

Mycotoxin concentration in salt-treated wet brewers' grains and effects of their substitution for soybean meal and corn silage

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ABSTRACT - This study investigated the occurrence of mycotoxins in wet brewers' grains (WBG) treated with salt (sodium chloride) and the intake, nutrient digestibility, and nitrogen use efficiency in lamb diets. Two experiments were conducted: first, WBG was distributed in plastic boxes and treated with no additive (control), sodium formate (3 g/kg of WBG), or three levels of salt—25, 30, and 35 g/kg of WBG. The WBG were stored at room temperature (17.7 ± 4.6 °C) for 27 days. In the second experiment, eight six-month-old male lambs were allotted to one of four total mixed ration (TMR) diets in a 4 × 4 double Latin square: 0, 10, 20, and 30% of WBG replacing corn silage and soybean meal. In the first experiment, the concentration of mycotoxins throughout the experiment was lower than the tolerance levels accepted by the European Community and Food and Drug Administration for animal feedstuffs. However, the control and sodium formate groups showed visual fungal development on the WBG surface from the sixth day and presented an unpleasant smell from day 12. In the salt treatments, fungal growth was observed on top of the WBG from day 12 and an unpleasant smell from day 15. Salt-treated WBG showed a lower pH than the control and sodium formate groups and decreased dry matter deterioration. In the second experiment, including up to 30% WBG in the TMR did not affect dry matter, organic matter, neutral detergent fiber, and acid detergent fiber intake or digestibility in lambs. However, the N digestibility and N use efficiency increased with WBG inclusion. These results suggest that salt can be used to increase the storage time of WBG up to 15 days and the inclusion of up to 30% WBG in TMR for lambs can improve N use efficiency without negatively affecting nutrient intake and digestibility.

Keywords: agroindustrial waste, digestibility, intake, mycotoxin

1. Introduction

The high costs of energy and protein ingredients present a challenge in the profitability of livestock systems. Wet brewers' grains (WBG), a mix of malted and mashed grains that are remnants of the beer brewing process, are typically available year-round and are often a low-cost material for farmers located near breweries. With an energy value of 85–90% of corn grain and a high rumen-undegradable protein content (NASEM, 2016), this byproduct is a potential cost- and nutrient-effective feed replacement for soybean and corn in ruminant diets. However, because of its high moisture content (70–85%), WBG are susceptible to rapid aerobic microbial deterioration (Mussatto et al., 2006), limiting their on-farm feedout period. Spoilage of WBG due to microbial deterioration has been reported to occur within two days in warm and humid environmental

conditions, causing large dry matter (DM) losses and reduced nutrient digestibility (Wang et al., 2014a). Recent studies have indicated that adding common salt to fresh WBG can increase its shelf life and reduce nutrient losses via inhibiting mold growth (Hatungimana and Erickson, 2019; Hatungimana et al., 2021).

Mycotoxin contamination is a major concern associated with using brewery byproducts in animal nutrition. Mycotoxins are secondary metabolites produced by mycotoxigenic fungi that are harmful to animals and humans (Bennett and Klich, 2003). Mycotoxins that accumulate in animal tissues, such as aflatoxins and ochratoxin A, receive special attention owing to cross contamination when ingested by humans (Mastanjević et al., 2019). In ruminants, intake of feedstuffs contaminated with mycotoxins has been associated with severe organ pathologies such as production losses, infertility, cancer, and animal death (Fink-Gremmels, 2008; Gallo et al., 2015; Hartinger et al., 2022). Aflatoxins, fumonisins, trichothecenes, ochratoxin A, and zearalenone are the most significant mycotoxins found in beer chains (Mélotte, 2004). Although previous studies have analyzed the counts of molds and yeast in salt-treated WBG (Hatungimana and Erickson, 2019), these analyses have not identified the mycotoxin occurrence or concentration in the feedstuffs.

