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EFFECT OF CRUDE GLYCEROL FROM *JATROPHA CURCAS* L. OIL ON THE PRODUCTION AND QUALITY OF CATTLE MILK

EFECTO DEL GLICEROL CRUDO PROCEDENTE DE ACEITE DE *JATROPHA CURCAS* L. EN LA PRODUCCIÓN Y CALIDAD DE LA LECHE VACUNA

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To evaluate the effect of crude glycerol from *Jatropha curcas* L. oil on the production and quality of cattle milk, a total of 30 multiparous cows from Siboney de Cuba breed were used, with an average pre-parturition body condition of 3.04 and an average pre-parturition live weight of 487.87 kg. The animals had an average number of lactations of 3.17 and between seven and 21 days after the start of milking. A completely random design with two treatments and 15 repetitions was applied. Each animal was considered a repetition. The treatments were: control) 1 kg of wheat bran during milking, without glycerol; treatment 2) 1 kg of wheat bran during milking + 200 mL of glycerol during the first 15 days, dose which was increased to 400 mL during the last 45 days of the trial). The animals grazed in areas of pitilla (*Sporobolus indicus* L.) with permanent access to water. The glycerol under study was characterized by a density of 1.02 g/mL, methanol content of 2.6 %, ignition point above 65 ^{0}C and pH of 8.74. It showed a dark brown color and sweet taste. These indicators are in accordance with the quality specification properties of crude glycerol, established by international standards. The average milk production/cow/day was higher (P=0.0001) in the treatment with the glycerol additive (3.43 vs. 2.20 L for the control), as well as the total milk production (P=0.0159). It is concluded that crude glycerol, obtained in the transesterification process of *J. curcas* oil, has physicochemical and organoleptic properties that make it suitable for their use in animal feeding as an additive. Its use increased milk production in Siboney Cuba cows without affecting quality indicators. Its application under production conditions and the development of new studies in other categories and species of animals are recommended.

Key words: *biofuel, glycerin, oilseeds, productive indicators*

Con el objetivo de evaluar el efecto del glicerol crudo procedente de aceite de *Jatropha curcas* L. en la producción y calidad de la leche vacuna, se utilizaron 30 vacas de la raza Siboney de Cuba, multíparas, con condición corporal promedio preparto de 3.04 y pesos vivos promedios preparto de 487.87 kg. Los animales presentaron un número de lactancias promedio de 3.17 y entre siete y 21 días de iniciado el ordeño. Se utilizó diseño completamente aleatorizado, con dos tratamientos y 15 repeticiones, cada animal se consideró una repetición. Los tratamientos fueron (Grupo control: cada animal consumió 1 kg de afrecho de trigo durante el ordeño, sin glicerol; Tratamiento 2: Cada animal consumió 1 kg de afrecho de trigo durante el ordeño + 200 mL de glicerol durante los primeros 15 días, dosis que se aumentó a 400 mL durante los últimos 45 días del ensayo). Los animales pastaron en áreas de pitilla (*Sporobolus indicus* L.) con accesibilidad al agua permanentemente. El glicerol en estudio se caracterizó por una densidad de 1.02 g/mL, contenido en metanol de 2.6 %, punto de inflamación superior a los 65 ^{0}C y el pH de 8.74. Mostró color marrón oscuro y sabor dulce. Estos indicadores concuerdan con las propiedades de especificación de calidad del glicerol crudo establecidas por las normas internacionales. La producción promedio de leche/vaca/día fue mayor (P=0.0001) en el tratamiento con el aditivo glicerol (3.43 vs. 2.20 L para el control) al igual que la producción de leche total (P=0.0159). Se concluye que el glicerol crudo obtenido en el proceso de transesterificación del aceite de *J. curcas* posee propiedades físico químicas y organolépticas que lo hacen apto para su utilización en la alimentación animal en forma de aditivo. Su utilización incrementó la producción de leche de vacas Siboney de Cuba, sin afectar los indicadores de calidad. Se recomienda su aplicación en condiciones de producción y el desarrollo de nuevos estudios en otras categorías y especies de animales.

Palabras clave: *biocombustible, glicerina, indicadores productivos, oleaginosas*

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Introduction

In recent decades, the global demand for the use of renewable energy sources has increased, with the aim of reducing the enormous environmental impacts and energy dependence currently caused by the excessive and indiscriminate use of fossil fuels (Zulqarnain *et al.* 2021).

