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RESEARCH ARTICLE

Effect of the consumption of a cocoa paste bar (*Theobroma cacao* L.) on metabolic indicators in university students

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ABSTRACT

The aim of this study was to determine the effect on metabolic indicators (total cholesterol, triacylglycerides, HDL, LDL and glycemia) in university students, after the daily consumption of 1.00 g of a cocoa paste bar (PCB) and 0.50 g of a white chocolate bar (BCB) during two months. The population was characterized according to their sex, weight, height, body mass index (BMI), physical activity and diet. The pre- and post-metabolic levels were measured in the control group (BCB) and experimental group (BPC). The students have normal weight (22.90 kg/m^2 and 24.99 kg/m^2), average physical activity was predominat (55.60 %) and their nutrition was inadequate. The study groups presented metabolic levels within the normal parameter. However, the consumption of 1 g of PCB for two months increases HDL levels (36.94 ± 13.42 mg/dL vs 59.74 ± 9.41 mg/dL), an effect that is highly desirable in a food.

Keywords: Chocolate; Glucose; Lipid profile; *Theobroma cacao* L

INTRODUCTION

In Mexico, most of the students who enroll in the University are from other municipalities or states, so they have to enter a period of social and cultural adaptation, which affects their lifestyle, mainly in their diet, so there is an increase in the consumption of processed foods, which provide high calories and low content of bioactive compounds, in addition, this population performs little physical activity, so if these parameters are not modified, in the future it will affect a risk of suffering from noncommunicable diseases, such as diabetes and dyslipidemia (Barba 2018). The prevalence of some metabolic syndrome in the Mexican population over the age of 20 is one of the causes that concern organizations and institutions involved on health (Shamah et al. 2020). That is why organizations and institutions involved in health, suggest the implementation of first level prevention programs, which include the consumption of food with bioactive compounds, one option could be the cocoa (Adams et al. 2016).

In Mexico, 28,000 tons of cocoa are produced each year (INEGI, 2021) and consumption per person is 700 g per year (Procuraduría Federal del Consumidor, 2018). It is mainly used for the preparation of cocoa paste, which is the product of fermentation, drying, roasting, grinding and mixing, conching and tempering, this product is pressed to extract its derivatives: cocoa butter, cocoa liquor and cocoa powder (residue), and these are used for the diversity of chocolates (Adams et al. 2016), which decrease its bioactive compounds such as antioxidants and unsaturated fatty acids (Fox et al. 2019). Because of this, dark or bitter chocolate, which has a percentage of 50.00% to 99.00% of cocoa paste plus sugar, is highly demanded by consumers, since its intake has a positive effect on blood pressure, insulin resistance and the reduction of lipid deposits (Rull et al. 2015; Fox et al. 2019), for which cardioprotective, anti-

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inflammatory, anti-obesity and neuroprotective properties are attributed (Rodríguez et al. 2019), compared to white chocolate, which does not contain cocoa and has a lower content of unsaturated fatty acids (Directive 2000/36/EC of the European Parliament and of the Council 2000). As evidenced in a study comparing the effect of consuming dark chocolate and white chocolate without cocoa, it was observed that consuming dark chocolate improved cognitive function, while white chocolate did not have this effect (Sumiyoshi et al. 2019).

Studies have been carried out to investigate the effect of cocoa consumption on health, in one study a moderate consumption of chocolate (7 to 15 g of cocoa per day) was associated with a lower frequency of chronic diseases (Rodríguez et al. 2019). It was also observed in postmenopausal women that an intake of 10 g of cocoa-rich chocolate causes a decrease in blood pressure and improves cardiovascular health (García et al. 2020). In addition to its potential use as an anticancer agent, which has been shown in vitro, but more research is needed in this regard (Zamanian and Rezaei, 2019).

Therefore, the aim of this work was to evaluate the effect of daily consumption during two months of 1.00 g of cocoa paste bar (*Theobroma cacao* L.) and 0.50 g of white chocolate bar on metabolic indicators (total cholesterol, triacylglycerides, HDL cholesterol, LDL and glycemia) in university students.

MATERIALS AND METHODS

This essay was a randomized controlled clinical trial. It was carried out at the Institute of Health Sciences (ICSa) of the Universidad Autónoma del Estado de Hidalgo (UAEH).

