

http://periodicos.uem.br/ojs/acta ISSN on-line: 1807-8672 Doi: 10.4025/actascianimsci.v47i1.72261



# Performance, carcass yield, and meat quality of broilers fed a diet containing *Bacillus thuringiensis* var. *israelensis*

Paola de Freitas Feltrin<sup>1</sup>, Aline Piccini Roll<sup>1</sup>, Camila Von Mühlen<sup>1</sup>, Fabio Pereira Leivas Leite<sup>2</sup>, Eduardo Gonçalves Xavier<sup>1</sup>, Fernando Rutz<sup>1</sup> and Victor Fernando Büttow Roll<sup>1\*</sup>

<sup>1</sup>Departamento de Zootecnia, Faculdade de Agronomia Eliseu Maciel, Universidade Federal de Pelotas, Rua Gomes Carneiro, 1, 96010-610, Balsa, Pelotas, Rio Grande do Sul, Brasil. <sup>2</sup>Universidade Federal de Pelotas, Pelotas, Rio Grande do Sul, Brasil. \*Author for correspondence. E-mail: roll98@ufpel.edu.br

**ABSTRACT.** This study aimed to evaluate the effect of *Bacillus thuringiensis* var. *israelensis* (Bti) on the performance, organ weights, carcass yield, and meat quality of chickens. A total of 288 male and female one-day-old Cobb chicks were either fed or not fed with Bti at a dose of 1x108 CFU per gram of feed for a period of 42 days resulting in a 2x2 factorial scheme (Bti x sex) with four treatments and 12 replicates of six birds each. The treatments were: T1 - Males without Bti; T2 - Females without Bti; T3 - Males with Bti; and T4 - Females with Bti. Performance variables were assessed weekly. At the end of the trial, 48 chickens (12 birds per treatment) were euthanized to determine carcass yield, edible organ yield, and meat quality. The results indicate that none of the variables were significantly affected by Bti inclusion in the diet. Male chickens, regardless of the BTI factor, showed higher body weight gain and feed intake at 1-21, 22-41, and 1-42 days of age, as well as better feed conversion in the 1-21-day period, in addition to higher live weight, liver weight, and thigh and drumstick weight. In summary, the inclusion of *Bacillus thuringiensis* var. *israelensis* in the chickens' diet did not yield positive results, indicating that more studies are needed to optimize its use in poultry production. The performance differences observed between the sexes, with males outperforming females, are consistent with those reported in the poultry industry and scientific literature.

**Keywords:** broilers; feed intake; feed conversion; live weight; probiotics.

Received on May 18, 2024. Accepted on August 21, 2024.

#### Introduction

Since the 1950s, antibiotics and chemotherapeutics have been added to animal feed, raising concerns about the emergence of antibiotic-resistant bacterial strains (Stachelek et al., 2021). In this context, alternatives to the use of antibiotics are constantly being developed in the research field, and among them is the use of probiotics in the feeding of chickens. Early and consistent success was achieved through the usage of continuous culture-generated mixtures of cecum competitive exclusion cultures to restrict the establishment of *Salmonella* in young chickens (Nisbet et al., 1994).

The probiotics have different mechanisms of action, with competition exclusion, direct antagonism, stimulation of the immune system, nutritional effect, and reduction of ammonia production as a highlight (Reis & Vieites, 2019). Various microorganisms are used as probiotics, including lactic acid bacteria, non-lactic acid bacteria, and yeasts (Coppola & Gil-Turnes, 2004). According to Reis and Vieites (2019), the main microorganisms used are from the *genera Lactobacillus*, *Bifidobacterium*, *Enterococcus*, *Streptococcus*, *Bacillus*, and also yeasts, which can be used individually or in combination with one another.

*Bacillus* spores are widely used as probiotics and competitive exclusion agents in humans and animals, which sets them apart from other species of microorganisms used in their vegetative form (Ricke & Saengkerdsub, 2015). Moreover, they produce several extracellular enzymes that increase food digestibility, as well as many antimicrobial compounds. The species also stimulates the immune system of host animals, thereby improving growth performance, feed conversion rate, and meat quality (Lee et al., 2012). The most well-known *Bacillus* species is *Bacillus cereus*, but apart from it, other species have been used as probiotics, such as *Bacillus licheniformis*, *B. pumilus*, *B. clausii*, *B. coagulans*, *B. thuringiensis*, among others (Hong et al., 2005).

*Bacillus thuringiensis* var. *israelensis* (Bti), belonging to the *Bacillaceae* family, is a Gram-positive, sporulating, strict aerobe, entomopathogenic rod, and stands out as the main bacterium used in biological control (Angelo et al., 2010). Approximately 90% of the global bioinsecticide market consists of products based on this bacterium (Vilas-Bôas et al., 2007). However, its probiotic properties are not yet well described,

Page 2 of 9 Feltrin et al.

making it extremely important to assess its action in chickens to advance sustainable animal treatment and reduce the use of chemically synthesized products. Therefore, the purpose of this study was to evaluate the effect of *Bacillus thuringiensis* var. *israelensis* on the performance, organ and carcass yield, and meat quality of chickens.

