Kimchi cabbage (*Brassica rapa* L.) by-products treated with calcium oxide and alkaline hydrogen peroxide as feed ingredient for Holstein steers

A-Rang Son[1#], Seon-Ho Kim[1#], Raniel A. Valencia[1,2,3], Chang-Dae Jeong[1], Mahfuzul Islam[1], Chul-Ju Yang[2,4] and Sang-Suk Lee[1]*

[1]*Ruminant Nutrition and Anaerobe Laboratory, Department of Animal Science and Technology, Sunchon National University, Suncheon 57922, Korea*
[2]*Interdisciplinary Program in IT-Bio Convergence System (BK 21 plus), Sunchon National University, Suncheon 57922, Korea*
[3]*Department of Animal Science, College of Agriculture, Central Luzon State University, Science City of Muñoz 3120, Philippines*
[4]*Animal Nutrition and Feed Science Laboratory, Department of Animal Science and Technology, Sunchon National University, Suncheon 57922, Korea*
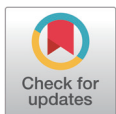
*Corresponding author
Sang-Suk Lee
Ruminant Nutrition and Anaerobe Laboratory, Department of Animal Science and Technology, Sunchon National University, Suncheon 57922, Korea.
Tel: +82-61-750-3237
E-mail: rumen@scnu.ac.kr

ORCID
A-Rang Son
https://orcid.org/0000-0002-3896-0950

Abstract

This study aimed to investigate the effects of Kimchi cabbage by-products either treated or untreated with calcium oxide (CaO) and alkaline hydrogen peroxide (AHP) as substitutional ingredient of total mixed ration (TMR) on *in vitro* fermentation, *in situ* disappearance and growth performance of Holstein steers. Cannulated Holstein (600 ± 47 kg) was used for both the *in vitro* and *in situ* experiments. The treatments used were TMR only (CON), TMR + 30% Kimchi cabbage by-products fresh matter (FM) basis (TC), TMR + 30% Kimchi cabbage by-products FM basis + 5% CaO FM basis (TCC), and TMR + 30% Kimchi cabbage by-products FM basis + 5% CaO FM basis + 3.22% AHP FM basis (TCCA). For *in vivo* experiment, thirty-four Holstein steers (273 ± 45 kg) were subjected to a 150-day feeding trial, divided into two groups: CON and TC. In the *in vitro* experiment, pH of TCCA was greatest (*p* < 0.05) among other treatments at all incubation times. Ammonia nitrogen and volatile fatty acid concentrations were not significantly different for each treatment. However, butyrate was greater (*p* < 0.05) in TCC and CON than in both TC and TCCA. During *in situ* experiment, the dry matter (DM) disappearance was greatest (*p* < 0.05) in TCCA among other treatments. Also, disappearance of neutral detergent fiber (NDF) and acid detergent fiber (ADF) were observed greatest (*p* > 0.05) in TCCA treatment. In the in vivo experiment, average daily gain (ADG) did not differ between CON and TC. In blood profile analysis, alanine aminotransferase, aspartate aminotransferase, glucose, total cholesterol, high-density lipoprotein cholesterol, low-density lipoprotein cholesterol, and total protein concentration were not significantly different between treatments. But, creatinine concentration was greater (*p* < 0.05) in TC than

Seon-Ho Kim
https://orcid.org/0000-0002-9350-1853
Raniel A. Valencia
https://orcid.org/0000-0002-3577-9187
Chang-Dae Jeong
https://orcid.org/0000-0001-6567-4397
Mahfuzul Islam
https://orcid.org/0000-0001-6010-140X
Chul-Ju Yang
https://orcid.org/0000-0003-3510-3096
Sang-Suk Lee
https://orcid.org/0000-0003-1540-7041

in CON. Overall results suggest that Kimchi cabbage by-products either treated or untreated with CaO and AHP can be used as substitutional ingredient in TMR for Holstein steers.

