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*Effect of probiotic supplementation on the performance and the composition of the intestinal microflora in broilers

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ABSTRACT

This paper investigates the influence of probiotic VEBAC on the intestinal microflora composition and the growth (the weight gain, feed conversion rate) of broiler chickens. Eighty Avian 24K male chickens were divided into 2 groups, 40 birds in each. The aim of this research was to determine the probiotic influence on the weight gain, feed conversion ratio and intestinal microflora content of the chickens. First group was a control group (without VEBAC in drinking water), while the second one was experimental (with addition of VEBAC - 3 g/100 l water). After six weeks of fattening, average live weight in the control group was 1956.10±15.03 g and in the experimental group 2168.25±54.24 g (P<0.01). Feed conversion was 2.16±0.26 and 2.02±0.28 kg/kg respectively. The results of bacteriological analyses of intestinal microflora point out the conclusion that lactic bacteria Enterococcus faecium M-74, which were consumed by broilers from the experimental groups through drinking water, resulted in reduction of bacteria from family Enterobacteriaceae and bacteria Escherichia coli, Staphylococcus aureus and Enterococcus faecalis.
(Keywords: broiler, probiotic, performance traits, intestinal microflora, *Enterococcus faecium* M-74)

ÖSSZEFOGLALÁS

Probiotikus készítmények adagolásának hatása a brojlerek telejesítményére és a bél mikroflóra összetételére

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A VEBAC probiotikum hatását vizsgáltuk brojlerek növekedésére, takarmány hasznosítására, illetve a bél mikroflóra összetételére. 80 Avian 24K brojler kakast két 40-es csoportra osztottunk. A kontroll csoport nem kapott VEBAC-ot az ivóvízben, a kísérleti csoport ivóvize viszont literenként 3 g probiotikumot tartalmazott feloldva. Hat hét hizlalás után a kontroll csoport átlagos élőtömege 1956±15,03 g, a kísérleti csoporté pedig 2168,25±54,24 g volt (P<0,01). A takarmány hasznosítás a két csoportban 2,13±0,26, illetve 2,02 ±0,28 kg/kg volt. A béltartalom mikroflóra analízise a következőket mutatta ki: az Enterococcus faecium M-74 tejsavbaktérium, melyet a kísérleti csoport brojlerei fogyasztottak az ivóvízen keresztül,

*A szerkesztőbizottság megjegyzése: a tanulmány érdekes, de a kis egyedszám miatt az eredmény fenntartással fogadható.

csökkentette az Enterobacteriaceae családhoz tartozó, valamint a Escherichia coli, a Staphylococcus aureus és az Enterococcus faecalis baktériumok számát.
(Kulcsszavak: brojler, probiotikum, teljesítmény vizsgálatok, bél mikroflóra, *Enterococcus faecium* M-74)

