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High-Protein bar Supplemented with Chia Seed Improves Lipidemic Parameters in Wistar Rats

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Abstract — *Chia (Salvia hispanical.) seeds are known to have high content of polyunsaturated fatty acids (PUFA) and fiber. This study aimed to evaluate the effect of a High-Protein Bar (PB) supplemented with chia seed added to the feed on the organs, tissues, and biochemical parameters of male Wistar adult rats (n=32) divided into four groups (n=8), namely group I (ration + 20% chia seeds); group II (ration + PB without chia seeds); group III (ration + 20% PB containing 15% chia seed); group IV (ration + 20% PB containing 20% chia seeds). The shelf-life of PBs was assessed during 45 days in terms of texture, color, and antioxidant activity using the β-carotene/linoleic acid assay. The centesimal composition of the formulations showed a significantly higher value of fiber offered to group I. Animals of groups III and IV showed a lower consumption of the ration (p<0.05), while those of group I lower weight of the heart as well as of retroperitoneal, epididymal and perirenal tissues (p<0.05). The biochemical parameters showed a significant improvement (p<0.05) in testosterone levels in groups that received the rations partially replaced by chia seed-containing PB. In addition, group II, which received the ration enriched with PB without chia seed, showed the highest serum triacylglycerol value, highlighting the important role of chia seeds on lipidemic parameters. It is worth mentioning that more in-depth studies must be carried out to validate the results obtained in the current study.*

I. INTRODUCTION

Food intake is closely related to health, not only in terms of quantity but also of composition and quality of the diet (Quirk et al., 2013). With the high number of people with obesity and non-communicable chronic diseases (NCDs), researchers and public agencies from various countries have adopted strategies to raise awareness in the attempt to reduce these indexes. In the 1980s, Japan began the first strategy, through the use of functional foods, intending to prevent NCDs and improve their quality of life. Functional foods are foods that can reduce health risks and, therefore, should be consumed daily (Siró et al., 2008).

Research has considered chia (*Salvia hispanica* L.) as a functional food thanks to its high nutritional value, which depends on the planting, harvesting, storage conditions, and seed processing after harvest. Its chemical composition includes proteins of high biological value, a high content of polyunsaturated fatty acids such as omega 3 and 6(Julio et al., 2015)and a high content of dietary fibers which stimulate satiety and improve digestive system function, culminating in the reduction of body weight (Clark and Duncan, 2017). Studies have shown the role of chia seed in improving dyslipidemia, insulin resistance, and intramuscular lipid metabolism, as well as in inhibiting the lipogenic pathway(Ferreira et al., 2020).

High-protein bars (PBs) were initially designed with the main purpose of supplying nutritional deficiencies to military and physical exercise practitioners.The aim offood industry research and development (R&D) is to create new products and launch them on the market, and due the demand for healthy and nutritive foods, PBs are nowadays a good option for supplying fiber, proteins, vitamin, and mineral needs of consumers (Bosquesi et al., 2016).

Although there are several PBs on the market, sugar-free formulations are still little commercialized in Brazil. In this context, Veggi et al. (2018)studied sugar-free PB formulations containing different chia seed proportions as a source of fibers, among whichthat containing20%chia seed wasthe most accepted bythe panelists. Therefore, the present study aimed to investigate the effects of a) storage on physicochemical quality of two different PB formulations and b) intake of a diet based on PBs enriched with chia seedson tissue and biochemical parameters of healthy, sedentary eutrophic rats.

II. MATERIALS AND METHODS

Stability Study

In a previous study, Veggi et al. (2018) developed PB formulations supplemented with chia seeds in different proportions, namely10, 15, and 20% (PB2),and one without chia seeds to serve as a control (PB1). The centesimal composition of formulations developed by Veggi et al (2018) showed around 20% of moisture, 2.4% of ashes, 20% -23%of proteins, 20% of lipids, 12 - 22% of fibers, and 14% - 26% of carbohydrates. In the present study, the stability of bothPB1 and PB2,which was the most accepted in the previous study, was assessed, after different storage times, i.e., 0 (T0), 7 (T1), 15 (T2), 30 (T3) and 45 days (T4), in terms of physicochemical parameters such as color, texture, pH, water activity and antioxidant activity by the β-carotene/linolenic acid assay. For this purpose, the PBs were stored at 25 °C in an incubator for biochemical oxygen demand (BOD) testing, model TE-371(Tecnal, Piracicaba, SP, Brazil).

Antioxidant activity by the β-carotene/linolenic acid assay

The antioxidant activity of PB formulations was assessed by the β-carotene/linoleic acid assay previously described byRufino et al. (2006).The absorbance of samples was measured at 470 nm with a UV-Vis spectrophotometer, model UV-1800 (Shimadzu, Kyoto, Japan). All analyses were done in triplicate.The results were expressed as β-carotene oxidation inhibition percentage (% I), which was calculated,according to the following equations, as the decrease in sample absorbance (As) in relation to that(Ac) of a 6-hydroxy-2,5,7,8-tetramethylchroman-2-carboxylic acid (Trolox) solution used as a control:

