# Effects of the different feeding types on growth performance, carcass characteristics, and meat composition of Holstein steers

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# 사료 급여 방식이 Holstein 거세우의 사양 성적, 도체 특성 및 육질에 미치는 영향

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Abstract This study was conducted at a farm in hwacheon-gun, to evaluate the effects of different feeding types on growth performance, carcass characteristics, and meat composition of Holstein steers for seven months. Fifteen steers were randomly assigned to one of three dietary groups: T1 (concentrate feed + ryegrass straw), T2 (total mixed fermentation [TMF] + concentrate feed), and T3 (TMF). Average daily gain and dry matter intake were lower in T3 group than in T1 and T2 groups, but the differences were not significant. Concentrations of blood urea nitrogen in plasma were higher in T1 and T2 groups than in T3 group (p <0.05). Carcass weight, rib eye area, marbling score, and meat quality grade were slightly, but not significantly, higher in T2 group than in T1 and T3 groups. The amino acids composition and physicochemical characteristics in the sirloin were similar among the treatment groups. The content of eicosenoic acid in the sirloin was higher in T1 group than in T3 group (p  $\langle$  0.05). Thus, the findings of this study indicate that a sequential feeding method using TMF and concentrate feed had some positive effect on carcass weight, rib eye area and marbling score without any negative effect on growth performance and meat composition of Holstein steers.

요 약 본 연구는 사료 급여 방식이 Holstein 거세우의 사양 성적, 도체 특성 및 육질에 미치는 영향을 조사하기 위해 화천군 소재 목장에서 7개월간 수행되었다. Holstein 거세우 15두는 T1구(배합사료 + ryegrass straw), T2구(total mixed fermentation [TMF] + 배합사료) 및 T3구(TMF)로 각각 5두씩 배치하였다. 일당증체량 및 건물섭취량은 T1구 및 T2에 비해 T3구에서 낮았지만, 통계적인 유의성은 없었다. 혈청 요소태질소의 농도는 T3구 비해 T1구 및 T2에서 높았다(p<0.05). 도체중, 등심단면적, 근내지방도 및 육질 등급은 T1구 및 T3에 비해 T2에서 높은 경향을 보였지만, 통계적인 유의성은 인정되지 않았다. 등심의 아미노산 조성 및 물리화학적 특성은 처리구간 유사한 결과를 보였다. 등심 의 eicosenoic acid의 함량은 T3구에 비해 T1구에서 높았다 (p < 0.05). 따라서 본 연구의 결과에서 TMF와 배합사료 의 순차적인 혼합 급여 방식은 홀스타인 거세우의 사양 성적 및 육질에 대한 부정적인 영향 없이 도체중, 등심단면적 및 근내지방도에 일부 긍정적인 영향을 미치는 것으로 판단된다.

Keywords: Holstein Steers, Total Mixed Fermentation, Concentrate Feed, Growth Performance, Carcass Characteristics, Meat Composition

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Received April 29, 2024 Accepted July 5, 2024

Revised June 11, 2024 Published July 31, 2024

# 1. Introduction

With the recent spread of health-conscious consumerism, negative perceptions of high-marbling beef with relatively high fat content have been increasing. As a result, more consumers are selecting intermediate beef (adequate marbling) with a relatively high muscle ratio, even though the fat content is rather low compared to high-marbling beef which have higher prices and fat contents [1]. When subjected to the same feeding protocol over the same length of time, Holstein steers have lower muscle fat than Hanwoo steers; however Holstein steers have a higher lean meat percentage and are more suitable for producing low-fat and high-protein beef. In general, Hanwoo steers are generally slaughtered at approximately 31 months of age, whereas Holstein steers are slaughtered between 20 and 24 months of age [2]. In addition, Holstein steers have a higher feed intake than Hanwoo steers, so if they are fed for a long time like Hanwoo steers, it is highly likely that the feed cost will increase and consequently decrease profitability. Therefore, there is a need for a study on establishing a shorter raising period and changing the feeding method for the production of intermediate beef, which is preferred by consumers.

The total mixed ration (TMR) feeding method has the effect of increasing the uniformity of growth performance and carcass grade among steers, when compared to the concentrate feed-based feeding method. Moreover, the TMR can stabilize rumen fermentation and reduce feed costs by using agricultural by-products [3,4]. As more farmers use TMR produced in-house, the proportion of TMR is gradually increasing, with formula feed production reaching 7 million tons and TMR around 3 million tons in the last five years. The TMR feeding system, which is widely applied when dealing with Holstein lactating cows, has rapidly spread to Hanwoo

steers in recent years, and comparative studies between the concentrate feed feeding method and the TMR feeding method are actively being conducted for Hanwoo steers [5]. In addition, we have observed a progressive increase in the interest and use of TMF with improved palatability and preservability gained from the introduction of a microbial fermentation process [6]. In Korea, in the case of Holstein steers, some farms provide TMR or TMF during the growing period due to the increase in feed cost, but most farms use a concentrate feed for the purpose of shortening the fattening period and improving the average daily gain (ADG) through the supply of high energy/protein. For this reason, it is difficult to find studies on TMF or TMF/concentrate feed for Holstein steers. Therefore, this study was conducted to investigate the effect of different feeding types (concentrate feed or/and TMF) on the growth performance, carcass characteristics, and meat composition of Holstein steers.

