Contents of macro- and microelements in blood serum and breast muscle of broiler chickens subjected to different variants of pre-slaughter handling

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ASTRACT: This experiment was aimed at determining contents of Ca, Mg, Na, K, P, and Fe in blood serum and breast muscles of broiler chickens COBB 500 subjected to different variants of pre-slaughter handling in the summer period: no transport (N–T); transport to a distance of 100 km (T–100) and 200 km (T–200). The complete pre-slaughter handling consisted of the following stages: catching the birds, weighing, loading to containers, transport and waiting for slaughter, unloading and weighing after the transport. In the N–T group, transport and weighing after the transport were excluded from the pre-handling management. Stress factors occurring in the pre-slaughter handling evoked a detrimental effect on the bodies of both males and females and appeared to affect concentrations of Ca, Mg, Na, K, P, and Fe in blood serum and breast muscles of the broiler chickens. An upward tendency was observed in serum levels of those elements after transport to a distance of 100 km which was, however, followed by their decline. An opposite tendency was demonstrated in breast muscle. Taking into account the sex of the chickens, differences were observed in contents of macro- and microelements between males and females, both in their blood sera and breast muscles.

Keywords: chickens; stress; pre-slaughter handling; macro- and microelements

During pre-slaughter handling, poultry is exposed to a variety of co-acting stimuli: sudden displacement, vehicle movement, speeding up, vibrations, jolts, noise, lack of feed and water, restriction of natural behaviours, disturbance of flock hierarchy, restriction of living space, exposure to pain, and change of transport microclimate (Duncan, 1989; Broom, 1990; Mitchell and Kettlewell, 1998). The accumulation of such a great number of stress factors in such a short period of time results in diminished immunity of birds as well as in physiological and morphological changes in their bodies. Those effects are primarily manifested by changes in the blood composition, changes in the structure of muscle tissue, body weight losses and increasing death rate as well as by damage to bird carcasses and deterioration of their meat quality (Kettlewell, 1989; Weeks and Nicol, 2000; Warriss et al., 2005; Večerek et al., 2006; Wojcik, 2007).

A high number of stress stimuli acting in the pre-slaughter handling disturb an organism's homeostasis. This, in turn, leads to a loss of macro- and microelements, deficiency of which in meat may affect its quality (Mowat, 1994; Podgorski et al., 2001; Truchlinski et al., 2007). Available literature provides no data on any trial of monitoring changes in contents of macro- and microelements simultaneously in blood and meat of poultry subjected to the action of various stress-bearing factors. Hence, the study was undertaken to determine the contents of Ca, Mg, Na, K, P, and Fe in blood serum and breast muscles of broiler chickens subjected to different variants of pre-slaughter handling in the summer season.

MATERIAL AND METHODS

The experimental material was broiler chickens COBB 500 reared under controlled environmental conditions up to 42 days of age. The chickens were fed *ad libitum* with loose industrial feed mixtures for broiler chickens: Starter (1–7 days of rearing), Grower – 1 and 2 (8–35 days of rearing), and Finisher (the last 7 days of rearing). Various pre-slaughter handling techniques were applied to male and female chicks:

- N–T – no transport (30 males, 30 females);
- T–100 – transport to a distance of 100 km (30 males, 30 females);
- T–200 – transport to a distance of 200 km (30 males, 30 females).

The complete pre-slaughter handling consisted of the following stages: catching the birds, weighing, loading to containers, transport and waiting for slaughter, unloading and weighing. In turn, pre-slaughter handling without transport (N–T group) involved: catching the birds, weighing, loading to containers, waiting for slaughter, and unloading.

On the day preceding slaughter, at 9.00 p.m., feed mixtures were taken away and the birds had only access to water. On the next day, at 7.00 a.m., the chicks were weighed and randomly divided into sub-groups, based on the birds' sex and the applied pre-slaughter handling procedure. Broilers (males and females separately) were placed in 18 plastic perforated containers (30 × 60 × 90 cm) designed for poultry transportation, 10 birds per container. The chickens from the N–T group were slaughtered after 8.00 a.m. Transport of the birds began at 8.00 a.m., and proceeded in a vehicle adjusted to poultry transportation. After travelling 100 km, the vehicle reached the laboratory at around 10.30 a.m., and birds from the T–100 group were unloaded. Next, the vehicle drove another 100 km and returned to the laboratory at around 1.00 p.m. Each time, the vehicle drove the same route at an average speed of 55 km/h.

