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THE EFFECT OF CALCIUM AND MAGNESIUM SUPPLEMENTATION ON PERFORMANCE AND BONE STRENGTH OF BROILER CHICKENS

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ABSTRACT
Aim of the experiment was evaluation of the effect of reduced calcium and magnesium content in the broiler chickens diet on its parameters of fattening, bone strength and calcium and magnesium content in liver. The trial was performed with cockerels of Ross 308 hybrid (n = 160) which were fattened in cage batteries from day 11th to 36th day of age. Cockerels were divided into 4 groups (differ in various intake levels of calcium and magnesium) in four replications. The maize-wheat-soybean basal diet contained 2.33 g Ca and 1.58 g Mg per kilogram. Calcium was added by $CaCO_3$ and magnesium by $MgSO_4$. Control group (C) received feed mixture with added $CaCO_3$ in dose of 19.49 g.kg^{-1} and 0.41 g.kg^{-1} of $MgSO_4$. Three experimental groups contain added $CaCO_3$ in dose of 11.83 g.kg^{-1} and 0 g.kg^{-1} $MgSO_4$ (group Exp1); $CaCO_3$ 11.83 g.kg^{-1} and 0.41 g.kg^{-1} $MgSO_4$ (group Exp2); $CaCO_3$ 19.49 g.kg^{-1} and 0 g.kg^{-1} $MgSO_4$ (group Exp3), respectively. The feed consumption was daily monitored and the cockerels were weighed twice a week. At the end of the study the experimental animals were weighted and slaughtered by decapitation. The weight of carcasses, liver and proportion of breast and thigh muscle was determined in the selected chickens (n = 24). The atomic absorption spectrometry was used for Ca and Mg evaluation in liver tissues. Bone strength parameter was measured at the femur bone. The statistically significant differences (p >0.05) were not detected between control and experimental groups in the case of studied parameters of fattening, bone strength and calcium and magnesium content in the chicken´s liver. Based on the obtained results it could be concluded the reduction of determined elements in the chicken diet did not deteriorate parameters of yield, elements content in liver tissue as well as the bone strength of broiler chickens.

Keywords: poultry nutrition; Ross 308; liver; $CaCO_3$; $MgSO_4$

INTRODUCTION
Calcium (Ca) has important biological functions and must be provided in adequate amounts **(Peters and Mahan 2008)**. Inadequate calcium intake may affect bone mineral content, muscle function and other functions of minerals in the body **(Peters and Mahan 2008; Horky, 2015)**. Unfortunately, the mineral requirements of broiler chickens, as determined by several organizations 10 – 20 years ago, may not support optimal chicken performance in today's strain **(Ruttanavut and Yamauchi 2010)**. It is known that the genetic makeup of the bird influences the utilization of Ca and thereby its requirement. Therefore, it is presumed that the requirement of Ca may not be the same as reported in earlier studies to meet the demand for highly productive birds **(Shafey et al., 1990; Hurwitz et al., 1995)**. Magnesium (Mg) metabolism is closely associated with Ca and phosphorus **(McDonald et al., 2011; Horky et al. 2016)**. **Yang et al. (2012)** reported that dietary $MgSO_4$ supplementation significantly prevented heat stress-induced oxidative damage and improved growth performance in broilers. Some minerals such as zinc (Zn), and Mg are strongly associated with the antioxidant defense of organism **(Sahin et al., 2006)**. Recommended nutrient content by **Zelenka et al. (2007)** indicates the delivered amount of calcium and magnesium in feed mixtures for fattening chickens 9 g.kg^{-1} of calcium and 0.5 g.kg^{-1} of magnesium. Magnesium is one of the most abundant divalent cation in living cells and plays a vital role in many cellular processes. Magnesium deficiency or reduction in dietary intake of Mg^{2+} is strongly correlated with numerous metabolic abnormalities and chronic diseases including diabetes, ischemic heart disease and hypertension in which the accumulation of reactive oxygen species (ROS) is commonly observed **(Chakraborti et al., 2002; Guerrero-Romero and Rodriguez-Moran, 2002; Song et al., 2005; Valko et al., 2007; Bo and Pisu, 2008; Nevrkla et al., 2014; Nevrkla et al., 2016, Horky, 2014; Horky et al. 2012)**.

