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## Effect of extrusion temperature, moisture and sunflower oil content on the functional properties and digestibility of bovine cattle feeds

Efecto de la temperatura de extrusión, humedad y contenido del aceite de girasol sobre las propiedades funcionales y digestibilidad de alimentos para ganado bovino

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### ABSTRACT

Preparation of extruded products with high oil content, presents a technological challenge, due oil decreases specific mechanic force but also acts as a lubricant, and forms starch-lipid complexes; thus, decreasing starch gelatinization. This research aimed to evaluate the effect of temperature, moisture, and sunflower oil content, on the extrusion process of bovine cattle feed. Two main ingredients were used for each diet: alfalfa (*Medicago sativa* L.), and bean (*Phaseolus vulgaris* L.). The obtained results showed that high temperature, moisture, and oil content, decreased bulk density, and hardness ( $P < 0.05$ ). Oil content-temperature interaction increased both bulk density and hardness, while moisture-oil content interaction increased ( $P < 0.05$ ) hardness. Optimization was performed based on the physicochemical characteristics of commercial feeds, showing that the best bean diets were obtained at 121°C, 14% moisture content with 0% sunflower oil; 120°C and 16% moisture content with 3.5% sunflower oil; and, 142°C and 15% moisture content with 7% sunflower oil. Effective degradability ranged from 87.4 - 90.4% for all extruded diets; and none of them showed significant differences between bean and alfalfa ( $P < 0.05$ ), which opens a high potential opportunity of producing high concentrations of CLA from sunflower oil at a ruminal level.

**Keywords:** Bovine cattle feed, extruded, digestibility, sunflower oil.

### RESUMEN

La preparación de productos extruidos con alto contenido de aceite presenta un desafío tecnológico, debido a que el aceite disminuye la fuerza mecánica específica pero también actúa como lubricante y forma complejos de almidón y lípidos; disminuyendo así la gelatinización del almidón. El objetivo de esta investigación fue evaluar el efecto de la temperatura, la humedad y el contenido de aceite de girasol en el proceso de extrusión de alimento para ganado bovino. Se usaron dos ingredientes principales para cada dieta: alfalfa (*Medicago sativa* L.) y frijol (*Phaseolus vulgaris* L.). Los resultados obtenidos mostraron que la alta temperatura, la humedad y el contenido de aceite, disminuyeron la densidad aparente y la dureza ( $P < 0.05$ ). La interacción entre el contenido de aceite y la temperatura

aumentó tanto la densidad aparente como la dureza, mientras que la interacción entre el contenido de humedad y aceite aumentó la dureza ( $P < 0.05$ ). La optimización se realizó con base en las características fisicoquímicas de alimentos comerciales, mostrando que las mejores dietas de frijol se obtuvieron a 121°C, 14% de contenido de humedad con 0% de aceite de girasol; 120°C y 16% de contenido de humedad con 3.5% de aceite de girasol; y, 142°C y 15% de contenido de humedad con 7% de aceite de girasol. La degradabilidad efectiva varió de 87.4 a 90.4% para todas las dietas extruidas; y ninguno de ellos mostró diferencias significativas entre el frijol y la alfalfa ( $P < 0.05$ ), lo que abre una gran oportunidad potencial de producir altas concentraciones de CLA a partir del aceite de girasol a nivel ruminal.

