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Mussel (Mytilus Galloprovincialis) By-Product Protein Attenuates Hypertension, Renal Dysfunction, and Metabolic Disturbances in Obese Rats

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ABSTRACT: Obesity is associated with an increased risk of arterial hypertension and renal dysfunction, but the protective value of aquaculture by-product proteins remains poorly documented. This study evaluated the effects of a protein concentrate extracted from by-products of the Mediterranean mussel Mytilus galloprovincialis on blood pressure and renal function in Wistar rats made obese through high-fat feeding. Twenty-four male rats were allocated to three groups (n = 8): Control, Obese (high-fat diet containing 35% fat for 13 weeks), and Treated obese (mussel protein supplementation for 4 weeks). The high-fat diet induced marked hypertension (systolic blood pressure: 169.5 ± 10.2 vs 117.3 ± 2.3 mmHg), cardiac and aortic hypertrophy, glomerular hyperfiltration, elevated proteinuria and microalbuminuria, and an increased vascular resistance index. Mussel protein supplementation reduced systolic blood pressure by 25%, normalized heart and aorta weight, corrected the hyperfiltration, and significantly reduced proteinuria and urinary uric acid relative to the untreated obese group ($p < 0.05$). The protein concentrate was compositionally standardized, containing 80.40 ± 0.32% crude protein (N × 6.25) and a complete essential amino acid profile (85.21 ± 1.32 g amino acids/100 g protein), with glutamic acid, glycine, aspartic acid, taurine, and leucine as the predominant residues. Proteins extracted from M. galloprovincialis by-products thus exert a cardiovascular and renal protective effect in the obese rat, plausibly linked to their amino acid and taurine content, supporting the development of this concentrate as a standardized nutraceutical or dietary supplement ingredient, in line with circular economy principles.

1. INTRODUCTION

Obesity is today one of the leading public health challenges worldwide, with a steadily increasing prevalence in both developed and low- or middle-income countries (Bray et al., 2017). Beyond its impact on quality of life, it represents a major risk factor for chronic non-communicable diseases, notably type 2 diabetes, dyslipidemia, cardiovascular disease, and chronic kidney disease (Blüher, 2019).

Among the most frequent complications of obesity, arterial hypertension occupies a prominent place: more than half of cases of essential hypertension are directly or indirectly related to overweight and obesity (Boutari et al., 2023). Its pathophysiology is complex and multifactorial. Expansion of visceral adipose tissue promotes excessive secretion of pro-inflammatory adipokines (TNF-α, IL-6, leptin) at the expense of adiponectin, contributing to a chronic low-grade inflammatory state, endothelial dysfunction, and progressive vascular remodeling (Hotamisligil, 2017; Saltiel and Olefsky, 2017). At the same time, obesity persistently activates the sympathetic nervous system and the renin-angiotensin-aldosterone system (RAAS), promoting

vasoconstriction, sodium and water retention, and, ultimately, established arterial hypertension (Hall et al., 2015). This prolonged hypertension leads to structural alterations of target organs left ventricular hypertrophy, arterial stiffening, glomerular hyperfiltration followed by progressive decline in renal function contributing to a self-perpetuating cardiorenal syndrome (Hall et al., 2015; D'Agati et al., 2016).

Given the limitations of conventional pharmacological treatments, marine-derived bioactive proteins and peptides appear to be a promising nutritional avenue. Their antihypertensive activity relies mainly on inhibition of angiotensin-converting enzyme (ACE), but is often accompanied by complementary antioxidant and anti-inflammatory properties (Ngo et al., 2012; Kim and Wijesekara, 2010). The valorization of fisheries and aquaculture by-products long considered industrial waste fits squarely within this logic, transforming these by-products into high-value functional ingredients (Ferraro et al., 2010).

Mytilus galloprovincialis, a major aquaculture species in the Mediterranean, is rich in high-nutritional-value proteins and bioactive compounds (Gerdol and Venier, 2015). Protein hydrolysates from blue mussel (Mytilus edulis) have already shown an antihypertensive effect in spontaneously hypertensive rats, associated with modulation of renal gene expression involved in coagulation and lipid metabolism (Feng et al., 2015). However, in vivo studies specifically addressing proteins extracted from M. galloprovincialis by-products as opposed to purified hydrolysates in a model of obesity associated with hypertension remain extremely limited, particularly when it comes to simultaneously assessing blood pressure, renal function, and urinary biomarkers.

Beyond their pharmacological interest, marine-derived proteins and peptides are increasingly considered as candidate ingredients for nutraceutical and dietary supplement development, provided that their nutrient composition can be characterized and standardized in a manner analogous to pharmaceutical-grade quality control (Li-Chan, 2015; Vettorazzi et al., 2020). Compositional standardization in particular the crude protein content and the amino acid profile, including the content of taurine, a sulfur-containing amino acid increasingly recognized for its cardiovascular and antihypertensive properties (Santulli et al., 2023; Sun et al., 2016) is a prerequisite for regulatory acceptance and for establishing a reproducible dose-response relationship, rather than treating such by-products merely as an undifferentiated alternative protein source (Cunha et al., 2021).

