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Fatty acid profile and meat quality of muscles from crossbred Angus-Simmental, Wagyu-Simmental, and Chinese Simmental cattles

Running head: breed differences in fatty acid profile and meat quality

Abstract

This study assessed breed differences in fatty acid composition and meat quality of Longissimus thoracis et lumborum (LTL) and semitendinosus (SE) of Angus x Chinese Simmental (AS), Wagyu x Chinese Simmental (WS), and Chinese Simmental (CS). CS (n=9), AS (n=9) and WS (n=9) were randomly selected from a herd of 80 bulls which were fed and managed under similar conditions. Fatty acid profile and meat quality parameters were analyzed in duplicate. Significant breed difference was observed in fatty acid and meat quality profiles. AS exhibited significantly ($P < 0.05$) lower C16:0 and higher C18:1n9c compared with CS. AS breed also had a tendency ($P < 0.10$) to lower total SFA, improve C18:3n3 and total UFA compared with CS. Crossbreed of AS and WS had significantly ($P < 0.05$) improved the lightness, redness, and yellowness of muscles, and lowered cooking loss, pressing loss, and shear force compared with CS. These results indicated that fatty acid composition and meat quality generally differed among breeds, although the differences were not always similar in different tissues. Fatty acid composition, meat color, water holding capacity, and tenderness favored AS over CS. Thus, Angus cattle might be used to improve fatty acid and meat quality profiles of CS, and AS might contain better nutritive value, organoleptic properties, and flavor, and could be potentially developed as an ideal commercial crossbreed.

Keywords: Angus; Chinese Simmental; Wagyu; Crossbreed; fatty acid; meat quality.

Introduction

In recent year, significant changes have taken place with respect to beef consumption in China. In 1996, the per capita consumption of beef was 2.82 kg, which increased to 5.33 kg in 2014. The emphases on healthy life style and dietary habit of consumers have increased the demand for more flavorful and healthier meat.(Resurreccion, 2004)

Beef fatty acid composition has received increasing attention due to their correlation for nutritional value, meat quality, palatability, and associated roles in human health.(Wood et al., 2008) It has been proven in previous studies that eating quality, sensory properties, meat color, and shelf life are affected by the variety and amount of fatty acids in beef muscles.(Calkins and Hodgen, 2007; Wood et al., 2004) For example, oleic acid (C18:1n-9) has positive correlation with beef flavour, while the ratio of monounsaturated to saturated fatty acids (MUFA : SFA) affects the taste and texture of beef.(Garmyn et al., 2011)

Both non-genetic (feedstuff, fatness and age) and genetic (breed, sex and genotype) factors affect the fatty acid profile of meat.(De Smet et al., 2004; Malau-Aduli et al., 2000) Breed is among the factors with a major influence on the fatty acid profile and meat quality of beef.(Nuernberg et al., 2005) Breed differences in fatty acid compositions have been reported in the intramuscular fat of Angus, Hereford and their crossbreed,(Papaleo Mazzucco et al., 2016) subcutaneous and intramuscular fat of Wagyu and Aberdeen Angus steers,(May et al., 1993) intramuscular triacyglycerol and polar lipids of Simmental and Aberdeen Angus steers,(Itoh et al., 1999) and intramuscular fat of Charolais, Hereford, Aberdeen Angus, and Simmental bulls.(Bures et al., 2006) Therefore, it is likely that selecting genetically superior cattle can improve the contents of beneficial fatty acids and meat quality.

Currently, Chinese Simmental, with its larger body size, fast growth and low intramuscular fat content features, is one of the most abundant breeds in western China. Angus and Wagyu beefs are the two most well-known breeds which are both known for their superior marbled appearance together with excellent favour, tenderness and meat color.(Maltin et al., 2007) The present study aimed to determine breed differences in fatty acid profile and meat quality of Longissimus thoracis et lumborum (LTL) and semitendinosus (SE) muscles of Angus x Chinese Simmental (AS) F_1 bulls, Wagyu x Chinese Simmental (WS) F_1 bulls, and Chinese Simmental (CS). We hypothesized that the composition of fatty acids and the quality of meat in Chinese Simmental could be improved by crossbreeding with Angus or Wagyu.

Material and Methods

Animal and harvest

This study was approved by Animal Care and Use Committee of Gansu Agricultural University (Approved No. 2012-2-159). All animal procedures were consistent with the Regulations for the Administration of Affairs Concerning Experimental Animals (The State Science and Technology Commission of P.R. China, 1988). Animals were harvested in conformity with the national standards of humane food animal harvesting and processing. Chinese Simmental bulls (CS, n=9), Angus (♂) x Chinese Simmental (♀) F_1 bulls (AS, n=9) and Wagyu (♂) x Chinese Simmental (♀) F_1 bulls (WS, n=9) were randomly selected from a herd of 80 bulls for a 180 d feeding trial after 14 d of conditioning period. All animals were fed and managed under similar conditions at JinChang. Animals at different growth periods were fed according to NRC requirements for the class and weight of the animals (Table 1). Both AS and WS were bred by artificial insemination with Angus and Wagyu sperm from American bulls.

