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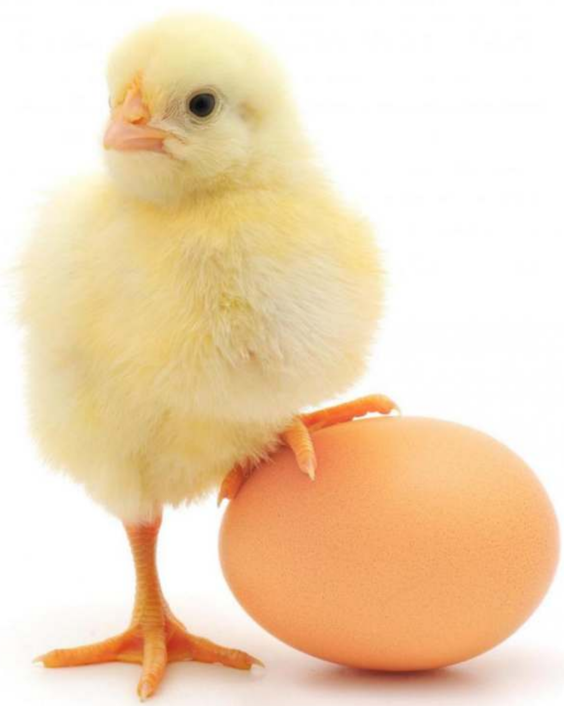

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Effect of Malic Acid on Growth Performance, Carcass Characteristics, and Feed Efficiency in the Broiler Chickens

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Abstract: This study was performed with growing chicken (1 to 56 d of age) to evaluate the effects of malic acid on broiler chicken performance. Malic acid was added to the water and offered to chicken freely from first to end of experiment with constant concentration. The treatments were zero (as a control), 0.05, 0.10, and 0.15 percent of malic acid which dissolved in water and given to them in waterer pan. The effect of treatments was investigated using 192 broiler chickens. The experimental design was a completely randomized with four treatments and four replicates. Each of four replicates was contain 12 pieces of chicken. Corn and soybean based diet were fed in mesh form, and the chickens had free access to diet and water. At the end of trial, two birds (one male and another female which selected phenotypically) from each pen were slaughtered. Blood sample has been taken just before slaughter of birds for serum analyzing. No significant difference ($P > 0.05$) was observed between treatments for weight gain and feed conversion, but treatment with 0.15% malate showed lowest feed intake ($P = 0.0590$). Highest water consumption has been observed in water with 0.05% malate concentration. This amount was 3.82% higher than control ($P = 0.0769$) meanwhile water with 0.15% malate showed lowest water consumption among malic acid treatments. The difference between 0.05 and 0.15 percent of malate was 3.76% for water consumption ($P = 0.0811$). Reduction of serum cholesterol was observed in birds on 0.05% malate ($P<0.05$), meanwhile highest serum glucose was belong to 0.10% malate concentration ($P<0.05$). Added malate to the water resulted in differences in liver composition. Chicks given 0.15% added malate had lowest liver dry matter and glycogen, and highest protein ($P<0.05$). Result showed significant interaction between treatment and sex for percentage yield of breast ($P = 0.0466$).

Key words: Broiler, malate, carcass composition, liver, cholesterol, triacylglycerol

Introduction

Malic acid is formed in metabolic cycles in the cells of plants and animals, including chickens. Peripheral malate derives from feed sources and from synthesis in the citric acid cycle. It plays an important role in generating mitochondrial ATP both under aerobic (Cheeseman and Clark, 1988) and hypoxic (Hoehl *et al.*, 1987; Wiesner *et al.*, 1988) conditions. Under aerobic conditions, the oxidation of malate to oxaloacetate provides reducing equivalents to the mitochondria by the malate-aspartate redox shuttle (Cheeseman and Clark, 1988). Under anaerobic conditions, with an excess of cytosolic reducing equivalents, inhibition of glycolysis occurs. By its simultaneous reduction to succinate and oxidation to oxaloacetate, malate is capable of removing cytosolic reducing equivalents, thereby reversing inhibition of glycolysis (Hoehl *et al.*, 1987; Wiesner *et al.*, 1988; Wilson and Cascarano, 1970). One mole of ATP is formed for each mole of malate reduced to succinate via fumarate (Hoehl *et al.*, 1987), and 3 moles of ATP for each mole of malate oxidized to oxaloacetate.

