



## ANIMAL SCIENCE

# Changes on the physicochemical and fatty acid profile of meat induced by inclusion of biscuit bran in lamb diet

RENATA T. ALENCAR, WILDER H.O. VEGA, LUIZA N.C. DA SILVA, HÉLIO H.A. COSTA, MICHELLE O.M. PARENTE, LISIANE D. DE LIMA & ALINE V. LANDIM

**Abstract:** The animal feed science is in constant search for new products that bring economic return, without harming the quality of the final product. This study aimed to evaluate the effect of increasing levels of substitution of corn by biscuit bran in lamb diet upon the fatty acid profile of its meat and its physicochemical and sensory characteristics. Twenty-four male lambs divided in four treatments were used. The treatments consisted in increasing levels (0, 15, 30, and 45%) of substitution of corn by biscuit bran in lamb diet. The significance of the treatments was determined by ANOVA and the adjusted means were compared by Tukey test at 5%. The effects were determined by linear and quadratic responses. The use of up to 45% biscuit bran sweet type did not modify the physical and sensory characteristics of meat, just as it did not affect nutrient and dry matter intakes and animal performance. A replacement of 45%, reduces the cholesterol ( $P = 0.03$ ) and the total content of saturated fatty acid ( $P = 0.002$ ), not modifying other physicochemical characteristics. The replacement of corn by biscuit bran sweet type in the feeding represents an alternative nutritional strategy for sheep meat production with desirable organoleptic and quality characteristics.

**Key words:** Cholesterol, circular economy, co-product, Morada Nova breed.

## INTRODUCTION

The Brazilian semi-arid region is the most densely populated dry land region, with more than 53 million inhabitants. The traditional production of sheep occurs mainly on an extensive basis, depending on the native vegetation of caatinga, which results in a low performance (IBGE-Instituto Brasileiro de Geografia e Estatística 2018).

The search for good quality alternative feeds for the formulation of lower cost rations that allow the best economic adjustment of the producer is always justified. In this perspective, the use of co-products from the food industry, among them the biscuit bran (BB), is presented as an option.

Biscuit bran is a co-product of worldwide distribution. It is produced from the leftovers of the biscuit manufacturing process. It presents in its composition a caloric content similar to corn (86.6 % and 87.7% of Total Digestible Nutrients, respectively), making it a viable alternative energy source, provided the animal performance, the quality of the final product, and a constant supply are maintained (Corassa et al. 2014). Similar to corn, the BB is relatively low in protein and high in starch, which can affect forage utilization negatively, especially in diets based on lower-quality forages.

Red meat has been an essential component in the human diet, being a source of proteins, vitamins, minerals, and fatty acids. However, it

has been criticized for its nutritional value due to the usual high amounts of saturated fatty acids (Wood 2017), presenting a high correlation with cardiovascular diseases, obesity, and hypercholesterolemia, resulting in a search for food with health-promoting properties (Santos et al. 2013, Sacks et al. 2017).

There is no information available about feeding ruminants with biscuit bran and its effect on meat quality. We have hypothesized that there is possibility of replacing corn by sweet biscuit bran, without prejudice to the performance and meat of the lambs. Thus, the aim of this study was to evaluate the effects of increasing levels of dietary biscuit bran in substitution of corn on the qualitative characteristics of meat from Morada Nova lambs.

## MATERIALS AND METHODS

The animal research was conducted in accordance with the institutional committee on Animal Use of Universidade Estadual Vale do Acaraú (CEUA/UVA - No. 006.09.015.UVA.504.02).

### Location, animals and experimental design

The study was conducted in the Experimental Center of Universidade Estadual Vale do Acaraú -UVA, Sobral, Ceará, in the Brazilian semi-arid region, 3°36' S, 40°18' W, 56 m.a.s.l. A total of twenty-four Morada Nova non-castrated male lambs, from single born with approximately 120 days old and a mean initial body weight (BW) of  $17.1 \pm 3.74$  kg were used in this study. The animals were individually housed in covered pens with a concrete floor. Lambs were distributed in a randomized complete design; six animals were assigned to 4 treatments. Experimental diets were randomly allocated to pens and so, therefore, the animal was the experimental unit.

### Chemical composition analysis of ingredients

The diets were well homogenized and offered in two daily meals at 8:00 and 16:00 hours. To favor conditions of diet selectivity offered by the experimental units, 15% of leftovers in natural matter were established per day. The lambs had free access to freshwater and mineral salt. Before the first meal was offered, the leftovers were collected and weighed individually for daily intake adjustment. Individual samples of each feed offered and the leftovers of each animal were collected weekly, about 300 g of each, packed in identified plastic bags and stored in a freezer at -20 °C. At the end of the trial, diet samples were thawed and then ground through a 1 mm Wiley Mill screen (Marconi, Piracicaba, SP, Brazil) and the dry matter (DM) (Method 934.01), organic matter (Method 924.05), ether extract (Method 930.29), and total nitrogen (N; Method 984.13) were determined according to the AOAC (2012). Crude protein was calculated by multiplying the total nitrogen by 6.25. The food concentration of total digestible nutrients (TDN) was estimated according to Weiss (1999). The moisture (method 930.15), ash (method 920.153), and crude protein (Method 928.08) contents of the meat were determined according to AOAC (2012). Neutral detergent fiber (NDF) was analyzed sequentially, according to Van Soest et al. (1991).