The present study therefore aimed to evaluate the antihypertensive and nephroprotective effects of a protein concentrate extracted from aquaculture by-products of Mytilus galloprovincialis, considered here as a candidate standardized nutraceutical ingredient, in Wistar rats made obese through high-fat feeding. Specifically, the effects of this supplementation were assessed on: (i) hemodynamic parameters, including systolic, diastolic, and mean arterial blood pressure (SBP, DBP, MAP), pulse pressure (PP), and the vascular resistance index (VRI); (ii) morphometric parameters of hypertension target organs, namely heart, kidney, and aortic weight and their corresponding cardiac, renal, and aortic indices; (iii) renal function, including serum urea and creatinine, and creatinine clearance as an estimate of glomerular filtration rate (GFR); and (iv) urinary biomarkers, including diuresis, proteinuria, microalbuminuria, glucosuria, urinary urea, creatinine, and uric acid, natriuresis and kaliuresis, and the derived protein-to-creatinine (PCR) and albumin-to-creatinine (ACR) ratios. and (v) the crude protein content and amino acid composition of the protein concentrate itself, in order to characterize its potential as a standardized dietary supplement ingredient.

2. MATERIAL AND METHODS

2.1. Preparation of mussel proteins

Undersized, damaged, and non-commercial specimens of Mytilus galloprovincialis were collected in March 2024 from a commercial mussel farm located in Kristel (35°49'18"N, 0°28'59"W), Oran, Algeria. After collection, the samples were transported to the laboratory under refrigerated conditions (4°C). The edible tissues were manually separated from the shells, thoroughly washed with distilled water, and dried in a ventilated oven at 60°C until constant weight.

Protein extraction was performed using a protocol developed in our laboratory and adapted from the method described by Guillaume et al. (Guillaume et al., 1999). Briefly, the dried mussel tissues were subjected to heat treatment (80–85°C for 20 min), followed by mechanical pressing and centrifugation to recover the protein-rich fraction. The resulting protein sediment was dried at 40–45°C for 24 h, finely ground, and subsequently defatted by Soxhlet extraction using n-hexane at 50°C for 5 h to obtain a protein concentrate (PC). The final protein concentrate, characterized by a protein-to-lipid ratio of 25.45, was stored at −20°C until incorporation into the experimental diets.

2.2. Chemical and amino acid composition of the protein concentrate

The crude protein content of the protein concentrate was determined by the Kjeldahl method (N × 6.25). The amino acid composition was determined by HPLC after acid hydrolysis (6 N HCl, 110 °C, 24 h) and pre-column derivatization with ortho-phthalaldehyde (OPA) (Roth, 1971). Taurine, a non-proteinogenic sulfur-containing amino acid, was quantified using the same procedure and expressed separately from the proteinogenic amino acid total. Analyses were performed in triplicate (n = 3), and results are expressed as mean ± SD, both in g/100 g protein and as a percentage of total amino acids. Amino acid essentiality was assigned according to the FAO/WHO (FAO/WHO, 2013) reference pattern for dietary protein quality evaluation.

2.3. Animals and ethical approval

Twenty-four male Wistar rats (8–10 weeks old) were acclimatized for one week and then housed individually under controlled conditions (22 ± 2 °C, relative humidity 50–60%, 12 h/12 h light/dark cycle), with free access to water and food. All experimental procedures were conducted in accordance with the Guide for the Care and Use of Laboratory Animals and the ARRIVE 2.0 guidelines (Percie du Sert et al., 2020), and were approved by the Institutional Animal Ethics Committee (Approval No. 991, December 10, 2020). The ethics approval certificate was issued by the President of the Ethics and Deontology Committee of the University of Oran 1 Ahmed Ben Bella, Oran, Algeria.

2.4. Experimental protocol

Following a one-week acclimatization period, rats were randomly assigned to three experimental groups (n = 8 per group): a Control (C) group fed a standard diet; an Obese (Ob) group fed a high-fat diet (35% fat) for 13 weeks; and a Treated obese (Ob-Pcm) group fed the same high-fat diet for 13 weeks followed by 4 weeks of dietary intervention in which casein was completely replaced with mussel by-product protein concentrate as an isonitrogenous protein source. The total experimental period was 17 weeks. At the end of the protocol, animals were individually housed in metabolic cages for 24-hour urine collection before sacrifice.

The control, Ob, and Ob-Pcm diets were formulated to provide equivalent energy density within each dietary category while differing in fat content and protein source (Table 1). The HFD and HFD-Mpc diets were hyperlipidic, providing 19.08 MJ/kg diet (4,558 kcal/kg). In the HFD-Mpc diet, mussel by-product protein concentrate completely replaced casein while maintaining the same total dietary protein content (200 g/kg diet). All diets were prepared in powdered form and provided ad libitum throughout the experimental period.

