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EFFICACY OF PLANT EXTRACT ON PERFORMANCE AND MORPHOLOGICAL AND HISTOCHEMICAL PATTERNS OF DIGESTIVE TRACT WALL IN CHICKENS

DJELOTVORNOST BILJNOG EKSTRAKTA NA PROIZVODNA SVOJSTVA I MORFOLOŠKIH I HISTOKEMIJSKIH UZORAKA STIJENKE PROBAVNOG TRAKTA U PILIĆA

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SUMMARY

The ban on the use of antibiotics as antimicrobial performance promoters in animal Nutrition has been in force since 1986 in Sweden and since 1999 in EU countries. In the European Union only three feed antibiotics are still permitted - Salinomycin-Na, Fiavophospholipol and Avilamycin and general ban is expected at the end of 2005. At present coccidiostatics are not included in the list of banned antibiotics for poultry.

As substitutes for feed antibiotics different feed additives can be used. However, their activities, mechanisms and effectiveness can not bee fully compared to the antibiotics. The most effective in antimicrobial operation are acidifiers, probiotics, prebiotics, peptide concentrates, herbs and plant extracts.

Dried herbs contain numerous biologically active substances (Bakhiet and Adams, 1995; Baratta et al., 1998; Best, 2000; Dorman and Deans, 2000). Their nutritional value and potency depend on the plant species, soil quality and weather conditions during growth, harvest time and technology as well as the conservation (drying) technique used. Thus, standarization of herbal preparations is very difficult to obtain.

However, these preparations, chemically extrac-ted from different parts of plants contain precisely defined active compounds which allows standarization of these mixtures and their protection against harmful chemical and mechanical activities.

The sources of biologically active plant substances are very rich and numerous. In some countries, for example in China, over 6000 different species of spices and herbs are used, but even more than 12 thousand different plant species are used for medicinal purposes (Chen et al., 2003). The most recognized herbs are presented in Table 1.

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Table 1. Plant extract activities
Tablica 1. Djelovanje biljnog ekstrakta

Plant species Vrsta biljke	Utilized parts Korišteni dijelovi	Main active compounds Glavni aktivni sastojci	Reported properties - Navodna svojstva
Aromatic spices - Aromatične mirodije			
Nutmeg - Orašac	Seeds - Sjemenke	Sabinene	Digestion stimulant, antidiarrhoeic - Potiče probavu, protiv proljeva
Cinnamon - Cimet	Barks - Kora	Cinnamaldehyde	Appetite and digestion stimulant, antiseptic activity Potiče apetit i probavu, antiseptičko djelovanje
Clove - Klinčić	Cloves - Cvjetić	Eugenol	Appetite and digestion stimulant, antiseptic activity Potiče apetit i probavu, antisept. djelov.
Cardamon - Kardamon	Seeds - Sjemenke	Cineole	Appetite and digestion stimulant Potiče apetit i probavu
Coriander - Korijandar	Leaves, seeds - Listovi, sjemenke	Linalool	Digestion stimulant - Potiče probavu
Cumin - Kim	Seeds - Sjemenke	Cuminaldehyde	Digestion stimulant, carminative, milk-producing Potiče probavu, protiv nadutosti, proizvodi mlijeko
Anise - Anis	Seeds - Sjemenke	Anethole	Digestion stimulant, milk-producing Potiče probavu, proizvodi mlijeko
Celery - Celer	Seeds, leaves - Sjemenke, listovi	Phtalides	Appetite and digestion stimulant Stimulira apetit i probavu
Parsley - Peršin	Leaves - Listovi	Apiol	Appetite and digestion stimulant, antiseptic Potiče apetit i probavu, antiseptik
Fenugreek - Kozji rog	Seeds - Sjemenke	Trigonelline	Appetite stimulant - Potiče apetit
Aromatic herbs and spices - Aromatične trave i mirodije			
Garlic - Češnjak	Bulbs - Glavice	Allicin	Digestion stimulant, antiseptic Potiče probavu, antiseptik
Rosemary - Ružmarin	Leaves - Listovi	Cineole	Digestion stimulant, antiseptic, antioxidant Potiče probavu, antiseptik antioksidant
Thyme - Majčina dušica	Whole plants - Čitava biljka	Thymol	Digestion stimulant, antiseptic, antioxidant Potiče prob., antiseptik, antioksidant
Sage - Žalfija	Leaves - Listovi	Cineol	Digestion stimulant, antiseptic Potiče probavu, antiseptik
Bay laurel - Lovor	Leaves - Listovi	Cineol	Appetite and digestion stimulant, antiseptic Potiče apetit i probavu, antiseptik
Peppermint Ljuta metvica	Leaves - Listovi	Menthol	Appetite and digestion stimulant, antiseptic Potiče apetit i probavu, antiseptik
Pungent spices - Ljute mirodije			
Capsicum - Paprika (Meksička papričica)	Fruits - Plodovi	Capsaicin	Antidiarrhoeic, anti-inflammatory, digestion stimulant, tonic - Protiv proljeva, protuupalno potiče probavu, tonik
Pepper - Papar	Fruits - Plodovi	Pipeline	Digestion stimulant - Potiče probavu
Horseradish - Hren	Roots - Korijen	Sinigrine, Allyl isothiocyanate	Digestion stimulant - Potiče probavu
Mustard - Gorušica	Seeds - Sjemenke	Sinigrine, Allyl isothiocyanate	Digestion stimulant - Potiče probavu
Ginger - Đumbir	Rhizomes - Podanci	Zingerole	Gastric stimulant - Potiče rad želuca

