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Original Research Article

Dietary supplementation of *Lonicera macranthoides* leaf powder improves amino acid profiles in serum and longissimus thoracis muscle of growing-finishing pigs

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ABSTRACT

A 60-days feeding trial was conducted to determine the effect of dietary supplementation of *Lonicera macranthoides* leaf (LML) on growth performance, meat quality, and free amino acid profiles in growing-finishing pigs. *L. macranthoides* leaves were harvested, air-dried and milled to powder. A total of 288 crossbred barrows (Large White × Landrace) with an average initial BW of 54.0 ± 3.4 kg were randomly allocated to 3 treatment groups and fed either a basal diet or a diet supplemented with 0.5% or 1.0% LML powder. Results showed that diet supplemented with LML powder did not affect average daily feed intake (ADFI) and feed: gain ratio ($P > 0.05$) in growing-finishing pigs. Moreover, diet supplemented with LML powder showed no significant effects on carcass traits ($P > 0.05$) including dressing percentage, backfat thickness and loin muscle area, and meat quality traits ($P > 0.05$) including meat color, intramuscular fat and cooking loss. However, diet supplemented with LML powder significantly improved free amino acid profiles in serum and longissimus thoracis muscle in growing-finishing pigs. Most importantly, dietary LML powder increased total free amino acids content ($P < 0.001$) and essential free amino acid content ($P < 0.001$) in longissimus thoracis muscle. These results indicate that LML has the potential to improve the nutritional value of meat through improving free amino acid profiles.

1. Introduction

Meat is the best source for high biological value protein and some of the most important micronutrients such as essential amino acids (EAA) (Biesalski, 2005). Conjugated linoleic acid, n-3 fatty acids, vegetable proteins, and herbs are added in animal diets as feed additive to improve the quality and nutritional value of meat (Hanczakowska et al., 2015; Kouba et al., 2003). However, much attention has been paid to improve the intramuscular fat content and fatty acid profiles whereas the amino acid composition of meat has been neglected. In fact, the content of EAA is an important factor in determining the nutritional value of meat (Pereira and Vicente, 2013) and the composition, and the content of free amino acids provide importantly the taste properties of meat (Kato et al., 1988).

The Chinese herbal medicine is often used as feed additive to maintain good health and improve livestock performance (Kong et al., 2011). For years, the beneficial effects of Chinese herbal medicine on the maintenance of good health in animal and humans and on the improvement of amino acid metabolism of pigs have

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been declared (Kong et al., 2011). *Lonicera macranthoides*, commonly known as *Lonicerae Flos* (Shanyinhua in Chinese) was recorded in the Chinese Pharmacopoeia (Edition, 2010). Based on its character of nature, safety, low cost and sweet taste (Wang et al., 2009; Liu et al., 2013), it would be a potential feed additive for pigs. Moreover, our laboratory found that chlorogenic acid, one of the main bioactive compounds of *L. macranthoides* leaf (LML) (Sun et al., 2011), beneficially modulate energy metabolism and amino acid metabolism (Ruan et al., 2014). Therefore, we conducted the present study to investigate the effects of LML on amino acid profiles and meat quality traits in growing-finishing pigs.

2. Materials and methods

2.1. Chemical analysis of powder of L. macranthoides leaves

L. macranthoides leaf was obtained from Longhui County in Hunan Province (supplied by Hunan Longhui Science and Technology Industrial Park, Investment & Development Co., Ltd, Longhui, China). Leaves were air-dried at 25°C and then milled to pass through a 2 mm sieve. The LML were then analyzed in triplicate for dry matter (DM) content (AOAC, 2005; method No. 930.15), ash (AOAC, 2005; method No. 942.05) and nitrogen (N) (AOAC, 2005; method No. 984.13). Crude protein (CP) was then calculated as N × 6.25. Neutral detergent fiber (NDF) and acid detergent fiber (ADF) were determined according to previous method (Van Soest et al., 1991). Calcium (Ca) was analyzed by an atomic absorption spectrophotometer procedure (AOAC, Method 968.08) after wet digestion sample preparation (AOAC Method 935.13). Phosphorus (P) was analyzed using a colorimetric procedure (AOAC, Method 931.01). Basic nutritional compositions of the dried powder of LML are presented in Table 1.

2.2. Animals diets and experimental design

The experimental protocol was approved by the Protocol Management and Review Committee of the institute of Subtropical Agriculture, Chinese Academy of Science and Pigs were cared for and slaughtered according to the guidelines of the institute of Subtropical Agriculture on Animal Care (Changsha, China).