#### Material and methods

The study was carried out at the animal facility of the Laboratory of Teaching and Zootechnical Experimentation Prof. Dr. Renato Rodrigues Peixoto – UFPEL, following the principles of the Helsinki Declaration and approved by the Animal Experimentation Ethics Committee (CEEA) UFPEL, under protocol number 9053. A total of 288 one-day-old broiler chicks (both males and females) from the Cobb commercial lineage were used. The birds were randomly assigned in a 2x2 factorial scheme (Bti x Sex), resulting in four treatments with 12 replicates of six birds each. Each replicate was housed in an experimental pen (1 m²) with a 10 cm deep rice husk bedding, and they had *ad libitum* access to feed and water for 42 days. The diets were based on corn and soybean meal, meeting the nutritional requirements of chickens for the initial (1-20 days), growth (21-35 days), and final (36-42 days) phases, as outlined in Table 1. The treatments were: T1 - Males without Bti; T2 - Females without Bti; T3 - Males with Bti; and T4 - Females with Bti.

	Starter Diet*	Grower Diet**	Finisher Diet**
Ingredient (kg)	(1-20 days)	(21-35 days)	(36-42 days)
Corn	57.07	61.05	64.41
Soybean meal	38.03	33.51	30.6
Oil	2.5	3.2	3.0
Calcium carbonate	1.40	1.33	1.20
Salt	0.43	0.42	0.39
L-Lisine	0.164	0.169	0.148
DL-Methionine	0.322	0.256	0.192
L-Treonine	0.085	0.065	0.060
Total	100.0	100.0	100.0
Calculated composition			
Metabolizable energy (Kcal Kg <sup>-1</sup> )	2980	3070	3100
Crude protein (%)	20.8	19.0	18.0
Calcium (%)	0.95	0.96	0.90
Sodium (%)	0.20	0.19	0.18
Available phosphorus (%)	0.46	0.41	0.41
Digestible Met + Cys (%)	0.95	0.90	0.86
Digestible lysine (%)	1.20	1.05	0.98
Digestible Threonine (%)	0.85	1324	1261

Table 1. Nutritional composition and calculated values of the experimental diets

The *Bacillus thuringiensis* var. *israelensis* (Bti) utilized in this study is part of the microorganism collection of the Microbiology Laboratory of the Biotechnology Centre at the Federal University of Pelotas (UFPel). For production, Bti was inoculated on Brain Heart Infusion agar (BHI agar, Difco) and incubated for 24 hours at 28°C. Subsequent to growth, isolated colonies were inoculated into 200 mL of BHI medium and incubated in an orbital shaker at 200 rpm for 24 hours. The cultures were then transferred into 4 L of Nutrient Yeast Extract Salt Medium (NYSM medium) at 28°C with agitation (250 rpm) and aeration (1vvm) for 72 hours. The cells were harvested by centrifugation at  $6000 \times g$  for 20 minutes and concentrated in a final volume of 1 L of phosphate-buffered saline (PBS). The Bti was placed on top of the mixture of the ingredients in a proportion of 1 L of concentrated Bti culture added to each 100 kg of feed, resulting in a final concentration of 1 x  $10^8$  Colony-Forming Units (CFU) per gram of feed, administered daily for 42 days. The Bti was incorporated into the diet on a weekly basis, followed by quantification in order to assess cell viability and confirm the appropriate concentration. Once prepared, the diets were stored in separate containers for each trial to allow a better control of feed consumption for each repetition.

<sup>\*</sup>Starter: Choline 7,430 mg; Folic acid 127.76 mg; Vitamin E 311.72 IU; Vitamin K3 26.28 mg; Vitamin B2 85.41 mg; Vitamin B6 43.80 mg; Iron 1,800 mg; Zinc 1,667 mg; Vitamin D3 38,180 IU; Vitamin A 172,170 IU; Vitamin B1 26.28 mg; Copper 300 mg; Iodine 20 mg; Nicotinic acid 569.40 mg; Pantothenic acid 255.65 mg; Vitamin B12 222.20 mcg; Manganese 2,100 mg; Selenium 10 mg; Calcium 247 g; Phosphorus 110.57 g. \*\*Grower: Choline (Min) 4,780 mg; Folic acid (Min) 58.56 mg; Vitamin E (Min) 365.85 IU; Vitamin K3 (Min) 25.18 mg; Vitamin B2 (Min) 80 mg; Vitamin B6 (Min) 44.36 mg; Iron (Min) 1,800 mg; Zinc (Min) 1,667 mg; Vitamin D3 (Min) 25,182 IU; Vitamin A (Min) 110,040 IU; Vitamin B1 (Min) 24.27 mg; Copper (Min) 300 mg; Iodine (Min) 20 mg; Nicotinic acid (Min) 482.34 mg; Pantothenic acid (Min) 226.35 mg; Vitamin B12 (Min) 203.85 mcg; Manganese (Min) 2,100 mg; Selenium (Min) 10 mg; Calcium (Min) 221.7 g; Phosphorus (Min) 98.70 g.

