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Effect of dietary mulberry *(Morus alba)* leaves on performance parameters and quality of breast meat of broilers

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ABSTRACT

The purpose of this study was to investigate the effect of the dietary mulberry leaves given to broiler chicken on their performance and on the quality of the breast meat. The experiment was conducted for 4 weeks, on 114 broilers, aged 21 days. The broilers were assigned to 3 groups (C, E1 and E2) with 38 animals per group. The diets for groups E1 and E2 also included mulberry leaves 2 and 5%, respectively. The birds had free access to the feed and water.

The average daily feed intake (g/day/broiler), average daily weight gain (g/day/broiler), final weight (g) and feed conversion ratio (g feed/g gain) were not significantly different among the 3 groups. The fatty acid content of the breast muscle had the following evolution: the concentration of saturated fatty acids decreased, while the concentration of polyunsaturated fatty acids increased by 14.53% in groups E1 and 25.58% in E2 compared to the control group, the difference between groups E2 and C being significant. The cholesterol concentration decreased in the experimental groups, the difference between groups E2 and C also being significant. The inclusion of up to 5% mulberry leaves in broiler diets did not have adverse effects on broiler performance, while it increased the quality of the breast muscle by decreasing the concentration of saturated fatty acids, omega 6 (n-6) to omega 3 (n-3) ratio, and also the concentration of cholesterol.

Key words: Broiler, Chicken breast, Cholesterol, Fatty acids, Mulberry leaves, Performance

The production cost of poultry production increased substantially recently with the higher prices of the feeding ingredients, particularly for soybeans and corn (Raghavan 2009). Thus, seeking for low cost local feed sources with similar feeding values, which to replace partially the common ingredients has never been more pressing (Wong and Tan 2009).

The use of mulberry leaves in poultry feeding is an alternative because they have a higher feeding value than other forages; they have high dry matter, protein and minerals, a high level of available digestible energy and a low fibre content. The amino acids composition of the mulberry leaves showed that they are a good source of essential amino acids, lysine (1.88%) and leucine (2.55%) particularly (Al-Kirshi *et al.* 2009). At the same time, they are rich in ascorbic acid and contain carotene, vitamin B_1, folic acid, folinic acid and vitamin D. Compared to soybeans, the main protein source for intensive poultry production, the mulberry leaves have a higher level of amino acids, which may compensate the adverse effect of the fibre on monogastric animals, on broilers particularly (Aya°an and Baylan 2012, Freddie Simol *et al.* 2012).

The effects of the dietary mulberry leaves were studied and reported recently for ruminants (Kandylis *et al.* 2009, Pal *et al.* 2010) and poultry (broilers, layers) (Olteanu *et al.* 2012). Thus, Chowdary *et al.* (2009), Dorigan *et al*. (2011) and Freddie Simol *et al.* (2012) investigated their effects on the production parameters and health state of broilers, while Jang *et al*. (2008) and Park and Kim (2010) studied effect of dietary mulberry leaves on the quality of breast muscle in terms of antioxidative potential during storage. Since it is known that high cholesterol concentration in broiler meat is detrimental to human health, Suwannee Saenthaweesuk (2009) and Sunder *et al.* (2011) investigated the effect of dietary mulberry leaves extracts used to decrease blood cholesterol concentration in broilers.

The mulberry leaves are a by-product from silkworm production, with high feeding potential, and given the serious condition of sericulture in Romania nowadays, study aimed to investigate the effect of dietary mulberry leaves given to broiler chickens on their performance and on the quality of breast meat.

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MATERIALS AND METHODS

The experiment was conducted for 4 weeks, on 21 day-old, 114 broilers with an average initial body weight of 488.92 g, and with no significant differences. The broilers were assigned to 3 groups (C, E1 and E2) with 38 animals per group, housed in 3-tier batteries, which allowed the daily record of the ingesta and excreta. The birds had free access to the feed and water. The light regimen was of 16 h/day, and environmental temperature was maintained to 22°C throughout the experimental period.