#### 2. Materials and Methods

# 2.1 Animals, treatments, and management

This study was conducted at a farm in Hwacheon-gun, using fifteen Holstein steers for seven months  $(430.5\pm46.3 \text{ kg}, 12.2\pm0.9 \text{ months})$  of age).

Based on the nutrient requirements of Holstein steers, the feeding amount was based on the age of the experimental animals: T1 was fed 13 to 15 kg of concentrate feed and 2-3 kg of ryegrass straw, T2 was fed 18 to 20 kg of TMF with top-dressed5 kg of concentrate feed, and T3 was fed 24 to 26 kg of TMF. The steers were arranged into three pens according to their treatment group. Each pen was 5 × 10 m in size and covered with 20 cm of sawdust.

The concentrate feed, TMF, and ryegrass straw were fed twice daily (08:00 and 17:00). Water was always freely available, and other feeding

management tasks were conducted in accordance with the practices of the farm. The chemical compositions, as well as the carbohydrate and protein fractions of the experimental diets, are presented in Tables 1 and 2.

# 2.2 Growth performance and plasma metabolites

Feed intake and residual quantity were measured daily before morning feeding to calculate the actual feed intake. ADG was calculated based on the number of days in each month, while dry matter intake (DMI) and ADG were used to calculate the feed conversion ratio (FCR).

The chemical compositions of the experimental diets were analyzed using the standard methods of the AOAC [7]. Neutral detergent fiber (NDF) and acid detergent fiber (ADF) were analyzed according to the methods of Van Soest et al. [8] using a filter bag (Ankom F57, Ankom Technology, Macedon, NY, USA).

Starch was analyzed using the methods of Hall [9]. Soluble protein (SolP) was analyzed following the methods of Krishnamoorthy et al. [10]. Neutral detergent insoluble crude protein (NDICP) and acid detergent insoluble crude protein (ADICP) were analyzed following the methods of Licitra et al. [11] and the carbohydrate and protein fractions of the Cornell net carbohydrate and protein system (CNCPS) were evaluated based on the measured contents of carbohydrates and proteins, using the methods outlined by Fox et al. [12].

Blood samples for the analyses of plasma metabolites were taken at 2-month intervals from the jugular vein of the steers, using an 18-gauge needle and a blood collection tube coated with heparin (Vacutainer, Becton-Dickinson, Franklin Lakes, NJ, USA). Blood samples were stored in an ice box and transferred to the laboratory within 6 h of collection.

Table 1. Ingredient and chemical composition of experimental diets

Item	Concentrate feed	TMF	Ryegrass straw
···· Ingredie	nt composition	n (%) ·····	
Corn grain	34.50	38.50	-
Wheat grain	10.00	-	-
Cane molasses	4.00	-	-
Wheat flour	-	-	-
Wheat bran	4.00	-	-
Lupin	2.50	-	
Whole cottonseed	2.50	-	
Corn gluten feed	20.00	15.00	-
Dried corn distiller's drains with solubles	2.50	-	-
Soybean meal	5.00	-	-
Coconut meal	-	-	-
Palm kernel meal	10.00	-	-
Rapeseed meal	1.00	-	-
Oligosaccharide by-product	-	6.00	-
Mushroom by-product	-	17.00	-
Soysauce by-product	-	7.00	-
Barley haylage	-	6.00	-
Rice straw haylage	-	-	-
Saccharomyces cerevisiae (liquid)	-	3.30	-
Ca salts of fatty acids	0.20	-	-
Salt dehydrated	0.60	-	-
Limestone	2.37	0.70	-
Sodium bicarbonate	0.50	0.50	-
Vitamin premix <sup>1</sup>	0.10	-	-
Mineral premix <sup>2</sup>	0.10	-	-
Feed additives	0.13	-	-

···· Chemical composition (DM basis, %) ····

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Dry matter	$87.85 \pm 0.34$	$62.82 \pm 0.4$	$88.35 \pm 1.25$
Crude protein	$15.59 \pm 0.73$	$15.80 \pm 0.25$	$6.90 \pm 0.29$
Ether extract	3.11±0.56	$3.05 \pm 0.21$	$1.20 \pm 0.12$
Crude fiber	$4.75 \pm 0.71$	16.56±0.32	$41.08 \pm 0.19$
Neutral detergent fiber	19.73±2.51	$32.49 \pm 3.30$	$74.51 \pm 1.21$
Acid detergent fiber	11.52±2.18	18.13±4.16	$42.64 \pm 0.32$
Crude ash	$6.77 \pm 1.42$	$8.77 \pm 0.61$	$6.58 \pm 0.11$
Total digestible nutrients	83.67±0.57	81.74±0.63	$42.89 \pm 0.27$

