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## The effect of mixed herb extracts on selected stress parameters in broiler chickens of three genetic lines

### Introduction

Stress is present in every living animal organism as a result of stimulation from environmental factors. It is an integral part of life, vital to life, and the normal adaptation of the body (Selye, 1936). Stress most often occurs due to unfavourable living conditions or a persisting situation that causes it (stressor), where the problem cannot be solved. Although stress is a response assisting the body in adapting to ambient conditions, it reduces welfare and thus productivity of animals, and often has a negative effect on the quality of animal products (Puvadolpirod, Thaxton, 2000b).

In states of stress, the sympathetic-adrenal-medullary, hypothalamo-pituitary-adrenal, and hypothalamo-pituitary-thyroid systems are stimulated. The levels of catecholamines (Post et al., 2003), corticosterone (Puvadolpirod, Thaxton, 2000a,b) and the heterophil to lymphocyte ratio increase in blood (Scanes, 2016). Intensive livestock production contributes to stress, behavioural and physiological abnormalities, as well as many injuries and diseases of animals. That is why, in many countries, notably the European Union, efforts are made to make extreme systems of livestock production more welfare friendly. Currently, particular interest is given to medicinal plants and herbal preparations, which show broad and diverse biological and health properties. Scientific literature reports many studies concerning the beneficial effect of dried herbs as a biocomponent of concentrate feeds or as extracts and infusions on the gastrointestinal, endocrine, and immune systems (Nasir, Grashorn, 2010; Wallace et al., 2010). Furthermore, they also have bacteriostatic, antiviral, sedative, and oxidation-reduction action (Wang et al., 2008; Wallace et al., 2010; Voljč et al., 2013). The increased interest in medicinal plants and their potential use in animal husbandry is also due to the fact that they are relatively safe and require no withdrawal period. Skomorucha and Sosnówka-Czajka (2013) and Zhang et al. (2013) reported that some

herbs and herbal extracts may reduce the body's physiological response to stress associated with adverse housing conditions.

Therefore, the aim of the study was to determine the effect of adding a mixture of anti-stress herbs to drinking water on the level of stress hormones (noradrenaline, adrenaline, corticosterone) and the heterophil to lymphocyte ratio in the blood of intensively reared broiler chickens of three genetic lines.

## Material and methods

The experiments were performed at a poultry farm of the National Research Institute of Animal Production in Aleksandrowice in Poland with 360 broiler chickens of three genetic groups. On the first day of life, broiler chicks were weighed, tagged, and divided into group I – 'Ross 308', group II – 'Cobb 500', and group III – 'Hubbard Flex' broilers. Each group had three subgroups. Birds were kept for 42 days on litter at a stocking density less than 33 kg/m<sup>2</sup>. All groups had uniform environmental (temperature, air humidity, light regime) and feeding conditions. Birds were fed *ad libitum* starter (ME 3000 kcal, CP 21.0%), grower (ME 3100 kcal, CP 19.8%), and finisher diets (ME 3100 kcal, CP 18.5%) based on concentrates at 1–3, 4–5 and 6 weeks of age, respectively. Birds had free access to water drinkers throughout the study. In each group from 21 to 35 days of rearing, water drinkers were supplemented for 5 h/day (08:00–13:00 hours) with a tincture from mixed herbs at 2 ml/l water. The herb mixture contained 30% chamomile (*Matricaria chamomilla* L.), 10% oregano (*Origanum vulgare* L.), 10% yarrow (*Achillea millefolium* L.), 10% knotgrass (*Poligonum aviculare* L.), 10% valerian (*Valeriana officinalis* L.), and 20% inflorescence of large-leaved lime (*Tilia platyphyllos* L.). This mixture was selected based on the present authors' earlier research. The tincture from mixed herbs was made by a professional herbal company and has been certified to conform to the company's quality standards. At 21, 28, 35, and 42 days of age, blood was collected from 10 birds per group to determine the levels of corticosterone (by enzyme immunoassay), noradrenaline, and adrenaline (by radioimmunoassay). Blood smears were made from the collected blood to count lymphocytes and heterophils and to calculate the heterophil to lymphocyte ratio (H:L). Lymphocytes and heterophils were counted under a Nikon YS 100 microscope after May-Grünwald and Giemsa staining.

The results were statistically analysed by an analysis of variance, and significant differences were estimated with Duncan's test. The statistical calculations were performed with Statgraphics plus 6.0. Effects were considered significant at a probability of  $p \leq 0.05$  and  $p \leq 0.01$ .