INTRODUCTION

Probiotics are often used in feeding of poultry in intensive rearing systems. Beneficial effects of probiotics were observed in toxin neutralisation, prevention of development and multiplication of specific bacteria, change in microbial metabolism and immunity stimulation (*Fuller*, 1989). It was found that probiotics enhance mineral absorption, synthesis and absorption of vitamins, especially of B-vitamin group, which are important for normal function of nervous system and have positive effect on stress. Positive effects resulted in increased body weight, better feed conversion and decreased mortality. *Kumprecht et al.* (1983) investigated the efficiency of probiotic *Enterococcus faecium* M-74 on daily weight gain, feed consumption, lactic bacteria and *Escherichia coli* content in intestinal chyme. After 49 days of fattening, chickens' live weight in experimental group was 49 g, or 3.05% higher than in the control group. Feed conversion of the control group was 2.35 kg, in comparison to the 2.38 kg of the experimental group. Authors stated that *Enterococcus faecium* M-74 significantly increased number of *Lactobacillus spp.* and *Streptococcus spp.* and decreased *Escherichia coli* in the intestine content, which explains better weight gain in the experimental group. Research results of *Hinton et al.* (1991), *Corrier et al.* (1992), *Cox et al.* (1992), *Nuotio et al.* (1992), as well as of *Schneitz and Nuotio* (1992) proved that usage of different probiotic microorganisms significantly decreased colonisation of *Salmonella spp.* in chicken intestines. *Kumprecht et al.* (1994) explored the influence of different probiotics on weight gain, feed conversion efficiency, lactic acid content in chyme of caecum, as well as the presence of *Escherichia coli*. Chickens from the 1st group were fed with diets containing yeast *Saccharomyces cerevisiae* var. *ovalis*. Chickens of the 2nd experimental group were given diets with *Enterococcus faecium* M-74, while chickens from 3rd group were given equal amount of *Saccharomyces cerevisiae* var. *ovalis* and *Enterococcus faecium* M-74. Authors stated that chickens from experimental groups were 5% heavier at the end of the fattening process, while feed conversion efficiency was 7% better. When compared to the control group, experimental group had significantly higher concentration of lactic acid in chyme of the caecum. Feeding of chickens with diets containing probiotics positively influenced cellulase activities and reduction of *Escherichia coli* by 58-74%. Comparing experimental groups showed that the best results regarding mentioned indicators were achieved in the group that was fed with diets with addition of *Saccharomyces cerevisae* and *Enterococcus faecium* M-74 combination. The aim of this study was to investigate the effect of probiotic preparation VEBAC containing lyophilised bacteria *Enterococcus faecium* M-74, on intestinal microflora content and growth of fattening chickens.

MATERIALS AND METHODS

Eighty Avian 24K male chickens were divided into 2 groups, with 40 birds in each. First group was a control group (without VEBAC in drinking water), while the second group was experimental (with addition of VEBAC - 3 g/100 l water). Probiotic VEBAC® is a trademark manufactured by Krka-Novo Mesto (Croatia) and contains 5×10^9 g^{-1} of stabile lactic bacteria *Enterococcus faecium* M-74. From 1st to 21st experimental day chickens

were fed with ST_1 grower starter diet containing 22.18% crude proteins and 12.30 MJ/kg ME. From 22nd to 42nd day chickens were fed with ST_2 diet containing 18.66% crude protein and 12.10 MJ/kg ME (*Table 1*). Throughout the experiment food and water were provided *ad libitum*.

Table 1

Composition of diets

Ingredients(1), %	Diet(2) ST_1	Diet(2) ST_2
Maize(3)	58.7	69.6
Extr. Soybean meal(4)	25.5	18.5
Extr. Sunflower meal(5)	3.0	3.0
Fish meal(6)	7.0	4.5
Sunflower oil(7)	2.0	-
Dicalcium phosphate(8)	1.0	1.5
Limestone(9)	2.0	2.0
Salt(10)	0.3	0.4
*Premix	0.5	0.5
Calculated nutrient composition(11), %		
Crude protein(12)	22.18	18.66
Ether extract(13)	4.09	3.39
Crude fibres(14)	3.45	3.48
Lysine(15)	1.24	0.95
Methionine(16)	0.41	0.35
Na	0.21	0.22
Ca	1.38	1.39
P total(17)	0.67	0.68
ME MJ/kg	12.30	12.10

*1 kg premix contain (*1 kg premix tartalmaz*): A 3.6 mg (12000 IU); D_3 0.05 mg (2000 IU); E 30 mg; K_3 2.5 mg; B_1 1.5 mg; B_2 6.0 mg; B_6 4.0 mg; B_{12} 0.015 mg; pantothenic acid 15 mg; nicotinic acid 20 mg; folic acid 0.5 mg; choline chloride 500 mg; Fe 30 mg; Cu 4.0 mg; Mn 80 mg; Zn 40 mg; Co 0.10 mg; Se 0.15 mg; antioxidant(a*ntioxidáns*) 147 mg; lysine (l*izin*) 1000 mg and methionine (*metionin*) 500 mg.

