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Comparative Study among Whey Protein, AminoAcids andChia Seeds on Enhancing thePerformance of Endurance Sports of Rats

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Abstract

The present work was conducted to study the comparative among whey protein, amino acids and chia seedsinenhancing the performance of endurance sports in rats.The 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 group. Groups from 2-4 were fed on a basal diet containing 10% whey protein, amino acids and chia seeds, 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 carbohydrates followed by fats and protein with values of 39.5, 30 and 21, respectively. Chia seeds fatty acid contains the highest value of linoleic acid followed by γ-linolenic acid. Feeding rats led to a significant increase in creatinine kinase, lactate dehydrogenase, serumalkaline phosphatase (ALP), ammonia, and glutathione-S-transferase (GST) in all tested groups compared withthe control group.

While there wasa significant decrease in serum glucose and Malondialdehyde in tested groups. There wasa significant decrease in serum aspartate aminotransferase (AST) in groups fed on whey protein and amino acids,while was no difference in group was fed on chia seeds. There was a significant decrease in creatinine and uric acid in the group that was fed on chia seeds, results recorded significant increase in serum alanine transaminase in the groups fed on amino acids and chia seeds, while it records a significant decrease in group fed on whey protein. The highest increase in serum creatinine recorded in the group was 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.

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

Introduction

Asuccessful 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 ***Sigalet 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 Nahuati 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 ***Tabeenet al.,(2023).***Therefore,this study was carried out to investigate the comparative among whey protein, amino acids and chia seeds supplementation on endurance sports in rats.

Materials and Methods

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.

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)***andDPPH radical-scavenging activity assayed according to the method of***Brand et al., (1995).***

Preparation of the 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:

Forty-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 on diet containing whey protein, amino acids and chia seeds10 % 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 ***Khaburet 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***Jiaoyanet 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 methoddes cribed by***Trinder,(1969).***Serum creatine kinase was determined according to ***Tietz,(1976).***Serum lactatedehydrogenase was determined according to ***Vassault,(1986).***Serum aspartate transaminase, alanine transaminase and Alkaline Phosphates(AST,ALTandALP) were determined according to***Schumann,(2002), Sherwin, (1984)and Belfield* and *Goldberg, (1971),***respectively**.** Serum ammonia, uric acid concentration, urea nitrogen and creatin-inewere determined according to ***Konitzer and Voigt,(1963), Fossatietal.,(1980), Patton and Crouch, (1977) and Murray and***

Kaplan, (1984),respectivel.Serum malondialdehyde (MDA) and glutathione-S-transferase (GST) were determined by the method of ***Ohkawa etal., (1979) and Habiget al.,(1974).***

Data was statistically analyzed using analysis of variance (ANOVA) at $p<0.05$ according to ***Sendecor and Cochran, (1986).***

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 sostudies 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, rosemarinic, 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, kaempferol and rosemarinic, respectively**.*Jiauret al., (2017*)**reported that phenolics exist in the soluble and insoluble-bound forms sochia 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***Labancaet 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 tested groups (2, 3 and 4) which were fed on diet contain whey protein, amino acids and chia seeds as compared to the 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 which treated with 10% whey protein followed by G3 & G4 which fed on a diet containing 10 % amino acids and 10% chia seeds. Respectively. While the results showed significant decrease in FER in all tested groups (2, 3 and 4) which were fed on diet containing whey protein, amino acids and chia seeds compared with the 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 which were fed on a diet containing 10% amino acids and 10% chia seeds. respectively.The highest increase in BWG recorded in G4 were fed on 10 % chia seed diet.

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 isin 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 on a diet containing whey protein compared to the control group with mean value 62.00 vs.58.33 mg/dL. While it was non- significant change in group 3 which fed on amino acids, compared with the control group with mean values 56.33 vs. 58.33 mg/dL. On the other hand, group 4 which was fed on diet containing chia seeds recorded significant decrease in the mean values of serum glucose compared with control group with mean values 38.00 vs. 58.33 mg/dL.The highest value recorded in (G2) which was fed on diet containing whey protein followed by rats fed on amino acids (G3) and chia seeds (G4), respectively. ***Maryam et al., (2022)***reported that whey protein (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, and 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 for 8 weeks ***Mariana et al.,(2019)***therefore**,** these data in parallel with current research.

The effect of whey protein, amino acids and chia seeds.Oncreatinine kinase(CK)in rats is presented in Table (5). Results showed a 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 diet containing chia seed followed by groups (2 & 3) were fed on diet containing whey protein and amino acid, respectively. ***Zhiqianget al., (2022)*** showed that after 8 weeks of exercise, the heart rate of the 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, The 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 supplements rich in omega-3 fatty acids, beta-sitosterol and campesterol were the major phytosterols in chia seeds supplements. 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 thatwhey protein supplement(WPS)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 levels compared to the control group.

