Fattening turkeys with the addition of lactic acid and copper

Kuna-Broniowska Izabela†, Makarska Ewa‡, Bożena Nowakowicz-Dębek§ and Halina Pawlak∥

†Department of Applied Mathematics and Computer Science, University of Life Sciences in Lublin, Lublin, Poland; ‡Department of Chemistry, University of Live Sciences in Lublin, Lublin, Poland; §Department of Animal Hygiene and Environment, University of Life Sciences in Lublin, Lublin, Poland; ∥Department of Technology Fundamentals, University of Life Sciences in Lublin, Lublin, Poland

ABSTRACT
The paper presents results of research on the effects of different doses of copper in the form of CuSO₄·5H₂O and Cu chelate and lysine (1:2), also with 0.4% lactic acid in drinking water on weight gain of turkeys. The study sample consists of 20 experimental groups of 120 birds each. The addition of lactic acid to the feed (excluding copper) contributed slightly to the reduction of the weight gain of turkeys. In contrast, a significant decrease in body weight gain of turkeys was observed, using lactic acid along with inorganic form of copper. Turkeys fed with the feed containing only organic form of copper at a dose of 20 mg reached the greatest weight gain. Lactic acid and different doses and forms of copper significantly differentiate the body weight gain of turkeys. The combination of the lactic acid and copper added resulted in lower weight gains of birds; it was only the administration of the 50 mg copper dose in organic form that increased the weight gain to the level comparable to the weight gains obtained for birds fed without the lactic acid addition. The use of feed supplements requires the selection of an appropriate dose to the form of copper.

1. Introduction
Copper, despite a small demand by turkeys amounting to circa 2–4 mg per kg⁻¹ of feed, conditions the proper course of a number of processes and functions, that is, forming collagen cross linkages, skeleton mineralization, haem biosynthesis, oxidative phosphorylation, glucose and cholesterol metabolism, immunological functions, central nervous system functioning and a lot of others (Burkitt 2001; Samanta et al. 2011; Gaetke et al. 2014). Copper is often added to poultry feeds in increased quantities for preventive purposes because it stimulates the weight gain (Pesti & Bakalli 1996). In practice, however, there is often a copper deficiency in turkeys, with the reason being the presence of phytates and phosphates in feed as well as the antagonism with some chemical elements, for example, between Zn and Cu (Pang & Applegate 2007). It is for that reason copper resorption from the digestive system can take place using various mechanisms, making it difficult to determine (Kashani et al. 1986; Makarski & Zadura 2006a).

The zinc resorption process in the digestive system takes place primarily in the small intestine, where this chemical element is combined with the adequate peptide carrier. Further Zn transport can take place in the already created form or after oxidation. Then zinc is transported to the liver, from where it passes to the blood stream. In poultry the largest quantities of Zn are accumulated in the liver, kidneys and bones, which have a specific capability of accumulation of this component. After exceeding the maximum tissue capacity for Zn when it is continued to be supplied to the organism, we can observe its increased concentration in the blood plasma, which is followed by toxic symptoms (Kidd et al. 1996). The common practice in the course of industrial breeding of turkeys is the addition of the lactic acid on the silicon carrier to the feed or directly to the drinking water in small quantities, usually at 0.2%–0.3%. The lactic acid is a strong prebiotic, which, by lowering the pH to 5.0–6.8 in individual sections of the digestive system, creates favourable conditions for the colonization of useful bacteria as well as aids the resorption of mineral components. As indicated by the research conducted by Byrd et al. (2001) and Higgins et al. (2007), the addition of lactic acid in drinking water significantly reduces the growth of Salmonella enteritidis and S. typhimurium.
in the digestive system and lowers their contents in the excreted uric acid.

The aim of the research was the determination of the impact of the administration of copper at varying doses in the form of CuSO₄·5H₂O and Cu chelate with lysine in the composition with the lactic acid at the concentration of 0.4% in drinking water on the weight gain of turkeys.

