Effect of cellulolytic *Ruminococcus albus* KU-F152 and non-cellulolytic *Selenomonas ruminantium* S137 supplementation on feedlot performance, carcass characteristics and meat quality of Holstein crossbred steers

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**Abstract**

The effect was determined of cellulolytic *Ruminococcus albus* KU-F152 and non-cellulolytic *Selenomonas ruminantium* S137 on the digestibility, feedlot performance, carcass characteristics and meat quality of Holstein crossbred steers. Eight steers were randomly inoculated with *R. albus* KU-F152 and *S. ruminantium* S137, and eight steers were used as the non-inoculated control. The steers were fed a Total mixed ration (TMR) diet (2:1:79 ratio of corn silage and concentrate) that was offered *ad libitum* for 6 months. The results showed that the inoculation of *R. albus* KU-F152 and *S. ruminantium* S137 had no positive effect on the growth performance of Holstein steers; however, the inoculation increased the feed conversion ratio (FCR) compared with the control. The ruminal fermentation results demonstrated that *R. albus* and *S. ruminantium* inoculation significantly increased total volatile fatty acid (VFA), levels and increased the marbling score of meat as carcass characteristics. Regarding meat quality characteristics, *R. albus* and *S. ruminantium* inoculation increased the meat fat and reduced the thawing loss. Furthermore, the meat of the treatment group had a significantly lower shear force (tender meat) and a lighter colour than that of the non-inoculated control.

**Keywords:** Dairy steers; Feedlot; Meat quality; Bacteria supplementation; *Ruminococcus albus* KU-F152; *Selenomonas ruminantium* S137

1. Introduction

Rumen bacteria play important roles in digesting feed, especially the fibre, by increasing the solubility of crystalline cellulose [29]. Cellulose is the most abundant component of plant cell walls [20]. Fibrous feed is the main energy source for ruminants and has an important role in the development of calf digestive organs [9]. The digestibility of cellulose was improved by the introduction of highly cellulolytic strains of bacteria in the rumen and cellulolytic bacteria belonging to the *Fibrobacter succinogenes*, *Ruminococcus flavefaciens* and *R. albus* groups [20]. However, cellulolytic bacteria will interact synergistically with non-cellulolytic bacteria, and Sawanon and Kobayashi [27] and Sawanon et al. [28] found that non-cellulolytic *Selenomonas ruminantium* improves fibre digestion when co-cultured with *R. flavefaciens* or *F. succinogenes*. Piamya et al. [24] found that co-culture of *R. albus* KU-F152 and *S. ruminantium* S137 resulted in a higher dry matter (DM) and Neutral detergent fiber (NDF) digestibility of fibrous feed compared with monoculture. Other studies have demonstrated the cellulolytic interaction of various bacterial combinations, such as *R. flavefaciens* or *F. succinogenes*, with non-cellulolytic *S. ruminantium* or *P. ruminicola* [14, 27, 28]. These researchers reported an interesting relationship between non-cellulolytic bacteria and cellulolytic bacteria in ruminal fibre.
degradation, as non-cellulolytic bacteria species can activate cellulolytic bacteria through nutritive interactions, including hydrogen transfer, ammonium, vitamin or cellulose gene expression as cross feeding of degradation and fermentation products derived from plant fibre [14, 16]. Wolin et al. [30] reported that cellulolytic bacteria such as *F. succinogenes*, *R. flavefaciens* and *R. albus* produced succinate during fibre digestion, and non-cellulolytic *S. ruminantium* converted succinate (succinate-decarboxylation) into propionate. The propionate is used in the cattle liver for glucose production, with the glucose from propionate being the preferred substrate for deposition of intramuscular fat, such as marbling, a key component in determining meat quality grade [10]. In addition, non-cellulolytic bacteria can reduce the negative effects caused by rapid ruminal fermentation of feed with a high concentrate ratio, which results in a pH decrease due to the accumulation of organic acids in the rumen and a shift in the delicate balance of rumen microorganisms [19]. The growth of cellulolytic bacteria is inhibited at a ruminal pH below 6.0, and the lactate-producer bacterium, *Streptococcus bovis*, is replaced instead by *Lactobacillus* spp. at a ruminal pH below than 5.4, causing excess lactate accumulation and cow death from acidosis. Lactate-utilizing bacteria, such as *Megasphaera elsdenii* and *S. ruminantium* S137, begin to proliferate in response to lactic acid accumulation and remove it by converting it to volatile fatty acids (VFAs) that can be absorbed in the rumen [28, 32].

