THE EFFECT OF ADDING HERBAL EXTRACTS TO DRINKING WATER ON BODY TEMPERATURE, LEVEL OF THYROID HORMONES AND H:L RATIO IN THE BLOOD OF BROILER CHICKENS EXPOSED TO ELEVATED AMBIENT TEMPERATURE*

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Abstract

The aim of the study was to determine the effect of supplementing drinking water with extracts from lemon balm, sage and nettle on body temperature, level of thyroid hormones and the heterophil to lymphocyte (H:L) ratio in the blood of broiler chickens exposed to elevated rearing temperature. One-day-old Ross 308 chicks were divided into four groups: group I (CON) and experimental groups II (LB), III (S) and IV (N), in which, from 22 to 42 days of rearing, drinkers were supplemented with lemon balm extract, sage extract or nettle extract (2 ml/l water), respectively. In addition, at 5 weeks of growth, all the groups were exposed to elevated ambient temperature (up to 30°C) for 5 days, after which the recommended thermal conditions were reinstated. During the study, mortality, rectal temperature and radiated temperature of the broilers were monitored. Blood was collected from 10 birds per group to determine the concentration of thyroid hormones (T_3 and T_4) and to make blood smears. The H:L ratio was determined based on the percentage of heterophils (H) and lymphocytes (L). The herbal extracts from lemon balm, sage and nettle, added to drinking water at 2 ml/l, reduced the blood level of the thyroid hormone (triiodothyronine) and rectal temperature in the experimental broilers during the initial period of thermal stress. Chickens receiving the nettle extract were also characterised by lower radiated temperature of the unfeathered body, a lower H:L ratio in the blood during the increase in ambient temperature, and the lowest mortality percentage. It can therefore be considered that the dietary supplementation of nettle, in the form and concentration used in this study, had the most favourable effect on the physiological status of the birds (body temperature, level of thyroid hormones and H:L ratio) exposed to elevated ambient temperatures (30°C), and thus on their welfare.

Key words: herbal extracts, elevated ambient temperature, physiological indicators, broiler chickens

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High ambient temperature is a major stressor for farm animals (Attia and Hassan, 2017; Abd El-Hack et al., 2020). Modern lines of broiler chickens are particularly sensitive to high air temperatures (Song et al., 2018; Attia et al., 2018 a; Cheng et al., 2019) because their rapid growth, high body weight and good feed conversion increase the amount of heat energy they produce (Giloh et al., 2012; Lara and Rostagno, 2013; Wang et al., 2020), which inhibits the body's adaptation to adverse thermal conditions (Attia et al., 2017 a). The thermal comfort zone for homeothermic animals is characterised by a range of environmental temperatures, within which animals have minimal and nearly constant energy expenditure for maintaining body temperature (Cândido et al., 2020). According to Olanrewaju et al. (2010), the body temperature of an adult chicken is 40.6 to 41.7°C, and the thermoneutral zone that allows chickens to maintain their body temperature is 18 to 24°C. When birds are exposed to temperatures above their thermoneutral zone, heat exchange between the bird and its environment is impeded, which is reflected in altered physiological and metabolic reactions such as: endocrine disruption (Jiang et al., 2020), electrolyte imbalance (Attia et al., 2011), elevated body temperature (Giloh et al., 2012; Skomorucha and Sosnówka-Czajka, 2017; Vermette et al., 2017), increased heterophil to lymphocyte ratio (Vermette et al., 2017), immunosuppression (Xu et al., 2018), oxidative stress (Song et al., 2018), behavioural changes (Olanrewaju et al., 2010; Lara and Rostagno, 2013) and compromised productivity and meat quality (Olanrewaju et al., 2010; Saeed et al., 2019). According to many authors, high ambient temperatures cause substantial economic losses due to reduced weight gains and increased mortality of the birds (Wang et al., 2018; Saeed et al., 2019; Jiang et al., 2020).

An important role in thermoregulation and nutrient metabolism is played by the thyroid hormones thyroxine (T_4) and triiodothyronine (T_3), the level of which is associated among others with ambient temperature (Giloh et al., 2012; Jiang et al., 2020). Regulation of the concentration of T_3 and T_4 in the body is essential for maintenance of body temperature via energy metabolism in homeothermic animals (Jiang et al., 2020). In general, birds exposed to elevated ambient temperatures were reported to show reduced blood T_3 levels (Sohail et al., 2010; Giloh et al., 2012; Mack et al., 2013; Skomorucha and Sosnówka-Czajka, 2017), and decreased (Sohail et al., 2010), increased (Giloh et al., 2012; Skomorucha and Sosnówka-Czajka, 2017) or unchanged T_4 levels (Mack et al., 2013).

