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Effects of Dietary Glycine Betaine on Growth and Pork Quality of Finishing Pigs

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ABSTRACT : This study was carried out to compare the growth performance and quality properties of pork from finishing pigs fed different levels of betaine. A total 120 female pigs (Landrace×Yorkshire×Duroc) were fed either a control commercial diet or the control diet supplemented with 2, 4 and 6% betaine for 31 days. The average daily feed intake (ADFI) of the 2% diet was lower than of the other treatment groups. The average daily gain (ADG) for pigs fed betaine diets was significantly higher ($p<0.05$) compared with non-supplemented diets. Feed conversion ratio (FCR) of pigs fed betaine diets was significantly lower ($p<0.05$) compared with non-supplemented diets. pH of loin and ham samples were not significantly different between dietary groups, whereas CIE a* (redness) of pork loin was increased by dietary betaine. Also, the shear force value of loin was significantly higher ($p<0.05$) in pigs given dietary betaine compared with non-supplemented diets, but no significant differences were found in cooking loss by the loin among diets with different levels of betaine ($p>0.05$). Dietary supplementation with betaine decreased total cholesterol concentrations in blood, and increased saturated fatty acid and decreased unsaturated fatty acid levels in muscle. Pigs supplemented with betaine had increased betaine concentrations in the loin muscle. It was concluded that dietary betaine supplementation of finishing pigs can improve growth performance and reduce blood cholesterol concentrations. It was also concluded that dietary betaine produced detectable betaine concentrations in the lion muscle. (**Key Words** : Betaine, Growth Performance, Muscle Types, Betaine Concentration, Pork Quality, Pig)

INTRODUCTION

Betaine (glycine betaine, trimethylglycine) is a naturally occurring, nontoxic amino acid derivative that is commonly found in a large number of plant and animal species (Saarinen et al., 2001). Betaine plays an important function in amino acid and lipid metabolism and antibody production in practical animal production (Verreschi et al., 2000; Kim et al., 2003; Zulkifli et al., 2004). Much of the research with betaine in animals has evaluated effects on growth and carcass traits, and usually different levels of betaine have been fed. Matthews et al. (2001) and Lawrence et al. (2002) reported that addition of betaine to the diet of finishing pigs may result in improved leanness and carcass quality. Betaine has also been suggested for swine for reduction of carcass backfat and for increasing lean mass, as well as for improving feed efficiency (Campell et al., 1995; Cromwell et al., 1999).

Currently, the pig industry is focused on improving pork quality. Matthews et al. (1998) reported that subjective color of the loin muscle in pigs fed 0.125% betaine was decreased. Hur et al. (2007) reported that dietary betaine may improve leanness and redness in different pork muscle parts such as belly, picnic shoulder and ham, but another study found that betaine increased backfat thickness and decreased *longissimus* muscle area (Haydon et al., 1995). Also, Feng et al. (2006) reported that dietary betaine may improve leanness and hepatic betaine-homocysteine methyltransferase (BHMT) activity in barrows fed 0.125% betaine was increased.

Pigs exhibit high plasma levels of cortisol and catecholamines in response to stress (D'Souza et al., 1998). Some studies showed oxidative stress effect of metallothionein (MT) in pigs (Li et al., 2007). Betaine has also been shown to protect cells from osmotic stress. Bagnasco et al. (1986) reported a high level of betaine in cells of the inner medulla of the kidney, which may indicate that betaine plays an important role in intracellular osmotic balance. Lever et al. (2004) reported that betaine is actively

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Table 1. Ingredients and chemical composition of experimental diet (as-fed basis)

Item	Experimental diet
Ingredients	
Yellow corn	67.32
Soybean meal	23.60
Limestone	0.43
Tricalcium phosphate	1.92
Salt	0.30
Vitamin*	0.10
Mineral**	0.10
Animal fat	2.16
Molasses	4.00
Lysine	0.07
Total	100.00
Chemical composition[1]	
Digestible energy (kcal/kg)	3,400.00
Crude protein (%)	16.00
Lysine (%)	0.90

* Vitamin: vit A, 4,000 IU; vit D_3, 800 IU; vit E, 15 IU; vit B_3, 2 mg; thiamin, 8 mg; riboflavin, 2 mg; vit B_{12}, 16 mg, pantothenic acid, 11 mg; niacin, 20 mg; biotin, 0.02 mg.

