FULL ARTICLE

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Wet citrus pulp in finishing diets for feedlot lambs: performance and hepatic enzyme concentration

Polpa cítrica úmida na terminação de cordeiros confinados: desempenho e concentração de enzimas hepáticas

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ABSTRACT

Pectin industry generates a citrus pulp residue compounded by peduncle, endocarp, juice vesicles, columella, seeds and tiny fractions of epicarp and mesocarp of citrus fruits, denominated Wet Citrus Pulp (WCP), which has not yet been tested as food for lamb's nutrition. Thus, this study aimed to determine the effect of partial replacement of ground corn by WCP in high-concentrate diets on the performance and hepatic enzyme concentration of feedlot lambs. Forty-two male lambs (15 Santa Inês and 27 ½ Dorper × Santa Inês), with 24.7 ± 1.5 kg of BW and 60 ± 5 d of age was assigned to a randomized complete block design. Within blocks (n = 14), lambs were randomly assigned to 1 of 3 treatments: 0WCP (control) – diet containing 75.5% ground corn without WCP; 20WCP – diet containing 20% WCP in replacement of ground corn, and 40WCP – diet containing 40% WCP in replacement of ground corn. The experiment lasted 70 days, which was split in 3 experimental periods (14 days of diets' adaptation and 2 sub-periods of 28 days each). Statistical analyses were performed using the MIXED procedure of the SAS. Orthogonal polynomials for diet response were determined by linear and quadratic effects. There was a quadratic effect for DM, CP, ash, ether extract and NFC intake The highest DM and CP intake was observed for lambs fed 20WCP, however, the control diet increased the ash, ether extract and NFC intake. The increased levels of WCP decreased the ADG and feed efficiency (FE) during the adaptation period, however, did not affect the ADG and FE on periods 1 and 2. Consequently, the increased levels of WCP inclusion decreased linearly the BW. There was no effect of WCP inclusion in diets on Gamma-Glutamyl Transferase (GGT) concentration. However, there was a linear increase for Aspartate Aminotransferase (AST) concentration during the adaptation period, but without difference in other periods. In conclusion, adding up to 40% of WCP in finishing diets for feedlot lambs decrease performance during adaptation period, compromising the final body weight, but without damages effects on liver enzymes.

Keywords: By-product. Performance. Feed efficiency.

RESUMO

A indústria de produção de pectina gera um resíduo composto por pedúnculo, endocarpo, vesículas de suco, columela, sementes e minúsculas frações de epicarpo e mesocarpo de frutas cítricas, denominada Polpa Cítrica Úmida (WCP), que ainda não foi testada como alimento na nutrição de ovinos. Sendo assim, este estudo teve como objetivo avaliar o efeito da substituição parcial do milho pela WCP em dietas contendo elevado teor de concentrado sobre o desempenho e a concentração de enzimas hepáticas de cordeiros confinados. Quarenta e dois cordeiros machos (15 Santa Inês e 27 ½ Dorper x Santa Inês), com 24,7 ± 1,5 kg de peso corporal e 60 ± 5 dias de idade foram distribuídos em delineamento de blocos completos casualizados. Dentro de cada bloco (n = 14), os cordeiros foram distribuídos aleatoriamente entre os tratamentos: 0WCP – dieta contendo 75,5% de milho moído, sem a inclusão de WCP; 20WCP – dieta contendo 20% de WCP em substituição ao milho moído; e 40WCP – dieta contendo 40% de WCP em substituição ao milho moído. O experimento teve duração de 70 dias, sendo dividido em 3 períodos experimentais (14 dias de adaptação as dietas e 2 períodos de 28 dias cada). As análises estatísticas foram realizadas utilizando o procedimento MIXED do SAS. Os polinômios ortogonais lineares e quadráticos foram utilizados para avaliar o efeito das dietas. Houve efeito quadrático

