

≪Research Note≫

Nutritive Value of Mulberry Leaf Meal and its Effect on the Performance of 35-70-Day-Old Geese

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Availability of feed crops for animal production is decreasing, creating a need to identify alternative food sources. With their high protein content, mulberry leaves are a likely candidate for feed supplementation and have been trialed on pigs and chickens, but little is known about their effect as a feed supplement on geese. Here, we determine the nutritive value of mulberry leaf meal (MLM), measure the digestibility of energy and amino acid of MLM in male Sichuan white geese, and evaluate the performance of these geese fed an MLM-supplemented diet. The composition of MLM was as follows: gross energy 4.94 Mcal/kg, crude protein 18.81%, ether extract 11.65%, crude fiber 12.45%, calcium 2.46%, phosphorous 0.24% and amino acids 0.26-1.92% (all % on a dry matter basis). Using the emptying then force-feeding method on 24 geese aged 194 days, we measured the apparent metabolizable energy of MLM as 1.58 Mcal/kg (on a dry matter basis), and the true total tract digestibility of the amino acids in MLM as 50.54-79.98%. We then randomly allocated a further 210 geese aged 35 days to one of five dietary treatments (control diet alone or supplemented with 4%, 8%, 12% or 16% MLM). Each treatment contained six replicate pens of seven birds per pen, and birds were maintained on their treatment until 70 days of age. Geese fed diets containing MLM exhibited lower weight gains, elevated feed consumption and an increased feed to gain ratio ($P \le 0.05$) compared with geese fed the control diet. Moreover, geese fed diets supplemented with MLM all experienced diarrhea, reduced amounts of subcutaneous fat and lower percentages of skin and abdominal fat ($P \le 0.05$) compared with control geese. In conclusion, MLM should be used with caution as a feed supplement for geese.

Key words: carcass yields, chemical composition, digestibility, geese, growth performance, mulberry leaf meal J. Poult. Sci., 54: 41-46, 2017

Introduction

Availability of feed crops is decreasing because of the demands of an expanding human population and greatly increased levels of biofuel production. As a result, utilization of unconventional feed sources, such as tree leaves, for animal production has attracted widespread attention. Mulberry (*Morus spp.*) species are widely distributed throughout Asia, Europe, Africa and the Americas, and have been used in animal production since the late 1980s (Sánchez, 2002). The leaves of mulberries are highly palatable and easily digestible (70–90%) for herbivores and can also be fed to monogastrics (Sánchez, 2000). The crude protein content of mulberry leaves and young stems ranges

Correspondence: Prof. Xiangwei Peng, Poultry Science Institute, Chongqing Academy of Animal Sciences, China. from 15–28%, values that are similar to most legume forages (Sánchez, 2000). The nutritive value of mulberry leaves as a protein source has been previously estimated for beef cattle (Huyen *et al.*, 2012), sheep (Kandylis *et al.*, 2009), pigs (Ly *et al.*, 2001), laying hens and broilers (Al-Kirshi *et al.*, 2009).

Goose meat is a source of high-quality protein for human consumption that is also rich in both unsaturated and essential fatty acids but low in cholesterol (Schmid, 2010.). As such, there is a growing interest worldwide in increasing goose production. According to FAO statistics, more than 6. 8 billion geese were raised for meat production worldwide in 2013. Given the high nutritive value of mulberry leaves and the efficiency of roughage consumption by geese, mulberry leaves might be used as a common feed for geese. However, little information exists regarding the potential value of mulberry leaves in goose production. Here, we determine the chemical composition of mulberry leaf meal (MLM), measure its digestibility in geese, and evaluate its effect on

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Received: April 27, 2016, Accepted: June 30, 2016

Released Online Advance Publication: August 25, 2016

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goose performance.

Materials and Methods

Animal Ethics

This study was approved by the animal care and welfare committee of the Chongqing Academy of Animal Science (CAAS), China. All geese used in this experiment were obtained from the goose-breeding center of CAAS.

