Research Article

Evaluation of the Physicochemical Properties of an Extruded Cattle Feed Added with Huizache (*Vachellia Schaffneri*) and its Impact on *In Vitro* Digestibility

García Domínguez María Fernanda[1], Páez Lerma Jesús Bernardo[1], Reyes Jáquez Damián[1], Carrete Carreón Francisco Oscar[2], Rosas Guerra María Inés[1], Araiza Rosales Elia Esther[2]*

[1]Tecnológico Nacional de México/Instituto Tecnológico de Durango, Blvd. Felipe Pescador 1830, Nueva Vizcaya, 34080 Durango, Dgo; [2]Universidad Juárez del Estado de Durango, Facultad de Medicina Veterinaria y Zootecnia. Carretera al Mezquital Km 11.5. Durango, Dgo.

Abstract | This research aimed to develop and evaluate a balanced feed with the addition of huizache (*Vachellia schaffneri*). Eight formulations including huizache (HUI) in different percentages (0-10%) were evaluated. Subsequently, a D-optimal design was used with the extruder outlet temperature (90-150 °C, X_1), humidity (14-18%, X_2) and HUI concentration (0-10%, X_3) as factors. The evaluated responses were bulk density (BD), expansion index (EI), water absorption index (WAI), water solubility index (WSI), and hardness (H), as well as total phenols (TP), condensed tannins (CT) and *in vitro* dry matter digestibility (IVDMD). With the superimposition of response surfaces, a numerical optimization of the extrusion process was conducted based on the selected model. The optimal processing conditions for the elaboration of the extruded product were X_1=150 °C, X_2=14% humidity and X_3=10% HUI, with an EI of 1.107, BD of 1060.79 kg/m^3, H of 145.2 N, WAI of 3.51 g/g, WSI of 7.58%, TP of 0.246%, CT of 0.173% and IVDMD of 93.82%. According to the results, HUI is an ingredient that can be included in the formulation of extruded products for animal consumption, as it could improve rumen fermentation.

Keywords | Additive, Animal feeding, Balanced feed, Temperature, Humidity, Extrusion

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***Correspondence** | Araiza Rosales Elia Esther, Universidad Juárez del Estado de Durango, Facultad de Medicina Veterinaria y Zootecnia. Carretera al Mezquital Km 11.5. Durango, Dgo; **Email:** e_araiza2002@hotmail.com
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INTRODUCTION

The current population growth requires increases in livestock production to meet the food demand (INEC, 2018). According to the FAO (2017), by 2050, the population will exceed the alarming figure of 9 billion people. However, the increase in agricultural production implies a greater exploitation of natural wealth (Carro *et al*., 2018), which is increasingly constrained by the increase in greenhouse gases that cause climate change (Garcia *et al*., 2020). In the search for alternatives to avoid competition to produce food for consumption, the limitations of planting areas, in addition to reducing the environmental impact, developing non-conventional livestock feeds has been chosen by incorporating plants with little or no use in cattle feed (Holguín *et al*., 2020). Using these tree or shrub plants to develop livestock feed could benefit arid areas susceptible to food shortages without becoming competition for human food (Pámanes *et al*., 2021). Huizache (HUI) is a shrubby legume distributed in Mexico, whose leaves and pods are used as a nutritional supplement in animal feed (Zarza *et al*., 2020) for its protein content that allows it to be used in diets to produce livestock species (Gómez, 2019). The use of plants such as HUI could reduce dependence on the use of external ingredients, thus minimizing damage to natural resources. Technologies such as extrusion make possible to explore the use of various inputs for feed formulation (Verhelts, 2019). Extrusion is a versatile and modern process that can transform agricultural products into fully cooked food products (Cotacallapa *et al*., 2021) using high processing temperatures in relatively short times (da Silva *et al*., 2018), without significantly altering the nutritional value of the food (Leonard *et al*., 2020). Therefore, this research aimed to evaluate the effect of temperature, moisture content and HUI content of an extruded cattle feed on its physicochemical properties and *in vitro* digestibility.

MATERIALS AND METHODS

Ingredients

HUI (*Vachellia schaffneri*) was collected from Francisco I. Madero, Durango, México in March 2021. The branches were sampled to collect leaves; the leaves were dried in a forced-air oven until constant weight at 55 °C, and the leaves were ground in a knife mill fitted with a 1 mm mesh to obtain a homogeneous sample for further analysis. Flour, soybean paste, soybean husks, distillers' dried grains, ground corn (MM) and molasses were purchased from commercial feed stores in Durango, Mexico.