**Palabras clave:** alimento para ganado bovino, digestibilidad, aceite de girasol.

## INTRODUCTION

Cattle's breeding is one of the main cornerstones of the global economy, being usually sustained from grassland, which lately has been affected by many problems, particularly, dry seasons ([Petherick, 2005](#)). A feasible option to help to solve this problem is to use pinto beans (*Phaseolus vulgaris* L.) as an ingredient for animals' feed. Small, cracked, or split beans are considered an agro-industrial by-product, not suitable for human consumption. Bean has a good protein (18 - 24%) and energetic (60 - 65%) content, but it also has the disadvantage of anti-nutritional factors (phytic acid, condensed tannins, polyphenols, trypsin inhibitors, chymotrypsin,  $\alpha$ -amylase inhibitors, and haemagglutinating activity) presence ([Iniestra-González et al., 2005](#)). However, these factors can be inhibited by thermal processing, such as extrusion ([González-Valadez et al., 2008](#)). Sunflower oil is rich in linoleic acid content ([El-Saidy et al., 2011](#)), which is transformed by ruminants into conjugated linoleic acid (CLA); a common term referring to all isomers of an octadecanoic carboxylic acid with unsaturation in different positions. CLA presents several health benefits: it helps to decrease weight-loss promoting muscle mass growth, and also, it is anti-carcinogenic ([Pariza et al., 2001](#)). CLA content in bovine milk ranges from 6 to 16 mg/ g lipids, being lower in meat ([Chillard et al., 2007](#)).

Recommended daily CLA consumption is 3 g/d for a 70 kg person, having the highest consumption rates in Australia (1.5 g/d) and Germany (0.5 g/d) ([Poulson et al., 2004](#)). Studies showed an increase of 1.5 g/100 lipids g in milk obtained from cattle fed with 11.2% of sunflower seeds and an increase of 0.8 g/100 lipids g from cattle fed with 5% of a mixture of sunflower oil and fish oil (3:1) ([Abu-Ghazaleh and Holmes, 2006](#)). Extrusion has a wide range of food applications ([Singh et al., 2007](#)), representing a good processing alternative, due to its economic feasibility since crop residues are underutilized in many developing countries. This research aimed to study the effect of temperature (T), moisture content (MC), and sunflower oil content (OC) on the extrusion process to elaborate bovine cattle feed, which could be reflected in milk and meat CLA content for human consumption.

## MATERIALS AND METHODS

### Experimental diets

Two diets were elaborated with 10% of bean flour (BD) or 10% of alfalfa flour (AD). Agroindustrial Saltillo bean (*Phaseolus vulgaris*) residue (small and cracked beans) was used, as well as alfalfa (*Medicago sativa*), cornflour (*Zea mays*), cane molasses, Mine-Gan, Química Industrial Agropecuaria S.A. de C.V., México), soybean meal flour (47.7% crude protein), sunflower oil and CaCO<sub>3</sub>. All ingredients were milled and sieved (< 2 mm). Three OC/corn flour percentages ratios were used: 0:55, 3.5:51.5 or 7:48. All other ingredients were kept constant for AD and BD: cane molasses: 5%; soymeal: 5%; CaCO<sub>3</sub>: 2%; and, glandless cottonseed meal: 23%.

### Chemical composition

The chemical composition of raw materials, extrudates, and two commercial diets were determined following ([AOAC, 2019](#)) standards.

### Extrusion

Samples were processed with a Brabender laboratory simple-screw extruder (Model 2523, 3/4" L/D - 25:1 ratio, C. W., Disburg, Germany), with four heating zones. The first three heating zones had a constant T; 90, 100, and 110°C, respectively. The fourth heating zone varied (120, 135, and 150°C), according to experimental design. The screw compression force was 1:1 and the internal diameter of the exit die was 6 mm. Before extrusion, all ingredients were mixed and conditioned to MC of 14, 16, or 18%, following experimental design. After processing, extruded samples were cooled at room T for 4 h and stored in sealed polyurethane bags at 4°C for further analysis.

### Experimental design and data analysis

A factorial design with three independent variables was performed for both diets: OC (X<sub>1</sub>) [0, 3.5 and 7%], T (X<sub>2</sub>) [120, 135 and 150°C] and MC (X<sub>3</sub>) [14, 16 and 18%] before extrusion. Response variables were: expansion index (EI), bulk density (D), water absorption index (WAI), water solubility index (WSI), and hardness (H). Surface response methodology was applied to the experimental data using Design Expert 7.0<sup>®</sup> software and results were analyzed by multiple quadratic regressions. Statistical analysis and variance analyzes (ANOVA) for each response were performed using Statistica 7.0<sup>®</sup>.