Chemical Composition of MLM

Mature mulberry leaves were obtained from the Sericulture Institute of CAAS and then sun-dried and crushed into powder form. Samples of MLM were analyzed for dry matter (AOAC 4.1.06; AOAC 2000), gross energy (PARR 6400 calorimeter, Moline IL), crude protein (AOAC 990.03; AOAC 2000), ether extract (AOAC 920.39; AOAC 2000), crude fiber (AOAC 978.10; AOAC 2000), calcium (AOAC 4.8.03; AOAC 2000) and total phosphorous (AOAC 3.4.11; AOAC 2000). The methionine and cystine contents of MLM were analyzed according to the method recommended by Xie et al. (2004). Briefly, methionine and cystine in MLM were oxidized by adding 88% formic acid and 30% hydrogen peroxide at a ratio of 9:1, and then hydrolyzed at 110°C with 6 M HCl for 24 h. The hydrolyzate was then pH adjusted to 2.2 and analyzed using an amino acid analyzer (L-8900, Hitachi, Tokyo, Japan). Tryptophan and other amino acids were determined according to the method supported by the Standardization Administration of China (2000). Tryptophan was determined using alkali hydrolysis, where each sample was hydrolyzed with 4 M LiOH at 110°C for 20 h, then sodium citrate of pH 2.2 and 6M HCl were added. Samples were centrifuged at $1520 \times g$ for 8 min at 4°C, filtered using a 0.22-µm filter membrane (Micrio PSE Polyethersulfone, Jinteng, Tianjin, China), and the supernatants analyzed using High Performance Liquid Chromatography (Agilent 1260, Agilent, Santa Clara, CA, USA). The remaining amino acids in MLM were hydrolyzed with 6 M HCl at 110°C for 24 h, then analyzed by ion-exchange chromatography with an Amino Acid Analyzer (L-8900, Hitachi, Tokyo, Japan).

MLM Digestibility in Geese

Digestibility of the energy and amino acid contents of MLM in geese was measured using the "Sibbald method" with minor modifications (Sibbald, 1976). Briefly, 24 male Sichuan white geese aged 194 days with an average body weight of 3.5 kg were allocated according to initial weight to receive an MLM-supplemented feed or a control feed with no nitrogen supplementation (n=12 per group) (Table 1). Feathers within 5 cm of each bird's vent were removed to expose the skin and a special plastic retainer was sutured to the exposed skin. Birds were kept individually in metal cages ($56 \text{ cm} \times 36 \text{ cm} \times 60 \text{ cm}$) and fed with a standard feed for 48 hours. Birds were then assigned to the MLM or nonnitrogen treatment groups, and fed an MLM-supplemented or corn starch diet (Table 1), respectively, ad libitum for 24 h. After a 24 h fast, birds in the MLM groups were tube-fed 65 g of MLM, and those in the non-nitrogen group were tube-fed 65 g of corn starch. All geese had unrestricted access to

Table 1. Diet ingredients in Experiment 1 (% as fed)

Itam	Diet		
Item	MLM	corn starch	
Mulberry leaf meal	94.5	_	
Corn starch	_	94.5	
Mineral and vitamin premix ¹	0.25	0.25	
Salt	0.30	0.30	
Total	100.00	100.00	

¹Vitamin mineral premix contained the following minerals in milligrams per kilogram of diet: Cu, 8; Fe, 85; Zn, 80; Mn, 85; Se, 0.3; I, 0.4 and the following vitamin per kilogram of diet: vitamin A, 2500 IU; vitamin D₃, 2000 IU; vitamin E, 10 IU; vitamin K₃, 2 mg; thiamine, 1.5 mg; riboflavin, 10 mg; pyridoxine hydrochloride, 3 mg; cobalamin, 0.02 mg; pantothenic acid, 10 mg; nicotinic acid, 50 mg; folic acid, 1 mg; biotin, 0.15 mg; and choline chloride, 1000 mg.