Population sample

For this study, 38 students (27 women and 11 men) from the ICSa of the Bachelor's Degree in Nutrition at UAEH were included: those students among 19-25 years, women and men who studied from the third to eighth semester of the Bachelor's degree, who presented a normal or altered lipid profile and glycemia level, and those who signed informed consent. For the exclusion criteria: they were pregnant women, students with some allergy and intolerance to cocoa derivatives, with pharmacological and dietetic treatments, and students with an ingestion of polyphenol supplements and who suffer from migraine. In the elimination criteria: they were the students who withdrew voluntarily, and those who did not finish the intervention. The study was approved by the Research Ethics Committee of the ICSa of the UAEH (CEI-2020-005).

Supply of the cocoa paste and white chocolate bars

In a previous study, a cocoa paste bar was produced. The fermented and dried cocoa beans (*Theobroma cacao* L.) were purchased at the Mayordomo store in the City of Puebla, Puebla. The cocoa beans were washed and roasted (115 °C for 5 min), then the epicarp was manually removed. The beans were ground in a manual grain mill (Tisamatic, Mexico City, Mexico). The paste obtained was melted (58 °C for 5 min) and deposited in 1.00 g molds. The molds were placed in a refrigerator (7 °C) (Image 1). Subsequently, the cocoa paste bar was analyzed for fatty acids and phenolic compounds (Table 1). On the other hand, white chocolate was purchased from a commercial brand and to make the bar, it was deposited in 0.50 g molds. The chemical composition of white chocolate is shown in Table 1.

Collection of blood sample

The extraction of the blood sample was carried out at the beginning and at the end of the study, the biochemical analysis of the blood (lipid profile and glycemia) was performed according to the technique and the materials required and indicated by the Mexican standard (NOM- 253-SSA1-2012) for the disposal of human blood and its components for therapeutic purposes (NOM 2012).

Determination of metabolic indicators

Plasma and blood serum were separated and an SPRINREACT® brand enzyme kit (Girona, Spain) was used to determine the metabolic indicators (glucose, triacylglycerides, total cholesterol and high-density lipoprotein HDL) and the analysis of each of the indicators followed the manufacturer's methodological instructions. The determination of low-density lipoprotein LDL was through Friedewald's formula reported by Garzón (2006).

Diet quality

The quality of the students' diet was carried out by applying a questionnaire called 24-hour remainder, where their daily food intake [type, frequency and amount (g)] was specified for three days during two months, two assigned

Table 1: Chemical characteristics of white chocolate trademark (WC) and chocolate paste made by our work team (CP).

Nutrient (g/100 g)	WC	CP
Energy (Kcal)	130.00	3.60
Protein (g/100 g)	< 1.00	13.29
Lipids (g/100 g)	7.00	53.00
Saturated (g/100 g)	7.00	0.04
Monounsaturated (g/100 g)	NR	82.00
Polyunsaturated (g/100 g)	NR	9.06
Carbohydrates (g/100 g)	16.00	28.09
Fiber (g/100 g)	< 1.00	UN
Sodium (g/100 g)	5.00	UN
Phenols (mg EAG/100 g)	NR	1379.75

NR: Not reported. UN: Undetermined.

