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Cholesterol content and fatty acid composition in *Longissimus dorsi* muscle of purebred and crossbred pigs

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Abstract The fat and cholesterol contents, and fatty acid composition in *Longissimus dorsi* (LD) muscle differ between purebred and crossbred pigs was determined. Duroc purebred (D, n=10), two-way crossbred (Largewhite x Landrace; LWLR, n = 10), and three-way crossbred [Duroc x (Largewhite x Landrace); DLWLR, n=10] pigs were used in this study. Each group consisted of 2 genders (5 barrows and 5 gilts). All pigs were reared under the same housing and feeding conditions for 6 months until slaughtered at 110 ± 5 kg live weight, following which, LD muscle was collected from the left side of each carcass for subsequent analyses. The results showed that D had higher (P<0.01) fat percentage than LWLR and DLWLR. Neither pig group nor gender affected cholesterol content (P > 0.05). For fatty acid composition, D presented highest concentrations of C14:0, C16:0, C16:1, C18:0, C18:1n9c, C18:2n6c, C18:2n6c, C20:1, SFA (saturated fatty acid), MUFA (monounsaturated fatty acid), and PUFA (polyunsaturated fatty acid) than those recorded in samples obtained from DLWLR and LWLR (P<0.01). DLWLR had significantly higher concentrations of C16:1, C18:1n9c and MUFA (P<0.05), with a tendency of higher concentration of C16:0, C18:0, C18:2n6c, C20:1, SFA, and PUFA than LWLR (P<0.1). The ratio of unsaturated to saturated fatty acid (P/S) was more favorable in LWLR compared with those of D and DLWLR (P<0.01). There was a significant group x gender interaction for the concentration of C23:0 (P<0.05). In Duroc purebred pigs, higher amount of C23:0 was observed in gilts than that in the barrow. However, barrow of DLWLR showed higher C23:0 than the amount recorded in the gilts. Fat percentage positively correlated with the concentrations of C14:0, C16:0, C16:1, C18:0, C18:1n9c, C18:2n6c, C20:1, C23:0, SFA, MUFA, PUFA, and P/S (P <0.01) but negatively correlated with C23:0 (P<0.05).

Keywords: Purebred pig, Crossbred pig, Cholesterol content, Fatty acid composition

Introduction

Meat quality is determined by properties such as pH, color, tenderness and juiceness. Intramuscular fat (IMF) is known to influence the eating quality attributes particularly tenderness, juiceness, and flavour (Lonergan *et al*., 2007; Wood, *et al*., 2004). The level of fatness also has an effect on the fatty acid composition as the contents of saturated (SFA) and monounsaturated (MUFA)

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fatty acids increase faster with increasing fatness than does the content of polyunsaturated (PUFA), resulting in a decrease in the polyunsaturated/saturated fatty acids (P/S) ratio (De Smet *et al*., 2004). Meat is a major source of fat in diet, fatty acid composition in intramuscular fat especially of SFA, which have been implicated in diseases such as cardiovascular diseases (CVD). A risk of CVD is significant reduction by replacing SFAs with unsaturated fats, especially PUFA (Wang and Hu, 2017). These authors also state that both n-6 and n-3 polyunsaturated fatty acids are associated with lower CVD risk. Improving pork overall eating quality is very important for the pork industry to enhance the competiveness of any pork market. There are many researchers try to improve intramuscular fat in pork because it influences on many aspects of eating quality. Breeds of pig is one of the most important possibility to increase IMF, especially Duroc (D) breed that had higher marbling than other breeds (Choi *et al*., 2016).

In pig industry, the three-way crossbred pigs are mainly used for commercial pig production due to they have more great production efficiency than purebred and two-way crossbreds (Nelson and Robison, 1976). Duroc is usually included in the crossbreeding system for its paternal traits as it has fast growth, better feed efficiency, higher meat in the carcass, and better meat quality (Choi *et al*., 2014). The maternal line is crossbred pig between Largwhite (LW) and Landrace (LR) giving inherited of fertility, milking ability, and litter size (Kim *et al*., 2006). However, there is not much in formation about cholesterol content and fatty acid composition of Duroc purebred and its crossbreds. Therefore, the aim of this study was conducted to investigate whether three-way crossbred pig can have the superia performance in terms of high intramuscular fat as its Duroc paternal line or not. This study also investigated whether Duroc purebred had different cholesterol content and fatty acid composition when compared to two-way (Largwhite x Landrace; LWLR) and three-way crossbred [Duroc x (Largewhite x Landrace); DLWLR].