**Tab. 1.** The level of stress hormones in the blood of broiler chickens; values shown as different letters within the line differ significantly according to the Duncan test at  $p \leq 0.05$ ;  $n = 10$ 

Hormones [ng/ml]	Days of rearing excrement	Group			SEM
		Ross 308	Cobb 500	Hubbard Flex	
Noradrenaline	21	121.56	132.16	146.68	27.94
	28	64.54	70.58	118.34	18.83
	35	87.94	101.49	122.68	16.12
	42	99.93a	50.31b	98.78a	16.69
Adrenaline	21	53.18	146.44	133.34	39.66
	28	78.96	60.67	127.39	25.93
	35	78.38	154.85	164.72	30.50
	42	76.19	34.03	64.17	17.90
Corticosterone	21	11.85	14.03	12.14	2.56
	28	17.10	17.33	15.58	4.33
	35	14.93	18.74	15.98	2.15
	42	19.65	19.41	20.09	2.39

Note: group I – ‘Ross 308’, group II – ‘Cobb 500’, and group III – ‘Hubbard Flex’ broilers; SEM – standard error of the mean

## Results

On day 42 of the experiment, the noradrenaline concentration was lowest in the blood of ‘Cobb 500’ compared to ‘Ross 308’ and ‘Hubbard Flex’ broilers ( $p \leq 0.05$ ) (Tab. 1). No statistically significant differences in the adrenaline and corticosterone levels were found between the experimental groups. On day 21 of age, ‘Cobb 500’ broiler chickens were characterised by the highest heterophil percentage and lowest lymphocyte percentage compared to the other genetic groups ( $p \leq 0.01$ ), which translated into a difference in the H:L ratio between these groups ( $p \leq 0.01$ ) (Tab. 2). On day 42 of age, the difference in the percentage of heterophils and lymphocytes, and also in the H:L ratio became apparent between ‘Ross 308’ and ‘Cobb 500’ chickens ( $p \leq 0.01$ ).

## Discussion

A decisive role in the stress mechanism, or the body’s response to environmental stimuli, is played, among others, by the stress hormones corticosterone, adrenaline, and noradrenaline (Puvadolpirod, Thaxton, 2000a; Pohle, Cheng, 2009). Catecholamines (adrenaline and noradrenaline) are involved in numerous physiological and pathological processes, and their release into the bloodstream may increase blood pressure, heart beat, and muscle tension, in addition to increasing blood glucose levels by stimulating the conversion of hepatic glycogen to glucose (Virden et al., 2007; Kober et al., 2010).

**Tab. 2.** Heterophil and lymphocyte levels (%) in the blood of broiler chickens; values shown as different letters within the line differ significantly according to the Duncan test at  $p \leq 0.01$ ;  $n = 10$ 

Item	Days of rearing	Group			SEM
		Ross 308	Cobb 500	Hubbard Flex	
heterophils	21	33.90a	47.30b	34.70a	2.23
lymphocytes		61.00a	46.30b	60.80a	1.85
H:L		0.57a	1.04b	0.58a	0.06
heterophils	28	29.20	31.80	34.80	2.61
lymphocytes		64.90	60.50	58.70	3.18
H:L		0.47	0.56	0.64	0.07
heterophils	35	41.90	37.70	35.30	3.99
lymphocytes		52.80	55.00	58.50	4.11
H:L		1.35	0.71	0.65	0.37
heterophils	42	37.00a	21.10b	27.90	3.17
lymphocytes		56.10a	71.10b	62.60	3.47
H:L		0.74a	0.31b	0.49	0.09

Note: group I – ‘Ross 308’, group II – ‘Cobb 500’, and group III – ‘Hubbard Flex’ broilers; SEM – standard error of the mean

Cheng et al. (2001) and Pohle and Cheng (2009) report that changes in adrenaline and noradrenaline blood levels may serve as an indicator of welfare and coping with stress. In our study, ‘Cobb 500’ broiler chickens responded most favourably to drinking water with a mixed herb extract, which was reflected in the lowest level of noradrenaline and a tendency for lower blood adrenaline compared to ‘Ross 308’ and ‘Hubbard Flex’ chickens. The beneficial effect of adding mixed herbs to water on ‘Cobb 500’ chickens was also reflected in the lower H:L ratio, especially when compared with ‘Ross 308’ chickens. Likewise, Najafi and Torki (2010) found a significant decrease in the amount of heterophils and an increase in the amount of lymphocytes in the blood of broiler chickens in response to a diet with herbs. In turn, Yesilbag et al. (2012) showed increased heterophil percentage as well as increased H:L percentage ratio in the blood of birds fed a diet supplemented with an extract from oregano and rosemary essential oils compared to the control group. Long-term stress results in the corticosterone stress hormone being released into the blood of birds (Virden et al., 2007). A high level of this hormone adversely affects the body and may induce cardiovascular diseases, hypercholesterolemia, damage to the digestive tract, changes in immune function, as well as metabolic changes (Puvadolpirod, Thaxton, 2000c; Virden et al., 2007). Kannan et al. (1997) report that a lower blood corticosterone concentration is generally indicative of the body’s lower susceptibility to stressors. However, in our study, we did not observe any effect of supplementing the herb mixture on a lower level of this hormone in the blood in any of the chickens from the experimental groups.

