Health effects of peptides obtained from hydrolysed chicken by-products by the action of *Bromelia pinguin* and *B. karatas* proteases in Wistar rats induced with metabolic syndrome

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Abstract

Metabolic syndrome (MS) is considered a major public health problem because it is associated with the development of cardiovascular disease and type 2 diabetes. Bioactive peptides can play an important role in the prevention and treatment of MS. The possible health effects of peptides obtained from hydrolysed chicken by-products (CH) by the action of plant proteases from *Bromelia pinguin* (BP), *B. karatas* (BK), and bromelain (BRO) were evaluated in a model of induced MS. Thirty male Wistar rats were randomised into the following groups: (1) standard diet (STD); (2) induction of MS with a hypercaloric diet (MS+CH); (3) CH-BP 200 mg CH/kg; (4) CH-BK 200 mg CH/kg; (5) CH-BRO 200 mg CH/kg; and (6) carnosine (CAR) 50 mg of carnosine/kg of body weight. The CH decreased the glucose levels ($p < 0.05$) and improved the lipid profile ($p < 0.05$) in the serum of the groups with induced MS. Liver lesions were attenuated with a decrease in hepatic enzymatic activities ($p < 0.05$), and the accumulation of lipid inclusions in the liver decreased. The data showed that CH and the use of proteases to obtain peptides with health effects could be a good therapeutic alternative for individuals with MS.

Introduction

The incidence of metabolic syndrome (MS) is increasing worldwide (Bjørnshave and Hermansen, 2014). MS is characterised by a combination of metabolic disorders related to abdominal obesity, dyslipidaemia, impaired glucose homeostasis, and hypertension. These conditions put an individual at risk of developing type 2 diabetes (T2D) and cardiovascular disease (CVD) (Gancheva *et al.,* 2017). Because MS has become an important public health problem in recent years, it is, therefore, necessary to study its pathophysiology to understand its origin and identify alternatives therapies that could be used to reduce the health parameters altered by MS (Lawson and Cheverud, 2012).

Bioactive peptides derived from different protein sources can play an important role in the prevention and treatment of MS and its complications, since they have antihypertensive, antioxidant, antidiabetic, antimicrobial, and anti-inflammatory properties with promising applications in the food and pharmaceutical industries (Toldrá *et al.*, 2016; Daliri *et al.*, 2017; Bravo *et al.*, 2019; Mas-Capdevila *et al.,* 2019). Chicken by-products have strong potential for the production of these biomolecules of interest (Lasekan *et al.*, 2013). It has been shown that chicken by-product hydrolysates (CH) have different biological activities beneficial to health, by generating anti-inflammatory (Khiari *et al.*, 2014) and antihypertensive (Onuh *et al.*, 2016; Mas-Capdevila *et al.*, 2018; 2019) activities, enhancing the expression of genes that participate in the regulation of lipid metabolism (Yang *et al.*, 2014), decreasing oxidative stress by increasing endogenous antioxidant activity (Chou *et al.*, 2014), and

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attenuating metabolic alterations related to non-alcoholic fatty liver (Lin *et al.*, 2017) and liver fibrosis (Chen *et al.*, 2017).

In recent years, numerous studies have focused on proteolytic plant extracts to obtain hydrolysates and/or bioactive peptides. The fruits *Bromelia pinguin* and *B. karatas* are considered to be a source of plant proteases with promising properties for industrial and therapeutic applications, and to obtain bioactive peptides (Mazorra-Manzano *et al.*, 2018). Recent studies have shown that hydrolysed bioactive peptides can be obtained from proteases extracted from *B. pinguin* and *B. karatas*, and from other sources of proteins (Meza-Espinoza *et al.*, 2018; Romero-Garay *et al.*, 2020). The objective of the present work was to evaluate the health effects of peptides obtained from CH by the action of plant proteases of *B. pinguin* (BP), *B. karatas* (BK), and bromelain (BRO), in a model of induced MS, on the different biochemical markers and hepatocellular lesions that were altered by the development and prevalence of MS.

Materials and methods

Hydrolysed chicken by-products

Extraction and pre-purification of enzymes from the fruits of *B. pinguin* and *B. karatas* was carried out following the method described by Garcia-Magaña *et al.* (2018) and Romero-Garay *et al.* (2020), later to be applied in poultry by-products (viscera: 44.5% (w/w), heart: 44.5% (w/w), and blood: 11% (w/w)), and hydrolysates were obtained following the method proposed by Romero-Garay *et al.* (2020). The degree of hydrolysis of the hydrolysates was 22.9, 8.8, and 5.1% for CH-BP, CH-BK, and CH-BRO, respectively (Romero-Garay *et al.*, 2020). The hydrolysates were frozen at -20°C, and then lyophilised for subsequent use.

