

# Effect of a plant preparation Citrosept on selected immunity indices in blood of slaughter turkey hens

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## Abstract

**Introduction and objective.** The objective of this study was to determine the effect of *per os* administration of 3 various dosages of a Citrosept preparation (a grapefruit extract) to growing turkey hens on changes in their selected haematological and immunological blood indices. An attempt was also undertaken to select the most efficient dose of the preparation with respect to the mentioned indices in turkey hens.

**Materials and methods.** The experiment was conducted on 180 turkey hens allocated at random to 4 groups, 45 birds in each group. Samples of their full blood were analyzed for haematological indices, such as red blood cell count (RBC), haemoglobin content (Hb), haematocrit value (Ht), and white blood cell count (WBC). Samples of blood plasma were assayed to determine the activity of lysozyme (chamber-diffusive method) and heterophils capability to reduce nitro blue tetrazolium (stimulated and spontaneous NBT test). Phagocytic activity of leucocytes against *Staphylococcus aureus* 209P strain was assessed and expressed as the percentage of phagocytic cells (% PC) and phagocytic index (PI).

**Results.** The administration of the grapefruit extract to turkey hens with drinking water caused a significant increase in haemoglobin content in blood, as well as an increase in non-specific humoral immunity marker (activity of lysozyme) and non-specific cellular immunity marker (percentage of phagocytic cells;  $P \leq 0.05$ ).

**Conclusions.** The results obtained enabled the positive evaluation of the advisability of applying the Citrosept preparation in the feeding of turkey hens at the age of 6–9 weeks. Among the doses examined, the most efficient with respect to the stimulation of the non-specific humoral and cellular immunity was the dose of 0.021 ml/kg of body weight.

## Key words

Citrosept, slaughter turkeys, hamatological indices, immunological blood marker

## INTRODUCTION

An increasing interest has been observed recently in the use of natural preparations that stimulate the immune responses of birds. In view of a diversified pharmacological activity of white grapefruit, an attempt was made to apply a natural preparation – Citrosept, an extract from pulp, stones and albedo of this plant, to study the haematological and immunological blood indices of birds. The extract from grapefruit was discovered in 1980 by an American immunologist, Jacob Haich, and rapidly aroused interest, mainly in the medical world but also in other branches, e.g., agriculture, breeding, therapeutics or the cosmetic and food industries. The therapeutic properties of the white grapefruit extract are due, most of all, to its antibacterial, antimycotic and antiviral activity, as well as to the feasibility of its application in prophylaxis and therapy. The extract from grapefruit is capable of eradicating or inhibiting the growth of many pathogenic bacteria, fungi, viruses and unicellular parasites. Research has shown that effects of the grapefruit extract on microorganisms consist in the destruction of the cytoplasmic membrane. Active constituents of the extract cause damage to structures of this membrane and hinder

amino acid synthesis by a microorganism. Simultaneously, low-molecular cellular structures are sucked out of the microorganism. This neutralizes the pathogen and eventually causes its death. In the case of grapefruit extract application, the time span of this process is significantly shorter than on the use of other preparations eradicating microorganisms [1–3].

Fruits of white grapefruit constitute a rich source of biologically-active compounds including, among others, flavonoids (0.015 mg·ml<sup>-1</sup>) and phenolic acids (o-dihydroxyphenols – 0.199 mg·ml<sup>-1</sup>), both completing an everyday diet, as well as vitamin C (1 g per 100 ml of preparation) [4]. The content of flavonoids in the analyzed preparation is of great significance because they strengthen the liver, intestines and cardiovascular system. They also enhance vitamin absorption and increase body immunity and efficiency. Until now, a few tens of substances belonging to different chemical groups with defined pharmacological activity have been identified in white grapefruit. Of these, an important group is constituted by the naturally occurring flora flavonoid, abundant in compounds, including: flavones, flavonols and flavanones. They act synergistically (enhance the activity of one another) with vitamin C and protect it against oxidation. Flavonoids occur in the pulp, external pericarp and seeds of grapefruit. They are represented by the following compounds: nobiletin, scutellarein, tangeretin, sinensetin, and heptametoxyflavone. Apart from flavonoids, white grapefruits are also rich in flavanones: naringenin and

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its glycosides: naringin and narirutin, as well as hesperidin, neohesperidin, poncirin, and others [3, 5, 6, 7].