Wet brewers' grains have generally been included in up to 30% of the ration DM as a substitute, primarily for soybean meal (Murdock et al., 1981; Miyazawa et al., 2007; Imaizumi et al., 2015) or corn silage in ruminant diets (Firkins et al., 2002; Stefanello et al., 2019) without negatively affecting intake and digestibility. Owing to its high energy and protein content, WBG have the potential to replace soybean meal and corn silage; however, the effect concomitantly substituting corn silage and soybean meal with WBG on the intake and nutrient digestibility in ruminant diets is unknown.

This study was designed to investigate the effect of storing WBG with common salt on the nutritional composition and concentration of mycotoxins and the effect of feeding diets with increasing WBG levels in place of soybean meal and corn silage on intake and *in vivo* nutrient digestibility in sheep. We hypothesized that adding common salt would decrease the WBG DM deterioration and reduce mycotoxin concentration and that WBG could replace up to 30% of soybean meal and corn silage without negatively affecting intake and apparent total tract nutrient digestibility in sheep.

2. Material and Methods

The experiments were conducted in Lages, SC, Brazil (50.18° W, 27.47° S; 920 m above sea level). All procedures were approved by the Ethics Committee on Animal Use (CEUA; case number CEUA 9148230921).

2.1. Trial 1: Effect of salt storage on the chemical composition and mycotoxin concentration in WBG

Wet brewers' grains were obtained from a local brewery, and fresh samples were collected to determine its initial chemical composition. Fresh WBG was transferred to 50-L plastic boxes without lids (3,000 cm² open surface), not compacted, and distributed into five treatments with three replications in a completely randomized design. The treatments were as follows: WBG without additive (control), sodium formate (commercial preservative, SF, 98% purity, 3 g/kg of fresh WBG), and salt (sodium chloride) in proportions of 2.5, 3.0, and 3.5% of fresh WBG (S2.5, S3.0, and S3.5, respectively). Each box was filled with twenty kilograms of fresh WBG, mixed with the respective treatments and stored at room temperature for 30 days (17.7±4.6 °C) in a covered warehouse. A sample of approximately 400 g was collected from each experimental unit every three days. The content of each experimental unit was thoroughly mixed during 1 min, then the samples were taken from the top, intermediate, and bottom layer of the boxes. The pH of the samples was measured with a digital pH meter immediately after sampling, after which the samples were divided into two subsamples (for chemical composition analyses and mycotoxin quantification) and frozen at -20 °C

until further use. The temperature of WBG stored in the boxes was measured every three days using a portable digital skewer thermometer.

All samples were dried at 55 °C for 72 h, ground through a 1-mm screen, and stored in plastic bags. For mycotoxin analysis, three composite samples were assembled per treatment: one from the initial period of the experiment (days 0, 3, and 6), one from the intermediate period (days 9, 12, and 15), and one from the final period (days 18, 21, and 27) to obtain the mycotoxin concentration in the WBG throughout the experimental period.

2.2. Trial 2: Increasing WBG levels in sheep diets—intake and digestibility

The WBG used in this experiment belonged to the same batch of that used in the previous experiment. To preserve the material from deterioration, WBG were placed in plastic bags and kept frozen at -20 °C. Prior to use in diets, bags containing WBG were removed from the freezer and thawed at room temperature. Total mixed ration (TMR) diets were randomly assigned to eight crossbreed Poll Dorset × Milchschaaf castrated male lambs (58.5±5.2 kg body weight) and placed in metabolic cages with free access to water. The treatments were arranged in a replicate 4 × 4 Latin square design with four 14-day experimental periods, consisting of nine days for diet adaptation and five days for data and sample collection. The treatments consisted of replacing corn silage and soybean meal with WBG at four different levels (DM basis): control diet with no WBG inclusion (WBG₀), 10% WBG (WBG₁₀), 20% WBG (WBG₂₀), and 30% WBG (WBG₃₀). Diets were formulated to meet the nutritional requirements of sheep using the Small Ruminant Nutrition System computational package. In the WBG₃₀ treatment, the WBG completely replaced soybean meal and corn silage in the diet. All dietary ingredients and chemical compositions are listed in Table 1.