Animals and experimental design

Animals were provided by BIOINVERT S.A. (Mexico City). Thirty adults male Wistar rats (8 - 10 weeks old) weighing 110 ± 30 g were used in this experiment; they were kept in a 12 h light/12 h dark cycle at a temperature of 23 ± 1°C and 60 ± 10% relative humidity. The approved experimental protocol (M00.2./471/2019) and the protocols of the Institutional Committee for the Care and Use of Animals according to the Mexican Standards (NOM-062-ZOO-1999) were followed. During the first

week, the animals were fed with a standard diet (STD): 49% carbohydrates (p/p), 23% protein (p/p), 3% fat (p/p), 6% fibre (w/w), and 7% ash (w/w) (Nutricubo Purina), with water *ad libitum*.

The protocol proposed by Morales-Ávila *et al.* (2020) was followed to develop a murine model with metabolic syndrome (MS), by making modifications in the diet, and inducing hyperglycaemia. Glucose tolerance tests were performed at the end of the induction period by oral administration (gavage) of 2.5 g dextrose/kg body weight. After dextrose administration, blood glucose concentrations were measured at 0, 30, 60, and 90 min. The STD control group had a concentration of 83.11 mg/dL at the beginning of the test, reaching maximum values of 115.0 mg/dL at 30 min after consumption of oral dextrose, and returning to baseline levels at 60 min. The animals with induced MS reached peak concentrations of 207.83 mg/dL at 30 min, which decreased to 164.5 mg/dL up to 90 min without returning to the initial value of 139 mg/dL. The development of hyperglycaemia can be diagnosed by the presence of classic signs and symptoms such as high fasting blood glucose levels, and/or an altered glucose tolerance curve.

The healthy control group was fed STD and water *ad libitum* throughout the experiment. To induce MS, the rats were fed with a hypercaloric diet (HC/21% fat), using 58% STD diet as a base supplemented with 19% animal fat (w/w), 12% sucrose (w/w), and 11% egg (w/w); for induction of hyperglycaemia, two intraperitoneal injections of streptozotocin (STZ; Sigma-Aldrich, St. Louis, MO; 40 mg/kg, per application per week) dissolved in citrate buffer (pH 4.5) were administered.

At the end of the induction period (five weeks), the MS rats were randomly divided into five groups (*n* = 5/group): group 1 = continued on the SDT diet; group 2 = with MS on HC diet; group 3 = with MS with oral doses of CH-BP (200 mg/kg); group 4 = group with MS with oral doses of CH-BK (200 mg/kg); group 5 = with MS with oral doses of CH-BRO (200 mg/kg); and group 6 = with MS with oral doses of CAR (50 mg/kg); CAR was used in the sixth murine group because it is a commercial peptide serving as a reference. Oral doses via gastric gavage of CH were dissolved in water for human consumption, and administered in doses of 0.3 mL on seven days of the week for the entire experimental period.

Food intake and body weight were monitored every week during the experiment; after five weeks, the animals were euthanised after 12 h of fasting to obtain blood and hepatic tissue. Euthanasia was performed according to the Manual on the Use and Care of Experimental Animals (NOM-062-ZOO-1999). Analyses of biochemical markers were carried out on the serum obtained from the blood of each rat (4,500 rpm for 5 min at 4°C). Tissue samples of liver were collected by dissection and immersed in a physiologic solution of formalin (formaldehyde: 10% (v/v), NaCl: 0.9% (w/v)) to prevent deterioration of the tissue.

Analysis of biochemical markers in plasma
Liver enzyme activity

The enzymatic activities of liver aspartate transaminase (AST), alanine transaminase (ALT), alkaline phosphatase (AP), and γ-glutamyl transpeptidase (GGT) were assessed following the methods of the International Federation of Clinical Chemistry (IFCC) using enzymatic colorimetric kits (SPINREACT, Girona, Spain) with a Biosystem auto-analyser (BTS-350, Barcelona, Spain). The results were expressed as units of activity per litre (U/L).