Each week, the body weight gain (BWG) and feed consumption of all birds, as well as any remaining feed, were measured. The feed conversion ratio (FCR) was calculated using the formula FCR = FC/BWG, in which FCR represents the feed conversion ratio, FC stands for feed consumption, and BWG for body weight gain. After the 42-day experiment, 48 chickens were chosen and euthanized, with 12 birds per treatment group (6 males and 6 females), selected based on their body weights matching the average of their respective group. The birds were fasted for 8 hours before euthanasia, which was performed in the Animal Science Experimentation Laboratory using an inhalational anesthetic (Isoflurane) in an euthanasia chamber. After verifying unconsciousness, the birds were exsanguinated by jugular section. This process was carried out to assess organ and carcass yield, meat quality, and intestinal morphometry.

The birds were gutted, and the weight of the edible viscera (heart, liver, and gizzard) was measured to determine the absolute and relative organ weight, calculated as (organ weight/live bird weight) \* 100. Abdominal fat, which includes the adipose tissue in the abdominal area, was also weighed, and its percentage was calculated using the same formula of the edible viscera.

The yield of the plucked and gutted carcass, excluding feet, head, and neck, along with individual cuts, was determined relative to the live weight of the bird at the time of slaughter. Each carcass was weighed to separate the thigh, drumstick, winglet, wing tip, back, and breast cuts, all of which retained their skin and bones. The relative weight of each cut was calculated using the formula (cut weight/live bird weight) \* 100.

To evaluate meat quality, 12 samples per treatment (6 males and 6 females) of boneless, skinless breast meat were selected and thawed at 4°C for 24 hours. The water holding capacity (WHC) was assessed using the filter paper press method, in which a sample with approximately 5 grams of breast meat was subjected to 1 kg of pressure for 5 minutes (Sierra, 1973). Press losses were determined by the difference in weight and were expressed as a percentage of the initial weight. Cooking losses were determined by weighing raw breast samples of approximately 150 g, wrapping them in aluminium foil, and grilling them on an electric grill (Britânia, Grill Press 180) until the internal temperature reached 85°C. The cooking loss was calculated by the difference in sample weight before and after cooking and expressed as a percentage of the initial sample weight. The drip losses were measured by conditioning raw breast samples of approximately 5g under refrigeration (4°C) for 5 days in plastic tubes designed for dripping. After this period of 5 days, samples were reweighed, and the change in their weight was due to the fact that there was drip loss. In addition, the calculation utilized was expressed in percentage.

The pH level was measured using a digital pH meter (Marte MB 10) with a penetration electrode, directly inserting the electrode into the sample. Shear force was verified using a TA XT Plus texture analyzer. Colour determination involved measuring three distinct points on the breast to obtain the average value using a Chroma Meter CR-300 colorimeter (Minolta, Osaka, Japan), with readings of L\* (luminance), a\* (red-green component), and b\* (yellow-blue component) in the CIELAB colour space.

The following mathematical model was used for the statistical analysis:  $Y_{ijkl} = \mu + A_i + \beta_j + A\beta_{ij} + E_{ijkl}$ , in which:  $\mu$  = overall mean;  $A_i$  = probiotic (*Bacillus thuringiensis*) effect (i =1,2);  $\beta_j$  = sex effect (j = 1,2);  $A\beta_{ij}$  = interaction (probiotic x sex); and  $E_{ijkl}$  = random error. The data were analyzed by ANOVA with Bti and sex as fixed factors using the R package emmeans (R Core Team, 2020). When a significant F-value was observed, mean values were compared using the Tukey test with a significance level of p < 0.05.

## **Results and discussion**

It was hypothesized that adding Bti to the diets would enhance the performance of male or female chickens. No significant interaction between Bti and sex factors was found for the performance variables, and no differences (p > 0.05) were noted regarding the Bti factor alone (Table 2). Statistical differences (p < 0.05) were observed concerning the sex factor during 1 to 21 days, 21 to 42 days, and 1 to 42 days. Male chickens exhibited higher body weight gain and feed consumption than females. During the 1 to 21-day period, the best feed conversion (p < 0.05) was attributed to males (1.49) compared to females (1.60).

In various studies, researchers explored the influence of different *Bacillus* strains on chicken performance. Meurer et al. (2010) observed improved body weight gain and feed conversion when evaluating the use of *Bacillus subtilis* C-3102 in the diets of Cobb chickens from day 1 to 42. Sen et al. (2012) reported increased body weight gain and improved feed conversion with addition of *Bacillus subtilis* LS 1-2 to the diets of Ross chickens from day 1 to 21 and from day 22 to 35.

Page 4 of 9 Feltrin et al.

