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Effects of a Low-Crude Protein Diet Supplemented with Rumen-Protected Lysine and Methionine on Growth Performance and Nitrogen Excretion in Japanese Black Steers

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Abstract

To suppress the environmental problems caused by livestock manure, we attempted to reduce the amount of nitrogen excreted in urine and feces by feeding low-crude protein (CP) diets to Japanese Black steers. The dietary CP contents were 12%-13% on a dry-matter basis in the control diet (CON, n = 4, initial body weight [BW] 268 kg) and 11% in the low-CP diet with amino acids (LCPAA, n = 4, initial BW 276 kg). The LCPAA diets were supplemented with rumen-protected lysine and rumen-protected methionine during the early and middle fattening stages. The nitrogen excretion in urine was significantly lower in the LCPAA-fed than CON-fed steers at the early ($P < 0.01$) and middle ($P < 0.05$) fattening stages. In both cases, the plasma urea nitrogen concentrations were mostly lower in LCPAA- than CON-fed steers. The BW gain during the early fattening stage tended to be lower in LCPAA- than CON-fed steers ($P < 0.10$), but the dietary CP content affected neither the carcass weight nor the lean weight. The dietary CP content had no adverse effect on carcass traits. These results indicate that in Japanese Black steers, the 11% CP diet throughout the fattening period suppressed BW gain during the early period but reduced nitrogen excretion.

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

Introduction

Nitrogen excreted in livestock feces and urine contributes to a range of environmental issues, including water pollution from nitrate nitrogen as well as global warming and ozone layer depletion from nitrous oxide emissions (Lashof & Ahuja 1990, Hooda et al. 2000, Ravishankara et al. 2009). Reducing the nitrogen excretions from livestock could help protect both the local and global environments.

Dietary manipulation has been reported to reduce nitrogen excretion in feces and urine, with one method involving a combination of dietary protein reduction and amino acid addition (Gerber et al. 2013). Although the research regarding low-protein diets supplemented with amino acids for pigs and poultry is relatively advanced, there are few studies on such supplementation in ruminants.

In ruminants, crystalline amino acids are degraded in the rumen. Accordingly, several studies have employed infusions of amino acids into the abomasum or feeding of rumen-protected amino acids, revealing that the amino acids lysine and methionine decrease nitrogen excretion (Greenwood & Titgemeyer 2000, Batista et al. 2016) and increase weight gain (Oke et al. 1986, Klemesrud et al. 2000, Xue et al. 2011) in growing cattle. In the Japanese fattening system, low-protein diets supplemented with rumen-protected lysine and rumen-protected methionine

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are fed to Holstein steers, reducing nitrogen excretion without affecting productivity (Kamiya et al. 2021).

Japanese Black cattle, the primary type of beef cattle raised in Japan, have a slower rate of weight gain, more intramuscular fat deposits, and lower protein requirements during the fattening period than Holstein cattle (NARO 2009, Albrecht et al. 2011). Therefore, the feeding method for fattening Japanese Black cattle is markedly different from that for Holstein cattle. Regarding the crude protein (CP) requirement of fattening steers weighing 300 kg, the dietary CP content for Holstein steers gaining 1.5 kg per day is 15% on a dry-matter (DM) basis. In contrast, Japanese Black steers that gain 1.0 kg per day have a dietary CP content of 13% on a DM basis (NARO 2009). Thus, the dietary CP content of Japanese Black steers is usually lower than that of Holstein steers, and it is necessary to investigate diets with lower CP contents than previously described (Kamiya et al. 2021). In addition, because Japanese Black steers generally have more marbling than Holstein steers (Yamada 2022), producers and researchers pay close attention to the effects of various feeds on carcass traits that impact the commercial value of the steers. However, to our knowledge, there have been no investigations of the effects of low-CP diets supplemented with rumen-protected amino acids on the fattening performance of and nitrogen excretion by Japanese Black fattening cattle. Previous reports have suggested that lysine or methionine are growth-limiting amino acids in cattle (Klemesrud et al. 2000, Xue et al. 2011). Therefore, supplementation with these amino acids is predicted to reduce the negative effects of low-CP diets on cattle growth. The purpose of this study was to investigate the effects of protein-content reduction in fattening diets supplemented with rumen-protected lysine and rumen-protected methionine on the feed intake, body weight gain, carcass traits, and nitrogen excretion of Japanese Black steers.