Glucose, total protein, albumin, urea, and other metabolites

The concentrations of glucose, total protein, albumin, urea, creatinine, uric acid, and bilirubin (total and direct) were evaluated following the methods of the International Federation of Clinical Chemistry (IFCC) using enzymatic colorimetric kits (SPINREACT, Girona, Spain) with the Biosystem auto-analyser (BTS-350, Barcelona, Spain). The results were expressed in milligrams per decilitre (mg/dL).

Lipid profile

Total cholesterol, triglycerides, and high-density lipoprotein cholesterol (HDL-C) were evaluated using enzymatic colorimetric kits (SPINREACT, Girona, Spain) with the Biosystem auto-analyser (BTS-350, Barcelona, Spain). The fractions of low-density lipoprotein cholesterol (LDL-C) and very low-density lipoprotein cholesterol (VLDL-C) were determined using the Friedewald equations (Siri-Tarino and Krauss, 2016). The results were expressed in milligrams per decilitre (mg/dL).

Histologic analysis

Liver samples were fixed by conventional histologic techniques into paraffin blocks using a KEDEE automatic tissue processor (KD-TS3A, Zhejiang, China). The tissues were cut into 5-µm sections with a Leica microtome (RM2125RTS, Nussloch, Germany), and stained with haematoxylin and eosin (Al Sayed *et al.*, 2016). The sections were analysed using an OMAX digital light microscope (M837ZL-C180U3, Kent, WA, USA).

Statistical analysis

Statistical analysis of the experimental data was performed using one-way analysis of variance (ANOVA, $p < 0.05$). Differences between means were evaluated using the Tukey-Kramer multiple comparison test ($p < 0.05$). The Kruskal-Wallis test ($p < 0.05$) and Dunn's multiple comparison ($p < 0.05$) were used for non-normal data. Statistical analysis was performed using NCSS 2007 software (NCSS LLC, Kaysville, UT).

Results and discussion

Effect of CH on liver enzyme activity

The effects of CH on hepatic enzyme activity in the groups with MS are presented in Figure 1. The control group (MS+HC) showed higher values for liver enzyme activities (ALT, AST, and AP), which could be related to the level of damage generated in the cells with induction of MS.

Figure 1A shows the serum levels of ALT; the groups with MS dosed with the hydrolysates presented different results. The CH-BRO group had the highest percentage decrease in ALT (26.2%) with activity of 61.2 U/L, and followed by the CH-BP group at 17.1% (68.7 U/L); compared with the control group MS+HC ($p < 0.05$) which had a percentage decrease similar to the comparative group (CAR) at 26% (61.3 U/L). The groups with MS dosed with CH did not show significant differences from the STD control group ($p > 0.05$), except for the CH-BK group.

Figure 1B shows the serum AST values. The CH-BK (94.07 U/L) and CH-BRO (94.33 U/L) groups showed a greater decrease in AST activity when compared with the other groups with MS treated with CH, followed by the CH-BP group (110.2 U/L), presenting significant differences when compared with the control group MS+HC ($p < 0.05$). The groups dosed with CH did not show significant

differences when compared with the control group (STD) and the comparative group (CAR).

Figures 1C and 1D present the plasma levels of GGT and AP. Serum GGT levels were not different between the groups ($p > 0.05$). Regarding the levels of AP, the groups dosed with CH did not present significant differences when compared with the STD control group; the CH-BRO group had the best results with a 20.6% decrease in AP activity with values of 285.46 U/L, when compared with the MS+HC control group. With respect to the treatments, the group dosed with CAR presented a greater decreased in AP activity of 34.7% ($p < 0.05$).

Central obesity, type 2 diabetes mellitus, hypertension, non-alcoholic fatty liver, hyperglycaemia, and hyperlipidaemia can be considered manifestations of MS (Bugianesi, 2005). The presence of MS might favour the generation of hepatocellular lesions that are related to increased serum levels of liver enzymes (ALT, AST, GGT, and AP). AST and ALT are found in the cytoplasm and mitochondria of hepatocytes, and an increase in their activity indicates apoptosis or cell lysis (Fishbein *et al.*, 2003); as the liver is damaged, liver cells release AST, ALT, and PA (Lin *et al.*, 2017).