### Performance trial

The animals were weighed at the beginning of the adaptation period to adjust the amount of leftovers and to calculate the consumption in grams per metabolic unit, ( $\text{g}/\text{kgLW}^{0.75}$ ) and weekly to calculate daily consumption and feed conversion, respecting a fasting period of 16 hours before weighing. Intakes in  $\text{g}/\text{kgLW}^{0.75}$  and percentage of live weight (% LW), dry matter, organic matter, crude protein, and neutral detergent fiber fractions were determined, as

well as the initial live weight (ILW), final live weight (FLW), average daily weight gain (DWG) and feed conversion (FC) were evaluated.

### Treatments, and animal slaughter

The experimental diets (NRC 2007) were characterized by increasing levels of biscuit bran replacing corn (Tables I and II), as follows: (1) 0% (0 BB, without biscuit bran); (2) 15% of biscuit bran replacing corn (15 BB); (3) 30% of biscuit bran replacing corn (30 BB); and (4) 45% of biscuit bran replacing corn (45 BB). The diets were formulated according to NRC (2007), with a forage:concentrate ratio of 30:70, with Tifton 85 hay being the forage source and the concentrate was formulated using ground corn, soybean, biscuit bran (BB), and limestone (Table II).

The sweet biscuits which made up the biscuit bran were purchased at a bakery factory (Coelho LTDA, Sobral, Ceará, Brazil). These products were adequate for animal consumption. The biscuits were standardized using only the sweet type, the same trade mark and without filling or additional additive. They went through the uniform milling process at 3mm in a Willey type mill (TECNAL®- TE-650, Piracicaba, SP, Brazil) and were adequately stored in plastic drums, cleaned, and identified, until the moment of

their use. During diet formulation, corn was coarsely ground and mixed with soybean meal, biscuit bran, and limestone.

The concentrate and hay were weighed separately using an electronic scale (Welmy, BCW 6/15/30, Santa Bárbara d'Oeste, SP, Brazil), mixed, and offered daily to the animals. In advance, after 60 days of confinement, the animals were slaughtered, presenting an average weight of  $27.5 \pm 3.1$  kg, carcass mean weight of 13.53 kg, and carcass yield of 48.58%. The lambs were slaughtered after a fasting period of 16 hours.

The stunning was performed following the rules of Industrial and Sanitary Inspection of Products of Animal Origin - RIISPOA (MAPA 2017). After skinning and evisceration, the carcasses were placed in a cold chamber at 4°C for 24 hours. The temperature and pH were measured in the semimembranosus muscle, at 0 and 24 hours after the slaughter, using a digital potentiometer (DIGIMED, 300M, São Paulo, Brazil). The *Longissimus thoracis et lumborum* (LTL) was removed from the left half of the carcass. It was then divided into three subsamples, vacuum packed, and frozen at -20 °C until subsequent fatty acid (FA) determination, and performing of sensory, chemical and physical analyses.

**Table I. Chemical-bromatological composition of ingredients.**

Nutrients (% DM)	Ingredients			
	Hay	Corn grain	Soybean meal	Biscuit Bran
Dry matter	94.81	97.03	97.71	97.71
Organic matter	91.40	98.18	92.91	98.29
Crude protein	7.77	9.92	55.63	9.48
Ethereal extract	1.34	6.80	2.30	6.10
Total digestible nutrients	45.12	66.10	56.06	91.05

DM = dry matter.

**Table II.** Proportion, chemical-bromatological composition and fatty acid profile of the ingredients and the experimental diets.

Ingredients, % DM <sup>a</sup>	Ingredients		Diets <sup>b</sup> Levels of BB (%)			
	Corn	BB	0%	15%	30%	45%
Tifton 85 -grass hay	-	-	30.00	30.00	30.00	30.00
Ground corn	-	-	41.81	35.54	29.34	23.0
Soybean meal	-	-	27.7	27.7	27.7	27.7
Biscuit Bran	-	-	0.0	6.34	12.55	18.86
Limestone	-	-	0.50	0.50	0.50	0.50
<i>Chemical composition, %</i>						
Dry matter	96.8	98.0	96.62	96.67	96.71	96.74
Organic matter	98.8	98.6	94.27	94.24	94.33	94.31
Crude protein	11.5	11.0	21.82	21.78	21.84	21.69
Ethereal extract	4.8	6.4	3.91	3.89	3.87	3.88
NDFap	-	-	32.72	32.14	31.16	31.04
TDN	96.8	98.0	81.05	81.41	80.87	80.78
<i>Saturated Fatty Acids</i>						
14:0	0.02	13.15	0.32	1.15	1.98	2.81
16:0	15.38	33.57	20.81	21.96	23.09	24.25
18:0	1.92	5.00	3.05	3.25	3.44	3.64
20:0	0.45	0.22	0.76	0.75	0.74	0.73
22:0	0.17	0.07	0.74	0.73	0.73	0.72
24:0	0.25	0.71	0.99	1.01	1.04	1.07
Sum	18.21	52.72	26.68	28.87	31.02	33.21
<i>Unsaturated fatty acids</i>						
c-9 18:1	29.50	32.04	18.57	18.74	18.90	19.05
c-11 18:1	0.55	15.11	0.62	0.58	0.55	0.51
20:1 n-9	0.00	0.54	0.00	0.03	0.06	0.10
18:2 n-6c	50.76	15.11	42.87	40.61	38.38	36.12
18:3 n-3	0.98	0.11	11.25	11.19	11.13	11.08
Sum	81.79	47.80	73.32	71.12	68.97	66.78

<sup>a</sup>Ingredients based on dry matter: TDN = Total digestible nutrients; NDFap = Neutral detergent fiber corrected for ash and protein;

<sup>b</sup>Diets: Levels of substitution of corn by biscuit bran: 0%; 15%; 30% and 45%; BB = biscuit bran.

