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Non-ruminants

Effects of chestnut (*Castanea sativa*) meal supplementation on growth performance, carcass characteristics, and meat quality of pigs

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ABSTRACT - This study examined the effect of chestnut (*Castanea sativa*) meal (CNM) on growth performance, carcass characteristics, and meat quality of pigs. Thirty-two crossbred pigs were randomly allocated equally into four groups and fed one of four diets containing 0, 30, 50, or 100 g kg^{-1} DM CNM for 49 days. The animals were housed individually into the cage, fed the diet *ad libitum*, and allowed to access water freely. Feed efficiency decreased quadratically, while carcass yield decreased linearly with increasing CNM supplementation. The highest pH and the lowest drip loss were found in 30 g kg^{-1} of CNM supplementation, respectively, while no effects on fatty acid profiles were observed in all treatments. The addition of CNM at 30 g kg^{-1} into the diets could improve feed efficiency and reduce drip loss in meat.

Key Words: carcass quality, growth factor, pig

Introduction

Dietary supplementation of antibiotics for animals are prohibited in most countries because of the potential risk of developing antibiotic resistance. Consequently, various alternative approaches have been suggested for the use of natural additives to improve growth performance and meat quality. Chestnut has been used as an animal feed supplement (Liu et al., 2011; Prevolnik et al., 2012; Liu et al., 2013). Unlike other nuts and seeds, sweet chestnut (a cool season crop) has low calories with rich minerals, vitamins, and monounsaturated fatty acid (FA) (De Vasconcelos et al., 2010). In particular, water-soluble extract of sweet chestnut wood (*Castanea sativa*) from Southern Europe provided antimicrobial phytochemicals and increased the production of broiler chicks due to the improvements of manure quality, litter characteristics, and pathogen populations (Hooge et al., 2012). Gai et al. (2011) also reported that application of chestnut in animal diet enhanced the growth performance of young poultry and rabbit caused by the improvement of gastrointestinal microflora stability. In ruminant, application of chestnut reduced methane emission (Liu et al., 2011) and increased antioxidant enzyme (Liu et al., 2013). Frankič and Salobir (2011) suggested that sweet chestnut wood extract could be included in pig diets as a source of natural antioxidants. These previous studies indicated the beneficial effect of chestnut on animal health. In addition, chestnut in the diet of the Celta pig breed resulted in higher concentrations of unsaturated FA (Domínguez et al., 2012). Lee et al. (2016) suggested that supplementation of chestnut meal could be replaced up to 5% for pig considering feed intake and digestibility. However, limited studies were conducted to estimate the effect of chestnut meal on not only growth performance, but also on meat quality of pigs. Therefore, the objective of this study was to assess the effect of chestnut meal (CNM) supplement on growth performance, blood metabolites, carcass characteristics, and meat quality of pigs.

Material and Methods

This study was conducted in Changnyeong-gun, Gyeongsangnam-do, South Korea (35°27'08.0" N, 128°27'11.4"E). The animals were cared according to the guidelines of the Animal Care and Use Committee and approved by the Local Animal Care Committee on animal

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use (case no. BACC-02013-008). Thirty-two crossbred pigs (Landrace × Yorkshire × Duroc; mixed sex; 16 weeks of age) with an average body weight of 67.78±2 kg were randomly distributed into four treatments with eight pigs in each. Pigs were kept in individual pens equipped with a one-hole feeder and a nipple drinker to provide feed and water, respectively. The housing facility had automatic environmental control system, and the floor was fully slatted with concrete or plastic panels. The trial was continued for 49 days. Concentrate mixture (pig grower diet) was provided as basal diet *ad libitum* (Table 1). The CNM was purchased from Hapcheon Agricultural Co-operative Society (Hapcheon, South Korea). In the feeding trial, the basal diet was supplemented with CNM at 0, 30, 50, and 100 g kg^{-1} DM.

