Praca oryginalna *Original paper*

Effect of water supplemented with a herbal product on the internal organs of broiler chickens

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Summary

We analyzed the effect of a herbal (phytoncides) supplement on the morphology of internal organs and intestinal morphometry of broiler chickens. The study was conducted on 60 birds divided into 3 groups (n = 20): one control and two experimental. Starting at 3 weeks of age, chickens from the experimental groups were given water with the addition of a preparation based on concentrated natural components in two doses: 1 and 2 ml/l. Group 1 was given the supplement twice for 3 and 5 days, and group 2 once for 3 days. After slaughter at the age of 40 days, the proventriculus, gizzard, duodenum, jejunum, ileum, cecum, liver, spleen, kidneys, bursa of Fabricius (BF), lungs, and pectoral muscles were sampled for histological analyses. Additionally, villi height (VH) and crypt depth (CD) in the duodenum, jejunum, and ileum were measured. The results did not show statistically significant differences between the groups in the number of histological lesions in the organs and tissues sampled. Morphometric evaluation showed statistically significant differences between the groups ($p < 0.05$) for VH and CD in the duodenum and ileum.

Keywords: broiler chicken, water, herbal supplement, histopathology, morphometric changes, small intestine

The assurance of water quality and safety in the poultry production industry is a key factor for birds' performance and the safety of meat and meat products. On one hand, drinking water can become a source of serious microbiological and chemical hazards. On the other hand, in poultry production, it can be used as a medium for the distribution of drugs and supplements. Poultry, kept in flocks, are frequently given a solution of herbs in their drinking water on a daily basis to ensure their health and prevent diseases (14). Numerous phytogenic compounds applied this way show antimicrobial, antifungal, and antioxidant properties (22), as well as preventative, therapeutic, and immunomodulatory effects on humoral and cell-mediated immunity in chickens and turkeys or immunomodulatory effects against protozoa (9, 17, 30). This is due to the fact that some plants and their extracts contain volatile oils that can alleviate coccidiosis and reduce its severity (5). There are also reports showing that plant and herbal supplementation of water given to poultry prevents natural infections by viral diseases (29) and may result in enhanced productivity, growth performance, welfare, feed efficiency, and, ultimately, poultry meat quality (1, 20, 27, 28). Plant and herbal treatment of poultry can also contribute to decreased ammonia production (14). Thus, herbal and plant-based drugs

and supplements can ensure all aspects of One Health, mainly because of the absence of drug resistance development and their high efficacy. However, there are proven limits to broiler performance with respect to intestinal health (8) and internal organ pathology (21). It is also noteworthy that phytochemicals have not been established as essential nutrients and, when in excess, could be detrimental (7), which makes it important to study their effects.

The aim of our research was to verify the histopathological and morphometric patterns of selected organs and tissues of broiler chickens after supplementation of drinking water with an authorized preparation containing natural plant and herbal components.

Material and methods

Water supplement. The phytoadditive (Poland) used in this study is a dietary supplement authorized for use in poultry and designed for the control of *Eimeria* spp. and anaerobic bacteria. It contains glycerin, sorbitol, and sensory additives, i.e. 51.75% of herbal extracts – products processed from spices and herbs (*Sinapis alba* L., *Piper nigrum* L.), products from the processing of plants (*Acorus calamus* L., *Saponaria officinalis* L.), as well as thymol 0.5%, methyl salicylate 0.5%, eucalyptus oil 0.5%, anise oil 0.5%, eugenol/isoeugenol 0.5%, garlic oil 0.5%, and mustard oil 0.5%. Dosage, as recommended by the manufacturer in broiler chicken: 1-1.5 l/1000 l of drinking water for 2 to 5 days, depending on the intended effect (preventive/supporting digestion and absorption).

Animals, supplementation, and management. A total of 60 Ross 308 male broiler chickens were randomly divided into three groups (B1, B2, and B3) of 20 birds each. They were housed in isolated pens in a biosafety level 3 facility. Water and commercial feed were provided *ad libitum*. At the age of 3 weeks, the birds from groups B1 and B2 were administered multiherbal supplement in drinking water. Group B1 was given supplemented drinking water first for 3 days (1 ml/l) and then for 5 days (2 ml/l) with a 9-day interval between the water supplementation procedures. Group B2 was given supplemented drinking water for 3 days (1 ml/l). Group B3 was the control group. None of the birds died during the experiment. At the age of 40 days, all birds were slaughtered in a separate production process in a slaughterhouse registered according to the EU law (24). The carcasses were weighed, and tissue samples were collected from 6 random birds from each of the three groups.