Starter mixtures contained 21.7% and grower mixtures 19.8% of crude protein. Mixtures were not supplemented with any conventional feed antibiotic or alternative substances; there only the coccidiostatic (Diclazuril) was applied.

For morphological and histochemical investigations from each treatment 7 birds aged 21 and 7 aged 42 days were selected, killed and the gastrointestinal tract was prepared for morphological and histochemical examinations. Immediately after animals death rings about 7 mm thick were taken from the middle area of the glandular stomach and 15 mm long pieces of small intestine from its middle area. Samples of tissues were placed in 7% neutral formol solution and shaked for better fixation. Paraffin slides of 4 μm thick were stained with hematoxylin (H) and eosin (E). Slides were examined under Axiophot-Zeiss microscope coupling with a camera and Multi Scan Base program. In each of the intestine slides, 6 well oriented villi and crypts were measured. The middle value for each bird and then for each group was established.

In the stomach thickness of the glandular layer and total amount of the mucous were measured. Besides, in order to determine the intensity and composition of mucous secretion, Alcian Blue (pH 2.5)-PAS (AB-PAS) method was used for acid and neutral mucopoly-saccharides differentiation and Aldehyde Fuchsine - Alcian Blue for sulfomucin and sialomucins differentiation (Lamb and Reid, 1969).

The supplementation of mixtures containing maize or wheat+barley with plant extract improved the feed conversion, digestibility of nutrients as well as decreased the fungus number, however these differences were insignificant. The improvement of concentration of Lactobacillus was stated only in groups fed mixtures based on wheat and barley and supplemented with XTRACT.

Moderate hyperplasia of the deep layer of proventriculus occured in 21 d-old animals fed diet supplemented with XT. This layer was also higher in birds fed maizediet. Glandular layer hyperplasia may indicate the increase of secretory activity and/or express the adaptation mechanisms. These dependences were not observed in 42 d-old chickens.

