EFFECT OF WATER ACIDIFICATION ON SOME MORPHOLOGICAL, DIGESTIVE AND PRODUCTION TRAITS IN BROILER CHICKENS

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Summary

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The objective of the study was to determine the effect of acidifier added to drinking water on growth rate, performance index, flock uniformity, weight of edible giblets and immune organs, fermentation process in the caecum and excretion of dry matter and crude protein in broiler chicks. One hundred one day old broiler chicks were assigned in two equal groups. Birds of the test group were supplied with drinking water with the addition of acidifying preparation (in amount of 2 mL/L during the whole experiment).Acidification of drinking water had positive effect on growth rate during finisher phase (P<0.05) and reduction of crude protein in faeces (P<0.001). Although not statistically significant, water acidification increased flock body weight uniformity. No significant effect of water acidification were observed on performance index, weight of edible giblets and immune organs, pH and concentration of short chain fatty acids in caecum content as well as content of dry matter in faeces.

Key words: caecal fermentation, chickens, excretion, flock uniformity, growth rate, immune organs

INTRODUCTION

After the European Union has restricted the use of antibiotics as growth stimulators in animal production in 2006, the search for more suitable and safer alternatives became of particular interest. Antibiotics have been used for preventing diseases and improvement of growth performance. However, this continuous use of antibiotics resulted in development of drug-resistant bacteria, drug residues in the body of animals, and imbalance of normal microflora. Different natural substances, such as probiotics, prebiotics (Wang *et al*., 2016), phytobiotics (Abd El-Hakim *et al*., 2009; Hassan *et al.*, 2015), enzymes (Ao *et al*., 2009; Benţea *et al.,* 2010), humic substances (Šamudovská & Demeterová, 2010), and acidifiers (Byrd

et al., 2001; Eftekhari *et al*., 2015), can be used as feed additives and may improve health of animals, reduce microbial pathogens (e.g. *Escherichia coli*, *Salmonella* or *Campylobacter*) without the use of antibiotics, and enhance nutrition digestibility for better performance and to decrease the accumulation of ammonia in premises.

High nitrogen excretion is one of most serious environmental problems in intensive poultry-raising. This nitrogen is in the litter converted through microbial fermentation to volatile ammonia, whose higher concentration in the air of stud areas negatively affects health and performance of animals as well as health of farm staff (Abd El-Hakim *et al*., 2009). It was found that in the poultry-raising, 18 per cent of fed nitrogen is released to the atmosphere in the form of ammonia (Patterson, 2005). Experiments with low-protein diets supplemented with amino acids were performed to reduce nitrogen excretion. The depression of dietary crude protein levels caused the increment of abdominal fat; the growth performance and carcass yield of broiler chickens were also affected (Bregendahl *et al.*, 2002; Yonemochi *et al.*, 2003).

One of the possibilities to lower the ammonia excretion in poultry-raising, might be the application of acidifying substances to diets or drinking water. Acidifiers (mainly organic acids and their salts) are additives which can be added to animal diets as a suitable replacement for antibiotic growth promoters. By their supplementation the buffering capacity of feed and the pH of gastrointestinal tract are lowered, the digestibility of proteins is improved and growth and multiplication of pathogenic microorganisms causing diarrhoea, are restricted. Among most used organic acids are the lactic acid, fumaric acid, citric acid, propionic acid,

butyric acid, formic acid, acetic acid and sorbic acid. Acidifiers are usually not composed of a single acid, but a combination of two or more. Also inorganic acids, such as hydrochloric acid and phosphoric acid, might be used as acidifiers, they lower the pH but they were found to be ineffective (Dhama *et al*., 2014; Krisham & Narang, 2014). It was observed in many studies that the organic acids addition to the diets of broiler chicks may positively influence growth, nutrient utilisation and the microbial population of gastrointestinal tract (Abdel-Fattah *et al*., 2008; Ao *et al*., 2009; Hassan *et al*., 2010; Mohamed *et al*., 2014; Hassan *et al*., 2015). However, the acidification of diets may cause corrosion of metal tools and cans for preparation and storing of diets (Zhu *et al*., 2014). This negative effect can be reduced by application of organic acids into drinking water, what may have similar positive effect on performance of broiler chicks (Pesti *et al*., 2004; Sultan *et al*., 2015). Alzawqari *et al*., (2013) reported that the addition of organic acid into drinking water 8 h before slaughter might help to reduce gizzard, caecal and faecal contamination by pathogenic bacteria and reduce microbial loads on poultry carcasses.

