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## POTENTIAL USE OF THE STINGING NETTLE AS AN ENHANCER OF WEIGHT GAIN AND INNATE IMMUNE RESPONSE IN BROILER CHICKENS

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**Abstract.** Researches were conducted to investigate the *in vivo* effect of an nettle plant alcohol extract on the phagocytosis and body weight in 48 days old broiler chickens (n=34). The birds were divided into three groups: subcutaneously injected with 0.5 ml of saline (control, n = 12), with 0.5 ml of alcohol (group II, n=11) or with 0.5 ml of alcoholic nettle extract (group III, n=11). Sterile heparinized blood, sampled on days 0, 7 and 13, was processed by an *in vitro* carbon particle inclusion test, the phagocytosis being expressed in optical density units. The birds were weighed simultaneously with the blood sampling. We concluded that a single nettle extract treatment does not significantly influence the phagocytosis, while the second administration intensified it. The alcoholic nettle extract had a beneficial effect on the body weight of broiler chickens.

**Key words:** Broiler chickens; Nettle extract; Phagocytosis; Body weight.

### INTRODUCTION

Poultry industry represents one of the economically most important branches of animal production, where appropriate productive levels can be obtained only by the use of technologies that ensure and preserve birds' health and alleviate stress. The effects exerted by the alcoholic vegetal extract on birds as well as their role in increasing production in different farmed species are of great interest not only for veterinarians, but also for farmers and food processors (Wang, M. et al. 2001; Harata, K. et al. 2001).

Researches conducted to establish the activity of several vegetal extracts on the immune system were connected with major problems their use could solve (Pusztai, A. et al. 1993; Pop, M. et al. 1990; Spînu, M. et al. 1992). Changes in classical therapies by the use of plant extracts could enhance the innate and adaptive immune responses and improve animal production levels, via their combined, innate and adaptive immune responses stimulating activity (Spînu, M. et al. 1998). Beneficial effects of the nettle plant have been studied in detail in both human and veterinary medicine (Ionescu, S. and Savopol, 1977; Radu, I., Andronescu, 1984; Randall et al., 1999).

This study aimed to investigate the potential *in vivo* influence of an alcoholic nettle plant extract on the non-specific cell-mediated immune response (phagocytosis) and changes in body weight in broiler chickens.

### MATERIALS AND METHODS

Broiler chickens (n=34), aged 48 days, were divided into three groups: a) group I, untreated control, injected with 0.5 ml of saline, n=12; b) group II, injected with 0.5 ml of 70° alcohol, n=11; and c) group III, injected with 0.5 ml of alcoholic nettle extraction, n=11. The birds were injected by subcutaneous route.

Injections were performed twice, on day 0 (beginning of the experiment) and after 7 days. Blood for the immunological tests was sampled three times, on days 0, 7 and 13, on heparin (50 IU/ml) and processed within maximum 4 hours after sampling.

**Phagocytic activity.** 0.5 ml aliquots of heparinized blood were co-incubated at 37°C with 1.5 ml of a supernatant of India ink, in sterile tubes. At 0, 30 and 60 minutes of incubation, 150 ml of the mixture were transferred to 3 ml of saline. Finally, all the tubes containing saline + blood + India ink were centrifuged 10 minutes at 1500 rpm. Supernatant optical densities, rendering 0, 30 and 60 minutes of incubation for each sample, were read in 96 well plates, using a multichannel spectrophotometer, at 535 nm wavelength. The results were expressed in optical density units (ODU) and subsequently Ln values were calculated. Phagocytic activity was calculated as the difference between the natural logarithms of the optical densities of the phagocytosis at 0–30-min and 30-60-min.

**Body weight.** Chickens were weighed at the beginning of the experiment as well as on days 7 and 13 and the changes in body weight were recorded.