**Table 2.** Mean (± SE) body weight gain (BWG), feed consumption (FC) and feed conversion (FCR) of male and female chickens fed diet containing *Bacillus thuringiensis* var. *israelensis* (Bti) in the periods of 1-21 days, 21-42 days, and 1-42 days.

		BWG (g)			FC (g)			FCR	
Item	1-21 days	21-42 days	1-42 days	1-21 days	21-42 days	1-42 days	1-21 days	21-42 days	1-42 days
Effect of Bti									_
Without Bti	658	1415	2072	1016	2561	3577	1.57	1.83	1.74
With Bti	668	1321	1989	1005	2512	3517	1.52	1.97	1.80
Effect of sex									
Male	701 <sup>a</sup>	1496ª	2197ª	1038a	2735a	3773 <sup>a</sup>	$1.49^{a}$	1.89	1.74
Female	$625^{\rm b}$	$1239^{b}$	1864 <sup>b</sup>	983 <sup>b</sup>	$2337^{\rm b}$	$3320^{\rm b}$	$1.60^{\rm b}$	1.92	1.80
SEM	17.1	46.9	55.7	12.3	41.7	49.4	0.03	0.07	0.04
P-value									_
Bti	0.67	0.16	0.30	0.54	0.40	0.39	0.26	0.19	0.39
Sex	0.003	0.0003	0.0001	0.003	< 0.0001	< 0.0001	0.03	0.80	0.38
Bti:Sex	0.36	0.66	0.51	0.99	0.84	0.86	0.21	0.66	0.48

\*Different letters in the column indicate significant differences (p < 0.05) between the means according to the Tukey test. SEM: standard error of the mean.

Marubashi et al. (2013) evaluated the addition of *Bacillus subtilis* C-3102 to the diets of Ross 308 male chickens, considering a 3.3% body weight gain improvement from day 1 to 21 and a 3.0% feed conversion improvement from day 22 to 42. Over the entire 42-day period, supplemented birds exhibited 2.6% better feed conversion. Bai et al. (2017) supplemented *Bacillus subtilis fmbj* to the diets of Arbor Acres male chickens, reporting increased body weight gain, feed consumption and improved feed conversion from day 21 to 42 and from day 1 to 42.

In our study, Bti addition did not significantly affect the chickens' performance, aligning with Domingues et al. (2014), who noticed no impact on body weight gain, feed consumption or feed conversion up to 42 days with *Bacillus subtilis* supplementation to the diets of Cobb male chickens. Most probiotics used in chickens' diets show effectiveness within daily intake levels of 10<sup>7</sup> to 10<sup>9</sup> CFU g<sup>-1</sup> (Patterson & Burkholder, 2003; Mountzouris et al., 2010), and the dosage in our study falls within these recommended limits. The reasons for the lack of effectiveness of Bti in improving the chickens' performance are uncertain. One reason could be the lack of viability of Bti in the diet. However, we can discard this hypothesis because all of the weekly diet samples showed that the microorganisms were viable. The efficacy of such products depends on the quantity and characteristics of the microorganisms used, therefore, it is challenging to make direct comparisons between studies.

The performance disparities observed between sexes, with males outperforming females, were anticipated and are common in poultry farming. Stringhini et al. (2003) and Api et al. (2017) also noted superior performance of males due to their higher feed consumption rate, resulting in greater muscle tissue deposition and body weight gain. Male chickens exhibit faster growth because of their higher feed consumption rate, resulting in greater muscle tissue deposition and body weight gain (Bertechini, 2006).

No significant interaction effect between Bti and sex factors was found for organ weights and abdominal fat. Additionally, no statistical differences (p > 0.05) were noted in organ weights due to Bti addition (Table 3). However, regarding the sex factor, it was evident that males had a greater liver weight compared to females (38.2 g vs. 33.4 g; p < 0.05).

**Table 3.** Mean (± SE) organ weights (g) at 42 days of age of male and female chickens fed diet containing *Bacillus thuringiensis* var. *israelensis (Bti)*.

Item	Gizzard	Liver	Abdominal Fat	Heart
Effect of Bti				
Without Bti	46.6	37.0	20.6	10.03
With Bti	43.5	34.6	18.3	9.96
Effect of sex				
Male	47.1	38.2a	17.4	10.98
Female	43.0	$33.4^{b}$	21.5	9.01
SEM	1.83	1.5	1.64	0.46
P-value				
Bti	0.23	0.27	0.32	0.91
Sex	0.12	0.03	0.08	0.004
Bti:Sex	0.65	0.14	0.23	0.58

<sup>\*</sup>Different letters in the column indicate significant differences (p < 0.05) between the means according to the Tukey test. SEM: standard error of the mean.

A study by Manafi et al. (2018) made an evaluation of a multispecies probiotic containing four Bacillus species and *Saccharomyces boulardii* (Microguard®) in the diets of Ross 308 male chickens at 42 days, in which lower liver and abdominal fat weights were reported compared to the control group, differing from the findings of this study. Santos et al. (2005) and Schettino et al. (2006) observed higher liver weights in females, contrasting with our results.