<sup>1</sup>Vitamin premix provided the following quantities of vitamins per kilogram of the diet: vitamin A, 10,000 IU; vitamin D<sub>3</sub>, 1,500 IU; vitamin E, 25 IU.; <sup>2</sup>Mineral premix provided the following quantities of minerals per kilogram of the diet: Fe, 50 mg; Cu, 7mg; Zn, 30 mg; Mn, 24 mg; I, 0.6 mg; Co, 0.15 mg; Se, 0.15 mg.; In this study, the raw materials comprised mainly inexpensive by-products and domestic forage (rice straw and whole crop barley haylage) for the production of low-cost TMF. This was done in order to reduce feed cost, which is a problem when rearing Holstein beef steers. TMF was prepared by considering the ratio of crude protein and carbohydrate fractions with a slow degradation rate in the rumen, and an hourly degradation rate of 20% or less obtained through a preliminary test (unpublished). TMF was carried out through a fermentation process at 38 °C for 48 h after mixing all raw materials.

Table 2. Carbohydrate and protein fractions of experimental diets

Item	Concentrate feed	TMF
Starch (% DM)	34.90	19.20
Sugar (% DM)	5.10	2.40
NFC <sup>1</sup> (% DM)	47.90	35.42
Lignin (% DM)	2.73	4.43
SolP <sup>2</sup> (% DM)	5.40	6.70
NDICP <sup>3</sup> (% DM)	1.41	2.33
ADICP4 (% DM)	1.07	1.50
Carbohydrate fraction		
CA <sup>5</sup> (% CHO)	7.06	3.35
CB1 <sup>6</sup> (% CHO)	48.33	26.78
CB2 <sup>7</sup> (% CHO)	10.94	17.28
CB3 <sup>8</sup> (% CHO)	24.7	32.76
CC9 (% CHO)	9.07	19.83
Protein fraction		
PA <sup>10</sup> +PB1 <sup>11</sup> (% CP)	51.40	36.20
PB2 <sup>12</sup> (% CP)	40.18	44.60
PB3 <sup>13</sup> (% CP)	2.00	10.00
PC <sup>14</sup> (% CP)	6.42	9.20
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<sup>1</sup>NFC, non-fiber carbohydrate: <sup>2</sup>SOLP, soluble protein: <sup>3</sup>NDICP, neutral detergent insoluble protein: <sup>4</sup>ADICP, acid detergent insoluble protein: <sup>5</sup>CA: fast fermented: <sup>6</sup>CB: moderate fermented: <sup>7</sup>CB2: intermediately fermented: <sup>8</sup>CB3: slowly fermented: <sup>9</sup>CC: unfermentable: <sup>10</sup>PA: non-protein nitrogen: <sup>11</sup>PB1: rapidly degradable protein: <sup>12</sup>PB2: intermediately degradable protein: <sup>13</sup>PB3: intermediately degradable protein: <sup>14</sup>PC: unavailable protein.

Blood samples were centrifuged at 1,250  $\times$  g for 10 min to separate the plasma and analyzed using an automatic blood analyzer (Hitachi 7020, Hitachi Ltd., Tokyo, Japan). The analyses included glucose (GLU), non-esterified fatty acid (NEFA), blood urea nitrogen (BUN), albumin (ALB), total protein (TP), cholesterol (CHO), triglycerides (TG), aspartate-amino-transferase (AST), alanine-amino-transaminase (ALT),  $\gamma$ -glutamyl-transferase (GGT), calcium (Ca), phosphorus (P), magnesium(Mg).

# 2.3 Carcass characteristics and meat composition

At the end of the experimental period (20 months of age), all steers were slaughtered at the local slaughterhouse to assess carcass yield and quality traits. Carcass evaluation was performed, on meat from the 13<sup>th</sup> rib section from the left

side of each carcass, by meat graders using the criteria provided by the Korean carcass grading system [13]. Meat quality traits were measured for marbling score, meat color, fat color, texture, and maturity. Yield traits were measured for carcass weight, back fat thickness, and the size of the rib eye area. The carcass yield index (YI) was calculated according to the following equation: YI =  $(68.184 - [0.625 \times \text{back fat thickness {mm}}]) + [0.130 \times \text{rib eye area {cm}}^2] - [0.024 \times \text{carcass weight {kg}}]) + 3.23.$ 

Evaluation of the physicochemical composition of beef from Holstein steers: Sirloin samples from cold carcasses of steers were used for meat composition analysis. The carcass was transported to the laboratory, and its quality was analyzed in a low-temperature room (5 °C) after the removal of fat, connective tissue, and blood.

The chemical composition of the sirloin was measured according to the standard methods of the AOAC [7]. To measure the pH of the sirloin, approximately 10 g of sirloin was cut into small pieces and homogenized with 90 mL of distilled water (PolyTron PT-2500 E, Kinematica, Lucerne, Switzerland). The pH values were then measured immediately after homogenization using a pH meter (Orion 230A, Thermo Fisher Scientific Inc., Waltham, MA, USA).