Materials and Methods

Animals

The purebred Duroc (D), two-way crossbred (LWLR), and three-way crossbred (DLWLR) were used in this experiment. A total of 30 pigs, 5 barrows and 5 gilts in each pig group were raised and fed at the same condition for 6 months prior to slaughter at 110±5 kg live weight. After slaughter, *Longissimus dorsi* (LD) muscle was taken from each left carcass for analyzing fat content, cholesterol content, and fatty acid composition.

Proximate analyses and cholesterol determination

The moisture and fat content of muscle was determined according to the AOAC (2005) methods.

For cholesterol determination, sample preparation and saponification was based on the method of Du and Ahn, (2002) with slightly modification. Briefly, a 0.4 g of ground meat was weighed into a 50 ml screw-cap test tube, and then 10 mL of saponification reagent prepared by freshly mixing ethanol and 33% (w/v) KOH solution at a ratio of 94:6, 0.5 mL of 20% ascorbic acid, and 50 µL of 5α-cholestane solution (1 µg/µL in hexane) was added immediately. The sample was homogenized with a polytron for 5 sec at full speed, capped, and then incubated for 1 hour at 50 ℃ in a waterbath. After cooling in ice water for 10 min, 10 mL HPLC water and 10 mL hexane were added. Tubes were capped tightly and then the contents were mixed thoroughly by shaking. After 15 hours for phase separation, the sample tubes were centrifuged at 2,500 rpm at 4 ℃ 15 min. The hexane layer containing unsaponifiables was carefully transferred to a scintillation vial and evaporated to dryness under nitrogen flow. The dried sample was redissolved in 200 µl ethyl acetate, and then analyzed cholesterol content by gas chromatography (Agilent 7890, USA) fitted with a flame ionization detector (FID) using a capillary GC column (Zebron ZB-5, Phenomenex, Torrance, CA, USA) (30 m × 0.25 mm x 0.1 µm film thickness). The gas chromatography conditions were as follows: injected temperature, 260 ℃; detector temperature, 285 ℃; carrier gas, He; split ratio, 5 : 1 ; temperature program, initial temperature 200 ℃, followed by an increase of 10 ℃/ min to 260 ℃, and then 5 ℃/min to 285 ℃. Cholesterol identification was made by comparing the relative retention time of peaks from samples with standards from SIGMA (USA). The internal standard used was 5α-cholestane.

Fatty acid analysis

Fatty acid analysis was determined as previously described by Raes *et al*. (2001). In brief, lipids were extracted from fresh meat using chloroform/methanol (2:1, vol/vol). Nonadecanoic acid (19:0) was added as an internal standard. The fatty acid methyl esters (FAME) were analyzed by gas chromatography using a fused silica capillary column (model SP^{TM}-2560, Supelco, Bellfonte, PA) for FAME (100 m × 0.25 mm x 0.2 µm film thickness). The gas chromatography conditions were as follows: injected temperature, 240 ℃; detector temperature, 260 ℃; carrier gas, He; split ratio, 10 : 1 ; temperature program, initial temperature 60 ℃, followed by an increase of 20 ℃/ min to 170 ℃, 5 ℃/min to 220 ℃ then 2 ℃/min to 240 ℃. Fatty acid

methyl ester samples were identified by comparing the retention times of FAME peaks from samples with those of standards obtained from Supelco (Supelco 37 Component Fame Mix 47885, Sigma–Aldrich, Bellefonte, PA). Identification of the peak included fatty acids between C14:0 and C22:6. Fatty acids were expressed as mg/100 g fresh meat. The following fatty acid combinations and ratios were calculated: total saturated fatty acids (SFA), total mono-unsaturated fatty acids (MUFA), polyunsaturated fatty acids (PUFA), PUFA/SFA ratio (P/S) and n-6/n-3 ratio.

Statistical analysis

Analysis of variance was generated by using the GLM procedure (SAS Institute Inc., 2003) with pig group and gender as the main effects with interaction. Least squares means were separated using the probability of difference option (PDIFF), and the results were considered significant difference when $P<0.05$. The relationships between study traits were evaluated by Pearson correlation coefficients.

Results

There was no significant group x gender interactions for the study traits except C 23:0. Therefore, only main effects are presented in Table 1 and Table 2. Neither group of pig nor gender affected the concentration of moisture, cholesterol, C15:0, and C23:0 ($P>0.05$). Duroc purebred had significantly higher fat percentage than LWLR and DLWLR ($P<0.01$) as shown in Table 1. In this study showed that the Duroc purebred pig had the highest fat percentage than two-way crossbred and three-way crossbred.