2. Material and methods

2.1. Animals and measurements

The research involved 2400 turkeys of the heavy BUT-9 type, which were kept under the same zoohygienic conditions pursuant to the standards as provided for turkeys for fattening and remained under continuous veterinary care. Throughout the entire experiment, the birds were fed with typical all-mash compound feed and had continuous access to water.

In the second week of rearing, the turkeys were divided into 20 experimental groups (Table 2). The turkeys are assigned to the groups due to the homogeneity of group averages of their body weight. They were held in the same building, along with the increasing of the weight of the birds; the available surface was systematically widened so that turkeys have had easy access to feed and water. Throughout the experiment the weekly weight gains of turkeys were recorded as well as the feed consumption and the death rate. This study’s experiments were approved by the Local Ethical Committee (permission no. 42/2002) of Lublin (Poland).

2.2. Statistical analyses

The experimental data obtained were subjected to statistical analysis using the software Statistica 5.0. The experiment was set up in the triple cross-validation system, the examined factors being: copper form – two levels, copper dose – five levels and the lactic acid additive – two levels. In total, 20 combinations of the examined factors were created that were used in feeding of one of the 20 groups of ringed turkey males. The turkeys were fed with the foodstuff with or without the lactic acid addition accompanied by organic or inorganic form of copper, which was administered in the following doses: 0, 5, 10, 30 and 50 mg. Two control groups or birds were identified:

1. Birds fed with the lactic acid addition – Group 1.
2. Birds fed without copper addition and without lactic acid addition – Group 11.

The linear model of the experiment can be presented as follows:

\[ y_{ijk} = \mu + \alpha_j + \beta_k + \gamma_l + (\alpha\beta)_{jk} + (\alpha\gamma)_{jl} + (\beta\gamma)_{kl} + e_{ijkl} \]

where \( A \) – copper form: organic, inorganic; \( B \) – copper doses: 0, 5, 10, 30 and 50 mg; \( C \) – lactic acid additive at 0%, 0.4%; \( \alpha_j \) – effect of the \( j \)th copper form, \( j = 1, 2 \); \( \beta_k \) – effect of the \( k \)th copper dose, \( k = 1, 2, 3, 4 \) and 5; \( \gamma_l \) – effect of the \( l \)th addition, \( l = 1, 2 \); \( (\alpha\beta)_{jk} \) – effect of synergy of the \( j \)th copper form with the \( k \)th copper form; \( (\alpha\gamma)_{jl} \) – effect of synergy of the \( j \)th copper form with the \( l \)th addition; \( (\beta\gamma)_{kl} \) – effect of synergy of the \( k \)th copper dose with the \( l \)th addition and \( e_{ijkl} \) – experimental error.

The variance analysis was used to compare the weight gains in turkeys in reference to non-zero copper doses, whereas control levels were included in the contrast analysis. The orthogonal contrasts method was used for planned comparisons regarding comparisons with control objects and comparisons for the lactic acid addition.

3. Results and discussion

The nutrition experiment was conducted in order to examine the impact of various doses of two copper forms and the lactic acid addition on the turkey weight gains. The weight gains were measured for the same birds throughout the five-week period. No bird deaths were recorded. The initial body weights of turkeys in the examined groups (1–20) differed and therefore in the statistical analysis, weekly absolute weight gains of birds were examined as well as percentage of weight gains when compared to the initial weights.