After the transport, the chickens were weighed, their blood samples were collected from a wing vein and then they were slaughtered. Slaughter of the experimental chickens was conducted under laboratory conditions following all procedures applied to birds in a slaughterhouse (permission of the Local Ethical Commission No. 3/N of the 23rd January 2004). After chilling, the samples (ca. 150 g) of right breast muscle were collected from carcasses for laboratory analyses.

Feed mixtures, blood serum and breast muscles were analyzed to determine the concentrations of calcium (Ca), magnesium (Mg), phosphorus (P), sodium (Na), potassium (K) and iron (Fe). The content of macro- and microelements in blood serum and breast muscles was determined in triplicate, in a mixed sample collected from chicks placed in each of 18 containers. The biological material was subjected to wet mineralization and ash was hot dissolved in a 1 M solution of nitric acid. The resultant mineralizates were analyzed for contents of Ca, Mg and Fe by the method of atomic absorption flame spectrometry. Determinations were carried out with the use of an atomic absorption spectrometer Unicam 939 Solar – England, equipped in Optimus data basis, background correction (deuterium discharge lamp), appropriate cathode lamps, graphite furnace Unicam GF 90 and autosampler FS 90. Contents of Na and K were determined by the emission technique (acetylene-air flame). Assays were conducted by means of an atomic emission spectrometer Pye Unicam SP 2900 – England (Whiteside and Miner, 1984). The content of phosphorus was analyzed by the colorimetric method (Zegarska et al., 2000). Absorbance was measured with a spectrophotometer VIS 6000 – by KRÜSS – OPTRONIC, Germany, at a wavelength of $\lambda = 610$ nm.

The data collected for levels of macro- and microelements in blood serum and breast muscle of the broiler chickens was processed by a two-way analysis of variance in orthogonal design (ANOVA). The statistical analysis of data involved determination of arithmetic means ($\bar{x}$) and standard deviations (± SD). The significance of differences between mean values computed for particular levels of experimental factors was determined by Duncan's test. All calculations were made with Statistica 8.0 PL software.

RESULTS AND DISCUSSION

Meeting requirements for mineral compounds is determined by contents of minerals in feed mixtures, their availability as well as by correlations between individual macro- and microelements (Shelton and Southern, 2006). Contents of the analysed mac-

Table 1. Changes in the levels of macro- and microelements in feed mixtures

Macro- and microelements	Starter 110	Grower 114	Grower 115	Finisher 118
Calcium (mg/g)	8.98	7.38	5.62	6.55
Magnesium (mg/g)	1.624	1.650	1.625	1.715
Phosphorus (mg/g)	5.44	5.27	4.96	4.90
Sodium (mg/g)	1.436	1.340	1.442	1.215
Potassium (mg/g)	9.26	8.94	8.92	8.37
Iron (mg/kg)	142.0	148.9	132.2	121.4

ro- and microelements in feed mixtures (Table 1) were consistent with demands of chicken broilers and amounted to (on average): Ca – 7.13 mg/g, Mg – 1.65 mg/g, P – 5.14 mg/g, Na – 1.36 mg/g, K – 8.87 mg/g, and Fe – 136.13 mg per kg.

Mineral compounds present in cells and tissues of animals serve a variety of functions in their bodies. Sodium, potassium and calcium, occurring in tissues and cellular fluids in the form of electrolytes, affect the osmotic pressure and acid-base balance in the body. Potassium is additionally responsible for the proper functioning of neural and muscle tissue.