Materials and methods

1. Cattle management and carcass evaluation

Eight Japanese Black steers (average age 9 months) were assigned based on body weight (BW) and sire to a control group (CON, n = 4) or a group fed a low-CP diet supplemented with rumen-protected amino acids (LCPAA, n = 4). Since we expected that there would be a period when production could not be maintained with the CP contents set for the low-CP group, rumen-protecting amino acids were added to the low-CP group.

On average, the steers were kept from 9 to 28 months of age, in three stages of fattening: early (9-15 months of age), middle (16-22 months), and late (23-28 months). The steers were slaughtered in a slaughter facility 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. 21B078ILGS) and conducted according to the NARO Implementation Regulations on Animal Experiments.

2. Feeding management and feed analysis

The chemical composition and ingredients of the diets in the experiment are shown in Table 1. Rumen-protected lysine (AjiPro-L, Ajinomoto Co., Inc., Tokyo, Japan) and rumen-protected methionine (Mepron, Evonik Nutrition and Care GmbH, Hanau, Germany) were commercially available. Steers were raised in barns with sawdust bedding and individually fed the concentrate mix and hay separately according to the feeding program (Table 2). The steers were fed the concentrate mix twice daily (in the morning and evening) and hay once daily (in the morning). Water and mineral blocks were available ad libitum. Refusals were collected and measured daily before the morning feeding. The body weight of each steer was measured about once a week before the morning feeding.

The DM contents of the diets were measured by forced air drying at 105°C for 16 hr (Barbosa et al. 2015). To determine the chemical composition of each diet, the CP, acid detergent fiber, neutral detergent fiber, and starch were chemically analyzed, and the total digestible nutrients (TDN) value was estimated. The CP content was measured by the macro-Kjeldahl method using an automatic analyzer (KjeltecTM 8400, FOSS, Hilleroed, Denmark) (AOAC 1990). The acid detergent fiber and neutral detergent fiber contents were measured by the detergent method using a raw fiber extractor (FIWE 6, VELP Scientifica, Usmate Velate, Italy) (Van Soest et al. 1991). The total starch content was measured by the amyloglucosidase/α-amylase method (Total starch assay kit, Megazyme, Ltd., Wicklow, Ireland). The TDN of each concentrate mix was estimated based on the feed ingredient values from the Standard Tables of Feed Composition in Japan (NARO 2010). The TDN of the timothy hay was calculated using the following formula (Otsuki 2001):

$$TDN = 87.09 - 0.752 \times ADF$$

where TDN is the total digestible nutrients (% of DM) and ADF is the acid detergent fiber (% of DM).

Metabolizable lysine and methionine in the early and middle fattening stages were designed using AMTS

Table 1. Chemical composition and ingredients of experimental diets

	Concentrate mix for the early stage		Concentrate mix for the middle stage		Concentrate mix for the late stage		Timothy hay
	CON	LCPAA	CON	LCPAA	CON	LCPAA	
Dry matter, % of FM	88.5	88.3	88.1	88.0	87.4	87.4	90.5
Chemical composition, % of DM							
TDN[a]	83.2	83.6	84.3	84.6	84.5	84.7	57.8
CP	15.6	12.3	14.2	11.9	13.0	11.7	7.6
ADF	7.3	6.8	7.0	6.6	6.5	6.3	38.9
NDF	22.4	22.3	20.9	20.8	20.2	20.1	64.5
Starch	47.1	52.6	48.5	52.2	51.5	53.6	1.5
Ingredients, % of FM							
Corn	28.4	35.9	36.7	42.2	39.9	42.8	-
Barley	37.2	37.2	33.3	33.3	33.1	33.1	-
Rice	0.8	0.8	0.8	0.8	0.8	0.8	-
Wheat bran	16.9	16.9	15.2	15.2	15.1	15.1	-
Soybean meal	8.6	0.8	6.8	1.1	4.0	1.1	-
Other by-products[b]	6.9	6.9	6.1	6.1	6.1	6.1	-
Supplement[c]	1.2	1.2	1.1	1.1	1.1	1.1	-
Rumen-protected lysine	-	0.23	-	0.23	-	-	-
Rumen-protected methionine	-	0.02	-	0.02	-	-	-