$$Ac = Abs_{initial} - Abs_{final} \quad (1)$$

$$As = Abs_{initial} - Abs_{final} \quad (2)$$

$$\%\, I = \frac{A_C - A_S}{A_C} \times 100 \quad (3)$$

Objective color analysis

The objective color analysis of samples was performed with aportable spectrophotometer, model CM-700d(Konica Minolta, Tokyo, Japan), calibrated to a white standard, on the coordinates L* (lightness), a* (red-green component), and b* (yellow-blue component) according to the CIELab system methods. After exposition for 1h at room temperature(24 ± 1 ºC),six measurements were taken at three different points of each sample, and the average values were used for statistical analysis. In particular, the value of L* indicates the position of the point on the vertical axis of luminosity, the value of a*the intensity ofthe green (-) to red (+) component, and the value of b* that of blue (-) to yellow (+) component of light. The saturation index (C*) and hue angle (h*) were calculated bythe equations:

$$C^* = (a^{*2} + b^{*2})^{1/2} \quad (4)$$

$$h^* = \tan^{-1}(a^*/b^*) \quad (5)$$

Texture

Compression analysis after the samples had been left for 1 h at room temperature. It was performed with the TA.XT.PLUStexturometer (Texture Analyzer Stable Micro System Inc., Surrey, England), with a 5kg load cell, probe P/20P, and speed of 1mms^{-1}.For each treatment, 6 readings were performed, and the results were expressed as strength (N).

pH measurement

The pH of samples was measured by direct potentiometry using a digital potentiometer, model HI

2221 (HANNA Instruments, Woonsocket, RI, USA), according to themethod 943.71 of the Association of Official Analytical Chemists (AOAC, 2012).

Water activity

Water activity (A_w) was determined using a water activity meter, modelAquaLab 4TE 02 (Decagon Devices, Pullman, WA, USA), according to the method 978.18 of AOAC (2012).

Proximate feed composition

Centesimal analysis was carried out according to the methods described by the AOAC (2012). Moisture was determined gravimetricallyat 105 °C usingan oven, model400/2ND-300 (Nova Ética, Vargem Grande Paulista, SP, Brazil),according to the 925.09 method, ashes by incineration at 550 °C of the residue in a furnace, model D21 (Quimis, Diadema, SP, Brazil), according to the 923.03 method, lipids by a Soxhlet apparatus, modelTE 044 (Tecnal, Piracicaba, SP, Brazil), according to the method 920.39, proteins by a modified Kjeldahl 991.20 method (model TE 0363, Tecnal) usingthe 6.25 conversion factor, and crude fibers according to 044/IV (Instituto Adolfo Lutz, 2008).

Animal study

Healthy, sedentary, eutrophic, male Wistar rats (*Rattus norvegicus*), aged 3 months andwith weight between 250 and 300g (n=32), were used in this study. Initially, all animals underwent a five-day adaptation to the experimental environment (animal room) and received water and a standard semi-purified commercial diet (Presence Purina, São Paulo, SP, Brazil)*ad libitum*. The animals were housed individually in cages and kept under room temperature and 12-hlight-dark cycle. The study was approved by the Animal Use Ethics Committee (CEUA) of the Federal University of Mato Grosso, Campus of Cuiabá (CEUA Process n. 23108.209001 / 2017-71).

Experimental Design

The animals were subdivided into four groups (n=8) to assess the effect of four differentdiets formulated using standard rat feed (PresencePurina), partially replaced by chia seed or PB supplemented with chia seed (Table 1),on tissue, biochemical,and clinical parameters ofmale adult rats.

Over the 32-dayexperiments, the average water intake (mL/24h)and food intake (g/24h), calculated by the weight of leftovers the following day, were recorded,as well as the animal body mass three times a week.

Table 1.The different treatment diets offered to the different animal groups

Group	Diet
I	Ration replaced by 20%chia seeds
II	Ration replaced by 20% of *PB withoutchia seeds
III	Ration replaced by 20% of PB containing 15% chia seeds
IV	Ration replaced by20% of PB containing 20% chia seeds

*Formulations of the protein bars (PBs) can be accessed in Materials and Method and also in Veggiet al. (2018).

Sample collection and analysis

After the animals had fasted for 12 h, they were euthanized by inhalation of an excess of ethylic ether followed by decapitation, and blood sample was collected in tubes with clot activator/EDTA/EDTA K3. The determinations of glucose, lipid profile (TG, HDL, LDL, and total cholesterol), insulin, glycated hemoglobin, total blood proteins, albumin, andanabolic hormones GH and testosterone were carried out with an automatic biochemical analyzer, modelLabmaxPlenno (Labtest, Belo Horizonte, MG, Brazil). Hormonal cortisol as a stress biomarker was evaluated by chemiluminescence using an immunoassay system, modelImmulite® 1000 (Siemens Healthineers, São Paulo, SP, Brazil). Heart, liver, stomach, kidneys, adipose tissues (retroperitoneal, omental, epididymal, inguinal, and perirenal subcutaneous), and muscle tissues (soleus, extensors, and gastrocnemius) were properly excised and weighed (wet weight).

Statistical Analysis

For each formulation, 5 repetitions were performed, and the physicochemical data were analyzed in triplicate. Data were submitted to the Shapiro-Wilk normality test. For statistical comparisons among treatments, analysis of variance (ANOVA) was applied for parametric data, followed by theT-student test, while the Wilcoxon test was used for non-parametric data. For statistical comparisons between the times of each treatment, ANOVA was used for parametric data, followed by Tukey's *post-hoc* test (p<0.05), while the Kruskal-Wallis test followed by *post-hoc*Nemenyi test was applied to non-parametric data.