**Objectives.** The objective of this study was to determine the effect of administration of the plant preparation Citrosept with drinking water to growing turkey hens on changes in the selected haematological indices and immunological blood markers. An attempt was also undertaken to select the most efficient dose of the preparation with respect to the mentioned indices in turkey hens.

## MATERIALS AND METHOD

**Experimental design.** The experiment was conducted on 180 turkey hens allocated at random to four groups, with 45 birds in each group. Each group was randomly divided into 3 repetition sub-groups of 15 birds. The birds were reared following zoohygienic standards for this species and breed of birds. From the beginning of the 6<sup>th</sup> week until the end of the 15<sup>th</sup> week of life, they were kept in pens, in the rooms with a litter rearing system. The C group was the control group that did not receive the experimental additive. In turn, turkey hens from groups II-IV were administered the plant preparation to drinking water in the following doses: Group II – 0.011 ml/kg b.w.; Group III – 0.021 ml/kg b.w.; Group IV – 0.042 ml/kg b.w. Throughout the study period, the birds from all groups were fed *ad libitum* with standard pelleted complete feed mixtures produced by the Animex company, adjusted to the age and developmental stage of the birds, accordingly to a programme covering 5 feeding sub-periods. Contents of basic nutrients in the feed mixtures corresponded to the binding guidelines of Poultry Feeding Standards [8].

The analyzed preparation was applied to turkey hens with fresh drinking water for the period of 28 days (6<sup>th</sup>-9<sup>th</sup> weeks of life). After 4 weeks of preparation administration, a 2-week break was made in supplementation (10<sup>th</sup>-11<sup>th</sup> week of life), during which the birds received pure water without the additive. After this break, the turkey hens were again administered the plant preparation with drinking water in identical doses (12<sup>th</sup>-15<sup>th</sup> week of life). At the end of the 9<sup>th</sup>, 11<sup>th</sup> and 15<sup>th</sup> week of life, blood was sampled from the basilica vein (*vena basilica*) from 6 turkeys from each repetitive subgroup for haematological and immunological analyses. The blood was sampled in the morning hours after 2-h fasting. EDTA was used as a blood anticoagulant in the analyses of the haematological indices.

**Blood indices evaluation.** The haematological analyses were conducted with the use of a Swelab AC 920 analyzer. The following haematological indices were determined in the study: red blood cell count (RBC), haemoglobin content (Hb), haematocrit value (Ht), and white blood cell count (WBC).

The non-specific humoral and cellular immunity was evaluated by determining the activity of lysozyme in blood plasma with the chamber-diffusive method in an agarose gel, and heterophils capability to reduce nitro blue tetrazolium (NBT test) in 2 variants – stimulated and spontaneous. Additional analyses were carried out for the phagocytic activity of leucocytes against *Staphylococcus aureus* 209P strain, expressed by the percentage of phagocytic cells (% PC) and phagocytic index (PI), i.e., the mean number of bacteria per one phagocytic cell [9, 10].

**Statistical analysis.** The results obtained were subjected to statistical analysis using STATISTICA 6.0 PL Software. The statistical significance of differences between mean values was estimated with a one-way analysis of variance (ANOVA) and *post-hoc* Duncan's test, assuming a significance level at  $P \leq 0.05$ .

## RESULTS AND DISCUSSION

Results of analyses of haematological blood indices of turkey hens are presented in Table 1. No significant differences were noted between the control group and the experimental groups with reference to the level of the erythropoietic system parameters, except for Hb level at the end of the 15<sup>th</sup> week of birds life.

**Table 1.** Haematological indices in blood of turkey hens at different ages: control group (C) and groups receiving Citrosept preparation (II-IV).