2.5. Animal Health Monitoring, Food Intake, and Safety Assessment

Throughout the experimental period, all animals were monitored daily for their general clinical condition, including behaviour, locomotor activity, grooming, food acceptance, and the occurrence of any signs of distress, morbidity, or adverse effects. Mortality was also recorded throughout the study. Body weight was measured weekly during the 13-week high-fat diet induction period and daily throughout the 4-week supplementation phase. Food intake was recorded daily during the supplementation period, enabling calculation of the feed efficiency ratio (FER; body weight gain/food intake), which served as an indirect indicator of dietary acceptability and short-term safety of the mussel protein concentrate. No treatment-related clinical abnormalities, behavioural alterations, or mortality were observed during the experimental period, supporting the good tolerability of the dietary intervention under the conditions of the present study.

2.6. Blood pressure and morphometric parameters

Systolic (SBP) and diastolic (DBP) blood pressure were measured by non-invasive tail-cuff plethysmography (CODA®, Kent Scientific Corporation) after acclimatizing the animals to the procedure, in a temperature-controlled room (28–30 °C). After sacrifice, the heart, kidneys, and thoracic aorta were dissected, rinsed, and weighed; cardiac, renal, and aortic indices were calculated by relating each organ weight to final body weight.

2.7. Renal function and urinary parameters

Serum urea and creatinine concentrations were determined using commercial kits, as were serum sodium and potassium concentrations. Creatinine clearance (mL/min), used here as a surrogate for glomerular filtration rate (GFR), was calculated as: Clearance = (urinary creatinine × urinary flow rate) / serum creatinine. Twenty-four-hour urine collections allowed measurement of diuresis, proteinuria, microalbuminuria, glucosuria, urea, creatinine, uric acid, and urinary sodium and

potassium. The urinary protein-to-creatinine ratio (PCR) and the urinary albumin-to-creatinine ratio (ACR) were calculated to correct for variations in urinary dilution.

Derived cardiovascular parameters mean arterial pressure (MAP = [SBP + 2×DBP]/3) and pulse pressure (PP = SBP − DBP) were also calculated. In addition to systolic, diastolic, and mean blood pressure, the CODA® VPR system directly records tail blood flow and tail blood volume for each measurement cycle (Daugherty et al., 2009). Based on this direct flow output, a vascular resistance index (VRI, arbitrary units) was computed for each animal as the ratio of mean arterial pressure to mean tail blood flow (VRI = MAP / tail blood flow), applying to the tail circulation the same pressure-flow relationship (resistance = pressure/flow) that defines total peripheral resistance in systemic hemodynamics (Trammel and Sapra, 2023). Because tail blood flow was recorded by the CODA® software in relative, non-calibrated units, VRI is likewise expressed in arbitrary units and should be interpreted as a within-study, relative index of tail vascular resistance rather than an absolute measurement of total peripheral resistance.

2.8. Statistical analysis

Data are presented as the mean ± standard error of the mean (SEM). The normality of data distribution was evaluated using the Shapiro–Wilk test. Statistical differences among the experimental groups were assessed by one-way analysis of variance (ANOVA), followed by Tukey's post hoc multiple comparison test whenever a significant overall effect was detected. Statistical analyses were performed using IBM SPSS Statistics for Windows, version 27.0 (IBM Corp., Armonk, NY, USA). All tests were two-tailed, and differences were considered statistically significant at $p < 0.05$. Levels of statistical significance were defined as follows: $^{**}p < 0.001$, $^{*}p < 0.01$, $p < 0.05$, and NS (not significant), $p \geq 0.05$.

3. RESULTS

3.1. General health status and food intake

No mortality and no overt clinical signs of toxicity (e.g., piloerection, lethargy, diarrhea) were observed in any experimental group throughout the 17-week protocol, including during the 4-week mussel protein supplementation phase. Food intake remained consistent with the ad libitum availability of the diets in all groups, and no signs of feed refusal were noted after the substitution of casein with mussel by-product protein concentrate, supporting the acceptability of the supplemented diet. Food intake during the 4-week supplementation phase averaged 22.8 ± 1.4, 19.7 ± 1.2, and 21.4 ± 1.1 g/day in the Control, Obese, and Obese + Pcm groups, respectively, with a corresponding feed efficiency ratio of 0.145 ± 0.011, 0.281 ± 0.018, and 0.188 ± 0.015 (Table 3), confirming that substitution of casein with mussel by-product protein concentrate did not impair feed intake or efficiency relative to the control diet.

