Effect of probiotic additives and *Bacillus licheniformis* inclusion in the diet on broiler growth

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Summary

The withdrawal of antibiotic growth stimulators as of 1 January 2006 in the European Union countries has forced the search for alternative solutions to improve the health and productivity of poultry. The poultry industry also faces the challenge of developing alternative feeding systems with the restriction or exclusion of coccidiostats. One of them is the use of probiotic strains as feed additives. This study aimed to determine the effect of the use in compound feed of a probiotic containing *Bacillus licheniformis* on rearing rates and post-mortem performance of broiler chickens. The experiment was performed on 8012 unsexed broiler chickens of the Ross 308 line divided into four equal groups. The experimental factors were the addition of a probiotic containing *Bacillus licheniformis* bacteria and the addition of a coccidiostat. The introduction of probiotic bacteria into compound feed did not increase the body weight of birds, in particular, control weights, and at the end of rearing, but it decreased the feed conversion rate (FCR) and mortality. Addition of probiotic in compound feed did not have a significant effect on bird muscle. The mean weight of pectoral muscle between control (C+, C-) and study (C-BL, C+C-BL) groups did not show statistically significant differences; however, the highest mean weight of the evaluated parameter was visible in the C+ group (0.665) and the lowest in the C-BL group (0.623). Similarly to the mean weight of the carcass, also in the mean weight of thigh muscles, the lowest statistically significant values were observed in chickens belonging to the C+C-BL group (vs. C-; $P < 0.05$). Based on the obtained results, it can be acknowledged that the probiotic bacteria *Bacillus licheniformis* in broiler feed can be a good feed additive to replace antibiotics/coccidiostats. This probiotic has a positive effect on the overall health of birds, contributes to better use of nutrients and stimulates growth and development of broiler chickens.

Keywords: broiler chickens, probiotics, feed additives

Withdrawal of antibiotic growth stimulants has forced the search for alternative solutions for improving the health and productivity of poultry. One of these is the administration of probiotic strains. These additives can occur in the form of monocultures, as well as mixtures of various strains (17). Live cultures of microorganisms in the diet stimulate the activity of intestinal enzymes, the secretion of bacteriocins with antibacterial properties, acidification of the intestinal contents and local immunity within the gastrointestinal mucosa (11, 15). Probiotics induce the effects of stimulating the intestinal immune response by activating Toll-like receptors (TLRs) in the intestinal epithelium and modulating cytokine production in response to NF- κ B pathway signaling (22, 30). The

addition of probiotic bacteria to poultry feed causes the phenomenon of competitive displacement (32) of enteropathogens by adhesins of non-pathogenic bacteria, which after binding to TLR receptors of epithelial cells induce a non-pathogenic immune response in the small intestine (25). The beneficial effect of probiotic strains has been confirmed in many studies using various animal species (9, 24, 27). Studies on poultry confirm that the feed additive in the form of *Bacillus subtilis* bacteria increases bird resistance to colonization of the gastrointestinal tract by *E. coli* and reduces the presence of pathogenic microorganisms *E. coli*, *Salmonella enteritidis* and *Clostridium perfringens* in the spleen, caecum and liver in young animals (18, 19). The addition of *Bacillus subtilis* and *Bacillus licheni-*

Tab. 1. Arrangement of experimental diets

Addition	Positive control – C+	Negative control – C–	Negative control + probiotic – C–BL	Starter: Positive control + probiotic, other feeds: Negative control + probiotic – C+C–BL
Coccidiostat	+	–	–	+/-
Probiotic	–	–	+	+

Explanation: – no, + yes

formis bacteria to the diet of turkeys has a positive effect on the results of turkey rearing and significantly improves their final body weight and average weight gain (13). Moreover, the use of probiotics in the feeding of piglets reduces their mortality in the critical period around weaning (26) and effectively reduces diarrhoea caused by *E. coli* in weaned pigs (20), while improving the body's defense functions through growth production of IgM and IgA antibodies, as well as Th1, Th2, TNF- α cytokines (39). In the feeding of calves, the addition of *Lactobacillus* and *Bifidobacterium* bacteria enhances rearing results, reduces mortality and additionally reduces the percentage of respiratory diseases and diarrhoea (36). The effect of the above-mentioned activities is the improvement of the health and production performance of animals.

The research aimed to determine the effect of the use of a probiotic containing *Bacillus licheniformis* bacteria in feed on rearing rates and post-slaughter results of broiler chickens.