### Optimization

Optimization was performed for each OC using a central composite design with three independent variables. Priority responses used for the optimization were: H 25 - 50 N, EI 1.0 - 1.1, minimum WSI, and maximum D.

### Functional Properties

EI and D were measured according to ([Gujska and Khan, 1990](#) and [Wang et al., 1993](#)), respectively. WAI and WSI were determined as outlined by ([Ding et al., 2005](#)). H was evaluated using a texture profile analyzer model TA-XT2 (Texture

Technologies Corp., Scarsdale, NY/Stable MicroSystems, Haslemere, Surrey, UK). In each trial, fifteen samples were sheared using a Warner Brazler blade probe (sensitivity of 1 kgf and 5 cm min<sup>-1</sup>) to evaluate breaking strength.

### In vitro digestibility and effective degradability

In vitro digestibility (IVD) was calculated using the Daisy II procedure (ANKOM, 2017); inoculum was prepared by diluting ruminal liquid obtained from a 459 kg rumen fistulated cow (criollo race) fed at free grazing, with a buffer solution 1:4 (v/v). Mean comparison was obtained using the Tukey test ( $P < 0.05$ ). Effective degradability (ED) was calculated following (Solanas *et al.*, 2004) method. Means differences (Duncan, 95%), correlations (Pearson), and t-tests (Student) were performed using Statistica 7.0<sup>®</sup>.

## RESULTS AND DISCUSSION

### Chemical composition

The chemical content of all ingredients (Table 1) is comparable to other reports (Reyes-Jáquez *et al.*, 2011), except for alfalfa's ash content, which is 2% above reported values, possibly due to a higher presence of fertilizers. Also, alfalfa's crude protein content was slightly higher than other reports (Coblentz and Hoffman, 2009). THE highest NFE was shown in pinto beans, thus increasing the extrusion capacity of the diets (Reyes-Jáquez *et al.*, 2012).

**Table 1. Chemical composition of ingredients before extrusion (g/ 100g DM).**

Ingredient	Ash	Crude protein	Crude fat	Crude fiber	NFE*
Alfalfa flour	10.6 ± 0.15	25.2 ± 1.68	0.6 ± 0.06	23.1 ± 1.29	40.3 ± 0.06
Pinto Saltillo Bean flour	3.9 ± 0.11	24.2 ± 0.38	0.9 ± 0.11	2.3 ± 0.19	68.6 ± 0.56
Soybean meal flour	6.9 ± 0.03	58.5 ± 0.53	1.8 ± 0.13	0.0 ± 0.00	32.8 ± 0.43
Corn	1.5 ± 0.04	9.2 ± 0.56	1.9 ± 0.00	2.9 ± 0.02	84.3 ± 0.64
Cottonseed meal	7.2 ± 0.72	52.5 ± 1.13	1.9 ± 0.14	7.6 ± 0.09	30.6 ± 1.80
Molasses	7.1 ± 0.78	5.8 ± 0.89	0.1 ± 0.02	0.5 ± 0.05	86.9 ± 3.96
Calcium carbonate	39.7 ± 1.86	0.1 ± 0.02	0.0 ± 0.00	0.0 ± 0.00	60.2 ± 2.97