3.2. Chemical composition of the protein concentrate

The protein concentrate contained 80.40 ± 0.32% crude protein (N × 6.25) and 85.21 ± 1.32 g amino acids/100 g protein (Table 2). Glutamic acid, glycine, aspartic acid, taurine, and leucine were the predominant residues, and all nine essential amino acids were present, with methionine as the first limiting amino acid (FAO/WHO, 2013). This amino acid profile, and in particular the taurine content (6.60 ± 0.12 g/100 g protein), provides a compositional basis for considering the concentrate as a candidate functional ingredient for nutraceutical or dietary supplement development (Li-Chan, 2015; Santulli et al., 2023).

3.3. Morphometric parameters

After thirteen weeks of high-fat feeding, obese rats displayed a significant increase in final body weight, weight gain, and heart, kidney, and aorta weight compared with the control group ($p < 0.05$; Table 3), reflecting experimental obesity associated with hypertrophy of hypertension target organs. Mussel protein supplementation for four weeks significantly reduced final body weight and heart, kidney, and aorta weight relative to the untreated obese group, with values approaching those of the control group (Figure 1).

3.4. Hemodynamic parameters

The high-fat diet induced a marked elevation in systolic, diastolic, and mean blood pressure, as well as pulse pressure ($p < 0.01$; Table 4, Figure 2). Treatment with mussel proteins reduced SBP by 25% relative to the obese group (127.0 ± 5.8 vs 169.5 ± 10.2 mmHg), with parallel reductions in DBP and MAP to values statistically comparable to the control group.

3.5. Renal function

Obese rats showed a significant increase in serum creatinine and a markedly elevated creatinine clearance compared with controls (Table 5, Figure 3). This increase in clearance rather than a decrease is consistent with the phenomenon of early glomerular hyperfiltration, widely described in obese subjects prior to the onset of established renal insufficiency (Basolo et al., 2023; D'Agati et al., 2016). Treatment with mussel proteins normalized serum creatinine and brought creatinine clearance to values statistically indistinguishable from the control group ($p < 0.05$ vs Ob), suggesting a correction of this hyperfiltration rather than a simple linear improvement in renal function.

3.6. Urinary parameters

Obesity was associated with a marked increase in proteinuria, urinary creatinine excretion, urinary uric acid, and the albumin-to-creatinine ratio (ACR), as well as disturbances in natriuresis and kaliuresis relative to the control group (Table 6, Figures 4, 5, 6). Mussel protein supplementation significantly reduced proteinuria (−36% vs Ob) and urinary uric acid (−76% vs Ob), with partial normalization of the PCR and ACR indices. Absolute urinary excretion of sodium and potassium was higher in the Ob-Pcm group than in the two other groups, a pattern consistent with the increased protein and osmotic load of the supplemented diet.

3.7. Vascular resistance index

The vascular resistance index (VRI, arbitrary units) was significantly increased in obese rats compared with controls (1.89 ± 0.14 vs 1.12 ± 0.08), consistent with the increased vascular afterload associated with obesity-induced hypertension. Mussel protein supplementation significantly reduced the VRI (1.24 ± 0.14 vs Ob), although not fully back to control levels, with all three groups remaining statistically distinct from one another ($p < 0.05$; Table 7, Figure 7).

4. DISCUSSION

This study shows that a protein concentrate extracted from Mytilus galloprovincialis by-products reduces blood pressure, limits cardiac and aortic hypertrophy, and improves renal function in the obese rat. These results confirm, in a new experimental model, the cardioprotective and nephroprotective potential already reported for other marine proteins, while extending it for the first time to a mussel-farming by-product rather than a purified hydrolysate. Beyond these physiological effects, the compositional data obtained here a high, standardized crude protein content and a complete essential amino acid profile rich in taurine support the further development of this concentrate as a candidate nutraceutical ingredient or dietary supplement, rather than merely as an alternative protein source (Li-Chan 2015; Vettorazzi et al. 2020).

The blood pressure reduction observed after supplementation is consistent with the most extensively documented mechanism for marine peptides: inhibition of angiotensin-converting enzyme (ACE), which limits angiotensin II formation and thereby reduces vasoconstriction, aldosterone secretion, and sodium and water retention (Pujiastuti et al., 2019). This mechanism was not measured directly in our study, an important limitation discussed below, but it is supported by seminal work on blue mussel (Mytilus edulis) hydrolysates, which showed a significant reduction in systolic and diastolic blood pressure in spontaneously hypertensive rats after 28 days of oral administration (Feng et al., 2015). Part of the effect observed may also relate to antioxidant and vasoprotective action: oxidative stress reduces nitric oxide (NO) bioavailability and promotes endothelial dysfunction, while several marine peptides have shown the capacity to restore this NO-dependent pathway. Collectively, ACE inhibition, improved NO bioavailability, and attenuation of oxidative and inflammatory stress represent plausible, non-mutually-exclusive mechanisms through which M. galloprovincialis proteins may lower blood pressure and protect the kidney in this model; direct mechanistic confirmation (e.g., ACE-inhibitory activity assay, nitrite/NO quantification, oxidative stress and inflammatory markers) is identified as a priority for future work (see Limitations below).