Aim of the experiment was evaluation of the effect of reduced calcium and magnesium content in the diet of broiler chicken on parameters of fattening, bone strength and calcium and magnesium content in the chicken´s liver.

MATERIAL AND METHODOLOGY

Experimental birds, diets and treatments

The trial was performed with cockerels of Ross 308 hybrid (n = 160) which were fattened in cage batteries from day 11th to 36th day of age. Cockerels were divided into 4 groups in four replications. Prior to formulating the diets, feed components were analyzed for Ca and Mg contents and the data were used to formulate the experimental diets. The basal diet contained 2.33 g Ca and 1.58 g Mg per kilogram. The composition of the basal diet is shown in Table 1.

Calcium was added by $CaCO_3$ and magnesium by $MgSO_4$. Control group (C) received feed mixture with added $CaCO_3$ in dose of 19.485 $g.kg^{-1}$ and 0.407 $g.kg^{-1}$ of $MgSO_4$. Three experimental groups (Exp1; Exp2; Exp3) contain added $CaCO_3$ and $MgSO_4$ in doses reported in Table 2.

The crumbly feed mixture was supplied *ad-libitum* and its consumption was recorded every day. Access to drinking water was also *ad-libitum*. Weighing of chickens was carried two times a week. Microclimate and lighting regime were modified according to the technological instructions for Ross 308. The values of microclimate were recorded every day.

Evaluating of carcass quality

The experimental animals were weighted and slaughtered by decapitation in the age of 36 days. The weight of selected carcases (n = 24) was determined. The carcase was free of neck, feather, offal and feet. After liver dissection, the livers were weighted and the percentage of the live weight of chicken was calculated. Subsequently, the skin from breast was removed and breast meat was deboned. The skin from legs was also removed and the legs were deboned. After breast and legs dissection, the muscles were weighted and the percentage of the live weight of chicken was calculated.

Determination of calcium and magnesium from liver tissue

Samples were mineralized by nitric acid with addition of hydrogen peroxide. The mineralization was carried out in microwave decomposition closed system Ethos 1 (Milestone S.r.l., Italy). After cooling, the mineralized samples were quantitatively transferred to 25 ml volumetric flask and fill up to the volume by demineralized water. The content of Ca and Mg in the mineralized samples was determined using atomic absorption spectrometry. The protocol was carried out using ContrAA 700 (Analytik Jena AG, Germany).

Table 1 Composition of the basal diet.

Ingredients	**%**
Maize	34
Wheat	31
Soybean meal	26
Sunflower oil	4
Vitamin-mineral premix*	2
Experimental premix**	2.5
Chromium oxide	0.5
Nutrient composition	
Dry matter	90
N-Substances	20.66
Ether extract	5.89
Crude fiber	3.14
Ash	5.53
Lysine	1.19
Methionine	0.58
Non-phytate P	0.30

*Note: premix content of one kg: lysine 101.65 $g.kg^{-1}$, methionine 135.63 $g.kg^{-1}$, threonine 51.22 $g.kg^{-1}$, calcium 68.31 $g.kg^{-1}$, phosphorus 98.19 $g.kg^{-1}$, natrium 62.89 $g.kg^{-1}$, magnesium 4.7 $g.kg^{-1}$, sulphur 0.39 $g.kg^{-1}$, chlorine 119.69 $g.kg^{-1}$, copper 752.5 $mg.kg^{-1}$, iron 3768.6 $mg.kg^{-1}$, zinc 3400 $mg.kg^{-1}$, manganese 6046.07 $mg.kg^{-1}$, cobalt 11 $mg.kg^{-1}$, iodine 47.95 $mg.kg^{-1}$, selenium 8.96 $mg.kg^{-1}$, retinol 680000 IU, cholecalciferol 250000 IU, alfatocoferol 2250 $mg.kg^{-1}$, K3 74.8 $mg.kg^{-1}$, B1 206.44 $mg.kg^{-1}$, B2 344 $mg.kg^{-1}$, B6 300.44 $mg.kg^{-1}$, B12 1999.2 $mg.kg^{-1}$, biotin 11 $mg.kg^{-1}$, niacinamid 1793.4 $mg.kg^{-1}$, calcium pantothenate 676.2 $mg.kg^{-1}$, folic acid 82.8 $mg.kg^{-1}$, choline chloride 9000 $mg.kg^{-1}$.

