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INFLUENCE OF THE PREBIOTIC SALGARD AND A HERB MIXTURE ON PEKIN DUCKLINGS IN ORGANIC POULTRY PRODUCTION: II. HISTOLOGICAL AND MICROBIOLOGICAL INVESTIGATION

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Abstract: The purpose of this investigation was to study the influence of the prebiotic Salgard and an herb mixture (rosemary, thyme, basil, oregano and cinnamon) on the histostructure of some internal organs and on the intestinal microflora of Pekin ducklings in an organic production system. Seventy two Pekin ducklings distributed randomly into 3 groups of 24 birds each and sexed (12 ♂and 12 ♀) were used as followed: group I (control) fed a standard diet; group II – fed the same diet supplemented with the prebiotic Salgard at a concentration of 0.15 %, and group III – fed the same diet supplemented with 0.15 % of a herb mixture in an equal proportion (0.03% of each herb – rosemary, thyme, basil, oregano and cinnamon). At slaughter, material for histological examination was obtained from the ileum, the caeca, the liver and the kidneys of birds. Faecal content from the ileum and the caeca were submitted to microbiological analysis. The addition of Salgard in a concentration of 0.15 % to the diet of Pekin ducklings contributed to significant increasing the length of the villi intestinales ($P<0.001$) and diameter of intestinal glands ($P<0.01$) in the ileum and epithelial height ($P<0.05$) in the caeca, as well as with a reduction of pathogenic intestinal microorganisms in the birds that received the prebiotic and herb mixture.

Key words: duck, prebiotic Salgard, herb mixture, histological characteristics, intestinal microflora

Introduction

The organic production system is in harmony with natural poultry rearing conditions and therefore, a prerequisite for a high level of welfare. Today as growth promoters in poultry nutrition, which have positive effect on poultry growth and feed conversion are in use probiotics, prebiotics, enzymes, acidifiers, antioxidants and phytogene additives *(Perić et al., 2009)*.

The prebiotic Salgard is provided by Optivite LTD, UK and composed of propionic acid (20,000 mg/kg), ammonium propionate (85,000 mg/kg), ammonium format (160,000 mg/kg) and formic acid (35,000 mg/kg). Salgard is a feed treatment that helps protect against bacterial and fungal challenges *(Акyurek et al., 2011)*. The prebiotics has a microbicide effect on *Escherichia coli*, *Campylobacter* spp., as well as some Gram-positive microorganisms such as *Staphylococcus* spp, *Streptococcus* spp., *Listeria* spp. and *Clostridium* spp. At the same time it protects the beneficial microflora – *Lactobacillus* spp., *Bifidobacterium* spp. and *Bacteroides* spp. in the animal intestinal tract, resulting in improved health and development, higher utilization of feeds, and a positive effect on their productive qualities in organic animal farming conditions *(Griggs and Jacob, 2005; Biggs et al., 2007; Levic et al., 2008)*. The supplementation with herbs and spices in organic production is an important alternative for improving the health and welfare of animals and poultry. Their active substances stimulate non-specific resistance, increase the appetite and feed conversion, thus increasing productivity *(Loo and Richard, 1992; Jamroz et al., 2006; Mikulski et al., 2008; Frankič et al., 2009)*.

Furthermore, certain herbs (rosemary, thyme, basil, oregano) possess strong anti-inflammatory, antistress and antioxidant properties *(Jamroz et al., 2003, 2006; Lee et al., 2003; Bampidis et al., 2005; Mikulski et al., 2008 ; Windisch et al., 2008)*.

Cinnamon oil and its constituents cinnamaldehyde and eugenol have antibacterial activity against *Escherichia coli*, *Pseudomonas aeruginosa, Enterococus faecalis, Staphylococcus aureus, Staphylococcus epidermis*, *Salmonella sp*. and Parahemolyticus *(Chang et al., 2001)*.

The purpose of our research was to study the influence of the prebiotic Salgard and the herb mixture (rosemary, thyme, basil, oregano and cinnamon) on the histomorphological structure of some internal organs and on the intestinal microflora of Pekin ducklings in an organic production system.

Materials and Methods

The study was carried out in the poultry farm of Department of Animal Science at the Agricultural University – Plovdiv.