For the measurement of cooking loss of the sirloin, 1.0 cm-thick steaks were placed in a polyethylene bag and heated in a water bath at 75 °C for 40 min, before being cooled at room temperature for 30 min. The percentage of cooking loss was determined by the difference in steak weights taken before and after cooking.

Water holding capacity (WHC) of the sirloin was measured according to the procedure outlined by Hofmann and White [14]. Briefly, a 0.3 g sample of muscle was placed in a filter-press device and compressed for 5 min. After this process, WHC was calculated from duplicate samples as the ratio of the pressed-meat area to the total area using an area-line meter (Super

PLANIX-a, Tamaya Technics Inc., Tokyo, Japan).

Meat surface color of the sirloin was measured using a Chorma meter (Colorimeter CR-300, Minolta Co., Osaka, Japan) immediately after removing the meat from the polyethylene bag. The color values of L\* (lightness), a\* (redness), and b\* (yellowness) were repeatedly measured in the same manner. The standard white plate had a Y value = 93.60, an x value = 0.3134, and a y value = 0.3194.

Myoglobin content of the sirloin was analyzed according to Krzywicki's method [15] for the relative concentrations (%) of DeoxyMb, OxyMb, and MetMb. Five grams of the sample and 25 mL of 40 mm potassium phosphate buffer (pH 6.8, 4 °C) were placed in a 50 mL centrifuge tube, homogenized at 5,000  $\times$  g and 4 °C for 10 s, and then left at 4 °C for 1 h. After centrifugation  $(1,250 \times g, 4 \, ^{\circ}\text{C}, 30 \, \text{min}), 200 \, \mu\text{L} \text{ of the supernatant}$ was dispensed into a 96-well plate, and absorbance was measured at 525 nm, 545 nm, 565 nm, and 572 nm using a spectrophotometer (Spectronics 21D, B&L, NY, USA). Finally, the concentrations of DeoxyMb and OxyMb were calculated using Krzywicki's equation [15], and the concentration of MetMb was calculated as follows: 100 - [DeoxyMb (%) + OxyMb (%)].

The amino acid content of the sirloin was analyzed using HPLC (Waters<sup>®</sup> 510 Pump; Waters<sup>®</sup> Automated Gradient Controller; Waters<sup>®</sup> 486 Tunable Absorbance Detector; Waters<sup>®</sup> Temperature Control Module, California, USA) by pre-treating samples according to the method of Mason et al. [16].

The fatty acid composition of the sirloin was analysed according to Folch's method [17]. After weighing 0.5 g of a sample in a 30 mL tube, 20 mL of a chloroform-methanol (2:1) solution, and 5 mL of a 0.88% NaCl solution were added. The mixture was then shaken for 5 min and incubated at 4 °C for 36 h. After 36 h, the lower layer was transferred to a 25 mL tube after centrifugation (1,250  $\times$  g, 4 °C, 30 min) and the

organic solvent was blown out with nitrogen gas. Subsequently, 0.5 N methanolic NaOH was added to 1 mL of the lower layer, heated for 15 min, and cooled. After this, 2 mL of 14% BF<sub>3</sub>-methanol was added before the mixture was heated for 15 min, cooled, and 1 mL of heptane and 2 mL of saturated NaCl solution were added. The tube was then shaken and then left at room temperature for more than 40 min. Thereafter, the supernatant was taken with a fine pipette, transferred to a vial, and the fatty acid content was analysed using a gas chromatography (Shimadzu-17A, Shimadzu, Kyoto, Japan).

# 2.4 Statistical analyses

All experimental data like as growth performacne, blood metabolites, carcass and meat composition were analysed according to the independent variable (feeding type) using the GLM procedure in the SAS package 9.1 software program (SAS Institute Inc., Carey, NC, USA). Significant differences among the treatment groups were analysed by Tukey's test at the 95% significance level.

#### 3. Results and Discussion

Table 3 shows the effect of the different feeding types on the growth performance of Holstein steers.

The ADG, DMI, and TDN intakes were lowest in the T3 group compared to the T1 and T2 groups, but there were no statistically significant differences among them. The FCR was lower in T1 and T2 than in T3, but this difference was not statistically significant.

TMR (TMF) is known to yield positive effects compared to concentrate feed [3,4] because of the effects of maintaining pH homeostasis [18], reducing metabolic diseases [19], and improving DMI [20]. However, this study revealed a tendency for the ADG to decrease due to TMF

Table 3. Effects of the different feeding types on growth performance of Holstein steers.

