Polish Journal of Veterinary Sciences Vol. 15, No. 2 (2012), 227-232

DOI 10.2478/v10181-011-0138-0

Original article

The effect of different dietary sodium levels on blood mineral concentrations and tibia mineralization in turkeys

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Abstract

The objective of this study was to determine the effect of different dietary levels of sodium in diets with and without sodium chloride on mineral metabolism, including blood electrolyte levels and tibia mineralization parameters, in young turkeys (to six weeks of age). The influence of diets with a low (L), medium (M) and high (H) sodium content, at 0.34, 1.34 and 2.82 g/kg respectively, was compared. The content of chloride and potassium in turkey diets (1.7 – 5.9 and 11 g/kg, respectively) was above the recommended levels. The sodium-deficient diet L decreased the serum concentrations of sodium, chloride and phosphorus, and it increased the serum levels of calcium and magnesium in turkeys, compared with groups M and H. Relative to group L, different dietary sodium levels in groups M and H contributed to a similar increase in the body weights of birds (1.06 vs. 1.46 and 1.44 kg, $p<0.001$) and in the absolute (4.60 vs. 6.83 and 6.62 g, $p<0.001$) and relative tibia weight (0.42 vs. 0.46 and 0.46% body weight, $p=0.031$). No significant differences were found between groups with respect to the content of ash, calcium and phosphorus in tibia dry matter. Supplemental sodium increased the bone density index (from 50.6 to 68.4 and 66.3 mg/mm in groups L, M and H, respectively, $p<0.001$), the maximum bending moment (from 5.27 to 7.40 and 7.33 N/mm, $p=0.002$) and the minimum breaking strength of tibia (from 0.136 to 0.191 and 0.189, $p=0.002$).

In conclusion, our study indicates that the applied dietary treatment with a moderate sodium level (1.34 g/kg) resulted in a rate of bird growth and tibia mineralization similar to those observed with the treatment with much higher Na content (2.82 g/kg).

Key words: NaCl supplementation, blood electrolytes, tibia mineralization, turkey

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Introduction

Leg deformities and abnormalities, which adversely affect the welfare and growth rate of birds, are among the most common health problems encountered in intensive poultry farming (Nääs et al. 2009). In turkeys, leg problems often result from selection for a rapid increase in body weight and breast muscle yield (Crespo et al. 2000). The cortical bone of fast-growing meat-type chickens has been found to be less well mineralized and more porous than that of the slow-growing strain (Williams et al. 2000). The bones of fast-growing broilers have been also shown to contain less ash than the bones of slow-growing birds (Leterrier and Nys 1992). Research results also suggest that femoral and tibial structural deformation and fractures in turkeys may be associated with insufficient skeletal adaptation to heavy body weight (Crespo et al. 2000, Tatara et al. 2011).

The most common reason for insufficient bone mineralization are mineral metabolism disorders, in particular a deficiency of calcium and phosphorus (Kestin et al. 2001, Rao et al. 2003, Venäläinen et al. 2006) as well as the deficiency or poor absorption of vitamin D_3 (Faruga et al. 2009). According to Mayne et al. (2007), a high litter moisture content may lead to footpad dermatitis in growing turkeys. The dietary sodium intake is often increased in broilers to stimulate their growth (Vieira et al. 2003, Borges et al. 2003), which leads to higher water consumption levels (Murakami et al. 2000, Mushtaq et al. 2007).

Sodium, potassium and chloride ions are responsible for maintaining the acid-base balance and normal blood and tissue pH levels, in particular under thermal stress conditions (Borges et al. 2003, Zhou et al. 2011). The physiological functions of sodium include also the regulation of enzyme activity and tissue protein synthesis (Olanrewaju et al. 2007), which is why an increase in dietary sodium supply is expected to promote the growth of broiler chickens (Watkins et al. 2005, Mushtaq et al. 2007). Sodium metabolism disorders lead to a decrease in the ash content of bones, thus exerting a negative effect on the bone mineralization process (Murakami et al. 1997). Sodium-calcium interactions also play a vital role in the pathogenesis of skeletal muscle damage in broiler chickens (Sandercock and Mitchell 2004). The influence of different dietary sodium levels on the growth rate and skeletal stability of turkeys remains scarcely investigated.

The aim of this study was to determine the effect of a different sodium content (low, medium and high) of diets on mineral metabolism, including blood electrolyte levels and tibia mineralization parameters, in young turkeys (to six weeks of age).