Formulation/Experimental Treatments

A balanced feed of 12% crude protein (CP) was formulated for fattening cattle (NRC, 2001) with the inclusion of HUI (X_3) in percentages of 0-10%, according to the experimental design. The ingredients used for the formulation of the feed are shown in Table 1. The proportion of ingredients that remained constant in the formulation consisted of 21% soybean husks, 4% distillers' dried grains, 6% cane molasses, 5% soybean paste, 3.5% flour, and 3.5% wheat bran. The HUI substituted ground corn (GC) so that eight different treatments with different HUI and GC ratios were evaluated: 57:0, 56.94:0.06, 53.62:3.38, 53.43:3.57, 51.07:5.93, 51.01:5.99, 50.84:6.16 and 47:10.

Chemical Composition

The determination of CP, dry matter (DM), ash (ASH) and ether extract (EE) of ingredients and feeds was performed using AOAC (2019) standard methods. Cell wall components such as neutral detergent fibre (NDF), acid detergent fibre (ADF) and lignin (LIG) were determined using an ANKOM Fiber Analyzer (ANKOM Technologies, USA) and according to the calculations proposed by Van Soest *et al.* (1991). Non-structural carbohydrates (NSC) were determined by difference using Equation 1.

$$NSC=[100-(PC+EE+ASH+NDF)]$$

Extrusion Process

The extrusion of the treatments was conducted using a Brabender single-screw extruder (Duisburg Germany) with a 1:1 compression ratio, screw speed at 150 rpm, feed speed at 45 rpm, a 6 mm output die and four heating zones, with the first three at constant temperatures of 90, 100 and 110 °C, respectively, while the fourth temperature (X_1) was varied according to the experimental design. The treatments' moisture content (X_2) was adjusted before the extrusion process following the experimental design. The desired humidity level was adjusted by sprinkling distilled water into the ingredients and mixing it by hand for 15 minutes. Moistened mixtures were refrigerated at 4 °C and stored in sealed polyurethane bags for at least 12 hours before being extruded. Upon completion, the extruded samples were placed in trays to cool down at room temperature for 1 hour. They were then packed in polyurethane bags and refrigerated for further analysis.

Table 1: Chemical composition of ingredients in experimental treatments [g/100 g].

Ingredients	MS	CE	PC	USA	FDN	FDA	LIG
Huizache	93.41±2.769	6.61±0.002	14.01±0.263	1.34±0.265	39.54±1.981	32.86±1.91	8.15±0.685
Ground corn	89.34±0.417	0.74±0.377	6.53±0.304	1.89±0.0003	13.13±0.125	3.18±0.079	-
Wheat bran	89.72±0.876	6.44±0.312	15.23±0.195	1.46±0.021	36.83±1.25	13.35±0.566	1.69±0.142
Soybean husks	89.31±2.838	4.41±0.375	12.39±0.287	1.01±0.012	58.39±0.075	44.47±0.395	1.34±0.169
Soybean paste	90.51±0.257	7.14±0.551	40.57±0.922	0.98±0.036	5.31±0.483	3.34±0.243	0.34±0.056
Distiller's grains	90.51±0.257	7.16±0.169	27.49±0.103	4.61±0.079	40.65±0.221	14.23±0.221	1.93±0.444
Flour mill	87.82±0.278	6.94±0.291	41.93±0.855	3.82±0.078	27.15±0.936	17.77±0.646	4.23±1.082
Molasses	76.25±2.756	10.72±0.397	5.36±0.359	-	-	-	-

MS: dry matter; **PC:** crude protein; **EE:** ethereal extract; **CE:** ash; **NDF:** neutral detergent fiber; **FDA:** acid detergent fiber; **LIG:** lignin. Results are expressed as the mean ± standard deviation.

Experimental Design and Data Analysis

A D-optimal experimental design with three independent variables was conducted using the Design-Expert 13.0 software (State-Ease Inc., Minneapolis, MN, USA), and 15 experimental treatments were obtained. The evaluated factors were X_1 = die outlet temperature [90-150 °C], X_2 = moisture content [14-18%] and X_3 = HUI content [0-10%]. The responses of the experimental design were expansion index (EI), bulk density (BD), hardness (H), water absorption index (WAI), water solubility index (WSI), total phenols (TP), condensed tannins (CT) and *in vitro* dry matter digestibility (IVDMD. The results were analyzed by multiple quadratic regressions (Equation 2). Regression coefficients were obtained by fitting the experimental data to the quadratic model. The statistical significance of the coefficients (p<0.05) was evaluated through an analysis of variance for each response. A numerical optimization was performed through the methodology of overlapping response surfaces for each treatment.