Intraperitoneal injection of malic acid to rats in amounts of 7.5 mg per kg body weight resulted in elevated mitochondrial malate followed by increased mitochondrial respiration, increased mitochondrial uptake and utilization of key substrates for ATP formation, (Bobyleva-Guarriero and Lardy, 1986). Under malate deficiency, increased proteolysis and transamination of several amino acids occur in order to increase mitochondrial malate levels through gluconeogenesis (Bobyleva-Guarriero *et al.*, 1989). Also, liver mitochondrial phosphoenolpyruvate carboxykinase may play an important role in generating malate from phosphoenolpyruvate and bicarbonate by a reversal of the activity of this enzyme under conditions of increased gluconeogenesis. This mechanism of malate production has been demonstrated in rabbit liver (Carlsen *et al.*, 1988). The reversal of this enzymatic reaction favors hepatic lipogenesis. Therefore, chronic malate deficiency could play a role in certain types of hyperlipidemia. In humans as well as in other animals tested, when there is increased demand for ATP, there is also an increased demand and utilization of malate (Abraham and Flechas, 1992).

The use of a metabolite of the citric acid cycle to correct an enzymatic deficiency of the respiratory chain has proven successful in the case of NADH coenzyme QI0 oxidoreductase deficiency (Kobayashi *et al.*, 1987).

The evidence by which exogenous malic acid may affect on chick performance is lacking. Therefore, the current

Table 1: Influence of malic acid concentration on the final body weight, feed intake and feed conversion

	Malic acid concentration (%)				
	0	0.05	0.10	0.15	SEM*
Body weight 1 (g)	2386	2455	2417	2340	42.6
Feed intake (g)	5029	5092	5030	4874	74.1
Feed conversion (feed:gain)	2.11	2.08	2.08	2.08	0.0221

[1]Final body weight in 56d old broiler chicken. [2]ab Means in row with no common superscript differ significantly (P<0.05).
*Standard error of the mean.

study was conducted to determine the effects of malic acid consumption on chicken performance, carcass characteristics and blood serum triacylglycerols and cholesterol concentration.

Materials and Methods

Chickens and treatments: One hundred and ninety two 1-d-old commercial broiler chickens (Ross, Iranian agency) were housed in floor pens containing litter composed of wood shaving and received a corn-based starter diet. Four or five days after hatching, the chicks were sorted and those with extreme weights discarded. After sorting, the chicks were randomly assigned to 16 pens each consisting of 12 birds. The room temperature was gradually decreased from 32°C at d1 to 24°C at d 22. The chicks were fed with three type diets consisted starter, grower and finisher. The light was continuous during the experiment. The corn-based diet was formulated according to the nutritional requirements for chickens. Diet was fed in mesh form and contained no growth factors, coccidiostats, exogenous enzymes, or antibiotics. Malic acid was added to the water and offered to chicken freely from first to end of experiment with constant concentration in entire experiment. The treatments were zero (as a control), 0.05, 0.10, and 0.15 percent of malic acid which dissolved in water and given to them in waterer pan. Feed and water were supplied *ad libitum* throughout the entire experiment. Body weights were determined at 14, 21, 42 and 56 d of age, but is reported in final weight. Feed intake was recorded for each pen during the entire experiment and water consumption was recorded for each pen from day 21 to day 56.

Carcass characteristics and blood samples collection: At the end of the experiment (56 d of age), two birds (one male and another female which selected phenotypically) of each pen were bled by cutting the carotid artery and blood has been taken from these artery. The blood samples were centrifuged and serum was harvested and stored at -80°C.
The carcass feather's removal was accomplished in a free-action picker after subscalding at approximately 60°C. Heads and shanks were removed, and the remaining carcasses were dissected to breast, thigh, wings, liver, abdominal fat and weighed. The percentage yield of each part was calculated on the basis of carcass weight. The meat samples (Pectoralis major, Pectoralis minor, and Sartorius) were used for determination of fat, protein, ash and glycogen. The samples were minced two times and then homogenized and kept at -25°C.

Chemical measurements: Total fat content of meat and liver was determined by extraction of samples with petroleum ether. The determination of nitrogen in meat and liver was performed with the macro-Kjeldahl method. The glycogen content in meat and liver samples were measured as described by Djawdan *et al.*, 1998.
Serum samples were also analyzed for triacylglycerols using an enzymatic and colorimetric procedure (Kit 10-525, Ziestchem Diagnostic kit, Tehran, Iran) and for cholesterol by an enzymatic procedure (Kit 10-508, Ziestchem Diagnostic kit, Tehran, Iran) and for glucose by an colorimetric procedure (Kit 10-518, Ziestchem Diagnostic kit, Tehran, Iran).