**Experimental premix: Content different levels of $CaCO_3$ and $MgSO_4$ according to Table 2.

Table 2 Addition of $CaCO_3$ and $MgSO_4$ ($g.kg^{-1}$) and total levels of Ca and Mg ($g.kg^{-1}$) in the diet.

	C	**Exp1**	**Exp2**	**Exp3**
$CaCO_3$	19.485	11.832	11.832	19.485
$MgSO_4$	0.407	0	0.407	0
Total Ca	9	6	6	9
Total Mg	2.33	1.58	2.33	1.58

Figure 1 Bone strength measurment.

The N_2O/C_2H_2 flame was used for the Ca and Mg determination. The samples and standard solutions were diluted in 1% HCl, the ionizing buffer KCl was used. For Ca and Mg analysis, the $LaCl_3$ as a displacing agent was used. The wavelength was set on λ = 239.8559 nm for Ca determination and λ = 202.582 nm for Mg determination.

Bone strength measurement

For the measurement was used a universal apparatus for measuring physical characteristics - TIRATEST 27025 (Germany). The device allows measurements of different materials in tension, compressive and flexural strength. To test the strength of bone was chosen three-point bending. Loading speed was 100 mm / min until fracture of bone. Bone strength parameter was measured at the femur bone.

Statistical analysis

Data has been processed by Microsoft Excel (USA) and STATISTICA.CZ, version 12.0 (CZ). The results were expressed as mean ± standard deviation. One-way analysis (ANOVA) was used. Sheffe´s test was applied to defined statistical differences and the differences between groups were considered significant at p <0.05.

RESULTS AND DISCUSSION

During the experiment, the highest average feed consumption 4.65 kg per chicken was estimated in the group Exp2 (Table 3). The lowest feed consumption was estimated in the control group (4.47 kg per chicken. The differences of the feed consumption between both groups were not statistically significant (p >0.05). The determined feed consumption is lower than Akter et al described **(Akter et al. 2016)**. It could be concluded the change of Ca and Mg content in the feed mixture did not affect the feed consumption significantly. This result is in agreement with **Akter et al. (2016)** who described the influence of low content of Ca in feed mixture which affects the increase intake of feed mixture by broiler chickens.

At the end of trial was observed non-significant (p >0.05) higher average weight of chickens (2 126 g) in Exp2 group (Table 4). Whereas **Hoeven-Hangoor et al. (2013)** presented the addition of 0.255 g.kg^{-1} MgSO4 was affected the average liveweight of chickens 2355g on 36th day of life. **Akter et al. (2016)** showed the addition 10 g.kg^{-1} of calcium in comparison with v 6 g.kg^{-1} in the feed mixture highly influenced the decrease of liveweight gain. Similar results were published by **Rama Rao et al. (2006)** and **Singh et al. (2013)**. However, we did not notice the described trend in our experiment. Whereas **Liu et al. (2007)** found the addition of magnesium to the feed mixture did not influence the average liveweight and feed intake in chickens.

Table 5 present the carcass yield. The highest carcass yield was found in the Exp1 group (73.62%) but differences between groups were not significant (p >0.05). Carcass yield, stated in the technological procedure for Ross 308 **(Aviagen Group 2014)**, is the 71.72% for 2,000 g of live weight. The higher breast and leg meat yield was found in the Exp1group. According to the manual of hybrid Ross 308 **(Aviagen Group 2014)** 21.20% of breast muscle at 2,000 g of live weight is stated. The same manual **(Aviagen Group 2014)** indicates a yield of leg meat 16.01% for 2,000 g live weight. The differences among groups in slaughtering yields were not statistically significant (p >0.05). Our results are in a good agreement with **Akter et al. (2016)**, who found the different amount of calcium in the feed mixture did not affect of the carcase yield and the proportion of breast and thigh muscle in tested chickens. On the contrary of **Salmanzadeh et al. (2012)** observed in their experiment that glucose and glucose + magnesium administration significantly increased the breast muscle size of broiler chickens.

