The Application of Probiotics, Essential Oils and their Combination in the Poultry Production

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Abstract

The aim of this experiment was to observe the effect of the combination of probiotics and essential oils on the production of broilers and microbiological properties in the conditions of welfare. The experiment was carried out on broiler type Cobb 500. The welfare and the body weight of broiler chickens were evaluated at the end of the experiment. The average body weight of broiler chickens at the end of the experiment in the control group was 1567.2 g. while in trial group (feedstuff enriched with combination of 0, 05 % *Origanum aetheroleum* - essential oil and 0, 10 % probiotics – *Enterococcus faecium* CECT 4515) was 1607.20 g. The differences between trial group and control group were not highly significant ($P<0.05$). Samples of caecal chime were obtained and the number of *Enterococcus sp*. from 5.03 to 6.25 log cfu.g^{-1}, the number of *Lactobacillus sp*. from 6.12 to 6.67 log cfu.g^{-1}, and the number of *Enterobacteriaceae sp*. from 6.57 to 7.25 log cfu.g^{-1} was determined.

Keywords: broiler, essential oil, *Enterococcus faecium*, feedstuff, *Origanum aetheroleum*

1. Introduction

Essential oils have recently emerged as alternatives to antibiotics in animal production. There are commercially available products. Essential oils derived mainly from spices and herbs and their pure compounds have been shown to have antimicrobial effects *in vitro* [1]. In addition to their antimicrobial activity, essential oils possess various biological activities such as acting as antioxidants [2], being hypocholesterolemic [3, 4], affecting flavor], stimulating the digestion process [5]. Essential oils are already marketed for use in animal production and are claimed to be "digestive enhancers" [6]. Essential oils have been reported to affect rumen microbial activity, the effects being positive. Commercial preparation of essential oils did not affect the protozoal numbers in the rumen but did increase the bacterial population, possibly leading to increased nitrogen availability to the host [7]. An antioxidant effect of essential oils in broiler chickens has been reported as well [8]. The latter study [9], reported that oregano essential oils exerted antioxidant property in meats and abdominal fat, pointing at the incorporation of the protective antioxidant components of the essential oil into the membrane. Probiotics are currently used in the sense opposed to antibiotics [10]. Probiotic preparations are regarded as substances which affect the most favorable yield and animal health [11]. The use of probiotics have been reported to regulate physiological processes which affect animal growth, performance, increasing resistance to infectious diseases and improves health [12].

Following the facts from the scientific literature, the scientific research contribution was the adding of Origanum aetheroleum - essential oil in combination with probiotics – *Enterococcus faecium* into the feedstuff for chickens in relation to their production and microbiological parameters.

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2. Materials and methods

We divided the broilers in to two groups. These groups differed with feed addition, which was used in feed mixtures:
CG (control group) – soy-grain type feed mixtures with no added probiotics and essential oil.
In the trial group (TG) feedstuff enriched with combination of 0, 05 %*Origanum aetheroleum* - essential oil and 0, 10 % probiotics – *Enterococcus faecium* CECT 4515 were used.
In the experiment with fattening chickens, we have focused on observing the impact of combination of probiotics – *Enterococcus faecium* and *Origanum aetheroleum* - essential oil in feedstuff.
Enterococcus faecium CECT 4515 is included in the probiotical preparative Fecinor protected by layers of polysaccharides, which are allowed to pass the stomach, protection is against its pH and gall salts, also partially are protected against high temperatures.
The characteristics and content *Origanum aetheroleum* - essential oil was determined by the use of gas chromatography (chromatograph, type 8015-91-6): relative density 0.915-0.975 $g.cm^{-3}$, effective compound of carvacrol 57.0 %.
In the experiments, one day old broilers of meat-type COBB 500 have been used. The trials were carried out in a poultry farms in the hall with the possibility of feeding of 24 000 broiler chickens.
Boxes were created at the entrance door. Each box was intended for one group. Boxes have between separated by perforated netting and plastic fence between them. In each box were located 100 broiler chickens. Size of the area in each frame allowed unrestricted access to feed and water (as well as for natural activities) for each broiler chicken. Chickens were reared on deep litter material. The bottom layer of bedding was up to 8 cm and consisted of wood chips and the top layer consisted of 5 cm high straw. The total fattening period was divided into three phases:
a. Starter intended for chickens aged from 1 to 18 day during which the chicks were fed the starter feed mixture (HYD-01);
b. growth for chicks aged 19 to 31 days with growth forage mixture HYD-02,
c. final, for chicks aged 32 to 38 days with final forage mixture HYD-03.
They were usually served mixed compound feed for chickens for fattening with the balanced content of nutrients and metabolizable energy in accordance with their needs.
In the experiments, we have monitored the following indicators:
- Body weight of broilers at the end of the experiment (on a balance-type weighing Kern ECB 20K20 d with an accuracy of ± 0.1 grams).
Monitored microbiological parameters:
The number of cfu (colony forming units) of *Enterococcus* sp. on Slanetz-Bartley agar after 48 to 72 hours incubation at 37 ^{o}C. The number of cfu of *Lactobacillus* sp. on MRS agar after the incubation took from 48 to 72 hours at the temperature 37 ^{o}C. The number of cfu of *Enterobacteriaceae* sp. on McConkey agar after 48 to 72 hours at 37 ^{o}C.
In evaluating the results we used the plate dilution method. Basic dilution was: 1 g Chym + 99 ml saline (0.85% NaCl) by decimal dilution system. Basic dilution (10^{-1}), we prepared by mixing 5 g sample and 45 ml saline or 10 g sample and 90 ml of normal saline. The basic dilution was prepared further by decimal dilution system. Samples have been incubated on the surface or embedded. The inoculated Petri dishes were cultivated in an incubator, bottom up. Temperature and time, was adjusted according to the group of cultivated microorganisms. After the cultivation, we counted colonies grown on culture medium in Petri dishes. To calculate $CFU.g^{-1}$ (Colony Forming Units), we used the following formula (which takes into account Petri dishes of two consecutive dilutions):
$N = \sum C / [(n1 + 0.1\ n2).\ D]$; $\sum C$ - the sum of characteristic colonies on selected dishes;n1 - number of dishes of 1 dilution used to calculate; n2 - number of dishes of 2 dilution used to calculate; d - dilution factor is identical to the 1st dilution used [17]. For the inoculation we used dilutions 10^{-4}, 10^{-5} and 10^{-6}. Applied inoculation and cultivation methods are given in Table 1. The results were evaluated according to basic statistical characteristics.