Diets were provided twice a day (08:00 and 17:00 h) as a TMR. The quantity provided was adjusted daily based on the intake observed on the previous day, allowing a surplus of 10%. Diet and refusal samples were collected daily during the last five days of each experimental period, including a

Table 1 - Ingredients and nutrient composition of treatment diets

Ingredient	Treatment (g/kg dry matter) ¹			
	WBG ₀	WBG ₁₀	WBG ₂₀	WBG ₃₀
Tyfton 85 hay (<i>Cynodon</i> spp)	301	301	301	300
Corn silage	301	201	100	0.0
Wet brewers' grains (WBG)	0.0	101	201	300
Corn ground (grain)	203	261	299	333
Soybean meal	122	62.1	28.4	0.0
Molasses	4.9	4.9	5.0	4.9
Megalac®	9.1	8.5	8.9	8.5
Sodium bicarbonate	9.8	9.9	9.2	9.9
Mineral mixture	30	30	31	31
Urea	7.7	7.8	4.3	0.0
Calcitic limestone	4.9	4.9	5.0	4.9
Dicalcium phosphate	7.0	7.1	7.1	7.1
Chemical composition				
Dry matter (g/kg fresh matter)	510	476	444	387
Organic matter	933	931	933	934
Crude protein	123	139	150	151
Neutral detergent fiber	439	456	494	510
Acid detergent fiber	240	228	217	222
Metabolizable energy (Mcal/kg)	2.36	2.38	2.40	2.38

¹ WBG₀ - without inclusion of WBG; WBG₁₀ - 10% inclusion of WBG; WBG₂₀ - 20% inclusion of WBG; WBG₃₀ - 30% inclusion of WBG.

subsample of 10% of the total daily feces produced by each animal, which was dried in a 60 °C forced-air oven for 72 h and ground through a 1-mm screen using a Wiley mill. Diet samples were divided by treatment and period, and samples of refusals and feces by animal and period, and were stored until further analysis. The total urine volume of each animal was measured and collected daily in containers containing 100 mL of a 3.6 M sulfuric acid solution (urine pH < 2) during the last five days of each experimental period. After measuring the volume, a 10% aliquot of the total urine was collected and transferred to 200-mL flasks; the volume was completed with distilled water and then frozen at -20 °C for further analysis.

2.3. Laboratory analysis

Dry matter content was determined via drying samples at 105 °C for 24 h. Ashes were measured via combustion in a muffle furnace at 550 °C for 4 h, and the organic matter (OM) was quantified based on the mass difference. The total nitrogen (N) content was measured via the Dumas combustion method 968.06 (AOAC, 2019) using Leco FP 528 equipment (LC, Leco Corporation, MI, USA). Neutral detergent fiber (NDF) was determined according to a previously reported method (Mertens, 2002), except that the samples were weighed in filter bags and treated with neutral detergent using an ANKOM A220 system (ANKOM Technology, NY, USA). This analysis included a heat-stable α -amylase and residual ash but did not include sodium sulfite. Acid detergent fiber (ADF) was analyzed according to method 973.18 of the AOAC (AOAC, 2019). Mycotoxin occurrence was determined using a liquid chromatography–tandem mass spectrometry (LC-MS/MS)-based multi-mycotoxin method. The samples were analyzed for aflatoxins (B1, B2, G1, and G2), deoxynivalenol (DON), fumonisins (B1 and B2), nivalenol, ochratoxin A, and zearalenone.

2.4. Statistical analysis

Data were analyzed using the MIXED procedure in SAS (Statistical Analysis System, v.9.2). For the first experiment, data on mycotoxin concentrations were analyzed descriptively, whereas the chemical composition and pH were analyzed according to the following model:

$$Y_{ijk} = \mu + P_i + T_j + T \times P_k + e_{ijk}$$

in which Y_{ijk} = variable of interest, μ = overall mean, P_i = random effect of period (i = number of days after storage), T_j = fixed effect of treatment, $T \times P_k$ = interaction treatment $\times$ period, and e_{ijk} = residual error. For the second experiment, data were analyzed using a model that included the random effects of animals and fixed effects of treatment and period. Significance was determined at $P \leq 0.05$ and tendency at $P \leq 0.10$.