The broilers received a basal diet made of corn, soybean meal, gluten and oil (Table 1). The experimental diets replaced part of corn, soybean meal and gluten from the control diet by ground mulberry leaves (2% for E1 and 5% for E2). The dietary content of main nutrients, primarily the limiting amino acids, was in agreement with feeding requirements of the species and with the age of broilers (Table 1). Thus, diets provided in average 18.50% protein, 16.50 MJ/kg energy, 1.18% lysine, 0.48% methionine, 1.58% leucine and 0.72% threonine, as essential amino acids. Each diet was formulated using the chemical composition determinations performed on raw ingredients using mathematical model for poultry diets formulation (Burlacu *et al*. 1999), in agreement with feeding requirements recommended for intensive production of broilers.

The following parameters were monitored throughout the experimental period: average daily feed intake, average daily weight gain and feed conversion ratio. Six broilers from each group were slaughtered at the end of the experiment and breast muscle samples were collected and analysed for quality parameters.

The following chemical determinations were performed: dry matter (DM) using the gravimetric method; crude protein (CP) using the Kjeldahl method; ether extractives (EE) by extraction in organic solvents; crude fibre (CF) by alkali and acid hydrolysis; ash, using the gravimetric method; calcium by titrimetric method; phosphorus by spectrophotometry; amino acids by high performance liquid chromatography; fatty acids using gas chromatography and cholesterol by gas chromatography. The statistics processing used Origin 5.0 software, using the T-test (two populations), at 0.05 level of significance.

RESULTS AND DISCUSSION

Chemical composition and the fatty acid composition of the mulberry leaves are given Table 2. The high protein (24.70%), minerals (3.24% Ca and 0.25% P) and energy (17.14 MJ/kg) content, as well as the lack of antinutritional factors showed that they have a high feeding value and that they can also be used to feed the animal stock, besides the silkworms.

The amino acids content of mulberry leaves (1.68% lysine, 1.73% leucine and 1.53% threonine) showed that they are a good source of amino acids, particularly of essential amino acids. These results are in agreement with

Table 1. Diet formulation and chemical analysis results

	C	E1	E2
Ingredients			
Corn (%)	62.58	61.65	59.02
Soybean meal (%)	27.00	24.90	23.80
Gluten (%)	3.00	2.50	2.50
Oil (%)	2.50	4.20	5.00
Ground mulberry leaves (%)	0.00	2.00	5.00
Monocalcium phosphate (%)	1.50	1.50	1.50
Calcium carbonate (%)	1.70	1.53	1.43
Salt (%)	0.30	0.30	0.30
Methionine (%)	0.17	0.18	0.19
Lysine (%)	0.19	0.18	0.20
Choline (%)	0.06	0.06	0.06
Vitamin-mineral premix*(%)	1.00	1.00	1.00
Total	100	100	100
Chemical analysis results			
DM (%)	87.54	87.63	88.04
Crude protein (%)	18.12	18.73	18.53
Ether extractives (%)	6.02	6.20	6.11
Crude fibre (%)	3.67	4.07	4.23
Ash (%)	5.26	5.36	5.59
Calcium (%)	0.99	0.99	1.00
Phosphorus (%)	0.71	0.74	0.71
mino acids			
Lysine (%)	1.23	1.10	1.22
Methionine (%)	0.47	0.49	0.50
Cystine (%)	0.35	0.24	0.25
Threonine (%)	0.72	0.73	0.72
Glycine (%)	0.59	0.63	0.64
Serine (%)	1.03	0.85	0.96
Leucine (%)	1.56	1.58	1.61
Isoleucine (%)	0.81	0.85	0.85
Valine (%)	0.84	0.70	0.74
Arginine (%)	1.28	1.19	1.16
Phenylalanine (%)	0.93	0.83	0.81
Tyrosine (%)	0.67	0.64	0.66
Gross energy (Mj/kg)	16.55	16.53	16.75

*Content per kg diet: vitamin A-11000IU; vitamin D3-2000IU; vitamin E-27IU, vitamin K3-3mg; vitamin B_1-2mg; vitamin B_2-4mg; pantothenic acid -14.85mg; nicotinic acid -27mg; vitamin B_6-3mg; vitamin B_7–0.04mg; vitamin B_9-1mg; vitamin B_{12}-0,018mg; vitamin C-20mg; Mn-80mg; Fe-80mg; Cu-5mg; Zn-60mg; Co-0.37mg; I-1.52mg; Se-0.18 mg, coccidiostatic.