Table 2. Main sources of several bioactive compounds
Tablica 2. Glavni izvori nekoliko biodjelatnih sastojaka

Active substance - Djelatna tvar	Source of active substance - Izvor djelatne tvari
Anethole	Green anise, fennel - Zeleni anis, komorač
Borneol	Rosmarin, thyme - Ružmarin, majčina dušica
Carvon	Spearmint, carvi, aneth - Zelena metvica, kim
Caryophyllene	Clove, pepper, sage - Klinčić, papar, žalfija
Cinnamaldehyde	Cinnamon - Cimet
Cineole (Eucalyptol)	Eucalyptus, laurel, cardamone - Eukaliptus, lovor, kardamon
Cuminaldehyde	Cumin - Kim
Estragole	Estragon, basil, fennel - Estragon, bosiljak, komorač
Eugenol	Clove, cinnamon leafs - Klinčić, listovi cimeta
Linalol	Basil, thyme, orange flower, sage, coriander, laurel - Bosiljak, majčina dušica, narančin cvijet, korijandar, lovor
Allicin	Garlic - Češnjak
Sabinene	Pepper, nutmeg, carrot, cardamone - Paprika, orašac, mrkva, kardamon
Terpineol	Orange flower, rosmarin - Narančin cvjet, ružmarin
Thymol	Thyme, Oregano - Majčina dušica, origano
Carvacrol	Oregano - Origano

In 21 d-old animals fed maize the thickest mucous membrane was observed, after 42 d the differences equalized and the mucous was even thicker in birds fed wheat+barley. No essential differences were found between groups. Original results of those investigations are presented in the work of Jamroz et al., (2003).

The mode of action of bioactive compounds (Table 3) present in plants, especially their pharmacological effects is well recognized in human medicine, however in livestock production, where fast effects of application of those extracts are expected, there is a lack of exact data and the amount of precise scientific evaluation still remains insufficient.

Some graphically presented aspects of active plant substances (Fig. 1) indicate that improvement of the activity of endogenous enzymes in GI tract the increase of nutrients absorption, the balance of the microbial population in intestines as well as the modification of short chain fatty acids production may be an important and beneficial factor in animal nutrition.

Antioxidative activity of some plant substances (Table 4) may display important implications, for instance, in feeding adult animals (Lee et al., 1995; Valenzuela, 1995; Baratha et al., 1998; Lopez-Bote et al., 1998; Grassmann et al., 2000; Tschirch, 2000).

Fig. 1. Step-by-step mode of action of plant active substances

Slika 1. Način djelovanja biljnih djelatnih tvari korak po korak

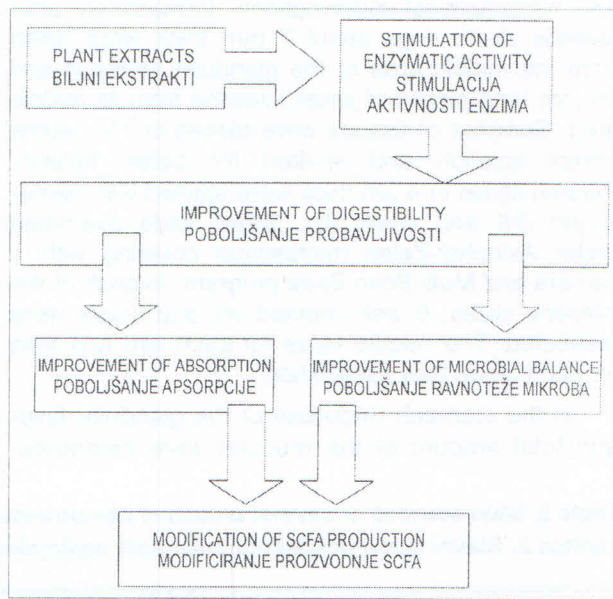


Table 3. Mode of action of plant active substances:

Tablica 3. Način djelovanja djelatnih biljnih tvari:

- improvement of endogenous enzymes secretion poboljšanje izlučivanja endogenih enzima;-
- stimulation of appetite, digestibility and absorption of nutrients - poticanje apetita, probavljivost i apsorpcija hranjivih tvari;
- better balance of microbial flora, reduction of E. coli and Clostridium population - bolja ravnoteža mikrobske flore, smanjenje E.coli i Clostridium populacije;
- stimulation of Lactobacillus spp. proliferation - poticanje širenja Lactobacillus spp.;
- protection of intestinal micro villi - zaštita od crijevnih mikroresica;
- cellular antioxidative function - stanična antioksidacijska funkcija;
- antibacterial and antiviral activity - antibakterijsko i antivirusno djelovanje;
- antidiarrhoeic functions - protuproljevna funkcija;
- stimulation of immunity - poticanje imuniteta.

(Molcyar and Narasinham, 1986; Deans and Ritchie, 1987; Hili et al., 1997; Firouzi et al., 1998; Lis-Balchin and Deans, 1998; Liu-Fengtlua et al., 1998; Gollnisch and Halle, 2001; Jamroz et al., 2003)

Table 4. Antioxidant activity of cinnamon extracts
Tablica 4. Antioksidantno djelovanje ekstrakata cimeta

Antioxidant analysis - Antioksidantna analiza	
Determined by the beta-carotene/linoleic acid system - Određeno sustavom beta-karotin/linolna kiselina	
Test substance - Testirana tvar	Inhibition of oxidation Inhibicija oksidacije (%)
No additive - Bez aditiva	3.5
Cinnamon Extract 1 (ether extraction) - Ekstrakt cimeta 1 (ekstrakcija eterom)	96.5
Cinnamon Extract 2 (methane extraction) - Ekstrakt cimeta 2 (ekstrakcija metanom)	67.2
Cinnamon Extract 3 (aqueous extraction) - Ekstrakt cimeta 3 (vodena ekstrakcija)	87.5
BHT (positive control) - BHT (pozitivna kontrola)	80.5

Estimated in Pancosma, 2000 - Procijenjeno u Pancosma, 2000.

Regarding the problem of using alternative feed additives as important in animal nutrition in our Department of Animal Nutrition of Agricultural University investigations on the mode of action of some bioactive of plant origin substances were carried out. The synthesis of some results obtained in these experiments are presented in this paper. In our experiments the XTRACT* preparation (see below) was used.

	Content g/100g Sadržaj g/100 g	Action - Djelovanje
Carvacrol (oregano) Carvacrol (origano)	4.95	Stimulation of enzyme activity - Poticanje aktivnosti enzima Stimulation of Lactobacillus spp. proliferation - Poticanje razmnažanja Lactobacillus spp Improvement of SCFA production - Poboljšanje proizvodnje SCFA
Cinnamaldehyde (cinnamon) Cinnamaldehyde (cimet)	2.97	Protection of intestinal microvilli - Zaštita od crijevnih mikrovila Stimulation of Lactobacillus spp. proliferation Poticanje razmnažanja Lactobacil. spp Improvement of SCFA production - Poboljšanje proizvodnje SCFA Improvement of intestinal content acidity (pH) - Poboljšanje crijevnog sadržaja kiseline (pH)
Capsaicin (mexican pepper) Capsaicin (meksička paprika)	1.98	Stimulation of enzyme activity - Poticanje aktivnosti enzima Protection of intestinal micro villi - Zaštita od crijevnih microvilla Stimulation of SCFA production - Poticanje proizvodnje SCFA Stimulation of Lactobacillus spp. proliferation - Poticanje razmnažanja Lactobac. spp

* XTRACT preparation; standarized and stabilized in starch gel matrix, coated with rapeseed fat; product of AXISS France
* XTRACT pripravak; standardiziran i stabiliziran u matrici škrobnog gela, obložen masnoćom repičinog sjemena; proizvod AXISS-a, Francuska

Some activities, earlier known in human pharmacology, were expected: from the ingredients of the preparation

Carvacrol (oregano) - stimulation of Lactobacillus proliferation and volatile fatty acid production;

Cinnamaldehyde (cinnamon) villi protection through improvement of its intercellular antioxidant activity;

Capsaicin (paprika) acting on the stimulation of digestive enzymes activity.