According to Nivia-Osuna *et al.* (2020), with the rise of global biofuel production, especially biodiesel, as a sustainable source of a new energy resource, the alternative of its production from oilseed plants arises, which represent various benefits related to its biodegradability. These are cleaner plants and are part of a technological progress that seeks to limit greenhouse gas emissions and reduce environmental pollution.

Among the challenges that the biodiesel industry faces is the creation of new markets for the use of glycerol, a byproduct of the biodiesel manufacturing process in an amount of approximately 10 %, by weight of the triglycerides that make up the oils and fats used as raw matter (Melero *et al.* 2012). Due to its properties, glycerol is a primary raw matter in the food, medical and cosmetic industries (Posada and Cardona 2010). However, glycerol from biodiesel production has contaminants that make difficult its use in these industries that require high purity standards (Menéndez *et al.* 2023).

In 2006, the US Food and Drug Administration (FDA 2006) recognized glycerol as safe for use in concentrates for animal feeding. There are several studies that use this by-product as an alternative energy source in ruminants feeding, because it is available in the animal for the production of short-chain fatty acids at ruminal level (Ogborn 2006, Wang *et al.* 2009, Van-Cleef *et al.* 2016 and Nivia-Osuna *et al.* 2020).

In Guantánamo province, Cuba, a research project is being carried out with financing from the Swiss Agency for Development and Cooperation (SDC), which has among its purposes the promotion of integrated agricultural systems, in which biofuels and animal food are produced from non-edible oilseed plants, including *J. curcas* L., which stands out for its potential as a source of natural and renewable raw matters, is not classified as human or animal food and shows resistance to extreme natural conditions (Agrawal *et al.* 2023). The Guantanamo biodiesel plant produces 400 L of biodiesel/day, equivalent to 105,600 L/year. These production volumes generate approximately 9,715.2 kg of glycerol/year.

There are no basic studies available that offer safety about the nutritive value of glycerol, which would allow its use in ruminant feeding as a result of the biodiesel production process. Therefore, the objective of this research was to evaluate the effect of crude glycerol, derived from *J. curcas* L. oil, on the production and quality of milk from Siboney de Cuba cows.

Materials and Methods

Physicochemical characterization of crude glycerol from J. curcas L. oil

The vegetable oil used in the process of obtaining glycerol came from *J. curcas*, originally from Cabo Verde, harvested in the southern coastal area of Guantánamo province. After harvesting, the ripe fruits were dried in the sun and shelled to obtain the seeds. For oil extraction, the seeds were pressed with an expeller machine with a power of 7.5 kW, speed of 1,400 rpm and capacity of 200 kg of seeds per hour. The crude oil obtained was filtered through a filter press, which guaranteed a product of 25 microns. Subsequently, the oil was subjected to a heating process at 105 ºC to extract all soluble and volatile impurities, including water (Piloto *et al.* 2021).

Glycerol was obtained during the biodiesel production process through an oil transesterification process, according to Sotolongo *et al.* (2021), with methanol and sodium hydroxide (NaOH) as a catalyst, at the Paraguay Biodiesel Plant, belonging to LABIOFAM, in Guantánamo.

The physicochemical characterization study of glycerol was carried out at "Centro de Estudio de Energía y Refrigeración de la Facultad de Energía Mecánica e Industrial de la Universidad de Oriente".The physicochemical and organoleptic properties determined, the test method, as well as the standards of the American Association of Materials Testing used, are shown in table 1. These properties were determined in triplicate.

Productive and milk quality indicators in cows supplemented with the mixture of wheat bran and glycerol

Location: The research was carried out at San Rafael dairy, km 18 on the highway to Santiago de Cuba, belonging to Unidad Empresarial de Base (UEB) Burene, Empresa Pecuaria Iván Rodríguez, Niceto Pérez municipality, Guantánamo province.

Animals: A total of thirty multiparous dairy cows of Siboney de Cuba breed were used, with an average pre-parturition body condition of 3.04 and an average pre-parturition live weight of 487.87 kg. The animals had an average number of lactations of 3.17 and between seven and 21 days after the start of milking.

Treatments and experimental design: A pre-experimental phase was carried out with the purpose of adapting the animals to the diets during 14 d and, subsequently, the research lasted 60 d. Analysis of variance was performed according to a completely random design with two treatments and 15 repetitions.