Seventy two Pekin ducklings were sexed, individually weighed and distributed randomly into 3 groups of 24 birds (1 control and 2 treatments)(12 ♂and 12 ♀). All birds were fed with diet containing ME – 12.4 MJ/kg and CP – 18.6 % during the starter period (1-28 day of age) and diet containing ME – 12.7 MJ/kg and CP – 16 % during the finisher period (29-63 day of age) and only varied in the feed additives . The ducklings from group I (control) fed a standard diet; group II – fed the same dietsupplemented with the prebiotic Salgard produced by Optivite LTD, Nottinghamshire, UK at a concentration of 0.15 %, and group III – fed the same diet supplemented with 0.15 % of a herb mixture in an equal proportion (0.03% of each herb - rosemary (*Rosmarinus officinalis)*, thyme (*Thymus serpyllum)*, basil (*Ocimum basilicum)*, oregano (*Origanum vulgare L.)*, cinnamon (*Cinnamomum verum)*. All herbs were produced by Bioset LTD, Plovdiv, Bulgaria. The Salgard and herb mixture were added in diet from the 1st day of age to the end of the experiment..

Histological examinations. The material for the histological study – pieces of about 1 cm^3 in size, was obtained from different parts of the intestines (middle part of ileum and both caeca), the liver and the kidneys immediately after the birds were slaughtered – (six birds from each group – 3 males and 3 females). The samples were immediately placed into 10% neutral formalin. After fixation, the samples were washed with running water, dehydrated in an alcohol series, cleared in xylene, and embedded in paraffin. The formed blocks were cut with a paraffin microtome "Reichert." The 6 μm tick sections were stained with hematoxylin and eosin. The observation, micro morphometric examination and photographing were done on a "Hund" microscope. The metric study of the preparations was performed with a standardized eye piece-micrometer. The following parameters were measured – length of villi intestinals, epithelial height of lamina epithelialis, diameter of intestinal glands. It was made 10 measurements per parameter from each slaughtered bird

Microbiological examinations. The faecal content of the ileum and caeca was examined microbiologically by means of routine laboratory methods for isolation, identification, and typizations by genus and species. A part of microbial isolates were identified on the semi-automated identification system CRYSTAL (Becton Dickinson) for enterobacteria and staphylococci, as well as on the API-20 NE system.

Statistical analyses. Micro morphometric histological examinations were expressed as a mean and standard error. Data were subjected to one-way analysis of variance (ANOVA) using GraphPad InStat 3.06 software to determine the level of significance among mean values Tukey's HSD test was performed as a post-hoc test after ANOVA.

Results and Discussion

Light microscope examination revealed that the ileal wall of the ducklings from the three groups was composed of 4 layers: tunica mucosa, tunica submucosa, tunica muscularis, and tunica serosa. The mucous coat is formed of three sublayers: lamina epithelialis – a single-layer columnar epithelium, lamina propria – loose connecting tissue, in which her intestinal glands are located, and lamina muscularis – smooth muscle tissue, which goes into the villi and attains almost up to their tips. The submucosa is a very thin layer whereas the musculature is made of a thicker internal and a thinner external smooth muscle layers. The outermost layer of the intestine is the serous coat – visceral peritoneum.

The micro morphometrical measurments study (Table 1) also shows that the ileum's mucosa forms relatively high villi intestinales, and they are the highest in the ducklings from the group II (Figure 1), while in groups I and III the differences were insignificant (Figure 2). A similar tendency was observed in the height of lamina epithelialis and the diameter of intestinal glands.

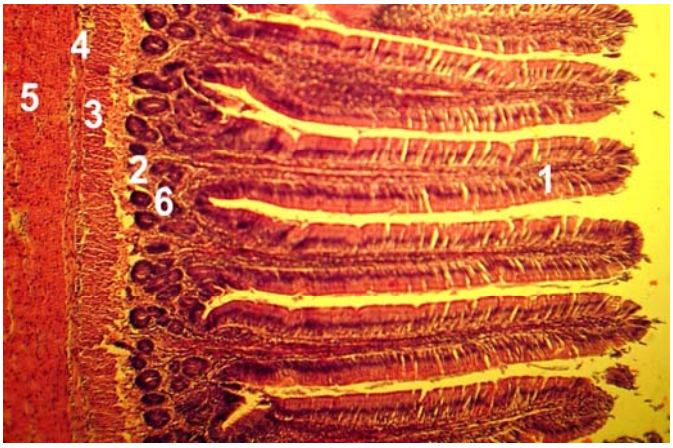

Figure 1. Cross section through the ileal wall (II group): 1 – l. epithelialis. 2 – l. propria. 3 – l. muscularis. 4 – t. submucosa. 5 – t. muscularis. 6 – intestinal glands (bar = 100 μm).