drinking water and light was provided on a 24 h light/0 h dark cycle. To prevent damage to the mucosa of the esophagus during tube-feeding, water was added to the dietary pellets to produce a paste. At the time of tube-feeding, a bottle cut to a length of 3 cm with a collection bag was screwed to the sutured plastic lids for excreta collection, which lasted for 24 h. Excreta samples were dried at 65°C and their energy and amino acid contents measured using the methods outlined above. The apparent metabolizable energy of excreta samples was calculated using the following equation:

$$AME(Mcal/kg) = \frac{GE_{diet} - GE_{excreta}}{GE_{diet}} \times GE_{MLM}$$

Where GE_{MLM} was the gross energy of MLM (Mcal/kg), GE_{diet} was the total energy intake originating from the tube-fed MLM (Mcal), $GE_{excreta}$ is the total energy output in the excreta when tube-feeding MLM (Mcal).

True total tract digestibility (TTTD) of amino acids contained in MLM was calculated using the following equation:

$$\frac{\mathrm{AI}_{\mathrm{MLM}}-\mathrm{AO}_{\mathrm{MLM}-\mathrm{excreta}}+\mathrm{AO}_{\mathrm{corn\ starch}-\mathrm{excreta}}}{\mathrm{AI}_{\mathrm{MLM}}}\times100,$$

where AI_{MLM} is the total amino acid intake from the tubefed MLM (g), $AO_{MLM-excreta}$ is the total amino acid output in the excreta when tube-feeding MLM (g), and $AO_{com \ starch-excreta}$ is the total amino acid output in the excreta when tubefeeding corn starch (g).

Effect of MLM on Goose Performance

TTTD(%) =

To evaluate the effect of MLM-supplementation on goose performance, 210 male Sichuan white geese aged 35 days with an initial average body weight of 1740 g were randomly allocated to one of five treatments, each consisting of six replicate pens with seven birds per pen. The treatments were as follows: 0% MLM, 4% MLM, 8% MLM, 12% MLM and 16% MLM, where all diets were iso-energy and iso-nitrogen. Compositions and chemical analyses of the diets are shown in Table 2. Birds were kept in plastic wire-floor pens with dimensions of 150 cm \times 200 cm \times 60 cm. All birds

ets (% as fed	
12%	16%
MLM	MLM
55.00	53.80
14.28	12.01
2.55	3.40
12 00	16 00

Table 2. Ingredients and chemical composition of the diets (% as fed)

Item	Control	4% MLM	8% MLM	12% MLM	16% MLM
Ingredient					
Corn	59.14	58.64	56.83	55.00	53.80
Soy bean meal	19.69	15.86	15.12	14.28	12.01
Fish meal	1.00	2.50	2.50	2.55	3.40
Mulberry leaf meal		4.00	8.00	12.00	16.00
Alfalfa meal	13.59	12.41	10.62	8.90	7.40
Soy bean oil	2.88	3.16	3.65	4.11	4.48
Lysine · HCl	0.11	0.10	0.10	0.10	0.09
Dl-Methionine	0.23	0.23	0.23	0.23	0.23
L-Tryptophan	0.08	0.08	0.08	0.08	0.09
L-Arginine · HCl	0.08	0.13	0.14	0.15	0.16
Salt	0.30	0.30	0.30	0.30	0.30
Limestone	1.10	0.84	0.68	0.50	0.30
Hydrophosphate	1.56	1.50	1.50	1.55	1.50
Mineral and vitamin premix ¹	0.25	0.25	0.25	0.25	0.25
Total	100.00	100.00	100.00	100.00	100.00
Calculated composition					
Metabolisable energy					
(MJ/kg)	11.92	11.92	11.92	11.92	11.92
Crude protein	16.02	16.02	16.02	16.02	16.02
Crude fibre	5.10	5.10	5.11	5.10	5.10
Calcium	1.04	1.04	1.04	1.04	1.04
Total phosphorous	0.64	0.64	0.64	0.64	0.64
Available phosphorous	0.44	0.45	0.45	0.44	0.45
Lysine	0.85	0.85	0.85	0.85	0.85
Methionine	0.48	0.48	0.48	0.48	0.48
Cysteine	0.24	0.20	0.21	0.22	0.22
Threonine	0.62	0.61	0.61	0.61	0.61
Tryptophan	0.27	0.27	0.27	0.27	0.27
Arginine	1.00	0.99	1.00	1.00	1.00