Furthermore, the inoculation or supplementation of rumen bacteria in rearing cattle may increase performance, such as feed intake, feed conversion and body weight [11, 15]. While the inoculation of rumen bacteria could increase the feed intake of Holstein caves during the weaning period [26], few studies have reported the effect of inoculation of rumen bacteria on performance. However, there has been no report on bacterial application in feedlot Holstein steers. Therefore, the objective of this experiment was to evaluate the effect of cellulolytic *R. albus* KU-F152 and non-cellulolytic *S. ruminantium* S137 supplementation on the feedlot performance, carcass characteristics and meat quality of Holstein steers.

2. Material and methods

2.1. Bacterial and basal media preparation

Cellulolytic *R. albus* KU-F152 from buffalo rumen [5, 25] and non-cellulolytic *S. ruminantium* S137 from sheep rumen [27] were used. *R. albus* KU-F152 was pre-cultured in 10 mL basal medium containing cellulose and cellobiose at 0.5% (w/v) as carbon sources under anaerobic conditions for maintaining bacteria. One hundred millilitres of basal medium were prepared with the following composition .75 mL of mineral solution I (0.6 g of K$_2$HPO$_4$ in 100 mL of distilled water) and mineral solution II (1.2 g of NaCl, 1.2 g of (NH$_4$)$_2$SO$_4$ 0.6 g of KH$_2$PO$_4$, 1.2 g of CaCl$_2$, 0.25 g of MgSO$_4$⋅7H$_2$O and 100 mL of distilled water), 0.1 mL of 0.1% resazurin, 0.1 g of L-cysteine-HCl-H$_2$O, 0.2 g of bactopeptone, 0.12 g of yeast extract, 0.5 g of glucose, 0.5 g of cellobiose, 30 mL of clarified rumen fluid, and 50 mL of distilled water, and the pH was adjusted to 6.8 with 1 N NaOH before addition of 5 mL of 8% Na$_2$CO$_3$ [28]. After incubation at 38°C for 24 hr, 2.5 mL of *R. albus* KU-F152 suspension was propagated in 250 mL of the same basal medium at 38°C for 24 hr and adjusted to an optical density (OD) of 0.5 (3.85 × 10$^7$ CFU/mL) with anaerobic dilution solution for inoculation [27].

*S. ruminantium* S137 was pre-cultured in 10 mL basal medium containing 0.1% (w/v) glucose. One hundred millilitres of basal medium were prepared with the following composition .75 mL of mineral solution I (0.6 g of K$_3$PO$_4$ in 100 mL of distilled water) and mineral solution II (1.2 g of NaCl, 1.2 g of (NH$_4$)$_2$SO$_4$ 0.6 g of KH$_2$PO$_4$, 1.2 g of CaCl$_2$, 0.25 g of MgSO$_4$⋅7H$_2$O and 100 mL of distilled water), 0.1 mL of 0.1% resazurin, 0.1 g of L-cysteine-HCl-H$_2$O, 0.2 g of bactopeptone, 0.12 g of yeast extract, 0.1 g of glucose, 30 mL of clarified rumen fluid, and 50 mL of distilled water, and the pH was adjusted to 6.8 with 1 N NaOH before addition of 5 mL of 8% Na$_2$CO$_3$ [28]. After incubation at 38°C for 4 hr, 2.5 mL of *S. ruminantium* S137 suspension was propagated in 250 mL of the same basal medium at 38°C for 4 hr and adjusted to 0.5 OD (4.25 × 10$^7$ CFU/mL) with anaerobic dilution solution for inoculation [27].

