

Lipid peroxidation as the additional indicator of transport stress in broilers

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Abstract

Stressful conditions (transport, temperature fluctuations, infections and excessive crowding) lead to dysfunction of immunological mechanisms in birds. They influence the lipid balance what manifests itself through the increase of the malonodialdehyde (MDA) level, reacting with the 2-thiobarbituric acid (TBA), what is an indicator of lipid peroxidation. In the case of birds, the classical indicator of the stressful reaction is the heterophile/lymphocyte ratio (H/L), which changes depending on the stress intensity.

The present study defined the influence of road transport on lipid peroxidation process as the additional indicator of stress in broilers. The investigation was conducted on 10 broilers Ross 308 (39 days old), which were transported on the distance of 70 km. The birds were clinically healthy, free of parasites and free of *Salmonella spp.* infection in the beginning of the experiment. The determination of the TBA-reactive substances (TBARS) in serum was conducted according to the method proposed by Ledwożyw et al. (1986).

The analysis of the results with a Student t-test showed the statistically significant difference ($P \leq 0.05$) in the TBA-reactive substances level before and directly after the birds transportation.

Key words: transport stress, chicken, lipid peroxidation, TBARS.

Introduction

The creation of highly reactive toxic oxygen forms and the mechanisms of their neutralization via either enzymatic or non-enzymatic way determine the balance in the organisms. Disturbance of this balance leads to pathological changes. Stressful situations (transport, temperature fluctuations, infections and excessive crowding) lead to dysfunction's of immunological mechanism. They influence the lipid balance what manifests itself with the growth of malonodialdehyde (MDA) level, reacting with the 2-thiobarbituric acid (TBA), which is the indicator of lipid per-

oxidation process (Balogh et al. 2001). In the case of birds, the basic indicator of the stress reaction is the heterophile:lymphocyte ratio (H:L), which changes depending on the stress intensity (Gross and Siegel 1983, Mills et al. 1993, Hester et al. 1996, Collette et al. 2000, Puvadolpirod and Thaxton 2000, El-Iethey et al. 2001).

Many observations suggest that heat and transport stress induce lipid peroxidation in animal tissue and that this results in production of free radicals (Sahin et al. 2002). Oxidative stress in the body occurs when the production of free radicals overwhelms the antioxidant defense systems and oxidative damage of cells

is the result. In recent years reduction in the level of plasma antioxidants such as vitamin E, as well as increase in oxidized glutathione and products of lipid peroxidations, have been used as biomarkers of oxidative injury (Chew 1996). These changes can provide insights into pathophysiology and clinical situation as they may be useful to assess the severity of the injury.

Highly reactive free radicals are generated during the normal cellular metabolism and from the ingestion/inhalation of environmental pollutants and drugs. These free radicals, when allowed to accumulate, are capable of destroying the integrity of cellular membranes, enzymes and nuclear DNA.

The aim of this study was to define the influence of the road transport (70 km during two hours) on lipid peroxidation as the additional indicator of stress in broilers.

Materials and Methods

The investigation was carried out on 10 chicken broilers Ross 308 (39 days old), which were transported over 70 km distance during two hours. The experiment was conducted in April and the ambient temperature was 12°C. The birds were clinically healthy, free of parasites and free of *Salmonella spp.* infection. They were kept in separate cages during the transportation. The blood samples from the wing vein were taken for examination before and directly after the transportation. Blood samples were centrifuged and the serum stored at -20°C until analysis.

The serum concentrations of lipid peroxidation products obtained before and after transportation were evaluated by measuring the TBA-reactive substances concentrations according to the method proposed by Ledwożyw et al. (1986). The absorbance of the butanol layer was measured at 532 nm (Ultrospec 2000, Pharmacia, Sweden). The result were calculated using a standard curve prepared with different dilutions of malondialdehyde and expressed in $\mu\text{mol}/\text{protein}$ content (Kankofer 2000).

Total protein concentration in examined sera were measured with a biuret method (Cormay Total protein 60) kit using the LP 400 photometer (Dr Bruno Lange, GmbH) with filter OD = 546 nm.

The means of the measured parameters were calculated and compared using Student's t-test and analysis of correlation (Statistica 5. 0.).

Results

The analysis of the results with the Student t-test showed the statistically significant difference ($P \leq 0.05$) in the levels of the TBA-reactive substances before and directly after the birds transportation (Table 1). TBA-reactive substance levels were statisti-

cally significantly higher ($P \leq 0.05$) after transporting in all examined birds (0.117 ± 0.023 vs. 0.092 ± 0.028). In single animals (bird nr 1, 6, 9) significantly higher difference were observed between TBA-reactive substances concentrations in sera obtained after and before transport ($0.044 \mu\text{mol}/\text{protein}$, $0.048 \mu\text{mol}/\text{protein}$, $0.052 \mu\text{mol}/\text{protein}$) then in the remaining sera.

