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Development and Evaluation of an Extruded Balanced Food for Sheep Based on Cottonseed Meal (*Gossypium hirsutum*)

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Abstract

The objective of this research was to evaluate the effect of the content of cottonseed meal (*Gossypium hirsutum*) and the processing variables on the functional properties and the content of gossypol of an extruded feed for sheep (*Ovis aries*). The diet was balanced according to the requirements of fattening Dorper sheep breed under 1 year. The extrusion process was optimized using a surface response methodology, with four independent variables: temperature in the last heating zone (120–160°C), moisture content (14–18%), screw speed (120 rpm–180 rpm), and cottonseed meal content ($9 \text{ g}^{-27} \text{ g } 100 \text{ g}^{-1}$), in a single screw extruder. The optimal food had 27.25% crude protein, 4.24% crude fat, 12.21% crude fiber, 46.95% nitrogen-free extract, and 9.35% ash. The composition of essential amino acids in the optimal diet was 1.00 g kg^{-1} of lysine, 1.25 g kg^{-1} of phenylalanine, 2.04 g kg^{-1} of leucine, 0.87 g kg^{-1} of isoleucine, 0.98 g kg^{-1} of threonine, 1.15 g kg^{-1} of valine, and 0.65 g kg^{-1} of histidine. The fatty acids present in the highest concentration in the optimal diet were 2.14% linoleic acid, 1.11% oleic acid, and 0.81% palmitic acid. The gossypol content of the optimal diet was less than 0.1%, which ensures the safety of cottonseed meal as a protein source. The optimum conditions of the extrusion process were 120°C temperature, 120 rpm screw speed, 14.00% humidity, and $27 \text{ g } 100 \text{ g}^{-1}$ cottonseed meal.

Keywords: cottonseed meal, sheep, balanced feed, extrusion, gossypol

1. Introduction

Even though new livestock farming technologies are constantly being developed, worldwide many grazing animals feed on pastures, grasslands, crop residues, etc. due to their low input costs and better resilience to market fluctuations [1]. In Mexico and

South America, the increased demand for sheep meat due to historical and cultural traditions generates an attractive market that has led to the intensification of sheep livestock production. In these regions, most producers use a grazing scheme for their animals while a small sector feeds them under stable weight-gain systems. In these conditions, sheep production requires high reproductive efficiency and low feeding costs. Balanced food is a necessity not only for the animal but also for the producer because it allows storage for long periods, provisioning in times of shortage, saving time in preparation, and ease of handling when feeding animals. Cottonseed meal is a by-product of cotton used for animal feed as it is rich in oil and protein. However, the gossypol content limits the use of cotton seeds in animal feed. High levels of free gossypol may be responsible for acute clinical signs of gossypol poisoning that include shortness of breath, decreased body weight gain, anorexia, weakness, apathy, and death after several days [2]. Gossypol is a phenolic compound with a molecular weight of 518.55 Dalton; it is a yellow, crystalline pigment, insoluble in water, soluble in acetone and chloroform, and is produced by the glands of the cotton plant [3]. Gossypol exists in the cotton plant as a defense agent and is responsible for toxicity problems associated with excessive feeding of cottonseed meals in animals [2]. In addition to animal toxicity, it has been reported to have anticancer, antiviral, and male infertility effects [2]. Ruminants can digest gossypol better than monogastric animals, so cottonseed meal is only used up to 23% in ruminant feed, due to the presence of gossypol, resulting in limited use [4]. Preventive procedures to limit the toxicity of gossypol involve the treatment of the cottonseed product to reduce the concentration of free gossypol with the most common treatment: thermal processing.

Extrusion has been described as a continuous-flow reactor capable of processing biopolymers and ingredient mixtures at high temperatures, pressures, and shear forces at low humidity. In addition, extrusion has a lower processing cost compared to other thermal processing, and being a continuous process, it has been used to modify functional properties [5]. The objective of this research was to produce an extruded feed for ruminants based on cottonseed meal and to evaluate its functional properties as well as the gossypol content.