This study was conducted at the Experiment Farm of Hunan Jiahe Agriculture & Stock Raising Co., Ltd (Changsha, China). A total of 288 crossbred barrows (Large White × Landrace) with an average initial body weight (BW) of 54.0 ± 3.4 kg were used in the 60-d growth experiment. Pigs were randomly allocated to 3 treatment groups with 8 pens per treatment and 12 pigs per pen. Pigs were fed either a basal diet (control) or a diet supplemented with 0.5% or 1.0% powder of LML. The basal diets were formulated to meet National Research Council (2012) recommendation (Table 2). Pigs were housed in partially slotted and concrete floor facility in 24 adjacent pens which were equipped with a self-feeder and nipple drinker to allow ad libitum access to feed and water throughout the experimental period.

During the experiment, body weight was recorded every month and feed consumption was recorded every week. One pig was randomly chosen from each pen and bled by anterior vena cava puncture at the end of the experiment. Thereafter, the samples were centrifuged at 2,500 × *g* at 4°C for 10 min, and serum samples were stored at −20°C until analysis for free amino acid composition. Immediately after slaughtering, longissimus thoracis muscle resected from the 13th/14th rib from the right side of the carcass were used for the analysis of meat quality and were stored at −80°C for the analysis of free amino acid concentrations.

Table 1
Basic nutritional composition in dried powder of *Lonicera macranthoides* leaf.

EE, MJ/kg	CP, %	ADF, %	NDE, %	Ca, %	P, %	Ash, %
4.1	7.8	32.7	47.5	0.75	0.14	5.9

EE = energy; CP = crude protein; ADF = acid detergent fiber; NDF = neutral detergent fiber.

Table 2
Ingredient and nutrient levels of basal diet (DM basis).

Item	Content, %
Ingredient	
Corn	73.40
Expanded Soybean	10.54
Enzymatically decomposed Soybean meal	5.65
Soybean oil	5.80
L-lysine 98%	0.40
L-methionine	0.06
L-threonine	0.15
Premix[1]	4.00
Nutrient levels[2]	
CP	15.09
Ca	0.55
TP	0.56
AP	0.39
DE, MJ/kg	14.20

CP = crude protein; TP = total phosphorus; AP = available phosphorus.
[1] Content of premix (‰): polymineral 25; carnitine 0.5; Vitamin A, 4,640,000 IU; Vitamin D_3, 870,000 IU; Vitamin E, 20,500 IU; Vitamin B_{12}, 5; monosodium glutamate 2.5; butylated hydroxytoluene 5; calcium formate 12.5; $Fe_2(SO_3)_3$ 5; $MgSO_3$ 10; Cr 5; Calcium Phosphate 285; limestone 300; chaff 91.5; NaCl 75; feed carrier with zeolite powder.
[2] Nutrient levels were calculated values.

2.3. Meat sampling and quality evaluation

Average backfat thickness was defined as the average thickness of 1st and last rib and last-lumbar backfat. Carcass was cut between the 6th and 7th rib, then loin muscle area was measured and calculated as previous methods (Li et al., 2015) did. The pH values of the longissimus thoracis muscle were measured at 45 min (pH_{45}) and 24 h (pH_{24}) post mortem by a MP120 Mettler Toledo pH meter (Mettler-Toledo, GmbH, Schwarzenbach, Switzerland) previously calibrated at pH 9.182, 6.864 and 4.003. The color of longissimus thoracis muscle was assessed objectively in triplicate using a Minolta Chroma Meter CR-300 (Minolta Co. Ltd, Osaka, Japan) from a freshly cut surface using the parameters L* (brightness), a* (redness) and b* (yellowness). The cooking loss was determined according to previous methods (Kayan et al., 2011). A loin cube was taken from the longissimus thoracis muscle slice after being trimmed of fat and connective tissue, then was weighed, placed in a polyethylene bag and incubated in water at 75°C for 50 min. The bag was then immersed in flowing water at room temperature for 30 min and the solid portion was re-weighed. Intramuscular fat content of samples were assessed as crude fat on longissimus thoracis muscle by Soxhlet extraction.

