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THE PROBIOTIC MIXTURE X FEEDING EFFECT ON THE GROWTH AND DEVELOPMENT OF BROILER CHICKEN DIGESTIVE TRACT

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Abstract
The issue of antibiotic resistance has become more pressing in the last decades. Therefore, substitutes for antibiotics are being sought. The aim of our study was to evaluate the effect of the mixture x of lactic acid bacteria on development of the broiler chicken digestive tract and the growth. The study was organised in three trials. In each trial, 260 one day old Ross 308 broiler chicks (males and females) were obtained from a commercial hatchery. They were randomly divided in two groups – the control group and the probiotic group. The dietary treatment was basal diet for the control group and basal diet + the mixture X of lactic acid bacteria 4 g 10 kg^{-1} for the probiotic group. Broilers were raised till day 35. All broilers were weighted on the day 1, 7, 14, 21, 28, 35 and 10 birds per treatment were randomly selected and killed by cervical dislocation. The gastrointestinal tract was excised (proventriculus, gizzard, intestines) and weighed with content. Overall, this study achieved significant results of the body weight results in the probiotic and the control groups, 2,835.7g ±161.74 and 2,828.02±115.64, respectively. The body weight of chickens and their gastrointestinal tract parts (proventriculus, gizzard, intestines) did not differ between the probiotic and control groups ($p > 0.05$).
Key words: body weight, *Lactobacillus farciminis*, *Lactobacillus rhamnosus,* poultry, Ross 308.

Introduction

The use of antibiotics in poultry, as in other agricultural sectors, has been very widespread since the discovery of antibiotics. The main uses of antibiotics in poultry are the treatment and prevention of diseases, as well as growth promoters (Abudabos, Al-Batshan, & Murshed, 2015; Al-Khalaifa *et al.*, 2019; Reuben *et al*., 2021; Wang *et al*., 2016).

The issue of antibiotic resistance has become more pressing in the last decades. There are studies linking the emergence of antibiotic resistance to the use of antibiotics as growth promoters in the poultry industry (Cosby *et al*., 2015).

The European Union has banned the use of human antibiotics in animals as growth promoters since 2006 (Djuric, 2005). Since that ban, producers in the European Union have been facing various problems, such as reduced growth rates, dysbacteriosis and enteritis caused by various pathogens (Palamidi, 2016; Reuben, 2021). Therefore, substitutes for antibiotics are being sought. The beneficial effects of probiotics, prebiotics, acidifiers and phytogenic substances etc. on birds have been studied very actively (Abudabos, Al-Batshan, & Murshed, 2015).

Probiotics have been shown to inhibit the development and growth of pathogens and to improve the intestinal microflora. Colonization of the intestinal tract by beneficial bacteria, such as *Lactobacillus spp.*, *Bifidobacterium spp.* etc., reduces attachment sites and nutrients to the pathogenic microflora. Probiotics promote the development and growth of intestinal beneficial bacteria in the intestinal tract, thus improving the functioning of the intestinal barrier (Adhikari & Kim, 2017; Jha, 2020; Reuben *et al*., 2021) and improving feed digestibility. Due to the ability of probiotics to suppress the pathogenic microflora, it is possible to prevent the development of various diseases, such as salmonellosis, campylobacteriosis and coccidiosis (Markowiak & Slizewska, 2018). Probiotic bacteria have been shown to stimulate and enhance the immune system (Adhikari & Kim, 2017; Ajuwon, 2016; Ebeid, Al-Homidan, & Fathi, 2021). Studies have been reported that the use of probiotics improves the histo-morphology of the intestinal tract, especially crypt depth and villus height, thus increasing the nutrient absorption surface. It significantly improves the percentage of moisture, protein and ash in the meat (Ebeid, Al-Homidan, & Fathi, 2021). Probiotics have been shown to improve blood biochemistry, such as lowering cholesterol (Jha, 2020; Reuben *et al*., 2021).

