Trop J Nat Prod Res, January 2025; 9(1): 50 - 57

ISSN 2616-0684 (Print)
ISSN 2616-0692 (Electronic)

Tropical Journal of Natural Product Research

Available online at https://www.tjnpr.org

Original Research Article

The Effect of a High-Whey Protein Diet Combined With *Lactobacillus acidophilus* on Insulin Resistance, Intestinal Microbiota, and the Histology of the Liver, Spleen, Kidneys, and Colon

Aicha Sbaihia [1], Bouasria Benbouziane[1]*, Soumia Keddari[1], Mohamed C. Bentahar[2], Hiba Fedal[3]

[1]*Laboratory of Bioeconomics, Food safety and Health, Faculty of Natural Sciences and Life, Abdelhamid Ibn Badis University, BP 188, 27000, Mostaganem, Algeria*
[2]*Laboratory of Applied Animal Physiology, Faculty of Natural Sciences and Life, Abdelhamid Ibn Badis University, BP 188, 27000, Mostaganem, Algeria*
[3]*Laboratory of Anatomical pathology, Sisters BEDJ Hospital, 02000, Bensouna city, Chlef, Algeria.*

ARTICLE INFO

Article history:
Received 12 October 2024
Revised 16 October 2024
Accepted 11 December 2024
Published online 01 February 2025

ABSTRACT

The diet profoundly impacts the microbial ecology in the gastrointestinal tract and the majority of biological functions. This study attempts to discover the effects of chronic high-protein diets (HPD) and Lactic Acid Bacteria (LAB) on the liver, spleen, kidneys, and colon histology, with a special focus on their interactions with the gut flora. In a 12-week treatment program, which categorised four groups of obese rats, each receiving either a normal diet (14% whey protein) or a high-protein diet (50% whey protein), with and without the inclusion of *Lactobacillus acidophilus* (LAB). The HPD diet significantly increased liver transaminases (AST (78.29 U/L) and ALT (66.43 U/L)), while normal diets raised insulin levels (0.95 µU/mL); however, their combination with LAB improved HbA1c (2.5) and insulin levels (0.25µU/mL). HPD caused infiltrations in the muscular layers of the colon and hypertrophy of the glomeruli, as well as dilation and congestion of the capillary sinuses, not to mention the significant infiltration in the splenic red and white pulps. Normal diets caused fatty deposits in the kidneys and hyalinisation of the tubules, as well as lymphatic aggregations in the intestinal crypts and damage to epithelial tissue. The consumption of high-protein diets in association with LAB (10%) significantly increased the number of *Lactobacillus* compared to the group on normal diets and *Clostridium_sensu_stricto_1* in HPD without LAB association (12%). The association of LAB to the HPD diet improves their impact on general physiological functions and specifically improves gut microbiota.

Keywords: High protein diet, *Lactobacillus acidophilus*, Obese rat, Gut microbiota

Introduction

A prevalent disease that impacts nations at all levels of development is obesity. It ranks as the second most common cause of premature death globally. Because diets heavy in simple carbs and saturated fats are widely accepted, along with sedentary lifestyles, the incidence of obesity is fast rising.[1] A healthy diet is one of the elements that can be controlled in developing metabolic syndrome. Milk and dairy products are abundant in protein, calcium, phosphorus, potassium, and magnesium. Milk proteins consist of vital amino acids, especially branched-chain amino acids.[2] The popularity of high-protein diets for controlling body weight has contributed to their increased usage in recent years. The liver is the main organ that breaks down macronutrients in the body, and it is also connected to the development of several disorders.[3]

*Corresponding author. E mail: bouasria.benbouziane@univ-mosta.dz

Tel: +213552761195

Citation: Sbaihia A, Benbouziane B, Keddari S, Bentahar MC, Fedal H. The Effect of a High-Whey Protein Diet Combined With *Lactobacillus acidophilus* on Insulin Resistance, Intestinal Microbiota, and the Histology of the Liver, Spleen, Kidneys, and Colon. Trop J Nat Prod Res. 2025; 9(1): 50 – 57. https://doi.org/10.26538/tjnpr/v9i1.8

Official Journal of Natural Product Research Group, Faculty of Pharmacy, University of Benin, Benin City, Nigeria

But other tissues or organs, such as conventional immune organs, could also have a role in the genesis of inflammatory processes.[4] In addition, obesity has an impact on the structure and function of the spleen. In obese individuals, the spleen often shows a significant buildup of fat and disrupted immune responses.[5] Prior research has indicated that excessive use of whey protein can negatively affect long-term health. The unfavourable effects include a rise in the occurrence of acne,[6] microbiota dysfunction,[7] and changes in the regular metabolism of the kidneys,[8] and liver.[9]

Moreover, adding spices to rats' meals might improve the recovery of heart and liver damage brought on by consuming diets that promote metabolic syndrome by reducing serum enzyme activities and alleviating dyslipidemia.[10] The spleen is classified as a secondary lymphoid organ, where T and B cells undergo antigen-dependent proliferation and differentiation. When an animal is complete, the histological nature of the spleen matches the normal characteristics of the species. The spleen cords protrude from a capsule that surrounds the organ externally. The pulp of the spleen is red and white. The reticular cells that surround the blood cells make up the red pulp. The splenic lymph nodes and the arteriolar lymphoid sheath that envelops them make up the white pulp.[11]

In the last ten years, research into the billions of microorganisms (microbiota) that live inside the human body has significantly increased their interactions with their hosts. These previously overlooked individuals have been acknowledged for their role in various physiological functions of the host, including metabolism, immunity, cardiovascular function, and neuronal development. Any disruption or dysfunction in their structure or function can disrupt the balance between the microbes and the host, potentially leading to disease.[12] Hence, comprehending the significance of gut microbiota in the

ISSN 2616-0684 (Print)
ISSN 2616-0692 (Electronic)

progression of human ailments is crucial. Long-term consumption of a diet high in casein and proteins has impacted the liver's metabolic pathways, increasing triglyceride (TG) deposition, inflammation, and enzyme activation in response to increased oxidative stress and pH imbalance.[3] The majority of the amino acids released from consumed protein during proteolysis are either transported to peripheral tissues for protein synthesis or to the liver for gluconeogenesis, where they eventually break down and create ammonia as a byproduct. Hepatic encephalopathy, which arises when the liver's urea cycle performance is impaired, is one of the clinical disorders where elevated ammonia concentrations in the brain significantly reduce neuronal activity.[13]
All previous studies have extensively examined the effects of high-protein diets and the impact of *Lactobacillus sp.* on physiology and the microbiota separately, and this highlights the originality of this work, where we studied the importance of their association with various vital functions. Our study aims to investigate the effect of long-term consumption of the described diets, with or without LAB, on the physiology of the liver, spleen, colon, and kidneys, as well as their effects on insulin resistance, especially their interactions with the gut microbiota.