Stringhini et al. (2003) and Api et al. (2017) noted that females exhibited a higher abdominal fat yield, a trend not observed in this study. From 35 days onwards, females tend to accumulate more fat in the carcass (Bertechini, 2006). Additionally, abdominal fat is considered one of the factors influencing feed conversion, since adipose tissue deposition reduces feeding efficiency.

There was no significant interaction effect between Bti and sex, and no statistical effect of Bti on live weight, eviscerated carcass weight, and carcass yield (Table 4). Male chickens exhibited a higher live weight compared to females (2319 g vs. 2088 g; p < 0.05), as expected due to their superior body weight gain.

Domingues et al. (2014), using *Bacillus subtilis*, also did not find significant differences in chicken carcass yield and parts. Moreover, other studies investigating various probiotic dosages have reported variations in performance and carcass response, indicating that the optimal concentrations of probiotics in chicken feed may differ based on the microorganisms included in the product (Pourakbari et al., 2016).

**Table 4.** Mean (± SE) live weight, eviscerated carcass weight and carcass yield at 42 days of age of male and female chickens fed diet containing *Bacillus thuringiensis* var. *israelensis* (Bti).

Item	Live Weight (g)	Eviscerated Carcass (g)	Carcass Yield (%)	
Effect of Bti				
Without Bti	2256	1634	72.4	
With Bti	2152	1567	72.6	
Effect of sex				
Male	2319 <sup>a</sup>	1675	72	
Female	2088 <sup>b</sup>	1525	73	
SEM	77.4	58.8	0.36	
P-value				
Bti	0.35	0.42	0.76	
Sex	0.04	0.08	0.06	
Bti:Sex	0.24	0.22	0.35	

<sup>\*</sup>Different letters in the column indicate significant differences (p < 0.05) between the means according to the Tukey test. SEM: standard error of the mean.

No significant interaction effect between Bti and sex factors, and no effect of Bti alone (p > 0.05) were noted on the weights of the drumstick, thigh, wingette, wingtip, back, and breast. Male chickens exhibited higher drumstick and thigh weights compared to females (116.6 g vs. 99.3 g; 132 g vs. 108 g, respectively; p < 0.05), as expected due to their greater body weight gain and live weight (Table 5).

**Table 5.** Mean (± SE) cut weights (g) at 42 days of age in male and female chickens fed diet containing *Bacillus thuringiensis* var. *israelensis* (Bti).

Item	drumstick	thigh	wingette	wingtip	back	breast
Effect of Bti						
Without Bti	112	125	49.7	38.6	392	539
With Bti	104	115	47.6	36.8	413	522
Effect of sex						
Male	116.6 <sup>a</sup>	132a	49.2	39.3	434	558
Female	99.3 <sup>b</sup>	$108^{\rm b}$	48.1	36.1	371	503
SEM	4.13	4.85	1.9	1.35	25.3	23.7
P-value						
Bti	0.23	0.15	0.43	0.35	0.57	0.62
Sex	0.004	0.0008	0.66	0.10	0.08	0.10
Bti:Sex	0.25	0.05	0.19	0.79	0.54	0.57

 $<sup>^*</sup>$ Different letters in the column indicate significant differences (p < 0.05) between the means according to the Tukey test. SEM: standard error of the mean.

In a study by Manafi et al. (2018), supplementing male chickens of the Ross 308 lineage with a multispecies probiotic containing four *Bacillus* species and *Saccharomyces boulardii* (Microguard®) at 42 days resulted in a lower relative breast weight compared to the control group. Domingues et al. (2014), evaluating *Bacillus subtilis* supplementation in the diets of male Cobb chickens at 42 days, found no statistical differences in drumstick and thigh, wing, and breast yields. These findings are consistent with the results of this study.

Page 6 of 9 Feltrin et al.

A significant interaction between Bti and sex was noted only for cooking loss (p < 0.05), as shown in Table 6. Non-Bti males had the highest cooking loss percentage (31.2%), while non-Bti females had the lowest cooking loss percentage (25.9%). Males and females receiving Bti showed similar cooking losses of 27.7 and 27.6%, respectively. This interaction suggests a sex-specific response to Bti in terms of cooking loss, indicating that male chickens were more adversely affected by the lack of Bti than female chickens.

No statistical differences (p > 0.05) were observed due to the Bti and sex effects alone on pH values, water holding capacity, cooking loss, drip loss, and shear force results. The significant differences in cooking losses between males and females were explained by the interaction effect with Bti.

**Table 6.** Mean (± SE) pH, water holding capacity (WHC), cooking loss (CL), drip loss (DL) and shear force (SF) of meat at 42 days of age from male and female chickens fed diet containing *Bacillus thuringiensis* var. *israelensis* (Bti).

