

# Poultry-beneficial solid-state *Bacillus amyloliquefaciens* B-1895 fermented soybean formulation

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Birds were given a new formulation of the *Bacillus amyloliquefaciens* B-1895 solid-state fermented soybean that retained the spores of the aforementioned organism. Mass dynamics, feed flow rate and broiler performance were observed to evaluate the efficacy of the formulation. At each time point, the live mass was greater than that of the control group, reaching a difference of 7–8% by day 28. A difference of 5.3–8.8% was observed in feed conversion per kilogram live mass (1.97 kg in the controls as compared with 1.81–1.87 kg in experimental groups). This indicates a positive effect of the *B. amyloliquefaciens* B-1895 formulation on the live mass of broilers as well as on feed consumption.

**Key words:** *Bacillus amyloliquefaciens*, probiotic, solid-state fermentation

The World Health Organization defines probiotics as live microorganisms capable of delivering scientifically measurable positive effects to eukaryotic organisms when administered in adequate quantities [1]. In poultry, many farmers still use antibiotics for control of pathogenic microbiota. However, probiotics can efficiently control pathogens and stimulate the growth of birds [2]. This makes probiotics attractive for organic poultry production. In the poultry industry, it is essential that the method of application be simple, preferably in the form of feed additives [3]. Usually, probiotic formulations for poultry are prepared using liquid phase fermentation followed by freeze-drying or spray drying. This is a cost-effective approach.

Recently, *Bacillus* spp. emerged in livestock

applications, especially in the poultry industry [4–6]. Bacilli are known for their remarkable adaptability to diverse conditions; their spores can withstand harsh environmental stress, have a long shelf life and thus can be stored in a state of dehydration [7, 8]. Moreover, *Bacillus* spp., including *B. amyloliquefaciens* can be found in the normal intestinal microbiota of poultry and are capable of germinating and re-sporulating in the gastrointestinal tract of chickens [5, 9].

Ushakova et al. [10] indicated the importance of biofilms and their promising application in medical and veterinary probiotic formulations, specifically those generated by *B. subtilis* and other spore-forming probiotic microorganisms [11]. These produce nutritionally-functional products capable of strong antagonistic activity to pathogens [12], technological plasticity and high enzymatic activity [13].

The present study aimed to estimate the feasibility of soybean solid-state fermentation by *Bacillus amyloliquefaciens* B-1895 for the production of a cost-effective formulation for application as a poultry dietary supplement.

*B. amyloliquefaciens* B-1895 (soil isolate) was obtained from the Russian National Collection of

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Industrial Microorganisms, Moscow, Russia (RNCIM). It is a nonpathogenic microorganism with a reported high level proteolytic activity [14]. Luria-Bertani (LB) medium (Difco, Detroit, MI, USA) was used to propagate the strain under laboratory conditions. The cells were grown aerobically either on a solid agar plate or in a liquid broth, with agitation. The microorganism was stored at 4°C until use.

One kilogram (dry weight) of the Don-21 variety of soybean (39% protein, purchased from the I.G. Kalinenko All-Russia Research Institute of Crops, Zernograd, Rostov Region, Russia) was washed with tap water and then soaked overnight in two volumes of tap water. The following day, the water was drained, and the beans were autoclaved for 15 min at 121°C. The beans were cooled down to 60°C, and 10 ml overnight culture of *B. amyloliquefaciens* B-1895 ( $10^8$  CFU/ml) in LB were added and mixed thoroughly. Inoculated beans were placed in a layer of 3–5 cm in a sterile tray with a non-hermetic lid and incubated for 24 hours at 42°C. Then the beans (50% moisture), coated with bacterial biofilm ( $3 \times 10^{11}$  CFU/g), were ground in a meat grinder (Kenwood 700 MG, Watford, UK). The resulting mass was placed in open trays in a layer of 1–2 cm and incubated for 48–72 hours at 45°C in a ventilated oven (until dried; 5% moisture). After drying, the solid product granules with  $9 \times 10^{11}$  CFU/g of B-1895 cells were milled in a coffee grinder (KMM 30, Braun, Kronberg, Germany). To evaluate survivability of the B-1895 spores on the granules, they were stored for one year at room temperature. After one year storage, this formulation contained  $5 \times 10^{11}$  spores of *B. amyloliquefaciens* B-1895. The spore content (100%) was confirmed by the plate count of the formulation before and after exposure to heat (100°C, 20 min).

