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COMPARATIVE ANALYSIS OF ACETIC AND CITRIC ACID ON INTERNAL MILIEU OF BROILER CHICKENS

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ABSTRACT

The aim of the present study was to analyse the effect of two organic acids (acetic and citric acid) inclusion on serum parameters and the level of antioxidant status of broiler chickens. Some organic acidifiers reduce the growth of many intestinal bacteria, reduce intestinal colonisation and reduce infectious processes, decrease inflammatory processes at the intestinal mucosa, increase villus height and function of secretion, digestion and absorption of nutrients. Broiler chickens hybrid Ross 308 (n=180) were divided into 3 groups: one control (C) and two experimental groups (E1, E2). Experimental animals received acetic and citric acid per os in water in single dose 0.25% for 42 days. After 42 days of feeding blood samples were collected (n=10 in each group). Significant decrease of serum triglycerides in citric acid group when compared with the control group was recorded. Acetic acid administration resulted in increased sodium level. Significant increase of albumin content in both experimental groups and increase of bilirubin content in citric group was recorded. Acids administration had no significant effect on other serum and antioxidant parameters. Acetic and citric acid had no harmful influenced on internal milieu of broiler chickens. The research on the field of organic acid will be worthy of further investigation.

Keywords: acetic acid; citric acid; serum parameters; antioxidants; broiler chickens

INTRODUCTION

Organic acids are weak acids and do not dissociate completely in water. They are widely distributed in nature as normal constituents of plants or animal tissues and formed through microbial fermentation of carbohydrates mainly in the large intestine **(Partanen and Mroz, 1999)**. Organic acids such as citric and acetic acids have successfully been supplemented in feed in broiler production **(Kirchgessner and Roth, 1982; Hassanpour et al., 2009)** also as alternatives to antibiotic growth promoters **(Gunal et al., 2006)**. They have mainly been used in order to sanitize the feed having problem with bacterial infection **(Thompson and Hinton, 1997)**. Several organic acids have been reported to improve growth performance, feed efficiency and mineral absorption when supplemented in nonruminant diets **(Boling et al., 2000)**, are able to inhibit microorganism growth in the food, and consequently to preserve the microbial balance in the gastrointestinal tract and improve the solubuility of the feed ingredients, digestion and absorption of nutrients **(Brown and Southern, 1985; Boling et al., 2000; Farran et al., 2005; Yesilbag and Colpan, 2006; Deepa et al., 2011)**. Citric acid is a weak organic acid and has been reported to cause a decrease in the pH of intestinal contents by contributing hydrogen ions to the intestinal environment in chickens **(Brown and Southern, 1985)** thus may have antimicrobial effects **(Barnhart et al., 1999)**. It has been reported that citric acid improves phytate P utilization in broiler chickens **(Snow et al., 2004; Rafacz-Livingston et al., 2005)**, increased the feed intake and lowered feed conversion ratio **(Deepa et al., 2011)**, improved weight gain **(Nezhad et al., 2007)**, feed efficiency and feed consumption, increased the immune status of the broilers **(Abdel-Fattah et al., 2008)**.

It was reported that vetch soaked in 1% acetic acid at room temperature for 24 h improved performance of broilers and laying hens **(Farran et al., 2001)** and at 40°C for 24 h enhanced hen performance **(Farran et al., 2005)**. **Adenkola et al. (2008)** reported that acetic acid may be of value in combating adverse effects of stress in turkeys reared during the hot-dry season.

Aerobic organisms are exposed to reactive oxygen species (ROS). In healthy organisms, their production is balanced by the antioxidant defence system. Antioxidants within cells, cell membranes and extracellular fluids can neutralize excessive ROS formation (**Halliwell, 2007)**. Cells seem to use several systems for protection against oxidative stress **(Sedlak and Snyder, 2004)**. Examples include repair enzymes (to repair damaged biomolecules), preventative antioxidants as albumin (to prevent the formation of free radicals) and scavenging antioxidants as bilirubin (to remove reactive species once formed). Data obtained from the literature indicated that only certain food components are protective against ROS (reactive oxygen species) in humans and animals **(Chakraborty et al., 2009)**. Our previous results reported that various feed additives have antioxidant properties and affect the internal milieu of poultry **(Capcarova et al., 2008; 2010a, b; 2012)**.

Many studies consider mainly the effect of organic acid on chicken performance, production parameter and its antimicrobial effects. To our knowledge only few data regarding the effect of organic acids as acetic and citric acids on serum parameters and antioxidant status of chicken blood are available. Base on this our study was designed to determine the effect of acetic and citric acids on internal milieu of chickens.