In relation to the results, the treatments with CH and CAR improved progressive liver damage characterised by a decrease in serum values of AST, ALT, and AP, in the groups with MS (Figure 1). Peptides < 10 kDa from chicken liver show similar results, generating a decrease in serum levels of AST, ALT, and AP in murine models with liver damage (Chen *et al.*, 2017; Lin *et al.*, 2017). It has been suggested that supplementation with CH could attenuate the increased levels of AST and ALT (Chen *et al.*, 2017) linked to the content of bioactive compounds (taurine, carnosine, and anserine). Similarly, the hepatoprotective effect of CH in murine models with oxidative stress (Chou *et al.*, 2014) on a high fat diet (Yang *et al.*, 2014), and in murine models supplemented with taurine (Balkan *et al.*, 2001), has been demonstrated.
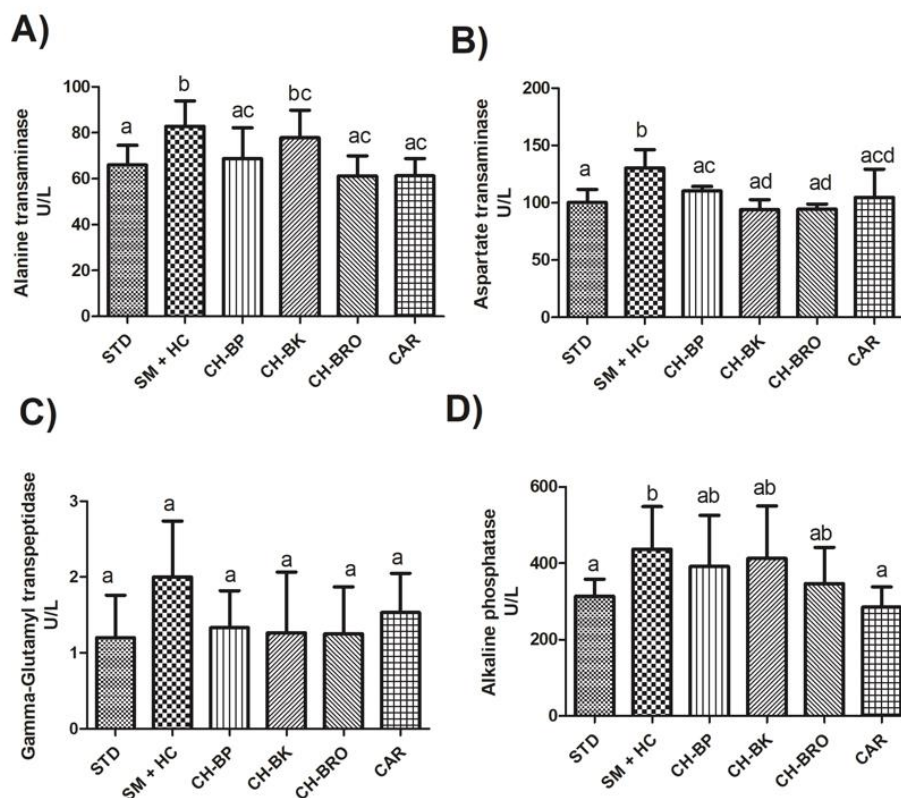

Figure 1. Effect of chicken by-products hydrolysates on the enzymatic activities and serum biochemical values of the groups with metabolic syndrome. Different lowercase letters indicate significant differences ($p < 0.05$). STD = standard diet; MS = metabolic syndrome; HC = hypercaloric diet; CH = chicken by-product hydrolysates; BP = *B. pinguin*; BK = *B. karatas*; BRO = bromelain; and CAR = carnosine.

Effect of CH on growth values, plasma glucose, total protein, albumin, urea, and other metabolites

The effect of CH on growth values (body weight and food intake), glucose levels, and other serum biochemical markers are presented in Table 1. Body weight and food intake among the groups treated with CH did not show significant differences during the experimental period ($p > 0.05$) (Table 1).

Regarding the results obtained from the groups with MS treated with CH, the glucose levels in the CH-BP group did not show significant differences when compared with the STD control group ($p > 0.05$), presenting serum values of 150.4 mg/dL (Table 1) and generating a decrease in plasma glucose of 18% when compared with the MS+HC control group. All the treatments with CH in the groups with MS did not show significant differences between them and in comparison with the CAR group; however, the CH-BP group generated a greater decrease. The regulatory capacity of CH on the decrease in blood glucose levels might be related to the inhibition of GLUT2 (glucose transporter 2) and SGLT1 (sodium-dependent glucose transporter 1) (Mojica *et al.*, 2017), improvement of glucose uptake in muscle cells and its uptake in blood (Roblet *et al.*, 2016), inhibitory activity of α-glucosidase (Yu *et al.*, 2012; Fang *et al.*, 2017), and inhibitory activity of dipeptidyl peptidase-4 (Wang *et al.*, 2015; 2019).

