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Effects of Dietary Supplementation of *Lactobacillus farciminis* and *Lactobacillus rhamnosus* on Growth and Production Indicators of Broiler Chickens

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ABSTRACT

In response to the 2006 EU ban on the use of antibiotics as growth promoters, researchers have sought alternatives, leading to a focus on the beneficial effects of probiotics on chickens. The aim of this study was to evaluate the effect of the probiotic mixture containing *Lactobacillus* (*L.*) *farciminis* CNCM-I-3699 and *Lactobacillus rhamnosus* CNCM-I-3698 on the growth, production indicators, and edible organs of broiler chickens. Three trials were conducted, each consisting of 260 newly hatched Ross 308 broiler chicks (males and females) from a commercial hatchery, randomly allocated into control (n = 130) and probiotic-supplemented groups (n = 130). The dietary treatments were basal diet for the control group and basal diet + the mixture of *L. farciminis* CNCM-I-3699 (2.10^{10} GU/g) and *L. rhamnosus* CNCM-I-3698 (2.10^{10} GU/g) at a rate of 4g/10kg of diet for the probiotic supplemented group. Broilers were raised until day 35 of age, and their body weight and feed intake were recorded on days 1, 7, 14, 21, 28, and 35. All broiler chickens were weighed on the first day. The investigated parameters included average weight gain, feed conversion ratio, cumulative feed intake, and the European Broiler Index. Daily mortality was recorded. The average organ's relative weight was calculated for each group on days 1, 7, 14, 21, 28, and 35. Although both groups yielded positive results regarding growth and production indicators, no significant differences were observed between the two groups, suggesting that probiotics may not provide expected outcomes when appropriate conditions and age-related requirements are met. The probiotic-supplemented group exhibited significantly accelerated growth in the heart and liver. However, relative organ weights did not differ significantly between the groups.

Keywords: Body weight, Edible organs, Poultry, Probiotic, Productivity

INTRODUCTION

Poultry industry in the European Union (EU) is one of the fastest-growing agricultural industries. In Latvia, the poultry industry is growing very fast due to an ever-growing need for affordable and good-quality protein. Among meat products, chicken is the most consumed meat. The EU is one of the largest poultry meat producers and a net exporter of poultry products, with an annual production of around 13.4 million tons (European Commission, 2022).

Since 2006, when the EU banned antibiotic use as growth promoters in animal feed to help fatten livestock, meeting consumer demand for quality products has become challenging (Cogliani et al., 2011). Thus, there has been extensive research on the beneficial effects of prebiotics, probiotics, and acidifiers on chickens to improve their health. The aim was to reduce the necessity for antimicrobial medicine and improve commercial productivity (Abudabos et al., 2015; Babazadeh and Asasi, 2021; Marwi et al., 2021; Mirzaei et al., 2022).

A chicken's intestinal tract holds a microbial profile, including *Lactobacillus* (*L.*) spp., which is sensitive to stress, making chickens prone to health issues and productivity (Patterson and Burkholder, 2003). Probiotics help maintain gut microbial flora, improve resistance against harmful pathogens, and enhance the overall development and growth of animals (Wang et al., 2016;

Shah et al., 2021; Agustono et al., 2022). Chen et al. (2018) found that adding *L. rhamnosus* strain to the feed of broiler chickens improved live weight and average daily growth. The ability of *L. rhamnosus* to improve growth has been reported in current studies (Bampidis et al., 2020b; Fesseha et al., 2021). Similar findings have been reported for *L. farciminis* (Bampidis et al., 2020a), which improves stress resilience, leading to improved intestine health and growth (Ait-Belgnaoui et al., 2006). On the contrary, probiotics have no significant effect on live weight in broiler chickens (Qorbanpour et al., 2018; Zhu et al., 2020; Atsuti et al., 2022).