The effect of whey protein, amino acids and chia seeds on LDH in rats is 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 the control group with mean values 187.5, 231.6 and 190 vs. 166.67 mg/dL, respectively. feeding rats in (G 3) with diet containing 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-chyuanet 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 thedietary supplement of chia seeds was rich in omega-3 fatty acids. Beta-sitosterol and campesterol were the major phytosterols in chia seeds oil dietary supplements. 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 whichwere in a line with the research findings.The comparison between the control group, and groups fed with diets containing whey protein, amino acids and chia seeds in the level of aspartate amino transferaseAST, alanine amino transferaseALT and alkaline phosphatase ALP inrats, are summarized in Table (7). The results showed that, feeding rats on amino acids led to a significant increase $p<0.05$ in serum ALT enzyme, as compared to the control group, which fed on a basal diet, with a mean value 28.0 vs. 20.3 u/L. While it recorded a non-significant increase in the group that fed on chia seeds compared with the control group with a mean value 21.2 vs. 20.3 u/L. the group fed on whey protein showed a significant decrease compared to the control group fed on the basal diet with mean value 14.75 vs. 20.3 u/L.Feeding rats on chia seeds showed non-significant differences in serum AST compared with the control group fed on a basal diet witha 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 a diet contains whey protein showed a significant increase compared with the 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 an increase in serum ALP enzyme, as compared to the negative control group. The best results in this parameter were recorded for the group fed on a 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 withnon-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 our findings.***Mariana et al., (2019)***showed that a total of 20 proteins were cataloged in chia seed, 12 of which were involved in the regular metabolic processes of the plant cells. However, eight proteins were specifically related to the 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.

Deyanget 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 in 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 is 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 in rats is presented in Table (8). Results showed that all groups 2-4 were Fed on whey protein, amino acids and chia seeds led to a significant increase $p<0.05$ in serum ammonia, as compared to the control group fed on basal diet 89.25,90.00 and 76.50 vs 66.33umol/L, respectively.From the data group (3) was fed on amino acids recorded the highest increase in serum ammonia followed by group (2) fed on whey protein and group (4) which fed on a diet containing chia seed, respectively. ***Jeddidiahand 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 chlorideso, research results are in harmony with these findings.***Namroudet 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 the 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 a reduction of CP to 17%. Supplementing Glycine and Glutamine increased plasma and excretory uric acid level in spite of decreasing blood ammonia concentration.Resultsin 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) was fed on a diet containing amino acids led to a significant increase $p<0.05$ in serum urea nitrogen, as compared to the control group fed on a basal diet by mean value 39.00 Vs. 22.00 mg/dl. While it led to a significant decrease in groups (2&4) that were fed on diet containing whey protein and chia seeds compared with the 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 containing 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 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 the 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 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 the group fed on chia seed showed a significant decrease in uric acid serum compare with the 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 was fed on 10% amino acids. Followed by group 2 was fed on a diet containing 10% whey protein.

Danielle et al., (2021)showed thatproteins, 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 the 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 onwhey protein, amino acids and chia seeds on MDA. Results show significant decrease in 3 groups (2,3& 4) fed on diet containing whey protein, amino acids and chia seeds, respectively. compare with the control group was fed on basal diet with mean values 00.77, 00.78& 00.59 vs. 00.82umol/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 onwhey protein, amino acids and chia seeds on GST (U/L). Results show significant increase in 3 groups (2,3& 4) that were fed on whey protein, amino acids and chia seeds, respectively, as compared with the control group was fed on a 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 thatdietary 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, withboth rats and humans, that whey products can boost cellular antioxidant biomarkers.Therefore,these results werein parallel with current findings.***Rafaela et al., (2015)*** reported that rats fed on a 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 theHFF 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 by 35% and 47%, respectively, compared to the 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 arethese resultsagree with the 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-linoleic 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
Chromogenic 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
Control	18.56	42.5 ± 7.15^{a}	0.082 ± 0.007^{a}
10% Whey protein	17.42	8.33 ± 1.06^{c}	0.017 ± 0.009^{c}
10% Amino Acids	15.66	13.33 ± 2.11^{c}	0.030 ± 0.004^{c}
10% Chia seeds	19.24	35.00 ± 4.75^{b}	0.065 ± 0.009^{b}

Values are expressed as mean ± 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)
Control	58.33 ± 04.91^{b}
10% Whey protein	62.00 ± 04.02^{a}
10% Amino acids	56.33 ± 07.31^{b}
10% Chia seeds	38.00 ± 05.28^{c}

Values are expressed as mean ± SE.

