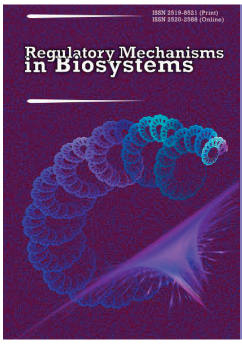

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Changes in protein and mineral metabolism in broiler chickens with perosis

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Perosis is one of the most common leg pathologies in broiler chickens, during the period of intense weight gain – at the age of 14–35 days. Due to manganese deficiency, the number of sick birds can reach up to 5% of the flock. These studies were carried out in order to establish changes in some indicators of protein, macro- and micromineral metabolism in the blood serum of clinically healthy broiler chickens and birds with perosis at 14, 21 and 28 days of age. A batch of 2,000 Cobb-500 crossbred broiler chickens was selected. Two groups of chickens were directly involved in the research, in which blood was taken at the age of 14, 21 and 28 days: healthy birds and those with perosis signs. Clinical studies showed that 8.0% of chickens on 28th day suffered from perosis. Trace mineral biochemical parameters of serum and blood of broiler chickens with perosis on the 28th day of life significantly differed from those of healthy birds (manganese and zinc). It was found that on the 28th day of life the weight of chickens with perosis was reduced by 42.7%, causing a loss of weight 88 kg per batch of 2000 birds, with a consumption of feed 140 kg. The obtained data will allow the development of early perosis prevention schemes in broiler chickens, which will help manage production losses and increase its profitability. On farms, to prevent the occurrence of perosis, it is necessary to take into account the technological factors of the production of compound feed. Also, an increase in the level of total protein and albumin in serum in the blood may indicate inflammatory processes and dehydration of the body. Therefore, it is better to site a sick bird separately for rearing or hand over to a sanitary culling.

Keywords: broiler chickens; perosis; protein metabolism; mineral metabolism; zinc; manganese.

Introduction

Legs dysfunction is one of the most common and important problems in broiler chicken rearing on industrial and individual farms. They are caused by various factors: bone, tissue and nerve disorders, as well as violations of the technology of rearing conditions. Deficiency, and sometimes excessive amounts of essential minerals, plays a leading role in the development of leg diseases of poultry (Sanotra et al., 2001; Waldenstedt, 2006). Insufficient intake of vitamins, macro- and microelements, protein, amino acids, as well as unbalanced levels of fatty acids and reduction of total feed consumption are causes of disorders of skeletal development in birds (Cook, 2000; Oviedo-Rondón et al., 2006).

Perosis (manganese rickets, slipped tendon) is a disease characterized by deformity and inversion of the joints, as well as the violation of the bone and ligament formation process. As a result, changes in the anatomical position of the calf tendon, which emerges from its condyle, develop; the foot rotates at a right angle, similar to a dislocated leg (Greenacre, 2015; Olgun, 2017). This disease is mostly manifested in broiler chickens during the period of intensive weight gain – at the age of 14–35 days. (Weeks & Butterworth., 2004).

The main causes of perosis are deficiencies of manganese, zinc (Zoidis et al., 2020), choline (Farina et al., 2017) or biotin in a feed. However, most often in broilers, this disease is associated with a lack of manganese in feed, rapid weight gain of birds and high poultry density in the chicken coop (Greenacre, 2015; Priyanka et al., 2018). Increased levels of calcium, phosphorus and sodium in the diet of poultry with insufficient manganese content, increases the development of perosis (Saeeda, 2014). The development of this disease is also affected by a lack of niacin, riboflavin (Trzeciak et al., 2014), folic acid, cyanocobalamin and the wrong ratio in the diet of acidic and alkaline equivalents, an excess of iodine and molybdenum – manganese antagonists (Manohar & Kanagaraju, 2015). Manganese is one of the main trace elements, which is present in all body tissues and is the basis for normal metabolism of amino acids, lipids, proteins, carbohydrates (Widiyanto et al., 2013; Tufarelli & Laudadio, 2017), and is important in bone development (Liu et al., 2015). Manganese deficiency reduces the synthesis of fatty acids, proteins and methionine, disrupts the metabolism of nicotinic acid and reduces the level of alkaline phosphatase and phosphorus in the blood. Redox processes and metabolism of ascorbic, folic and pantothenic acids are disturbed, which leads to a decrease in tissue respiration activity. Skeletal growth is slowed down, bone formation is disturbed, joints are deformed (Weeks & Butterworth., 2004). In Ukraine (Chudak et al., 2010) and countries of near (Pavlović et al., 2020) and far abroad (Das et al., 2008; Gabdo et al., 2017), small poultry farms use their own feed mostly. However, due to frequent reliance on cheap and low-quality equipment and the use of mainly homegrown raw materials or defective vitamin-mineral-amino acid premix, the feed as a result does not meet the necessary standards (Oviedo-Rondón et al., 2006). Thus, the manifestation of perosis due to a deficiency of manganese and choline in the diet can be up to 5% of poultry, and in severe cases – up to 10% (Julian, 2005). This leads to early culling of poultry and shortage of products, which causes material damage to the economy due to reduced profitability (Dinev, 2012).