### Physical analyses and calculations

In order to determine the water holding capacity (WHC) of the meat using the pressure method, LTL was thawed in a 4 °C refrigerator for 24 h. Samples (500 ± 20 mg) were placed on filter paper between two acrylic plates, and a 10 kg

weight was placed on top of the plates for 5 min. The results are expressed as percentages relative to the initial weight, as follows:  $WHC = 100 - [(IW - FW)/IW \times 100]$ , where IW = initial weight and FW = final weight. To measure cooking weight loss (CWL), LL steaks were weighed and cooked

in an electric oven (BRANSTEMP, Gril 84, São Paulo, Brazil), preheated (170 °C) for 16 minutes (until the sample internal temperature reached 71 °C). The CWL was calculated as the difference between the weight of the sticks before and after oven broiling. Subsequently, the same samples were used for the measurement of the shear force (SF). For meat texture evaluation through SF, the sample was analyzed in the texturometer (TA-XT /Express Enhanced, Hamilton, MA, USA), equipped with a blade (1 mm) type Warner Bratzler, operating at 20cm/min; and the peak shear force required to cut the sample was recorded in kilogram-force per square centimeter ( $1 \text{ kgf/cm}^2 = 0.09806649 \text{ newton / millimeter}^2$ ).

The color determination was performed using a colorimeter, Minolta Chroma Meter Model CR-400/410 with CIE L\*, a\*, b\* system, where L\* determines the lightness, a\* the intensity of red, and b\* the intensity of yellow (Girolami et al. 2013). Thirty minutes before the evaluations, the samples already thawed and kept at 4 °C, were removed from the vacuum packaging and exposed to air for oxygenation of the superficial myoglobin, and after this period six readings were performed per treatment.

### **Lipid extraction, cholesterol and fatty acid analyses**

Lipids were extracted and quantified following the methodology described by Folch et al. 1957 and the spectrophotometer (METERTEC, model SP-818, Brazil) reading was performed using the methodology of Hartman & Lago (1973). The cholesterol content was determined using a HPLC (High Performance Liquid Chromatography), according to the methodology described by Bragagnolo & Rodriguez-Amaya (2001). The fatty acids were separated and determined using gas chromatograph (GCMS-QP5050A, Shimadzu, Brazil) coupled to a flame ionization detector,

equipped with a capillary column of fused silica (60 m x 0.53 mm; 1µ film thickness). The mobile phase consisted of helium C-50 at a flow of 9 psig. Fatty acids were identified using Sigma reference standards and quantified using the internal standard.

All indexes and ratios were calculated based on standardized formulas worldwide. The ratio between hypocholesterolemic and hypercholesterolemic fatty acids followed using the formula  $h/H = (C18:1 \text{ cis-9} + C18:1 \text{ trans-15} + \text{PUFA } \omega\text{-6} + \text{PUFA } \omega\text{-3}) / (C12:0 + C14:0 + C16:0)$ ; The Desirable Fatty Acids (DFA) were obtained from the sum:  $\text{DFA} = \text{MUFA (monounsaturated fatty acids)} + \text{PUFA (polyunsaturated fatty acids)} + C18:0$  (Santos-Silva et al. 2002, Borghi et al. 2016, de Abreu et al. 2019).

### **Sensory evaluation**

The sensorial analysis were performed according to procedures described by Landim et al. (2011). The characteristics were evaluated through a team of 10 trained panelists, composed by five men and five women, with ages varying from 19 to 33 years. The intensity of each attribute was evaluated on a non-structured scale of nine centimeters, anchored at the ends with terms that express intensity. The samples were thawed at 10 °C, 24 hours before the test, diced (approximately 2 cm<sup>3</sup>) and baked in a preheated (170 °C) electric oven (BRANSTEMP, Gril 84, São Paulo, Brazil) for 16 minutes (until the sample internal temperature reached 75 °C). Once cooked, the samples were packed in aluminum paper, wrapped in a preheated beaker and covered with watch glass (to avoid the loss of volatiles). Each evaluator was subjected to three sessions, receiving in each one of them a sample of each treatment, codified in three-digit random numbers and served with a balanced position, accompanied by cracker biscuits and mineral water for cleaning of the palate

between one sample and another. Sensations included, color (perception of intensity of meat color), aroma (sensation of smell liberated by the sample during chewing), taste (sensation of flavor liberated by the sample during chewing), softness (perception of force necessary to cut the sample when bitten) and juiciness (perception of quantity of liquid liberated by the sample of meat in the mouth). Color varied from pale (1) from extremely dark (9), aroma from absent (1) to strong (9), taste from nothing tasty (1) to extremely tasty (9), softness varied from extremely soft (1) to extremely tough (9) and juiciness from extremely dry (1) to extremely succulent (9).

### Statistical analysis

All continuous variables were subjected to the Shapiro-Wilk and Bartlett tests and attended the assumptions of normality and homogeneity of variances. The dry matter, nutrient intake, performance, physicochemical characteristics and fatty acid content of the meat were analyzed using the mixed model procedures, with treatment (diet) as a fixed factor. The effect of the treatments was determined by analysis of variance and the adjusted means were compared by Tukey HSD test at  $p \leq 0.05$ . An orthogonal partition of the sum of the square of treatments into linear ( $-3 \times m_1 - 1 \times m_2 + 1 \times m_3 + 3 \times m_4$ , where  $m$  = means of treatment) and quadratic ( $1 \times m_1 - 1 \times m_2 - 1 \times m_3 + 1 \times m_4$ , where  $m$  = means of treatment) degree effects was obtained. For the sensorial attributes, the Kruskal-Wallis test was used (provides a one way-ANOVA, with the "panelist" as a random factor). Results are reported as means  $\pm$  SEM. In the analysis, the following general linear model was used:

$$Y_{ijk} = \mu + T_i + (P_{ij}) + e_{ijk}$$

Where,  $Y_{ijk}$  = value observed in treatment  $i$  and animal  $j$ ;  $\mu$  = general mean;  $i$  = effect of

treatment (replacement level of corn by biscuit bran: 0%, 15%, 30% and 45%);  $P_{ij}$  = panelist effect (only in sensorial traits);  $e_{ijk}$  = random error.