The effect of probiotics on animal feed consumption has not been fully confirmed. Several studies have shown that the addition of different types and doses of probiotics did not affect feed consumption indicators (Awad et al., 2009; Sugiharto et al., 2018; Atsuti et al., 2022) or did not reduce feed consumption and feed conversion rate (FCR) in broiler chickens (Zhu et al., 2020; Agustono et al., 2022). However, some studies have indicated that the dietary inclusion of *Lactobacillus* spp. (*L. farciminis*, and *L. rhamnosus*) significantly reduce daily feed consumption and FCR (Hussein and Selim, 2018; Bampidis et al., 2020b).

Higher body weight (BW) affects inner organ development, but this connection has not always been observed. Shah et al. (2021) have shown that probiotics (*E. faecium* and *P. acidilactici*) could improve live weight gain, but not the relative weight of inner organs (liver, heart, gizzard). The addition of a mixture of *Bacillus* spp. to the diet does not affect the weight of inner organs (heart, liver, gizzard) as well as live weight (Sugiharto et al., 2018). Atsuti et al. (2022) found that adding *L. casei* to the feed did not impact the final weight, but the live weight increased. It could increase the relative weight of the spleen and heart, but reduce the relative weight of the liver.

Although there are studies on probiotics, such as *L. farcimins, L. rhamnosus*, *Lactobacillus* species of Kefir, and their impact on chickens' growth, clear information is missing on whether it impacts feed consumption, FCR, and inner organ weight (Awad et al., 2009; Vahdatpour and Babazadeh, 2016).

Therefore, the objective of the present study was to evaluate the effects of the probiotic mixture containing *L. farciminis* CNCM-I-3699 and *L. rhamnosus* CNCM-I-3698 on the growth, production indicators, and edible weight of organs in broiler chickens.

MATERIALS AND METHODS

Ethical approval

All issues related to growing the chickens were conducted according to the Republic of Latvia Cabinet Regulation No. 98, 2010, "Welfare Requirements for Keeping and Use of Chicken for Meat Production" (Republic of Latvia, Cabinet Regulation No. 98, 2010). The study was approved by the Research Committee of the Faculty of Veterinary Medicine, Latvia University of Life Sciences and Technologies, Latvia (protocol No.2021/1).

Experimental design and management of broiler chickens

The study was conducted from April to December 2021 and organized into three trials. The study was performed at the Clinical Research Center, Faculty of Veterinary Medicine, Latvia University of Life Sciences and Technologies, Jelgava, Latvia.

A total of 260 Ross 308 (*Gallus gallus*) newly hatched broiler chicks (female and male) were obtained from a commercial hatchery located in Kekava, Latvia (total number of 780 chicks). The broilers were weighed and completely randomly divided into two groups, namely the control group (Con, n = 130 with an initial weight of 44.64 ± 1.92 g) and the probiotic-supplemented group (ProL, n = 130 with an initial weight of 45.40 ± 2.03 g). Broiler chickens were raised until day 35. Broiler chickens were not vaccinated during the study. Strict biosecurity requirements were followed, including closed rooms, disposable clothing, gloves, shoes, disinfection barriers, and the presence of people only during chicken feeding (twice daily). Mortality was recorded once a day. At the end of the study, the total mortality rate was recorded as 2-5%. The cause and pattern of death were recorded.

The chickens were divided into two separate enclosed pens, commonly referred to as bio-chambers. Each pen was designed with a deep litter system featuring a floor made of clean pine and spruce shavings. The size of each pen was 9 m^3. The rooms were equipped with full microclimate control (temperature, humidity, and air supply, control of incoming and outgoing air composition, and light mode), as well as video surveillance. The lighting setting on the first day was 23 hours of light and an hour of darkness (23 hours/1 hour). Afterward, the darkness hours were slowly extended to 6 hours until day 26 (18 hours/6 hours). In the last week of the study, the dark time was gradually reduced until it reached 20 hours of light and 4 hours of darkness (20 hours / 4 hours). The

ambient temperature for the initial day was 33-34℃, and as the chickens grew, it slowly decreased to 22℃ until the end of the study. The 24-hour lighting and temperature regime were created based on previous studies and the Ros 308 breeder guidelines (Cassuce et al., 2013; Broiler management manual ROSS, 2018).