$T1^1$	$T2^2$	$T3^3$	SEM	Pr⟩F
431.1	430.2	430.2	11.95	1.00
716.2	704.8	674.2	16.90	0.61
1.31	1.26	1.12	0.04	0.15
15.85	15.79	14.55	0.34	-
13.67	4.37	-	1.85	-
-	11.41	14.55	0.63	-
2.18	-	-	0.01	-
12.37	12.98	11.89	0.49	0.56
12.21	12.71	13.21	0.41	0.64
	431.1 716.2 1.31 15.85 13.67 - 2.18 12.37	431.1 430.2 716.2 704.8 1.31 1.26 15.85 15.79 13.67 4.37 - 11.41 2.18 - 12.37 12.98	431.1 430.2 430.2 716.2 704.8 674.2 1.31 1.26 1.12 15.85 15.79 14.55 13.67 4.37 - - 11.41 14.55 2.18 12.37 12.98 11.89	15.85 15.79 14.55 0.34 13.67 4.37 - 1.85 - 11.41 14.55 0.63 2.18 0.01 12.37 12.98 11.89 0.49

 <sup>1.2.3</sup>In this and all other tables, T1: concentrate feed + ryegrass straw; T2: concentrate feed (top dressing) + TMF, T3: TMF.
 4ADG: average daily gain; 5DM: dry matter; 6TMF: total mixed fermentation; 7FCR: feed conversion ratio.

feeding. This is considered to be the cause of the lack of energy for weight gain because of the decrease in DMI. It has been reported that the feed energy level in the late fattening period affects the ADG and the higher the TDN level, the higher the ADG [21-23]. The results of this study were supported by Rodrigo et al. [24] who stated that the higher the amount of fiber supplied through the feed, the lower the feed intake. That is due to the chemical composition

of TMF, which has a higher ratio of fiber (from roughage and by-products) compared to the concentrate feed. Therefore, it is considered that the decrease in feed intake and digestibility leads to insufficient energy supply, thereby affecting the ADG and FCR of steers. However, in the results of this study, the ADG between the T1 (fed concentrate feed alone) and T2 (fed TMF and concentrate feed) groups was similar. In light of this, and also considering the ADG and feed cost, it is considered that the TMF or concentrate feed fed by mixing at a certain ratio rather than alone, can have a positive effect on the growth performance of Holstein steers.

Table 4 shows the effect of the different feeding types on the plasma metabolites of Holstein steers. Regardless of the treatment groups, plasma GLU concentration tended to decrease during the  $2^{\rm nd}$  period compared to the  $1^{\rm st}$  period, and NEFA concentration tended to increase; however, neither of these changes were statistically significant. The difference in plasma BUN concentrations among treatment groups during the  $1^{\rm st}$  period was small, but in the  $2^{\rm nd}$  period the concentration had decreased in T3 group when compared to T1 and T2 groups (p < 0.05).

Table 4. Effects of the different feeding types on blood metabolites concentrations of Holstein steers

Item	1 <sup>st</sup> period (0 day)					2 <sup>nd</sup> p	period (217	days)		
item	T1	T2	Т3	SEM	Pr⟩F	T1	T2	Т3	SEM	Pr⟩F
GLU <sup>1</sup> (mg/dL)	67.40	65.20	65.00	6.05	0.43	63.80	59.00	62.60	4.24	0.13
NEFA <sup>2</sup> (uEq/L)	186.00	192.00	181.20	10.28	0.29	221.40	229.00	269.75	34.23	0.15
BUN <sup>3</sup> (mg/dL)	13.04	15.62	13.56	0.68	0.14	$14.74^{a}$	$14.24^{a}$	$10.80^{b}$	0.70	0.03
ALB <sup>4</sup> (g/dL)	3.62	3.04	3.10	0.18	0.10	3.76	3.80	3.74	0.03	0.78
$TP^5$ (g/dL)	6.22	6.20	6.58	0.22	0.42	7.16	7.08	7.12	0.08	0.92
CHO <sup>6</sup> (mg/dL)	197.40	209.20	189.00	13.73	0.30	175.80	146.00	130.60	5.82	0.23
$TG^7$ (mg/dL)	21.40	25.60	24.40	2.69	0.43	26.80	22.80	21.40	1.62	0.54
AST <sup>8</sup> (U/L)	71.36	75.6	68.82	2.35	0.52	102.86	108.61	99.43	3.89	0.65
ALT <sup>9</sup> (U/L)	21.25	19.85	22.89	1.03	0.53	34.82	37.25	36.43	1.24	0.75
GGT <sup>10</sup> (U/L)	26.20	29.22	24.62	1.8	0.6	32.2	37.2	30.58	2.12	0.39
Ca <sup>11</sup> (mg/dL)	8.64	8.40	8.98	0.38	0.15	8.96	8.82	8.84	0.06	0.59
$P^{12}$ (mg/dL)	7.08	7.84	7.86	0.36	0.37	7.40	7.84	8.00	0.19	0.45
Mg <sup>13</sup> (mg/dL)	2.30	2.16	2.10	0.09	0.68	2.24	2.16	2.34	0.03	0.08

<sup>1</sup>GLU: glucose: <sup>2</sup>NEFA: non-esterified fatty acid: <sup>3</sup>BUN: blood urea nitrogen: <sup>4</sup>ALB: albumin: <sup>5</sup>TP: total protein: <sup>6</sup>CHO: cholesterol: <sup>7</sup>TG: triglycerides: <sup>8</sup>AST: aspartate-amino-transferase: <sup>9</sup>ALT: alanine-amino-transaminase: <sup>10</sup>GGT: γ-glutamyl-transferase: <sup>11</sup>Ca: calcium; <sup>12</sup>P: phosphorus: <sup>13</sup>Mg: magnesium.