Keywords: Kimchi cabbage by-products, Calcium oxide, Alkaline hydrogen peroxide, Total mixed ration

INTRODUCTION

The demand for livestock products has increased over the years; thus, a huge quantity of feed resources for livestock production is needed [1], leading to feedstuff scarcity and higher feed costs [2,3]. The use of alternative feed resources [1] and sustainable feed formulations, defined as nutritional and economic feed optimization [4], could play an important role in overcoming this challenge. Accordingly, the use of the total mixed ration (TMR) in feeding systems, which can reduce feed costs by utilizing cheap feedstuff from agricultural by-products, is an alternative to the existing production system [5] and also reduce environmental pollution [6]. By-products are also beneficial in alleviating fermentation in the rumen while providing high energy levels to the rations by means of their special chemical properties that are not acquired in ordinary feed grains or forages [1,6].

The most common by-products in agriculture, specifically in Korea, are the by-products of the Kimchi cabbage (Chinese cabbage or Korean traditional *Baechu*) which is regarded as an important vegetable for making kimchi. From 2011 to 2017, the total annual production of both Chinese cabbage and cabbage in the country was approximately two and a half million tons, which was estimated to be the fourth-largest producer of cabbage in the world [7]. However, up to 30% of the total production is discarded as waste during the processes of production, postharvest storage, transportation, and the further processing of the Chinese cabbage [7,8]. The disposal rate is also very high in the wholesale market, where the cabbages are brought before their distribution to retailers or intermediate merchants [7]. Thus, several studies have been conducted that aimed at determining the efficient utilization of Chinese cabbage and its by-products as feed ingredients for ruminant diets [7,9]. However, the Chinese cabbage by-products has a high water content of approximately over 90%, which reduces its usability as a livestock feed as it can readily be decomposed by putrefying microorganisms [7]. In addition, most agricultural by-products show poor digestibility due to the cell wall structure that protects nutrients from microbial digestion in the rumen [10,11]. Research throughout the years has revealed that disappearance can be improved by the addition of feed additives such as sodium hydroxide (NaOH), which induces the breakage of the bonds between lignin and hemicellulose [10,11]. Nonetheless, it is necessary to find alternatives due to the high cost, handling hazards, and environmental pollution associated with NaOH [12]. Calcium hydroxide (CaO) and alkaline hydrogen peroxide (AHP) as alkaline agent feed additives, can be used as substitutes for NaOH, because they can increase the digestibility and feed intake of ruminants by altering the structure of lignin removal from the lignocellulose [13–15]. Additionally, these alkali agents have been found to initiate the distension of the cellulose fibers that could upsurge the internal surface area, allowing the cellulases to contact the substrate, resulting in greater hydrolysis in the amorphous region and consequently increasing the proportion of crystalline cellulose [13,16]. These effects enable rumen microbes to attack the structural carbohydrates more easily; hence, greater disappearance and intake could be achieved [13]. Therefore, this study was conducted to investigate the effects of Kimchi cabbage by-products either treated or untreated with CaO and AHP as substitutional ingredient of TMR on *in vitro* fermentation, *in situ* disappearance and growth performance of Holstein steers.

MATERIALS AND METHODS

All experimental procedures were performed in accordance with the Animal Experimental Guidelines provided by the Sunchon National University Institutional Animal Care and Use Committee (SCNU-IACUC), Korea. The experimental protocol was approved by the SCNU-IACUC (Approval number: SCNU IACUC-2018-01).

Preparation of experimental diet and chemical analysis

The TMR was formulated based on the guideline of National Research Council (NRC) [17] for beef cattle and was prepared by mixing timothy hay, alfalfa hay, lupine seed, corn, corn gluten feed, whole cottonseed, tall fescue, molasses, salt, vitamin-mineral mix, protected fat, and limestone (Table 1). By-products of Kimchi cabbage were collected in Suncheon NongHyup Namdo Kimchi (Suncheon, Korea). Treatments used for the *in vitro* and *in situ* experiments include: TMR only (CON), TMR + 30% Kimchi cabbage by-products (TC), TMR + 30% Kimchi cabbage by-products + 5% CaO (TCC), and TMR + 30% Kimchi cabbage by-products + 5% CaO + 3.22% AHP (TCCA), fresh matter basis. The inclusion rate of alkali agents were based on Nuñez et al. [18]. For the *in vivo* experiments, CON and TC were used as treatments. The Kimchi cabbage by-products (4.68% of dry matter [DM] basis) were treated with powdered CaO (1% of DM basis) and/or AHP (0.64% of DM basis) by manual mixing in a plastic container for approximately 1 h. The treated Kimchi cabbage by-products were subsequently mixed with the TMR. The ingredients and chemical compositions of experimental TMRs were presented in Table 1. The chemical compositions evaluated were crude protein (CP), crude fiber (CF), ether extract (EE), calcium, phosphorus, neutral detergent fiber (NDF), and total digestible nutrients (TDN). Proximate analysis was performed according to the Association of Official Analytical Chemists [19] method. CF were analyzed using the method of Van Soest et al. [20].