[a] The TDN of concentrate mix was estimated according to the Standard Tables of Feed Composition in Japan (NARO 2010). The TDN of timothy hay was calculated from 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
ADF: acid detergent fiber; CON: control; CP: crude protein; DM: dry matter; FM: fresh matter; LCPAA: the low-crude protein diet with amino acids; NDF: Neutral detergent fiber; TDN: total digestible nutrients

Table 2. Feeding program for fattening Japanese Black steers

	Age, months		
	9-15	16-22	23-28
Concentrate mix for the early stage of fattening	5.0-8.0		
Concentrate mix for the middle stage of fattening		8.5[a]-9.4	
Concentrate mix for the late stage of fattening			9.4-10.0
Timothy hay	3.0	2.5	2.5

The data are in kgFM/day.
[a] Mixture of the concentrate mix for the early fattening stage and the concentrate mix for the middle stage
FM: fresh matter

Cattle Pro ver. 4.11.x (2020, AMTS LLC, Groton, NY, USA) to be approximately the same for the CON and LCPAA groups. Intakes of metabolizable lysine and metabolizable methionine were estimated based on the National Academies of Sciences, Engineering, and Medicine (NASEM) method of summing the absorbed amino acids supplied by ruminally undegradable protein and ruminal bacteria (NASEM 2016). The metabolizable intakes were estimated for each amino acid as follows:

$$MAA = RUPAA \times 0.8 + MCPAA \times 0.8 \times 0.8$$

$$RUPAA = (CP/100) \times (RUP/100) \times (FEEDAA/100) \times DMI$$

$$MCPAA = (42.73+TDNI \times 0.087) \times (MCAA/100)$$

where MAA is the metabolizable amino acid intake (g/day), RUPAA is the amino acid intake derived from the ruminally undegradable protein (g/day), MCPAA is the amino acid intake derived from the microbial crude protein (g/day), CP is the dietary crude protein content (% of DM), RUP is the ruminally undegradable protein content in the CP (% of CP), FEEDAA is the composition of the amino acid in feed (% of protein), DMI is the dry-matter intake (gDM/day), TDNI is the total digestible nutrient intake (g/day), and MCAA is the composition of the amino acid in the ruminal bacteria (% of microbial crude protein). The intakes of lysine and methionine derived from the microbial crude protein (MCP) were calculated assuming that lysine and methionine constituted 7.9% and 2.6% of the amino acid composition of ruminal bacteria, respectively.

3. Blood sampling and analysis

Blood samples were collected monthly from 8 months of age (pre-experiment) to 27 months from the jugular vein into heparinized tubes before the morning feeding. The plasma was separated by centrifugation at 1,760 g for 20 min at 4°C and stored at −30°C until analyzed. The plasma urea nitrogen concentration was determined based on an enzymatic method with urease, using an automated biochemical analyzer (Dri-chem 3500V, FUJIFILM Co., Tokyo, Japan).

4. Nitrogen balance trials

We measured the nitrogen excretion and nitrogen retention at 11 months of age in the early stage of fattening, 19 months in the middle stage, and 26 months in the late stage, collecting 80 mL of spot fresh urine and a fecal grab sample from each steer in the morning and evening for three consecutive days, as reported by Baldwin et al. (1983). The urine samples were frozen at −30°C immediately after collection, and all samples for each steer were mixed before analysis. The fecal samples were refrigerated at 4°C immediately after collection, and all samples for each steer were mixed before analysis.