The lactic acid addition did not affect significantly the turkey weight gains when compared to the control groups (Table 2, Groups 1 and 11), but the combination of the acid with copper resulted in decreased weight gain results (Groups 2–9). The highest weight gains were achieved at 0.4% of the lactic acid addition to the bird feed at the highest copper dose (50 mg – Group 10) in organic form, whereas the lowest gains were obtained at 5 mg copper dose in both forms (Group 2 and 6). Moreover, for both forms of copper (organic and inorganic), body weight gains of turkeys did not change uniformly with a change of dose of copper. The changes to the weight of turkeys fed without the addition of lactic acid (Groups 11–20) did not take place in an analogical way as in the case of acid addition; in this case, the lowest and highest weight gains were observed at 20 mg dose of copper in the inorganic and organic form, respectively. The weight gains of turkeys fed with the lactic acid addition and non-zero copper dose (the exception being 50 mg dose) were lower than the weight gains of turkeys fed with analogical copper doses without lactic acid. Most probably the combination of the lactic acid and copper added to the lower weight gains of birds, it was only the administration of the 50 mg copper dose in organic form (Group 10) that increased the weight gain to the level comparable to the weight gains obtained for birds fed without the lactic acid addition (Figure 1, Table 2, Groups 12–20). The weight gains in birds after five weeks of fattening were equalized for the organic and inorganic form at the highest copper dose, that is, 50 mg administered without the lactic acid addition as well as for organic copper with the addition of this acid (Figure 2). The results of the weight gains of turkey fed with the feed supplemented with lactic acid were worse when compared to the birds that were fed without that additive (the exception – 50 mg dose in the organic form). The most significant differences between the gains for the organic and inorganic form of copper were observed for the 20 mg dose without the presence of the lactic acid in feed, whereas in the case of addition of this acid to the feed, most significant differences between the weight gains were observed for the 50 mg copper dose.
The results for the ‘0’ dose concern the average weight gain in two control groups (number 1 and 11); the weight gains in these groups were at the similar level (1521.30 and 1502.63), which allowed us to combine them (Figure 3). The research did not involve the 10 mg copper dose in inorganic form; therefore, the plot does not contain the result for this form of copper.

The average turkey weight gains at various copper doses in organic form were higher when compared to the average weight gains of turkeys at the relevant doses of copper in inorganic form. The analysis of variance, presented in Table 1, do not take into account the dose 10 g of copper and the control. The omitted levels of factors are analyzed in planned comparisons. A significant differentiation of turkey weight gains was found, due to the interaction between various forms and various doses of copper with or without the addition of lactic acid (Table 1, $F = 2.85$, $p$-value = .04). Weight gain of birds fed with the addition of the various forms of copper with or without the addition of lactic acid is not uniformly shaped with a change in the dose of copper. Due to the significance of the correlation between the three investigated factors are not verified hypotheses about the individual impact of these factors on the body weight gain of turkeys. The significance of copper form and significance of lactic acid, found by using the $F$ test, may be the result of interaction between the forms of copper and the doses of copper with or without lactic acid addition in the diet (Table 1, $F = 4.90$, $p$-value = .03, $F = 30.79$, $p$-value = .00 respectively). However, in the graphs (Figures 1 and 3) a small variability of weight gain due to these factors can be observed. Using the post-hoc comparison, three homogeneous groups of mean values were created (Table 2).

The 5 mg doses of Cu in Groups 2 and 6 are considered the lowest in inorganic form and organic form, respectively. Group 5 is connected with the highest copper dose in inorganic form – 50 mg. Groups 18 and 19 are the groups of turkeys fed with the addition of 20 and 30 mg copper doses in organic form.

- The first homogeneous group does not contain the average weight gain values of the birds fed with the addition of the organic copper form in the 20 mg and 30 mg doses (Groups 18 and 19).
- The second homogeneous group does not contain the weight gains of the birds fed with the addition of copper in the lowest dose of 5 mg (Groups 2 and 6) and the average value for the weight gains of the birds fed with copper addition of organic copper in 20 mg doses (Group 18).
- The third group did not include the average weight gains of birds fed with copper addition in the lowest dose of 5 mg both in organic and inorganic form and the average weight gain of birds fed with the lactic acid addition and 50 mg copper dose in inorganic form.
- The lowest weight gains were obtained with small copper doses both in organic and inorganic form (Groups 2 and 6) disregarding lactic acid addition. The weight gains of turkeys fed with the addition of lactic acid only (Group 1) were higher when compared to the weight gains of turkeys fed with the addition of lactic acid and inorganic form of copper (Groups 2, 3, 4 and 5).
- Weight gains recorded in the control group and in the groups fed with the addition of only lactic acid were in the middle of the average weight gain values of the turkey groups covered by the research (Groups 1 and 11).

The highest weight gains were observed in turkeys fed with the addition of organic form of copper in 20 and 30 mg doses (Groups 18 and 19).