All statistical analyses were conducted using IBM SPSS Statistics 22 for Windows (SPSS Inc., IBM Corporation, NY, USA).

## RESULTS

Table III shown the dry matter and nutrients intake and the performance of lambs feed with BB in replacement by corn. Any of the intake and performance traits accessed had effect of the diets ( $p > 0.05$ ). In addition, it was observed that the performance of lambs fed with different levels of BB also did not differ from animals fed the standard corn-based diet ( $p > 0.05$ ).

The physicochemical characteristics and sensory attributes are shown in Table IV. The levels of replacement of corn by BB have not affected the moisture, ash, protein, and lipid contents of the muscle. Besides that, cholesterol content decreased linearly with the addition of BB ( $P = 0.04$ ). In the physical aspects, the lightness variable ( $L^*$ ) had a quadratic effect ( $P = 0.03$ ) with the replacement of corn by BB in the diets, with a minimum value (41.32) corresponding to the level of 45% of BB. Cooking loss (CL), water retention capacity (WRC) and shear force (SF) were not influenced by diet ( $p > 0.05$ ) (Table IV). The inclusion levels of BB in the diet have not affected any of the sensorial attributes of meat. The average scores were observed ranging from 7.23 to 7.83, or "I liked regularly" to "I liked moderately", indicating satisfactory results.

The effects of the increasing levels of BB on the *longissimus lumborum* muscle fatty acid (FA) composition, in g/100 g, are shown in Table V. Twenty-three different fatty acids were obtained from the LTL muscle of the Morada Nova lambs. Nine saturated fatty acids (SFA), seven monounsaturated fatty acids (MUFA), and

**Table III. Dry matter and nutrient intake and performance lambs fed with biscuit bran (BB).**

Traits <sup>a</sup>	Levels of BB <sup>b</sup> (%)				SEM <sup>3c</sup>	P-Value <sup>d</sup>	
	0	15	30	0		L	Q
DMI, (g/kg LW <sup>0.75</sup> )	78.22	82.63	88.15	81.41	3.201	0.06	0.10
DMI, (%LW)	3.65	3.84	4.09	3.75	0.205	0.17	0.21
DMI (g/day)	744	875	822	820	29.131	0.55	0.50
OMI, (g/kg LW <sup>0.75</sup> )	73.25	77.25	82.63	76.48	2.962	0.06	0.10
OMI (%LW)	7.32	7.72	8.27	7.64	0.297	0.06	0.10
OMI (g/day)	703	829	779	777	27.533	0.55	0.49
CPI (g/kg LW <sup>0.75</sup> )	20.13	20.85	22.13	20.65	0.775	0.13	0.17
CPI (%LW)	0.94	0.97	1.03	0.95	0.043	0.25	0.27
DWG (g/day)	173	188	196	204	10.832	0.33	0.62
ILW (kg)	17.04	19.18	17.05	17.27	0.994	0.84	0.90
FLW (kg)	26.76	29.92	27.93	28.68	0.867	0.65	0.74
FR	4.98	4.61	4.30	4.24	0.363	0.44	0.73

<sup>a</sup>Traits: DMI = Dry matter intake; OMI = Organic matter intake; CPI = Crude protein intake; DWG = Daily weight gain; ILW = Initial live weight; FLW = Final live weight; FR = Feed Ratio.

<sup>b</sup>Levels of substitution of corn by biscuit bran: 0%; 15%; 30% and 45%;

<sup>c</sup>SEM = standard error of the mean;

<sup>d</sup>P-Value: L = linear effect; Q = quadratic effect.

seven polyunsaturated fatty acids (PUFA) were identified. The BB addition induced changes in FA profile, with a quadratic effect on proportions of 14:0 ( $p < 0.001$ ), 15:0 ( $P = 0.022$ ), 17:0 ( $P = 0.001$ ), 22:0 ( $P < 0.001$ ) and consequently, total saturated fatty acid (SFA,  $p < 0.002$ ). The total monounsaturated fatty acids (MUFA) also had a positive quadratic effect ( $p < 0.001$ ), because especially the quadratic effects of BB addition on 18:1 *n-9 cis* ( $p < 0.01$ ), the major MUFA. Additionally, the others minors MUFA, as *c-9* 14:1 ( $P = 0.012$ ), 16:1 ( $P = 0.041$ ) and 22:1 *n-9* ( $P = 0.003$ ) also had a quadratic effect, while the 20:1 *n-9* proportion increased linearly ( $P = 0.010$ ) and *c-9* 17:1 and *t-8* 18:1 ( $P = 0.043$ ) decreased linearly ( $p < 0.001$ ) with dietary BB addition. Regarding PUFA content, the BB addition had only a quadratic effect on 22:6 *n-3*, the minor PUFA ( $P = 0.006$ ), but did not change ( $p > 0.05$ ) the major FA (C18:2 *n-6*)

and, consequently the total polyunsaturated fatty acids (FA) also unchanged.