Histopathological evaluation. Samples of internal organs, i.e. proventriculus, gizzard, duodenum, jejunum, ileum, cecum, liver, spleen, kidneys, bursa of Fabricius (BF), lungs, and pectoral muscles, were fixed in 10% neutralized formalin and embedded in paraffin blocks. The sections (4 μm thick) were stained with haematoxilin and eosin (HE). They were imaged with a Panoramic Scanner MIDI 3DHISTECH (3DHISTECH, Hungary). The microscopic pattern was evaluated subjectively by two pathologists.

Morphometric analysis. Villi height (VH) and crypt depth CD) in the duodenum, jejunum, and ileum were measured from the tip of the villi to the top of the crypt and from the base of the villi to the submucosa, respectively. Each section was imaged with a Panoramic Scanner MIDI (3DHISTECH, Hungary). The measurement data for the alimentary system were prepared with the Panoramic Viewer software (3DHISTECH, Hungary).

Statistical analysis. Statistical analysis was performed with the Statistica 13 PL software (StatSoft Inc). In order to compare differences in the number of microscopic changes in internal organs and tissues, the difference test was used for two structure indices (fractions). The results were considered statistically significant at $p < 0.05$. The Shapiro-Wilk test was used to examine the distribution of variables obtained in morphometric analyses, and the Leven test determined the homogeneity of their variance. The Kruskal-Wallis test and analysis of variance were used to compare differences between the means, and multiple comparison tests were used as well. The variables are presented in box-whisker plots. The results were considered statistically significant at $p < 0.05$.

Results and discussion

Body weight. After defeathering the whole carcass, average body weights were 2.44 kg in group B1, 2.52 kg in group B2, and 2.65 kg in group B3. There were no statistically significant differences in body weight between the groups.

Histopathological findings. A total of 72 samples of 12 different organs and tissues were analyzed. Only samples of the cecum were free of histopathological lesions in all groups. All other organs showed variously distributed retrogressive, progressive, and circulatory lesions, including inflammatory patterns. The kidneys showed 6 different pathological patterns, including retrogressive, inflammatory, and circulatory lesions. Five different lesions were also noted in the duodenum and liver, which additionally showed proliferation of bile ducts. Retrogressive changes were most frequent. They were present in 9 different organs, followed by inflammatory lesions present in 7 organs. Circulatory lesions were visible in 4 organs, and progressive ones in 3 organs. However, no significant differences in the number of particular lesions were observed between the groups. A detailed distribution of microscopic lesions in different groups is presented in Table 1.

Morphometric analyses. Morphometric analysis of particular segments of the alimentary tract showed statistically significant differences for VH in the duodenum between groups B1 and B2 and between B1 and B3 ($p < 0.05$). There was no statistical difference between groups B2 and B3 ($p > 0.05$, Fig. 1).

Statistically significant differences between all groups were observed in terms of duodenal CD ($p < 0.05$, Fig. 2). Similar results were obtained for VH and CD in the ileum. There were statistically significant differences in VH between groups B1 and B2 and between B1 and B3 ($p < 0.05$). There was no statistical difference between groups B2 and B3 ($p > 0.05$, Fig. 3).

Statistically significant differences between all groups were observed for CD in the ileum ($p < 0.05$, Fig. 4). Morphometric analysis of the jejunum did not

Tab. 1. Types of histopathological lesions found in organs and tissues in all chicken groups (n = 6)

