

Comparative Study Among Whey Protein, Amino Acids and Chia Seeds on Enhancing the Performance of Endurance Sports of Rats

Ahmed A. Ameen¹, Omneya W. Darwish², Haggag M. Hamdy³

Nutrition and Food Science Department, Faculty of Home Economics, Helwan University

Corresponding author: Omneya W. Darwish

Email: omneyawdarwish@gmail.com

Abstract

The present work was conducted to study the comparative among whey protein, amino acids and chia seeds on enhancing the performance of endurance sports in rats. Chemical composition and fatty acid content of chia seeds were analyzed. Forty-eight albino rats (Sprague Dawley Strain) (150±5g) were divided into four groups (12 of each). The first main group fed on basal diet for 28 days and served as -ve control. Groups from 2-4 were fed on basal diet with 10% whey protein, amino acids and chia seeds supplementation, respectively for 28 days. All 4 groups had the same swimming protocol for 28 days. The chemical composition of chia seeds showed that the highest content was carbohydrate followed by fats and protein as values 39.5, 30 and 21, respectively. Chia seeds fatty acid contains highest value of linoleic acid followed by γ -linolenic acid. Feeding rats led to significant increase in creatinine kinase, lactate dehydrogenase, serum alkaline phosphatase (ALP), ammonia, and glutathione-S-transferase (GST) in all tested groups compared with -ve control group. While there was significant decrease in serum glucose and Malondealdehyde in tested groups. There was significant decrease in serum aspartate aminotransferase (AST) in groups were fed on whey protein and amino acids. While was no differ in group were fed on chia seeds. There was significant decrease in creatinine and uric acid in group were fed on chia seeds, results recorded significant increase in serum alanine transaminase in groups were fed on amino acids and chia seeds, while it records significant decrease in group fed on whey protein. The highest increase in serum creatine recorded in group were fed on amino acids. According to the above results chia seeds have a beneficial effect on enhancing the performance of endurance sports in rats which may be used with human beings.

Keywords: chia seeds, Amino Acids, whey protein, and endurance sport.

1. Introduction

A successful swimming performance is a multi-factorial accomplishment, resulting from a complex interaction of physical, biomechanical, physiological and psychological factors, all of which are strongly affected by the special medium of water as well as by genetic factors. The nature of competitive swimming is unique, as most of the competitive events last less than four minutes. Yet training regimens have an endurance nature (many hours and many kilometers of swimming every day), which makes it impossible to classify swimming by definitions of aerobic-type or anaerobic-type events, as in track and field sports Sigal et al., (2022).

Whey protein (WP) is the milk serum that has been separated from the milk during cheese manufacture. Specifically, whey is formed by the action of chymosin after casein curd formation. Whey is composed of vitamins, lactose, minerals, fat (in small amounts) and soluble proteins. In bovine milk, 80% of protein is in the form of casein, with WP accounting for the remaining 20%, which includes β -lactoglobulin (β -Lg), α -lactalbumin (α -Lac), bovine serum albumin (BSA) and a small amount of lactoferrin (Lf). Whey protein can also improve vascular function, inflammation, glucose tolerance

and lipid metabolism, especially of triacylglycerol (TAG) and cholesterol. WP has a stronger satiety effect than other protein sources, including casein. This important property is primarily due to the high content of branched-chain amino acids (BCAAs), such as leucine, isoleucine, tryptophan and valine Serena et al., (2023).

Amino acids are functional and structural units of protein, nutritionally classified into two groups: non-essential (synthesized in the body) and essential amino acids (cannot be synthesized rapidly enough to meet the metabolic requirement). Amino acids play vital physiological roles in the body. After absorption, amino acids are assembled and metabolized to form proteins that are used to build different body tissues. Ten amino acids classified as essential (lysine, methionine, tryptophan, threonine, arginine, isoleucine, leucine, histidine, phenylalanine and valine) Mahmoud et al., (2021).

Chia (*Salvia hispanica*), derived from the Nahuatl word "chian" which means oily, is one such super seed belonging to flowering herbaceous plant of family Lamiaceae. The seed is flat, oval shaped and white to brownish in color and contains high proportions of the essential fatty acid, α -linolenic acid, which is associated with maintaining healthy cholesterol level, brain development and immune system. Besides rich fatty acid profile, it contains

significant amount of dietary fiber, and the seed exudes a mucilaginous polysaccharide when placed in aqueous medium which promotes digestion. Therefore, both chia seeds and its mucilage are nutrient-rich food sources. Moreover, chia is believed to be free of toxins, allergens and other anti-nutritional factors. The profuse number of bioactive compounds in chia makes it as a suitable nutritional supplement. Besides, chia is also approved as a Novel Food by the European Parliament and Council of Europe in 2009 Tabeen et al., (2023). Therefore, this study was carried out in order to investigate the comparative among whey protein, amino acids and chia seeds supplementation on endurance sports in rats.

2. Materials and Methods

Materials

Whey protein concentrate isolate, casein, vitamins, minerals, cellulose, choline chloride, amino acid supplements purchased from EL-Gomhoria company, Cairo, Egypt. Chia seeds (*Salvia hispanica* L., family Lamiaceae), starch and soy oil obtained from Local market in Cairo, Egypt. Kits for biochemical analysis obtained from Gamma-Trade, Giza, Egypt. Male albino rat (n= 48), weighting an average of (150±5g) purchased from Helwan Farm of Experimental Animals, Ministry of Health, Helwan, Cairo, Egypt.

Methods

Preparation of Chia Seeds

The dried seeds grinded using a coffee grinder into a fine powder and frozen at -20 °C till used.

Chemical Analysis of Chia Seeds

Chemical analysis and fatty acid content of chia seeds was conducted in Food Analysis Unit, Agricultural Research Center, Egypt. Chemical composition was determined according to A.O.A.C., (2012). Phenolic compounds were determined by HPLC according to Goupy et al., (1999) and DPPH radical-scavenging activity assayed according to the method of Brand et al., (1995).