Furthermore, plasma ALB and TP concentrations tended to increase in all treatment groups during the 2<sup>nd</sup> period compared to the 1<sup>st</sup> period, but there was no statistically significant difference among the treatment groups. The concentration of CHO and TG in the plasma tended to increase as the intake of concentrate feed increased, but, again, no statistically significant difference was observed. The concentrations of AST, ALT, and GGT in the plasma tended to increase during the 2<sup>nd</sup> period when compared to the 1<sup>st</sup> period, suggesting that the effect of the feeding type was small. Furthermore, it was found that the CA, P, and MG concentrations in the plasma remained relatively constant throughout the experimental period.

It is known that the plasma metabolites of cattle are affected by various factors such as age, breed, season, disease, feed, and stress [25,26]. Plasma GLU and NEFA are known to have an inverse relationship [27]; therefore, as energy intake increases, GLU concentration increases while NEFA concentration decreases. In addition, it has been reported that plasma CHO and TG also increase with fat content, energy level, and feed intake [28]. In this study, plasma NEFA concentration in T3 group (fed TMR alone) showed a tendency to increase, while CHO and TG concentrations tended to be higher in T1 group (fed concentrate feed alone). The present results suggest a trend similar to the results of the previous studies [27,28]. In addition, the increase in NEFA concentration regardless of the feeding type is considered to be due to the increase in maintenance energy demand [29].

Conversely, plasma BUN concentration mainly increases with higher protein intake [29,30]; therefore it is affected by the protein content and feed intake. In this study, the reason for the decrease in plasma BUN concentration in steers that were fed TMF alone, was thought to be because the protein content of TMF was lower than that of the concentrate feed. From the

results of this study, for the most ideal nutrient balance and metabolic function associated with fattening Holstein steer, it is considered that concentrate feed alone or concentrate feed mixed with TMF is desirable rather than TMF alone.

Table 5. Effects of the different feeding types on carcass characteristics of Holstein steers

Item	T1	T2	T3	SEM	Pr⟩F
Yield traits <sup>1</sup>					
Carcass weight (kg)	378.20	410.40	370.80	9.67	0.22
Rib eye area (cm²)	68.80	73.40	70.40	1.73	0.58
Back fat thickness (mm)	6.00	5.20	4.00	0.57	0.18
Yield index	65.55	63.75	65.18	0.54	0.38
Yield grade (A:B:C, %)	0:100:0	0:80:20	20:80:0	-	-
Quality traits <sup>2</sup>					
Marbling score	1.80	2.20	1.60	0.26	0.66
Meat color	4.60	4.40	4.60	0.13	0.80
Fat color	2.00	2.00	2.00	-	-
Maturity	4.60	4.40	4.60	0.13	0.80
Quality grade (1:2:3, %)	20:20:6	0:80:20	0:40:60	-	-

Area was measured from sirloin taken at 13<sup>th</sup> rib and back fat thickness was also measured at 13<sup>th</sup> rib: Yield index was calculated using the following equation: 68.184 - (0.625 × back fat thickness (mm)) + (0.130 × rib eye area (cm²)) - (0.024 × dressed weight amount (kg)): Carcass yield grades from C (low yield) to A (high yield).

<sup>2</sup>Grading ranges are 1 to 9 for marbling score with higher numbers for better quality (1 = devoid, 9 = abundant); meat color (1 = bright red, 7 = dark red); fat color (1 = creamy white, 7 = yellowish); texture (1 = soft, 3 = firm); quality grades from 3 (low quality) to 1<sup>++</sup> (very high quality).

Table 5 shows the effect of the different feeding types on the carcass characteristics of Holstein steers. Carcass weight was higher in the T2 group than in the T1 and T3 groups, and back fat thickness was the thickest in the T1 group. these differences were Nevertheless, statistically significant. The rib eye area tended to be greater in the T2 group than in the T1 and T3 groups, but no statistically significant difference was observed. Regarding the marbling score, the order of highest to lowest was as follows: T2 group, then T3 group, and lastly the T1 group. Meanwhile, there was no difference among treatment groups with regard to meat

color, fat color, and texture. The appearance rate of meat quality grade, which ranked grade 2 or higher, was higher in the T2 group than in the T1 and T3 groups.

