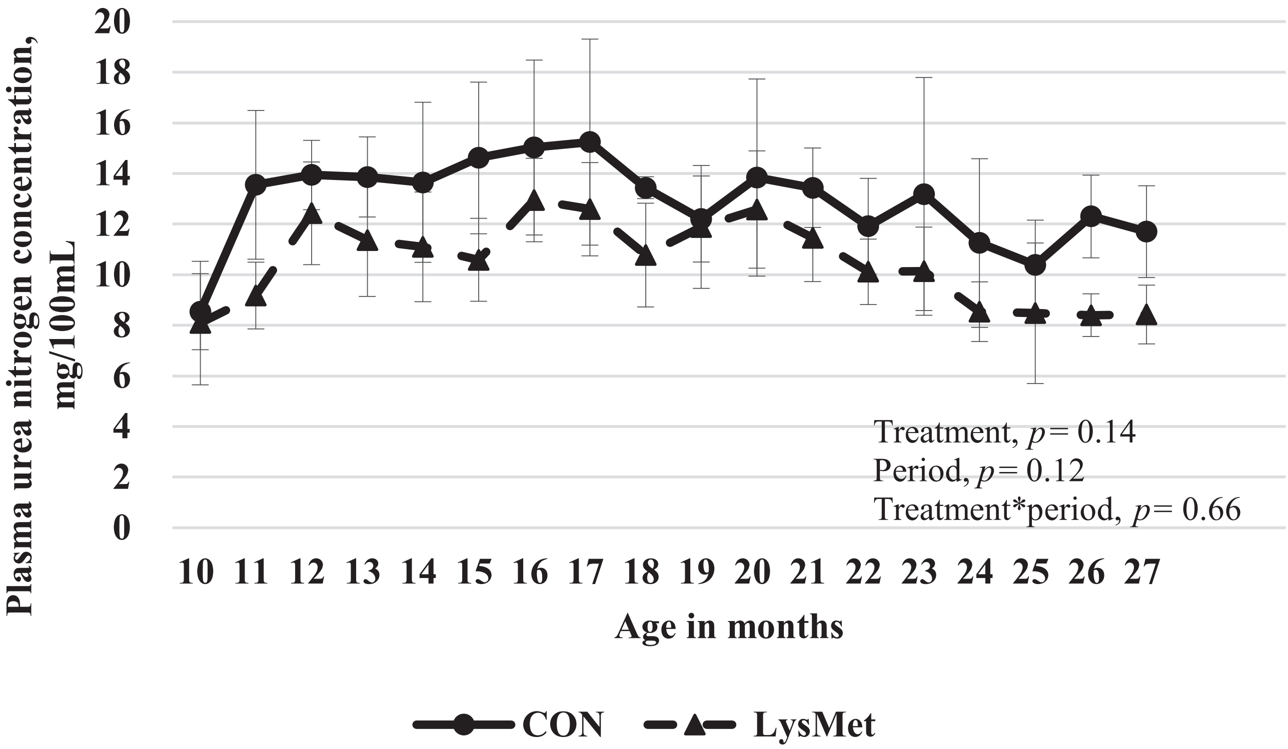

Fig. 1. Plasma urea nitrogen concentration of Japanese Black steers fed conventional or moderate crude protein diets supplemented with rumen-protected lysine and methionine

Values are mean ± SD. CON: control (●, n= 4), LysMet: moderate-protein diet with rumen-protected lysine and methionine (▲, n= 4)

Optimizing feed composition by reducing dietary protein levels and improving feed efficiency can reduce nitrogen excretion from livestock waste. This approach not only mitigates the environmental burden associated with nitrogen emissions but also helps alleviate the global feed protein shortage. The findings of the present study indicate that the use of rumen-protected lysine and methionine combined with a dietary regimen consisting of 12% CP during the early fattening stage and 11% CP during the middle fattening stage leads to the production of high-quality beef and decreases nitrogen excretion in Japanese Black steers.

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Table 5. Carcass traits of Japanese Black steers fed conventional or moderate crude protein diets supplemented with rumen-protected lysine and methionine

	CON	LysMet	p-value
Carcass weight, kg	502 ± 18	494 ± 47	0.76
Tissue weight in half-carcass[a], kg			
Lean	129 ± 16	132 ± 1	0.99
Fat	101 ± 10	92 ± 9	0.28
Bone	22 ± 2	24 ± 2	0.41
Others	1 ± 0	1 ± 0	0.25
Carcass traits			
Rib eye area, cm^2	70.8 ± 2.8	73.1 ± 11.1	0.71
Rib thickness, cm	7.5 ± 0.2	7.6 ± 1.4	0.92
Subcutaneous fat thickness, cm	3.1 ± 0.5	2.6 ± 0.3	0.18
Yield score	74.6 ± 0.4	75.4 ± 1.9	0.44
BMS number	9.5 ± 1.3	9.8 ± 1.0	0.77
BCS number	3.5 ± 0.6	3.3 ± 0.5	0.54
BFS number	2.8 ± 1.0	2.8 ± 0.5	1.00
Beef meat brightness	5.0 ± 0.0	5.0 ± 0.0	-
Beef meat firmness	5.0 ± 0.0	5.0 ± 0.0	-
Beef meat texture	5.0 ± 0.0	5.0 ± 0.0	-
Beef fat brightness and quality	5.0 ± 0.0	5.0 ± 0.0	-

Values are mean ± SD. [a] These values are the weights of lean meat, fat, and bone in the left half of the carcass. BCS: beef color standard; BFS: beef fat color standard; BMS: beef marbling standard; CON: control; LysMet: moderate-protein diet with rumen-protected lysine and methionine

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