Materials and Methods

The experiment was carried out at the Research Laboratory of the Department of Poultry Science, University of Warmia and Mazury in Olsztyn, on 168 female BUT-10 turkeys kept for six weeks in three-tier battery cages, each with a floor area of 0.5 m^2 (14 birds/1 m^2). The turkeys were randomly divided into three groups of eight replicates, each of seven birds. The temperature and lighting program was consistent with the recommendations of the British United Turkeys Ltd (2009). The birds were fed *ad libitum*, and they had free access to water. The composition and nutritional value of basal starter (1 – 21 days) and grower (22 – 42 days) diets are presented in Table 1. Group L turkeys were fed low-sodium diets without the addition of NaCl. Group M and H birds received diets supplemented with 0.254% and 0.636% NaCl, respectively. Sodium chloride was added to diets M and H in the form of a 1% premix, which was prepared under laboratory conditions. Samples of experimental diets were assayed for the content of sodium and potassium (by atomic absorption spectrometry) and chloride by the biamperometric technique (Jankowski et al. 2011a). The analyses were

Table 1. Composition and calculated nutrient content of basal starter (1-21 days) and grower (22-42 days) diets, %.

Specification	1 – 21 days	22 – 42 days
Composition, %		
Wheat	26.22	34.47
Maize	20.00	18.00
Soybean	41.70	36.50
Potato protein	5.00	4.45
Soybean oil	2.17	2.20
Limestone	1.62	1.45
Monocalcium phosphate	2.27	2.02
L-Lysine 99 MonohydroCL	0.28	0.25
DL-Methionine 99	0.24	0.21
Premix[1]	0.50	0.45
Nutritional value		
ME, kcal/kg	2801	2851
Crude protein, %	27.99	25.98
Lysine, %	1.75	1.58
Methionine, %	0.66	0.60
Met + Cys, %	1.10	1.02
Threonine, %	1.08	0.98
Tryptophan, %	0.35	0.32
Ca, %	1.26	1.12
Available P, %	0.65	0.60
Na, %	0.03	0.02

[1] content per kg of premix: vitamin A – 2 400 000 IU, vitamin D_3 – 1 000 000 IU, vitamin E – 20 g, vitamin K_3 – 1.2 g, vitamin B_1 – 1 g, vitamin B_2 – 1.6 g, vitamin B_6 – 1.2 g, vitamin B_{12} – 6 mg, biotin (H) – 60 mg, Fe – 12 g, Mn – 24 g, Zn – 22 g, Cu – 4 g, J – 600 mg, Se – 60 mg, pantothenic acid – 5 g, nicotinic acid – 16 g, folic acid – 600 mg, choline chloride – 80 g.

performed at the National Feed Laboratory in Lublin, and their results were used to calculate the dietary electrolyte balance (DEB), with the following formula: DEB = Na mEq/kg + K mEq/kg – Cl mEq/kg (Mongin 1981).

After 42 days of feeding, all turkeys were weighed individually and seven birds representing an average body weight for each group were selected for blood sampling from the wing vein to determine the concentrations of selected elements. The analyses were performed by the Analytical Laboratory at the Municipal Hospital in Olsztyn, using the COBAS INTEGRA 400 PLUS system (Roche). The concentrations of Ca, P and Mg were determined by the colorimetric method, and the levels of Na, K and Cl by indirect potentiometry (Tykałowski et al. 2011).

After electrical stunning, the birds were decapitated and their tibias were removed and cleaned of soft tissue. The left tibias were weighed and set aside for later mineralization. The right tibias were measured to determine their length, perimeter and diameter, and set aside for strength tests. The bones were kept deep-frozen (-25°C) until analysis.

To determine the content of ash, calcium and phosphorus, the tibias were dried, and samples with a weight of ca. 1 g and a length of 2 cm were collected from the middle part of the bones. The samples were mineralized in a VELP DK 20 electric aluminum heating block with selectable temperatures (VELP Scientifica, Italy). Analytical samples were prepared together with test samples. The analytical samples were mineralized in a mixture (3:1) of nitric acid and perchloric acid (Merck, Germany). The calcium content of mineralizates was determined by flame atomic absorption spectrometry (acetylene-air flame). The analysis was performed with the use of a Unicam 939 Solar atomic absorption spectrophotometer (United Kingdom) equipped with an Optimus data station, background correction system (deuterium lamp) and cathode lamps (Whiteside and Miner 1984). The phosphorus content of mineralizates was determined by colorimetry, using ammonium molybdate, sodium sulfate and hydroquinone (Tietz 1986). Absorbance was measured with the use of a VIS 6000 spectrophotometer (Krüss-Optronic, Germany) at a wavelength of $\lambda = 610$ mm.