In vitro fermentation and *in situ* disappearance

Cannulated Holstein with body weight of 600 ± 47 kg, housed at animal facility in Suncheon National University located in Suncheon-si, Jeollanam-do, Korea, was used for the *in vitro* and *in situ* experiments. Rumen fluid was collected before morning feeding and was obtained by squeezing the ruminal contents of the animal through the four layers of a surgical gauze and collected in a stainless-steel vacuum bottle. After collection, the filtrates were subsequently sealed, stored at 39℃, and directly transported to the laboratory. The buffer used in this study consisted of (per L) 0.45 g dipotassium phosphate (K_2HPO_4), 0.45 g potassium dihydrogen phosphate (KH_2PO_4), 0.9 g ammonium sulfate ($[NH_4]2SO_4$), 0.12 g calcium chloride dihydrate ($CaCl_2·2H_2O$), 0.9 g sodium chloride (NaCl), 0.19 g magnesium sulfate heptahydrate ($MgSO_4·7H_2O$), 1.0 g trypticase peptone, 1.0 g yeast extract, and 0.6 g L-cysteine hydrochloride [21]. The prepared buffer was autoclaved for 15 min at 121℃ and maintained in a 39℃ water bath, adjusted to pH 6.9 using 10 N NaOH, and dispensed anaerobically by continuous bubbling of CO_2 gas. Meanwhile, the collected rumen fluid was strained again using a 4-layer surgical gauze placed in a funnel and the filtrate was allowed to flow into a 1-L graduated cylinder, with continuous bubbling of the CO_2 gas. The filtrated rumen fluid was then mixed with the prepared buffer medium at a ratio of 1:3 and allowed to bubble for 30 min. The pH was adjusted again to 6.9, before filling the serum bottle with the buffered rumen fluid. The serum bottle (160 mL capacity) was prepared by adding 1 g of weighed substrate of each treatment with three replications. One hundred milliliter of buffered rumen fluid was filled into individual serum bottles containing the substrate, under anaerobic conditions [22]. The bottles were subsequently sealed with rubber septum stoppers and aluminum caps and incubated at 39℃ for 6,

Table 1. Ingredients and chemical compositions of experimental TMRs (DM basis, %)

	Treatments[1]			
	CON	TC	TCC	TCCA
Ingredients				
Timothy hay	20.12	10.21	4.74	4.18
Alfalfa hay	4.69	6.08	11.28	11.28
Lupine seed	6.23	6.19	6.17	6.17
Corn	40.42	40.21	40.07	39.99
Corn gluten feed	16.41	16.33	16.27	16.27
Whole cottonseed	3.02	3.00	2.99	2.99
Tall fescue	2.85	7.08	7.06	7.06
Molasses	5.04	5.01	5.00	5.00
Salt	0.31	0.31	0.31	0.31
Vitamin-mineral mix[2]	0.28	0.28	0.28	0.28
Protected fat	0.00	0.00	0.15	0.15
Limestone	0.63	0.62	0.00	0.00
Kimchi cabbage byproducts	0.00	4.68	4.68	4.68
CaO	0.00	0.00	1.00	1.00
AHP	0.00	0.00	0.00	0.64
Total	100.00	100.00	100.00	100.00
Chemical compositions				
DM (% of as fed basis)	65.65	66.26	68.80	69.97
Crude protein	14.78	14.83	14.35	14.24
Crude fiber	13.18	12.94	12.71	12.83
Ether extract	4.30	4.20	4.38	4.32
Calcium	0.50	0.60	1.21	1.23
Phosphorus	0.30	0.56	0.56	0.56
NDF	28.30	27.60	26.40	26.40
Total digestible nutrients	82.90	82.50	82.71	82.70

[1]TMR only (CON); TMR + 30% Kimchi cabbage by-products (TC); TMR + 30% Kimchi cabbage by-products + 5% CaO (TCC); TMR + 30% Kimchi cabbage by-products + 5% CaO + 3.22% AHP (TCCA), fresh matter basis.