The *B. amyloliquefaciens* B-1895 formulation contained protein, 45.9% (dry weight, State Standard GOST 10846-91), fat, 10.3% (dry weight, State Standard GOST 29033-91), and moisture, no more than 61.3% (State Standard GOST 10856-96). It was tested in the poultry plant of “Roster” Ltd. (Georgia). Thirty-three 1-day-old “Ros-308” chickens (no vaccinations or other treatments prior to the study), divided in 3 groups (11 birds per group), were used in the study. Group 1 represented a control group; birds in this group were given an antibiotic (10 mg per 1 kg of the feed (Table 1), cloxacillin, SYVA, Leon, Spain) for the first 3 days, with drinking water and combined feeding (start, grower, finish) during the entire breeding period. Group 2 was an experimental group, which received the same feed during the entire period and the *B. amyloliquefaciens* B-1895 formulation

Table 1. Poultry feed formulation

Variable components	Age (days)		
	0–11	11–24	25–slaughter
Crude protein (%)	22.8	21.9	21.2
Energy (kcal/kg)	307	316	320
Lysine (%)	1.42	0.82	0.7
Methionine (%)	0.49	0.43	0.38
Methionine + cystine (%)	1.06	0.95	0.81
Calcium (%)	0.98	0.94	0.84
Phosphorus (%)	0.47	0.44	0.4
Vitamin A (IU/kg)	13,500	10,000	10,000
Vitamin D3 (IU/kg)	4,950	3,900	3,900
Vitamin E (IU/kg)	73	48	47

Major constituents remaining unchanged for the entire feeding period: yellow maize (50–55%), soy meal (28–33%), sunflower oil (2–4%), monocalcium phosphate (0.2%).

(0.5 g/1 kg feed) without cloxacillin from the first day of the experiment. Experimental group 3 received the same amount of the formulation as Group 2; in addition, this group received cloxacillin for the first 3 days.

The birds were held in KBU-3 (Russia) battery cages for up to a 30 day period. Maintenance and microclimate parameters were kept constant for all three groups. During the study period, the following observations were carried out: i) live mass dynamics at 1, 7, 14, 21, and 28 days (by individual weight), ii) daily food consumption (the food of one day and the remaining on the second day were weighed daily on electronic scales) and iii) observance of a decreased number of birds and determination of reasons for the decline.

Recently, studies involving *Bacillus* spp. indicating their benefits in turkey poults [2] and broilers [15] have been increasing. Broiler birds, as compared with other fowl, are more susceptible to gastro-intestinal diseases. The reason for this is their very short period of breeding (35–36 days), which prevents full development of immune systems. The lack of intestine-beneficial microflora, especially in the early days of life, increases the vulnerability of these birds to viral and microbial infections. Therefore, the present study was designed to evaluate a formulation containing *B. amyloliquefaciens* B-1895 for possible application in broiler feeding.