2. Materials and methods

2.1 Diet formulation

A diet for fattening Dorper sheep breed (under 1 year, not castrated) was balanced using WinFeed 2.8© (1999–2004) program with the projected nutritional characteristics shown in **Table 1**. For the formulation of the treatments, cottonseed meal (*Gossypium hirsutum*) (CSM), dry molasses, soybean meal (44% protein), nixtamalized corn (*Zea Maize*) (NC), and dried distillers' grains with solubles (30% protein) (DDGS) were used, which were purchased from the main animal food shops in the municipality of Durango, Mexico. CSM, dry molasses, soybean meal, NC, and DDGS were subjected to grinding in a commercial coffee mill to reduce the particle size, which was sieved using a 40 mesh. The ratio of ingredients consisted of 12 g 100 g⁻¹ of soybean meal, 15 g 100 g⁻¹ of DDGS, 7 g 100 g⁻¹ of dry molasses, and 30 g 100 g⁻¹ of NC that were kept constant. Five different diets with different ratios of CSM and NC were evaluated according to the experimental design: 0:36, 9:27, 18:18, 27:9, and 36:0 g of CSM: g of NC 100 g⁻¹, respectively.

Nutritional characteristics	Composition [%]
Dry matter	211.905
Crude protein	28.65
Energy	1368.3 (Kcal/kg)
Neutral detergent fiber	6.48
Lysine	0.424
Methionine	0.144
Calcium	0.041
Phosphorus	0.162

Table 1.
Projected nutritional characteristics of the formulation using WinFeed 2.8©.

2.2 Extrusion processing

The extrusion of the treatments was performed using a single screw laboratory extruder (compression ratio 1:1) Brabender brand Model 20DN/8–235-00 (Duisburg, Germany), ¾” L/D – 25:1 ratio with the following characteristics: four heating zones (90, 100, and 110°C for the first, second and third zone, respectively, and the fourth one was adjusted according to the experimental design), screw compression ratio 1:1, screw diameter of 19 mm and exit die diameter of 6 mm i.d. Before extrusion, formulated mixtures were prepared, and moisture content was adjusted following the experimental design. The desired moisture level was adjusted by spraying distilled water onto the mix of ingredients, which was then hand-mixed for 15 min and conditioned for 12 h in closed plastic containers at 4°C. Three separate extrusion runs were carried out for each treatment. Extruded treatments were cooled down at room temperature for 1 h and stored in sealed polyurethane bags at 4°C for further analyses.

2.3 Experimental design and data analyses

A rotatable central composite experimental design ($\alpha = 2$) with four independent variables was performed (**Table 2**) and 27 treatments were generated. The responses were expansion index (EI), bulk density (BD), penetration force (PF), water absorption index (WAI), water solubility index (WSI), and water activity (WA). Numerical optimization was performed using the superimposition of surface response for each

Variables	Levels				
	- α	-1	0	+1	+ α
Temperature [°C]	100	120	140	160	180
Moisture content [%]	12	14	16	18	20
Screw speed [rpm]	90	120	150	180	210
Cottonseed meal content [g 100 g ⁻¹]	0	9	18	27	36

Table 2.
Levels of independent variables.

treatment (Design Expert Version 13.0). Experimental data was adjusted to quadratic models, and regression coefficients were obtained. Statistical significances of the regressions' terms were examined by variance analyses (ANOVA) for each response ($p < 0.05$).

2.4 Determination of physical and functional properties

2.4.1 Expansion index and bulk density

Ten randomly selected extruded samples of each treatment were measured in diameter (d) and length (L). Each sample was taken three measurements of the diameter, the average value was calculated and then the extruded diameter was divided by the diameter of the hole of the exit die placed in the extruder nozzle using a vernier. Then each extruded (Pm) was weighed to determine the density using Eq. 1 [6].

$$\text{Density} = \frac{Pm}{\left(\pi \left(\frac{d}{2}\right)^2 L\right)} \quad (1)$$

2.4.2 Penetration force

The determination of the penetration force of the extrudates was performed using a Universal Texture Analyzer TA-XT2 (Texture Technologies Corp., Scarsdale, NY/ Stable MicroSystems, Haslemere, Surrey, UK) using a Warner Bratzler blade. A total of 15 samples were measured per treatment, at a speed of 1 mm s^{-1} , recording the average of the maximum penetration force.