The structure of the caeca wall in the three groups of ducklings is typical for this part of the digestive system. It contains the same 4 layers observed in the ileum. The birds from group II and group III have an increased presence of leucocytes in the propria of the mucosa and in the submucosa (Figure 3). There is no visible difference in the structure of the caeca wall between birds which were fed with Salgard and herbal mixture. The data from the micro morphometrical study indicates that Pekin ducklings, which received the prebiotic Salgard, villi intestinal reach the greatest height (Table 1). The epithelium height and the

diameter of the intestinal glands in this group are also larger, compared to the groups I and III, but the differences are insignificant.

Table 1. Micromorphometri data from the examination of the ileum and caeca (n=10) in ducklings from the different groups

Group	Ileum			Caecum		
	Length of villi, µm	Epithelial height, µm	Glands diameter, µm	Length of villi, µm	Epithelial height, µm	Glands diameter, µm
Group I (control)	892.4±12.5 a_1	57.7±1.3	55.1±0.6 b_2	404.4±10.3	56.8±0.6 b_4. c_1	59.2±1.78
Group II	962.2±16.3 a_1. b_1	59.5±0.7	58.6±0.9 b_2. b_3	418.6±9.4	59.6±0.8 b_4	62.2±1.7
Group III	904.6±15.0 b_1	57.8±1.3	55.8±1.0 b_3	405.2±7.1	58.8±0.8 c_1	60.4±1.6

Note: $P<0.001$ at a_1- a_1; $P<0.01$ at b_1- b_1. b_2- b_2. b_3- b_3. b_4- b_4; $P<0.05$ at c_1- c_1 in the same vertical rank

Light microscope examination of the liver showed no significant differences in the structure of its parenchyma between the different groups of birds. The liver lobules were not clearly differentiated due to the small amount of interstitial connective tissue around them (Figure 4). The glandular tubules were circumferentially located around the central vein and were built from polygonal hepatocytes, with an average diameter of 10-13 µm.

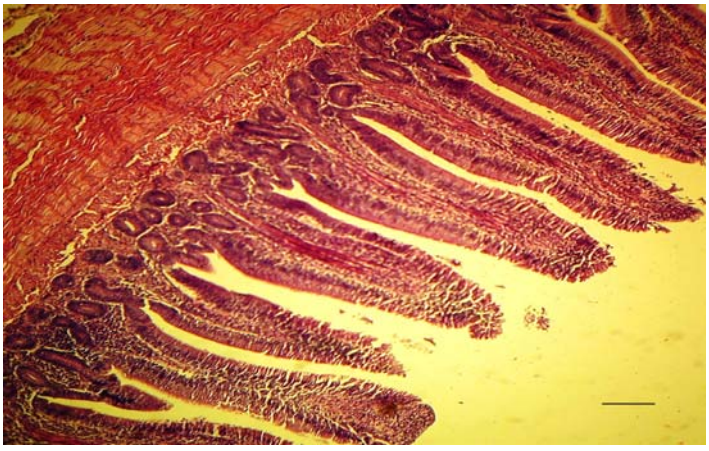

Figure 2. Cross section through the ileal wall (I group): The well-shaped round intestinal villi of the mucous coat could be observed (bar=70 µm).

The histological examination of the kidneys did not reveal any structural differences between the three groups of ducklings could be discovered. The kidney lobules were well differentiated, and each had well formed cortex and medulla (Figure 5).

There is significantly positive effect of prebiotics on performance and height of intestinal villus in small intestines of broilers *(Žikić et al., 2008)*. *Awad et al. (2009)* concluded that dietary treatments influenced the histomorphological parameters of small intestinal villi. The addition of either probiotic or synbiotic increased ($P < 0.05$) the villus height:crypt depth ratio and villus height in both duodenum and ileum. The duodenal crypt depth remained unaffected ($P > 0.05$). However, the ileal crypt depth was decreased by dietary supplementations compared with control.

Garcia et al. (2007) established that the diets with 5,000 and 10,000 ppm of formic acid, and with 200 ppm of plant extract based on a blend of oregano, cinnamon, and pepper essential oils had positive effect on the intestine mucosa and growth performance in broilers.

The results from the microbiological study (Table 2) showed that the addition of Salgard in the birds' diet did not result in impaired content of the intestinal cenosis, which could be expressed by pathological disturbances. The presence of the same microbial varieties from the beneficial microflora, established in healthy untreated birds, was proved. A relative yet transient decrease in the amount of *Bifidobacterium* spp., *Enterobacter* spp., *Enterococcus* spp. and *Clostridium* spp. microorganisms was also evident in the caeca and the ileum of the birds that received the prebiotic.

