

DIETARY EFFECT OF MULBERRY LEAF (*Morus alba*) MEAL ON GROWTH PERFORMANCE AND SERUM CHOLESTEROL LEVEL OF BROILER CHICKENS

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ABSTRACT

To investigate the effect of dietary mulberry leaf meal on body weight, feed conversion efficiency and blood cholesterol level, 240 day-old broiler chicks (Cobb 500) were divided into 6 treatments, each with 4 replications (10 birds/ per replicate) and offered manually prepared diets supplemented with 2.5, 3.5, 4.5% mulberry leaf meal (MLM), MLM Extract and 0.5% for a period of six weeks. Average body weight (g) gain increased ($P > 0.05$) at 2.5 or 4.5% supplementation of MLM and with MLM extract compared to control and antibiotic group. Feed conversion ratio was better at 4.5% supplementation (1.67) and on addition of MLM extract (1.63) compare to control (1.79). Total cholesterol, HDL-cholesterol and triglyceride of broiler chicks in different dietary treatments of during experimental periods were non-significant at day 10 to 15 but total cholesterol and triglyceride decreased significantly ($P > 0.05$) at d 15 to 22 compared to control and antibiotic group. Significant ($P < 0.05$) reduction of total cholesterol and triglyceride with supplementation of mulberry leaf meal at 2.5, 3.5, 4.5% MLM, MLM extract were also observed at d 22 to 42 compared to control and antibiotic group. The result suggests that inclusion of mulberry leaf meal (both 3.5% powder and extract) may be used to formulate low-cost broiler grower diet in order to produce low-cholesterol broiler meat.

Key Words: Mulberry Leaf Meal (MLM), Antibiotic, Broiler, Serum cholesterol

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INTRODUCTION

The cost of feed constitutes is the major proportion between 60-75% of the total cost of poultry production and protein cost account for over 40% of the total feed cost in livestock and poultry farming (Ojewola et al., 2005). Besides, the price of conventional protein feeds resources such as groundnut cake, fish meal and soybean meal are in high cost and cannot permit profit maximization in poultry ventures. In view of this, current research interest in the poultry industry is aimed at finding alternatives to this elusive feed ingredient. The list of possible feed alternatives includes tree fodder mulberry leaves (*Morus alba*) as a source of dietary protein for commercial livestock and poultry operations. Mulberry grows well in the tropics and subtropics, and is reported to have excellent nutritional value as forage. It is grown extensively for its leaves, which are used for raising silkworms in the sericulture industry. Mulberry leaves are rich in protein (15-35%), minerals (2.42-4.71% Ca, 0.23-0.97% P) and metabolizable energy (1,130-2,240 kcal kg⁻¹) with absence of or negligible anti-nutritional factors (Omar et al., 1999; Sarita et al., 2006). Mulberry leaves contain β -carotene, which can be converted with varying efficiency by animals to vitamin A and the xanthophylls, which can be a good source of the pigmentation of egg yolk (Sarita et al., 2006). Excellent results have been obtained with using mulberry leaves as ruminant feed (Rojas and Benavides, 1994; Gonzalez et al., 1996; Omar et al., 1999). Information on feeding mulberry to non-ruminants is scanty but it has been used in pigs (Trigueros and Villalta, 1997), laying hens (Narayana and Setty, 1977), and rabbits (Deshmukh et al., 1993). Dot et al. (2000) 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 effect of mulberry leaves inclusion in poultry diets on production performance, cholesterol and triglyceride in blood, meat and egg.