1. táblázat: A takarmány összetétele

Komponensek(1), Takarmány(2), Kukorica(3), Extr. Szójadara(4), Extr. napraforgódara(5), Halliszt(6), Napraforgó olaj(7), dikalcium-foszfát(8), Takarmánymész(9), Só(10), Számított táplálóanyag tartalom(11), Nyersfehérje(12), Nyerszsír(13), Nyersrost(14), Lizin(15), Metionin(16), Összes foszfor(17)

Every week chickens were individually weighted. After that, average weekly weight gains and growth rates were calculated for each group. Feed consumption was monitored simultaneously. Feed consumption and feed conversion ratio were presented for each week of the fattening period and for the whole fattening period. Weekly growth rates (GR_W) were calculated using the following equations:

$$GR_W = (y_i - y_{i-1}) / y_{i-1}$$

where: i = 1... 6 weeks, y_i = chickens' weight at the end of i^{th} week.

Average growth rates for the groups of chickens were calculated as a geometric means of weekly gain rates:

$$GR_A = \sqrt[6]{gr_1 \cdot gr_2 \cdot gr_3 \cdot gr_4 \cdot gr_5 \cdot gr_6}$$

For the purpose of microorganism investigation in the small intestine 3 chickens from each group were sacrificed after 42 days of fattening. Bacteriological analysis determined the following groups of microorganisms:

- *Enterobacteriaceae* on EE Broth Mosel and Violet red Bile Glucose Agar (VRBG) – 19-24 hours incubation, temperature 37°C, oxydase-negative colonies;
- *Escherichia coli* on peptonic water and Mac Conkey Agar, 18-24 hours incubation, temperature 37°C, fluorescence, indole positive test;
- *Enterococcus faecalis* on Azide Dextrose Broth and Kanamycin Aesculin Azide Agar, 24 hours incubation, temperature 37°C;
- *Staphylococcus aureus* on Giolitti Cantoni Bujon and Baird Parker Base Agar, 48 hours incubation, temperature 37°C; coagulase-positive black colonies;
- *Bacillus spp*. on Plate–count agar, pasteurisation 10 minutes at 70 °C, 72 hours incubation, temperature 30 °C, catalase-positive rods;
- *Clostridium spp.* - DRCM agar + overlay, pasteurisation 10 minutes at 70 °C, 48 hours incubation, temperature 37 °C, anaerobic, black colonies.

Bacterial colonies were identified and counted while average number of live bacteria in gram of original content of small intestine was calculated by multiplication of counted colonies by dilution factor. Dilution factor is a reciprocal value of dilution exponent. Such value is expressed as CFU g^{-1} (Colony Forming Units), i.e. units that form colonies.

Data processing was completed by the statistical program SAS, 6.12. version.

RESULTS AND DISCUSSION

From the data presented in the *Table 2* it is obvious that throughout the whole fattening period, chickens that received VEBAC in the drinking water achieved higher average live weights when compared to the chickens from the experimental group, without VEBAC in the drinking water. In comparison to the control group, broilers from the experimental group achieved higher average live weight from experimental to 6th week by 15 g, 44 g, 127 g, 163 g and 212 g. Statistically significant differences in achieved live weights were established among broilers from the control and experimental group ($P<0.01$).

The results obtained in the experimental group of chickens that had added probiotic VEBAC, were in accordance with the research results of *Kumprecht et al*. (1991, 1994) and *Kumprecht and Zobač* (1992). These results confirmed the fact that addition of probiotic during fattening process influenced the enhancement of broilers' performances. While above mentioned authors stated that usage of probiotic in the fattening process could increase live weights by 3.4-6.3%, in this study live weights were enhanced by 10.8% at the end of the 6th week.

Average weekly weight gains are also indicators of positive effect of VEBAC probiotic added in the drinking water (*Table 3*). From 1st to 6th week they were higher for 3.3%, 5.2%, 9.4%, 21.0%, 8.3% and 10.5% in the experimental group of broilers. According to the calculated values for growth rates, efficiency of VEBAC was obvious during the whole investigated fattening period.