The nitrogen contents of the urine and fecal samples were analyzed by the macro-Kjeldahl method, as was used for the CP analysis of feed. The urinary creatinine concentration was measured using the Jaffe method (LabAssay Creatinine, FUJIFILM Wako Pure Chemical Co., Ltd., Osaka, Japan). The urine volume was estimated using the creatinine concentration as an internal marker (Chizzotti et al. 2008), with a daily urinary excretion of creatinine of 26 mg/kg BW (Whittet et al. 2004). The urinary nitrogen excretion was estimated as follows:

$$UNE = 26 \times BW/Cr \times (UN/100) \times 1{,}000$$

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

The feed and fecal acid-insoluble ash (AIA) concentrations were measured by the 4 N hydrochloric acid method (Terada et al. 1979). Fecal weights were estimated using the AIA concentration as an internal marker (Sales & Janssens 2003). The fecal nitrogen excretion was estimated as follows:

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

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

5. Carcass weight and traits

One day after slaughter, each carcass was incised between the sixth and seventh ribs, and the carcass traits were evaluated by the Japan Meat Grading Association. Association members measured the rib eye area, rib thickness, and subcutaneous fat thickness at the incision plane between the sixth and seventh ribs. They then calculated the yield score using the following formula:

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

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 bounded by the longissimus thoracis fascia line. The rib thickness was measured as the length from the thoracic side of the pleura to the dorsal side of the latissimus dorsi muscle at approximately the center of the full rib length. The subcutaneous fat thickness was measured as the length from the dorsal side of the latissimus dorsi muscle to the carcass surface on a line raised perpendicular to the carcass surface from the side edge of the iliocostalis muscle.

The association also evaluated 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. The marbling evaluation score was expressed as a beef marbling standard (BMS) number from 1 to 12; the higher the number, the more marbling is present. The meat color score was expressed by a beef color standard (BCS)

number from 1 to 7; the higher the number, the darker the meat color. The fat color evaluation was expressed as a beef fat color standard (BFS) score from 1 to 5; the higher the value, the yellower the fat. The meat brightness, firmness, texture, and fat brightness and quality were scored from 1 to 5; the higher the value, the better the quality. A half carcass was dissected two or three days after slaughter and weighed for lean meat, fat, bones, and other qualities. The lean meat contained intramuscular fat.

6. Statistical analyses

The plasma urea nitrogen concentration was tested using a linear mixed model with treatment, period, and interaction of treatment and period as fixed effects, and cattle as a random effect (R ver. 4.1.0). Because there was a significant interaction between treatment and period, these data were compared between groups separately for each month of age. Differences in parameters between groups were tested by an analysis of variance (ANOVA: R ver. 4.1.0). Probability (P) values < 0.05 and < 0.10 were considered to indicate significance and a tendency, respectively.

Results

1. Body weight and feed intake

Table 3 summarizes the effects of the low-CP diet supplemented with rumen-protected lysine and methionine on the BW and feed intake of steers. There was no significant difference in initial BW between the CON and LCPAA groups at the early, middle, or late stages of fattening, and no significant between-group difference in the final BW before slaughter. The dietary CP content did not affect the DM and TDN intakes during the early, middle, or late stages. As a result, the gain-to-feed ratio was not affected by the dietary CP content during any fattening stage.

The starch intake during the early fattening stage was significantly higher in the LCPAA group than the CON group ($P < 0.05$). However, no significant difference was observed in the middle or late stages. The CP intake was significantly lower in the LCPAA group than in the CON group during the early stage of fattening ($P < 0.01$). It tended to be lower in the LCPAA group in the middle stage ($P < 0.10$), but no significant difference was observed after that. There was no difference in the intake of metabolizable lysine or metabolizable methionine between the CON and LCPAA groups. The BW gain during the early stage of fattening tended to be lower in the LCPAA group than in the CON group ($P < 0.10$), but no significant between-group difference was observed at the middle and late stages. The dietary CP contents of the CON group were 13.3% DM, 12.8% DM, and 12.1% DM during the early, middle, and late stages of fattening, respectively. The dietary CP content of the LCPAA group was 11.2% DM at each stage.

2. Plasma urea nitrogen concentrations

The plasma urea nitrogen concentrations were significantly lower or tended to be lower in the LCPAA group than the CON group during the early and middle stages of fattening, except at 17 and 21 months of age (Fig. 1). There was no statistically significant difference in the plasma urea nitrogen concentration between the CON and LCPAA groups during the late fattening stage.