In beef cattle, an increase in the amount of energy supplied through feed helps to improve intramuscular fat (marbling), but may cause an increase in the back fat thickness or a decrease in the rib eye area [31]. In this study, it is thought that while the back fat thickness was thicker, the rib eye area was smaller due to the feeding of concentrate feed with higher energy compared to TMF. Nevertheless, feeding TMF alone can help to reduce back fat thickness, but because intramuscular fat (marbling) deposition may also be reduced, it is considered desirable to supply additional concentrate feed. In previous studies on Hanwoo steers, carcass weight, rib eye area, and marbling score increased when TMF was supplemented [3,4,32], which differs from the results of this study. Meanwhile, an increase in energy derived from starch and non-fibrous carbohydrates, has been reported to help improve intramuscular fat [33]. Therefore, as shown in the results of this study, it is believed that the system of feeding concentrate feed mixed with TMF rather than TMF alone can have a positive effect on meat quality improvement without an excessive increase in back fat thickness.

Table 6 shows the effect of the feeding types on the physicochemical characteristics of the sirloin of Holstein steers. The moisture content of the sirloin was lower in the T2 group than in the T1 and T3 groups, while the crude protein content was higher in the T2 group than in the T1 and T3 groups. Nevertheless, these differences were not statistically significant. Ether extract content was higher in the T2 group than in the T1 and T3 groups, but no statistically significant difference was observed. Additionally, the feeding type had little effect on the pH, WHC, and heating loss of the sirloin.

Table 6. Effects of the different feeding types on physicochemical characteristics in the sirloin of Holstein steers

Item	T1	T2	Т3	SEM	Pr⟩F
Dry matter (%)	72.31	67.94	70.48	1.08	0.26
Crude protein (%)	6.25	10.17	8.56	0.83	0.16
Ether extract (%)	18.10	20.41	16.90	0.87	0.26
Crude ash (%)	1.06	0.98	1.04	0.06	0.85
рН	5.61	5.58	5.58	0.02	0.81
Water holding capacity (%)	58.93	58.33	58.96	0.48	0.85
Cooking loss (%)	30.63	30.36	28.79	0.41	0.15

In general, it has been reported that moisture and crude protein content of carcasses decrease, while the content of ether extract increases proportionally to increases in the marbling score and meat quality grade [34-36]. In addition, when the meat quality grade is high, the WHC increases [37], and it has also been reported that WHC and cooking loss have a proportional relationship [38]. In the results of this study, the group fed concentrate feed mixed with TMF, which had excellent meat quality grades, showed lower moisture and crude protein content, but higher crude fat content compared to the concentrate feed group or TMF group. This illustrates a trend similar to that of the previous study. However, because the difference in meat quality grade among treatment groups was not large, the effect on WHC and cooking loss was considered to be small. Therefore, from the results of this study, it is thought that in Holstein steers, the physicochemical characteristics are affected by the meat quality grade (marbling score) of the carcasses rather than the feeding

Table 7 shows the effect of the different feeding types on the meat color of the sirloin in Holstein steers. The brightness and redness of the sirloin were higher in the T2 group than in the T1 and T3 groups, but there were no statistically significant differences among them. Meanwhile, the yellowness was similar among

treatment groups. Furthermore, there was little effect of the feeding type on the ratio of deoxymyoglobin, oxymyoglobin, and metmyoglobin in the sirloin.

Table 7. Effects of the different feeding types on surface colors and myoglobin forms in the sirloin of Holstein steers

Item	T1	T2	Т3	SEM	Pr⟩F
L* (Lightness)	34.71	35.17	33.81	0.44	0.48
a* (Redness)	18.38	19.11	18.11	0.27	0.32
b* (Yellowness)	9.69	9.90	9.46	0.15	0.51
Deoxymyoglobin (%)	16.14	17.80	18.23	0.78	0.55
Oxymyoglobin (%)	63.61	62.62	63.54	1.16	0.94
Metmyoglobin (%)	20.25	19.58	18.24	1.24	0.82

The change in meat color is affected by optical characteristics such as connective tissue mass, muscle mass, and reflected light [39]. In particular, the brightness [40] and redness [41] of the sirloin increases in proportion to the intramuscular fat (marbling) level. In the results of this study, the difference among the treatment groups regarding marbling score was not large; however, the lightness and redness tended to be higher in the T2 group (fed concentrate feed mixed with TMF), which had the highest marbling score. This occurrence is considered similar to the results of the previous study [40,41]. Notably, the myoglobin of the sirloin is finally oxidized to metmyoglobin through an oxidation step [42]. In the result of this study, the difference among treatment groups in the ratio of the myoglobin form was small; thus, it is thought that the effect of the feeding type on meat color and myoglobin concentration in the sirloin of Holstein steers was small.