## RESULTS AND DISCUSSIONS

The *in vitro* phagocytic test allows making the evaluation of recognition and afterwards the inclusion of non-specific particles (Obertreis, B. et al. 1996; Dumitru, C. et al. 1996). This mechanism is essential for initiating the specific immune response, being followed by either destruction or removal of foreign materials or antigenic processing. Attempts to clarify the chemical profile of stinging nettle and to provide an insight into the mechanisms by which the immune response is improved showed that *Urtica dioica* extracts increased the activity of non-stimulated intestinal epithelial cells, inducing the enhanced secretion of protein-1 and growth-related oncogene, also stimulating MyD88/NF- $\kappa$ B/p38 signaling, and enhancing intestinal steady-state defense. These results recommend the stinging nettle as a candidate for the phytopharmaceuticals or dietary supplements for co-treatment of various inflammatory diseases (Francišković, M. et al. 2017).

Administration of the injectable alcoholic nettle extract to broiler chickens led to changes in phagocytosis, an important component of the innate cell-mediated immune response (figures 1 to 3).

The analysis of the data obtained from the first sampling (fig. 1) showed that mean optical density values in the control group were lower than those in the alcohol or nettle extraction injected groups, nevertheless, all the groups showed a statistically non-significantly different, but high phagocytosis. In all groups, the Ln of the ODU values during the first reading interval (0 to 30 min) indicated that the phagocytosis increased, when compared to the second interval ( $p < 0.05$ , group II).

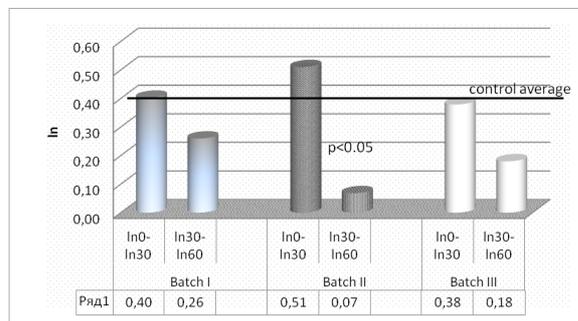


Figure 1. *In vitro* phagocytic activity at the first sampling ( $x \pm s$ )

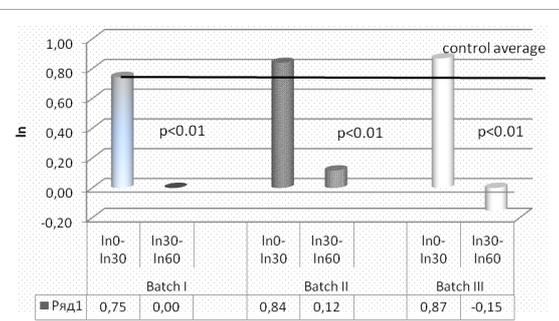


Figure 2. *In vitro* phagocytic activity at the second sampling ( $x \pm s$ )

After 7 days, the phagocytic activity decreased the most in the control group (fig. 2) when compared to the first sampling ( $0.150 \pm 0.069$  ODU versus  $0.081 \pm 0.048$  ODU). Similar results were obtained in the other two groups, probably due to the manipulation and injection stresses, the nettle extract and the alcohol alleviating this decrease (0.069 ODU for group I, 0.033 ODU for group II and 0.048 ODU for group III). By the end of the experiment, the phagocytic activity significantly ( $p < 0.01$ ) increased in all groups compared to both first and second samplings. The increase was much higher in the nettle extract injected group, compared to similar results in the other two groups (fig. 3), underlining the beneficial effect of the alcoholic nettle extract on the non-specific cell mediated immune response in broiler chickens.

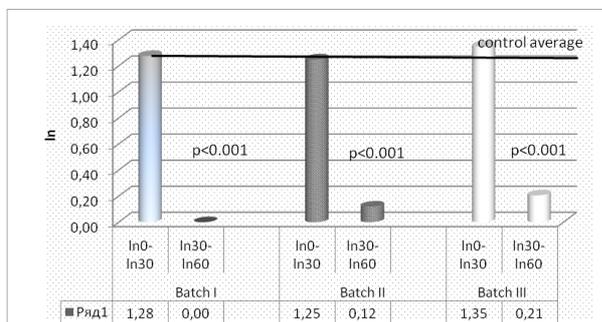
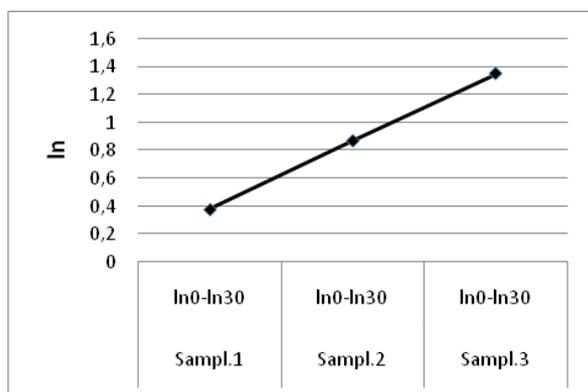