The objective of this experiment was to study the influence of drinking water acidification on growth performance, some carcass characteristics, processes of digestive tract and excretion in broiler chicks.

MATERIALS AND METHODS

The experiment was carried out in the barns of University of Veterinary Medicine in Košice in compliance with the EU regulations concerning the protection of experimental animals. The experiment

was carried out with the consent of the institutional Animal Care and University Ethics Committee.

One hundred unsexed one day old broiler chicks (Ross 308) were used in 42-days growing trial. Chicks were weighed, randomly assigned in two equal groups and housed on deep bedding in agreement with the technological instruction for Ross 308 chicks, with controlled light, temperature, animal hygiene and feeding regime. The stocking density didn't exceed 33 kg/m^2 as per Council directive 2007/43/ EC (Anonymous, 2007). The average initial body weight of chicks was 42 g. The control group received normal drinking water (pH 7.37) during the experiment. The test group received drinking water enriched with a commercially available acidifier (Schaumacid Drink$^®$ – a blend of ascorbic acid, lignosulphonic acid, lactic acid, ammonium formate and ammonium propionate; Schaumann) in amount of 2 mL/L of water (pH 3.98) during the whole experiment. Broiler in both groups have been fed with a starter diet during 1st–2nd week, then with a grower diet during 3rd– 5th week and then with a finisher diet during the 6th week. Diets were based on corn, soybean meal and wheat (Table 1). No antibiotic growth promoters or anti-coccidial drugs were used in the diets. All

Table 1. Composition of diets

	Starter diet	Grower diet	Finisher diet
Ingredients (%)			
Corn	43.50	50.00	50.00
Soybean meal	36.00	33.00	31.00
Wheat	12.10	9.00	10.40
Vegetable oil	4.00	4.00	5.00
Limestone	2.00	1.60	1.50
Vitamin and Mineral premix	2.00[1]	2.00[2]	2.00[3]
Lysine	0.4	0.4	0.1
Chemical composition (%)			
Dry matter	89.69	90.02	89.39
Crude protein	25.00	23.05	21.87
Ether extract	7.01	7.19	8.03
Crude fibre	3.67	4.43	4.26
Crude ash	8.23	6.69	6.60
Metabolisable energy (MJ.kg^{-1})	13.30	13.30	13.50

[1]Vitamin and Mineral premix (per kg): Ca 95 g, P 135 g, Na 75 g, Mg 5 g, DL-methionine 80 g, vit.A 600,000 IU, D$_3$ 135,000 IU, E 900 mg, K$_3$ 150 mg, panthotenic acid 600 mg, niacin 4000 mg, cholin chloride 20,000 mg, B$_6$ 150 mg, B$_{12}$ 900 µg, biotin 3000 µg, folic acid 76,000 µg, vit. C 2000 mg, Fe 1500 mg, Cu 500 mg, Zn 3000 mg, Mn 5000 mg, I 25 mg, Se 23 mg, Co 10 mg; [2]Vitamin and Mineral premix (per kg): Ca 100 g, P 135 g, Na 75 g, Mg 5 g, DL-methionine 80 g, vit. A 425,000 IU, D$_3$ 84,000 IU, E 900 mg, K$_3$ 100 mg, pantotenic acid 420 mg, niacin 3400 mg, cholin chloride 14,200 mg, B$_6$ 100 mg, B$_{12}$ 640 µg, biotin 2150 µg, folic acid 54,500 µg, vit.C 1400 mg, Fe 1500 mg, Cu 500 mg, Zn 3000 mg, Mn 5000 mg, I 25 mg, Se 23 mg, Co 10 mg; [3]Vitamin and Mineral premix (per kg): Ca 110 g, P 145 g, Na 75 g, Mg 9 g, DL-methionine 55 g, vit. A 370,000 IU, D$_3$ 135,000 IU, E 900 mg, K$_3$ 95 mg, panthotenic acid 370 mg, niacin 3880 mg, cholin chloride 14,000 mg, B$_6$ 95 mg, B$_{12}$ 560 µg, biotin 1850 µg, folic acid 47,000 µg, vit.C 1240 mg, Fe 1500 mg, Cu 500 mg, Zn 3000 mg, Mn 5000 mg, I 25 mg, Se 23 mg, Co 10 mg

birds were given free access to feed and water. The lighting schedule was 24 h of light per day.