| Parameter                                                | Control            | Experimental groups |                    |                   |       | SEM |
|----------------------------------------------------------|--------------------|---------------------|--------------------|-------------------|-------|-----|
|                                                          | C                  | II                  | III                | IV                |       |     |
| 9 weeks of age                                           |                    |                     |                    |                   |       |     |
| Red blood cell count, 10 <sup>12</sup> ·l <sup>-1</sup>  | 2.21               | 2.25                | 2.28               | 2.23              | 0.046 |     |
| Haemoglobin, mmol·l <sup>-1</sup>                        | 8.34 <sup>ab</sup> | 8.53 <sup>ab</sup>  | 8.21 <sup>b</sup>  | 8.90 <sup>a</sup> | 0.111 |     |
| Haematocrit, %                                           | 30.8               | 32.6                | 32.5               | 30.2              | 0.577 |     |
| White blood cell count, 10 <sup>9</sup> ·l <sup>-1</sup> | 23.7               | 24.0                | 24.7               | 23.4              | 0.464 |     |
| 11 weeks of age                                          |                    |                     |                    |                   |       |     |
| Red blood cell count, 10 <sup>12</sup> ·l <sup>-1</sup>  | 2.26               | 2.30                | 2.38               | 2.40              | 0.053 |     |
| Haemoglobin, mmol·l <sup>-1</sup>                        | 8.11               | 8.40                | 8.23               | 8.55              | 0.220 |     |
| Haematocrit, %                                           | 33.6               | 33.4                | 33.9               | 34.2              | 0.213 |     |
| White blood cell count, 10 <sup>9</sup> ·l <sup>-1</sup> | 27.9               | 29.2                | 28.6               | 27.8              | 0.378 |     |
| 15 weeks of age                                          |                    |                     |                    |                   |       |     |
| Red blood cell count, 10 <sup>12</sup> ·l <sup>-1</sup>  | 2.50               | 2.45                | 2.59               | 2.58              | 0.055 |     |
| Haemoglobin, mmol·l <sup>-1</sup>                        | 8.99 <sup>b</sup>  | 9.12 <sup>b</sup>   | 10.02 <sup>a</sup> | 9.96 <sup>a</sup> | 0.165 |     |
| Haematocrit, %                                           | 33.7               | 32.9                | 33.6               | 33.0              | 0.458 |     |
| White blood cell count, 10 <sup>9</sup> ·l <sup>-1</sup> | 29.4               | 29.8                | 30.4               | 30.6              | 0.458 |     |

<sup>a, b</sup> Means within row marked with different letters differ significantly at  $P \leq 0.05$ .

The application of the Citrosept preparation caused a statistically significant increase in haemoglobin by over 11% and 10% in groups of turkey hens receiving the initial (Group III) and the highest (Group IV) dose of the preparation, compared to the control group ( $P \leq 0.05$ ). The results of haemoglobin level analysis indicate that the increase in the value of this blood parameter in the experimental groups was due to the presence of easily-available iron in the preparation that is indispensable for haemoglobin synthesis. According to Zaporowska (2002), the high bioavailability of iron is determined by significant quantities of vitamin C and organic acids in the raw material, that facilitate the absorption of this element [11].

Investigations carried out by other authors with slaughter turkey hens also demonstrated a positive impact of biostimulants prepared based on Krantz aloe, chokeberry, Gins-seng, purple coneflower, common knotgrass, yarrow and garlic, added to drinking water for the birds, mainly on Hb level and Ht value [12, 13, 14, 15, 16].

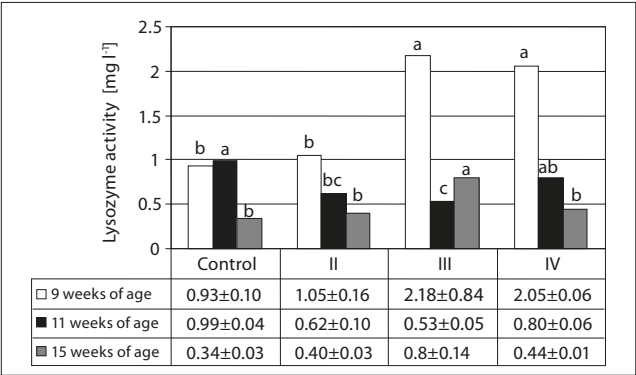
After 10 weeks of the study (15<sup>th</sup> week of rearing), in all experimental groups of turkey hens, a growing tendency was

noted in the WBC value, compared to the control group. This tendency was higher by over 1%, 3%, and 4% than in the control group (Tab. 1). No data was found, however, in the available literature on the mechanisms responsible for the increased count of white cells in blood upon the addition of a grapefruit extract, nor on changes in the other haematological indices in blood of turkeys; therefore, these issues should be addressed in future studies.

