Lambs Fed Diets Containing By-Product from Coconut Processing: Histomorphometry Characteristics in the Digestive and Renal Systems

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Research Article

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Abstract

This study aimed to evaluate the histological characteristics in the digestive and renal systems of lambs fed diets containing coconut by-product (CB). A total of 35 male lambs with an initial weight of 16.9 ± 2.93 kg were distributed in a completely randomized design with five levels of CB in the diet (0; 4.8; 9.6; 14.4 and 19.2% in total dry matter). Samples of the liver, kidney, rumen, and intestine were histomorphometrically evaluated and the data were submitted to regression analysis, at a 5% error probability. The inclusion of CB linearly decreased the dry matter intake and caused a quadratic effect for the height of ruminal papillae, absorption area, epithelium thickness, as well as for average daily gain. The inclusion of CB linearly increased the mucous layer and reduced the submucosal layer, as well as promoted a decrease in goblet cells in the small intestine. The inclusion of BC did not influence hepatic glycogen, additionally, the histopathological examination did not reveal liver damage or congestion, vacuolization, and necrosis of the renal tissue. Therefore, our results indicate that CB can be included in lambs diet up to the level of 7.2% without causing changes in the histomorphometry characteristics of the gastrointestinal tract and changes in liver and kidney tissue that compromise animal performance.

Introduction

The use of agro-industrial by-products generated through fruit processing in tropical regions is a strategy to make animal production more efficient and minimize costs associated with food. The coconut tree (*Cocos nucifera* L.) is a widespread plant throughout the world; however, its commercial exploitation is predominantly present in the tropics (Foale and Harries, 2011; Nayar, 2017). The processing of this fruit generates a large amount of waste, such as the coconut processing by-product (CB), which is obtained by scraping the film from the solid endosperm.

The inclusion of CB can strategically be used to attend to the energy requirements of lambs because of its high lipids content, considering that supplying energy in form of lipids improves animal performance and reduces feed costs (NRC, 2007; Toral et al., 2009). On the other hand, the increase of lipids content in diets may impact the ruminal fermentation, providing morphological changes in the ruminal mucosa, and affecting animal performance (Barboza et al., 2019; Gallo et al., 2019).

Studies in rats demonstrate an association between increased lipid intake and kidney damage (Declèves et al., 2014; Jang et al., 2020). The effect of lipid intake in the liver was evaluated in sheep, where the lipid intake was related to an increase in liver mass, where the authors discussed the role of this organ on lipid metabolism (Medeiros et al., 2008; Moreno et al., 2011). However, there are no reports on the histopathological characterization of these organs in lambs fed with CB. Therefore, we hypothesize that the high levels of CB included in the diet may affect the histomorphometry in organs of lambs. In this sense, this study aimed to evaluate the histological characteristics in the digestive and renal systems of lambs fed diets containing CB.

Material And Methods

The study was conducted according to ethical standards and approved by the Animal Use Ethics Committee of the Federal University of Alagoas (License No. 01/2018). The trial was carried out in the Federal University of Alagoas - Campus Arapiraca, located in the municipality of Arapiraca, Alagoas, Brazil, located under the geographical coordinates 9 ° 45'6 " S, 36 ° 39'37 " W, with an altitude of 280 meters. The climate is tropical with a dry season (Koppen classification).

Thirty-five undefined breed lambs, intact males, with a mean age of 4 months and body weight (BW) of 16.9 ± 2.93 kg were used in this study. The experimental area was composed of individual stalls, with dimensions of 1.0 m × 1.6 m, with access to individual drinking fountains feeders. Before the beginning of the experiment, all animals were identified and submitted to the control of ectoparasites and endoparasites (Cydectin®) and vaccinated against clostridiosis (Poli-Star).

