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The Effects of Dietary Mulberry Leaves (*Morus alba* L.) on Chicken Performance, Carcass, Egg Quality and Cholesterol Content of Meat and Egg

Paichok PANJA*

Department of Agricultural Technology, Faculty of Science and Technology, Thammasat University, Rangsit Campus, Pathumthani 12121, Thailand

(*Corresponding author's e-mail: paichok@tu.ac.th)

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Abstract

Experiment I (Broilers)

The experimental design was completely randomized. Two hundred and forty of 3 week old male broilers were divided into 5 groups with 4 replicates of 12 birds each. The protein and metabolizable energy content of basal diets were 20 % and 3,000 kcal/kg, respectively. The basal diets were supplemented with mulberry leaves at 0, 0.5, 1.0, 1.5 and 2.0 % of diet. The results demonstrated that feed intake, weight gain, feed efficiency, nitrogen and energy intake were not significantly different among treatments ($P > 0.05$). The carcass quality showed that carcass weight, dressing percentage and percent of abdominal fat pad were also not significantly different ($P > 0.05$). The blood cholesterol and triglyceride were significant ($P < 0.05$) lower at higher levels of mulberry leaves. However, the cholesterol content in thigh meat was not significantly different among treatments ($P > 0.05$).

Experiment II (Layers)

The experimental design was completely randomized and two hundred of 27 week old laying hens were divided into 5 groups with 4 replicates of 10 birds each. The protein and metabolizable energy content of basal diet were 16 % and 2,750 kcal/kg, respectively. The basal diets were supplemented with mulberry leaves at 0, 0.5, 1.0, 1.5 and 2.0 % of diet. The results found that feed intake, egg weight, egg mass, and egg quality were not significantly different among treatments ($P > 0.05$). The blood cholesterol was found to decrease ($P < 0.05$) at 0.5, 1.0 and 2.0 % of mulberry leaves inclusion. Likewise, triglyceride was also found to decrease ($P < 0.05$) at 0.5 to 1.5 % of inclusion. In addition, yolk cholesterol content was found to decrease and was significantly different ($P < 0.05$) at 2 % of inclusion.

Keywords: Mulberry leaves, performance, carcass, egg quality, cholesterol

Introduction

Mulberry leaves (*Morus alba* L.) have been used to feed silkworms but today are used to produce tea products. Mulberry leaves contain protein about 19 - 22 % dry matter [1]. Moreover, it has a lot of xanthophyll [2]. Recently it is reported that green tea (over 10 cups a day) has effects to decrease total-cholesterol, triglyceride and increase HDL-cholesterol which resulted in an atherogenic decrease [3]. Dot *et al.* [4] demonstrated that the intake of mulberry leaves reduced the concentration of serum lipids and atheromatous thickening of arterial intima in hypercholesterolemic rabbits.

Although much work has been done on the utilization of rats, mice and rabbits, reports on the use of mulberry leaves in poultry feeds are limited. Thus there is a need to study the mulberry leaves supplementation in poultry diets on production

performance, carcass quality, egg quality, and cholesterol and triglyceride in blood, meat and egg.

Materials and methods

Animals and diets

Experiment I

Two hundred and forty male broilers were fed a commercial diet containing 3,000 kcal/kg metabolizable energy and 23 % crude protein. Four 100-W incandescent light bulbs provided continuous lighting during blooding. Feed and water were allowed *ad libitum.* At three weeks of age, the chicks were randomly divided into 5 groups with 4 replications of 12 birds each. They were housed in 20 pens with natural ventilation. Five experimental diets, including a basal diet (**Table 1**) were formulated. To this feed was added mulberry leaves (ML) meal supplement at different levels: 0 % (control group with out ML), 0.5, 1.0, 1.5 and 2.0 % of diet. The diets were isonitrogenous (20 %) and isocaloric (3,000 kcal/kg). The diets were fed *ad libitum* in mash form for a further period of four weeks. The feed was added in small portions twice daily to avoid excessive feed accumulation and rancidity and wastage. Body weight and feed consumption data were collected once a week, on a group basis. At the end of the experiment, on day 49, the final weight of the chicks was taken. For carcass analysis, 4 birds of each replicate were selected at random and individually weighed.

Carcass separation

Carcasses were sectioned into breast and thigh portions as described by [5]. Four birds were selected at random from each replicate for slaughter by bleeding.

Table 1 Nutrient composition of experimental diet (broilers).

