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Department of Biochemistry and Toxicology, University of Life Sciences in Lublin,
Akademicka 13, 20-950 Lublin, e-mail: jerzy.truchlinski@up.lublin.pl

JERZY TRUCHLIŃSKI, KATARZYNA OGNIK, KATARZYNA ROSTEK

Influence of hawthorn preparation administration as a fodder additive on antioxidation indicators in turkey hens

Wpływ podawania preparatu z głogu jako dodatku paszowego na wskaźniki antyoksydacyjne krwi indyczek

Summary. The paper aimed at applying the hawthorn preparation Interactum crataegi at the dose of 0.35 ml/kg b.w./daily as an additive to drinking water for turkey hens. The study was carried out using 6-week-old turkey-hens of heavy BIG 6 type. Birds were divided into two groups: control and experimental, in which turkey hens were administered plant-origin hawthorn preparation in the amount of 0.35 ml/kg b.w./daily. For the whole experiment, birds had ad libitum standard full-dose mixtures (Provimi Polska) according to a program including 5 feeding periods. Superoxide dismutase and catalase activities, total antioxidant potential of a plasma, as well vitamin C and Fe, Zn, Cu contents were determined in turkey-hen's blood. The increase of the total antioxidant potential (FRAP) in experimental birds' blood along with superoxide dismutase (SOD) activity were recorded at the end of the experiments. The superoxide dismutase activity increase was correlated with copper concentration increase.

Keywords: hawthorn preparation, antioxidation indices, turkey hens

INTRODUCTION

A tendency to replace the antibiotic growth stimulators and coccidiostatic agents with natural fodder additives has been recently observed. According to a definition, herbs are wild plants, originated from natural habitats, or obtained from field cultivations. The plant's parts with the highest accumulation of biologically active substances are considered as herbal material [Zięba 2000, Truchliński 2006].

Applying herbs in a diet, dietetics, prophylaxis and treatment of some human's and animal's disorders is known for a long time; however, herbs used for animal feeding are usually remains from the processing of herbs for medicinal agents. Active substances present in herbs modify and stimulate the functioning of both human and animal organisms.

Addition of some plants or taste-flavor additives can contribute to the tastiness improvement, hence better fodder uptake and larger body weight gains. Studies conducted by Faruga and Jankowski [1996], who tested the effects of 0.5% or 1% addition of herbal mixture Melamix, confirmed that statement. Achieved results indicate better utilization of a fodder by experimental-group birds as well as higher efficiency of leg and breast muscles. Moreover, it was found the cholesterol level decrease in plasma of birds fed that way. Apparently higher sensory assessment of the roasted meat of experimental group as compared to the control was the main virtue of herb addition. The meat was characterized by higher juiciness and crispness, desired taste and flavor. Other study conducted by Faruga and Jankowski [1996] applying 0.5% or 1% herbal mixtures (pepper mint, birch leaves, nettle, chamomile, yarrow, birdweed) vs. another mixture, in which roots and leaves of comfrey replaced birch and mint leaves, confirmed earlier findings.

Administering the diet with 1% addition of herbal mixture Vipromix [Faruga and Pudyszak 1999] composed of mint, horsetails, caraway seeds, chamomile, savory, birdweed, and sweet flag rhizomes had positive effects on healthiness, which manifested as decreased mortality amongst experimental bird groups. Another virtue of that mixture was lower utilization of a fodder per 1 kg of body weight gain as well as higher gains as compared to the control birds. Čermak *et al*. (1996) achieved similar results when used Biostrong preparation that made the improvements in bird's condition (decrease of their mortality in experimental group). It also strengthened the immune response towards prophylactic vaccination [Fabczak *et al*. 1998].

The dietetic value of a meat and other animal-origin products becomes higher due to herbs in a diet of swine and poultry [Fritz and Grela 1995]. Better production effects using herbal mixtures rather than single herb species were achieved in numerous experiments. The set of plants can be selected in such a way to get expected health and production effects or intensify any desired traits. It was reported that not every plant species is readily consumed by animals. Swine dislikes sweet flag rhizomes, wild thyme, mistletoe, wormwood, and goat's rue [Fritz and Grela 1995, Grela 2001].