Preparation of Basal Diet

The experimental diets were formulated according to the method of Reeves et al., (1993). Salt mixture and vitamin mixture were formulated according to Hegsted et al., (1941) and Campbell, (1963), respectively.

Experimental Design

Twenty-eight rats weighting approximately (150± 5 g) were kept in standard cages at room temperature (25±3 °C) with a 12 h dark/light cycle. They were left for 7 days as adaptation period and they allowed to feed standard laboratory food and water ad libitum. After the adaptation period, rats divided into four main groups (12 rats of each). Group 1 was fed on basal diet for 4 weeks and served as negative control group. Groups 2- 4 were as the same of -ve control and fed whey protein, amino acids and chia seeds 10

% of each, respectively for 4 weeks. All four groups had the same swimming protocol. The swimming protocol was for 10 min every day during 1st week, 15 min every day during 2nd week, 20 min every day during 3rd week and 25 min every day for 4th week. Swimming tank was manufactured using the method of Khabur et al., (2013).

Rats were weighted weekly and feed intake (FI) was recorded daily all over the experimental period. At the end of the experiment, body weight gain (BWG) and feed efficiency ratio (FER) were calculated according to the method of Chapman et al., (1959). All rats were exposed to the exhaustive swimming test, the time when rats failed to rise to the upper surface of water to breathe and after 7s Jiaoyan et al., (2011), then all experimental rats were sacrificed. Blood samples were collected from each rat and centrifuged at 3000rpm for 15 min to obtain serum for the biochemical analysis.

Serum glucose was determined according to the method described by Trinder, (1969). Serum creatine kinase was determined according to Tietz, (1976). Serum lactate dehydrogenase was determined according to Vassault, (1986). Serum aspartate transaminase, alanine transaminase and Alkaline Phosphates (AST, ALT and ALP) were determined according to Schumann, (2002), Sherwin, (1984) and Belfield and Goldberg, (1971), respectively. Serum ammonia, uric acid concentration, urea nitrogen and creatinine were determined according to Konitzer and Voigt, (1963), Fossati et al., (1980), Patton and Crouch, (1977) and Murray and Kaplan, (1984), respectively. Serum malondialdehyde (MDA) and glutathione-S-transferase (GST) were determined by the method of Ohkawa et al., (1979) and Habig et al., (1974). Data was statistically analyzed using analysis of variance (ANOVA) at p<0.05 according to Sendecor and Cochran, (1986).

3. Results and Discussion

The chemical composition as well as fatty acid content of chia seeds was illustrated in Table (1). The percentage of carbohydrate, protein, lipid, ash and crude fiber in chia seeds were 39.50, 21.00, 30.00, 6.20 and 3.30%, respectively. From these data, it could be observed that, the percent of carbohydrate was high in chia seeds, followed by total lipid and protein, respectively. Chia seeds contains palmitic acid, palmitoleic acid, γ - linolenic acid (GLA) and linolenic acid with values 0.24, 4.81, 25.46 and 69.49%, respectively. It can be observed that chia seeds contain high concentration of linolenic acid followed by γ - linolenic acid.

Valdivia-López and Tecante, (2015) reported that, carbohydrates content for 26–41% of the weight of *S. hispanica* seeds. The seeds contain about 30–34% of dietary fiber, protein makes up 15–25% of the weight of *S. hispanica* seeds. While, Silva et al., (2016) reported that chia seeds contain a high content of fats (30–33%), carbohydrates (26–41%), dietary fiber (18–30%), proteins (15–25%), vitamins, minerals, and antioxidants. These results near this

research obtained findings.

γ -linolenic acid acts in several ways to exert its effects, including the modulation of eicosanoids (PGs, LTs) and cytokines, and by regulating genes that affect apoptosis and cell growth. GLA is functionally EFA because it can correct the symptoms of EFA deficiency so studies have confirmed its anti-inflammatory properties of GLA Rezapour-Firouzi, (2017).

An intact inner-mitochondrial membrane made of the phospholipid, cardiolipin, is required for producing ATP. The optimal functional configuration of cardiolipin is enriched with four linoleic acid side chains. Linoleic Acid enriched cardiolipin provides the scaffold for the electron transport chain proteins to efficiently conduct ATP synthesis. Because humans cannot endogenously synthesize linoleic acid, diet is the sole source of linoleic acid to synthesize 4 linoleoyl-cardiolipin. During oxidative phosphorylation involving the electron transport chain, cardiolipin is remodeled by exchanging oxidized linoleic acid for new non-oxidized linoleic acid molecules. In rodent models, loss of 4-linoleoyl-cardiolipin and accumulation of linoleic acid-poor cardiolipin species in cardiac and skeletal muscles allow proton 'leakage', reduce ATP production and impair cardiac or skeletal muscle function. In addition, linoleic acid levels were associated with lower ectopic lipids in muscle, reduced insulin resistance and risk for type 2 diabetes. Randomized controlled trials that have evaluated the effect of dietary linoleic acid supplementation on cardiometabolic and physiologic outcomes revealed linoleic acid increased lean mass while reducing markers of inflammation and ectopic lipid content of the liver; however, intramuscular triglycerides were not measured. Problematically, older adults have lower levels of serum linoleic acid and intakes might be decreasing as edible oils that were rich in linoleic acid are being replaced with oils containing minimal amounts Martha et al., (2022).

Phenolic components of chia seeds were illustrated in Table (2). The gallic acid, vanillic acid, chlorogenic acid, β -coumaric, hesperidin, myricetin, quercetin, rosmarinic, apigenin and kaempferol content in chia seeds were 0.55, 2.94, 1.68, 1.36, 3.57, 6.75, 142.11, 3.61, 2.30 and 4.73%, respectively. From these data, it could be observed that, the percent of quercetin was high in chia seeds, followed by myricetin and rosmarinic, respectively.

Jiaur et al., (2017) reported that phenolics exist in the soluble and insoluble-bound forms so chia seeds provide a viable source of functional food ingredients or nutraceuticals with protective antioxidant potential, but further research is required to confirm their anti-obesity and anti-diabetic effects. Chemical compounds, such as caffeic acid, ferulic acid, chlorogenic acid, rosmarinic acid, and flavonoids (quercetin, kaempferol, daidzein, etc.) biological activities vary from antioxidant, anti-aging, and anti-hypertensive to anti-cancerogenic and anti-inflammatory Labanca et al., (2017).