[2]Vitamin-mineral mix contained vit. A 2,650,000 IU, vit. D3 530,000 IU, vit. E 1,050 IU, niacin 10,000 mg, Mn 4,400 mg, Zn 4,400 mg, Fe 13,200 mg, Cu 2,200 mg, iodine 440 mg, and Co, 440 mg/kg of Grobic-DC provided from Bayer Health Care (Leverkusen, Germany).

TMR, total mixed ration; DM, dry matter; CaO, calcium oxide; AHP, alkaline hydrogen peroxide; NDF, neutral detergent fiber.

12, and 24 h with shaking (100 rpm) [23].

Moreover, samples from each treatment were dried in an oven for 48 h at 80℃ and ground to pass through a 1 mm screen [24]. The samples weighing 2 g (DM basis) were placed in nylon bags (5 × 10 cm; 45 μm pore size), and the bag openings were tied with nylon strings. Then, the bags were submerged in hot water (39℃–40℃) for approximately 30 min, and then deep fermentation in the rumen fistula of the cannulated animal immediately after morning feeding. The incubation times were 0, 3, 6, 9, 12, and 24 h, with six replications per treatment. After each incubation, the nylon bag was removed from the rumen cannula, immersed in clear water, and then washed without heating using a crude fiber analyzer (Ankom 220, Ankom Technology, Macedon, NY, USA). Samples for each incubation time were dried at 80℃ for 48 h in a dry oven immediately after washing.

Analysis of *in vitro* rumen fermentation and *in situ* disappearance

A press and sensor machine (EA-6, Laurel Electronics, Costa Mesa, CA, USA) was used to measure the total gas (TG). The pH was determined using the Schott ® Instrumets Lab 860 (SI Analytics GmbH, Mainz, Germany) after opening each serum bottle. Samples of fermentation were also collected in 1.5 mL cryotubes and deep frozen at −80℃. These samples were later thawed at room temperature and then centrifuged at 13,000×g for 15 min at 4℃ using a Micro 17TR centrifuge (Hanil Science Industrial, Incheon, Korea) and the supernatant was used for ammonia nitrogen (NH_3-N) and volatile fatty acids (VFA) analysis [22]. Using the Libra S22 spectrophotometer (Biochrom, Cambridge, UK) at an absorbance of 630 nm, NH_3-N concentration was measured according to the methods developed by Chaney and Marbach [25]. VFA were analyzed using high performance liquid chromatography (Agilent Technologies1200 series, Agilent Technologies, Santa Clara, CA, USA) with a UV detector (210 nm and 220 nm) and a Metacarb87H (Agilent Technologies) column using 0.0085 N H_2SO_4 as a buffer at a flow rate of 0.6 mL/min and temperature column of 35℃. The DM disappearance in the rumen was calculated according to the protocol of Van Emon et al. [26]. For NDF and acid detergent fiber (ADF) analysis, ANKOM 220 Fiber Analyzer (AnkomTechnology) based on the Van Soest et al. [20] method was used.

Growth performance and blood profiling of Holstein steers (*in vivo*)

Thirty-four heads of Holstein steers (273 ± 45 kg) were used as experimental animals in this study for 150 d; animals were housed in the farm located in Gimje-si, Jeollanam-do, Korea. The animals were selected randomly and equally distributed into two groups (CON and TC). The steers were housed in steel-constructed pens (5 m × 10 m) with two or three steers per pen. The animals were fed twice a day (08:00 and 18:00) and water was made available *ad libitum*. The *in vivo* data included the body weight, average daily gain (ADG), and feed conversion ratio (FCR). We offered the same amount of TMR as group feeding (10 kg fresh matter basis/steer/d) for all steers throughout the feeding trial and no orts were observed. The ADG was calculated by subtracting the initial weight to the final weight and divided by the experimental period. The FCR was calculated by dividing the total input of the given feed by the total weight gain. At the end of the feeding trial, blood samples were collected 3 h after morning feeding from the jugular vein in 5 mL BD Vacutainer® SST™ II Advance (BD Vacutainer Systems, Plymouth, UK), left at room temperature for 30 min, and subsequently centrifuged at 1,300×g for 10 min at 4℃ to obtain the serum. Blood parameters such as alanine aminotransferase (ALT), aspartate aminotransferase (AST), glucose, total cholesterol, high density lipoprotein (HDL) cholesterol, low density lipoprotein (LDL) cholesterol, creatinine, blood urea nitrogen (BUN), and total protein were analyzed using an automated blood biochemical analyzer (Hitachi 747, Hitachi, Tokyo, Japan).