Materials and Methods

Animals and Experimental Design
Thirty-two healthy male Wistar rats were acquired from the Pasteur Institute in Algiers, Algeria. Following the quarantine period, the rats were placed in cages within a room that had good air circulation. The room was set to a 12-hour light and 12-hour dark cycle. The rats had unfettered access to food and beverages. The protocol adhered to the criteria of the National Institutes of Health (NIH). All animal experiments conducted at the University of Abdelhamid Ibn Badis, Mostaganem, have been approved by the local ethics council for the institution's animal care, with Approval No. 2019-013. The rats were provided with a high-fat diet containing 30% animal fat, following the AIN-93M (American Institute of Nutrition) rodent diet composition, as shown in Table 1. This diet was maintained for 12 weeks until the rats reached a body weight range of 350-450 grams. The rats were split up into four groups and randomly allocated to either a high-protein diet or a normal diet. The rats were categorised into four diet groups, with 8 rats in each group. The diet groups were normal diet (Control: 14% whey protein), normal diet with lactic acid bacteria (LAB) (NL: 14% whey protein + LAB (*L. acidophilus*)), high protein diet (PD: 50% whey protein), and high protein diet with LAB (PDL: 50% whey protein + LAB (*L. acidophilus*)). The constituents of the whey protein diet were identical to those of the control diet, with the exception that half of the dietary starch was substituted with whey protein. Each rat was given fresh food three times a week by replacing the food container for each rat.
The bacterial cell was incubated for a minimum of 19 hours to achieve a concentration of $5x10^8$ CFUs, as determined by the absorbance value (OD) and CFUs measured throughout the 24-hour incubation period. The collected bacterial cells were centrifuged at 3500 rpm for 10 minutes, and the liquid portion was discarded. The rats in each subgroup were administered the LAB (*L. acidophilus*) orally via a gavage tube at a dosage of $5x10^8$ CFU/mL/day, with a volume of 1 mL each day.

Table 1: Composition of different dietary components.

Ingrédients (g/Kg)	Control	NL	PD	PDL
Proteins (whey protein isolate 97%)[1]	140	140	500	500
Starch flour[2]	572.7	572.7	212.7	212.7
sucrose [3]	100	100	100	100
Cellulose (son de blé)[4]	100	100	100	100
Soybean oïl[5]	40	40	40	40
Minerals mix , AIN 93-M[6]	35	35	35	35
Vitamins mix , AIN 93-V[7]	10	10	10	10
Choline [8]	2.3	2.3	2.3	2.3
Energies (kcal/g)	3610.8	3610.8	3610.8	3610.8

[1] Pure Whey Isolate™ 97, BULK, a company registered in England & Wales (company number 05654661) with registered address: Unit 1 Gunfleet Business Park, Brunel Way, Colchester, Essex, CO4 9QX, UK. Registered in the UK for VAT No. GB 254 5648 84.
[2,3,4] Sigma Aldrich S9765-500G, S9378-1KG, C6288-250G.Allemagne.
[5] Biomérieux, Allemagne.
[6,7] MP Biomedical, US.
[8] BULK, a company registered in England & Wales (company number 05654661) with registered address: Unit 1 Gunfleet Business Park, Brunel Way, Colchester, Essex, CO4 9QX, UK. Registered in the UK for VAT No. GB 254 5648 84.
-Control: 14% whey protein
-NL: 14% whey protein + LAB (*L. acidophilus*)
-PD: 50% whey protein
-PDL: 50% whey protein + LAB (*L. acidophilus*)

Measurement of Biochemical Parameters
Before sacrifice, I mL of blood was obtained from the animals' jugular veins using the Waynforth technique.[14] After that, the blood sample was centrifuged for 15 minutes at 2500 rpm and 4 °C. The serum was kept in storage at -20 °C for further examination. The enzymatic determination of aspartate aminotransferase (AST) and alanine aminotransferase (ALT) was performed using the Automate VITROS 350, US. The HPLC Automate VITROS 350 (USA) was utilised for the quantification of HbA1c. Insulin was quantified using the ECLIA method (VITROS ECI, USA). Insulin resistance was assessed using the Homeostasis Model Assessment (HOMA). The HOMA index was obtained by multiplying the fasting insulin (measured in mU/L) by the fasting glucose (measured in mmol/L) and then dividing the result by 22.5.[15]

The HOMA-IR index was calculated using the following formula:

$$\boldsymbol{HOMA-IR} = \frac{fasting\ insulin\ (mU/L)\ \boldsymbol{X} fasting\ glucose\ (mmol/L)}{22.5}$$

Sample preparation and histopathological analysis
The rats were sacrificed under chloroform anaesthesia, and the vital organs were exercised: large intestine, small intestine, liver, spleen, and kidneys, and the length of the colon and weight were recorded. Some of the tissues were stored in a 10% formalin solution, and others were covered in paraffin and sliced. The slices were then stained with hematoxylin and eosin (H&E) to assess the level of inflammation through a microscope (B-292, OPTIKA, Italy).

ISSN 2616-0684 (Print)
ISSN 2616-0692 (Electronic)

Collection of Stool Samples from Albino Rats and 16S rRNA gene amplicon sequencing

Fresh faecal samples were obtained from the cage floor immediately after the rats defecated on the final day of the treatment period.[16] NOVOGENE China carried out this part of the work. The DNA extraction from the intestinal contents was conducted following the guidelines provided by a stool DNA mini-kit (Qiagen, Hilden, Germany). The 16S RNA gene's global bacterial region V3-V4 was amplified using PCR barcode primers 338 F (5-ACTCCTACGGGAGGCAGCAG-3) and 806 R (5-GGACTACHVGGGTWTCTAAT-3). One FastPfu buffer, 250 μM dNTP, 0.1 μM of each primer, one unit of FastPfu Polymerase (Beijing TransGen Biotech, Beijing, China), and ten nanograms of template DNA were used in the 20 μL solution for the PCR. The reaction mixture was heated to 95 ℃ for two minutes to perform the polymerase chain reaction (PCR). After that, there were thirty cycles of denaturation (30 seconds at 95 ℃), annealing (30 seconds at 55 ℃), and extension (30 seconds at 72 ℃). The last extension phase was carried out for five minutes at 72 ℃. The desired areas were amplified with certain primers attached to barcodes using 2% agarose gel electrophoresis, and the PCR products of the right size were selected. Each sample's PCR results were mixed in equal amounts before being put through end-repair, A-tailing, and ligation using Illumina adapters. The libraries were processed using a paired-end Illumina platform, resulting in the generation of 250 bp paired-end raw reads. The DNA library in the experiment was assessed using Qubit and real-time PCR for quantification, and a bioanalyser was used to analyse the size distribution of the library. Using Illumina platforms, the measured libraries were merged and evaluated following the required quantity of data and the specified library concentration.

Statistical analysis

Data is presented as means ± standard error of the mean. An analysis of variance (ANOVA) with one factor was conducted. When the *P*-values were significantly different, the Tukey post-hoc test was applied with a significance level of $p<0.05$. The statistical analyses were performed using Prism Graphpad Software, version 9.5.1. (San Diego, California, USA,2023).