Item	pН	WHC (%)	CL (%)	DL (%)	SF (Kg)
Effect of Bti					
Without Bti	5.87	30.9	28.6	10.20	3.58
With Bti	5.86	26.9	27.6	9.52	2.89
Effect of sex					
Male	5.89	30.6	$29.4^{a}$	10.45	3.34
Female	5.84	27.1	$26.8^{b}$	9.27	3.13
SEM	0.02	1.77	0.78	0.96	0.37
P-value					
Bti	0.89	0.11	0.39	0.62	0.20
Sex	0.21	0.17	0.02	0.40	0.69
Bti:Sex	0.06	0.10	0.02	0.43	0.43

<sup>\*</sup>Different letters in the column indicate significant differences (p < 0.05) between the means according to the Tukey test. SEM: standard error of the mean.

Dalólio et al. (2015), supplementing Cobb chickens with *Bacillus subtilis*, *Bifidobacterium bifidum*, *Enterococcus faecium*, and *Lactobacillus acidophilus* for 42 days, did not observe any effects of supplementation on meat quality characteristics. Conversely, Bai et al. (2017) found that drip loss, cooking loss, and shear force were influenced by *Bacillus subtilis fmbj* provided in the diet. The evaluation of water holding capacity and drip loss is crucial as they can impact the juiciness, tenderness and flavour of the meat (Chen et al., 2012). Additionally, water holding capacity is linked with cooking losses, in which higher liquid retention in meat leads to lower losses during heating. Meat texture is also a significant factor determined by shear force (Alves et al., 2016). Zhou et al. (2010) reported a positive effect of feeding *Baccilus coagulans ZjU0616* in chicken diets on shear force and drip loss, which was not observed in this study. The average pH of the breast meat obtained from different treatments in this experiment falls within the range of 5.70 to 5.96, considered normal for chicken meat (Van Laack et al., 2000).

Table 7 shows the results of skin and meat colour, in which no statistical differences (p > 0.05) were observed due to both Bti and sex effects. However, a significant interaction (p < 0.05) was noted between Bti and sex for the yellow-blue component ( $b^*$ ) of the skin. Male chickens receiving Bti exhibited a lower  $b^*$  value (20.3) followed by females not receiving (22.1), while males not receiving Bti and females receiving Bti had higher  $b^*$  values of the skin (24.4 and 24.0, respectively). These results indicate that the presence of Bti significantly lowered the  $b^*$  values in male chickens, while female chickens did not show a similar reduction. Bai et al. (2017) discovered that supplementation with *Bacillus subtilis fmbJ* significantly enhanced  $L^*$ ,  $a^*$ , and  $b^*$  values, differing from the findings in this study.

**Table 7.** Mean (± SE) skin colour and meat colour at 42 days of age of male and female chickens fed diet containing *Bacillus* thuringiensis var. israelensis (Bti).

Item	L*skin	a*skin	b*skin	L*meat	a*meat	b*meat
Effect of Bti						
Without Bti	67.9	1.35	23.3	56.9	-0.001	12.4
With Bti	67.6	-0.26	22.2	56.0	-0.141	12.4
Effect of sex						
Male	68.2	1.20	22.4	56.3	-0.217	11.9
Female	67.3	-0.11	23.1	56.6	0.074	12.9
SEM	0.55	0.90	0.83	0.87	0.41	0.56
P-value						
Bti	0.73	0.23	0.39	0.45	0.81	0.98
Sex	0.29	0.32	0.53	0.82	0.62	0.20
Bti:Sex	0.26	0.64	0.01	0.75	0.39	0.23

<sup>\*</sup>SEM: standard error of the mean. L= lightness, with 0 being black and 100 being white. a= colour's position between red and green. b= colour's position between yellow and blue.

Meat colour is the first characteristic noticed during purchase, particularly for boneless products, and it is also used as an indicator of quality, often associated with freshness (Muchenje et al., 2009). Furthermore, meat colour is primarily influenced by the myoglobin pigment, the concentration of which depends on factors such as species, animal age, muscle anatomical position, diet, pre-slaughter conditions, and muscle oxidation and oxygenation state (Lima Júnior et al., 2011).

### Conclusion

The inclusion of *Bacillus thuringiensis* var. *israelensis* in the chickens' diet did not yield positive results, indicating that more studies are needed to optimize its use in poultry production. The performance differences observed between the sexes, with males outperforming females, are consistent with those reported in the poultry industry and scientific literature.

## Acknowledgments

The author Paola de Freitas Feltrin received support through a Ph.D. scholarship from the CAPES Foundation (Coordination for the Improvement of Higher Education Personnel). The author Aline Piccini Roll was supported by CAPES through a Postdoctoral scholarship (PNPD-CAPES). The authors Fabio Pereira Leivas Leite, Eduardo Gonçalves Xavier, and Victor Fernando Büttow Roll were supported by grants from the National Council for Scientific and Technological Development - CNPq (CNPq/Research Productivity).