para o consumo de matéria seca, PB, cinzas, extrato etéreo e CNF. A maior ingestão de MS e PB foi observada para os cordeiros alimentados com 20WCP, entretanto, a dieta controle aumentou o consumo de cinzas, extrato etéreo e CNF. O aumento na inclusão de WCP diminuiu o ganho médio diário e a eficiência alimentar durante o período de adaptação, entretanto, não afetou essas variáveis durante os períodos 1 e 2. Consequentemente, a inclusão de WCP reduziu o peso corporal ao final do período experimental. Não houve efeito das dietas na concentração de gama-glutamil transferase (GGT). Entretanto, houve aumento linear na concentração de aspartato aminotransferase (AST) durante o período de adaptação. Em conclusão, a inclusão de até 40% de WCP nas dietas de terminação para cordeiros confinados diminuiu o desempenho durante o período de adaptação, comprometendo o peso corporal ao final do período experimental, entretanto, não comprometeu a afetou as enzimas hepáticas.

Palavras-chave: Co-produtos. Desempenho. Eficiência alimentar.

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Introduction

Rearing lambs in feedlot conditions can increase body weight gain, reduce the age at slaughter and increase the meat quality (Bampidis & Robinson, 2006). Brazil is an important corn producer and it is the main energy ingredient used in feedlot diets, however, corn has an expensive cost because of the high global demand. As a result, the use of agro industrial by-products, such as a corn replacement, can maintain the performance and reduce production costs in high-energy diets (Silva et al., 2002).

The citrus processing industry plays an important role in the agro-industrial sector especially in tropical and subtropical regions. Citrus by-products represent an important food resource for animal nutrition, it is a high-energy by-product and can be used to replace corn in finishing diets for feedlot lambs (Rodrigues et al., 2008).

The higher pectin content results in a fast rumen fermentation providing energy for microbial growth, which may increase animal performance (Ferreira et al., 2020; Oltramari et al., 2016; Polizel et al., 2016). Additionally, the high NDF digestibility helps to control the rumen fermentation when citrus pulp replaces corn (DePeters et al., 1997; Hall et al., 1988).

Citrus pulp has been most commonly used in ruminants' feed in pellet form. However, due to the high cost of drying, alternatives have been obtained for the use of citrus by-products in its natural form (Pereira et al., 2008; Porcionato et al., 2004). During the production of pectin, citrus pulp is washed with water, which produces unusable solids including peduncle, endocarp, juice vesicles, columella, seeds and tiny fractions of epicarp and mesocarp from the citrus fruit, denominated as Wet Citrus Pulp (WCP) (Leite et al., 2017; Peixoto et al., 2015). However, there are few studies evaluating the inclusion of WCP in feedlot lamb diets

It was hypothesize that ground corn may be partially replaced by WCP in high-concentrate diets for feedlot lambs without compromising the performance and the hepatic health of the lambs.

Thus, this study evaluated the partial replacement of corn by WCP on the performance and hepatic enzymatic concentration (AST and GGT) of feedlot lambs.

Material and Methods

The experiment was carried out at the System facilities of the Intensive Production of Sheep and Goats (SIPOC) of Animal Science Department, "Luiz de Queiroz" College of Agriculture, University of São Paulo (USP), located in Piracicaba – São Paulo (22° 42' 24" S and 47° 37' 53" W), Brazil. The experiment was reviewed and approved by the Animal Care and Use Committee at the same institution (Number 2019-08).

Forty-two male lambs were used, 15 Santa Inês and 27 ½ Dorper × Santa Inês, with an initial body weight (BW) of 24.7 ± 1.5 kg and 60 ± 5 d of age. The lambs were kept in an individual tie-stall system, with covered stalls, with feeder and waterers. At the onset of the experiment, the lambs were weighed after 14-hour solids fasting.

All lambs were dewormed with 1.0% moxidectin (Cydectin, Fort Dodge Animal Health, Campinas, SP, Brazil) at a dosage of 1 mL/50 kg BW and received a supplemental injection of vitamin A, D, and E (ADE, Zoetis Indústria de

Produtos Veterinários Ltda., Guarulhos, SP, Brazil) before beginning of the experiment.