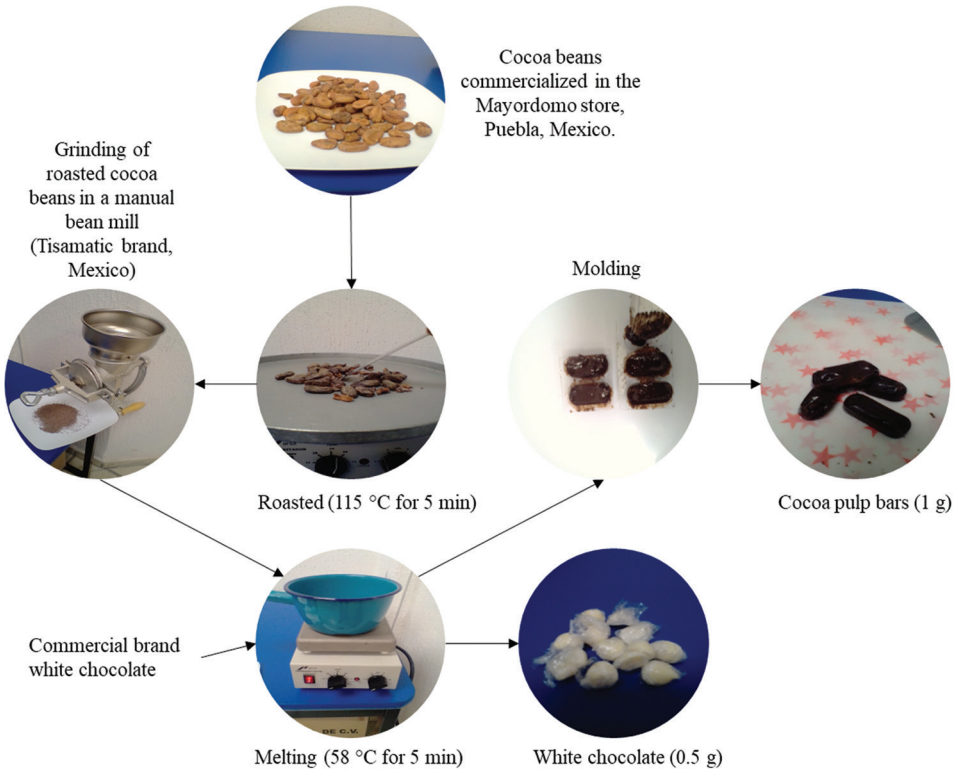

Image 1. Process of obtaining cocoa paste bar (*Theobroma cacao* L.) and white chocolate bar

working days and and one weekend day, and to determine the nutritional composition of their diet, a food processor nutrition analysis software ESHA Research (Oregon, United States) was used, in which was used the recommended daily intake parameter for adults over 18 years (INCMNSZ 2015).

Physical activity

The physical activity record was made by applying a questionaire (before the intervention of the cocoa paste bar and the white chocolate bar) according to the specifications of the International Physical Activity (2016), which provides information on the time spent to perform physical activity in MET-s (Metabolic Equivalent) per minute of walking per week.

Statistic analysis

The analysis of the results was carried out withstatistical program SPSS © (Statistical Package for Social Studies) version 19.0 for Windows 10 (IBM Corp. Released 2013. Armonk, NY: IBM Corp), applying the Shapirto-Wilk test to make the analysis of normality of the study groups. To analyze the variables of weight, height and body mass index (before the consumption of the white chocolate bars and cocoa paste), to determine the dependent variables, to compare the pre- and post-means of each one of the groups and to know the significant difference ($p < 0.05$) of glucose, cholesterol, triacylglycerides, HDL and LDL the Student t-test was applied.

RESULTS

Sample population

In the population characteristics results, there is predominance of women in both groups (control and experimental), due to the fact that they are the majority in the Nutrition Degree (Table 2) and because of their morphology (hormonal factors) (Martínez-Roldan 2005), there are differences in the results of the age, weight and height indicators.

Regarding the physical activity performed by the students of the Nutrition Degree, it is mainly in the classification of medium (control group) and high (experimental group) (Table 2). The results of the average physical activity (control group) are similar to the percentage (54.8%) indicated by Castañeda-Vázquez et al. (2016).

In the results of diet, it was determined that the students of the Nutrition Degree, their diet is unbalanced, insufficient in quality and quantity of nutrients, where the control group consumes a higher content of macromolecules and energy, compared to the experimental group.

Effect of the consumption of cocoa paste on the metabolic indicators

Table 3 shows the results of the metabolic indicators of the control and experimental groups with the consumption

of 0.50 g of white chocolate and 1.00 g of cocoa paste, respectively.

As to the blood glucose concentration, it is observed that there was a significant difference in the control group compared to the experimental group, in the last one it is due to a lower consumption of carbohydrates. However, the groups that participated in this work have a normal blood glucose level (< 140 mg/dL or 7.8 mmol/L) (American Heart Association 2020).

In the results in the concentration of triacylglycerides, in both groups it is observed that they presented an increase in these constituents, due to the increase in lipids in their diet. For other hand, the student population of the Nutrition Degree is within desirable levels of triacylglycerides (< 150 mg/dL) (American Heart Association 2020).

The results of the total cholesterol concentration in the control group increased compared to the experimental group (Table 3), but he levels are within what is recommended as normal with < 200 mg/dL (American Heart Association 2020).