Feedstuffs	Percentage
Corn meal	59.93
Palm oil	1.56
Soybean meal (44 %)	35.23
Calcium carbonate	1.65
Hyperphosphate	1.04
DL-methionine	0.08
L-lysine	0.11
Vitamin - mineral premix	0.40
Total	100.00
Calculated analysis	
Protein (%)	20.00
Metabolizable energy (kcal/kg feed)	3,000.00
Fat (%)	4.00
Fiber (%)	3.64
Calcium (%)	1.06
Available phosphorus (%)	0.44
Lysine (%)	1.17
Methionine + cysteine (%)	0.72

The thigh portion was cut through the junction of thigh muscle at the pelvic girdle to the hip joints disjointing the femur following the method of [6]. The thigh meat samples were taken after removal of the skin and subcutaneous fat deposits. No attempt was made to separate the intramuscular fat from muscle tissue as adopted by [7]. Uncooked muscle tissue from the thighs was pooled. The samples were ground separately and immediately placed in plastic containers and stored at −20 °C until analyzed.

Experiment II

At 27 weeks, two hundred layers (Isa brown) were randomly divided into 5 groups, each group had 4 replicates with 10 birds per replicates. Five experimental diets containing 0, 0.5, 1.0, 1.5 and 2.0 % mulberry leaves were formulated. The basal diet with 0 level mulberry leaves acted as the control (**Table 2**). To this feed was added mulberry leaves (ML) meal supplement at different levels: 0 % (control group without ML), 0.5, 1.0, 1.5 and 2.0 % of diet. All diets were isonitrogeneous (16 %) and isocaloric (2,750 kcal/kg). The diets were fed *ad libitum* in mash form for a further period of ten weeks. Feed was added in small portions twice daily to avoid excessive feed accumulation and rancidity and wastage.

Feed intake and egg production data were collected once a week and daily respectively on a group basis. Four eggs per replicates were collected at random to determine egg quality.

Cholesterols and triglycerides content in plasma

At the end of the experiment, four birds of each replicates were selected at random and blood taken from the jugular vein and centrifuged at 3,000 rpm for 20 min. The blood plasma was stored at –30 °C until analyzed. The cholesterols and triglycerides were determined using the technique developed by [8].

Cholesterols content in meat and egg

Meat and eggs cholesterol were determined using the technique developed by [9].

Statistical analysis

The data collected were subjected to both analysis of variance and Duncan's Multiple range test (the differences between treatments) using the Statistical Analysis System (SAS) 1996 [10].

Table 2 Nutrient composition of experimental diet (layers).

Feedstuffs	**Percentage**
Corn meal	45.81
Rice bran	20.00
Leucaena leaf meal	3.50
Corn gluten	3.00
Soybean meal (44 %)	12.65
Fish meal (60 %)	4.05
Calcium carbonate	9.40
Mono-dicalcium phosphate	0.78
Salt	0.20
DL-methionine	0.51
Vitamin - mineral premix	0.10
Total	100.00
Calculated analysis	
Protein (%)	16.00
Metabolizable energy (kcal/kg feed)	2,750.00
Fat (%)	5.40
Fiber (%)	4.50
Calcium (%)	4.20
Available phosphorus (%)	0.45
Lysine (%)	0.78
Methionine + Cysteine (%)	0.63
Tryptophan	0.16

Results and discussion

Experiment I

Production performance

Feed consumption, weight gain, feed efficiency, nitrogen and energy intakes were not significantly different among treatments ($P > 0.05$) (**Table 3**). This might be because all diets were isocaloric and isonitrogenous [11]. These results are similar to Seeang [12] who reported that the supplementation of mulberry leaves in the layer diets had no effects on feed intake. Besides, Panja [13] showed that native chicken and hybrid native chicken which received the same diet also had no effects on the feed consumption.

In general the body weight gain tended to decrease with increasing levels of mulberry leaves which is probably due to the high tannin and high fiber contents of the mulberry leaves used in the experiment. Fuller *et al.* [14-15] demonstrated that chick growth had a negative relationship with dietary tannin content though [16] found no such relationship. Vohra and Kratzer [17] showed that growth of chicks was affected when they consumed high fiber diets. Feed conversion ratios were slightly worse in chicks fed mulberry leaves supplemented diets. This might account for the decrease in weight gain.

Carcass quality

Dressing percentage and abdominal fat pad are given in **Table 4**. They were not significantly different among treatments ($P > 0.05$). This fact seems to match theoretically, because a close relationship between them is well known.

Table 3 Effect of mulberry leaves supplementation on broiler performance.