Cumulative values of feed consumption and feed conversion from 1st to 6th week according to the groups are shown in *Table 4*. Broilers from the experimental group, which were given VEBAC in drinking water until 6th week, consumed on average 162 g or 3.93% more feed than broilers from the control group. More efficient feed conversion in the experimental group (6.05%) was in accordance with the results obtained by *Kumprecht et al.* (1994), *Kumprecht and Zobač* (1998).

It is also obvious that broilers from the experimental group achieved higher feed conversion ratio (2.02 kg/kg) when compared to broilers of the 1st group (2.16 kg/kg).

Table 2

Average live weights of the chickens during the performences trials

Age(1) (weeks)(2)	**control group(3)n = 40**	**experimental group(3) n = 40**
1st day(4)	43.05 ± 0.68	43.10$^{n.s}$ ± 0.49
1	133.75 ± 4.79	136.25$^{n.s.}$ ± 3.72
2	344.31 ± 3.60	359.28** ± 5.19
3	642.52 ± 16.01	686.45** ± 12.79
4	1042.63 ± 29.92	1169.71** ± 19.50
5	1477.42 ± 25.22	1640.38** ± 30.06
6	1956.10 ± 45.03	2168.25** ± 54.24

$^{n.s.}$=not significant (*nem szignifikáns*) (P>0.05); **P<0.01

2. táblázat: A csirkék átlagos tömege a hizlalás alatt

Kor(1), Hetek(2), Csoport(3), Első nap(4)

Table 3

Weekly weight gain and growth rate of broilers

Age(1) (weeks) (2)	**Weight gain(3), g**		**GR_W(5)**	
	control group(4)	experimental group(4)	control group(4)	experimental group(4)
1	90 ± 2.15	93** ± 2.33	2.11	2.16
2	212 ± 4.05	223** ± 1.12	1.57	1.64
3	298 ± 11.32	326** ± 13.22	0.84	0.91
4	399 ± 20.15	483** ± 21.05	0.62	0.70
5	435 ± 25.10	471** ± 24.18	0.42	0.40
6	478 ± 25.80	528** ± 26.16	0.32	0.32

Growth rate (*növekedési arány*)(5) **P<0.01

3. táblázat: A brojlerek hetenkénti tömeggyarapodása és növekedési aránya

Kor(1), Hetek(2), Tömeggyarapodás(3), Csoport(4)

Table 4

Results of the performance trials

Age(1) (weeks)(2)	Feed consumption(3), g		Feed conversion(5), g/g	
	control group(4)	experimental group(4)	control group(4)	experimental group(4)
1	116 ± 2.33	123** ± 2.38	1.29 ±0.02	1.32n.s. ± 0.01
2	375 ± 6.10	358** ± 6.20	1.77 ±0.04	1.60n.s. ± 0.03
3	550 ± 20.80	561** ± 20.49	1.84 ±0.05	1.72n.s. ± 0.04
4	810 ± 22.35	892** ± 21.35	2.03 ±0.05	1.85n.s. ± 0.06
5	1051 ± 30.21	1091** ± 27.14	2.42 ±0.07	2.32n.s. ± 0.07
6	1223 ± 30.44	1262* ± 32.33	2.56 ±0.05	2.39n.s. ± 0.10
Total(6)	4125	4287		
Mean(7)			2.16 ±0.26	2.16n.s. ± 0.26

n.s.=non significant (n*em szignifikáns*) (P>0.05), *P<0.05, **P<0.01

4. táblázat: A teljesítményvizsgálatok eredményei

Kor(1), Hetek(2), Takarmányfelvétel(3), Csoport(4), Takarmány értékesítés(5), Összesen(6), Átlag(7)

The results of bacteriological analysis of broilers' intestinal microflora are presented in *Table 5*. Probiotic VEBAC, which contained 5×10^9 g^{-1} of stabile lactic bacteria *Enterococcus faecium* M-74, had different effect on colonisation of digestive system by microorganisms.