Of crucial significance is also magnesium that activates a number of enzymes indispensable in

Table 2. Changes in the levels of macro- and microelements in blood serum

Macro- and microelements	Statistical measure	Pre-slaughter handling (I)			Sex (II)		Significance of interactions I × II
		N–T	T–100	T–200	male	female	
Number	n	18	18	18	27	27	54
Calcium (μg/g)	$\bar{x}$	101.81	103.67	103.27	101.17	104.66^{x}	–
	SD	7.36	3.42	6.05	5.50	5.63	
Magnesium (μg/g)	$\bar{x}$	21.27^{B}	$23.18^{A,a}$	$22.46^{A,b}$	22.63^{x}	21.99	–
	SD	1.17	0.91	1.19	1.26	1.36	
Phosphorus (μg/g)	$\bar{x}$	$185.12^{B,b}$	195.23^{A}	192.96^{a}	190.39	191.82	–
	SD	14.81	8.73	5.07	9.73	12.38	
Sodium (mg/g)	$\bar{x}$	$3.12^{B,b}$	3.30^{A}	3.27^{a}	3.25	3.22	–
	SD	0.22	0.16	0.12	0.20	0.18	
Potassium (μg/g)	$\bar{x}$	190.99^{B}	205.53^{A}	196.94	192.77	202.88^{x}	XX
	SD	23.42	8.23	13.29	19.20	13.07	
Iron (μg/g)	$\bar{x}$	1.21^{B}	1.33^{A}	1.15^{B}	1.23	1.23	XX
	SD	0.10	0.15	0.12	0.19	0.09	

the values designated by different superscripts are significantly different: $^{A,B}P \leq 0.01$ (pre-slaughter handling)
$^{a,b}P \leq 0.05$ (pre-slaughter handling); $^{xx}P \leq 0.01$ (sex); $^{x}P \leq 0.05$ (sex)
XX – significance of interactions at a level of $P \leq 0.01$
X – significance of interactions at a level of $P \leq 0.05$
N–T – no transport
T–100 – transport to a distance of 100 km
T–200 – transport to a distance of 200 km

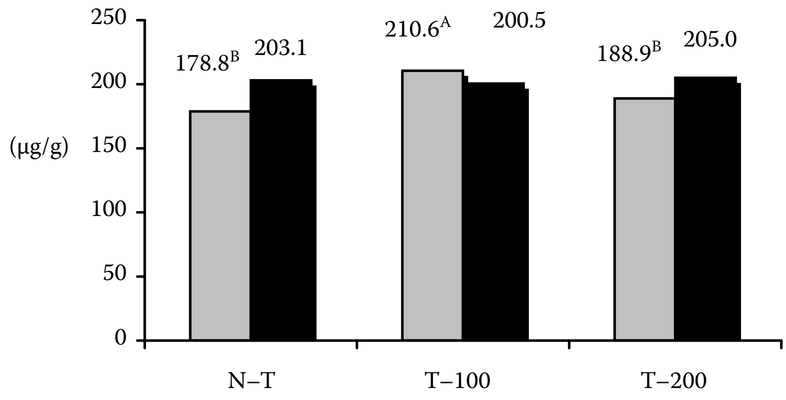

Figure 1. Co-effects of pre-slaughter handling and sex on the serum level of potassium in broiler chickens
☐ male
■ female
$^{A,B}P \leq 0.01$

carbohydrate and phosphorus-calcium metabolism as well as serves an important function in the contraction process of muscles, including the cardiac muscle, and maintains the heartbeat at a normal level. Besides, it influences neuro-muscular excitability, stimulates defence mechanisms of the body and exerts a sedating effect. Ample research has demonstrated that stress stimuli provoke such changes in bodily matter metabolism that re-evoke or intensify magnesium deficiency in blood and muscles (Siegel, 1995; Bláhová et al., 2007).

Iron is a constituent of multiple enzymes and metalloproteins that participate in redox processes. Iron bound in blood in the form of haemoglobin participates in oxygen transfer from lungs to tissues. In turn, in muscles it is a constituent of myoglobin – a red pigment of muscles that collects oxygen from red blood cells and utilizes it for the work of muscles.

