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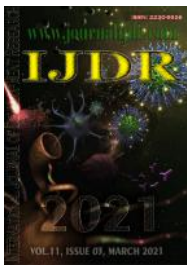

RESEARCH ARTICLE **OPEN ACCESS**

PERFORMANCE OF CONFINED LAMBS WITHWHOLE GRAIN MAIZE AND OATS DIETS SUPPLEMENTED WITH BACILLUS AMYLOLIQUEFACIENS

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ABSTRACT

The objective of this study was to investigate the effect of *Bacillus amyloliquefaciens* supplementation on the feed of confined lambs over performance, hematological, biochemical and ruminal health parameters. In order to perform the investigation, 24 females of the Texel x Ile de France breeds, aged 150-210 days, weaned, weighing 20.56 ± 5 kg, were randomly divided into two groups: Control group, which received concentrate at 3.5% of live weight; and the *Bacillus* Group, which received concentrate at 3.5% of live weight inoculated with *B. amyloliquefaciens*. The experimental dose of bacteria used was 2.0 x 10^6 cfu per kg of live weight. The results indicated that the supplementation of *B. amyloliquefaciens* promoted 13% higher Average Daily Gain compared to the control group, although there was no difference for weight and carcass yield between the groups. Supplementation with *B. amyloliquefaciens* was not harmful to the ruminal health of the animals.

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INTRODUCTION

Sheep farming is an activity spread throughout the Brazilian territory and plays an important role in the economical parameters. According to Brazilian Institute of Geography and Statistics (IBGE), sheep sales grew by about 50% in 2017 comparedto 2006, from 2.28 million heads to 3.37 million heads sold, generating a movement of approximately US$125 million. Within the activities of sheep farming are the production of milk, wool and meat(VIANA, 2008), the latter being an important share of the market(HERMUCHE et al., 2012). Currently, a more intensive system has been adopted for finishing lambs, such as confinement, aiming at the production of younger animals for slaughter. Young lambstend to present better performance, and consequently, the final product has reduced seasonality and greater uniformity (Gallo et al., 2014). According to Rogério et al. (2018), the high concentrated diet (HCD) is an alternative feed applied in order to improve the productivity of the animal's system. Some advantages are cost reduction inthe production of roughage; decrease in the area destined for planting pastures; higher consumption of dry matter (DM), increase in animal feed efficiency; anticipation of the slaughter season, better finishing and uniformity of the carcass. Although HCDs bring these benefits, their use in some cases can cause health problems such as ruminal and systemic acidosis, laminitis and ruminitis, and can lead to decreased performance of animals under confinement(PAULINO et al., 2014). However, these problems can be reduced by the use of probiotics in food, which are microbial supplements that significantly increase the digestibility and therapeutic value of food according to Fueler (1989). In addition, probioticshave already improved feed conversion, weight gain and performance of confined animals (NETO et al., 2020; APPELT et al., 2010). Within the group of bacteria with probiotic capacity, *Bacillus amyloliquefaciens* is mentioned.Le et al. (2017) observed an enhancement in performance, digestibility, dry matter consumption, daily weight gain and milk production when applying these bacteria in diets for sheep. However, only few studies have been conducted on the influence of *B. amyloliquefaciens* over confined sheep (LE et al., 2017). Thus, this study aimed to study the effects of adding *B. amyloliquefaciens* to the diet of confined lambs with a high content of concentrate (88%) in terms of performance, hematological parameters, serum biochemicals and ruminal health.

MATERIALS AND METHODS

The experiment was carried out in the Center-South region of the state of Paraná, Brazil, in the city of Guarapuava. Twenty-four female lambs from the crossing of Texel x Ile de France breeds were used, aging between 150-210 days old and weaned. The animals were confined in a shed, with 2m x 2m stalls, with suspended slatted floors. Each stall contained three troughs (for concentrate, hay and mineral salt) and a drinking fountain. Feeding was done at 8 am. For feeding the animals, the diet consisted in a concentrate formulated at 2% of the live weight (LW) of corn, 1% of the LW of soybean meal, 0.5% of the LW of black oat grains and 0.5% of the LW of barley straw. The nutritional composition of the total feed is described in

Table 1, with the supplementation of mineral salt *ad libitum* (Matsuda®). The food offered to the animals was calculated based on the requirements described by Cabral et al. (2008) for this category, with contents of crude protein (CP) in 16.7%, total digestible nutrients (TDN) in 78.9 %, neutral detergent fiber (NDF) in 24.4% and acid detergent fiber (ADF) in 12.8%.