2.4. Free amino acid analysis

About 100 mg samples of longissimus thoracis muscle were dissolved in 50% methanol solution at 4°C for 30 min and centrifuged at 10, 000 × *g* for 10 min, then the supernatant was filtered through glass wool for further analysis (Li et al., 2015). Serum for free amino acid analysis was pretreatment as previously described (Kong et al., 2009). Briefly, 1 mL of serum and 2.5 mL of 7.5% trichloracetic acid were mixed thoroughly, then centrifuged at 12, 000 × *g* at 4°C for 15 min and the supernatant was filtered through a 0.45 μm membrane. Then a volume of 40 μL supernatant for each samples were labeled with iTRAQ reagents (AA 45/32 kit; Applied Biosystems) as recommended by the manufacturer, and analyzed on an Applied Biosystems 3200 Q TRAP LC/MS/MS system equipped with a RP-C18 column.

2.5. Statistical analysis

Data obtained were analyzed using one-way ANOVA using the SAS 8.2 software package (SAS Institute, Inc.). Variability of all the data was expressed as standard deviation (SD). Differences between significant mean values were compared using Duncan's multiple range test. Results were considered statistically significant at $P < 0.05$ and were considered tending to be significant at $0.05 \le P < 0.1$.

3. Results

3.1. Growth performance, carcass and meat quality traits

Effects of LML powder on growth performance in growing-finishing pigs are shown in Table 3. Throughout the 60 days of experiment, there was no significant difference in weight gain, feed: gain ratio ($P > 0.05$) or feed intake ($0.05 \le P < 0.1$) between the control and LML groups.

Effects of LML powder on carcass and meat quality traits in growing-finishing pigs are shown in Table 4. No significant difference was observed in carcass traits including dressing percentage, loin muscle area and backfat thickness ($P > 0.05$) between the control and LML treatment groups. Moreover, there was no difference in meat quality traits including cooking loss, pH_{45} value and muscle color (L^*, a^*, and b^*) between the control and LML treatment groups, except that the inclusion of LML powder significantly increased the pH_{24} value ($P < 0.05$) and tended to increase intramuscular fat content ($0.05 \le P < 0.1$).

3.2. Free amino acid profiles in serum and longissimus thoracis muscle

Effects of LML on serum free amino acid concentrations in growing-finishing pigs are shown in Table 5. Lysine concentration was lower while proline and aginine concentrations were higher in pigs fed diet supplemented with LML powder than those pigs fed basal diet ($P < 0.05$). Cysteine and glutamic acid concentrations were higher in pigs fed diet supplemented with 1% LML powder (HLML) than those pigs fed basal diet ($P < 0.05$) while pigs fed diet supplemented with 0.5% LML powder (LLML) had no such difference ($P > 0.05$). Dietary LML had no significant effects on total essential free amino acid (EAA) concentration ($P > 0.05$) and total non-essential free amino acid (NEAA) concentration ($P > 0.05$), as well as the ratio of total EAA to total NEAA ($P > 0.05$). However, serum total free AA concentration was higher in pigs fed diet supplemented with LML than those pigs fed basal diet ($P < 0.05$).

Effects of LML on free amino acid concentrations of longissimus thoracis muscle in growing-finishing pigs are shown in Table 6. Arginine ($P < 0.001$), valine ($P < 0.05$) and glycine ($P < 0.05$) concentrations were higher in pigs fed diet supplemented with LML than those pigs fed basal diet. Total EAA concentration ($P < 0.001$), the ratio of total EAA to total NEAA ($P < 0.001$) and total free AA concentration ($P < 0.001$) were higher in longissimus thoracis muscle of pigs fed diet supplemented with LML than those pigs fed basal diet, while no difference was observed in total NEAA concentration ($P > 0.05$).

Table 3
Effect of dietary powder of *Lonicera macranthoides* leaves on growth performance in growing-finishing pigs.

Item	Control	LLML	HLML
IBW, kg	54.2 ± 4.4	54.1 ± 3.3	54.1 ± 6.2
FBW, kg	112.0 ± 4.2	111.6 ± 4.2	110.2 ± 4.6
ADFI, kg/d	2.86 ± 0.15*	2.66 ± 0.17#	2.79 ± 0.11*#
ADG, kg/d	0.97 ± 0.06	0.95 ± 0.11	0.94 ± 0.07
F:G	2.94 ± 0.13	2.84 ± 0.25	2.95 ± 0.31

IBW = initial body weight; FBW = final body weight; F:G = feed:gain ratio; LLML = low *L. macranthoides* leaf, 0.5% of basal diet; HLML = high *L. macranthoides* leaf, 1% of basal diet.
*,# Within a row, means with different superscript tend to be significantly different ($0.05 \le P < 0.1$). Data presented as Mean ± SD. $n = 8$ per diet.

Table 4
Effect of dietary powder of *Lonicera macranthoides* leaves on carcass and meat quality traits in growing-finishing pigs.