Diet and supplementation of *Lactobacillus* spp.

Fresh drinking water was provided *ad libitum* in nipple drinking lines. The basal diet provided to both the Con and ProL groups of chickens was identical and formulated based on the specific age of the chicks. Starter feed was provided from hatching to day 10, grower feed from day 11 to 24, and finisher feed from day 25 until the end of the study. The main sources of protein in the basal diet were wheat grain, soybean, and rapeseed. The analytical composition of the feed is summarized in Table 1. The ration was prepared based on the Ross 308 breeding guidelines (Broiler Management Manual ROSS, 2018). The starter diet contained wheat grains, soy sprouts, vegetable oil, corn gluten, monocalcium phosphate, fish meal, calcium carbonate, sodium chloride, and sodium sulfate (2975 kcal metabolizable energy [ME]). The grower (3075 kcal ME) and the finisher diets (3195 kcal ME) contained no fish meal but rapeseed and fatty acids.

In the study, the mixture (Lot No.: 92024406) of lactic acid bacteria 4g/10kg was manually added to the ProL feed. The mixture was a bioactive substances complex based on heat-inactivated co-culture of probiotic strains, *L. farciminis* CNCM-I-3699 (2.10^{10} GU/g) and *L. rhamnosus* CNCM-I-3698 (2.10^{10} GU/g) in association with their environment (functional metabolites and bioactive peptides). This complex was obtained from thermally processed fermented milk (heat-treated bacteria retain their cell wall structure) and stabilized on the cereal-based carrier. The product was stored according to the manufacturer's requirements (in a cool, dry, well-ventilated area), with a shelf life of 18 months from the manufacturing date.

To assess the consumption of the supplemented feed containing lactic acid bacteria by the broilers, and to determine the viability of these bacteria post-consumption, the results obtained from the broiler crops in both groups were compared. Immediately after the completion of each trial, data regarding the quantity of lactic acid was collected. A total of 15 samples were taken from each group, with 15 samples from the ProL group and 15 samples from the Con group. The contents of crops were examined at the Scientific Laboratory of Biotechnologies, Molecular Biology and Microbiology Department, Latvia University of Life Sciences and Technologies.

Table 1. Analytical composition of the basal diet balanced for broiler chickens

Components	Starter diet	Grower diet	Finisher diet
Metabolizable energy (kcal)	2975	3075	3195
Protein (%)	22.5	21.50	19.50
Fibre (%)	2.40	2.86	2.83
Fat (%)	4.24	5.20	7.22
Ash (%)	4.32	4.73	3.68
Carbohydrates (%)	46.06	46.71	45.53
Water (%)	12.82	11.08	10.98
Other ingredients	5.46	6.12	8.27
Lysine (%)	1.36	1.20	1.14
Methionine (%)	0.84	0.60	0.85
Minerals			
Ca (%)	0.96	1.00	0.78
Na (%)	0.35	0.16	0.19
P (%)	0.50	0.50	0.50
Vitamins			
A (U/kg)	16,900	14,300	13,000
D_3 (U/kg)	6,500	5,500	5,000
E (mg/kg)	104,0	88.0	80.0
Micronutrients			
FeSO4 (mg/kg)	22.1	18.7	17.0
Ca $(IO_3)_2$ (mg/kg)	1.63	1.38	1.25
CuSO4 (mg/kg)	20.8	17.6	16.0
MnO_2 (mg/kg)	156.0	132.0	120.0
ZnO (mg/kg)	117.0	99.0	90.0
Na_2SeO_3 (mg/kg)	0.39	0.33	0.30

Ca: Calcium, Na: Sodium, P: Phosphorus

Growth performance, production indicators, and edible organs

All broiler chickens were weighed on the first day and subsequently on days 7, 14, 21, 28, and 35 in both the Con and ProL groups using calibrated scales called Soehnl with an accuracy of ± 1 g. The average BW was calculated for each group. The amount of feed prepared for both study groups (Con and ProL) was the same. It was extended twice a day in the feeders with suitably sized openings, reducing the possibility of the chickens scavenging it in the litter. The amount of food consumed by each group was determined once a week by weighing the amount of uneaten food left in the feeders from both groups.

