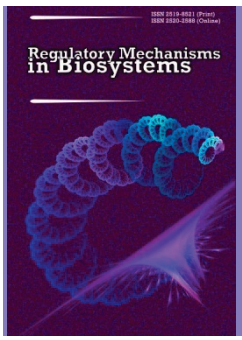

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Efficacy of manganese pantothenate and lysinate chelates for prevention of perosis in broiler chickens

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Perosis is a common metabolic disease of industrial birds, especially broiler chickens. It leads to a violation of the balance of biotic substances in the body of chickens, which is clinically manifested by the curvature of the limbs, reduced mobility, and, consequently, reduced profitability of meat production. Prevention of perosis is possible provided that chickens receive a sufficient amount of manganese in a biologically available form. Studies were conducted to determine the efficiency of use of manganese chelates (pantothenate and lysinate) for prevention of perosis in broiler chickens. Efficacy was confirmed by examining changes in the clinical state, indicators of protein and mineral metabolism, as well as meat productivity of birds. For the experiment, broiler chickens of the Cobb-500 cross were taken at the age of 14 days. The birds of the control group received a standard diet, and the chickens from two experimental groups additionally received manganese pantothenate and lysinate with water during the critical period for the development of perosis – 14–28 days old. After 14 days of administration of manganese pantothenate and lysinate, the weight of the experimental birds at the age of 28 days was greater by 133.6 g (+11.0%) and 142.2 g (+11.7%), respectively, in comparison with poultry of the control group. Additional provision of manganese pantothenate and lysinate to chickens of the experimental groups contributed to an increase in the blood serum total protein concentration by 11.0% and 12.8 %, albumin – by 10.1% and 8.2%, magnesium – by 8.1% and 9.0% and manganese – by 29.6% and 26.9%, respectively, compared with indicies of the control group birds. The use of manganese chelates in the form of pantothenate (0.2 mL/L of water) and a lysinate (0.5 mL/L) during the 14–28th days of broiler chickens' rearing provides 100% prevention of perosis. This reduces the death of broiler chickens, increases body weight, and, as a result, significantly increases the profitability of meat production.

Keywords: protein metabolism; mineral metabolism; amino acids; trace elements; poultry.

Introduction

Due to the high growth rate of modern crossbreed meat poultry and insufficient mineral supply, there are more frequent cases of limb pathology in broiler chickens (Dinev, 2012). Nutrient requirements for chicks can vary by sex, rearing phase, and cross-breeding (Pacheco et al., 2017). Trace elements such as zinc and manganese are essential for the physiological processes of bone tissue development of all animals (Richards et al., 2010; Bomko et al., 2018). Manganese (Mn) is an important micronutrient in bird feeding, which is necessary for the prevention of perosis, improving the bearing and strength of the eggshell (Aschner & Aschner, 2005; Tufarelli & Laudadio, 2017). It is necessary for normal immune function, regulation of blood sugar and cellular energy, reproduction, digestion, bone growth, and helps protect against free radicals (Liu et al., 2015). Also, a no less important element for poultry is zinc. It is a component of about 300 enzymes and acts as their activator (lecithinase, arginase, some peptidases) (Britanico et al., 2012). Zinc enhances the effect on mineralization, formation and increase in bone mass and synthesis of keratin and collagen proteins that form the feather cover (Pacheco et al., 2017).

Perosis in broiler chickens occurs due to the low content of the manganese in the diet (Liu et al., 2015) and manifests itself at the age of 14–28 days (Dinev, 2012). This trace element deficiency in the diet of chickens can occur not only when it is insufficiently contained in the feed, but also when the feed production technology is violated (Oviedo-Rondón et al., 2006). Although the problem of perosis and possible ways of solving it are well known to scientists, this disease remains an important focus of scientific work of scientists from different countries of the world: the USA (Lilburn, 2021), Brazil (Noetzold et al., 2020; Santiago et al., 2020), China (Wang et al., 2020; Zhang et al., 2020), Poland (Matuszewski et al., 2020). To prevent trace elements' deficiencies, it is customary to add them to the diet in the form of inorganic salts (Stanaćev et al., 2014). The assimilability of these inorganic forms varies within 5–20%, while organic (chelate compounds) – 80–90% (Britanico et al., 2012). Organic trace elements in the chelated forms have several advantages: they are stable in the gastrointestinal tract and are protected from the formation of complexes with other food components that inhibit their absorption (Vieira, 2008). Also, the addition of minerals in the chelated form improves their transport and absorption in the birds' intestines (Ramos-Vidales, 2019). Despite the high level of bioavailability in comparison with non-organic trace elements, their chelated forms are much more expensive, therefore it is not economically feasible to replace them by 100% in the diet of chickens on small farms.