Statistical analysis

All the data obtained in this experiment were collected for each incubation period, and statistical analysis was performed using the standard statistical analysis system (SAS) version 9.1 statistical package (SAS Institute, Cary, NC, USA). Using the general linear model (GLM), data were analyzed by analysis of variance (ANOVA). Duncan's multiple range test was used to identify differences between and among control and treatment group. To indicate significance between mean, $p < 0.05$ was considered.

RESULTS AND DISCUSSION

In vitro fermentation and *in situ* disappearance

The effects of different dietary treatments on pH, TG, NH_3-N, and VFA during *in vitro* rumen fermentation are shown in Table 2. The *in vitro* pH was greater ($p < 0.05$) in TCCA and TCC groups at all the incubation times than the two other groups. The reason of high pH might be due to the influence of the alkaline substances such as CaO and AHP, which were added to the Kimchi cabbage by-products. Similarly, Boukila et al. [27] reported that feeding lambs with high dietary $Ca(OH)_2$ appears to delay a drop in ruminal pH. In addition, Schroeder et al. [28] stated that diets containing supplementary CaO had a greater pH than those that did not apply any additive. This is due to the fact that CaO and AHP are alkaline agents [27]. Nuñez et al. [18] stated that alkalizers can neutralize acidity as well as increase the pH. The greater pH of feed entering the gut may have buffered initial ruminal pH in cow supplied with supplementary CaO [28]. The amount of gas generated in all treatment groups increased with prolonged incubation time, and was greater ($p < 0.05$) in the CON, TC, and TCC groups with 220.33 mL, 217.00 mL, and 212.33 mL, respectively,

Table 2. Effects of Kimchi cabbage by-products either treated or untreated with CaO and AHP as substitutional ingredient of TMR on *in vitro* pH, total gas, NH_3-N and VFA concentrations of Holstein steers

Item	Time (h)	Treatments[1]				SEM	*p*-value
		CON	TC	TCC	TCCA		
pH	6	6.41[c]	6.40[c]	6.49[b]	6.53[a]	0.01	< 0.01
	12	6.33[b]	6.32[b]	6.43[a]	6.44[a]	0.01	< 0.01
	24	6.18[c]	6.16[c]	6.28[b]	6.37[a]	0.01	< 0.01
Total gas (mL)	6	92.33[ab]	101.33[a]	80.33[bc]	76.00[c]	3.60	< 0.01
	12	167.67[a]	162.67[a]	150.67[b]	147.00[b]	2.69	< 0.01
	24	220.33[a]	217.00[a]	212.33[a]	197.33[b]	3.33	< 0.05
NH_3-N (mg/dL)	6	15.51	15.89	15.36	13.92	0.68	0.35
	12	17.04	18.29	16.34	16.90	0.97	0.60
	24	17.42	18.42	15.90	16.39	0.48	0.06
Acetate (mM)	6	35.84	36.33	36.98	36.60	0.53	0.57
	12	40.26	40.94	38.87	38.28	0.68	0.10
	24	44.93	45.26	46.12	47.66	1.36	0.64
Propionate (mM)	6	15.22[a]	14.45[a]	13.18[b]	13.04[b]	0.27	< 0.01
	12	15.89	16.34	15.51	15.99	0.22	0.24
	24	18.18	17.39	17.97	18.20	0.33	0.40
Butyrate (mM)	6	8.79[a]	8.28[a]	4.66[b]	4.86[b]	0.26	< 0.01
	12	13.45[bc]	12.35[c]	17.26[a]	15.30[b]	0.58	< 0.01
	24	22.02[a]	18.44[b]	23.54[a]	18.76[b]	0.56	< 0.01
A:P ratio	6	2.21[b]	2.52[ab]	2.81[a]	2.81[a]	0.08	< 0.05
	12	2.53	2.51	2.51	2.39	0.06	0.45
	24	2.47	2.60	2.57	2.62	0.05	0.39
Total VFA (mM)	6	61.01[a]	59.07[a]	54.81[b]	54.50[b]	0.68	< 0.01
	12	69.61	69.63	71.61	69.58	0.91	0.49
	24	85.14	81.10	87.63	84.62	2.01	0.37

[1]TMR only (CON); TMR + 30% Kimchi cabbage by-products (TC); TMR + 30% Kimchi cabbage by-products + 5% CaO (TCC); TMR + 30% Kimchi cabbage by-products + 5% CaO + 3.22% AHP (TCCA), fresh matter basis.

