Rev. Bras. Saúde Prod. Anim., Salvador, v.22, 01 - 12, e2122192021, 2021
http://dx.doi.org/10.1590/S1519-99402122192021

Animal Nutrition
Received on: 18/01/2021
Accepted on: 14/06/2021

Ingestive behavior of lambs fed with guava agro-industrial waste (*Psidium guajava*)

Comportamento ingestivo de cordeiros alimentados com resíduo agroindustrial de goiaba (Psidium guajava)

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ABSTRACT

The objective of this work was to evaluate the effect of increasing levels of guava agro-industrial waste (GAW) on the ingestive behavior of Santa Inês lambs. Forty non-castrated sheep of the Santa Inês breed were used, at an initial weight of 21.33 ± 2.62 kg, and at the age of 120 days, distributed in a completely randomized design, with five treatments and fed with increasing GAW levels (0.0, 7.5, 15.0, 22.5 and 30.0%). The average daily gain (ADG) showed orthogonal contrast (P <0.05), in which the animals that ingested the control diet had lower performances. The animals with GAW added to the diet obtained more significant weight gains reflected by a better feed conversion. Among the variables, idleness, feeding, and total chewing time had a significant effect (P<0.05); animals spent more time idle at the 30.0% inclusion level and less time in the control group. However, the means of the 7.5, 15.0, and 22.5% GAW inclusion levels were statistically similar to the control group and the 30.0% level. There was a significant effect (P<0.05) on feed efficiency, with the lowest values being presented for the control group and the highest values at the 30% inclusion level of GAW. The 30.0% GAW feed

ISSN 1519 9940

for lambs in confinement reduces feed time, and total chewing prolongs idleness and increases feed efficiency without compromising dry matter intake, neutral detergent fiber intake, and ADG.
Keywords: alimentary efficiency, alternative feed, ethology, GAW, idle

RESUMO

O objetivo deste trabalho foi avaliar o efeito da inclusão de níveis crescentes de resíduo agroindustrial de goiabeira (RAG) no comportamento ingestivo de cordeiros Santa Inês. Foram utilizados 40 cordeiros não castradas da raça Santa Inês, com peso inicial de 21,33 ± 2,62 kg e idade de 120 dias, distribuídos em delineamento inteiramente casualizado, com 5 tratamentos e alimentados com níveis crescentes de GAW (0,0; 7,5, 15,0, 22,5 e 30,0%). O ganho médio diário (GMD) apresentou contraste ortogonal (P <0,05), no qual os animais que ingeriram a dieta controle tiveram desempenho inferior. Os animais com GAW adicionado à dieta obtiveram maiores ganhos de peso refletidos em uma melhor conversão alimentar. Entre as variáveis, ociosidade, alimentação e tempo total de mastigação tiveram efeito significativo (P <0,05); os animais passaram mais tempo ociosos no nível de inclusão de 30,0% e menos tempo no grupo controle. No entanto, as médias dos níveis de inclusão de 7,5, 15,0 e 22,5% no GAW foram estatisticamente semelhantes às do grupo controle e ao nível de 30,0%. Houve efeito significativo (P <0,05) na eficiência alimentar, com os menores valores sendo apresentados para o grupo controle e os maiores valores no nível de inclusão de 30% do GAW. A alimentação de 30,0% GAW para cordeiros em confinamento reduz o tempo de alimentação e mastigação total, prolonga a ociosidade e aumenta a eficiência alimentar sem comprometer o consumo de matéria seca e de fibra em detergente neutron e GMD.
Palavras-chave: alimentação alternative, eficiência alimentar, etologia, ocioso, RAG

INTRODUCTION

Animal behavior is a way of looking at the entire production system, including the animal's activities in its social and physical environment (Custodio et al., 2017). The most studied parameters to evaluate the ingestive behavior are feeding time, rumination and leisure, feed efficiency and rumination, the number of merciful chews per meal bolus, time spent chewing ruminal cheeses, and several merciful chews per day (Burger et al., 2000). Ingestion behavior estimates are reported as relevant tools in dietary assessments, allowing for adjusting the administration of small ruminant rations to obtain the best productive performance (Barros et al., 2014; Nicory et al., 2015; Sá et al., 2015; Silva et al., 2016). The use of alternative products in sheep feed did not affect the consumption of MS and NDF, nor did they modify the ingestive behavior of these animals.