Experimental design: The experimental design was performed in randomized blocks (RBD), with two treatments and twelve replications. The animals went through an adaptation period of 14 days in order to acclimate to the environment, food and sanitary management. After 3 days of arrival, all animals underwent coproparasitological and hematological tests. From the results of these exams, deworming of all animals was performed as they presented an Egg Per Gram count (EPG) above 800 in feces. The vermifuge used was Nitroxinil 34%, subcutaneously, at a dosage of 6.8 mg per animal weight kg. After the adaptation period, the experiment started. Two groups were separated by randomization: Control Group (CG) with 12 animals which received only concentrate and barley straw;and *Bacillus* Group (BG) with 12 animals which received concentrate inoculated with the experimental dose 2.0 x 10^6 cfu of *B. amyloliquefaciens* per kg of LW and barley straw daily. The animals were weighed every 15 days in order to readjust the diet and assess performance. The average daily weight gain of the animals (ADG) was determined after the adaptation period, starting from the first weighing. The calculation was done by subtracting the updated weight from the previous weight, then dividing the result by the number of days between weighings. The animals remained in the experiment until reaching the target weight of 40 ± 2 kg, then proceeded to slaughter in a refrigerator, under the state inspection regime (SIR) of Paraná, Brazil.

Bacillus amyloliquefaciens: The *B. amyloliquefaciens* used was in liquid culture sealed in plastic packaging. The amount of probiotic used to inoculate the feed was calculated in the proportion of 10 mL of *B. amyloliquefaciens* for each 1 kg of corn, providing an experimental dose of 2.0 x10^6 cfu per kg of LW. The concentrate was weighed once a week, and the probiotic was inoculated with the aid of sterile plastic syringes in a plastic package with the amount of corn for each bay in the BG, stirred vigorously in order to be evenly incorporated. Then the corn was incorporated with the other ingredients of the concentrate, mixed again for a new homogenization. The plastic bags were then identified with the stall number and stored in a room next to the shed. Every morning the feed packs were provided to the animals.

Ruminal Health Assessment: Immediately after slaughter, the rumen dorsal sac was pierced using a sterile scalpel, and approximately 100 ml of ruminal fluid was collected to determine: pH, color, odor, consistency and time of bacterial activity. The pH of the ruminal fluid was measured with a glass electrode using a Hanna® bench pH meter. After each measurement, the electrode was cleaned with distilled water, in order to not occur interferences with the results. For the determination of color (corn yellow, straw yellow, dark yellow, pale yellow and brown), odor (aromatic, slightly disgusting, disgusting and acidic) and consistency (liquid, slightly viscous, consistent and firm) a sub-sample of ruminal liquid was obtained and all samples were analyzed by the same person through the sense organs (DIAZ GONZÁLEZ et al., 2000).The bacterial activity time was calculated through the methylene blue reduction test. Approximately 10 ml of rumen liquid were filtered through a double layer of gauze and inserted in sterilized pots properly identified, 0.5 ml of methylene blue at 0.02% was added to the liquid and the time took for the methylene blue solutionto disappear completelywas measured (ROSENBERGER, 1993). To determine the amount of ciliated protozoa, the methodology described by Dehority, Damron and McLaren (1983) was used in duplicate.