In this study, regarding the fatty acid ratios related to health indices, the best results were obtained with the inclusion of 45% of BB in the diet (Table VI). Only PUFA / SFA had no effect.

## DISCUSSION

The use of new dietary strategies in animal nutrition needs to validate its effects on products quality. This study explores the use of biscuit bran in lamb feeding and its effects on meat quality as a main focus; however, aware that nutrient intake and animal performance are decisive factors in the use or not of a feed, some aspects are discussed on these topics. The higher CMS obtained in this experiment (815.8 g DM per day) compared to that suggested

**Table IV. Physicochemical characteristics and sensory attributes of the Longissimus thoracis et lumborum muscle of sheep fed with a diet containing biscuit bran (BB).**

Traits <sup>a</sup>	Levels of BB (%)				SEM <sup>b</sup>	P-Value <sup>c</sup>	
	0	15	30	45		L	Q
<i>Chemical composition (%)</i>							
Moisture	74.95	75.30	75.22	74.56	0.047	0.66	0.37
Ash	1.12	1.12	1.06	1.07	0.015	0.32	0.59
Protein	23.66	23.46	25.21	24.59	0.054	0.17	0.36
Lipids	2.56	2.12	2.34	1.79	0.203	0.08	0.79
Cholesterol, mg/100g	75.09 <sup>a</sup>	65.98 <sup>ab</sup>	63.30 <sup>b</sup>	62.46 <sup>b</sup>	0.139	0.03	0.23
<i>Physical composition</i>							
Initial pH	6.74	6.66	6.65	6.77	0.020	0.55	0.29
Final pH	5.78	5.70	5.75	5.74	0.013	0.96	0.76
L*	44.15 <sup>ab</sup>	45.22 <sup>a</sup>	45.05 <sup>a</sup>	41.32 <sup>b</sup>	0.083	0.10	0.03
a*	15.19	14.24	16.21	14.02	0.068	0.94	0.62
b*	5.46	4.91	6.75	5.18	0.047	0.83	0.80
CL, %	41.96	42.42	41.17	40.25	0.075	0.14	0.21
WHC, %	56.02	57.22	56.70	54.40	0.090	0.71	0.41
SF, Kg/cm <sup>2</sup>	3.91	3.85	3.65	3.82	0.034	0.99	0.91
<i>Sensory attributes</i>							
Color	7.37	7.77	7.63	7.40	0.101	-	-
aroma	7.63	7.77	7.73	7.40	0.091	-	-
Taste	7.40	7.83	7.30	7.23	0.120	-	-
Softness	7.60	7.63	7.40	7.47	0.108	-	-
Juiciness	7.30	7.57	7.30	7.30	0.114	-	-

<sup>a</sup>Traits: L\*, a\* and b\* = lightness, intensity and the chromaticity of the red and yellow colors, respectively; CL = cooking loss; WHC = water holding capacity; SF = Shear Force.

<sup>b</sup>SEM = standard error of the mean;

<sup>c</sup>P-Value: L = linear effect; Q = quadratic effect;

<sup>ab</sup>Means between diets in the same row with different superscripts indicate differences at  $P \leq 0.05$  in the Tukey HSD test.

by the NRC (2007), (610 g DM per day) can be attributed to the low NDF content of the diets. Since low levels of NDF do not inhibit CMS. It was probably the energy demand of the animals that contributed to the modulation of the CMS (Lu et al. 2005). This intake pattern was observed at all levels of inclusion, including for the standard diet, where only corn was used; thus, the use of BB would be recommended, given its lower cost in relation of corn. The values of DWG and FC showed that the inclusion of BB in the lamb diets in the finishing phase does not affect the performance. The values obtained were consistent with that projected in the diet formulation (NRC 2007).

According to the Regulation No 1924/2006 of the European Parliament and Council (EC), (Reuterswärd 2007), foods might be labeled as low fat if its fat content is lower than 3g/100g. Regarding these requirements, the meat obtained in this study might be classified as lean, low in fat, and with a high content of others nutrients (Table IV).

The mean values for pH are 6.70 and 5.67 (0h and 24h, respectively), being within the average expected for *post mortem* sheep meat (Hajji et al. 2016). Thus, it exhibited adequate oxidative capacity and absence of pre-slaughter stress, showing that the transformation of muscle in meat occurred within the expected range. The absence of diet effect on the initial pH suggests

**Table V. Fatty acid profile (g/100 g of muscle) of Longissimus thoracis et lumborum muscle of lambs fed with biscuit bran (BB).**

