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Research Article

Bioefficacy and Safety Assessment of Protein Isolates Obtained from Nonconventional Sources

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Nonconventional protein isolates were prepared from defatted oilseeds (sesame, flaxseed, and canola). Bioevaluation was performed via growth study parameters i.e., protein efficiency ratio (PER), net protein ratio (NPR), and relative net protein ratio (RNPR). The highest values for these parameters were recorded in sesame protein isolates (SPIs) followed by canola protein isolates (CPIs) and flaxseed protein isolates (FPIs). Nitrogen balance study parameters represented maximum true digestibility (TD) in SPI trailed by FPI and CPI. However, biological value (BV) was found higher in FPI. Similarly, the highest value for net protein utilization (NPU) was noticed in FPI tracked by SPI and CPI. Moreover, safety assessment of protein isolates was also performed including serum protein and kidney and liver function tests. All these parameters showed nonsignificant variations among the tested protein isolates. The outcomes of research explicated that these protein isolates can play a pivotal role to increase the protein level of individuals.

1. Introduction

High quality proteins play an imperative role in maintaining better health of an individual. Purposely, the proteins obtained from animal sources are of high quality as compared to plant sources [1]. Though animal proteins exhibit high quality; nevertheless, they are more expensive than vegetable proteins. Owing to high cost and comparative dearth of food with animal proteins, it has become inevitable to find some new sources of better quality protein [2]. Additionally, increasing cost and insufficient provision of animal proteins have diverted the interest of researchers towards high protein oilseeds. These nonconventional protein sources also hold interactive properties with other food components such as water and lipids [3].

The sesame (*Sesamum indicum* L.) is an imperative oilseed crop that belongs to the *Pedaliaceae* family mostly cultivated in tropical areas. Globally, it is commonly known as beniseed, gingelly, sim sim, and til. The chemical composition of sesame seed revealed that it contains 25.8–26.9% protein, 2.50–3.90% fiber, 2.00–5.59% ash, and 10.10-17.90% carbohydrate [4]. The flaxseed (*Linum usitatissimum*) belonging to *Linaceae* family is commonly known as "Alsi" in Indopak. Generally, flaxseeds comprised of about 7.7% moisture, 20% protein, 41% fat, 28% fiber, and 3.4% ash [5]. Canola (*Brassica napus* L.) is an oilseed crop that was extensively cultivated in Canada and is currently being grown throughout the world including the Indo-Pak subcontinent. The defatted canola meal contains about 35-36 g/100 g protein as well as 12 g/100 g crude fiber contents along with some important minerals and vitamins [6].

The bioavailability of proteins depends on processing technology. According to Sá et al. [2], bioavailability of protein is evaluated by growth as well as nitrogen balance studies. Numerous foods are analyzed through *in vivo* assay to assess their ability for growth and usual metabolic activity. The protein efficiency elucidates the quality of protein destined for human consumption particularly growing infants and children [7].

The nutritional potential of protein isolates plays a vital role in growth and maintenance of body especially in infants and young children. In various research studies, rat bioassay has been used for efficacy purpose. Bioevaluation of protein encompasses the parameters such as protein efficiency ratio (PER), net protein ratio (NPR), and relative net protein ratio (RNPR) whereas nitrogen balance study comprises of biological value (BV), net protein utilization (NPU), and true digestibility (TD) [8]. Accordingly, food consumed by experimental animals differs with the protein level in the diet and the amount needed for physiological and metabolic functions. The utilization of protein isolates as food ingredients in the manufacturing of different food products requires prior safety assessment [9]. For this purpose, the researchers in this study focused on experimental rats that are fed on test diets containing the protein isolates and later these animals are subjected to serum protein analysis as well as kidney and liver function tests with the aim to determine the safety levels of protein isolates under study. The hypothesis being tested in this study was that the protein isolates are safe to be utilized for human consumption with no adverse health effects.