** Mineral: Cu, 130 mg; Fe, 175 mg; Zn, 100 mg; Mn, 90 mg; I, 0.3 mg; Co, 0.5 mg; Se, 0.2 mg.

The vitamins and minerals were provided per kilogram of complete diet.

[1] All of the data are analytical values except for digestible energy.

accumulated by many mammalian cells under hypertonic conditions. Furthermore, pigs supplemented with betaine had detectable betaine in muscle tissue (Matthews et al., 2001). Thus, these osmo-protectant properties of betaine could affect pork quality, assuming that betaine is accumulated in muscle tissue. However, very little research has been conducted to evaluate the effects of betaine on pork quality in different muscle types and its accumulation in muscle tissue of the pig.

Therefore, the purpose of this study was to determine whether betaine accumulation in muscle tissue is affected by feeding level, and how these levels of betaine affect pork quality in the different muscle types and the growth performance of pigs from 65 to 100 kg.

MATERIALS AND METHODS

Animal diet and experimental design

A total of 120 crossbred (Landrace×Yorkshire×Duroc) female pigs with an average initial weight of 65 kg were randomly allotted to four dietary treatments on the basis of weight. Three replications of ten pigs per replicate were used for each treatment group. The group diets included a corn-soybean meal basal (Table 1) supplemented with 0, 0.2, 0.4 and 0.6% betaine (v/w) for 31 days. Betaine (99% pure) was purchased from a commercial bio-chemical company (Jin-Bio, Jinju, Korea). Experimental diets and water were provided on an *ad libitum* basis throughout the experiment. The pigs were weighed and feed intake was determined weekly up to 100 kg body weight. Average daily gain (ADG), average daily feed intake (ADFI), and feed conversion ratio (FCR) were calculated for all pigs for the experimented period. Pigs were slaughtered at approximately 100 kg live weight and transported to the local slaughter house for electrical stunning followed by exsanguination. After slaughter, each pork carcass was chilled for 24 h at 2-4°C (cold-boned), and samples of loin and ham muscles were dissected from each carcass of deboned pork. All skin, subcutaneous fat and visible connective tissue were removed from fresh pork loin and ham muscles before evaluation for different quality parameters.

Muscle pH and fat content

The pH was measured using a digital pH meter (Model 520A, Orion, USA). Approximately 10 g of sample was cut into small pieces and 90 ml of distilled water was added. A slurry was then made using an homogenizer and the pH was recorded. The pH meter was calibrated daily with standard buffers of pH 4.0 and 7.0 at 25°C. Loin samples from each animal were taken for analysis of fat content by ether extraction (AOAC, 2000).

Meat color

Color (CIE L*, a*, b*) was measured using a Minolta colormeter (CR-400, Tokyo, Japan), with measurements standardized with respect to the white calibration plate. Five readings were taken from the surface of each sample.

Cooking loss and shear force determination

The weight of each sample was taken before and after cooking to determine cooking loss, which was defined as the cooked weight divided by uncooked weight multiplied by 100. Shear force samples were cut using a 1.27-cm core taken parallel to the muscle fiber, heated at 75°C in a water bath, and then cooled to room temperature. Samples were sheared perpendicular to the grain of the muscle fiber using an Instron University Testing Machine (Model 100) fitted with a Warner-Bratzler shearing device with a cross-head speed of 100 mm/mm and a 10 kg load cell.

