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EFFECT OF PROBIOTICS ON PROTEIN PRODUCTION IN FATTENING CHICKEN MEAT

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ABSTRACT

In this work the influence of probiotic preparations added into the water or feed on protein production in the thigh and the breast muscle of chickens was observed. Probiotics were applied into drink water for the Hubbard JV (the 1st experiment) and Hybro (the 2nd experiment) chickens and into the feed of crossbred ROSS 308 chickens (the 3rd experiment). Probiotics were produced on the base of microorganisms of *Lactobacillus fermentum* (1. experiment), *Enterococcus faecium* (2. experiment) and *Bacillus subtilis* and *Bacillus licheniformis* (3. experiment). Time of feeding was 42 days. A feed-stuff was used either in the dry form (the 1st and the 2nd experiment) or in granular form (the 3rd experiment). The nutritive contents were the same in each experiment, except the 3rd experiment, which was designed on the plant base. Probiotics increased the protein content in the breast muscle of all crossbreds, but this increase was not statistically significant. In the thigh muscle, protein content was increased only in crossbred Hybro and ROSS 308 chicken as compared to the control group. The highest protein content was in Hubbard JV chickens in both groups (control and experimental). In term of higher content of proteins in the breast and the thigh muscle we assume that the probiotic supplementation of the drink water may be more effective than its application into the feed.

Key words: protein; muscle; *Lactobacillus fermentum; Enterococcus faecium; Bacillus subtilis* and *Bacillus licheniformis*

INTRODUCTION

The feed-stuffs assigned on the diet of poultry are enriched by probiotics and enzymatic preparations. They are used as substitute additives. Probiotic microorganisms suppress expansion of enteropathogenic organisms. Probiotics increase digestibility and utilization of the feed-stuff and participate on optimal growth performance (Shahhani et al., 1989; Nahashon et al. 1992; Kumprecht and Zobač, 1998; Hai and Blaha, 2000; Andersson et al., 2001; Haščík et al., 2004, 2005a; Angelovičová, 1997; Angelovičová et al., 2005). Bedford and Schulz (1998) have observed that using the application of the enzymatic preparations into the feed for monogastric animals it is possible to increase utility with reduction in quantity of the feed-stuff. They observed that the use of exogenous enzymatic preparations can reduce negative responses of some antigens, which are components of nitrogenous substances and sugars. Probiotic bacteria have been defined as "live microbial food supplements which beneficially affect the host by improving the intestinal microbial balance" (Fuller, 1989). Probiotic bacteria are increasingly utilized in human food as well as in animal feed products (Fuller, 1999; Sanders and Huis in't veld, 1999). However, composition of the intestinal microbiota is poorly known, which hinders the understanding of the probiotic functions (Tannock, 2005). A probiotic strain should be of host origin, non-pathogenic, technologically suitable for industrial processes, acid- and bile-fast, adhere to the gut epithelial tissue, persist in the gastrointestinal tract for short period, produce antimicrobial substances, modulate immune responses and influence the metabolic activities of the gut (Kačániová et al., 2006).

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Haščík et al. (2007) observed that the probiotic preparations which were made from effective microorganisms can increase economical profit from the poultry production.

In our work we aimed at evaluation and comparison of protein production in the breast and the thigh muscle of broiler chickens which were fed by various types of probiotic preparations.