2.4.3 Water absorption index and water solubility index

The extrudates of each treatment were ground in a commercial coffee mill to a particle size of mesh 40. In a pre-weighed centrifuge tube, 1 g of sample per treatment was weighed, and 10 mL of distilled water was added, stirred for 30 minutes, and centrifuged at 3000 rpm for 15 min. The supernatant was decanted and evaporated to dryness in a convective stove at 97°C ; the residue was weighed, and the WSI was calculated using Eq. 2. After decanting the supernatant, the remaining sediment in the tube was weighed to calculate the WAI using Eq. 3 [7]. Both analyses were evaluated in triplicate.

$$\text{WSI} = \frac{\text{Weight of the dry supernatant}}{\text{Weight of dry sample}} \times 100 (\%) \quad (2)$$

$$\text{WAI} = \frac{\text{Sediment weight}}{\text{Weight of dry sample}} \text{ (g de H}_2\text{O g}^{-1} \text{ of sample)} \quad (3)$$

2.4.4 Water activity

The water activity was measured using HygroLab C1 equipment (ROTRONIC, Measurement solutions, Process sensing technologies), each treatment was evaluated in duplicate with an accuracy of ± 0.003 .

2.4.5 Proximal chemical analysis

The moisture content was evaluated by drying the sample in a stove at 105°C until it reached constant weight (925.10, [8]). The ash content was determined by calcination of the sample in an oven-muffle at 550°C until obtaining constant weight (923.03, [8]). The protein content was determined from the composition of the total nitrogen in the samples, using the Kjeldahl technique, according to AOAC Method 910.87 (2019). The crude fat content of the sample was determined using the hot fat extraction method, Soxhlet equipment and petroleum ether (40–60°C), according to method 920.39, AOAC [8]. Neutral detergent fiber and acid detergent fiber contents were evaluated following the procedures proposed by Van Soest et al. [9]. The nitrogen-free extract was obtained by difference from the obtained values of moisture, crude protein, crude fat, and ash (Eq. 4).

$$\%NFE = 100 - (\%Moisture + \%Crude\ protein + \%Crude\ fat + \%Crude\ fiber + \%Ash)$$

(4)

2.5 Determination of gossypol concentration

The concentration of gossypol was obtained following the official Mexican standard NOM-Y-217-A-1982 to analyze free gossypol in cottonseed meal for animal feed using a factorial design where the variables were temperature (120, 140, and 160°C) and moisture content (14, 16, and 18%).

2.6 Mineral determination

From the optimal treatment, 500 mg were taken and washed at 150°C with 15 mL of concentrated HNO₃ and 2 mL of 70% HClO₄. The samples were dried at 120°C and the residues were dissolved in 10 mL of a 4.0% HNO₃–1% HClO₄ solution. The mineral content of each sample was determined inductively by argon plasma emissions by atomic spectroscopy.

2.7 Fatty acids determination

From the optimal treatment, samples were extracted using an ASE 200 system (Dionex, Idstein, Germany) using 11 mL extraction cells. An azeotropic mixture of cyclohexane and ethyl acetate was used as a solvent. The conditions used were temperature, 80°C; pressure, 10 MPa; preheating, 0 min; heating, 5 min; static, 10 min; flow, 60%; purging, 120 s; and 2 cycles. The remaining fraction was condensed with a broken evaporator (180 mbar, 30°C) and then evaporated using a stream of nitrogen [10]. The samples were analyzed in a Hewlett-Packard 5890 series II gas chromatograph. A capillary column covered with 100% cyanopropyl polysiloxane (CP-Sil 88, 50 m × 0.25 mm, 0.20 μm, Chrompack, Middelburg, The Netherlands), started at 60°C (waiting time of 1 min), increased in intervals of 7°C min⁻¹ until reaching 180°C, then 3°C min⁻¹ to 200°C (waiting time 1 min) and finally 10°C min⁻¹ to 230°C (waiting time 10 min). Helium was used as carrier gas at a constant flow of 1.3 mL/min. Nitrogen was used as the makeup gas.