The experiment design consisted of a randomized complete block, with 3 treatments (diets) and 14 blocks. The blocks were defined according to the weight, breed and age of the lambs. The experiment lasted 70 days, and was split in 3 experimental periods, 14 days of diets' adaptation and 2 sub-periods of 28 days each (period 1 and 2).

The experimental diets were defined by the inclusion of WCP in partially replaced of the ground corn. The experimental diets were as follows: **0WCP** (control) – diet containing 75.5% ground corn without WCP; **20WCP** - diet containing 20% WCP in replacement of ground corn, and **40WCP** - diet containing 40% WCP in replacement of ground corn. The diets were formulated according to National Research Council (2007) for growing lambs. Composition and chemical analyses of the experimental diets are shown in Table 1. The WCP used in the present experiment was composed by 15.74% of DM (as fed), 7.55% CP, 0.61% of ash, 55.40% NDF, 24.20% ADF, 3.27% ether extract and 33.17% NFC (DM basis).

The diets adaptation began including 20% of WCP in DM content, and every 3 days it was increased by 10% until reaching 40% of WCP in DM content.

Corn and *Coastcross* hay were coarsely ground using a grinder (Nogueira DPM – 4 mill, Itapira, SP, Brazil) with a screen of 10 mm sieve. The ingredients were mixed daily using a horizontal mixer with a 500 kg capacity (Lucato, Limeira, SP, Brazil). Total mixed diets were fed every other day at 08:30 h, and animals were allowed *ad libitum* access to feed and fresh water. The feed amount offered was calculated according to previous intake, adjusting when needed, so refused feed did not exceed 15% of daily intake. Every two days, the orts were collected and weighed on an electronic scale with an accuracy of 1 g (Marte AC-10K; São Paulo, SP, Brazil). The samples of the orts and fed offer were storage every week at -18 °C for later analysis.

Table 1 – Ingredients and chemical composition of experimental diets with citrus pulp (Piracicaba, 2019)

Item	Diets[1]		
	0WCP	20WCP	40WCP
Ingredients, %			
Coastcross hay	10.0	10.0	10.0
Corn	75.5	55.2	34.8
WCP	0.0	20.0	40.0
Soybean meal	11.2	11.5	11.9
Mineral	1.5	1.5	1.5
Urea	0.3	0.3	0.3
Limestone	1.0	1.0	1.0
Ammonium chloride	0.5	0.5	0.5
Chemical composition, %			
Dry matter	88.9	47.5	32.8
NFC	58.2	50.1	41.9
NDF	17.3	26.1	34.9
ADF	6.3	10.5	14.7
EE	2.8	2.8	2.9
CP	15.1	14.9	14.8
Ash	6.6	6.1	5.5
TDN	80.2	78.3	77.2

[1]0WCP = control diet (no WCP inclusion); 20WCP = diet containing 20% of WCP (DM basis); 40WCP = diet containing 40% of WCP (DM basis); NFC = Non-fibrous carbohydrates; NDF = neutral detergent fiber; ADF = Acid detergent fiber; EE = ether extract; CP = crude protein; TDN = Total nutrients digestible.

Lambs were weighed after a 14 h fast on days 0, 14, 42 and 70 of the experimental period to determine the average daily gain (ADG) and feed efficiency (FE; g of BW/kg of feed).

On days 0, 14, 42 and 70 of each experiment sub-period, blood samples from each lambs were collected from the jugular vein puncture. The blood was collected intro Vacutainer tubes without anticoagulant (Vacuette®, Americana, SP, Brazil). The samples were duly identified, and immediately centrifuged at 2,000 × g at 4 °C for 15 minutes. After centrifugation, 2 aliquots were obtained from the serum and were stored separately at -18 °C, in order to determine the concentration of aspartate aminotransferase (AST) and gamma-glutamyl transferase (GGT).