AS bulls were sired by 5 Angus bulls (Frozen semen numbers 014AN00365, 7AN00437, 14AN00513, 7AN00358, 7AN00437), and WS bulls were sired by 5 Wagyu bulls (Frozen semen numbers KSNJHN12050400, KSNJHN120416008, KSNJHN120409008, KSNJHN120423008, KSNJHN120410008). CS bulls were chosen from the progeny from 100 heads CS sire. At December 23th, 2018, all animals were transported to a commercial facility 97 km from the research center in Wuwei, and slaughtered after 0 min lairage time. Carcasses were chilled at 4 ℃ for 72 h. After aging, LTL and SE muscles were obtained from the left side of each animal carcass, individually vacuum packed, identified by animal number, and frozen at −20 °C until the time at which analyses were performed. All samples were analyzed in duplicate.

Fatty acid analysis

Analysis of fatty acid composition in muscles was conducted following the previously published protocol with some modification.(O'Fallon et al., 2007) Samples were uniformly distributed by grinding in liquid nitrogen. One gram of each sample was placed into a 16 x 125 mm screw-cap Pyrex culture tube, added with 5.3 mL of MeOH, and 0.7 ml of 10 N KOH in water. Then, the tube was incubated in a water bath at 55 ℃ for 2 h with vigorous shaking for 10 s every 20 min to promote proper permeation, dissolution, and hydrolysis. After incubation, the samples were cooled to below room temperature in a cold water bath. Then, 0.58 mL of 24N H_2SO_4 in water was added, and the tubes were mixed by inversion. Once the precipitate of K_2SO_4 was present, the samples were incubated again in a water bath at 55 ℃ for 2 h with hand-shaking for 10 s every 20 min. After fatty acid methyl esters (FAME) synthesized, the samples were cooled again in a cold water bath. Then, samples were added with 3 mL of hexane, and the tubes were vortexed on a multitube vortex for 5 min followed by 5 min centrifugalization in a tabletop centrifuge. The hexane layer containing the FAME was collected and placed into a gas chromatography (GC) vial. The vial was capped and placed at -20 ℃ until GC analysis. Gas chromatography (model 6890 N, Aglient Technology, Wilmington, DE, USA) was used to separate and quantify the derivatized methyl ester of fatty acids. A fused-silica column (SP-2560; Sigma-Aldrich, Co., St. Louis, MO), with 100 m × 0.25 mm × 0.2 μm film thickness, was applied for the chromatographic separations. Carrier gas was nitrogen, with a split ratio of 100:1 and a column flow rate of 1 mL/min. The injector temperature was set at 260 ℃. The temperature of the gas chromatograph column oven was initially programmed at 140 ℃ for 4 min and then increased at a rate of 4 ℃/min from 140 ℃ to 230 ℃, 2 ℃/min from 230 ℃ to 240 ℃ and then maintained at 240 ℃ for 10 min. Thirty-seven FAME preparations (Supelco 37 Component FAME mix standard, Sigma, St. Louis, MO) were injected respectively to relate the peaks to known FAMEs. The concentrations of each fatty acid from areas under the peaks, which were those adjacent to FAME in the standard mixture, were calculated using the retention times. The fatty acid concentration was expressed as the percentage of an individual fatty acid in the total fatty acid composition.

Meat quality evaluation

The pH values were measured directly in LTL muscle (at the 3rd and 4th reciprocal thoracic vertebrae) and in SE muscle (at a designated position) using a portable pH Meter HI98103 (Beijing Taiyasaifu Co., Ltd, Beijing, China). The pH values given in the table were the averages of three measurements of each carcass. The meat color was assessed using a Minolta colorimeter (Chroma Meter CR-400, Minolta Camera Co. Ltd., Osaka, Japan) to determine color coordinate values for *L**- (lightness), *a** (redness), and $b*$ (yellowness) following procedures of the Commission International de l'Eclairage (CIE). Reading of each of the $L*$, $a*$, and $b*$ values were taken at 3 spots on the surface, and each spot was repeated 4 times per 15 cm^2. The values were averaged to obtain a representative reading of the surface color.

Meat samples with 2.5 cm thick of similar geometry were applied for determination of retort cooking lost. Samples were weighed, wrapped in a heat-resistant vacuum bag, and then cooked in a constant temperature water bath of 80 ℃ to a final internal temperature of 70 ℃. Internal temperature was monitored with a thermometer (with diameter of 0.5 cm) inserted into the geometric center of the samples. At the final temperature, each sample was cooled in room temperature to 20 ℃, dried with filter paper, and weighed. Raw and final sample weights were used to determine retort cooking loss.

Approximately 30 g of steak with similar geometry were weighed, and placed into a steamer of 100 ℃ for 30 min. Then, samples were cooled to room temperature, and weighed again. The difference between raw and heated weights was recorded as moist cooking lost and expressed as a proportion of the raw weight.