$$Y= B_0+B_1\,X_1+B_2X_2+B_3\,X_3+B_{11}\,X_1^2+B_{22}\,X_2^2+B_{33}\,-_3^2+B_{12}\,X_1\,X_2+B_{13}\,X_1\,X_3+B_{23}\,X_2\,X_3$$

Physicochemical Properties

Expansion index and bulk density: The technique described by Oke *et al.* (2013) was used to determine EI and BD. For BD, ten extruded samples were randomly selected and then the average value of the diameter (d) was obtained with three measurements of each extruded. After that, the length (l) was measured, and each extruded sample (Pm) was weighed. The BD was obtained using Equation 3. For EI, the average value of the diameter was divided by the diameter of the outlet die hole.

$$Densidad = \frac{Pm}{\pi(\frac{d}{2})^2 l}$$

Hardness: H was evaluated using a Universal Texture Analyzer TA-XT2 (Texture Technologies Corp., Scarsdale, NY/Stable MicroSystems, Haslemere, Surrey, UK) with the Warner Bratzler blade. Fifteen extruded samples were measured per treatment, at a speed of 1 mm/s, recording the average of the maximum hardness.

Water absorption index and water solubility index: WAI and WSI were evaluated as described by Ding *et al.* (2005). One gram of ground product was sifted into a No. 40 mesh (0.420 mm). The sample was mixed in 10 mL of distilled water, then stirred for 30 min and centrifuged at 3000 x for 15 min. The resulting supernatant was decanted into a previously tared vessel. With the gel's weight obtained after the supernatant removal, the WAI was calculated as the unit weight of the solids on a dry basis and expressed as grams of water retained per gram after removal of the sample supernatant (g/g), while WSI was obtained by evaporating the supernatant and was expressed as the weight of the dry solids in the supernatant as a percentage of the original weight of the sample (%).

Phenolic compounds: To obtain the extracts, 0.5 g of sample was mixed and agitated in 45 mL of 70% ethanol for 24 h at room temperature. The extracts were filtered and dried until the ethanol was removed in the extraction hood. Yields were calculated based on the dry sample.

Total Phenols and Condensed Tannins

TP were determined using the Folin-Ciocalteu method, and CT were determined using the vanillin method, both described by Heimler *et al.* (2005), using gallic acid and catechin as standards, respectively.

In Vitro Dry Matter Digestibility

IVDMD was determined with a Daisy digester (ANKOM Technology, Fairport, NY, USA) according to the manufacturer's manual of techniques (ANKOM, 2017) using an inoculum prepared from buffer solutions and liquid extracted from a fistulated bovine in a 2:1 ratio.

RESULTS AND DISCUSSION

Chemical Composition of Ingredients

Table 1 shows the chemical composition of the feed ingredients. The CP content of the HUI was 14%, which differs from other authors (Zapata *et al*., 2020: Araiza *et al*., 2022a), who analyzed the foliage of the huizache and found a CP content of 17%. However, these variations in CP content may be due to the place of origin of the collected material (Velázquez *et al*., 2011). According to Mamani and Cotacallapa (2018), the CP content is variable in different harvest sites, attributed to factors related to soil fertility and the phenological state of the plant, so, based on its collection, it still needs to have its maximum nutritional potential. On the other hand, the CP content of HUI was higher than that of ground corn, so the substitution of HUI for ground corn makes a protein contribution to the feed.

Physicochemical Properties

Table 2 shows the regression coefficients obtained from the responses of the extruded samples.

Expansion Index and Bulk Density

EI and BD are related to product expansion and are inversely related since the increase in EI results in a decrease in BD and vice versa. According to the regression coefficients in Table 2, X_2 had a significant negative effect on the IE (p<0.05) in its linear term, which is consistent with Lourenço *et al.* (2016), who found that EI decreased due to

Table 2: Regression coefficients of responses of extruded HUI treatments.