The groups treated with CH did not present significant differences ($p > 0.05$) in the protein content when compared with the MS+HC control group; only the CH-BP group presented higher values of this parameter in relation to the CAR group and the STD control group ($p < 0.05$). However, the percentage protein increase was not high. With regard to albumin levels, no significant difference was observed between the control groups (STD and MS+HC) and the groups dosed with hydrolysates (CH-BP, CH-BK, and CH-BRO) ($p < 0.05$). The liver synthesises and excretes most plasma proteins (albumin) circulating in the body involved in the transport of nutrients, lipid metabolism, and excretion of unwanted metabolites (Jin *et al.*, 2016). Based on the results, it is suggested that CHs do not alter protein synthesis metabolism.

In relation to the concentration of urea in plasma, the groups dosed with hydrolysates and peptides (CH-BP, 18.8 mg/dL; CH-BRO, 21.3 mg/dL; and CAR, 16.7 mg/dL) do not present

significant differences ($p > 0.05$) when compared with the MS+HC group (18.5 mg/dL). The CH-BK group (13.6 mg/dL) presents low serum levels when compared with the other groups, and the values were similar to the STD control group (10.8 mg/dL) (Table 1). Serum creatinine levels were not different between the groups ($p < 0.05$). With respect to uric acid values, no significant differences ($p > 0.05$) were observed between the groups dosed with CH-BP peptides (2.2 mg/dL) and the MS+HC control group (2.1 mg/dL).

Regarding the hydrolysate and peptide treatments, the CH-BP and CAR groups (3.01 mg/dL) did not present significant differences ($p > 0.05$). A slight significant increase ($p < 0.05$) of uric acid was observed in the CH-BK and CH-BRO groups. Urea, creatinine, and uric acid levels are useful for the control of renal function in patients with MS and diabetes (Becerra-Verdín *et al.*, 2019; Morales-Ávila *et al.*, 2020). Based on the results, it is suggested that CHs do not have a negative effect on kidney function. With respect to the levels of total bilirubin (BT) and bilirubin (BD), the CH-BRO group presented higher values of BT, however, the levels of BD in serum were not different between the other groups ($p < 0.05$). It was also observed that the increase was minimal. BT and BD reflect a hepatobiliary balance in relation to the production/excretion of bilirubin; change in these processes has been associated with pathologies such as obesity, type 2 diabetes mellitus, and MS (Lee *et al.*, 2017). Based on the results, the groups treated with CH did not show a negative effect on the levels of biochemical markers, synthesis activities of the liver, and kidney function.

Effect of CH on the lipid profile

The results of the effect of CH on the lipid profile of the groups with MS are presented in Table 2. The groups with MS treated with CH that presented a greater decrease in total cholesterol levels (TC) were CH-BK (2.2 mg/dL) and CH-BRO (42.1 mg/dL), which differed significantly ($p < 0.05$) from the MS+HC control group and the CAR group, which presented the highest levels of TC (Table 2). The CH-BP group did not present significant differences ($p > 0.05$) with respect to the levels of TC (50.2 mg/dL) and peptides (9.2 mg/dL) in the CAR group. Increased levels of HDL-C in the CH groups (9.4 mg/dL in the CH-BP group) were observed, which

Table 1. Effect of chicken by-products hydrolysates (CH) on growth values and serum biochemical values of the groups treated with metabolic syndrome (MS).