The effect of whey protein, amino acids and chia seeds on feed intake (FI), body weight gain (BWG) and feed efficiency ratio (FER) of rats presented in Table (3). Results showed that there was significant decrease in BWG in all groups (2, 3 and 4) were fed on whey protein, amino acids and chia seeds compared to the negative control group with mean values 8.33, 13.33 and 35.00 vs. 42.5g, respectively. Above data show that the highest decrease record in G2 was fed on 10% whey protein followed by G3 & G4 were fed on 10 % amino acids and 10% chia seeds. Respectively. While the results showed significant decrease in FER in all groups (2, 3 and 4) were fed on whey protein, amino acids and chia seeds compared with negative control group which fed on basal diet with mean values 0.017, 0.030 and 0.065 vs. 0.082, respectively. Data show the highest decrease record in G2 was fed on 10 % whey protein followed by G3 & G4 were fed on 10 % amino acids and 10% chia seeds. Respectively. The highest increase in BWG recorded in G4 were fed on 10 % chia seed diet that increase may be cause of that 100g of chia seeds contain 30g of fats & 39g of carbohydrate.

Paige et al., (2014) prove that body weight and body fat were significantly decreased from baseline in the whey protein ratio (WPR) within-group analyses. The effects of WP were more favourable when compared with carbohydrates than protein sources other than whey. Results from the subgroup analyses indicated a statistically significant increase in lean body mass among studies that included a resistance exercise component along with WP provision so this is in parallel with these results.

Fei and Feifan (2022) showed that alterations in dietary essential amino acids intake result in improvements in fat and weight loss in rodents, and each has its distinct mechanism. For example, leucine deprivation increases energy expenditure, reduces food intake and fat mass, primarily through regulation of the general control nonderepressible 2 (GCN2) and mammalian target of rapamycin (mTOR) signaling. Methionine restriction by 80% decreases fat mass and body weight while developing hyperphagia, primarily through fibroblast growth factor 21 (FGF-21) signaling. Some effects of diets with different protein levels on energy homeostasis are mediated by similar mechanisms.

Fabian et al., (2020) reported that there was a significant increase in body weight changes between week I and IV for the group 1 that was fed with fructose/lard and rat pellet only. There was a gradual increase in body weight of the rats fed with chia seeds/extract, however the difference between groups was not significant. These all results were in harmony with this research findings.

The effect of whey protein, amino acids and chia seeds on serum glucose (mg/dl) of rats presented in Table (4). Serum glucose was significant increase in group 2 which fed whey protein compared to the

negative control group with mean value 62.00 vs. 38.00 mg/dL. While it was non-significant decrease in group 3 which fed on amino acids, compared with negative control group with mean values 56.33 vs. 58.33 mg/dL. And recorded significant decrease in group 4 were fed on chia seeds compared with negative control group with mean values 38.00 vs. 58.33 mg/dL. The highest value recorded in (G2) which was fed on whey protein followed by rats fed on amino acids (G3) and chia seeds (G4), respectively.

Maryam et al., (2022) reported that WP decreased postprandial glucose incremental area under the curve (iAUC) and increased iAUCs of insulin and incretin hormones. Whey protein affects glycemic control mainly through stimulating insulin and incretins secretion, slowing gastric emptying, and appetite suppression.

Ryan et al., (2022) showed that both aerobic exercise and whey protein can improve glucose regulation. There were no differences for insulin, C-peptide, glucagon, Gastric inhibitory polypeptide (GIP) and glucagon-like peptide-1 (GLP-1) between trials for the remaining duration of the oral glucose tolerance tests (OGTT). Glucose responses during an oral glucose tolerance test were improved. There were no additional improvements in glucose responses when vigorous-intensity aerobic exercise was combined with whey protein.

Nick et al., (2020) showed that amino acid profiles are significantly altered in individuals with metabolic disorders, specifically obesity, type 2 diabetes and metabolic syndrome. For example, valine, isoleucine, glutamic acid and proline levels increased in these metabolic disorders, while glycine decreased in one study. As mentioned above, these same amino acids are also associated with impaired fasting blood glucose in patients with hypertension. As mentioned above, these amino acids are also elevated in patients with β blocker induced impaired fasting glucose. Branched-chain amino acids provide an interesting association with diabetes.

In animal studies, both, chia seed and chia flour (heat-treated and untreated), reduced plasma glucose in normal Wistar rats after 14 days of treatment. The consumption of chia seeds had improved glucose and insulin tolerance. These results may be associated with the expression of heat shock proteins (HSP70, HSP25) and peroxisome proliferator-activated receptor-g coactivator-1a (PGC-1a) in skeletal muscle. Both of these proteins protect against insulin intolerance, increase control of energy homeostasis and glucose metabolism. Similar results were observed in Wistar rats fed with a high-fat and high-carbohydrate diet and 5% of chia seeds during 8 weeks Mariana et al., (2019) therefore, these data in parallel with current research.

The effect of whey protein, amino acids and chia seeds on creatinine kinase (CK) on rats presented in Table (5). Results showed significant increase in creatinine kinase in all groups (2, 3 & 4) which fed on

whey protein, amino acids and chia seeds compared to the negative control group were fed on basal diet with mean values 106.00, 102.33 and 136.00 vs. 47.00 mg/dL. The highest value recorded in (G 4) was fed on chia seed followed by groups (2 & 3) were fed on whey protein and amino acid, respectively. Zhiqiang et al., (2022) showed that after 8 weeks exercise, the heart rate of group supplemented with whey protein recovered faster, and the exercise indexes showed significant improvement compared with those before the experiment; $P < 0.05$ in the comparison of the performance of push-up, shuttle run and 800 m freestyle between group supplemented with whey protein and group not supplemented. Group supplemented with whey protein had better routine blood indicator values and significantly lower blood lactic acid, creatine kinase, and blood urea nitrogen values than another group after the experiment.