3. Results

3.1. Trial 1: Effect of salt storage on the nutritional composition and mycotoxin concentration in WBG

No interaction of treatment $\times$ number of days after storage was observed for any of the measured nutritional composition parameters ($P > 0.05$; Figure 1). Compared with control and SF, adding salt increased the DM and ash contents and decreased the NDF, ADF, and crude protein (CP) contents of WBG during the 27-day storage period ($P < 0.001$). An interaction of treatment $\times$ number of days after storage was observed for the WBG pH during the experimental period ($P < 0.001$). In treatments with salt addition, the pH of the stocked material decreased over time compared with that in control and SF (Figure 2). No interaction of treatment $\times$ number of days after storage ($P = 0.42$) or treatment ($P = 0.75$) effect was observed on WBG temperature during the experiment; however, time had a significant effect on this variable, decreasing approximately 15 °C after three days of storage ($P < 0.001$).

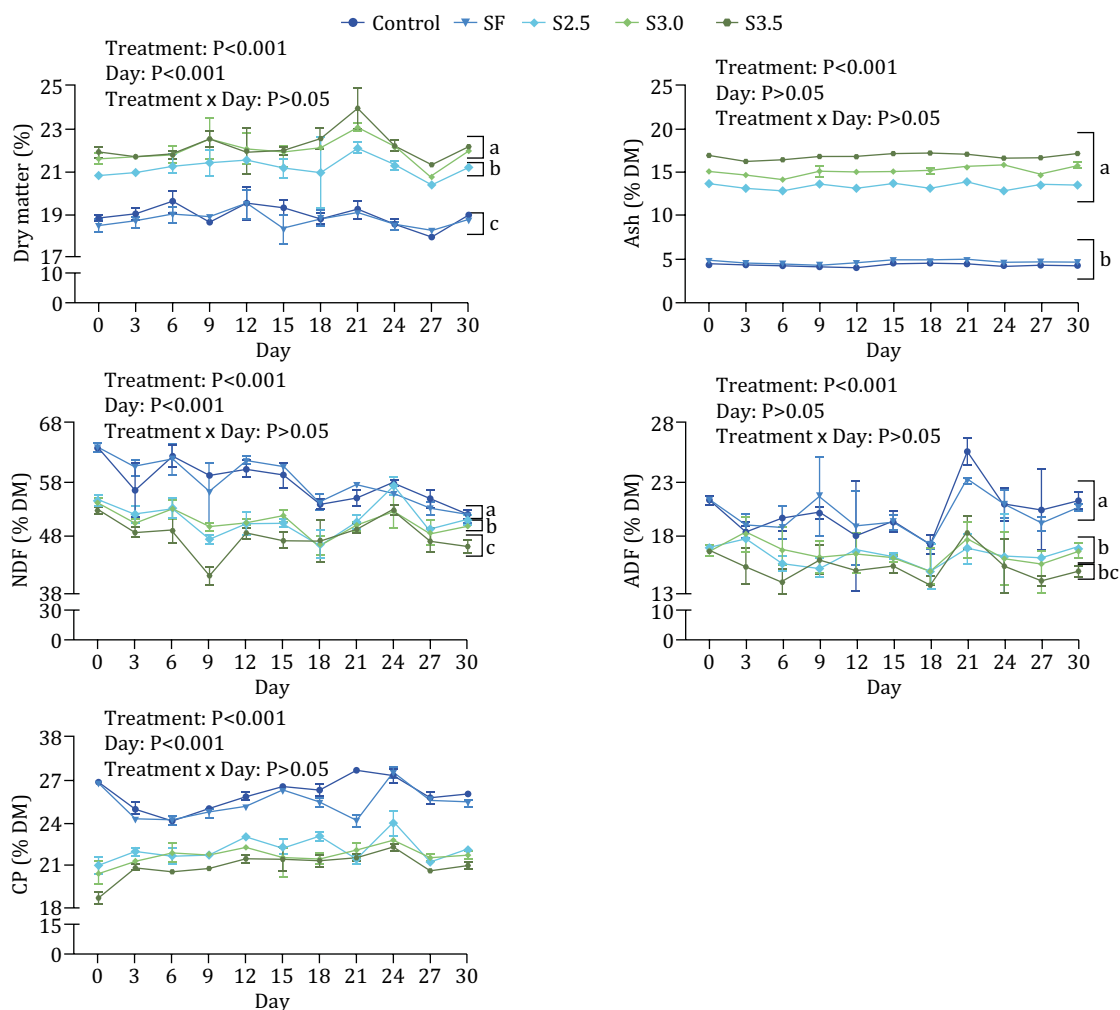


Figure 1 - Effect of treatment with no additives (control), sodium formate (SF), 2.5% salt (S2.5), 3% salt (S3.0), or 3.5% salt (S3.5) (fresh matter basis) on the chemical composition of wet brewers' grains stored for 27 days.

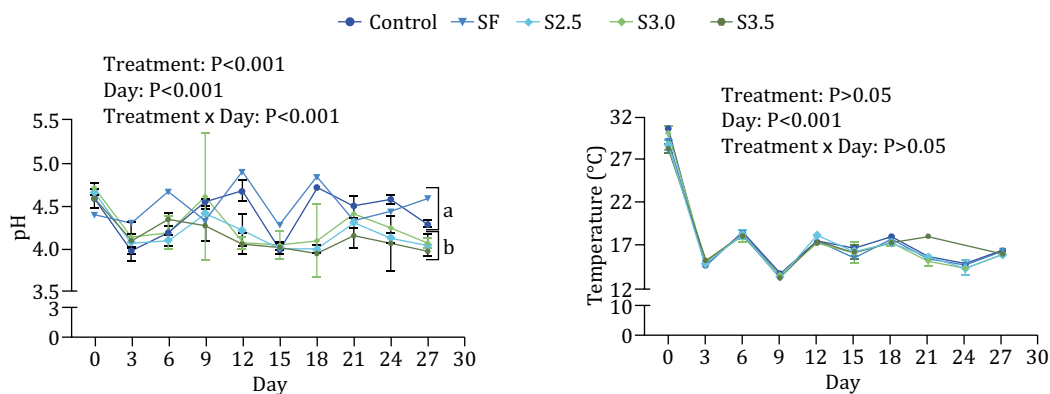