Total Fatty acids	Levels of BB <sup>b</sup> (%)				SEM <sup>c</sup>	P-Value <sup>d</sup>	
	0	15	30	45		L	Q
<i>Saturated</i>							
14:0	7.21 <sup>b</sup>	9.35 <sup>ab</sup>	9.99 <sup>a</sup>	0.55 <sup>c</sup>	0.224	<0.001	<0.001
15:0	0.77 <sup>ab</sup>	0.70 <sup>b</sup>	1.12 <sup>a</sup>	0.22 <sup>c</sup>	0.071	0.094	0.022
16:0	22.86 <sup>a</sup>	20.94 <sup>ab</sup>	21.90 <sup>a</sup>	15.76 <sup>b</sup>	0.528	0.026	0.166
17:0	0.57 <sup>b</sup>	0.59 <sup>b</sup>	0.65 <sup>a</sup>	0.35 <sup>c</sup>	0.037	0.033	0.014
18:0	13.72	14.79	13.43	13.53	0.431	0.744	0.661
20:0	-	-	-	0.62	-	-	-
21:0	-	-	-	0.02	-	-	-
22:0	0.12 <sup>c</sup>	0.32 <sup>ab</sup>	0.40 <sup>a</sup>	0.21 <sup>bc</sup>	0.032	0.111	<0.001
24:0	0.00	0.12 <sup>A</sup>	0.00	0.02 <sup>b</sup>	0.022	0.580	0.067
Sum	45.27 <sup>a</sup>	46.82 <sup>a</sup>	47.49 <sup>a</sup>	31.28 <sup>b</sup>	1.897	0.002	0.002
<i>Monounsaturated</i>							
c-9 14:1	0.13 <sup>ab</sup>	0.20 <sup>a</sup>	0.37 <sup>a</sup>	0.06 <sup>c</sup>	0.041	0.924	0.012
c-9 16:1	1.55 <sup>ab</sup>	1.35 <sup>b</sup>	1.84 <sup>a</sup>	0.77 <sup>c</sup>	0.109	0.064	0.041
c-9 17:1	0.66 <sup>a</sup>	0.49 <sup>b</sup>	0.56 <sup>ab</sup>	0.22 <sup>c</sup>	0.047	<0.001	0.197
t-11 C18:1	1.01 <sup>a</sup>	0.83 <sup>a</sup>	0.81 <sup>a</sup>	0.43 <sup>b</sup>	0.097	0.047	0.594
c-9 C18:1	43.07 <sup>b</sup>	42.29 <sup>b</sup>	41.37 <sup>b</sup>	56.81 <sup>a</sup>	1.409	<0.001	<0.001
20:1 n-9	0.00 <sup>c</sup>	0.17 <sup>b</sup>	0.17 <sup>b</sup>	0.18 <sup>a</sup>	0.041	0.010	0.119
22:1 n-9	1.47 <sup>b</sup>	1.69 <sup>a</sup>	1.73 <sup>a</sup>	0.44 <sup>c</sup>	0.158	0.005	0.003
Sum	47.90 <sup>b</sup>	47.10 <sup>b</sup>	46.85 <sup>b</sup>	59.46 <sup>a</sup>	1.331	<0.001	<0.001
<i>Polyunsaturated</i>							
18:2 n-6 t	-	-	-	0.47	-	-	-
18:2 n-6	6.69	5.31	4.97	7.84	1.694	0.717	0.278
18:3n-3	0.14	0.40	0.39	0.37	0.601	0.061	0.106
20:2	-	-	-	0.22	-	-	-
22:2	-	-	-	0.13	-	-	-
20:5				0.03			
22:6n-3	0.00 <sup>c</sup>	0.37 <sup>a</sup>	0.31 <sup>a</sup>	0.18 <sup>b</sup>	0.049	0.211	0.006
Sum	6.82	6.08	5.66	9.26	1.545	0.459	0.227
SCDi- 17 <sup>a</sup>	52.99 <sup>a</sup>	44.99 <sup>b</sup>	46.20 <sup>b</sup>	37.31 <sup>c</sup>	1.752	<0.001	0.865

<sup>a</sup>SCDi-17: Stearoyl-CoA desaturase activity index, computed as  $c9-17:1/(c9-17:1+17:0) \times 100$ .

<sup>b</sup>Levels of substitution of corn by biscuit bran: 0%; 15%; 30% and 45%

<sup>c</sup>SEM = standard error of the mean;

<sup>d</sup>P-Value: L = linear effect; Q = quadratic effect;

<sup>abc</sup> Means in the same line followed by distinct letters are different by the Tukey HSD test ( $p \leq 0.05$ ).

that regardless of diet, the glycogen content resulted in the same acidity in the muscle, one-hour *post mortem*. The lower final pH confirmed the fact that adequate-energy diets protect animals against potentially glycogen-depleting

stressors (Velasco et al. 2004). This fact favors a longer shelf life.

No variation in dry matter, ash, and protein contents was found in this study, probably due to the similar composition of the experimental

**Table VI. Effects and standard errors of health indices of meat in lambs fed with biscuit bran (BB).**

Fatty acids ratio <sup>a</sup>	Levels of BB <sup>b</sup> (%)					P-Value <sup>d</sup>	
	0	15	30	45	SEM <sup>c</sup>	L	Q
DFA	68.87 <sup>b</sup>	67.97 <sup>b</sup>	65.94 <sup>b</sup>	82.25 <sup>a</sup>	0.001	0.02	<0.01
PUFA/SFA	0.18	0.13	0.12	0.39	0.086	0.24	0.07
MUFA/SFA	1.08 <sup>b</sup>	1.01 <sup>b</sup>	0.99 <sup>b</sup>	2.14 <sup>a</sup>	0.021	0.01	0.04
(C18:0+C18:1) / C16:0	2.50 <sup>b</sup>	2.73 <sup>b</sup>	2.50 <sup>b</sup>	5.12 <sup>a</sup>	0.005	<0.01	0.08
h/H	1.50 <sup>b</sup>	1.45 <sup>b</sup>	1.34 <sup>b</sup>	4.13 <sup>a</sup>	0.011	0.01	<0.01

<sup>a</sup>Fatty acids ratios: DFA = Desirable fatty acids (MUFA+PUFA+C18:0); PUFA/SFA = Ratio between the polyunsaturated fatty and saturated acids; MUFA/SFA = Ratio between the monounsaturated fatty and saturated acids; (C18:0+C18:1)/C16:0 = Ratio of stearic, oleic and palmitic fatty acids; h/H = Ratio of hypocholesterolemic and hypercholesterolemic fatty acids.

<sup>b</sup>Levels of substitution of corn by biscuit bran: 0%; 15%; 30% and 45%.