Raw samples of 1.0 cm thick were used for the determination of pressing lost. Samples were weighed to 0.001 g, wrapped with gauze, and then sandwiched between 18 layers of filter paper with good water absorption, top to bottom. A weight of 35 kg was applied for 5 min and weight was recorded immediately after press. The difference between initial weight and post pressing weights was recorded as pressing lost and expressed as a proportion of the initial weight.

Meat samples with a center temperature of 0 ~ 4 ℃ were obtained, cooked in a constant temperature water bath of 80 ℃ to an internal temperature of 70 ℃. At the final temperature, samples were removed from the bath and cooled to an internal temperature of 0 ~ 4 ℃. At least three 1.27 cm diameter cores were removed from each sample parallel to the muscle fiber orientation. A peak shear force was obtained for each core perpendicular to muscle fiber orientation with a TA-XT Plus Texture Analyzer (Stable Micro System, Godalming, UK) equipped with a Warner-Bratzler shear head, and the value reported for each sample was the average of at least three evaluated cores.

Statistical analysis

The effect of breeds and tissues on fatty acid composition was assessed using PROC MIXED (SAS, USA). The linear model used was:

$Yijk = \mu + Si + Gj + SGij + ek\ (ij)$,

where:

Yijk is the observed value of the kth animal in the ith breeds and jth tissues, μ is the mean value common to all observations, Si the fixed effects of the ith breeds, Gj the fixed effects of the jth tissues, SGij the fixed interaction between the ith breeds and jth tissues, and ek (ij) is the random deviation of the kth animal in the ith breeds and jth tissues. The differences among means from different breeds were determined using one-way analysis of variance (ANOVA). For all variables analyzed, a *P-value* of < 0.05 or < 0.01 was considered as statistically significance, while $0.05 < P < 0.10$ was identified as a trend.

Results and Discussion

Slaughter traits

A summary of slaughter traits was given in Table 2. No significant difference was found in slaughter weight, body side length, heart girth, chest width, and cannon circumference among the three breeds. CS bulls showed significantly higher values *($P < 0.05$)* of height at withers compared with WS. Chest depth was significantly higher *($P < 0.05$)* in CS bulls than in AS bulls. Also, CS bulls had significantly larger *($P < 0.05$)* hind leg circumference ($P < 0.05$) compared with AS and WS breeds. It was observed that the carcass traits of AS and WS crossbreeds were not superior to CS bulls. Compared with Wagyu and Angus, CS breed has larger birth weight, rapid growth rate, and later maturing characteristics.(Bures et al., 2006) Thus, crossbreeding CS with Wagyu and Angus might not lead to significant crossbreeding effect in carcass traits.(Papaleo Mazzucco et al., 2016)Fatty acid composition

The intramuscular saturated fatty acid (SFA) composition of the LTL and SE muscles in the three breeds was presented in Table 3. Total SFA took up approximately 50% of all fatty acids in AS, WS, and CS breeds, with palmitic acid (C16:0), stearic acid (C18:0), and myristic acid (C14:0) together dominantly comprised more than 90% of total SFA. Similar profiles were also presented in other literatures investigating Wagyu,(Kazala et al., 1999; Mir et al., 2000) Angus,(Purchas et al., 2005) Yak,(Zhang et al., 2009) and other crossbred beefs.(Coleman et al., 2016)

Breed difference was expressed in several fatty acids. C16:0 was significantly higher *($P < 0.05$)* in CS compared with AS breed in SE muscle, while C14:0 tended *($P < 0.1$)* to be higher in CS than in AS in LTL muscle. It is generally accepted that some SFA that are commonly found in meat, especially C16:0 and C14:0, raise the total cholesterol and low-density lipoprotein, and are thus risk factors in coronary heart disease.(Erkkilä et al., 2008; Webb and O'Neill, 2008) Thus, AS breed, with lower proportion of C16:0 and C14:0, might be more beneficial to human health. WS was found to have significantly higher *($P < 0.05$)* heptadecanoic acid (C17:0) compared with AS and higher *($P < 0.01$)* lignoceric acid (C24:0) compared with CS in LTL muscle. In addition, AS tended to have lower *($P < 0.1$)* caproic acid (C6:0) than CS, lower *($P < 0.1$)* lauric acid (C12:0) than WS, and lower *($P < 0.1$)* total SFA than CS in LTL muscle. SFA is recognized as a critical predisposing factor in the development of cardiovascular diseases, and is implicated in cancers, obesity, diabetes and other health problems.(Briggs et al., 2017; Pighin et al., 2016) Therefore, dietary recommendation promote foods that are low in saturated fat. Taken together, crossbreed of Angus (♂) x Simmental (♀) might have a preferable SFA profile that is more satisfied for the need of modern consumers than Wagyu (♂) x Simmental (♀) and Simmental (♀), with significant lower C16:0 and C17:0, and a tendency to lower C6:0, C12:0, C14:0, and total SFA content.