For the animal testing data were evaluated for normality test using the Kolmogorov-Smirnov method. Parametric data were subjected to analysis of variance (ANOVA), followed by Tukey's *post-hoc* test, while non-parametric data were analyzed using the Scott-Knott test.

For calculating differences between means, the R version 3.4.1 program was used. The effect size test was based on *a priori* testing (F-value ≤ 0.10: small; F-value0.10< F-value≤ 0.25: medium; F-value0.26< F-value≤ 0.40: large), performed by the G-power program, version 3.1.9.2.

III. RESULTS

Protein Bar Stability Study

Table 2 shows the stability profile of the two protein bar formulations (PB1 e PB2) developed by Veggi et al. (2018). A decrease in antioxidant protection during the shelf-life assaywas observed, so that, notably, after 30 days of storage the antioxidant activity was no longer detected.

Table 2. Physicochemical parameters of Protein Bars assessed over 45 days of storage in the BOD chamber at 25 ºC. Storage time (days):0 (T0), 7 (T1), 15 (T2), 30 (T3), 45 (T4)

Parameter	Storage time (days)	Formulation		*p*-value
		PB1	PB2	
%I**	T0	37.14 ± 5.75^{a}	44.09 ± 8.58^{a}	>0.05
	T1	47.96 ± 5.94^{a}	45.70 ± 7.92^{a}	<0.05
	T2	60.35 ± 3.02^{b}	59.01 ± 1.08^{b}	<0.05
	T3	12.53 ± 1.62^{c}	15.73 ± 1.40^{c}	<0.05
	T4	nd^{d}	nd^{d}	<0.05
L^{*}*	T0	66.62 ± 1.63^{b}	61.66 ± 1.59^{ab}	<0.05
	T1	68.88 ± 0.85^{a}	63.01 ± 0.85^{a}	<0.05
	T2	69.28 ± 0.73^{a}	61.60 ± 0.73^{ab}	<0.05
	T3	67.80 ± 0.85^{ab}	60.38 ± 0.85^{bc}	<0.05
	T4	65.65 ± 1.25^{bc}	59.99 ± 1.25^{bc}	<0.05
a^{*}	T0	7.22 ± 0.43^{bc}	6.59 ± 0.58^{b}	<0.05
	T1	6.92 ± 0.23^{cd}	5.54 ± 0.29^{c}	<0.05
	T2	6.65 ± 0.20^{d}	5.39 ± 0.50^{c}	<0.05
	T3	7.49 ± 0.29^{b}	7.94 ± 0.70^{a}	>0.05
	T4	7.88 ± 0.29^{a}	7.04 ± 0.55^{b}	<0.05
b^{*}*	T0	35.35 ± 1.42^{a}	35.18 ± 1.91^{b}	<0.05
	T1	35.62 ± 0.41^{a}	31.03 ± 1.58^{ab}	<0.05
	T2	34.91 ± 0.82^{ab}	38.13 ± 1.53^{a}	<0.05
	T3	34.21 ± 0.60^{b}	31.00 ± 0.94^{ab}	<0.05
	T4	34.20 ± 0.77^{b}	30.35 ± 1.24^{a}	<0.05
C^{*}*	T0	36.08 ± 1.40^{ab}	33.53 ± 1.90^{a}	<0.05
	T1	36.29 ± 0.40^{a}	31.52 ± 1.63^{ab}	<0.05
	T2	35.54 ± 0.86^{ac}	31.11 ± 1.55^{b}	<0.05
	T3	35.02 ± 0.58^{c}	32.01 ± 1.56^{ab}	<0.05
	T4	35.09 ± 0.79^{bc}	31.15 ± 1.27^{b}	<0.05
h^{*}(°)*	T0	78.44 ± 0.76^{ab}	78.65 ± 0.10^{ab}	0.54
	T1	78.10 ± 0.38^{a}	79.86 ± 0.57^{a}	<0.05
	T2	79.21 ± 0.19^{a}	80.02 ± 0.78^{a}	<0.05
	T3	77.64 ± 0.51^{bc}	75.63 ± 1.08^{b}	<0.05

	T4	77.06 ± 0.34^{c}	76.94 ± 0.86^{b}	0.85
Strength (N)*	**T0**	10.66 ± 1.84^{c}	9.68 ± 1.62^{b}	0.64
	T1	12.94 ± 3.82^{bc}	15.41 ± 2.50^{a}	0.20
	T2	13.93 ± 1.33^{ab}	15.54 ± 2.29^{a}	0.02
	T3	15.77 ± 1.92^{a}	9.91 ± 1.74^{b}	0.01
	T4	15.09 ± 2.15^{ab}	8.58 ± 1.19^{b}	<0.05
pH*	**T0**	5.90 ± 0.07^{a}	5.91 ± 0.07^{a}	0.11
	T1	5.72 ± 0.06^{b}	5.71 ± 0.05^{ac}	0.55
	T2	5.67 ± 0.07^{b}	5.65 ± 0.05^{c}	0.15
	T3	5.85 ± 0.04^{a}	5.85 ± 0.04^{ab}	0.04
	T4	5.90 ± 0.28^{a}	5.89 ± 0.27^{ab}	0.77
A_w^{**}	**T0**	0.85 ± 0.02^{b}	0.84 ± 0.02^{b}	0.19
	T1	0.87 ± 0.01^{a}	0.87 ± 0.01^{a}	0.81
	T2	0.86 ± 0.01^{b}	0.85 ± 0.02^{b}	0.16
	T3	0.85 ± 0.02^{b}	0.84 ± 0.02^{b}	0.72
	T4	0.85 ± 0.01^{b}	0.85 ± 0.01^{b}	0.43