Phosphorus participates in the transmission of neural stimuli and is a constituent of cellular membranes and soft tissues. It is also active in a number of metabolic processes and chemical reactions, thus facilitating e.g. the release of energy from proteins, carbohydrates and lipids, especially in the case of body exposure to stress-bearing factors.

The optimal contents of minerals in a feed mixture enable the proper functioning of an organism and good production performance (Sembartowicz et al., 2003; Gergely et al., 2006). In contrast, stress factors like high temperature of environment or transport affect the diversification of macro- and microelements in blood serum (Večerek et al., 2002; Orowicz et al., 2004; Bláhová et al., 2007) or muscles (Suchý et al., 2002; Niess et al., 2005).

The highest serum levels of macro- and microelements (Table 2) were observed in the group of chickens transported to a distance of 100 km: Mg – 23.18 μg/g, P – 195.23 μg/g, Na – 3.30 mg/g, K – 205.53 μg/g, and Fe – 1.33 μg/g, as compared to non-transported chickens (21.27 μg/g, 185.12 μg/g, 3.12 mg/g, 190.99 μg/g, 1.21 μg/g, respectively) and to birds transported to a distance of 200 km (22.46 μg/g, 192.96 μg/g, 3.27 mg/g, 196.94 μg/g, and 1.15 μg/g respectively). The level of calcium appeared to be negligibly higher in the group of chickens transported to a distance of 100 km (103.67 μg/g) in

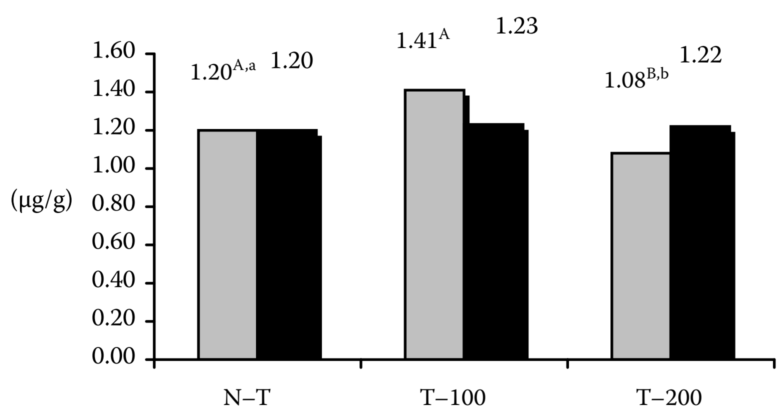

Figure 2. Co-effects of pre-slaughter handling and sex on the serum level of iron in broiler chickens
☐ male
■ female
$^{A,B}P \leq 0.01$
$^{a,b}P \leq 0.05$

Table 3. Changes in the levels of macro- and microelements in breast muscle

Macro- and microelements	Statistical measure	Pre-slaughter handling (I)			Sex (II)		Significance of interactions I × II
		N–T	T–100	T–200	male	female	
Number	n	18	18	18	27	27	54
Calcium (µg/g)	$\bar{x}$	39.95	39.01	40.70	38.06	41.71	–
	SD	7.64	4.52	8.04	3.65	8.63	
Magnesium (µg/g)	$\bar{x}$	307.32	303.22^{b}	313.05^{a}	308.60	307.13	–
	SD	13.47	8.94	9.82	10.80	12.24	
Phosphorus (mg/g)	$\bar{x}$	2.44	2.44	2.50	2.44	2.48^{x}	–
	SD	0.09	0.08	0.10	0.09	0.09	
Sodium (µg/g)	$\bar{x}$	387.87^{A}	371.89^{B,a}	360.16^{B,b}	379.41^{xx}	367.21	–
	SD	18.59	18.75	12.16	18.67	19.86	
Potassium (mg/g)	$\bar{x}$	4.04	3.99	4.02	3.99	4.04	–
	SD	0.13	0.10	0.12	0.10	0.13	
Iron (µg/g)	$\bar{x}$	3.49	3.41	3.42	3.41	3.47	–
	SD	0.21	0.30	0.28	0.23	0.30	

The values designated by different letters are significantly different: $^{A,B}P \leq 0.01$ (pre-slaughter handling)
$^{a,b}P \leq 0.05$ (pre-slaughter handling); $^{xx}P \leq 0.01$ (sex); $^{x}P \leq 0.05$ (sex)
N–T – no transport
T–100 – transport to a distance of 100 km
T–200 – transport to a distance of 200 km

respect of the two other groups (N–T – 101.81 µg/g and T–200 – 103.27 µg/g).