2.8 Determination of amino acids

The optimal treatment was analyzed to determine the amino acid content using an Agilent 1260 Infinity chromatograph equipped with a microdegassifier (G1379B), a 1260 binary pump (G1312B), a multiple wavelength standard detector (G1315C), and a Zorbax Eclipse-AAA column (150 mm × 4.6 mm, 5 µm, internal particle diameter, Agilent Technologies, Santa Clara, CA). The samples were freeze-dried, ground, and hydrolyzed. A total of 1 g of sample was weighed and HCl was added. The samples were hydrolyzed for 24 h to 110°C. After hydrolysis, the samples were vacuum evaporated, and the hydrolysates were reconstituted in 2 mL of HCl.

3. Results and discussion

3.1 Determination of physical and functional properties of extruded treatments

Table 3 shows the regression coefficients of the extruded treatments. The expansion index (EI) was negatively affected ($p \leq 0.05$) by the temperature in its quadratic term, possibly because high temperatures in extrusion cause degradation of starch molecules and result in reduced expansion [11]. The cottonseed meal content had a negative effect ($p \leq 0.05$) because the complexes that form proteins with starch and fiber disrupt the cutting force as a result of the interactions of the components; protein molecules could affect the gelatinization process in different ways depending on their ability to retain water and their ability to interact with starch molecules and surface granules [12]. The decrease in EI can be attributed to the fact that, during the extrusion process at high temperatures, starch undergoes further degradation and may become more dextrinized, reducing the EI values that are accentuated in mixtures with low starch content. The water absorption index (WAI) was positively affected ($p \leq 0.05$) by temperature, this being a property that indicates the amount of water retained by starch since a proliferation of hydrophilic sites allows greater accessibility of water to interact through hydrogen bonds [13]. The temperature-humidity interaction had a significant positive effect ($p \leq 0.05$) since water acts as a plasticizer during extrusion cooking, thus reducing the degradation of starch granules and resulting in a greater capacity for water absorption [14]; in addition, high temperatures increase the degradation and dextrinization of starch [15]. As the temperature increases, hydrogen bonds decrease just like the hydration of the ionic groups, so a denatured protein generally binds 10% more water than its native equivalent, in addition to increasing the surface area of proteins. However, it should also be borne in mind that the aggregation phenomenon may occur, increasing protein-protein interactions, and thus decreasing its water-binding capacity. For the water solubility index (WSI), humidity has a significant negative effect ($p \leq 0.05$). WSI measures the number of soluble components released from starch after extrusion and is related to the degree of polymerization of starch occurring within the extruder [16]. The low moisture content decreases the gelatinization of the starch because high temperatures decrease the humidity, lowering the availability of water for the starch granules. Sobuñola et al. [15] state that this is due to the interactions between starch, protein, fiber, and lipids. These interactions can increase the molecular weight of the complex formed causing a decrease in the solubility index. Pardhi et al. [16] report that high humidity levels result in low levels of WSI in the extrudate. Jong-Bang et al. [17] showed that a low humidity together with a high speed decreases the WSI, however, increasing the temperature increases the WSI, due to the depolymerization of the