Table 2. Chemical composition of the mulberry leaves

Nutrients (% DM)	Values
Crude protein	24.70
Ether extractives	1.59
Crude fibre	12.86
Ash	14.78
Gross energy, MJ/kg DM	17.14
Amino acids (% DM)	
Aspartic acid	3.27
Glutamic acid	2.93
Serine	1.14
Glycine	0.86
Threonine	1.53
Arginine	1.13
Alanine	1.39
Tyrosine	0.69
Valine	0.85
Phenylalanine	0.98
Isoleucine	0.91
Leucine	1.73
Lysine	1.68
Cystine	0.20
Methionine	0.34
Fatty acids (% fat)	
Myristic acid C14:0	1.37
Myristoleic acid C14:1	0.64
Pentadecanoic acid C15:0	2.42
Pentadecenoic acid C15:1	7.24
Palmitic acid C16:0	25.22
Palmitoleic acid C16:1	2.35
Heptadecanoic acid C17:0	0.44
Stearic acid C18:0	5.36
Oleic acid C18:1n9	3.37
Linoleic acid C18:2n6	13.50
α linolenic acid C18:3n3	29.07
Arachidic acid C20:0	1.87
Octadecatetraenoic acid C18:4n3	0.20
Eicosatrienoic acid C20:3n6	0.81
Arachidonic acid C20:4n6	0.53
Minerals	
Calcium,% DM	3.24
Phosphorus,% DM	0.25
Iron, mg/kg DM	30.22
Copper, mg/kg DM	7.36
Manganese, mg/kg DM	61.25
Zinc, mg/kg DM	38.92
Cadmium, mg/kg DM	0.43

Table 3. Broiler performance (average values) ($\bar{X}\pm S_{\bar{X}}$)

Item	C	E1	E2
Average daily feed intake (g/day/broiler)	136.59 ±31.49	134.86 ±35.62	134.07 ±34.82
Initial weight (g)	485.96 ±21.80	496.92 ±27.02	483.28 ±30.60
Final weight (g)	2222.50 ±122.48	2247.36 ±93.12	2226.99 ±141.51
Average daily weight gain (g/day/broiler)	62.02 ±4.60	62.52 ±4.97	62.25 ±5.50
Feed conversion ratio (g feed/ g gain)	2.20 ±0.14	2.16 ±0.15	2.15 ±0.13

the data reported by Al-Kirshi *et al.* (2009). The mulberry leaves also had a rather large content of monounsaturated fatty acids (7.24% pentadecenoic acid, 2.35% palmitoleic acid and 3.37% oleic acid) and of polyunsaturated fatty acids (13.50 linoleic acid and 29.07% linolenic acid).

The use of 2% (E1) and 5% (E2) mulberry leaves to feed the broilers aged 21 days did not yield significant differences between the experimental groups and the control group in terms of average daily feed intake (g/broiler/day), average daily weight gain (g/broiler/day), final weight (g) and feed conversion ratio (g feed/g gain) (Table 3). This meant that the dietary mulberry leaves did not have an adverse effect on broiler performance. Ortiz *et al.* (2010) reported similar results using 8% dietary mulberry leaves given to broilers aged 35 days, while Chowdary *et al*. (2009) who used 5, 10, 15 and 20% dietary mulberry leaves noticed that the lowest FCR value was noticed for the 10% rate of inclusion, suggesting that this level would lower the production costs.

The results regarding content of main nutrients of broiler breast (Table 4) showed no significant differences between the control and experimental groups, being of about 22–23% protein, 1.1–1.5% fat and 1.1% ash.