2. Materials and Methods

2.1. Preparation of Raw Material. Oilseeds i.e., sesame (TS-5), flaxseed (Chandni), and canola (Faisal canola) were procured from Ayub Agriculture Research Institute (AARI), Faisalabad, Pakistan. The seeds were initially cleaned and then grounded to fine powder. Moreover, the conventional solvent (hexane) method was employed to extract oil from the oil seeds using the Soxtec system (Model: H-2 1045 Extraction Unit, Hoganas, Sweden) [10]. Resulting defatted oilseeds were dried and stored for further processing.

2.2. Protein Isolates Preparation. For the preparation of protein isolates, defatted oilseeds were dissolved in distilled water (1/10) with pH 9.5. Furthermore, centrifugation was carried out at 4000 rpm for 20 min to separate the supernatant. Afterwards, the pH of the collected supernatant was set at 4.5 following recentrifugation, neutralization, and freeze drying [6].

2.3. Bioevaluation Protocols. Bioevaluation of oilseed protein isolates was conducted by feeding respective diets to different groups of Sprague–Dawley rats. The study was approved by the Directorate of Graduate Studies of the University of Agriculture, Faisalabad, Pakistan. The control diets consisted of soy, casein, and no protein diet (Table 1). Commercial soy protein isolate and casein had protein content 93.48% and 96.35%, respectively. The diets were made isonitrogenous by maintaining the protein content at 10% level. Likewise, vitamins and minerals mixtures were added. The nitrogen-free mixture was comprised of sucrose, cellulose, and corn starch.

Table 1: Experimental diets.

Diet constituents (g)	Diets				
	SPI	FPI	CPI	Soy	Casein
Sesame protein isolates	11.09	—	—	—	—
Flaxseed protein isolates	—	11.58	—	—	—
Canola protein isolates	—	—	11.14	—	—
Soy protein	—	—	—	10.69	—
Casein	—	—	—	—	10.37
Corn oil	5.0	5.0	5.0	5.0	5.0
Mineral mixture	5.0	5.0	5.0	5.0	5.0
Vitamin mixture	1.0	1.0	1.0	1.0	1.0
N-free mixture	77.91	77.42	77.86	78.31	78.63
Total diet weight (g)	100	100	100	100	100

2.4. Housing of Rats. For bioevaluation study, thirty male Sprague–Dawley rats with uniform weight and size were housed in the Animal Room of NIFSAT, UAF. Initially, rats ($n = 5$) were divided into six groups. The rats were given respective diets for 10 days. The temperature (23 ± 2°C) and humidity (50 ± 5%) were maintained throughout the experimental period. The spilled diet and feces were collected on daily basis. At the termination of trial, overnight fasted rats were decapitated using sharp knife, and their bodies were allowed to dry till constant weight by placing in a hot air oven (105°C). The dried carcass was grinded, and its nitrogen content was estimated [11].

2.5. Feed Intake and Body Weight Gain. Feed intake of experimental rats was calculated on daily basis by eliminating spilled diet from the total diet consumed during the entire experimental period. Gain in body weight of each group was calculated for growth index with regard to respective diet [12].

2.6. Protein Quality Evaluation. Net feed intake and body weight gain were employed to determine growth study parameters such as protein efficiency ratio (PER), net protein ratio (NPR), and relative net protein ratio (RNPR). The spilled diet, feces, urinary outputs, and dried rat bodies were analyzed for nitrogen content to estimate nitrogen balance study parameters such as true digestibility (TD), biological value (BV), and net protein utilization (NPU) [13].

2.7. Safety Assessment. Safety assessment was conducted via serum protein as well as kidney and liver function tests.

2.7.1. Serum Protein. Serum total protein, albumin, globulin, and albumin/globulin ratio were estimated by following the procedures described by [14].

2.7.2. Liver and Kidney Functioning Tests. The sera of rats were subjected to liver function tests via enzymatic evaluation i.e., alanine aminotransferase (ALT), aspartate amino transferase (AST), and alkaline phosphatase (ALP). Moreover, for kidney functioning tests, the GLDH method was used to analyze the serum urea while creatinine was estimated by following the Jaffe-method using commercial kits [15].