Figure 2 - Effect of treatment with no additives (control), sodium formate (SF), 2.5% salt (S2.5), 3% salt (S3.0), or 3.5% salt (S3.5) (fresh matter basis) on the pH and temperature of wet brewers' grains stored for 27 days.

All WBG samples showed individual mycotoxin levels below the quantification limits of the LC-MS/MS-based method throughout the 27-day storage period. Thus, no statistical analysis was possible for the effect of treatment and storage period on individual mycotoxin concentrations. Mycotoxin data were averaged by treatment and compared with the maximum tolerance levels for individual mycotoxins accepted by the European Union (EC, 2002) and Food and Drug Administration (FDA, 2000) for animal feedstuffs (Table 2).

Table 2 - Mycotoxin concentrations in wet brewers' grains (WBG) and the European Union (EU) and Food and Drug Administration (FDA) maximum tolerance levels for mycotoxins in animal feedstuffs¹

Mycotoxin	Method quantification limit (µg/kg)	Concentration of mycotoxins (µg/kg) ²					Maximum tolerance levels (µg/kg)	
		Control	SF	S _{2.5}	S _{3.0}	S _{3.5}	EU	FDA
Aflatoxins (B1, B2, G1, G2)	1	< 1	< 1	< 1	< 1	< 1	5 to 20	20
Deoxynivalenol	200	< 200	< 200	< 200	< 200	< 200	8,000	5,000 – 10,000
Fumonisin (B1, B2)	125	< 125	< 125	< 125	< 125	< 125	50,000	20,000 – 100,000
Nivalenol	100	< 100	< 100	< 100	< 100	< 100	NR	NR
Ochratoxin A	2	< 2	< 2	< 2	< 2	< 2	50 – 250	NR
Zearalenone	20	< 20	< 20	< 20	< 20	< 20	250 – 2,000	NR

NR - not regulated.