The aim of our research was to study changes in protein, macro- and micromineral metabolism in broiler chickens with perosis.

Materials and methods

The research protocol of the current study was approved by the Ethics Committee of the Bila Tserkva National Agrarian University (Approval number: 5.03.2019 No. 3). The study was conducted in 2019 on the basis of the Research Institute of Internal Animal Diseases and in a broiler chickens raising complex of the Training and Production Center of the

Bila Tserkva National Agrarian University. For the study, a batch of 2,000 Cobb-500 crossbred broiler chickens 14 days old was selected. Among them, 40 clinically healthy broiler chickens and 40 with signs of perosis were selected for the analysis of serum biochemical parameters at 14, 21 and 28 days of age. Chickens of this age were selected because of the fast growth which occurs at this period (Yildiz et al., 2009), which causes the greatest vulnerability to pathologies of the legs (Knowles et al., 2008). At the beginning of the study, the chickens were kept on a straw litter; the keeping density was 15 birds/m^2. The length of daylight was 16 hours of light and 8 hours of darkness. The air temperature on the 14th day of growing was 27.5 °C, on the 21st day 25 °C and on the 28th 23 °C (at a relative humidity of 50% in all periods). Prior to the study, the chickens were vaccinated against Gamboro disease, Newcastle disease and infectious bronchitis. On the 20th day of their lives, they were revaccinated for Newcastle disease.

Feeding of broiler chickens was carried out according to the technological map with compound feed of our own production: starter (14–21 days of life), grower (22–35 days), from 36 days to slaughter – finisher. Trace elements were added to feed with a premix in the form of inorganic salts (Table 1, 2). Compound feed on the farm was produced using a line (capacity 1000 kg/h) type MS-001n-PP (LLC "Knyazha Avila", Ukraine).

Table 1
The composition of feed for clinically healthy broiler chickens and those with perosis

Ingredients g/kg of feed	Prestarter (0–10 days)	Starter (11–21 days)	Grower (22–35 days)	Finisher (36–45 days)
Corn	400	308	350	409
Wheat	184	140	150	100
Soybean oilcake (39% of raw protein)	0	402	314	285
Sunflower meal (34% of raw protein)	100	40	70	80
Soybean meal (60% of raw protein)	200	0	0	0
Fishmeal (60% of raw protein)	60	0	0	0
Sunflower oil	11	28	37	47
Limestone	6	0	0	0
Chalk	7	18	15	15
Monocalcium phosphate	7	14	14	14
Premix 5 %	25	50	50	50

Table 2
The nutrient value of 1 kg of mixture feed for clinically healthy broiler chickens and those with perosis

Characteristics		Prestarter (0-10 days)	Starter (11–21 days)	Grower (22–35 days)	Finisher (36–45 days)
Nutrition (calculated)	Exchange energy, kcal/100 g	291	296	309	316
	Raw protein, %	22.7	22.6	20.4	19.6
	Calcium, %	1.0	1.0	0.9	0.9
	Useful phosphorus, %	0.4	0.4	0.4	0.4
Trace elements (defined)	Zinc, mg/k g	80.6	95.4	73.7	64.8
	Manganese, mg/kg	110.6	105.8	88.7	84.1
	Cuprum, mg/kg	10.5	9.8	10.7	8.8
	Iron, mg/kg	267.1	174.3	121.4	128.6
	Selenium, mg/kg	0.4	0.4	0.3	0.3
	Cobalt, mg/kg	0.5	0.8	0.7	0.9

