The Effect of Probiotics on the Microbial Properties and Growth Performance of Broiler Chickens

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Abstract

In this study was examined the probiotic effect of the Enterococcus faecium CECT 4515 on the production growth and health of broiler chickens in conditions of welfare. The animals had a free access to feedstuff and water. The experiment was carried out on broiler types Cobb 500. The welfare, body weight of broiler chickens at the end of each cycle was interpreted. The breeding was kept in the terms of welfare. The viability in trial group (fed with feedstuff enriched with probiotic Enterococcus faecium CECT 4515) was 100% while in the control group the viability was 97 %. The average body weight at the end of experiment of chickens in control group was 1567.20 g while the average body weight of chickens in trial group was 1580.8 g. The difference between these two groups was not significant (P>0.05). Samples of caecal chime were acquired and the number of Enterococcus sp. from 6.52to 6.59 log cfu.g-1, the number of Lactobacillus sp. from 8.55 to 8.75 log cfu.g-1, and the number of Enterobacteriaceae sp. from 7.45 to 7.81 log cfu.g-1 was determined in the trial group.

Keywords: Body weight, caecum properties, intestinal microflora, probiotics.

1. Introduction

Food processors and consumers have expressed a desire to reduce the use of synthetic chemicals in food preservation. Common culinary herbs, spices and aromatic plants that exhibit antimicrobial activity could provide sources of acceptable, natural alternatives [1]. An aternative to the essential oils is also the pobiotics. Todays probiotics are defined as biopreparations that contain living cells or metabolites of stabilized autochthonous micro-organisms that optimize the colonization and composition of gut microflora in both animals and humans and have a stimulative effect on digestive processes and the immunity of the macro-organism [2]. Probiotics display several ways of action: antagonistic action towards pathogen bacteria by secretion of products which inhibit their development, such as bacteriocins, organic acids and hydrogen peroxide; the other way is competitive exclusion which represents competition for locations to adhere to the intestinal mucous membranes and in this way pathogen micro-organisms are prevented from inhabiting the digestive tract, and the third way is competition for nutritious substances [3]. In this way, they create conditions in intestines which favour useful and inhibit the development of pathogen bacteria [4]. Their effect on production results reflects in reduction of risk of diseases [5], they improve the function of the immune system [6] and exhibit significant influence on morpho-functional characteristics of intestines [7]. These effects lead to growth of broiler chickens [8], improvement of feed conversion and reduced mortality [9]. The addition of probiotic to the diet has been found to improve egg production, food conversion ratio [10,11] and food consumption [12].On the other hand, no positive results could be established in application of probiotic preparations in fattening of broilers in studies by certain number of researchers [13]. In this study

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we are trying to do a further investigation of above mentioned statements.

2. Materials and methods

In the experiment with broilers type Cobb 500 our goal was to monitor the influence of probiotics in two experimental groups, which were enriched with probiotic compound in the following scheme: CG (control group) - soy-grain type feed mixtures with no added probiotics and TG (test group) - soy-grain type feed mixtures with the addition of 0.10% of probiotics– Enterococcus faecium CECT 4515. Enterococcus faecium CECT 4515 is included in 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.

In the experiment with fattening chickens, we have focused on observing the impact of probiotics – Enterococcus faecium in feedstuff.

In the experiment, 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: starter, intended for chickens aged from 1 to 18 day during which the chicks were fed the starter feed mixture (HYD-01), growth for chicks aged 19 to 31 days with growth forage mixture HYD-02, 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).

Watched microbiological parameters:

The number of cfu (colony forming units) of Enterococcus sp. on Slanetz-Bartley agar after the incubation took from 48 to 72 hours at the temperature 37˚C. The number of cfu of Lactobacillus sp. on MRS agar after the incubation took from 48 to 72 hours at the temperature 37˚C. The number of cfu Enterobacteriaceae sp. on McConkey agar after the incubation took from 48 to 72 hours at the temperature 37˚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, we prepared further by decimal dilution system. Samples have finished vaccine, or embedded. Inoculated Petri dishes in an incubator, we cultivate bottom up. Temperature and time, was adjusted according to the group of cultivated microorganisms. After the cultivation, we counted colonies on the dishes. To calculate CFU.g-1 (Colony Forming Units), we used the following formula (which takes into account the bowls 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 [14]; For the inoculation we used dilutions 10-4, 10-5 and 10-6. Used inoculation and cultivation methods are given in Table 1. The results were evaluated according to basic statistical characteristics.