Each animal was considered a repetition, for a total of 15 per treatment. They were identified by the names and ear tags placed on the left ear. Each treatment was made up of

Table 1. Indicators, test methods and quality standards (according to test methods) to carry out the physicochemical characterization of glycerol from *J. curcas* oil

Indicators	Unit	Test method	Crude glycerol (international standards)
Water content (Karl Fischer)	% (m/m)	UNE-EN 12937:2000	4.3
Viscosity 40 ºC	mm²/s	ASTM D445-09 (ASTM International 2019)	69.0
Density at 15 ºC	g/mL	ASTM D1198-99 (ASTM International 2019)	1.2595
Ignition point °C	°C	ASTM D92-05ª (ASTM International 2019)	< 65
Refraction index at 20 ºC		NC ISO 6320. 2013. 202013220202013	1.47-1.57
pH		NC 528.2009	4-9
Cooper corrosion		ASTM D-130 (ASTM International 2019)	-
Humidity	%	NC ISO 662. 2001	1- 28.70
Ashes	% (m/m)	ASTM D5468-95 (ASTM International 2019)	5.1
Gross calorific value	MJ/kg	ASTM D5468-95 (ASTM International 2019)	18.20
Glycerol content	% (m/m)	ASTM D7591(ASTM International 2019)	79
Methanol content	% (m/m)	UNE-EN. 14110	2.6
Organoleptic characteristics	Color Taste	NRSP312 Medicine of plant origin	Viscous liquid odorless

representative animals with the same number of lactations, milking weeks and productive history, with the aim of reducing experimental error and variation factors not related to diet. The latter considered as the only factor of variation in productive results.

Treatment 1. Control group, each animal intake 1 kg of wheat bran during milking, without glycerol.

Treatment 2. Each animal intake 1 kg of wheat bran during milking + 200 mL of glycerol during the first 15 d, a dose that was increased to 400 mL during the last 45 d of the test.

The wheat bran used in the research had 88 % dry matter (DM) and 14.8, 6.0, 0.12 and 0.8 % crude protein (CP), crude fiber (CF), calcium (Ca) and phosphorus (P), respectively, as well as 4.60 MJ/kg DM of energy. Glycerol from treatment 2 was daily mixed, manually, with wheat bran to ensure adequate homogenization of both products.

Milking took place between 5:00 and 7:00 a.m. From that time until 5:00 p.m. the animals grazed in collective stables. The predominant grass in them was pitilla (*Sporobolus indicus* L.), whose nutritional composition was characterized by having 26.3 % of DM and 7.2, 28.5, 0.44 and 0.2 % of CP, FB, Ca and P, respectively, as well as 4.999 MJ/kg DM of energy.

Productive indicators: Daily milk production was quantified for each animal in each treatment and then the production of each experimental group was averaged. While for total milk production all milk produced in the 60 d of experimentation for each treatment was added.

Milk quality indicators: For milk quality studies, five random samples were taken from each treatment, to which potassium dichromate was added at a rate of 0.06 g/100 mL of milk and they were kept refrigerated for later sending to the laboratory. The acidity indicators were determined by titration with 0.1 N NaOH (NC 71, 2021); pH, by potentiometry; density, using a Quevenne lactodensimeter (NC 119, 2022); total solids and fat percentage, using the near-infrared spectroscopy method (FIL-141:B,1997) using the MILKSan 104 A/S Foss Electric.

The productive indicators evaluated were analyzed using the statistical package SAS 9.2 (2010).

Results and Discussion

Physicochemical characterization of crude glycerol from Jatropha curcas oil

The results of the physicochemical properties of crude glycerol from *J. curcas* oil and its comparison with the quality specification properties of crude glycerol reported in the literature are shown in table 2. Its composition does not differ from those reported in the international literature.

The physicochemical characteristics of crude glycerol have been widely described in the international literature (Nivia-Osuna *et al.* 2020 and Jadan *et al.* 2023). Glycerol is classified as a stable and multifunctional compound. According to its physicochemical properties, it is considered a highly hygroscopic polyalcohol, with neutral pH, chemically stable under normal storage and handling conditions. It is odorless, viscous, sweet to the

Table 2. Physicochemical characterization of crude glycerol obtained in the transesterification process of *J. curcas L.* oil.