The enzymatic activity of AST was determined according to Ladue & Wroblewski (1956), using a commercial kit AS 3804 of the Randox® brand. The enzymatic activity of GGT was determined according to the colorimetric method described by Szasz (1974), using a commercial kit GT 3817 of the Randox® brand.

Samples of WCP, experimental diets and orts were dried in a forced-air oven at 60 °C (Association of Official Analytical Chemists, 1990; method #930.15) and ground using a Wiley mill (Marconi, Piracicaba, SP, Brazil) with a 1.0-mm sieve. Association of Official Analytical Chemists, 1990; #934.01). The final DM content was determined after oven-drying at 105 °C for 24 h, and ash concentration was determined by incinerating the samples in a muffle furnace at 550 °C for 4 h (Association of Official Analytical Chemists, 1990; method #942.05). Total nitrogen concentration was determined using a Leco TruMac N (Leco Corp., St. Joseph, MI, USA) according to the Association of Official Analytical Chemists (1990; method #968.0). Crude protein (CP) was obtained by multiplying the total N content by 6.25. Sequential detergent fiber analyses were used to determine NDF (Van Soest et al., 1991) and ADF (Goering & Van Soest, 1970) concentration in an Ankon 2000 fiber analyzer (Ankom Tech. Corp., Fairport, NY, USA). The NDF was determined using heat-stable α-amylase and sodium sulfite. The fat concentration was determined by a supercritical carbon dioxide extraction system (Leco TFE2000,

Leco, Corp.). Non-fibrous carbohydrates (NFC) were estimated according to the equation: NFC = 100 – (NDF + CP + EE + Ash). The total digestible nutrients (TDN) were calcuted according to Weiss et al. (1992): TDN = CPdigested + (EEdigested × 2.25) + NDFdigested + NFCdigested.

For all the variables analyzed herein, animal was considered the experimental unit and all the data were analyzed using the PROC MIXED procedure of SAS 9.3 (SAS Inst., Inc., Cary, NC). All data were submitted to the Levene test to verify the homogeneity of variances, the Shapiro-Wilk test to check the normality of the residuals and the removal of "outliers".

The BW was analyzed including the treatments (levels of WCP inclusion) as fixed effect, with block as a random effect. The ADG, nutrient intake, FE and hepatic enzymes were analyzed as repeated measurements over time (period), including the treatments (levels of WCP inclusion), period and treatment × period as a fixed effect and with block and animal as a random effect. The covariance structure was defined according to the smallest Akaike Information Criterion. Treatment means were calculated using the LSMEANS option. Orthogonal polynomials for diet response were determined by linear and quadratic effects. For all the data, significance was set at $P \leq 0.05$.

Results

There was a quadratic effect of diets on the DM ($P = 0.02$), CP ($P = 0.02$), ash ($P = 0.02$), ether extract ($P = 0.01$) and NFC ($P = 0.01$) intake (Table 2). The highest DM and CP intake was observed for lambs fed 20WCP, however, the animals fed control diet increased the ash, ether extract and NFC intake. There was a diet and period interaction for NDF and ADF intake. There was a linear increase in the NDF intake during the adaptation ($P < 0.01$) and period 1 ($P < 0.01$; Figure 1). However, a quadratic effect was observed in period 2 ($P = 0.05$). Similarly, there was a quadratic effect of diets on the ADF intake during adaptation ($P = 0.03$) and in period 1 ($P = 0.04$; Figure 1). The inclusion of WCP increased linearly ($P < 0.01$) the ADF intake in period 2. There was a period effect for nutrient intake ($P < 0.01$), and the lowest intake was observed during the adaptation period. In addition, an increase in the nutrient intake was observed from the first to the second experimental period.