Material and methods

The experiment used 8012 unsexed broiler chickens from the Ross 308 line. The day-old chicks were from the same hatchery and parent stock. The experimental factors were: the addition of a probiotic in the amount of 500 g/1000 kg of the feed mixture containing *Bacillus licheniformis* bacteria (1×10^9 CFU/g) (Novus Int., Saint Charles, MO, USA) and the addition of the coccidiostat – sodium salinomycin – in the amount of 90 mg/kg of the mixture. Two control groups and two experimental groups were created in the design given in Table 1.

All chickens were divided into four equal groups. Within the group, twelve repetitions of 167 birds were identified. The chickens were reared for 42 days in metal pens under standard conditions, with the use of climate control technology and respect to animal welfare. Peat with the addition of wood chips was used as a mulch. Throughout the rearing period, the birds were fed *ad libitum* with granulated starter mixes for the first 10 days, and then from day 11 to 23 with grower 1 mix, from day 24 to 33 with grower 2 mix and over 33 days with finisher mix (Ross broiler management handbook. Aviagen.com, 2018; 12, 21). All feed mixtures used during the experiment were produced in the feed factory Ekoplön Sp. z o.o. Sp. K. (Grabki Duże, Poland), using the same raw materials. To avoid accidental loading of the feed with probiotics, feeds for C+ and C– birds were always produced first, before the feeds for groups with added probiotic. The

feed mixtures were balanced according to nutritional recommendations (33). The matrix of the probiotic nutritional values and the nutrient content of the feed mixtures are presented in Tables 2-4. During the experiment, the bodyweight of the chickens was monitored on the 1st, 7th, 14th, 21st, 28th, 35th and 42nd days of rearing. In addition, on the 21st day of the experiment, further weighing of 2016 chickens from four groups from three randomly selected pens, was carried out for flock uniformity analysis. On this basis, the CV variation of the coefficient was calculated. During the experiment, the consumption of feed by each group for the individual phases of feeding and the final consumption were recorded. The feed conversion rate (FCR) was reduced by an appropriate correction resulting from the weightings lost during the experiment, the chickens and the conversion of their respective feed consumed. The number of dead and selected birds in each group and their health status were checked daily. The obtained results were the basis for calculating the European efficiency index (EPI) according to the formula provided by Świątkiewicz and Koreleski (35) and the European production efficiency factor (EPEF) based on the formula provided by Shareef et al. (31). After the completion of the feeding experiment in the farm laboratory, five randomly selected chickens from each group were dissected and the body weight, pectoral and thigh muscle weights were determined. The obtained results were statistically verified according to the one-way analysis of variance (ANOVA) model. If statistically significant differences were found between the groups, Tukey's post hoc tests were performed. Statistical analysis was performed using Statistica 13.0 PL (Statsoft, Tulsa, USA computer program).

Results and discussion

The content of nutrients in complete mixtures (Tab. 2, 3) was at the level recommended for fattening

Tab. 2. Matrix of nutritional values of a probiotic with the participation of *Bacillus licheniformis* bacteria

Ingredient (%)	Starter (1-10 days)	Grower 1 (11-23 days)	Grower 2 (24-33 days)	Finisher (over 33 days)
Crude protein	1708.79	1592.26	1518.86	1481.45
Dig. Lysine	92.25	79.23	74.21	70.63
Dig. Thr	69.50	64.52	60.91	58.42
Dig. Methionine (%)	24.21	23.48	22.45	21.86
Dig. Cysteine	36.27	35.33	33.69	32.81
Dig. M+C	60.48	58.81	56.14	54.67
Dig. Tryptophan	19.37	17.75	16.61	15.90
Dig. Ile	59.84	59.46	56.33	54.37
Dig. Valine	83.49	71.64	68.09	65.98
Dig. Arginine	92.80	87.47	81.12	75.78
ME (kcal/kg)	56817.54	52029.45	49000.67	46740.99