	Level of Mulberry Leaves (%)					C.V.	F-test
	0	0.5	1.0	1.5	2.0		
Feed intake (g/bird/day)	115.9	121.83	121.73	115.32	120.39	3.19	NS
Weight gain (g/bird/day)	54.67	54.99	56.96	51.44	53.06	4.60	NS
Feed conversion ratio(feed : gain)	2.11	2.22	2.14	2.24	2.27	2.75	NS
Nitrogen intake (g/bird/day)	3.70	3.90	3.90	3.69	3.85	3.15	NS
Energy intake (kcal/bird/day)	346.77	365.49	365.19	345.96	361.17	3.19	NS

NS: non-significant at 95 %

Cholesterol and triglyceride content in plasma

The effects of dietary mulberry leaves on plasma cholesterol and triglyceride are given in Table 5. Total cholesterol and triglyceride content were lower for groups fed mulberry leaves diets compared to the control ($P < 0.05$). This is perhaps because of its crude fiber content. Balmer and Zilversmit [18] reported that fiber is an indigestible feed component affecting cholesterol metabolism and concentration of cholesterol in blood. Tasi *et al.* [19] reported serum cholesterol levels in rats decreased as dietary fiber content increased. Similar results were observed in laying hens [20]. Similarly, Kawrhung [21] reported that rabbits fed a high cholesterol diet and mulberry leaves at 2.5 %, showed a decrease in the levels of cholesterol in their blood by a half during 10 weeks.

Table 4 Effect of mulberry leaves supplementation on carcass quality.

	Level of Mulberry Leaves (%)					C.V.	F-test
	0	0.5	1.0	1.5	2.0		
Live weight (kg/bird)	2.35	2.32	2.35	2.19	2.18	4.00	NS
Carcass weight (kg/bird)	1.78	1.71	1.71	1.62	1.62	6.44	NS
Dressing percentage (%)	75.84	73.57	72.68	74.06	74.03	8.32	NS
Abdominal fat pad (% of body weight)	1.02	0.95	0.90	1.06	1.1	6.32	NS

NS: non-significant at 95 %

Table 5 Effect of supplementary mulberry leaves on plasma cholesterol and triglyceride content.

	Level of Mulberry Leaves (%)					C.V.	F-test
	0	0.5	1.0	1.5	2.0		
Cholesterol (mg/dl)	134.50^{a}	125.50^{c}	129.50^{b}	125.50^{c}	110.50^{d}	0.57	*
Triglyceride (mg/dl)	75.50^{a}	67.00^{b}	63.00^{c}	63.50^{c}	58.50^{d}	1.20	*

Different superscripts in the same row are significantly different at the 5 % level

Cholesterol content of thigh meat

The cholesterol content of thigh meat is shown in **Table 6**. The content was not significantly different among treatments ($P > 0.05$). Changes in plasma cholesterol belong to the "fast turnover cholesterol pool" [23] the muscle cholesterol pool comprises a slow turnover pool and equilibrates slowly with the plasma cholesterol pool. The muscle cholesterol pool is larger and perhaps less active and it may take a longer period to show a significant reduction of cholesterol levels. Therefore, a possible explanation is that cholesterol is usually associated with adipose tissue, which is more abundant in thigh meat. Also, thigh muscle has much more slow-twitch fibers. Slow-twitch fibers have many more mitochondria, their mitochondria are bigger, and the metabolic rate is much faster in comparison to fast-twitch fibers.

Slow-twitch sarcoplasmic reticula are found to contain two to three times as much cholesterol as fast-twitch *Caudofemoralis* sarcoplasmic reticula in rabbits [24]. The high cholesterol concentration reduces membrane fluidity, lowers Ca^{+} –ATPase activity [25], and regulates contraction and relaxation rates.

Table 6 Effect of mulberry leaves supplementation on cholesterol of thigh meat.

	Level of Mulberry Leaves (%)					C.V.	F-test
	0	0.5	1.0	1.5	2.0		
Cholesterol (mg/100g)	75.50	79.50	82.50	83.00	82.00	1.37	NS

NS: non-significant at 95 %

Experiment II

Production performance

Feed intake, egg production, egg weight and egg mass are given in **Table 7**. Feed intake and egg production were not significantly different among treatments. This is because the diets were isonitrogenous and isocaloric [11,12]. Egg weight and egg mass were also not significantly different among treatments ($P > 0.05$) because almost all the egg weight consists of yolk and albumen [26] and yolk and albumen weight were not significantly different (**Table 8**). This comes from the fact that egg mass was calculated from egg weight and egg production.