Feed samples were collected weekly (200 g) across the experiment. The collected feed samples were composited by treatments and subsampled (1 kg) for the analysis. The chemical compositions were analyzed according to the AOAC methods (AOAC, 1990). Daily feed intake was measured individually. Live weight was measured on days 0 and 49 of feeding trial to determine average daily gain (ADG) and feed efficiency. Feed efficiency was determined by dividing total gain with total feed intake during 49 days (gain/intake). Blood sample was collected from *vena cava cranialis* using a K_3EDTA vacuum tube at 49 days of feeding trial, after then, centrifuged at 3000 ×*g* for 15 min to separate serum. Blood glucose and blood urea nitrogen were measured using a blood chemistry analyzer (VET Test 8008, IDEXX, USA). White blood cell and red blood cell were analyzed using an automatic blood analyzer (Celltac α MEK-6450K, Nihon Kohden, Japan).

Table 1 - Ingredients and chemical compositions of basal diets (g kg^{-1} DM)

Item	Basal diet
Ingredient	
Ground corn	485
Soybean meal	319
Rice bran	50
Tallow	48
Lupine	32
Molasses	30
Calcium phosphate	16
Lysine	5
Mineral premix[1]	6
Methionine	5
NaCl	3
Vitamin primix[2]	1
Chemical composition[3]	
Dry matter	872.4
Crude protein	146.1
Ether extract	35.1
Ash	18.9
ME (kcal/kg)	3,100

DM - dry matter; ME - metabolizable energy.
[1] One kilogram of the diet contained the following: Fe, 70 mg; Cu, 50 mg; Zn, 25 mg; Mn, 30 mg; I, 0.7 mg; Co, 0.5 mg; Se, 0.26 mg.
[2] One kilogram of the diet contained the following: vitamin A, 16,000 IU; vitamin D3, 3,000 IU; vitamin E, 40 IU; vitamin B1, 2.5 mg; vitamin B2, 20 mg; vitamin B6, 4 mg; vitamin B12, 0.076 mg; vitamin K3, 2.5 mg; pantothenic acid, 40 mg; niacin, 75 mg; biotin 0.15 mg; folic acid, 0.65 mg; ethoxyquin, 12 mg.
[3] Values represent results of three samples, each assayed in triplicate.

At the end of the feeding trial, all animals were slaughtered, being approved by Ministry of Agriculture, Food and Rural Affairs, South Korea. After slaughter, all cold carcasses were chilled at 2 °C for 24 h, and carcass characteristics (carcass weight, carcass yield, and back fat thickness) were measured by the guidelines of the Animal Products Grading Service, South Korea (APGS, 2007).

The loin from *Longissimus dorsi* (2 kg) was obtained from each animal using a perpendicular cut to the backbone between the 7th and 8th ribs. The loin pH was measured using a pH meter (Mettler Toledo Co., MP 230, Switzerland) and color using a Minolta Chroma Meter CR-300 (Minolta Co., Ltd., Tokyo, Japan) standardized with white calibration plate (y = 93.5; x = 0.3132, y = 0.3198), respectively. Cooking loss was determined by calculating the weight loss during cooking. Sample in a plastic bag was boiled in water bath at 90 °C for 30 min, and then, it was calculated as the percentage loss based on the initial sample weight. Drip loss was determined according to the methods of Jama et al. (2008) and was measured as the change in percent weight after 24 h storage at 4 °C. For water-holding capacity (WHC), 10 g loin samples were placed into a propylene centrifugal vial and heated in water bath for 30 min at 70 °C. After cooling to room temperature, the sample was centrifuged at 1000 ×*g* for 10 min at 4 °C to determine the amount of gravy. Shear force (3 cm [width] × 1 cm [length] strips) was measured using the Instron Rheometer (CR-311; Sun Scientific Co., Tokyo, Japan). All samples were sheared perpendicular to the direction of muscle fiber by using a 50 kg transducer, a crosshead speed of 100 mm/min, and a load range of 20 kg.