Fatty acid composition

Extracted fat (80 mg) and 0.4 mg of tricosanoic acid methyl esters (0.4 mg/ml hexane, international standard) were placed into a screw-capped test tube and solvent was removed under nitrogen. One ml of 0.5 N NaOH (in methanol) was added, the sample hydrolyzed for 7 min at 90°C, and then cooled to room temperature over 5 min. Free fatty acid was methylated for 10 min at 90°C with addition of 1 ml 4% H_2SO_4, and then cooled at room temperature for 30 min. Two ml of hexane and distilled

Table 2. Effect of dietary glycine betaine on growth performance of finishing pigs[1]

Item	Glycine betaine (%)			
	0	0.2	0.4	0.6
Initial weight (kg)	65.91±3.39	67.27±4.40	65.55±3.46	67.30±2.49
Final weight (kg)	94.00±3.67	96.50±4.50	101.37±1.21	100.60±1.44
Average daily feed intake (kg/d)	3.12±0.57[a]	2.82±0.41[b]	3.22±0.52[a]	3.22±0.60[a]
Average daily gain (kg/d)	0.91±0.04[d]	0.94±0.04[c]	1.16±0.08[a]	1.07±0.04[b]
Feed conversion ratio	3.45±0.15[a]	3.00±0.15[b]	2.80±0.19[c]	3.01±0.12[b]

[1] Data are means of three replicates of five pigs per replicate.
[a, b, c] Means in the same row with different superscript letters are significantly different ($p<0.05$).

water was added, and 1 ml of supernatant was collected and stored in a freezer (-20°C) until analysis. To determine the content of conjugated linoleic acid esters and total fatty acid, 0.5 μl of collected sample was introduced to the split injection port of a gas chromatograph (GA-17A, Shimadzu, Japan). The gas chromatography conditions were as follows: Initial temperature of column started at 180°C, and was heated to 230°C at 1.5°C/min. Temperature was maintained for 2 min, while temperature of injector and detector was set to 240 and 260°C, respectively. Each fatty acid was identified in the form of a methyl ester by comparing the retention times with the standard acquired from Sigma (Sigma, St. Louis, MO). Identification of the peak included fatty acids between 14:0 and 18:3. The weight percentage of each fatty acid in the total detected fatty acids was adopted as a measurement value.

Cholesterol analysis

Five pigs were randomly selected from each treatment and blood samples were collected via the anterior vena cava at initiation and on day 31 of the experiment. Pigs were held without feed for 12 h before bleeding. After collection, blood samples were transferred into vacuum tubes (Becton Dickinson Vacutainer Systems, Franklin Lakes, NJ) and immediately stored in a refrigerator at -20°C. Samples were then centrifuged at 2,000×g for 30 min and serum was separated. The total cholesterol concentration in serum was determined in an automatic analyzer (Hitachi 747, Japan) by direct enzymatic kits (Boehringer Mannheim, Germany).

Tissue betaine analysis

Approximately 45 min post-mortem, tissue samples were collected from loin muscle for all treatments of pigs. The muscle tissues were stored at -20°C until analysis. Muscle tissue (1 g) was homogenized in 2 ml of 0.1 M sodium acetate buffer (pH 6). Protein was precipitated by adding 1.5 ml of 40% (w/v) trichloroacetetic acid. The solution was mixed on a vortex and then placed on ice for 30 min. The sample was then centrifuged at 5,000×g for 10 min. The supernatant was extracted twice with an equal volume of diethyl ether to remove trichloroacetic acid and to adjust the pH to 3.0. The sample was then diluted to 5 ml with water, filtered through a 0.2 μm PVDF filter, and stored at -20°C. Betaine separation was performed using a cation exchange column of Ca^{2+} type (Aminex HPX-87C, Bio-Red, Hercules, CA). The column temperature was 85°C. The HPLC conditions were: 0.004 M $Ca(NO_3)_2$ with a flow rate of 0.6 ml/min; injection volume was 50 μl; run time was 45 min.