Responses	Coefficients										
	Intercept	Linear			Interaction			Quadratic			R^2
	b_0	X_1	X_2	X_3	X_1X_2	X_1X_3	X_2X_3	X_1^2	X_2^2	X_3^2	
EI	1.1	-0.003	-0.022	0.001	0.010	-0.009	0.008	-0.005	0.023	0.003	0.489
BD	1175.13	-39.24	26.95	19.98	-13.16	-13.09	-37.2	-10.55	8.91	14.01	0.801
H	126.13	-7.24	11.52	5.45	-5.9	7.21	-6.56	6.98	6.77	-1.02	0.603
WSI	9.78	-0.235	-0.004	-0.215	-0.097	-0.514	0.509	0.015	-0.377	-0.466	0.628
WAI	3.55	0.203	0.177	-0.108	-0.098	-0.065	-0.003	0.063	-0.044	0.033	0.656
IVDMD	89.26	0.986	-1.45	1.61	-0.219	-0.608	-0.641	1.6	0.056	-1.39	0.882
TC	0.118	-0.005	-0.001	0.054	0.002	-0.004	0.005	0.003	0.004	0.01	0.917
TF	0.199	-0.007	-0.003	0.078	-0.001	-0.022	-0.001	-0.003	-0.013	0.008	0.986

X_1: temperature (°C); **X2:** humidity (%); **X3:** Huizache content (%). **EI:** expansion index (-); **BD:** bulk density kg/m^3; **H:** hardness (N); **WSI:** water solubility index (%); **WAI:** water absorption index (g/g); **IVDMD:** *in vitro* dry matter digestibility (%); **TC:** condensed tannins (%); **TF:** total phenols (%).

an increase in humidity, showing that humidity was a factor with a predominant effect on EI and, therefore, on BD (Ding *et al*., 2005). In turn, X_2 had a positive effect on BD ($p<0.05$). In addition, the elasticity of the dough is reduced as there is higher moisture content through the plasticization of the molten material, which consequently results in less gelatinization (Nkubana *et al*., 2020), thus reducing expansion and, in turn, increasing density and hardness. On the other hand, during the extrusion process, there is a sudden pressure drop when the treatment comes out of the extruder, due to the change from high pressure to atmospheric pressure conditions (Altan *et al*., 2008). In this process, the water nucleates, causing the formation of bubbles in the extruded material, generating an expansion due to the sudden evaporation of moisture caused by the pressure drop (Kamarudin *et al*., 2018). However, an increase in X_2 causes the bubbles within the extruded to collapse after expansion at the matrix outlet due to reduced viscosity (Kantrong *et al*., 2018), as it has been reported that in products with lower humidity, being more viscous, they have a more significant expansion than those with higher humidity (Delgado *et al*., 2021). As the pressure differential is higher for products with low moisture due to the drag generating more pressure on the die (Oke *et al*., 2013), EI decreases when the moisture content increases. X_1 showed a significant negative effect on BD ($p<0.05$), while X_3 had a positive effect ($p>0.05$) on BD (both in their linear terms), which is consistent with previous researches (Bisharat *et al*., 2013; Sharma *et al*., 2017). The increase in X_3 increased BD, as the protein and rigid fiber of the HUI flour prevented the expansion of the air bubbles and reduced the formation and retention of expanded air pockets due to the rupture of the cell walls (Ravindran *et al*., 2011; Pérez *et al*., 2017), which was probably caused by the increased friction between the fed material and the surface of the barrel as a result of the increase in viscous dissipation of the mass (Martinez *et al*., 2011). As fibre- and protein-rich materials are added to extruded products, density is expected to increase. As for X_1, the high extrusion temperature and shear force generate heat that causes the water to overheat as the extruded exits the die (Gopirajah and Muthukumarappan, 2018), which encourages the formation of bubbles and generates a lower viscosity, forming a lighter structure and therefore less dense and hard (Fletcher *et al*., 1985; Jiangping *et al*., 2017).

Hardness

H is a property defined as the maximum force needed to penetrate a product by a probe (Charunuch *et al*., 2014). According to the analysis, X_2 has a significant positive effect on its linear term on H ($p<0.05$), which indicates that as humidity increases from 14 to 18%, hardness increases, which is consistent with the positive effect of humidity on BD and the negative effect on EI that was previously described. Sharma *et al*. (2017) found that higher humidity significantly increased H since this factor participates as a plasticizer of the matter found within the extruder, which results in lower viscosity and a decrease in energy dissipation. Therefore, bubble growth is compressed at the outlet, generating a more rigid product.