MATERIAL AND METHODOLOGY

Animals and diets

The experiment was conducted on broiler chickens, hybrid Ross 308 (n=180). Each group included 60 chickens. Chickens (no sexed) were divided into three groups (control - C and two experimental groups E1 - E2). Experimental chickens received an organic acids inclusion in water as follows: E1 - acetic acid in concentration 0.25%; E2 - citric acid in concentration 0.25%. The group of chickens received feed mixture without organic acid addition served as control.

The feeding period lasted 42 days. Chickens were fed ad libitum with complete feed mixture (CFM) (Biofeed a.s., Kolarovo, Slovakia) as follows: CFM starter (powdery form) from Day 1 till Day 21 and CFM grower (granula form) from Day 22 till Day 42. Water was provided *ad libitum*. Ingrediens and nutrient composition of diets is shown in Table 1. CFM starter contains of cereal grains, soybean meal, fish products, minerals, vitamin-mineral premix and CFM grower includes cereal grains, products and by-products from oil plants, soybean meal, fish products, minerals, vitamin-mineral premix.

Animals were kept in thermoneutral hall (from Day 1 33 °C until 21 °C at the end). In closed hall thermo aggregate was installed and experimental conditions with defined temperature and humidity were simulated by sensor. Simulated conditions were continually monitored using electronic recorder (Hivus s.r.o., Zilina, Slovak Republic). Animals were stabled cage technology (MBD). The measurements of the cage were 75x50 cm (0.370 m^2). The experiment was realized in approved breeding hall of Department of Poultry and Small Animal Husbandry in Nitra, Zobor unit.

Chickens were healthy and their condition was judged as good at the commencement of the experiment. Conditions of animals care, manipulation and use corresponded with the instruction of ethical commission. Care and use of animals and experimental devices met the requirements of the certificate of Authorization to Experiment on Living Animals no. SK PC 30008 (State Veterinary and Food Institute of Slovak Republic).

Blood sampling and analyses

After 42 days of feeding blood samples were collected (n=10 in each group). The blood serum was separated from whole blood by centrifugation at 3000g for 30 min. The concentrations of serum parameters: glucose, total cholesterol, total proteins, triglycerides, calcium (Ca), phosphorus (P), magnesium (Mg), sodium (Na), potassium (K), chlorides (Cl), alanine aminotransferase (ALT), alkaline phosphatase (ALP) and gamma glutamyl transferase (GGT) in blood serum of broiler chickens were analysed. Ecoline kits on automatic analzyer Microlab 300 (Merck, Germany), spectrophotometer Genesys 10 (Thermo Fisher Scientific Inc., USA) were used according to manufacturer condition.

The activity of antioxidant enzyme superoxide dismutase (SOD), glutathione peroxidase (GSH-Px), bilirubin, and albumins content were assayed by spectrophotometer Genesys 10 (using antioxidant RANDOX kits (Randox Labs., Crumlin, UK) according to the manufacturer's instructions.

Statistical analysis

SAS software and Sigma Plot 11.0 (Jandel, Corte Madera, USA) were used to conduct statistical analyses. One-way ANOVA was used to calculate basic statistic characteristics and to determine significant differences among the groups. Data presented are given as mean and standard deviation (SD). Differences were compared for statistical significance at the level $P < 0.05$.

RESULTS AND DISCUSSION

Organic acids given in animal diet improved digestibility of proteins and amino acids and the absorption of minerals **(Omogbenigum et al., 2003)**. Citric and acetic acids have been examined many times for its efficacy in improving growth performance, however little work has detected their effects on internal milieu of animals. The poultry sector is searching for new feed additives in order to improve the feed efficiency and the animal health **(Shane, 1999)**. Among various compounds, organic acids are promising alternatives **(Hyden, 2000)**.

Table 1 Diet composition of feed mixture KKZ HYD-01 and HYD-02

Ingredient	KKZ HYD-01	KKZ HYD-02
Dry matter (g.kg-1)	917.3	913.3
Crude protein (g.kg-1)	211.3	199.7
Fat (g.kg-1)	25.5	23.0
Starch (g.kg-1)	413.0	434.8
Total sugar (g.kg-1)	49.5	31.7
ME (MJ)	11.69	11.56
Ca (g.kg-1)	12.121	8.207
P (g.kg-1)	7.833	6.834

Ca, calcium; P, phosphorus

Table 2 Effect of organic acids on serum parameters of chickens

Parameter	C	E1	E2
Glucose (mmol.L^{-1})	11.31 ±0.60	11.09 ±0.72	11.29 ±0.56
Triglycerides (mmol.L^{-1})	0.78 ±0.16^{a}	0.68 ±0.15	0.60 ±0.09^{b}
Cholesterol (mmol.L^{-1})	5.60 ±0.61	5.41 ±0.54	5.72 ±0.23
Total proteins (g.L^{-1})	37.85 ±2.86	36.45 ±3.27	39.20 ±3.40

C - control group (without organic acid supplement); E1 - E2 experimental groups with organic acid addition, values shown as means ± SD, superscripts with different letters $^{(a-b)}$ within the same row differ significantly (*P* <0.05).