Statistical analysis

Data were analyzed by analysis of variance (ANOVA) using the Statistical Analysis System (SAS) program. If a significant difference was detected, Duncan's multiple range test was employed to determine significance between treatments. Significance level was established at $p<0.05$. Orthogonal polynomials to determine the linear and quadratic effects were used for the increasing dietary betaine levels.

RESULTS AND DICUSSION

Growth performance

Table 2 shows the effect of dietary supplementation with different levels of betaine on growth performance of finishing pigs. The final weights did not show significant differences among the diets with different levels of betaine ($p>0.05$). Dietary supplementation with 0.2% betaine resulted in significantly lower ADFI compared to other treatment groups. The ADG for pigs fed betaine diets was significantly higher ($p<0.05$) than for pigs fed the non-supplemented diet; pigs fed the 0.4% level of betaine had the highest ADG. The FCR of pigs fed betaine diets was significantly lower ($p<0.05$) than the non-supplemented diet (control), and the control had highest the FCR among the treatments. This result indicated that betaine supplementation could improve growth performance and feed efficiency. Previous reports found that feed intake was reduced in gilts fed with 0.125% betaine (Matthews et al., 1998). Numerous researchers have reported that ADG and FCR were improved when a betaine-supplemented diet was fed to pigs (Campell et al., 1995; Yu et al., 2004).

Table 3. Effect of dietary glycine betaine on muscle pH, color, shear force, cooking loss, tissue betaine and cholesterol concentrations of finishing pigs[1]

Item	Glycine betaine (%)			
	0	0.2	0.4	0.6
Loin				
pH (postmortem 24-h)	5.60±0.07	5.55±0.02	5.57±0.04	5.61±0.02
Lightness (CIE L*)	53.13±1.53	53.53±1.41	51.37±1.41	53.39±0.46
Redness (CIE a*)	8.48±0.21^{b}	10.04±0.19^{a}	8.72±0.41^{ab}	9.45±0.65^{ab}
Yellowness (CIE b*)	3.42±0.41	4.04±0.37	4.46±0.45	4.39±0.26
Shear force (kg/cm^2)	3.40±0.07^{a}	3.09±0.05^{b}	2.67±0.09^{c}	2.42±0.09^{c}
Cooking loss (%)	20.87±0.68	23.84±0.84	22.63±1.03	21.32±1.83
Crude fat (%)	2.68±0.10	2.66±0.24	2.57±0.17	2.82±0.28
Ham				
pH (postmortem 24-h)	5.69±0.10	5.70±0.09	5.74±0.05	5.76±0.04
Lightness (CIE L*)	43.45±0.86^{ab}	36.90±3.95^{b}	45.45±1.66^{a}	40.24±1.46^{ab}
Redness (CIE a*)	14.68±1.11	17.40±1.36	15.14±1.58	17.95±0.93
Yellowness (CIE b*)	4.05±0.36^{c}	5.10±0.12^{ab}	4.83±0.32^{b}	5.73±0.17^{a}
Shear force (kg/cm^2)	4.82±0.26	4.45±0.22	4.40±0.13	4.29±0.09
Cooking loss (%)	22.51±0.50^{b}	26.56±0.90^{a}	26.19±0.54^{a}	24.12±0.49^{a}
Tissue betaine and total cholesterol concentrations				
Tissue betaine (loin, mg/g)	0.08±0.01^{b}	0.16±0.02^{a}	0.17±0.04^{a}	0. 19±0.01^{a}
Total cholesterol (mg/dl)	42.96±0.13^{a}	39.14±0.11^{b}	40.95±0.08^{ab}	39.96±0.05^{ab}

[1] Data are means of three replicates of five pigs per replicate.

[a, b, c] Means in the same row with different superscript letters are significantly different ($p<0.05$).