Employing orthogonal contrasts, five planned comparisons were carried out:

1. Comparison of the average weight gain with the addition of lactic acid and copper in inorganic form against the average weight gain with the addition of just lactic acid (Groups 2–5 against Group 1).
2. Comparison of the average weight gain with the addition of lactic acid and copper in organic form against the average weight gain with the addition of just lactic acid (Groups of birds 6–10 against Group 1).
3. Comparison of the average weight gain with the addition of copper in inorganic form to the average weight gain of the group of birds fed without additions (Groups of birds 12–15 against control Group 11).

### Table 1. Three-factor analysis of variance for the weight gains (after five weeks) of turkeys fed with the addition of various doses of two copper forms with or without the lactic acid.

| Source of variation                  | $F$     | $p$   |
|-------------------------------------|---------|-------|
| Copper doses                         | 1.56    | .20   |
| Copper form                          | 4.90*   | .03   |
| Lactic acid                         | 30.79** | .00   |
| Copper doses × copper form           | 0.33    | .80   |
| Copper doses × lactic acid           | 0.83    | .48   |
| Copper form × lactic acid           | 0.26    | .61   |
| Copper doses × copper form × lactic acid | 2.65*   | .04   |

*Rejecting the null hypothesis on the significance level .05.
**Rejecting the null hypothesis on the significance level .01.

### Table 2. Homogeneous groups for the mean weight gains of turkeys after five weeks of fattening.

| Supplements                        | Groups number | Gains [g] | Sets of means |
|------------------------------------|---------------|-----------|---------------|
| 5 mg inorganic copper lactic acid  | 2             | 1337      | +             |
| 5 mg organic copper and lactic acid| 6             | 1346      | +             |
| 50 mg inorganic copper and lactic acid | 5      | 1364.3    | + a           |
| 30 mg inorganic copper and lactic acid | 4      | 1397.9    | + a a         |
| 10 mg organic copper and lactic acid | 7      | 1400.16   | + a a a       |
| 30 mg organic copper and lactic acid | 9      | 1408.6    | + a           |
| 20 mg organic copper and lactic acid | 8      | 1429.24   | + a a         |
| 20 mg inorganic copper and lactic acid | 3      | 1480.8    | + a a a       |
| 20 mg inorganic copper             | 13         | 1493.4    | + a a a       |
| Control- without supplements       | 11          | 1502.63   | + a a a       |
| 5 mg inorganic copper              | 12          | 1509.28   | + a a a       |
| Lactic acid without copper         | 1           | 1521.3    | + a a a       |
| 30 mg inorganic copper             | 14          | 1529.25   | + a a a       |
| 10 mg organic copper               | 17          | 1557.55   | + a a a       |
| 50 mg organic copper               | 20          | 1568.35   | + a a a       |
| 50 mg copper and lactic acid       | 10          | 1573.6    | + a a a       |
| 50 mg inorganic copper             | 15          | 1573.63   | + a a a       |
| 5 mg organic copper                | 16          | 1584.40   | + a a a       |
| 30 mg organic copper               | 19          | 1603.25   | + a a a       |
| 20 mg organic copper               | 18          | 1634.25   | + a a a       |

*Belongingness the mean weight gain in the groups (1–20) to the one of three sets of homogeneous means.
Comparison of the average weight gain with the addition of copper in organic form against the average weight gain of the birds' group fed without additions (Groups of birds 16–20 against control Group 11).

Comparison of the average weight gain with the addition of copper in inorganic form against the average weight gain with the addition copper in organic form (Groups of birds 12–15 against Groups 16–20, Table 3).

At the significance level of .05, it was found that only two comparisons are significant, namely:

- Comparison of various doses of inorganic form of copper with lactic acid addition against the addition of just acid. The average weight gain of birds from the group fed with the addition of lactic acid was lower than the average weight gain of birds from the groups fed with acid addition and supplemental copper in inorganic form (contrast 1).
- Comparison of average weight gains with the addition of copper in inorganic form against the average weight gain with the addition of copper in organic form. The average weight gain of birds from the groups fed with inorganic form of copper addition was significantly lower when compared to the average weight gain of birds from the groups fed with the addition of copper in organic form (contrast 5).
- The remaining comparisons were found insignificant.