Each private broiler chicken farm has its program for infectious and non-infectious poultry diseases prevention. But farms do not always have the opportunity to manufacture or purchase compound feed with a new composition of trace elements. Also, it is difficult to add and effectively mix the trace element supplements using low quality equipment (Oviedo-Rondón et al., 2006). Therefore, it remains relevant for small poultry farms to add critical minerals with water using the dosing pumps, which allows them to be accurately dosed without significant expenditures of time, labour, and funds (Whaley, 2004).

The study aimed to determine the effectiveness of manganese pantothenate and lysinate for perosis prevention, determining changes in the clinical state, biochemical blood parameters, and meat productivity of broiler chickens.

Materials and methods

The study was conducted in 2020 at the Training and Production Centre poultry farm, and on the basis of the Scientific Research Institute of Internal Diseases of Animals of the Bila Tserkva National Agrarian University. The research protocol of the current study was approved by the Ethics Committee of the Bila Tserkva National Agrarian University (Approval number: 20.08.2019, No. 7).

Before the experiment, we selected 300 chickens aged 1-day Cobb-500 cross for subsequent formation of control and experimental groups. Before they reached the age of 14 days, we conducted a daily clinical examination of these chickens. For the experiment, three groups (according to the principle of analogues, from the one batch) of 14-day-old healthy broiler chickens were selected – a control group and two experimental ones (n = 50). This age period was chosen due to the intensity of the bone and cartilaginous tissue development; chickens 14–28 days old are the most vulnerable to perosis (Knowles, 2008).

At the beginning of the experiment, broiler chickens were kept in coops stocked with a density of 14 birds/m^2, on a bed of straw. The air temperature for chickens on the 14th day of rearing was 27.5 °C, on the 21st day – 25 °C, and on the 28th day – 23 °C (with a relative humidity of 50% during the entire period). The light cycle for the birds was 16 hours of light and 8 hours of darkness. Throughout the experiment, the chickens had free access to food and water. By the beginning of the experiment, the chickens were vaccinated against Newcastle and Gumboro diseases, and infectious bronchitis according to the preventive measures program developed at the farm. On the 20th day of life, they were re-vaccinated against Newcastle disease.

For the experiment, we chose the manganese chelates – pantothenate and lysinate. For the first experimental group we used a vitamin-amino acid (pantetonate) manganese supplement – based on the glycine and pantothenic acid (at a dose of 0.2 mL/L of water); for the second experimental group – based on the lysine, at a dose of 0.5 mL/L of water (TS U 24.1-30931207-011-2007, Private enterprise "Kronos-Agro", Ukraine). Chelated manganese was fed to the experimental broiler chickens at the age of 14–28 days old (Table 1).

Feeding of the broiler chickens was carried out according to the technological chart with the compound feed of the farm's production: the starter (14–21th days), the grower (22–35th days), and the finisher (from the 36th day until slaughter). The feed compositions were made based on the corn, soybeans, and sunflower meal from the plant materials of local cultivation (Table 2).

Table 1
Experiment scheme for broiler chickens of control and experimental groups (n = 50)

Ingredients	Broiler chickens 14-28 days old, n = 50		
	Control group	I experimental group with Mn-pantothenate	II experimental group with Mn-lysine
Inorganic Mn (with feed, basic diet), mg	115	90	90
Mn-pantothenate (with water), mL	0.0	0.2	0.0
Mn-lysine (with water), mL	0.0	0.0	0.5

Table 2
Composition and nutritional value of standard feed for broiler chickens of control and experimental groups

Characteristics	Starter, 11–21 days	Grower, 22–35 days	Finisher, 36–45 days
Corn, g/kg	400	400	420
Sunflower meal, g/kg (33% of raw protein)	60	58	90
Wheat, g/kg	176.2	193.4	190.2
Soy full fat extruded, g/kg (34% of raw protein)	88.0	168.6	184.0
Soybean meal, g/kg (41% of raw protein)	225.8	130.0	65.8
Premix, g/kg	50	50	50
Nutrition (counted)			
Exchange energy, kcal/100 g	311	318	319
Raw protein, %	19.6	18.6	17.5
Calcium, %	0.84	0.76	0.77
Available phosphorus, %	0.42	0.38	0.38
Trace elements (defined)			
Zinc, mg/kg	100	100	100
Manganese, mg/kg	90	90	90

A clinical study and weighing of the birds of the control and experimental groups were carried out before the blood samples were taken on the 14th, 21st, and 28th days of rearing. The severity of symptoms in broiler chickens was determined using diagnostic criteria for perosis assessment: I degree – slightly displaced calf tendons; II degree – displaced calf tendons and III degree – displaced calf tendons, enlarged hocks, and twisted legs.