After 42 days of fattening, 1.72×10^7 CFU g^{-1} live bacteria from family *Enterobacteriaceae* were found in the control group in 1 gram of intestinal content. On the other hand, reduction of these coliform bacteria was observed and their presence was 1.39×10^6 CFU g^{-1}, i.e. 90% less in relation to the control group.

Table 5

Comparative presence of microorganisms in the control and experimental group (in 1 g of original content of small intestine) (CFV g^{-1})

Microorganisms(1)	control group(2)	experimental group(2)
Enterobacteriaceae	1.72×10^7	1.39×10^6
Escherichia coli	1.36×10^6	2.72×10^5
Enterococcus faecalis	8.83×10^2	8.05×10^2
Staphylococcus aureus	4.92×10^6	1.73×10^6
Bacillus spp.	5.73×10^4	4.61×10^4
Clostridium spp.	1.15×10^3	1.04×10^3

5. táblázat: 1 g béltartalomra vonatkoztatott élő baktériumok mennyisége a kontroll és a kísérleti csoportban

Mikroorganizmusok(1), Csoport(2)

Application of probiotic in experimental group resulted also in significant decrease of *Escherichia coli*: from $1.36^{\times}10^{6}$ CFU g^{-1} to $2.72^{\times}10^{5}$ CFU g^{-1} colonies in gram of intestinal content.

Very high efficiency of *Enterococcus faecium* M-74 was observed regarding the vitality of pathogen bacterium *Staphylococcus aureus*; in experimental group their number was 1.73×10^{6} CFU g^{-1} of the intestine content, i.e. almost 64% less than in control group.

Probiotic VEBAC had limited effect on *Enterococcus faecalis* bacteria in the experimental group of broilers. Their presence was determined in amounts of 8.83×10^{2} CFU g^{-1} in control and 8.05×10^{2} CFU g^{-1} in experimental group.

Sporogenic bacteria from genus *Bacillus spp.* and *Clostridium spp.* showed marked resistance to probiotic VEBAC. Their presence remained almost the same in the digestive system of the broilers in both investigated groups.

By this manner VEBAC ensured the balance of the microflora and stimulated other bacterial species to produce nutrients that positively influenced fattening traits of broilers. These results are in accordance with those of *Barrow* (1992), *Bogut et al.* (1998, 2000), *Milaković et al.* (1999).

CONCLUSIONS

On the basis of research results of VEBAC probiotic addition in drinking water on Avian 24 broilers, the following conclusions can be drawn:

- Average live weights of the experimental broiler group (3 g/100 lit. VEBAC), at the age of 42 days, were 10.8% higher than those of the control group, which did not receive probiotic in the drinking water.
- Added probiotic improved feed conversion ratio by 6.48% in the experimental group of broilers. Throughout the whole fattening period, these broilers consumed 3.93% more feed than the broilers from the control group.
- Bacteriological analyses of intestinal microflora pointed out the reduction of live bacteria from family *Enterobacteriaceae* in the experimental group in 1 gram of intestinal content, which contained 90% less bacteria, when compared to the control group.
- Experimental group had less *Escherichia coli* in gram of intestinal content (2.72×10^{5} CFU g^{-1}) when compared to the control group (1.36×10^{6} CFU g^{-1}).
- In comparison to the control group, in the experimental group presence of *Staphylococcus aureus* as pathogenic bacteria in the intestinal content was lowered for 64%.
- Probiotic VEBAC had minor influence on bacteria *Enterococcus faecalis* in the experimental group (8.05×10^{2} CFU g^{-1}) than in the control group (8.83×10^{2} CFU g^{-1}).
- Sporogenic bacteria from genuses Bacillus spp. and Clostridium spp. showed a strong resistance, which was concluded upon their presence in digestive system, that was almost the same in both groups of broilers.

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