

## The influence of *Enterococcus faecium* M-74 bacteria on bone mineralization in chickens

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### Abstract

Comparative feed and group trials were performed to study the influence of *Enterococcus faecium* M-74 bacteria (a basic ingredient of the Lactiferm L-5 probiotic) in feed mixtures on calcium, phosphorus and magnesium resorption in the broilers' thighbones. The experiment included 300 ROSS 208 broilers. Up to the age of 42 days they were fed with the mixtures HYD 01, HYD 02 and HYD 03, which differed in animal protein and plant protein contents. The experimental feed mixtures for groups G1 and G2 contained 14.5 % - 14.9 % animal proteins, the mixtures for groups G3 and G4 contained only plant proteins. To the feed of groups G2 and G4 5 mg/kg Lactiferm L-5 was added. The control group C was fed with a commercial mixture, which contained 23.7 % - 24.5 % animal proteins without the Lactiferm L-5 addition. On the 21st day a significant difference was observed in calcium content of the thighbones. On the 42nd day this difference no longer persisted. The phosphorus and magnesium contents had the highest average values (phosphorus in the G4 group, magnesium in the G2 group) when fed with feed mixtures which included Lactiferm L-5. This observation indicates a possible positive influence of *Enterococcus faecium* M-74 on phosphorus and magnesium resorption in the bones, which probably will be effective only in the last phase of fattening.

**Key words:** broiler, thighbone, probiotic, plant protein, resorption, calcium, phosphorus, magnesium

### Introduction

The application of probiotics can positively affect the intestinal microflora, which has an important influence on the metabolic processes of nutrients' digestion and on immunity (Netherwood et al., 1999). The widely used probiotic Lactiferm contains as active component the *Enterococcus faecium* M-74 bacteria, which is characterised by low generation age and high stability in the intestines. It triggers intensive production of L-lactic acid and inhibits the growth of enterotoxic bacteria. Its application is most essential for the younger chicken. Positive influence of Lactiferm on the health of monogastric animals was observed by many authors (Chmelníčná, 2000, Hejlíček, Soukupová, 1995, Šišák et al., 1995 and others). For proper bone growth of the young organism, optimal feeding, adequate treatment and good health is mandatory. The goal of our work was to study the influence of *Enterococcus faecium* M-74 on bone mineralization.

### Material and Methods

A comparative feed and group experiment, which was carried out at the Institute of Veterinary Hygiene included 300 ROSS 208 broilers, which were partitioned into four experimental groups and one control group. The broilers up to the age of 42 days were initially fed with the feed mixture HYD 01 (to the 21st day), then with HYD 02 (to the 35th day) and finally with HYD 03, which differed in animal protein and plant protein contents. Properly feeding the chicken initially demanded an abundant supply of proteins, in later stages of their lives energy considerations were more relevant. The experimental feed mixtures for groups G1 and G2 contained animal proteins, the mixtures for the groups G3 and G4 contained only plant proteins. To the feed mixtures of the groups G2 and G4 Lactiferm L-5 in a concentration of 5mg/kg was added. The control group C was fed with a commercial mixture, which contained animal proteins without Lactiferm. The trial scheme is shown in Table 1. The active component of Lactiferm L-5 is a culture of *Enterococcus faecium* M-74

bacteria in the concentration  $5.10^9 \text{ g}^{-1}$ . Characteristics of the trial were obtained after sacrificing 6 broilers (3 males and 3 females from each group) after 21 and 42 days. The concentrations of calcium, phosphorus and magnesium in the thighbones were analysed via gas absorption spectrophotometry. Summary statistics and significance verification of differences between groups were evaluated via two-sample nonpaired t-tests with the Statgraphic 5.0 package and are documented in Table 2 for the end of the feeding period.

## Results and Discussion

The contents of calcium in the bones were considerably different among the groups. On the 21st day, the lowest average content of calcium was observed in G4, the highest in G3 (+ 13.1 %,  $P < 0.05$ ). Significant differences ( $P < 0.05$ ) were observed between G4 and G2 and an insignificant difference ( $P < 0.1$ ) was registered between G4 and G1. All groups except G2 experienced a decrease of the average calcium content in the thighbone in the fattening period. The highest average value was reached in G4, the lowest in the control group C (-12,8 %). All differences after 42 days were insignificant. These results are consistent with observations by Horniaková and Krivánek (2001).

The average phosphorus content in the bones after 21 days varied from  $91043.7 \text{ mg.kg}^{-1}$  (G1) to  $98960.5 \text{ mg.kg}^{-1}$  (G4), but the differences were insignificant. At the end of the feeding period the highest average phosphorus content was recorded in G4, the lowest in G1 (-13.2 %,  $P < 0.01$ ). These results are in accordance with insignificant differences observed by Horniaková and Krivánek (2001) in another experiment.

The changes of magnesium content in all groups followed a similar tendency with the exception of the group G2. On the 21st day the values spanned the interval  $3477.00 \text{ mg.kg}^{-1}$  (G2) –  $3777.33 \text{ mg.kg}^{-1}$  (G4). Differences were insignificant.