Table 7 Effect of mulberry leaves supplementation on productive performance of layer.

	Level of Mulberry Leaves (%)					C.V.	F-test
	0	0.5	1.0	1.5	2.0		
Feed intake (g/bird/day)	99.00	98.75	97.25	98.00	95.75	4.12	NS
Egg production (%)	91.98	90.13	93.44	90.74	92.61	3.60	NS
Egg weight (g/egg)	56.35	56.90	54.37	56.45	56.06	3.32	NS
Egg mass (g/bird/day)	51.83	51.28	50.80	51.22	51.92	4.53	NS

NS: non-significant at 95 %

Egg quality

Albumen height, haugh unit, yolk color, shell color, shell thickness, albumen weight and yolk weight are given in **Table 8**. They were not significantly different among treatments ($P > 0.05$). The control diet of this trial had corn gluten, which had a lot of carotene. So, mulberry leaves do not effect yolk color though [12] found, it had an effect because the control diet of Samrhan had no corn gluten.

Shell colour is controlled by several genes that regulate the deposition of pigments derived from the haem porphyrin ring. Hens laying brown eggs possess different alleles at several loci that provide code for the deposition of protoporphyrin in the region of the shell. Since only a few loci code for the proteins are involved in the metabolic pathway regulation, the brown shell colour is a highly heritable trait [27].

Cholesterol and triglyceride content in plasma

Total cholesterol and triglyceride content were lower significantly different ($P < 0.05$) for groups fed mulberry leaves diets compared to the control (**Table 9**). The reason was discussed in trial 1.

Cholesterol of yolk

Total cholesterol was lower significantly different ($P < 0.05$) at 2 % of mulberry leaves in the diet compared to the control (**Table 10**). Though, the groups at 0.5 - 1.5 % were not significantly different, it tended to decrease. However, cholesterol in yolk decreased less than in the blood because, the relative resistance of the egg composition to alterations in diet apparently reflects the nutritional and structural requirements for the development of the embryo [28].

Table 8 Effect of mulberry leaves supplementation on egg quality.

	Level of Mulberry Leaves (%)					C.V.	F-test
	0	0.5	1.0	1.5	2.0		
Albumen height (mm)	7.84	7.99	7.69	7.87	7.92	7.77	NS
Haugh unit	89.25	90.75	89.50	89.25	90.25	3.50	NS
Yolk colour	11.85	11.92	11.90	11.84	11.95	0.96	NS
Shell colour	34.42	36.44	33.55	36.98	35.55	6.31	NS
Shell thickness (mm.)	0.375	0.377	0.397	0.382	0.388	2.36	NS
Shell weight (g/egg)	6.95	6.83	6.98	6.92	6.74	3.62	NS
Albumen weight (g/egg)	35.70	36.58	34.55	36.35	35.81	4.33	NS
Yolk weight (g/egg)	13.70	13.49	12.84	13.18	13.51	4.32	NS

NS: non-significant at 95 %

Table 9 Effect of mulberry leaves supplementation on plasma cholesterol and triglyceride content.

	Level of Mulberry Leaves (%)					C.V.	F-test
	0	0.5	1.0	1.5	2.0		
Cholesterol (mg/dl)	93.00^{a}	87.50^{b}	82.00^{c}	90.50^{ab}	78.00^{d}	1.37	*
Triglyceride (mg/dl)	834.00^{b}	786.00^{ac}	739.00^{d}	784.00^{c}	810.50^{b}	1.20	*

Different superscripts in the same row show significant differences at the 5 % level

Table 10 Effect of mulberry leaves supplementation on yolk cholesterol.

	Level of Mulberry Leaves (%)					C.V.	F-test
	0	0.5	1.0	1.5	2.0		
Cholesterol (mg/100g)	$1,116^{a}$	$1,108^{a}$	$1,049^{ab}$	$1,032^{ab}$	960^{b}	4.54	*

Different superscripts in the same row show significant differences at the 5 % level

Conclusions

Broilers

Mulberry leaves supplements at 0 - 2 % were not affected on performance and carcass quality. The cholesterol and triglycerides in blood were found to decrease at higher levels of mulberry leaves. However, thigh meat cholesterol was not significantly different among treatments ($P > 0.05$).

Layers

The mulberry leaves supplements also do not affect the productive performance and egg quality. Blood cholesterol and triglyceride were found to decrease at higher levels of mulberry leaves inclusion. Yolk cholesterol was also found to decrease and was significantly different at 2 % inclusion.

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