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ORIGINAL PAPER

The effect of medicinal plants and plant extracted oils on broiler duodenum morphology and immunological profile

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Abstract

It was previously reported that essential oils from aromatic plants have an antimicrobial activity against many bacterial pathogens [13]. We have conducted an in vivo experiment to study the effect of some aromatic plants and in particular to investigate the effect of oils extracted from these plants at the immune level and duodenal morphology. During the experiment 90 chicken broilers were divided in three experimental groups: control group (C), group 1 (G1) and group 2 (G2). The chicken broilers from group G1 had received feed with 0.05% incorporated oils extracted from savory (Satureja hortenis), mint (Mentha piperita) and sea-buckthorn (Hippophae rhamnoides). Group G2 received a premix of plants (savory, mint and sea-buckthorn) during daily feeding. The control group (C) received normal feed with no supplements. The amount of lysozyme detected at group G1 was doubled (28.55 mcg/cm^3) compared to G2 (13.2 mcg/cm^3) and the control (11.42 mcg/cm^3). The incorporation of extracted oils in food resulted in a powerful stimulation of intestinal mucous membrane, manifested by development of intestinal villi, the hypertrophy of villi, hyperplasic hypertrophy of capillary network and the stimulation of leukocytes infiltration. The muscular hypertrophic processes and leukocyte infiltration are visible in the endomesium and perimesium of the muscular tunic. Microscopical images of the G2 group taken from the duodenum sections suggest the stimulation of angiogenesis. The processes are however of smaller intensity in the G1 group. This work shows that essential oils extracted from plants improve the immune response and also are able to cause changes of the duodenal mucosa with beneficial effects for the animal.

Keywords: medicinal plants, plant extracted oils, duodenum morphology, immunological profile, broiler

Introduction

With the development and wide use of synthetic and semi-synthetic antibiotics, pros and cons have been experienced throughout the last 50 years which have been directed research back to natural antimicrobial products as indispensable resources [5]. Consequently there is considerable research interest in the possible use of natural products, such as essential oils and extracts of edible and medicinal plants, herbs and spices, for the development of new additives in animal feeding. Aromatic plants like Sea-buckthorn contain flavonoids described previously as stimulators of the immune response, these dietary flavones having an effect against microbial infection [6,8,9] showed that the essential oil extracted from *Satureja hortensis* reversed oxidative damage to rat lymphocytes induced by hydrogen peroxide. Regarding the effect of these extracts on animal growth performance the results are contradicting most of the existing data showing no effect. One example of a positive effect is reported in pigs fed with diets containing 100 mg fennel oil/kg. The decrease of percentual

feed intake was significant [14]. Extracted oils have been previously used to test the effect on broilers growth performance and at 7 % in the diet did not improved weight gain [1]. Weight gain is probably related to other feed components as suggested for laying hens where the diet must contain a high level of amino acids to get an increase in egg weight from the addition of corn oil in the diet [1]. The abdominal fat in broilers fed linseed oil was reduced as a consequence of higher lipid oxidation despite higher synthesis of endogenous fatty acids [2]. It was also suggested that feeding chickens polyunsaturated fatty acids, in comparison to dietary saturated fatty acids reduced the amount of both abdominal fat and skin fat [4]. It was also suggested that in broilers fed diets rich in saturated fatty acids higher insulin levels were detected [3]. Supplementation of the diet with 2% palm kernel oil significantly elevated blood phospholipids concentration, but depressed the accumulation of the other lipid fractions in both organs and the blood of birds [11] [3] reported that broilers fed oxidised fat showed a significantly increased concentration of tocopherols, lutein, beta-carotene, and retinol in plasma and tissue. The effect of medicinal plants and oils extracted from these plants as immunological stimulators and especially the effect at microscopic level is not very well known. In this experiment the immunological effect of essential oils extracted from savory (*Satureja hortenis*), mint (*Mentha piperita*) and sea-buckthorn (*Hippophae rhamnoides*) as well as the changes within the duodenal wall structure were studied.