The intramuscular unsaturated fatty acid (UFA) composition of the LTL and SE muscles in the three breeds was presented in Table 4. Total UFA ranged from 46.03% to 50.50% in LTL muscle, and from 50.24% to 53.35% in SE muscle. Monounsaturated fatty acids (MUFA) comprised the largest proportion of UFA, with oleic acid (C18:1n9c) being the most abundant. These results are in consistent with other studies on beef.(Blanco et al., 2009; Domingo et al., 2015; Papaleo Mazzucco et al., 2016) Previous investigation demonstrated that C18:1n9c could reduce LDL cholesterol to prevent arteriosclerosis without decreasing the level of the beneficial HDL cholesterol in humans.(Enser et al., 1998) C18:1n9 is suggested to be positively associated with the softness of fat.(Vahmani et al., 2015) Also, higher proportion of C18:1n9c could improve the sensory quality of beef.(Van Ba et al., 2013) Significant breed difference was detected in the value of C18:1n9c. AS expressed significantly higher ($P < 0.05$) proportion of C18:1n9c compared with CS breed, which might be an advantage for AS breed.

In addition, breed difference tended to exerted in several UFAs. For SE muscle, AS tended ($P < 0.10$) to have higher myristoleic acid (C14:1) compared with WS, and higher linolenic acid (C18:3n3) compared with CS; while WS tended ($P < 0.10$) to have higher cis-10-pentadecenoic acid (C15:1) than AS and CS, and higher cis-13,16-docosadienoic acid (C22:2) than AS. C18:3n3 is one of the polyunsaturated fatty acid (PUFA) considered good for human health.(Widmann et al., 2011) Here, the tendency of higher C18:3n3 in AS breed was in consistent with some previous investigations which reported higher ($P < 0.01$) C18:3n3 content in Aberdeen Angus relative to Charolais, Simmental, and Hereford bulls.(Bures et al., 2006; Itoh et al., 1999) For LTL muscle, C16:1 and C22:1n9 had a tendency ($P < 0.10$) to be higher in CS than in AS breed. WS tended ($P < 0.10$) to be higher in C20:1 and C22:2 compared with AS. While C24:1 and total UFA tended ($P < 0.10$) to be higher in AS than in CS breed. The tendency of higher total UFA proportion in AS might be attributed to the significantly higher percentage of C18:1n9c in AS compared with CS. UFA have a certain protective effect against the cardiovascular disease, and could delay the occurrence of atherosclerosis disease.(Nogi et al., 2011) Thus, ongoing efforts have been put into improving the UFA profile in beef to provide a more desirable beef product for consumers' need. These data suggested that the content of C18:1n9c, C18:3n3, and total UFA in Chinese Simmental could be enhanced by cross-breeding with Angus cattle due to positive heterosis, and Angus x Simmental breed might be a better choice both for flavor and health.

To evaluate the nutritional properties of intramuscular fat, the ratio of PUFA/SFA, n-6/n-3, SFA/UFA, and MUFA/PUFA was determined (Table 5). Breed difference was observed in the ratio of SFA/UFA and MUFA/PUFA in LTL muscle. AS presented significantly lower ($P < 0.05$) SFA/UFA ratio compared with CS. The ratio of MUFA/PUFA was significantly lower ($P < 0.05$) in WS than in CS. High ratio of SFA/UFA is believed to have strong correlation with many pathological states in humans, such as increased risks of vascular and coronary diseases.(Calder and J Deckelbaum, 2003) Thus, lower ratio SFA is preferable.(Piot et al., 1998) It is suggested that to minimize the intake of SFA and enhance the intake of PUFA can minimize the risk of cardiovascular diseases.(Hoffman and Wiklund, 2006; Wood JD et al., 2003) Thus, Many have focused on producing meat with a higher ratio of PUFA/SFA.(Wood et al., 2004) The PUFA/SFA ratio in this study showed mean values ranged from 0.14 to 0.29, which were lower than the recommendations (0.45) of the British Department of Health (1994).(Department of Health, 1994) However, beef typically has a ratio of 0.1,(Enser, 2000) and similar values were found for this ratio in other purebred and crossbred beef.(Bermingham et al., 2018; Bhuiyan et al., 2017; Domingo et al., 2015; Piao et al., 2019) Significant breed difference did not express in the PUFA/SFA ratio. Yet, AS had a numerically highest value of 0.29 in SE muscle, which might be an advantage.

An excessive amount of n-3 PUFAs and a high n-6/n-3 ratio implicate in the promotion of many diseases.(Przybylski and Hopkins, 2015) PUFA from the n-6 series are involved in the synthesis of eicosanoids biologically active in very small quantities and with properties much more inflammatory than eicosanoids from the n-3 series. (Simopoulos, 2002) Thus, nutritional guideline recommends to minimize the intake of n-6 fatty acids relative to n-3 fatty acids.(Department of Health, 1994) The obtained n-6/n-3 ratio in this study ranged from 5.34 to 12.79, which was all exceed nutritional recommendations of 0.45.(Department of Health, 1994) The results obtained in other studies assessing Galician Blond,(Bispo et al., 2010) Belgian Blue and Limousin,(Cuvelier et al., 2006) and crossbreed of Holstein with Gallega, Limousine, and Belgian Blue,(Domingo et al., 2015) showed similar behavior and were higher than those showed in this work. Significant breed difference did not express in the n-6/n-3 ratio. Yet, AS had a numerically lowest value of 5.34 in LTL muscle. Thus, AS breed might have slight edge than WS and CS in the context of human health.