From each group of chickens, 20 birds were randomly selected for blood sampling and subsequent analysis. Blood samples for the study were taken by the intravital puncture method from the axillary vein, 2.5 mL from each bird (Kelly et al., 2013; Sakara et al., 2018), into Vacutainer tubes (Becton Dickinson, England) with a blood coagulation activator and gel. Then the tubes with blood samples were kept for 30 minutes at room temperature, until the clot compartment. The samples were centrifuged at 3000 rpm for 10 min until the final separation of the serum from the blood cells.

The concentration determination of total protein, albumin, total calcium, inorganic phosphorus, magnesium, was carried out according to the manufacturer's instructions, using "Filisit-diagnostics" reagents (Filisit-diagnostics, Ukraine) with the Stat Fax 1904+ biochemical analyser (Awareness Technology, USA). The study of the manganese and zinc content in the poultry blood serum was carried out by atomic absorption spectrophotometry using the Shimadzu AA-6650 device with an electrothermal atomizer (Shimadzu Corporation, Kyoto, Japan). Dilution of standards and serum was carried out according to the recommendations for the device using (Shimadzu Corporation. Atomic absorption spectrophotometry cookbook. Section 1: Basic Conditions of Analysis of Atomic Absorption Spectrophotometry).

The content of microelements (Zn, Mn) was investigated in compound feeds for all periods of feeding (pre-starter, starter, grower, finisher) at the Laboratory of Toxicological Monitoring of the Department of Toxicology, Safety and Quality of Agricultural Products of the National Research Centre "Institute of Experimental and Clinical Veterinary Medicine" using the method of X-ray fluorescence analysis following the manuals for the device Spectroscan-Max (Spectron, Russian Federation).

The results were determined as mean ± standard error (x ± SE). A Bonferroni-corrected ANOVA was used to determine the difference between the samples. The results were considered reliable at $P < 0.05$. The results of the studies were statistically calculated using the Statistica 10 program (StatSoft Inc., USA, 2011).

Results

It was found that in birds of all groups at the age of 14 days old (beginning of the study), visible mucous membranes were a pale pink colour, disorientation, and fragility with a disproportionate plumage growth were

observed. In all three groups, no chickens with perosis were found. At the end of the experiment, 28 day old chickens from two experimental groups were active, willingly ate feed, and consumed water, the plumage of the birds was tightly attached to the body. It should also be noted that there were no chickens with perosis found among those that had consumed manganese pantothenate and lysinate with water. In the birds of the control group, pronounced dishevelled plumage, pallor of mucous membranes, growth retardation, and diarrhea were noted. Perosis developed in 4 chickens of the control group (8%), with the second stage of legs' damage.

According to the weighing results, it was found that after 7 days of drinking manganese pantothenate and lysinate, the average body weight of chickens in the experimental groups was greater than that of the control group by 8.7% and 9.4% ($P < 0.001$; Table 3), respectively. At the end of the experiment, on the 28th day of the chickens' life, the difference in body weight increased by 11.0% and 11.7%, respectively ($P < 0.001$).

We found that after 7 days of consumption of manganese lysinate, the total protein concentration in the chickens' blood serum was 8.8% higher than that of the birds in the control group ($P < 0.01$; Table 4). After 14 days of consumption of manganese pantothenate and lysinate, the total protein content was higher by 11.0% and 12.8% respectively, in comparison with the indicators of the control group, who received the standard diet ($P < 0.001$).

Table 3

Dynamics of weight gain in broiler chickens of control and experimental groups ($x \pm SE$, $n = 50$)

Group	Age of broiler chickens		
	14th days	21st days	28th days
Control	400.7 ± 17.6	704.8 ± 22.9°°°	1075.5 ± 34.5°°°
I experimental group with Mn-pantothenate	406.4 ± 14.5	772.3 ± 24.8* °°°	1209.1 ± 28.4*** °°°
II experimental group with Mn-lysine	392.3 ± 23.2	777.6 ± 24.9** °°°	1217.7 ± 33.6*** °°°

Note: * – $P < 0.05$, ** – $P < 0.01$, *** – $P < 0.001$ compared to control; °°° – $P < 0.001$ compared to the beginning of the experiment.