Herbs can be applied in a single form or as mixtures [Kinal *et al*. 1998] at the amounts up to 5% for adult birds. Higher herb percentage makes higher crude fiber level in a mixture, thus it can have negative consequences of poultry production effects. The crude fiber content in dried herbs is 23%–32%. Herbs also contain proteins (9.7%–16.1%), fats (1.2%–12.4%), and no-nitrogen extractable substances (23.4%–46.2%) [Mazanowski 2008].

Herbs have been used for poultry fodder additives for many years. Up-to-date, antibiotics that are at present eliminated from mixtures and replaced with alternative products (e.g. herbs) were applied as growth stimulators [Fritz *et al*. 1990]. Using the herbs in broiler-chicken feeding mainly aims at improving their metabolism rate and health. It can be achieved by means of lowering the population of pathogenic bacterial flora and stimulation of development of desired microorganisms that contribute to better nutrient utilization as well as to decrease of ammonia and hydrosulfides emission [Fritz *et al*. 1990, 1992]. Herbs affect the slaughter traits of broiler-chicken carcasses. According to many authors, they make lower fat adiposity [Fritz *et al*. 1990, 1999, Czaja and Gornowicz 2004]. Fritz *et al*. [1995] also found a positive influence of yarrow mixture with St. John's-wort on carcass skin color, and 1% garlic addition elevated the organoleptic assessment of slaughter broiler-chicken's meat. Kinal *et al*. [1998] reported similar re-

sults applying balm and yarrow in their study. These herbs had positive effects on carcass skin color and meat sensory features.

Truchliński *et al*. [2006], who applied the garlic extract, observed significant changes in concentrations of some elements at blood plasma and tissues of turkey hens. They also recorded the increase of Mg, Fe, Zn, and Cu concentrations, along with the higher retention of Cu, Fe, and Zn in the liver, and Mn, Mg, Cu, and Zn in feathers.

Herbs can modify the meat chemical composition, namely protein and fat contents. Czaja and Gornowicz [2004] performed the experiments involving broiler-chickens fed with a fodder with 1–2% herbal mixture (pot marigold, St. John's-wort, chamomile, pepper mint, and common nettle) to analyze the composition of chicken's muscles. The largest protein level (23.74%) was found in pectoral muscles of bird's group fed with a mixture containing the highest herb percentage. Herb addition to mixtures applied in chicken's diet also affects their blood physiological indices. Schleicher *et al*. [1998] reported increased leukocyte and glucose levels in blood of chickens given with caraway and chamomile additives. Fritz *et al*. [1994] found that herb addition enhanced the alanine aminotransferase activity (ALAT) in bird's blood plasma, which was confirmed by Kinal *et al*. [1998]. These authors in earlier works proved that erythrocyte and hematocrit levels increased in blood of chickens fed with a herbal-enriched fodder mixture [Fritz *et al*. 1990]. Herbs are in a position of changing the length of some digestive tract fragments: e.g. small intestine. Schleicher *et al*. [1998] and Fritz *et al*. [1994] reported apparent elongation of small intestine at chickens administered with herbal extracts. Moreover, they found morphological differences manifesting as some elongation of intestinal villi, hence enhancing the intestine's absorption area. The increase of the lymphatic system activity in small intestine wall was also recorded [Fritz *et al*. 1999]. Kapica *et al*. [2006] also observed positive effects of herbs on digestion processes at poultry (addition of melissa, hop, linden, mint, pansy, and nettle) manifesting as the increase of the total protein and the decrease of proteolytic enzymes activities.

Very good results in feeding the hatching hens were recorded due to dried nettle administration [Dubińska and Faruga 1967, Wężyk *et al*. 1993]. Sembratowicz [2004], who used yarrow, purple coneflower, birdweed, and nettle extracts in turkey hen's diet, reported the influences of these herbs on bird's immune system. Administering the Biostymin (water extract of Krantz aloe) and Bioaron C (extract made of chokeberry juice, vitamin C addition, and aloes extract) stimulated the immune system. Furthermore, Biostymin addition caused better body weight gains at lower fodder utilization.