Live body weight (BW) and feed consumption were observed weekly. Growth rate, performance index and flock uniformity was calculated according to the equations: Growth rate = {(final BW – initial BW) / [0.5 × (initial BW + final BW)]} x 100; Performance index = (BW / feed conversion ratio) × 100; Flock uniformity (%) = 100 – [(standard deviation / BW mean) × 100].

At the age of 35 days, eight birds from each group were weighed and slaughtered by cervical dislocation to determine relative weight of edible giblets (liver, heart) and immune organs (spleen, bursa of Fabricius), and to determine pH and concentration of short chain fatty acids in caecum contents. The pH value of caecum contents was determined by pH-meter (Consort C830, Belgium). Relative organ weight was calculated as a percentage of live body weight. The faeces were collected thrice a day every day during the fifth week. The collection of faeces from random chickens in each group was made on clean solid base immediately after excretion to eliminate any contamination with raw feed or feathers. Composite samples from each group in appropriate amounts were frozen and kept at −18°C until analysis for dry matter and crude protein content.

The concentration of fatty acids (acetic acid, propionic acid, butyric acid, lactic acid) was analysed by isotachophoresis using a two-capillary isotachophoretic analyser (EA100, VILLA LABECO, Slovak Republic).

Diets were ground through a 1 mm screen in preparation for chemical analysis. Dry matter, crude protein, ether extract, crude fiber, crude ash in the diets and dry matter and crude protein in the faeces were analysed according to Association of Official Analytical Chemists (AOAC 2001).

Statistical evaluation of the effects of acidifier on body weight, growth rate, flock uniformity, performance index, relative weight of organs, indicators of the fermentation process and content of dry matter and crude protein in faeces of chicks was done by unpaired *t*-test. Results were expressed as means ± standard error of the means (SEM).

RESULTS

Evaluating growth rate of chicks in respective trial phases, a significantly higher growth rate of chicks in trial group than in the control group (P<0.05; Table 2) was found in finisher phase (36th–42nd day). The addition of acidifier to drinking water had no significant influence on growth of chicks in starter (1st–14th day) and grower (15th–35th day) phase of trial. Similarly,

Table 2. Effect of drinking water acidification on growth rate

	Control			Test		
1st–14th day	156.8	±	0.75	157.2	±	0.44
15th–35th day	139.6	±	1.21	136.8	±	0.14
1st–35th day	191.6	±	0.07	191.2	±	0.09
36th–42nd day	24.9	±	0.35[a]	27.0	±	0.52[b]
1st–42nd day	193.4	±	0.06	193.3	±	0.05

Note: [ab] significant differences (P<0.05).

Table 3. Effect of drinking water acidification on flock uniformity and performance index

	Control			Test		
Flock uniformity (%)						
35th day	82.25	±	1.76	87.59	±	1.14
42nd day	83.85	±	4.11	86.97	±	1.15
Performance index						
35th day	119.25	±	3.48	115.66	±	2.35
42nd day	140.10	±	4.23	135.70	±	3.14

Table 4. Effect of drinking water acidification on relative weight of organs (% of live body weight)

	Control			Test		
Edible giblets						
Liver	2.015	±	0.129	1.956	±	0.058
Heart	0.588	±	0.035	0.586	±	0.015
Immune organs						
Spleen	0.099	±	0.005	0.100	±	0.006
Bursa of Fabricius	0.266	±	0.016	0.276	±	0.017

Table 5. Effect of drinking water acidification on pH and concentration of short chain fatty acids in the caecum content

	Control			Test		
pH	6.93	±	0.07	6.86	±	0.06
Acetic acid (mmol.L^{-1})	145.95	±	8.31	154.08	±	10.86
Propionic acid (mmol.L^{-1})	27.22	±	2.01	30.10	±	3.33
Butyric acid (mmol.L^{-1})	8.78	±	0.97	11.39	±	1.98
Lactic acid (mmol.L^{-1})	29.18	±	3.42	32.23	±	2.69

Table 6. Effect of drinking water acidification on content of dry matter and crude protein in faeces

	Control			Test		
Dry matter (g)	166.9	±	0.59	165.6	±	0.92
Crude protein (g.kg^{-1} dry matter)	309.5	±	3.36[a]	288.2	±	0.44[b]

Note: [ab] significant differences (P < 0.001).

the growth rate of chicks was not significantly influenced by the effect of tested additive during period of 35 days and during the whole trial period (1st–42nd day).