Blood samples for blood count and serum biochemical analysis: During the morning (7:30 am to 8:30 am), about 5 mL of blood was collected from each animal by puncture of the cephalic vein. The amount obtained was divided into two tubes, one with EDTA to perform the blood count and the other without anticoagulant for biochemical analysis. The blood count was performed by the Hematological Analyzer SDH-3 VET®. Tubes without anticoagulant were centrifuged (Spinlab®) at 2.53 RPM for 10 minutes to obtain the serum, which was divided into two aliquots, packed in polypropylene microtubes and kept at -20 ° C. The biochemical tests performed were Total Protein, Urea, Aspartate Aminotransferase, Albumin, Creatine, Glucose, Total Cholesterol and Triglycerides with Labtest® kits, which were analyzed in a semi-automatic spectrophotometer (BioPlus 200®). Blood analyzes were carried out at the beginning of the confinement and when the animals left for slaughter.

Carcass yield: The warm carcass weight was used to calculate the carcass yield, divided by the weight of the animal when leaving the property and multiplied by 100 to obtain the percentage value.

Statistical analysis

The data were submitted to analysis of variance with a significance level of 5%, by the F-Test. The analyzes were performed using the SISVAR® statistical program.

RESULTS

Animal performance: Slaughter was divided into three batches, the first batch of 7 animals, 39 days of confinement, the second batch of 9 animals, 59 days of confinement and the third batch of 8 animals, 67 days of confinement. Performance data are shown in Table 2. It is possible to notice that the BG group had an average ADG 13% higher than the CG. The animal weight averages of the BG remained superior to the CG during the evaluated period. The BG animals consumed about 1.5% less dry matter and reached their target weight 4 days before the animals in the CG.

Table 1. Nutritional composition of the total feed provided for Texel x Ile de France confined lambs supplemented withBacillus amyloliquefaciens. Guarapuava, Paraná, Brazil 2020

Parameter	%
Dry Matter (DM), % Natural Matter	88.47
CP % DM	16.7
TDN % DM	78.9
NDF % DM	24.4
ADF % DM	12.8
Mineral Matter % DM	1.8

Table 2. Average values for the parameters ADG (kg), Weight (kg) and Calculation of Difference of the BG and CG groups average weights of Texel x Ile de France confined lambssupplemented with *Bacillus amyloliquefaciens*. Guarapuava, Paraná, Brazil 2020

Groups	Initial Weight	Final Weight	ADG
CG	20.3	38.3	0.372
BG	21.2	40.0	0.422
VC%	10.21	5.56	14.69
Difference*	0.9	1.7	0.050

*Difference between the average weight (kg) of BG and CG.

Serum hematological and biochemical analyzes: For hematological analyzes, there was no difference (p> 0.05) between groups for the variables: Hematocrit (HT), Red Blood Cells (RBC), Leukocytes (LEUK) and Platelets (PLAT). There was a statistical difference (p <0.05) only for Hemoglobin (HB) referring to the period of the animals exiting the confinement, where the BG group presented higher values than the CG group, but both groups are within the reference values. As shown in Table 3, both groups showed mean serum values for hematological parameters within the reference for the species (PUGH & BAIRD, 2012). For serum metabolic analyzes (Table 5) there was no statistical difference (p> 0.05) between groups for the parameters: Glucose, Albumin, Total Proteins (TP), Globulin

Table 3. Average hematological values between the BG and CG groups of Texel x Ile de France confined lambs supplemented with *Bacillus amyloliquefaciens*. Guarapuava, Paraná, Brazil 2020.

Group	Period	HT	HB	RBC	LEUK	PLAT
		%	g/dL	10^6/ µL	10^3/µL	10^3
CG	Entry	28.5	11.1	9.8	7574.0	699
	Exit	32.3	13.5b	11.1	5502.0	545
BG	Entry	29.794	11.763	10.479	6188.0	655
	Exit	34.989	14.475a	12.287	5113.0	508
VC % Entry		14.91	15.31	14.17	20.95	25.47
VC% Exit		7.91	5.45	11.61	30.52	56.95
RV*		27-45	9-15	9-15	4000-2000	205-705

Average values with different letters in the column differ from each other in the 5% F-Test; CG: Control Group; GB: *Bacillus* Group; *RV=Reference values according to Pugh and Baird (2012).