Obtained results were used to calculate average weekly/daily weight gain, daily feed intake (FI), cumulative feed intake (CFI), and consumed feed quantity on 1 kg live weight in consecutive study periods of 1-7, 8-14, 15-21, 22-28, 29-35 days. Slaughtering was performed every 7 days for inner organ analysis, and pathological sampling was performed. The initial broiler count in each group was 130 on the first day of the experiment. The broiler count gradually decreased over time, with 115 chickens on day 7, 100 on day 14, 85 on day 21, 70 on day 28, and 55 on day 35. The FCR was calculated using the formula described by Chen et al. (2018). The European Broiler Index (EBI) was calculated by following the formula described by Banaszak et al. (2022).

Average grams gained/day of rearing × % survival rate)/FCR × 10

On days 1, 7, 14, 21, 28, and 35 of age, 45 chickens per treatment (15 chickens per treatment in each replicate) were randomly selected and slaughtered by cervical dislocation. The edible organs were removed (liver, heart, gizzard and weighed and weighed using calibrated scales (Kern EW 420 - 3NM [± 0.01g], Germany). Average organ relative weight (percentage of each chicken's live weight) was calculated for each group.

Statistical analysis

MS Excel and R-Studio were used for the statistical data analysis to determine production indicators, organ weight, and live weight data. The relative weight of each organ was determined by calculating the percentage of proportion between each organ and the chicken's live weight. For each indicator within the group and age, mean and standard deviation (SD) were calculated. To determine whether the results between the two groups were significantly different, the student's t-test was used. P values less than 0.05 ($p<0.05$) were considered statistically significant.

RESULTS

Growth performance and production indicators

Impacts of probiotics containing two strains of *Lactobacillus* spp. (*L. farciminis* CNCM-I-3699 and *L. rhamnosus* CNCM-I-3698) on the growth factors, feed consumption indicators, and organ development were determined every 7 days (on days 7, 14, 21, 28, and 35 of the study). The mean number of lactobacilli over three trials from the broiler crops was determined to be 11.22×10^5 for the ProL group and 8.42×10^5 for the Con group. The higher volume of lactic acid bacteria contents in ProL groups crops presented evidence that these broilers consumed supplemented feed with *L. farciminis* and *L. rhamnosus* and that these bacteria can grow and multiply in the digestive tract.

The initial live weight of broilers showed no significant difference between the two groups, meaning that the raw data was the same and could not affect the results (Table 2). The initial weight plays a crucial role in the further development of broiler chickens. On day 7, the live weight for broiler chickens of both groups increased 3.5 times, with no significant differences between groups. During this period, there was an increase in the ProL broiler chickens' weight by 164.48 ± 12.75 g, while the Con group showed an increase of 166.68 ± 17.11 g. The average daily weight gain for the ProL was 23.50 ± 1.82 g, and for the Con, it was 23.81 ± 2.44 g. In the next two weeks, the broiler chicken weight in both groups almost doubled, and on day 28 of the study, the weight reached 1962.12 ± 100.52 g (average daily weight increased by 111.03 ± 9.54 g) in the ProL group and 1957.71 ± 101.94 g (average daily weight increased by 112.73 ± 8.90 g) in the Con group.