Table 3
Blood biochemical parameters and body weight in broiler chickens: clinically healthy and with perosis (n = 20)

Indicator	14 days old		21 days old		28 days old	
	clinically healthy	with perosis	clinically healthy	with perosis	clinically healthy	with perosis
Body weight, g	419.3 ± 7.6	266.7 ± 9.5***	817.6 ± 11.5	588.7 ± 25.4***	1304.8 ± 22.2	747.8 ± 22.4***
Total protein, g/L	30.9 ± 1.3	36.4 ± 2.4*	30.0 ± 1.0	38.8 ± 1.9***	31.6 ± 1.5	38.4 ± 1.8***
Albumins, g/L	17.9 ± 1.1	21.9 ± 0.7**	18.8 ± 0.8	21.5 ± 0.9**	19.1 ± 1.8	23.5 ± 0.7*
Total calcium, mmol/ L	2.72 ± 0.08	2.71 ± 0.09	2.62 ± 0.06	2.74 ± 0.04	2.62 ± 0.06	2.81 ± 0.05**
Inorganic phosphorus, mmol/L	1.91 ± 0.08	2.03 ± 0.08	2.23 ± 0.07	2.03 ± 0.10	2.21 ± 0.06	2.01 ± 0.09*
Magnesium, mmol/L	0.93 ± 0.03	1.02 ± 0.05	0.91 ± 0.03	0.91 ± 0.04	0.92 ± 0.02	1.11 ± 0.03***
Manganese, µmol/L	2.3 ± 0.1	1.5 ± 0.1***	2.4 ± 0.2	1.3 ± 0.1***	2.4 ± 0.2	1.1 ± 0.1***
Zinc, µmol/L	29.0 ± 1.8	18.4 ± 1.1***	26.0 ± 1.3	19.1 ± 0.7***	26.3 ± 0.9	18.9 ± 0.9***

Note: * – P < 0.05; ** – P < 0.01; *** – P < 0.001 relatively clinically healthy birds with Bonferroni correction.

Water supply for poultry was provided through a system of nipple watering at the rate of 12 birds/nipple. Feed distribution was performed using an automated line. Poultry access to water and feed was unrestricted throughout the rearing period. The severity of symptoms in broiler chickens was determined using diagnostic criteria for perosis assessment: first degree – slightly displaced calf tendons, second degree – displaced calf tendons and third degree – displaced calf tendons, enlarged hocks and twisted legs (Zhaojun et al., 2013). The body weight of broiler chickens – clinically healthy and those with perosis was determined before blood sampling at the age of 14-, 21- and 28 days.

Blood samples for the study were taken using Vacutainer tubes (Becton Dickinson, England) with coagulation activator and gel by the method of lifelong puncture of the axillary vein (Kelly & Alworth, 2013; Sakara et al., 2018). Sampling was performed at 8 am on the 14th, 21st, and 28th days of rearing. Thereafter, the blood tubes were incubated for 30 min at room temperature (20–25 °C) until the clot separation process began. The liquid substance (blood serum) was centrifuged at 3000 rpm for 10 min until the final separation of the serum from the blood formed elements. The content of total protein, albumins, total calcium, inorganic phosphorus, magnesium, cholesterol, triglycerides, uric acid were determined in the blood serum in accordance with the manufacturer's instructions, Phyllisit-Diagnostics reagents (Ukraine) using a biochemical analyzer Stat Fax 1904+ (Awareness Technology, USA). Studies of the content of manganese, zinc, iron, cuprum in the serum of poultry were performed by atomic absorption spectrophotometry on a device Shimadzu AA-6650 (Shimadzu, Kyoto, Japan) with an electrothermal atomizer. To dilute the standards we used 0.1% solution of nitric acid (high purity) (Lachema, Czech Republic). Dilution of standards and serum was performed according to the manuals for work on the device. The content of microelements (Zn, Mn, Cu, Fe, Se, Co) was investigated in feeds for all periods of feeding (prestart, start, grower, finisher) in the Laboratory of Toxicological Monitoring, Department of Toxicology, Safety and Quality of Agricultural Products of the National Research Center "Institute of Experimental and Clinical Veterinary Medicine" (certificate of certification No. 75.00-033/2019, valid until 20.02.2022) using the method of X-ray fluorescence analysis in accordance with 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 studies were statistically calculated using the Statistica 10 program (StatSoft Inc., USA, 2011).