Fatty acids content of broiler breast are given in Table 5. The *cis* oleic acid ($C_{18:1n9}$), monounsaturated acid, is the most widespread of all fatty acids from natural fats. In many fats, oleic acid counts for more than half of the total weight of acids, and just a few fats have less than 10% oleic acid. The oleic acid does not lack completely from any fat investigated so far. The proportion of oleic acid in broiler breast samples was lower in experimental groups: by 5.23% in E1 and by 9.43% in group E2, compared to the control group.

The differences between group E2 and C were significant ($P\leq0.05$), data similar to the reports of Komprda *et al.* (1999).

Also very widespread, but not in amounts as large as the *cis* oleic acid, is the palmitoleic acid (C_{16}-1n). The proportion of palmitoleic acid in the broiler breast samples was lower in the experimental groups: by 29.07% in E1 and by 32.77% in group E2, compared to the control group, the differences between group E2 and C being significant ($P\leq0.05$). The values are lower than those reported by

Table 4. Content of the main nutrients in the fresh broiler breast (average values) $\bar{X}\pm S_{\bar{X}}$

Item	Protein (%)	Fat (%)	Ash (%)
C	23.416±0.527	1.597±0.887	1.102±0.108
E 1	22.766±1.090	1.233±0.251	1.151±0.087
E 2	23.004±0.836	1.138±0.339	1.147±0.130

Table 5. Fatty acids concentration in the broiler breast (average values) ($\bar{X}\pm S_{\bar{X}}$) (% fat)

Item	C	E1	E2
Capric acid C10:0	0.03±0.03	0.32±0.24	0.06±0.08
Myristic acid C14:0	0.60±0.10	0.51±0.05	0.45±0.08
Myristoleic acid C14:1	0.13±0.03	0.10±0.02	0.08±0.04
Pentadecanoic acid C15:0	0.15±0.13	0.16±0.19	0.12±0.05
Pentadecenoic acid C15:1	0.51±0.18	0.82±0.24	0.78±0.16
Palmitic acid C16:0	22.31^{c}±2.37	20.41±1.11	18.54^{a}±2.17
Palmitoleic acid C16:1	4.27^{c}±0.84	3.44±0.57	2.87^{a}±0.52
Heptadecanoic acid C17:0	0.15±0.03	0.18±0.07	0.16±0.05
Heptadecenoic acid C17:1	0.17±0.11	0.37±0.16	0.29±0.14
Stearic acid C18:0	6.90±0.38	7.14±0.47	7.22±0.37
Trans oleic acid C18:1n11	0.24±0.04	0.24±0.02	0.26±0.05
Cis oleic acid C18:1n9	32.34^{c}±1.79	29.68±2.45	29.29^{a}±1.75
Cis linoleic acid C18:2n6	27.12^{c}±3.57	30.23±2.09	33.69^{a}±2.88
γ Linolenic acid C18:3n6	0.20±0.06	0.21±0.04	0.22±0.04
α Linolenic acid C18:3n3	0.53±0.09	0.61±0.10	0.63±0.08
Conjugated linoleic acid C18:2	0.37±0.12	0.38±0.10	0.30±0.09
Octadecatetraenoic acid C18:4n3	0.39±0.09	0.46±0.07	0.49±0.09
Eicosadienoic acid C20:2n6	0.34±0.11	0.37±0.05	0.33±0.04
Arachidonic acid C20:4n6	1.78^{c}±0.61	2.64±0.83	2.66^{a}±0.58
Docosapentaenoic acid C22:5n3	0.50±0.22	0.80±0.26	0.81±0.22
Docosahexaenoic acid C22:6n3	0.01±0.03	0.08±0.18	0.11±0.11
Other fatty acids	0.99±0.30	0.88±0.30	0.66±0.37

^{a,c} Significant differences (P≤0.05) from C and E2.

Komprda *et al.* (1999).