The ProL group chickens at this age reached 2835.70 g ± 161.74 g with an average daily weight gain of 79.72 g ± 4.62 g and an overall weight gain of 2790.30 g ± 161.57 g during the study. In comparison, the Con group indicated lower results, an average weight of 2828.02 g ± 115.64 g, a daily gain of 79.53g ± 3.29 g, and an overall weight gain of 2783.38 g ± 115.17g during the study. The average results of daily weight gain and overall weight gain did not differ significantly between groups ($p > 0.05$). The EBI for ProL group chickens also reached 621.27 ± 49.38 at the end of the study, while it was slightly lower in the Con group and reached 619.73 ± 37.40 ($p > 0.05$).

Although there was no significant difference between the ProL and Con group of chickens regarding feed consumption indicators ($p > 0.05$), there was a visible tendency for better results in the experimental group when calculating the average values throughout the study (1-35 per day), supplementing feed with the probiotics reduces FCR by 0.78%, cumulatively consumed feed by 0.51%, and the feed consumed per 1 kg of live weight gain decreases by 0.70% compared with the Con group (Table 3).

Table 2. The effect of dietary supplementation of *Lactobacillus farciminis* and *Lactobacillus rhamnosus* on the growth performance of broiler chickens during 35 days

Parameter	Time (day)	Values of growth indicators (mean ± SD)		P value
		Probiotic group	Control group	
Body weight (g)	First day	45.40 ± 2.03	44.64 ± 1.92	0.331
	7	209.89 ± 14.30	211.32 ± 18.55	0.460
	14	588.86 ± 49.50	582.43 ± 43.46	0.437
	21	1184.88 ± 114.55	1168.62 ± 91.11	0.428
	28	1962.12 ± 100.52	1957.71 ± 101.94	0.480
	35	2835.70 ± 161.74	2828.02 ± 115.64	0.475
Average weekly weight gain (g/chicken/week)	1-7	164.48 ± 12.75	166.68 ± 17.11	0.434
	8-14	378.97 ± 35.22	371.11 ± 25.45	0.385
	15-21	596.02 ± 65.10	586.19 ± 48.88	0.422
	22-28	777.24 ± 66.77	789.09 ± 62.33	0.417
	29-35	873.59 ± 70.92	870.31 ± 38.63	0.474
	1-35	2790.30 ± 161.57	2783.38 ± 115.17	0.477
Average daily weight gain (g/chicken/day)	1-7	23.50 ± 1.82	23.81 ± 2.44	0.434
	8-14	54.14 ± 5.03	53.02 ± 3.64	0.385
	15-21	85.15 ± 9.30	83.74 ± 6.98	0.422
	22-28	111.03 ± 9.54	112.73 ± 8.90	0.417
	29-35	124.80 ± 10.13	124.33 ± 5.52	0.474
	1-35	79.72 ± 4.62	79.53 ± 3.29	0.477
European broiler index	1-35	621.27 ± 49.38	619.73 ± 37.40	0.484

SD: Standard deviation

Table 3. The effect of dietary supplementation of *Lactobacillus farciminis* and *Lactobacillus rhamnosus* on feed consumption indicators of broiler chickens during 35 days

Parameter	Time (day)	Feed consumption indicators (mean ± SD)		P value
		Probiotic group	Control group	
Feed intake (g/chicken/day)	1-7	31.18 ± 0.71	31.32 ± 0.74	0.414
	8-14	59.83 ± 2.39	60.15 ± 1.97	0.435
	15-21	97.96 ± 2.51	97.63 ± 3.54	0.451
	22-28	146.01 ± 7.58	145.90 ± 5.82	0.492
	29-35	179.58 ± 10.10	182.20 ± 7.63	0.369
	1-35	102.91 ± 2.94	103.44 ± 1.89	0.404
Feed conversion ratio	1-7	1.04 ± 0.08	1.04 ± 0.11	0.499
	8-14	1.09 ± 0.07	1.10 ± 0.08	0.397
	15-21	1.12 ± 0.08	1.14 ± 0.07	0.417
	22-28	1.20 ± 0.05	1.20 ± 0.05	0.472
	29-35	1.27 ± 0.04	1.28 ± 0.03	0.378
	1-35	1.27 ± 0.04	1.28 ± 0.03	0.378
Cumulative feed intake (g/chicken)	1-7	218.26 ± 4.96	219.22 ± 5.20	0.414
	1-14	637.09 ± 18.05	640.24 ± 15.88	0.416
	1-21	1322.79 ± 35.51	1323.63 ± 39.49	0.490
	1-28	2344.88 ± 32.88	2344.93 ± 24.24	0.499
	1-35	3601.94 ± 102.98	3620.33 ± 66.08	0.404