For fatty acid composition (Table 2), D had the highest concentration of C14:0, C16:0, C16:1, C18:0, C18:1n9c, C18:2n6c, C18:2n6c, C20:1, SFA, MUFA, and PUFA followed by DLWLR and LWLR ($P<0.01$). DLWLR had significantly higher concentration of C16:1, C18:1n9c, and MUFA and also had a trend to have higher concentration of C16:0, C18:0, C18:2n6c, C20:1, SFA, and PUFA than LWLR ($P<0.1$).

In this study, barrow tended to have higher fat percentage than gilt. While gender had little effect on fatty acid composition except barrow had higher C18:0 than gilt ($P<0.05$).

There was a significant group x gender interaction for the concentration of C23:0 ($P<0.05$) as shown in Figure 1. In Duroc purebred pigs, higher amount of C23:0 was observed in gilts than that in the barrow. However, barrow of DLWLR shown higher C23:0 than the amount recorded in the gilts.

Fat percentage had a significant correlation with C14:0 (r = 0.77, P < 0.01), C16:0 (r = 0.78, <0.01), C16:1 (r = 0.77, P < 0.01), C18:0 (r = 0.78, P < 0.01), C18:1n9c (r = 0.78, P < 0.01), C18:2n6C (r = 0.71, P < 0.01), C20:1 (r = 0.76, P < 0.01), C23:0 (r = -0.38, P < 0.05), SFA (r = 0.78, P < 0.01), MUFA (r = 0.78, P < 0.01), PUFA (r = 0.71, P < 0.01), P/S (r = 0.68, P < 0.01).

Table 1. Effect of group and gender on the concentration of moisture, fat, and cholesterol in *Longissimus dorsi* muscle of pigs

Trait	Group[1]			Gender		P-Value		
	D	LWLR	DLWLR	Gilts	Barrows	Group	Gender	Group * Gender
Moisture (%)	73.86±0.43	74.12±0.49	73.96±0.52	74.23±0.39	73.73±0.40	0.873	0.365	0.966
Fat (%)	2.06±0.15 [a]	0.92±0.15 [b]	1.34±0.15 [b]	1.27±0.12	1.62±0.12	<.0001	0.061	0.189
Cholesterol[2]	46.34±4.84	55.15±4.01	59.15±4.25	51.58±3.40	55.51±3.74	0.159	0.432	0.934

[a,b]LSMeans±SE with different superscription within each main effect in the same row differ (p<0.01)
[1/] D : Duroc, LWLR: LargeWhite×Landrace, DLWLR: Duroc × LargeWhite ×Landrace
[2]/mg/100g fresh meat

Table 2. Effect of group and gender on fatty acid compositon (mg/100g) in *Longissimus dorsi* muscle of pigs

Trait[1]	Group			Gender		P-Value		
	D	LWLR	DLWLR	Female	Male	Group	Gender	Group*Gender
C14:0	44.22±3.28[a]	15.82±3.09 [b]	23.08±3.09 [b]	25.94±2.52	29.47±2.62	<0.0001	0.368	0.561
C15:0	15.32±1.52	14.36±1.43	13.46±1.43	14.04±1.17	14.71±1.22	0.670	0.679	0.118
C16:0	722.21±49.15 [a]	290.50±46.34[b]	415.01±46.34[b]	430.21±37.84	521.60±39.38	<0.0001	0.123	0.375
C16:1	92.77±6.12 [a]	41.18±5.77 [c]	59.95±5.77[b]	62.44±4.71	66.83±4.91	<0.0001	0.523	0.650
C18:0	381.32±26.30[a]	134.09±27.79[b]	205.95±24.79[b]	207.90±20.24[d]	273.00±21.07[e]	<0.0001	0.044	0.189
C18:1n9c	1060.98±68.85[a]	459.99±64.91[c]	665.64±64.91[b]	668.52±53.00	789.21±55.16	<0.0001	0.139	0.497
C18:2n6C	198.75±7.77 [a]	124.44±7.33[b]	143.46±7.33[b]	151.71±5.98	159.38±6.23	<0.0001	0.378	0.974
C20:1	21.38±1.9[a]	5.87±1.80[b]	10.79±1.80[b]	11.04±1.47	14.32±1.53	<0.0001	0.141	0.721
C23:0	30.54±1.44	30.95±1.36	30.46±1.36	32.12±1.11	29.17±1.16	0.952	0.102	0.028
SFA[1]	1193.65±78.15[a]	485.74±73.68[b]	688.00±73.68[b]	710.24±60.16	868.10±62.62	<0.0001	0.095	0.342
MUFA[2]	1175.15±76.36[a]	507.05±71.99[c]	736.38±71.99[b]	742.00±58.78	870.38±61.18	<0.0001	0.155	0.525
PUFA[2]	198.75±7.77[a]	124.44±7.33[b]	143.46±7.33[b]	151.71±5.98	159.38±6.23	<0.0001	0.377	0.974
P/S[2]	0.18±0.01 [b]	0.28±0.01[a]	0.21±0.01 [b]	0.23±0.01	0.20±0.01	0.0004	0.132	0.358