Values expressed as means ± standard deviations. Means followed by the same letter in same column do not differ statistically. *The Wilcoxon test, and **Student's t-test wereused to compare the difference among the means. PB1 = Protein bar without chia seeds; PB2 = Protein bar with 20% chia seeds;%I = β-carotene oxidation inhibition percentage; C* = saturation index; L* = lightness; a* = intensity of the green (-) to red (+) component of light; b* = intensity of the blue (-) to yellow (+) component of light; h* = hue angle; A_w = water activity; nd = not detected.

The reactant used in the total antioxidant capacity assayis linoleic acid, in which one of the hydrogen atoms of one of the methylene groups is removed leaving the acid free radical ready to attack β-carotene molecules; consequently, its double bond is destroyed resulting in the formation of orange products and a decrease in absorbance at 470nm. Table 2 shows the protective activity of inhibiting the autoxidation of PB formulations over 30 days of storage; for this reason, a comparison test of means among the different storage times was not applied.

No differences between the PB samples were observed when considering pH and A_w over the shelf-life study ($p>0.05$) compared to the initial (T0) and final (T4) times of storage, while a statistically significant reduction of PB2 compression ($p<0.05$) was detected only at T4.

As for the color parameters, the intensity of the green-red component of light (a*) showed a statistically significant increase ($p<0.05$) during storage of PB1,which acquired a reddish color at the end of treatment.On the other hand, the intensity of the blue-yellow component (b*)for PB1 and PB2 showed significant decreases ($p<0.05$) either between them at the same storage time or among the different storage times in the same treatment, showing a general reduction in yellow color. Color saturation (C*) was significantly different ($p<0.05$) between PB1 and PB2,with PB1 showing a higher color purity compared to PB2. Regarding the tone, which is represented by the hue angle (h*) being a near brown color, samples did not differ from the initial to the final time of storage ($p>0.05$).

Proximate feed composition

The diet fed to animals in group I, with 20% chia seeds added to the ration, showed the highest amount of fiber ($p <0.05$), while that for group II, with 20% PB,showed the lowest one due to the absence of chia grains (Table 3). As expected, intermediate values of this content were detected in the diets for groups III and IV, which were prepared by adding 20% PB containing 15 and 20% chia seeds to the ration (Veggi et al., 2018), respectively.

Table 3. Proximate analysis of diets prepared for the different groups of test animals (male Wistar rats) by partially replacing the ration by chia seeds or protein bar

Group	Moisture	Ashes	Proteins	Lipids	Fibers	Carbohydrates
I	16.58±0.71[a]	6.54±0.28[a]	26.62±0.72[a]	4.33±1.23[a]	15.97±1.33[a]	29.94±1.50[a]
II	14.29±2.62[a]	5.72±0.17[c]	27.43±1.80[a]	6.47±1.20[a]	8.70±0.30[c]	37.47±0.45[a]
III	17.57±0.34[a]	5.94±0.12[bc]	28.18±1.64[a]	5.82±0.21[a]	10.59±0.71[bc]	31.89±2.10[a]
IV	16.83±0.60[a]	6.30±0.12[ab]	28.24±0.83[a]	5.35±0.23[a]	12.89±1.03[b]	30.39±2.01[a]
***p*-value**	0.07	<0.05	0.44	0.09	<0.05	0.53

Values expressed as means ± standard deviations. Means followed by the same letter do not differ statistically. The Tukey test was applied at the 1% probabilitylevel to compare the difference among the means. Group I = 80% ration + 20% chia seed; group II = 80% ration + 20% protein bar without chia seeds; group III = 80% ration + 20% PB containing 15% chia seed; group IV = 80% ration + 20% PB containing 20% chia seeds.

Whereas all diets exhibited similar contents of lipids and proteins, the ash content was lower in the diet containing PB without chia seeds, suggesting a positive role of seeds in the mineral content of diets.

General parameters

Figure 1 shows the values of daily intakes of diet and water as well as that of the animal body weight along the32-day experiment. Groups I and III showed similar values of water intake ($p > 0.05$) over the 32 days of the experiment. There was a progressive increase in the body weight of all the animals along the time up to 27 days, after which the growth ceased. Similarly, there was a generalized increase ($p < 0.05$) in the feed intake of all groups, but no significant differences were detected among groups ($p > 0.05$) from the 27th day onwards.