2.8. Statistical Analysis. The collected data were statistically analyzed using Statistical Package (Costat-2003, CoHort, v6.1.). Accordingly, the level of significance was estimated by analysis of variance (ANOVA) using completely randomized design (CRD) followed by a least significant difference (LSD) test for post hoc comparison of means [16].

3. Results

3.1. Bio-Efficacy Study. The protein quality of defatted oilseed protein isolates was assessed through bio-efficacy trial. In this context, Sprague–Dawley rats were used as test animals and soy and casein as reference diets. The growth study parameters comprised of protein efficiency ratio (PER), net protein ratio (NPR), and relative net protein ratio (RNPR). Whilst, nitrogen balance study parameters consist of true digestibility (TD), biological value (BV), and net protein utilization (NPU).

3.2. Growth Study Parameters

3.2.1. Protein Efficiency Ratio (PER). Statistical analysis revealed significant difference between the groups with respect to PER consuming different protein rich diets. The present results revealed that the highest PER was observed for the SPI diet (2.14 ± 0.10) trailed by CPI (2.09 ± 0.06) while, minimum PER was recorded for the FPI diet (1.98 ± 0.07). Nonetheless, reference diets exhibited PER values as 2.74 ± 0.12 (casein) and 2.52 ± 0.09 (soy) (Table 2).

3.2.2. Net Protein Ratio (NPR). The results of the present study regarding net protein ratio (NPR) showed significant difference in NPR. It was observed that SPI-based diet exhibited the highest NPR (5.03 ± 0.26) followed by CPI (4.98 ± 0.11) and FPI (4.67 ± 0.13). However, the reference protein diets i.e., soy and casein exhibited NPR values 5.52 ± 0.13 and 5.93 ± 0.19, respectively (Table 2).

3.2.3. Relative Net Protein Ratio (RNPR). The RNPR was calculated via reference protein (casein as 100). RNPR is a ratio of NPR values of oilseed protein isolates and the standard protein. The means for RNPR of test diets ranged from 78.75 for flaxseed protein isolates to 84.82 for sesame protein isolates while 83.98 RNPR was noticed for Canola protein isolates. Moreover, the reference diets Soy and Casein indicated 93.09 and 100 RNPR, respectively (Table 2). The SPI-based group revealed appreciable nitrogen balance that indicated greater nitrogen intake as compared to fecal and urinary excretion.

Table 2: Growth study parameters of test diets.

Diets	PER	NPR	RNPR[‡]
SPI	2.14 ± 0.10^{c}	5.03 ± 0.26^{c}	84.82
FPI	1.98 ± 0.07^{e}	4.67 ± 0.13^{d}	78.75
CPI	2.09 ± 0.06^{d}	4.98 ± 0.11^{c}	83.98
Soy	2.52 ± 0.09^{b}	5.52 ± 0.13^{b}	93.09
Casein	2.74 ± 0.12^{a}	5.93 ± 0.19^{a}	100

Means with different letters in a column have significant difference, RNPR = relative net protein ratio, and [‡]calculated from standard protein casein as 100.

3.3. Nitrogen Balance Study. It includes true digestibility (TD), biological value (BV), and net protein utilization (NPU).

3.3.1. True Digestibility (TD). Significant variations were seen amongst the groups with respect to TD. The present results indicated that the highest TD was observed for SPI-based diet 77.23 ± 3.20 followed by FPI and CPI as 72.47 ± 2.27 and 70.34 ± 2.10%, respectively (Table 3). Moreover, the reference diets containing soy and casein reflected TD as 90.05 ± 2.87 and 91.34 ± 4.39, correspondingly. The maximum TD for SPI indicated exceptional digestibility as well as absorption of protein in the body in comparison with other protein isolates.