Results and Discussion

High-protein diets (HP) are popular in Western nations, particularly when they are linked to physical activity, weight loss, or maintenance. On the other hand, nothing is known about the long-term health consequences of using these diets. For this reason, it is necessary to examine the impacts of extended exposure to a high-protein diet in healthy animals. Our diet consisted of high-protein whey. When compared to other protein sources, milk proteins are thought to have a high nutritional value due to their excellent digestion and comparatively large concentrations of important amino acids.[3] It is challenging to accurately attribute the observed metabolic effects to protein content alone because of the study's concomitant reduction in dietary carbohydrates. However, the levels of sucrose and other dietary components, including fats, vitamins, minerals, and fibres, were kept constant to avoid interfering with these components in our study.

There was a significant difference in serum AST levels ($p < 0.0001$, R^2= 0.90) between normal diet groups (Control and NL) and high protein diet groups (PD and PDL). The significant differences in ALT levels were between the control and PDL groups ($p < 0.0001$, R^2=0.95). The PDL group had the highest ALT, and the PD and PDL groups had the highest AST values (Table 2). The liver's feedback pathway to the intestine through the secretion of bile and antibodies, as well as the portal vein's ability to carry products directly from the intestine to the liver, define the gut-liver axis, which is the interaction between the intestine and its microbiota and the liver. The gut-liver axis' balance depends on the microbiological community's regulation. Through a variety of pathways, including increased hepatic lipid metabolism, enhanced alcohol generation, increased intestinal permeability, bacterial translocation, intestinal bacterial proliferation, imbalance of the gut microbiota, and decreased bile output, the gut microbiota influences the liver.[17] The increase in AST and ALT levels, which are serum markers of liver damage, supports the notion that animals fed a high-protein diet had hepatic cell injury, even though the animals were at an early stage of fatty liver disease because obvious signs of macrovascular fat deposition were not seen. As reported by Mohmmad-Desoky,[1] obesity has led to an increase in AST and ALT levels in both the control and NL groups. Also, it shows different lesions in the tissues of the liver and spleen, as well as massive infiltration of lymphocytic cells around the portal veins. After three months of a high-protein diet, there was a chronic increase in protein consumption, which led to an upregulation of three separate aspects of hepatic amino acid metabolism: (i) the urea cycle, (ii) deamination, and (iii) the import of amino acids into the cell. This is in line with the well-established effect of increased amino acid flow to the liver, which raises blood urea levels and urea production. This metabolic change is associated with increased liver amino acid deamination to remove nitrogen and maintain nitrogen balance.[18] Griffin *et al.*[12] published control simulation findings that demonstrated blood ammonia levels at equilibrium within normal physiological limits. Blood ammonia levels rose by 59% in response to a 72% increase in protein consumption. Blood ammonia levels were raised by 41–130% in liver cirrhosis models, depending on the amount of protein consumed in the diet. In simulations of heterozygous individuals with a loss-of-function allele of the carbamoyl phosphate synthetase I (CPS I) gene involved in the urea cycle, blood ammonia levels more than quadrupled (rising from around 18 to 60 μM depending on dietary protein consumption). It's noteworthy to note that, in our study, administering 200 mg/kg of whey protein isolate over eight weeks resulted in greater liver damage scores when compared to a control diet. Another research revealed hepatocellular injury and nuclei loss in healthy rats treated with 0.3 g/kg of whey protein concentrate (WPC-80) for 21 days. A larger dosage of WPC-80 (0.5 g/kg) was reported to cause more significant harm in the rats. Nonetheless, the histological study revealed no evidence of fibrosis or inflammation.[19] The data shown in Table 2 indicate that high protein and normal diets have no significant (p =0.46, R^2= 0.18) effect on liver weight among the test animals. Furthermore, the data shows that there was a significant (p =0.008, R^2= 0.61) increase in spleen weight in control and NL groups in comparison with PD and PDL groups. The histological examination under an optical microscope shows that the liver has a lobular structure composed of hepatocyte cells arranged in trabecular patterns. Figure 1a shows that the parenchyma exhibits a chronic diffuse inflammatory response, primarily consisting of lymphocytes with moderate density. The inflammation is mainly located around the portal areas. The presence of a small number of binucleated hepatocytes in certain areas shows the regeneration of the liver tissue, accompanied by a few vacuolar cells. Occasionally, the sinus capillaries and the center-lobular vein exhibit dilation and congestion. The NL group (Figure 1b) shows lobules consisting of interconnected and separated hepatocyte pathways with frequently congested and dilated sinus capillaries. The central lobular veins are congested, while the remaining parenchyma shows moderate and varied chronic inflammation. Hepatocytes are infrequently binucleated, occasionally exhibiting granular cytoplasm. In the PD group (Figure 1c), the liver tissue was extensively altered by an inflammatory response characterised by the presence of mononuclear cells, mainly lymphocytes, with occasional accumulation around the portal area. The occurrence of binucleated hepatocytes was infrequent. Additionally, there was congestion and dilation of the sinusoidal capillaries and central veins within the lobules. Figure 1d shows the liver tissue that consists of hepatocytes arranged in trabecular patterns. This tissue was affected by a mild inflammatory response, primarily consisting of lymphocytes. The sinus capillaries in the liver appear congested and dilated. The histological investigation of the spleen shows a splenic parenchyma, which is enclosed by a fibrous capsule. The conjunctive trajectories that support the parenchyma come from this capsule. The parenchyma in question was composed of two distinct components. As shown in Figure 2a (Control group), the first is a white pulp, which can vary in size due to either atrophy or hypertrophy. This white pulp is comprised of distributed lymph nodules, which are surrounded by a congestive arteriola with a thick wall. The second component is a red pulp, which is a loosely structured and highly vascularised tissue known as Billroth cordons. This red pulp

ISSN 2616-0684 (Print)
ISSN 2616-0692 (Electronic)

also contains macrophages. The typical structure of the parenchyma was significantly modified due to the presence of a widespread infiltration of lymphocytes. Figure 2b (NL group) shows a splenic parenchyma enclosed by a thin connective tissue capsule. The white pulp often consists of atrophic and widely spread lymph nodules arranged around a central arteriole, while the red pulp comprises congested sinusoidal capillaries. Furthermore, in the PD group, the white pulp (Figure 2c), which is disorganized in its arrangement, was observed inside the parenchyma. The lymph nodules were shrunken and were bordered by an arteriole with a hardened wall. Finally, the parenchyma of the PDL group (Figure 2d) was composed of a white pulp, which consists of enlarged lymph nodules surrounding a thick and congested arteriole. The red pulp contains numerous sinus capillaries, some of which may be dilated and congested. The remaining portion of the red pulp shows infiltration of lymphocytes throughout.

Table 2: Comparison between means, standard errors and *P* value of AST, ALT, Insulin, HbA1c, HOMA IR, liver weight and spleen weight in all groups.