Meat quality

Results related to meat quality were presented in Table 6. The pH values were measured 72 h post mortem. In LTL muscle, the pH value was significantly higher $(P < 0.05)$ in AS and WS compared with CS. Both AS and WS breeds had a pH value over 6, which exceeded the normal range for beef (5.4-5.8).(Mueller et al., 2019; Zheng et al., 2018) Preslaughter conditions, stress, muscle physiology, and breed might be associated with these atypical pHs.(Oliveira et al., 2012) The pH values in SE muscle ranged between 5.65 and 5.86, which was within normal range. Significant breed difference also expressed in the meat color profile. WS showed significantly higher $(P < 0.05)$ CIE L^*- (lightness) compared with CS, higher $(P < 0.05)$ CIE a^* (redness) and CIE b^* (yellowness) compared with AS and CS in LTL muscle. While AS had significantly higher $(P < 0.05)$ CIE a^* (redness) and CIE b^* (yellowness) compared with CS in SE muscle. Meat color is a dominant factor that affects consumer acceptance, purchasing decisions, and satisfaction, since meat color is used as an indicator of freshness and wholesomeness.(Lawrie, 2006; Mancini and Hunt,

2005) Results from this study indicated that crossbreeds of AS and WS could produce visually more appealing meat with lighter, more yellow-red and a more saturated colour.

Water holding capacity is known as the ability of muscle to bind water under a given set of conditions, which is related to sensory characteristics of meat regarding flavor and juiciness, and even economic efficiency.(Lawrie, 2006) Significant breed differences were exhibited for water holding capacity parameters. Cooking losses in this study remained between 11.23% and 34.87%, within the normal range for beef.(Muchenje et al., 2009) AS and WS exhibited significantly lower *($P < 0.05$)* retort cooking loss, pressing loss, and moist cooking loss compared with CS in LTL muscle. WS showed significantly lower *($P < 0.05$)* pressing loss compared with CS, and AS had significantly lower *($P < 0.05$)* moist cooking loss compared with CS in SE muscle. Cooking loss and pressing loss are both negatively associated with the water holding capacity and are used as indicators of meat juiciness.(Cao et al., 2019) There results suggested that AS and WS crossbreeds might improve the water holding capacity and juiciness of LTL and SE muscles.

Tenderness is the most important determinant of meat quality, which can be quantified by the Warner-Bratzler shear force test.(Cao et al., 2019; Przybylski and Hopkins, 2015) The mean shear force found in this study ranged from 2.20 to 4.46 kg/cm^2, which was within the limit for the tenderness in beef (4.5 kg/cm2).(Belew et al., 2003) Besides, significant breed differences were for shear force values. AS and WS had significantly lower *($P < 0.05$)* shear force compared with CS in both LTL and SE muscles, and more than a 1.5-fold decrease was observed in the shear force of AS compared with CS. As tenderness increased with a decrease in shear force,(Bhuiyan et al., 2017) AS and WS crossbreeds might produce more tender meat, with AS has a slight edge over WS.

Conclusion

Collectively, breed difference exists in fatty acid profile and meat quality from beefs of different muscles, indicating that it may be possible to crossbred Angus or Wagyu with Chinese Simmental to enhance the quality of beef. For meat quality, both Wagyu x Chinese Simmental and Angus x Chinese Simmental crossbreed improved meat color, water holding capacity, and tenderness of Chinese Simmental. Considering fatty acid profile, crossbreed of Angus x Chinese Simmental maybe a preferable choice with significantly less palmitic acid (C16:0), more oleic acid (C18:1n9c), and a tendency to lower total SFA and improve total UFA, to provide consumers a healthier beef product with more juiciness and tenderness. However, many factors, such as slaughter weight, gender, age, feedstock ect., can affect the fatty acid composition and meat quality in tissues, meaning that future research is needed to evaluate the effect of these factors have on fatty acids and meat quality in Chinese Simmental crossbred to verify our results.