Table 1: Monitored microbiological parameters

Microorganism cultivated	Growth media	Inoculation mode	Used dilution	Type of organism	Cultivation temperature	Time of cultivation
Enterobacteriaceae sp.	McConkey agar	Pouring plate	10^{-5}-10^{-6}	aerobic	37 °C	48 – 72 h
Enterococcus sp.	Slanetz-Bartley agar	Pouring plate	10^{-4}-10^{-5}	aerobic	37 °C	48 – 72 h
Lactobacillus sp.	MRS agar	Pouring plate	10^{-4}-10^{-5}	aerobic	37 °C	24 h

McConkey agar – Biomark laboratories, Pune (India)
Slanetz-Bartley agar, MRS agar – Imuna, Šarišské Michaľany

3. Results and discussion

The observed effects of essential oils on growth performance in chickens are either positive [13, 14] or non-significant [15]. The inclusion levels varied from 20 to 200 ppm. When the effect was positive, weight gain and feed intake were increased whereas the feed: gain ratio was lowered when compared to control. On the other hand, [16] reported that when dietary oregano essential oils, at the concentrations of 50 and 100 ppm, were fed to broiler chickens for a period of 38 days hardly any effects on body weight and feed conversion ratio could be demonstrated. [17] reported that dietary carvacrol and thymol, at levels of 150 ppm, did not influence body-weight gain. In another experiment with broilers were also found a lack of effect of thymol on growth performance and digestive enzyme activity when fed at a level of 100 ppm for a period of 6 weeks [18].

Body weight of chickens at the end of the experiment

Body weight of chickens ranged from 1567.20 g (control group) to 1607, 20 (trial group) Differences in body weight of chickens at the end of the experiment were not statistically significant (P> 0.05).

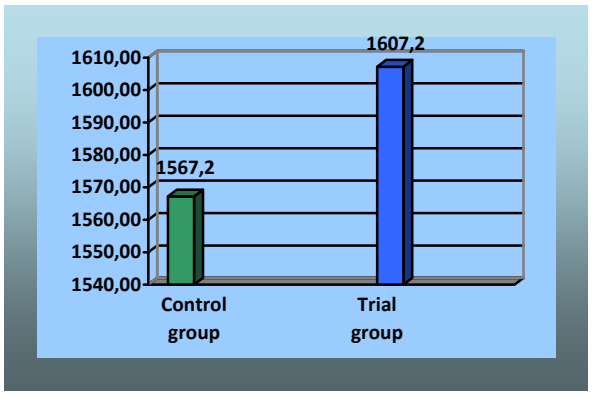

Figure 1: Body weight of broilers at the end of the experiment (g)

Table 2: Mathematics and statistical evaluation of results of broilers weight at the end of the experiment and differences between groups

Group	n	s[g]	v_k	Control group	Trial group
Control group	100	131.87	9.58		-
Trial group	100	184.34	11.66	-	

Scheffe´s test at significance level $P_{0.05}$; $^{-}P>0.05$

[19] observed health and overall production of broilers after vaccination against coccidia in combination with the organic component Orego-Stim (Origanum vulgare). Allowance of Orego-Stim (Origanum vulgare) affected weight gain and feed intake of chickens during the first 58 days, but feed conversion was not affected.

