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EFFECT OF WATER SUPPLEMENTATION WITH HERBAL EXTRACTS ON BROILER CHICKEN WELFARE*

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Abstract

This study investigated the effect of adding extracts from selected herbs to water on alleviation of broiler stress associated with intensive production, and thus on improvement of welfare. In experimental groups (II, III and IV), alcoholic extracts from chamomile inflorescence (*Matricaria chamomilla* L.), lemon balm (*Melissa officinalis* L.) or from St John's wort (*Hypericum perforatum* L.), respectively, were added to water drinkers (2 ml · l^{-1} water) from 21 to 35 days of rearing for 5 h/day. Throughout the experiment, body weight, feed and water intake and number of dead birds were recorded once a week. At 21, 28, 35 and 42 days of rearing, blood was collected from 7 birds in each group to determine the levels of corticosterone, cholesterol, glucose, and the immunoglobulin complex. The response of birds to the herbal additives was positive. The herb extracts contributed to a decrease in cholesterol level and an increase in the level of the immunoglobulin complex in the blood. Supplementation of water with chamomile and St John's wort extracts contributed to an increase in body weight, while the extracts from lemon balm and St John's wort also had a positive effect on broiler survival. The results obtained indicate that out of the three herbs chosen for the experiment, St John's wort extract proved the most efficient in relieving the body's physiological response to stress, and thus in improving welfare.

Key words: broiler chickens, herb extracts, productivity, corticosterone, blood biochemical parameters

The basic principles of farm animal welfare, elaborated by the British Farm Animal Welfare Council, are found in the Code of Recommendations for the Welfare of Livestock, which states that the conditions under which animals are kept should guarantee freedom from hunger and thirst, freedom from discomfort, freedom from

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pain, injury or disease, freedom to express natural behaviour, and freedom from fear and distress (Sokołowicz et al., 2009). However, despite the many requirements set out in the legislation concerning the detailed conditions of animal housing and protection, intensive husbandry continues to cause considerable discomfort to birds, often giving rise to stress reactions that disturb the body's internal homeostasis. Therefore, many countries, notably the European Union, are making efforts to adopt less intensive farming practices that are more welfare-oriented and protect the environment.

One of the tools to evaluate welfare levels in farm animals is to measure their stress responses (Buijs et al., 2009). Puvadolpirod and Thaxton (2000) report that the most popular indicator of avian stress is an increase in blood corticosterone level. According to the same authors, the body's physiological response to stress is also reflected in increased plasma cholesterol and glucose levels. Compromised welfare also has many negative consequences for production and makes birds less resistant, which contributes to a number of diseases (Skomorucha et al., 2004; Campo et al., 2005). As a result, the search continues to find ways of alleviating stress as well as the emotional and somatic responses to large-flock management.

In recent years, various feed additives have been increasingly used to improve animal feeding efficiency, health status, and body condition. These include herbs and herbal components containing beneficial active substances such as volatile oils, tannins, glucosides, flavonoids, terpenes, mucilages and organic acids, which show anti-stress, anti-bacterial, anti-viral, anti-fungal and immunostimulatory action, encourage the secretion of digestive enzymes, thus improving the appetite of animals, and maintain the body's physiological balance (Bölükbaşı et al., 2008; Nasir and Grashorn, 2010; Wallace et al., 2010).

Bölükbaşı et al. (2008) demonstrated that thyme, sage and rosemary oils added to laying hen feeds improved feed conversion and increased egg weight. Kwiecień et al. (2006) observed a positive effect of a 2% dietary herb supplement on the body weight and liver weight of broiler chickens. Meanwhile, Hertrampf (2001) reported a positive effect of adding oregano extract to water on the production results of broiler chickens.

Herbs are also natural immunostimulants (Roth-Maier et al., 2005; Nasir and Grashorn, 2010). A positive effect on the health status of birds is also produced by hypocholesterolemic herbs. Volatile oils found in plants can inhibit the activity of the liver enzyme (HMG-CoA reductase) that regulates the amount of synthesized cholesterol and thus reduce its blood levels (Bölükbaşı et al., 2008).

Herbs are also thought to reduce the body's susceptibility to stress. Essential oils found in chamomile, lemon balm and St John's wort have sedative and anti-stress effects. However, there are no studies available in the literature on the use of these herbs to reduce stress in broiler chickens.