	Control	NL	PD	PDL	P value	R^2
AST(U/L)	63.5 ± 1.79	58.38 ± 3.59	78.29 ± 1.7**	73.37 ± 0.85**	P<0.0001	0.90
ALT(U/L)	34.88 ± 0.72**	52.13 ± 3.15	52.93 ± 1.50	66.43 ± 1.78**	P<0.0001	0.95
Insulin(µU/ml)	0.95 ± 0.15**	0.25 ± 0.04	0.29 ± 0.01	0.28 ± 0.01	P<0.0001	0.90
HbA1c	3.60 ± 0.71	2.5 ± 0.25	3.71 ± 0.27*	3.92 ± 0.14*	P =0.017	0.55
HOMA IR	0.24 ± 0.05**	0.06 ± 0.01	0.07 ± 0.003	0.07 ± 0.01	P<0.0001	0.84
Liver weight(g)	11.88 ± 1.44	12.84 ± 1.27	12.78 ± 1.51	10.98 ± 0.76	P =0.46	0.18
Spleen weight(g)	1.17 ± 0.24**	1.03 ± 0.08*	0.7 ± 0.07	0.82 ± 0.08	P =0.008	0.61

*: significant; **: highly significant.
-Control: 14% whey protein
-NL: 14% whey protein + LAB (*L. acidophilus*)
-PD: 50% whey protein
-PDL: 50% whey protein + LAB (*L. acidophilus*)

Abdominal obesity may also account for the rise in splenic diameter in SM patients in the current investigation, as, after controlling for other confounding variables, there was a significant link between splenic length diameter (SLD) and waist circumference. Obesity-related intracellular lipid deposits in the spleen and sinusoidal dilatation may account for this connection.[20] Gallagher and his colleagues,[21] recently documented a drop in spleen size following weight loss, which lends credence to this argument. Both people and rats have shown signs of glucose homeostasis following splenectomy. The splenectomy performed in the current investigation at 21 days led to decreases in plasma.[22, 23] Resistance training with a plant-based protein diet could play a key role in maintaining the immune responses of the spleen throughout ageing. However, a diet rich in animal proteins is suggested to decrease the immune activity of the spleen.[24]

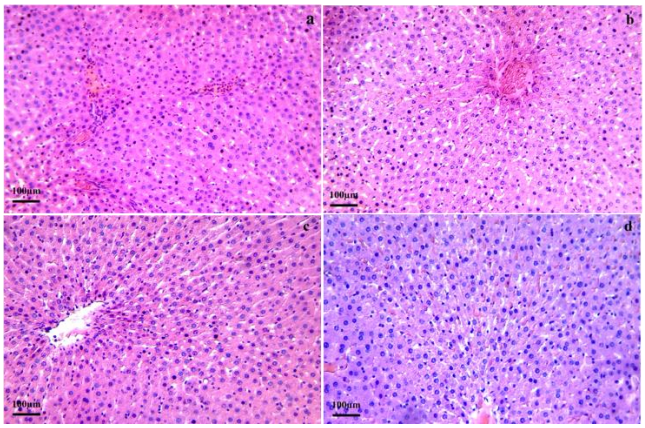

Figure 1: Photo of a histological sections taken from the optical microscope representing the liver, including (a) Control: 14% whey protein, (b) NL: 14% whey protein + LAB (*L. acidophilus*), (c) PD: 50% whey protein, (d) PDL: 50% whey protein + LAB (*L. acidophilus*).

There was a significant difference in serum insulin levels ($p < 0.0001$, $R^2= 0.90$). The control group represents the highest value (0.95 µU/mL). NL, PD, and PDL groups had a lower insulin level. PD and PDL groups had significant and the highest HbA1c (p =0.017, $R^2= 0.55$) levels. Thus, the control group revealed a significantly high value of HOMA-IR ($p < 0.0001$, $R^2= 0.84$) (Table 2). Consequently, our study suggests that chronic high-protein diet intake in healthy individuals may reduce insulin sensitivity. These findings are comparable to those of Rietman *et al*.[25] Another study reports that treating patients with polycystic ovarian syndrome (PCOS) with a low-carb, high-protein diet lowered their insulin levels.[26] The results reported by Sun *et al.*[27] show higher levels of HbA1c compared to the results of the current experiment. Chronic consumption of high-fat diets exacerbates obesity, insulin resistance, and systemic inflammation. The microarchitecture of the jaw can also be damaged by obesity brought on by a high-fat diet and insulin resistance, which has decreased mineral composition and bone formation rates and increased osteoclast-mediated bone resorption.[28] The primary source of protein in the HPD in this study was whey protein, which has a high concentration of branched-chain amino acids (BCAA). According to Newgard, the combination of decreased insulin sensitivity and glucose utilisation is enhanced by lipids and BCAAs.[29] Consequently, the HPD displays insulinotropic traits, which, over time, may culminate in insulin resistance.[30]

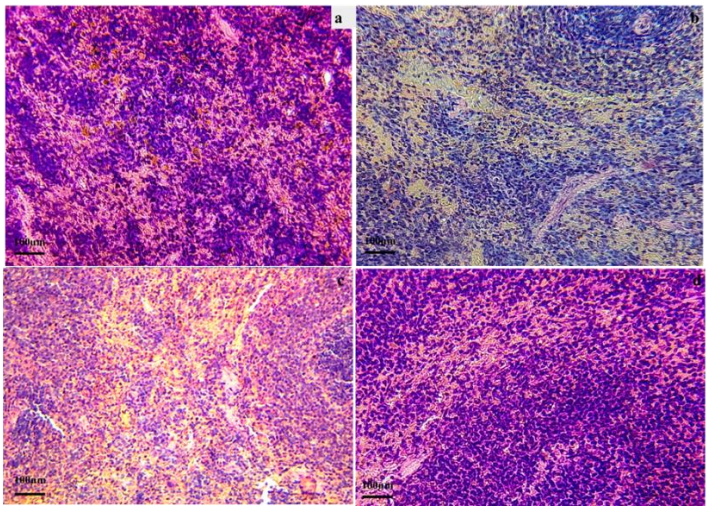

Figure 2: Photo of a histological slice taken from the optical microscope representing the spleen, including (a) Control: 14% whey protein, (b) NL: 14% whey protein + LAB (*L. acidophilus*), (c) PD: 50% whey protein, (d) PDL: 50% whey protein + LAB (*L. acidophilus*).

ISSN 2616-0684 (Print)
ISSN 2616-0692 (Electronic)