Table 4

Blood biochemical parameters in broiler chickens of control and experimental groups ($x \pm SE$, $n = 20$)

Indicator	Groups	Age of broiler chickens		
		14th days	21st days	28th days
Total protein, g/L	Control	29.5 ± 0.7	29.0 ± 1.0	30.6 ± 0.6
	I experimental group with Mn-pantothenate	30.0 ± 0.4	30.4 ± 0.8	34.4 ± 0.7°°° ***
	II experimental group with Mn-lysine	30.6 ± 0.6	31.8 ± 0.5**	35.1 ± 0.9°°° ***
Albumin, g/L	Control	16.6 ± 0.78	16.3 ± 0.3	16.8 ± 0.6
	I experimental group with Mn-pantothenate	17.2 ± 0.9	18.0 ± 0.3	18.7 ± 1.0*
	II experimental group with Mn-lysine	16.6 ± 0.4	17.4 ± 0.4	18.3 ± 0.4* °°°
Magnesium, mmol/L	Control	0.96 ± 0.04	0.95 ± 0.02	0.91 ± 0.04
	I experimental group with Mn-pantothenate	0.87 ± 0.03	0.98 ± 0.02°°	0.99 ± 0.02* °°
	II experimental group with Mn-lysine	0.88 ± 0.05	1.00 ± 0.10** °	1.00 ± 0.10* °
Manganese, µmol/L	Control	1.9 ± 0.1	1.9 ± 0.2	1.9 ± 0.1
	I experimental group with Mn-pantothenate	1.7 ± 0.1	2.1 ± 0.1 °°	2.7 ± 0.3** °°°
	II experimental group with Mn-lysine	1.7 ± 0.2	2.0 ± 0.2	2.6 ± 0.3** °°
Zinc, µmol/L	Control	25.4 ± 1.2	24.8 ± 0.8	25.2 ± 1.4
	I experimental group with Mn-pantothenate	25.8 ± 1.4	28.7 ± 1.1**	27.8 ± 1.1
	II experimental group with Mn-lysine	26.5 ± 0.9	27.1 ± 1.3	27.0 ± 1.3

Note: * – $P < 0.05$, ** – $P < 0.01$, *** – $P < 0.001$ compared to control; ° – $P < 0.05$, °° – $P < 0.01$, °°° – $P < 0.001$ compared to the beginning of the experiment.

It was found that after 14 days of manganese pantothenate and lysinate consumption, the level of albumin in the broiler chickens' blood serum had increased by 10.2% and 8.2%, respectively, compared with the control indices ($P < 0.05$). The concentration of magnesium in the broiler chickens' blood serum at the end of the study in the experimental groups, which received manganese pantothenate and lysinate, was 8.1% and 9.0% respectively higher than in birds of the control group ($P < 0.05$; Table 4). The level of manganese in the blood serum of chickens after 14 days of this mineral pantothenate's application increased by 29.6% ($P < 0.01$; Table 4) compared with the indicators of the control group. Also, significant changes were diagnosed by the additional drinking of manganese lysinate, after 14 days the concentration of this trace element in the blood serum of the experimental birds was 26.9% higher than in the control group ($P < 0.01$). As for zinc, significant changes in its concentration in the broiler chickens' blood serum were recorded after 7 days of consumption of manganese pantothenate. Thus, the zinc content was 13.6% higher than in the blood serum of the control group birds ($P < 0.01$).

Discussion

To increase the bioavailability of trace elements in industrial poultry feed, various forms of compounds are used: nanoparticles (Matuszewski et al., 2020), inulin prebiotics (Ahmad et al., 2021), chelates (Meng et al., 2021), and nanoaquachelates (Nguyen et al., 2019). Chelates are a special group of complex chemical compounds that have been known to researchers for more than eight decades. They are heterocyclic compounds in which a metal ion is bound to ligands (amino acids, peptides, etc.). The most stable complexes have the structure of five- and six-membered rings (Vieira, 2008; Stanaćev et al., 2014). In the research of Meng et al. (2021), manganese methionine hydroxy analogue chelated was used as a chelate; Saldanha et al. (2020) used manganese proteinate; Yaqoob et al. (2020) used manganese glycinate; Ji et al. (2006) used manganese glycinate and methionate. As a result, all researchers noted that the use of manganese in the chelates' form led to improved health, productivity, or blood composition of experimental birds. In the current study, the use of manganese pantothenate (0.2 mL/L of water) and lysinate (0.5 mL/L) during 14–28th days of broiler chickens' rearing provided 100% prevention of perosis. Also, those measures contributed to the body weight gain of birds by 11.0% and 11.7% ($P < 0.001$), respectively.