Statistical analysis: The complete randomized model was used to analyze data for weight gain, feed intake, feed conversion and water intake. In this regard, four treatments offered to chicken in four replicates individually. The experimental design for blood metabolite and carcass characteristics investigation was a completely randomized one with a 4×2 factorial arrangement of treatments. Each of four treatments was replicated four times per sex (n=4). The data were analyzed using general linear model procedure of SAS (SAS User's Guide, 1988). Duncan's multiple range test (SAS User's Guide, 1988) (P<0.05) was used to test the significance of difference between means and in some case non-orthogonal contrasts were used to determine treatment differences. Values are given as means, and the homogeneity of variance was checked.

Results and Discussion

Growth Performance Table 1 summarizes the effects of different levels of malic acid on live weight, feed intake and feed conversion. Higher live weight (69 g) in chicken on treatment with 0.05% malic acid concentration to compare with control group did not showed significant difference (P=0.2722). Feed intake was reduced 4.5% (P=0.059) using treatment with 0.15% malic acid concentration in water to compare with control and in

Table 2: Influence of malic acid concentration on the serum triacylglycerols, total cholesterol and glucose concentration

	Malic acid concentration (%)					Sex		
	0	0.05	0.10	0.15	SEM†	Male	Female	P*
Triacylglycerol (mg/dl)	50.3[b]	74.6[a]	50.5[b]	36.4[b]	5.30	50.4	50.5	0.3477
Cholesterol (mg/dl)	85.0[a]	69.4[b]	79.9[ab]	82.8[ab]	4.80	85.7	72.9	0.0134
Glucose (mg/dl)	255.3[b]	259.3[ab]	272.8[a]	268.8[ab]	4.88	270.1	257.9	0.0364

ab Means in row with no common superscript differ significantly (P<0.05). † Standard error of the mean. * Probability.

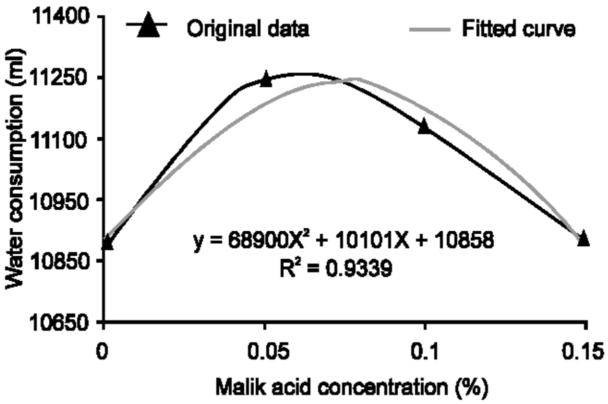

Fig. 1: Total water consumption (per chick) during day 21 to day 56. The equation is related to the fitted curve

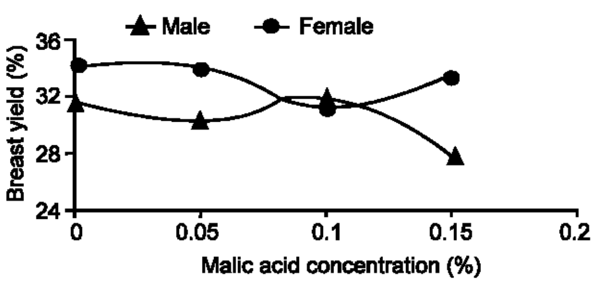

Fig. 2: The interaction between sex and treatment (malic acid concentration) for breast yield (P=0.0466)

this regard, no significant differences (P>0.05) in feed conversion was observed among treatments, through the entire experiment. As a limit literature for effect of malic acid consumption in poultry, knowledge about the role of dietary malate with this type offering is lacking. In rats, the oral administration of potassium malate increases anaerobic endurance, measured by swimming time prior to exhaustion, without a concomitant increase in carbohydrate and oxygen utilization (Dunaev *et al.*, 1988). This effect of malate showed a dose-response relationship with doubling of swimming time. However, at a higher dosage, a decrease in effectiveness of malate as observed probably due to depletion of other key substances. The above study suggest that malate has carbohydrate effects.

Total water consumption from day 21 to day 56 has been shown in Fig. 1. Using of the 0.05% malic acid in water caused increasing water consumption by 3.82% to compare with control group (P=0.0769), but increasing malic acid concentration to 0.15% in water reduced water consumption by 3.76% to compare treatment with 0.05% malic acid concentration (P=0.0811). This type reaction of chickens to the water consumption produced curvilinear shape which is explain as a second-degree parabola characterized by one change in the direction of water consumption as malic acid concentration increases. At a glance, the reason for the reduction of water consumption may reflect to the higher acidity in water which contain 0.15% malic acid concentration. But as a mentioned by Fuerst and Kare (1962) birds have a wide range of tolerance for acidity and alkalinity in their drinking water and they would accept strong mineral acid solution over extended periods of time. For this reason, probably metabolic feed back control may acts as a mechanism for water consumption.