Morphological lesions	Group B1	Group B2	Group B3
Proventriculus		Number of birds	
infiltration of lymphoid cells in glands	6	5	6
necrosis of glandular epithelium	2	0	4
replacement of glandular epithelium with ductal epithelium	6	6	6
Gizzard		Number of birds	
focal infiltration of lymphoid cells in mucosa	2	2	0
focal disruption of koilin layer	1	0	0
Duodenum		Number of birds	
congestion of the mucosa	0	2	0
focal infiltration of lymphoid cells in mucosa	5	0	3
focal infiltration of heterophils in mucosa	0	1	1
focal necrosis of crypts epithelium	3	0	2
focal presence of coccidia	1	0	0
Jejunum		Number of birds	
congestion of the mucosa	1	0	1
focal infiltration of lymphoid cells in mucosa	0	0	1
focal infiltration of heterophils in mucosa	0	0	1
focal necrosis of crypts epithelium	0	0	1
Ileum		Number of birds	
focal infiltration of lymphoid cells in mucosa	0	1	0
Cecum		Number of birds	
no lesions	0	0	0
Liver		Number of birds	
congestion	0	2	2
fatty degeneration of hepatocytes	1	0	1
infiltration of lymphoid cells around blood vessels and bile ducts	3	3	2
infiltration of heterophils around blood vessels and bile ducts	2	2	2
proliferation of bile ducts	1	0	0
Kidney		Number of birds	
congestion	2	3	3
mesangioproliferative glomerulopathy	1	2	3
focal necrosis of convoluted tubules epithelium with heterophils infiltration	0	2	0
focal interstitial infiltration of lymphoid cells	0	0	1
multifocal interstitial infiltration of lymphoid cells	0	0	1
focal interstitial infiltration of heterophils	0	0	2
Spleen		Number of birds	
congestion	0	1	0
atrophy of lymphoid tissue	2	4	3
reticular cells hyperplasia	1	3	3
Bursa of Fabicius		Number of birds	
atrophy of lymphoid tissue	0	1	1
Lungs		Number of birds	
congestion	1	1	1
cartilaginous nodule	2	3	1
Pectoral muscle		Number of birds	
focal defragmentation of muscle fibres	4	5	4
focal necrosis of muscle fibres with inflation of lymphoid cells	1	0	3
multifocal necrosis of muscle fibres with infiltration of lymphoid cells	1	1	1
fibromuscular dysplasia in artery	1	0	0

show any statistically significant differences between the groups in terms of VH and CD.

Most studies show a positive effect of natural feed supplementation with a single natural additive in different forms (10, 15, 16, 31). These additives are proven to be a good substitute for antimicrobial growth promoters that are not allowed in animal food production in the EU. This is why much attention is paid to production parameters when natural supplements are verified and used. Any additional effects that are not directly related to production efficiency, because of the short life span of broiler chicken, are not widely studied. The scarcity in evidence for the beneficial effects of phytoadditive compounds in terms of nutrient digestibility and gut function, when used in broilers, has already been noted (23). Data on supplementation of water are also scarcer than those on supplementation of feed, and there are very few reports on water supplementation with a mixture of natural additives for broiler chickens.

There are no published data on the effects of the multiherbal supplement tested in this study, but it consists mostly of herbal extracts proven to be beneficial feed additives (11). Most of them are reported to have positive effects on poultry performance and health as digestion stimulants and growth promoters, as well as hepatoprotective

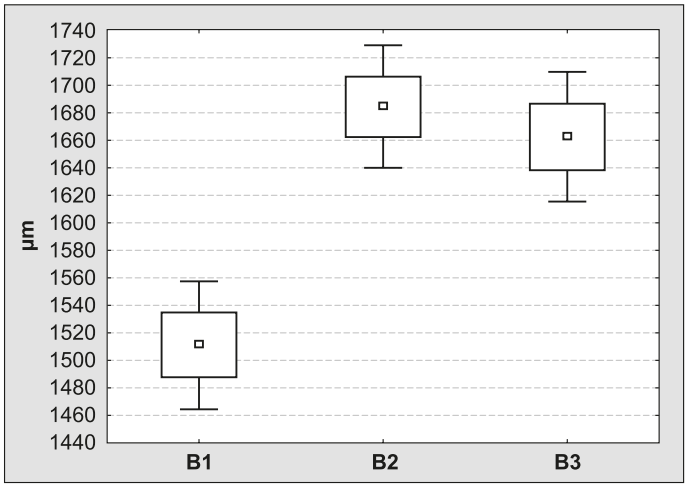

Fig. 1. Distribution of the means of VH in the duodenum in all experimental chicken groups