Therefore, the aim of the present study was to determine the effect of water supplementation with herb extracts from chamomile (*Matricaria chamomilla* L.), lemon balm (*Melissa officinalis* L.) and St John's wort (*Hypericum perforatum* L.) on alleviating stress associated with intensive production in broiler chickens, and thus on improving their welfare.

Material and methods

The experiment was carried out at a poultry farm in Aleksandrowice on 650 Ross 308 broiler chickens from the Poultry Hatchery in Łężkowice. On the first day of life, after weighing and tagging, chicks were allocated to four experimental groups, each having three replicates.

In group I (control), broiler chickens received drinking water without herb extracts throughout rearing. In groups II, III and IV, from 21 to 35 days of rearing for 5 h/day (from 0800 to 1300 h), water for drinking was supplemented with alcoholic extracts respectively from chamomile inflorescence (*Matricaria chamomilla* L.), lemon balm (*Melissa officinalis* L.) and St John's wort (*Hypericum perforatum* L.) at 2 ml · l^{-1} water.

Birds were kept for 42 days on litter at a stocking density of 12 chickens/m^2, which did not exceed 33 kg/m^2 (Regulation of the Minister of Agriculture and Rural Development, Official Journal 2010.56.344). All the groups had standardized environmental (temperature, air humidity, light programme) and feeding conditions.

Chickens received *ad libitum* concentrate-based starter diet until 3 weeks, grower diet from 4 to 5 weeks, and finisher diet at 6 weeks of age (Table 1). Throughout the experiment, birds were allowed free access to waterers.

Table 1. Ingredient composition (%) and nutritive value of starter, grower and finisher diets

Item	Diet	
	Starter (1–21 days)	Grower-Finisher (22–42 days)
Maize	55.35	47.21
Soybean meal	37.5	33.75
Wheat	-	10.00
Rapeseed oil	2.90	4.80
Ground limestone	1.10	1.15
Dicalcium phosphate	2.10	2.00
Sodium chloride	0.30	0.30
Vitamin-mineral premix[1,2,3]	0.50	0.50
L-Lysine	0.03	0.09
DL-Methionine	0.22	0.20
Nutritive value per kg		
Crude protein (%)	22.00	20.5
Metabolisable energy (MJ)	12.5	13.0
Lysine (g)	12.0	11.5
Methionine (g)	5.50	5.20
Calcium (g)	9.50	9.30
Phosphorus (g)	4.30	4.10

[1]Supplements per kg starter diet: vit. A 13 500 IU; vit. D_3 3 600 IU; vit. E 45 mg; vit. B_1 3.25 mg; vit B_2 7.5 mg; vit. B_6 5 mg; vit. B_{12} 32.5 mcg; vit. K_3 3 mg; biotin 150 mcg; nicotinic acid 45 mg; calcium pantothenate 15 mg; folic acid 1.5 mg; choline chloride 447.6 mg; Mn 100 mg; Cu 1.75 mg; Fe 76.5 mg; Se 0.275 mg; I 1 mg; Zn 75 mg; Co 0.4 mg; Seldox HM (antioxidant) 25 mg; Clinacox 0.5 % 1 mg.

[2]Supplements per kg grower diet: vit. A 12 000 IU; vit. D_3 3 250 IU; vit. E 40 mg; vit. B_1 2 mg; vit. B_2 7.3 mg; vit. B_6 4,25 mg; vit. B_{12} 30 mcg; vit. K_3 2.25 mg; biotin 100 mcg; nicotinic acid 40 mg; calcium pantothenate 12 mg; folic acid 1.0 mg; choline chloride 335.7 mg; Mn 100 mg; Cu 1.5 mg; Fe 65 mg; Se 0.25 mg; I 0.9 mg; Zn 65 mg; Co 0.4 mg; Seldox HM (antioxidant) 25 mg; Coxidin 200 100 mg.