## References

- Alves, M. G. N., Albuquerque, L. F., & Batista, A. S. M. (2016). Qualidade da carne de frangos de corte. *Essentia*, *17*(2), 64-86.
- Angelo, E. A., Vilas-Boas, G. T., & Castro-Gómez, R. J. H. (2010). *Bacillus thuringiensis*: características gerais e fermentação. *Semina: Ciências Agrárias*. *31*(4) 945-958. https://doi.org/10.5433/1679-0359.2010v31n4p945
- Api, I., Takahashi, S. E., Mendes, A. S., Paixão, S. J., Refati, R., & Restelatto, R. (2017). Efeito da sexagem e linhagens sobre o desempenho e rendimento de carcaça de frangos de corte. *Ciência Animal Brasileira*, *18*, 1-10. https://doi.org/10.1590/1089-6891v18e-32691
- Bai, K., Huang, Q., Zhang, J., He, J., Zhang, L., & Wang, T. (2017). Supplemental effects of probiotic *Bacillus subtilis* fmbJ on growth performance, antioxidant capacity, and meat quality of broiler chickens. *Poultry Science*, *96*(1), 74-82. https://doi.org/10.3382/ps/pew246
- Bertechini, A. G. (2006). Nutrição de Monogástricos. Editora UFLA.
- Chen, H., Do ng, X., Yao, Z., Xu, B., Zhen, S., Li, C., & Li, X. (2012). Effects of prechilling parameters on water-holding capacity of chilled pork and optimization of prechilling parameters using response surface methodology. *Journal of Animal Science*, *90*(8), 2836-2841. https://doi.org/10.2527/jas.2011-4239
- Coppola, M. D. M., & Gil-Turnes, C. (2004). Probióticos e resposta imune. *Ciência Rural*, *34*(4), 1297-1303. https://doi.org/10.1590/S0103-84782004000400056
- Dalólio, F. S., Moreira, J., Valadares, L. R., Nunes, P. B., Vaz, D., Pereira, H. J., Pires, A.V., & Da Cruz, P. J. (2015). Aditivos alternativos ao uso de antimicrobianos na alimentação de frangos de corte. *Revista Brasileira de Agropecuária Sustentável*, *5*(1), 86-94. https://doi.org/10.21206/rbas.v5i1.281
- Domingues, C. H. F., Santos, E. T., Castiblanco, D. M. C., Quadros, T., Petrolli, T., Duarte, K. F., & Junqueira, O. M. (2014). Avaliação do desempenho e rendimento de carcaça de frangos de corte alimentados com dietas contendo probiótico nas diferentes fases de criação. *Revista Agrocientífica*, 1(1), 7-16.
- Hong, H. A., Duc, L. H., & Cutting, S. M. (2005). The use of bacterial spore formers as probiotics. *FEMS Microbiology Reviews*, *29*(4), 813-835. https://doi.org/10.1016/j.femsre.2004.12.001
- Lee, J., Park, I., Choi, Y., & Cho, J. (2012). Bacillus strains as feed additives: In vitro evaluation of its potential probiotic properties. *Revista Colombiana de Ciências Pecuárias*, *25*(4), 577-585.
- Lima Júnior, D. M., Rangel, A. H. N., Urbano, S. A., Maciel, M. V., & Amaro, L. P. A. (2011). Alguns aspectos qualitativos da carne bovina: uma revisão. *Acta Veterinaria Brasilica*, *5*(4), 351-358.

Page 8 of 9 Feltrin et al.

Manafi, M., Hedayati, M., & Mirzaie, S. (2018). Probiotic Bacillus species and *Saccharomyces boulardii* improve performance, gut histology and immunity in broiler chickens. *South African Journal of Animal Science*, 48(2), 379-389. https://doi.org/10.4314/sajas.v48i2.19