Table 4. Average serum metabolic values of biochemical analyzes, triglycerides (TRI), and Aspartate Aminotransferase (AST) performed inTexel x Ile de France confined lambs supplemented with *Bacillus amyloliquefaciens*. Guarapuava, Paraná, Brazil 2020

Group	Period	TRI	Glucose	Albumin	TP	Globulin	CHOL	UREA	AST
		mg/dL	mg/dL	mg/dL	g/dL	mg/dL	mg/dL	mg/dL	U/L
CG	Entry	38.25a	52.625	2.155	6.288	4.125	37.125	37.250	97.75
	Exit	15.857	55.429	2.546	7.014	4.469	42.714	53.143	120.00
BC	Entry	28.00b	54.250	2.165	6.100	3.935	38.625	36.000	103.63
	Exit	16.25	57.750	2.631	7.163	4.531	35.125	54.125	146.50
VC% Entry		25.69	12.81	16.76	13.36	19.77	26.87	58.74	22.35
VC% Exit		53.38	18.95	13.94	8.19	17.60	20.31	23.73	44.15
RV*		17.6-24	50-80	2.4-3.0	6.0-7.5	3.5-5.7	52-76	17-43	60-280

Average values with different letters in the column differ from each other in the 5% F-Test; CG: Control Group; GB: *Bacillus* Group; *RV=Reference values according to Kaneko 2008.

(GLOB), Cholesterol (CHOL), Urea and Aspartate Aminotransferase (AST). Except for Triglycerides (TRI) referring to the period of the animals entering the confinement ($p < 0.05$), wherein the animals in the CG group showed higher values than the BG group. For the animals exiting period, there was no statistical difference for TRI between the groups ($p > 0.05$). The levels of glucose, albumin, TP, blood cells and AST remained within the reference values for the species (KANEKO, 2008) during the experiment period, except for CHOL and urea.The animals of both groups showed values below the reference values for cholesterolduring the periods of entry and exit, but they did not differ from each other ($p < 0.05$).The animals of both groups presented higher values than the reference values for urea in the exit period, but they did not differ from each other ($p < 0.05$).

Ruminal health: There were no statistical differences ($p > 0.05$) between groups for the parameters: pH, Color, Odor, Consistency, Bacterial Activity Time and Number of Ciliated Protozoa.

Carcass yield: Carcass weight and yield showed no statistically significant difference ($p > 0.05$) between groups.