[3]Supplements per kg finisher diet: vit. A 10 500 IU; vit. D_3 2 500 IU; vit. E 40 mg; vit. B_1 1.5 mg; vit B_2 5.5 mg; vit. B_6 3.25 mg; vit. B_{12} 25 mcg; vit. K_3 2 mg; biotin 100 mcg; nicotinic acid 35 mg; calcium pantothenate 12 mg; folic acid 1.0 mg; choline chloride 298.4 mg; Mn 80 mg; Cu 8 mg; Fe 60 mg; Se 0.2 mg; J 0.8 mg; Zn 50 mg; Co 0.4 mg; Seldox HM (antioxidant) 25 mg.

Alcoholic herb extracts were produced by a professional herbal company and were certified to conform with quality standards (ZN-08/NX/519, ZN-07/NX/525, ZN-08/NX/545).

During the experiment, body weight, feed and water consumption, and number of dead birds were recorded at weekly intervals. At 21, 28, 35 and 42 days of rearing, blood was collected from 7 birds in each group to determine the levels of corticosterone, cholesterol, glucose, and the immunoglobulin complex. Corticosterone was determined using immunoassay (Diagnostic System Laboratories, USA) and Sirio S reader. The concentration of blood biochemical parameters was analysed with Epol 20 photometer using reagent kits and Alpha Diagnostics procedures. The concentration of immunoglobulins was determined by the method of Lowry as modified by Ślebodziński et al. (1982).

The results were analysed statistically by analysis of variance and significant differences were estimated with Duncan's test. The statistical calculations were performed with Statgraphics plus 6.0.

Results

The production results of broiler chicks are shown in Table 2. At 35 days of rearing, broilers from groups II and IV were characterized by higher body weight compared to control birds ($P \leq 0.05$). No statistically significant differences were found in feed conversion (kg/kg gain) and water use per kg feed. Chickens from groups III and IV exhibited a 100% survival rate in the grower period.

Table 2. Production results of broiler chickens

Item	Days of rearing	Group				SEM
		I Control	II Chamomile	III Lemon balm	IV St John's wort	
Body weight (g)	21	821.43	851.43	845.94	876.80	20.07
	28	1362.09	1425.71	1416.53	1424.00	30.77
	35	1949.14 a	2089.39 b	2034.65 ab	2072.80 b	41.02
	42	2447.81	2597.14	2501.10	2578.70	54.30
Feed conversion ratio (kg/kg gain)	1–21	1.36	1.33	1.33	1.35	0.05
	22–42	2.61	2.72	2.73	2.63	0.13
	1–42	2.06	2.09	2.09	2.05	0.05
Water use (l) per kg feed	1–21	2.05	2.09	2.08	2.10	0.04
	22–42	2.27	2.19	2.24	2.22	0.03
	1–42	2.20	2.16	2.20	2.18	0.02
Mortality (%)	1–21	2.22	2.96	2.22	4.47	-
	22–42	1.48	1.48	0	0	-
	1–42	3.70	4.44	2.22	4.47	-

a, b – values in rows with different letters differ significantly ($P \leq 0.05$).

A highly significant difference in blood corticosterone level between the control group and group IV was noted on day 42 of the experiment, and a significant one between groups II and IV (Table 3).

Table 4 shows the level of blood biochemical parameters in broiler chickens. When analysing blood glucose levels, a statistically significant difference was found on day 42 of the experiment between groups II and IV. On day 35, blood cholesterol level was significantly lower in chickens from experimental groups compared to control ones. On day 42 of rearing, blood cholesterol level was the lowest in chickens from group IV compared to the other groups ($P \leq 0.01$ and $P \leq 0.05$).

Table 3. Blood corticosterone levels in broiler chickens (ng · ml^{-1})

Days of rearing	Group				SEM
	I Control	II Chamomile	III Lemon balm	IV St John's wort	
21	12.24	16.57	16.04	14.57	2.37
28	10.08	11.23	10.01	11.04	1.32
35	7.36	10.10	11.23	14.92	1.59
42	12.11 A	10.65 a	10.27 AB	9.60 Bb	1.47

a, b – values in rows with different letters differ significantly ($P \leq 0.05$).
A, B – values in rows with different letters differ highly significantly ($P \leq 0.01$).

On day 28 of the experiment, the highest level of the immunoglobulin complex was noted in chickens from group III and the lowest in chickens from group I ($P \leq 0.01$). On day 35 of rearing, the level of the immunoglobulin complex was significantly higher in chickens from the group receiving St John's wort extract in water compared to control chickens.