In the first experiment, in which broiler chickens were fed on diets without additive (negative control) and supplemented with feed antibiotic (10 ppm) or 150 and 300 ppm of plant extract some beneficial results were obtained:

- improvement of the body weight of 21 day-old chickens by 5.4% (150 ppm) or 8.1 (300 ppm) - $P<0.01$ in comparison to the negative control group;
- feed conversion by 3.1 or 7.1% better than in the control ($P<0.05$);
- slightly diversified performance in older birds;
- similar apparent ileal digestibility of amino acids in "extract" and supplemented with feed antibiotics groups.

The purpose of the second experiment was to determine the influence of plant extract preparation on:

- apparent digestibility of nutrients,
- number of microorganisms in the intestine content,
- endogenous enzymes activity,
- morphology as well as histochemical changes in stomach and ileum walls in chickens.

The one-day old male Hubbard HI-Y broiler hybrids were randomly divided into four dietary treatments (each in seven subgroups - 12 animals). Chickens were kept in battery cages in the standard environmental conditions. The feed mixtures consisted of grains with different NSP content (Table 5).

Mobilization of mucocytes in the superficial epithelium and increased secretion of neutral mucopolysaccharides with a small amount of sialomucins or/and local cells disruption with releasing large amount/of mucus were observed in both groups of 21 d old birds fed XT (Fig. 2-5). This could explain the protective properties of plant extracts related to micro villi.

In some places the cells with acid-mucopolysaccharides, which in control animals were present only in the superficial gland to 1/2-2/3 of its height, appeared in the superficial epithelium. It may suggest the accelerated cell migration in superficial glands or change in mucocyte differentiation.

Table 5. Experimental diets in second experiment
Tablica 5. Pokusno hranjenje u drugom pokusu

Group I - Skupina I	Group II - Skupina II	Group III - Skupina III	Group IV - Skupina IV
Maize (58%) Kukuruz (58%)		Wheat + barley (30 + 30%) - Pšenica + ječam (30 + 30%)	
Without plant extract Bez biljnog ekstrakta	With 100 ppm plant extract Sa 100 ppm biljnog ekstrakta	Without plant extract Bez biljnog ekstrakta	With 100 ppm plant extract Sa 100 ppm biljnog ekstrakta

- better breast muscle share in chickens fed supplemented feed ($P<0.05$);
- lower number of fungi and Clostridium perfringens and E. coli (CFU) in experimental groups than in the control ($P<0.05$);
- positively affected (1.1-3.5%) nitrogen and apparent ileal digestibility of nutrients and amino acids in very young chickens;

Figures 2-4 (all preparations and photos made by Houszka, 2003)
Slike 2-4. Sve pripravke i fotografije načinio Houszka, 2003.

Fig. 2. Increased release of mucus composed of neutral and acid mucopolysaccharides. Proventriculus. Gr. IV-2LAB-PAS

Slika 2. Povećano oslobađanje mukoze koja se sastoji od neutralnih i kiselih mukopolisaharida. Proventriculus. Gr. IV - 21 AB-PAS

Fig. 3. Disintegration of superficial epithelium with massive mucus release. Proventriculus. Gr. II and IV-21. H.E.