The females were characterized by a higher ($P \leq 0.05$) content of Ca (104.66 µg/g) and K (202.88 µg per g) in blood serum as compared to the males (101.17 µg/g and 192.77 µg/g, respectively). In turn, the level of Mg turned out to be higher in males (22.63 µg/g, $P \leq 0.05$) than in the females (21.99 µg/g).

Highly significant correlations were demonstrated in serum levels of potassium (Figure 1) and iron (Figure 2). They were due to a higher content of those elements in males transported to a distance of 100 km (potassium – 210.6 µg/g, iron – 1.41 µg per g) as compared to the non-transported ones (potassium – 178.8 µg/g, iron – 1.20 µg/g) and those transported to a distance of 200 km (potassium – 188.9 µg/g, iron – 1.08 µg/g).

The content of sodium (Na) in breast muscle (Table 3) was observed to decrease along with an increasing distance of transport, and thus along with continually growing stress: N–T – 387.87 µg/g; T–100 – 371.89 µg/g, and T–200 – 360.16 µg/g. In addition, breast muscles of the males were characterised by a higher level of Na (379.41 µg/g, $P \leq 0.01$) as compared to the females (367.21 µg/g). In the case of Mg, its level in breast muscle was higher in the chickens transported to a distance of 200 km (313.05 µg/g, $P \leq 0.05$), in respect of chickens transported to a distance of 100 km (303.22 µg/g) and negligibly higher than in the non-transported birds (307.32 µg/g). The content of phosphorus (P) in the breast muscle of females was higher (2.48 mg/g, $P \leq 0.05$) as compared to that of the males (2.44 mg per g).

The reported study demonstrated an upward tendency in serum levels of elements in chickens transported to a distance of 100 km, which was then followed by their decrease. In breast muscle that tendency turned out to be opposite. Such changes in the levels of macro- and microelements in blood serum and breast muscle may be due to losses of water from the body during transport in the summer period. Water losses likely resulted from increased temperature and relative humidity in the containers used for bird transportation (Kettlewell, 1989; Mitchell and Kettlewell, 1998; Wójcik, 2007). This, in turn, evokes an enhanced activity of the thermoregulatory system counteracting the overheating of birds which, by increasing heat release by evaporation from the respiratory airways, removes water from their bodies. During the transport of poultry, when watering the birds or supplementation of their water losses are impossible, dehydration of their bodies is likely to occur, which in turn may lead to an elevated content of solid elements of blood and dry matter of muscles and, consequently, increased levels of macro- and microelements. The diminishing serum levels of minerals along with the extension of the transport distance to 200 km may indicate the utilization of macro-and microelements for the organism's defence against increasing stress (Truchlinski et al., 2007).

CONCLUSIONS

Summarizing the experiment, it may be concluded that pre-slaughter handling affects the content of macro- and microelements in both blood serum and breast muscle of chicken broilers. An upward tendency was observed in their level after transport to a distance of 100 km, which was then followed by their decline. An opposite tendency was observed in breast muscle. In respect of the sex of birds, levels of macro- and microelements were found to differ between males and females, both in their blood serum and breast muscle.

REFERENCES

Bláhová J., Dobšíková R., Straková E., Suchý P. (2007): Effect of low environmental temperature on performance and blood system in broiler chickens (*Gallus domesticus*). Acta Veterinaria Brno, 76, S17–S23.

Broom D.M. (1990): Effects of handling and transport on laying hens. World's Poultry Science Journal, 46, 48–50.