The lambs were distributed in a completely randomized design, the diets consisted of five levels of CB (0; 4.8; 9.6; 14.4 and 19.2%) in dry matter (DM), with seven animals for level. The experimental period lasted 86 days, comprising 15 of adaptation to diets and facilities, and 71 days of data collection. The diets were composed of bagasse of sugar cane, corn, soybean meal, and CB (Tables 1 and 2), and were formulated to meet the nutritional requirements of lambs weighing 22.5 kg of BW, to an average daily gain of 200 g, according to the NRC (2007). The roughage: concentrate ratio was 33:67. The DM intake (DMI) of each lamb was estimated based on the measurements of food and orts. Food was provided *ad libitum* twice a day, the orts of each animal were weighed daily, and the offer was adjusted based on measurements from the two previous days, allowing for orts of approximately 12%. To measure the average daily gain (ADG) the lambs were weighed at the beginning and the end of the period.

Table 1
Chemical composition of ingredients used in diets containing coconut by-products.

Composition	Sugar cane bagasse	Corn	Soybean meal	Coconut by-product
Dry matter[1]	688	886	887	942
Organic matter[2]	963	987	942	972
Ash[2]	37	13	58	28
Crude protein[2]	16	74	431	184
Ethereal extract[2]	8	58	40	394
Neutral detergent fiber[2, 3]	791	139	123	255
Acid detergent fiber [2, 3]	524	24	71	113
Lignin [2]	82	4	1	38
Non-fibrous carbohydrates [2, 3]	148	717	348	140
Total carbohydrates [2]	939	856	471	394

[1]g/kg natural matter; [2]g/kg dry matter; [3] Corrected for ash and protein.

Table 2
Proportion of ingredients and chemical-chemical composition of experimental diets containing coconut by-products.

Ingredients[2]	Inclusion of the coconut by-product (%)				
	0	**4.8**	**9.6**	**14.4**	**19.2**
Sugar cane bagasse	333	333	333	333	333
Coconut by-product	0	48	96	144	192
Corn	382	342	302	262	221
Soybean meal	247	240	232	225	217
Calcitic limestone	3	3	3	3	3
Urea	10	10	10	10	10
Common salt	16	16	16	16	16
Mineral salt	10	10	10	10	10
Total	1000	1000	1000	1000	1000
	Chemical Composition				
Dry matter[1]	824	827	829	832	835
Organic matter[2]	941	940	940	940	939
Ash[2]	59	60	60	60	61
Crude protein[2]	167	169	172	174	177
Ethereal extract[2]	35	51	67	83	99
Neutral detergent fiber[2,3]	347	353	358	364	369
Acid detergent fiber[2,3]	201	205	209	213	217
Non-fibrous carbohydrates[2,3]	418	394	369	344	319
Total carbohydrates[2]	765	746	727	708	689

[1]g/kg natural matter; [2]g/kg dry matter; [3]Corrected for ash and protein; Protein equivalent of urea = 2820 g/kg of dry matter.

At the end of the experimental period, the lambs were randomly distributed in a slaughter order and submitted to 16 h of fasting. At the time of slaughter, the lambs were weighted, desensitized by the penetrative percussive method with the aid of a captive dart pistol, suspended by the hind limbs by ropes, and bled by splitting the carotid arteries and jugular veins according to the humane slaughter rules of the Ministry of Agriculture, Livestock, and Supply (2000).

The animals were manually skinned manually according to the methodology of Cezar and Sousa (2007), the internal components of the pelvic, abdominal, and thoracic cavities were removed, and then fragments no larger than 0.5 cm^3 of the organs were collected: liver (left lateral lobe) and kidney (cortical area), and fragments no larger than 1 cm from the organs: rumen (dorsal sac wall) and small intestine (middle portion of the duodenum), which were fixed in 10% formaldehyde, and packed in identified containers. These fragments were removed from the same topographic portion in all lambs.

Histomorphometric and histopathological analyzes were performed at the Animal Histology Laboratory, of the Agricultural Sciences Center of the Federal University of Paraíba, and the histological processing was performed according to the methodology described by Barboza et al. (2019), Lima et al. (2019), and Silva et al. (2020a).