The current ongoing global warming generates increasing losses in the poultry industry due to hyperthermia in broiler chickens. There is therefore a need for finding practical methods to alleviate the negative impacts of heat stress in birds. Many authors have reported positive effects of the dietary inclusion of vitamins, minerals, amino acids, prebiotics and probiotics on heat-stressed poultry (Kumar et al., 2017; Cheng et al., 2019). Increasing interest is devoted to all kinds of plant-derived preparations (phytobiotics), which are easily available, cheap, safe for use, and contain antioxidants that regulate the metabolism and performance of birds exposed to heat stress (Abd El-Hack et al., 2020). Attia and Al-Harthi (2015) and El-Shoukary et al. (2014) observed that supplementing 1% *Nigella sativa* seeds to the diets of broiler chickens exposed to elevated ambient temperatures, increased their daily weight gains, considerably reduced the number of panting birds, and decreased the

corticosterone and T_3 levels compared to the control group. In turn, Rehman et al. (2018) showed the dietary inclusion of 2% ginger led to considerable improvements in blood parameters and body weight gains of broiler chickens exposed to heat stress. The beneficial effects of herbal supplements on the body of broiler chickens reared under high ambient temperature were also reported by Ragab et al. (2013), Attia et al. (2017 b; 2018 b), Sadeghi and Moghaddam (2018), and Song et al. (2018).

Common and highly valued herbs include lemon balm, sage and nettle, which show various health-promoting benefits, and thanks to the presence of essential oils they have sedative and anti-stress effects. Furthermore, these plants show strong antioxidant properties (Poulios et al., 2020; Bonetti et al., 2016; Kasapidou et al., 2014), which makes them useful for reducing stress induced by high air temperature. However, the literature reports no information concerning the use of these herbs to alleviate the effects of heat stress on the avian body.

Therefore, the aim of the present study was to determine the effect of adding lemon balm, sage and nettle extracts to drinking water on the body temperature, level of thyroid hormones, and the heterophil to lymphocyte ratio (H:L) in the blood of broiler chickens exposed to high ambient temperature.

Material and methods

Experimental site

The experiment was conducted at a poultry farm of the National Research Institute of Animal Production in Aleksandrowice.

Birds and experimental design

The experimental material consisted of 640 day-old Ross 308 broiler chicks from the Poultry Hatchery in Łęzkowice. At one day of age, the chicks were weighed, identified with tags and assigned to four groups (160 birds per group), each having four subgroups (40 birds per subgroups). Group I was the control (CON), and in groups II (LB), III (S) and IV (N) water drinkers were supplemented from 22 to 42 days of age with liquid alcoholic extract from lemon balm (*Melissa officinalis* L.), sage (*Salvia officinalis* L.) and nettle (*Urtica dioica* L.), respectively, at 2 ml/l water (Skomorucha and Sosnowka-Czajka, 2013). The experimental herbs were chosen mainly for their content of antioxidant substances (essential oils, flavonoids, polyphenolic acids, β -carotenoids, chlorophyll (a and b), xanthophylls and vitamins C and E). Additionally, birds in all the four groups were exposed to high ambient temperature (30°C) from 30 to 35 days of age and normal thermal conditions (20–18°C) from 36 to 42 days of age. The birds were raised for 42 days on litter at a stocking density less than 33 kg/m² (Regulation of the Minister of Agriculture and Rural Development, Journal of Laws 2010 no. 56 item 344). The lighting schedule was 23L:1D for the first 7 days, and then 20L:4D from 8 to 38 days and 23L:1D from 39 to 42 days. The chickens were fed *ad libitum* starter (up to week 3), grower (weeks 4 to 5) and finisher diets (week 6) based on concentrates (Table 1). Feed was fed from feed hop-

pers. Drinking water supplemented with herbal extract was provided in bell drinkers. The water were manually replenished twice a day. Throughout rearing, the broilers had free access to the feed and water drinkers.

Ethanol herb extracts were produced by a professional herbal company and were certified to conform with in-house quality standard (ZN-16/NX/900, ZN-07/NX/523, ZN-11/NX/546).

The experiment was approved by the Second Local Ethics Committee for Animal Experiments in Kraków (no. 1205/2015).