Table 1. TBA-reactive substances (TBARS) concentrations ($\mu\text{mol}/\text{protein}$) in sera obtained before and after the transportation from broilers.

Number of broiler	TBARS $\mu\text{mol}/\text{protein}$	
	before transport	after transport
1.	0.054	0.098
2.	0.063	0.098
3.	0.084	0.090
4.	0.089	0.112
5.	0.103	0.106
6.	0.068	0.116
7.	0.091	0.129
8.	0.106	0.107
9.	0.107	0.159
10.	0.150	0.150
Mean	0.092	0.117*
SD	0.0276	0.0228

* - significance of differences at $P \leq 0.05$ between groups

The statistical analysis showed the significant difference ($P \leq 0.05$) in the total protein level (g/l) in sera obtained before and after the transportation (Fig. 1). The highest results were observed in bird nr 2 (40.7 g/l) and nr 9 (40.3 g/l) before transport and in bird nr 2 (37.1 g/l) after transport. The mean protein level was 33.79 g/l before transportation and 29.05 g/l after transportation.

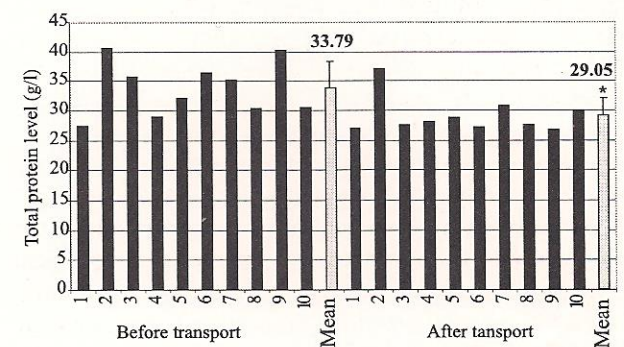


Fig. 1. Total protein level (g/l) (Mean \pm SD) in sera obtained before and after the transportation from broilers.

* - significance of differences at $P \leq 0.05$ between groups

Discussion

Lipid peroxidation processes may be detected by the determination of their intermediates and metabolites. The most common assays are based on the measuring of the level of TBA-reactive substances, conjugated dienes hydroperoxides.

Many studies showed a correlation between stress reaction and increase of lipid peroxidation activity in plasma and serum. The increase of TBARS level observed in serum is one of the major indicators of this process (Scarpellini et al. 1994, Matsumoto et al. 1999).

The analysis of the increase of TBA-reactive substances in examined sera showed the significant role of transport stress on lipid peroxidation. Results obtained in our experiment are in major part similar to results obtained by other authors, who evaluated the influence of different stress factors on TBARS level (Zhang and Piantadosi 1991, Matsumoto et al. 1999). These observations suggest that transport stress induce lipid peroxidation process in animal tissues. Probably the physical effort during transport, which is connected with standing on the platform, keeping the balance and lack of the possibility to change position can influence the metabolic processes in muscles. The presence of these processes was shown as an effect of physical effort in humans and animals during exercises (Ji et al. 1998, Kanter 1998).

A reduction in habitual levels of physical activity has been shown to lead to impairment of immune responses to infections and stress. Oxidative stress may occur during exhausting physical exercises as a result of acute increase of oxygen metabolism. Either maximal or supramaximal exercises cause sudden excess of oxygen free radical production in skeletal muscles which can cause cell damage. The classical example of these phenomena are changes in skeletal or cardiac muscle, where prolonged and exhaustive exercise may cause damage of sarcoplasmic membranes, impairment of muscle contractility, disturbance of myofibril structure and biochemical changes which include elevation of blood creatine kinase and lactate dehydrogenase. Prolonged and exhaustive exercise effects the number of mitochondria in working muscle groups as well as increases the activity of enzymes involved in oxidative phosphorylation in the respiratory chain. Prolonged physical activity also influences DNA in the nuclei of working myocytes. It is emphasized that moderate, submaximal physical training is beneficial, protecting skeletal muscles from free radical damage by increasing the SOD-1, Mn-SOD, GPx-SH and catalase activity.

Reactive oxygen species, although unavoidable intermediates, may have positive and negative effects on tissues and cells. There are some evidences that imbalance between production and neutralization of re-

active oxygen species may lead to peroxidative changes in cells and disturbances in their metabolism.