SD: Standard deviation

Table 4. The effect of dietary supplementation of *Lactobacillus farciminis* and *Lactobacillus rhamnosus* on organ relative weight of broiler chickens during 35 days

Trial days	Organ	Probiotic group (Mean percentage ± SD)	Control group (Mean percentage ± SD)	P value
1	Liver	4.00 ± 0.48	3.93 ± 0.52	0.303
	Heart	0.72 ± 0.09	0.67 ± 0.07	0.006
	Gizzard	7.53 ± 0.74	7.62 ± 0.57	0.316
7	Liver	4.83 ± 0.48	4.41 ± 0.64	0.050
	Heart	0.73 ± 0.08	0.71 ± 0.11	0.276
	Gizzard	4.78 ± 0.65	4.77 ± 0.78	0.492
14	Liver	3.58 ± 0.27	3.58 ± 0.38	0.482
	Heart	0.70 ± 0.11	0.64 ± 0.08	0.014
	Gizzard	3.77 ± 0.37	3.67 ± 0.40	0.159
21	Liver	2.82 ± 0.26	2.74 ± 0.36	0.174
	Heart	0.55 ± 0.07	0.54 ± 0.11	0.322
	Gizzard	2.94 ± 0.45	2.98 ± 0.47	0.375
28	Liver	2.56 ± 0.29	2.47 ± 0.23	0.107
	Heart	0.47 ± 0.09	0.48 ± 0.05	0.180
	Gizzard	2.39 ± 0.32	2.36 ± 0.33	0.357
35	Liver	2.11 ± 0.26	2.18 ± 0.21	0.127
	Heart	0.46 ± 0.05	0.46 ± 0.05	0.487
	Gizzard	1.81 ± 0.46	1.85 ± 0.51	0.389

SD: Standard deviation

Edible organs

On the first day of the study, the two groups did not differ significantly in terms of liver and gizzard weight ($p > 0.05$). Only the heart weight on the first day was significantly higher in the ProL group than in the Con group ($p < 0.05$). The chickens appeared to be in good health upon visual examination, and when samples of their organs were collected for pathoanatomical analysis, no visible abnormalities or pathologies were detected. On day 7, liver weight in ProL group chickens was significantly higher in the Con group than the ProL and Con groups, with percentages of 4.83% and 4.41%, respectively ($p < 0.05$). On day 14, it was found that the relative weight of the liver and gizzard did not differ significantly between the two groups ($p > 0.05$). The relative heart weight in the ProL group was significantly higher than the Con group ($p < 0.05$).

In the remaining phases of the study (days 21 and 28) and at the end of the study (day 35), no significant differences were observed between the two groups regarding the relative weights of organs ($p > 0.05$, Table 4).