MATERIAL AND METHODS

The experiment was divided into three parts. As a biological material combinations of hybrid chickens of the meat type Hubbard JV (the 1st part), Hybro (the2nd part) and Ross 308 (the 3rd part) were used. Cage technology was used for poultry breeding during the feeding experiment. The experiments were conducted in the experimental hall of the Department of Poultry Science and Small Animals Husbandry at SPU in Nitra. The 1-day old chickens from each part (1st, 2nd, 3rd.) were pooled and divided into two groups (control, experimental) at 60 chickens per group. Ten chickens were placed to each cage (26.6 chickens x m^{-2}). The broiler chickens from the 1st and the 2nd experiment were fed with commercial feedstuff HYD-01 (21 days) and since the 22nd day to 42nd day - with the HYD-05 feedstuff. In the 1st experiment we applied the probiotic preparation based on *Lactobacillus fermentum* which contains $1x10^8$ CFU per 1g of medium. The measurement of probiotics is shown in table 2. In the 2nd experiment we used the probiotic preparation based on *Enterococcus faecium*. It contained 2 x 10^{10} CFU per 1g of medium. The measurement of probiotics is shown in table 3. Both preparations were applied into the water. In the 3rd experiment we used the probiotic preparation based on *Bacillus subtilis* and *Bacillus licheniformis* at the rate of 400 g x t^{-1} or 1.28 x 10^6 CFU. t^{-1} respectively, which was added into the feedstuff.

The feeding period was 42 days for each group. The chickens were fed ad libitum. Nutrient content in 1 kg of HYD-01 and HYD-02 mixtures was similar in each experiment (tab. 1). The broiler chickens in the 3rd experiment were fed by granulated feedstuff which was based on plant constituents.

Table 1: Nutrient content in 1 kg mixtures of HYD-01 and HYD-02

Formulation feed mixtures in %	HYD-01 –starter	HYD-02 -growth
Proteins in g	222,18	208,25
Metabolizable energy in MJ	11,907	12,065
Lysine in g	13,10	12,80
Methionine + cysteine in g	8,414	8,539
Calcium in g	9,09	8,41
Non phytated phosphorus in g	3,09	3,74

For the evaluation of protein content in the broilers meat we picked up 30 chickens from each group. Most valuable parts of carcass, the breast muscle without skin and the thigh muscle with skin and subcutaneous fat were removed.

The basic chemical composition was measured using INFRATEC1256 (NSR) device. Contents of dry matter and proteins were expressed in g x 100 g^{-1}.

Basic statistical characteristics (arithmetical average, standard deviation, min, max and variation index) were evaluated by the SAS 8.2 statistical program. Differences between groups in the experiment were estimated by the t-test.

Table 2: Measurement of drinking water and probiotic preparation in experimental group per day (the 1st part of experiment)

Week	Count of broilers	Amount of drinking water in l	Measurement of probiotic (ml)
1	60	2,5	6,6
2	60	3,5	6,6
3	60	4,6	3,7
4	60	6,7	3,7
5	60	8,6	3,7
6	60	10,6	3,70

Table 3: Measurement of drinking water and probiotic preparation in experimental group per day (the 2nd part experiment)

Week	Count of broilers	Amount of drinking water in l	Measurement of probiotic (ml)
1	60	2,28	0,72
2	60	3,42	0,30
3	60	4,50	0,30
4	60	6,00	0,30
5	60	7,80	0,30
6	60	9,60	0,30

RESULTS AND DISCUSSION

Variability in chemical composition of the meat is relatively high at comparison of several livestock species and the wildlife animals. One of the ways how to influence the quality of the meat is animal nutrition or, eventually, application of new trends in poultry breeding. In recent time, probiotic preparations based on various strains of microorganisms, belong to such new trend.

Table 4: Chemical composition of breast muscles (1st , 2nd , 3rd part of experiment)

Indicator	Part of experiment	Group	n	$\bar{x}$	s	min.	max.	v %	Statistical evaluation
Dry matter (g.100g^{-1})	1.	Control	30	25,32	0,31	25,00	25,70	1,23	K:P $^{-}$
		Experimental	30	25,86	0,40	25,50	26,50	1,56	
	2.	Control	30	25,55	0,32	25,10	26,20	1,27	K:P $^{-}$
		Experimental	30	25,74	0,52	24,80	26,40	2,03	
	3.	Control	30	24,64	0,43	24,01	25,50	1,73	K:P $^{-}$
		Experimental	30	24,35	0,29	23,92	24,72	1,21	
Proteins (g.100g^{-1})	1.	Control	30	22,56	0,21	22,20	22,70	0,92	K:P $^{-}$
		Experimental	30	22,84	0,43	22,20	23,40	1,87	
	2.	Control	30	22,78	0,29	22,10	23,20	1,26	K:P $^{-}$
		Experimental	30	23,07	0,25	22,50	23,30	1,06	
	3.	Control	30	21,95	0,44	21,00	22,50	1,99	K:P $^{-}$
		Exprimental	30	22,05	0,24	21,70	22,40	1,07	