The histological examination of the control group kidneys (Figure 3a) reveals renal parenchyma composed of a cortex mostly consisting of shrunken and hardened tubules on the outer edge, with reduced glomeruli size. The spinal region was predominantly invaded by adipose tissue. Furthermore, Figure 3b (NL group) reveals renal parenchyma composed of a cortex including tubules, some of which have walls that were hyalinised. The glomeruli were abundant with congested blood arteries. Figure 3c (PD group) shows that the renal parenchyma consists of tubules that vary in appearance, ranging from regular to atrophic, with reduced visibility. The glomeruli, which are part of the renal parenchyma, also show signs of atrophy. Additionally, there was evidence of a mild diffuse inflammatory reaction affecting the parenchyma. Moreover, Figure 3d (PDL group) showed a renal parenchyma composed of a cortex with hyalinised tubules at the outside edge and atrophic tubules in the core. The glomeruli were also atrophied. The histological examination of the control group (Figure 4a) reveals a colic wall composed of a thin mucous membrane lined by a slightly damaged simple prismatic epithelium. The epithelium was folded into elongated crypts within a fibro-vascular chorion, and there were scattered inflammatory cells of the lymphocyte type present. There was a small reduction in the number of goblet cells. Lymphocytes often aggregate in crypts and intercellular spaces in certain locations. The muscle is mildly invaded by one of the most commonly disseminated inflammatory cells, which are lymphatic cells. A hematoxylin and eosin-stained histological analysis of rat colon tissue (NL group) reveals that the colonic wall has a superficial mucosa made up of a single layer of cylindrical epithelial cells organised in formations resembling villi. The mucosa was moderately damaged and thin, and it extended into the depth of the tissue, forming occasionally enlarged crypts. Surrounding the mucosa is a slightly inflamed fibro-vascular layer. The muscle is typically slender (Figure 4b). The histology of the PD group animals colon (Figure 4c) reveals that the colic wall was composed of a mucous membrane with a damaged epithelium. The epithelium may form cellular rosettes, giving it a marguerite field appearance. The presence of caliciform cells was moderate. The chorion, which is the connective tissue layer, was both fibro-vascular and edematous. It was infiltrated by an inflammatory reaction primarily consisting of lymphocytes. Occasionally, these lymphocytes are found in small scattered lymphatic follicles. The cryptic areas, which are small cavities, may be disorganised and irregularly dilated in some cases. The sub-serous was composed of fibro-fat tissue that was dense and highly supplied with blood vessels. The PDL (Figure 4d) group presents that the colonic wall was composed of mucosa with areas of atrophic surface epithelium that were slightly damaged, forming cellular rosettes within an inflammatory connective tissue layer. The submucosa is the site of a significant and diverse inflammatory response, characterised by the presence of inflammatory cells that are typically seen in clusters or lymphatic follicles with thin muscles. The findings displayed in Figure 5 demonstrate that the administration and regimens of LAB affect the microbiota's biodiversity both directly and indirectly. Figures 5a and 5b show a significant increase in *UGC-005* and a significant (p <0.05) presence of *Clostridia_UGC-014*, *Clostridium_sensu_stricto_1*, *Romboustia*, and the *Christensenellaceae_R-7* group. Except that *NK4A214_group*, *Allobaculum*, *Ruminococcaceae*, and *Lactobacillus* were more numerous in the control group compared to the NL group. Figures 5c and 5d show a diversity that differs from that of the normal diet groups (control and NL), where it was found that *Clostridium_sensu_stricto_1* increased significantly (p =0.04) in the PD and PDL groups. For *Romboustia* and *Lactobacillus*, there was a significant increase in the PDL group, whereas, in the PD group, it was due to the association of LAB. A high-protein diet caused a less favourable kidney profile, particularly concerning urinary and morphological markers, which could increase the risk of developing long-term kidney diseases.[31] The mild kidney damage (45%) observed in the high-fat and high-fructose diet (HFHFD) manifested as hydropic degeneration, haemorrhages, indications of inflammation, and proliferation of glomerular capillaries. In contrast, the group that had a regular diet (SD) only displayed minor lesions (7%).[32] Pet treats enriched with Lactobacillus have changed the feline microbiota (*Peptostreptococcaceae, Lactobacillus, Blautia, and Enterobacteriaceae*), which in turn controls the microbial processes involved in phenylalanine metabolism and lowers dangerous levels of Indoxyl Sulfate (IS). Their abundance can influence the efficacy of Lactobacillus isolates in enhancing gut-derived metabolites and kidney function.[33] Renxu Lai indicated that the highest category of protein consumption did not have a significant association with the risk of colorectal cancer compared to the lowest category. The risk of colon cancer is not significantly correlated with dietary protein intake. In the examination of subgroups based on the kind of research design, the kind of protein (plant or animal), sex, and geographic areas, the connection was also not statistically significant. [34]

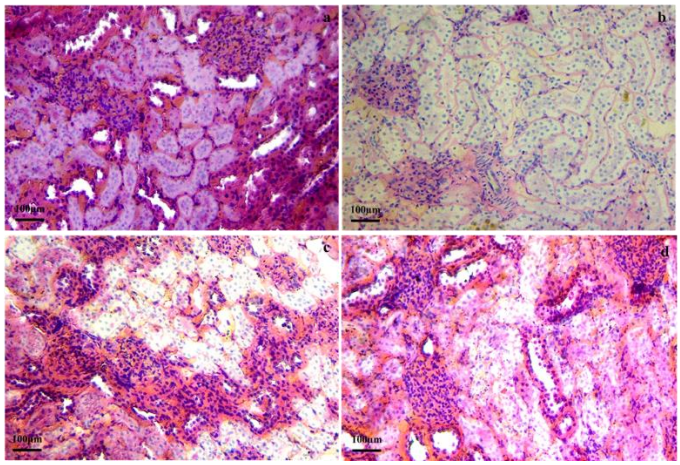

Figure 3: Photo of a histological slice taken from the optical microscope representing the kidney, including (a) Control: 14% whey protein, (b) NL: 14% whey protein + LAB (*L. acidophilus*), (c) PD: 50% whey protein, (d) PDL: 50% whey protein + LAB (*L. acidophilus*).

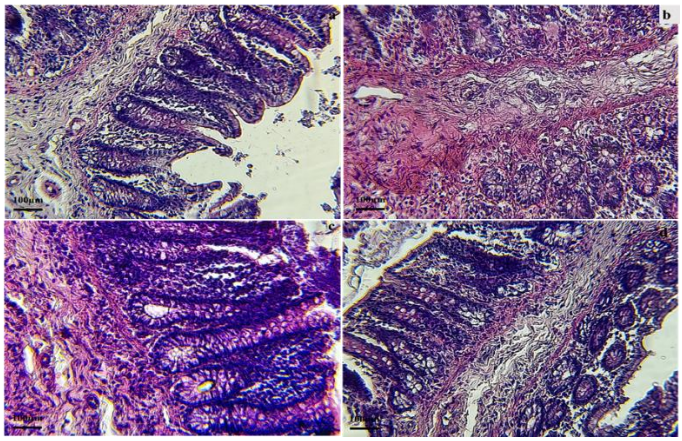

Figure 4: Photo of a histological slice taken from the optical microscope representing the colon, including (a) Control: 14% whey protein, (b) NL: 14

According to our findings, a high-sugar diet consumed over an extended period causes obesity and dynamic alterations in the faecal microbiota in taxa linked to metabolic diseases. In addition, *Lactobacillaceae* belongs to the family of bacteria that produce lactic acid and have several advantageous properties. These include probiotic properties, the ability to induce lactose intolerance, relief, immunomodulation, resistance to bile acids, and the generation of bacteriocins.[35] Protein fermentation results in metabolites like H_2S, ammonia, phenols, indoles, and branched-chain fatty acids. These compounds have harmful effects on the health of the colon, including thinning of the mucosal barrier, increased permeability of the colon, damage to colonocyte DNA, and disturbances in colonocyte growth and metabolism.[36] Carbs are normally absorbed and digested in the small intestine, with the proximal portion of the large intestine merely