Indicators	Unit	*Jatropha curcas* L. oil Glycerol	Crude Glycerol (international standards)
Water content, karl Fischer	%, m/m	3.80 ± 0.02	4.3
Viscosity, 40 ºC	mm, ²/s	47.07 ± 0.50	42.41 - 69.01
Density, 15 ºC	g/mL	1.02 ± 0.00	1.01- 1.26
Ignition point, °C	°C	71.00 ± 1.00	< 65
Refraction index, 20 ºC		1.58 ± 0.00	1.47-1.57
pH		8.74 ± 0.04	4-9
Cooper corrosion		1a	-
Humidity	%	18.00 ± 1.00	1- 28.70
Ashes	%, m/m	4.50 ± 0.02	5.1
Gross calorific value	MJ/kg	18.62 ± 0.00	18.20
Glycerol content	%, m/m	76.50 ± 0.01	79
Methanol content	%, mm (m/m)	2.6± 0.01	2.6
Organoleptic characteristics	Color Taste	Dark Brown Sweet	Dark coffee - dark Brown Sweet

taste, soluble in water and other polar solvents, insoluble in hydrocarbons and of low environmental toxicity. In addition, it has humectants properties and high energy content. It has a high boiling point and viscosity, caused by the hydrogen bonds formed between its molecules (Jadan *et al.* 2023).

The physicochemical characteristics of the glycerol under study coincide with these properties. However, it was observed that its ignition point is slightly higher than that found in the literature with a magnitude higher than 65 ºC (table 2). This could be related to the lower amount of methanol used in the transesterification process (Sotolongo *et al.* 2021). The refraction index was very close to the upper limit of the values found in the literature.

The consulted literature contains pH values for glycerol from various vegetable oils, which are characterized by being acidic or alkaline with values below and above the range established by the quality specifications for crude glycerol (Zulqarnain *et al.* 2021 and Menéndez *et al*. 2023). However, the pH of the glycerol under study was in the range of crude glycerol quality specifications with a basic or alkaline pH magnitude.

Copper corrosion was (a for crude glycerol from *J. curcas*, corroborating its basic or alkaline character, being a non-corrosive substance. There were no reports of this indicator in the available literature for glycerol from other vegetable oils.

The methanol content of glycerol from *J. curcas* oil was 2.6 % (table 2), a value that coincides with that referred to in the international standards. According to FDA (2006), methanol contents higher than 0.5 % in diets for young and monogastric ruminants could be unsafe, due to its harmful effects, since methanol is metabolized in the liver, passing through formaldehyde, formic acid and finally, CO_2 and water. The formic acid metabolism is slow, so it accumulates in the body and produces metabolic acidosis.

The toxic and limiting effect of methanol intake is most frequently seen on monogastric or preruminant animals (Galvani 2008). However, in older ruminants the possible use of methanol to produce methane from its fermentation in the rumen by some methanogenic bacteria has been showed (EFSA 2010). Since the levels mentioned above are metabolized by ruminal bacteria and transformed into methane, they do not cause damage to ruminant animals that intake the product.

In the study, glycerol was subjected to purification treatment, where the previously heated glycerol phase sample was neutralized with 40 % acetic acid until reaching pH 7. The neutralized sample was subsequently heated to 75 °C to eliminate the methanol content (Sotolongo *et al.* 2021).

The organoleptic properties, such as color and taste of the crude glycerol under study, correspond to what has been reported in the literature and coincide with Menéndez *et al.* (2023) studies, who evaluated these properties in glycerol resulting from the transesterification process of other vegetable oils.

The properties of crude glycerol, obtained in the transesterification process of *J. curcas* oil at LABIOFAM Enterprise in Guantanamo province, have physicochemical and organoleptic properties similar to those of crude glycerol from other vegetable oils and are in accordance with the international standards for specifying the quality of crude glycerol, so it can be used in animal feeding.

Productive and milk quality indicators in cows supplemented with the mixture of wheat bran and glycerol

A vital indicator for assessing the effectiveness of a diet and the productivity of a dairy cow system is milk production. Table 3 shows the productive results in the dairy cows under study. The average milk production/cow/d and total milk production showed differences between the treatments. The highest value was observed in the treatment with the glycerol additive ($P<0.05$).

This result could be related to the gluconeogenic effect of glycerol as a food supplement, which induces an increase in energy availability for milk production from its fermentation in the rumen to propionate, mainly (Van Cleef *et al.* 2016). This supplement can participate with 30-72 % for the synthesis of glucose in the liver tissue, a contribution that depends on the amount of non-structural carbohydrates contained in the diet and the dry matter intake (Melero *et al.* 2012). In a study by Hidalgo-Hernández *et al.* (2018), it was shown that lipolytic bacteria, as well as *Selenomonas ruminantium* and *S. dextrinosolvens*, are the groups with the greatest participation in the fermentation of glycerol to propionic acid.