There was an interaction between diet and period on the ADG ($P < 0.01$) and FE ($P < 0.01$). Analyzing the data within each period, the increased levels of WCP decreased ($P < 0.01$) the ADG during the adaptation period, however, did not affect the ADG on periods 1 and 2 (Figure 2). In addition, the WCP inclusion decreased the FE ($P < 0.01$) during the adaptation period, without affect the FE on period 1 and 2 (Figure 2).

The increased inclusion of WCP decreased linearly the BW at the end of adaptation period ($P < 0.01$), period 1 ($P < 0.01$) and period 2 ($P = 0.01$).

There was no treatment effect on the GGT concentration (Figure 3). There was an interaction between diet and period for AST concentration ($P = 0.04$). There was a linear increase ($P = 0.01$) for in AST concentration during the adaptation period in the experimental diets, and no effect was observed in the periods 1 and 2 (Figure 3). There was a period effect on GGT ($P = 0.02$) and AST ($P < 0.01$) concentration, with increased blood concentrations of GGT and AST throughout the experiment.

Table 2 – Dry matter intake, nutrient intake, average daily gain, feed efficiency and BW of lambs fed the experimental diets of citrus pulp (Piracicaba, 2019)

Item[4]	Diets[1]			SEM[2]	*P*-value[3]			
	0WCP	20WCP	40WCP		L	Q	P	D × P
Intake, g/d								
DM	919.3	1004.8	940.2	38.18	0.55	0.02	<0.01	0.55
CP	138.9	150.1	139.3	5.71	0.94	0.02	<0.01	0.57
NDF	158.9	286.8	373.7	10.99	<0.01	0.04	<0.01	<0.01
ADF	57.8	80.6	90.9	3.05	<0.01	0.02	<0.01	0.03
Ash	60.7	60.5	51.1	2.29	<0.01	0.02	<0.01	0.41
Ether extract	25.26	25.02	20.75	0.93	<0.01	0.01	<0.01	0.34
NFC	542.9	492.0	361.5	18.48	<0.01	0.01	<0.01	0.08
ADG, g	285.4	245.4	217.3	13.19	<0.01	0.69	<0.01	<0.01
FE, gain/feed	0.33	0.24	0.23	0.01	<0.01	0.01	0.14	<0.01
BW, kg								
Initial	24.66	24.65	24.66	1.43	1.00	0.97	-	-
Adaptation, 14d	29.07	27.20	25.88	1.60	<0.01	0.58	-	-
Period 1, 42d	36.69	35.11	33.96	1.69	<0.01	0.78	-	-
Period 2, 70d	44.21	42.71	41.09	1.70	0.01	0.95	-	-

[1]0WCP = control diet (no WCP inclusion); 20WCP = diet containing 20% of WCP (DM basis); 40WCP = diet containing 40% of WCP (DM basis); [2]SEM = standard error of the means; [3]L = linear effect; Q = quadratic effect; P = period effect; D × P = diet and period interaction; [4]DM = dry matter; CP = crude protein; NDF = neutral detergent fiber; ADF = acid detergent fiber; NFC = non-fiber carbohydrate; ADG = average daily gain; FE = feed efficiency; BW = body weight.