Number of Lactobacillus sp. varied in the trial group in the range from 8.05 log CFU.g-1 to 8.71 log CFU.g-1. The highest average number of log CFU.g-1 Lactobacillus sp. was detected in the trial group – 8.43 log CFU.g-1. Compared with the control group, we observed higher numbers of Lactobacillus sp. Which states for a positive effect of the used probiotic.

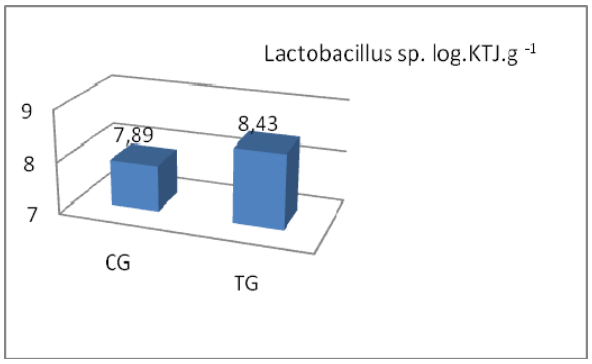

Figure 2 Average numbers of Lactobacillus sp. in the intestinal tract CG (control group) – no addition of probiotics and TG (trial group) - addition of 0.10 % of probiotics and 0.05% of essential oil

The number of Enterococcus sp.was in the trial group in a range from 6.54 to 7.48 log CFU.g-1.

The highest average number of log CFU.g-1 of Enterococcus sp. was observed in the control group (7.15 log KTJ.g-1), where they were fed chicken feed without the addition of probiotics and essential oil. Numbers of Enterococcus sp. in the control group were significantly lower.

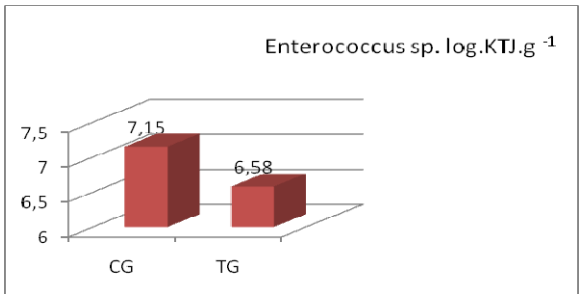

Figure 3 Average numbers of Enterococcus sp. in the intestinal tract CG (control group) – no addition of probiotics and TG (trial group) - addition of 0.10 % of probiotics and 0.05% of essential oil

The number of Enterobacteriaceae sp. ranged from 7.03 to 8.13 log CFU.g-1. The highest average number of log CFU.g-1 Enterobacteriaceae sp. was recorded in the control group (8.13 log CFU.g-1). The numbers of CFU of Enterobacteriaceae sp. were in the trial group significantly lower.

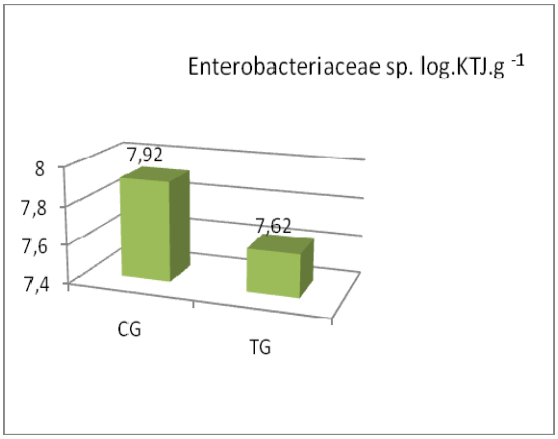

Figure 4 Average numbers of Enterobacteriaceae sp. in the intestinal tract CG (control group) – no addition of probiotics and TG (trial group) - addition of 0.10 % of probiotics and 0.05% of essential oil

4. Conclusions

The aim of this study was to evaluate the role of essential oils in combination with probiotics as the possible alternatives to antibiotics in poultry nutrition in relation to their production and microbiological parameters.

We used the feedstuff enriched with combination of 0, 05 % *Origanum aetheroleum* - essential oil and 0, 10 % probiotics – *Enterococcus faecium* CECT 4515. In our experiment with broilers type Cobb 500 were measured body weight of broilers at the end of the experiment from 1567.20 g (trial groups) to 1589.60 g (control group), which were not statistically significant (P> 0.05).

The findings of our experiments indicate that the use of combination of probiotics and essential oils have a favorable effect on the intestinal tract as the numbers of lactobacillus were significantly higher compared with the control group. The numbers of Enterococcus sp. were lower which may be due to the the type of experimental animals or the strain of used microorganism. The number of Enterobacteriaceae sp. were significantly lower compare with the control group.

Acknowledgements

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