2.2. Animals, experimental design and feeding

The experiment was conducted at the Ruminant Research Unit, Department of Animal Science, Faculty of Agriculture at Kamphaeng Saen, Kasetsart University, Nakhon Pathom, Thailand. Sixteen Holstein crossbred steers with an average age of 18 months (mean live weight ± standard deviation, 448.61 ± 51.50 kg) were vaccinated against foot and mouth disease, and dewormed to remove endo- and ectoparasites. The steers were kept in individual pens (3.0 × 4.5 m). Then, eight steers were randomly inoculated with 250 mL of *R. albus* KU-F152 and 250 mL of *S. ruminantium* S137 using an anaerobic technique at 14 days intervals. Eight steers were supplemented with 500 mL of medium without bacteria medium as the control.
The steers were fed a TMR diet (Table 1) *ad libitum* for 6 months. The diet was provided twice per day (07.00 AM and 16.00 PM). The experiment lasted 184 days. At the end of the study, all steers were deprived of feed but allowed free access to water. The steers were housed in collective pens for approximately 12 hr.

**Table 1** Ingredient composition and nutrient content of TMR diet

| Item                                      | Amount  |
|-------------------------------------------|---------|
| Ingredient of TMR diet, % dry matter      |         |
| Corn silage                               | 21.19   |
| Molasses                                  | 6.63    |
| Urea (46-0-0)                             | 1.50    |
| Cassava chip                              | 30.00   |
| Expeller pressed palm kernel meal         | 20.90   |
| Soybean meal (44% CP)                     | 7.85    |
| Ground corn                               | 10.11   |
| Premix for beef cattle<sup>1</sup>        | 0.50    |
| Sulphur                                   | 0.30    |
| Salt                                      | 0.50    |
| Dicalcium phosphate P-18                  | 1.20    |
| Sodium carbonate                          | 0.10    |
| Nutrient content of TMR diet, % dry matter|         |
| Crude protein                             | 14.78   |
| Crude fat                                 | 1.26    |
| Crude fibre                               | 17.21   |
| Ash                                       | 6.78    |
| Calcium                                   | 0.75    |
| Phosphorus                                | 0.47    |
| NDF                                       | 42.92   |
| ADF                                       | 24.57   |
| NFE                                       | 56.97   |
| TDN                                       | 66.98   |
| Gross energy (GE) Mcal/kg                 | 3,236.32|

<sup>1</sup> Vitamin A = 2,160,000 IU, vitamin B3 = 100,000 IU, vitamin E = 5,000 IU, Mn = 8.5 g, Zn = 6.4 g, Cu = 1.6 g, Mg = 16 g, Co = 320 mg, I = 800 mg, Se = 32 m.

### 2.3. Performance determination

Dry matter intake (DMI), average daily gain (ADG) and the feed conversion ratio (FCR) were measured to obtain animal performance data. DMI was measured by the difference between steer weights before successive feeding times less the weight of any residual feed recorded for individual animals every morning. Steers were weighed at the beginning of the study and then every 30 days (successive periods) during the experiment. The means of the initial, final and successive body weights for each treatment were recorded throughout the trial at the same intervals to determine the ADG and FCR.
2.4. Rumen fermentation characteristics

Ruminal fluid was sampled from sixteen steers at the end of the experiment using suction via the mouth. The tube was passed over the back of the tongue and into the oesophagus, and a vacuum pump was used to apply suction to draw the rumen liquid into the sampling bottle. Ruminal fluid samples were taken 4 hr after the morning feeding. Ruminal fluid pH was measured immediately using a portable pH meter (Oakton pH Testr 30, USA). One millilitre of the rumen samples was centrifuged at 4,000 × g for 10 min and the supernatant was analysed for ammonia nitrogen (NH$_3$-N) using the phenol-hypochlorite method modified from Weatherburn [30] with a spectrophotometer (Thermo Scientific, Helios Zeta ultraviolet-visible (UV-VIS) model, USA). To determine VFAs, 1 mL ruminal fluid was mixed with 0.2 mL metaphosphoric acid solution and 40 μL crotonic acid solution. After incubation overnight at 4 °C, samples were centrifuged at 12,000 × g for 10 min. The supernatant was collected for analysis using gas chromatography as described by Sawanon et al. [28].