Muscle pH, color, fat content, shear force and cooking loss

Table 3 shows the effect of dietary supplementation with different levels of betaine on muscle pH, color, fat content, shear force and cooking loss in different pork muscle of finishing pigs. In this study, pH of loin and ham was not significantly different on all dietary treatments. In agreement with our result in finishing pigs, Yu et al. (2004) reported that there were no significant effects on pH value. The lightness (CIE L*) and yellowness (CIE b*) were not significantly different among the dietary treatments ($p>0.05$), whereas redness (CIE a*) of loin muscle was significantly higher on 0.2% dietary betaine than in pigs fed non-supplemented diets ($p<0.05$). Also, redness was not significantly affected in the 0.4% and 0.6% betaine groups compared to the non-supplemented diet, although it was numerically higher in pigs fed all levels of betaine. However, the redness of ham muscle was not significantly different among the dietary treatments ($p>0.05$) and dietary supplementation with betaine produced significantly higher yellowness than the non-supplemented diet. Some studies showed a negative effect of betaine on meat color. Matthews et al. (2001) did not observe any effects on color in the loin muscle of pigs fed betaine. Øverland et al. (1999) reported that subjective color was paler in pigs fed betaine. Also, fat content of loin muscle was not significantly different among the dietary treatments ($p>0.05$). Yu et al. (2004) reported that betaine was effective in increasing total lipid of finishing pork. Schrama et al. (2003) also reported that betaine was associated with the deposition of fat in *longissimus* muscle of pork, but this was not found in our study.

There were significant differences in shear force values among the loin samples. The shear force values were significantly lower ($p<0.05$) on dietary betaine than on the non-supplemented diet. There was a linear decrease in shear force value with increasing levels of dietary betaine that was significantly lower in the dietary 0.6% supplemented treatment. Therefore, the significant decrease in shear force values from dietary supplementation with betaine could possibly be due to the tenderizing effects of betaine. In this study, different shear force values of loin samples were expected because researchers had shown that a strong positive relationship between shear force and water-holding capacity existed in meat (Joo et al., 1999; Alvarado and Sams, 2000). However, no significant differences were found in cooking loss among the loins from different levels of betaine supplementation ($p>0.05$). Shear force value of ham was not significantly different among the dietary groups, and the data on the non-supplemented and 0.6% betaine diets showed significant differences with lower cooking loss than on 0.2 and 0.4% betaine ($p<0.05$).

Total cholesterol and tissue betaine contents

As shown in Table 3, dietary supplementation with 0.2% betaine results in significantly lower total cholesterol in pigs when compared with the non-supplemented diet ($p<0.05$). Although serum total cholesterol was not

Table 4. Effect of dietary glycine betaine on fatty acid composition (%) of finishing pigs[1]

Item	Glycine betaine (%)			
	0	0.2	0.4	0.6
Loin				
Myristic acid	1.29±0.06	1.28±0.13	1.32±0.12	1.35±0.15
Palmitic acid	26.22±0.22^{c}	27.31±0.54^{a}	26.59±0.28^{bc}	27.23±0.27^{ab}
Palmitoleic acid	3.03±0.55^{b}	3.02±0.22^{b}	3.97±0.07^{a}	3.66±0.11^{a}
Stearic acid	14.64±0.48	14.90±0.49	14.50±0.41	14.35±0.33
Oleic acid	50.51±1.37	49.00±0.54	50.30±0.33	50.01±0.90
Linoleic acid	2.10±0.73	2.09±0.70	1.22±0.51	1.31±0.10
Linolenic acid	0.18±0.03^{b}	0.50±0.17^{a}	0.14±0.03^{b}	0.22±0.12^{b}
Archidonic acid	2.03±0.95	1.90±0.30	1.95±0.62	1.87±0.12
SFA[2]	42.14±0.51^{b}	43.49±0.60^{a}	42.42±0.78^{ab}	42.94±0.63^{ab}
USFA[3]	57.86±0.51^{a}	56.51±0.60^{b}	57.58±0.78^{ab}	57.06±0.63^{ab}
Ham				
Myristic acid	0.98±0.09	1.03±0.08	0.95±0.03	1.14±0.34
Palmitic acid	21.81±0.25^{c}	25.28±0.46^{a}	23.64±0.71^{b}	22.80±0.82^{bc}
Palmitoleic acid	2.95±0.53	2.14±0.88	2.58±0.28	2.81±0.46
Stearic acid	13.38±0.25^{c}	15.27±0.18^{b}	21.39±0.59^{a}	15.20±1.04^{b}
Oleic acid	47.34±0.17^{a}	48.22±0.69^{a}	38.18±0.51^{b}	46.86±1.32^{a}
Linoleic acid	12.12±0.71^{a}	7.22±0.23^{c}	11.21±0.76^{ab}	10.10±0.71^{c}
Linolenic acid	0.16±0.04	0.26±0.12	0.35±0.32	0.31±0.11
Archidonic acid	1.26±0.47	0.58±0.37	1.70±1.05	0.77±0.27
SFA	36.17±0.26^{d}	41.58±0.34^{b}	45.98±1.00^{a}	39.13±1.69^{c}
USFA	63.83±0.26^{a}	58.42±0.34^{c}	54.02±1.00^{d}	60.87±1.68^{b}

