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The influence of fattening periods on chemical composition, fatty acid profile, cholesterol and ribonucleotide content of Charolaise crossbred steers

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Abstract The chemical composition, fatty acid profile, cholesterol, and ribonucleotide content of *longissimus thoracis* muscle from Charolaise crossbred steers fattened for 12 and 15 months were investigated. Chemical composition did not differ between the two fattening periods. Fat content was 4.38 and 4.66 %, moisture content 72.79 and 71.14 %, ash content 1.24 and 1.23 %, and protein content 23.60 and 23.73 % for fattening periods of 12 and 15 months, respectively. Fatty acid composition was not affected by fattening period, except that C15:0 content was higher in steers fattened for 12 months than in steers fattened for 15 months ($P < 0.05$). There was no effect of fattening period on cholesterol content ($P > 0.05$). As for the effect of fattening period on ribonucleotide content, only guanosine monophosphate (GMP) of steers fattened for 12 months was significantly higher than those of steers fattened for 15 months ($P < 0.05$).

Keywords: Meat quality, Meat flavor, Fattening period, Crossbred steer

Introduction

Beef is a popular protein food among consumers. It has been reported that more and more cattle are being raised in Thailand and the demand for beef is increasing every year (Office of agricultural economics, 2020). Beef not only

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provides consumers with high nutritional value, but also a unique taste and flavor that satisfy consumers (Macleod, 1998). Bunmee *et al.* (2018) found that growth performance and meat quality are influenced by breed, nutrition, and management. Therefore, various *Bos taurus* are imported to be used as terminal sires for crossbreeding with indigenous or other Bos indicus breeds. The most important terminal sire breeds for producing high quality crosses of beef cattle in Thailand are Charolais (Bureau of Biotechnology in Livestock Production, 2018). Nutrition and management influence meat quality; the fattening period for the production of high quality beef in Thailand, especially at Pon Yang Kham Livestock Breeding Cooperatives Ltd. in Sakon Nakhon Province, Thailand, is at least 12 months. Kheawrod *et al.* (2020) reported that the lean and bone content of steer carcasses fattened for 15 months was significantly higher than those fattened for 12 months, but the fat content was not significantly different. Conversely, Nogalski *et al.* (2018) reported that bulls and steers slaughtered at 18 months of age had higher fat content and lower bone content than those slaughtered at 15 months of age.

Currently, beef producers still face the problem of variable beef quality, especially insufficient marbling. To produce high quality beef with high marbling, a longer fattening period might be required. Chemical composition, such as nutrient composition, fatty acid composition, cholesterol, and ribonucleotide content of meat, are important factors affecting the quality of meat in terms of nutritional value and meat flavor, and there is also limited information on the influence of fattening duration on this chemical composition. Therefore, the aim of this study was to determine and compare the effects of 12 and 15 months fattening duration on the nutrient composition, fatty acid composition, cholesterol, and ribonucleotide content of Charolaise crossbred steers.

Materials and methods

Animals and feeding

The study was conducted in a Pon Yang Kham Livestock Breeding Cooperatives Ltd. in Sakon Nakhon Province, Thailand. The animals in this study were 12 offsprings from Brahman or Brahman x Native dams inseminated with frozen semen from the same Charolais sire. The crossbred bulls were castrated before the start of the fattening period with the Burdizzo. Steers were fattened at 18-24 months of age with body weight estimated at approximately 380 kg by measuring chest girth. The 6 steers were fattened for 12 months and the other 6 steers were fattened for 15 months. They were reared

under the same housing and feeding conditions in the cooperative farm in Sakon Nakhon Province. The steers were fed ad libitum with a 12.8% protein concentrate and roughage with fresh grass and straw. After 6 months of fattening, molasses was added to the diet. All cattle were weighed and slaughtered at the end of the fattening period. The average slaughter weight was 604.34 kg and 724.49 kg for steers fattened for 12 and 15 months, respectively. After slaughter, the muscles of *Longissimus thoracis* (LT) were taken to analyse the nutrient composition, fatty acid composition, cholesterol, and ribonucleotide content.