3. Nitrogen excretion

The results of the nitrogen balance trial are shown in Table 4. No effects of the dietary CP content were observed on the fecal nitrogen excretion or the nitrogen retention at the early and middle stages of fattening. However, the nitrogen intake ($P < 0.01$), urinary nitrogen excretion ($P < 0.01$), and total nitrogen excretion ($P < 0.05$) at the early stage were significantly lower in the LCPAA group than in the CON group. In the middle stage, the nitrogen intake and total nitrogen excretion tended to be lower in the LCPAA group than in the CON group ($P < 0.10$), and the urinary nitrogen excretion was significantly lower ($P < 0.05$) in the LCPAA group. No significant between-group differences were observed in nitrogen intake, fecal nitrogen excretion, urinary nitrogen excretion, total nitrogen excretion, or nitrogen retention in the late stage.

4. Carcass weight and traits

The differences in the dietary CP content did not affect the carcass weight or the weights of lean meat, fat, or bone in the half-carcasses (Table 5). There were no significant differences between the CON and LCPAA groups in the rib eye area, rib thickness, or subcutaneous fat thickness at the incision plane between the sixth and seventh ribs. The BMS number tended to be higher in the LCPAA group than in the CON group ($P < 0.10$). However, there were no significant between-group differences in the BCS number, BFS number, meat brightness, meat firmness, meat texture, or fat brightness and quality.

Discussion

Feeding low-CP diets supplemented with rumen-protected lysine and methionine to Japanese Black steers during the fattening period did not affect the DM or TDN intakes, BW, or the gain-to-feed ratio. The

Table 3. Effects of a low-crude protein diet supplemented with rumen-protected lysine and methionine on the body weight and feed intake of Japanese Black steers

		CON	LCPAA	*P*-value
Body weight, kg	Initial (early stage)	268 ± 64	276 ± 18	0.80
	Initial (middle stage)	475 ± 82	454 ± 22	0.64
	Initial (late stage)	667 ± 114	624 ± 31	0.49
	Final	774 ± 138	742 ± 23	0.67
Body weight gain, kg/day	Early stage	1.06 ± 0.14	0.91 ± 0.06	0.09
	Middle stage	0.88 ± 0.15	0.78 ± 0.06	0.27
	Late stage	0.72 ± 0.23	0.77 ± 0.14	0.75
	Total	0.90 ± 0.16	0.82 ± 0.04	0.40
DM intake, kg/day	Early stage	7.6 ± 0.7	7.2 ± 0.2	0.32
	Middle stage	8.0 ± 1.4	7.4 ± 0.4	0.46
	Late stage	8.1 ± 1.7	8.4 ± 0.7	0.82
	Total	7.9 ± 1.2	7.6 ± 0.4	0.66
Gain-to-feed ratio	Early stage	0.140 ± 0.014	0.127 ± 0.010	0.15
	Middle stage	0.110 ± 0.008	0.106 ± 0.012	0.54
	Late stage	0.088 ± 0.018	0.091 ± 0.011	0.74
	Total	0.114 ± 0.009	0.108 ± 0.005	0.34
TDN intake, kg/ day	Early stage	5.8 ± 0.5	5.6 ± 0.1	0.48
	Middle stage	6.4 ± 1.1	6.0 ± 0.3	0.59
	Late stage	6.5 ± 1.3	6.7 ± 0.5	0.71
	Total	6.2 ± 0.9	6.1 ± 0.3	0.83
Starch intake, kg/ day	Early stage	2.6 ± 0.2	2.9 ± 0.1	0.01
	Middle stage	3.2 ± 0.5	3.4 ± 0.2	0.52
	Late stage	3.3 ± 0.7	3.7 ± 0.3	0.31
	Total	3.0 ± 0.4	3.3 ± 0.2	0.23
CP intake, kg/day	Early stage	1.01 ± 0.08	0.80 ± 0.01	< 0.01
	Middle stage	1.03 ± 0.18	0.84 ± 0.04	0.08
	Late stage	0.98 ± 0.21	0.94 ± 0.06	0.68
	Total	1.01 ± 0.15	0.85 ± 0.04	0.08
Dietary CP content, %[a]	Early stage	13.3 ± 0.2	11.2 ± 0.1	< 0.01
	Middle stage	12.8 ± 0.1	11.2 ± 0.1	< 0.01
	Late stage	12.1 ± 0.2	11.2 ± 0.2	< 0.01
	Total	12.8 ± 0.1	11.2 ± 0.1	< 0.01
Metabolizable lysine intake, g/day	Early stage	37.7 ± 2.8	39.0 ± 0.6	0.40
	Middle stage	40.3 ± 6.7	41.7 ± 1.9	0.71
	Late stage	40.4 ± 7.8	41.3 ± 2.9	0.83
	Total	39.4 ± 5.5	40.7 ± 1.6	0.66
Metabolizable methionine intake, g/day	Early stage	12.1 ± 0.9	12.0 ± 0.2	0.82
	Middle stage	13.2 ± 2.2	13.0 ± 0.6	0.88
	Late stage	13.4 ± 2.6	14.0 ± 1.0	0.71
	Total	12.9 ± 1.8	12.9 ± 0.6	0.95