Water Absorption Index and Water Solubility Index

The WAI describes the degree of starch gelatinization as it relates to the amount of water that is absorbed by starch granules when they are in excess water (Alcázar and Almeida, 2015), so it depends on the hydrophilic groups that are free to bind to water molecules and also on the ability of macromolecules to form a gel (Néder *et al*., 2016). WSI is defined as the number of soluble polysaccharides released from the granules after the addition of excess water and indicates the degree of degradation of the molecular components (Rodríguez *et al*., 2011). A high WSI reflects the presence of solutes from gelatinization and is sometimes attributed to starch dextrinization. In contrast, a high WAI indicates the presence of large starch fragments due to lower starch degradation (Ravindran *et al*., 2011). The positive coefficients of X_1 and X_2 in their linear terms had a significant effect on WAI ($p < 0.05$), while X_3 had a negative effect on WAI ($p < 0.05$).

The effect of X_1 on the WAI is consistent with other reports (Singh and Muthukumarappan, 2014; de Cruz *et al*., 2015). At elevated temperatures, the crystalline structure of the starch is destroyed, which allows it to retain more water (Pérez *et al*., 2019) due to an increase of exposed hydroxyl groups (Zhang *et al*., 2014), forming an expanded matrix that absorbs more water by separating the amylopectin and amylose branches (Jiangping *et al*., 2017). On the other hand, the positive linear coefficient of humidity indicates that the increase in humidity from 14 to 18% also increases WAI, which is consistent with other researches where the WAI increases with a higher moisture content (Kothakota, 2013; Gat and Ananthanarayan, 2015). Leonard *et al*. (2015) and Gulati *et al*. (2016) explained that higher humidity coupled with higher temperature causes the viscosity of the material to be lower, which promotes extensive internal mixing and uniform heating that improves starch gelatinization and increases WAI.

A lower WAI due to the increase in X_3 in the extrudates may be attributed to the higher fiber input by the HUI and the reduction of starch, which was observed by Ajita and Jha (2017), where they found a lower WAI in extruded plants with a higher percentage of wheat bran, since the substitution of starch for the fiber component, decreases starch gelatinization. In addition, starch gelatinization was probably limited by an increased interaction between the hydrophilic groups available in the protein with the starch caused by the increase in HUI (Sharma *et al*., 2017).

Regarding WSI, the interaction of the X_1X_3 and X_2X_3 factors had a negative and positive effect on the WSI ($p < 0.05$), respectively. For WSI, its decrease could be attributed to an increase in temperature since starch depolymerization did not occur at high temperatures (Atienzo-Lazos *et al*., 2011). Reyes-Jáquez *et al*. (2012) reported that high temperatures reduce solubility as hydrophobic groups within the protein structure are exposed due to denaturation. On the other hand, an increase in WSI due to a higher moisture content in the treatment could be attributed to the reduction of lateral expansion because of the melting of the material, as observed by Kothakota (2013). However, it has also been found that there is an increase in WSI at lower moisture content because of more significant starch degradation (Singh *et al*., 2007). An increase in WSI with a higher moisture content was observed by Prabhakar *et al*. (2017) in extruded citrus residues.

Total Phenols and Condensed Tannins

The extrusion process uses short-term cooking. However, the phenolic compounds in the treatments are subjected to varying degrees of heating and moisture content (Yaĝci y Göĝûş, 2009), which are enough to cause changes in those compounds.

Table 2 shows that X_1 and X_3 in their linear terms had a significant effect on TP ($p<0.05$), just as interaction X_1X_3 and X_2 in their quadratic term had a significant effect on TP ($p<0.05$). Polyphenols are compounds that are very susceptible to oxidation/degradation during extrusion due to the thermomechanical conditions involved in the process (Obradovic *et al*., 2015), because high temperatures accelerate the degradative oxidation of TP (Chaparro *et al*., 2009) since phenolic compounds are thermolabile. Their nature can be altered at temperatures above 80 °C (Sharma *et al*., 2012). Extrusion can alter the molecular structure of these compounds as well as reduce their chemical reactivity (Patterson, 2017), as phenolic compounds can undergo decarboxylation of phenolic acids during extrusion (Brennan *et al*., 2011) due to higher melting temperature and moisture content (Pedrosa *et al*., 2021), which can reduce their extractability by promoting the polymerization of phenols (Wang *et al*., 2014), such as the formation of a complex protein matrix that makes the difficult to extract (Almirudis, 2018). The extrusion process denatures proteins that lead to open structures that promote tannin-protein interaction, thus causing the formation of complexes that reduce their extractability (Brennan *et al*., 2011). The negative effect of X_2 on TP content may be due to the increased generation of moist heat, which is more destructive and produces a synergistic effect with temperature (Yaĝci and Göĝûş, 2009).