¹ All WBG samples showed individual mycotoxin levels below the quantification limits of the liquid chromatography–tandem mass spectrometry (LC-MS/MS)-based method along the 27-day experimental period. Thus, no statistical comparison was possible between treatments and periods (days of storage). Values were averaged by treatment and compared to the threshold levels permitted by the EU and FDA for individual mycotoxins.

² Control - no additives; SF - sodium formate (3 g/kg fresh WBG); S_{2.5} - 2.5% common salt; S_{3.0} - 3% common salt; S_{3.5} - 3.5% common salt.

3.2. Trial 2: Progressive inclusion of WBG in sheep diets—intake and digestibility

Compared with the control, increasing WBG levels showed no effects on the replacement of corn silage and soybean meal in the diet on DM, OM, NDF, and ADF intake or digestibility (Table 3). The DM, OM, NDF, and ADF intake values averaged at 1.57, 1.44, 0.708, and 0.342 kg/day, respectively, whereas the DM, OM, NDF, and ADF digestibility values were 0.643, 0.670, 0.563, and 0.575, respectively. The average digestible OM intake was 0.96 kg/day.

Nitrogen intake and N retention tended to increase ($P = 0.06$), whereas urinary N excretion did not differ between the treated WBG compared with WBG₀ ($P = 0.11$; Table 4). Nitrogen fecal excretion was the lowest in WBG₃₀ compared with other treatments ($P < 0.01$); N apparent digestibility was greater in WBG₂₀ and WBG₃₀ than in WBG₀ and WBG₁₀ ($P = 0.02$). Nitrogen use efficiency increased in all treatments with WBG compared with that in the control ($P < 0.05$).

Table 3 - Intake and apparent digestibility of non-nitrogenous compounds with the progressive inclusion of wet brewers' grains (WBG) as a substitute for corn silage and soybean meal in lamb diets

Parameter	Treatment ¹				SE	P-value
	WBG ₀	WBG ₁₀	WBG ₂₀	WBG ₃₀		
Intake (kg/day)						
Dry matter	1.47	1.53	1.64	1.53	0.276	0.74
Organic matter (OM)	1.37	1.42	1.53	1.43	0.258	0.74
Neutral detergent fiber	0.600	0.689	0.802	0.741	0.152	0.20
Acid detergent fiber	0.343	0.346	0.365	0.314	0.059	0.54
Digestible OM	0.97	0.95	1.02	0.90	0.221	0.83
Digestibility						
Dry matter	0.68	0.64	0.64	0.61	0.059	0.27
Organic matter	0.71	0.67	0.67	0.63	0.056	0.26
Neutral detergent fiber	0.57	0.56	0.59	0.53	0.124	0.79
Acid detergent fiber	0.66	0.57	0.52	0.55	0.094	0.16

SE - standard error.

¹ WBG₀ - without inclusion of WBG; WBG₁₀ - 10% inclusion of WBG; WBG₂₀ - 20% inclusion of WBG; WBG₃₀ - 30% inclusion of WBG.

Table 4 - Intake, digestibility, and N use efficiency (NUE) with the progressive inclusion of wet brewers' grains (WBG) as a substitute for corn silage and soybean meal in lamb diets

Parameter	Treatment ¹				SE	P-value
	WBG ₀	WBG ₁₀	WBG ₂₀	WBG ₃₀		
N intake (g/day)	31.3	37.0	42.4	37.3	4.80	0.06
N excretion (g/day)						
Urinary	15.7	7.1	8.8	11.7	3.76	0.11
Fecal	9.8b	11.9a	11.0ab	8.3c	0.99	<0.01
N apparent digestibility	0.68b	0.67b	0.74a	0.78a	0.05	0.02
N retention (g/day)	5.8	18.0	22.6	17.2	7.09	0.06
NUE (g/g intake)	0.17b	0.48a	0.55a	0.47a	0.14	0.03

SE - standard error.