Response	Intercept	Lineal				Quadratic				Interactions					
	b ₀	X ₁	X ₂	X ₃	X ₄	X ₁ ²	X ₂ ²	X ₃ ²	X ₄ ²	X ₁ X ₂	X ₁ X ₃	X ₁ X ₄	X ₂ X ₃	X ₂ X ₄	X ₃ X ₄
EI	1.29E+00	1.85E-04	-1.42E-02	2.06E-02	-1.81E-03	-6.06E-02*	-3.00E-02	-4.93E-02	-5.22E-02*	-2.55E-02	-8.13E-03	-1.89E-02	-1.44E-02	4.75E-02	-1.86E-02
BD	1.56E+03	4.21E+02	-3.80E+02	-4.08E+02	4.43E+02	1.04E+02	1.55E+02	-2.22E+01	8.11E+01	-5.12E+02	-6.22E+02	6.49E+02	5.58E+02	-5.94E+02	-5.87E+02
PF	1.00E+02	3.54E+00	1.07E+00	-3.03E+00	2.22E+00	3.35E+00	-1.91E+00	-6.01E-01	2.72E+00	-1.55E+00	-5.01E+00	-9.44E-01	2.05E+00	1.59E+00	-1.18E+00
WAI	3.48E+00	3.69E-02*	-2.64E-03	-1.15E-02	-3.74E-03	1.40E-02	-6.49E-04	-1.82E-02	-3.19E-03	4.63E-03	-4.17E-02*	4.74E-02	-6.84E-05	-6.68E-03	-1.70E-02
WSI	1.05E+01	9.65E-01	9.78E-01	-1.10E+00*	5.63E-01	5.53E-01	4.46E-01	4.72E-01	5.47E-01	9.90E-01	-1.05E+00	6.13E-01	-1.09E+00	-2.26E-01	-1.05E-01
AW	6.65E-01	-1.86E-02*	3.04E-03	3.58E-02*	-1.49E-03	-1.55E-02	-2.35E-03	2.86E-03	-7.95E-03	-6.42E-03	2.17E-02	1.08E-02	-7.44E-03	-4.93E-03	-5.93E-04

EI = expansion index, BD = bulk density, WAI = water absorption index, WSI = water solubility index, AW = water activity. X₁ = Temperature (°C), X₂ = screw speed (rpm), X₃ = moisture content (%), X₄ = cottonseed meal content (%), *indicates statistical significance (p > 0.05).

Table 3.
Regression coefficients of the surface response models.

starch and other macromolecules present in the mixture, which leads to the reduction of the chain's amylose and amylopectin.

3.2 Numerical optimization

The numerical optimization was performed by a superimposition of surface response method, obtaining the following conditions: 120°C temperature, 120 rpm screw speed, 14% moisture content, and 27:9 g of CSM: g of NC 100 g⁻¹; the responses obtained for the optimal feed were: 150.75 N of penetration force, 3.48 g g⁻¹ of water absorption index, 11.79% of water solubility index and 0.62 of water activity. The criteria used for the optimization of the factors were to minimize temperature, moisture content, screw speed and to maximize cottonseed meal content (by substituting soybean meal) to reduce processing and formulation costs.

3.3 Proximal chemical analysis

The optimal formulation of the diet consisted of 12 g 100 g⁻¹ soybean meal, 15 g 100 g⁻¹ DDGS, 7 g 100 g⁻¹ molasses, 30 g 100 g⁻¹ nixtamalized corn, and 27:9 g of CSM: g of NC 100 g⁻¹ cottonseed meal, meet the nutritional requirements for sheep <1 year [18], and it was compared against some similar commercial foods (**Table 4**).

The high protein content of the food is desirable because the feeding of ruminants is supplemented by fodder in percentages of 60% balanced food and 40% fodder, since fodder usually has very low nutritional value, especially in developing countries during dry seasons. For instance, mature grasses only have crude protein levels of 3.5–8%. As an example, during the early dry season in Samoa (May, June, and July), crude protein content in batiki bluegrass dramatically decreases and ranges only between 3% and 9% [19]. Also, the supply of amino acids depends on the protein content in the diet, from the transfer through the rumen to the intestines as undegraded vegetable protein and microbial protein, and its absorption in the small intestine; furthermore, cottonseed meal has a better response compared to other protein sources, such as hay, for animal fattening [20]. National Research Council [18] indicates that energy is the most limiting factor in the nutrition of small ruminants, an energy deficiency will lead to low production, poor reproduction, high mortality, and susceptibility to diseases and parasites. Minerals play an important role in the functioning of the body's cells as they promote the health of the skin and promote growth. The type of carbohydrate found in the diet conditions the development of the type of flora suitable for fermentation and the pH adjustment to its ideal range. Thus, a starch-rich ration is fermented by an amylolytic flora that performs best at pH from 5.5 to 6.0. Fiber, as a nutrient,