The most informative finding of this study concerns creatinine clearance, which was markedly increased not decreased in untreated obese rats, before being brought back to values comparable to controls after supplementation. This pattern is the classic signature of early glomerular hyperfiltration, now recognized as a direct pathogenic mechanism linking obesity and kidney disease: in obese subjects without primary renal disease, glomerular filtration rate increases with body weight, and this hyperfiltration precedes and predicts the subsequent decline in renal function rather than being its consequence (Basolo et al., 2023). Proposed mechanisms include increased renal plasma flow, reduced afferent vascular resistance, activation of the renin-angiotensin-aldosterone system, and chronic low-grade inflammation a picture consistent with obesity-related glomerulopathy,

which is already well characterized histopathologically (D'Agati et al., 2016). The correction of this hyperfiltration after treatment, together with the reduction in proteinuria and urinary uric acid, suggests that mussel proteins limit progression toward structural glomerular damage rather than correcting an already established renal insufficiency. Comparable observations have been reported in obese Zucker rats receiving intact or hydrolyzed proteins from blue whiting (Micromesistius poutassou), with attenuation of hypertension progression and improvement in urinary markers of renal function (Drotningsvik et al., 2021). The concomitant increase in FENa and FEK observed in untreated obese rats provides further support for this interpretation: a disproportionately elevated fractional excretion of sodium and potassium, despite lower absolute natriuresis, points to reduced tubular reabsorptive capacity relative to the markedly increased filtered load, rather than a primary tubular secretory defect. The partial, but incomplete, correction of FENa and FEK after mussel protein supplementation mirrors the partial correction of proteinuria and uric acid excretion, and is consistent with an amelioration of the early functional changes associated with hyperfiltration rather than a full reversal of glomerulotubular dysfunction.

The effects observed could partly be explained by the concentrate's richness in arginine the substrate for endothelial nitric oxide synthase and thus for vasorelaxation as well as in taurine, whose antihypertensive and vasoprotective action is well documented (Abebe and Mozaffari, 2011). This is consistent with more recent evidence, including a randomized, double-blind, placebo-controlled trial in prehypertensive adults in which 1.6 g/day of taurine significantly lowered clinic and ambulatory blood pressure and improved vascular function (Sun et al., 2016), and with recent reviews positioning taurine as a leading candidate for cardiovascular-oriented dietary supplementation (Santulli et al., 2023). Our study used total proteins rather than hydrolysates or purified peptides; it is plausible that gastrointestinal digestion released bioactive peptides in vivo capable of acting on several pathophysiological pathways simultaneously, a hypothesis consistent with a recent report showing that a tuna protein hydrolysate corrects hypertension, cardiac and vascular hypertrophy, oxidative stress, and NO bioavailability in rats fed a high-fat diet, through coordinated modulation of the AT1R/NOX2, eNOS, and Nrf2/HO-1 pathways (Maneesai et al., 2023). This parallel suggests that M. galloprovincialis proteins could act through similar mechanisms, although this remains to be confirmed directly. Because taurine represented 6.60 ± 0.12 g/100 g protein of the concentrate characterized here (Table 2), a level comparable to that reported in other mussel-derived hydrolysates explored for nutraceutical use (Cunha et al., 2021), it constitutes a plausible, quantifiable marker for the future standardization of this ingredient.

The present study has several strengths that enhance the scientific relevance of its findings. To the best of our knowledge, it is among the first to investigate the effects of proteins extracted from Mytilus galloprovincialis aquaculture by-products on obesity-associated hypertension. Unlike most previous studies on marine antihypertensive peptides, which have mainly focused on purified enzymatic protein hydrolysates tested in spontaneously hypertensive rats (SHR), our work evaluated non-hydrolyzed dietary proteins directly extracted from aquaculture by-products, thereby avoiding the need for costly enzymatic hydrolysis. This original approach provides not only scientific and nutritional relevance but also practical value for the development of sustainable functional ingredients.

Another major strength of this study is the use of a well-established high-fat diet-induced obesity model that reproduces several key features of obesity-related cardiometabolic disorders, including hypertension, renal dysfunction, and structural alterations of target organs. In addition, the study adopted an integrated experimental approach by simultaneously assessing hemodynamic parameters (systolic and diastolic blood pressure, mean arterial pressure, and pulse pressure), morphometric parameters (heart, kidney, and aortic weights together with their corresponding organ indices), renal function (serum urea, serum creatinine, and creatinine clearance), and a broad panel of urinary biomarkers, including proteinuria, microalbuminuria, glucosuria, urinary creatinine, urinary urea, urinary uric acid, natriuresis, and kaliuresis.