3.3.2. Biological Value (BV). Biological value serves as an indicator of protein quality and reflects the absorbance of protein from food that ultimately plays its role in body growth. Statistically significant means values regarding BV indicated highest value for FPI (69.35 ± 3.47%), whilst the diets containing CPI and SPI exhibited 67.66 ± 2.59 and 63.94 ± 2.50% BV, respectively (Table 3). Nevertheless, the BV for soy and casein based diets were 91.55 ± 2.46 and 92.72 ± 3.10%, correspondingly. Higher biological value noted in FPI as compared to other protein isolates indicates improved profile, digestibility, and bioavailability of amino acids.

3.3.3. Net Protein Utilization (NPU). The results were statistically significant for NPU among the groups. Mean values regarding NPU indicated that the highest NPU (50.26 ± 2.44%) was observed for FPI-based diet, while 49.38 ± 1.58 and 47.59 ± 1.85% for SPI and CPI, respectively. Moreover, soy-and casein-based diets performed better than other diets with NPU values 82.44 ± 2.07 and 84.69 ± 3.47%, correspondingly (Table 3).

3.4. Safety Assessment of Defatted Oilseed Protein Isolates

3.4.1. Serum Protein Analysis. The result values regarding serum total protein were ranging from 6.45 ± 0.27 to 6.86 ± 0.67 g/dL. These results clearly indicated that the

Table 3: Nitrogen balance study parameters of experimental diets.

Diets	TD (%)	BV (%)	NPU (%)
SPI	77.23 ± 3.20^{c}	63.94 ± 2.50^{d}	49.38 ± 1.58^{cd}
FPI	72.47 ± 2.27^{cd}	69.35 ± 3.47^{c}	50.26 ± 2.44^{c}
CPI	70.34 ± 2.10^{d}	67.66 ± 2.59^{cd}	47.59 ± 1.85^{d}
Soy	90.05 ± 2.87^{b}	91.55 ± 2.46^{b}	82.44 ± 2.07^{b}
Casein	91.34 ± 4.39^{a}	92.72 ± 3.10^{a}	84.69 ± 3.47^{a}

Means with different letter in a column have significant difference. SPI = Sesame protein isolates. FPI = Flaxseed protein isolates. CPI = Canola protein isolates.

highest protein was observed in a group fed on SPI-based diet 6.79 ± 0.32 g/dL trailed by CPI and FPI as 6.66 ± 0.51 and 6.45 ± 0.27, respectively. However, for reference diets, i.e., soy and casein the values were 6.78 ± 0.38 and 6.86 ± 0.67, correspondingly. Serum albumin concentration was in the range of 3.08 ± 0.35 to 3.16 ± 0.11 g/dL for different experimental groups. Likewise, the globulin was observed to be varying from 2.91 ± 0.13 to 3.04 ± 0.13 g/dL. Furthermore, A/G ratio ranged from 1.04 ± 0.09 to 1.07 ± 0.08 for the experimental diets (Table 4).

3.4.2. Renal Functioning Tests. The production of urea in the body is related to the amino acid deamination. Mean values for renal functioning parameters revealed nonmomentous variation in rats fed on different experimental diets. The means for urea content indicated that it ranged from 14.85 ± 0.70 to 15.35 ± 0.62 mg/dL while creatinine varied from 0.30 ± 0.02 to 0.33 ± 0.01 mg/dL (Table 5). There were no statistically significant variations amongst the groups depicting similar renal functioning.

3.4.3. Hepatic Functioning Tests. Mean values indicated nonsignificant differences among various diets containing defatted oilseed protein isolates with respect to hepatic health biomarkers. The means revealed that the enzymes activities i.e. ALT, AST, and ALP varied as 38.92 ± 2.90 to 40.10 ± 2.64, 76.48 ± 9.92 to 78.45 ± 5.65, and 144.66 ± 10.90 to 146.22 ± 6.91 U/L, respectively. The rats fed on protein isolates based experimental diets exhibited comparable results for liver enzymes with control (Table 5).

4. Discussion

Growth parameters study is a promising tool to estimate nutritional attributes of test proteins [2]. The PER is dependent upon the amount of essential amino acids as well as the capability of body to digest them. The results of current exploration regarding protein efficiency ratio are in harmony with the outcomes of Bae et al. [17]. They delineated that the PER exhibited a decreasing trend (2.70-2.53) in fish diets with increasing levels of defatted sesame seed meal. Likewise, Adeniyi et al. [18] documented PER 1.91 for sesame seed in diets fed to rats. Furthermore, 2.60 PER was recorded for *Lupinus* species [19].