The determination of pH and thiobarbituric acid reactive substances (TBARS). were repeated at 1, 3, 5, and 7 days of storage at 4 °C. For pH determination, 3 g samples were taken from the loin and homogenized in a blender (IKA T25-B, Malaysia) for 1 min using 27 mL of distilled water. The pH was measured using a pH meter (Mettler Toledo Co., MP 230, Switzerland). The TBARS were assessed according to the procedure described by Buege and Aust (1978). Sample of 5 g was weighted into a 50 mL test tube and homogenized with 15 mL of deionized distilled water using a Plytron homogenizer for 10 s at the highest speed (T25basic, IKA, Selangor, Malaysia). The sample homogenate (1 mL) was transferred to a disposable test tube, and butylated hydroxyanisole (10%, 50 uL)

and thiobarbituric acid/trichloroacetic acid (TBA/TCA) solutions (2 mL) were added. The sample was mixed using vortex mixer, and then incubated in boiling water bath for 15 min to develop color. The sample was cooled down and then the absorbance read at 531 nm with a blank containing 1 mL of double distilled water and 2 mL of TBA/TCA solution. The amounts of TBARS were expressed as mg of malondialdehyde (MDA) per kg of sample.

In sensory analysis, the meat was judged by 10 panelists. The panelists were selected and trained according to the procedures of Meilgaard et al. (1999). Meat was cooked to an internal temperature of 70 °C in water bath. The raw meat was evaluated for meat color, marbling score, and overall acceptability, while cooked meat was evaluated for meat color, aroma, flavor, juiciness, and overall acceptability by using a 9-point hedonic scale, in which 1 = extremely bad or dislike extremely and 9 = extremely good or like extremely. Pork loin (2 g) was freeze dried (FreeZone 12plus, LABCONCO, Kansas City, USA) and methylated using the direct methylation method described by Jenkins et al. (2001). The FA methyl esters were analyzed using gas chromatography (GA-17A, Shimadzu, Tokyo, Japan) with a CP-Sil88 column (100 m × 0.25 mm × 0.2 um; Chrompack, Middelburg, Netherlands). The carrier gas was nitrogen, and the temperatures of injector and detector were maintained at 230 °C. The oven temperature was started at 120 °C, ended at 220 °C, and maintained for 10 min. The FA profiles (14:0 to 24:1) were determined by comparing the retention times with FA methyl ester standards and expressed as a percent of the total FA.

All data were tested by ANOVA using the GLM procedure of the SAS (Statistical Analysis System, version 8.2) package program. The IML procedure of SAS was used to generate coefficients for the testing of linear, quadratic, and cubic effects of treatments with unequal spacing. Polynomial contrasts (linear, quadratic, and cubic effects) were used to evaluate the effects of increasing CNM level. The Tukey test was also used to identify differences among treatments and storage periods of pH and TBARS. Values of $P<0.05$ were considered significant.

Results

Growth performance, blood metabolites, and carcass characteristics were not influenced by CNM levels, except for feed efficiency (Table 2). Feed efficiency was quadratically changed ($P = 0.014$) and higher ($P<0.05$) in supplementation of 30 g kg^{-1} of CNM than that in supplementations of 0 and 100 g kg^{-1} CNM. In addition, carcass yield reduced linearly ($P = 0.046$) with increasing levels of dietary CNM. The red blood cells increased lineally with increasing dietary level of CNM, while the other blood metabolites were not affected.

Meat lightness, cooking loss, WHC, and shear force were not affected by CNM supplementation, except for pH and drip loss (Table 3). However, redness ($P = 0.048$) and yellowness ($P = 0.037$) of pork loin were changed cubically as the levels of CNM increased.

The pH values of pork loin were affected by the treatments at 1, 5, and 7 days of storage ($P<0.05$) at 4 °C, which was highest in 30 g kg^{-1} of CNM supplementation (Table 4). In contrast, TBARS values ($P = 0.048$) reduced linearly with increasing levels of dietary CNM on day 5 of storage.

Table 2 - Effects of supplemented levels of chestnut meal (CNM) on growth performance, blood metabolites, and carcass characteristics of pigs