The addition of acidifier to drinking water led to moderate increase of chicken weight uniformity on 35th and 42nd day of trial in comparison to control group,

though the difference (6.49%, 3.72%, respectively) was not significant (Table 3). Similarly, the performance index was not significantly influenced by the acidification of drinking water (Table 3).

The relative weight of internal organs is presented in Table 4. Concerning data of edible giblets (liver, heart) and immune

organs (spleen, bursa of Fabicius), it was noticed that drinking water acidification showed no significant effect.

The values of the monitored indicators of the fermentation process in the caecum are shown in Table 5. The pH value was not affected, although concentration of each short chain fatty acid was higher in the experimental group than in the control group. The total content of short chain fatty acids was higher by 7.90% in the experimental group (227.80 mmol.L^{-1}) than in the control group (211.13 mmol.L^{-1}). However, the differences between the groups were not statistically significant.

Content of dry matter in chicken faeces of trial group was comparable to that in controls (Table 6). Concentration of crude protein in dry matter of faeces of chicks in trial group was significantly lower than in control group (P<0.001).

DISCUSSION

The results of our experiment indicate that acidification of drinking water significantly improved growth rate of broiler chicks only in the sixth week of the experiment (during finisher phase). Performance index was not significantly affected. Our findings support the result of Eftekhari *et al.* (2015) who reported that acidified drinking water with an acidifier product, containing lactic acid, formic acid, propionic acid, sorbic acid, and citric acid, had a positive effect on growth performance, but only in the starter phase. Abdel-Mageed (2012) found that supplementation of butyric acid to the diets with recommended protein and recommended energy for Japanese quails (according to NCR) had no significantly effect on final body weight, growth rate and performance index for the whole period. In contrast, the addition of butyric acid to the diets

with low protein and low energy significantly improved final body weight, growth rate and performance index of Japanese quails for the whole period. Similar findings were reported by Abdel-Fattah *et al.* (2008). Chicks fed diets containing acetic acid, citric acid or lactic acid had significantly higher final body weight and significantly higher values of performance index than chicks fed diets without acids. Sultan *et al.* (2015) recorded that body weight of broilers increased linearly by using different levels of organic acid blend (citric acid, lactic acid, $CuSO_4$, and phosphoric acid) added to the drinking water. On the other hand, the reduction of water pH from 7.4 to 4.5 with formic acid supplementation significantly decreased body weights of broilers at 21st and 42nd day of age (Açıkgöz *et al.*, 2011).

According to the results of the present experiment, acidification of drinking water had non-significant but positive effect on flock uniformity. Improvement in flock body weight uniformity is one of the most important economical factors in broiler production. This is because birds from a more uniform flock cause less disruptions for the machinery during slaughter and downstream carcass processing (Fasina & Olowo, 2013).

The relative weight of edible giblets (liver, heart) and immune organs (spleen, bursa of Fabricius) was not significantly affected in our experiment. Our results are in agreement with the findings of Eftekhari *et al.* (2015) who indicated that acidified drinking water (with above mentioned acidifier product) had no effect on weight of edible giblets and immune organs. These results confirmed those of Haq *et al.* (2014) who found that dietary acidification with citric acid had no effect on the relative weight of liver and heart. Also, Abdel-Fattah *et al.* (2008) reported simi-

lar findings. The relative weight of edible giblets was not significantly affected by addition of organic acids to the diets, but supplemental organic acids significantly increased the relative weight of immune organs (bursa and thymus). However, this effect was not attained for the relative weight of spleen. Mohamed *et al*. (2014) observed increase in the relative weight of bursa and spleen by diets supplementation with commercial product of organic acids (fumaric acid, calcium formate, calcium propionate, potassium sorbate and hydrogenated vegetable oil). These results might imply that organic acids can lead to improvement of the immune response and diseases resistance. On the other hand, Abdel-Mageed (2012) found that addition of butyric acid to the diets had no significant effect on the relative weight of immune organs, except the weight of thymus when the butyric acid was added to the diets with low protein and low energy. The addition of butyric acid to the diet with low protein and low energy resulted in the increase of the relative weight of edible giblets, too.