Item	Control	LLML	HLML
Dressing percentage, %	68.5 ± 0.8	67.0 ± 0.5	68.7 ± 1.2
Loin muscle area, cm^2	53.6 ± 7.1	55.1 ± 7.7	50.6 ± 4.1
Backfat thickness, cm	1.80 ± 0.39	1.80 ± 0.61	1.70 ± 0.36
pH_{45}	6.43 ± 0.41	6.28 ± 0.07	6.16 ± 0.19
pH_{24}	5.17 ± 0.12[a]	5.36 ± 0.10[b]	5.36 ± 0.08[b]
Cooking loss, %	48.8 ± 1.4	48.6 ± 1.1	47.8 ± 1.2
Intramuscular fat, %	2.17 ± 0.28*	2.42 ± 0.14#	2.41 ± 0.19#
Color			
L^*	48.3 ± 1.0	47.9 ± 1.2	46.1 ± 2.0
a^*	13.0 ± 1.3	13.8 ± 0.7	13.8 ± 0.6
b^*	5.61 ± 0.8	5.90 ± 0.42	5.08 ± 0.46

LLML = low *L. macranthoides* leaf, 0.5% of basal diet; HLML = high *L. macranthoides* leaf, 1% of basal diet.
a,b Within a row, means with different superscript letters are different ($P < 0.05$); *,# within a row, means with different superscript letters tend to be significantly different ($0.05 \le P < 0.1$); Data presented as Mean ± SD. $n = 8$ per diet.

Table 5
Effect of dietary powder of *Lonicera macranthoides* leaves on serum free amino acid concentration (μmol/L) in growing-finishing pigs.

Item	Control	LLML	HLML
EAA			
Arg	306 ± 30[a]	421 ± 113[b]	457 ± 127[b]
Lys	166 ± 51[a]	57 ± 12[b]	78 ± 14[b]
Met	45 ± 5	50 ± 7	53 ± 4
Phe	79 ± 14	83 ± 24	95 ± 9
Leu	201 ± 52	210 ± 38	220 ± 30
Ile	114 ± 4[a]	116 ± 38[ab]	134 ± 5[b]
Thr	134 ± 40	122 ± 37	132 ± 48
Val	348 ± 40	318 ± 47	355 ± 9
NEAA			
Cys	50 ± 7[a]	57 ± 17[ab]	74 ± 13[b]
Glu	153 ± 7[a]	176 ± 21[ab]	196 ± 41[b]
Gly	1,063 ± 247	1,042 ± 192	823 ± 113
Ala	226 ± 64	231 ± 68	236 ± 22
Ser	89 ± 13	98 ± 13	102 ± 15
Pro	279 ± 81[a]	402 ± 19[b]	390 ± 30[b]
Tyr	91 ± 14	82 ± 15	99 ± 4
Total EAA	1,392 ± 121	1,376 ± 89	1,524 ± 103
Total NEAA	1,953 ± 148	2,092 ± 151	1,923 ± 93
EAA:NEAA	0.71 ± 0.04	0.67 ± 0.05	0.79 ± 0.07
Total AA	3,329 ± 64[a]	3,473 ± 86[b]	3,441 ± 97[b]

EAA = essential amino acids; NEAA = non-essential amino acids; LLML = low *L. macranthoides* leaf, 0.5% of basal diet; HLML = high *L. macranthoides* leaf, 1% of basal diet.
a,b Within a row, means with different superscript letters are different ($P < 0.05$); Data presented as Mean ± SD. $n = 8$ per diet.

4. Discussion

L. macranthoides has been a commonly used Chinese Herbal medicine for almost 1,500 years. Accumulating evidence shows

Table 6
Effect of dietary powder of *Lonicera macranthoides* leaves on free amino acid concentration (μg/g) in longissimus thoracis muscle of growing-finishing pigs.