[a-c]Means with different superscripts in the same row differ significantly ($p < 0.05$).

CaO, calcium oxide; AHP, alkaline hydrogen peroxide; TMR, total mixed ration; NH_3-N, ammonia-nitrogen; VFA, volatile fatty acid; A:P ratio, acetate to propionate ratio.

during 24 h incubation, than in TCCA. In the *in vitro* fermentation, the amount of gas generated is used as an important indicator for determining fermentation properties in the rumen along with the pH [29]. This means that decomposition and fermentation of the substrate were effectively carried out in the course of fermentation [30]. Ammonia-nitrogen concentrations were lower in TCC and TCCA at 24 h of incubation with 15.90 mg/dL and 16.39 mg/dL, respectively ($p > 0.05$) than in CON and TC. Chaudhry [31] reported that concentration of NH_3-N in rumen was lower in straw treated with CaO, NaOH, and NaOH + AHP than untreated straw. Accordingly, the decrease in the NH_3-N was presumably a consequence of an increase in the available energy, and rumen microbes utilize ammonia during the enhanced degradation of straw components after chemical treatments [31]. It was reported that the NH_3-N concentration should be 5 mg/dL or more to ensure and maintain maximum microbial protein synthesis [32], while the intake and disappearance of the DM are maximized when the concentration is maintained at 20 mg/dL [33]. Acetate was greater in TCCA (47.66 mM) after 24 h followed by TCC, TC, and CON with 46.12 mM, 45.26 mM, and 44.93 mM, respectively ($p > 0.05$). In contrast, propionate was greater ($p < 0.05$) in CON and TC at 6 h incubation with 15.22 mM and 14.45 mM, respectively, than TCC (13.18 mM) and TCCA (13.04 mM). Schroeder et al. [28] reported that ruminal propionate concentration was decreased while acetate concentration was increased when CaO was added to the diet due to the improvement in forage fiber disappearance. On the other hand, butyrate was greater ($p < 0.05$) in TCC at 12 and 24 h of incubation with 17.26 mM and 23.54 mM, respectively, than the other treatments. Nuñez et al. [18], reported that butyrate increased linearly as CaO inclusion increased, indicating that the efficiency of dietary energy utilization was improved by the treatment. At 24 h of incubation, TCCA had the greatest acetate to propionate ratio (A:P ratio), numerically, compared to the other treatment but there were no significant differences. Nuñez et al. [18] reported that the A:P ratio increased linearly with increasing CaO inclusion due to the increased fiber disappearance. Total VFA concentration was greater ($p < 0.05$) in TC at 6 h than TCC and TCCA, but was not significantly different in CON. At 24 h of incubation, there was no significant difference among the treatments but numerically greater in TCC. This might be due to the improved degradation of fiber with the influenced of alkaline treatment [18].

The effect of different dietary treatments on DM, NDF, and ADF disappearance through *in situ* experiment is shown in Table 3. The DM disappearance gradually increased in all treatments after 3 h of incubation, and was greater ($p < 0.05$) in TCCA with 79.53% disappearance at 24 h than the other treatments. Wanapat et al. [34] reported that alkaline agent like CaO improves DM disappearance of roughage. Moreover, Chaudry [35] reported that AHP was the most effective treatment for improving the degradation of wheat straw. According to Khejornsart et al. [14] and Carvalho et al. [15], disappearance and feed intake of ruminants can be improved by alkaline treatment because it alters the structure of lignin removed from lignocellulose. In connection with Rezende et al. [36] stated that the removal of lignin from the inside parts of the cell wall damaged and weakened the cell morphology by separating the cell bundles, thus resulting in long cellular structures which are connected in the longitudinal direction. Wanapat et al. [34] stated that the application of alkaline agents to substrates can chemically break the ester bonds of lignin, hemicellulose, and cellulose, and physically swell the structural fibers. The NDF and ADF disappearance in the TCCA treatment was numerically greater than in the other treatments after 24 h of incubation with 40.77% and 35.57%, respectively, which indicates improvement in rumen fermentation and disappearance when CaO and AHP were added. Nuñez et al. [18] stated that the linear increase in NDF and ADF disappearance was due to the increase in CaO inclusion, which enhances ruminal fiber fermentation. Chaudhry [31] reported that when CaO and AHP were incorporated into rice straw, it effectively removes barriers that limit the digestion of structural