Nutrient composition analysis

The nutrient content of the LT muscle was assessed using the AOAC (2005) methodology. The ash content was measured by burning in a 550℃ furnace, and the dry matter content was assessed by drying in a 100℃ oven. The fat content was determined using an extraction with petroleum ether in a Soxtec 416 apparatus (Model Gerhardt analytical system, Königswinter, Germany). A Speed digester (Model K-439, Buchi, Thailand) and a Distillation Unit equipment were used to assess the crude protein content (Model B-324, Buchi. Thailand).

Fatty acid analysis

Fatty acid composition was determined according to the method of Raes *et al.* (2001). The lipid extraction processes were performed using chloroform as described by Folch (1957). The internal standard, methyl nonadecanoate (SFA-013N, Accu Standard, New Haven, CT, USA) was added during extraction process. The gas chromatography (7890B, Agilent, Santa Clara, CA, USA) with a fused silica capillary column (model SPTM-2560, Supelco, Bellfonte, PA, USA) for FAME (100 m×0.25 mm×0.2 μm film thickness) was used to analyse fatty acid methyl esters (FAME).

Cholesterol analysis

Cholesterol measurement was performed using the method outlined by Du and Ahn (2002). The frozen samples (after immersion in liquid nitrogen) were pulverized using grinder (WSG30E, Waring, USA). Ground breast meat of 0.4 g was extracted with 10 mL of saponification reagent (ethanol: 33% KOH (w/v): 20 % ascorbic acid (94:6:0.5). A 50 μL of 5 α-cholestane solution (1 μg/μL in hexane) was subsequently added as an internal standard. The gas

chromatography (7890B, Agilent, Santa Clara, CA, USA) was used to analyse cholesterol content.

Ribonucleotide analysis

According to the method of Tikk *et al.* (2006), one gram of pulverized sirloin muscle in 6 mL of cold 0.6 M perchloric acid was homogenized at 23,319 ×g for 10 sec (T25 Ultra-Turrax®, Ika). The homogenate was left on ice for 15 min and then neutralized by adding 5.4 mL of 0.8 M KOH and 0.25 mL of KH_2PO_4 buffer. The pH of mixed sample was adjusted to 7 with 0.8 M KOH and the volume was finally made up to 15 mL with HPLC water. After centrifugation at 10,000 ×g for 10 min at 4^{o}C (Scanspeed 1580R, Labogene, Lillerod, Denmark), 1 mL of supernatant was aspirated to a small tube and frozen at –80^{o}C. After thawing the frozen sample and centrifuging at 10,000 ×g for 5 min at 4 ^{o}C (Scanspeed 1580R, Labogene, Denmark), the supernatants were then analyzed for IMP, inosine, hypoxanthine, and GMP using the HPLC (Chromaster, Hitachi, Tokyo, Japan) fitted with a UV detector (210 nm). A stationary phase was the TSK Gel Amide-80 column (Tosoh, Tokyo, Japan) while the eluent phase consisted of a buffer containing acetonitrile: KH_2PO_4, 70:30. The content of ribonucleotide was quantified based on a standard curve using external standards (57510 Inosine-5-monophosphate disodium salt hydrate, 14125 Inosine, H9377 Hypoxanthine, and G8377 Guanosine-5-monophosphate disodium salt hydrate, Sigma-Aldrich, St. Louis, MO, USA).

Statistical analysis

Data were expressed as mean ± standard deviation. Differences were tested by two-tailed independent t-test to compare the amount of nutrient composition, fatty acid composition, cholesterol, and ribonucleotide contents of meat which came from Charolaise crossbred steers fattened for 12 and 15 months. The values $P < 0.05$ were considered statistically significant. Statistical analysis was done using SPSS version 17 (SPSS Inc, Chicago, IL, USA).

Results

The nutrient composition of LT of crossbred steers fattened for 12 and 15 months is shown in Table 1. There were no statistically significant differences ($P > 0.05$) with fat content of 4.38% and 4.66%, moisture content of 72.79% and 71.14%, ash content of 1.24% and 1.23%, and protein content of 23.60%

and 23.73% in LT muscles of steers fattened for 12 and 15 months, respectively.

Table 1. Effect of fattening period on chemical composition of *Longissimus thoracis* of Charolais crossbred steers

Traits	Fattening period		P-value
	12 months	15 months	
Fat (%)	4.38 ± 0.65	4.66 ± 0.47	0.599
Moisture (%)	72.79 ± 4.74	71.14 ± 0.65	0.439
Ash (%)	1.24 ± 0.09	1.23 ± 0.04	0.878
Protine (%)	23.60 ± 1.21	23.73 ± 0.25	0.820

Cholesterol content and fatty acid composition are shown in Table 2 and there were no statistically significant differences ($p > 0.05$). The cholesterol content in LT muscle of crossbred steers fattened for 12 and 15 months was 135.88 and 136.29 mg/100 g, respectively.