All these good properties of probiotics described above contribute to the improvement of growth rates - weight gain, feed conversion, etc. Shah *et al*. (2020) has shown that feeding probiotics has improved live weight gain at all stages of the bird's development. Positive results have been obtained Chen *et al.* (2018) and Wang *et al.* (2016), as well as Awad *et al*. (2009), who added *Lactobacillus sp.* product to the diet, achieved a higher live weight and live weight gain of broilers on the last day of the study compared to the control group. The absolute and relative weight of the proventriculus and the relative weight of the gizzard did not differ significantly between the groups. The effectiveness of probiotics, which also contain *Lactobacillus spp.* bacteria, has been described, where weight gain is observed in the final growth phase (Palamidi, 2016). Positive results in weight gain were also shown in male strain ISA brown, to which *Lactobacillus acidophilus*, *Lactobacillus plantarum* and *Bifidobacterium spp*. in the feed were added (Agustono *et al*., 2022). In contrast, the results of the study, such as the addition of a mixture of *Bacillus spp.* to the diet, did not affect the live weight and feed conversion of broilers at the end of the study (Sugiharto *et al*., 2018). The study that used *Lactobacillus* strains also did not show a positive effect on live body wight and feed conversion (Olnood *et al*., 2015). It is explained that the effects of beneficial bacteria may depend on the age, sex of the animal, as well as microclimatic conditions, feed composition, etc. Under favorable conditions, where the animal is not exposed to the risk of disease, stress, the addition of probiotics to the feed may not give the expected results (Baurhoo, Phillip, & Ruiz-Feria, 2007; Ebeid, Al-Homidan, & Fathi, 2021).

Therefore, the aim of our study was to evaluate the effect of the mixture x of lactic acid bacteria on development of the broiler chicken digestive tract and the body weight.

Materials and Methods

Experimental design and management of broiler chickens

The study was conducted from April to December, 2021. The study was organised in three trials. The first trial was from April 21 to May 26. The second trial was from June 22 to July 27. The third trial was from November 10 to December 14. The study was performed at the Faculty of Veterinary Medicine, Latvia University of Life Sciences and Technologies, Jelgava, Latvia.

In each trial, 260 one day old Ross 308 broiler chicks (males and females) were obtained from a commercial hatchery. They were weighted and randomly divided in two groups – the control group and the probiotic group. The birds were placed in closed and ventilated similar pens, on a deep litter system of wood shavings. The lighting program was 23h light and 1h dark at the first day. Afterwards, the dark hours were slowly extended to 18h light and 6h dark from day 7 till the day 26. Afterwards, dark hours were slowly reduced to 20h light and 4h dark till the end of the study. The temperature of the first week of life was 33-34 °C, and it was slowly decreased to 22 °C until the end of experiment. Fresh drinking water was provided *ad libitum*. The dietary treatments was basal diet for the control group and basal diet + the mixture X of lactic acid bacteria 4 g 10 kg^{-1} for the probiotic group. The mixture X is a bio active substances complex based on probiotic strains of heat-inactivated lactic acid bacteria – *Lactobacillus farciminis* CNCM-I-3699 – 2.10^{10} CFU/g and *Lactobacillus rhamnosus* CNCM-I-3698 – 2.10^{10} CFU/g, which is activated upon entering the digestive tract. Also in other authors' works, these lactic acid bacteria strains are mentioned as probiotics used in animal feed as an additive (Tareb, Bernardeau & Vernoux, 2015; Tareb *et al.*, 2015). The mixture is in a powder form, stable at room temperature.

The broilers were fed with Starter diet from day 0 to day 10, Grower diet from day 11 to day 24 and Finisher diet from day 25 till the end of the study. The main sources of the protein in the basal diet are wheat grain, soyabean and rape. The analytical composition of the feed is summarized in Table 1.

oilers were raised till day 35. All broilers were weighted in day of the placing and afterwards on the day 7, 14, 21, 28, 35 all birds of both groups with, used calibrated scales 'Soehnl' (±1g), average body weight was calculated for each group. At the day 1, 7, 14, 21, 28, 35 of age 15 birds per treatment were randomly selected and killed by cervical dislocation. The gastrointestinal tract was excised (proventriculus,

Table 1

Analytical composition of basal diet

Components	Starter diet, %	Grower diet, %	Finisher diet, %
Crude protein	22.50	21.50	19.50
Crude fiber	2.40	2.86	2.83
Crude fat	4.24	5.20	7.22
Crude ash	4.32	4.73	3.68
Lysine	1.36	1.20	1.14
Methionine	0.84	0.60	0.85
Calcium (Ca)	0.96	1.00	0.78
Sodium (Na)	0.35	0.16	0.19
Phosphorus (P)	0.50	0.50	0.50

gizzard, intestines) and weighed with content, used calibrated scales 'Kern EW 420-3NM' (±0.01g), average organ relative weight (percentage of each bird's live weight) was calculated for each group.

Statistical data analysis

The assumption of normal data distribution was assessed using the Shapiro–Wilk test and visual inspection of their histograms and normal Q–Q plots. The assumption of homogeneity of variances was tested using the Levene test. To determine whether there were statistically significant differences between three independent groups, we used the Kruskal–Wallis H test with pairwise comparisons using Dunn's procedure with Bonferroni adjustment. To determine whether there were statistically significant differences between two independent groups, we used the independent samples T test or Mann-Whitney U test.