Materials and Method

Experimental Design and Husbandry

The experiment was performed in the department of Animal Feeding and Nutrition, Banat University of Agricultural Sciences, Timisoara. We have used 90 chicken broilers of ROSS 308, divided in three experimental groups, with 30 chicks per group. Chicks were vaccinated for infectious bronchitis, and Marek's disease at the hatchery. Water and lighting were continuous through the trial. Feed and water were offered for ad libitum consumption throughout the experimental period. The experiment was conducted for 6 weeks, from hatch to 42 days.

As shown in Table 1, group G1 received essentials oils of savory, sea-buckthorn and mint at a dietary inclusion rate of 0.05%, while group G2 received the same diet plus 2% plant premix [12]. These quantities were similar during the experiment. The control group received none of these supplements. Through the experimental period of 0-3 weeks diets were formulated to contain 3200 kcal/kg, 23.63 % protein and 4.12 % cellulose. For the period 3 to 6 weeks diets were formulated to contain 3212 kcal/kg, 20.16 % crude protein and % cellulose. Diets were formulated according with [10] in order to satisfy broilers nutritive requirements. The nutritive characteristics of the diets are shown in Table 3. The difference between experimental groups was made by the addition of extracted oils or medicinal plants supplement (table 2).

Table 1. Experimental design

0 – 3 weeks		
Control	**G1**	**G2**
Diet 0-3 weeks	Diet 0-3 weeks + 0.05% essentials oils savory, box thorn and mint	Diet 0-3 weeks + 2% plant premix (savory, box thorn and mint)
3-6 weeks		
Diet 3-6 weeks	Diet 3-6 weeks + 0.05% essentials oils savory, box thorn and mint	Diet 0-3 weeks + 2% plant premix (savory, box thorn and mint)

Table 2 Supplement structure

G1	G2
Oils extracted from: savory, mint, sea-buckthorn, and acidifying	Plant premix of: savory, mint, sea-buckthorn and acidifying

Table 3

Component	%
0- 3 weeks	
Corn	54,18
Soy bean meal	34
Fishmeal	5
DL methionine	0,27
Sunflower oil	3,5
Calcium carbonate	1
Di-calcium phosphate	0,8
Salt	0,25
Vitamins and minerals premix	1
Nutritional content	
Energy (kcal/kg)	3200
Crude protein (%)	23,63
Lysine (%)	1,25
Methionine (%)	0,9
Threonine (%)	0,42
Calcium (%)	1
Phosphorus (%)	0,9
3-6 weeks	
Corn	60,26
Soy bean meal	31
Fish meal	2
DL methionine	0,19
Sunflower oil	3,5
Calcium carbonate	1
Di-calcium phosphate	0,8
Salt	0,25
Vitamins and minerals premix	1
Nutritional content	
Energy (kcal/kg)	3212
Crude protein (%)	20,16
Lysine (%)	1,05
Methionine (%)	0,72
Calcium (%)	0,9
Phosphorus (%)	0,6

In order to establish the feed intake for the experimental the allocated food was weight at the beginning and at the end of the experiment (6 weeks). These data was necessary to calculate the feed intake for each experimental period as well as the average daily gain.
In order to test the immunomodulator we have measured the following:

- Serical properdine (17.5-20 mg / 100 ml serum),
- Lysozyme (10.5-15 μg / cm ^{3}serum)
- Leucograme

The leucograme values of reference at broiler chickens are: lymphocytes – 63% ± 10%, segmental neutrofiles 27% ± 6 %, eosinofiles 2.2 ± 1 %, basofiles – 1.3 % ±0.8 %, monocytes 4.1%±1 %.

The hystological examination of intestinal wall was made by covering the stages of sampling, washing, inclusion, cutting and coloring with hematoxiline-eosine, respectively Mallory trycromic coloration.