NFE= Nitrogen free extract

### Functional Properties

All regression coefficients of EI are low (Table 2), possibly because NFE (Table 1) is not high enough to expand, and, process conditions did not yield a significant pressure differential, thus influencing only shaping, not expansion. These results are similar to others (Reyes-Jáquez *et al.*, 2011), where similar diets were extruded at the same T but with higher MC (18 - 22%) and without oil. Oil adding in extrudates decreases starch gelatinization due to lower shear stress applied upon the mixture since it acts as a lubricant; it also promotes lipid-starch complexes' formation, restraining water-starch interaction, resulting in lower EI (Liu *et al.*, 2006). D and H of AD and BD presented significant negative coefficients in all lineal terms (Table 2). High OC percentages generate a less structured and more fragile matrix, because of

lipid-starch complexes' formation (Abu-Hardan *et al.*, 2011); also, high crude fiber content contributes to the fragility of the extruded matrixes (Reyes-Jáquez *et al.*, 2012). High lipid content in extrudates decelerates starch retrogradation and reduces H, adding the fact that high T further degrades starch granules, even leading to dextrinization and creating a less dense and easier to disrupt matrix (Rodríguez-Miranda *et al.*, 2012). High MC generates a higher amount of steam, producing an expanded volume when the mixture is exposed to a pressure differential, resulting in the rupture of matrixes' cellular walls and creating a more porous product with low D and H ( $P < 0.05$ ) (Reyes-Jáquez *et al.*, 2012). OC-T interaction presented a significant positive effect ( $P < 0.05$ ) on D and H, due high concentrations of oil generate more lipid-starch complexes, although high T could lead to break such complexes, allowing starch gelatinization and retrogradation (De Pilli *et al.*, 2011). OC-MC interaction had a positive effect ( $P < 0.05$ ) on H; (Hernández-Hernández *et al.*, 2011) proposed a model of starch and  $\alpha$ -lysophosphatidylcholine (LPC) complexes, indicating a strong competition among lipids and water to bond with starch, and inferring that high MC and OC, are likely to bond starch and water, gelatinizing it and obtaining denser and harder matrixes.

**Table 2. Regression coefficients obtained from quadratic response surface models for bean and alfalfa diets.  $X_1$  = oil content,  $X_2$  = temperature,  $X_3$  = moisture content.**

Responses	Intercept	Lineal			Quadratic			Interactions			R <sup>2</sup>
	b <sub>0</sub>	X <sub>1</sub>	X <sub>2</sub>	X <sub>3</sub>	X <sub>1</sub> <sup>2</sup>	X <sub>2</sub> <sup>2</sup>	X <sub>3</sub> <sup>2</sup>	X <sub>1</sub> X <sub>2</sub>	X <sub>1</sub> X <sub>3</sub>	X <sub>2</sub> X <sub>3</sub>	
EI-AD	1.088	-0.023*	-0.047*	-0.018*	-0.02*	-0.046*	-0.009*	-0.019+	0.014*	-0.009*	0.537
EI-BD	1.058	-0.02*	-0.044*	0.005	0.004	-0.03*	0.004*	-0.008	0.004*	0.022	0.384
D-AD	988.805	-47.65*	-32.52*	-12.06*	3.999	48.497*	8.27	57.404*	-16.93*	34.478*	0.457
D-BD	1100.499	-53.71*	-42.53*	-17.54*	5.711*	17.027*	-34.43*	13.287*	-10.09*	2.722	0.439
H-AD	34.232	-20.65*	-4.932*	-7.829*	16.34*	3.006*	0.804	8.319*	3.542*	1.237	0.697
H-BD	34.636	-19.41*	-2.922*	-5.873*	15.54*	1.389	3.54*	6.134*	2.242*	1.224	0.706
WAI-AD	2.688	-0.072*	0.19*	-0.006	-0.003	0.076	-0.08	0.051	0	0.054	0.549
WAI-BD	2.273	-0.133*	0.065*	-0.013	0.012	0.006	0.007	0.028	-0.011	0.033	0.422
WSI-AD	12.024	-0.375	-0.868	0.224*	-0.05	-0.882*	0.586*	0.17	0.076	0.099	0.547
WSI-BD	11.54	0.489*	-0.234*	-0.214*	0.096	-0.11	0.339*	-0.247*	-0.191	0.194	0.452