<sup>c</sup>SEM = standard error of the mean;

<sup>d</sup>P-Value: L = linear effect; Q = quadratic effect;

<sup>a,b</sup>Means between diets in the same row with different superscripts indicate differences at  $p \leq 0.01$  in the Tukey HSD test.

diets. The mean value of meat protein in the present study (above 23%) is higher than what has been discussed in the literature for Morada Nova lambs, with an average of 20% (Costa et al. 2011, Silva et al. 2016). Thus, this food might be labeled as a rich protein food (Reuterswärd 2007).

The color of the meat is an important factor for the buying behavior (Wood 2017). The lightness is related to structural attributes of the muscle, whereas color attributes (redness and yellowness) tend to be strongly associated with the pigment myoglobin (Hughes et al. 2014). Despite the quadratic effect observed in the lightness  $-L^*$  trait, we have considered this factor as irrelevant in terms of deciding or not to buy the product, since this and the other values (without effect of the level of replacement) were within the accepted values for sheep meat (Khlijji et al. 2010). Redness ( $a^*$ ) and yellowness ( $b^*$ ) were not influenced by the inclusion of BB. Results are within the range mentioned by Khlijji et al. (2010), with variations of 30.03 to 49.47 in the value of  $L^*$ ; from 8.24 to 23.53 in the value of  $a^*$ , intensity of red; and from 3.38 to 11.10 for  $b^*$ , intensity of yellow. Similar results obtained

in these characteristics might be the result of similar myoglobin and iron contents.

The average shear force observed in this study was 3.81 kgf/cm<sup>2</sup>, the meat is classified as middle softness, according to the classification of Cezar & Sousa (2018), which varies from soft (2.28 to 3.63 kgf/cm<sup>2</sup>), middle softness (3.64 to 5.44 kgf/cm<sup>2</sup>) to hard and extremely hard (above 5.44 kgf/cm<sup>2</sup>). This result was in line with other studies with Morada Nova lambs (Silva et al. 2016). Among the characteristics that contribute to the meat quality, softness is considered of great importance for the acceptability of the product and for consumer satisfaction (Wood 2017, Chikwanha et al. 2018).

The experimental diets have not altered the WHC and CL. This result shows a balance between WHC and CL, which is important at the time of tasting and for meat yield in consumption. These quality factors are related to pre and post cooking of meat, directly influencing the juiciness during chewing (Wood 2017).

Although, the sensory characteristics might vary according to changes in pH drop and profile of fatty acids (Ribeiro et al. 2011), the replacement of corn by BB has not affected the sensory acceptance of meat by the consumer.

Additionally, this results were in line with the values reported by other authors (Borghi et al. 2016, Cirne et al. 2017).

The profile of human diet has gone through several changes. Currently, there is an increase in the search for lean meats, since dietary factors play a critical role in the development of cardiovascular diseases (CVD). In this perspective, the high consumption of meat has been positively associated to a high risk of morbidity and mortality due to CVD, because animal fats are mainly saturated (Sacks et al. 2017). In this study, the SFA, especially 14:0 proportion in meat reduced drastically when 45% of BB was added. The proportion of C16:0 also decreased with BB addition, although to a lesser extent. The BB is rich in SFA, and the sum of C14:0 and C16:0 comprises almost 50% of the BB's FA composition that resulted in a slightly dietary increased in their concentrations with increasing levels of BB. However, the meat content of these SFA had a quadratic effect, with lower value when 45% BB was added. In blood of rats, both 12:0 and 14:0 acid disappeared more rapidly than palmitic acid, suggesting the fast oxidation of these FA (Goransson 1965). In the same way, the decrease in saturated fatty acids digestibility from 12:0 to 18:0 also was reported in previously studies with ruminants (Steele & Moore 1968, Andrews & Lewis 1970, Dohme et al. 2004). So, these results suggest that 14:0 is intensively oxidized or elongated to 16:0 rather than stored in adipose tissue. The same type of response for 12:0 and 14:0 FA was recently reported in lambs fed high 12:0 babassu oil (Parente et al. 2020). Therefore, the possibility of elongation of FA chain due the BB addition cannot be discarded. Some minors FA's (20:0, 21:0, 22:2 and 24:2) were found only in meat from lambs fed 45% of BB. So, adding to the fact of greater medium-chain fatty acid (MCFAs) digestion, there is the possibility that some of them had been

used to be elongated in others FA's. These MCFAs also have the potential to adversely affect ruminal ciliate protozoa, several bacterial species, and methanogens (Henderson 1973, Soliva et al. 2003, Dohme et al. 2008), knowing odd chain FA are synthesized by microbes in the rumen and the low 15:0 and 17:0 proportions indicates a reduced microbial activity (Dohme et al. 2004), the quadratic effect on the minors SFA C15:0 and C17:0 are properly understood in this study. In this study, the BB caused a decreasing of the SFAs and cholesterol in meat, constituting an animal nutritional management strategy in favor of the compositional quality of meat.

It has been proposed that *De novo* lipogenesis (DNL) of SFA are preferentially desaturated by SCD in adipocytes compared with exogenous SFA (Collins et al. 2010), and probably because of this, the stearyl-CoA desaturase activity (SCD) reduced linearly with BB addition, which resulted in the effects on *c*-9 C14:1, *c*-9 C16:1 and *c*-9 C17:1 found. The increasing levels of BB addition also decreased linearly the *t*-11 18:1 proportion because the higher dietary SFA in BB diets, in contrast to reduced dietary unsaturated fatty acids (UFA), especially C18:2 *n*-6 proportion. In ruminant edible fats, *t*-11 18:1 can be formed in the rumen ecosystem through the incomplete biohydrogenation ruminal process and/or from 18:0, especially through SCD activity in adipocyte. Thus, whenever an accumulation of trans-18:1 in the rumen is found, the basic explanation has been that this is a consequence of changes in rumen microbiota resulting in less activity of the final reductive step of the biohydrogenation rate (BHR), (Bessa et al. 2015). In this study, the slightly increased in dietary SFA, in opposite to decreased UFA as BB was added, resulted in a possible lower BHR and consequently, the intermediate products production, as *t*-11 18:1.