Antibiotics as feed additives have been used for years. Antibiotics have also been widely used in animal feed in many other countries although a number of individual countries and the European Union have restricted the sub therapeutic use of some antibiotics (Aarestrup, 2000). So, there is a great interest in developing natural alternatives to antibiotic growth promoters in order to maintain both bird performance and health (Cross et al., 2007). As a result, numerous medicinal herbs have been suggested to livestock producers as alternatives to antibiotic growth promoters (Doyle, 2001). Several researchers have reported the possibility of growth-promoting and antioxidative effects for some traditional medicinal herbs (Liu et al., 2006). These medicinal herbs are considered to be natural products, so consumers may willingly allow them to be included in livestock feeds. Thus the objective of the present experiment was to evaluate the effect of graded level of mulberry leaf meal in the broiler diets in order to observe the performance, feed efficiency and cholesterol level.

MATERIALS AND METHODS

Preparation of *Morus alba* leaf powder

Morus alba was collected from local area of Dinajpur district in Bangladesh. The leaves were sundried, coarsely powdered manually and then directly mixed with manually prepared diets in appropriate doses (Table 1, 2 & 3).

Preparation of other feed ingredients

Sundried and grinded corns, meat meal, bone meal, rice polish, soybean meal, soybean oil and other feed items were collected from local market of Dinajpur, Bangladesh and then directly mixed with manually prepared diets in appropriate doses (Table 1, 2 & 3). Vitamin premix used in the formulated diets was made of reputed veterinary Medicine Company.

Birds and experimental design

A total of 240 one day-old broiler chick (Cobb 500) were reared at brooding house to adjust with the environmental condition up to 10 days. After that, chicks were randomly allocated to six dietary treatment groups having 40 birds in each group; each treatment was composed of four replicates with 10 birds in each in a complete randomized design. The birds were housed on floor and routinely managed as any other commercial broiler flock. Heating was provided by a single electric brooder, where the initial temperature was set at 32 °C and decreased by 2 °C per week to final temperature of 20 °C at the end of experiment.

Experimental diets

The experimental diets in mash form and drinking water was provided *adlibitum*. All the diets were formulated manually to meet the nutrient requirements of broiler (NRC, 1994). The chicks were fed starter diet from 1 to 10 days, grower diet from 11-20 days and a finisher diet from 21 to 42 days old broiler (Table 1, 2 & 3). Diets were analyzed for dry matter, crude protein, crude fibre and crude fat according to the AOAC (1980) methods. The experimental diets were designed as T₀= control, T₁= 2.5% MLM, T₂= 3.5% MLM, T₃= 4.5% MLM, T₄= MLM Extract and T₅= 0.5% antibiotic (oxytetracycline, Trade name ®, Renata Ltd)

Observation of birds

All birds were examined twice daily for any visible physical changes like restlessness, lordosis, abnormal gait, vices and depression as well as changes of feeding style during treatment. All birds were vaccinated against Newcastle and Gumboro as per instruction of the manufacturers.

The performance trial

Initial body weight was recorded before the on set of the trial. Then body weight and feed consumption were recorded daily. Final weight of the birds was also recorded. These data were used to calculate body weight gain and feed conversion ratio.

Blood collection and estimation of serum lipid profile

The blood was collected at the last date of the experiment from each group (3 birds) with a syringe and needle directly through wing vein puncture without using any anticoagulant. After centrifugation of the clotted blood, the supernatant was carefully collected by a micropipette and preserved in eppendorf vial. The collected serum was stored at -15°C until estimation of total cholesterol, high-density lipoprotein (HDL)-cholesterol and triglycerides using lipid profile kit (Crescent Diagnostics).

Statistical Analysis

The data was analyzed by using the MSTATC program. Differences among treatments, when significant, were also ordered using Tukey's test (Kuehl, 1994). Statements of statistical significance were based on $P < 0.05$ or $P < 0.01$.