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Table 1. Feedlot rations for all breeds

Feedstuff(%)	Stage weight(Kg)						
	270-315	315-360	360-405	405-450	450-495	496-540	540-585
Corn	53.71	74.28	78.75	81.38	84.49	86.64	75.47
Flax	30.34	19.27	14.66	10.29	7.71	5.48	12.14
Mountain flour	1.15	0.76	0.61	0.45	0.35	0.3	0.47
Salt	4.19	-	-	-	-	-	-
Calcium hydrophosphate	-	-	0.09	0.16	0.2	0.2	-
Bicarb	2.23	-	-	1.42	1.45	1.48	2.38
[1]Premix	8.91	5.69	5.89	5.68	5.81	5.9	9.54
Total	100	100	100	100	100	100	100
Nutritional standard							
ADG(kg/d)	1.125	1.35	1.35	1.35	1.35	1.35	1.125
CP(% DM)	11.6	12.29	11.34	10.71	10.08	9.66	9.59
TDN(% DM)	70	76.98	77	76.85	76.86	76.87	72.3
NEm(Mcal/100 kg)	166.81	186.67	186.67	186.67	186.67	186.67	172.67
NEg(Mcal/100kg)	108.87	134.77	124.76	125.11	125.09	125.08	113.09
Ca(% DM)	0.53	0.49	0.44	0.39	0.35	0.32	0.29
P (% DM)	0.27	0.25	0.24	0.23	0.22	0.21	0.21
DMI (Kg/d)	6.75	7.425	8.19	8.955	9.675	10.395	10.62

ADG, average daily gain; CP, crude protein; TDN, total digestible nutrient; NE, net energy; Ca, calcium; P, phosphorus; DMI, dry matter intake.

[1] Vitamin-mineral premix: A,D 3, E, Mn, Zn, Fe, Cu, Se, I, Co.

Table 2: Least squares means and standard errors for slaughter traits of Angus x Chinese Simmental, Wagyu x Chinese, and Chinese Simmental in longissimus dorsi and semitendinosus muscles.

Item	AS	WS	CS	P-Values
Slaughter weight (kg)	602.44±59.95	600.00±76.11	586.22±44.38	
Height at withers (cm)	129.06±4.32^{ab}	125.44±4.10^{b}	129.44±3.47^{a}	**
Body side length (cm)	153.67±8.31	151.28±8.05	148.44±6.84	
Heart girth (cm)	206.33±8.90	204.33±8.90	209.67±7.52	
Chest depth (cm)	69.11±2.84^{b}	69.56±3.64^{ab}	72.67±3.35^{a}	**
Chest width (cm)	50.94±2.38	53.44±4.13	54.33±5.87	
Hind leg circumference (cm)	53.89±2.57^{b}	56.11±4.70^{b}	62.11±2.57^{a}	**
Cannon circumference (cm)	20.60±1.42	20.78±1.63	20.39±1.36	

AS, Angus x Chinese Simmental; WS, Wagyu x Chinese; CS, Chinese Simmental; LTL, longissimus thoracis et lumborum; SE, semitendinosus muscles.

* P < 0.1; ** P< 0.05; ***P < 0.01.

^{a,b} Values in the same line with different capital letter superscripts mean samples have significant difference. The same as below.

Table 3: Least squares means and standard errors for saturated fatty acids compositions of Angus x Chinese Simmental, Wagyu x Chinese, and Chinese Simmental in longissimus dorsi and semitendinosus muscles.

Item	Tissue	AS	WS	CS	P-Values
C4:0	LTL	0.09±0.02	0.06±0.03	0.10±0.25	
	SE	0.05±0.02	0.06±0.03	0.09±0.03	
C6:0	LTL	0.04±0.10^{b}	0.02±0.12^{b}	0.33±0.11^{a}	*
	SE	0.09±0.10	0.06±0.12	0.00±0.00	
C8:0	LTL	0.02±0.03	0.05±0.04	0.09±0.04	
	SE	0.09±0.03	0.07±0.04	0.07±0.04	
C10:0	LTL	0.06±0.02	0.05±0.03	0.10±0.03	
	SE	0.13±0.02	0.07±0.03	0.10±0.03	
C11:0	LTL	0.04±0.01^{a}	0.03±0.01^{ab}	0.01±0.01^{b}	**
	SE	0.01±0.01	0.02±0.01	0.01±0.01	
C12:0	LTL	0.19±0.06^{b}	0.34±0.07^{a}	0.23±0.06^{ab}	*
	SE	0.10±0.06	0.09±0.07	0.12±0.07	
C13:0	LTL	0.31±0.14	0.13±0.17	0.23±0.16	
	SE	0.83±0.14^{a}	0.44±0.18^{b}	0.65±0.17^{ab}	*
C14:0	LTL	1.41±0.27^{b}	1.54±0.32^{ab}	2.17±0.30^{a}	*
	SE	1.74±0.27	1.56±0.35	1.91±0.32	
C15:0	LTL	0.43±0.07^{ab}	0.55±0.08^{a}	0.33±0.08^{b}	*
	SE	0.50±0.07	0.45±0.09	0.55±0.08	
C16:0	LTL	26.56±0.81	26.66±0.97	27.38±0.91	
	SE	23.66±0.81^{b}	25.53±1.05^{ab}	26.80±0.97^{a}	**
C17:0	LTL	0.72±0.24^{b}	1.64±0.29^{a}	0.97±0.27^{ab}	**
	SE	1.21±0.24	1.26±0.31	1.27±0.28	
C18:0	LTL	20.41±1.05	21.33±1.26	21.71±1.18	
	SE	0.00±0.00	0.00±0.00	0.00±0.00	
C20:0	LTL	0.29±0.76	0.27±0.91	1.96±0.85	
	SE	1.33±0.76	0.28±0.96	0.00±0.00	
C21:0	LTL	0.18±0.07	0.20±0.08	0.23±0.08	
	SE	0.36±0.07	0.33±0.09	0.22±0.08	
C22:0	LTL	0.57±0.11	0.44±0.14	0.40±0.13	
	SE	0.30±0.11	0.31±0.14	0.37±0.14	
C23:0	LTL	0.81±0.40	1.04±0.48	0.49±0.45	
	SE	1.72±0.40	1.69±0.52	1.81±0.48	
C24:0	LTL	0.13±0.07^{ab}	0.40±0.09^{a}	0.07±0.08^{b}	***
	SE	0.26±0.07	0.17±0.09	0.25±0.09	
SFA	LTL	52.26±1.63^{b}	54.77±1.95^{ab}	56.81±1.83^{a}	*
	SE	49.18±1.63	49.31±2.09	52.95±1.94	