The search for alternative feeds for ruminants such as agro-industrial by-products may be an alternative method for reducing the expenses associated with using concentrate (Alves et al., 2010; Azevedo et al., 2013). Accordingly, agro-industrial wastes could be expected to have a favorable economic effect and a reduction of environmental encumbrance. The agro-industrial waste (seeds) from the guava

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(Psidium guajava) has 87.06% of unsaturated fatty acid and 63.94 g $100g^{-1}$ of total dietary fiber (Uchoa-Thomaz et al., 2014). The primary determinant for greater inclusion of GAW in feed is tannin, lignin, saponin, and phytic acid, which are anti-nutritional factors found mainly in the guava seeds (Maniyan et al., 2015). The GAW contains 2 – 4% condensed tannins, which may benefit protein metabolism in ruminants. Excess tannin in animal feed can reduce palatability, feed intake, and dry matter digestibility due to the formation of complexes and the inhibition of enzymes along the digestive tract (Acamovic and Brooker, 2005).

Our study hypothesizes that the use of guava agro-industrial residue, due to the presence of tannin in its composition, interferes with the ingestive behavior of ruminants. Therefore, the objective of this work was to evaluate the effect of increasing levels of guava agro-industrial residue on the ingestive behavior of Santa Inês lambs.

MATERIAL AND METHODS

The Animal Ethics Committee approved this study of the Federal University of Paraiba (UFPB), Brazil (protocol no. 2305/14). The experiment was conducted at the Federal University of Paraíba, Campus at Bananeiras-Paraíba, Brazil (altitude 552 m, latitude 6° 41' 11", longitude 35° 37' 41"). Air temperature (black globe temperature, BGT) was 24.97 ºC, and relative humidity (RH) was 76.48% in the stalls. Twenty non-castrated sheep of the Santa Inês breed were used, at an average initial weight of 21.33 ± 2.62 kg and an average age of 120 days. The animals were divided into individual stalls (1.50 m^2) with slatted and suspended floors. The animals had free access to feed and water. The experiment lasted 63 days, 15 of which were for adaptation to the feeds, facilities, and management. Daily weight gain (ADG) was calculated using the following equation (Gowane et al., 2015): ADG = (Final live weight (kg) – Initial weight (kg))/Days in feedlot (48 days)

Tifton 85 hay (*Cynodondactylon* L.) was substituted for dehydrated and ground GAW at levels of 0, 7.5, 15.0, 22.5, and 30.0% of the dry matter (DM) in diets that contained ground corn, soybean meal, and a vitamin and mineral supplement (Table 1).

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Table1. Percentage and bromatological composition of experimental diets.

Ingredient (g kg^{-1} DM.)	Levels of inclusion (%)				
	0.0	7.5	15.0	22.5	30.0
Guava agro-industrial waste (GAW)[1]	0.0	75.0	150	225	300
Tifton hay	500	425	350	275	200
Ground corn	310	310	310	310	310
Soybean meal	170	170	170	170	170
Mineral supplement[2]	15.0	15.0	15.0	15.0	15.0
Calciticlimestone	5.00	5.00	5.00	5.00	5.00
Chemical composition					
Dry matter, DM (g kg^{-1} as fed)	888	888	889	889	890
Crude protein. CP (g kg^{-1} DM)	154	154	154	154	154
Ethereal extract. EE (g kg^{-1} DM)	31.3	37.9	44.5	51.1	57.7
Neutral detergent fiber. NDF (g kg^{-1} DM)	489	484	479	474	468
Fiber in acid detergent. FAD (g kg^{-1} DM)	249	262	276	290	304
Ash (g kg^{-1} DM)	64.5	60.3	56.2	52.0	47.8
Total Carbohydrates. TC (g kg^{-1} DM)	748	744	741	766	734
Non-fibrous carbohydrates. NFC (g kg^{-1} DM)	258	260	262	292	265
Total tannins. TT (g kg^{-1} DM)	0.0	5.0	9.9	14.9	19.8
Lignin[3] (g kg^{-1} DM)	33.7	44.4	55.0	65.7	76.3
Metabolizable energy. ME (Mcal/kg DM)	2.48	2.44	2.39	2.34	2.30

[1]GAW composition: DM - 908; CP - 91.8; EE - 107; NDF - 730; ADF - 620; Ash – 21.3; TC – 779; NFC – 48.7; Tannin - 6.60%; [2]Composition of mineral supplement. per kg: P: 70.0 g; Ca: 140 g; Na: 148 g; S: 12 g; Mg: 1.32 mg; F: 700 mg; Zn: 4.70 mg; Mn: 3.69 mg; Fe: 2.20 mg; Co: 140 mg; I: 61.0 mg; Se: 15.0 mg; Monensinasódica: 100 mg[3] Lignin (19.7%)

The GAW, which is essentially composed of seeds, was donated by Palmeiron (Belo Jardim - Brazil). The drying was carried out in the sun until an average content of 10% humidity was reached. After dehydration, the GAW was ground for greater homogeneity of the ration and better availability of nutrients.