As a result of stress some immune cells migrate to the skin of the ear and the stressed animal become better prepared for anti-inflammatory response. However, the duration or intensity of the stress can also affect the viability and reactivity of the immune cells. When the stress is chronic or too severe, released stress hormones may ultimately cause destruction of leukocytes or the immune tissues themselves, instead of enhancing the overall response (Brinkmann and Kristofic 1995). For example, chronic restraint stress in mice causes thymic involution and T-lymphocyte apoptosis (Tarcic et al. 1998).

The birds before transport had significantly higher total protein levels. Protein synthesis during heat or transport stress is directed towards cell preservation (Lindquist 1986). Many proteins reduced in the time of transport stress influence.

In conclusion, this study shows that short road transport in broilers induces changes in the level of TBA-reactive substances (TBARS) and total protein levels as a response to stress. Stress in birds, as well as in other animals, involves a cascade of physiological adaptative responses. Stress may be considered as a possible condition through which different risk factors may exert effect on the development of this abnormal behavior in broilers.

Results of the present revealed that the monitoring of lipid peroxidation may be used as the additional indicator of transport stress in chicken.

References

- Balogh N, Gaal T, Husveth F, Vajdovich P (2001) Rate of lipid peroxidation in brain and liver tissues and the total antioxidant status of blood plasma in developing chicks. *Acta Vet Hung* 49: 197-202.
- Brinkmann V, Kristofic C (1995) Regulation by corticosteroids of the Th1 and Th2 cytokine production in human CD4+ effector T cells generated from CD45RO- and CD45RO+ subsets. *J Immunol* 155: 3322-3328.
- Chew BP (1996) Importance of antioxidant vitamins in immunity and health in animals. *Anim Feed Sci Technol* 59: 103-114.
- Collette JC, Millam JR, Klasing KC, Wakenell PC (2000) Neonatal handling of Amazon parrots alters the stress response and immune function. *Appl Anim Behav Sci* 66: 335-349.
- El-Iethy H, Jungi TW, Huber-Eicher B (2001) Effects of feeding corticosterone and housing conditions on feather pecking in laying hens (*Gallus gallus domesticus*). *Physiol Behav* 73: 243-251.
- Gross WB, Siegel HS (1983) Evaluation of the heterophil/lymphocyte ratio as a measure of stress in chickens. *Avian Dis* 27: 972-979.
- Hester PY, Muir WM, Craig JV, Albright JL (1996) Group selection for adaptation to multiple-hen cages: hematology and adrenal function. *Poult Sci* 75: 1295-1307.

- Ji LL, Leeuwenburgh C, Gore M, Fiebig R, Hollander J, Bejma J (1998) Oxidative stress and aging. Role of exercise and its influences on antioxidant systems. *Ann N Y Acad Sci* 854: 102-117.
- Kankofer M (2000) Antioxidative defence mechanisms in bovine placenta and their importance for placental release. *Reprod Dom Anim* 35: 229-233.
- Kanter M (1998) Free radicals, exercise and antioxidant supplementation. *Proc Nutr Soc* 1: 9-13.
- Ledwożyw A, Michalak J, Stępień A, Kądziołka A (1986) The relationship between plasma triglycerides, cholesterol, total lipids and lipid peroxidation products during human arteriosclerosis. *Clin Chim Acta* 155: 275-284.
- Lindquist S (1986) The heat shock response. *Ann Rev Biochem* 55: 1151-1191.
- Matsumoto K, Yobimoto K, Thi Thu Huong N, Abdel-Fattah M, Van Hien T, Watanabe H (1999) Psychological stress-induced enhancement of brain lipid peroxidation via nitric oxide system and its modulation by anxiolytic and anxiogenic drugs in mice. *Brain Res* 893: 74-84.
- Mills AD, Jones RB, Williams JB (1993) Responses to isolation in Japanese quail genetically selected for high or low sociality. *Physiol Behav* 53: 183-189.
- Puvadolpirod S, Thaxton JP (2000) Model of physiological stress in chickens 1. Response parameters. *Poult Sci* 79: 363-369.
- Sahin K, Sahin N, Sari M, Gursu MF (2002) Effects of vitamins E and A supplementation on lipid peroxidation and concentration of some mineral in broilers reared under heat stress (32°C). *Nutr Res* 22: 723-731.
- Scarpellini F, Sbracia M, Scarpellini L (1994) Psychological stress and lipoperoxidation in miscarriage. *Ann N Y Acad Sci* 709: 210-213.
- Tarcic N, Ovadia H, Weiss DW, Weidenfeld J (1998) Restraint stress-induced thymic involution and cell apoptosis are dependent on endogenous glucocorticoids. *J Neuroimmunol* 82: 40-46.
- Zhang J, Piantadosi CA (1991) Mitochondrial oxidative stress after carbon monoxide hypoxia in the rat brain. *J Clin Invest* 90: 1193-1199.