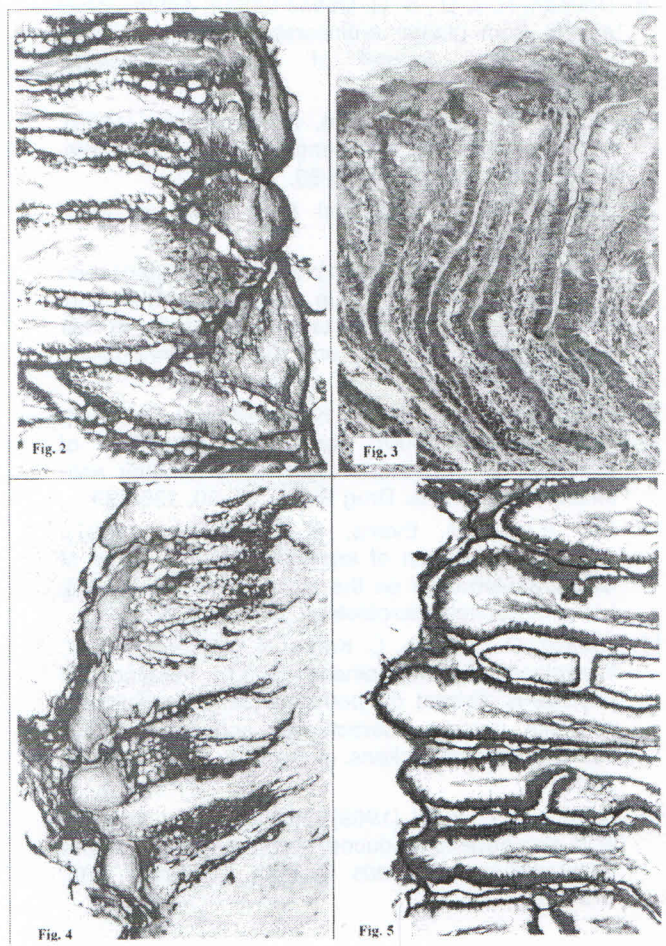
Slika 3. Raspadanje/dezintegracija površinskog epitela s obilnim izlučivanjem mukoze. Proventriculus II i IV - 21 H.E.

Fig. 4. Massive release of mucous material in whole depth of the mucous. Proventriculus. Gr. IV-21.AB-PAS.

Slika 4. Obilno izlučivanje mukoznog materijala u čitavoj dubini mukoze. Proventriculus. Gr. IV - 21 AB-PAS

Fig. 5. Increased synthesis and accumulation of mixed (AB+; PAS+) mucus material and limited secretion activity in cells of superficial epithelium. Proventriculus. Gr. IV - 21. AB-PAS.

Slika 5. Povećana sinteza i akumulacija miješanog mukoznog materijala (AB +; PAS+) i ograničena aktivnost izlučivanja u stanicama površinskog epitela. Proventriculus. Gr. IV - 21 AB-FAB



Folds of the proventriculum mucous in animals fed XT, fused in to large unshaped structures, which limited its functional capacity. As neutral mucopolysaccharides positive cells of the superficial epithelium show marked secretory ability with high sensibility to different agents action, the sulfomucins were secreted in a small amount by cells of the bottom of the glands.

In groups fed XTRACT the mucous secretion intensity was a little higher. Described morphological changes disturbed influence of extract on the digestive tract well seen after 21 d, however compensatory mechanisms reduced effect of long-term plant extract application.

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SAŽETAK

Zabrana upotrebe antibiotika kao podupirača antimikrobskog djelovanja u hranidbi životinja postoji od 1986. u Švedskoj a od 1999. u zemljama EU. U Europskoj Uniji još su dozvoljena samo tri antibiotika u hrani - Salynomycin-Na, Flavophospholipol i Avilamycin, a opća se zabrana predviđa za kraj 2005. Za sada kokcidiostatici nisu uključeni u popis zabranjenih antibiotika za perad.

Kao nadomjestak antibioticima u hrani mogu se upotrijebiti razni dodaci. Međutim, njihovo se djelovanje, mehanizmi i djelotvornost ne mogu potpuno usporediti s antibioticima. Najdjelotvorniji su u antimikrobskom djelovanju zakiseljivači, probiotici, prebiotici, koncentrati peptida, aromatične trave i biljni ekstrakti.