Results and Discussion

Comparative analysis of the trials showed that the data come from one population ($p > 0.05$). Therefore, to increase the power of analysis and the precision of the results obtained, we combined data from three trials.

The results about the average body weight are summarized in Table 2. The initial body weight for the probiotic and the control groups is considered to be bred appropriately and did not differ between the probiotic and the control groups ($p > 0.05$), meaning the output data will not affect further results. The initial body weight is very important factor in broiler production. Mendes et al. (2011) have studied that birds with an initial weight of 39.29-41.30g at 42 days of age weigh 1.98% more than birds with an initial weight of 39.9-41.3g. However, Patbandha *et al.* (2017) have studied that chickens with high initial body weight (47.76g ±0.37) gained significantly more weight (19.65g, $P \leq 0.05$) than those with low initial weight (41.24g ±0.23) up to day 15, but body weight did not differ on later age among the groups.

We see a tendency for the probiotic group to gain weight slightly faster than the control group, but basically body weight did not differ between probiotic and control groups on all weighting days ($p > 0.05$). Similar results were obtained by Olnood et al. (2015), when the mixture of *Lactobacillus* strains on a basal diet did not increase live weight and feed conversion at the end of the study.

The results about the relative weight of gastrointestinal tract parts are summarized in Table 3. There are various trends. The relative weight of the proventriculus is slightly higher in the probiotic group than in the control group up to day 14, but later in the age the weight is equalized between groups and in the control group it is slightly milder than in the probiotic group at the end of the study. The relative weight of the gizzard on day 21 is slightly higher in the probiotic group than in the control group, but does not differ on other weighing days. Intestinal relative weight increases to day 7 in both groups and then gradually decreases until the end of the study. The relative weight of the gastrointestinal tract remains relatively high until day 7 of age in the probiotic group and the control group, 16.61g ±1.74 and 16.76g ±2.12, respectively, it decreases with increasing body weight.

In general, the weight of relative gastrointestinal tract parts did not differ between the probiotic and control groups ($p > 0.05$). Overall, this study achieved very good results in both groups. This could be explained by the fact that in favourable conditions, where the bird is not exposed to the risk of disease, stress, as in the case of our study, the addition of probiotics to the feed may not give the expected results. Various factors that could affect the results have been described in the literature, such as the age, sex of the bird, as well as microclimatic conditions, feed composition, etc. (Baurhoo, Phillip, & Ruiz-Feria, 2007).

We compared these results with Awad *et al.* (2009) study (Awad study). Used the same broiler cross in both studies – Ross 308. Housing conditions were similar, like bedding were wood shawings, broilers were raised till day 35. The *Lactobacillus*

Table 2

Body weight

Trial day	Probiotic group		Control group		p
	mean value, g	SD	mean value, g	SD	
Initial weight (n=130)	45.40	2.03	44.64	1.92	0.331
Day 7 (n=115)	209.89	14.30	211.32	18.55	0.460
Day 14 (n=100)	588.86	49.50	582.43	43.46	0.437
Day 21 (n=85)	1,184.88	114.55	1,168.62	91.11	0.428
Day 28 (n=70)	1,962.612	100.52	1,957.71	101.94	0.480
Day 35 (n=55)	2,835.7	161.74	2,828.02	115.64	0.475

Table 3

Organ relative weight

Trial day	Parameter	Probiotic group (n=15)		Control group (n=15)		p
		Mean value, %	SD	Mean value, %	SD	
Day 1	Gastrointestinal tract	16.9	1.91	16.77	1.18	0.742
	Proventriculus	1.02	0.09	1.04	0.07	0.4
	Gizzard	7.48	0.79	7.62	0.57	0.442
	Intestines	8.41	1.34	8.11	0.94	0.332
Day 7	Gastrointestinal tract	16.61	1.74	16.76	2.12	0.763
	Proventriculus	0.96	0.18	0.92	0.13	0.377
	Gizzard	4.74	0.68	4.77	0.78	0.852
	Intestines	10.92	1.3	11.07	1.69	0.696
Day 14	Gastrointestinal tract	13.34	1.02	13.45	1.2	0.693
	Proventriculus	0.71	0.15	0.64	0.09	0.058
	Gizzard	3.77	0.34	3.67	0.4	0.317
	Intestines	8.85	0.83	9.14	1.06	0.246
Day 21	Gastrointestinal tract	11.08	1.5	10.8	1.19	0.423
	Proventriculus	0.5	0.08	0.52	0.08	0.49
	Gizzard	2.94	0.45	2.05	1.11	0.051
	Intestines	7.64	1.19	8.11	1.91	0.254
Day 28	Gastrointestinal tract	9.04	0.58	9.04	0.76	0.986
	Proventriculus	0.41	0.06	0.43	0.1	0.274
	Gizzard	2.36	0.38	2.36	0.39	0.941
	Intestines	6.28	0.6	6.25	0.72	0.87
Day 35	Gastrointestinal tract	7.94	1.22	7.81	1.45	0.707
	Proventriculus	0.36	0.05	0.4	0.11	0.099
	Gizzard	1.81	0.51	1.85	0.51	0.777
	Intestines	5.77	1.19	5.56	1.32	0.532

spp. products for probiotic groups are used in both studies.