It was found that the growth rate of the tested group followed the polynomial function as depicted in Fig. 1. The weight gain results are presented in Table 2. There was a loss of one bird in Group 1 due to physical trauma. As for bird live mass dynamics, the efficiency of the formulation is evident from the obtained results. In the first week of breeding, the live mass of the birds in the experimental group exceeded that of the birds

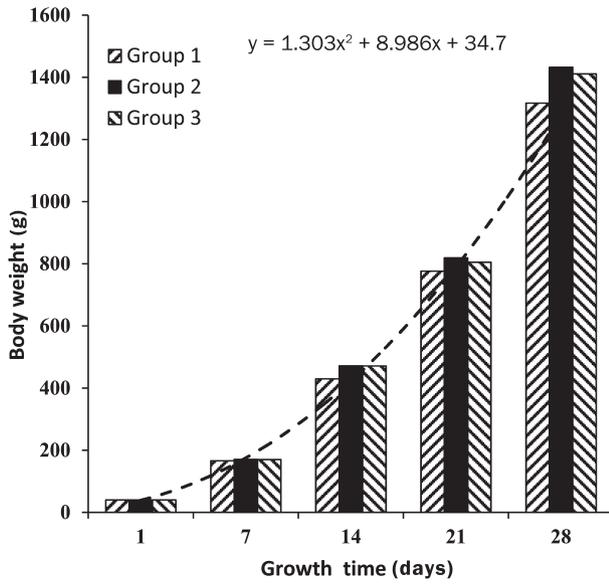


Fig. 1. Growth dynamics of broiler chickens.

in the control group by 3.0%. In the second week, the difference reached 9.5%, and at 28 days, the mass difference reached 7–8% in favor of the experimental group. There were no statistically significant differences in the growth parameters of experimental groups 2 and 3. Differences were compared by the Student's t-test and

considered significant when  $p \leq 0.05$ . This suggests that the formulation containing *B. amyloliquefaciens* B-1895 can be used alone or in combination with antibiotics.

The food consumption results are summarized in Table 3. Despite the fact that the feed flow rates of the three groups were nearly the same (2,600–2,650 g), there were observed differences in feed conversion per 1 kg live mass, which was 1.97 kg in the control group and 1.81–1.87 kg in the experimental groups, 5.3–8.8% less. Differences were compared by the Student's t-test and considered significant when  $p \leq 0.05$ . Thus, the formulation containing *B. amyloliquefaciens* B-1895 had a positive effect on the live mass of broilers and on feed consumption. Soybeans are rich in proteins and lipids. It is presumed that cultivation of microorganisms on their surface can also stimulate various beneficial proteolytic activities. Our observations are in agreement with the recent findings by Molnár et al. [16], who showed that the weight of broiler chickens receiving feed supplemented with *B. subtilis* was significantly higher and the feed consumption was better than that of the control group regardless of the microbial load in the formulations. In addition, the birds from the groups fed *B. subtilis*-supplemented diets had significantly increased antibody responses to vaccination against Newcastle virus [16]. This indicates that although spore-forming probiotics may be metabolically dormant prior to administration,

Table 2. Dynamics of the live weight of broiler chickens

Group	Index	Age (days)				
		1	7	14	21	28
1	n	11	11	10	10	10
	Average weight (g)	40	166	430	776	1,317
	Standard deviation	<1	9	32	58	35
2	n	11	11	11	11	11
	Average weight (g)	40	171	472	819	1,432
	Standard deviation	<1	10	40	53	42
	p (t-test)*	NT	0.21	0.015	0.096	0.0001
3	n	11	11	11	11	11
	Average weight (g)	40	170	471	805	1411
	Standard deviation	<1	8	21	55	34
	p (t-test)*	NT	0.81	0.004	0.26	0.0001

\* Statistical significance of differences from group 1.

Table 3. Feed consumption by broiler chickens

Index	Unit	Group		
		1	2	3
Feed flow rate on 1 bird	kg	2.60	2.60	2.65
Feed flow rate for 1 kg growth	kg	1.97	1.81	1.87

they may germinate in the gastrointestinal tract of chicks and become viable and functional probiotics. However, further work is required to determine the specific function(s) of the *B. amyloliquefaciens* B-1895 fermented product as a probiotic formulation. In addition to studying this formulation in comparison with products resulting from microbial processing of the same substrate by different microorganisms, it will be important to measure other parameters such as the potential differences in bacterial populations in feces and enzymatic activity of the probiotic strain.

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