Table 3. Effects of Kimchi cabbage by-products either treated or untreated with CaO and AHP as substitutional ingredient of TMR on *in situ* DM, NDF and ADF disappearance (%)

Item	Time (h)	Treatments[1]				SEM	*p*-value
		CON	TC	TCC	TCCA		
DM disappearance (%)	0	31.16[b]	34.64[a]	30.63[b]	30.15[b]	0.35	< 0.01
	3	53.54[c]	57.65[a]	54.67[b]	53.86[c]	0.16	< 0.01
	6	60.51	61.78	60.75	62.01	0.56	0.25
	9	62.50[bc]	64.49[ab]	61.44[c]	64.98[a]	0.59	< 0.05
	12	63.58[b]	67.85[a]	64.78[b]	68.14[a]	0.69	< 0.01
	24	72.23[b]	74.94[b]	75.12[b]	79.53[a]	0.93	< 0.01
NDF disappearance (%)	3	5.12	5.58	9.10	11.92	2.32	0.39
	6	18.35	11.13	11.94	19.93	3.07	0.30
	9	21.71	16.09	17.19	24.85	2.52	0.18
	12	26.98	22.31	24.30	23.71	3.59	0.87
	24	37.24	37.49	38.48	40.77	2.16	0.77
ADF disappearance (%)	3	1.98	3.59	8.18	6.96	1.80	0.31
	6	10.70	4.08	13.85	12.90	3.47	0.50
	9	11.41	7.68	16.87	15.48	3.31	0.55
	12	12.23	14.95	19.06	16.10	2.65	0.48
	24	25.68	24.29	31.14	35.57	2.45	0.21

[1]TMR only (CON); TMR + 30% Kimchi cabbage by-products (TC); TMR + 30% Kimchi cabbage by-products + 5% CaO (TCC); TMR + 30% Kimchi cabbage by-products + 5% CaO + 3.22% AHP (TCCA), fresh matter basis.

[a-c]Means with different superscripts in the same row differ significantly (*p* < 0.05).

CaO, calcium oxide; AHP, alkaline hydrogen peroxide; TMR, total mixed ration; DM, dry matter; NDF, neutral detergent fiber; ADF, acid detergent fiber; A:P ratio, acetate to propionate ratio.

carbohydrates. In addition, Castañón-Rodríguez et al. [16] revealed that supplementation with alkaline agents caused swelling of cellulose fibers that could increase the internal surface area, allowing cellulases to have direct contact with the substrates, allowing a greater level of hydrolysis in the amorphous region, and consequently increasing the proportion of crystalline cellulose. Thus, concentration of alkaline agents can effectively improve the breakdown of ester bonds between NDF and ADF and physically swollen structural fibers [37], which permits rumen microbes to breakdown the structural carbohydrates without difficulty [16].

Growth performance and blood profile of Holstein steers (*in vivo*)

The effect of dietary treatment on the growth performance of Holstein steers was shown in Table 4. The initial weight of the experimental animals was 273.06 kg in CON and 272.29 kg in TC. At the end of the 150-d feeding trial, the final body weight was 417.82 kg in CON and 413.88 kg in TC, but there was no significant difference. Results were the same with Song et al. [7], during their 12-wk feeding trial, the final body weight of steers were not significantly different between treatments. The ADG and FCR were not significantly different between treatments. It is determined by the number of factors, such as the composition and quality of the feed [38]; thus, the greater the feed quality, the greater the ADG and FCR [39]. The effect of dietary treatments on Holstein steers blood profiles was shown in Table 5. The blood ALT, AST, and HDL cholesterol were numerically greater (*p* > 0.05) in CON with 26.00 U/L, 70.00 U/L, and 112.71 mg/dL, respectively, than in the TC group. Glucose, total cholesterol, LDL cholesterol, and total protein were numerically greater (*p* > 0.05) in TC with 89.47 mg/dL, 135.94 mg/dL, 25.35 mg/dL, and 6.58 g/dL, respectively, than in CON. Briefly, ALT, AST, HDL, glucose, total cholesterol, LDL, and total protein were