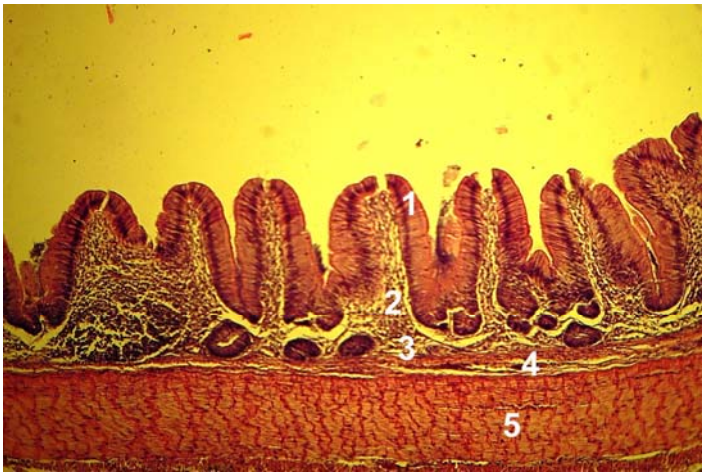

Figure 3. Cross section through the caecal wall (II group): 1 – l. epithelialis. 2 – l. propria. 3 – l. muscularis. 4 – t. submucosa. 5 – t. muscularis; (bar = 100 µm)

The prebiotic has a protective effect on the beneficial microflora and stimulates its development – *Lactobacillus* spp. and *Bacteroides* spp., improving digestion and the nutirent utilization. Salgard has a relatively broad antimicrobial effect on *E. coli*, *Campylobacter* spp, *Listeria* spp. and *Clostridium* spp.

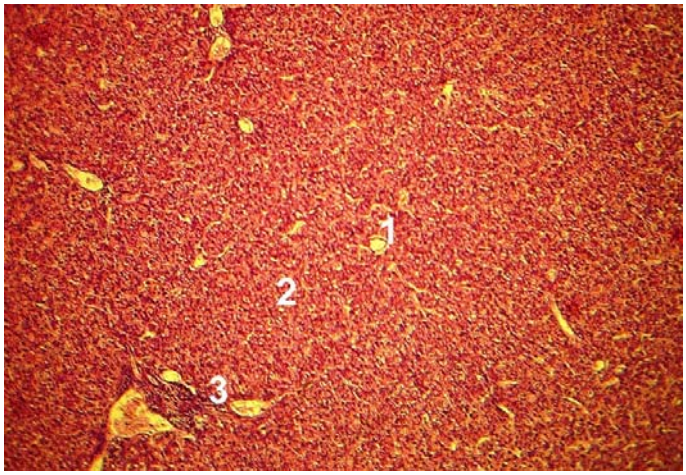

Figure 4. Liver of a duckling from the group II: 1 – v. centralis. 2 – hepatocytes. 3 – interlobular loose connective tissue with artery. vein and biliary duct located within; (bar = 100 µm)

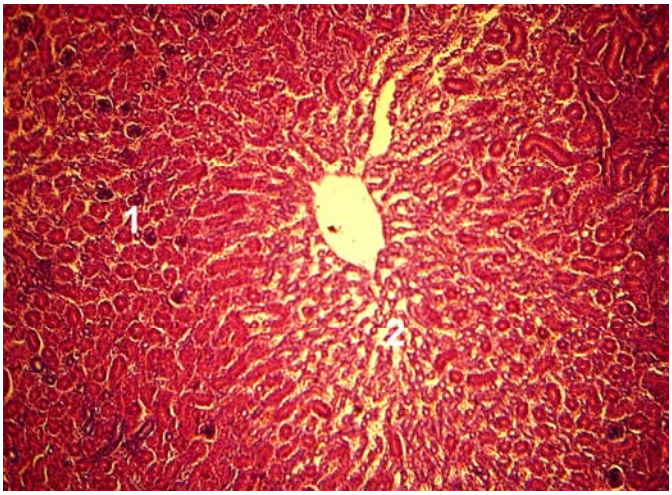

Figure 5. Kidney of a duckling from the group II: 1 –cortex. 2 – core (bar = 100 µm)