In summary, the herb extract had the most favourable effect on relieving the body’s physiological response to stress, and thus on improving the welfare of ‘Cobb 500’ com-

pared to 'Ross 308' and 'Hubbard Flex' broilers. It can therefore be considered that active substances in herbs have varying effects on the body of broiler chickens of different origin.

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## Abstract

The aim of the study was to determine the effect of adding a mixture of anti-stress herbs to drinking water on stress hormone levels and the heterophil to lymphocyte ratio in the blood of intensively reared broiler chickens of three genetic lines. The experiment was performed with 'Ross 308', 'Cobb 500', and 'Hubbard Flex' broiler chickens, which were divided into three experimental groups. Birds were maintained on litter for 42 days at a stocking density less than 33 kg/m<sup>2</sup>. All groups were provided with the same environmental and feeding conditions. In each group from 21 to 35 days of rearing, water drinkers were supplemented for 5 h/day (08:00–13:00 hours) with an alcoholic extract from mixed herbs (30% chamomile, 10% oregano, 10% yarrow, 10% knotgrass, 10% valerian, 20% inflorescence of large-leaved lime) at 2 ml/l water. At 21, 28, 35, and 42 days of rearing, blood was collected from 10 birds per group to determine corticosterone, noradrenaline, and adrenaline levels. The heterophil to lymphocyte ratio (H:L) was also calculated. On day 42 of the study, 500 broiler chickens were characterised by the lowest noradrenaline concentration and a lower H:L ratio compared to 'Ross 308' and 'Hubbard Flex' ( $p \leq 0.05$ ) and 'Ross 308' chickens ( $p \leq 0.01$ ), respectively. It was concluded from the study that the herb extract had the most favourable effect on relieving the body's physiological response to stress, and thus on improving welfare in 'Cobb 500' compared to 'Ross 308' and 'Hubbard Flex' broilers. It can therefore be considered that active substances in herbs have varying effects on the body of broiler chickens of different origin.

**Key words:** adrenaline, broiler chickens, corticosterone, H:L, mixed herb extract, noradrenaline

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## Wpływ ekstraktu z mieszanki ziół na wybrane parametry stresu kurcząt brojlerów trzech linii genetycznych

### Streszczenie

Celem badań było określenie wpływu dodatku do wody pitnej ekstraktu z mieszanki ziół wykazujących właściwości antystresowe na poziom hormonów stresu oraz stosunek heterofilii do limfocytów we krwi kurcząt brojlerów trzech linii genetycznych utrzymywanych w systemie intensywnym. Doświadczenie przeprowadzono na kurczątach brojlerach: 'Ross 308', 'Cobb 500' oraz 'Hubbard Flex', które przydzielono do trzech grup doświadczalnych. Ptaki utrzymywano przez okres 42 dni, na ściółce o obsadzie nie przekraczającej 33 kg/m<sup>2</sup>. Wszystkie grupy miały ujednoczone warunki środowiskowe oraz żywieniowe. W każdej grupie od 21 do 35 dnia odchowu przez 5 h/dobę (od 8.00–13.00) dodawano do poidel z wodą ekstrakt spirytusowy z mieszanki ziół (30% rumianku pospolitego, 10% ziela lebidki pospolitej, 10% ziela krwawnika pospolitego, 10% ziela rdestu ptasiego, 10 % kozłka lekarskiego, 20% kwiatostanu lipy szerokolistnej) w ilości 2 ml/l wody. W 21, 28, 35 oraz 42 dniu odchowu pobrano krew od 10 ptaków z grupy i oznaczono poziom: kortykosteronu, noradrenaliny i adrenaliny a także obliczono stosunek heterofilii do limfocytów (H:L). Kurczęta brojlerzy 'Cobb 500' charakteryzowały się w 42 dniu doświadczenia najniższym poziomem noradrenaliny oraz węższym stosunkiem H:L w porównaniu odpowiednio z kurczętami 'Ross 308' i 'Hubbard Flex' przy

$p \leq 0,05$  i kurczętami 'Ross 308' przy  $p \leq 0,01$ . Na podstawie badań stwierdzono, że najkorzystniejszy wpływ podawanego ekstraktu z mieszanki ziół na łagodzenie fizjologicznej reakcji organizmu na stres, a tym samym na poprawę dobrostanu stwierdzono w przypadku kurcząt brojlerów 'Cobb 500' w porównaniu z kurczętami 'Ross 308' i 'Hubbard Flex'. Można uznać, że substancje czynne zawarte w ziołach mają zróżnicowany wpływ na organizm kurcząt brojlerów różnego pochodzenia.

**Słowa kluczowe:** adrenalina, ekstrakt z mieszanki ziół, H:L, kortykosteron, kurczęta brojlery, noradrenalina

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