- Marubashi, T., Gracia, M., Esteve-Garcia, E., & Piskoríková, M. (2013). The efficacy of the probiotic feed additive Calsporin<sup>®</sup> (Bacillus subtilis C-3102) in broilers: combined analysis of four different studies. *Journal of Applied Animal Nutrition*, 1.
- Meurer, R. F. P., Leal, P. C., Rocha, C. D., Bueno, I. J., Maiorka, A., & Dahlke, F. (2010). Evaluation of the use of probiotics in diets with or without growth promoters for broiler chicks. *Revista Brasileira de Zootecnia*, 39(12), 2687-2690. https://doi.org/10.1590/S1516-35982010001200019
- Mountzouris, K. C., Tsitrsikos, P., Palamidi, I., Arvaniti, A., Mohnl, M., Schatzmayr, G., & Fegeros, K. (2010). Effects of probiotic inclusion levels in broiler nutrition on growth performance, nutrient digestibility, plasma immunoglobulins, and cecal microflora composition. *Poultry Science*, *89*, 58-67. https://doi.org/10.3382/ps.2009-00308
- Muchenje, V., Dzama, K., Chimonyo, M., Strydom, P. E., Hugo, A., & Raats, J. G. (2009). Some biochemical aspects pertaining to beef eating quality and consumer health: A review. *Food Chemistry*, *112*(2), 279-289. https://doi.org/10.1016/j.foodchem.2008.05.103
- Nisbet, D. J., Ricke, S. C., Scanlan, C. M., Corrier, D. E., Hollister, A. G., & Deloach, J. R. (1994). Inoculation of broiler chicks with a continuous-flow derived bacterial culture facilitates early cecal bacterial colonization and increases resistance to Salmonella typhimurium. *Journal of Food Protection, 57*(1), 12-15. https://doi.org/10.4315/0362-028X-57.1.12
- Patterson, J. A., & Burkholder, K. M. (2003). Application of prebiotics and probiotics in poultry production. *Poultry Science*, 82(4), 627-631. https://doi.org/10.1093/ps/82.4.627
- Pourakbari, M., Seidavi, A., Asadpour, L., & Martínez, A. (2016). Probiotic level effects on growth performance, carcass traits, blood parameters, cecal microbiota, and immune response of broilers. *Anais da Academia Brasileira de Ciências*, 88(2), 1011-1021. https://doi.org/10.1590/0001-3765201620150071
- R Core Team. (2020). *R: A language and environment for statistical computing*. R Foundation for Statistical Computing. https://www.R-project.org/
- Reis, T. L., & Vieites, F. M. (2019). Antibiotic, prebiotic, probiotic and symbiotic in feeeds of broiler chickens and laying hens. *Ciência Animal*, *29*(3), 133-147.
- Ricke, S. C., & Saengkerdsub, S. (2015). Bacillus probiotics and biologicals for improving animal and human health: current applications and future prospects. *Beneficial microbes in fermented and functional foods, 1*, 341-360.
- Santos, A. L., Sakomura, N. K., Freitas, E. R., Fortes, C. M. L. S., Carrilho, E. N. V. M., & Fernandes, J. B. K. (2005). Estudo do crescimento, desempenho, rendimento de carcaça e qualidade de carne de três linhagens de frango de corte. *Revista Brasileira de Zootecnia*, *34*(5), 1589-1598. https://doi.org/10.1590/S1516-35982005000500020
- Schettino, D. N., Cançado, S. V., Baião, N. C., Lara, L. J. C., Figueiredo, T. C., & Santos, W. L. M. (2006). Efeito do período de jejum pré-abate sobre o rendimento de carcaça de frango de corte. *Arquivo Brasileiro de Medicina Veterinária e Zootecnia*, *58*(5), 918-924. https://doi.org/10.1590/S0102-09352006000500030
- Sen, S., Ingale, S. L., Kim, Y. W., Kim, J. S., Kim, K. H., Lohakare, J. D., Kim, E. K., Kim, H. S., Ryu, M. H., Kwon, K., & Chae, B. J. (2012). Effect of supplementation of *Bacillus subtilis* LS 1-2 to broiler diets on growth performance, nutrient retention, caecal microbiology and small intestinal morphology. *Research in Veterinary Science*, *93*(1), 264-268. https://doi.org/10.1016/j.rvsc.2011.05.021
- Sierra, I. (1973). Producción de cordero joven y pesado en la raza Rasa Aragonesa. *Revista del Instituto de Economía y Producciones Ganaderas del Ebro*, *18*, 28.
- Stachelek, M., Zalewska, M., Kawecka-Grochocka, E., Sakowski, T., & Bagnicka, E. (2021). Overcoming bacterial resistance to antibiotics: the urgent need. *Annals of Animal Science*, *21*(1), 63-87. https://doi.org/10.2478/aoas-2020-0098
- Stringhini, J. H, Laboissiére, M., Muramatsu, K., Leandro, N. S. M., & Café, M. B. (2003). Avaliação do desempenho e rendimento de carcaça de quatro linhagens de frangos de corte criadas em Goiás. *Revista Brasileira de Zootecnia*, *32*(1), 183-190. https://doi.org/10.1590/S1516-35982003000100023

- Van Laack, R. L., Liu, C. H., Smith, M. O., & Loveday, H. D. (2000). Characteristics of pale, soft, exudative broiler breast meat. Poultry Science, *79*(7), 1057-1061. https://doi.org/10.1093/ps/79.7.1057
- Vilas-Bôas, G. T., Peruca, A. P. S., & Arantes, O. M. N. (2007). Biology and taxonomy of Bacillus cereus, Bacillus anthracis and Bacillus thuringiensis. *Canadian Journal of Microbiology*, *53*(6), 673-687. https://doi.org/10.1139/W07-029
- Zhou, X., Wang, Y., Gu, Q., Li, W. (2010). Effect of dietary probiotic, Bacillus coagulans, on growth performance, chemical composition, and meat quality of Guangxi Yellow chicken. *Poultry Science*, *89*(3), 588-593. https://doi.org/10.3382/ps.2009-00319