Blood serum metabolite: The data related to serum metabolite are shown in Table 2. Significant increase (P<0.05) of triacylglycerol was observed for chicks on 0.05 percent malic acid. These data showed no significant difference (P>0.05) between control and 0.10 and 0.15 percent malic acid for the serum triacylglycerols concentration. The condition for cholesterol is completely different. Birds on malic acid treatment showed lower (P<0.05) or same levels of serum cholesterol. In this case significant difference was observed between the sex (P=0.0134). Changes in blood and liver cholesterol contents are more frequently observed, perhaps because blood (serum) and liver cholesterol belong to the "fast turnover cholesterol pool". Some researcher (Chobanian and Hollander, 1962; Field *et al.*, 1960; Konjufca *et al.*, 1997; Wagner and Clarkson, 1974) has been reported that age and sex have a bearing on the cholesterol metabolism and cholesterol concentration in pigeons. No significant trend was found in relation to increase malic acid dosage for blood metabolites. The highest triacylglycerols and the lowest cholesterol are belonged to birds on 0.05% malic acid treatment and with increasing malic acid dosage to 0.10%, the condition

Table 3: Influence of malic acid concentration on carcass characteristics

	Malic acid concentration (%)					Sex		
	0	0.05	0.10	0.15	SEM†	Male	Female	P*
Fresh carcass[1] (%)	67.93	70.89	71.21	68.82	1.489	68.27	71.15	0.3470
Thigh (%)	29.74	31.02	30.01	29.94	0.635	30.47	29.89	0.5280
Breast (%)	32.67	21.95	31.43	30.57	0.796	30.31	32.99	0.2357
Wings (%)	12.07	11.75	11.69	12.07	0.257	11.96	11.83	0.6274
Abdominal fat (%)	3.08	2.50	2.92	2.84	0.346	2.71	2.96	0.68511

Carcass yields as a percentage of live weight. ab Means in row with no common superscript differ significantly (P<0.05).
† Standard error of the mean. * Probability.

Table 4: Effect of malic acid concentration on meat composition in the birds

	Malic acid concentration (%)					Sex		
	0	0.05	0.10	0.15	SEM†	Male	Female	P*
Moisture (%)	69.83	69.91	70.28	69.92	0.671	70.49	69.48	0.9682
Protein (%)	19.48	20.36	20.96	20.00	0.571	20.70	19.70	0.3395
Ether extract (%)	8.56	7.53	6.24	8.12	1.113	6.61	8.61	0.5088
Glycogen (%)	0.79	0.88	0.94	0.84	0.061	0.92	0.81	0.3385
Ash (%)	1.34	1.32	1.57	1.12	0.153	1.27	1.40	0.5239

ab Means in row with no common superscript differ significantly (P<0.05). † Standard error of the mean. * Probability.

(serum metabolites) regressed to the normal. Then with increasing malic acid dosage to 0.15%, the serum triacylglycerols reduced about 27% to compare control group, but serum cholesterol was remained relatively constant. This type fluctuation in response to the increasing malic acid dosage partly refer to this aspect that at a higher dosage, a decrease in effectiveness of malate as observed probably due to depletion of other key substances. This result agrees with the finding of other researchers who has mentioned adequate amounts of the B vitamins (thiamine and riboflavin) (Lehninger, 1978), vitamin B_6 (Cheeseman and Clark, 1988) and magnesium (Taroni and Di Donato, 1988) are required for normal activity of malate enzyme in different metabolic path ways in the body where malate plays a key role.

No significant difference (P>0.05) was detected between treatments on malic acid consumption for serum glucose concentration (Table 2), but 7% more serum glucose concentration (P<0.05) in birds on 0.10% malic acid treatment to compare control group showed higher gluconeogenesis in these birds. In this regard other researchers reported that the relatively small amounts of exogenous malate are required to increase mitochondrial oxidative phosphorylation and ATP production. Once an elevated mitochondrial malate concentration is attained, it may support an increased rate of substrate transport into the mitochondria without depleting its own matrix concentration, for malate is regenerated in the tricarboxylic acid cycle during the oxidation of the substrates with which it exchanges (Bobyleva-Guarriero and Lardy, 1986; Hoehl *et al.*, 1987). Malate is not only oxidized to oxaloacetate (Belikova *et al.*, 1988) but also reduced to succinate (Hoehl *et al.*, 1987; Wiesner *et al.*, 1988). Succinate may use for gluconeogenesis or at least reduce proteolysis and transamination of several amino acids which is occur in order to increase through gluconeogenesis (Bobyleva-Guarriero, 1989). The above studies suggest that malate has carbohydrate and oxygen-sparing effects (Abraham and Flechas, 1992).