Table 4. Level of biochemical blood parameters in broiler chickens

Item	Days of rearing	Group				SEM
		I Control	II Chamomile	III Lemon balm	IV St John's wort	
Glucose (mmol/l)	21	10.21	10.33	10.50	10.12	0.27
	28	11.07	10.72	10.42	10.76	0.28
	35	10.64	10.96	11.33	10.77	0.29
	42	10.22 ab	10.72 a	10.39 ab	9.87 b	0.26
Cholesterol (mmol/l)	21	2.74	2.91	2.70	2.98	0.11
	28	3.06	2.89	3.07	2.95	0.12
	35	3.55 a	2.94 b	2.95 b	2.92 b	0.19
	42	3.14 B	2.98 b	3.07 B	2.56 Aa	0.12
Immunoglobulin complex (g/l)	21	14.6	12.1	14.7	13.7	1.10
	28	10.7 Aa	13.5 bc	15.1 Bb	12.7 ac	0.80
	35	14.7 a	15.5 ab	16.1 ab	16.8 b	0.69
	42	16.1	14.6	14.3	15.5	0.65

a, b – values in rows with different letters differ significantly ($P \leq 0.05$).
A, B – values in rows with different letters differ highly significantly ($P \leq 0.01$).

Discussion

The literature reports that compromised welfare associated with intensive husbandry may negatively affect poultry production results (Sosnówka-Czajka et al., 2003; Skomorucha et al., 2004; Campo et al., 2005). According to Lee et al. (2003 a), herbs and herb extracts may alleviate the effects of stress associated with unfavourable rearing conditions. In our study, differences in the body weight of broiler chickens were found in the second week of herb supplementation in water. Compared to the control group, chickens from the experimental groups receiving chamomile extract and St John's wort were heavier by 140 and 124 g, respectively. On day 42 of the experiment, chickens from the experimental groups showed a tendency for greater body weight compared to chickens not supplemented with herb extracts. Many studies confirm a positive effect of adding herb preparations to poultry diets on their body weight (Bampidis et al., 2005; El-Bagir et al., 2006; Kwiecień et al., 2006; Mohammed and Abbas, 2009; Toghyani et al., 2010). According to Hippenstiel et al. (2011), this is related to the antibacterial effect of volatile oils that help to improve bacterial flora in the digestive tract, which increases the production of digestive enzymes and improves the quality of digestion. However, some publications report negative effects of the active substances in herbs on production results (Lee et al., 2003 b; Khalaji et al., 2011). A study by Schleicher et al. (1996) demonstrated that better effects are achieved when using a mixture of properly chosen herbs than when the same herbs are individually added to the diet. Brzóska et al. (2010) reports that feeding a herb mixture to chickens had no effect on their body weight, feed consumption and feed conversion. Likewise in our study, adding extracts from single herbs to water had no effect on feed conversion and water use by broiler chickens.

Schleicher et al. (1998) report that chamomile or dandelion added to the diet of broiler chickens reduced their mortality. In our study, we found a positive effect of lemon balm and St John's wort on broiler survival during the period when birds received water with extracts from these herbs.

The intensive husbandry usually gives rise to considerable stress, thus reducing welfare levels in birds (Bessei, 2005; Skomorucha et al., 2007). As a result of stress, the avian body undergoes many physiological changes, including increases in the plasma levels of corticosterone, glucose and cholesterol (Puvadolpirod and Thaxton, 2000). In our study, the extracts from chamomile, lemon balm and St John's wort, added to water from 21 to 35 days of rearing had no effect on lowering blood corticosterone levels in broiler chickens during this period. However, on the last day of the experiment, this hormone had a lower concentration in the blood of chickens which received water with St John's wort extract compared to the control group and the group receiving chamomile extract. As regards blood cholesterol content, it was lower in the blood of broiler chickens from the experimental groups compared to the control group on the last day on which water with individual herbs was supplemented. On day 42 of rearing, the lowest cholesterol level was found in the blood of broiler chickens receiving St John's wort extract. According to Majewska et al. (2007), plant and herb supplements added to poultry diets may reduce blood cholesterol levels and improve the health status of birds.