Item	Supplemented level of CNM (g kg^{-1} DM)				SEM	Contrast		
	0	30	50	100		Linear	Quadratic	Cubic
Growth performance								
Initial body weight (kg)	67.9	68.1	67.3	67.3	1.97	0.794	0.988	0.792
Final body weight (kg)	114.8	119.9	115.0	115.4	2.89	0.854	0.134	0.223
Average daily gain (kg)	0.91	1.07	1.01	0.98	0.028	0.628	0.082	0.323
Daily feed intake (kg)	2.21	2.21	2.20	2.24	0.013	0.411	0.183	0.689
Feed efficiency (gain/intake)	0.41b	0.48a	0.46ab	0.44b	0.060	0.875	0.014	0.255
Blood metabolites (mg/dL)								
Blood glucose	96.3	96.4	96.9	95.0	4.86	0.817	0.863	0.765
Blood urea nitrogen	14.3	14.4	16.8	15.1	1.27	0.529	0.363	0.281
White blood cell	18.8	18.5	18.5	18.6	1.26	0.966	0.889	0.999
Red blood cell	6.13	6.36	6.61	6.92	0.275	0.049	0.872	0.845
Carcass characteristics								
Carcass weight (kg)	83.9	87.8	82.2	82.9	2.19	0.467	0.399	0.210
Carcass yield (%)	73.1	73.2	72.3	71.7	1.88	0.046	0.566	0.557
Back fat thickness (mm)	20.5	21.4	19.4	21.8	0.86	0.431	0.366	0.117

DM - dry matter; SEM - standard error of the mean.
a-b - Means with different letters in the same row significantly differ at $P<0.05$.

For sensory evaluations, CNM treatments affected cubically (P = 0.016) meat color of cooked meat, especially it was highest in supplementations of 50 and 100 g kg^{-1} CNM (Table 5). Pork sensory scores of flavor (P = 0.033) and off-flavor (P = 0.050) were improved linearly by increases of CNM supplementation levels, while juiciness score and overall acceptability of cooked meat were not affected.

For FA profiles of pork loin, no significant differences (P>0.05) were observed in concentrations of individual FA, saturated FA, and unsaturated FA (Table 6). However, C16:1n-7 concentration (P = 0.021) was changed cubically as the levels of CNM increased.

Discussion

No significant differences were found among the treatments for growth performance, blood metabolites, and carcass characteristics. Similar to the present study, Štukelj et al. (2010) observed no improvement in growth performance and blood parameters when growing pigs were fed diet with organic acids and tannin. Prevolnik et al. (2012) also showed that feeding pigs with 0.2% sweet chestnut wood extracts had no effect on growth performance or carcass quality. Antongiovanni et al. (2007) observed that 250 g 100 kg^{-1} tannin in the diets of growing pigs did not improve growth performance. However, feed efficiency (P = 0.014) was affected quadratically as the levels of CNM increased. This result in the present study might be due to an increase in ADG (Quadratic, P = 0.082). For blood parameters, the only changes were an increased concentration of red blood cell when CNM was included in diets or as CNM levels increased. Blood glucose and blood urea nitrogen were within reference ranges (Mitruka and Rawnsley, 1977).

In the current study, pig diet supplementation with 30 g kg^{-1} resulted in a higher pH value in loin muscle compared with 0 and 100 g kg^{-1} CNM supplementation levels (P<0.05). In general, the pH is closely related to tenderness and WHC in meat (Bouton et al., 1971; Li et al.,

Table 3 - Effects of supplemented levels of chestnut meal on the pH, meat color, and physicochemical characteristics of pork loin

Item	Supplemented level of CNM (g kg^{-1} DM)				SEM	Contrast		
	0	30	50	100		Linear	Quadratic	Cubic
pH	5.57b	5.66a	5.58ab	5.57b	0.013	0.971	0.825	0.981
Meat color[1]								
L*	55.9	54.1	56.6	55.8	2.510	0.725	0.780	0.054
a*	6.69	5.89	7.36	7.12	1.337	0.268	0.847	0.048
b*	4.32	3.34	4.39	4.32	1.290	0.554	0.323	0.037
Physicochemical characteristics								
Cooking loss (%)	41.6	40.9	41.7	40.4	1.844	0.252	0.696	0.312
Drip loss (%)	5.17a	2.88b	3.23ab	3.32ab	2.515	0.241	0.161	0.466
WHC (%)	67.5	74.9	73.5	72.5	9.847	0.703	0.153	0.569
Shear force (kg/cm^2)	4.06	3.74	3.86	4.01	0.823	0.956	0.400	0.885

DM - dry matter; WHC - water-holding capacity; SEM - standard error of the mean.
a-b - Means with different letters in the same row significantly differ at P<0.05.
[1] L* = lightness; a* = redness; b* = yellowness.