RESULTS AND DISCUSSION

Body weight gain, feed consumption and feed efficiency of the birds

The average body weight gain and total feed consumption of the birds fed different formulated MLM and antibiotic diets has been shown in table 4. The results shows that average body weight gain did not increased significantly while supplemented with different doses of MLM and antibiotic diets. However, average body weight gain increased more than 15% in MLM supplemented diets compared to control (T_0) and antibiotic fed group (T_5). This result is in agreement with Panja (2013) who observed non significant improvement of body weight gain in broilers supplemented with mulberry leaves at 0, 0.5, 1.0, 1.5 and 2.0 % of diet. This might be because all diets were isocaloric and isonitrogenous (Tan et al., 1988). The average feed consumption ranged 2300-2700g was also non-significant among treatments. This result is similar to Seeang (2001) and Simol et al. (2012) who reported that the supplementation of mulberry leaves in the layer diets had no effects on feed intake. Besides, Panja (2000) showed that native chicken and hybrid native chicken which received the same diet also had no effects on the feed consumption.

The feed conversion ratio (FCR) of birds fed different diets has been indicated in the Table 4. The results indicate that FCR could not affect by the supplemented diets. However, FCR was better at T_4 (1.63) followed by T_3 (1.67), T_1 (1.80), T_2 (1.81), T_0 (1.83) and T_5 (1.90). Similar results were also obtained by Simol et al. (2012) who showed that mulberry leaf powder can substitute up to 30% of commercial feed without any adverse effect on the feed intake, growth and FCR of the broiler chicken. But improvement of FCR might be due to stimulation of digestive enzymes followed by better digestion and utilization of feed. Moreover, dietary interactions between fat-protein, protein-minerals or minerals-fats may create the differences. It is known that high fibre content reduces feed intake in broilers (Janssen and Carré, 1985) but the better amino acid composition in mulberry leaves (Al-Kirshi et al., 2010) could have compensated for this effect.

Serum lipid profile contents of broilers

The effects of MLM supplemented diets on serum lipids like total cholesterol, HDL-cholesterol, and triglycerides of broilers are shown in table 5. Total cholesterol, HDL-cholesterol and triglyceride of broiler chicks in different dietary treatments during experimental periods were almost statistically similar and the differences were not significant ($P < 0.05$) from 10 to 15 days. However, from the table (5), it was observed that the values of total cholesterol level in T_3 , T_2 , T_1 , T_4 groups were 130.7, 91.67 mg dl⁻¹, 141.7 and 116.3 respectively. All the values were found to be lower than that of control groups T_0 (168.0 mg dl⁻¹), T_5 (164.3 mg dl⁻¹) and significantly differing from other groups. In the T_2 and T_4 groups i.e. both 3.5% MLM supplemented and their acetone extract was found total cholesterol content significantly lower than that of control fed groups. Total cholesterol and triglyceride were significantly reduced compared to control and antibiotic group (Table 5). Similar results were reported by Panja (2000) who supplemented with mulberry leaves at 0, 0.5, 1.0, 1.5 and 2.0 % of diet in broiler. This is perhaps because of its crude fiber content. Balmer and Zilversmit (1974) reported that fiber is an indigestible feed component affecting cholesterol metabolism and concentration of cholesterol in blood. Tasi et al. (1976) reported serum cholesterol levels in rats decreased as dietary fiber content increased. Similar results were observed in laying hens (Menge et al., 1974). Similarly, Kawrhung (1996) 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.

CONCLUSION

From above observation, it may be concluded that the MLM supplemented diets had limited effect on mortality rate and had no detrimental effect on fat content. The performances of broiler i.e., final body weight, feed intake, and feed conversion ratio were improved by feeding MLM supplemented diets at different levels. The positive effect of these was on serum lipids. Among the MLM supplemented diets, our findings suggest that supplementation of both 3.5% MLM powder and extracts of MLM has high potential as commercial applications for production of low-cholesterol and healthy broilers.