Statistical evaluation: - = P ≥0,05, n – number of pieces, $\bar{x}$ - average, s - standard deviation, min. – minimal value, max. – maximal value, v – variation index [%]

Table 5: Chemical composition of thigh muscles (1st , 2nd , 3rd part of experiment)

Indicator	Part of experiment	Group	n	$\bar{x}$	s	min.	max.	v %	Statistical evaluation
Dry matter (g.100g^{-1})	1.	Control	30	31,18	1,82	29,40	34,20	5,85	K:P $^{-}$
		Experimental	30	31,26	0,70	30,50	32,40	2,25	
	2.	Control	30	33,19	2,07	30,40	35,60	623	K:P $^{-}$
		Experimental	30	30,54	0,97	28,70	31,80	3,18	
	3.	Control	30	27,68	1,40	25,26	29,55	5,07	K:P $^{+}$
		Experimental	30	29,44	1,70	27,56	31,60	5,78	
Proteins (g.100g^{-1})	1.	Control	30	18,34	0,57	17,50	19,00	3,10	K:P $^{-}$
		Experimental	30	18,06	0,09	17,90	18,10	0,50	
	2.	Control	30	17,52	0,66	16,60	18,50	3,76	K:P $^{-}$
		Experimental	30	18,01	0,27	17,60	18,40	1,49	
	3.	Control	30	17,00	0,37	16,50	17,60	2,20	K:P $^{-}$
		Experimental	30	17,18	0,35	16,60	17,60	2,05	

Statistical evaluation: - = P ≥0,05, n – number of pieces, $\bar{x}$ - average, s - standard deviation, min. – minimal value, max. – maximal value, v – variation index [%]

Measured values of protein and dry matter contents in our experiment are shown in tables 4 and 5. On the basis of experimental data we can state that the dry matter content in the breast muscles ranged from 24.35 g.100g^{-1} (the 3rd part of experimental group) to 25.86 (1st part of experimental group) per 100g of meat sample. The lowest value of the dry matter count without application of probiotics was noticed at hybrid combination Ross 308 and the value was 24.64 g x 100g^{-1}. The highest value 25.35 g x 100g^{-1} was noticed at hybrid combination Hybro.

After the application of probiotic preparations the highest dry matter count in breast muscles was noticed at hybrid preparation Hubbard JV (25.86 g x 100g^{-1}) with the application of probiotic *Lactobacillus fermentum* base; the lowest value (24.35 g x 100g^{-1}) - with the application of probiotic *Bacillus subtilis* and *Bacillus licheniformis* base was noticed at hybrid combination Ross 308. Our obtained values are comparable with the data published by Uhrín et al. (1993) or Mojto and Palanská (1997). In their experiments with the meat from the breast muscles of the chickens the values ranged from 25.36 to 26.19 g x 100g^{-1}.

The dry matter count in the thigh muscles was in each experimental group higher than in groups of the meat from the breast muscles. It is due to presence of skin and subcutaneous fat in the samples from the thigh part of the carcass. The lowest value (27.68 g x $100g^{-1}$) was measured in the control group at 3rd part of control (the group with hybrid combination of Hybro chickens). The highest value - 33.19 g.$100g^{-1}$ (the 2st part – control group) was measured in Hubbard JV chickens.

After application of the *Lactobacillus fermentum* base probiotic preparation the highest value (31.26 g x$100g^{-1}$) of the dry matter counts was noticed in the thigh muscle in hybrid combination Hubbard JV. The measured values of the dry matter content in thigh musculature with skin and subcutaneous fat after application of probiotic preparations are lower in groups with hybrid combinations Ross 308 chickens and higher in the group with the hybrid combination the Hubbard JV and Hybro chickens. These values are comparable with the results published by Horváthová (1989), where the values ranged from 32.95 to 37.66 g x$100g^{-1}$.