Doha et al., (2022) revealed that dietary supplement rich in omega-3 fatty acids, beta-sitosterol and campesterol were the major phytosterols in chia seeds supplement. Rats fed on the high-fat diet showed significant elevation in inflammatory markers, oxidative stress, dyslipidemia, and cardiac enzymes in association with the elevation of kidney function compared with normal rats. Administration of both doses of dietary supplement significantly improved all the studied biochemical parameters. The high dose of the dietary supplement was promising in the reduction of inflammatory markers, oxidative stress, and improved dyslipidemia in accordance with the reduction of all cardiac enzymes and kidney function.

Fui-Ching et al., (2019) found that whey protein supplement (WPS) has significantly overall increased the level of essential amino acids level and branched-chain amino acids compared to the control group (without WPS). Moreover, was observed to decrease myoglobin level and creatine kinase level compared to the control group.

The effect of whey protein, amino acids and chia seeds on LDH on rats presented in Table (6). Feeding rats on whey protein, amino acids and chia seeds led to significant increase $p < 0.05$ in LDH, as compared to negative group with mean values 187.5, 231.6 and 190 vs. 166.67 mg/dL, respectively. feeding rats in (G 3) with diet contain amino acids recorded the highest significant increase followed by (G 4) were fed on chia seeds then (G 2) were fed on whey protein, respectively.

Wen-chyuan et al., (2014) reported that WP supplementation significantly decreased final body, muscle, liver, brown adipose tissue, and kidney weight and relative weight (%) of muscle, liver, and BAT as well as levels of aspartate aminotransferase, lactate dehydrogenase, creatine kinase, and uric acid. In addition, WP supplementation slightly increased endurance time and significantly increased grip strength and levels of albumin and total protein. WP supplementation improved exercise performance, body composition, and biochemical

assessments in mice and may be an effective ergogenic aid in aerobic exercise training.

Shaimaa and Ibrahim, (2022) reported that dietary supplement of chia seeds was rich in omega-3 fatty acids. Beta-sitosterol and campesterol were the major phytosterols in chia seeds oil dietary supplement. Rats fed on the high-fat diet showed significant elevation in inflammatory markers, oxidative stress, dyslipidemia, and cardiac enzymes in association with the elevation of kidney function compared with normal rats which was in a line with the research findings.

The comparison between the -ve control group, and groups fed with diets contain whey protein, amino acids and chia seeds in the level of aspartate amino transferase AST, alanine amino transferase ALT and alkaline phosphatase ALP on rats, are summarized in Table (7). The results showed that, feeding rats on amino acids led to significant increase $p < 0.05$ in serum ALT enzyme, as compared to the negative control group, which fed on basal diet, with mean value 28.0 vs. 20.3 u/L. While it recorded non-significant increase in group which fed on chia seeds compared with negative control group with mean value 21.2 vs. 20.3 u/L. Group which fed on whey protein showed significant decrease compared to negative group fed on basal diet with mean value 14.75 vs. 20.3 u/L. Feeding rats on chia seeds showed non-significant differ in serum AST compared with negative control group fed on basal diet with mean value 107.67 u/L for both groups. While it showed significant decrease in group 2 & 3 which fed on whey protein & amino acids compared with negative control group fed on basal diet. With mean values 87.5 & 95.67 vs. 107.67 u/L. Diet contains amino acids & chia seeds showed non-significant increase in the mean value of serum ALP enzyme compared with negative control group fed on basal diet with mean values 105.0 & 108.5 vs. 80.0 u/L. while diet contain whey protein showed significant increase compared with negative control group with mean values 85.0 vs. 80.0 u/L that of the negative control group. The highest decrease in serum ALT & AST recorded in (G 2) which fed on whey protein. All groups (2, 3 and 4) showed increase in serum ALP enzyme, as compared to the negative control group. The best results in this parameter recorded for the group fed on whey protein diet.

Emily et al., (2021) reported that a 35 g whey preload would improve insulin sensitivity and glucose handling while reducing biomarkers associated with non-alcoholic fatty liver disease (NAFLD). Alanine aminotransferase (ALT), and aspartate aminotransferase (AST) remained unchanged, but "day" had an effect on the AST:ALT ratio, whereas triglycerides and sex hormone binding globulin overall were greater in the PCOS group. Current results were in agreement with these researches.

Mariana et al., (2019) showed that a total of 20 proteins were cataloged in chia seed, 12 of those were involved in the regular metabolic processes of the plant cells. However, eight proteins were

specifically related to production and storage of plant lipids, thus explaining the high concentration of lipids in chia seeds (around 30%), especially omega-3 fatty acids (around 20%). The analyses of amino acid sequences showed peptides with bioactive potential, including dipeptidyl peptidase-IV inhibitors, angiotensin-converting enzyme inhibitors, and antioxidant capacity. These results correlated with the main health benefits of whole chia seed in humans such as antioxidant capacity, and hypotensive, hypoglycemic, and anticholesterolemic effects. Such relation can be associated with chia protein and peptide compositions and therefore needs further investigation in vitro and in vivo.

Deyang et al., (2021) reported that low-protein diets promote metabolic health in rodents and humans, and the benefits of low-protein diets are recapitulated by specifically reducing dietary levels of the three branched-chain amino acids (BCAAs), leucine, isoleucine, and valine. Here, the research demonstrates that each BCAA has distinct metabolic effects. A low isoleucine diet reprograms liver and adipose metabolism, increasing hepatic insulin sensitivity and ketogenesis and increasing energy expenditure, activating the Fibroblast growth factor 21 (FGF21)-utilizing uncoupling protein 1 (UCP1) axis. Reducing valine induces similar but more modest metabolic effects, whereas these effects are absent with low leucine. Reducing isoleucine or valine rapidly restores metabolic health to diet-induced obese mice. Finally, the research demonstrates that variation in dietary isoleucine levels helps explain body mass index differences in humans. Results reveal that isoleucine as a key regulator of metabolic health and the adverse metabolic response to dietary BCAAs and suggest reducing dietary isoleucine as a new approach to treating and preventing obesity and diabetes.