The diet was provided with a forage: concentrate ratio of 50:50 to provide a gain of 250g/day, as recommended by the NRC (2007). It was calculated to have about 15.0% CP, and 2.40 kcal/kg concentrated metabolizable energy in the diet. The experimental diet was offered *ad libitum* at 07:30 and 16:30 as a complete mixture.

The feed was offered, and the leftovers were weighed daily to calculate the voluntary consumption and readjustment of the quantity offered, establishing 10% of leftovers based on the dry matter. The mean of the differences between the total amount of nutrients in the diet offered and the number of nutrients in the leftovers was used to estimate the nutrient intake. Consumption of DM was calculated concerning live weight (LW) and metabolic weight ($MW^{0.75}$). Water was offered to each sheep daily using 5 L buckets placed next to feeding troughs. Average daily water intake (ADWI) was measured to the nearest 10 mL. Loss of water due to evaporation was assessed by measuring the volume of water lost from an identical bucket placed beyond the reach of sheep (Mdletshe et al., 2017).

During the period in the feedlot (48 days), three visual evaluations were performed (for the last three weekends before slaughter, i.e., 31, 38, and 45 days

of confinement). The animals were evaluated for 24hour (08:00 to 08:00), with an interval of 5 min (Carvalho et al., 2006) in a direct fashion. The behavioral variables were: feeding (chewing of starter in mouth), ruminating (chewing regurgitated food, either in standing or in lying position), idleness (Standing without any movement or behavior), drinking (swallow the water), mastication (It is the sum of feeding times and the time of rumination), others (defecation, urination, drinking) activities (Nicory et al., 2015).

The results referring to the efficiency of the ingestive behavior, obtained by the methodology described by Burger et al. (2000), were obtained by the relations:

(a) FE = DMI/FT; (b) FE = NDFI/FT; (c) RE = DMI/RT; (d) RE = NDFI/RT; (e) TCT = FT + RT, in which: FE = feeding efficiency (g DM/min); DMI = dry matter intake (g DM/min); FT = feeding time (min/day); RE = rumination efficiency (g DM/min); NDFI = Neutral detergent fiber intake (g NDF/min); RT = rumination time (min/day); TCT = total chewing time (min/day).

There was also continuous observation of the number of times the animal defecated, urinated, and sought water, through adopting visual observation of the animals for 24 hours, which trained observers in an alternation system performed, strategically positioned so as not to promote changes in the routine of the animals.

The experimental design was a completely randomized design, with five treatments and eight replications. Data were subjected to analysis of variance (ANOVA), and the averages were compared by Tukey test at 5% probability through the PROC GLM and regression analysis using the SAS® program (2003).

RESULTS AND DISCUSSION

The DM and NDF were not influenced by the treatments (P>0.05). Among the behavioral measures, idle, feeding, and TCT had a significant effect (P<0.05) on the inclusion level of GAW. The animals spent more time idle at the 30.0% inclusion level and less time in the control group. However, the means at the 7.5, 15.0 and 22.5% GAW inclusion levels were statistically similar to the control group and the 30.0% level (Table 2).

The average daily gain (ADG) showed orthogonal contrast (P <0.05), in which the animals that ingested the control diet had lower performances. The animals with GAW added to the diet obtained more significant weight gains reflected by a better feed conversion. They were showing that GAW had a positive influence on growth parameters, regardless of the level of inclusion in the diet.

Table 2. Ingestive behavior of sheep Santa Inês fed with increasing levels of guava agro-industrial waste. (GAW.)

Variable	Levels of inclusion (%)					SEM	*P* Value	
	0.0	7.5	15.0	22.5	30.0		Linear	Quadr
Intake								
DM (kg day^{-1})	1.20	1.33	1.26	1.31	1.34	0.02	0.126	0.429
NDF (kg day^{-1})	0.60	0.65	0.61	0.67	0.63	0.01	0.324	0.233
ADG (kg)	269.94*	329.89	327.25	338.03	317.13	0.05	0.099	0.037[1]
Behavioral measure (min/day)								
Rumination	533	548	542	547	535	13.6	0.963	0.910
Idle	609b*	663ab	696ab	708ab	739.4a	12.7	0.001[2]	0.232
Feeding	283a*	216ab	188ab	174ab	155b	13.5	0.001[3]	0.524
Drinking	14.4	12.5	13.1	10.6	10.6	3.11	0.447	0.201
TCT	815a*	764ab	714b	721ab	690b	12.6	0.001[4]	0.292