The dry matter count in carcasses of broilers is closely associated with the count of proteins and lipids. The lowest count of proteins in 100g of the breast muscles was 21.25 g x $100g^{-1}$ in the control group at 3rd part of experiment (hybrid combination Ross 308) and the highest was 23,07 g x $100g^{-1}$ at hybrid combination Hybro with probiotic application of *Enterococcus faecium* base. In control groups (without application of probiotic preparations) a similar tendency as in the experimental groups was observed. The lowest value (21.95 g.$100g^{-1}$) of proteins was noticed at hybrid combination Ross 308 and the highest (22.78 g.$100g^{-1}$) at hybrid combination Hybro.

The increase of the protein count in the breast muscles in experimental groups indicates possible effect of the probiotic preparations on production of proteins in musculature of broilers. This suggestion is in agreement with Haščík et al. (2005b) statement, even though statistical differences between experimental groups were not significant ($P \geq 0.05$). The measured protein counts in the breast muscles of broilers in the 1st and the 2nd part of the experiment are up to the mark, whereas the similar results were published by Uhrín et al. (1993), where the values ranged from 22.65 to 23.40 g x $100g^{-1}$.

The results which were obtained at the 3rd part of the experiment indicate on the reduced protein count in both experimental groups. Decreased values of the protein count are probably caused by nutrition. The broilers in this part of experiment were fed with food-stuff based only on plant constituents. The effect of the application of probiotic preparations in experimental groups is expressed in the increase of protein count about 0,10 g x $100g^{-1}$ compared to the control group. The statistical differences were not significant ($P \geq 0.05$).

In samples from the thigh part (regarded as „red meat") protein counts were increased compared to the values measured in samples from the breast parts. This was noticed in each group of experimental parts (from the 1st to the 3rd). These results are comparable with the results of Haščík et al. (2005b). The value of the protein count was the lowest (17.00 g x $100g^{-1}$) in control group at the 3rd part of the experiment (the Ross 308 chickens) and the highest (18.34 g.$100g^{-1}$) was in control group at the 1st part of the experiment (the Hubbard JV chickens).

After the application of probiotic preparations the highest value (18.06 g x $100g^{-1}$) of the protein counts was noticed in the thigh muscles at hybrid combination Hubbard JV and the lowest (17.18 g x $100g^{-1}$) - at hybrid combination Ross 308. In the thigh musculature, like to the breast musculature, the probiotic effect on the total count of proteins was not statistically confirmed. Our results are consistent with the observations of Haščík et al. (2005b), but they differ from the data of Uhrín et al. (1993).

A positive fact found in our experiment is, that at hybrid combinations, Hybro and Ross 308 showed an increasing tendency of the protein counts; in the hybrid combination Hubbard JV chickens, the values of the protein counts were not significantly decreased. On the basis of obtained results we can state that from different probiotics tested in our experiment the most effective was a preparation based on *Enterococcus faecium*, whilst probiotic preparations based on *Bacillus subtilis* and *Bacillus licheniformis* were less effective. The efficiency of the probiotic preparations in the nutrition of broilers is higher at their application into the water than if it is supplied into the complete food-stuff.

SUMMARY

The results of our experiment and results of other authors demonstrate, that by the application of probiotic preparations in the nutrition of poultry we can increase the production indicators, improve health state of the broiler chickens and, finally, we can positive influence the quality of the chicken meat. For consumers is very important to know, that the musculature without skin and subcutaneous fat contains more proteins and less fat than musculature with mentioned attributes. Poultry, which is fed with the right balanced food-stuffs, enriched with probiotic preparations, is one of the way how to increase the supply of essential proteins for consumers. On the other hand, consummation of poultry meat of this sort may have positive effects on cardiovascular system and prevention of cancer diseases. Well-balanced consummation of the poultry meat can lead to longevity of consumers.

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