¹ WBG₀ - without inclusion of WBG; WBG₁₀ - 10% inclusion of WBG; WBG₂₀ - 20% inclusion of WBG; WBG₃₀ - 30% inclusion of WBG.

Means followed by the same letter do not differ by Tukey's test (P<0.05).

4. Discussion

4.1. Effect of salt on WBG preservation

The low mycotoxin levels in WBG, even with visible fungal growth, may be explained partially by the microorganisms colonizing WBG, which were probably non-mycotoxin-producing molds and yeasts. The presence of visible fungal biomass in feedstuff does not necessarily indicate mycotoxin contamination. This may be attributed to secondary metabolism in fungal species being usually activated by environmental factors (Harmon and Phipps, 2022). Fungi produce mycotoxins under stress conditions, primarily temperature stress, or to combat external agents, such as fungicides. Magan et al. (2003) reported that the ideal conditions for deoxynivalenol production occurred at an average temperature of 25 °C and that it was significantly greater at this temperature than at 15 °C, which may have contributed to the low mycotoxin levels in this study, as the average temperature of all treatments during the experimental period was 15.9 °C. Noteworthy, the higher initial temperature measured on the WBG on day 0 might have been influenced by its temperature at arrival, because WBG were delivered from the brewing industry at high temperature. Non-limiting levels of fumonisins in feedstuffs with visible fungal development have been previously reported (Cavaglieri et al., 2009; Gonzalez Pereyra et al., 2011). Regardless of the low levels of mycotoxins observed throughout the storage period, the control and SF showed visual fungal development on the WBG surface from day 6 of the experiment and, from day 12, they presented an unpleasant smell. For the salt treatments, fungal growth on top of the WBG was observed from day 12 onwards and an unpleasant smell from day 15. Thus, it is reasonable to assume that the unpleasant smell of the WBG may significantly affect palatability and limit its inclusion in animal diets after these storage period. This study did not evaluate neither the occurrence of biogenic amines nor the effectiveness of salt in preventing its formation. Biogenic amines are a group of nitrogen compounds formed mainly by decarboxylation of amino acids during microbiological degradation of foods in fermentation processes (Santos, 1996). These compounds can be toxic to animals and humans if ingested at high levels. Previous studies reported common salt to be effective in controlling the formation of biogenic amines through the inhibition of bacterial enzymatic activity and prevention of bacterial growth (Wang et al., 2014b).

The increase in DM and ash contents with salt inclusion was expected because common salt has high DM and mineral content. Similarly, the highest concentrations of CP, NDF, and ADF in the treatments without salt can be explained by OM dilution with salt. The increase in DM content with salt addition may also be explained by the lower pH levels in the salt-treated samples compared with control and SF, because pH differences may lead to different activities of microorganisms responsible for DM deterioration (Lowes et al., 2000). Studies have reported that pH values lower than 4.0 avoid the proliferation of undesirable microorganisms (Moriel et al., 2015). In the present study, the small variation on pH values intra treatments do not have an apparent explanation; however, most pH

values remained below 4.0 in the salt treatments, different from what was observed in control and SF. Thus, the proliferation of spoilage-inducing microorganisms can be inferred to have been partly inhibited by salt, which resulted in greater DM conservation, particularly at the inclusion 2.5% level. The lack of effect of SF on the chemical composition and pH of WBG compared with the control in our study is not clear, but could be a consequence of the high-water content of the WBG coupled with their storage under ambient conditions in open boxes. The absence of significant changes in the WBG chemical composition throughout the storage period, regardless of treatment, suggests that medium- or long-term storage and feeding of WBG will be limited by organoleptic factors rather than nutritional characteristics or mycotoxin contamination. It is also important to highlight that decreased feed intake by sheep eating diets with salt on the short (Masters et al., 2005) or long term (Blache et al., 2007) have been reported with sodium levels greater than 50 g/kg DM. In the current study, the sodium level in treatment S2.5 was not greater than 10 g/kg of WBG, which would be nearly 3 g/kg in a diet with 30% of WBG on a DM basis.