2.5. Carcass characteristics

All Holstein crossbred steers were weighed at the end of the experiment (mean live weight ± standard deviation, 635.41 ± 38 kg BW and then deprived of feed but allowed free access to water before being humanely slaughtered after a fasting period of approximately 12 hr according to Islamic tradition by severing the jugular veins, carotid arteries, trachea and the oesophagus. The blood was allowed to drain from the carcass. The weights of the intestine, rumen, reticulum, omasum and abomasum were recorded. Abdominal fat was removed from the abdomen and weighed. The fat covering the kidneys was removed and weighed. The warm carcass weight was taken shortly after slaughter. The pH level of the carcass was measured at 1 hr, 24 hr and 7 days post mortem from the muscles of the lumbar region (between the 4th and 5th lumbar vertebrae) using a portable meter with a penetrating electrode probe (TESTO205 pH meter; Testo Pty Ltd., Croydon South, VIC, Australia) according to the techniques of Orellana et al. [23]. After slaughtering, each carcass was divided into two equal longitudinal halves.

The back fat thickness was measured using callipers on the longissimus dorsi muscle between the 12th and 13th ribs at three-quarters of the length of the loin eye muscle from the chine (backbone) according to Orellana et al. [23].

The rib eye area was measured as the cut surface of the rib eye muscle between the 12th and 13th ribs by tracing the outline onto tracing paper and measuring the area using an LI-3100 Area Meter (LI-3100, Li-COR Biosciences, Lincoln, NE, USA) according to the methods of Cacere et al. [7].

The dressing percentage was measured after the carcass had been chilled at 0–4 °C for 7 days and then weighed to determine the cold carcass weight. The dressing percentage was calculated using the formula of dressing percentage = (cold carcass weight × 100) / live weight before slaughter [6].

The marbling score was evaluated by estimating the amount of intramuscular fat visible on the cut surface of the rib eye muscle between the 12th and 13th ribs using a photographic standard scale of five values (1 = devoid, 2 = slight, 3 = small, 4 = moderate and 5 = abundant) after chilling for 7 days according to the Thai Agricultural Commodity and Food Standard [22].

2.6. Meat quality

One kilogram of the longissimus dorsi muscle between the 12th and 13th ribs was sampled to determine meat quality after preservation at 0–4 °C for 7 day.

Meat colour was measured after the meat samples of the longissimus dorsi muscle were cut at a thickness of 2.5 cm and then exposed to air for 30 min. After muscle oxygenation, the colour was measured using a colour meter (Hunter Lab Mini Scan EZ, 4500 L, Reston, VA, USA) to determine the colorimetric index of chromaticity. The components of L* (lightness), a* (red-green) and b* (yellowness) are measured in the Hunter colour system and were assessed at three different points on the muscle surface [8].

The drip loss (%) was evaluated from a meat sample with a 2.5 cm thickness that was weighed before storage (W1). The meat was then covered with a white cloth and hung at 2–4 °C for 24 hr. The meat weight was measured after storage (W2). The percentage of drip loss was calculated using the formula drip loss (%) = (W1 - W2) × 100 / W1 [4].

The thawing loss (%) was evaluated from a meat sample with a 2.5 cm thickness that was kept at -20 °C for 24 hr and then weighed (W1). The meat was defrosted to 4 °C having been frozen at -20 °C and then weighed (W1). The percentage of thawing loss was calculated using the formula thawing loss (%) = (W1 - W2) × 100 / W1 [12].
The cooking loss (%) was evaluated from a meat sample with a 2.5 cm thickness that was kept at 4 °C for 24 hr and weighed before boiling (W1). The samples were placed in a plastic vacuum-sealed bag to protect the meat from steam forming inside the bag. The meat in the bag was cooked in hot steam until it reached a defined internal temperature of 72 °C; then, it was removed from the steam and cooled to room temperature. The meat was weighed after boiling (W2) and the percentage of cooking loss was calculated using the formula cooking loss (%) = (W1 - W2) × 100 /W1 [12].

The shear force (SF) was evaluated after core samples for cooking loss were used to determine tenderness by measuring the Warner-Bratzler shear force (WBS). The sample was punched parallel to the muscle fibres using a steel hollow-core device with a diameter of 1.0 cm to obtain six pieces from each muscle sample. The shear force test was performed using a Warner-Bratzler Shear Device (Challion; GH Electronics Co.; San Francisco, CA, USA). The samples were sheared across the fibre axis using a V-shaped cutting blade with a shearing velocity of 20 cm/min. The WBS value from each muscle sample was recorded and the average value was used for evaluation [17].