For liver and kidney, 6 photomicrographs per animal were digitized, totaling 42 samples per diet for each organ, using the × 40 objective. In each of the photomicrographs, the observer looked for histopathological changes in the components of the nephron (renal corpuscle, proximal twisted tubules, Henle loop, and distal twisted tubules) to check for possible kidney damage caused by the inclusion of CB in the diets.

The samples of ingredients and orts were pre-dried at 55° C for 72 h, in a forced air circulation oven and ground in a knife mill, with pore sieves of 1 mm and 2 mm. The determinations of DM (method AOAC 934.01), mineral matter (MM; method AOAC 942.05), crude protein (CP, method AOAC 954.01), ether extract (EE; method AOAC 920.39), and lignin (method AOAC 973.18) were carried out by the AOAC (2019). The neutral detergent fiber (NDF) and acid detergent fiber (ADF) were determined according to Mertens (2002). The NDF and ADF were corrected for ash and protein according to Licitra et al. (1996).

For the rumen and small intestine histomorphometry, the × 40 objective was used. For the rumen variables: height of the papilla (from the base to the apex) and width of the papilla (in the middle region) 5 measurements were taken per lamb, totaling 35 samples per diet. For the thickness of the muscular layer, epithelial thickness, and epithelial keratinization, 7 measurements were made per lamb, totaling 49 samples per diet. For the duodenum variables: mucosal and submucosal layer thickness, 6 measurements were made per lamb, totaling 42 samples per diet. For the variables mentioned above, hematoxylin-eosin histological staining was used. For the measurement of the number of goblet cells in the duodenum in each animal, the number of goblet cells in 2000 µm linear intestinal epithelium was counted, under the histochemical staining of periodic acid from Schiff (PAS), using 4 areas of 500 µm.

For statistical analysis, the data were submitted to regression analysis using (PROC REG) of SAS - *University Edition*, and the regression analysis (linear and quadratic models) obtained was considered significant when $P < 0.05$, with the models being chosen based on the values of the determination coefficients and biological behavior.

Results

The inclusion of CB promoted a decrease in the DMI (Figure 1; $P<0.05$). A quadratic effect ($P<0.05$) was observed for the ADG with a maximum value of 164.1 g/day estimated for the inclusion of 3.6% of CB. The variables related to rumen morphometry were influenced ($P<0.05$) by the inclusion of CB, except for the width of the ruminal papilla (Table 3). The inclusion of CB up to 19.2% of the diet resulted in a quadratic effect ($P<0.05$) for epithelial thickness, epithelial keratinization, and papilla height and, also for the rumen absorption area. The rumen muscle layer thickness decreased linearly ($P<0.05$) with the inclusion of CB.

Table 3
Morphometric measurements of the ruminal mucosa of lambs fed diets containing coconut by-products

Variables	Inclusion of the coconut by-product (%)					SEM	*P*-valor	
	0	4.8	9.6	14.4	19.2		L	Q
Papilla height. μm	1182	1111	1111	892	708	35.2	<0.01	<0.01
Papilla width. μm	369	365	362	364	362	4.27	0.62	0.77
Absorption area. μm^2	437884	406335	402860	327898	258279	13868	<0.01	<0.05[2]
Epithelial thickness.μm	155.7	138.7	165.9	143.8	121.3	3.53	<0.01	0.02[3]
Muscle layer. μm	1224.2	1106.7	1089.8	1014.2	991.4	39.8	<0.04[4]	0.69
Epithelial keratinization. μm	38.7	29.6	26.2	24.0	20.0	1.23	<0.01	0.04[5]

[1] $Y = 1170.8 + 2.24x - 1.38x^2$ ($R^2 = 0.97$); [2] $Y = 432325 - 1988.7x - 359.8x^2$ ($R^2 = 0.93$); [3] $Y = 149.1 + 2.28x - 0.188x^2$ ($R^2 = 0.58$); [4] $Y = 1180.4 - 10.76x$ ($R^2 = 0.81$); [5] $Y = 37.496 - 1.36x + 0.027x^2$ ($R^2 = 0.91$).