The course of metabolic processes in turkeys depends on the presence of copper. The European Commission allows using copper for all species pursuant to Directive 70/524/EEC (European Commission 2003). However, it is recommended that the levels should be adjusted in such a way as to minimize negative effect on the environment. The increased copper contents in the feedingstuff stimulate the weight gain of birds and improves the use of the feedingstuff; it stimulates tissue protein biosynthesis (Harms 1986; Makarski & Zadura 2006b). The Cu...
supplementation also performed in other species. It is concluded that supplemental Cu might improve growth performance in the finishing bulls and decrease the prevalence of lameness (Fagari-Nobijari et al. 2013). The addition of copper, disregarding its dose and chemical form, increases the accumulation of this element in turkey livers. This supplementation does not lead though to significant changes in the enzymes activity in blood serum when compared to the control group, but it significantly affects lipid markers in blood serum (Makarski & Gortat 2011). The authors, by adding copper in the dose of 10 and 20 mg, obtained statistically insignificant weight gains of turkeys. But in own research, by adding 20 and 30 mg of the organic form of copper, the highest weight gains of birds were obtained. In other research, Makarski and Zadura (2006b), by supplementing the birds with 10 mg of organic copper, obtained significant weight gains of birds when compared to the control group. Dmoch and Polonis (2010), by supplementing turkeys with 50 mg of copper with drinking water in the form of chelate, obtained a higher weight of birds only in the 15th week of fattening when compared to other groups. The dead weight higher by 7% was obtained in the group supplemented with 30 mg of copper in the form of copper chelate with lysine, which correlates with own research. Higher copper levels in feeding stuffs are reflected in the higher concentration in tissues, which, as indicated by Makarski et al. (2014), can constitute a valuable source of this element in everyday human diet and, consequently, can prevent diseases related to Cu deficiency. Probiotic milk acid bacteria show positive effects in the bird’s digestive system. Wajda et al. (2010), while conducting research on lactic acid bacteria in turkeys, obtained higher daily weight gains and lower feedingsuff consumption, but it was the case only in the first 12 weeks of turkeys’ life. The addition of these bacteria with lactose decreased bird’s death rate. However, the results obtained following the supplementation with lactic acid in own research were in the middle of the average values of the recorded weight gains. Similar results were obtained by Butelkis et al. (2008) while testing lactic acid bacteria of the Pediococcus acidilactici 18/5M strain. The intestinal flora in turkeys is the first significant barrier protecting organisms against adverse environmental effects. Therefore, it is justified that probiotic preparations be searched for and that their level should be adjusted to the feeding period and environmental conditions.

4. Conclusions

(1) The use of copper and lactic acid as feed supplements requires the adjustment of an appropriate dose to the form of copper.

(2) The combination of copper with 0.4% lactic acid, except the organic form of copper in the 50 mg dose, influences reduction of the body weight gains of turkeys in relation to the nutrition without supplementation.

(3) The most favourable combination of copper with 0.4% lactic acid is the 50 mg dose of organic form.

Disclosure statement
No potential conflict of interest was reported by the authors.

References
Burkitt MJ. 2001. A critical overview of the chemistry of copper-dependent low density lipoprotein oxidation: roles of lipid hydroperoxides, alpha-tocopherol, thiols, and ceruloplasmin. Arch Biochem Biophys. 394:117–135.
Buteikis G, Matusevičius P, Januškevičius A, Jankowski J, Mikulski D, Blok J, Kozłowski K. 2008. Use of synbiotic preparations in turkey diets and their effect on growth performance. Vet Zoot. 43:14–19.

Byrd JA, Hargis BM, Calla Well DJ, Bailey RH, Herron KL, Mcreynolds JL, Brewer RL, Anderson RC, Bischoff KM, Callaway R, Kubena LF. 2001. Effect of lactic acid administration in the drinking water during pre-slaughter feed withdrawal on Salmonella and Campylobacter contamination of broilers. Poultry Sci. 80:278–283.