Table 3 Effect of organic acids on serum mineral parameters of chickens

Parameter (mmol.L^{-1})	C	E1	E2
Ca	2.73 ±0.22	2.55 ±0.29	2.77 ±0.20
P	2.34 ±0.19	2.29 ±0.25	2.36 ±0.17
Mg	1.02 ±0.301	0.92 ±0.14	1.01 ±0.16
Na	145.6 ±2.6^{b}	146.3 ±1.8	148.9 ±3.3^{a}
K	5.26 ±0.61	5.19 ±0.28	5.26 ±0.45
Cl	115.5 ±3.0	114.8 ±1.6	116.5 ±2.3

Ca - calcium, P - phosphorus, Ma - magnesium, Na - sodium, K - potassium, Cl - chlorides, C - control group (without organic acid supplement); E1 - E2 experimental groups with organic acid addition, values shown as means ± SD, superscripts with different letters $^{(a-b)}$ within the same row differ significantly (*P* <0.05).

Table 4 Effect of organic acids on enzymatic activity of chickens

Parameter (µkat.L^{-1})	C	E1	E2
ALT	0.10 ±0.03	0.09 ±0.02	0.11 ±0.02
ALP	34.96 ±13.71	34.47 ±6.58	42.24 ±15.84
GGT	0.37 ±0.07	0.34 ±0.10	0.29 ±0.10

ALT - alanine aminotransferase, GGT - gamma glutamyl transferase, ALP - alkaline phosphatase, C - control group (without organic acid supplement); E1 - E2 experimental groups with organic acid addition, values shown as means ± SD, differences were not significant (*P* >0.05)

Table 5 Effect of organic acids on antioxidant parameters of chickens

Parameter	C	E1	E2
SOD (U.ml^{-1})	15.36±2.11	13.17±2.52	16.32±2.91
GSH-Px (U.L^{-1})	524.35±69.49	526.35±59.65	495.37±77.26
Albumins (g.L^{-1})	13.41±0.93^{b}	14.26±1.06^{b}	15.60±1.02^{a}
Bilirubin (mmol.L^{-1})	3.37±0.72^{b}	5.05±2.21	6.66±3.24^{a}

SOD - superoxid dismutase, GSH-Px - glutathion peroxidase, Fe - iron, Cu - copper, E1 - E4 experimental groups with propolis addition in various doses, values shown as means ± SD, superscripts with different letters $^{(a-b)}$ within the same row differ significantly (*P* <0.05).

Effect of organic acids on serum parameters of broiler chickens

Results of this study are shown in Table 2. Dietary supplementation with both acids resulted in decrease in blood triglycerides content of broiler chickens. Statistical analyse showed significant difference (P <0.05) between the control and E2 (citric acid) group. Increase of glucose in acetic acid group in comparison with the control group was insignificant (P >0.05). Blood glucose, cholesterol and total proteins were not influenced by organic acids inclusion. Serum cholesterol content was not influenced by acids inclusion. In the study with mice a mixture of thiamine, arginine, caffeine, and citric acid resulted in decrease of triglyceride content **(Muroyama et al., 2003)**. The results of **Štukelj et al. (2010)** indicated that the diet with citric acid had no deleterious effects on various blood parameters of pigs and no changes have been determined. Adding of acidifier to the chicken diet caused decrease of serum cholesterol, total lipids and triglycerides **(Abdel-Fattah et al., 2008)**. **El-Afifi et al. (2001)** reported no significant effect on serum lipids after citric acid treatment. The discrepancies in literature may be due to different time of feeding, kind of animal involved in the experiments, concentration of acid and environmental conditions. The mechanism of action through organic acids can decrease serum triglyceride content and possibly other parameters of lipid profile may be interpreted through influence in decreasing the microbial intracellular pH. Thus, inhibits the action of important enzymes and forces the bacterial cell to use energy to release the acid protons, leading to an intracellular accumulation of acid anions **(Abdel-Fattah et al., 2008)**.