Glycerol, as an energy supplement, has been evaluated in diets for dairy cattle, at different stages of lactation, mainly in the transition period (Ogborn 2006 and Carvalho *et al.* 2011) and early and mid lactation (Wang *et al*. 2009), as energy supplement (Gaillard *et al.* 2018). However, the effects of glycerol feeding on milk production and composition have not been sufficiently conclusive (Nivia-Osuna *et al.* 2020).

Several studies show that there is variability in the effect of glycerol administration on ruminal pH and the proportion of acetate, propionate, butyrate and valerate, which affects milk production. However, its response depends on the dose and the rate of disappearance at ruminal level (Van Cleef *et al.* 2016).

Lee *et al.* (2011) provide 200 g of glycerol to rumen-cannulated steers fed a diet of alfalfa and corn silage. These authors found a decrease in the acetate:propionate ratio, due to higher propionate production.

According to Ogborn (2006), the effects of glycerol on milk production are observed when levels higher than 6 % are used. However, Bodarski *et al.* (2005) increased milk production with small doses of glycerol in cows during the first 10 weeks of lactation, which could be related to the increase in dry matter intake.

The results obtained in this research are in agreement with those obtained by Olivares-Palma *et al.* (2013), who showed that supplementation with 1500 g of crude glycerol produced an increase of approximately 8 % more in milk production. Khalid and Al-Anbari (2023) also found an increase in milk production with the addition of 150 mL of glycerol in Holstein cows.

There were no differences in any of the milk components studied at the beginning of the experiment before supplying glycerol (table 4), nor after 60 d of experimentation (table 5).

In the scientific literature consulted, it was observed that the milk components are generally not affected by the use of glycerol, with the exception of some researchers where effects on the percentage of milk fat have been found.

Table 3. Effect of crude glycerol from *Jatropha curcas* L. oil on milk production in Siboney de Cuba cows, supplemented with wheat bran

Indicators	Treatments		S.E ±	P
	Control	Control + glycerol		
Average milk production/cow/d during the experiment, L	2.20	3.43	0.11	0.0001
Total milk production per treatment, L	1320.00	2058.00	13.78	0.0159

Table 4. Effect of crude glycerol from *Jatropha curcas* L. oil on milk quality of Siboney de Cuba cows (day 1 of the experiment).

Indicators	Treatments		S.E±	P
	Control	Control + glycerol		
Acidity	0.139	0.144	0.010	0.3513
Density, g/L	1.033	1.035	0.020	0.6272
pH	7.10	7.10	0.03	0.3495
Total solids , %	11.40	11.43	0.12	0.5290
Fat, %	3.50	3.50	0.2	0.3814

Table 5. Effect of crude glycerol from *Jatropha curcas* L. oil on milk quality of Siboney de Cuba cows (day 60 of the experiment)

Indicators	Treatments		S.E ±	P
	Control	Control + glycerol		
Acidity	0.141	0.148	0.010	0.3824
Density, g/L	1.033	1.035	0.020	0.6751
pH	7.20	7.20	0.01	0.3425
Total solids, %	11.41	11.45	0.13	0.6780
Fat, %	3.50	3.50	0.02	0.3637

Shin *et al.* (2012) modified the fat concentration, with higher values when using 5 % glycerol. Meanwhile, Suarez Ariza (2020), when evaluating the effect of glycerol on cows whose basal diet consisted of *Brachiaria decumbes* grazing and corn silage, there were no found changes in the composition and quality of the milk. Khalid and Al-Anbari (2023) were no found changes in the percentages of milk components and recently Da Rosa *et al.* (2024), when evaluating the effect of glycerol on grazing cows of *Lolium multiflorum* Lam. also there were no found changes in the composition and quality of milk at different lactation periods.

The percentage of fat obtained in the research corresponds to the values reported for the crosses (Holstein × Zebu) and the expected response with a basically fibrous diet, with predominance of acetic fermentation at the ruminal level (Hernández and Ponce 2003). The amount of forage in the cow's diet is a determining factor in the concentration of fat in milk and its importance lies in the fact that it is the main means of ensuring the precursors of fat synthesis at satisfactory levels. These precursors are obtained from diet and adipose tissue. The SCFA are synthesized in the mammary gland from acetate and β-hydroxybutyrate (Mayburgh *et al.* 2012), both compounds derived from the fermentation of the main fibrous component of the diet.

Conclusions

It is concluded that the crude glycerol obtained in the transesterification process of *J. curcas* oil has physicochemical and organoleptic properties that make it suitable for their use in animal feeding as an additive. Its use increased milk production in Siboney de Cuba cows, without affecting quality indicators. Its application in production conditions and the development of new studies in other categories and species of animals are recommended.

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