The effect of whey protein, amino acids and chia seeds on serum ammonia on rats presented in Table (8). Results showed that all groups 2-4 were Fed on whey protein, amino acids and chia seeds led to significant increase $p < 0.05$ in serum ammonia, as compared to negative group fed on basal diet 89.25, 90.00 and 76.50 vs 66.33 umol/L, respectively. From the data group (3) were fed on amino acids recorded the highest increase in serum ammonia followed by group (2) fed on whey protein and group (4) were fed on chia seed, respectively.

Jeddiah and Patrick, (2019) reported that increasing dietary protein intake by 72% resulted in a 59% increase in blood ammonia levels. Simulations of liver cirrhosis increased blood ammonia levels by 41 to 130% depending upon the level of dietary protein intake. Simulations of heterozygous individuals carrying a loss of function allele of the urea cycle carbamoyl phosphate synthetase I (CPS1) gene resulted in more than a tripling of blood ammonia levels. The viability of differentiated SH-SY5Y cells was decreased by 14% by the addition of a slightly higher amount of ammonium chloride so,

research results in harmony with these findings.

Namroud et al., (2008) reported that decreasing dietary crude protein (CP) below 19% depressed performance and appetite and increased fat deposition in the whole body and abdominal cavity significantly. Adding the glycine and glutamine mixtures to low-crude protein diets improved performance and decreased fat deposition. Uric acid, moisture, and acidity of excreta were decreased by reduction of dietary CP; excretory ammonia level was increased in 17% CP diets. Blood ammonia level was increased and plasma uric acid was decreased with reduction of CP to 17%. Supplementing Glycine and Glutamine increased plasma and excretory uric acid level in spite of decreasing blood ammonia concentration. Results in harmony with these findings.

Results in Table (9) illustrate the effect of feeding on whey protein, amino acids and chia seeds on kidney function (serum uric acid, urea nitrogen and creatinine) of rats. Effect of feeding rats on BUN (mg/dL), Results show that group (3) were fed on diet contain amino acids led to significant increase $p < 0.05$ in serum urea nitrogen, as compared to the negative control group fed on basal diet by mean value 39.00 Vs. 22.00 mg/dl. While it led to significant decrease in group (2&4) were fed on whey protein and chia seeds compared with negative control group was fed on basal diet by mean values (20.33 & 17.75 vs. 22.00 mg/dl, respectively. From above data, it shows the highest decrease in serum uric acid recorded in (G 4) fed on chia seeds followed by (G 2) fed on whey protein. Effect on Feeding rats on diet contain whey protein, amino acids and chia seeds on serum creatinine. Results show feeding rats on amino acids led to significant increase $p < 0.05$ in serum creatinine, as compared to the negative control group fed on basal diet 00.99 vs. 00.61 mg/dl. while it causes non-significant increase in group were fed on whey protein compare with negative control group by mean value 00.68 vs. 00.61 mg/dl. Feeding rats on chia seeds led to significant decrease in mean value compared with group were fed on basal diet (negative control group) by value 00.54 vs. 00.61 mg/dl. From above data, it shows the highest increase in serum creatinine found in (G 3) were fed on 10% amino acids followed by (G 2) fed on 10% whey protein compare with negative control group. Effect on Feeding rats on diet contain whey protein, amino acids and chia seeds on uric acid. Results show rats in group 3 were fed on amino acids recorded significant increase in values of serum uric acid compare with negative group were fed on basal diet with mean values 01.45 vs. 00.80 mg/dl, followed by group were fed on whey protein which recorded significant increase in mean values of serum uric acid compare with negative control group were fed on basal diet with mean values 00.96 vs. 00.80 mg/dl. While group were fed on chia seed showed significant decrease in uric acid serum compare with negative control group fed on basal diet with mean

values 00.69 vs. 00.80 mg/dl. respectively. It means that the highest increase in serum uric acid recorded in group 3 were fed on 10% amino acids. Followed by group 2 were fed on diet contain 10% whey protein.

Danielle et al., (2021) showed that proteins, especially plant proteins, may reduce inflammation among adults with chronic kidney disease (CKD). There was a statistically significant decrease when comparing animal proteins to unspecified proteins in C-reactive protein (CRP) levels among dialysis participants, favoring unspecified proteins. Furthermore, animal proteins (eggs, red meat) showed increasing trends in CRP levels compared to whey protein isolate. Caution must be considered regarding these results as controlled, non-randomized, trials were included in the analysis, which may have contributed to high risk of bias. Future research should focus on protein types and the impact they have on kidney disease progression and inflammation markers. This results in agreement with current results.

Results in Table (10) illustrate the effect of feeding on whey protein, amino acids and chia seeds anti-oxidant markers malondialdehyde (MDA), glutathione-S-transferase (GST) of rats. Effect of feeding rats on whey protein, amino acids and chia seeds on MDA. Results show significant decrease in 3 groups (2,3 & 4) were fed on whey protein, amino acids and chia seeds, respectively. compare with negative control group was fed on basal diet with mean values 00.77, 00.78 & 00.59 vs. 00.82 $\mu\text{mol/L}$, respectively. Data above show that the highest decrease in serum MDA recorded in (G 4) was fed on chia seed, followed by (G 2) fed on whey protein, then (G 3) was fed on amino acids, respectively. Effect of feeding rats on whey protein, amino acids and chia seeds on GST (U/L). Results show significant increase in 3 groups (2,3 & 4) were fed on whey protein, amino acids and chia seeds, respectively. compare with negative control group was fed on basal diet with mean values 56.25, 59.67 & 58.25 vs. 48.00 U/L, respectively. Data above show that the highest increase in serum GST recorded in (G 4) was fed on chia seeds, followed by (G 3) fed on amino acids, then (G 2) was fed on whey protein, respectively.