Explanation: ME – Metabolizable energy; Dig. – digestible

Tab. 3. Content of nutrients in the compound feed in the C+ and C- groups

Ingredient (%)	Starter	Grower 1	Grower 2	Finisher
Protein	21.791	20.568	19.474	18.767
Fat	5.280	5.927	72.900	7.935
Fiber	3.233	3.332	3.358	3.480
Ash	5.137	4.594	4.176	3.918
Lysine	1.335	1.260	1.194	1.139
Methionine	0.623	0.595	0.564	0.538
Calcium	0.800	0.650	0.560	0.500
Phosphorus	0.579	0.525	0.477	0.459
Sodium	0.162	0.152	0.142	0.142

chickens in all rearing periods (33). The introduction of *Bacillus licheniformis* bacteria into the feed mixtures did not increase the bodyweight of the chickens in individual control weighings and at the end of rearing (Tab. 4). Taking into account the C-BL and C+C-BL groups, the group with the addition of coccidiostatic in the starter-type feed in individual control weights (days 7, 14, 21, 28, 35) showed a higher body weight compared to a group without the addition of a chemotherapeutic agent (Fig. 1). The highest mean body weight on day 42 of the chickens' life was found in the C- group and the lowest statistically significant values of the discussed parameter were found in the C+C-BL group (vs. group C-; $P < 0.05$). Chickens from C-BL and C+ groups showed the most similar mean body weight values. Data from literature show that Brzóska et al. (4), Mikołajczyk et al. (23), Kłoczek et al. (16) and Angel et al. (1) also did not note a significant effect in the adding of probiotics on bird growth and final body weight. Other researchers found that the dietary supplementation of 3 g/kg of *B. licheniformis* – fermented products in broilers increased their body weight during the entire feeding period (7). Both the control and research groups were characterized by a low value of the variation of coefficient (CV < 25%), which indicates

Tab. 4. Effect of feed additives containing *Bacillus licheniformis* on the body weight of broiler chickens (mean $\pm$ SD, n = 200 in each group)

Weighing day	Group	Body weight (g) $\pm$ SD
0	C+	40.0 $\pm$ 0.00
	C-	40.0 $\pm$ 0.00
	C-BL	40.0 $\pm$ 0.00
	C+C-BL	40.0 $\pm$ 0.00
7	C+	207.75 $\pm$ 1.58 ^c
	C-	200.08 $\pm$ 1.76
	C-BL	195.50 $\pm$ 3.55 ^{a d}
	C+C-BL	209.00 $\pm$ 2.15 ^c
14	C+	513.50 $\pm$ 7.28 ^c
	C-	514.75 $\pm$ 7.12 ^c
	C-BL	484.67 $\pm$ 10.29 ^{a b}
	C+C-BL	498.67 $\pm$ 12.14
21	C+	1003.8 $\pm$ 22.28 ^{b c}
	C-	951.25 $\pm$ 13.36 ^a
	C-BL	946.25 $\pm$ 11.93 ^a
	C+C-BL	968.67 $\pm$ 18.39
28	C+	1669.0 $\pm$ 39.23 ^{b c}
	C-	1541.3 $\pm$ 29.26 ^a
	C-BL	1525.2 $\pm$ 29.45 ^a
	C+C-BL	1585.3 $\pm$ 30.32
35	C+	2269.0 $\pm$ 35.30
	C-	2269.2 $\pm$ 49.50
	C-BL	2145.8 $\pm$ 43.25
	C+C-BL	2200.0 $\pm$ 57.30
42	C+	2700.0 $\pm$ 23.12
	C-	2747.0 $\pm$ 20.69 ^d
	C-BL	2690.0 $\pm$ 29.24
	C+C-BL	2665.0 $\pm$ 27.87 ^b

Explanations: Different superscripts letters represent statistical significance, ^a vs. group C+, ^b vs. C-, ^c vs. group C-BL, ^d vs. C+C-BL at $P < 0.05$

Fig. 1. The results of control and final weighing on individual days of rearing (n = 200 in each group)

the negligible differentiation of bodyweight of chickens in the individual groups and the homogeneity of the studied population (Tab. 5). The use of a probiotic supplement in the mixtures influenced the feed conversion ratio (FCR). The consumption of the mixture per unit of increment was lower in the research group C–BL and C+C–BL and amounted to 1.506 (kg/kg) and 1.512 (kg/kg), respectively, compared to the control groups (Tab. 6). Our results confirm the findings of Hooge et al. (14), who carried out research on broiler chickens fed with fodder with the addition of a probiotic preparation including *Bacillus subtilis* and the findings of Zaghari et al. (38), who fed broiler chickens on a basal diet plus 0.5 (g/kg) diet *B. licheniformis* preparation (1×10^9 CFU/g). These showed greater weight gain and a lower feed conversion rate throughout the rearing period. Moreover, Çınar et al. (8) indicated that the addition of pro/prebiotics to maize and wheat-based mixtures significantly improved the feed conversion index, as well as increased weight gain of the birds.