Carcass characteristics: The mean percentage of carcass parts in different treatments is depicted in Table 3. The percentages of carcass yield, which numerically is higher in birds on malic acid treatments, were not significant (P>0.05). In this regard, no significant effect (P>0.05) of treatments on carcass parts could be found, but the data analyzing showed significant (P=0.0466) interaction among sex and treatment for breast yield (Fig. 2). Higher percentage of breast parts in female birds may refer to the less deposition fat or higher protein in this part. Higher protein is associated with higher water, which is altogether increased breast weight. In agreement with results of present study (Young *et al.*, 2001) has been reported that female birds yielded larger proportions of breast. The difference between two sexes comes to adjustment in birds on 0.10% malic acid concentration. In this treatment breast yield percentage showed same amount among two sexes (Fig. 2). For higher dosage of malic acid (0.15%) the situation again regressed to the normal condition, where male birds produced less breast yield to compare with females. The reason for this type interaction is not completely understood. Generally this is concluded that the reaction of male birds to malic acid dosage in 0.10%

Table 5: Effect of malic acid concentration on liver composition in the birds

	Malic acid concentration (%)					Sex		
	0	0.05	0.10	0.15	SEM†	Male	Female	P*
Dry matter (%)	29.0[a]	28.6[ab]	28.2[ab]	27.5[b]	0.42	27.9	28.7	0.0936
Glycogen (%)	11.2[ab]	12.5[a]	10.0[ab]	8.4[b]	1.29	9.9	11.2	0.1645
Crude protein (%)	65.8[b]	65.8[b]	70.3[ab]	73.1[a]	1.68	66.9	70.6	0.0336
Ether extract (%)	17.1[a]	15.4[ab]	13.6[ab]	12.4[b]	1.61	13.3	15.9	0.1267
Ash (%)	5.89	6.32	6.23	6.01	0.221	6.12	6.11	0.4667

ab Means in row with no common superscript differ significantly (P<0.05). † Standard error of the mean. * Probability.

concentration is better than female.

The data in relation of chemical composition of meat samples (Pectoralis major, Pectoralis minor, and Sartorius) are presented in Table 4. As the results are shown higher numerically protein and glycogen and lower fat percentage in meat samples belonged to the birds on malic acid treatments, but this difference was not statistically significant (P>0.05). In this regard, the difference between male and female birds also was not significant (P>0.05).

Liver composition in chickens has been shown in Table 5. Higher dry matter and fat content along with lower protein content in the liver were belonged to the birds on control treatment (P<0.05). Lowest dry matter and glycogen (P<0.05) and highest protein content in the liver have been shown in birds on 0.15% malate concentration. Effect of sex, only was showed for protein concentration (P = 0.0336). Birds have the ability to store large quantities of excess energy (in the form of triacylglycerols) in liver, adipose tissue and in yolk of developing oocytes (Hermier, 1997). Lipogenesis (i.e., the conversion of glucose to triacylglycerols) takes place primarily in the liver of birds (Leveille *et al*., 1975) and involves a series of linked, enzyme-catalyzed reactions including glycolysis, the citric acid cycle and fatty acid synthesis. Hepatic lipogenesis is subject to both nutritional and hormonal control and this metabolic process is highly responsive to changes in the diet (Hillgartner *et al*., 1995; Kersten, 2001). Adipose tissue serves primarily as a storage site for lipid with little lipogenesis occurring in this tissue (Hermier, 1997). Differential lipogenic capacity of liver vs. adipose tissue in birds is a function of the expression of a key transcription factor, sterol regulatory element binding protein-1 (Richards *et al*., 2003). In another study, Latour *et al*. (1994) has been reported that chicks given 7% added lard had lower liver fat throughout the trial than chicks fed no added lard. Additionally, these chicks had a higher body protein content at 6 and 7 d of age. With respect to the above studies, the role of malic acid to changing metabolic pathway may result to higher glycogen synthesis or lower lipid production, which is completely affected by malic acid dosage. More research is need for better understanding of dietary malic acid role on chicken performance and its interaction with other key substance such as B vitamins or magnesium.

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