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

Table (5):

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

Groups	CK (mg/dL)
Control	47.00 ± 03.21[c]
10% Whey protein	106.00 ± 12.17[b]
10 % Amino acids	102.33 ± 04.06[b]
10 % Chia seeds	136.00 ± 06.65[a]

Values are expressed as mean ± 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)
Control	166.67 ± 01.76[c]
10% Whey protein	187.50 ± 05.98[b]
10% Amino acids	231.67 ± 17.29[a]
10% Chia seeds	190.00 ± 04.76[b]

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)
Control	20.33±00.88^{b}	107.67±02.03^{a}	80.00 ± 10.58^{b}
10% Whey protein	14.75±01.65^{c}	87.50 ± 05.33^{c}	85.00 ± 07.93^{b}
10% Amino acids	28.00±03.51^{a}	95.67 ± 05.78^{b}	105.00± 08.08^{a}
10% Chia seeds	21.25±01.25^{b}	107.00±08.21^{a}	108.50± 04.21^{a}

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)
Control	66.33 ± 02.91^{c}
10% Whey protein	89.25 ± 07.62^{a}
10% Amino acids	90.00 ± 04.51^{a}
10% Chia seeds	76.50 ± 02.40^{b}

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)
Control	22.00±02.08^{b}	00.61± 00.06^{b}	00.80 ± 00.07^{c}
10% Wheyprotein	20.33±00.85^{b}	00.68 ± 00.03^{b}	00.96± 00.13^{b}
10%Amino acids	39.00±05.69^{a}	00.99 ± 00.06^{a}	01.45 ± 00.03^{a}
10% Chia seeds	17.75±02.32^{c}	00.54 ± 00.07^{c}	00.69 ± 00.21^{d}

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)
Control	00.82± 00.06^{a}	48.00 ± 04.58^{c}
10% Whey protein	00.77± 00.02^{b}	56.25 ± 04.89^{b}
10% Amino Acids	00.78 ± 00.10^{b}	59.67 ± 02.03^{b}
10% Chia seeds	00.59± 00.03^{c}	68.25 ± 02.87^{a}

Values are expressed as mean ± SE.

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

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دراسة مقارنة بين بروتين شرش اللبن والأحماض الأمينية وبذور الشيا لتحسين أداء رياضات التحمل للفئران

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الملخص العربى

أجريت هذه الدراسةللمقارنة بين بروتين شرش اللبن ومكملات الأحماض الأمينية وبذور الشيا على تعزيز أداء رياضات التحمل في الفئران. تم تقسيم 48 فأر ألبينو (Sprague Dawley Strain)(150 ± 5 جم) إلى أربع مجموعات (12فأر لكل مجموعة). المجموعة الرئيسية الأولى تغذت على الغذاء الأساسي كمجموعة ضابطة سالبة لدة 28 يوما. تم تغذية باقي المجموعات من المجموعة الثانية الي الرابعة على الغذاء الأساسي مضاف له 10٪ من بروتين شرش اللبن ومكملات الأحماض الأمينية وبذور الشيا على التوالي لمدة 28 يومًا. تم تطبيق بروتوكول مماثل للسباحة على الأربع مجموعات لمدة 28 يومًا.، وتم تقدير التركيب الكيميائي لبذور الشيا والاحماض الدهنية الموجودة بها. أظهرت النتائج أن أعلى محتوى من المكونات كان الكربوهيدرات تليها الدهون ثم البروتين بقيم 39.5 و30 و21 على التوالي وكذلك احتوت بذور الشيا على حمض اللينوليك يليه حمض جاما لينوليك كأكبر نسب موجودة بالبذور.أدت تغذية الفئران في الثلاث مجموعات المختبرة على بروتين شرش اللبن، الاحماض الامينية وبذور الشيا على التوالي، إلى زيادة معنوية في creatine kinase،lactate dehydrogenase،ALP، ammonia, GSTمقارنة بالمجموعة الضابطة السالبة. بينما أدت تغذية مجموعتي الاحماض الامينية وبذور الشيا الي انخفاض معنوي في نسبة الجلوكوز في الدم مقارنة بالمجموعة الضابطة السالبة. كان هناك انخفاض معنوي في مصل (AST)في المجموعات التي تم تغذيتها على بروتين شرش اللبنوالأحماض الأمينية. بينما لم يكن هناك اختلاف في المجموعة التي تم تغذيتها على بذور الشيا. بينما أحدث انخفاض معنوي في creatinine وuric acidفي المجموعة التي تم تغذيتها على بذور الشيا، وسجلت النتائج زيادة معنوية في مصل Alanine Transaminase في مجموعات تم تغذيتها على الأحماض الأمينية وبذور الشيا،وسجلت انخفاضًا معنويًا في المجموعة التي تم تغذيتها على بروتين شرش اللبنمقارنة بالمجموعة الضابطة السالبة. تم تسجيل اعلىنسبة لمصل الكرياتين في المجموعة التي تم تغذيتها على الأحماض الأمينية مقارنة بالمجموعة الضابطة السالبة. أظهرت النتائج انخفاض معنوي في MDA في جميع المجموعات المختبرة مقارنة بالمجموعة الضابطة السالبة. وفقًا للنتائج، فإن بذور الشيا لها تأثير مفيد في تعزيز أداء رياضات التحمل في الفئران والتي يمكن استخدامها مع الرياضيين.

الكلمات المفتاحية: بذور الشيا، الأحماض الأمينية، بروتين مصل اللبن، ورياضة التحمل.