¹ which was identical to that in table 1.

had free access to pelleted feed and water. At 49–50 and 68–70 days of age, a feces score was estimated based on the following scoring system: 1=normal feces, 2=watery feces and 3=more severe watery feces. The diarrhea score was expressed as an average of the scores at 49–50 and 68–70 days of age. At 70 days of age, live weight gain for all geese was measured and recorded following a 12 h fast. Feed intake and feed/gain values were calculated throughout the experimental period. Two geese were selected according to the average body weight of corresponding pens and killed by cervical dislocation. Breast meat (including pectoralis major and pectoralis minor), leg meat (including thigh and drumstick), subcutaneous fat and skin and abdominal fat were removed from the carcasses, weighed, and expressed as relative to the live body weight at processing.

Statistical Analysis

Data on goose performance and carcass characteristics were subjected to one-way ANOVA using the GLM procedure of SAS (SAS Institute, 1999). Differences were considered significant at P < 0.05 and means were compared using Tukey's test. The experimental unit was the individual goose for the energy and amino acid digestibilities, and per

repeat for the growth and carcass indexes.

Results and Discussion

Chemical Composition of MLM and its Digestibility in Geese

The composition profile of MLM (Table 3) shows that it contains high levels of crude protein (18.81%). This result is similar to that of Sánchez (2000), who reported that the crude protein content of mulberry leaves ranges from 15 to 22% depending on the variety, age of the leaves and the growing conditions. Our results also show that MLM contains 18 different amino acids at levels of 0.26-1.92% (on a dry matter [DM] basis), indicating that MLM could be a good source of protein for animals. However, digestibility of MLM in geese in terms of energy and amino acids was low (Table 4), at 1.58 Mcal/kg DM for apparent metabolizable energy and 50.54-79.98% for true digestibility of amino acids. Among the amino acids, TTTD of MLM methionine, lysine, tryptophan and threonine in geese was 67.15%, 60.88%, 63.26% and 58.30%, respectively. Overall, digestibility in geese of amino acids from MLM was found to be lower than that recorded for chickens. Specifically, Al-Kirshi et al. (2009) reported

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Item	Total content (%)
Gross energy (Mcal/kg)	4.94
Crude protein	18.81
Ether extract	11.65
Crude fibre	12.45
Calcium	2.46
Phosphorous	0.24
Methionine	0.36
Lysine	1.11
Valine	0.62
Leucine	1.45
Isoleucine	0.74
Threonine	0.79
Phenylalanine	0.92
Arginine	0.89
Histidine	0.35
Glycine	0.99
Proline	0.88
Serine	0.80
Cystine	0.30
Alanine	1.09
Glutamate	1.92
Tyrosine	0.62
Aspartate	1.77
Tryptophan	0.26
1 1 1 1 1 1	

Table 3. The chemical composition of MLM $(as \% DM)^1$

Table 4.The digestibility of energy and amino acids ofMLM in geese of MLM in geese (as DM basis)1

Item	Value
AME (Mcal/kg)	1.58
True total tract digestibility of amino acids (%)	
Methionine	67.15
Cystine	62.03
Lysine	66.08
Tryptophan	63.26
Threonine	58.30
Arginine	79.98
Leucine	65.69
Isoleucine	55.63
Valine	55.32
Phenylalanine	66.77
Histidine	62.96
Glycine	50.54
Proline	64.59
Serine	60.80
Alanine	53.04
Glutamate	56.62
Tyrosine	62.51
Aspartate	67.86
1	