The highest liver weight was found in the control group compared to experimental groups (Table 5). Values are not statistically significant (p >0.05). Similar results were published by **Štenclová et al. (2016)**. They determined the low amount of zinc in the feed mixture significantly affect

Table 3 Feed consumption per chicken and trial.

Group	n	Mean (g) ±standard deviation
C	25	4465 ±270.5
Exp1	25	4579 ±269.6
Exp2	25	4649 ±309.4
Exp3	25	4610 ±274.8

Note: Differences between groups were not statistically significant (p >0.05).

Table 4 The average weight of broiler chickens at the beginning and end of the experiment.

Group	n	C	Exp1	Exp2	Exp3
		Mean (g) ±standard deviation			
Start of the trial	40	311 ±3.1	315 ±3.6	315 ±2.7	310 ±2.8
End of the trial	40	2029 ±37.2	1982 ±39.3	2126 ±42.0	2057 ±42.8

Note: Differences between groups were not statistically significant (p >0.05).

Table 5 Carcass yield.

Group	n	Carcass	Breast meat	Leg meat without bone	Liver
		Mean (%) ±standard deviation			
C	24	72.95 ±0.415	20.83 ±0.367	14.83 ±0.204	2.17 ±0.056
Exp1	24	73.62 ±0.397	21.69 ±0.348	15.23 ±0.172	2.02 ±0.061
Exp2	24	73.37 ±0.289	21.04 ±0.343	14.86 ±0.268	2.04 ±0.040
Exp3	24	73.34 ±0.339	20.76 ±0.415	15.04 ±0.209	2.10 ±0.052

Note: Differences between groups were not statistically significant (p >0.05).

Table 6 Concentration of Ca and Mg in the liver (mg.kg^{-1} fresh weight).

Group	n	Ca	Mg
		Mean (mg) ±standard deviation	
C	24	100.82 ±3.088	211.19 ±2.859
Exp1	24	97.24 ±2.269	208.91 ±3.724
Exp2	24	99.22 ±2.524	211.21 ±3.297
Exp3	24	99.27 ±2.495	208.82 ±2.802

Note: Differences between groups were not statistically significant (p >0.05).

Table 7 Bone strength.

Group	n	Mean (N) ±standard deviation
C	24	247.90 ±11.308
Exp1	24	271.12 ±11.373
Exp2	24	267.13 ±10.467
Exp3	24	266.90 ±7.722

Note: Differences between groups were not statistically significant (p >0.05).

the higher weight of liver in the control group in comparison with experimental group of chickens.

Table 6 shows average concentration of Ca and Mg in the liver (mg.kg^{-1} fresh weight). The highest content of calcium in liver was estimated in the control group of chickens. The experimental group Exp2 showed the highest content of magnesium. The differences among analyzed groups were not statistically significant (p >0.05). Our obtained results of Ca and Mg content in the liver tissue of broilers are comparable with results published by **Majewska et al. (2016)**. They observed the low calcium concentration (70.76 mg.kg^{-1}) and higher magnesium concentration (328.62 mg.kg^{-1}) in liver tissue of broiler chickens.

The highest average of leg bone strength was reached in the experimental group Exp2 (Table 7). However, the differences among examined groups were not statistically significant (p >0.05). **Askari et al (2015)** found the average leg bone strength 246 N/m^2, that responds to our results of control group. The leg bone strength was determined 221.82 N/m^2 in the case of group with reduced content of Ca-P in the feed mixture. In contrary to results obtained by **Swiątkiewicz et al. (2011)** the decreased level of calcium and phosphorus was tended to reduce breaking point of leg bone of broiler chicken.

CONCLUSION

From the obtained results is evident the reduced supplementation of calcium and selenium did not influence the evaluated parameters of fattened chickens. Based on our results could be concluded the reduced content of studied elements did not cause the reduction of feed intake, neither the parameters of fattening and bone strength. Prior to eventual introduction of low calcium and magnesium diet for fattened chicken is essential to carry out further studies for evaluation of possible interactions with other mineral elements.

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