The comprehensive evaluation was further strengthened by the calculation of complementary functional indices, including the protein-to-creatinine ratio (PCR), albumin-to-creatinine ratio (ACR), fractional excretion of sodium (FENa), fractional excretion of potassium (FEK), body mass index (BMI), and the Lee index. Together, these parameters provided a more comprehensive characterization of obesity-induced cardiorenal alterations and of the protective effects of mussel proteins.

Finally, replacing dietary casein with Mytilus galloprovincialis proteins under isocaloric and isonitrogenous conditions allowed the observed biological effects to be attributed primarily to the nutritional quality of the protein source rather than to differences in protein intake. Beyond its experimental relevance, this strategy also supports the principles of the circular economy and sustainable resource management by promoting the valorization of Mediterranean mussel aquaculture by-products that remain largely underutilized despite their high nutritional potential.

From a translational perspective, the value of this protein concentrate lies not only in its physiological effects but also in its suitability for standardization as a nutraceutical ingredient. Unlike whole mussel meat, whose composition varies with season, harvest site, and processing, protein concentrates and hydrolysates can be characterized and quality-controlled on the basis of defined analytical markers, crude protein content, amino acid profile, and the levels of specific bioactive amino acids such as taurine and arginine — a requirement increasingly emphasized in the regulatory and quality-control literature on nutraceuticals and functional food ingredients (Li-Chan, 2015; Vettorazzi et al., 2020). The compositional data reported here (Table 2) provide such a quantitative basis and position the concentrate as a candidate dietary supplement ingredient rather than a simple food substitute, consistent with recent work identifying mussel-derived protein hydrolysates as promising nutraceutical and functional food candidates (Cunha et al., 2021).

Several limitations should be noted. First, this study did not directly measure ACE-inhibitory activity, the bioavailability of peptides released after digestion, or the expression of genes involved in the RAAS, inflammation, or oxidative stress complementary approaches needed to confirm the mechanisms proposed here by analogy with the literature. In addition, histopathological evaluation of renal and adipose tissue (e.g., glomerular morphology, tubular injury, interstitial fibrosis, adipocyte size) was not performed in this study; such analyses would provide direct structural confirmation of the functional improvements reported here and are planned as a priority for follow-up work. Second, urinary creatinine excretion in the obese group was substantially higher than in the two other groups, a disproportionate difference compared with other parameters; although the corrected clearance remains interpretable, this value would benefit from reconfirmation against the source samples. Third, the study was conducted exclusively in male Wistar rats over a four-week treatment period; reproducibility in females, over longer durations, and ultimately in humans, remains to be established. Finally, identifying the specific bioactive peptides through peptidomic approaches (LC-MS/MS) and evaluating their ACE-inhibitory activity in vitro would be a natural next step for this work.

This study shows that proteins extracted from aquaculture by-products of Mytilus galloprovincialis reduce blood pressure, limit cardiac and aortic hypertrophy, and correct the early glomerular hyperfiltration associated with experimental obesity in the Wistar rat. These effects are accompanied by a reduction in proteinuria and urinary uric acid, suggesting a protective effect on glomerular integrity.

Beyond these physiological findings, the standardized composition of the concentrate 80.40% crude protein and a complete essential amino acid profile rich in taurine (Table 2) positions M. galloprovincialis by-product proteins as a promising candidate nutraceutical ingredient or dietary supplement, and not merely as a source of functional ingredients for food fortification, while valorizing a currently underexploited aquaculture by-product. Further work, including peptidomic characterization, direct measurement of anti-ACE activity, formal dietary supplement stability and quality-control studies, and human trials, will be needed to confirm this potential.

Ethical Considerations

All procedures were conducted in accordance with the Guide for the Care and Use of Laboratory Animals and the ARRIVE 2.0 guidelines (Percie du Sert et al., 2020), and were approved by the University Ethics and Deontology Committee, University of Oran 1 Ahmed Ben Bella, Oran, Algeria (Approval No. 991, December 10, 2020). The ethics approval certificate was issued by Professor Layadi Khaled, President of the University Ethics and Deontology Committee.

Data Availability

The data supporting this study are available from the corresponding author upon reasonable request

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TABLES

Table 1. Composition of the experimental diets (g/kg)

Ingredient	*Control (C)*	*Obese*	*Obese + Pcm*
Casein[2]	200	200	–
Mussel by-product protein[3]	–	–	200
Corn starch[4]	690	290	290
Sucrose[5]	–	50	50
Fat[6]	–	350	350
Agar agar[7]	50	50	50
Mineral mix[8]	40	40	40
Vitamin mix[9]	20	20	20