There is also beta-glucan on market; it is produced from baking yeasts cell walls. It affects the increase of lysozyme activity and increase of NTB-positive cells percentage in experiments with turkey hens. It also made the decrease of alanine and aspartate aminotransferases activities (ALT and AST) as well as the increase of HDL fraction share. Administering that preparation contributed to better body weight gains [Truchliński *et al*. 2005a]. Applying the Ginsengin 200 preparation (ginseng root extract) as an additive to drinking water for turkey hens influenced on a significant body weight gain at lower fodder utilization. The increase of the total leukocyte count in white-cell picture was recorded in these birds' blood, which can be considered as a positive effect of the preparation on the immune system. The decrease of falls among chickens and diminished population of bacteria cultures in their feces were also recorded.

MATERIAL AND METHODS

Studied material comprised of 6-week-old turkey hens of heavy BIG-6 type maintained since 6 till 16 week of life on a straw litter in cages. The type was chosen, because of current problems with their rearing - birds were ill due to stress conditions during maintenance, which is usual at turkey. Birds were kept under standard zoohygienic conditions according to recommendations set by Faruga and Jankowski [1996]. The turkey hen's health state was monitored by veterinary doctor for the whole experiment.

Study was realized under production conditions with isolated boxes for experimental groups. Birds were randomly divided into 2 experimental groups consisting of 40 turkey hens each (2 replications of 20 birds each).

Group I was the control. Birds of group II for 28 days were administered with water solution of hawthorn preparation (0.35 ml/kg b.w./daily). Intractum crataegi preparation was an aqueous extract prepred on the basis of *Crataegi inflorescentia intractum*. After 28 days, a 2-week break was applied, and then the preparation was given to turkey hens again for 28 days.

In order to determine the daily dose of tested additive, all birds were weighed once a week, and in addition, 10 turkey hens of every group – daily. Results were then averaged. For the whole experiment, turkey hens of all groups were *ad libitum* fed with full-dose mixtures (Provimi, Poland) in accordance with a program for 5 feeding cycles. Contents of general nutrients in a fodder corresponded to current recommendations of poultry feeding norms [Zalecenia żywieniowe... 2005].

At the end of the 9th, 11th, and 15th week of life, a blood for analyses was taken from the wing vein of turkey hens. The antioxidation enzymes activity was determined in erythrocytes by means of spectrophotometric method. The superoxide dismutase (SOD) activity measurements were made applying the adrenalin method according to Misr with modifications for 320 nm wavelength. The method was modified to get better selectivity of transient reaction products at that wavelength [Bartosz 1995]. Catalase (CAT) activity was determined according to Bartosz' method; antioxidation potential of plasma (FRAP) was assayed according to method by Benzie and Strain [1996] and Iris *et al*. [1996].

Vitamin C content in blood plasma was determined colorimetrically in a reaction with 2,6-dichlorphenylindophenol according to Omaye *et al*. [1979].

Copper (λ 324,8 nm), zinc (λ 213,9 nm) and iron (λ 248,3 nm) contents were determined in blood plasma samples by means of ASA technique in Central Apparatus Laboratory, University of Life Sciences in Lublin, by means of inflammable AAS technique UNICAM 939. First a 1g sample of each of the products being examined was taken. The samples were then burned down in muffle furnace in 550 ± 25^{o}C. Next the incineration ashes were dissolved in hydrochloric acid in crucibles. Received solution was filtrated into flasks and completed with water. This way stock solution was obtained. 10 × and 100 × diluted solutions were also prepared. The results are given in mg/kg fresh substance (f. s.)

Achieved numerical data were subjected to statistical analysis with a help of Statistica ver. 5 software. The single-factorial variance analysis ANOVA was applied at the significance level 0.05.

RESULTS AND DISCUSSION

Among enzymes with antioxidant properties, superoxide dismutase (SOD), glutathione peroxidase (PGx), and catalase (CAT) are most often used. Data upon SOD and CAT activities in turkey hen's blood plasma are presented in Table 1. Here performed study revealed the increase of SOD activity within 10 weeks of observations in the case of hawthorn preparation (37.4 U/ml) as compared to the control group (30.5 U/ml). Such increase of the enzyme activity level was statistically significant ($p < 0.05$). Although no influence of tested preparation on CAT activity was recorded. Research made by Ognik *et al*. [2004], who applied purple coneflower extract (Echinovit C) as an additive to drinking water for turkey hens, did not prove its effects on neither SOD or CAT activities in bird's blood plasma. Also own study results indicate the lack of dependence between plant-origin preparation made of hawthorn and CAT activity increase. No works upon the influence of hawthorn addition in turkey hens diet on antioxidation enzymes levels makes difficult to compare and to confront own results with other authors ones.