Alberto et al., (2018) reported that dietary antioxidants help the body to fight against free radicals and, therefore, avoid or reduce oxidative stress. Recently, proteins from milk whey liquid have been described as antioxidants. Whey products exhibit radical scavenging activity and reducing power. The antioxidant activity of whey proteins must not only survive processing, but also upper gut transit and arrival in the bloodstream, if whey products are to promote antioxidant levels in target organs. Studies reveal that direct cell exposure to whey samples increases intracellular antioxidants such as glutathione. However, the physiological relevance of these in vitro assays is questionable, and evidence is conflicting from dietary intervention

trials, with both rats and humans, that whey products can boost cellular antioxidant biomarkers. Therefore, these results were in parallel with current findings. Rafaela et al., (2015) reported that rats fed on basal diet; and others fed a high-fat and high-fructose (HFF) diet; chia seeds short (6-weeks) and long (12-weeks) treatments received an HFF diet with chia seed; chia oil short (6-weeks) and long (12-weeks) treatments received an HFF diet with chia oil. HFF diet induced weight gain, oxidative stress and lipid peroxidation in plasma and liver of animals. Compared to HFF group chia seed and chia oil (12 and 6 weeks) intake increased plasma reduced thiol (GSH) levels, plasma catalase (CAT) and glutathione peroxidase (GPx) activities. In the liver glutathione reductase (GRd) activity was enhanced, while CAT

and GPx activities did not change. There were no differences in plasma and liver superoxide dismutase activity among chia diets and HFF group. Chia (seed and oil) intake did not modify liver lipid peroxidation, but was able to reduce plasma thiobarbituric acid reactive substances (TBARS) and 8-isoprostane levels increased by HFF group. Plasma and hepatic antioxidant capacity values were increased in chia seed and oil groups about 35% and 47%, respectively, compared to HFF group. Chia groups presented similar antioxidant potential, regardless of treatment time. Dietary chia seed and oil reduced oxidative stress *in vivo*, since it improved antioxidant status and reduced lipid peroxidation in diet-induced obese rats. Which are these results agree with research findings.

Table (1): Chemical composition and fatty acids of chia seeds (g/100g)

Contents	g/100g	Fatty acid	%
Total carbohydrate	39.50	C16:0(palmitic acid)	0.24
Protein	21.00	C16:1(palmitoleic acid)	4.81
lipids	30.00	C18:3 (γ -linolenic acid)	25.46
Ash	6.20	C18:3(linolenic acid)	69.49
Crude Fiber	3.30	-	-

Table (2): phenolic components of chia seeds (mg/kg)

constituent	mg/100g
Gallic acid	0.55
Vanillic acid	2.94
Chlorogenic acid	1.68
P coumaric	1.36
Hesperidin	3.57
Myricetin	6.75
Quercetin	142.11
Rosemarinic	3.61
Apigenin	2.30
Kaempferol	4.73

Table (3): Effect of whey protein, amino acids and chia seeds on feed intake (FI), body weight gain (BWG) and feed efficiency ratio (FER)

Groups	FI (g/day)	BWG(g)	FER
-ve Control	18.56	42.5 \pm 7.15 ^a	0.082 \pm 0.007 ^a
10% Whey protein	17.42	8.33 \pm 1.06 ^c	0.017 \pm 0.009 ^c
10% Amino Acids	15.66	13.33 \pm 2.11 ^c	0.030 \pm 0.004 ^c
10% Chia seeds	19.24	35.00 \pm 4.75 ^b	0.065 \pm 0.009 ^b

Values are expressed as mean \pm SE.

Values which have different superscript letters in each column significantly differ at $p < 0.05$.

Table (4): Effect of feeding on whey protein, amino acids and chia seeds on serum glucose

groups	Glucose(mg/dL)
Negative control	58.33 \pm 04.91 ^b
10% Whey protein	62.00 \pm 04.02 ^a
10% Amino acids	56.33 \pm 07.31 ^b
10% Chia seeds	38.00 \pm 05.28 ^c

expressed as mean \pm SE.

Values which have different superscript letters in each column significantly differ at $p < 0.05$

Values are

Table (5): Effect of feeding on whey protein, amino acids and chia seeds on creatinine kinase (CK)

groups	CK (mg/dL)
Negative control	47.00 \pm 03.21 ^c
10% Whey protein	106.00 \pm 12.17 ^b
10 % Amino acids	102.33 \pm 04.06 ^b
10 % Chia seeds	136.00 \pm 06.65 ^a

Values are expressed as mean \pm SE.

Values which have different superscript letters in each column significantly differ at $p < 0.05$

Table (6): Effect of feeding on whey protein, Amino Acids and chia seeds on Lactate Dehydrogenase (LDH)

groups	Lactate dehydrogenase (mg/dL)
Negative control	166.67 ± 01.76c
10% Whey protein	187.50 ± 05.98b
10% Amino acids	231.67 ± 17.29a
10% Chia seeds	190.00 ± 04.76b

Values are expressed as mean ± SE.
Values which have different superscript letters in each column significantly differ at p<0.05

Table (7): Effect of feeding on whey protein, Amino Acids and chia seeds on liver enzymes

Groups	ALT(u/L)	AST (u/L)	ALP(u/L)
Negative control	20.33 ± 00.88b	107.67 ± 02.03a	80.00 ± 10.58b
10% Whey protein	14.75 ± 01.65c	87.50 ± 05.33c	85.00 ± 07.93b
10% Amino acids	28.00 ± 03.51a	95.67 ± 05.78b	105.00 ± 08.08a
10% Chia seeds	21.25 ± 01.25b	107.00 ± 08.21a	108.50 ± 04.21a

Values are expressed as mean ± SE.
Values which have different superscript letters in each column significantly differ at p<0.05

Table (8): Effect of feeding on whey protein, Amino Acids and chia seeds on Ammonia

groups	Ammonia (umol/L)
Negative control	66.33 ± 02.91c
10% Whey protein	89.25 ± 07.62a
10% Amino acids	90.00 ± 04.51a
10% Chia seeds	76.50 ± 02.40b

Values are expressed as mean ± SE.
Values which have different superscript letters in each column significantly differ at p<0.05