ISSN 2616-0684 (Print)
ISSN 2616-0692 (Electronic)

fermenting undigested carbs like fibres. Short-chain fatty acids (SCFAs), including butyrate, propionate, and acetate, are fermentation byproducts and serve as a principal energy source for colonocytes.[37] This study's results indicated increased lymphocyte infiltration in the submucosa of high-protein diet groups and decreased infiltration in regular diet groups. These results are similar to many studies conducted on inflammatory bowel diseases.[38, 39] Rats given HPD for two weeks did not experience changes in the colonic epithelium's DNA damage, epithelial regeneration, or barrier function. Nonetheless, the transcriptional profile seen in the colonocytes of rats given an HPD suggests a downregulation of pathways related to essential cellular functions such as glutathione metabolism, DNA repair, and NF-*κB* signalling.[40] According to this study, there was no discernible correlation between the highest category of dietary protein consumption and the risk of colon cancer.[33] Research examining the reactions of the mouse microbiota to high-protein diets has shown that elevated dietary protein significantly reduces diversity at the family and genus levels. High amounts of dietary protein have been demonstrated to influence the relative abundance of particular taxonomic groups and to affect the microbiota's overall taxonomic diversity.[41] They also noted that significant increases in *Clostridium*, unidentified *Clostridial*, and *Allobaculum* were associated with higher dietary protein intake, alongside notable declines in the taxa *Eubacterium*, *Akkermansia*, *Mucispirillum, Ruminococcus, Johnsonella, Alistipes, Butyrivibrio*, and *Blautia*. A separate study indicated that the prevalence of *Bacteroides* rose with an increase in dietary protein.[42] Nevertheless, the modelling conducted by Holmes et al. revealed a reduction in *Bacteroides* with a high protein diet, indicating a lack of agreement among research.[41] When mice are given isotopically tagged protein sources, *Bacteroides* consume more dietary nitrogen than other species, supporting the theory that *Bacteroides* are motivated by increasing nitrogen availability from dietary protein.[42] A recent research that tracked microbial activity in bioreactors injected with human faecal microbiota using isotopically heavy water lends more credence to the notion that some *Bacteroides* order groups benefit from increased dietary protein consumption.[43, 44] The researchers discovered that certain *Bacteroides* species exhibited increased activity in bioreactors supplied with a medium that mimicked a high-protein diet, in contrast to a medium simulating a high-fibre diet. Consuming probiotics alone is insufficient; maintaining proper intestinal homeostasis, lowering obesity, and enhancing well-being also require regular exercise and calorie restriction.[45] Recent data indicate that T2DM has a negative correlation with the genera *Bifidobacterium, Bacteroides, Faecalibacterium, Akkermansia*, and *Roseburia*, whereas it shows a positive correlation with the genera *Ruminococcus, Fusobacterium, and Blautia.* Insulin sensitivity in type 2 diabetes mellitus was enhanced by butyrate-producing short-chain fatty acid bacteria. Patients with T2DM were predominantly associated with an increase in specific infectious microbes, such as *Clostridium spp.*, while control samples were primarily enriched in butyrate-producing bacteria and *Lactobacillus spp.*[46]

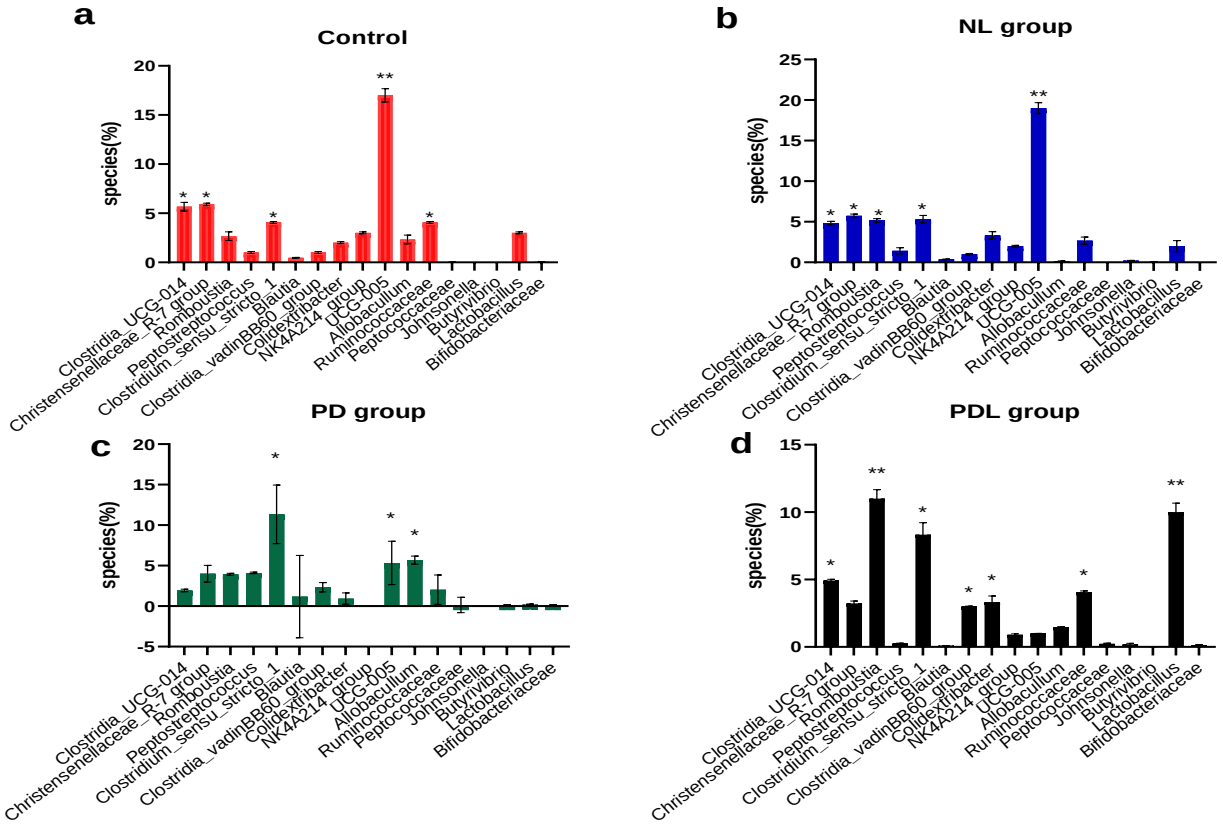

Figure 5: Relative quantity of fecal bacterial species evaluated by 16S rRNA gene amplicon sequencing, including (a) Control: 14% whey protein, (b) NL: 14% whey protein + LAB (*L. acidophilus*), (c) PD: 50% whey protein, (d) PDL: 50% whey protein + LAB (*L. acidophilus*). **: highly significant; *: significant.

Conclusion

This study investigates the effects of chronic high-protein diets (HPD) and Lactic Acid Bacteria (LAB) on the liver, spleen, kidneys, and colon histology in obese rats. The study found that HPD diets increased liver transaminases and insulin levels, while normal diets raised them. Combining HPD with LAB improved HbA1c and insulin levels. The study also found that high-protein diets with LAB increased *Lactobacillus sp.* compared to normal diets. The study needs more investigation into gut microbiota and the effect of their secondary metabolites on physiology.