Results and Discussion

Plant extracts effects may be due to a greater efficiency in the utilization of feed, resulting in enhanced growth. There is evidence to suggest that herbs, spices and various plant extracts have appetite- and digestion-stimulating properties and antimicrobial effects [7]. As previously reported in literature plants extracted oils had no effect on broiler growth performance [15]. In our experiments the addition of extracted oils from medicinal plants also had no effect ($p>0.10$) on the broiler growth performance during the experimental period (0-3 and 3-6 weeks). Body weight, average daily gain, average daily feed intake and gain-to-feed ratio were unaffected by the addition of these supplements. Data obtained during the experimental periods, regarding the production performance are presented in Table 4.

Table 4. The effect of extracted oils and medicinal plants on the main productive indices during the experimental periods of 0-3 and 3-6 weeks

Item	Groups		
	Control	G1	G2
0-3 weeks			
BW[1] (g)	827.8±18.13	772.59±20.64	829.56±16.71
ADG[2] (g)	37.56	34.93	37.64
ADFI[3] (g)	50.95	56.19	53.33
G:F[4]	0.737	0.621	0.705
3-6 weeks			
BW (g)	2225.23±67.74	2134.95±40.42	2122.13±52.86
ADG (g)	66.54	64.26	62.16
ADFI (g)	163.8	131.9	161.9
G:F	0.406	0.487	0.383

[1]BW = body weight
[2]ADG = average daily gain
[3]ADFI = average daily feed intake
[4]G:F = gain-to-feed ratio

In order to investigate the possibility of an immunological stimulation through the addition of extracted oils or medicinal plant premix a number of parameters closely related with the immune response were measured. The first two parameters tested were properdine and lysozyme. We have measured both at the end of experimental period (6 weeks). Properdine is a euglobulin in the fraction of beta and gamma globulin in the blood serum and together with the lysozyme plays an important role in the non-specific immunity. The leucocytes formula presented in table 5 didn't show significant differences between the three experimental groups.

Table 5. Haematological values of broiler chickens from experimental groups

Specification	Control	G1	G2
Lymphocytes	65.55±2.33	67.35±2.05	64.8±1.27
Neutrofils	30.65±0.63	32.75±0.49	32.2±0.14
Eosinofils	2.55±0.21	2.65±0.07	2.45±0.07
Basophils	1.05±0.21	1±0.14	0.95±0.07
Monocytes	3.05±0.63	2.35±0.21	2.9±0.56

As described in Figure 1 the addition of oils extracted from medicinal plants had an stimulatory effect on the levels of lysozyme and properdine in the blood serum. The amount of lysozyme in serum was 28.55 mcg/cm^3 in group G1 compared to 13.2 mcg/cm^3 in group G2. The control group had an amount of lysozyme in serum of 11.42 µg/cm^3. For properdine the G1 group had a detectable amount of 29.71 mcg/cm^3 and 22.35 mcg/cm^3 for group G2. The control group in this case had a detectable amount of 17.95 mcg/cm^3 (Fig. 1).

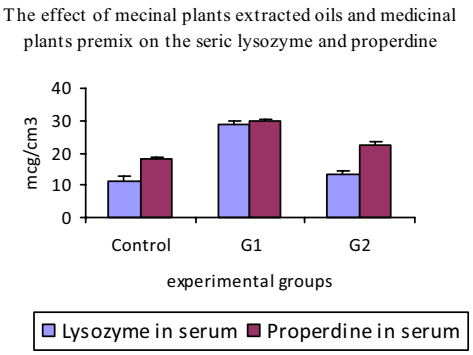

Fig. 1.

High values of serical properdine were observed in the G1 group, which were 65.51% higher compared to controls, followed by the G2 group, showing that changes in unspecific immune effectors are consequences of the actions of substances with immunomodulator effect of an exogenous nature.

A slight rise in the amount of lymphocytes in the G1 group was also observed, but not of the same magnitude as the previous two determinants. It is possible that if the study had been longer that these values would have been significantly increased, as this increase became obvious only after a period of stimulation in these substances.

To identify any possible changes at the level of duodenum we have taken microscopical sections of the intestinal wall in this region on the layers of the duodenum: the mucosa, submucosa and muscularis mucosa.