[a,b,c] LSMeans±SE with different superscription within each main effect in the same row differ (p<0.01)
[d, e] LSMeans±SE with different superscription within each main effect in the same row differ (p<0.05)
D : Duroc, LWLR: LargeWhite×Landrace, DLWLR: Duroc × LargeWhite ×Landrace
[1]/SFA (Saturated fatty acid) = C14:0 + C15:0 + C16:0 + C18:0 + C23:0
[2]/MUFA (Monounsaturated fatty acid) = C16:1 + C18:1n9c + C20:1
[3/]PUFA (Polyunsaturated fatty acid) = C18:2n6C
[4/]P/S = PUFA/SFA

Table 3. Correlation between % Fat and fatty acid composition

Trait	% Fat	
	r	P-value
C14:0	0.77	<.0001
C15:0	-0.13	0.489
C16:0	0.78	<.0001
C16:1	0.77	<.0001
C18:0	0.78	<.0001
C18:1n9c	0.78	<.0001
C18:2n6C	0.71	<.0001
C20:1	0.76	<.0001
C23:0	-0.38	0.0420
SFA	0.78	<.0001
MUFA	0.78	<.0001
PUFA	0.71	<.0001
P/S	0.68	<.0001

Discussion

In this study showed that the D purebred pig had the highest fat percentage than two-way crossbred and three-way crossbred. In agreement with Kim *et al*. (2006) and Choi *et al*. (2016) who reported that Duroc purebred had higher intramuscular fat than three-way crossbred.

Duroc purebred had higher fat content and also higher fatty acid composition than crossbred pigs. Choi *et al*. (2014) reported that Duroc purebred had higher fat percentage, C16:0 and SFA while had lower C20:1, total unsaturated fatty acids (USFA) and USFA/SFA than three-was crossbred (LandracxYorkshirexDuroc; LYD). Choi *et al.* (2016) also reported significantly higher intramuscular fat and SFA while lower MUFA, PUFA, n-3, and n-6 in Duroc purebred than LYD. Alonso *et al*. (2009) reported that three-way crosbred of Duroc sire line had higher intramuscular fat but lower PUFA and the ratio of unsaturated/saturated fatty acid (P/S) than Pietrain and Large White sire line.

Barrow tended to have higher fat percentage than gilt. Gender had little effect on fatty acid composition except barrow had higher C18:0 than gilt ($P<0.05$). Kim *et al.* (2018) reported no significant difference of intramuscular fat in loin between barrow and gilt, while barrow had significant higher SFA but lower n-6/n-3 than gilt. According to Alonso *et al*. (2009), Semimembranosus

muscle of barrows has lower C18:2n-6, C18:3n-3, C20:2n-6, and P/S than those of gilts ($P<0.05$), while other fatty acid compositions were not significantly different among genders, similar to the results of this study.

There was a significant group x gender interaction for the concentration of C23:0. In Duroc purebred pigs, higher amount of C23:0 was observed in gilts than that in the barrow. However, barrow of DLWLR shown higher C23:0 than the amount recorded in the gilts. This was different from Kim *et al*. (2018) and Alonso *et al*. (2009) that reported no significant interaction between different sire line of three-way crossbred pigs and gender on fatty acid composition.

Fat percentage had a significant correlation with C14:0, C16:0, C16:1, C18:0, C18:1n9c, C18:2n6C, C20:1, C23:0, SFA, MUFA, PUFA, and P/S. The study of Choi *et al*. (2016) showed the highest fat percentage and saturated fatty acid (C14:0, C16:0, C18:0) in Duroc purebred than in crossbred these results may support the positive correlation between the content of intramuscular fat and saturated fatty acid in this study.

It is concluded that the Duroc purebred pig had higher fat percentage, saturated and unsaturated fatty acid, MUFA, and PUFA than crossbred pig but P/S lower than LWLR crossbred. For gender effect, only C 18:0 of barrow was higher than gilt. There were significant correlation between fat percentage and fatty acid composition except with C15:0.

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