DISCUSSION

Growth performance and production indicators

In the present study, the initial weight of chickens did not differ significantly between the two groups. According to Mendes et al. (2011), broiler chickens with an initial weight range of 39.29-41.30 g at 42 days of age weigh approximately 1.98% more compared to chickens with an initial weight range of 34.4-35.22 g. Therefore, the initial weight of chickens has a significant impact (95% confidence) on the live weight of broiler chickens at the age of 42 days, meaning that broilers with a lower initial live weight cannot reach the same weight as chickens with a higher initial weight, even if they are grown under the same housing conditions. Similar results were achieved by Patbandha et al. (2017). They reported that BW increased significantly by 7.14% (10.62 g) and 5.52% (19.65 g) on days 8 and 15, respectively, in chickens with high baseline BW (47.76 ± 0.37), compared to chickens with a lower initial BW (41.24 ± 0.23 g). From day 15 until the end of the study (day 43), the weight did not differ significantly between the groups although the weight remained slightly higher until the end of the study in the group where the outgoing weight of broiler chickens was higher.

Although growth rates did not vary significantly between the two groups of the current study, they were numerically better in the ProL group (0.27% resulted in higher live weight, 0.24% increase in average daily growth, and 0.25% higher in the EBI at the end of the study). In a 42-day study conducted by Hosseini et al. (2013), Cobb broiler chickens supplemented with the

commercial probiotic of Protexin (which also included *L. rhamnosus*) did not achieve significantly higher live weight at all periods of the study (days 4, 14, 21, 28, 35, and 42). Meanwhile, in a 24-day study by Chen et al. (2018), white leghorn chickens fed with *L. rhamnosus* CF at the end of the study resulted in significantly higher live weight and average daily gain. Positive results were reported in a study by Fesseha et al. (2021), where the Sasso dual-purpose chicken was fed with *L. paracaseis* and *L. rhamnosus* feed supplement, achieving significantly higher live weight. The addition of probiotics containing *L. farciminis* to the feed also demonstrated a positive effect on live weight gain, as reported in a study by Bampidis et al. (2020a). Other studies using different probiotic compositions also showed greater increases in live weight (Awad et al., 2009; Wang et al., 2016; Shah et al., 2021). However, a significant increase in live weight was not gained through research with other probiotic bacteria (Qorbanpour et al., 2018; Sugiharto et al., 2018; Zhu et al., 2020).

Noticeably, results in both groups were also achieved in the EBI rates. Although no significant differences were found, this rate was 0.25% higher in the ProL group. Awad et al. (2009) conducted a 35-day study where Ross 308 chickens were supplemented with probiotics containing *Lactobacillus* spp., resulting in an EBI rate of 265. In contrast, Palamidi et al. (2016) added inactivated probiotics to the feed in their study, achieving an EBI rate of 305. The significant difference between these studies and the present study can be attributed to the average daily weight gain in the ProL and Con groups, which were 79.72 ± 4.62 g and 79.53 ± 3.29 g, respectively. Additionally, the ProL group exhibited an FCR of 1.27 ± 0.04, while the Con group had an FCR of 1.28 ± 0.03.

Despite not finding significant differences in feed consumption results, it is noteworthy that during 35 days of probiotic feeding, there was a notable improvement in feed conversion, with a 0.78% reduction. Additionally, there was a 0.51% decrease in cumulatively consumed feed and a 0.70% reduction in food consumed per 1kg of live weight gain.

Under production conditions where 40,000 broiler chickens are raised in a single group, these feed efficiency improvements lead to significant economic benefits. Specifically, there would be a reduction of approximately 400 kg in feed consumption per 1 kg of live weight gain and a total reduction of about 800-1000 kg per group. This represents a substantial economic advantage. Similar to the present study, Hosseini et al. (2013) conducted a 42-day study in which they investigated the effects of feeding the commercial probiotic supplement "Protexin," which included *L. rhamnosus*, on broiler chicken feed consumption. The study did not indicate a significant decrease in feed consumption as a result of probiotic supplementation. Some other studies have reported similar results, where feeding various probiotic bacteria did not lead to significant improvements in FCR and feed consumption rates (Sarangi et al., 2016; Atsuti et al., 2022). Better results were achieved by Chen et al. (2018), where broilers (white leghorns) were fed with *L. rhamnosus*. At the end of their study, a significantly reduced FCR was observed in *L. rhamnosus* group, compared to chickens that were not fed probiotics. Similarly, Fesseha et al. (2021) managed to reduce FCR, when the efficacy of *L. paracaseis* and *L. rhamnosus* was studied. A significant reduction in FCR has also been achieved by adding *Bacillus subtilis* and *L. acidophilus* in a 1:1 ratio to the basic diet by Zhu et al. (2020).