On the other hand, the consumption of 1.00 g of cocoa paste increased the HDL concentration, compared to the control group. It has been indicated that HDL levels > 45 mg/dL are related to a lower incidence of myocardial infarction or cardiovascular risk, with the recommended values for men and women of 45 mg/dL and 50 mg/dL respectively. Moreover, it is desirable for both gender to reach levels of 60 mg/dL (American Heart Association 2020), a value that was similar to that obtained in this work, with the consumption of 1.00 g of cocoa paste.

In the results of the LDL levels determined in the experimental group (which consumed 1.00 g of cocoa paste), a significant decrease was observed compared to the control group. However, both study groups are within a reduced risk level for ischemic heart disease (< 100 mg/dL) (American Heart Association 2020).

Table 2: Population characteristics.

Population	Control group (N=18)	Experimental group (N=20)
Age	20.83 ± 1.75	21.70 ± 2.49
Male	4	7
Female	14	13
Weight (Kg)	61.71 ± 16.22	66.43 ± 13.94
Height (m)	1.63 ± 0.08	1.62 ± 0.09
Body Mass Index (Kg m^{-2})	22.90 ± 4.04	24.90 ± 3.74
Physical activity (%)		
Low	16.70	10.00
Medium	55.60	20.00
High	11.10	35.00
No answer	16.70	35.00
Diet		
Energy (Kcal)	1759.89 ± 594.18	1655.73 ± 742.84
Carbohydrates (g)	208.44 ± 76.26	183.48 ± 99.49
Protein (g)	77.89 ± 38.61	72.91 ± 24.54
Lipids (g)	83.65 ± 34.55	70.57 ± 27.73

Values are mean of duplicate analyses ± DE.

DISCUSSION

Sample population

The students of the Nutrition Degree had a body mass index within the normal parameters (19.00 to 24.99 kg/m) (Centers for Disease Control and Prevention 2018), results that are different when compared with another study with university students conducted by Leyva-Soto (2018) who determined in this population a body mass index greater than 25 kg/m, and reported that this increment is due to a sedentary lifestyle, lack of responsibility and organization, which will impact in the future that this population has noncommunicable diseases, which are originated when there is a high intake of hypercaloric nutrients and a scarce physical activity, since these compounds are stored in white adipose tissue, which affects the ability of adipocytes to buffer the concentration of free fatty acids and glucose, so there is increased secretion of free fatty acids and pro-inflammatory adipokines, such as tumor necrosis factor alpha (TNFα), which has an impact on fatty acids free deposits are ectopically deposited in muscle, liver and pancreas, leading to hyperglycemia, hyperlipidemia and hyperinsulinemia respectively (Chu ang and McIntosh 2011).

Generally, the physical activity in university students is low as was reported in a study carried out on university students from different Faculties, Rodríguez-Rodriguéz

Table 3: Results of glucose, triacylglycerides, total cholesterol and HDL in control and experimental group.

	Control group			Experimental group		
	PRE	POST	*P value*	PRE	POST	*P value*
Glucose (mg/dL)	88.07 ± 12.35	92.94 ± 11.45	0.023*	110.49 ± 14.80	88.80 ± 18.80	0.001*
Triacylglycerides (mg/dL)	112.95 ± 30.12	144.76 ± 38.79	0.008*	105.60 ± 54.29	139.49 ± 56.65	0.027*
Cholesterol (mg/dL)	119.14 ± 36.58	127.11 ± 31.01	0.026*	127.99 ± 29.07	117.02 ± 20.61	0.130
HDL (mg/dL)	49.15 ± 5.49	46.72 ± 4.38	0.005*	36.94 ± 13.42	59.74 ± 9.41	0.000*
LDL (mg/dL)	47.40 ± 1.13	51.44 ± 1.22	0.005*	68.25 ± 1.21	29.38 ± 1.31	0.000*

Values are mean of duplicate analyses ± DE. *Significant difference $p < 0.05$.

et al. (2018) stated that this population is mostly sedentary, and very few have moderate or vigorous physical activity, so they are prone to increase long-term noncommunicable diseases, as stated by the WHO (2020) where he reports that at least 60% of the world's population, does not perform physical activity necessary to obtain health benefits, so if their lifestyle is not changed, will affect the incidence of noncommunicable diseases, because exercise has anti-inflammatory effects, reduces body weight, total body fat and visceral fat, leading to increased endocrine activity and a reduction in inflammatory biomarkers secreted by adipocytes and immune cells (Petros et al. 2004).