Table 2. Effect of fattening period on fatty acid composition and cholesterol of *Longissimus thoracis* of Charolais crossbred steers

Traits	Fattening period		P-value
	12 months	15 months	
Cholesterol[1]	135.88 ± 14.3	136.29 ± 15.48	0.963
Fatty acid[2]			
C12:0	0.69 ± 0.18	0.63 ± 0.36	0.734
C14:0	8.71 ± 1.25	7.45 ± 2.32	0.306
C14:1	8.67 ± 1.61	7.52 ± 2.92	0.452
C15:0	1.14 ± 0.08	0.73 ± 0.39	0.048
C16:0	19.02 ± 1.12	19.29 ± 1.33	0.728
C16:1	15.43 ± 1.56	15.11 ± 1.53	0.737
C17:0	3.23 ± 0.33	2.74 ± 0.47	0.080
C17:1	2.06 ± 0.34	1.65 ± 0.41	0.106
C18:0	7.23 ± 1.37	6.94 ± 1.27	0.723
C18:1n9c	31.47 ± 2.67	34.65 ± 5.04	0.239
C18:2n6c	1.37 ± 0.5	2.04 ± 0.99	0.207
C20:4n6	0.97 ± 0.54	1.27 ± 0.89	0.528
SFA	40.02 ± 2.49	37.77 ± 1.99	0.129
UFA	59.98 ± 2.49	62.23 ± 1.99	0.129
MUFA	57.63 ± 1.89	58.92 ± 3	0.429
PUFA	2.34 ± 0.99	3.31 ± 1.77	0.308
PUFA : SFA	1.51 ± 0.15	1.65 ± 0.14	0.128

[a,b] Means in rows with different superscripts are significant different at $p < 0.05$.
[1] mg/100g
[2] Percentage of total fatty acids
SFA = C12:0+ C14:0+ C15:0+ C16:0+ C17:0+ C18:0
UFA = C14:1+ C16:1 + C17:1 + C18:1n9c + C18:2n6c + C20:4n6
MUFA = C14:1+ C16:1 + C17:1 + C18:1n9c
PUFA = C18:2n6c + C20:4n6

There were no statistically significant differences in the amounts of most fatty acids in the LT muscle of both fattening periods, except for C15:0 fatty acid, which was significantly higher in the meat of 12-month fattened cattle than in the meat of 15-month fattened cattle ($P < 0.05$). It was also found that the amounts of SFA, UFA, MUFA, PUFA, and PUFA:SFA also did not show statistically significant differences ($P > 0.05$). When SFA and UFA were considered, it was found that the amount of UFA, an unsaturated healthy fatty acid, was higher than that of SFA, a saturated fatty acid. The level of C17:0 fatty acids tended to be higher in the meat of steers fattened for 12 months than in the meat of steers fattened for 15 months ($P=0.080$).

There were no statistically significant differences in the amounts of most fatty acids in the LT muscle of both fattening periods, except for C15:0 fatty acid, which was significantly higher in the meat of 12-month fattened cattle than in the meat of 15-month fattened cattle ($P < 0.05$). It was also found that the amounts of SFA, UFA, MUFA, PUFA, and PUFA:SFA also did not show statistically significant differences ($P > 0.05$). When SFA and UFA were considered, it was found that the amount of UFA, an unsaturated healthy fatty acid, was higher than that of SFA, a saturated fatty acid. The level of C17:0 fatty acids tended to be higher in the meat of steers fattened for 12 months than in the meat of steers fattened for 15 months ($P=0.080$).

The influence of the different fattening periods had no effect on the content of ribonucleotides, hypoxanthine, inosine, and IMP. However, GMP content was found to have statistically significant differences ($P< 0.05$) as shown in Table 3. After 12 months of fattening, GMP content in meat of crossbred steers was higher than steers fattened for 15 months.