Tibia breaking strength was measured with the use of a computerized Instron 4302 universal testing machine with head duty of up to 981 N and cross-head speed of 10 mm/min. The mechanical properties of tibias were determined in a three-point bending test (Feretti et al. 1993), at 40% total bone length. Load force was recorded during the test at 1/20 s intervals, and the maximum breaking force was determined. The breaking strength of tibias was expressed as the quotient of the maximum bending moment (W_f) and the cross-sectional area (A) of bones. The value of W_f was calculated with the following formula: $W_f = W_c \times L$, where: W_c – maximum breaking force, L – length. Similarly as in the works of other authors (Kwiecień and Winiarska-Mieczan 2008, Tatara et al. 2011), the cross-sectional area was determined using the formula: $3.14 \times [(HB\text{-}hb)/4]$, where: H – horizontal external diameter, h – horizontal internal diameter, B – vertical external diameter, b – vertical internal diameter. Cortical thickness was calculated as the difference between the values of H and h, and the bone density index was calculated as the quotient of tibia weight and length.

The results were processed statistically by one-way analysis of variance (ANOVA) in an orthogonal design. The significance of differences was estimated by Duncan's test. The calculations were performed using the Statistica software package ver. 8.1.

Results

The concentrations of Na, K and Cl in experimental diets and DEB values are shown in Table 2. The diets differed considerably with respect to Na content, slightly as regards Cl content, and no differences were found in K content. DEB was similar in all diets.

Table 2. Sodium chloride supplementation, sodium, potassium, chloride content (g/kg) and dietary electrolyte balance (DEB, mEq/kg) in experimental diets with low (L), medium (M) and high (H) supplementation of sodium.

Specification	Experimental group		
	L	M	H
NaCl addition, g/kg	0.0	2.54	6.36
Na addition, g/kg	0.0	1.0	2.5
Content in diet			
Sodium, g/kg	0.34	1.34	2.82
Potassium, g/kg	11.0	11.0	11.0
Chloride, g/kg	1.7	3.2	5.9
DEB, mEq/kg	242	249	246

Table 3 presents the concentrations of selected minerals in the blood serum of turkeys. In comparison with groups M and H, group L turkeys were characterized by lower levels of Na ($p<0.001$), Cl ($p=0.007$), P ($p=0.002$) and Mg ($p<0.001$). Significantly ($p=0.020$) higher serum Ca concentrations were noted in groups M and H. There were no significant differences between groups in serum K levels.

Table 3. Concentration of selected macroelements in the blood serum of turkeys.

Specification	Experimental group			SEM	p
	L	M	H		
Na, mmol/ l	141[b]	148[a]	148[a]	0.931	<0.001
K, mmol/l	3.56	3.55	3.27	0.178	0.781
Cl, mmol/l	105[b]	110[a]	110[a]	0.850	0.007
Ca, mg/dl	13.7[a]	12.8[b]	12.9[b]	0.157	0.020
P, mg/dl	6.30[b]	7.63[a]	7.77[a]	0.212	0.002
Mg, mg/dl	2.83[a]	2.37[b]	2.46[b]	0.055	<0.001

Values not sharing the same superscript letters within a column are different at $P \leq 0.01$

After six weeks of experimental feeding, group L turkeys had significantly (P<0.001) lower body weights (Table 4) as well as significantly lower absolute tibia weight (p=0.001) and relative tibia weight (P=0.031). No significant differences were found between groups with respect to the content of ash, Ca and P in tibia dry matter.

Table 4. Weight and mineral composition of turkey tibia.

	Experimental group			SEM	p
	L	M	H		
Body weight, kg	1.06[a]	1.46[b]	1.44[b]	0.043	<0.001
Tibia weight, g	4.60[b]	6.83[a]	6.62[a]	0.248	<0.001
Tibia relative weight, % BW	0.42[b]	0.46[a]	0.46[a]	0.008	0.031
Ash content, % DM	56.4	58.0	56.9	0.440	0.359
Ca content, % DM	22.4	23.3	22.2	0.244	0.179
P content, % DM	9.26	9.33	9.23	0.097	0.917

Table 5. Geometrical and mechanical properties of turkey tibia.