Intramuscular fat was measured according to the methods of AOAC [3]. The meat samples were analysed after freezing (-20 °C). The frozen sample was weighed before being the meat was freeze-dried. The freeze-dried sample was weighed and ground and the fat content was quantified using solvent extraction (petroleum ether, BP 40–60 °C) with a Soxhlet apparatus.

2.7. Data analysis

The data (n=8) on feedlot performance and carcass characteristics and meat quality were subjected to Student t-test. Differences in means were considered significant at $P < 0.05$. Values between $P > 0.05$ and $P ≤ 0.10$ indicated a trend, while values of $P > 0.10$ were not considered significant.

3. Results

3.1. Growth performance

The Holstein steers inoculated with cellulolytic R. albus KU-F152 and non-cellulolytic S. ruminantium S137 had a feed intake of 1.71% BW, which was not significantly different from control steers (1.80% BW). The inoculated steers had mean values for final weight of 659.50 kg, weight gain of 199.50 kg and average daily gain of 1.08 kg/d; these values were not significantly different from the control sample that had a final weight, weight gain and average daily gain of 631.33 kg, 171.17 kg and 0.93 kg/d, respectively. Moreover, R. albus KU-F152 and S. ruminantium S137 inoculation provided a FCR value for the steers of 9.72 that tended ($P = 0.08$) to be lower than that of the non-inoculated control with 10.11 (Table 2).

Table 2 Effect of cellulolytic Ruminococcus albus KU-F152 and non-cellulolytic Selenomonas ruminantium S137 supplementation on feedlot performance of Holstein crossbred steers.

| Item                 | Non-inoculated bacteria | Inoculated bacteria | SEM  | $P$-value |
|----------------------|-------------------------|---------------------|------|-----------|
| Initial weight (kg)  | 460.16                  | 459.50              | 21.83| 0.49      |
| Feed intake (%BW)    | 1.80                    | 1.71                | 0.07 | 0.25      |
| Final weight (kg)    | 631.33                  | 659.50              | 30.74| 0.27      |
| Weight gain (kg)     | 171.17                  | 199.50              | 8.87 | 0.26      |
| Average daily gain (kg/d) | 0.93                   | 1.08                | 0.14 | 0.26      |
| Feed conversion ratio | 10.11                   | 9.72                | 0.69 | 0.08      |

3.2. Rumen fermentation characteristics

Rumen pH and NH$_3$-N values of steers inoculated with R. albus KU-F152 and S. ruminantium S137 were 6.77 and 4.14 mg %, respectively, which were not significantly different to the pH (6.31) and NH$_3$-N (4.58 mg %) of the control. The total VFA of the inoculated treatment (55.44 mM/L) was significantly higher than that of the non-inoculated control (36.49 mM/L). The acetate, propionate and butyrate ratios of the inoculated treatment group were 75.63:13.48:10.90, respectively, which were not significantly different to the control (Table 3).
Table 3 Effects of cellulolytic *Ruminococcus albus* KU-F152 and non-cellulolytic *Selenomonas ruminantium* S137 supplementation on ruminal fermentation characteristics of Holstein crossbred steers.

| Item                        | Non-inoculated bacteria | Inoculated bacteria | SEM  | P-value |
|-----------------------------|-------------------------|---------------------|------|---------|
| pH                          | 6.31                    | 6.77                | 0.16 | 0.16    |
| NH₃-N (mg%)                 | 4.58                    | 4.14                | 0.37 | 0.42    |
| Total VFAs (mM/L)           | 36.49                   | 55.44               | 2.23 | 0.01    |
| Acetate (%)                 | 71.78                   | 75.63               | 2.69 | 0.29    |
| Propionate (%)              | 15.52                   | 13.48               | 1.09 | 0.42    |
| Butyrate (%)                | 12.13                   | 10.90               | 0.94 | 0.83    |
| Other (%)                   | 1.52                    | 1.94                | 0.21 | 0.14    |
| Acetate: Propionate ratio   | 4.63                    | 5.61                | 1.27 | 0.21    |

3.3. Carcass characteristics

The marbling score of steers inoculated with *R. albus* KU-F152 and *S. ruminantium* S137 was 1.80, which tended to be higher (*P* = 0.06) than that of the non-inoculated control (1.60). The back fat thickness, rib eye area, live weight at slaughter, hot carcass weight, dressing percentage, heart weight, liver weight, kidney weight, total fat, intestine weight, spleen weight, lung weight, rumen and reticulum weight, omasum weight, abomasum weight and stomach weight were not significantly different to the non-inoculated control (Table 4).