The degree of minerals' assimilation by the body varies significantly depending on the interaction between the trace elements, which can be either synergistic (as Zn and Mn) or antagonistic (as Zn and Cu) (Nollet et al., 2008; Gajula et al., 2011). Due to the high growth rate of highly productive broiler chicken crosses with an increase in the additional load on the bone structure, it became expedient to use high concentrations of manganese in the diet (Ji et al., 2006). Organic sources of manganese are more bioavailable than inorganic sources (Li et al., 2008). Khakpour et al. (2019) found that the ileum is the main site of manganese absorption, and manganese glycinate and its nano-form were more efficiently absorbed by the intestine than its other sources. That, in our opinion, explains the increase of the manganese concentration in the broiler chickens' blood serum after the use of chelates in our experiment.

Thus, from the beginning, the diet of birds contained the manganese concentration recommended for chickens of the appropriate age. However, it was the low bioavailability of this mineral in the inorganic form that led to a deficiency and, as a consequence, the perosis of chickens from the control group. If the concentration of inorganic manganese is increased for the prevention of perosis during the critical period of 14–28 days, the load

on both the chickens' organism and the environment in the future will increase (Zhu et al., 2019). Therefore, we did not consider this solution to the problem expedient. Sunder et al. (2006) have shown that the addition of organic manganese in the amount of 100 mg/kg of compound feed optimizes performance, mineralization, and immune response in broiler chickens. The use of organic sources of trace elements (Zn, Mn, and Se) in broiler chickens' diets increases their concentration in the blood and liver, but without changes in the composition of bones compared to using their inorganic forms. Feed additives formulated with 50% organic and 50% inorganic minerals are giving similar results (Lopes et al., 2018).

The results of another study (Mwangi et al., 2019) confirmed that feeding broiler chickens with a slight deficiency of manganese in the diet affects the growth rate and the concentration of this element in tissues. The addition of 75–100 mg/kg manganese to the main corn-soy diet increases overall resistance and reduces the fat deposition in broiler chickens (Ghosh et al., 2016). This trace element in organic form has a higher efficiency than inorganic (Mn-sulfate), and the methionine ligand promotes the absorption of the manganese better than glycine (Olgun et al., 2017). The research results mentioned above are consistent with our findings in the current study. The serum manganese content of broilers that received manganese chelates was higher by 29.6% and 26.9% ($P < 0.01$), respectively, compared with the indices in chickens fed a standard diet containing only inorganic manganese.

The amino acid lysine, which is the part of a lysinate, improves protein assimilation (Chang et al., 2018). Therefore, it could contribute to an increase in the weight and the concentration of total protein in the chickens' serum by 12.8% ($P < 0.001$), after using the manganese lysinate in our experiment. Similar research results were obtained by (Yuan et al., 2015). The increase in the albumin concentration due to the addition of lysine to a feed for laying hens is described in his studies by (Shahir et al., 2006). The increase in total protein content by 11.0% after the use of manganese pantothenate, in our opinion, was due to the glycine content (Siegert et al., 2019). Glycine and pantothenic acid affected the increase in the body weight when pantothenate solidified. Wang et al. (2016) in his research described the effectiveness of the use of pantothenic acid in increasing body weight gain and optimizing metabolism in birds.

Khillare et al. (2019) indicated in their research that introducing manganese glycinate into broiler chickens' diets helps to balance the concentration of metals in the blood. Thus, the blood serum indices of the poultry of the experimental groups in our studies demonstrated an increase in the concentration of magnesium by 8.1–9.0% ($P < 0.05$) at the end of the experiment. Talking about an upward trend of the zinc concentration in the blood serum of birds who received the manganese pantothenate similar results were obtained by (Sunder et al., 2013). They found that the addition of organic manganese 60 mg/kg to a feed significantly increases the absorption of zinc ($P < 0.01$).

Conclusions

The use of manganese chelates in the form of pantothenate (0.2 mL/L of water) and lysinate (0.5 mL/L) during 14–28th days of broiler chicken rearing not only provides 100% prevention of perosis but also contributes to the body weight gain of birds by 11.0% and 11.7% ($P < 0.001$), respectively. The use of manganese pantothenate and lysinate in the recommended doses increases the concentration of total protein in the blood serum by 11.0% and 12.8% ($P < 0.001$), albumin by 10.1% and 8.2% ($P < 0.05$), magnesium by 8.1% and 9.0% ($P < 0.05$) and manganese by 29.6% and 26.9% ($P < 0.01$), respectively, compared with the indices in chickens fed a standard diet containing only inorganic manganese.

The authors declare the absence of any conflict of interest.

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