Bölükbaşı et al. (2007) noted a decrease in blood cholesterol and triglyceride levels in laying hens in response to adding thymol oil at 300 mg/kg of feed. Similarly, Bölükbaşı et al. (2008) found lower blood cholesterol and triglyceride levels in laying hens fed a diet with 200 mg/kg of thyme, sage and rosemary oils compared to the control group. Different results were obtained by Sarica et al. (2005), who found no changes in total cholesterol concentration in the blood of broiler chickens after using a diet with thyme (1 g/kg of feed). Likewise, Bampidis et al. (2005) observed no changes in the amount of blood cholesterol in female turkeys fed an oregano-supplemented diet.

Dorhoi et al. (2006) report that ethanol extracts from garlic (*Allium sativum*), liquorice (*Glycyrrhiza glabra*), plantain (*Plantago major*) and sea buckthorn (*Hippophae rhamnoides*) have a beneficial effect on cell-mediated immunity in layers. Nasir and Grashorn (2010) report that *Echinacea angustifolia* extract induces a rise in serum immunoglobulin concentration of hens, thus improving their health and reducing mortality. Likewise, a dietary *Echinacea purpurea* supplement improves the immune status of broiler chickens and hens (Roth-Maier et al., 2005). In our study, we found a positive effect of adding a chamomile extract and a lemon balm extract to water on the blood level of the immunoglobulin complex in broiler chickens in the fourth week of growth. During the next week of water and herb extract administration to chickens, the immunoglobulin complex was higher in the blood of chickens receiving St John's wort compared to control chickens.

In summary, during the period when broilers were given drinking water with chamomile, lemon balm and St John's wort extracts at 2 $ml \cdot l^{-1}$, their organism responded positively to these experimental factors. Herb extracts contributed to a decrease in cholesterol level and an increase in the immunoglobulin complex in the blood of experimental birds compared to the control group. The chamomile and St John's wort extracts increased the body weight of the birds, while the lemon balm and St John's wort extracts also had a positive effect on broiler survival.

The present results indicate that out of the three herbs chosen for the experiment, St John's wort extract proved the most efficient in relieving the body's physiological response to stress, and thus in improving welfare.

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Wpływ dodatku do wody ekstraktów ziołowych na dobrostan kurcząt brojlerów

SUMMARY

Badano wpływ dodatku ekstraktów z wybranych ziół do wody na złagodzenie stresu kurcząt brojlerów związanego z intensywnym chowem, a w konsekwencji na poprawę dobrostanu. W grupach doświadczalnych (II, III i IV) kurczęta brojlery otrzymywały do poideł z wodą od 21. do 35. dnia odchowu przez 5 h/dobę ekstrakt spirytusowy odpowiednio z: kwiatostanu rumianku pospolitego (*Matricaria chamomilla* L.), ziela melisy lekarskiej (*Melisa offcinalis* L.) lub ziela dziurawca zwyczajnego (*Hypericum perforatum* L.) w ilości 2 ml·l^{-1} wody. W czasie doświadczenia co tydzień kontrolowano masę ciała kurcząt brojlerów, zużycie paszy i wody oraz liczbę kurcząt padłych. W 21., 28., 35. oraz 42. dniu odchowu pobrano krew od 7 ptaków z grupy w celu oznaczenia poziomu: kortykosteronu, cholesterolu, glukozy oraz kompleksu immunoglobulinowego. Obserwowano pozytywną reakcję organizmów ptaków na czynniki doświadczalne. Ekstrakty z ziół wpłynęły na obniżenie poziomu cholesterolu oraz wzrost poziomu kompleksu immunoglobulinowego we krwi ptaków. Dodatek ekstraktu z rumianku pospolitego i dziurawca zwyczajnego wpłynął na wzrost masy ciała ptaków, a ekstrakt z melisy lekarskiej i dziurawca zwyczajnego miał także pozytywny wpływ na przeżywalność kurcząt brojlerów.

Wyniki z przeprowadzonych badań wskazują, że z trzech wybranych do doświadczenia ziół najbardziej skuteczny w łagodzeniu fizjologicznej reakcji organizmu kurcząt brojlerów na stres, a tym samym na poprawę dobrostanu, okazał się ekstrakt z dziurawca zwyczajnego