Table 4 Effect of cellulolytic *Ruminococcus albus* KU-F152 and non-cellulolytic *Selenomonas ruminantium* S137 supplementation on carcass characteristics of Holstein crossbred steers.

| Item                        | Non-inoculated bacteria | Inoculated bacteria | SEM  | P-value |
|-----------------------------|-------------------------|---------------------|------|---------|
| Marbling score              | 1.60                    | 1.80                | 0.54 | 0.06    |
| Back fat thickness (cm)     | 1.12                    | 0.79                | 0.13 | 0.31    |
| Rib eye area (cm²)          | 51.83                   | 57.09               | 5.38 | 0.61    |
| Live weight at slaughter (kg)| 613.40                 | 638.40              | 23.04| 0.49    |
| Hot carcass weight (kg)     | 346.26                  | 355.12              | 18.56| 0.33    |
| Dressing percentage (%)     | 55.10                   | 54.29               | 0.70 | 0.72    |
| Heart (kg)                  | 2.22                    | 2.30                | 0.16 | 0.48    |
| Liver (kg)                  | 7.56                    | 7.92                | 0.82 | 0.43    |
| Kidney (kg)                 | 1.10                    | 1.28                | 0.14 | 0.26    |
| Spleen (kg)                 | 1.06                    | 1.24                | 0.13 | 0.22    |
| Lung (kg)                   | 4.84                    | 5.62                | 0.44 | 0.77    |
| Kidney fat (kg)             | 18.54                   | 16.34               | 2.83 | 0.74    |
| Total fat (kg)              | 55.26                   | 51.90               | 8.27 | 0.40    |
| Intestine (kg)              | 11.56                   | 11.28               | 1.50 | 0.18    |
| Rumen and reticulum (kg)    | 8.88                    | 10.02               | 1.03 | 0.10    |
| Omasum (kg)                 | 1.94                    | 1.96                | 0.12 | 0.15    |
| Abomasum (kg)               | 2.16                    | 2.23                | 0.31 | 0.50    |
| Stomach (kg)                | 12.98                   | 14.30               | 0.93 | 0.97    |
3.4. Meat quality

The meat pH values following inoculation with *R. albus* KU-F152 and *S. ruminantium* S137 were in the range 5.82–5.83 after preservation at 0–4 °C for 24 hr and 7 days, which were significantly higher than those of the non-inoculated control (5.57–5.62). The shear force and thawing loss of the inoculated treatments were 4.03 kg and 1.35%, respectively, which were significantly lower than the control (4.68 kg and 1.73%, respectively). Moreover, the meat fat (15.10%) of the inoculated treatment tended to be higher than for the meat fat (10.58%) of the non-inoculated control (*P* ≤ 0.09). The cooking loss and drip loss were not significantly different between the inoculated and control. Furthermore, the meat colour values of inoculated treatment were 43.42 L* (lightness), 13.93 a* (redness) and 14.35 b* (yellowness), which were higher than for the non-inoculated control (39.18 L*, 12.68 a* and 11.70 b*) as shown in Table 5.

Table 5 Effect of cellulolytic *Ruminococcus albus* KU-F152 and non-cellulolytic *Selenomonas ruminantium* S137 supplementation on meat quality of Holstein crossbred steers