Comparing initial body weight of chicken from the probiotic group significantly differs between this study (mean = 45.39 SD = 1.90) and mean value (mean = 40.85) of Awad study, a significant difference is 4.54, 95% CI [3.92 – 5.16], t = 14.92, df = 38, p< 0.001, d = 2.39. The same is with chicken from the control group on the 1st (0) day of experiment significantly differs between this study (mean = 44.81 SD = 2.08) and mean value (mean = 40.32) of Awad study, a significant difference is 4.49, 95% CI [3.82 – 5.17], t = 13.47, df = 38, p< 0.001, d = 2.16.

On the one hand, based on the available literature, it can be stated that the initial body weight is very important for the chicken to achieve a good increase in live weight during its lifetime. As it has been shown in Mendes et al. (2011) study, where birds with an initial weight of 39.29-41.30g at 42 days of age weigh 1.98% more than birds with an initial weight of 39.9-41.3g. On the other hand, Patbandha *et al.* (2017) have studied that chickens with high initial body weight gained significantly more weight (19.65g, p≤0.05) than those with low initial weight up to day 15, but body weight did not differ on later age among the groups.

At the end of the study, body weight of chicken from the probiotic group at day 35 of experiment significantly differs between this study (mean = 2,771.80, SD = 271.92) and mean value (mean = 1,765.51) of Awad study, a significant difference is 1,006.29, 95% CI [904.75-1,107.83], t = 20.27, df = 29, p< 0.001, d = 3.70, also the same with body weight of chicken from the control group at day 35 of experiment that significantly differs between this study (mean = 2,816.0, SD = 252.39) and mean value (mean = 1,753.64) of Awad study, a significant difference is 1,062.32, 95% CI [968.12-1,156.60], t = 23.05, df = 29, p< 0.001, d = 4.21. Other studies are available that have achieved a better body weight than study Awad. Shah *et al.* (2021) have described that body weight of the control group (CTL), probiotic group (CP) supplemented with commercial product that also contained lactic acid bacteria and probiotic group (SP) supplemented with *Enterococcus spp.* and *Pediococcus spp.* gained 2,293.75g 2,533.75g un 2,503.00g, respectively ($p<0.05$). Comparing the results of this study with the results of Shah *et al.* (2021) study, we still have gained better body weight on day 35.

There is no difference of chicken's proventriculus relative weight in the probiotic group at day 35 of the experiment between this study and Awad study, $p = 0.310$, as well as no difference in the control group, $p = 0.703$.

The relative weight of chicken's gizzard from the control group on day 35 of experiment significantly differs between this study (mean =1.85, SD = 0.51) and mean value (mean = 2.30) of Awad study, a significant difference is 0.45, 95% CI [0.26-0.65], t = - 4.825, df = 29, $p< 0.001$, d = 0.88. The relative weight of chicken's gizzard from the probiotic group on day 35 of experiment that significantly differs between this study (mean =1.81, SD = 0.46) and mean value (mean = 2.28) of Awad study, a significant difference is 0.47, 95% CI [0.30-0.64], t = - 5.563, df = 29, $p< 0.001$, d = 0.92. This could be explained by the fact that, as the bird grows and the muscle mass increases, the relative weight of the gizzard decreases in proportion. A large increase in live weight was achieved in this study, resulting in a lower relative weight of gizzard than in the Awad study, where such a high body weight was not achieved.

Conclusions

Exploring other studies, we can conclude that this study achieved very good body weight results in both groups, which could be explained by the favourable housing conditions (no risk of diseases, stress), the daily regime and initial body weight which contributed to this significant weight gain. Comparing with similar studies with *Lactobacillus spp.* products at the end of the study body weight of chicken from the probiotic group on day 35 of experiment significantly differ between this study and mean value of Awad study, also the same with body weight of chicken from the control group.

Various trends were observed in the study, but overall the weight of chickens and the relative weight of gastrointestinal tract parts did not differ between the experimental and control groups ($p > 0.05$). In order to evaluate the effect of mixture of lactobacilli x on the development of the digestive tract of broiler chickens, the study with histological samples should be continued.

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