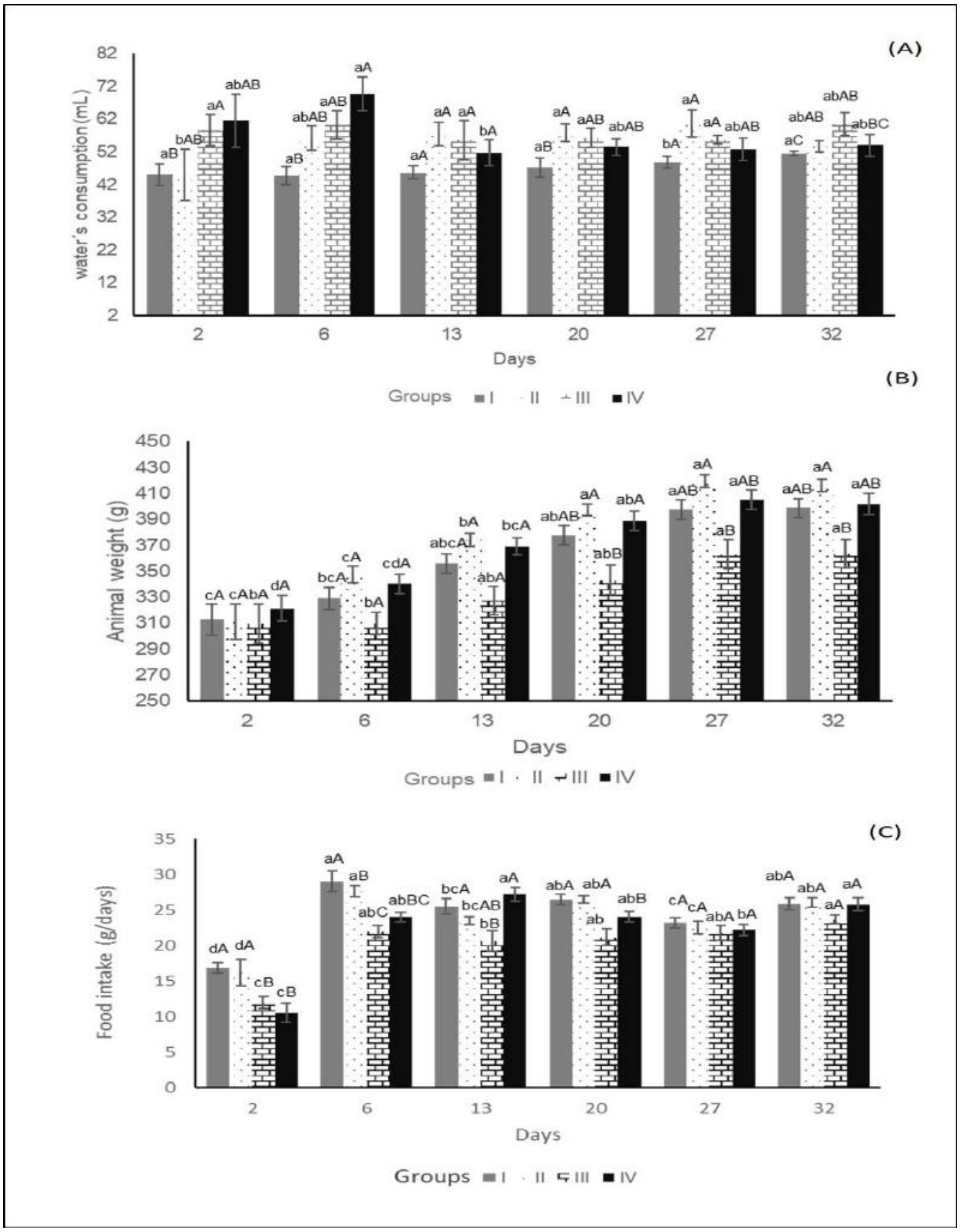

*Fig.1.(A) Food intake, (B)body weight, and (C) water intake in the different groups of test animals (male Wistar rats) from the 2nd to the 32nd day of the experiment. *Identical lowercase and capital letters indicate no statistically significant difference in each parameteralong the time in the same group and among the groups on the same day, respectively.Group I = 80% ration + 20% chia seed; group II = 80% ration + 20% protein bar without chia seeds; group III = 80% ration + 20% PB containing 15% chia seed; group IV = 80% ration + 20% PB containing 20% chia seeds.*

Fat and muscle organs and tissues

Group III presented lower weights of heart as well as retroperitoneal, epididymal and perirenal adipose tissues compared to group II, but with no statistically significant difference from groups I and IV, whilethe stomach weight in group III was lower than in group I only (Table 4).

Table 4. Weight of organs and tissues in the different groups of test animals (male Wistar rats).