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; LCPAA: the low-crude protein diet with amino acids; TDN: total digestible nutrients

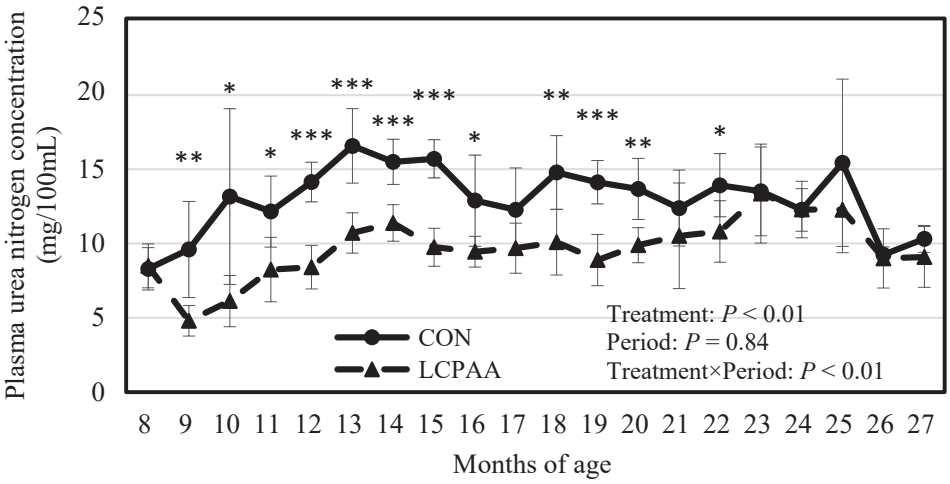

Fig. 1. Effects of the low-crude protein diet throughout the fattening period on the plasma urea nitrogen concentration of Japanese Black steers
CON: control (●, n= 4), LCPAA: the low-crude protein diet with amino acids (▲, n= 4)
Values are mean ± SD. **P* < 0.10, ***P* < 0.05, and ****P* < 0.01 between the CON and LCPAA groups

Table 4. Effects of a low-crude protein diet supplemented with rumen-protected lysine and methionine on the fecal and urinary nitrogen excreted by Japanese Black steers

	CON	LCPAA	*P*-value
At the early stage of fattening, g/day			
Nitrogen intake	140 ± 15	111 ± 3	< 0.01
Fecal nitrogen excretion	46 ± 10	40 ± 4	0.34
Urinary nitrogen excretion	60 ± 3	42 ± 6	< 0.01
Total nitrogen excretion	106 ± 9	82 ± 9	0.01
Nitrogen retention	35 ± 6	29 ± 8	0.30
At the middle stage of fattening, g/day			
Nitrogen intake	167 ± 32	129 ± 8	0.06
Fecal nitrogen excretion	48 ± 8	41 ± 5	0.19
Urinary nitrogen excretion	94 ± 15	74 ± 5	0.04
Total nitrogen excretion	143 ± 23	115 ± 4	0.06
Nitrogen retention	24 ± 9	14 ± 9	0.16
At the late stage of fattening, g/day			
Nitrogen intake	160 ± 32	138 ± 13	0.25
Fecal nitrogen excretion	41 ± 18	46 ± 11	0.68
Urinary nitrogen excretion	114 ± 16	93 ± 18	0.13
Total nitrogen excretion	155 ± 28	139 ± 21	0.38
Nitrogen retention	5 ± 7	–1 ± 16	0.57