Table 1. Ingredient composition (%) and nutritive value of the diets

Item	Diet	
	Starter ¹ 1–21 days	Grower ² , Finisher ³ 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	0.50	0.50
L-Lysine	0.03	0.09
DL-Methionine	0.22	0.20
Nutritive value per 1 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

¹Supplements per kg starter diet: vit. A 13 500 IU; vit. D₃ 3 600 IU; vit. E 45 mg; vit. B₁ 3.25 mg; vit. B₂ 7.5 mg; vit. B₆ 5 mg; vit. B₁₂ 32.5 mcg; vit. K₃ 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.

²Supplements per kg grower diet: vit. A 12 000 IU; vit. D₃ 3 250 IU; vit. E 40 mg; vit. B₁ 2 mg; vit. B₂ 7.3 mg; vit. B₆ 4.25 mg; vit. B₁₂ 30 mcg; vit. K₃ 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.

³Supplements per kg finisher diet: vit. A 10 500 IU; vit. D₃ 2 500 IU; vit. E 40 mg; vit. B₁ 1.5 mg; vit. B₂ 5.5 mg; vit. B₆ 3.25 mg; vit. B₁₂ 25 mcg; vit. K₃ 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; I 0.8 mg; Zn 50 mg; Co 0.4 mg; Seldox HM (antioxidant) 25 mg.

Data, sample collection and laboratory analyses

Bird mortality was monitored throughout the study. On days 31 and 35 (during exposure to high ambient temperature) and on day 40 of the experiment (five days after exposure to heat stress), blood was collected by decapitation from 10 birds per group, the concentration of thyroid hormones (T_3 and T_4) was determined, and blood smears were stained with a May-Grünwald Giemsa protocol. Stained leukocytes were counted under a Nikon YS 100 microscope by the differential count method. The percentage of heterophils (H) and lymphocytes (L) was used to calculate the H:L ratio. Thyroid hormones were determined in plasma by radioimmunoassay in an automatic gamma counter (LKB Wallace) using T_3 [^{125}I] (REF: RK-6CT1) and T_4 [^{125}I] (REF: RK-11CT1) RIA KIT (Institute of Isotopes Co., Ltd, Budapest, Hungary).

On the same dates (days 31, 35 and 40), rectal and radiation temperature of feathered (back) and nonfeathered parts (shanks) was measured in 10 birds per group. Rectal temperature was measured with a veterinary thermometer accurate to 0.1°C, and radiation temperature using a Cole-Parmer pyrometer. The pyrometer was placed 15 cm from the bird's skin.

Statistical analyses

The results were statistically analysed by one-way analysis of variance (ANOVA). The experimental unit was the individual bird. Significant differences for the means between the experimental groups were determined with Tukey's test. The effects were considered significant at a probability of $P < 0.05$ and $P < 0.01$. The statistical calculations were made using Statistica 12 software.

Results

Table 2 shows broiler mortality during rearing. Compared to the control birds, chickens from group N showed 0.84% and 1.67% lower mortality in the first and second rearing period, respectively. The lowest mortality during the whole rearing period was noted in group N compared to the other groups.

On day 35, when the ambient temperature was 10°C higher than recommended, the radiation temperature of feathered parts in chickens from group S differed from that of birds in groups LB and N at $P < 0.01$ (Table 3). Lower radiation temperature of nonfeathered parts on day 31 was characteristic of chickens from group N compared to the control group ($P < 0.05$). On day 35 of the study, radiation temperature of nonfeathered parts was noted in chickens from groups S and N in relation to the control birds ($P < 0.05$). Broiler chickens from the experimental groups exhibited lower rectal temperature on day 31 compared to the control birds at $P < 0.05$ (Table 3).

Thyroid hormone levels in the blood of the chickens are presented in Table 4. On day 31 of the study, T_3 concentration was highest in the control birds and lowest in the chickens from groups LB and N ($P < 0.01$). Differences in the T_3 level were also observed on the same day between groups CON and S, and between groups S and N ($P < 0.05$). The lowest T_4 level was noted in the blood of broilers from group

N compared to the other groups ($P<0.01$) on day 31. The difference in T_4 on that day also occurred between birds from groups LB and S ($P<0.05$). On day 35 of the study, the highest T_4 level was characteristic of chickens from group LB compared to those from groups S and N ($P<0.05$). On day 40, i.e. after exposure to high ambient temperature, the lowest T_4 concentration was observed in broilers from group N compared to birds from groups LB and S ($P<0.05$).

Table 2. Broiler mortality (%)

Days of rearing	Herbal extracts			
	CON	LB	S	N
1–21	1.67	3.33	2.0	0.830
22–42	2.50	1.67	3.00	0.830
1–42	4.17	5.00	5.00	1.67

CON – control – without herbal extract.