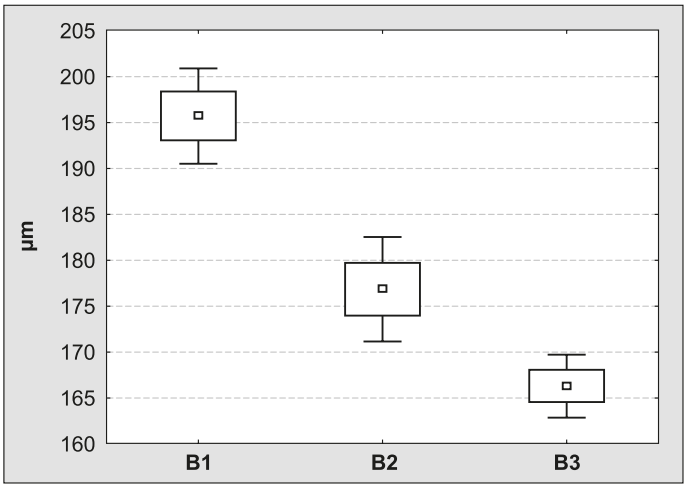

Fig. 2. Distribution of the means of CD in the duodenum in all experimental chicken groups

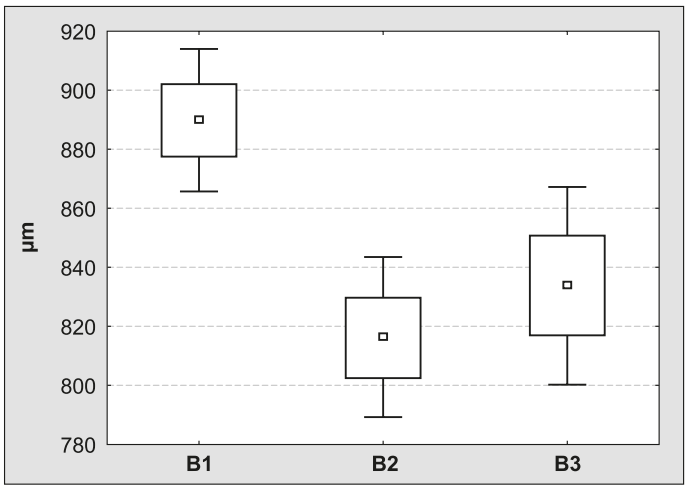

Fig. 3. Distribution of the means of VH in the ileum in all experimental groups

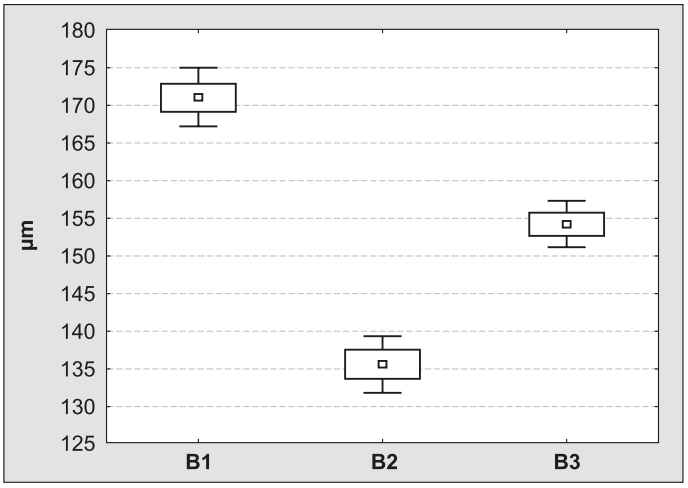

Fig. 4. Distribution of the means of CD in the ileum in all experimental chicken groups

and anti-ulcerogenic agents (33). Nevertheless, the efficacy of phytogenic applications in poultry depends on many factors. The most important ones are the combination of the active components, dosage, bird genetics, and overall diet composition. This was shown in studies on different products including commercial and non-commercial feed additives, which consisted of a single or a mix of different natural components (23), as in our experiment.

The supplement examined in this study did not show any statistically significant impact on the histopathological pattern of the organs and tissues analyzed. Our observations are similar to those by other authors supplementing water with silver nanoparticles (as a disinfectant) (18), anaerobic gut microbes (25), or commercial organic acid (6). These observations may point to water as a neutral and beneficial medium for supplements in terms of the health and condition of different internal organs of birds. Other authors, however, observed histopathological lesions in the liver and testicles after the administration of a phytogenic supplement in chicken through feed in 42-day trials (21).