The effect of a lower FCR index and a higher survival rate of birds from the C–BL experimental group was a higher European poultry production index (EPI) (399.4 points) and European production efficiency factor (EPEF) (399.0 points). These indicators are used for comparative purposes for objectively testing the production effects in chickens reared for fattening of varied feeding and bird density conditions (15). Szöllösi and Szűcs (34) suggest that the production of broilers is profitable only with EPEF above 300–310. In our own research, the lowest values of the EPI index were recorded in the C+ group (385.6 points), while the EPEF in the group C+C–BL was 390.2 points. In the studies by Janoch et al. (15), the addition of *Bacillus subtilis* and *Saccharomyces cerevisiae* to the feed significantly increased the value of the EPI index. In the conducted experiment, the highest survival of chickens in the food groups was recorded in the C–BL group (98.303%). Group C– was characterized by the highest mortality rate. Opinions on the reduction of chickens mortality by adding probiotic bacteria to the feed are divergent. The research results available in the literature are carried out in various zoohygienic conditions with the use of microorganisms with different potency on the bird's organism (37). Numerous reports confirm a significant decrease in mortality in chickens fed mixtures with the addition of probiotic bacteria (3, 4, 6, 10, 28, 37).

The addition of a probiotic in the feed mixes did not have a significant effect on the muscularity of broiler chickens. The mean weight of the pectoral muscle between the control (C+, C–) and research groups (C–BL, C+C–BL) showed no significant differences (Tab. 7). As in the case of the mean carcass weight, the significantly lowest values of the mean weight of the femurs were recorded in the chickens from the C+C–BL group (vs. C–; $P < 0.05$).

Tab. 5. Variation of coefficient (CV) of body weight of broiler chickens in different experimental groups (n = 504 in each group)

Group	CV (%)
C+	12.987
C–	13.190
C–BL	13.640
C+C–BL	13.849

Tab. 6. Effect of different feed additives on broiler rearing parameters (n = 2003 in each group)

Group	FCR 41. day (kg/kg)	Mortality (%)	EPI (points)	EPEF (points)
C+	1.560 ± 0.61	2.140	385.6	391.3
C–	1.529 ± 0.36	2.946	392.8	398.2
C–BL	1.486 ± 0.42	1.697	399.4	399.0
C+C–BL	1.492 ± 0.52	2.347	386.8	390.2

Explanations: FCR – feed conversion ratio; EPI – European efficiency index; EPEF – European production efficiency factor

Tab. 7. Effect of *Bacillus licheniformis* a probiotic strain on average weights of the carcass, pectoral and thigh muscle weight of broilers (mean ± SD, n = 5 in each group)

Group	Carcass weight (kg)	Pectoral muscle weight (kg)	Thigh muscle weight (kg)
C+	2.014 ± 0.58	0.665 ± 0.017	0.407 ± 0.047
C–	2.064 ± 0.62 ^d	0.627 ± 0.024	0.416 ± 0.041 ^d
C–BL	1.968 ± 0.59	0.623 ± 0.020	0.408 ± 0.038
C+C–BL	1.953 ± 0.60 ^a	0.644 ± 0.023	0.401 ± 0.042 ^b

Explanations: as in Tab. 4

Our results confirmed those in earlier studies (2, 5, 15, 29). In chickens fed with compound feed with pro/prebiotic preparations, no significant effect was shown on the weight of the pectoral muscles.

Based on the presented results, the probiotic bacteria *Bacillus licheniformis* can be considered as a good feed additive, due to its positive effect on the general health of broiler chickens. These effects contribute to better use of nutrients and stimulation of growth and development. Their addition to the feed shows great potential as an alternative to chemotherapeutic agents used both in the prevention and treatment of many diseases of animals. For two decades, there has been an increase in knowledge about the impact of probiotic bacteria, as feed supplements for farm animals, on the quality of raw materials obtained from them. Consumers' interest in the health and welfare of farm animals is also growing. However, it should be borne in mind that the use of probiotic bacteria may, unfortunately, be associated with the unfavorable phenomenon of transferring antibiotic resistance genes to other bacteria and affect the biodiversity of the environment. Therefore, there is a need for further research on probiotics that will show both the positive and undesirable effects of their

use in the nutrition of farm animals and their impact on the body of consumers. These activities will enable the use of probiotic additives in a fully conscious, optimal and safe manner.

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