LB – lemon balm extract.

S – sage extract.

N – nettle extract.

Table 3. Body temperature (°C) of broiler chickens (mean, $n = 10$)

Days of rearing	Herbal extracts				Pooled SEM
	CON	LB	S	N	
Radiation temperature of feathered parts					
31	38.6	37.0	37.8	37.0	0.610
35	37.7 AB	38.7 B	36.6 A	38.8 B	0.450
40	29.5	30.2	31.3	30.1	0.700
Radiation temperature of unfeathered parts					
31	40.3 a	39.7 ab	39.8 ab	39.1 b	0.270
35	40.6 a	40.4 ab	40.0 b	40.1 b	0.210
40	37.1	37.5	36.4	36.7	0.660
Rectal temperature					
31	42.2 a	41.9 b	41.9 b	41.9 b	0.080
35	42.0	42.0	42.1	42.1	0.100
40	41.5	40.6	41.3	41.3	0.500

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

A, B – values in rows with different letters differ highly significantly ($P<0.01$).

Pooled SEM – Pooled standard error of mean.

CON – control – without herbal extract.

LB – lemon balm extract.

S – sage extract.

N – nettle extract.

On day 35, broiler chickens from group N showed a lower H:L ratio than the control group and group LB at $P<0.01$ and $P<0.05$, respectively (Table 5).

Table 4. Plasma levels of triiodothyronine (T_3) and thyroxine (T_4) in the blood of broiler chickens (mean, $n = 10$)

Days of rearing	Herbal extracts				Pooled SEM
	CON	LB	S	N	
T ₃ (nmol/l)					
31	5.00 Aa	3.80 B	4.10 b	3.44 Ba	0.260
35	4.80	4.50	4.30	3.80	0.280
40	5.00	4.10	4.40	3.60	0.470
T ₄ (nmol/l)					
31	56.0 A	55.0 Aa	63.6 Ab	75.8 B	2.80
35	69.3 ab	73.8 a	60.3 b	62.1 b	3.68
40	63.8 ab	72.8 a	72.5 a	59.9 b	3.61

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

A, B – values in rows with different letters differ highly significantly ($P < 0.01$).

Pooled SEM – Pooled standard error of mean.

CON – control – without herbal extract.

LB – lemon balm extract.

S – sage extract.

N – nettle extract.

Table 5. H: L ratio in the blood of broiler chickens (mean, $n = 10$)

Days of rearing	Herbal extracts				Pooled SEM
	CON	LB	S	N	
31	0.800	0.700	0.600	0.600	0.130
35	1.06 A	0.810 a	0.710 ABab	0.420 Bb	0.130
40	0.720	0.510	0.690	0.510	0.110

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

A, B – values in rows with different letters differ highly significantly ($P < 0.01$).

Pooled SEM – Pooled standard error of mean.

CON – control – without herbal extract.

LB – lemon balm extract.

S – sage extract.

N – nettle extract.

Discussion

One of the body's responses to high ambient temperature is an increase in the body's radiation temperature (Skomorucha and Sosnówka-Czajka, 2017; Cândido et al., 2020). Vasodilation increases blood flow to the skin, especially to the nonfeathered areas, which leads to increased heat dissipation from the body via radiation, convection, and conduction (Giloh et al., 2012). Exposure of poultry to high ambient temperature may also increase rectal temperature (Giloh et al., 2012; Skomorucha and Sosnówka-Czajka, 2017; Attia and Hassan, 2017; Cândido et al., 2020), which, in turn, contributes to deaths in birds unable to regulate the body temperature during heat stress (Berrong and Washburn, 1998; Attia et al., 2017 a;

2018 a). In our study, broiler chickens from the groups supplemented with sage water extract and nettle showed lower radiation temperature of nonfeathered parts on day 31, and on days 31 and 35 of the study, respectively, compared to the control group. The experimental groups also showed lower rectal temperature on day 31 of rearing. According to Morovat et al. (2016), supplemental antioxidants in broiler diets slow down basal metabolism and cause a considerable reduction in the body temperature. The authors noted lower rectal temperature in broiler chickens supplemented with milk thistle extract and exposed to high ambient temperature compared to the control group. Likewise, Ragab et al. (2013) found that inclusion of fennel seeds at 1 or 2% of the diet of broilers exposed to heat stress conditions caused a considerable reduction in body temperature and bird mortality. Similar results were reported by Al-Sagan et al. (2020). However, in our study, the next measurement of rectal temperature on day 5 of the exposure to high ambient temperature (day 35) showed no differences between birds supplemented with herbal extracts in drinking water and the control birds. This may indicate that the experimental herbs mitigated the negative effects of high ambient temperature on the body only during the initial period. In turn, Baghban et al. (2016) observed no changes in rectal temperature of broiler chickens exposed to heat stress (32°C) for 8 hours daily in the second rearing period and receiving a diet supplemented with 0.5% turmeric or 0.5% cinnamon compared to the unsupplemented group. In our study, we found a positive effect of lemon balm and nettle on the survival of broilers during the period of supplementation (from days 22 to 42) compared to the control group.