[1] Data are means of three replicates of five pigs per replicate. [2] SFA: saturated fatty acid. [3] USFA: unsaturated fatty acid.
[a, b, c, d] Means in the same row with different superscript letters are significantly different ($p<0.05$).

significantly affected on the dietary 0.4 and 0.6% betaine compared to the non-supplemented diet, it was numerically lower in pigs fed all levels of betaine. Matthews et al. (1998) and Øverland et al. (1999) reported no effect of betaine on serum or plasma cholesterol concentration. Matthews et al. (2001) reported that plasma cholesterol was increased in pigs fed 0.125 and 0.250% betaine; however, our data indicate that total cholesterol was decreased in pigs fed the 0.2% level of betaine. Therefore, the effect on serum total cholesterol would seem to indicate that betaine affects lipid metabolism. Although this effect of betaine on cholesterol content in the current experiment is promising, more research is necessary.

One of the primary goals of this study was to determine whether dietary betaine supplementation would increase muscle tissue betaine and, if so, whether it would affect pork quality. Betaine concentrations were significantly higher ($p<0.05$) in pigs fed dietary betaine than when given a non-supplemented diet (Table 3). Matthews et al. (2001) reported no detectable betaine concentrations in pigs fed 0% betaine, whereas pigs supplemented with betaine had detectable concentrations in the loin muscle.

Fatty acid composition

Table 4 shows the effect of dietary supplementation with betaine on fatty acid composition in different pork muscle of finishing pigs. The proportion of palmitic acid was increased by dietary betaine, and palmitoleic acid also increased in loin muscle. Both palmitic and stearic acids were increased by dietary betaine, whereas linoleic acid in ham muscle was decreased by dietary betaine. Consequently, significant differences in the percentage of saturated (SFA) and unsaturated (USFA) fatty acids in ham samples were observed. In agreement with our result in finishing pigs, there are other reports that dietary betaine significantly decreased USFA and increased SFA (Fernandez et al., 1998), and increased the ratio of SFA and decreased USFA in different muscle types (Hur et al., 2007). While increasing SFAs in pig diets can direct the fat profile towards a more stable composition (Wood et al., 2003).

CONCLUSION

Results from this study demonstrated that betaine supplementation in the diet improved growth performance in finishing pigs. However, for pigs fed 0.6% betaine there were no significant effects on ADG and FRC compared with the 0.4% treatment. From the effects observed on meat qualities, such as fat content, lightness and cooking loss, we assume that dietary betaine may improve redness and tenderness. Also dietary supplementation with betaine decreased total cholesterol concentration in blood, and

increased SFA and decreased USFA levels in pork muscle. Especially, pigs supplemented with betaine had increased betaine concentrations in the loin muscle. Therefore, the addition of betaine to the diet of finishing pigs could result in improved pork quality, which is beneficial to the pork industry.

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