AS, Angus x Chinese Simmental; WS, Wagyu x Chinese; CS, Chinese Simmental; LTL, longissimus thoracis et lumborum; SE, semitendinosus muscles.

* P < 0.1; ** P< 0.05; ***P < 0.01.

^{a,b} Values in the same line with different capital letter superscripts mean samples have significant difference. The same as below.

Table 4: Least squares means and standard errors for unsaturated fatty acids composition of Angus x Chinese Simmental, Wagyu x Chinese, and Chinese Simmental in longissimus dorsi and semitendinosus muscles.

Item	Tissue	AS	WS	CS	P-Values
C14:1	LTL	0.30±0.10	0.39±0.12	0.35±0.11	
	SE	0.71±0.10^{a}	0.37±0.13^{b}	0.60±0.12^{ab}	*
C15:1	LTL	0.35±0.36	0.64±0.43	0.36±0.40	
	SE	0.73±0.36^{b}	1.90±0.47^{a}	0.58±0.43^{b}	*
C16:1	LTL	0.91±0.36^{b}	1.33±0.43^{ab}	1.91±0.40^{a}	*
	SE	1.82±0.36	1.09±0.47	0.98±0.43	
C17:1	LTL	0.55±0.11	0.54±0.13	0.57±0.12	
	SE	0.54±0.11	0.31±0.14	0.52±0.13	
C18:1N9T	LTL	1.10±0.21	1.20±0.25	1.18±0.23	
	SE	0.76±0.21	0.74±0.27	1.02±0.25	
C18:1N9C	LTL	37.19±1.43^{a}	33.82±1.71^{ab}	32.51±1.60^{b}	**
	SE	33.54±1.43	35.59±1.84	34.03±1.70	
C18:2N6T	LTL	3.00±0.86	2.66±1.02	2.57±0.96	
	SE	2.01±0.86	1.66±1.09	3.79±1.01	
C18:2N6C	LTL	2.68±0.87	3.02±1.04	2.99±0.97	
	SE	8.79±0.87	6.76±1.12	4.93±1.04	
C18:3N6	LTL	0.27±0.08	0.27±0.10	0.17±0.10	
	SE	0.15±0.08	0.27±0.11	0.33±0.10	
C20:1	LTL	0.16±0.06^{b}	0.32±0.07^{a}	0.19±0.07^{ab}	*
	SE	0.19±0.06	0.07±0.08	0.12±0.07	
C18:3N3	LTL	0.15±0.06	0.29±0.07	0.00±0.07	
	SE	0.26±0.06^{a}	0.31±0.08^{a}	0.10±0.07^{b}	*
C20:2	LTL	0.22±0.04	0.33±0.05	0.23±0.05	
	SE	0.13±0.04	0.16±0.05	0.23±0.05	
C20:3n6	LTL	0.75±0.25	0.72±0.30	0.32±0.28	
	SE	0.49±0.25	0.47±0.32	0.47±0.30	
C22:1n9	LTL	0.14±0.16^{b}	0.33±0.19^{ab}	0.59±0.18^{a}	*
	SE	0.63±0.16	0.33±0.20	0.45±0.19	
C20:3n3	LTL	0.49±0.19	0.15±0.23	0.23±0.21	
	SE	0.32±0.19	0.43±0.25	0.44±0.23	
C20:4n6	LTL	0.28±0.23	0.04±0.28	0.38±0.26	
	SE	0.73±0.23	0.21±0.30	0.73±0.28	
C22:2	LTL	0.10±0.05^{ab}	0.23±0.06^{a}	0.07±0.06^{b}	*
	SE	0.13±0.05^{ab}	0.26±0.07^{a}	0.10±0.06^{b}	*
C20:5n3	LTL	0.54±0.14	0.49±0.17	0.28±0.16	
	SE	0.38±0.14	0.27±0.18	0.38±0.17	
C24:1	LTL	0.80±0.15^{a}	0.75±0.18^{ab}	0.39±0.17^{b}	*
	SE	0.56±0.15	0.34±0.20	0.17±0.18	
C22:6n3	LTL	0.52±0.14	0.68±0.16	0.60±0.15	
	SE	0.47±0.14	0.39±0.17	0.58±0.16	
UFA	LTL	50.50±1.34^{a}	48.19±1.60^{ab}	46.03±1.50^{b}	*
	SE	53.35±1.34	51.95±1.73	50.24±1.60	
MUFA	LTL	41.50±1.49	39.31±1.78	38.05±1.67	
	SE	39.48±1.49	40.67±1.92	38.14±1.78	
PUFA	LTL	9.00±1.17	8.88±1.40	7.99±1.31	