Dmoch M, Polonisi A. 2010. Effect of dietary copper chelate and herbal mixture on slaughter turkey performance. Rocz Nauk PTZ. 6:73–82.

European Commission. 2003. Health and Consumer Protection Directorate-General Directorate C – Scientific Opinions. C2 – management of scientific committees; scientific co-operation and networks. Opinion of the scientific committee for animal nutrition on the use of copper in feedingstuffs.

Fagari-Nobijari H, Amanlou H, Dehghan-Banadaky M. 2013. The use of copper supplementation to improve growth performance and claw health in young Holstein bulls. J Agr Sci Technol. 15:77–86.

Gaetke LM, Chow-Johnson HS, Chow CK. 2014. Copper: toxicological relevance and mechanism. Arch Toxicol. 88:1929–1938.

Harms RH. 1986. Influence of three levels of copper on the performance of turkey poults with diets containing two sources of methionine. Poultry Sci. 66:721–724.

Higgins JP, Higgins SE, Vicente JL, Wolfenden AD, Tellez G, Hargis BM. 2007. Temporal effects of lactic acid bacteria probiotic culture on Salmonella in neonatal broilers. Poultry Sci. 86:1662–1666.

Kashani AB, Samie H, Emerick RJ, Carson CW. 1986. Effect of copper with three levels of sulfur containing amino acids in diets for turkeys. Poultry Sci. 65:1754–1759.

Kidd MT, Ferket PR, Qureshi MA. 1996. Zinc metabolism with special reference to its role in immunity. Word’s Poultry Sci J. 52:309–324.

Leach RM, Rosenblum CI, Amman MJ, Burdette J. 1990. Broiler chicks fed low-calcium diets. 2. Increased sensitivity to copper toxicity. Poultry Sci. 69:1905–1910.

Madej JA, Gawel A, Kuryszko J, Mazurkiewicz M. 1994. Studies on the pathological mechanism of lathyism as a cause of spontaneous rupture of arteries in turkeys. Med Weter. 50:558–561. Polish.

Makarski B, Gortat M. 2011. Effect of supplementation with copper in different chemical forms on selected physiological blood markers and content of minerals in selected tissues of turkeys. J Elementol. 16:591–602. doi:10.5601/jelem.2011.16.4.08.

Makarski B, Gortat M, Lechowski J, Zukiewicz-Sobczak W, Sobczak P, Zawiśłak K. 2014. Impact of copper (Cu) at the dose of 50 mg on haematological and biochemical blood parameters in turkeys, and level of Cu accumulation in the selected tissues as a source of information on product safety for consumers. Am Acad Emerg Med. 21:567–570.

Makarski B, Zadura A. 2006a. Changes in the content of mineral components in tissues of turkeys receiving a supplement of Cu chelate with lysine. J Elementol. 11:183–190.

Oestreicher P, Cousins RJ. 1985. Copper and zinc absorption in the rat: mechanism of mutual antagonism. J Nutr. 115:59–166.

Pang Y, Applegate TJ. 2007. Effect of dietary copper supplementation and copper source on digesta pH, calcium, zinc, and copper complex size in the gastrointestinal tract of broiler chicken. Poultry Sci. 86:531–537.

Pesti GM, Bakalli RI. 1996. Studies on the feeding of cupric sulfate pentahydrate and cupric citrate to broiler chickens. Poultry Sci. 75:1086–1091.

Santon A, Giannetto S, Sturniolo GC, Medici V, D’Jnca R, Irato P, Albergoni V. 2002. Interaction between Zn and Cu in LEC rats, and animal model of Wilson’s disease. Histochem Cell Biol. 117:275–281.

Standards of Poultry Feeding. 2005. Wyd. IFiŻZ, PAN Jabłonna. Polish.

Wajda S, Smiecińska K, Jankowski J, Matusievičius P, Buteikis G. 2010. The efficacy of lactic acid bacteria Pediococcus acidilactici, lactose and formic acid as dietary supplements for turkeys. Pol J Vet Sci. 13:45–51.