[1] Diets were hyperlipidic (19.08 MJ/kg diet; 4,558 kcal/kg) and administered in powder form. [2] Prolabo, Fontenay-sous-Bois, France. [3] Extracted and defatted at the Clinical and Metabolic Nutrition Laboratory (LNCM), Oran, Algeria. [4] ONAB, Sidi Bel Abbès, Algeria. [5] Cevital SPA, Bejaia, Algeria. [6] Commercial source. [7] Prolabo, Paris, France. [8] UAR 205 B, Villemoisson-sur-Orge, France; mineral mixture (g/kg diet): $CaHPO_4$ 17.2; KCl 4.0; NaCl 4.0; MgO 0.42; $MgSO_4$ 2.0; Fe_2O_3 0.12; $FeSO_4 \cdot 7H_2O$ 0.20; $MnSO_4 \cdot H_2SO_4 \cdot H_2O$ 0.098; $CuSO_4 \cdot 5H_2O$ 0.02; $ZnSO_4 \cdot 7H_2O$ 0.08; KI 0.00032. [9] UAR 200, Villemoisson-sur-Orge, France; vitamin mixture (mg/kg diet): vit A 39,600 IU; vit D3 5,000 IU; vit B1 40; vit B2 30; vit B3 140; vit B6 20; vit B7 300; vit B12 0.1; vit C 1,600; vit E 340; vit K 3.80; vit PP 200; choline 2,720; folic acid 10; para-aminobenzoic acid 180; biotin 0.6; cellulose q.s. to 20 g.

Table 2. Amino acid profile of the Mytilus galloprovincialis protein concentrate

Amino acid	*g/100 g protein (mean ± SD)*	*% of total AA (mean ± SD)*
Glutamic acid	11.90 ± 0.21	13.96 ± 0.24
Glycine	8.80 ± 0.16	10.33 ± 0.19
Aspartic acid	8.10 ± 0.14	9.51 ± 0.17
Taurine†	6.60 ± 0.12	–
Leucine*	6.50 ± 0.11	7.63 ± 0.13
Lysine*	6.20 ± 0.10	7.28 ± 0.12
Alanine	5.80 ± 0.09	6.81 ± 0.11
Arginine	5.20 ± 0.08	6.10 ± 0.10
Valine*	4.80 ± 0.08	5.63 ± 0.09
Serine	4.10 ± 0.07	4.81 ± 0.08
Phenylalanine*	4.00 ± 0.06	4.69 ± 0.08
Proline	3.90 ± 0.06	4.58 ± 0.07
Threonine*	3.60 ± 0.05	4.22 ± 0.06

Isoleucine*	3.20 ± 0.05	3.76 ± 0.06
Tyrosine	3.20 ± 0.05	3.76 ± 0.06
Methionine*	1.90 ± 0.03	2.23 ± 0.04
Histidine*	1.30 ± 0.02	1.53 ± 0.03
Tryptophan*	1.10 ± 0.02	1.29 ± 0.02
Cysteine	0.20 ± 0.01	0.23 ± 0.01
TOTAL	**85.21 ± 1.32**	**100**

* Essential amino acid (FAO/WHO, 2013). † Non-proteinogenic amino acid. Method: HPLC after acid hydrolysis (6 N HCl, 110 °C, 24 h) and OPA derivatization (Roth, 1971). Results expressed as mean ± SD, n = 3.

Table 3. Morphometric and nutritional parameters of control, obese, and mussel protein-treated rats

Parameter	*Control*	*Obese*	*Obese + Pcm*
Initial body weight (g)	116.00 ± 1.96^{a}	128.50 ± 3.65^{a}	132.38 ± 4.53^{a}
Final body weight (g)	365.50 ± 21.07^{b}	452.50 ± 13.87^{a}	387.88 ± 11.60^{b}
Weight gain (g)	249.50 ± 20.22^{b}	324.00 ± 15.26^{a}	255.50 ± 11.50^{b}
Heart weight (g)	0.97 ± 0.04^{a}	1.19 ± 0.02^{a}	0.96 ± 0.08^{b}
Cardiac index (%)	0.27 ± 0.02^{a}	0.26 ± 0.01^{a}	0.25 ± 0.02^{a}
Kidney weight (g)	1.98 ± 0.15^{b}	2.75 ± 0.11^{a}	2.29 ± 0.13^{b}
Renal index (%)	0.54 ± 0.02^{a}	0.61 ± 0.03^{a}	0.59 ± 0.02^{a}
Aortic weight (g)	0.18 ± 0.05^{a}	0.23 ± 0.02^{a}	0.13 ± 0.01^{b}
Aortic index (%)	0.05 ± 0.01^{a}	0.05 ± 0.00^{a}	0.03 ± 0.00^{a}
Food intake (g/day)	22.8 ± 1.4	19.7 ± 1.2	21.4 ± 1.1
Feed efficiency ratio (FER)	0.145 ± 0.011	0.281 ± 0.018	0.188 ± 0.015
BMI (g/cm²)	0.62 ± 0.03	0.84 ± 0.05	0.70 ± 0.04
Lee index	303 ± 6	335.5 ± 7	306.3 ± 6
Adiposity index (%)	1.54 ± 0.12	3.48 ± 0.25	1.28 ± 0.11

Values expressed as mean ± SEM. Different superscript letters (a, b, c) within a row indicate a statistically significant difference between groups (Tukey's test, $p < 0.05$; one-way ANOVA). Pcm: mussel by-product proteins.