¹ Values are the means of determined value 3 samples.

that digestibility of amino acids ranged from 65.54% for lysine to 81.99% for tryptophan in laying hens and from 65.64% for lysine to 80.85% for serine in broilers. In addition, the nutrient digestibilities of dry matter and neutral detergent fiber of mulberry leaves were higher in egg-layers than in broilers (Al-Kirshi *et al.*, 2009). The relatively low digestibility in geese compared with other types of poultry may be attributed partly to differences in experimental animal genotype and chemical determination methods.

Effect of MLM Supplementation on Goose Performance

As shown in Table 5, geese fed a diet containing MLM grew more slowly ($P \le 0.05$) and had a higher average feed intake $(P \le 0.05)$ and feed to gain ratio $(P \le 0.05)$ than those in the control group. These results differ from those of previous studies on broilers and laying hens. The inclusion of up to 5% MLM in broiler diets did not significantly affect the average daily feed intake, average daily weight gain, final weight or the feed to gain ratio in broilers (Margareta et al., 2015). Likewise, supplementing feed with concentrations of MLM as high as 6-10% did not negatively affect the performance or egg quality of laying hens (Al-Kirshi et al., 2010; Olteanu et al., 2012). However, it is noteworthy from our study in gees that increasing dietary MLM levels continuously aggravated diarrheal response in conjunction with lower daily weight gain and higher feed intake and feed to gain ratios (Table 5). This suggests that the decrease in growth performance may partly result from the diarrhea ¹Values are the means of 12 replicates of 1 birds.

caused by MLM consumption. Very high concentrations (1.5-2.5%) of alkaloidal sugar-mimic glycosidase inhibitors (4-dideoxy-1,4-imino-D-arabinitol, 1-deoxynojirimycin and 1,4-dideoxy-1,4-imino-D-ribitol) have been found in mulberry latex in several mulberry varieties (Konno et al., 2006). These compounds inhibit the hydrolysis of carbohydrates into glucose in the digestive tract and may thus cause hypertonic diarrhea. Recently, diarrhea was reported by Zhang et al. (2015) to occur in broilers and pigs as a result of mulberry leaf inclusion in feed. In their study, the loose stool rate increased from 10.2% to 21.2% in 3-month-old broilers fed a diet containing 1% mulberry juice, while supplementation with mulberry leaf powder (in which the mulberry juice is removed) instead of fresh mulberry (10%) leaves significantly reduced the occurrence of diarrhea and improved weight gain in piglets. Therefore, further study is needed to identify and remove substances in mulberry leaves that cause diarrhea in animals. Results from Zhang et al. (2015) and the present study suggest that diarrhea caused by mulberry leaf consumption is not species-specific. However, the severity of diarrhea caused by mulberry leaf consumption may vary by species. In the present study, low concentrations (4%) of MLM in geese feed caused severe diarrhea, while no diarrhea was reported in broilers or laying hens fed diets containing MLM concentrations of 5-10% in previous studies (Olteanu et al., 2012; Al-Kirshi et al., 2010). These findings suggest that geese may be more sensitive than chickens to the substances in mulberry leaves that cause diarrhea. Further study is needed to explain this variability in sensitivity to diarrhea-inducing substances in mulberry leaves among different species. Other toxic effects, such as steatosis, pro-

Item	Weight gain (g/ bird)	Feed intake (g/ bird)	Feed:gain (g:g)	Diarrhoea score
Control	55.93 ^a	215.16 ^b	3.85 ^c	1.0^{d}
4% MLM	47.02 ^b	329.65^{a}	7.00^{b}	2.1 ^c
8% MLM	44.33 ^{bc}	326.51 ^a	7.37 ^{bc}	2.1 ^c
12% MLM	41.79 ^{bc}	332.82^{a}	7.96 ^{bc}	2.5 ^b
16% MLM	38.69 ^c	358.57^{a}	9.27 ^c	2.9^{a}
Pooled SEM ²	1.35	11.9	0.41	0.10
P value	<0.001	<0.001	<0.001	<0.001

 Table 5.
 Effect of MLM on performance of geese from 35 days to 70 days of age¹

 $^{a-c}$ Means within a column with different superscripts are significantly different (*P* <0.05).

