

**Doctoral dissertation:**

**„Development and application of a preparation based on natural plant ingredients in the prevention of broiler chicken rearing”**

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**Abstract**

The aim of this study was to develop and evaluate the effectiveness of a preparation based on natural plant ingredients with phytobiotic properties in the prevention of broiler chicken rearing. Two prototypes of phytobiotic preparations were produced, consisting of a composition of micronized herbs or their extracts and peraclets. Prototype 1 was produced using the patented adiPHAG technology, which allows for the production of a stable and highly effective micellar emulsion in the duodenum. Its ingredients included methyl salicylate, menthol, anise oil, and eucalyptus oil. Prototype 2 of the preparation was developed based on processed spice and herbal products such as *Sinapis alba L.*, *Acorus calamus L.*, *Hedera helix L.*, *Curcuma longa L.* combined with thymol and menthol.

The prepared preparations were added to complete feed mixtures at quantities of 0.01% and 0.02%. The prepared feed mixtures were in powdered form and were produced in a commercial feed mill.

The study was conducted in an experimental chicken coop with 1280 commercial Ross 308 broilers. The birds were kept in a litter-based rearing hall in 40 pens divided into 5 experimental groups, each with 8 replicates. Three groups were tested with prototype preparations: group A with prototype 1 at 0.01%, group B with prototype 1 at 0.02%, and group C with prototype 2 at 0.01%. Two groups served as negative control (K) and positive control (N), containing a 0.01% additive of an existing commercial phytobiotic product on the market. The broiler chickens were kept until 35 days of age.

Statistically significant differences ( $P \leq 0.05$ ) in body weight were observed between groups B and N and group C during the first week of life. At 35 days of age, birds in groups A (2611.2 g) and B (2629.5 g) differed significantly in body weight from control groups K (2530.0 g) and N (2518.7 g). The body weight in group C (2574.4 g) was between the weights of groups A and B, as well as K and N. Group C had the lowest body weight from day 7 to day 21, and then it caught up with the control groups.

Statistically significant differences ( $P \leq 0.05$ ) in daily gains at 7 days were observed between chicks in groups B (22.65 g) and N (22.47 g) and C (20.95 g). In the remaining measurements, no statistically significant differences were found.

Regarding the FCR coefficient, it was significantly lower ( $P \leq 0.05$ ) between group B (0.888) and the other groups.

Feed intake at 21 days was significantly different ( $P \leq 0.05$ ) between groups C (1192.3 g) and K (1222.7 g), and at 35 days, between groups B (3719.4 g) and K (3581.6 g) and N (3571.3 g).

The synthetic EWW coefficient did not differ statistically between the groups, although it exceeded 500 points for groups A and B, indicating a very high efficiency in the growth.

Sensory analysis of the meat showed significant differences ( $P \leq 0.05$ ) in redness ( $a^*$ ) of leg muscles between groups K (3.44) and N (4.526) and yellowness ( $b^*$ ) of breast muscles between groups K (0.835) and N (1.860), as well as brightness ( $L^*$ ) for leg muscles between group C (54.22) and groups A (50.59) and B (50.62) and N (50.49). It was shown that the tested preparations did not affect the color of the meat, while prototype 2 affected the brightness coefficient. The prototype from the positive control group had a positive effect on redness ( $a^*$ ) and yellowness ( $b^*$ ) of the samples.

Thermal drip loss of breast muscles differed significantly ( $P \leq 0.05$ ) between groups A (30.06) and B (22.06), indicating a positive dose-dependent effect of the preparation. Thermal drip loss of leg muscles differed significantly ( $P \leq 0.05$ ) between group B (30.96) and groups K (23.83) and N (23.18).

There were no differences in pH, TBARS, or shear force values between the tested groups.

The results of the sensory evaluation of the meat parameters indicated differences ( $P \leq 0.05$ ) in the color of breast muscles between groups A (4.666) and B (4.000), and in the appearance of breast muscles between groups B (4.111) and C (4.111) and N (4.666). Differences were also observed in the smell and color of leg muscles between groups A (3.888) and K (4.638), as well as differences in the taste evaluation of leg muscles between group C (4.111) and N (4.638).

Antioxidant activity of the prototype preparations was lower than that of Trolox, with prototype 2 and the positive control group showing relatively low activity, while prototype 1 had the lowest activity within the tests.

Histological examination of the intestines showed a ratio of villi to crypt depth ranging from 1.39 (group B) to 21.6 (group A), with the lowest value achieved in group B. The surface

area of intestinal villi ranged from 207918  $\mu\text{m}^2$  (group K) to 15630587  $\mu\text{m}^2$  (group A). Crypt depth ranged from 72  $\mu\text{m}$  (group A) to 448  $\mu\text{m}$  (group C). Villus length reached values from 516  $\mu\text{m}$  (group B) to 2154  $\mu\text{m}$  (group A). Based on quantitative analysis, an increase in the absorptive surface area of the intestine was observed in group A, with the smallest increase in group C. Duodenal tissue of group A showed the strongest degree of restructuring and increased surface area for intestinal absorption. A significant increase in crypt depth was observed in group C, while group B exhibited intermediate features between groups A and C. Group N showed strong stimulation of the intestine for regeneration and the formation of new intestinal villi.

Immunohistometric evaluation indicated that the use of both prototype 1 and prototype 2 did not cause immunostimulation. The percentage of areas of the spleen and gut-associated lymphoid tissue (GALT) showing the presence of Bu-1+, CD4+, and CD8+ lymphocytes did not differ significantly compared to the control group. An exception was group C and the positive control, which caused a higher number of CD8+ cells in the spleen compared to the control group.

Based on the research results, the introduction of commercial prototype 1 of the preparation was recommended. It demonstrated effectiveness in a field observation where broiler chickens in the group receiving prototype 1 showed significantly lower ( $P \leq 0.05$ ) FCR values compared to the reference group.

Keywords: phytobiotics, phytoncides, broiler chickens, antibiotic growth promoters, herbal products.

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