For the control group mucosa contained villi with a medium height of approximately 1011.15 µ, with a slightly widened appearance (fig. 2) and coated with a tall monoded epithelium, with a medium height of 22.5µ. Intestinal glands can be seen attached to the villi (fig. 3) which consist of a large lumen, and epithelium as well as a high number of leukocytes, found even within the glandular lumen. Lax connective tissue from the villi and interglandular corrion connect both the lymphatic and capillary network and is loaded with many infiltrate cells.

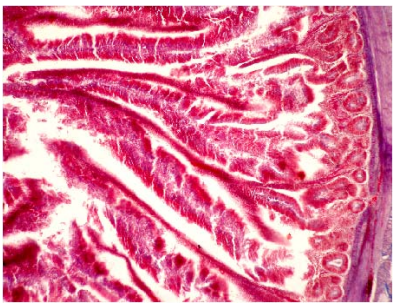

Fig. 2. Control lot – section through duodenum – general (100x; Mallory trichromical coloration)

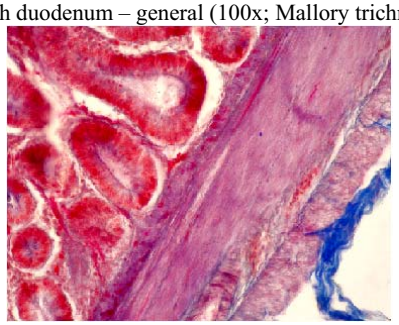

Fig. 3. Control lot – section through duodenum
– intestinal glands (100x; Mallory trichromical coloration)

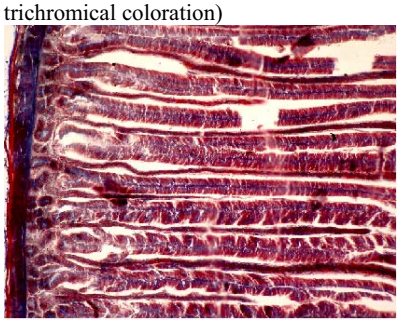

Fig. 4. Experimental lot with oils (ELO) – section through duodenum
– general (100x; Mallory trichromical coloration)

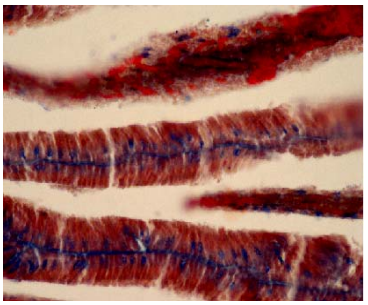

Fig. 5. Experimental lot with oils (ELO) – section through duodenum
– intestinal villositys (400x; Mallory trichromical coloration)

The capillary network shows evidence of hyperplasia and hypertrophy (fig. 6). In the G1 group, the intestinal glands of the duodenum appear to have a large lumen, are delimited by thin interglandular spaces, with the interglandular villi containing collagen fibres,

fibroblasts and leukocytes infiltrate. Also, a very large number of leukocytes are in transit between the glandular epithelial and villi (fig. 7). The interior muscular layer, made up of neat circular muscle fibres, in the endomysium and perimysium capillary ectasia and leukocytes infiltrates are shown (fig. 8). Also, the vascular packages from the structure of external perimysium, contains arteries, veins and capillaries with large lumens (fig. 9).

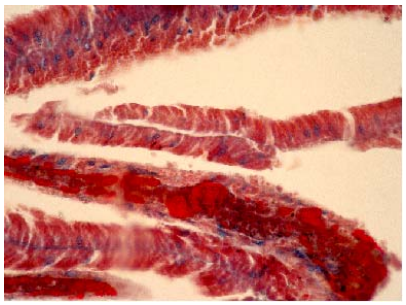

Fig. 6. Experimental lot with oils (ELO) – section through duodenum
– hypertrophycal capillary network (400x; Mallory trichromical coloration)