Table 8 shows the effect of the different feeding types on the amino acid composition of the sirloin of Holstein steers. There was no effect on the proportions of individual amino

Table 8. Effects of the different feeding types on amino acid composition in the sirloin of Holstein steers

Item	T1	T2	Т3	SEM	Pr⟩F
Aspartic acid (%)	9.64	9.80	9.86	0.15	0.86
Threonine (%)	3.66	3.01	2.90	0.18	0.20
Serine (%)	6.40	6.33	6.23	0.13	0.87
Glutamic acid (%)	4.63	4.85	4.94	0.07	0.24
Proline (%)	6.24	4.97	6.67	0.45	0.29
Glycine (%)	5.09	5.37	5.09	0.10	0.43
Alanine (%)	4.10	4.94	2.85	0.54	0.30
Valine (%)	3.98	3.97	3.91	0.20	0.99
Isoleucine (%)	4.09	3.02	3.19	0.33	0.39
Leucine (%)	3.55	3.47	3.58	0.07	0.85
Phenylalanine (%)	3.92	4.17	4.41	0.12	0.28
Tyrosine (%)	10.86	10.73	11.19	0.90	0.98
Histidine (%)	4.21	4.26	4.06	0.10	0.74
Lysine (%)	7.44	8.09	8.36	0.72	0.88
Arginine (%)	13.26	13.65	13.90	0.28	0.67
Methionine (%)	3.59	3.84	3.12	0.50	0.93
Cysteine (%)	5.11	6.11	5.73	1.26	0.95

acids, essential amino acids, and non-essential amino acids in the sirloin of Holstein steers. Kim et al. [43] reported that the amino acid composition of carcasses can vary depending on the amino acid content and the type of feed. In addition to this, Kang et al. [44] reported that the amino acid composition may change according to the difference in meat quality grade. Specifically, the higher the meat quality grade, the higher the methionine content of the sirloin. The results of this study show a tendency to increase the meat quality grade and methionine ratio by feeding concentrate feed, which is thought to be similar to the results of a previous study. Although there was no statistical significance in the results of this study, we found that the ratio of aspartic acid and glutamic acid, related to umami taste, tended to increase due to the feeding of TMF. This finding merits further research in this area.

Table 9. Effects of the different feeding types on fatty acids composition in the sirloin of Holstein steers

Item	T1	T2	Т3	SEM	Pr⟩F
C14:0 (Myristic acid, %)	3.31	3.52	3.40	0.13	0.87
C16:0 (Palmitic acid, %)	27.98	28.26	27.95	0.39	0.93
C16:1n7 (Palmitoleic acid, %)	5.67	5.48	5.95	0.20	0.69
C18:0 (Stearic acid)	10.40	10.85	10.58	0.28	0.83
C18:1n9 (Oleic acid, %)	49.87	49.54	49.29	0.47	0.90
C18:2n6 (Linoleic acid, %)	2.86	2.52	3.49	0.21	0.13
C20:1n9 (cis-11-Eicosenoic, %)	2.28 <sup>a</sup>	1.93 <sup>ab</sup>	1.69 <sup>b</sup>	0.09	0.02
C18:3n6 (y-linoleic acid, %)	0.08	0.10	0.10	0.00	0.12
C18:3n3 (Linolenic, %)	0.11	0.10	0.10	0.00	0.12
C20:4n6 (Arachidonic, %)	0.41	0.30	0.20	0.15	0.75
C20:5n3 (Eicosapentaenoic acid, %)	0.07	0.06	0.06	0.00	0.37
C22:4n6 (Docosatetraenoic Acid, %)	0.09	0.08	0.06	0.01	0.10
C22:6n3 (Docosahexaenoic acid, %)	0.05	0.04	0.04	0.00	0.37
SFA <sup>1</sup>	41.70	42.63	41.93	0.54	0.75
MUFA <sup>2</sup>	55.54	55.02	55.24	0.47	0.68
PUFA <sup>3</sup>	3.09	2.60	2.25	0.13	0.07

<sup>1</sup>SFA: saturated fatty acid; <sup>2</sup>MUFA: mono-unsaturated fatty acid; <sup>3</sup>PUFA: poly-unsaturated fatty acid.

Table 9 shows the effect of the different feeding type on the fatty acid composition of the sirloin of Holstein steers. The composition of palmitic acid, which accounts for the largest proportion of saturated fatty acids in the sirloin, tended to be lower in the T2 group than in the T1 and T3 groups, but the difference was not significant. The ratio of oleic acid tended to increase as the concentrate feed amount increased, but this difference was not statistically significant. The ratio of eicosenoic acid was found to be higher in the T1 group than in the T2 and T3 groups (p  $\langle$  0.05). The saturated fatty acid ratio was higher in the T2 group than in the

T1 and T3 groups, and unsaturated fatty acid ratio showed the highest values in the T1 group, but these differences were not statistically significant. Cho et al. [45] reported that the fatty acid composition of beef may vary depending on the type of feed. They noted that it was also affected by various factors such as breed, feed, and storage method. However, in the results of this study, the effect of the feeding types on the fatty acid composition of the sirloin was small, showing a different from a previous study.

The results obtained in this study revealed that only TMF appeared to have no positive effects on growth performance, carcass characteristics, and meat composition in Holstein steers. In contrast, a sequential feeding method including TMF and concentrate feed was found to have positive effects on TDN intake, carcass weight, and marbling score in Holstein steers.

## 4. Conclusion

Therefore, the sequential feeding method comprising TMF and concentrate feed can improve energy intake and carcass quality, without any negative effect on meat composition traits. Furthermore, a study with a larger number of animals and a longer trial period would be needed to more reliably determine the effect of feeding type on the productivity of Holstein steers.

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