Results

The 14-, 21-, and 28-days-old chickens, without signs of perosis, were mobile, eager to eat food and drink water, their crop was soft and moderately full, and their plumage was shiny and close to their bodies. Such chickens fed well, the skeleton was strong, the joints were not enlarged, and the legs were strong and straight, without pododermatitis and breast blisters.

Perosis was diagnosed in 42 of the 14 day old birds (2.1% of the batch). Among them, in 36 chickens there was a slightly displaced tendon, which characterizes the beginning of the perosis development; and in 6 chickens – a complete displacement of the tendon and an inverted limb. In most of the affected birds (76.2%) the development of this pathology was observed on the right leg.

Chickens with perosis were sedentary, with a marked lag in growth; the crop was poorly filled, and in 4 birds – it was completely empty. Their plumage was dull, disheveled, contaminated with excrement. Among the sick birds, 2 died before the 21st day, so the results of their blood tests were not taken into account later.

By the 21st day, the number of chickens with signs of perosis had increased by 42.8% and amounted to 72 birds (3.6% of the batch). Of these, 48 birds (2.4%) had a completely displaced tendon, 12 had a slightly displaced tendon, and 10 had a completely displaced tendon, enlarged hocks, and an inverted right leg. In birds with complete tendon displacement we noted reduced mobility, apathy, increased thirst, their plumage was brittle and dull; chickens with an inverted limb had an arthritis and breast blisters.

By the 28th day of the broiler chickens' life, the number of birds with perosis had increased to 160 birds (8.0% of the batch). In 124 chickens there was a displaced tendon, enlargement of the right hocks and inverted legs; in 6 birds – slightly displaced, in 28 – completely displaced tendon. Clinically, it was observed that birds with third-degree lesions lost their appetite; they suffred brown-green diarrhea, inflammation of the affected legs joints, decreased fatness, breast blisters. Complications in the form of pododermatitis were diagnosed in 70% of broiler chickens with perosis. Thus, the development of perosis in broiler chickens begins on the 14th day of life and can affect up to 8.0% of the population, with a subsequent negative impact on the condition of the carcasses.

We found that chickens with perosis had significantly lower body weight than healthy ones. For birds 14 days old, the body weight deficit was 36.4%, on the 21st day – 28.0%, and on the 28th – 42.7%. Analysing individual indicators, it was diagnosed that in 5 birds the body weight was even lower than 700 g. It was established that with 8.0% of poultry affected by perosis from a batch of 2000 chickens, the farm did not receive 88 kg of product weight with a consumption of 140 kg of feed (Table 3).

In chickens with clinical signs of perosis on the 14th day of life, there was an increase in the concentration of total protein in the serum by 17.8% compared with similar clinically healthy birds ($P < 0.05$; Table 3). On the 21st day, the protein content in healthy birds did not change, and in those with perosis there was a tendency to increase. Similar results were obtained from chickens on the 28th day of rearing; the total protein content was significantly higher by 21.5% in birds with perosis ($P < 0.001$). The content of albumin in the serum of poultry with perosis was significantly higher than that in clinically healthy chickens on the 14th day by 22.3% ($P < 0.01$) and on the 28th day by 23.0% ($P < 0.05$). We found that the concentration of total calcium in the serum of chickens with perosis on the 28th day was significantly higher by 7.7% than in clinically healthy birds ($P < 0.01$, Table 3). Broiler chickens with clinical signs of perosis on the 28th day also had a lower content of inorganic phosphorus in the serum (9.1%, $P < 0.05$). On the 28th day, there was an increase in magnesium content by 22.2% ($P < 0.001$) in the serum of chickens with perosis.

At the beginning of the study, in 14-day-old chickens with perosis, the content of manganese in the serum was 34.8% lower than in clinically healthy ($P < 0.001$; Table 3). It was found that on the 21st day of poultry rearing the difference was already 45.8% ($P < 0.001$), and on the 28th – 2.2 times ($P < 0.001$). The concentration of zinc in the serum of 14-day-old broiler chickens with perosis was lower by 36.6% than that in clinically healthy birds ($P < 0.001$). After 7 days, the content of this element in the serum of sick birds was lower by 26.5% ($P < 0.001$), and on the 28th day – by 28.1% less than in healthy birds ($P < 0.001$).