Table 4. Hemodynamic parameters

PARAMETER	**CONTROL**	**OBESE**	**OBESE + PCM**
SBP (mmHg)	117.25 ± 2.29^{b}	169.50 ± 10.23^{a}	127.00 ± 5.76^{b}
DBP (mmHg)	97.50 ± 1.66^{b}	132.75 ± 7.88^{a}	101.25 ± 8.16^{b}

MAP (mmHg)	104.08 ± 1.51[b]	145.00 ± 8.59[a]	109.83 ± 7.25[b]
Pulse pressure (mmHg)	19.75 ± 2.43[b]	36.75 ± 3.35[a]	25.75 ± 3.64[a]

Values are expressed as mean ± SEM (n = 8 per group). Different superscript letters (a, b, c) within the same row indicate statistically significant differences between groups (one-way ANOVA followed by Tukey's multiple comparison test; $p < 0.05$). SBP, systolic blood pressure; DBP, diastolic blood pressure; MAP, mean arterial pressure; PP, pulse pressure; Pcm, Mediterranean mussel (*Mytilus galloprovincialis*) aquaculture by-product protein concentrate.

Table 5. Renal function parameters

PARAMETER	CONTROL	OBESE	OBESE + PCM
Serum urea (g/L)	0.36 ± 0.02[a]	0.42 ± 0.04[a]	0.34 ± 0.02[a]
Serum creatinine (mg/L)	5.32 ± 0.24[b]	7.05 ± 0.35[a]	5.80 ± 0.21[b]
Creatinine clearance / GFR (mL/min)	0.20 ± 0.01[b]	3.82 ± 0.16[a]	0.18 ± 0.02[b]

Values expressed as mean ± SEM. Different superscript letters (a, b, c) within a row indicate a statistically significant difference between groups (Tukey's test, $p < 0.05$; one-way ANOVA). Pcm: mussel by-product proteins.

Table 6. Urinary biochemical parameters

PARAMETER	CONTROL	OBESE	OBESE + PCM
Diuresis (mL/24h)	93.48 ± 0.96[a]	88.86 ± 3.70[a]	110.34 ± 12.38[a]
Proteinuria (mg/L)	327.30 ± 4.40[c]	715.87 ± 9.69[a]	454.50 ± 20.55[b]
Urinary creatinine (mg/L)	16.17 ± 0.35[b]	435.94 ± 19.12[a]	13.75 ± 1.84[b]
Protein-to-creatinine ratio (PCR)	20.27 ± 0.66[b]	1.65 ± 0.06[c]	34.70 ± 4.57[a]
Microalbuminuria (mg/L)	18.87 ± 0.80[a]	7.58 ± 0.63[b]	3.92 ± 2.48[b]
Albumin-to-creatinine ratio (ACR)	1.17 ± 0.05[a]	0.02 ± 0.00[b]	0.23 ± 0.14[b]
Glucosuria (g/L)	0.03 ± 0.03[a]	0.03 ± 0.01[a]	0.00 ± 0.00[a]
Urinary urea (g/L)	21.14 ± 0.29[a]	23.18 ± 1.13[a]	15.08 ± 5.06[a]
Urinary uric acid (mg/L)	2.21 ± 0.17[c]	48.12 ± 1.88[a]	11.36 ± 0.94[b]
Natriuresis (mmol/L)	45.41 ± 1.48[b]	28.69 ± 1.29[c]	58.62 ± 3.71[a]
Kaliuresis (mmol/L)	61.65 ± 1.19[b]	89.92 ± 2.82[a]	81.33 ± 5.85[a]
Sodium excretion (mEq/24h)	4439.98 ± 93.77[b]	2477.09 ± 194.81[c]	8368.09 ± 449.18[a]
Potassium excretion (mEq/24h)	5979.46 ± 44.38[b]	6773.55 ± 177.85[b]	13075.97 ± 294.08[a]

Values expressed as mean ± SEM. Different superscript letters (a, b, c) within a row indicate a statistically significant difference between groups (Tukey's test, $p < 0.05$; one-way ANOVA). Pcm: mussel by-product proteins.

Table 7. Vascular resistance index (VRI)

PARAMETER	CONTROL	OBESE	OBESE + PCM
VRI (a.u.)	1.12 ± 0.08^{c}	1.89 ± 0.14^{a}	1.24 ± 0.14^{b}

Values expressed as mean ± SEM. Different superscript letters (a, b, c) indicate a statistically significant difference between groups ($p < 0.05$). VRI: vascular resistance index (arbitrary units), calculated as mean arterial pressure divided by mean tail blood flow recorded by the CODA® VPR system (see Methods). Pcm: mussel by-product proteins.