The inclusion of CB increased the growth of the mucous layer ($P<0.05$) and reduced the submucosal layer ($P<0.05$), as well as the goblet cells in the small intestine of lambs (Table 4). The inclusion of CB did not influence hepatic glycogen, and histopathological examination did not reveal liver damage or congestion, vacuolization, and necrosis of the renal tissue.

Table 4
Mean thickness of mucosa, submucosa and calyceal cells of the small intestine of lambs fed diets containing coconut by-product

Variables	Inclusion of the coconut by-product (%)					SEM	*P*-valor	
	0	4.8	9.6	14.4	19.2		L	Q
Mucosa. µm	470.8	470.0	508.6	519.8	521.1	9.03	0.016[1]	0.75
Submucosa. µm	1273.2	946.5	941.8	938.0	672.6	38.39	<0.001[2]	0.56
Goblet cells	93.0	91.1	70.1	68.4	67.9	3.25	<0.001[3]	0.35

[1]Y= 468 + 3.13x (R^2 = 0.86); [2]Y = 1195.8 - 25.16x (R^2 = 0.80); [3]Y = 92.7 - 1.52x (R^2 = 0.82).

Discussion

The histomorphometric characteristics of the rumen, intestine, kidneys, and liver of lambs fed with CB levels were assessed in the present study. Our results indicated that the intermediate levels of inclusion of CB in the diet of lambs do not alter the histomorphometric characteristics of the gastrointestinal tract and hepatic or renal changes, which could compromise the animal's performance.

The inclusion of CB resulted in a quadratic effect for ADG, this result is possibly a consequence of the decrease in the DMI with the inclusion of CB in the diet. The estimated inclusion of 7.2% of CB in the diet sustained the same ADG when compared to conventional diets in tropical regions using corn and soybean meal, which suggests that the evaluated by-product is an alternative that can be used as a substitute for grains in low quantities.

The quadratic effect for ruminal papilla height and epithelium thickness was possibly caused by the decrease in DMI and non-fibrous carbohydrates by lambs fed with higher amounts of CB, which possibly reduced the fermentable substrates by the ruminal microorganisms and may cause less production of the short-chain fatty acids at higher levels of CB inclusion (Rezaei et al., 2014; Shi et al., 2020). This fact can be explained by the absorption of the short-chain fatty acids, which stimulates the epithelial metabolism of the rumen and induces the growth of the epithelium (Baldwin et al., 2004; Wang et al., 2009), with the development of the height of the papillae strongly influenced by concentrations propionate and butyrate, as demonstrated by Suárez et al. (2006a,b), when observing greater development of the rumen mucosa in calves that were fed diets that provided higher concentrations of propionate and butyrate in the rumen.

A difference of 4.9% (56.8 µm) was estimated between the height of the ruminal papillae for the inclusion levels of 0% and 7.2% of CB in the diets, however, a more impressive decrease was observed in the papillae height, where the inclusion of 14.4% of CB promoted 18.1% of difference (202.19 µm), compared to the inclusion of 7.2% of CB. Possibly, up to the 7.2% level of CB inclusion, the propionate produced through the fermentation of glycerol released by the hydrolysis of triglycerides in CB stimulated the development of the papillae, and thus, combined with the increased availability of lipids in the small intestine, sustained similar performance when CB was not included in the diet, even with the decrease of non-fibrous carbohydrates in the diet.

Maintaining the height of the ruminal papillae is an important physiological process because even with the decrease in DMI, the quadratic effect found for the absorption area ensured that the products of rumen fermentation, which are important sources of energy for lambs, were absorbed and supply part of the lamb's energy requirements (Lesmeister et al., 2004). Since the NDF was constant between the levels evaluated, the decrease in the muscular layer of the rumen may be associated with an increase in the lipid content of the diets and a decrease in the DMI with the inclusion of CB, which resulted in a reduction in peristalsis and an increase in the duration of digesta retention (Owens and Basalan, 2016). This is because the intake of diets rich in EE stimulates the release of cholecystokinin (CCK), which in turn acts by reducing the motility of the rumen and small intestine (Reidelberger, 1994), and causes less hypertrophy and hyperplasia of the smooth muscle fibers that make up the rumen, leading to less development of the muscle layer compared to the 0% level (Suárez et al., 2006b). In other studies, the lower development of the rumen muscle layer was also associated with decreased ruminal motility when there were changes in the chemical composition of the evaluated diets (Wang et al., 2009; Lima et al., 2019).