Organweight(g)	Group				*p*-value	*F*-value
	I (n=8)	II (n=8)	III (n=8)	IV (n=7)		
Heart	1.37±0.60[ab]	1.41±0.04[a]	1.23±0.05[b]	1.40±0.03[ab]	0.04	0.25
Liver	13.45±0.62	13.64±0.49	11.91±0.59	13.07±0.61	0.16	0.45
Kidneys	3.26±0.16	3.13±0.08	2.86±0.14	3.06±0.13	0.17	0.45
Stomach	1.82±0.05[a]	1.77±0.05[ab]	1.55±0.08[b]	1.58±0.08[ab]	0.01	0.72
RAT	4.96±0.43[ab]	5.75±0.58[a]	3.40±0.42[b]	4.19±0.47[ab]	0.01	0.70
OAT*	0.44±0.09	0.61±0.07	0.40±0.09	0.52±0.10	0.3	0.38
EAT*	5.79±0.51[ab]	6.98±0.43[a]	4.63±0.62[b]	6.09±0.58[ab]	0.03	0.61
ISAT*	1.41±0.41	2.08±0.25	1.91±0.22	1.81±0.25	0.43	0.35
PAT	1.29±0.02[ab]	1.59±0.16[a]	0.92±0.10[b]	1.28±0.16[ab]	0.03	0.63
Soleus	0.37±0.02	0.38±0.01	0.35±0.03	0.39±0.02	0.61	0.37
EMT	0.27±0.03	0.18±0.02	0.22±0.02	0.23±0.02	0.08	0.65
GMT	4.63±0.25	4.87±0.06	4.43±0.26	4.79±0.18	0.46	0.34

Values expressed as means ± standard errors. Means followed by the same letter do not differ statistically. The Scott-Knott test was applied at the 1% probability level *The Tukey test was applied at the level of 1% probability. RAT = retroperitoneal adipose tissue, OAT = omental adipose tissue, EAT = epididymal adipose tissue, ISAT = inguinal subcutaneous adipose tissue, PAT = perirenal adipose tissue; EMT = Extensor muscle tissues, GMT = Gastrocnemius muscle tissue. Group I = 80% ration + 20% chia seed; group II = 80% ration + 20% protein bar without chia seeds; group III = 80% ration + 20% PB containing 15% chia seed; group IV = 80% ration + 20% PB containing 20% chia seeds.

Biochemical parameters

In the blood, group I showed the lowest glucose level, while group III the lowest high-density lipoprotein (HDL)level and group II the highest triglycerides (TAG) level. Moreover, groups III and IVwhose feed contained chia grain and protein bar in the diet showed the highest testosterone concentrations (Table 5).

Table 5.Effect of diets on blood biochemical parameters in the different groups of test animals (male Wistar rats)

Parameter	Group				*p*-value	*F*-value
	I (n=8)	II (n=8)	III (n=8)	IV (n=7)		
GLU (mg. dL^{-1})	111.89±3.78[b]	125.24±1.67[a]	137.37±3.70[a]	133.44± 3.16[a]	<0.05	0.85
TC (mg. dL^{-1})	99.35±4.74	92.56±3.78	90.32±4.30	94.54±3.44	0.47	0.27
HDL (mg. dL^{-1})	49.87±3.02[a]	41.63±0.99[ab]	40.12±0.10[b]	41.71±2.04[ab]	0.01	0.66
LDL (mg. dL^{-1})	31.62±2.69	22.46±2.88	29.90±3.22	33.18±2.28	0.06	0.53
TAG (mg. dL^{-1})	76.87±6.20[b]	128.00±10.40[a]	91.12±4.50[b]	92.14±6.90[b]	<0.05	1.13
TP (g.dL^{-1})	6.87±0.11	7.17±0.15	6.86±0.13	7.07±0.21	0.38	0.38
ALB (g.dL^{-1})	2.87±0.05	2.90±0.09	2.75±0.07	2.87±0.11	0.55	0.28
GLOB (g.dL^{-1})	4.00±0.08	4.27±0.08	4.11±0.14	4.20±0.14	0.34	0.45
HbA1c (%)	4.65±0.38	4.03±0.12	3.74±0.21	4.71±0.53	0.13	0.52
INS (μUI.dL^{-1})	1.55±0.07	1.42±0.05	1.81±0.20	1.58±0.07	0.13	0.52
IGF-I (ng. mL^{-1})	10.62±0.46	10.50±0.42	10.38±0.42	10.14±0.26	0.86	0.20
GH (ng. mL^{-1})	0.02±0.00	0.01±0.00	0.02±0.00	0.02±0.00	0.46	0

TES (ng. dL^{-1})	52.61±6.68^{bc}	49.54±8.47^{c}	92.95±5.68^{a}	82.81±11.25^{ab}	<0.05	0.25
COR (μg.dL^{-1}) *	0.89±0.04	0.96±0.12	0.90±0.03	0.90±0.03	0.86	0.17

Values expressed as means ± standard errors. Means followed by the same letter do not differ statistically. The Tukey test was applied at the 5% probability level. GLU = glucose; TC = total cholesterol; HDL = high density lipoprotein; LDL = low density lipoprotein; TAG = triglycerides; TP = total proteins; ALB = Albumin; GLOB = globulin; HbA1C = glycated hemoglobin; INS = insulin; IGF-I = insulin-1 growth factor; GH = growth hormone; TES = testosterone; COR = cortisol. Group I = 80% ration + 20% chia seed; group II = 80% ration + 20% protein bar without chia seeds; group III = 80% ration + 20% PB containing 15% chia seed; group IV = 80% ration + 20% PB containing 20% chia seeds.

IV. DISCUSSION

The aim of the present study was to determine the effects of a diet enriched with PB containing chia seeds on tissue and biochemical parameters in rats, and, to assess the effect of storage on physicochemical quality of two different PB formulations. Regarding the physical and physicochemical parameters analyzed during the shelf-life test, the addition of 20% of chia seeds affected the texture of PB2 during storage. PB1 streght remained the same throughout the storage period, while that of PB2 significantly decreased, which made it different from the other formulations after storage. On the contrary, (Zhu and Chan, 2018), when investigating the partial wheat replacement by chia seeds by up to 30% in bread baked with steam, observed an increase in sample hardness, which was attributed to the change in gluten formation due to wheat flour dilution by chia seeds.