Divi and Doerge (1996) report that flavonoids, which are strong antioxidants found in plants, inhibit thyroid peroxidase, the enzyme catalyzing biosynthesis of thyroid hormones. A decrease in plasma triiodothyronine improves the ability of birds to reduce heat production at high ambient temperatures (Yahav and McMurtry, 2001). Our results showed that during the initial period of high ambient temperature, broiler chickens supplemented with lemon balm, sage and nettle extracts in drinking water were characterised by lower blood T_3 levels compared to the unsupplemented group, which was reflected in the lower rectal temperature of these birds. Similarly, El-Shoukary et al. (2014) obtained a lower blood T_3 level in chickens supplemented with 1% black cumin seeds or 2% coriander seeds and exposed to heat stress (34°C) from 14 to 42 days of rearing. Another study showed T_3 concentration to increase in the blood of broiler chickens exposed to heat stress and fed a diet supplemented with natural antioxidants (Hassan et al., 2016; Wan et al., 2017; Sadeghi and Moghaddam, 2018). Sadeghi and Moghaddam (2018) found that dietary inclusion of ginger or garlic powder at 0.5% increased T_4 concentration in the blood of broiler chickens exposed to high ambient temperatures. The authors observed no effect of supplementing cinnamon powder at 0.5% on the blood level of this hormone in the experimental birds. In addition, El-Shoukary et al. (2014) and Wan et al. (2017) found no changes in T_4 concentration in the blood of chickens exposed to high ambient temperatures and supplemented with herbs. In our study, we observed T_4 blood concentration to increase in broiler chickens supplemented with nettle extract in drinking water on the second day of thermal challenge. In further studies, the inclusion of herbal extracts in drinking water did not have any effect on the T_4 blood level of the birds.

Heat stress contributes to lower lymphocyte production and higher heterophil production in the avian blood, which increases the H:L ratio (Vermette et al., 2017; Attia and Hassan, 2017; Attia et al., 2017 a; 2018 a; Xu et al., 2018). Therefore, the H:L ratio is used as a reliable indicator of stress in birds (Vermette et al., 2017; Attia et al., 2017 c). Najafi and Torki (2010) observed that a diet with 200 mg·kg⁻¹ of essential oils from thyme and cinnamon caused a significant decrease in heterophils and an increase in blood lymphocytes of birds compared to the group fed the diet without herbal extracts. In addition, Attia et al. (2017 b; 2018 b) observed similar results. In turn, Khafar et al. (2019) showed that a diet supplemented with essential oil from thyme at 150 and 200 mg·kg⁻¹ may effectively protect chickens from the negative effects of heat stress, among others by reducing the H:L ratio. Hassan et al. (2016) noted a reduction in the H:L ratio in the blood of broiler chickens exposed to high ambient temperature and receiving a diet with *Moringa oleifera* at 0.1, 0.2 and 0.3%. A reduction in the H:L ratio in the blood of chickens exposed to heat stress was also obtained by Hosseini et al. (2016) after dietary inclusion of essential oil from turmeric at 400 mg·kg⁻¹. In our study, the decreased H:L ratio in relation to the control group was obtained for birds supplemented with 2 ml/l nettle extract in drinking water.

In summing up, the extracts from lemon balm, sage and nettle added to drinking water at 2 ml/l caused, during the initial period of heat stress, a decrease in the blood level of thyroid hormone (triiodothyronine) and in rectal temperature of the experimental broiler chickens. This suggests that dietary inclusion of extracts from the studied herbs may alleviate the negative effects of short-term heat stress. Broilers receiving the nettle extract were also characterised by lower radiation temperature of featherless parts and a lower H:L ratio in the blood during the period of increasing ambient temperature, as well as by the lowest mortality percentage. It can therefore be concluded that dietary inclusion of nettle in the form and concentration used in our study had the most beneficial effect on the physiological status (body temperature, level of thyroid hormones and H:L ratio) of broiler chickens exposed to elevated ambient temperatures (30°C), and thus on their welfare.

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