The positive quadratic effect observed for *c*-9 18:1 content, even 18:0 proportion did not

change is explained by the large individual variability, more specific because two lambs fed 45% of BB. Only in meat from these two animals, 18:0 proportion was lower, suggesting that c-9 18:1 is a product from 18:0 by SCD activity (Bessa et al. 2015).

The health index results are in line with the SFA and MUFA contents, showing a positive effect of BB inclusion on the fatty acid profile of the meat. Of the five ratios associated with human health evaluated in this study, only the PUFA/SFA ratio was not influenced by diet. A linear effect was observed in the other ratios. We emphasize that this effect is mainly due to the lower percentage of SFA caused by the increasing inclusion of BB in the lamb diet. A higher MUFA/SFA ratio was observed at the level of 45% of BB (2.14). This result can be explained by the high concentration of oleic acid (56.81%).

The ratio (C18:0+C18:1)/C16:0 revealed the potential beneficial effects of lipids to human health resulting from the ingestion of red meats, reporting levels from 2.1 to 2.8% for sheep meat (Santos-Silva et al. 2002, Costa et al. 2009). In the present study, the level of 45% of BB in the diet presented the highest values, with an average of 5.12%. The ratio between hypocholesterolemic / hypercholesterolemic fatty acids (h/H), complements the evaluation of the fatty acid profile, where higher values are desirable. Its value reflects the risk of incidence of heart diseases for meat products (Sacks et al. 2017). In the present study, the h/H ratio presented the highest average, 4.1%, for the diet with 45% of BB. This result is explained by the lower content of hypercholesterolemic fatty acids. Several authors have reported h/H values between 0.39 to 2.1 (Santos-Silva et al. 2002, Borghi et al. 2016, de Abreu et al. 2019). It is desirable that the sheep meat presents in its composition a lower content of SFA and a higher content of PUFA, since the unsaturated fat is beneficial to human

health, reducing the risk of heart disease. In this study, there was a reduction up to 48.7% in saturated fatty acids. Therefore, at the level of 45%, only 564.2mg/100g of the fatty acids identified are considered harmful to human health.

In the present days, consumers are being “bombarded” with derogatory news about meat quality and production. In response, we believe that meat science needs adaptive approaches to oppose this trend, since there are nutrients that we need that are only found in meat. On the other hand, other nutrients, which are present in other foods, might also be found in meat, whose organoleptic characteristics are unique. Nutritional strategies as the one presented here show that the production of sheep meat with desirable organoleptic and quality characteristics is possible.

The replacement of corn by biscuit bran sweet type in the feeding of lambs represents an alternative nutritional strategy for sheep meat production with desirable organoleptic and quality characteristics. Additionally, their use up to 45% of corn replacement in the diet would favor the production of sheep meat with potential benefits for human health. The biscuit bran is recommended for sheep production in regions where the cost and availability of this product are favorable or where there is market demand for the meat characteristics presented here. Finally, our results suggest the need of further research in order to study the effect of total replacement of corn by biscuit bran in the physicochemical characteristics of sheep meat and their physiological and productive impacts.

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#### RENATA T. ALENCAR<sup>1</sup>

<https://orcid.org/0000-0001-8667-3272>

#### WILDER H.O. VEGA<sup>1</sup>

<https://orcid.org/0000-0001-9742-4501>

#### LUIZA N.C. DA SILVA<sup>1</sup>

<https://orcid.org/0000-0001-8922-8455>

#### HÉLIO H.A. COSTA<sup>2</sup>

<https://orcid.org/0000-0002-8875-8515>

#### MICHELLE O.M. PARENTE<sup>2</sup>

<https://orcid.org/0000-0001-6906-7455>

#### LISIANE D. DE LIMA<sup>3</sup>

<https://orcid.org/0000-0001-8543-0672>

#### ALINE V. LANDIM<sup>1</sup>

<https://orcid.org/0000-0002-4129-1161>

<sup>1</sup>Centro de Ciências Agrárias e Biológicas, Departamento de Ciência Animal, Universidade Estadual Vale do Acaraú / UVA, Betânia, 62040-370 Sobral, CE, Brazil

<sup>2</sup>Centro das Ciências Agrícolas e Ambientais,  
Universidade Federal do Maranhão, BR 222, Km  
04, s/n, 65080-805 Chapadinha, MA, Brazil

<sup>3</sup>Embrapa Caprinos e Ovinos, Fazenda Três Lagoas,  
Caixa Postal 71, 62010-970 Sobral, CE, Brazil

Correspondence to: **Wilder Hernando Ortiz Vega**

*E-mail: wilortvet@yahoo.es*

### **Author contributions**

All authors contributed to the work presented here, read and approved the final manuscript. Aline Veira Ladim, Hélio Henrique Araújo Costa and Lisiane Dorneles de Lima, designed the experiments. Wilder Hernando Ortiz Vega and Michelle de Oliveira Maia Parente analyzed the data and wrote the paper. Renata Teixeira Alencar and Luiza de Nazaré Carneiro da Silva, carried out the experiments in field and collected and tabulated data.