In the present study, the decrease in the hardness of the PB containing chia seeds can be ascribed to the mucilage covering the seeds, which is mainly composed of acid and/or neutral heteropolysaccharides and proteins with the property of forming colloidal solutions that, in contact with water, become viscous. During the shelf-life study, there was no increase in water activity in both formulations likely due, at least in part, to the ability of chia seed mucilage to bind outer water and to form constitutional, vicinal, and multilayered water in the samples.This may have reduced the molecular interaction among the ingredients, especially that between isolated soy protein and concentrated whey protein.

The method of assessing the antioxidant activity through the β-carotene/linoleic acid system evaluates the inhibition activity of free radicals generated by linolenic acid peroxidation, i.e., the ability to protect the sample in the oxidizing medium. Natural compounds with antioxidant activity have been used in several studies to develop new functional products (Jaster et al., 2018). In this study, the antioxidant activity of samples assessed by this method decreased during the 45-day storage. Both PB1 and PB2 formulations contained vitamin E, a lipophilic antioxidant agent, and citric acid, an antioxidant and hydrophilic/lipophilic chelating agent. Even with the presence of these antioxidants, the formulations did not prevent the oxidation of the β-carotene/linoleic acid system. For the formulation containing chia seeds, this fact can be explained by the high content of polyunsaturated fatty acids present in chia, which may have favored the formation of free radicals whose stabilization would have required greater antioxidant activity. Also, the PBs remained stored under the protection of light and refrigerated, which may have reduced oxidation. Morales et al. (2016)observed that lipid oxidation was accelerated in wheat-based biscuit formulations supplemented with different concentrations of chia seeds, which led to a reduction of the biscuit shelf-life.In this respect, it should be remembered that chia itself has a high antioxidant power due to the presence of polyphenols, flavonoids, and mainly vitamin E (Ding et al., 2018),which allowed reaching a protective activity of up to 79.3 %(Reyes-Caudillo et al., 2008).

In general, all the color parameters (L*, a*, b*, C* and h*) of PB were reduced by the addition of chia seeds, which means that PB2wasless reddish and yellowish than PB1 likely because the seeds hadthe characteristic brown “dots” of chia as well as an internal mass with uniform color. Other researchers who analyzed food products, such as bread and hams, made with chia seeds and flour, observed the same color behavior concerning parameters a* and b* compared to controls without chia (Ding et al., 2018). The hue angle (h*) was in the range of 70 and 100º, which corresponds to a position in the first quadrant, i.e., to a predominantly yellow color. The h* value of PB1 slightly decreased during the 45-day storage at 25 °C from 78.44°at the start to 77.06° at the end ($p<0.05$), while no statistically significant difference was observed for PB2.Such a scarce or negligible influence of protein replacement by chia seeds on the tonality parameter, as well as on the pH,agreeswith previous results of color attributes sensorially evaluated by the affective test using a structured 9-point hedonic scale(Veggi et al., 2018). These authors did in fact report acceptance rates of 86.44 and88.66% for PB1 and PB2, respectively, by untrained individuals, even though the purchase intent and global preference for the latter formulation were greater than for other samples without chia seeds or with lesser amounts of seeds.

Marineli et al. (2015a)reported that rats fed with chia seed and oil showed no reduction in body weight, nor an increase in abdominal fat, but a food intake decrease, while obese rats fed with chia seeds for 8 weeks had lower body weight associated with reduced retroperitoneal and omental adipose tissues(Poudyal et al., 2012). The non-statistically significant variations in food intake observed in the present study are consistent with the hypothesis of the latter authors that chia has properties that allow the redistribution of lipids in the body, resulting in a reduction in the accumulation of fat in tissues and, as a consequence, promoting a protective effect on several organs, including the liver.

Considering that chia is composed of approximately 30% of fiber, the lower blood glucose levels observed ingroup I (Table 5) may have been due to the greater content of chia seeds in their diet. In fact, fiber intake increases the viscosity of the intestinal mucosa, thus reducing the contact surface of glucose with the enterocyte, the postprandial glycemia and the insulin resistance (Pereira and Ludwig, 2001),in addition to promoting both fermentation and formation of short-chain fatty acids (Anderson et al., 2009). Similar results were obtained by da Silva et al. (2016), who fed ratswith a diet containing chia seeds and flour for a shorter time (28 days) than in the present study, and investigation showed longer time (6 and 12 weeks) of chia seeds supplementation in obese rats(Marineli et al., 2015b).The aforementioned phenomena also promote increases in satiety and lipid oxidation (i.e., reduction of adipose tissues),which can explain the reduced adipose tissue weight observed in group III. Several studies highlight the role played by chia, since it is known that it is rich in monounsaturated fatty acids, which are oxidized more quickly compared to saturated fatty acids; therefore, it is likely that the chia-based diet induced a high rate of basal energy expenditure as well as an increase in thermogenesis.