Effect of organic acids on serum mineral parameters of broiler chickens

The results are presented in Table 3. The addition of citric acid caused significant ($P < 0.05$) increase of Na content in comparison to the control group. Slight increase of this parameter was found also in E1 group (acetic acid), however without significant difference ($P > 0.05$). Other serum mineral parameters were not affecting by organic acids and the values of both experimental groups were similar to those found in the control group.

Citric acid is absorbed across the intestinal brush border membrane via a Na+-dependent transport mechanism that seems to be specific for tri- and dicarboxylic acids **(Wolffram et al., 1992)**.

Effect of organic acids on serum enzymatic activity of broiler chickens

Table 4 presents activity of selected enzymes ALT, ALP, GGT in blood serum of broiler chickens after organic acid supplementation. Among the enzymatic profile, there was no variation in enzymes activity in experimental groups when compared to the control group. Control group had similar level of enzymatic activity to those of the experimental groups and differences among the groups remained insignificant ($p > 0.05$).

Some authors reported significant changes of both acids on cholesterol, total proteins content, Ca and P content, enzymatic activity **(El-Afifi et al. 2001; Muroyama et al., 2003; Abdel-Fattah et al., 2008; Štukelj et al., 2010)**. The discrepancies in literature may be due to different time of feeding, kind of animal involved in the experiments, concentration of acid and environmental conditions.

Effect of organic acids on parameters of antioxidant status of broiler chickens

Addition of citric and acetic acids resulted in increase in albumins, bilirubin and D-3 hydroxybutyrate in chicken blood when compared to the control group (Table 5). Albumins content was increased in both experimental groups against the control, significantly ($P < 0.05$) in the groups with citric acid addition. Bilirubin concentration tended to increase in E1 group with comparison to the control group, however the differences remained insignificant ($P < 0.05$). In E2 group the increase in blood bilirubin was confirmed also statistically ($P < 0.05$). Activities of antioxidant enzymes (SOD, GSH-Px) in experimental groups were similar to those from control group and differences among the groups were insignificant ($P > 0.05$). SOD and GSH-Px are important antioxidant defences, as these enzymes are involved in the clearance of superoxide and hydrogen peroxide **(Mates and Sanchez-Jimenez, 1999)**. Our study demonstrated no diet related changes in SOD and GSH-Px activity. Similar results were reported by **Štukelj et al. (2010)** on pigs. Serum albumin an important protein that presents direct protective effects **(Bourdon et al., 1999)** represents a very abundant and important circulating antioxidant **(Roche et al., 2008).** In our study significant increase ($P < 0.05$) in the content of serum albumins in citric acid group E2 versus control group was measured. Acetic acid also increased albumins content when compared to the control group, however without significant difference ($P > 0.05$). **Yesilbag and Colpan (2006)** found that organic acid mixture in laying hen diet for 18 weeks significantly enhanced serum total protein and albumin concentrations what could be related to improvement of intestinal amino-acids absorption in acidic conditions that consequently enhances protein synthesis.

The combined evidence from animals and human studies indicates that bilirubin, member of the antioxidant family, is a major physiologic cytoprotectant and might alleviate oxidative stress in the blood **(Sedlak and Snyder, 2004)**. In our study both acids increased the bilirubin content in chickens against the control group, significantly ($P < 0.05$) in case of citric acid (E2 group). Organic acids can supress the growth of pathogenic bacteria, encourage the growth of beneficial microflora and ensure that the enzymes function is at maximal capacity **(Ghazalah et al., 2011)** The way of action of organic acids seems to be related to a reduction of pH in the upper intestinal tract, interfering with the growth of undesirable bacteria and modifying to intestinal flora **(Kirchgessner and Roth, 1982)**. An optimal balance in the intestinal flora is beneficial for health and development of the chickens **(Garrido et al., 2004)**. Some data indicated that addition of acetic and citric acids to the diet for broilers resulted in the increase of villus height of the small intestine. Organic acidifiers reduce the growth of many pathogenic or non-pathogenic intestinal bacteria, therefore reduce intestinal colonisation and reduce infectious processes, ultimately decrease inflammatory processes at the intestinal mucosa, which increase villus height and function of secretion, digestion and absorption of nutrients than can be appropriately performed by the mucosa **(Ghazalah et al., 2011)**. However, there is need to conduct more research in order to establish the suitability of adding organic acids to broilers diet **(Nourmohammadi et al., 2010)**.

CONCLUSION

In conclusion, these results show a beneficial effect of organic acids consumption, mainly citric acid, on serum triglycerides concentration, Na content and antioxidant status in broiler chickens. To widen this idea, more experiments should be performed with various combinations and doses of organic acids.

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