X_3 had a positive effect on CT ($p<0.05$) since increasing the HUI content from 0 to 10% has a more significant contribution to the CT content due to the presence of these compounds in the plant. In cattle feed, CT is desired when seeking to reduce the emission of gases such as methane, as long as they do not exceed 5.5% of the diet, due to possible harmful effects to the animal, such as reducing the voluntary consumption of food and the digestibility of nutrients (Núñez and Rodríguez, 2019). According to the obtained results, the extrudates are below this limit.

In Vitro Dry Matter Digestibility

X_1, X_2 and X_3 in their linear terms, had significant effects on the IVDMD ($p<0.05$), as well as the interaction of X_1X_3 and X_2X_3 and X_1 and X_3 in their quadratic terms ($p<0.05$) (Table 2). IVDMD of the extrudates ranged between 86.6-94.4%, with similar values in extruded cattle feed from other studies (Reyes-Jáquez *et al*., 2011; Delgado *et al*., 2020) and extruded pig feed (Araiza-Rosales *et al*., 2022b). Gelatinization of starch by the influence of temperature and humidity in the extrusion process (Donmez *et al*., 2021) makes it more susceptible to enzymatic hydrolysis due to the severity of the treatment, as it makes the starch granules easier to break down (Sun *et al*., 2019). In addition, the contact surface between the microbial enzymes and the substrate is enhanced by gelatinized starch and by mechanical breakdown of the plant cell wall (Sun *et al*., 2006). On the other hand, the degradability of the protein in the rumen is decreased by the partial denaturation of the protein (Reyes-Jáquez et al., 2011), which increases the amount of absorbable amino acids in the small intestine of cattle (Jenko *et al*., 2018), which is of utmost importance since the microbial protein does not me*et al* the requirements of the animal (Barchiesi *et al*., 2018). Razzaghi *et al*. (2016) compared the degradability of starch and protein in the rumen in unprocessed diets and diets processed by extrusion and pelletization in corn, wheat and soybean mixtures, whereby extrusion improved the digestibility value of feeds.

Numerical Optimization

Numerical optimization was performed by superimposing response surfaces (EI, BD, H, WAI, WSI, TP, CT, IVDMD). The optimal processing conditions were determined to obtain a high WAI, a lower WSA, a high IVDMD and a higher content of TF and CT, establishing as processing parameters the use of a high temperature, a low moisture content and a high HUI content. The optimal processing conditions for the feed were X_1=150 °C, X_2=14% moisture content and X_3=10% HUI content, with an EI of 1.107, BD of 1060.79 kg/m^3, H of 145.2 N, WAI of 3.51 g/g, WSI of 7.58%, TP of 0.246%, CT of 0.173% and IVDMD of 93.82%. The optimization results have similar values to commercial products shown in previous research (Reyes-Jáquez et al., 2011) or other optimizations in livestock feed (Delgado *et al*., 2020) so that extruded feed with the addition of HUI could be incorporated into the cattle feed with the addition of other forages. The chemical analysis was performed in the optimal extruded treatment with percentages of DM=89.24±0.485, ASH=4.44±0.038, CP=11.66±0.573, EE=1.48±0.107, NDF=26.288±0.591, ADF=15.97±0.604, LIG=1.34±0.084, NSC=56.07±0.225 and IVDMD of 94.46±0.652.

CONCLUSIONS AND RECOMMENDATIONS

The optimal processing conditions were obtained and according to the obtained results, the inclusion of HUI in cattle feed could replace conventional raw materials such as corn for food processing since the IVDMD did not show significant differences compared to commercial feeds, which suggests that HUI could be added to the formulation of extruded diets for bovine feed.

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NOVELTY STATEMENT

It is necessary to continue producing food for livestock in the face of the climate crisis and without compromising nutritional health. This study proposes the use of HUI as a non-conventional ingredient in the formulation of livestock feed with the use of extrusion to improve its nutritional quality.

AUTHOR'S CONTRIBUTIONS

Garcia Dominguez Maria Fernanda: Draft writing, investigation, analysis, data collection and curation.
Páez Lerma Jesús Bernardo: Conceptualization, Project management, supervision.
Reyes Jáquez Damián: Data analysis, methodology, visualization)
Carrete Carreón Francisco Oscar: Supervision, draft editing.
Rosas Guerra Maria Inés: Methodology, formal analysis)
Araiza Rosales Elia Esther: Data interpretation, conception, supervision.

Conflict of Interest

The authors declare that there is no conflict of interest related to the published article.

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