Sample	Moisture [%]	Total dry matter [%]	g 100 g ⁻¹				
			Crude protein	Crude fat	Crude fiber	Nitrogen free extract	Ashes
Optimal food	8.66	91.34	27.25	4.24	12.21	46.95	9.35
Commercial food	9.5	90.50	16.51	2.98	13.66	56.36	16.51
Requirement*	—	90	14.5	3	10	52	7.5

*Nutritional requirements for sheep less than 1-year-old [18].

Table 4.
Proximal chemical analysis of balanced optimal diet for sheep.

contributes to the maintenance of ruminal functioning, by acting as ruminal filling and stimulating ruminal physicochemical contractions and conditions.

3.4 Amino acids profile

Some vegetable proteins are deficient in sulfur amino acids (AA) compared to animal proteins and contain antinutritive factors. Nevertheless, by being supplemented with other proteins and physicochemical treatments, oilseed protein makes a significant contribution to human and animal dietary protein intake [21]. Extruded feeding increases the flow of AA to the duodenum of ruminants by 34% and increases the apparent absorption of AA in the small intestine by 58% [22]. The ruminant can synthesize arginine, although in insufficient quantities to meet the nutritional requirements, especially important during early growth or in reproductive stages. Aspartic acid and glutamic acid are rapidly metabolized and produce volatile fatty acids, histidine being one of the limiting amino acids in ruminants [23]. The optimal food meets most of the nutritional requirements of AA (**Table 5**). It was observed that both aspartic acid and glutamic acid are found in large amounts, which is desirable because they are metabolized very quickly and produce volatile fatty acids. Lysine contributes to weight improvement and its requirement in sheep is 2.78 g d^{-1} ; since the food contains 1 g kg^{-1} , it covers the daily needs of the animal. The ruminant can synthesize arginine, although in insufficient quantities to meet body requirements: 2.01 g d^{-1} [18].

Amino acid	Sample [g kg^{-1}]	Nutritional requirement [g d^{-1}] *
Lysine	1.00	2.78
Phenylalanine	1.25	3.9
Leucine	2.04	6.03
Isoleucine	0.87	4.01
Threonine	0.98	3
Valine	1.15	3.5
Histidine	0.65	1.01
Arginine	1.95	2.01
Glycine	1.00	—
Aspartic acid	2.33	—
Serine	1.31	—
Glutamic acid	4.52	—
Proline	1.38	—
Hydroxyproline	0.06	—
Alanine	1.25	—
Tyrosine	0.79	2.7

*Nutritional requirements for sheep less than 1-year-old [18].

Table 5.
Amino acid profile in the optimal food.

3.5 Fatty acids profile

Fatty acids are precursors that help the production of volatile fatty acids, being the main acetic, butyric, and propionic, which cover most of the energy requirements. Feed rations produce a lower concentration of volatile fatty acids compared to those based on concentrates with a high content of proteins or easily fermentable carbohydrates. The proportion of each of the volatile fatty acids in the mixture varies with the quality, quantity, and texture of the food ration components. Grain-based concentrates, which include heat and pressure treatment, are fermented more quickly and favor the production of propionic acid. This increase in digestibility occurs because during the previous treatment a certain degree of fragmentation of starch granules and partial hydrolysis of starch molecules occurs. The fatty acid profile of the optimal food consisted of 2.142% linoleic acid, 1.114% oleic acid, 0.122% stearic acid, 0.812% palmitic acid, 0.014% lauric acid, 0.015% myristic acid, and 0.016% palmitoleic acid. The optimal food provides an amount of linoleic acid above the nutritional requirements for sheep under 1 year (1.7 g d^{-1} , [18]), which can have a positive effect on the production of conjugated linoleic acid.