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Table 1: Composition of experimental starter diets fed to broilers

Items	Dietary level of Mulberry leaf meal (MLM)					
	T ₀ , kg (control)	T ₁ , kg (3.5% MLM)	T ₂ , kg (3.5% MLM)	T ₃ , kg (4.5% MLM)	T ₄ , kg (MLM Extract)	T ₅ , kg (0.5% Antibiotic)
Feed ingredients (kg/ 100 kg feed)						
Maize	52.00	50.00	50.00	50.00	50.00	50.00
Soybean meal	23.00	24.00	23.00	22.50	22.50	22.50
Rice polish	14.50	14.50	14.00	14.00	14.00	14.00
Soybean Oil	0.50	0.50	0.50	0.50	0.50	0.50
Salt	0.25	0.25	0.25	0.25	0.25	0.25
Methionine	0.25	0.25	0.25	0.25	0.25	0.25
Lysine	0.01	0.01	0.01	0.01	0.01	0.01
Growth promoter	0.50	0.50	0.50	0.50	0.50	0.50
Protein	9.00	7.50	8.00	7.500	7.500	8.00
MLM concentrate	0.00	2.50	3.50	4.50	3.50	0.00
Antibiotic	0.00	0.00	0.00	0.00	0.00	0.50
Vitamin Mineral Premix	0.25	0.25	0.250	0.25	0.25	0.25
Chemical composition						
ME(kcal kg ⁻¹)	3085	3120	3100.5	3080	3140	3095
CP(gm kg ⁻¹)	21.35	21.3	21.4	21.5	21.25	21.45
CF(gm kg ⁻¹)	3.75	3.77	3.72	3.75	3.77	3.77
Ca(gm kg ⁻¹)	1.12	1.13	1.12	1.11	1.13	1.12
P(gm kg ⁻¹)	0.56	0.56	0.56	0.54	0.58	0.56
Methionine (gm kg ⁻¹)	0.48	0.48	0.48	0.48	0.48	0.48
Lysine(gm kg ⁻¹)	1.18	1.18	1.19	1.18	1.19	1.19

Added broiler premix (Renata Animal Health Ltd.) @ 250 g per 100 kg which contained: vitamin A: 4800 IU; vitamin D: 960 IU; vitamin E: 9.2 mg; vitamin k₃: 800 mg; vitamin B₁: 600 mg; vitamin B₂: 2 mg; vitamin B₃: 12 mg; vitamin B₅: 3.2 mg; vitamin B₆: 1.8 mg; vitamin B₉: 2 mg; vitamin B₁₂: 0.004 mg; Co: 0.3 mg; Cu: 2.6 mg; Fe: 9.6 mg; I: 0.6 mg; Mn: 19.2 mg; Zn: 16 mg; Se: 0.48 mg; DL – Methionine: 20 mg; L- lysine:12 mg.

Table 2: Composition of the experimental grower diets fed to broilers

Items	Dietary level of Mulberry leaf meal (MLM)					
	T ₀ , kg (control)	T ₁ , kg (2.5% MLM)	T ₂ , kg (3.5% MLM)	T ₃ , kg (4.5% MLM)	T ₄ , kg (MLM Extract)	T ₅ , kg (0.5% Antibiotic)
Feed ingredients (kg/100 kg feed)						
Maize	53.00	52.00	52.00	52.00	52.50	53.00
Soybean meal	22.00	20.50	20.50	21.50	21.00	22.00
Rice polish	14.50	14.50	14.00	13.00	13.50	14.50
Soybean Oil	0.50	0.50	0.50	0.50	0.50	0.50
Salts	0.25	0.25	0.25	0.25	0.25	0.25
Methionine	0.25	0.25	0.25	0.25	0.25	0.25
Lysine	0.01	0.01	0.01	0.01	0.01	0.01
Growth promoter	0.50	0.50	0.50	0.50	0.50	0.50
Protein	9.00	9.00	8.50	7.50	8.50	9.00
MLM concentrate	0.00	2.50	3.50	4.50	3.50	0.00
Antibiotic	0.00	0.00	0.00	0.00	0.00	0.50
Vitamin Mineral Premix	0.25	0.25	0.25	0.25	0.25	0.25
Chemical composition						
ME(kcal kg ⁻¹)	3098	3130.5	3110.5	3080	3140	3107.5
CP(gm kg ⁻¹)	20.6	19.5	21	21.3	19.5	21.3
CF(gm kg ⁻¹)	3.77	3.78	3.76	3.77	3.78	3.78
Ca(gm kg ⁻¹)	1.18	1.15	1.16	1.15	1.16	1.13
P(gm kg ⁻¹)	0.59	0.57	0.58	0.57	0.58	0.57
Methionine(gm kg ⁻¹)	0.48	0.43	0.43	0.43	0.43	0.48
Lysine(gm kg ⁻¹)	1.05	1.06	1.05	1.05	1.06	1.05