The diet in university students is unbalanced, insufficient in quality and quantity of nutrients as was reported by De Piero (2015) who evaluated the food consumption (2253 kcal) and the profile of the diet of university students (86.90 g of protein, 73.30 g of lipids and 308.60 g of carbohydrates), indicating that their diet is insufficient in the quality of the nutrients they ingest, and their food intake is not within the recommended parameters (Rizo-Baeza et al. 2014). Regardless of age, sex and sociodemographic development of the place of residence, it has been indicated that poor eating habits are associated with a variety of chronic diseases, and may be an important factor in mortality from non-communicable diseases, and to prevent these conditions, it is suggested that adequate consumption of foods containing bioactive compounds (Lancet et al. 2019).

Effect of the consumption of cocoa paste on the metabolic indicators

The consumption of 1.00 g of cocoa mass bar influence blood glucose concentration, was due it contains 1379.75 mg EAG 100/g of phenols compared to white chocolate with 40.75 mg EAG 100/g (Lončarević et al. 2019). In another related study, it was observed that the population that consumed 96.00 mg of flavonoids per 100 g of dark chocolate had a lower risk of suffering from type 2 diabetes (Maskarinec et al. 2018), this may be due to the binding of phenols on the ligand of the receptor gamma activated by the proliferator of peroxisomes (PPAR-γ) (Kuroda et al. 2003), which exerts an important activity in the metabolism of glucose and fats, in addition, it has been shown that chronic inflammation subacute is a factor in the development of insulin resistance and diabetes (Miranda et al. 2015), since phenols inhibit the activation of mitogen-activated protein kinases (MAPK), nuclear factor kappa B (NF-κB) and activator protein (AP)-1, which may prevent the signaling of tumor necrosis factor-alpha (TNFα)/TNF receptor (TNFR) or lipopolysaccharide (LPS)/toll receptor (TLR) to MAPK kinases (MEK), extracellular signal-related enzyme kinase (ERK) and the terminal kinase c-Jun-NH2 (JNK), which decreases the activation of the inflammatory transcription factors NF-κB and AP-1, which are potent inducers of the expression of inflammatory genes [TNFα, interleukin (IL)-6, IL-8, IL-1β, and monocyte chemoattractant protein (MCP) -1] and negative regulators of insulin signaling [phosphorylation of serine residue 307 on the insulin receptor substrate (IRS) and protein tyrosine phosphatase (PTP) -1B], suppressing the insulin signaling required for the insulin-dependent glucose transport 4 (GLUT4) (Chuang and McIntosh, 2011).

On the other hand, in the cocoa paste bar, 82.00 g and 9.06 g of mono- and polyunsaturated fatty acids were quantified respectively, and it has been indicated that monounsaturated fatty acids have an anti-inflammatory effect, which prevents the activation of the NLRP3 inflammasome, which can be induced by saturated fatty acids in human monocytes/macrophages (L'homme et al. 2013). In addition, a diet high in monounsaturated fatty acids or the substitution of saturated for monounsaturated fatty acids induce changes in the distribution of abdominal fat, improve insulin sensitivity and postprandial oxidative stress in patients with metabolic syndrome (Finucane et al. 2015) due to the presence of phenols and unsaturated fatty acids in the cocoa paste bar, it can be a food that exerts benefits for insulin sensitivity, as reported by Perdomo et al. (2015) who demonstrated in a cardiovascular cell line that oleic acid does not generate and prevent the incidence of insulin resistance, since the activation of the PI3K enzyme and the AMPK-dependent mechanism were observed in the myotubes (Salvadó et al. 2013; Finucane et al. 2015; Perdomo et al. 2015) which prevents the phosphorylation of IRS-1 Ser and maintains the phosphorylation of IRS-1 Tyr in the presence of TNF-α or palmitate, favoring the activation of the PI3K pathway (Perdomo et al. 2015).