The quadratic effect observed for epithelial keratinization possibly reflects the decrease in the passage rate at the highest levels of CB inclusion, which induced a lower flow of the digest over the ruminal papillae, decreasing the abrasive effect on them, which led to a lower turnover rate cell in the outer layer of the epithelium, causing the keratin layer of the ruminal epithelium to decrease (Silva et al., 2020b). In the small intestine, the decrease in the passage rate possibly caused a linear decrease in the submucosal layer with the inclusion of CB. In this layer are located the Brunner glands that secrete an alkaline mucus to neutralize the pH of the region, and to protect the mucosa of the duodenum against harmful agents (Verdiglione and Montesi, 2019). Possibly, a decrease in the passage rate through this organ led to a decrease of mucus produced, thus causing a decrease in these glands, and consequently reducing the thickness of this layer (Lang and Tansy, 1983).

The amount of goblet cells in the intestine is considered a parameter of intestinal health, being better with the increase of these glands (Bueno et al., 2012). These cells are responsible for the mucin production, mucus responsible for assisting in peristalsis, in the mechanical protection of the intestinal epithelium and acting against infectious agents of the intestinal mucosa, and being a component of the intestinal glycocalyx, which helps in the food digestion. The lower passage rate in the higher levels of inclusion of CB may have decreased the proliferation of these cells for reducing the need to protect the epithelium against abrasion caused by the passage of the digesta, and thus, lesser need for mucus production.

The increase in the inclusion levels of CB increased the energy density of the diets, and the lipids started to represent an important source of energy to supply the lamb's energy demand. This event is evident with the increase of the intestinal mucous layer. This morphological modification allows the greater digestive capacity of the small intestine since a greater thickness of the mucosa indicates a greater height of the intestinal villus, because of the energy content of the diet and decreased passage rate (Montanholi et al., 2013; Lima et al., 2018). Thus, there was a greater area of contact between villi and nutrients and favored digestion and absorption to its maximum extent (Van Soest, 1994; Yansari et al., 2004; Gabriel et al., 2008). Despite apparently pointing to an increase in metabolic efficiency, there was no increase in ADG with the increased inclusion of CB, as there was a decrease of 65.3% in IDM between levels 0 and 19.2% of inclusion of CB.

The absence of liver and kidney damage in the lambs fed with CB may have occurred due to the absence of toxicological properties and anti-nutritional factors of the coconut (Lima et al., 2015). Based on this parameter, the inclusion of CB in all levels evaluated herein did not damage the liver and kidneys of lambs.

Therefore, our results indicate that CB can be included in diets for lambs up to the level of 7.2% without causing changes in the histomorphometric characteristics of the gastrointestinal tract and changes in liver and kidney tissue that compromise animal performance.

Declarations

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Competing interests

The authors declare that they have no competing interests.

Ethics approval

The study was conducted according to ethical standards and approved by the Animal Use Ethics Committee of the Federal University of Alagoas (License No. 01/2018).

Consent to participate

Not applicable.

Availability of data and material

The dataset generated or analyzed during the current study are available from the corresponding author upon reasonable request.

Author's contribution

The study was designed by Felipe José Santos da Silva, Dorgival Morais de Lima Júnior and Ariosvaldo Nunes de Medeiros. Felipe José Santos da Silva collected data in the field and laboratory and wrote the first draft. Beatriz Dantas Oliveira and Anaiane Pereira Souza improved and corrected the manuscript. Ricardo Romão Guerra, Vitor Visintin Silva de Almeida and Julimar do Sacramento Ribeiro contributed to the study. All the authors discussed the results and commented on the manuscript.