Table 2. Microbiological examination of faecal content from Pekin duckings

Group	Sample №	Intestine	*E. coli*	*Bifido bacterium*	*Proteus spp*	*Entero bacter* spp.	*Citro bacter* spp.	*Entero coccus spp.*	*Bacterio ides spp.*	*Clostri dium spp.*
Group I	1	caeca	(+)	(+)	(-)	(+)	(+)	(+)	(-)	(+)
	2		(+)	(+)	(-)	(+)	(+)	(-)	(-)	(-)
	3		(+)	(+)	(+)	(-)	(-)	(-)	(+)	(+)
	4	ileum	(+)	(-)	(-)	(-)	(-)	(-)	(-)	(+)
	5		(+)	(+)	(-)	(+)	(-)	(-)	(-)	(+)
	6		(+)	(-)	(-)	(-)	(-)	(-)	(-)	(-)
Group II	7	caeca	(+)	(+)	(+)	(-)	(-)	(-)	(-)	(-)
	8		(+)	(-)	(-)	(-)	(+)	(-)	(-)	(-)
	9		(+)	(+)	(-)	(-)	(+)	(-)	(-)	(-)
	10	ileum	(+)	(-)	(+)	(+)	(-)	(-)	(-)	(-)
	11		(+)	(-)	(-)	(-)	(-)	(+)	(-)	(-)
	12		(+)	(-)	(-)	(-)	(-)	(-)	(+)	(+)
Group III	13	caeca	(+)	(-)	(-)	(-)	(+)	(-)	(-)	(+)
	14		(+)	(+)	(+)	(+)	(-)	(+)	(-)	(+)
	15		(+)	(+)	(-)	(+)	(-)	(-)	(-)	(+)
	16	ileum	(+)	(+)	(-)	(-)	(+)	(+)	(-)	(+)
	17		(+)	(-)	(-)	(-)	(+)	(+)	(-)	(-)
	18		(+)	(+)	(+)	(-)	(-)	(+)	(-)	(-)

On the other, some aromatic plants (Chinese and Ceylon cinnamon, thyme, St. John's Wort etc.) possess antimicrobial properties against alimentary pathogens and thus suppress the growth and development of enteropathogenic strains of *E. coli*, *Salmonella* spp. and others *(Pasqua et al., 2006; Windisch et al., 2008; Frankič et al. 2009)*. The mentioned aromatic plants and their active substances alter the cell membrane structures of enteropathogenic strains, causing ion leakage out of the cells, and death of pathogens *(Windisch et al., 2008)*. The aldehyde contained in cinnamon (carvacrol) improves the growth of lactobacilli and thus increases the beneficial intestinal microflora *(Castillo et al., 2006)*.

Conclusion

The higher intestinal villi observed during micromorphological analysis and the insignificantly higher lining epithelia of the ileum and the caeca of the ducklings from the 2nd group were probably due to the added prebiotic.

The addition of Salgard and herb mixture to the diet of Pekin ducklings contributed to reduction of pathogenic intestinal microorganisms in the birds.

Supplementation of the feed with prebiotic and herb mixture did not cause significant differences in the histological structure of the liver and kidneys for the investigated period.

Uticaj prebiotika Salgard i biljne smeše u ishrani pekinških pačića u organskoj proizvodnji: II. Histološko i mikrobiološko ispitivanje

V. Gerzilov, G. Penchev, M. Lyutskanov, A. Bochukov, N. Bozakova, S. Popova-Ralcheva, V. Sredkova

Rezime

Cilj ovog ispitivanja je bilo praćenje uticaja prebiotika Salgarda i biljne smeše (ruzmarin, majčina dušica, bosiljak, origano i cimet) na histološku strukturu nekih unutrašnjih organa i intestinalne mikroflore pekinških pačića u organskom proizvodnom sistemu. U ovom istraživanju, 72 jednodnevna pačeta, podeljena su u 3 grupe od po 24 ptice u svakoj (12 ♂i 12 ♀) na sledeći način: grupa I (kontrola) hranjena standardnom hranom; grupa II – hranjena istom hranom uz dodatak prebiotika Salgard u koncentraciji od 0.15%, i grupa III – hranjena istom hranom uz dodatak 0.15% biljne smeše u jednakim proporcijama (0.03% svake biljke - ruzmarin, majčina dušica, bosiljak, origano i cimet).

Posle klanja, uziman je materijal za histološka ispitivanja iz ileuma, slepog creva, jetre i bubrega ptica. Fekalni sadržaj iz ileuma i slepog creva je podvrgnut mikrobiološkoj analizi.

Dodavanje Salgarda u koncentraciji od 0.15 % u obrok za pekinške pačiće je doprinelo signifikantnom povećanju dužine crevnih resica ($P<0.001$), prečnika crevnih žlezda ($P<0.01$) u ileumu, i visine epitelija ($P<0.05$) u slepom crevu, kao i smanjenja patogenih intestinalnih mikroorganizama kod grla koja su hranjena prebiotikom i biljnom smešom.

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