Besides above enzymes, non-enzymatic antioxidants such as vitamins A, C, E, uric acid, bilirubine, glutathione, malonic dialdehyde (MDA), and total antioxidant potential (FRAP), are also important. An apparent influence of administered hawthorn preparation on FRAP was recorded in the study (Tab. 1). Significant difference ($p < 0.05$) between groups was obvious at the 15th week of life amounting to 169 μmol/l at birds fed with hawthorn vs. 158.9 μmol/l at the control turkey hens. Among many minerals that take part in antioxidation reactions, transition metals ions (zinc, manganese, copper, selenium, and iron) are highly interesting, because they enter the catalytic centers of some enzymes. Deficiency of these elements in blood plasma sometimes leads to the decrease of enzymatic activity [Kleczkowski *et al*. 2004]. Contents of minerals (copper, iron and zinc) in blood plasma are presented in Table 2. Copper content at turkey hens given with hawthorn as a fodder additive at the amount of 0.35 ml/kg b.w./day was at much higher level than for control group. The highest level of the element was recorded in III group of experimental birds (27.8 μmol/l). In reference to the control (22 μmol/l), achieved difference appeared to be statistically significant. Ognik *et al*. [2004] observed similar dependence in blood plasma of turkey hens fed with 1,2,4-triazol derivative. In the case of iron, statistically significant difference was also present between III group of turkey hens that were administered with hawthorn preparation (38.4 μmol/l) and the control (33.2 μmol/l). Zinc contents – regardless of the blood intake routine – was at similar levels in all groups. Sembratowicz *et al*. [2004] reported similar facts when used Bioaceron C preparation as an additive to drinking water. Instead, Makarski and Polonis [2001], when feeding turkey hens with herbal mixture containing yarrow, pot marigold, *Capsella*, hawthorn, horsetails, nettle, and chokeberry pulp, achieved slightly different results: zinc and copper contents increased (20.88 mg dm^{-3} and 1.548 mg dm^{-3}, respectively) in blood plasma of turkey hens of experimental group as compared to the control (15.12 mg dm^{-3} and 1.007 mg dm^{-3}, respectively), as well as iron level significantly decreased (212.47 mg dm^{-3}) vs. control group (231.42 mg dm^{-3}).

Table 1. Levels of antioxidation indicators in blood plasma of 9-week-old turkey hens (4th, 6th, and 10th observation weeks) fed with hawthorn preparation
Tabela 1. Poziom wskaźników antyoksydacyjnych w surowicy krwi 9-tygodniowych indyczek (4 tydzień obserwacji, 5 tydzień i 10 tydzień) otrzymujących preparat z głogu

Specification Wyszczególnienie	Week Tydzień	Experimental groups Grupy doświadczalne	
		control kontrola	hawthorn (0.35 ml/kg b.w./day) głóg (0,35 ml/kg m.c./dzień)
SOD (U/cm^3) Dysmutaza ponadtlenkowa	4 6 10	21.4 ±5.30 32.4 ±8.21 30.5^b ±6.7	27.5 ±6.21 29.7 ±4.31 37.4^a ±9.12
CAT (U/cm^3) Katalaza	4 6 10	6.27 ±1.03 5.43 ±0.92 9.27 ±1.32	6.88 ±0.82 7.55 ±1.12 8.27 ±1.03
FRAP (µmol/l) Całkowity potencjał antyoksydacyjny osocza	4 6 10	150.0 ±31.2 142 ±28.9 158.9^b ±32.8	158.0 ±22.4 140.3 ±31.3 169.0^a ±27.5
Vitamin C (mg/l) Witamina C	4 6 10	0.22 ±0.04 0.20 ±0.05 0.25 ±0.04	0.24 ±0.02 0.25 ±0.05 0.29 ±0.05

a, b – $p < 0.05$ statistically significant difference
a, b – $p < 0,05$ różnice statystycznie istotne
± standard deviation
± odchylenie standardowe