Table 4 - Effects of supplemented levels of chestnut meal (CNM) on pH and TBARS of pork loins during storage periods at 4 °C

Item	Supplemented level of CNM (g kg^{-1} DM)				SEM	Contrast		
	0	30	50	100		Linear	Quadratic	Cubic
pH								
1[1]	5.57Bb	5.66Ba	5.58Bab	5.57Bb	0.113	0.971	0.825	0.981
3	5.73A	5.82A	5.74A	5.71A	0.102	0.635	0.322	0.491
5	5.69Aab	5.74ABa	5.60Bb	5.61Bb	0.150	0.398	0.528	0.767
7	5.68Aab	5.73ABa	5.59Bb	5.62Bb	0.144	0.433	0.465	0.680
TBARS (mg MDA kg^{-1} sample)								
1[1]	0.10	0.09	0.10	0.10	0.014	0.346	0.494	0.133
3	0.11	0.10	0.11	0.08	0.031	0.371	0.070	0.518
5	0.14	0.11	0.11	0.11	0.027	0.048	0.067	0.491
7	0.16	0.16	0.17	0.16	0.020	0.448	0.574	0.202

TBARS - thiobarbituric acid reactive substances; DM - dry matter; SEM - standard error of the mean; MDA - malondialdehyde.
A-B - Means with different uppercase letters in the same column significantly differ at P<0.05.
a-b - Means with different lowercase letters in the same row significantly differ at P<0.05.
[1] Storage days.

2013). Meat pH also has a profound effect on WHC (Honikel, 1987; Yoo et al., 2018), and meat pH significantly affects meat color (Swan and Boles, 2002). However, our observation did not support these findings. Meat pH is closely related to meat color. Meat with high pH has a more compact muscle structure, which limits oxygen diffusion and light absorption (Swan and Boles, 2002). However, redness ($P = 0.048$) and yellowness ($P = 0.037$) of pork loin were changed cubically as the levels of CNM increased. Drip loss in 30 g kg^{-1} CNM treatment was lower ($P<0.05$) than that in other groups. Andersen (2000) and Yoo et al. (2018) indicated drip loss or water-holding capacity as the most important quality characteristics for the processing industry. The development of drip loss over time is of great interest for the retail market for fresh meat, with respect to the length of display time of case-ready products without reduction in meat quality (Otto et al., 2006). The reason of decreased drip loss was unclear by the addition of CNM at 30 g kg^{-1} of diet in this study. However, CNM supplementation at 30 g kg^{-1} of diet could improve the interest of retail market of pork.

The pH values of stored meat decreased slightly with increasing levels of CNM over time. In terms of lipid oxidation, TBARS values in all treatments tended to increase with storage days. Moreover, the TBARS values on day 5 decreased linearly as the levels of CNM increased. The addition of 100 g kg^{-1} CNM showed pH and TBARS values similar to those of 50 g kg^{-1} CNM as storage days increased. In general, determining pH and TBARS is the most common method to evaluate the lipid oxidation in

Table 5 - Effects of supplemented levels of chestnut meal (CNM) on sensory evaluations of raw and cooked pork loin

Item	Supplemented level of CNM (g kg^{-1} DM)				SEM	Contrast		
	0	30	50	100		Linear	Quadratic	Cubic
Raw meat sensory scores[1]								
Meat color	4.81	4.88	4.88	4.81	0.433	0.956	0.691	0.962
Marbling score	4.39	4.38	4.41	4.55	0.704	0.627	0.807	0.976
Overall acceptability	4.64	4.67	4.77	4.66	0.448	0.930	0.617	0.737
Cooked meat sensory scores								
Meat color	4.85a	4.77b	5.30a	5.27a	0.337	0.005	0.061	0.016
Flavor	3.27	3.36	3.45	3.59	0.296	0.033	0.948	0.875
Off-flavor	1.80	1.84	1.96	1.98	0.197	0.050	0.622	0.411
Juiciness	4.34	4.50	4.48	4.59	0.474	0.334	0.792	0.761
Overall acceptability	4.82	5.05	4.89	4.75	0.369	0.470	0.224	0.362

DM - dry matter; SEM - standard error of the mean.
a-b - Means with different letters in the same row significantly differ at $P<0.05$.
[1] Sensory scores were assessed on 9-point scale, in which 1 = extremely bad or dislike extremely, and 9 = extremely good or like extremely.