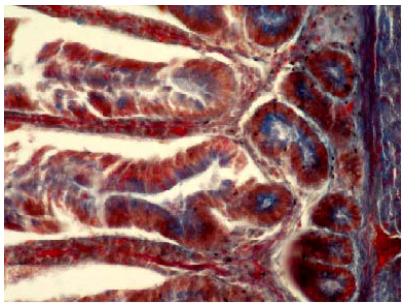

Fig.7. Experimental lot with oils (ELO) – section through duodenum
– hyperplasic hypertrophy of capillary network and leukocytes in transit (400x; Mallory trichromical coloration)

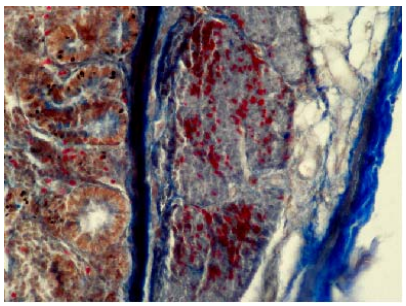

Fig. 8. Experimental lot with oils (ELO) – section through duodenum
–ectasiate capillary and leukocytes infiltrate in muscular tunic (400x; Mallory trichromical coloration)

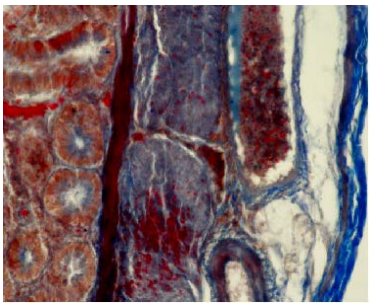

Fig. 9. Experimental lot with oils (ELO) – section through duodenum – ectasiate capillary and leukocytes infiltrate in mucous muscular tunics (400x; Mallory trichromical coloration)

For group G2 the intestinal mucous villi are very high (fig. 10) approximately. 1410μ, coated with a tall monolayer of epithelium (approximately. 23.5 μ), with an obvious ridged base. The enterocytes contain oval nuclei, placed in the basal third of the cell, with many calceiform cells dispersed among them.

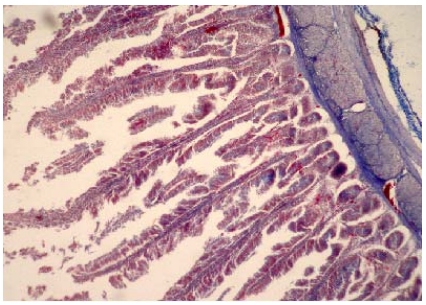

Fig. 10. Experimental lot with plants (ELP) – section through duodenum – intestinal villositys (100x; Mallory trichromical coloration)

The duodenal villi appears to be made up of a lax connective tissue, including the Bruke muscle, a hyperplastic capillary network and many migrating leukocytes. These images through the duodenum suggest that the angiogenesis process has been stimulated, judging by the presence of the capillary ectasia in the main villi and interglandular villi.

The capillary network appears to undergo both hyperplasia and hypertrophy and is seen to be powerfully stimulated by the lymphoid infiltrate (fig. 11). The muscle of the mucosa is formed from two thin layers of flat muscular fibres, and in the submucosa, the sanguine vessels have a large lumen. In this paper we report the positive effect of essential oils, extracted from aromatic plants, at the intestinal level having as a consequence an improved immunological response. This work provides the data regarding the changes in the microscopic structure of chicken duodenum as a consequence of aromatic plant extracted essential oils present in their feed. The histological changes presented here provide new information regarding the potential for using aromatic plants and aromatic plants oils in chicken feed.

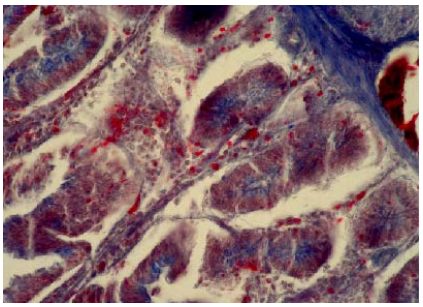

Fig. 11. Experimental lot with plants (ELP) – section through duodenum – hyperplasic hypertrophy of capillary network and stimulation of leukocytes infiltrate (400x; Mallory trichromical coloration)

Acknowledgements

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