Added broiler premix (Renata Animal Health Ltd.) @ 250 g per 100 kg which contained: vitamin A: 4800 IU; vitamin D: 960 IU; vitamin E: 9.2 mg; vitamin k₃: 800 mg; vitamin B₁: 600 mg; vitamin B₂: 2 mg; vitamin B₃: 12 mg; vitamin B₅: 3.2 mg; vitamin B₆: 1.8 mg; vitamin B₉: 2 mg; vitamin B₁₂: 0.004 mg; Co: 0.3 mg; Cu: 2.6 mg; Fe: 9.6 mg; I: 0.6 mg; Mn: 19.2 mg; Zn: 16 mg; Se: 0.48 mg; DL – Methionine: 20 mg; L- lysine:12 mg.

Table 3: Composition of the experimental finisher diets fed to broilers

Items	Dietary level of Mulberry leaf meal (MLM)					
	T ₀ , kg (control)	T ₁ , kg (2.5% MLM)	T ₂ , kg (3.5% MLM)	T ₃ , kg (4.5% MLM)	T ₄ , kg (MLM Extract)	T ₅ , kg (0.5% Antibiotic)
Feed ingredients (kg/ 100 kg feed)						
Maize	55.00	55.00	55.00	55.00	55.00	55.00
Soybean meal	20.00	20.00	20.00	21.50	19.00	20.50
Rice polish	14.50	14.50	12.50	11.00	13.00	13.50
Soybean Oil	0.50	0.50	0.50	0.50	0.50	0.50
Salt	0.25	0.25	0.25	0.25	0.25	0.25
Methionine	0.25	0.25	0.25	0.25	0.25	0.25
Lysine	0.02	0.02	0.02	0.02	0.02	0.02
Growth promoter	0.50	0.50	0.50	0.50	0.50	0.50
Protein	9.00	9.00	7.50	7.00	8.50	9.00
MLM concentrate	0.00	2.50	3.50	4.50	3.50	0.00
Antibiotic	0.00	0.00	0.00	0.00	0.00	0.50
Vitamin Mineral Premix	0.25	0.25	0.25	0.25	0.25	0.25
Chemical composition						
ME(kcal kg ⁻¹)	3115	3130.5	3140.5	3125	3170	3130
CP(gm kg ⁻¹)	19.5	18.5	18.5	19	18	19.5
CF(gm kg ⁻¹)	3.72	3.72	3.77	3.65	3.71	3.76
Ca(gm kg ⁻¹)	1.16	1.08	1.12	1.08	1.08	1.13
P(gm kg ⁻¹)	0.58	0.52	0.55	0.51	0.52	0.57
Methionine (gm kg ⁻¹)	0.4	0.4	0.4	0.4	0.4	0.4
Lysine(gm kg ⁻¹)	1.00	0.99	1.01	1.01	1.01	1.00

Added broiler premix (Renata Animal Health Ltd.) @ 250 g per 100 kg which contained: vitamin A: 4800 IU; vitamin D: 960 IU; vitamin E: 9.2 mg; vitamin K₃: 800 mg; vitamin B₁: 600 mg; vitamin B₂: 2 mg; vitamin B₃: 12 mg; vitamin B₅: 3.2 mg; vitamin B₆: 1.8 mg; vitamin B₉: 2 mg; vitamin B₁₂: 0.004 mg; Co: 0.3 mg; Cu: 2.6 mg; Fe: 9.6 mg; I: 0.6 mg; Mn: 19.2 mg; Zn: 16 mg; Se: 0.48 mg; DL – Methionine: 20 mg; L- lysine:12 mg.