Growth value	STD	MS+HC	CH-BP	CH-BK	CH-BRO	CAR
Initial body weight (g)	269.4 ± 26.4^a	298 ± 35.4^a	267.2 ± 38.2^a	285.4 ± 54.7^a	290.4 ± 77.0^a	296 ± 31.1^a
Final body weight (g)	321.2 ± 38.4^a	358.8 ± 42.6^a	329.6 ± 82.1^a	333.8 ± 73.1^a	331.5 ± 31^a	300.4 ± 29.1^a
Food intake (g/day)	21.7 ± 1.4^a	28.7 ± 3.3^b	24.7 ± 5.7^{ab}	24.3 ± 2.04^{ab}	22.7 ± 4.8^{ab}	23.1 ± 0.8^b
Biochemical value in serum (mg/dL)						
Glucose	131.96 ± 38.2^a	183.3 ± 38.6^b	150.4 ± 34.7^{ab}	191.7 ± 47.5^b	174.7 ± 30.3^b	155.2 ± 27.2^{ab}
Total protein	7.6 ± 0.9^a	8.1 ± 0.9^{ab}	9 ± 1.3^{bc}	8.3 ± 0.8^{abc}	8.5 ± 0.5^{abc}	7.26 ± 0.96^{ab}
Albumin	8.4 ± 1.1^a	8.2 ± 0.3^a	7.6 ± 0.7^a	8.2 ± 0.8^a	8.19 ± 0.3^a	8.3 ± 0.6^a
Urea	10.8 ± 3.1^a	18.5 ± 1.6^b	18.8 ± 1.4^b	13.6 ± 3.8^{ac}	21.3 ± 5.2^b	16.7 ± 5.04^{bc}
Creatinine	0.56 ± 0.04^a	0.58 ± 0.05^a	0.56 ± 0.050^a	0.56 ± 0.07^a	0.54 ± 0.09^a	0.59 ± 0.02^a
Uric acid	2.5 ± 0.5^a	2.1 ± 0.7^a	2.2 ± 0.4^b	3.5 ± 1.6^b	3.6 ± 0.7^b	3.01 ± 0.9^{ab}
Total bilirubin	0.08 ± 0.06^a	0.12 ± 0.07^{ab}	0.1 ± 0.09^{ab}	0.09 ± 0.05^{ab}	0.17 ± 0.07^b	0.14 ± 0.06^{ab}
Direct bilirubin	0.07 ± 0.05^a	0.7 ± 0.05^a	0.10 ± 0.04^a	0.13 ± 0.04^a	0.10 ± 0.06^a	0.13 ± 0.04^a

Values are means ± SD ($n = 5$). Means followed by different lowercase superscripts in a row are significantly different (ANOVA, $p < 0.05$). STD = standard diet; MS = metabolic syndrome; HC = hypercaloric diet; CH = chicken by-product hydrolysates; BP = *B. pinguin*; BK = *B. karatas*; BRO = bromelain; and CAR = carnosine.

Table 2. Effect of chicken by-products hydrolysates (CH) on the lipid profile of the groups with induction of metabolic syndrome (MS).

Lipid profile (mg/dL)	STD	MS+HC	CH-BP	CH-BK	CH-BRO	CAR
Total cholesterol	18.6 ± 4.5^a	102.5 ± 14.2^b	60.2 ± 14.6^c	42.2 ± 8.9^d	42.1 ± 17.9^d	50.2 ± 20.4^{cd}
HDL cholesterol	11.8 ± 2.9^a	4.8 ± 1.3^b	9.4 ± 1.9^a	8 ± 3.1^{ab}	8.2 ± 0.9^{ab}	9.2 ± 2.3^a
LDL cholesterol*	1.6 ± 0.5^a	82.8 ± 1.3^b	41.6 ± 1.9^c	9.5 ± 3.1^d	16.7 ± 0.9^e	31.9 ± 2.3^f
VLDL cholesterol*	7.0 ± 3.3^a	16.08 ± 5.8^b	9.16 ± 1.5^a	25.2 ± 6.8^c	16.6 ± 1.3^b	8.6 ± 1.18^a
Triglycerides	38.6 ± 12.1^a	67.8 ± 22.9^b	47.6 ± 5.5^a	122.2 ± 23.3^c	86 ± 8.4^b	45.6 ± 8.93^a

Values are means $\pm$ SD ($n = 5$). (^{a}) indicates the groups that did not obtain significant differences with the STD control group. (^{b}) indicates the groups that did not obtain significant differences with the metabolic syndrome (MS) control group. (^{c}) and (^{d}) indicate the groups that did not obtain significant differences between the different treatments. STD = standard diet; HC = hypercaloric diet; CH = chicken by-product hydrolysates; BP = *B. pinguin*; BK = *B. karatas*; BRO = bromelain; CAR = carnosine; HDL = high-density lipoprotein; LDL = low-density lipoprotein; and VLDL = very-low-density lipoprotein. (*) Values calculated using the Friedewald equations.