Table (9): Effect of feeding on whey protein, amino acids and chia seeds on kidney function

Groups	BUN (mg/dl)	Creatinine(mg/dl)	Uric acid (mg/dl)
Negative control	22.00 ± 02.08b	00.61 ± 00.06b	00.80 ± 00.07c
10% Whey protein	20.33 ± 00.85b	00.68 ± 00.03b	00.96 ± 00.13b
10% Amino acids	39.00 ± 05.69a	00.99 ± 00.06a	01.45 ± 00.03a
10% Chia seeds	17.75 ± 02.32c	00.54 ± 00.07c	00.69 ± 00.21d

Values are expressed as mean ± SE.
Values which have different superscript letters in each column significantly differ at p<0.05

Table (10): Effect of feeding on whey protein, Amino Acids and chia seeds on malonaldehyde (MDA) and Glutathione reductase

Groups	MDA (umol/L)	Glutathione reductase (U/L)
Negative control (-ve)	00.82 ± 00.06a	48.00 ± 04.58c
10% Whey protein	00.77 ± 00.02b	56.25 ± 04.89b
10% Amino Acids	00.78 ± 00.10b	59.67 ± 02.03b
10% Chia seeds	00.59 ± 00.03c	68.25 ± 02.87a

Values are expressed as mean ± SE.
Values which have different superscript letters in each column significantly differ at p<0.05

References

A.O.A.C. (2012): Association of Official Analytical Chemistry (A.O.A.C.) International, 19th ed., Gaithersburg, Maryland, USA.

Alberto R., Vitaly B., Phil M. and Linda G. (2018): Invited review: Whey proteins as antioxidants and promoters of cellular antioxidant pathways, *Journal of Dairy Science*; 101: 4747-4761.

Belfield A. and Goldberg D. (1971): Normal Ranges and Diagnostic Value of Serum 5'Nucleotidase and Alkaline Phosphatase Activities in Infancy. *Arch Dis Child*; 46:842-846.

Brand W., Cuvelier M. and Berset C. (1995): Use of a free-radical method to evaluate antioxidant activity. *LWT Food Sci. Techno.* 28; 25-30.

Campbell J. (1963): Evaluation of protein quality. National academy of science National research council publication, Washington, D.C. 1100, 31.

Chapman G., Gastilla R. and Campbell A. (1959): Evaluation of Protein in foods: 1- A Method for the determination of protein efficiency ratio. *Can. J. Biochem. Phys*; 37:679- 86.

Danielle F., Sofia A., Lucia E. and Jeanette M. (2021): Influence of Plant and Animal Proteins on Inflammation Markers among Adults with Chronic Kidney Disease: A Systematic Review and Meta-Analysis, *Nutrients*, 13(5), 1660.

Deyang Y., Nicole E., Cara L., Alexandra B., Michaela E. and Victoria F. (2021): The adverse metabolic effects of branched-chain amino acids are mediated by isoleucine and valine, cell metabolism,

33: 905-922.

Doha M., Shaimaa M., and Ibrahim H. (2022): Chia seeds oil enriched with phytosterols and mucilage as a cardioprotective dietary supplement towards inflammation, oxidative stress, and dyslipidaemia. *J Herbmед Pharmacol.* 11: 83-90.

Emily L., Manisha R., Shenavia B., Shane B. and Monique J. (2021): Whey Protein

Supplementation improves the glycaemic response and may reduce non-alcoholic fatty liver disease related biomarkers in women with polycystic ovary syndrome (PCOS). *Nutrients* 2021, 13(7), 2451.

Fabian D., Beatrice N., Andrew N. and Judith K. (2020): Effect of chia seeds (*Salvia hispanica*) on postprandial glycaemia, body weight and hematological parameters in rats fed a high fat and fructose diet, *Int. J. Biol. Chem. Sci.* 14(5): 1752-1762.

Fei x. and Feifan G. (2022): Impacts of essential amino acids on energy balance, *Molecular Metabolism*, 57, 101393.

Fossati P., Prencipe L. and Berti G. (1980): Enzymatic colorimetric method of determination of uric acid in serum. *Clin. Chem.* 26 (2): 227-273

Fui-Ching L., Tahir M., Hani F., Abdul H. and Amer H. (2019): Effectiveness of whey protein supplements on the serum levels of amino acid, creatinine kinase and myoglobin of athletes: a systematic review and meta-analysis. *Reviews* volume 8, Article number: 130.

Goupy P., Hugues M., Biovin P. and Amiot M. (1999): Antioxidant, composition and activity of barley (*Hordeum vulgare*) and malt extracts and of isolated phenolic compounds. *J. Sci. Food Agric.*, 79(12): 1625-1634.

Habig W., Michael J. and William B. (1974): Glutathione S-Transferases: The first enzymatic step in mercapturic acid formation, *Journal of biological chemistry*, 249:7130-7139.

Hegsted M., Mills C., Elvehjem C. and Hart E. (1941): Choline in the nutrition of chicks. *J. Biol. Chem.*, 138: 459-470.

Jeddiah W. and Patrick C. (2019): Effects of a high protein diet and liver disease in an in-silico model of human ammonia metabolism, *Theoretical Biology and Medical Modelling*:16, Article number: 11.

Jiaoyan R., Mouming Z., Haiyan W. and Lijun Y. (2011): Effects of supplementation with grass carp protein versus peptide on swimming endurance in mice, *J. of Nutrition*, 27:789-95.

Jiaur R., Adriano C. and Fereidoon S. (2017): Phenolic and polyphenolic profiles of chia seeds and their in vitro biological activities, *Journal of Functional Foods*, 35:622-634.

Khabour F., Alzoubi H., Alomari A., and Alzubi A. (2013): Changes in spatial memory and BDNF expression to simultaneous dietary restriction and forced exercise. *Brain Research Bulletin*, 90, 19–24

Konitzer K. and Voigt S. (1963): Direct determination of ammonia in blood and tissue

extracts

by means of the phenol by chlorite reaction *clin chem. Acta*,8,5.