	SE	13.86±1.17	11.18±1.50	12.00±1.39
N6	LTL	7.18±1.09	6.81±1.30	6.28±1.22
	SE	12.18±1.09	9.39±1.40	10.17±1.29
N3	LTL	1.71±0.26	1.61±0.31	1.26±0.29
	SE	1.43±0.26	1.37±0.33	1.85±0.30

AS, Angus x Chinese Simmental; WS, Wagyu x Chinese; CS, Chinese Simmental; LTL, longissimus thoracis et lumborum; SE, semitendinosus muscles.

* P < 0.1; ** P< 0.05.

[a,b] Values in the same line with different capital letter superscripts mean samples have significant difference.

Table 5: Least squares means and standard errors for fatty acids ratio of Angus x Chinese Simmental, Wagyu x Chinese, and Chinese Simmental in longissimus dorsi and semitendinosus muscles.

Item	Tissue	AS	WS	CS	P-Values
P/S	LTL	0.17±0.02	0.16±0.03	0.14±0.03	
	SE	0.29±0.02	0.23±0.03	0.23±0.03	
N6/N3	LTL	5.34±2.53	5.42±3.02	5.81±2.82	
	SE	9.72±2.53	8.56±3.25	12.79±3.01	
SFA/UFA	LTL	1.04±0.06^{b}	1.14±0.08^{ab}	1.26±0.07^{a}	**
	SE	0.94±0.06	0.98±0.08	1.04±0.07	
MUFA/PUFA	LTL	5.49±0.92^{ab}	4.80±1.09^{b}	8.05±1.02^{a}	**
	SE	2.96±0.92	3.77±1.18	3.57±1.09	

AS, Angus x Chinese Simmental; WS, Wagyu x Chinese; CS, Chinese Simmental; LTL, longissimus thoracis et lumborum; SE, semitendinosus muscles.

** $P < 0.05$.

^{a,b} Values in the same line with different capital letter superscripts mean samples have significant difference.

Table 6: Least squares means and standard errors for meat quality of Angus x Chinese Simmental, Wagyu x Chinese, and Chinese Simmental in longissimus dorsi and semitendinosus muscles.

Item	Tissue	AS	WS	CS	P-Values
pH	LTL	6.21±0.22^{a}	6.06±0.44^{a}	5.72±0.43^{b}	**
	SE	5.65±0.43	5.86±0.43	5.79±0.35	
L*	LTL	49.01±1.38^{ab}	49.79±2.06^{a}	48.72±1.51^{b}	**
	SE	49.30±1.04	49.24±1.53	48.81±1.16	
a*	LTL	7.18±1.68^{b}	8.73±1.70^{a}	6.08±1.81^{b}	**
	SE	5.60±1.77^{a}	5.46±1.31^{a}	4.26±1.03^{b}	**
b*	LTL	13.39±0.55^{b}	13.94±0.77^{a}	12.86±0.83^{c}	**
	SE	12.70±0.66^{a}	12.58±0.69^{ab}	11.89±2.20^{b}	**
Retort cooking loss (%)	LTL	29.27±6.21^{b}	30.15±6.92^{b}	34.87±6.42^{a}	**
	SE	30.68±6.85	32.09±5.60	33.36±7.11	
Pressing loss (%)	LTL	15.15±3.27^{ab}	13.71±4.02^{b}	15.47±6.16^{a}	**
	SE	12.58±4.06^{ab}	11.97±4.03^{b}	14.09±5.39^{a}	**
Moist cooking loss(%)	LTL	13.40±6.79^{b}	15.61±4.37^{b}	20.32±7.58^{a}	**
	SE	11.23±3.06^{b}	12.76±5.42^{ab}	16.02±6.35^{a}	**
Shear force (kg)	LTL	2.20±1.03^{b}	2.87±2.04^{b}	4.03±2.02^{a}	**
	SE	2.97±1.12^{b}	3.19±1.66^{b}	4.46±2.13^{a}	**

AS, Angus x Chinese Simmental; WS, Wagyu x Chinese; CS, Chinese Simmental; LTL, longissimus thoracis et lumborum; SE, semitendinosus muscles.

* $P < 0.1$; ** $P< 0.05$; ***$P < 0.01$.

^{a,b} Values in the same line with different capital letter superscripts mean samples have significant difference. The same as below.