\*Indicates significant difference ( $P < 0.05$ ). DA = alfalfa diet, DB = bean diet, EI = expansion index, H = hardness, D = bulk density, WSI = water solubility index and WAI = water absorption index

WAI is firstly related to the amount of absorbed water by the starch granules after swallowing in water excess and can be used as a gelatinization grade index (González-Valadez *et al.*, 2008); and, secondly, to the proteins hydrophilic balance in the mixture, which changes according to the denaturalization grade of proteins, where the extrusion process changes solubility profiles (Serrano *et al.*, 1998). Table 2 presents WAI regression analysis: OC and T linear terms affected ( $P < 0.05$ ) on WAI of both diets. Increasing OC decreases WAI due to lower water availability for

the starch granule. As for T, at high values, amylose and amylopectin chains form an expandable matrix that translates into higher water retention capacity. WSI is directly related to starch degradation happening inside the extruder (Gujaska and Khan, 1990). Table 2 presents WSI regression analysis: MC linear term, T, and MC quadratic terms of AD; and T, MC, and OC linear terms, MC quadratic term, T, and OC interaction term of BD, had a significant effect ( $P < 0.05$ ) on WSI. BD's T negative linear term and T and OC interaction indicate that with high T, proteins denaturalization exposes hydrophobic groups located in the interior, contributing to solubility diminish (Ikpeme *et al.*, 2010).

### Optimization

Optimal extrusion conditions were obtained at three different oil concentrations for each diet; AD (0% OC): 142°C, 18% MC; EI: 1.04, D: 1042.7 kg/m<sup>3</sup>, H: 50 N, WAI: 2.67 g/g, and, WSI: 10.5%; AD (3.5% OC): 131°C, 18% MC; EI: 1.04, D: 1021.7 kg/m<sup>3</sup>, H: 50 N, WAI: 2.67 g/g, and, WSI: 10.9%; AD (7% OC): 120°C, 18% MC; EI: 1.04, D: 1004.6 kg/m<sup>3</sup>, H: 25 N, WAI: 2.67 g/g, and, WSI: 10.4%; BD (0% OC): 121°C, 14% MC; EI: 1.09, D: 1125 kg/m<sup>3</sup>, H: 50 N, WAI: 2.34 g/g, and, WSI: 12.2%; BD (3.5% OC): 120°C, 16% MC; EI: 1.07, D: 1121.3 kg/m<sup>3</sup>, H: 50 N, WAI: 2.26 g/g, and, WSI: 12%; and, BD (7% OC): 142°C, 15% MC; EI: 1.02, D: 1101 kg/m<sup>3</sup>, H: 50 N, WAI: 2.27 g/g, and WSI: 11.6%. AD with 7% of OC had the lowest H, while this was not observed with BD. On the other hand, low T for BD with high OC was required compared to AD with low OC. BD needed high T to obtain optimal extrusion characteristics, specifically extrudates with 7% of OC compared to diets with 3 or 0% of OC. Optimization results have the required values to be commercialized due their similar characteristics compared to commercial products as shown in earlier publications (Reyes-Jáquez *et al.*, 2011). Chemical analysis was performed on BD optimal treatment with 7% of OC: MC: 10.7±0.22, protein: 17.6±0.63, fat: 10.7±0.11, fiber: 2.5±0.63 and ash: 8.7±0.18 g/100 g.

### In vitro digestibility and effective degradability

IVD of 60.7% or higher is a well-accepted digestibility (Coblentz and Hoffman, 2009), placing AD and BD above such values (Table 3). Excluding both commercial diets, all diets had IVD ranging from 85 to 89% without significant differences ( $P > 0.05$ ) among them. AD containing 0 or 3.5% of OC has a lower ( $P < 0.05$ ) IVD than both commercial diets. BD with 3.5 and 7% of OC had comparable IVD to both commercial diets, concluding that incorporating sunflower oil in the extruded diets, increases ( $P < 0.05$ ) IVD of BD. Table 3 shows the potential degradability (PD) and ED of all diets. PD describes the sum of soluble (A) and insoluble (B) fractions, being B, the maximum degradability peak achieved during 120 h. Table 3 showed that all EDs' values oscillated between 87.4 and 90.4% with no significant difference ( $P > 0.05$ ), which are acceptable values compared to other reports (Reyes-Jáquez *et al.*, 2011). It can be concluded that OC and anti-nutritional factors presence did not have a significant difference in IVD nor ED since they were inactivated by high T and shear stress during processing. Also, OC is inside permissible ranges for ruminants'