D.M. – Dry matter; N.D.F.- Neutral detergent fiber; A.D.G.- Average daily gain; T.C.T.- Total chewing time; S.E.M. – standar error means; Quadr = quadratic; Averages in rows followed by different letters are statistically different by the Tukey test (P <0.05).
*contrast orthogonal: control vs. levels of inclusion G.A.W.
[1]$Y=274.50+7.05x-0.19x^2$ ($R^2=0.90$)
[2]$Y=621(\pm20.2)+4.1(\pm1.1)X$ ($R^2=0.95$)
[3]$Y=262(\pm18.9)-3.97(\pm1.03)X$ ($R^2=0.89$)
[4]$Y=799(\pm18.8)-3.91(\pm1.02)X$ ($R^2=0.88$)

For the ADG, a quadratic equation was observed, with a maximum gain of 339.9 g / day, at the level of 18.55% inclusion of GAW in the diet. The ADG between treatments with GAW was 328 g / day, representing a 22% greater gain than the control treatment, which had a gain of 269.64 g / day. The ADG among the treatments with GAW was higher (31.2%) to the estimated weight gain of 250 g / day. However, this is explained since the CDM of the animals in this experiment was higher than that estimated by the NRC (2007). Another factor is related to the increase of EE in the diets that contained GAW, which was favored by the grinding process in order to be made available for digestion together with the other nutrients, consequently obtaining the excellent performance of the animals, since the FC among the treatments was 4.15 kg DM/kg A.D.G. Costa et al. (2018), concluded that the inclusion of GAW by up to 30% decreases the concentration of cholesterol and increases the concentrations of T3 and T4 in the blood, helping the metabolism of animals probably, because GAW is composed of unsaturated fatty acids, especially linoleic acid and oleic acid, with 77.3 and 9.4%, respectively, which may have contributed to the reduction of cholesterol through the process of ruminal biohydrogenation or due to the tannins present in GAW that can protect unsaturated fatty acid and make it available for absorption in the intestine (Uchôa-Thomaz et al. 2014).

The present study results are consistent with the findings by Hassan et al. (2016), who found GAW can be included in lamb diets at the 20% level, with no adverse effect on final live weight, weight gain, and feed conversion rate.

The animals fed a diet with 30.0% inclusion of GAW were less often to the trough than the control group. While the animals at the 7.5, 15.0, and 22.5% levels had statistically similar behavior. The lowest values for the TCT variable were found at levels of 15.0 and 30.0%.

The behavioral measures, idle, feeding, and TCT, presented significant orthogonal contrast (P<0.05) (Table 2), that is, with the inclusion of GAW in the diet, there was a difference in the responses of these measures compared to the control group. The linear increasing regressive behavior was observed for the idle measure with GAW in the animals' diet. The feeding and TCT measures presented a decreasing regressive effect with the inclusion of GAW. The time spent in rumination, idleness, and feeding activities agrees with Alves et al. (2010), regardless of animal species. Among the behavioral activities, idleness represented the most time.

Cirne et al. (2014), working with restricted concentrated diets, found that rumination time was lower than in our research and that the idle time was 64.26% higher. These authors attributed those results achieved due to particle size that did not have a greater need for rumination and high energy content in the diet. Our results differ from this because of the concentrate ratio and the particle size of the hay being around 2 cm.

The GAW used in this research was ground, which could have the nutrients encapsulated by the guava seed structure more available, primarily the ethereal extract representing 5% of the diet (Table 1). In this research, diets presented similar FDN with a mean value of 478.8 g kg^{-1} DM. The FDN was consistent with the stability in rumination activity performed by the animals in all treatments, since this parameter is considered a physiological characteristic that contributes to the digestion of dietary fiber and is triggered by, and is rhythmic with, the time of day that the diet is given (Gomes et al., 2012). However, the diet containing 30.0% GAW was 82.4% of particles smaller than 1.18 mm. (Nobre, 2017 unpublished data). According to Mertens (1997) and Zebelli et al. (2012), feed particles smaller than 1.18 mm pass through the rumen without rumination, and this is the minimum size to stimulate chewing activity. The decrease in particle size through milling would have increased the passage rate and, with the greater specific weight the, particles stayed longer in the ventral sac of the rumen, where there is a larger microbial population and possibility of more significant degradation, besides an increase in the surface area for exposure to microbial attack, which increases the digestion rate of potentially digestible plant cells (Gomes et al., 2012).