Table 2. Microelements contents (Cu, Fe, Zn) in turkey hens blood plasma
Tabela 2. Zawartość mikroelementów (Cu, Fe, Zn) w surowicy krwi indyczek

Specification Wyszczególnienie		Experimental groups Grupy doświadczalne	
		control – kontrola	hawthorn – głóg
Cu (µmol l^{-1})	1 2 3	23.8 ±2.82 21.6 ±1.35 22.0^b ±2.45	24.5 ±1.72 22.7 ±1.31 27.8^a ±3.0
Fe (µmol l^{-1})	1 2 3	32.4 ±2.23 32.2 ±2.79 33.2^b ±1.16	35.4 ±1.47 30.5 ±2.44 38.4^a ±2.04
Zn (µmol l^{-1})	1 2 3	27.7 ±2.78 29.3 ±3.58 31.4 ±1.74	27.8 ±2.32 28.7 ±1.53 32.7 ±2.32

a, b – $p < 0.05$ statistically significant difference
a, b – $p < 0,05$ różnice statystycznie istotne
± standard deviation
± odchylenie standardowe
1 – first blood intake, 2 – second blood intake, 3 – third blood intake
1 – pierwsze pobranie krwi, 2 – drugie pobranie krwi, 3 – trzecie pobranie krwi

Studies involving the plant-origin preparation made of hawthorn and given to turkey hens along with a fodder revealed the increase of superoxide dismutase activity, which was correlated with the copper content increase in blood plasma of tested birds. Other studies upon animals and people [Gehre *et al*. 1999, Bertineto *et al*. 2003] also indicate the presence of the dependence between copper concentration in blood plasma and SOD activity in erythrocytes. Available literature provides with a few data on antioxidation status of animal organisms administered with hawthorn as a fodder additive, and existing study results are very distinct, hence drawing univocal conclusions is very difficult.

CONCLUSIONS

1. Significant increase of superoxide dismutase (SOD) activity was recorded at turkey hens fed with the hawthorn preparation at the end of experiment.

2. Addition of the hawthorn preparation caused considerable increase of copper and iron contents in turkey hen's blood plasma.

3. Applying hawthorn preparation Interactum crataegi at the amount of 0.35 ml/kg b.w./day as an additive to drinking water for turkey hens made the increase of the total antioxidation potential (FRAP) in blood plasma of experimental birds.

4. Achieved results indicate the possibility to use tested preparation as a fodder additive in turkey hen's diet. However, it requires further verification at studies involving larger bird's populations as well as taking into account other ways of the preparation administration.

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Streszczenie. Celem pracy było zastosowanie preparatu z głogu *Interactum crataegi* w dawce 0,35 ml/kg m.c./dzień jako dodatku do wody pitnej dla indyczek. Badania wykonano na sześciotygodniowych indyczkach typu ciężkiego BIG 6. Ptaki podzielono na dwie grupy, grupę kontrolną i doświadczalną, w której indyczki otrzymywały wraz z wodą pitną roślinny preparat z głogu w ilości 0,35 ml/kg m.c./dzień. Podczas badań zwierzęta otrzymywały *ad libitum* standardowe mieszanki pełnoporcjowe firmy Provimi Polska, zgodnie z programem obejmującym 5 okresów żywieniowych. We krwi oznaczono aktywność dysmutazy ponadtlenkowej, katalazy, całkowity potencjał antyoksydacyjny osocza a także zawartość witaminy C oraz Zn, Cu i Fe. Pod koniec okresu doświadczalnego nastąpił wzrost poziomu całkowitego potencjału antyoksydacyjnego we krwi ptaków doświadczalnych oraz wzrost aktywności dysmutazy ponadtlenkowej. Wzrost aktywności dysmutazy ponadtlenkowej był skorelowany ze wzrostem poziomu miedzi.

Słowa kluczowe: preparat z głogu, wskaźniki antyoksydacyjne, indyczki