Abdel-Mageed (2012) reported that dietary acidification with butyric acid resulted in significant decrease of the pH value in ileum and caecum contents. Significantly decreased pH values of caecum contents after diet supplementation with benzoic acid was also noticed by Giannenas *et al*. (2014). Alzawqari *et al*. (2013) observed significant decrease of the pH in gizzard and caecum contents of broiler chickens that drank water acidified with citric acid (particularly at 4.5 and 6%) for 8 h during preslaughter feed withdrawal. Reduction of gastrointestinal pH is beneficial for the growth of favourable bacteria and unsuitable for the growth of pathogenic bacteria, which grow at a relatively higher pH such as coliforms (Lückstädt,

2007; Abdel-Mageed, 2012). In our experiment, the pH value in caecum content of test group chicks was not significantly affected. Similarly, Grashorn *et al*. (2013) who added organic acids preparation (calcium formiate, calcium propionate, benzoic acid, citric acid, fumaric acid, lactic acid and acetic acid) to the diets of chicks didn't observe any significant changes in the pH value of caecum content. However, they recorded the increase of short chain fatty acids contents (significantly for propionic and iso-butyric acid) in the experimental group than in the control group, except for butyric acid. Increased concentration of short chain fatty acids in the caecum content of test group was also observed in our study, but was not significantly affected.

The water acidification in the present study showed significant reduction in content of crude protein in faeces as compared to the control group. These results did not support the findings of Abd El-Hakim *et al*. (2009) who indicated that N content in faeces was not influenced by supplementation of citric acid to the diet. According to our results the reduction of crude protein in faeces may lead to decreased production of volatile ammonia through microbial fermentation in litter, however further experiments, including volatile ammonia measurements, should be performed to confirm this hypothesis.

A higher dry matter content in faeces may improve the microclimate in the poultry house, too. The lower water content in the litter limits microbial fermentation (Grashorn *et al*., 2013). In our experiment, dry matter content in faeces was not affected by acidification of drinking water. On the other hand, Grashorn *et al*. (2013) reported that the dry matter content of the chymus was insignificantly higher in all segments of the digestive

tract in the experimental group with acidified feed than in the control group.

Many studies show that the effect of acidifiers may vary and depends on the acidifying substances, their concentration, their application to the water or to the diet, and on used experimental diets. Several studies reported that organic acids have no effect on performance when chicks are housed in a clean environment (Abdel-Mageed, 2012). The mechanism how organic acids provide their positive effect on performance and health of animals, as reported by many studies, might by due their ability to acidify the content of digestive tract and regulate microbial flora in the gut. Lower pH in the stomach stimulates pepsinogen transformation into pepsin what leads to improved protein digestibility (Marín-Flamand *et al.*, 2014). Moreover, lower pH in gastrointestinal tract may positively affect the utilisation of minerals from diets (Abd El-Hakim *et al.*, 2009). Organic acids in dissociated form are responsible for modification of the pH, whereas undissociated organic acids can penetrate the bacterial cell wall, disturb the intracellular pH homeostasis, inhibit essential metabolic reactions, such as DNA formation and protein synthesis, and so inhibit the growth of pathogenic bacteria (Hardy, 2003; Krisham & Narang, 2014). Furthermore, reduced synthesis and secretion of corticosteroid hormones was reported by the supplementation of ascorbic acid, what alleviated the negative effect of heat stress on poultry performance. Ascorbic acid has also protective effect on pancreatic tissue against oxidative damage helping pancreas to function properly, thus improving retention of nutrients (Sahin & Sahin, 2002).

CONCLUSIONS

According to the present results, acidification of drinking water had positive effect on growth rate during finisher phase and reduction of crude protein in faeces. Although not statistically significant, water acidification increased flock body weight uniformity. Performance index, organ weights, pH and concentration of fatty acids in caecum content or content of dry matter in faeces were not significantly affected by water acidification.

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