differed significantly from the MS+HC control group ($p < 0.05$) and presented values similar to the STD control group (11.8 mg/dL). However, among the CH groups (200 mg/kg) and the CAR group, no significant differences ($p > 0.05$) were observed. The same behaviour was found with the serum triglyceride and VLDL-C levels. With respect to the LDL-C values, the CH-BK group showed a greater decrease in serum plasma levels, presenting significant differences ($p < 0.05$) when compared with the MS+HC control group and with the other groups treated with hydrolysates (CH-BP and CH-BRO) and peptides (CAR). The effect of CH on lipid metabolism could be mainly related to their content of peptides and amino acids. It has been shown that chicken liver hydrolysates could generate a decrease in the lipid profile in a model induced with obesity, mainly due to the content of taurine and carnosine (Yang *et al.*, 2014). Similarly, taurine supplementation has lipid-lowering effects because it generates greater production of faecal lipids and bile acids (Chang *et al.*, 2011). Bile acids facilitate biliary excretion of endogenous cholesterol metabolites and intestinal absorption of the main dietary lipids (triglycerides, phospholipids, and cholesterol esters). The effect of CHs on lipid metabolism are mainly related to their possible effect in decreasing lipid absorption by inhibiting the activity of pancreatic lipase, which contributes to lower absorption of exogenous lipids and the high binding activity to bile acids (Yang *et al.*, 2014; Siow *et al.*, 2016). Peptides with high binding capacity with bile acids have been

found to block the reabsorption of bile acids, and therefore decrease the level of cholesterol in the blood attributed to a possible hypocholesterolemic effect (Hosomi *et al.*, 2011; Lapphanichayakool *et al.*, 2017).

Histology

Liver photomicrographs of the groups of MS treated with CH are presented in contrast to control groups in Figure 2. Figure 2A shows the characteristic microanatomy of normal liver tissue (STD control group), where cords of polygonal hepatocytes were observed with round, centred achromatic nuclei and acidophilic cytoplasm, separated by sinusoidal capillaries, uniformly and evenly distributed, and radial flow into the central vein. Figure 2B shows the MS+HC control group, with a lack of radial distribution between the hepatocytes and sinusoidal capillaries, and accumulation of lipid inclusions around the central vein; lipid accumulation in hepatocytes, mainly as triglycerides, can generate lipotoxicity and possible damage (Zhang *et al.*, 2008). In addition, alterations in the architecture of the tissue were observed, which could be directly related to high levels of liver enzymes (ALT, AST, AP, and GGT).

The treatments with the CHs and CAR in rats with MS for five weeks showed a decrease in lipid inclusions, in addition to attenuation of the morphologic damage generated by the MS similar to that obtained with CAR, which is a commercial dipeptide (Figure 2C - 2F). The decrease in lipid inclusions observed in the groups treated with CH and

CAR when compared with the group with MS with a hypercaloric diet might be related to the improvement in the serum lipid profile, which could generate a decrease in the accumulation of lipids in the liver and lipid absorption and/or increased endogenous antioxidant capacity in the liver (Yang *et al.,* 2014).
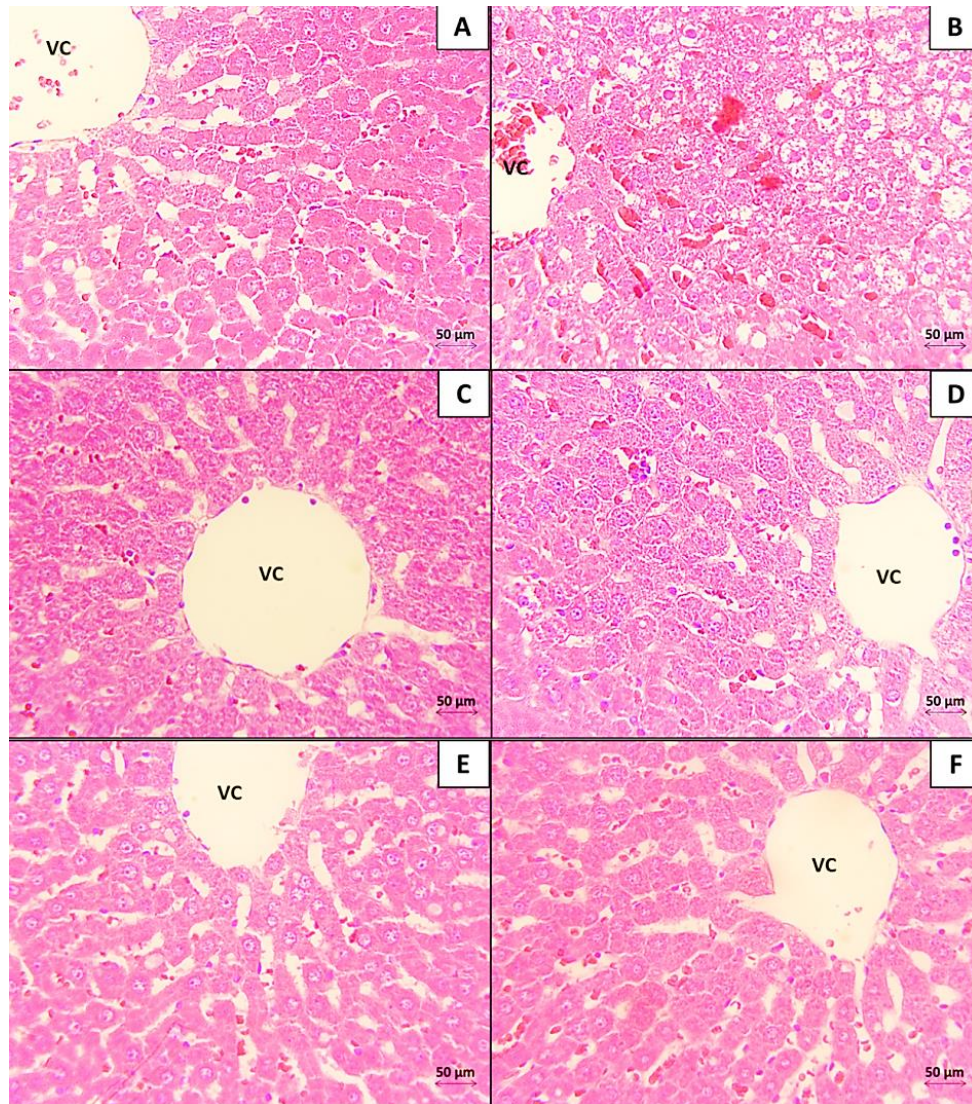