Table 1. Watched microbiological parameters

Cultivated type	Growth media	The way of innoculation	Used dilution	Type of organizm	Cultivation temperature	Tyme of cultivation
Enterobacteriaceae sp.	McConkey agar	inudate	10-5-10-6	aerobal	37 °C	48 – 72 h
Enterococcus sp.	Slanetz-Bartley agar	inudate	10-4-10-5	aerobal	37 °C	48 – 72 h
Lactobacillus sp.	MRS agar	inudate	10-4-10-5	aerobal	37 °C	24 h

McConkey agar – Biomark laboratories, Pune (India)

Slanetz-Bartley agar, MRS agar – Imuna, Šarišské Michaľany

3. Results and discussion

Probiotics are currently defined as substances or products containing sufficient numbers of viable microorganisms which, after having been implanted or after colonisation, change the microflora within a certain anatomical location of the host, and thus can manifest their beneficial effects on health [15]. The use of probiotic Enterococcus faecium CECT 4515 led to following resuls: Number of Lactobacillus sp. (Figure 1) varied in the trial group in the range from 8.55 log CFU.g-1 to 8.75 log CFU.g-1. The highest average number of log CFU.g-1 Lactobacillus sp. was detected in the trial group (8.59 log CFU.g-1) where they were fed chicken feed with the addition of probiotics. Compared with the control group, we observed higher numbers of Lactobacillus sp.These results suggest a possitive colonization of the intestine. Probioics as a living microbial probiotic culture can positively affect the host organism by improving its microbial balance [16]. Jin et al.[17] tested 12 Lactobacillus isolates for inhibition of 5 strains of Salmonella and found that all 12 Lactobacillus isolates inhibited the growth of the 5 strains of Salmonella.

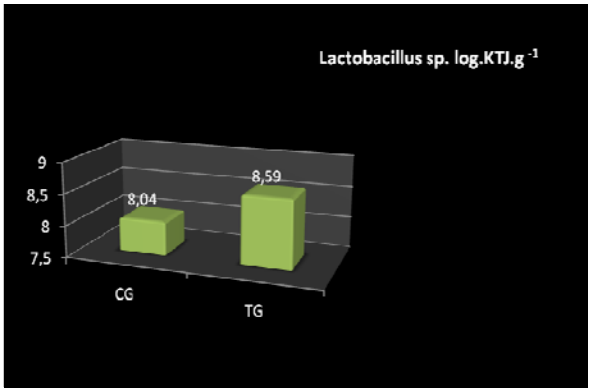

Figure 1. Average numbers of Lactobacillus sp. in intestinal tract CG (control group) – no addition of probiotics and TG (trial group) - addition of 0.10 % of probiotics

The number of Enterococcus sp. (Figure 2), was in the trial group in a range from 6.52 to 6.59 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), without the addition of probiotics. Numbers of Enterococcus sp. in the trial group were significantly lower. This fact may be a result a high competition in the intestinal tract of broilers.

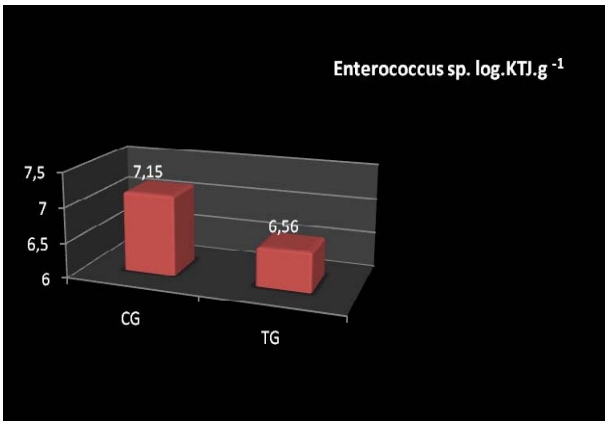

Figure 2: Average numbers of Enterococcus sp. in intestinal tract CG (control group) – no addition of probiotics and TG (trial group) - addition of 0.10 % of probiotics