Table 4: Growth performance of the birds fed on the experimental diets

Treatments	Average body weight (g)		Average weight gain (g)	Total feed Intake (g)	FCR
	Initial	Final			
T ₀	242.8	1550.0	1307.20	2350	1.83
T ₁	255.0	1600.0	1500.00	2700	1.80
T ₂	245.0	1650.0	1405.00	2550	1.81
T ₃	260.0	1750.0	1490.0	2500	1.67
T ₄	262.5	1850.0	1587.50	2600	1.63
T ₅	240.5	1500.50	1259.5	2400	1.90

Table 5: Serum lipid parameters in broilers fed different levels of MLM supplemented diets

Items	Day ^s	Dietary level of MLM						Level of Significance	
		T ₀ (control)	T ₁ (2.5%)	T ₂ (3.5%)	T ₃ (4.5%)	T ₄ (Extract)	T ₅ (Antibiotic)		
Total Cholesterol (mg dl ⁻¹)	10-	197.0 ^a	182.0 ^a	173.3 ^b	174.0 ^a	181.7 ^a	185.3 ^a	NS	
	15	±14.10	±4.58	±15.69	±9.53	±6.50	±24.00		
	15-	182.0 ^a	111.3 ^c ±13	125.3 ^b ±	120.7 ^{bc} ±11	120.7 ^{bc} ±5.	171.7 ^a		**
	22	±9.16	.65	4.04	.5	03	±7.63		
HDL-Cholesterol (mg dl ⁻¹)	22-	168.0 ^a ±	141.7 ^{bc} ±6	111.67 ^d ±	130.7 ^c ±30.	116.3 ^c ±	164.3 ^{ab} ±10.	*	
	42	6.24	.65	6.42	23	7.50	69		
	10-	43.33 ^a	49.00 ^a ±6.	38.33 ^a	48.33 ^a	46.00 ^a	51.33 ^a		NS
	15	±2.88	08	±4.16	±2.51	±6.08	±11.06		
Triglyceride (mg dl ⁻¹)	15-	56.33 ^a	52.33 ^{ab} ±8	48.00 ^{ab} ±2.	48.67 ^{ab} ±7.	47.67 ^{ab} ±5.	42.67 ^a	NS	
	22	±8.14	.73	64	02	03	±2.51		
	22-	46.00 ^{ab} ±	50.70 ^b	52.00 ^a	48.00 ^b	51.00 ^a ±	45.00 b±		*
	42	3.51	±1.52	±3.78	±4.58	6.65	6.24		
Triglyceride (mg dl ⁻¹)	10-	82.00 ^a ±	94.33 ^a ±14	89.00 ^a	74.0 ^a	83.33 ^a	85.3 ^a	NS	
	15	6.55	.64	±2.00	±9.53	±4.50	±24.00		
	15-	81.33 ^a ±	62.33 ^a	73.67 ^a	76.7 ^{bc} ±	72.50 ^a	82.0 ^a ±9.16		*
	22	1.52	±5.85	±5.68	11.59	±3.60			
Triglyceride (mg dl ⁻¹)	22-	102.0 ^c ±	77.00 ^c ±10	79.00 ^c	80.7 ^c	82.00 ^c	104.3 ^{ab} ±10.	*	
	42	3.00	.96	±4.58	±30.23	±7.54	69		

Different letters in Values are expressed as mean ± standard error of at least three replications each of which contains eight birds. Different letters in a row differ statistically significant (P <0.05). Similar letters in a row statistically non- significant (P >0.05).