	Experimental group			SEM	p
	L	M	H		
Tibia length, mm	90.8[b]	99.9[a]	99.8[a]	1.181	<0.001
Tibia perimeter, mm	18.20[b]	20.7[a]	20.8[a]	0.325	<0.001
Cross-sectional area, mm^2	14.7[b]	20.5[a]	20.2[a]	0.799	<0.001
Bone density index, mg/mm	50.6[b]	68.4[a]	66.3[a]	2.027	<0.001
Cortical thickness, mm	2.19[b]	2.92[a]	2.53[ab]	0.010	0.005
Maximum bending moment, N/mm	5.27[b]	7.40[a]	7.33[a]	0.305	0.002
Minimum breaking strength, KN	0.136[b]	0.191[a]	0.189[a]	0.008	0.002

In comparison with groups M and H, group L turkeys were marked by significantly lower parameters of tibia growth and mineralization (Table 5), including tibia length, perimeter and cross-sectional area (p<0.001 in all cases), bone density index, cortical thickness (p<0.001 and p=0.005, respectively), maximum bending moment and minimum breaking strength (p=0.002 in both cases).

Discussion

In the present study, experimental diets differed with respect to sodium content and, partially, chloride content, as chloride was supplied with sodium. In the basal diet, without sodium chloride, the sodium content was very low (0.34 g/kg), while the content of chloride (1.7 g/kg) and potassium (11 g/kg) was relatively high. The sodium content of diets was lower, whereas the content of Cl and K was higher than the respective nutrient requirements of young turkeys, determined at 1.7 g/kg for Na, 1.5 g/kg for Cl and 7 g/kg for K (NRC, 1994). The above was due to the low sodium content of cereals and soybean meal, the major components of experimental diets, accompanied by a high chloride content and a very high potassium content. Similar Na, Cl and K ratios in chicken diets without the addition of sodium chloride were also observed by other authors (Koreleski and Świątkiewicz 2010, Jankowski et al. 2011a,b). Due to a high potassium content, DEB was high (242 mqE/kg), similar to the value recommended for broiler chickens (Mongin 1981) and higher than the value of 211 mqE/kg recommended for turkeys, calculated based on the concentrations of Na, K and Cl in diets (NRC 1994).

Sodium supplementation had no effect on DEB, since it was accompanied by an increase in chloride content. The addition of sodium chloride to experimental diets increased the blood levels of sodium, chloride and phosphorus, it had no effect on potassium content, and it decreased the blood concentrations of calcium and magnesium. The observed changes were not determined by the amount of sodium added. A similar influence of different dietary sodium levels on blood mineral concentrations was noted in chickens (Tykałowski et al. 2011). In other experiments on chickens, an increase in DEB was followed by an increase in serum calcium concentrations (Olanrewaju et al. 2007), while the serum levels of sodium and potassium were determined by the chloride content rather than the sodium content of diets (Mushtaq et al. 2007).

Irrespective of its amount, supplemental sodium contributed to a significant increase in the body weights of turkeys at six weeks of age, and to an increase in absolute and relative tibia weight. Also in other experiments, broiler chickens responded by body weight gain to increased dietary sodium supply (Borges et al. 2003, Vieira et al. 2003, Jankowski et al. 2011a,b).

No significant differences were found between groups with regard to the content of ash, calcium and phosphorus in tibia dry matter, which indicates that a faster growth rate of turkeys fed diets with a medium and high sodium content did not decrease the concentrations of those elements in their tibial bones. Our results corroborate the findings of Mikulski et al. (2009) who reported an increase in the body weights of broiler chickens fed copper-supplemented diets, with no reduction in calcium and phosphorus concentrations in tibia dry matter. In another experiment involving chickens (Jankowski et al. 2011a), a significant increase in the levels of ash, calcium and phosphorus in tibia dry matter resulted from feeding a diet with a medium sodium content (1.2 g/kg feed offered at 15 – 35 days of age), while a further increase in dietary sodium supply decreased their content. In a study by Murakami et al. (1997), the ash content of bones tended to decrease as the sodium content of chicken diets increased. In a later experiment, the these authors (Murakami et al. 2000) demonstrated that a dietary sodium level of 0.15% was sufficient to maintain the maximum tibia ash content of male broilers.

In our study, the addition of sodium to experimental diets increased tibia length, cross-sectional area and perimeter as well as cortical thickness, which enhanced tibia elasticity (as manifested by higher values of the maximum bending moment) and increased its minimum breaking strength. Increased dietary sodium intake had neither a positive nor negative impact on the above parameters. In another experiment, the tibias of chickens fed a diet without additional sodium were significantly more prone to breaking, whereas high sodium supplementation levels decreased bone elasticity, as compared with birds receiving a diet with a medium sodium content.

Conclusions

In conclusion, our study indicates that the applied dietary treatment with moderate sodium level (1.34 g/kg), lower than the recommended level of 1.7 g/kg, resulted in a rate of bird growth and tibia mineralization similar to those observed with treatment with much higher Na content (2.82 g/kg).

Acknowledgments

This study was supported by the National Centre for Research and Development, Project No. R12 0096 06.

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