The number of Enterobacteriaceae sp. (Figure 3) ranged from 7.45 to 8.18 log CFU.g-1. The highest average number of log CFU.g-1 Enterobacteriaceae sp. was recorded in the control group (7.92 log CFU.g-1).The average numbers of Enterobacteriaceae sp. in trial group were slightly lower which indicates that the used probiotic moderates the intestinal microflora in a positive way. In a study looking at the effects of B. subtilis on Salmonella Enteritidis and Clostridium perfringens in young chickens, La Ragione and Woodward [18] showed that B. subtilis spores reduced colonization of the pathogens when the spores were administered 24 h prior to challenge with each of the pathogens.

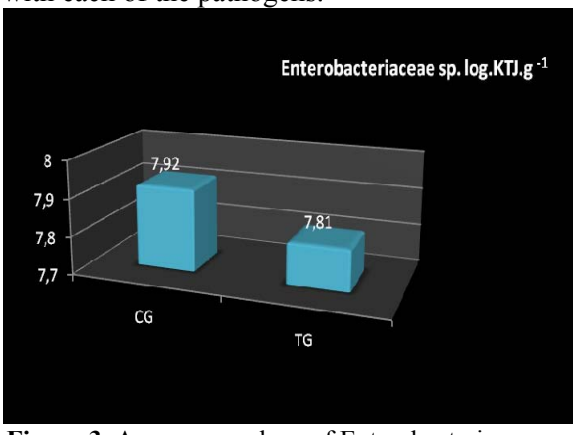

Figure 3. Average numbers of Enterobacteriacea sp. in intestinal tract CG (control group) – no addition of probiotics and TG (trial group) - addition of 0.10 % of probiotics

Body weight of chickens at the end of the experiment: Body weight of chickens ranged from 1567.20 g (CG) to 1580.80 g (TG). In the experiment monitored the broilers - Cobb 500 were differences in body weight of broilers not statistically significant (P> 0.05).

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]	vk	CG	TG
CG	100	131.87	9.58		-
TG	100	169.72	10.68	-	

Scheffe´s test at significance level P0.05; -P>0.05

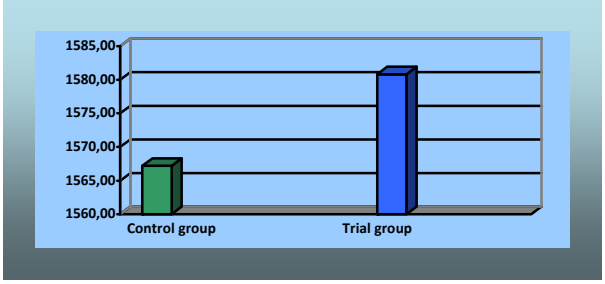

Figure 4. Body weight of broilers at the end of the experiment (g)

4. Conclusions

The results of this experiment suggest that the use of probiotics as a feed supplement has a appropriate effect on the populating of the digestive tract in fattening meat-type chickens, and can enhence a positive effect on their health and productive growth. In this experiment, we studied the impact of probiotics as a suitable feed supplement. We used the feedstuff enriched with 0.10% of probiotics. The obtained results suggest that the probiotic colonization positively affects the digestive tract and namely with the numbers of Enterococcus sp.and Lactobacillus sp. Which are higher compared with the numbers of control group. Compared with the control group were the numbers of Enterobacteriaceae sp. significantly lower. Numbers of Lactobacillus sp. ranged on average around 8.59 log CFU.g-1. We have also recorded the decreases of the amount of Enterococcus sp. in average of 6.56 log CFU.g-1, which may be due to the high competition in the digestive tract. These findings suggest a beneficial effect not only on the microflora of the intestine but a positive effect on the feed consumption and growth of broilers.