Consumption of a diet rich in sucrose for a long period of time (3 months) promotes dyslipidemia and insulin resistance (Oliva et al., 2013). Moreover, it is known that the increased availability of serum triglycerides and free fatty acids promotes lipid accumulation in non-adipose tissues, such as cardiac, hepatic, and skeletal muscle tissues, and lip toxicity, thus leading to cell dysfunction and death in non-adipose tissues(Schaffer, 2003). Chicco et al. (2009)did not observe any differences in blood glucose level after 3 weeks of administration of a diet rich in sucrose as well as a diet rich in both sucrose and chia seeds, while, after 2 months of ingestion, chia supplementation reduced visceral adipose tissue, dyslipidemia, and insulin resistance. In the present study, PBs containing 15 and 20% chia seeds led to satisfactory results concerning dyslipidemia after 4 weeks.

In a study performed by Ferreira et al. (2020), a diet rich in sucrose, with the replacement of corn oil by chia seeds as a lipid source, reduced the content of lipids in the skeletal muscle likely due to an increase in their oxidation. In fact,the groups that consumed chia seeds showed increased gene expression of carnitine palmitoyl transferase 1, increased levels of the receptors activated by the peroxisome proliferator (PPARα, PPARγ) and protein kinase activated by phosphorylated AMP, which is also a regulator of fatty acid metabolism. Additionally, the chia diet reduced isoforms of precursor proteins and mature forms of SREBP-1, a protein recognized for its lipogenic effect on skeletal and hepatic muscle tissue. Although analyses of molecular mechanisms were not performed in the present study, the findings of these authors may explain, at least in part, the positive effect of adding chia seeds to different diets, including sucrose rich diets.

The levels of total cholesterol and low-density lipids are related to the consumption of fibers, whose soluble fraction is associated with bile acids or cholesterol during the synthesis of intraluminal micelles, resulting in a decrease in liver cholesterol as well as standardization of LDL receptors, dispensing the LDL. The total cholesterol levels in all groups were higher than those observed by Molena-Fernandes et al. (2010) in rats supplemented for 35 days with brown and golden flaxseed flour, likely due to the presence of chocolate in all the PB formulations investigated in the present study.On the other hand, the group supplemented with chia had higher HDL levels perhaps owing to the influence of polyunsaturated fatty acids (PUFA) present in the chia seed; however, no decrease was observed inthe LDL level. In the present study, there was a reduction in triglycerides level in the groups that consumed chia supplemented feed, which was proportional to the increase in the content of chia seeds from 10 to 15% in PBs. Thus, the intake of diets containing chia seeds and PBs supplemented with chia seed, for a short period, improved lipid homeostasis in healthy and eutrophic animals, probably due to the different feeding structures, given the complexity of the food synergy and the interaction among stable compounds, which can greatly influence the bioavailability of nutrients.

It noteworthy that the groups supplemented with chia had higher testosterone levels (Table 5). It has been reported that anabolic androgenic steroids promote a decrease in serum HDL levels and an increase in the LDL one(Alquraini and Auchus, 2018). On the other hand, in the present study, there was an increase in HDL level like that observed in other studies carried out with male rats fed with diets supplemented with avocado oil and flaxseed flour (Abboud et al., 2015). These diverging results suggest that these effects greatly depend on the type of both steroid and supplemented matrix. Even though flaxseed grain resembles chia seeds because of its high contents of PUFA and fibers, male rats fed with a diet containing flaxseed had increased serum estradiol levels and no changes in that of testosterone compared to the control group (Corrêa et al., 2017). Studies carried out with men point out a link among low testosterone concentrations and insulin resistance, increased risk of diabetes mellitus, obesity, adverse lipid profile, metabolic syndrome, and cardiovascular risk (Dimopoulou et al., 2018). Hypogonadism, in addition to infertility, may be related to symptoms such as fatigue, weakness, decreased libido and energy, erectile dysfunction, reduced muscle, and bone mass and increased fat(Abboud et al., 2015). Therefore, the consumption of chia seedsappears to be more promising than thatof flaxseed, as it promoted loss of adipose tissue, maintained muscle mass, and decreased TAG levels in the present study.

In the present study, all biochemical parameters were obtained at values considered within the normal range for adult male rats (Melo et al., 2012). It is important to note that the findings of the present study contribute to the development of new food products, especially dietary foods that are free of sugar, a source of protein, and rich in fiber. The proposed formulations may have a significant role in the prevention of chronic non-communicable diseases since they allowed reductions in glycemia, triglyceridemia, and adipose tissues as well as an increase in serum HDL in sedentary eutrophic rats.

V. CONCLUSION

The results of this study showed that protein bars (PBs) supplemented with chia seeds were stable for 45 days of storage at room temperature,as confirmed by the maintenance of texture, pH, and water activity, which are important physicochemical parameters in monitoring the quality of foods during shelf life. These results are the premise for future investigations of product stability with a high-protein content supplemented with high fiber grains and monounsaturated fatty acids.

The consumption of feed partially replaced by chia seeds and PBs by rats, fora short period, proved to be an excellent alternative for reducing the weight of adipose tissues associated with the decrease in body weight, as well as for controlling serum levels of triglycerides and HDL. It is worth noting that the biochemical and molecular mechanisms involved in improving the lipid profile and reducing adipose tissue must be examined in future studies.

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