Item	Control	LLML	HLML
EAA			
Arg	1,161 ± 439[a]	4,014 ± 480[b]	4,412 ± 293[b]
Met	8 ± 3	7 ± 2	5 ± 1
Phe	18 ± 5	14 ± 2	18 ± 0.3
Ile	9 ± 0.3	8 ± 0.2	9 ± 0.7
Leu	15 ± 4	16 ± 2	19 ± 1
Lys	11 ± 3	13 ± 3	15 ± 1
Val	13 ± 2[a]	16 ± 2[b]	17 ± 2[b]
Tyr	214 ± 6	207 ± 34	217 ± 16
NEAA			
Glu	42 ± 6	44 ± 7	44 ± 4
Ser	11 ± 3	11 ± 2	12 ± 1
Gly	43 ± 6[a]	57 ± 8[b]	55 ± 5[b]
Thr	28 ± 5	25 ± 2	27 ± 1
Ala	33 ± 2	30 ± 6	28 ± 4
Cys	7 ± 1	8 ± 1	8 ± 1
Pro	74 ± 2	72 ± 10	75 ± 7
Total EAA	1,445 ± 467[a]	4,303 ± 387[b]	4,706 ± 594[b]
Total NEAA	241 ± 13	249 ± 9	251 ± 21
EAA:NEAA	6.0 ± 1.2[a]	16.8 ± 0.8[b]	18.9 ± 1.1[b]
Total AA	1,693 ± 389[a]	4,547 ± 405[b]	4,969 ± 558[b]

EAA = essential amino acids; NEAA = non-essential amino acids; LLML = low *L. macranthoides* leaf, 0.5% of basal diet; HLML = high *L. macranthoides* leaf, 1% of basal diet.
[a,b] Within a row, means with different superscript letters are different ($P < 0.05$); Data presented as Mean ± SD. $n = 8$ per diet.

that *L. macranthoides* exerts many biological effects, including anti-oxidant, anti-inflammatory and anti-bacterial properties (Hou et al., 2013). As *L. macranthoides* also tastes sweet and is nontoxic (Jiao et al., 2008; Sun et al., 2011), it could be a potential feed additive for livestock. Consequently, the present study for the first time reports the use of *L. macranthoides* as a feed additive, and its effects on growth performance, meat quality and amino acid profiles in growing-finishing pigs. In the present study, the results showed no changes in body weight, feed intake and F:G ratio when either 0.5% or 1% LML powder were supplemented in diets of growing-finishing pigs. Carcass traits such as dressing percentage, loin eye area, backfat thickness, and meat quality traits such as cooking loss, meat color and pH_{45} value were also not affected, except that pH_{24} value was significantly higher and intramuscular fat tended to increase in pigs supplemented with LML powder. Nevertheless, we found that free amino acid profiles in both serum and longissimus thoracis muscle were significantly changed after LML powder supplementation. These results are in line with previous results as the role of Chinese herbal medicine in amino acid metabolism in livestock has been well documented (Kong et al., 2009, 2011).

In the present study, our results showed that diet supplemented with 1% LML powder affected serum free amino acid concentrations such as arginine, lysine, cysteine, glutamic acid, proline concentrations and total free amino acid concentrations. These results are consistent with our previous studies (Ruan et al., 2014), in which we used metabolomics analysis and found that nitrogen metabolism and amino acid profiles were improved by chlorogenic acid which is one of the most important bioactive compounds in LML. Free amino acid composition is associated with meat flavor. Our results showed that diet supplemented with 1% LML powder increased arginine and valine concentration in longissimus thoracis muscle. These amino acids could react with reducing sugars to form Maillard reaction products contributing meat flavor (Imafidon and Spanier, 1994; Zhang et al., 2010). Specially, dietary LML also increased glycine concentration which is associated with sweet taste (Kato et al., 1988). Moreover, as fresh meat with higher free amino acid concentration could produce better Chinese smoke-dried meat (Chen et al., 2007), the meat of pigs fed diet supplemented with LML would be a better source.

Pork and pork products are the most important protein source for Chinese and the ratio between EAA/NEAA determines the biological values of pork proteins (Polidori et al., 2015). Our results showed that total EAA content and the ratio of EAA to NEAA were both significantly increased by LML supplementation. These results suggested that dietary LML could improve the nutritional value of meat in growing-finishing pigs. Most importantly, diet supplemented with 0.5% or 1% powder of LML increased the concentration of one of the most versatile amino acids, arginine by 246% and 280%, respectively. A possible reason for this remarkable increase might be that certain phytochemicals up-regulate the protein digestion and endogenous arginine synthesis (Kong et al., 2009). In addition, valine content was also increased by LML supplementation. This branched-chain amino acid is an important signaling molecule modulating intracellular cell signaling pathways and improving protein anabolism in meat (Rennie et al., 2006).

5. Conclusions

The present findings showed that dietary supplementation of LML powder could improve free amino acid profiles in serum and longissimus thoracis muscle in growing-finishing pigs. Especially, dietary LML increased EAA content and total free AA content in longissimus thoracis muscle, suggesting that LML could be a potential feed additive to improve the nutritional value of pork in growing-finishing pigs and diet supplemented with 0.5% LML would be proper.

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