consumption (Byers and Schelling, 1993). Extrusion T, MC, and OC affected negatively ( $P < 0.05$ ) D and H of both diets. However, increasing T and OC, and MC and OC, increases ( $P < 0.05$ ) H. Obtained IVD was 89.1 and 86.4% for BD and AD with 7% of OC, respectively. Obtained optimal extrusion conditions for BD with 7% of OC were 142°C and 15% of MC; while for AD with 7% of OC were 120°C and 18% of MC. ED was 87.5 and 87.4% for BD and AD with 7% of OC, respectively. Results showed that incorporating sunflower oil in the diets, increases ( $P < 0.05$ ) IVD, thus opening a high potential opportunity of producing high concentrations of CLA at the ruminal level.

**Table 3. Nonlinear regression coefficients of In Situ Degradability (ISD) and Effective degradability (ED) of bean and alfalfa extruded diets at three different oil concentrations: 0, 3.5, and 7%.**

Diet	Oil (%)	Coefficient			PD (%)	ED (%)	R <sup>2</sup>	IVD (%)
		A	B	C				
Alfalfa	0	0.54	0.41	0.07	95.2	88.2 <sup>a</sup>	0.99	85.2
Alfalfa	3.5	0.5	0.45	0.07	95.2	87.5 <sup>a</sup>	0.98	84.7
Alfalfa	7	0.51	0.44	0.07	94.7	87.4 <sup>a</sup>	0.96	86.4
Bean	0	0.6	0.36	0.07	96	89.7 <sup>a</sup>	0.98	86.3
Bean	3.5	0.6	0.36	0.06	96.2	90.4 <sup>a</sup>	0.98	89.4
Bean	7	0.49	0.47	0.08	95.9	87.5 <sup>a</sup>	0.97	89.1
Commercial 1	-	-	-	-	-	-	-	93.8
Commercial 2	-	-	-	-	-	-	-	91.5

Different letters indicate significant difference ( $P < 0.05$ ); Degradability times: 0 - 120 h. A = soluble fraction or quickly degradable, B = insoluble fraction but potentially degradable, C = degradation rate, t = feed residence time, considered 5% per hour, PD = potential degradability (A+B), ED = effective degradability rate at a constant feed's residence time in the animal (kp)  $0.05 \cdot h^{-1}$

### CONCLUSION

Extrusion temperature and moisture content affected negatively ( $P < 0.05$ ) D and H of extruded cattle feed. Likewise, D and H decreased ( $P < 0.05$ ) in extrudates, as oil content increased. However, increasing extrusion temperature and oil content, makes it possible to increase ( $P < 0.05$ ) hardness of extrudates, as well as increasing moisture and oil content. Obtained In Vitro Digestibility was 89.1% and 86.4% for bean and alfalfa diets, respectively. Obtained optimal extrusion conditions for a bovine cattle feed using pinto bean residues with 7% of sunflower oil were 142°C and 15% of moisture content. While for extrudates containing alfalfa with 7% of sunflower oil were 120°C and 18% of moisture content. Effective In Situ Degradability was 87.5% and 87.4% for bean and alfalfa diets, respectively. Results showed that the incorporation of sunflower oil in extruded diets, increases ( $P < 0.05$ ) In vitro digestibility of extruded alfalfa and pinto bean diets, opening a high potential opportunity to increase the production of organic CLA at ruminal level.

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