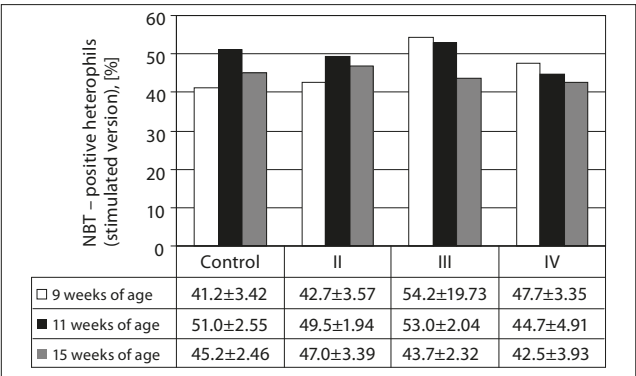
White blood cells are responsible for both the non-specific and specific immunity of the body. The non-specific immunity of a host body plays a significant role as the first line of defence against pathogens causing relatively little harm to the body, and possesses 2 types of defence mechanisms, i.e., humoral and cellular response. The humoral response involves especially the reaction to bacterial infections, whereas the cellular one – response to viral infections, as well as response during transplant rejection and in combating cancer cells. The cellular response is additionally responsible for delayed immune responses, including, e.g., inflammatory reaction [10, 17]. The ascending tendencies noted in the number of leucocytes in the experimental groups could result from the stimulation of non-specific immune responses, all the more that the grapefruit stones possess bioactive and therapeutic properties [3]. The results of research conducted by other authors also confirm the significant increase in WBC in blood of slaughter turkeys using immuno- and biostimulants, including fresh garlic, extracts and juices from purple coneflower, beta-glucan or aloe preparations [13, 15, 18, 19, 20]. The immunostimulatory effect of administering garlic-

derived substances on the specific and non-specific immunity was also observed in pregnant sows and their progeny in the form of increased values of such indicators as: white blood cell count in blood, activities of ceruloplasmine and lysozyme, and gamma-globulins level in blood [21, 22, 23].

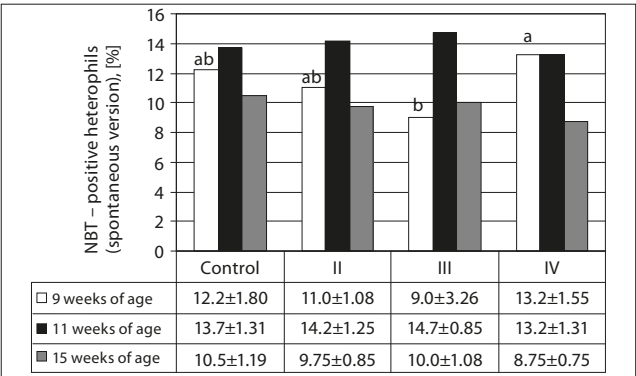
Results of assays of the immunological markers (Figs. 1, 2, 3, 4, 5) may point to the stimulation of the immune system of turkey hens from group III receiving the Citrosept preparation in a dose of 0.021 ml/kg b.w. A significant increase in the activity of lysozyme in blood plasma was



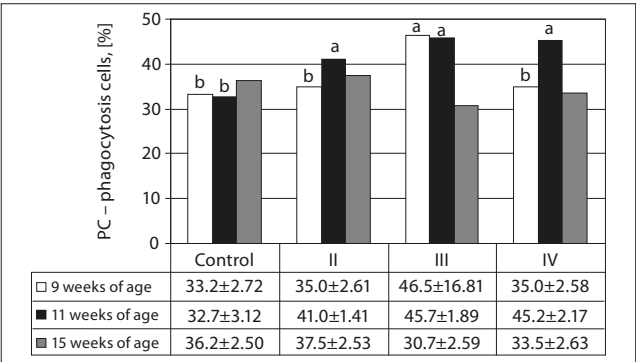
**Figure 1.** Activity of lysozyme in blood of turkey hens at different ages; control group (C) and groups receiving Citrosept preparation (II–IV).  $\bar{x} \pm \text{SEM}$   
a, b, c Different letters indicate statistically significant differences between control and experimental groups at different ages of turkey hens for  $P \leq 0.05$