There was a significant effect (P<0.05) on feed efficiency, with the lowest values being presented for the control group and the highest values at the 30.0% inclusion level of GAW Feeding efficiency also showed significant orthogonal contrast (P<0.05) that is, as GAW was included in lamb feed, feed efficiency increased. The FEDMI and FENDFI presented a positive linear regressive effect (Table 3).

Rev. Bras. Saúde Prod. Anim., Salvador, v.22, 01 - 12, e2122192021, 2021
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Table 3. Mean occurrences and SEM of feed efficiency (FE) and rumination efficiency (RE) of dry matter intake (DMI) and neutral detergent fiber intake (NDFI) of sheep Santa Inês fed with increasing levels of guava agroindustrial waste (GAW).

Efficiency	Levels of inclusion (%)					SEM	*P* Value	
(g h^{-1})	0.0	7.5	15.0	22.5	30.0		Linear	Quadr
FEDMI	264b*	396ab	480ab	622a	656a	42.4	0.001[1]	0.655
REDMI	143	150	152	154	156	4.89	0.413	0.798
FENDFI	128b	190ab	229ab	296a	309a	19.9	0.001[2]	0.620
RENDFI	69	72	72	73	73	2.31	0.563	0.789

SEM- standar error means; quadr = quadratic; [ab]Médias seguidas por letras distintas na mesma linha diferem entre si pelo teste de Tukey (P<0.05); *contrast orthogonal: control vs. levels of inclusion GAW
[1]Y=281(±62.6)+13.5±(3.41)X (R^2=0.97)
[2]Y=137(±29.7)+6.23(±1.61)X (R^2=0.97)

The addition of 30.0% of GAW adds 2% of tannin to the diet, and, probably, the amount was not enough to negatively interfere with the performance of the animals. Some authors state that < 50 g of condensed tannins/kg of DM is beneficial for animal performance (McMahon et al., 2000; Hervás et al., 2003; Min et al., 2003). The inclusion of only 25 g of condensed tannin/kg alfarroba pulp reduced growth rates in lambs (Priolo et al., 2000). However, when ingested at low doses (2 – 4% of DM), it can positively affect ruminant digestion (Mcsweeney et al., 2001). The nutritional effects and tannins used in the animals' diet may vary according to the chemical structure, molecular weight, amount of tannin ingested, animal species or category involved, and the energy and protein balance of the animals' diet (Frutos et al., 2004).

It was observed that there was no significant effect (P>0.05) of treatment on punctual defecation activity, however, urinating and drinking activity had a significant effect (P<0.05), showing that animals were more frequently at the drinking fountain and urinated more frequently at the 7.5% level of inclusion (Table 4).

Table 4. Distribution of the punctual activities defection, urination, drinking in several times/sheep/day comparing sheep Santa Inês fed with increasing levels of guava agroindustrial waste (GAW).

Activities	Levels of inclusion (%)					SEM.	*P*-Value	
	0.0	7.5	15.0	22.5	30.0		Linear	Quadr
Defeccion	15.5	16.4	13.0	14.0	16.1	0.46	0.644	0.086
Urination	11.2b	15.3a	13.0ab	13.22ab	13.7ab	0.46	0.382	0.220
Drinking	6.33ab	7.96a	5.25b	7.26ab	6.71ab	0.30	0.950	0.898

SEM – standard error means; Quadr= quadratic; Averages in rows followed by different letters are statistically different by the Tukey test (P <0.05); *contrast orthogonal: control vs. levels of inclusion GAW (P>0.05)

Animals that received a 30.0% GAW diet used 51.4% of the time per day in idle time and 10.8% of the time for feeding. However, control animals (0%

ISSN 1519 9940

GAW) spent 19.6% of the time feeding and 42.3% idle. At the 30.0% GAW level, the animals were 2.5 times more efficient than the control group (0% GAW) for FEDMI and 2.4 times more efficient for FENDFI. This higher efficiency can be attributed to the fact that feed was supplied with a mean particle size of 2 cm hay, favoring the chewing activity of the animals. In addition, the GAW was subjected to a grinding process, which resulted in particles of the same size as the standard concentrated feeds, which promoted a lower concentration of cell wall components in the diet.

Diets with a 30.0% GAW level for feedlot lambs reduce the time spent feeding and total chewing and prolonging idleness and increasing feed efficiency without compromising the intake of DM, NDF, and ADG by animals.

ACKNOWLEDGMENTS

The authors would like to thank the Universidade Federal da Paraíba for their technical assistance. CNPq and CAPES supported this study.

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