Figure 2. Histologic photomicrographs of liver sections (40× magnification) from the groups: **(A)** standard diet, **(B)** metabolic syndrome with hypercaloric diet, **(C)** chicken by-products hydrolysed by *B. pinguin* (200 mg/kg), **(D)** chicken by-products hydrolysed by *B. karatas* (200 mg/kg), **(E)** chicken by-products hydrolysed by bromelain (200 mg/kg), and **(F)** carnosine (50 mg/kg). VC = central vein.

The results are similar to those reported by Lin *et al*. (2017). They observed that liver lesions in the groups treated with chicken liver hydrolysates in a murine model of fatty liver improved by decreasing lipid inclusions and the levels of ALT and PA enzymes, leading to improved liver function. The possible role that peptides play in the attenuation of cellular lesions generated by induction of MS might be related to the regulation of genes involved in lipid metabolism (PPARα, RXRα, CPT1, and UCP2) and an increase in endogenous antioxidant activity (SOD and CAT); downregulation of lipogenesis and upregulation of the β-oxidation of fatty acids can contribute to decreased levels of serum triglyceride and decreased lipid inclusions in the liver (Yang *et al*., 2014). Similar results were observed in the present work. It has been shown that treatments with chicken liver hydrolysates could generate a therapeutic effect on liver lesions related to liver fibrosis through the improvement in endogenous antioxidant activities (SOD and CAT), a decrease in the expression of genes and proteins of TGF-β (transforming growth factor beta) (Chen *et al*., 2017), a decrease in the development of fatty alcoholic liver

through increased antioxidant capabilities, the regulation of lipid homeostasis through the positive regulation of β oxidation fatty acids, negative regulation of fatty acid synthesis, as well as decreasing inflammatory responses in the liver by decreasing cytokine secretion (Lin *et al.*, 2017). CAR has antioxidant and lipid-lowering capacity (Mong *et al.*, 2011), and provides an attenuating effect on liver lesions (Yan *et al.*, 2009). Ali *et al.* (2012) have reported that anserine could also have a hepatotoxic effect due to a decrease in oxidative stress.

Conclusion

The health effects of CH (200 mg/kg) were analysed in a murine model induced with MS. It was observed that CH decreased glucose levels, and improved the lipid profile. In addition, a therapeutic effect was observed on hepatic lesions associated with the decrease in enzymatic activities and the decrease in hepatic accumulation of lipid inclusions. Treatment with CH-BP showed results similar to those obtained with carnosine, which is a commercial dipeptide. The results reported in the present work allow us to consider that CH obtained through the use of plant enzymes could generate compounds with health effects, which in turn could potentially be used in the development of functional foods and/or nutraceuticals, in addition to promoting the recovery and use of fruits endemic to the Americas.

Acknowledgement

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