The mixture of plant extracts present in the multiherbal supplement used in our experiment affected the morphology of the intestinal tract in various ways, depending on the intestinal section. A comparison of its effects is presented in Table 2, which shows that the development of the intestinal tract in broiler chickens can be stimulated by the multiherbal preparation as a water supplement.

The health status of poultry and their intestinal condition is reflected by VH and CD. The absorption of nutrients is higher when VH increases and CD decreases. The producer's recommendation is to use the supplement once to improve digestion and absorption. Our

Tab. 2. The effect of water supplementation according to two different protocols on CD and VH compared with the control group

Measured parameter	Duodenum		Jejunum		Ileum	
	single application	double application	single application	double application	single application	double application
VH	no impact	decrease	no impact	no impact	no impact	increase
CD	increase	increase	no impact	no impact	decrease	increase

results do not fully confirm this effect/improvement. However, according to morphometric measurements, such improvement occurred to some moderate extent in the last segment of the small intestine. This result is consistent with the fact that the main role of the ileum is thought to be water and mineral absorption (26). We did not observe an increase in VH and CD in duodenal and jejunal sections, respectively. This is in contrast to results obtained in an experiment in which the addition of two different phytobiotics, i.e. *Persicaria odorata* and *Piper betle*, to feed caused an increase in VH and a decrease in CD in the duodenum and jejunum in chickens (4). Increased VH and CD were observed in other studies, for example in those in which feed was supplemented with safflower oil and vitamin C (2). The same authors reported increased VH and CD in the ileal segment in safflower-supplemented groups, which confirms the results of our experiment in the case of the double application of the supplement and the rule that larger villi have larger crypts to ensure their renewal (32). In another experiment, a commercial polyherbal additive given to Ross 308 broilers in the feed affected ileal morphology, which resulted in increased VH, while CD did not differ from the control group (12). Similar to our experiment, this protocol was conducted without any challenge or stress-induced conditions. According to the producer of the multiherbal supplement used in the study, re-supplementation is aimed at combating protozoan infestation that is related to the protozoan development cycle. Our results suggest that full treatment against protozoa may also bring additional benefits by improving absorption in the ileum. The morphology of the gastrointestinal tract during *Eimeria* infection, which is explained elsewhere (5), indicates that various conditions affecting VH, CD, and other parameters depend on the composition of the botanical supplements used.

Nevertheless, it must be noted that improvement in the development of the small intestine and its histomorphology greatly depends not only on substances present in the feed, but also on the form of the feed. In the current study, all birds were supplied with the same feed so that its composition, or formula, could not explain the effects noted. Some authors reported that the more mashed the feed, the smaller VH and CD (3). In this context, water appears to be a much less potent medium for supplement application than regular feed. However, its role in another kind of supplementation in poultry was reported as significant (13).

Another of our findings was a decrease in CD in the ileal part of the small intestine after a single water supplementation. Reduced CD may have multiple effects on the colonization by microorganisms responsible for many diseases of the digestive system. It is hypothesized that a smaller crypt size may alter the chemical and nutritional environment and thus inhibit microbial growth and settlement. It is also possible that other competitive microflora may inhabit smaller crypts (19) and prevent invasion by pathogenic bacteria. However, this finding of our experiment should be confirmed by further microbiological research because results vary significantly depending on the supplement or mix of supplements analyzed.

This study had some limitations. First, because information on the effects of the multiherbal water supplement on the morphology of different organs and tissues was lacking and the chance of success of our experiment was highly variable, a minimum sample size was established to find statistical significance. Second, we did not study the production parameters of the birds. Nevertheless, our experiment can be useful in designing large growth performance studies to validate herbal products authorized for use in poultry. Future studies should verify whether an increased VH in the ileum actually results in improved nutrient absorption. The effect of an increased CD on the inhibition of intestinal microflora should also be verified.

In conclusion, the supplementation of drinking water with the multiherbal preparation did not influence the histological pattern of the internal organs and tissues of broiler chickens. It modulated intestinal architecture, and its effect may be related to an improvement in intestinal functioning and a more efficient intestinal absorption.

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