4.2. Effect of progressive inclusion of WBG on nutrient intake and digestibility of lamb diets

The absence of differences in DM, OM, NDF, and ADF intake across treatments confirmed one of the hypotheses of the study that WBG could concomitantly replace corn silage and soybean meal in ruminant diets. The higher NDF and moisture content of WBG have already been associated with reduced DM intake when it is included in ruminant diets (Cabral Filho et al., 2007). Historically, increased NDF has been associated with decreased DM intake in ruminants because of physical restrictions (Mertens, 1994) and high-moisture content feeds have been shown to decrease feed intake, with a 10% increase in moisture leading to a decrease in DM intake of 0.2 kg/100 kg of body weight (Harmon and Phipps, 2022). However, our results indicated that including WBG up to 30% of the DM, which resulted in an increase in NDF content from 439 to 510 g/kg DM and a decrease in DM from 510 to 387 g/kg DM, did not impair DM intake in the lambs. Similarly, the absence of an effect of WBG on the apparent total-tract digestibility of DM, OM, NDF, and ADF between treatments confirms that including moderate quantities of WBG in TMR diets is recommended. Previous research has also reported no effects on OM digestibility of the inclusion of up to 33% of WBG in substitution of Tifton 85 hay (Cabral Filho et al., 2007). The same authors observed that when WBG was included at 67% DM, OM digestibility decreased by approximately 12%. The slightly greater value of ADF vs. NDF digestibility was unexpected. This result could be, at least partially, explained because the chemical analyses of NDF and ADF in feed and feces were performed including residual ash, which result in an overestimation of both NDF and ADF content. A greater content of neutral detergent insoluble ash could explain a greater underestimation of the apparent digestibility of NDF compared with ADF.

Another important finding of this study was the substantial improvement in N use efficiency with the dietary inclusion of WBG, which was driven by an increase in N digestibility and a tendency for reduction in urinary N excretion. The lower apparent N digestibility of soybean meal relative to that of WBG could be partly associated with the soybean variety (Lehmali and Jafari, 2019) or with a reduction in N digestibility during the processing of soybean meal (Parsons et al., 1992). Lower urinary N excretion with WBG can be attributed to the high rumen undegradable protein content of this byproduct (64% of CP; NASEM, 2016), which decreases rumen ammonia production and its consequent excretion in the form of urea N through urine.

5. Conclusions

In the present study, we found that mycotoxin contamination of WBG at harmful levels in animals did not occur for up to 27 days of storage. The inclusion of 2.5% common salt in WBG increased the on-farm feedout period of WBG. The inclusion of WBG up to 30% of dietary DM can replace total corn silage and soybean meal with WBG in TMR diets for lambs. At this inclusion level, the N use efficiency can be improved without negative effects on nutrient intake and digestibility.

Conflict of Interest

The authors declare no conflict of interest.

Author Contributions

Conceptualization: Hentz, F. **Data curation:** Silva, A. M. and Ribeiro-Filho, H. M. N. **Formal analysis:** Silva, A. M. **Funding acquisition:** Hentz, F. **Investigation:** Silva, A. M.; Dornelles, R. R.; Martini, M. I. and Silva, L. H. **Methodology:** Hentz, F. and Ribeiro-Filho, H. M. N. **Project administration:** Hentz, F. **Supervision:** Ribeiro-Filho, H. M. N. **Validation:** Ribeiro-Filho, H. M. N. **Writing – original draft:** Silva, A. M. **Writing – review & editing:** Hentz, F. and Ribeiro-Filho, H. M. N.

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