Values are mean ± SD.
Nitrogen excretion was measured at 11, 19, and 26 months of age during the early, middle, and late fattening stages, respectively.
CON: control; LCPAA: the low-crude protein diet with amino acids

Table 5. Effects of a low-crude protein diet supplemented with rumen-protected lysine and methionine on the carcass traits of Japanese Black steers

	CON	LCPAA	*P*-value
Carcass weight, kg	495 ± 89	480 ± 23	0.75
Tissue weight in half-carcass[a], kg			
Lean	129 ± 25	129 ± 5	0.99
Fat	94 ± 14	88 ± 8	0.44
Bone	25 ± 6	22 ± 1	0.43
Others	1 ± 0	1 ± 0	0.74
Carcass traits			
Rib eye area, cm^2	69.3 ± 12.3	72.5 ± 13.8	0.74
Rib thickness, cm	7.6 ± 1.0	7.7 ± 0.5	0.87
Subcutaneous fat thickness, cm	2.9 ± 1.0	3.0 ± 0.5	0.93
Yield score	74.7 ± 2.0	75.3 ± 1.4	0.60
BMS no.	8.3 ± 1.0	9.8 ± 1.0	0.07
BCS no.	3.5 ± 0.6	3.8 ± 0.5	0.54
BFS no.	3.0 ± 0.0	3.5 ± 0.6	0.13
Beef meat brightness	4.8 ± 0.5	5.0 ± 0.0	0.36
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; LCPAA: low-crude protein diet with amino acids

intakes of metabolizable lysine and metabolizable methionine were similar between the LCPAA and CON groups because rumen-protected lysine and rumen-protected methionine were added to the LCPAA diet. However, the BW gain during the early stage of fattening was lower in the steers fed a diet with a lower CP content. It has long been known that in raising beef cattle, a high dietary CP content increases the BW gain (Haskins et al. 1967, Braman et al. 1973, Byers & Moxon 1980, Perry et al. 1983). It has also been reported that adding a combination of rumen-protected lysine and rumen-protected methionine (Oke et al. 1986, Veira et al. 1991, Hussein & Berger 1995) or rumen-protected lysine alone (Klemesrud et al. 2000, Xue et al. 2011) to low-CP feeds increases the BW gain. However, in the present experiment, BW gain in the LCPAA group in the early fattening stage could not be maintained at the same rate as in the CON group. The disparity in the dietary CP content between the two groups was most pronounced during the early stage. The metabolizable protein content in the feed was required to be higher during the early stage of fattening than in the middle and late stages (NARO 2009) and therefore may have been deficient in the LCAPP group. On the other hand, DM digestibility was not affected in the early stage. However, fiber digestibility was lower in the LCPAA group than in the CON group (data not shown), suggesting a deficiency of degradable protein intake. To increase the BW gain in the LCPAA group during the early stage of fattening, it would thus be necessary to increase the dietary CP in that stage to more than 11% to increase the amount of nitrogen supplied to the rumen and the amount of metabolizable protein.

We also observed that the plasma urea nitrogen concentrations during the early and middle fattening stages were lower in the steers fed the diet with lower CP content. In several animal species, the blood urea nitrogen concentration is highly correlated with the urinary nitrogen excretion (Kohn et al. 2005). In feedlot cattle, the serum urea nitrogen concentration and urinary nitrogen excretion have both been shown to increase linearly when the dietary CP content is increased

(Vasconcelos et al. 2009). Even under the conditions of recent fattening programs in Japan, Holstein steers with low nitrogen excretion had low plasma urea nitrogen concentrations (Kamiya et al. 2020). Based on the plasma urea nitrogen concentrations observed in the present study, it can be expected that the urinary nitrogen excretion of Japanese Black steers during the early and middle stages of fattening will be lower in steers fed a diet with a lower CP content.