Table 4. Effects of Kimchi cabbage by-product as substitutional ingredient of TMR on growth performance of Holstein steers

Items	Treatment[1]		SEM	*p*-value
	CON	TC		
Initial body weight (kg)	273.06	272.29	5.49	0.92
Final body weight (kg)	417.82	413.88	9.74	0.78
ADG (kg)	0.97	0.94	0.08	0.85
FCR	11.74	11.93	1.10	0.90

[1]TMR only (CON); TMR + 30% Kimchi cabbage by-products (TC), fresh matter basis.

TMR, total mixed ration; ADG, average daily gain; FCR, feed conversion ratio.

Table 5. Effects of Kimchi cabbage by-product as substitutional ingredient of TMR on blood profiles of Holstein steers

Items	Treatment[1]		SEM	*p*-value
	CON	TC		
ALT (U/L)	26.00	25.71	0.74	0.78
AST (U/L)	70.00	69.77	2.36	0.96
Glucose (mg/dL)	86.18	89.47	1.74	0.19
Total cholesterol (mg/dL)	133.24	135.94	5.53	0.74
HDL (mg/dL)	112.71	110.41	2.42	0.51
LDL (mg/dL)	23.24	25.35	1.40	0.30
Creatinine (mg/dL)	0.80[b]	0.92[a]	0.04	< 0.05
BUN (mg/dL)	15.09[a]	13.04[b]	0.67	< 0.05
Total protein (g/dL)	6.31	6.58	0.10	0.06

[1]TMR only (CON); TMR + 30% Kimchi cabbage by-products (TC), fresh matter basis.

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

TMR, total mixed ration; ALT, alanine aminotransferase; AST, aspartate aminotransferase; HDL, high density lipoprotein; LDL, low density lipoprotein; BUN, blood urea nitrogen.

not affected by the change in TMR composition with the Kimchi cabbage by-product. Creatinine concentration was 0.92 mg/dL in TC, which was greater ($p < 0.05$) than CON group. Cao et al. [40] concluded that vegetable by-products such as Chinese cabbage have high nutritive value and in vitro DM disappearance and good potential as vegetable protein sources for ruminant, which might be the reason for the increase of creatinine concentration; since, increased protein intake contributes to greater muscle gains [41,42]. This creatinine, which is usually eliminated only via the kidneys, is produced as an end-product of muscle metabolism [43]. Costa e Silva et al. [44] reported that the increase in body weight is correlated with that of increase in creatine concentration. Thus, the relationship between muscle tissue or CP concentration and body weight could follow the similar relationship between the creatinine concentration and body weight [44]. Blood urea nitrogen was greater ($p < 0.05$) in the CON with 15.09 mg/dL than in the TC group. This BUN can be affected by different factors such as dietary N-to-energy ratio, level of forage intake, and protein degradability in the rumen [45]. Wanapat et al. [34] reported that the concentration of BUN is negatively correlated to the level of production of NH_3-N in the rumen, in which in the present study the production of NH_3-N was numerically greater in TC than in the CON group. Protein degradation occurs more rapidly than its synthesis or the imbalances of fermentable energy and available nitrogen, and thus ammonia accumulates in the rumen fluid and be absorbed into the blood where it is transported to the liver and converted to urea [46].

In conclusion, Kimchi cabbage by-products treated with CaO and AHP increased *in vitro*

pH, and butyrate production. It also improved the *in situ* DM, NDF, and ADF disappearance. Moreover, NH_3-N, acetate, and propionate production were not hampered by CaO and AHP treatment of Kimchi cabbage by-products. ADG, FCR, and blood profiles during *in vivo* were not affected by the TMR substituted by Kimchi cabbage by-products, but it increased the creatinine production. Therefore, overall results suggest that Kimchi cabbage by-products either treated or untreated with CaO and AHP can be a potent substitutional ingredient of TMR for Holstein steers.

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