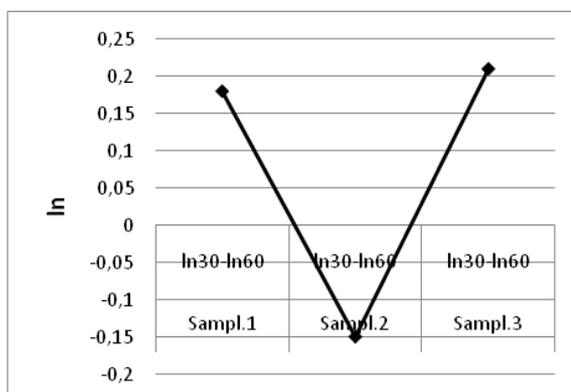
Figure 3. *In vitro* phagocytic activity at the third sampling ( $x \pm s$ )

Fig. 4 and 5 illustrate the differences between the intensity of phagocytosis during the experiment. The first reading period of 30 min after sampling better reflects the enhancement of phagocytosis by the nettle extract treatment in chickens.

Nettle was used as a dietary supplement in animals from ancient times. A study conducted on a total of 300 Ross-308 broiler chickens, to evaluate the effects of 0.5%, 1%, 1.5% and 2% *Urtica dioica* dietary supplement on growth performance, carcass characteristics, and blood biochemistry and immune parameters, indicated that 1% of nettle supplement significantly



**Figure. 4.** The continuously increasing trend of the phagocytic activity in the nettle extract treated group (0-30 min)



**Figure. 5.** Diminished dynamics of the phagocytic activity during the second reading period (30-60 min) in the nettle extract treated group

( $p < 0.05$ ) increased the body weight at 42 days of age, but did not change the immune related parameters among the treatments (Safamehr, A. et al. 2012).

Evaluating comparatively the effects of chicory (*Cichorium intybus*) and nettle (*Urtica dioica*) in 42 day old chickens, fed in different diets on growth performance, carcass characteristics and biochemical parameters, showed that both nettle and chicory, supplemented with enzyme had similar effects. Although these supplements appeared suitable for chicken diets, selection is necessary to obtain beneficial results (Safamehr, A. et al. 2013). Measurements of changes in body mass showed a gradual increase in body weight in all groups during two weeks.

**Table 1.** Changes in mean values of body weight (kg) during the experiment

	group I control			group II, alcohol			group III, nettle extract		
	Sampling I	Sampling II	Sampling III	Sampling I	Sampling II	Sampling III	Sampling I	Sampling II	Sampling III
Mean values	1.506	1.936	2.183	1.497	1.918	2.180	1.627	2.064	2.409
Standard deviation	0.143	0.204	0.206	0.166	0.169	0.182	0.203	0.286	0.356

While the mean total increase was 0.677 kg in the control group, in the birds treated with alcohol it reached 0.683 kg. The nettle extract exerted the most stimulating effect (0.782 kg), group III exceeding with 105 g the mean performances of the control group (table 1). These results supported the nettle extract potential to increase the weight gain, which could also be the result of an improved antimicrobial defense. The nettle extract exerted its positive effect in this experiment being administered not orally but by subcutaneous injection route, so that it could serve not only as a dietary supplement but also as an adjuvant in immune stimulation.

## CONCLUSIONS

1. Repeated alcoholic nettle extract treatments in broiler chickens improve not only the weight gain but also the non-specific cell-mediated immunity.

2. The oral route is not the only one by which the alcoholic nettle extract could improve the body condition and immunity. The subcutaneous administration route could increase the bioavailability of the active principles, avoiding digestion.

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