Labanca R., Svelander C., Eliasson L., Linhares R., Araújo B., Ahrné L. and Alminger M.

(2017): Supercritical carbon dioxide extraction and conventional extraction of chia seed oils: chemical composition and lipid oxidation. *Int. J. Res.* Available 2017, 4, 563–572.

Mahmoud A., Shaaban S., Mayada R., Ruchi T., Iqbal Y., Kumar K., and Izabela M. (2021):

Nutritional significance of amino acids, vitamins and minerals as nutraceuticals in poultry production and health—a comprehensive review. *Veterinary quarterly*, 41:1–29.

Mariana G., Hercia S. and Elvira G. (2019): Chia seed (*Salvia hispanica* L.) as a source of proteins and bioactive peptides with health benefits: a review, *Comprehensive Reviews in Food Science and Food Safety*, 18, 2.

Martha A., Brian C., Rayan M. and Peggy M. (2022): Linoleic Acid Intake and Physical

Function: Pilot Results from the Health ABC Energy Expenditure Sub-Study, *Adv Geriatr Med Res.* 4(1):1-15.

Maryam N., Bahram P., Pedram T. and Ali T. (2022): A systematic review of whey protein supplementation effects on human glycemic control: A mechanistic insight. *Diabetes & Metabolic Syndrome: Clinical Research & Reviews.* 16, 7.

Murray R. and Kaplan A. (1984): *Clin chem. The C.V. Mosby Co. St louis. Toronto. Princeton;* 1261-1266.

Namroud N., Shivazad M. and Zaghari M. (2008): Effects of Fortifying Low Crude Protein

diet with crystalline amino acids on performance, blood ammonia level, and excreta characteristics of broiler chicks, *Poultry Science*, 87, 11: 2250-2251.

Nick E., Max H. and Jace T. (2020): Amino acids in health and endocrine function, part of the advances in *Experimental Medicine and Biology* book series (AEMB, 1265).

Ohkawa H., Ohishi W. and Yagi K. (1979): Assay for lipid peroxidase in animal tissues by thiobarbituric acid reaction *biochem.* 95, 351.

Paige E., Dominik D. and Vanessa P. (2014): Effects of whey protein and resistance exercise on body composition: a meta-analysis of randomized controlled trials, *J Am Coll Nutr.*, 2014;33(2):163-75.

Patton J. and Crouch R. (1977): Enzymatic colorimetric method to determination urea in serum. *Anal. Chem.*, 49: 464.

Rafaela M., Sabrina A., Érica A. and Mário J. (2015): Antioxidant potential of dietary chia seed and oil (*Salvia hispanica* L.) in diet-induced obese rats, *Food Res Int*;76(Pt 3):666-674.

Rezapour-Firouzi S. (2017): Herbal Oil Supplement with Hot-Nature Diet for Multiple Sclerosis in Nutrition and Lifestyle in Neurological Autoimmune Diseases.

Ryan A., Emily Z., Todd J., Matthew L., Matthew F., Christopher J., Anthony A. and Vic B.

(2022): Whey protein improves glycemia during an oral glucose tolerance test compared to vigorous-intensity aerobic exercise in young adult men, *BMC Sports Science, Medicine and Rehabilitation*; 14, 147.

Reeves P. Nielsen F. and Fahmy G. (1993): AIN-93. Purified diets for laboratory rodents: Final reports of the American Institute of Nutrition ad hoc writing committee of reformulation of the AIN-76 A Rodent Diet. *J. Nutr.* 123:1939-51.

Serena B., Peter s., Kanishka N., John F. and Paul D. (2023): The 'Whey' to good health: Whey protein and its beneficial effect on metabolism, gut microbiota and mental health, *Trends in Food Science & Technology*.

Schumann G. (2002): IFCC reference procedure for aspartate aminotransferase; *CCLM.* 40(7): 725-733.

Sendecor, G. and Cochran, W. (1986): *Statistical methods* 7th ed; Iowa state university press, Ames, USA, 90.

Shaimaa M. and Ibrahim H. (2022): Chia seeds oil enriched with phytosterols and mucilage as a cardioprotective dietary supplement towards inflammation, oxidative stress, and dyslipidemia, *J Herbm Pharm.*;11: 83-90.

Sherwin J. (1984): Liver function. In: Kaplan LA, PESCE AJ, eds. *Clinical chemistry, theory, analysis, and correlation*. St louis: mosby: 420-438.

Sigal B., Alon E., Dan N., Leonid K., and Yoav M. (2022): Genetic characteristics of competitive swimmers: a review, *Biol Sport.* 39(1):157–170.

Silva C., Garcia V. and Zanette C. (2016): Chia (*Salvia hispanica* L.) oil extraction using different organic solvents: Oil yield, fatty acids profile and technological analysis of defatted meal. *Int. Food Res. J.*, 23, 998–1004.

Tabeen K., Tabasum F., Tahiya Q., Asima R., Ajaz M., Bazila N., and Syed Z. (2023): Biochemical, nutraceutical and phytochemical characterization of chia and basil seeds: A comparative study, *international journal of food properties*, 26, 1, 1–13.

Tietz N. (1976): *Fundamentals of clinical chemistry*. 2nd ed W.B Saunders Philadelphia.

Trinder, P. (1969): Determination of blood glucose using 4- aminophenazone. *J. Clin. Path.*, 22: 246.

Valdivia-López M. and Tecante A. (2015): Chia (*Salvia hispanica*): A Review of Native Mexican Seed and its Nutritional and Functional Properties. *Adv. Food Nutr. Res.* 75, 53–75.

Vassault A. (1986): *Ann. Biol. clin.*, 44,686.

Wen-chyuan C., Wen-ching H., Chien-chao C., Yukai C. and Chi-chang H. (2014): Whey Protein Improves Exercise Performance and Biochemical Profiles in Trained Mice, *Med Sci Sports Exerc.* 46(8): 1517–1524.

Zhiqiang C., Xueming L. and Xupeng P. (2022): Effects of whey protein supplementation on exercise capacity and fatigue resistance.

Medicina dello Sport;75(2):218-26.