The nitrogen excretion at the early and middle stages of fattening was lower in the group fed the diet with lower CP content. A previous study described that low-CP diets supplemented with lysine reduced the urinary nitrogen excretion and increased the nitrogen retention in growing Holstein steers (Batista et al. 2016). Other groups found that a rumen infusion of methionine reduced the urinary nitrogen excretion and increased the nitrogen retention in growing Holstein steers (Greenwood & Titgemeyer 2000, Löest et al. 2002, Schroeder et al. 2006). Depending on the feed composition, methionine and lysine are considered limiting amino acids in growing Holstein steers, and supplementation reduces urinary nitrogen excretion and increases nitrogen retention. In our present study on Japanese Black steers, the CP content in the diets in the early and middle stages was approximately two percent lower in the group fed the low-CP diet supplemented with rumen-protected lysine and rumen-protected methionine than the group fed the control diet. We observed that this diet modification reduced the nitrogen excretion by approximately 20%. We speculate that in this experiment, a significant factor in reducing nitrogen excretion was in decreasing the dietary CP content. Although we added rumen-protected lysine and rumen-protected methionine to suppress decreased beef production, the precise impact of these additions on the reduction of nitrogen excretion remains for future research to clarify.

According to the Greenhouse Gas Inventory Office of Japan (GIO), nitrous oxide emissions from manure are proportional to the amount of nitrogen excreted in urine and feces (GIO 2020). Reducing nitrogen excretion can thus also reduce nitrous oxide, a greenhouse gas (Bao et al. 2018, Eckard et al. 2010) that is a significant factor in the depletion of the ozone layer (Ravishankara et al. 2009). The decrease in nitrogen excretion that we observed in the LCPAA group in the early and middle fattening stages indicates that low-CP diets could help reduce the environmental impact of the beef industry.

The dietary CP content had no effect on the half-carcass lean weights in this study, indicating that lowering the dietary CP content did not negatively affect the quantitative meat productivity. There were also no effects of the dietary CP content on the rib eye area or rib thickness, which develop most during the early and middle stages of fattening. However, the BW gain in the steers fed the diet with a lower CP content was low during the early stage. Although the between-group difference in carcass weight was insignificant, the mean carcass weights of the CON and LCPAA groups (495 versus 480 kg, respectively) were not the same. Before LCPAA diets can be implemented at beef cattle farms, it will be necessary to improve the diets so that the BW gain during the initial stages of fattening can be maintained at the same level as by conventional methods.

Compared to the control group, the beef marbling standard was slightly higher in the group fed the diet with lower CP content. Glucose is essential for de novo fatty acid biosynthesis in the intramuscular adipose tissue of feedlot cattle (Smith et al. 2018). Our study, replaced most of the soybean meal with corn in the concentrate mix of the LCPAA group. As a result, the LCPAA group's concentrate mix feed had a higher starch content than the CON group. The LCPAA steers had 10% higher average starch intake than the CON steers, and the between-group difference was significant at the early fattening stage. Feeding high-starch diets at an early age increases intramuscular fat accumulation (Park et al. 2018). Therefore, starch intake is considered more critical than protein intake for marbling in fattening cattle. The difference in starch intake that we observed between the CON and LCPAA groups may have played a role in the effects of the feed composition on the intramuscular fat content in these cattle, but further experiments are needed to clarify this. Because the carcass yield and meat quality of both groups were typical for Japanese Black steers, feeding an 11% CP diet would not have had an adverse effect on qualitative productivity.

The nitrogen excreted in livestock waste causes such environmental problems as water pollution, global warming, and ozone layer depletion. We developed a feed that reduces the amount of nitrogen excreted by beef cattle. Although the developed diet reduced the protein intake in fattening cattle, the intakes of energy, metabolizable lysine, and metabolizable methionine were equivalent to those of the control diet, and the novel diet did not adversely affect the half-carcass lean weight or meat quality. However, this experiment did not include a negative control in which no amino acids were added. Thus, the effect of amino acid addition could not be isolated from other dietary effects in this experiment. Further experiments are needed to clarify the effects of amino acid addition alone during the early and middle stages of fattening on the feeding performance of Japanese Black steers. Our findings demonstrate that

feeding an 11% CP diet throughout the fattening period suppressed the BW gain during the early period in Japanese Black steers but reduced nitrogen excretion, an environmentally hazardous substance.

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