Table 6 - Effects of supplemented levels of chestnut meal (CNM) on fatty acid profiles (g 100 g^{-1} of fatty acid) of pork loin

Item	Supplemented level of CNM (g kg^{-1} DM)				SEM	Contrast		
	0	30	50	100		Linear	Quadratic	Cubic
C14:0	1.33	1.38	1.36	1.42	0.054	0.311	0.951	0.628
C16:0	24.5	24.5	24.7	24.7	0.360	0.710	0.871	0.679
C16:1n-7	2.79	3.10	2.73	2.91	0.112	0.871	0.784	0.021
C18:0	12.5	12.4	13.2	12.6	0.270	0.656	0.249	0.092
C18:1t-11	0.16	0.15	0.19	0.11	0.050	0.470	0.490	0.508
C18:1n-9	42.1	42.9	41.5	41.7	0.930	0.639	0.872	0.317
C18:2n-6	12.0	11.0	11.6	12.0	0.830	0.795	0.481	0.550
C18:3n-3	0.46	0.43	0.44	0.47	0.018	0.855	0.384	0.702
C20:1	0.66	0.62	0.63	0.65	0.030	0.442	0.199	0.538
C20:4n-6	2.30	2.28	2.33	2.23	0.334	0.902	0.908	0.901
C20:5n-3	0.10	0.10	0.10	0.10	0.012	0.714	0.965	0.615
C22:0	0.33	0.32	0.34	0.32	0.034	0.996	0.888	0.631
C22:1n-9	0.03	0.03	0.03	0.02	0.005	0.226	0.812	0.758
C22:5n-3	0.23	0.23	0.24	0.23	0.030	0.956	0.791	0.740
C22:6n-3	0.09	0.08	0.09	0.08	0.012	0.671	0.971	0.703
C24:1n-9	0.37	0.37	0.36	0.34	0.043	0.654	0.843	1.000
SFA	38.76	38.70	39.70	39.10	0.530	0.566	0.487	0.260
UFA	61.24	61.30	60.30	60.90	0.530	0.568	0.493	0.256
SFA:UFA	0.64	0.63	0.66	0.64	0.015	0.635	0.595	0.274

DM - dry matter; SFA - saturated fatty acid; UFA - unsaturated fatty acid; SEM - standard error of the mean.

meat and its products (Rhee and Ziprin, 2001). Extensive research has indicated that lipid oxidation stability represented by TBARS can be associated with a reduction in meat pH because of the presence of antioxidants (Xiong et al., 1993; Choi et al., 2010). This could be explained by the fact that chestnut meal has little biological activity or antioxidant effectiveness.

The supplementation levels of CNM had no effects on the sensory evaluation of raw and cooked pork loin. However, meat color in cooked meat sensory scores was affected by CNM supplementation levels in the present study. Flavor and off-flavor of cooked meat were also increased linearly by CNM supplementation levels. To the best of our knowledge, no previous studies have been performed with CNM on pigs. Therefore it is hard to explain the reasons of these sensory evaluation results in the present study, but dietary supplementation with CNM seems to be more desirable on color score.

In contrast with these results, Bermúdez et al. (2012) and Temperan et al. (2014) reported differences in saturated FA content in *Longissimus dorsi* from the chestnut diets with C16 content. In addition, Domínguez et al. (2015) reported that chestnut-fed pigs had higher unsaturated FA content and lower saturated FA than those fed feed. This is mainly due to the higher C18:1n-9 and lower C16:0 and C18:0 contents of pigs fed feed. It is unclear why the inclusion of CNM had no effect on FA profiles in the present study. However, these inconsistent results in the present study might be due to differences of CNM, fattening period, or tannin content. Therefore, further studies need to be conducted to clarify the effect of CNM supplement on pigs.

Conclusions

Supplementation of chestnut meal in pig diets has no beneficial effect on growth performance, blood metabolites, and carcass characteristics. However, it can increase feed efficiency and drip loss depending on supplementary level. In particular, addition of chestnut meal at 30 g kg^{-1} is suggested as dietary supplementation in pig diets.

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