| Item                        | Non-inoculated bacteria | Inoculated bacteria | SEM  | *P*-value |
|-----------------------------|-------------------------|---------------------|------|-----------|
| pH at 1 hr                  | 6.33                    | 6.36                | 0.11 | 0.22      |
| pH at 24 hr                 | 5.62                    | 5.82                | 0.10 | 0.03      |
| pH at 7 days                | 5.57                    | 5.83                | 0.12 | 0.03      |
| Shear force (kg)            | 4.68                    | 4.03                | 1.30 | 0.01      |
| Cooking loss (%)            | 30.90                   | 35.46               | 2.33 | 0.25      |
| Drip loss (%)               | 2.23                    | 1.94                | 0.31 | 0.52      |
| Thawing loss (%)            | 1.73                    | 1.35                | 0.41 | 0.02      |
| Meat fat (%)                | 10.58                   | 15.10               | 6.58 | 0.09      |
| Meat fat (%DM)              | 27.80                   | 29.04               | 5.52 | 0.19      |
| Moisture meat (%)           | 62.76                   | 65.20               | 1.61 | 0.45      |
| Meat colour                 |                         |                     |      |           |
| L*                          | 39.18                   | 43.42               | 2.08 | 0.05      |
| a*                          | 12.68                   | 13.93               | 1.00 | 0.87      |
| b*                          | 11.70                   | 14.35               | 1.23 | 0.04      |

* L* = lightness, a* = red-green and b* = yellowness

4. Discussion

The cellulolytic *R. albus* KU-F152 and non-cellulolytic *S. ruminantium* S137 supplementation had no negative effects on the feed intake, final weight, weight gain and ADG of Holstein steers. Prihantoro et al. [26] reported that the inoculation of ruminal bacterium increased the feed intake and ADG (19.47%) of Holstein calves during the weaning period. In the current study, *R. albus* and *S. ruminantium* inoculation increased the final weight, weight gain and ADG compared to the non-inoculated control by 4.46%, 16.45% and 16.13%, respectively. These results were due to *R. albus* KU-F152 and *S. ruminantium* S137 increasing the feed digestion efficiency [5, 27]. Similarly, Prihantoro et al. [26] reported that buffalo rumen bacteria resulted in a relative improvement in the feed digestive efficiency of Holstein calves during the weaning period. In the current study, *R. albus* KU-F152 and *S. ruminantium* S137 increased the total VFA content in the rumen fluid. This result was confirmed by Piamya et al. [24] who reported that the co-culture of *R. albus* KU-F152 and *S. ruminantium* S137 increased the total VFA concentration *in vitro*. The rumen microbial community plays an important role in carbohydrate fermentation and in providing energy to the host animal through VFAs [2]. The pH values in the rumen of the inoculated steers increased, indicating that *R. albus* KU-F152 and *S. ruminantium* S137 could improve rumen conditions. Similarly, Musa et al. [21] reported that rumen bacteria improved the pH conditions and microbial ecology in calf rumen.

*R. albus* and *S. ruminantium* inoculation increased the marbling score (12.5%) of Holstein steers in the carcass characteristics of the current study. Similarly, Abbas et al. [2] reported that the rumen microbial community led to increased marbling by manipulating the rumen bacteria that create fermentation by-products of VFAs. Regarding the meat qualities in the current study, the bacterial inoculation showed the pH values after preservation at 0–4 °C for 24
and 7 days were higher than those of the control. Meat that has high pH values can retain a larger amount of water, resulting in high juiciness, compared to that with low pH values [1]. Moreover, the meat of steers inoculated with *R. albus* KU-F152 and *S. ruminantium* S137 in the current study had a lower thawing loss and shear force than control meat and the decreased shear force resulted in enhanced meat tenderness (Table 5). Furthermore, inoculated steers had a higher meat fat percentage (42.72%) and higher colour values of L* (lightness) and b* (yellowness) than the controls. Meat lightness is the first factor that consumers use as an indication of freshness [18]. These results suggested that *R. albus* and *S. ruminantium* supplementation in the rumen could improve the meat quality of dairy steers.

5. Conclusion

The inoculation of cellulolytic *R. albus* KU-F152 and non-cellulolytic *S. ruminantium* S137 had no effect on the feedlot performance of Holstein steers fed TMR; however bacteria supplementation increased the FCR compared to the control. Bacteria supplementation increased the ruminal total VFA, marbling score and meat fat and reduced the thawing loss. Furthermore, the meat following bacteria supplementation had a significantly lower shear force (tender meat) and a lighter colour than that of the control.

Compliance with ethical standards

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Disclosure of conflict of interest

The authors declare there are no conflicts of interest.

Statement of ethical approval

This study was conducted at the animal farm of Kasetsart University in Thailand with the approval of Kasetsart University Animal Use Committee (ID number: ACKU 60-AGK-003).

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