3.6 Minerals profile

The minerals profile of the optimal food meets the nutritional requirements of most micro- and macro-minerals (Table 6). For a ruminant, the main macro-minerals in his diet are Fe, Cu, Zn, and Mn. Iron represents 0.33% of the hemoglobin molecule, being necessary for the transport of oxygen by the blood to the tissues, in addition to being involved in the synthesis of myoglobin (muscle constituent) and ferritin. The optimal food had 475 ppm of Fe, being the maximum upper limit of Fe of 500 ppm. Cu intervenes in the fertility, enzymatic activation, and as a growth factor, being the upper limit of Cu of 50 ppm. Zn is a constituent of the hoof; in addition to reducing stress, somatic cells restore epithelial and are a fertility factor in adult animals; the minimum and maximum requirements are 22 and 150 ppm, respectively [18].

The food showed a concentration of 1.67% and 1.064%, for Ca and P, respectively. National Research Council [18] reports that Ca and P requirements are 0.51% and

Mineral	Concentration	Requirements*
Calcium	1.67%	0.51% ¹
Phosphorus	0.77%	0.24% ¹
Sodium	0.31%	—
Magnesium	0.349%	—
Manganese	74.9 ppm	—
Iron	475 ppm	500 ppm ²
Zinc	58.1 ppm	22 ppm ²
Copper	9 ppm	50 ppm ²
Potassium	1.064%	—

*Nutritional requirements for sheep less than 1-year old (¹Molle and Landau, 2017; ²National Research Council, 2007).

Table 6.
Mineral content in the optimal food.

Sample	Moisture [%]	Temperature [°C]	Free gossypol [%]
1	14	140	0.2404
2	18	140	0.1320
3	14	160	0.1216
4	18	120	0.1278
5	14	120	0.0752
6	16	160	0.0895
7	18	160	0.1047
8	16	120	0.1096
9	16	140	0.0957

The safe level of gossypol in diets for ruminants less to 1 year old is 0.1% [25].

Table 7.
Content of free gossypol in the optimal food.

0.24%, respectively. These results cause possible hyperparathyroidism or urolithiasis to be questioned, since high levels of Ca reduce the use of other minerals, although it has been reported that if it is not exceeded 2% and 3%, for Ca and P, respectively, there would be no problem in the animals [24].

3.7 Gossypol

The free gossypol content in the analyzed samples of the optimal diet was below 0.1% (**Table 7**). It has been reported that it is not advisable to feed ruminants less than a year and a half with a diet with free gossypol content greater than 0.1%, while adult ruminants can feed with levels greater than 1% [25]. It is observed that samples 5, 6, and 9 are those with the lowest gossypol content, which are ideal for ruminants less than 1-year-old. Sample 5 has the same extrusion conditions as the optimal feed. It should be noted that sample 1, despite having a high temperature compared to sample 5, has the highest concentration of gossypol. It has been reported that the moisture content during extrusion helps the destruction of some aflatoxins, in addition to a high moisture concentration in the extruded product increases the loss of toxic factors [6]. According to Gomes et al. [4], cotton flour contains 0.1–0.4% free gossypol. The processing of cotton flour, especially at high temperatures, favors the reduction of gossypol and it has been suggested that diets containing up to 200 mg of free gossypol are safe for ruminants, while 400 mg is the limit for considering it toxic, and, at levels greater than 800 mg, causes death [4].

4. Conclusions

The obtained extruded optimal feed met the nutritional requirements of Dorper sheep <1 year, showing a good composition of amino acids, fatty acids, and minerals. Also, since the gossypol content was less than 0.1% in the diet, cottonseed meal might be a good alternative as a protein source to feed small ruminants at early development stages.

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