Conflict of Interest

The authors declare no conflict of interest.

Authors' Declaration

The authors hereby declare that the work presented in this article is original and that any liability for claims relating to the content of this article will be borne by them.

Acknowledgements

The authors wish to sincerely thank Dr. Bouasria Benbouziane, for his kind advice, passionate support, and insightful criticism of this study. Additionally, special thanks to the veterinarians, Drs. Benmehdi and Benameur F, who prepared the histology sections (the regional veterinary laboratory, Mostaganem, Algeria). A special thanks to Dr. Belahdji Fatima Zahra for her support and assistance.

Funding

Under the Research Project (PRFU), the Ministry of Higher Education and Scientific Research at the University of Abdelhamid Ibn Badis in Algeria provided funding for this study. Code: D00L01UN270120200002.

References

1. Desoky AMAA. The Potential Histological Effect of Experimental Obesity on The Liver of Male Albino Rats (Light and Electron Microscopic Study). Egypt J Hosp Med. 2022;89(1):5028–5039.
2. Kratz M, Baars T, Guyenet S. The relationship between high-fat dairy consumption and obesity, cardiovascular, and metabolic disease. Eur. J.Nutr. 2013;52(1):1-24.
3. Díaz-Rúa R, Keijer J, Palou A, van Schothorst EM, Oliver P. Long-term intake of a high-protein diet increases liver triacylglycerol deposition pathways and hepatic signs of injury in rats. J Nutr Biochem. 2017;46:39–48.
4. Speretta GF, Leite RD, Duarte ACDO. Obesidade, inflamação e exercício: foco sobre o TNF-*α* e IL-10. Rev Hosp Univ Pedro Ernesto. 2014;13:61–69. https://doi.org/10.12957/rhupe.2014.9807 .
5. Ley EJ, Singer MB, Clond MA, Johnson T, Bukur M, Chung R, Margulies DR, Salim A. Long-term effect of trauma splenectomy on blood glucose. J Surg Res. 2012;177:152–156. https://doi.org/10.1016/j.jss.2012.03.068.
6. Wakefield AP, House JD, Ogborn MR, Weiler HA, Aukema HM. A diet with 35% of energy from protein leads to kidney damage in female Sprague-Dawley rats. Br J Nutr. 2011;106:656–663. Doi: 10.1017/S0007114511000730.
7. Gurgen SG, Yucel AT, Karakus AC, Cecen D, Ozen G, Kocturk S. Usage of whey protein may cause liver damage via inflammatory and apoptotic responses. Hum Exp Toxicol.2014;28:1–11. doi: 10.1177/0960327114556787.
8. Pontes TC, Fernandes FGM, Trindade AS, Sobral Filho JF. Incidence of acne vulgaris in young adult users of protein-calorie supplements in the city of João Pessoa – PB. An Bras Dermatol.2013;88:907–912. doi: 10.1590/abd1806-4841.20132024.
9. Moreno-Pérez D, Bressa C, Bailén M, Hamed-Bousdar S, Naclerio F, Carmona M, Pérez M, González-Soltero R, Montalvo-Lominchar MG, Carabaña C, Larrosa M. Effect of a protein supplement on the gut microbiota of endurance athletes: A randomised, controlled, double-blind pilot study. Nutrients. 2018;10:1–16. https://doi.org/10.3390/nu10030337.
10. Siddiq A, Osibemhe M, Nura L, Maibulangu B. M. Cardio-and-Hepatoprotective Benefits of Some Spices in Wistar Rats Induced with Metabolic Syndrome. Trop J Nat Prod Res. 2022;6:1707–1714.
11. Aparicio VA, Nebot E, Porres JM., Ortega FB, Heredia JM, Lopez-Jurado M, Ramirez PA. Effects of high-whey-protein intake and resistance training on renal, bone and metabolic parameters in rats. Br J Nutr. 2011;105:836–845. Doi: 10.1017/S0007114510004393.
12. Eaimworawuthikul S, Tunapong W, Chunchai T, Suntornsaratoon P. Altered gut microbiota ameliorates bone pathology in the mandible of obese – insulin-resistant rats. Eur.J.Nutr.2020;59:1453–1462. https://doi.org/10.1007/s00394-019-02002-8
13. Griffin JWD, Bradshaw PC. Effects of a high protein diet and liver disease in an in silico model of human ammonia metabolism. Theor Biol Med Model. 2019;16:1–14. https://doi.org/10.1186/s12976-019-0109-1.
14. Adebolu TT, Adediwura D V., Aiyenuro EA. Antibacterial Activity of Sorghum "Ogi" on Diarrhoeagenic *Escherichia coli*. J Adv Microbiol. 2018;12:1–8. https://doi.org/10.9734/jamb/2018/44011.
15. Waynforth HB and Flecknell PA. Experimental and Surgical Technique in the Rat Second Edition Elsevier book 2014; Second Edi:1–377.
16. Belkacem S, Bammoune A, Bali MM, Yargui L. Evaluation of insulin sensitivity in type 2 diabetics. Alg J Med Heal Res. 2022;1:61–74.
17. Liu J, Gu Z, Song F, Zhang H, Zhao J, Chen W. *Lactobacillus plantarum ZS2058*, and *Lactobacillus rhamnosus GG* Use different mechanisms to prevent *Salmonella* infection in vivo. Front Microbiol. 2019;10:1–9.
18. Serra F, Bonet L, Palou A. Amino-acid-enzyme activities in brown and white adipose tissues and the liver of cafeteria rats. Effects of 24 hours starving. Arch Int Physiol Biochim. 1987;95:263–268
19. Reis CEG. Discussion of "Whey protein supplementation and its potentially adverse effects on health: a systematic review"—Unsubstantiated claims of adverse effects of whey protein supplementation on human kidney and liver function. Appl Physiol Nutr Metab. 2021; 46(1):90–91. https://doi.org/10.1139/ apnm-2020-0674 PMID: 33399522
20. Abd El-Aziz R, Naguib M, Rashed LA. Spleen size in patients with metabolic syndrome and its relation to metabolic and inflammatory parameters. Egypt J Intern Med. 2018;30:78–82. https://doi.org/10.4103/ejim.ejim_86_17.
21. Gallagher D, Kelley DE, Thornton J, Boxt L, Pi-Sunyer X, Lipkin E, et al. MRI Ancillary Study Group of the Look AHEAD Research Group. Changes in skeletal muscle and organ size after a weight-loss intervention in overweight and obese type 2 diabetic patients. Am J Clin Nutr. 2017; 105:78–84.
22. Madsen L, Myrmel LS, Fjære E, Liaset B, Kristiansen K, Madsen L. Links between Dietary Protein Sources, the Gut Microbiota, and Obesity. Front. physiol. 2017;8:1–12. https://doi.org/10.3389/fphys.2017.01047.
23. de Souza TA, de Souza DW, Siqueira BS, Rentz T, de Oliveria Emílio HR, Grassiolli S. Splenic participation in glycemic homeostasis in obese and non-obese male rats. Obes Res Clin Pract. 2020;14:479–486. https://doi.org/10.1016/j.orcp.2020.07.009.
24. Santana ES dos S, de Oliveira CA, Lima FIA, Nucci RAB, Fonseca FLA, Maifrino LBM. Effect of Resistance Training and Diet Intake on Spleen Structure of Ovariectomized Wistar Rats. J Heal Allied Sci NU. 2022;12:47–52. https://doi.org/10.1055/s-0041-1732812.
25. Rietman A, Schwarz J, Tomé D, Kok FJ, Mensink M. High dietary protein intake, reducing or eliciting insulin resistance? Eur J Clin Nutr. 2014;68:973–979. https://doi.org/10.1038/ejcn.2014.123.
26. Aryani HP, Santoso B, Purwanto B, Mudjanarko SW, Utomo B. The Effect of Low-Calorie High Protein Diet on Insulin, TNF-*α* and P38MAPK levels in insulin-resistant PCOS mice models. Syst Rev Pharm. 2020;11:597–605. https://doi.org/10.31838/srp.2020.10.89.