¹Values are the means of 6 replicates of 7 birds.

² SEM, standard error of the mean.

Table 6. Effect of MLM on carcass yield of geese at 70 days of age^{1,2}

Item	Breast meat (%)	Leg meat (%)	Subcutaneous fat + skin (%)	Abdominal fat (%)
Control	6.24	10.02	14.41 ^a	2.50^{a}
4% MLM	6.52	10.31	11.54 ^b	1.31 ^b
8% MLM	6.33	10.29	10.60^{b}	1.12 ^b
12% MLM	6.29	10.37	10.57 ^b	1.04 ^b
16% MLM	6.24	10	10.06 ^b	0.96^{b}
Pooled SEM ³	0.13	0.19	0.35	0.12
P value	0.975	0.96	<0.001	<0.001

^{a-b} Means within a column with different superscripts are significantly different ($P \le 0.05$).

¹ Values are the means of 6 replicates of 2 birds.

² The yield is calculated using the following equation: Yield=(breast meat, leg meat, subcutaneous fat + skin or abdominal fat weight) / processing live $BW \times 100$

³ SEM, standard error of the mean.

liferation of hepatic duct cells, and multiple necrosis, as well as reductions in the sizes of the hepatocyte nucleus and pancreatic acini, have also been reported in broilers fed diets containing 30% mulberry leaves (Dorigan *et al.*, 2011). Therefore, supplementation of any animal feed with MLM should be done with caution. At present, little information is available regarding the toxicity of mulberry leaves and further research is needed.

As shown in Table 6, the yields of breast meat and leg meat were not affected (P > 0.05) by supplementation with MLM. However, the yields of subcutaneous fat and skin and abdominal fat were significantly decreased by MLM supplementation (P < 0.05), with no significant differences detected among MLM treatments (P > 0.05; Table 6). These findings indicate that supplementation with any level of MLM from 4 to 16% per dry weight may decrease fat deposition in geese. The regulatory effects of MLM on lipid metabolism have been studied in several animal species. In pigs, the inclusion of 10% mulberry leaf in their diet led to a lower percentage of leaf lard and lower back-fat thickness (Li *et al.*, 2012). In poultry, mulberry leaves were found to regulate the metabolic activity of lipids, with concentrations of 3% MLM being the most effective in decreasing total cholesterol and LDL-cholesterol, while at the same time increasing HDL-cholesterol and glucose concentrations (Park *et al.*, 2012). Dietary inclusion of mulberry leaves decreased the concentration of saturated fatty acids, the omega 6 (n-6) to omega 3 (n-3) ratio and cholesterol in broilers (Margareta *et al.*, 2015). Likewise, the severe diarrhea caused by the addition of MLM to feed in the present study appeared to hinder the digestion, absorption and utilization of feed nutrients and thus had a detrimental effect on fat deposition in geese.

In conclusion, MLM contains high levels of crude protein and amino acids, making it a potential feed stuff for animals. However, in geese fed an MLM-supplemented diet, we found low digestibilities of MLM energy and amino acids and a high prevalence of diarrhea. Therefore, we recommend that MLM be used with caution as a feed supplement for geese.

Acknowledgments

This work was supported by the Special Fund for Agro-Scientific Research in the Public Interest (201303143), the Earmarked Fund for China Agriculture Research System (CARS-43), and the Chongqing Fundamental Research Project (14444, 15436 and 15402).

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