DISCUSSION

The results found in this workdemonstrate that the dose 2.0 x 10^6 cfu of *B. amyloliquefaciens* per kg of LW used had no effect on weight gain. However, animals supplemented with *B. amyloliquefaciens* showed13% higher ADGcompared to the animals of the CG, which made it possible to reach the slaughter weight 4 days before, in addition to BG consuming 1.5% less dry matter. This is a beneficial point, as it reduces spending on food, which represents 70% of the costs of confined animals (PACHECO et al., 2006). Besides, fewer days of confinement directly reflects in less emission of greenhouse gases, as the amount of methane eliminated per kg of meat produced is smaller (BERCHIELLI et al., 2012). The dose 2.0 x 10^6 cfu of *B. amyloliquefaciens* per kg of LW used is a low dose when compared to that used by Le et al. (2017), who tested the dose 2.85 x 10^9cfu of *B. amyloliquefaciens* per kg of LW in sheep.Le et al. noticed a higher consumption of dry matter and greater weight gain in the final third of gestation, in addition to heavier lambs during the first 21 days of life, which, according to the authors, could be related to better milk production by supplemented sheep. Du et al. (2018) used the dose 4 x 10^{10}cfu of *B. amyloliquefaciens* per kg of LW in calves with retarded growing and observed enhancement in weight gain, feed intake and feed conversion rate, also improving intestinal and ruminal development when compared to the group that did not receive supplementation. There is no data in the literature on doses of *B. amyloliquefaciens* for supplementation of lambs in confinement, therefore the dose used in this work was defined aiming a better cost benefit relationfor the producer. Research conducted with small ruminants has shown controversial results, as the mechanism of action of probiotics in animal performance and health has not been fully clarified (ABDEL- TAWAB et al., 2016). As for hematological parameters, Çetin et al. (2005) stated that probiotics have the ability to increase levels of HB, HT and RBC, preventing cases of anemia. The increase in HB concentration observed in the present study by the BG group may be related to the increase in intestinal iron absorption (TRINDAD, 1996). The mechanisms are not yet fully understood, however, some probiotics cause the intestinal pH to decrease, enhancing mineral solubilization and increasing the absorption of solubilized iron, which cross the cell membrane more easily according to Ybarra et al. (2001). Data on the impact of probiotics on serum metabolic parameters in small ruminants is still controversial (EL-KATCHA et al., 2016). Abdel-Sala et al. (2014) observed that lambs supplemented with a high dose of 4.0 x 10^9 cfu kg^{-1}using*Streptococcus thermophilus* and *Lactobacillus acidophilus* had a serum increase in total protein, globulin and albumin, while Arab et al. (2014) found a tendency to lower values in the dosage of total protein, albumin, cholesterol, triglycerides and glucose. In this study, no significant differences were found between the groups for the evaluated parameters, results similar to those observed by Soriani et al., (2013). There were also no significant differences regarding the assessment of ruminal health, which can be inferred that both probiotic and HCD did not cause deleterious ruminal changes. Le et al. (2016) found influence of supplementation with *B. amyloliquefaciens* at a dose of 2.85 x 10^9 cfu kg^{-1} on the ruminal pH of sheep, which remained more stable and close to neutrality. According to Schofield et al. (2016),*B. amyloliquefaciens* can exert influence on genes that impact rumen fermentation, including antimicrobials and genes involved with carbohydrate metabolism. However, according to the authors, the mechanisms of action are not fully understood and further studies are needed. In HCDs with high energy inclusion, such as the one used in the present study, a decrease in ruminal pH can occur, depressing the growth of bacteria and ciliated protozoa. According to Arcury and Mattos (1992), this decrease impairs the digestibility of the diet and decreases animal performance.

Therefore, the supplementation of probiotics can help to improve the rumen environment, reducing the deleterious effects of HCDs. Data found by Schofield et al. (2018) showed that *B. amyloliquefaciens* supplementation in sheep at a dose of 2.85 x 10^9 cfu kg^{-1} had the ability to modulate the rumen bacterial community. This modulation led to a greater stabilization of enzymes that degrade fiber in the rumen, allowing enhanced degradation of the diet and weight gain when compared to the control group. In this work, the use of whole corn and oat grain in the diet may also have helped to improve ruminal fermentative stability. According to Britton and Stock (1987), the passage rate and the release of starch from whole corn is slower when compared to ground corn, which results in lower peaks of fatty acid production and helps to avoid metabolic disorders. This may explain thefound of ruminal content close to the neutrality. Regarding carcass weight and yield, Gomes et al. (2009) found no difference in confined cattle receiving supplementation with *Saccharomyces cerevisiae* (5 x 10^7 cfu kg^{-1}). This data corroborate with the present study and with Neumann et al. (2016), which according to the authors may be related to the dose and the period of use. In general, one of the hypotheses for the results without statistically significant differences in this study may be related to the low dose used, which makes probiotic colonization and action difficult. The lack of consensus regarding the dosage and form of supply also impair more concrete information. Further studies are needed to observe the use of *B. amyloliquefaciens* in higher dietary concentrations in the finishing phase of lambs receiving HCD, since the data in the literature are still controversial.

Conclusion

Supplementation with *B. amyloliquefaciens* promoted average values of ADG 13% higher than the CG. Supplemented animals showed better hemoglobin values ($p < 0.05$), although there were no differences in serum biochemical values between groups. Supplementation with *B. amyloliquefaciens* was not detrimental to the ruminal health of the animals and there were no significant differences in weight and carcass yield between groups.

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