Table 3. Effect of fattening period on ribonucleotides content of *Longissimus thoracis* of Charolais crossbred steers

Ribonucleotide[1]	Fattening period		P-value
	12 months	15 months	
Hypoxanthine	19.75 ± 3.95	20.93 ± 5.1	0.664
Inosine	57.36 ± 5.01	59.58 ± 15.99	0.752
IMP	249.01 ± 23.37	267.95 ± 47.71	0.403
GMP	8.78 ± 3.83[a]	4.57 ± 1.09[b]	0.027

[a,b] Means in rows with different superscripts are significant different at $p < 0.05$.
[1] 1mg/100 g.

Discussion

The results showed that the different 12 and 15 months fattened periods did not affect the nutrient composition. The range of nutrient composition was 4.38-4.66% for fat content, 71.14-72.79% for moisture content, 1.23-1.24% for ash content, and 23.60-23.70 % for protein content. Maher *et al.* (2004) reported that meat of Charolais purebred at slaughter weight of 647 kg and age at 15 months had fat 1.03%, moisture 75.67%, protein 22.39%. Waritthitham *et al.* (2010) reported that Thai native crossed with Charolais with slaughter weight 553 kg and an average age 29 months had fat 2.36%, moisture 73.90%, ash 2.36%, and protein 22.36%, respectively. Nutrient composition of LT muscle of Charolaise crossbred steers in the current study were slightly different from other studies as aforemention, might be due to the effect of breed, fattening duration, and also diet.

In this study, fattening duration had no significant effect on cholesterol content, which was 135.88 and 136.29 mg/100 g at 12 and 15 months of fattening duration, with mean weights of 604.34 and 724.49 kg, respectively. The results differ from the study of Bureš *et al.* (2006), according to which the LT meat of Charolais slaughtered at 630 kg had cholesterol content of 63 mg/100 g. Chaiwang, *et al.* (2015) showed that 75% Charolais × 25% Thai indigenous crosses had a cholesterol content of 95.5 mg/100 g in *Longissimus dorsi* muscle at a slaughter weight of 649 kg.

In the current study, LT muscle of 12 month fattened period had significantly higher C15:0 and tended to have higher C17:0 than those from LT muscle of 15 month fattened period. Venn-Watson *et al.* (2020) reported that higher circulating concentrations of odd chain fatty acids (OCFAs), pentadecanoic acid (C15:0) and heptadecanoic acid (C17:0), are associated with lower risk of cardiometabolic disease and that higher dietary intake of OCFAs is associated with lower mortality. Therefore, the higher amount of C15:0 and C17:0 found in the shorter 12-month fattening period might be better for health-conscious consumers than a longer fattening period. In addition, the P:S ratio of steers from the 12-fattening period was 1.51, which could be better for health as mentioned by Kang *et al.* (2004). To reduce the risk of cardiovascular disease, the desired dietary P:S ratio should be about 1.0-1.5, so that the serum low-density lipoprotein cholesterol to high-density lipoprotein cholesterol (LDL-C/HDL-C) ratio can be kept within a reasonable range of no more than 5 (Kang *et al.*, 2005).

It is well known that during postmortem aging muscles are converted into meat, which serves as a food source. The taste and texture of meat are improved by postmortem aging. Nucleotide triphosphates such as adenosine triphosphate

(ATP) and guanosine triphosphate (GTP) are degraded during postmortem meat aging, producing umami-related chemicals such as IMP and guanosine monophosphate (GMP) (Muroya *et al.*, 2019; Tikk *et al.*, 2006). Adenosine triphosphate is degraded to adenosine diphosphate (ADP) and adenosine monophosphate (AMP), both of which are in turn degraded to IMP. When IMP is degraded, inosine and hypoxanthine are formed (Muroya *et al.*, 2019; Tikk *et al.*, 2006). Inosine is tasteless, but hypoxanthine has a bitter taste (Jones, 1969; Tikk *et al.*, 2006). In the current study, there were no significant differences in IMP, inosine and hypoxanthine, but GMP content was higher in the muscle of 12 months fattened steers than 15 months fattened steers. Therefore, the meat from the shorter 12-month fattening phase might be more palatable than the meat from the longer 15-month fattening phase.

In conclusion, the shorter 12-month fattening period may be more suitable for beef production, as there was no significant difference in intramuscular fat between the two fattening periods. However, the meat from the shorter 12-month fattening period had a better fatty acid composition and also had a high content of umami related GMP.

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