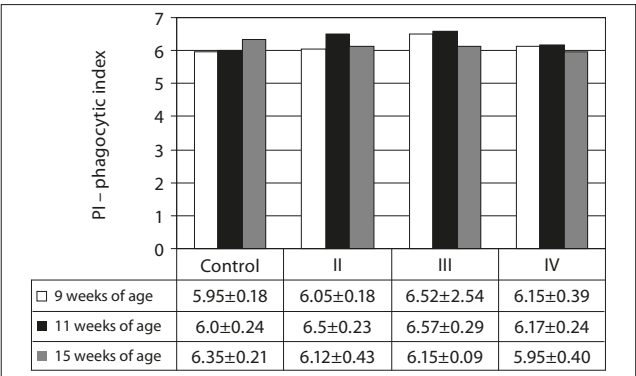
**Figure 2.** Percentage of reducing cells in stimulated test in blood of turkey hens at different ages; control group (C) and groups receiving Citrosept preparation (II–IV).  $\bar{x} \pm \text{SEM}$



**Figure 3.** Percentage of reducing cells in spontaneous test in blood of turkey hens at different ages; control group (C) and groups receiving Citrosept preparation (II–IV).  $\bar{x} \pm \text{SEM}$   
a, b Different letters indicate statistically significant differences between control and experimental groups at different ages of turkey hens for  $P \leq 0.05$



**Figure 4.** Percentage of phagocytic cells in blood of turkey hens at different ages; control group (C) and groups receiving Citrosept preparation (II–IV).  $\bar{x} \pm \text{SEM}$   
a, b Different letters indicate statistically significant differences between control and experimental groups at different ages of turkey hens for  $P \leq 0.05$



**Figure 5.** Phagocytic activity of leucocytes expressed as the phagocytic index in blood of turkey hens at different ages; control group (C) and groups receiving Citrosept preparation (II–IV).  $\bar{x} \pm \text{SEM}$

observed in this group of birds after 4 and 9 weeks of Citrosept administration ( $P \leq 0.05$ ). Also the phagocytic activity of leucocytes expressed by the percentage of phagocytic cells (% PC) in blood of 9-week-old turkey hens from group III demonstrated a statistically significantly higher value, by 40.1%, compared to the control group ( $P \leq 0.05$ ). Stimulation of non-specific immune responses was also observed in group IV of the birds after 4-week administration of the highest dose of the grapefruit extract to drinking water. The activity of lysozyme determined in this group was over twice as much as in the control group (Fig. 1); ( $P \leq 0.05$ ). This fact may be noted especially in young birds whose immune system is more susceptible to the effects of biologically-active compounds contained in grapefruit that are implicated to possess antibacterial and antimycotic properties, as well as to exert a positive effect on the growth of beneficial intestinal microflora [1, 2, 3, 5].

Owing to the significant role of the intestinal microflora in maintaining health status and high productivity of turkeys, the extract of grapefruit may arouse interest as a feed additive likely to constitute a significant factor inhibiting the proliferation of intestinal pathogens in poultry fed mixtures devoid of antibiotic growth stimulants. The immunostimulatory effect of herbal additives is a very important issue because ca. 75% of all cells of the immune system, i.e., 3% of body weight, are located in the alimentary tract [24]. The results obtained in the presented study indicate a positive effect of the initial (Group III) and the highest (Group IV) dose of the grapefruit extract (especially at the dose of 0.021 ml/kg b.w.) on the activity of lysozyme and phagocytic activity (% PC). Because many of the active compounds of grapefruit exhibit potential immunotrophic properties, the stimulatory effect of the preparation might have resulted from both the presence of plant components and the addition of vitamin C to the grapefruit preparation. Ascorbic acid is an indispensable element for the proper course of phagocytic reactions, it additionally stimulates interferon synthesis and lymphocyte proliferation, hence it could have played a significant role in this case (Schollenberger 1993). Worthy of note is the fact that the significant increase in those markers occurred usually at the end of the 4<sup>th</sup> week of observation (9<sup>th</sup> week of turkey hens life), which suggests that the shorter period of extract application to birds with drinking water is the most beneficial. In the feeding and prophylaxis of breeding animals, use is made increasingly often of the non-specific immunostimulation (immunopotentialization) in order to increase the natural (innate) immunity using natural immunomodulators. The effects of immunopotentialization include enhanced immune response, as well as extension of its duration, or both [26].

## CONCLUSIONS

The results obtained in this study enable the positive evaluation of the advisability of applying the Citrosept preparation in the feeding of turkey hens at the age of 6–9 weeks. Among the doses examined, the most efficient with respect to stimulation of the non-specific humoral and cellular immunity was the dose of 0.021 ml/kg of body weight.

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