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Figures

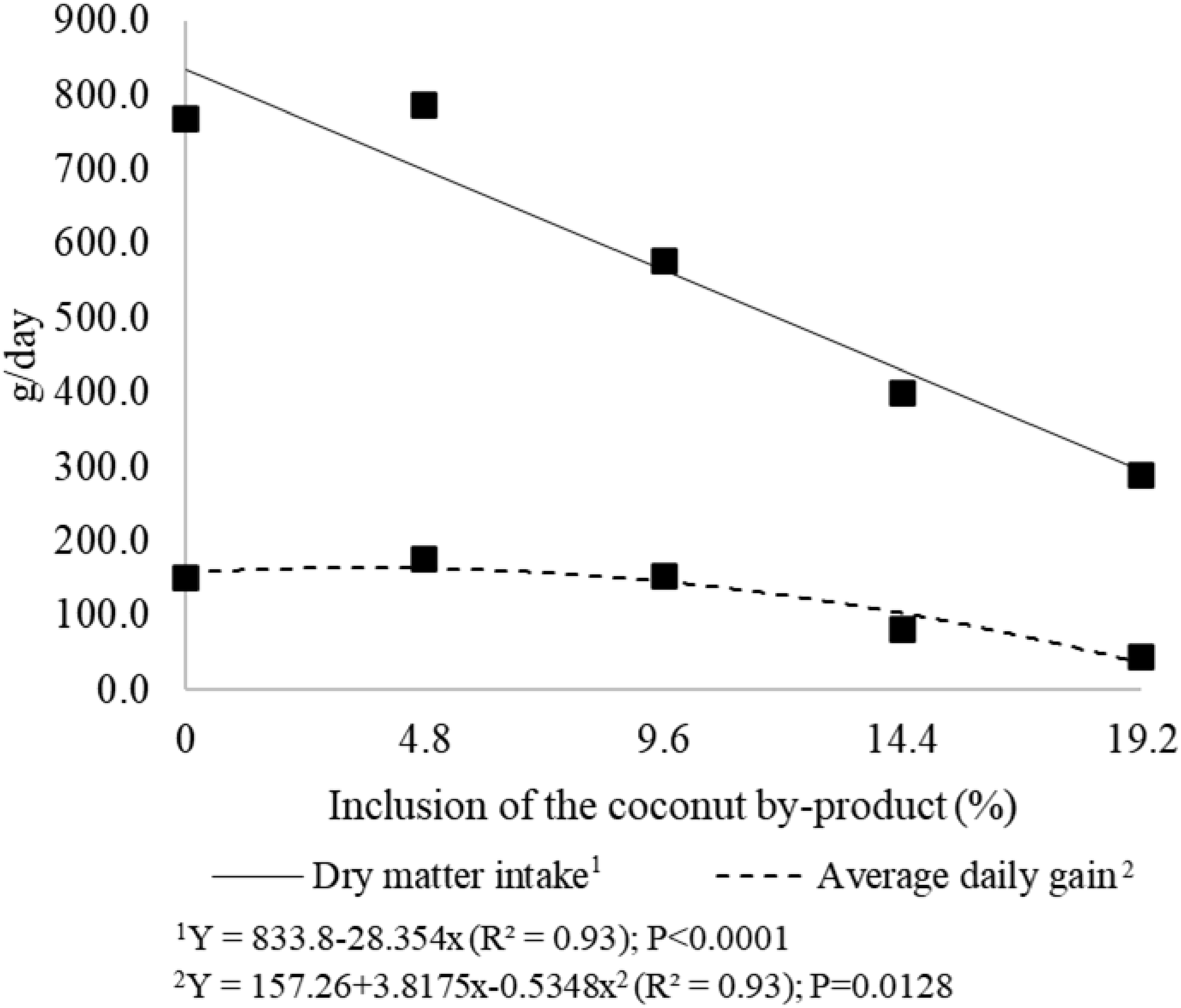

Figure 1

Dry matter intake and average daily gain (g/day) of lambs fed diets containing coconut by-products