Discussion

The rapid growth and development of the skeleton in modern crossbred broiler chickens is not accompanied by the development of strong enough legs that are fully capable of maintaining body weight, and subsequently leads to deformation of joints, tendons, etc. (Fleming, 2008). Genetics, maintenance, sanitation, comorbidities and, last but not least, feeding, affect the occurrence of leg disorders in birds (Waldenstedt, 2006). The body of the bird is not able to synthesize enough choline. Choline deficiency, as with manganese deficiency, can lead to slower growth and development, and as a result – to perosis in chickens (Selvam et al., 2018). Perosis can often be complicated by pododermatitis, which can cause bacterial infections. This negatively affects the health and productivity of broiler chickens (Hajilari et al., 2019).

In our opinion, the increase in the content of total protein in the blood serum of chickens with perosis is caused by the development of inflammatory processes in the body of the birds. Complications of pododermatitis were diagnosed in 70% of broiler chickens with perosis. It is known that due to the development of inflammatory processes at the site of injury, the concentration of total protein in the serum increases (Mayne, 2005; Hajilari et al., 2019). In the research results of Mondal et al. (2010), in the group of broilers which had low levels of manganese and zinc in the serum, the concentration of total protein was higher than in those which received a complete diet.

Calcium is well known for its role as a key element in many physiological functions of the body (Zhang et al., 2017). Together with phosphorus and magnesium, this vital element is actively involved in the formation of bone tissue (Shastak & Rodehutscord, 2015).

The increase in calcium and magnesium content in the serum of broiler chickens arose as a compensation for metabolic processes in bone tissue due to manganese deficiency (Zhaojun et al., 2013). Studies of Guo et al. (2019) are confirm that in pathologies of the legs, the content of total calcium in chickens increases and the level of inorganic phosphorus decreases. This is also confirmed by experiments of Huang et al. (2018) which showed that, compared with the control, birds with leg pathologies had elevated calcium levels and low serum phosphorus concentrations. In their studies of the effect of tibial dyschondroplasia on metabolic parameters in broiler chickens, Halit & Terim Kapakin (2012), indicate that in this pathology, the content of calcium and phosphorus in the serum is much lower than in healthy birds, and the concentration of magnesium does not change.

Mondal et al. (2010) in their studies showed that 16% of broilers fed a diet low in manganese and zinc, had development of leg pathology. According to the results of our studies, another clear sign of this disease is the lag of chickens in the body weight – by 42.7% on the 28th day of rearing. In our opinion, this is due to the fact that having inverted legs reduces the access to food and water consumption, which, in turn, leads to disruption of metabolic processes in the bird's body, the development of breast blisters and inflammation of the joints.

Thus, perosis leads to a complex of dysfunctions in the body of broiler chickens, which results in a lack of weight gain and early culling of birds due to deficient weight. That is why it can be argued that a properly designed program for the prevention of perosis during the rearing of broiler chickens is the key to its high profitability. It is important for farm owners who produce their own feed for broiler chickens to pay attention to the possibility of perosis development, as well as to use quality equipment and raw materials for mineral nutrition of poultry. After all, the thoroughness of mixing premixes with grain components of feed depends on the quality of equipment, and, as a result, the availability of vitamins, macro- and micronutrients and amino acids for birds changes.

Conclusions

Thus, perosis is a complex disease of broiler chickens, which, in addition to reducing the content of manganese in the blood of birds (2.2 times) and zinc (by 28.1%), leads to systemic metabolic disorders by the 28th day of their rearing. The concentration of protein (by 21.5%), total calcium (by 7.7%), magnesium (by 22.2%) and the concentration of inorganic phosphorus (by 9.1%) in the serum increased. Therefore, for effective prevention of perosis not only is the metabolism of manganese important, but also zinc and complete protein. By the 28th day of the chicken's life, 8.0% of the population is affected by perosis, their weight decreases by 42.7%. Because of this, early culling of birds is carried out, and their carcasses become substandard. As a result, the profitability of broiler meat production is reduced. In the future, such data will make it possible to develop schemes for early prevention of manganese deficiency (perosis) in broiler chickens.

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