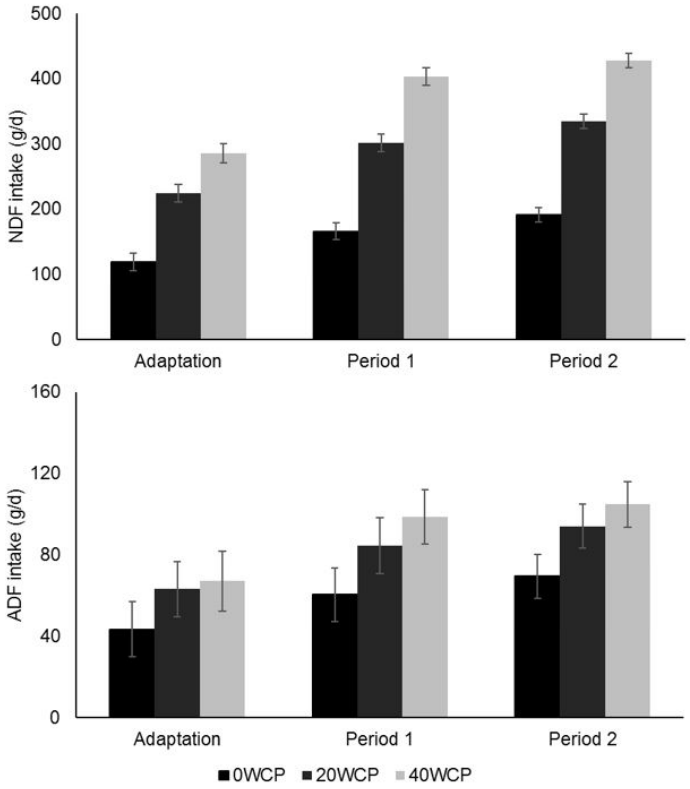

Figure 1 – Neutral detergent fiber (NDF) and acid detergent fiber (ADF) intake of lambs fed with the experimental diets of citrus pulp. There was interaction between diet and period. There was a linear increase for NDF intake during the adaptation ($P < 0.01$) and period 1 ($P < 0.01$). However, a quadratic effect was observed in period 2 ($P = 0.05$). Quadratic effect was observed for ADF intake during adaptation ($P = 0.03$) and period 1 ($P = 0.04$). In period 2, a linear increase ($P < 0.01$) was observed for the ADF intake. 0WCP = control diet (no WCP inclusion); 20WCP = diet containing 20% of WCP (DM basis); 40WCP = diet containing 40% of WCP (DM basis).

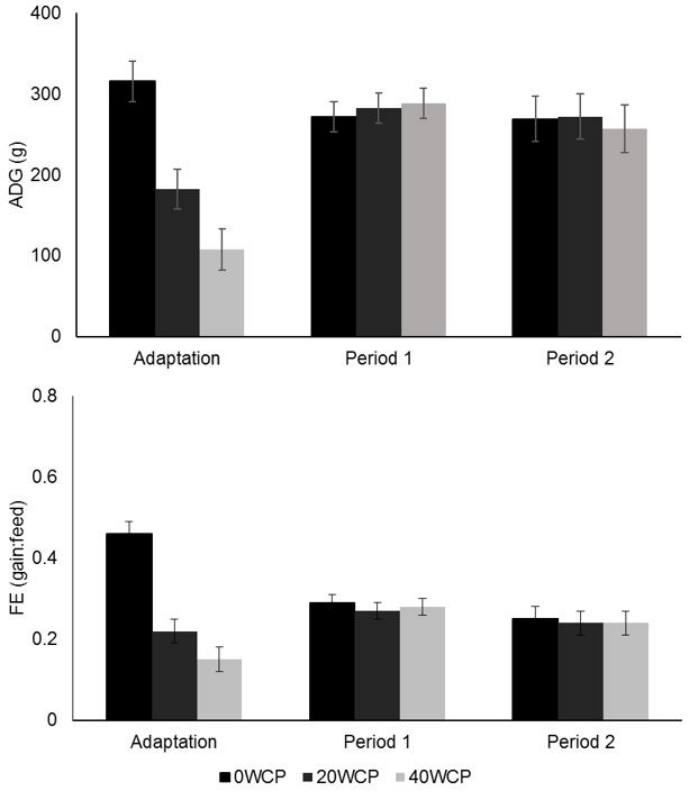

Figure 2 – Average daily gain (ADG) and feed efficiency (FE) of lambs fed with the experimental diets of citrus pulp. There was interaction between experimental diet and period for ADG and FE. There was a decreasing linear effect ($P < 0.01$) for ADG during the adaptation period, and no effect was observed in the other periods. Similarly, there was a linear reduction ($P < 0.01$) in feed efficiency with the inclusion of WCP during the adaptation period. 0WCP = control diet (no WCP inclusion); 20WCP = diet containing 20% of WCP (DM basis); 40WCP = diet containing 40% of WCP (DM basis).