Duncan I.J.H. (1989): The assessment of welfare during the handling and transport of broilers. In: Proc. 3rd European Symposium on Poultry Welfare. Tours, France, 93–107.

Gergely A., Kontraszti M., Herman A., Acs T., Gundel J., Palfy T., Mihok S., Lugasi A. (2006): Microelements in muscle tissues of turkeys kept on intensive and extensive farming systems. In: Procs. Trace Elements in the Food Chain, Budapest, Hungary, 436–440.

Kettlewell P.J. (1989): Physiological aspects of broiler transportation. World's Poultry Science Journal, 45, 219–227.

Mitchell M.A., Kettlewell P.J. (1998): Physiological stress and welfare of broiler chickens in transit: solutions not problems! Poultry Science, 77, 1803–1814.

Mowat D.N. (1994): Organic chromium: a new nutrient for stressed animals. In: Biotechnology in the Feed Industry. Proc. Alltech's 10th Annual Symposium. Nottingham University Press, UK, 275–282.

Niess E., Hovenjurgen M., Pfeffer E. (2005): Whole body concentrations of major minerals and of some trace elements in 3, 5 and 6 weeks old broiler chicks. Archiv fur Geflugelkunde, 69, 16–22.

Orowicz W., Brzezinska M., Gardzielewska J., Jakubowska M., Zielinska A. (2004): Content of some minerals in the blood of broiler chickens. Journal of Elementology, 9, 461–467.

Podgorski W., Trawinska B., Polonis A. (2001): Interdependence among some element contents in broiler-hen tissues. Folia Universitatis Agriculturae Stetinensis, 224 Zootechnica, 145–150.

Sembartowicz I., Makarski B., Cendrowska M., Truchlinski J. (2003): Influence of copper Bioplex, wheat bran and herbs on magnesium, calcium and vitamin F content in breast muscles of turkey hens. Annales Universitatis Mariae Curie-Sklodowska, Sectio EE, XXI, Poland, 68, 113–119.

Shelton J.L., Southern L.L. (2006): Effects of phytase addition with or without a trace mineral premix on growth performance, bone response variables, and tissue mineral concentrations in commercial broilers. Journal of Applied Poultry Research, 15, 94–102.

Siegel H.S. (1995): Stress, strains and resistance. British Poultry Science, 36, 3–22.

Suchý P., Jelínek P., Straková E., Hucl J. (2002): Chemical composition of muscles of hybrid broiler chickens during prolonged feeding. Czech Journal of Animal Science, 47, 511–518.

Truchlinski J., Ognik K., Sembratowicz I. (2007): Influence of prolonged and interrupted stress from crowding and cooling of turkey-hens on anti-oxidation indices of the blood. Medycyna Weterynaryjna, 63, 95–98.

Večerek V., Grbalová S., Voslařová E., Janáčková B., Malena M. (2006): Effects of travel distance and the season: of the year on death rates of broilers transported to poultry processing plants. Poultry Science, 85, 1881–1884.

Večerek V., Straková E., Suchý P., Voslarová E. (2002): Influence of high environmental temperature on production and haematological and biochemical indexes in broiler chickens. Czech Journal of Animal Science, 47, 176–182.

Warriss P.D., Pagazaurtundua A., Brown S.N. (2005): Relationship between maximum daily temperature and mortality of broiler chickens during transport and lairage. British Poultry Science, 46, 647–651.

Weeks C.A., Nicol C. (2000): Poultry handling and transport. In: Livestock handling and transport. CAB International, UK, 363–384.

Whiteside P., Miner B. (1984): Pye Unicam Atomic Absorption Data Book. Pye Unicam LTD. Cambridge, UK.

Wojcik A. (2007): The effect of the administration of chromium or herbs to broiler chickens on reducing the unfavorable effects of pre-slaughter handling. Dissertations and monographs. Wyd. UWM. Olsztyn, Poland, 125, 1–110.

Zegarska Z. (2000): Ćwiczenia z analizy żywności (pod red.). Wyd. UWM Olsztyn, Poland.

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