The concentration of triacylglycerides depends on the diet as indicated Espinoza et al. (2011) who analyzed the effect of cocoa products (2 to 12 weeks) in a population where their diet predominated saturated lipids, reported that there were no changes in the levels of triacylglycerides and HDL, results that are not similar to those obtained in this work. Dietary fatty acids have been indicated to influence the modulation of preadipocyte differentiation, and it has been shown that a diet with high concentrations of saturated fatty acids induces hyperplasia (proliferation of precursor cells) and hypertrophy of the adipose tissue (accumulation of triglycerides in preadipocytes) (Varela et al. 2013), since fatty acids exert an effect on the control of preadipocyte differentiation, which are regulated by gene expression mediated by receptors activated by proliferators peroxisomal (PPARγ), where fatty acids are endogenous ligands of PPARs, and most of them activate all PPAR isoforms (Chuang and McIntosh 2011). In another study

with a diet with a high content of acids saturated, mono- and polyunsaturated fats (Raz et al. 2013), differences were found in the results and it was indicated that this difference depend on the amount or sources of acids fatty acids, the time after the meal at which the concentrations were evaluated, the age, the people evaluated and the previous nutritional status of the participants or even due to the metabolism of fatty acids compared to other sources of lipids. In addition, ingested lipid sources not only contain saturated, mono- and polyunsaturated fatty acids, but also a wide variety of other nutrients, such as carbohydrates, fiber, protein, and other biologically active compounds, such as polyphenols and sterols, so they postprandial results could also have been influenced by the presence of these nutrients (Lozano et al. 2013).

The total cholesterol concentration in the control group increased, this effect in the control group may be due to the fact that the population did not have a control in the consumption of saturated fatty acids, and it has been shown that total cholesterol markers decrease when the intake, quantity and quality of saturated fatty acids are modified (Passos et al. 2019), since they activate the PPARα isoform, which also regulate the expression of genes for cholesterol synthesis and transport (Chuang and McIntosh 2011).

On the other hand, it has been reported that the health effect of HDL is that these chemical compounds protect the tissues and organs against the oxidation of LDL, because HDL transport paraoxonase-1 (PON1) that it is an antioxidant enzyme, which prevents the generation of oxidized pro-inflammatory lipids (lipid hydroperoxides and short-chain oxidized phospholipids) (Afonso and Spickett 2018). This biochemical mechanism is carried out since PON1 activates the enzyme HDL endothelial nitric oxide synthase (eNOS), in addition, it has been shown that HDL activates eNOS, initiating the intracellular kinase cascade associated with SR-B1 (Mineo et al. 2006). On the other hand, other mechanisms have been reported by which HDL can modulate inflammation, intervening in part with miR-223, since macrophages (J774) can transfer miR-223 to HDL (Vickers et al., 2011), that exert anti-inflammatory effects through the release of HDL-miR-223 and the translational repression of the intercellular adhesion molecule (ICAM)-1 in endothelial cells (Tabet et al. 2014).

The cocoa paste bar contains mainly phenols and also unsaturated fatty acids. These fatty acids can produce desirable health effects, due to the fact that they reduce the levels of total cholesterol, LDL-C and triacylglycerides, and increase the concentration of HDL-C (Nishi et al. 2014) as determined in this work to except for the levels of the triacylglycerides which have increased. Gilmore et al. (2011) carried out a nutritional intervention with a high content of monounsaturated fatty acids for 5 weeks, reporting an increase in the concentration of HDL-C and a decrease in the LDL-C: HDL-C ratio, similar to that obtained in this study, and indicated that it could be due to the activity of hepatic stearoyl-CoA desaturase-1 (SCD1) that can regulate plasma concentrations of triacylglycerides, since the mechanisms by which unsaturated fatty acids exert their effect on health are related to the concentration and size of lipoprotein particles and their metabolism (Lelis et al. 2016).

CONCLUSIONS

The students of the Nutrition Degree who participated in this work present normal weight, their physical activity is mainly medium. His quality of diet is unbalanced and his metabolic indicators are at a normal level. The daily consumption for two months of 1 g of the cocoa mass bar had a favorable effect on the HDL and LDL level.

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Authors' contributions

José Alberto Ariza Ortega, Nelly del Socorro Cruz Cansino and María Reyna Robles López designed and supervised the investigation. Luilli Antonio López Contreras, Esther Ramírez Moreno and Raúl René Robles de la Torre performed the data analysis. Imelda Sánchez Montoya and María Fernanda Escamilla Rosales performed the experimental part.

Disclosure statement

The authors declare no conflict of interest.

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