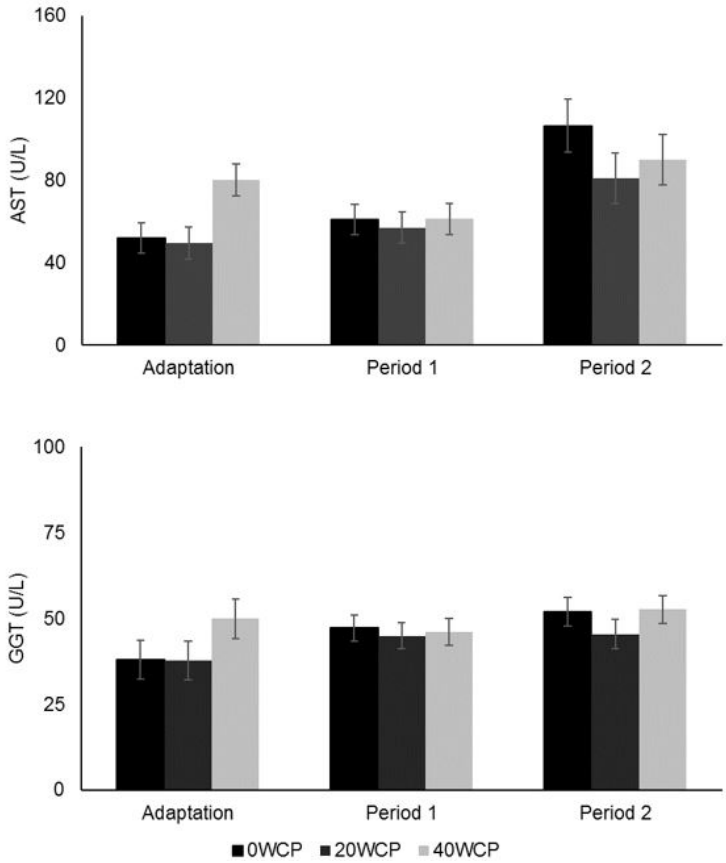

Figure 3 – Concentration of aspartate aminotransferase (AST) and gamma-glutamyl transferase (GGT) in lambs fed with the experimental diets of citrus pulp. There was an interaction between diet and period ($P = 0.04$) for AST concentration. A linear increase ($P = 0.01$) in the AST concentration during the adaptation period was observed in the experimental diets, and no effect was observed in the other periods. There was no effect of the diets in GGT concentration. 0WCP = control diet (no WCP inclusion); 20WCP = diet containing 20% of WCP (DM basis); 40WCP = diet containing 40% of WCP (DM basis).

Discussion

The replacement of ground corn by by-products in high-concentrate diets generally is done to minimize fluctuation of ruminal pH throughout the day, which may contribute to increase DMI and consequently ADG (Polizel et al., 2016). This study aimed to evaluate the inclusion of WCP as an energetic ingredient in diets for feedlot lambs. The data obtained in the preset study show that the partial replacement of ground corn by WCP was possible in feedlot lamb's diets because FE and ADG in 1st and 2nd period were similar among treatments (Figure 2). Nevertheless, the inclusion of WCP resulted in lower performance during the adaptation period, which induced a lower final BW.

The DMI levels observed in our experiment with the WCP diets (1.00 and 0.94 kg/day) were similarly with the values by National Research Council (2007) which recommended that lambs weighing 20 to 30 kg of BW intake 0.61 to 1.05 kg of DM/day.