The linolenic acid ($C_{18:2n6}$) is the most widespread of the polyunsaturated fatty acids. It can be found in the broiler breast in similar proportions with the oleic acid, followed by the arachidonic acid ($C_{20:4n6}$) and by the docosapentaenoic acid ($C_{22:5n3}$). The values of the linolenic acid were higher in the experimental groups than in the control group, by 11.47% in group E1, and by 24.20% in group E2, the latter being significantly (P≤0.05) higher than in the control group. These data were slightly higher than those reported by Komprda *et al.* (1999). The values of the arachidonic acid were also higher in the experimental groups

Table 6. Total saturated and unsaturated fatty acids and their ratio in the broiler breast

Item	Average values ($\bar{X}\pm S_{\bar{X}}$) (% fat)		
	C	E1	E2
SFA	30.13^{c}±2.88	28.72±0.89	26.54^{a}±2.59
MUFA	37.65^{c}±2.55	34.64±2.86	33.56^{a}±1.99
PUFA	31.24^{c}±5.00	35.78±3.62	39.23^{a}±4.28
PUFA/SFA	1.04	1.25	1.48
MUFA/SFA	1.25	1.21	1.26
Omega 6/Omega 3	31:1	17:1	18:1

^{a,c} Significant differences (P≤0.05) from C and E2.

than in the control group, by 48.31% in group E1, and by 49.44% in group E2, the latter being significantly (P≤0.05) higher than in the control group.

Among the saturated fatty acids determined in broiler breast samples, proportion of palmitic acid ($C_{16:0}$) were lower in the experimental groups than in the control group, by 8.52% in group E1 and by 16.89% in group E2, the latter being significantly (P≤0.05) lower than in the control group. These data were slightly lower than those reported by Komprda *et al.* (1999) and Criste *et al.* (2005).

Analysis of the experimental data showed that use of mulberry leaves in diets for broiler chicken decreased total content of saturated fatty acids by 4.68% in group E1 and by 11.92% in group E2 compared to the control group (Table 6). As the dietary proportion of mulberry leaves increased, the total proportion of polyunsaturated fatty acids in breast of broilers from groups E1 and E2 also increased by 14.53% and 25.58%, respectively, the latter difference being statistically significant (P≤0.05). These results are in agreement with the literature (Almeida *et al.* 2006).

The beneficial effects of PUFA depend on the ratio of omega 6 (n-6) to omega 3 (n-3) fatty acids, the ideal n-6:n-3 proportion being 4:1 (Tribole 2006). Nevertheless, presently this ratio varies from 20 to 30:1 in Western diets, which was also the result obtained in our experiment (Table 6). The use of the dietary mulberry leaves decreased omega 6 (n-6) to omega 3 (n-3) ratio from the broiler breast samples from 31:1 in the control group to 17: 1 in group E1 and 18:1 in group E2.

Several studies showed that use of mulberry leaves in broiler diets decreased level of serum cholesterol. Sunder *et al*. (2011) showed that use of 5% extract of *Morinda citrifolia* mulberry leaves in drinking water decreased significantly serum cholesterol compared to the control group. Panja (2013) noticed that use of 0–2% mulberry leaves in broiler diets decreased significantly serum cholesterol and triglycerides with the use of higher dietary proportions.

As expected, in our experiment, the higher concentrations of polyunsaturated fatty acids in the broiler breast samples from the experimental groups decreased the cholesterol level (Table 7), which was significantly (P≤0.05) lower in the experimental groups: by 18.5% in group E1,

Table 7. Cholesterol content in the fresh broiler breast muscle (average values) ($\bar{X}\pm S_{\bar{X}}$)

Item	%
C	$50.166^{b,c\pm}7.999$
E 1	$40.903^{a\pm}3.638$
E 2	$37.549^{a\pm}1.764$

[a,b,c] Significant differences (P≤0.05) from C, E1 and E2.

and by 25.2% in group E2 compared to the control group.

In conclusion, use of up to 5% mulberry leaves in the diets for broiler chicken aged 21 days did not influence adversely the bioproductive parameters, while it improved the quality of broiler breast meat by decreasing saturated fatty acids and cholesterol levels.

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