Overall, noticeable production ratings were obtained from both study groups with no significant differences. This could be explained by the fact that when the chickens are not exposed to the risk of disease and stress, as in the case of the current study, the addition of probiotics to the feed may not give the expected results. This shows the significance and importance of microclimate and housing on broiler chicken production and development (Baurhoo et al., 2007; Ebeid et al., 2021).

Edible organs

One of the objectives of the current research was to determine how the consumption of probiotics (*L. farciminis* and *L. rhamnosus*) affects the weight of edible organs since there are discrepancies in the literature about the effects of probiotic consumption and the growth of edible organs, such as the liver, heart, and gizzard. Some studies described a significant effect on the weight gain of these organs (Agustono et al., 2022). However, some authors found no significantly higher relative organ weights after feeding chickens probiotics (Awad et al., 2009; Shah et al., 2021). Some studies described that feed supplemented with probiotics could significantly decrease the relative weight of organs (Sugiharto et al., 2018).

In the current study, adding *L. farciminis* and *L. rhamnosus* to the feed increased heart weight in chickens of the ProL group at the age of 1 and 14 days and liver weight at the age of 7 days. However, in the remaining part of the study, no significant differences were observed in the relative weights of the heart, liver, and gizzard

between the two groups. Such a significant difference has not been found in the studies of other authors (Awad et al., 2009; Shah et al., 2021). Unlike the current study, the findings of Agustono et al. (2022) on the chicks from ISA brown strain fed by probiotics (containing *L. acidophilus*, *L. plantarum*, and *Bifidobacterium* spp.) indicated a significantly higher relative weight of the liver and heart at the end of the study. Some studies described that feed supplemented with probiotics could significantly decrease the relative weight of organs. Such results were described by Sugiharto et al. (2018), where the relative weight of the heart in the experimental groups of Lohman broiler chicks fed with multistrain probiotics in different concentrations was significantly lower than that of the Con group. Atsuti et al. (2022) found that chickens' liver weight was significantly lower than that of the Con group when increasing the amount of probiotics in the feed.

CONCLUSION

The broiler chickens that received the appropriate possible age-related housing conditions then feed supplemented with *L. farciminis* (CNCM-I-3699) and *L. rhamnosus* (CNCM-I-3698) mixtures from hatching to 35 days of age did not significantly improve production rates. However, some production indicators, such as body weight gain, EBI, FCR, and cumulative feed intake, were higher in the experimental group than in the control group. Regarding the ProL group, significantly higher weight gain of the liver was observed at the age of 7 days, and weight gain of the heart was observed at the age of 14 days. No significant differences in the weight of edible organs were observed at later age stages. It can be concluded that the difference between the heart and the liver weight could not be due to probiotic supplementation.

In order to emphasize that these results are important and that the effects of the consumption of probiotics cannot be evaluated unambiguously by other authors, research in this direction should be continued using histological, histochemical, and immunohistochemical examinations.

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Authors' contribution

Sabine Eglite contributed to data collection, database creation, and preparation of the manuscript. Lauma Mancevica participated in data collection, compilation, and preparation of the manuscript. Aija Ilgaza did the statistical analysis, managed the whole process, and made corrections. All authors checked and approved the final version of the manuscript for publishing in the present journal.

Competing interests

The authors have declared that no competing interest exists.

Ethical consideration

All authors have checked the ethical issues, including plagiarism, consent to publish, misconduct, data fabrication and/or falsification, double publication and/or submission, and redundancy.

Availability of data and materials

The data of this study are available from the corresponding author upon reasonable request.

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