The quadratic effect of diets observed in DMI when WCP was added, in which lambs in 20WCP treatment had higher DMI, probably was due to physicochemical characteristics of WCP. The inclusion of WCP in diet increased NDF and decreased NFC, ethereal extract and TDN of the diets. The increases in DMI in 20WCP treatment may be a way to compensate the lower energy density of the ration when compared to the control diet. In contrast, when 40% of WCP was added, the increase in the NDF may have resulted in a rumen fill limits to feed intake (Waldo, 1986). The increase in NDF of high digestibility in high starch diets normally increase animal performance (Ferreira et al., 2011; Polizel et al., 2016). In addition, the lower DMI for animals fed 40WCP diet may be due to the large water content in the WCP. The high water content in the diet increase the diet's volume, increasing the size of meal, which induces rumen-reticulum repletion, promoting consumption control by the physical mechanism (Allen, 2000; Allen et al., 2005; Mertens, 1997).

Regarding to nutrient intake, the increase on NDF and ADF intake can be explained by the WCP composition, whereas it contains a large amount of NDF and ADF compared with ground corn. Pectin is a complex structural carbohydrate with high and rapid ruminal degradation, and has as its forming unit galacturonic acid, and the

fermentation of pectin in the rumen provides higher productions of acetate and quickly available energy to the ruminal microorganisms. Besides that, higher acetate may also prevent abrupt ruminal pH drop, thus reducing the risk of acidosis by promoting a more favorable ruminal environment (Peixoto et al., 2015; Rodrigues et al., 2008).

The ADG obtained in the present study was similar to that described in the literature for Santa Inês and Dorper × Santa Inês weaned lambs (Ferreira et al., 2011; Polizel et al., 2017). However, our data suggested that is necessary to adapt the lambs to the inclusion of the WCP in high-concentrate diet. There is a minimal time required for adaptation of the ruminants to the diets which is affected by the physical and chemical characteristics of the ingredients, particle size, fermentable carbohydrates, fat or protein content and characteristics, environment and eating behavior. Thus, time is necessary to complete the expression of the new intake pattern concerning a new diet (Baumont et al., 2000; Pulina et al., 2013). Indeed, most research indicates that ruminant total-tract digestibility ordinarily adjusts to diets within 10 to 14 days (Borucki Castro et al., 2008; Ginane et al., 2015; Xu et al., 2018). The data from the present study demonstrate that lambs exhibited lower performance during adaptation of the diets containing WCP. However, after adaptation the performance of lambs fed WCP diets was similar to that of lambs fed control diet (based on ground corn), presenting the same ADG and FE.

Regarding lambs' body weight, the lower ADG of lambs fed diets containing WCP during adaptation resulted in lower BW at the end of the initial 14 d of the feedlot. Although the ADG of period 1 and 2 were equal between the treatments, the BW difference resulting from the adaptation period may explain the lower final body weight of the lambs that received WCP.

Serum activity of hepatic enzymes, such as AST and GGT (Figure 3) are used to diagnose hepatic impairment in domestic animals. The nature of hepatic pathology, whether necrotic or cholestatic, can be determined by the simultaneous analysis of the serum activity of the AST and GGT enzymes (Minervino et al., 2008). Some stimulation of exogenous origin, such as high concentrate feed and abrupt changes in diets, can cause damage to liver cells and the leakage of enzymes into the blood, increasing their levels (Braun et al., 1983; Moreira et al., 2012). In this study, the experimental diets presented specific differences in the concentration of AST during the adaptation period. However, none of lambs had higher AST and GGT than normal (Kaneko et al., 2008). Thus, no lamb developed liver injury during the experiment.

Conclusion

The inclusion of 20 or 40% of WCP in partial substitution of ground corn in diets for feedlot lambs diet can be recommended, as long as the animals are adequately adapted to receive the diets, because the adaptation period was the time when the inclusion of WCP negatively affected lambs' performance. In addition, the inclusion of 20 or 40% WCP may be recommended as it does not impair the liver health of feedlot lambs.

Conflict of Interest

We have no conflict of interest to declare.

Ethics Statement

The ethics committee approval certificate is attached (CEUA nº 2019-08).

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