An interesting fact was manifest on the 42nd day of feeding. The magnesium content in G2 had risen 6 % against the control group C and remained at this higher average value when compared with the other experimental groups, which underwent a rapid decrease of magnesium content. It is also interesting, that the phosphorus and calcium resorption followed a similar trend. Horniaková and Krivánek (2001) did not find any significant elevation in magnesium content in the thighbones after 42 days of the feeding with feed mixtures containing *Enterococcus faecium* M-74. In this trial insignificant differences were recorded ( $P < 0.1$ ) between the groups G1 and G2.

## Conclusion

On the 21st day significant differences were found in calcium content. On the 42nd day these differences were insignificant among the control group and the experimental groups. Concerning the phosphorus and magnesium content high average values (in case of phosphorus in the G4 group and in case of magnesium in the G2 group) were detected. Groups G2 and G4 were fed with feed mixtures containing the Lactiferm L-5 probiotic. This fact refers to a possible positive influence of *Enterococcus faecium* M-74 on the phosphorus and magnesium resorption into the bones, which will probably be manifest only in the fattening period. The addition of the probiotic was more effective in the groups where chickens were fed with feed mixtures containing plant proteins.

## References

HEJLÍČEK, K., SOUKUPOVÁ, A. (1995) Experiences with the Application of the Probiotic Lactiferm for Prevention and Suppression of Infections caused by *Clostridium perfringens* in Pigs and Poultry. International Conference: Probiotics in the Nutrition of Animals. Journal of Animal Nutrition, 40/9, p. 429-430.

**HORNIÁKOVÁ, E.** (1999) Fattening of Broilers with Different Ratios of Animal Components and Probiotics. Proceedings of Conf. XVII: Most Recent Knowledge on Production and Usage of Feeds, Feed Mixtures and Feed Additives in the Feeding of Animals with Future Perspectives. Research Conference of the Feeding Research Institute, Ivánka at Dunaji, pp. 210-213.

**HORNIÁKOVÁ, E., KRIVÁNEK, L.** (2001) Influence of experimental feed mixtures for broilers considering the utilisation of calcium, phosphorus and magnesium. Proceedings of the Conference: Risk Factors of the Food Chain. International Research Conf., Nitra, pp. 56-58.

**CHMELNIČNÁ, Ľ.** (2000) Poultry Growth Stimulation with Probiotics. SAU Monography, Nitra, 69 pp.

**NETHERWOOD, T., GILBERT, H.J., PARKER, D.S., O'DONNELL, A.G.** (1999) Probiotics shown to change bacterial community structure in the avian gastrointestinal tract. Applied and Environmental Microbiology. 65/11, p. 5134-5138.

**ŠIŠÁK, F., LÁNY, P., ČERNÍK, J., LOJEK, A.** (1995) Testing the Efficiency of Probiotics concerning *Salmonella* Contamination in Chickens. International Conference: Probiotics in the Nutrition of Animals. Journal of Animal Nutrition, 40/9, p. 429-430.

Trial Scheme Table 1

	HYD 01					HYD 02					HYD 03				
	C	G1	G2	G3	G4	C	G1	G2	G3	G4	C	G1	G2	G3	G4
X	+	+		-		+	+		-		+			-	
Y	23.70	14.50		0.00		24.50	14.90		0.00		15.56			0.00	
Z	-	-	+	-	+	-	-	+	-	+	-	-	+	-	+

X – presence of animal proteins in feed mixture

Y – share of animal proteins in percent

Z – addition of 5 mg/kg Lactiferm L-5

Calcium, phosphorus and magnesium in the thighbones after 42 days (mg.kg<sup>-1</sup>) Table 2

Group	Average	Standard deviation	Coefficient of variance	Minimum	Maximum	Significance
Ca						
C	75617.70	15022.00	19.8655	58823	97058	-
G1	82381.40	9823.04	11.9238	70833	93939	-
G2	79478.20	9921.56	12.4834	72020	100214	-
G3	82865.30	16588.60	20.0188	61267	96632	-
G4	86713.70	11570.70	13.3435	66152	97142	-
P						
C	101251.00	10302.00	10.2762	89191	113462	-
G1	95144.70	7653.73	8.0443	80000	100929	G4 +++
G2	108481.00	18059.80	16.6480	89286	130250	-
G3	100421.00	16448.70	16.3798	85338	126889	-
G4	109638.00	7688.65	7.0127	98387	116385	G1 +++
Mg						
C	3230.83	301.827	9.3421	2893	3624	-
G1	2975.33	321.277	10.7980	2410	3251	G2 +
G2	3293.00	208.777	6.3400	2956	3505	G1 +
G3	3139.17	128.859	4.1049	3042	3327	-
G4	3215.00	185.114	5.7572	2922	3441	-

### Efficacy and safety of a novel phytase in turkeys fed diets to market weight