Acknowledgements

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References

1. Delaquis, P. J., Stanich, K., Girard, B., Mazza, G., Antimicrobial activity of individual and mixed fractions of dill, cilantro, coriander and eucalyptus essential oils, In: International Journal of Food Microbiology, 2002., 74, 101– 109
2. Fuller R. J.: Probiotics. The Scientific Basis. Chapman and Hall, London, New York 1992, 8, 398.
3. Patterson, J. A., Burkholder, M.,K. (): Application of prebiotics and probiotics in poultry production. Poultry Science, 2003, 82, 627-631
4. Line, E.,J., Bailey, S.,J., Cox, N. A., Stern, N. J., Tompkins, T., Effect of yeast-supplemented feed on Salmonella and Campylobacter populations in broilers, Poultry Science, 1998, 77, 405-410
5. Mountozouris, K. C., Tsirtsikos, P., Kalamara, E., Nitsch, S., Schatzmayr, G., Fegeros, K., Evaluation of the Efficacy of a Probiotic Containing Lactobacillus, Bifidobacterium, Enterococcus, and Pediococcus Strains in Promoting Broiler Performance and Modulating Cecal Microflora Composition and Metabolic Activities. Poultry Science, 2007, 86, 309-317
6. Kabir, S. M. L., Rahman, M. M., Rahman, M. B., Rahman, M. M., Ahmed S.U., The dynamics of probiotics on growth performance and immune response in broilers. International Journal of Poultry Science, 2004, 3, 361-364
7. Yang, Y., Iji, P.A., Choct, M., Dietary modulation of gut microflora in broiler chickens: a review of the role of six kinds of alternatives to in-feed antibiotics. World's Poultry Science Journal, 2009, 65, 97-114
8. Li, X., Qiang, L., Liu Xu, C. H., Effects of supplementation of fructooligosaccharide and/or Bacillus subtilis to diets on performance and on intestinal microflora in broilers. Archiv fur Tierzucht, 2008, 51, 1, 64-70
9. Mohan, B., Kadirvel, R., Natarajan, A., Bhaskaran, M., Effect of probiotic supplementation on growth, nitrogen utilisation and serum cholesterol in broilers. British Poultry Science, 1996, 37, 395-401
10. Abdulrahim, S. M., Haddadin, M. S. Y., Hashlamoun, E. A. R., Robinson, R. K., The influence of Lactabacillus acidophilus and bacitracin on layer performance of chickens and cholesterol content of plasma and egg yolk. British Poultry Science, 1996, 37, 341-346
11. Tortuero, F., Fernandez, E., Effects of inclusion of microbial cultures in barley-based diets fed to laying hens. Animal Feed Science and Technology, 1995, 53, 255

12 .Nahashon, S. N., Nakaue, H. S., Mirosh, L. W., Production variables and nutritient retention in Single Comb White Leghorn laying pullets fed diets supplemented with direct-fed microbials. Poultry Science, 1994a, 73, 1699-1711

13. Maiolin, R., Fioretti, A., Menna, L. F., Meo, C., Research of the efficiency of probiotics in diets for broiler chickens. Nutrition Abstract and Review Series B, 1992, 62, 482

14. STN EN ISO 4833, 1997, Mikrobiológia potravín a krmív. Horizontálna metóda na stanovenie počtu mikroorganizmov. Metóda počítania kolónií kultivovaných pri 30 °C

15. Schrezenmeir, J., De Vresse, M., Probiotics, prebiotics, and synbiotics - approaching and definition. American Journal of Clinical Nutrition, 2001, 73, 361-364

16. Tannock, G. W., 2002, Probiotics and prebiotics: Where are we going? Otago : Caister Academic Press, 2002, pp. 336

17.Jin, L. Z., Ho, Y. W., Abdullah, N., Ali, M. A., and Jalaludin, S., Antagonistic effects of intestinal Lactobacillus isolates on pathogens of chicken. Lett. Appl. Microbiol., 1996, 23, 67–71

18. La Ragione, R. M., Casula, G., Cutting, S. M., and Woodward, M. J., Bacillus subtilis spores competitively exclude Escherichia coli O78:K80 in poultry. Vet. Microbiol., 2001, 79, 133–142