27. Sun P, Huang L, Shuai P, Wan Z, Liu Yingying, Xue J, Liu Yuping. Effect of a High Protein, Low Glycemic Index Dietary Intervention on Metabolic Dysfunction-Associated Fatty Liver Disease: A Randomized Controlled Trial. Front Nutr. 2022;9:1–12. https://doi.org/10.3389/fnut.2022.863834.
28. Eaimworawuthikul S, Tunapong W, Chunchai T, Yasom S, Wanchai K. Effects of probiotics, prebiotics or synbiotics on jawbone in obese- insulin-resistant rats. Eur J Nutr. 2019;58:2801–2810. https://doi.org/10.1007/s00394-018-1829-4.
29. Newgard, C.B. Interplay between lipids and branched-chain amino acids in the development of insulin resistance. Cell Metab. 2012,15, 606–614.
30. Medeiros CS, Neto IV de S, Silva KKS, Cantuária APC, Rezende TMB, Franco OL, Marqueti R de C, Freitas-Lima LC, Araujo RC, Yildirim A, Mackenzie R, Almeida JA. The effects of high-protein diet and resistance training on glucose control and inflammatory profile of visceral adipose tissue in rats. Nutrients. 2021;6:1–13. https://doi.org/10.3390/nu13061969.
31. Aparicio VA, Nebot E, Moral RG, Porres JM, Sánchez C. High-protein diets and renal status in rats. Nutr Hosp. 2013;28:232–237. https://doi.org/10.3305/nh.2013.28.1.6165.
32. Yustisia I, Tandiari D, Cangara MH, Hamid F, Daud NA. A high-fat, high-fructose diet-induced hepatic steatosis, renal lesions, dyslipidemia, and hyperuricemia in non-obese rats. Heliyon. 2022;8:e10896. https://doi.org/10.1016/j.heliyon.2022.e10896.
33. Tsai CW, Huang HW, Lee YJ, Chen MJ. Investigating the Efficacy of Kidney-Protective *Lactobacillus* Mixture-Containing Pet Treats in Feline Chronic Kidney Disease and Its Possible Mechanism. Animals. 2024;14:1–16. https://doi.org/10.3390/ani14040630.
34. Lai R, Bian Z, Lin H, Ren J, Zhou H, Guo H. The association between dietary protein intake and colorectal cancer risk : a meta-analysis. World J Surg Oncol. 2017:1–9. https://doi.org/10.1186/s12957-017-1241-1.
35. Yu Z, Nan F, Wang LY, Jiang H, Chen W, Jiang Y. Effects of a high-protein diet on glycemic control, insulin resistance, and blood pressure in type 2 diabetes: A systematic review and meta-analysis of randomised controlled trials. Clin Nutr. 2020;39:1724–1734. https://doi.org/10.1016/j.clnu.2019.08.008.
36. Cui X, Kim E. Dual Effects of High Protein Diet on Mouse Skin and Colonic Inflammation. Clin Nutr Res. 2018;7:56–68.
37. Bingham SA, Pignatelli B, Pollock JR, Ellul A, Malaveille C, Gross G, Runswick S, Cummings JH, O'Neill IK. Does increased endogenous formation of N-nitroso compounds in the human colon explain the association between red meat and colon cancer? J. Carcinog. 1996;17:515-523.
38. Kokou F, Sarropoulou E, Cotou E, Rigos G, Henry M, Alexis M. Effects of fish meal replacement by a soybean protein on growth, histology, selected immune and oxidative status markers of Gilthead Sea bream, *Sparus aurata*. J World Aquac Soc. 2015;46(2):115–128.
39. Estensoro I, Ballester-Lozano G, Benedito-Palos L, Grammes F, Martos-Sitcha JA, Mydland L-T, et al. Dietary butyrate helps to restore the intestinal status of a marine teleost (*Sparus aurata*) fed extreme diets low in fish meal and fish oil. PLoS One. 2016;11(11):1–21
40. Beaumont M, Andriamihaja M, Armand L, Grauso M, Jaffrézic F, Laloë D, Moroldo M, Davila A, Tomé D, Blachier F, Lan A. Epithelial response to a high-protein diet in rat colon. BMC Genom. 2017;18:1–14. https://doi.org/10.1186/s12864-017-3514-z.
41. Holmes AJ, Chew YV, Colakoglu F, Cliff JB, Klaassens E, Read MN, Solon-Biet SM, McMahon AC, Cogger VC, Ruohonen K, Raubenheimer D, Le Couteur DG, Simpson SJ. Diet-Microbiome Interactions in Health Are Controlled by Intestinal Nitrogen Source Constraints. Cell Metab. 2017;25:140–151. https://doi.org/10.1016/j.cmet.2016.10.021.
42. Reese AT, Pereira FC, Schintlmeister A, Berry D, Wagner M, Hale LP, et al. Microbial nitrogen limitation in the mammalian large intestine. Nat Microbiol. 2018;3:1441–1450. https://doi.org/10.1038/s41564-018-0267-7.
43. Bartlett A, Kleiner M. Dietary protein and the intestinal microbiota : An understudied relationship. iScience. 2022;25:105313. https://doi.org/10.1016/j.isci.2022.105313.
44. Starke R, Oliphant K, Jehmlich N, Schäpe SS, Sachsenberg T, Kohlbacher O, Allen-Vercoe E, von Bergen M. Tracing incorporation of heavy water into proteins for species-specific metabolic activity in complex communities. J Proteomics. 2020;222:1–10. https://doi.org/10.1016/j.jprot.2020.103791
45. Okediya CK, Oyewale JO, Okediya TT, Ajayi AS, Olasehinde GI. Effect of *Lactobacillus acidophilus* and *Lactobacillus plantarum* on Weight Reduction in Obese Rats. Trop J Nat Prod Res. 2021;5:759–762.
46. Oluwaloni Folusho O, Yakubu Omolara F, Adebayo Abiodun H, Koyejo Oluwatosin D, Lawal Adekunle K. Review of the Gut Microbiota Dynamics in Type-2 Diabetes Mellitus (T2DM): A Focus on Human-Based Studies. Trop J Nat Prod Res. 2023;7(6):3059–3079.