Moreover, Katoch and Bhatia [20] explicated that the PER of flaxseed meal was slightly lower as compared to soy meal. In another research investigation, Yuksel [21] recorded 1.87 protein efficiency ratio for diet having 16% partially defatted flaxseed flour. Previously, El-Enzi et al. [22] described that defatted flaxseed meal indicated 2.21 PER. The instant outcomes regarding protein efficiency ratio of CPI are in agreement with the results presented by Hassaan et al. [23], and they explicated that the PER ranged from 1.83 to 2.33 for different canola meal-based diets. Later, Arrutia et al. [24] revealed that canola protein isolates (CPI) exhibited PER of 2.64. Likewise, Wanasundara et al. [25] also reported the similar protein efficiency ratio (2.64) for rapeseed flour.

Thirunathan and Manickavasagan [26] documented that NPR for raw and extruded pea varied from 2.9 to 2.5. One of their peers, Santiago et al. [27] reported NPR for diets containing almond, cashew nut, and peanut as 3.53, 3.17, and 2.91, correspondingly. In another research investigation, 3.04 NPR was noticed for complementary diet containing germinated cowpea, maize, and sesame seeds [28]. Moreover, the current outcomes were in contrast with the previous findings of Kaur et al. [29] who noticed NPR of extruded flaxseed meal as 3.22. This contrast might exist due to compositional difference between extruded meal and protein isolates. Furthermore, they observed RNPR (58.6) for extruded flaxseed meal. In an earlier research exploration, RNPR (64) in pea protein concentrates was noted. Later, a group of researchers worked on nutritional quality of pea and delineated RNPR (59.6) for raw pea, whilst RNPR values for extruded pea ranged from 50.6 to 54.2 [30].

Nitrogen balance study includes true digestibility (TD), biological value (BV), and net protein utilization (NPU). The protein digestibility depends on the quantity of limiting as well as essential amino acids in the tested diets. Previously, Sarkar et al. [31] elaborated that animal protein exhibit higher digestibility (90–99%) as compared to plant protein (70–90%). The results revealed lower true digestibility for the tested diets as compared to casein; nonetheless, it was higher than certain other cereal proteins. In a research study, it was elucidated that extruded flaxseed meal exhibited 73% TD [29]. Current results for TD of canola protein isolates are in contrast with the findings of Kaplan et al. [32] who documented 93.3% TD, and Wanasundara et al. [25], reported 95% true digestibility for canola protein isolates. Previously, Kapravelou et al. [33] documented 87% TD for cowpea protein isolates.

El-Enzi et al. [22] reported the BV of defatted sesame meal as 67.73%. Moreover, the biological value for defatted flaxseed meal was noted as 76.35%. Later, Li et al. [34] documented 64.60% BV for unleavened flat bread diet comprising of 16% partially defatted flaxseed flour. Moreover, Kaur et al. [29] indicated 80.0% BV for extruded flaxseed meal. Earlier, Beszterda and Nogala–Kałucka [35] corroborated the biological value of 87% for mustard protein isolates while BV for defatted meal was reported as 86%. In the nutshell, oilseed protein isolates exhibit better quality proteins with balanced amino acid profile that can play an imperative role in improving the growth of test animals.

The instant results for NPU are in corroboration with the findings of [36], and they documented that sesame seeds

Table 4: Serum protein profile of experimental rats.

Diets	Total protein (g/dL)	Albumin (g/dL)	Globulin (g/dL)	A/G ratio
SPI	6.79 ± 0.32	3.12 ± 0.25	3.01 ± 0.25	1.04 ± 0.09
FPI	6.45 ± 0.27	3.08 ± 0.35	2.95 ± 0.18	1.05 ± 0.07
CPI	6.66 ± 0.51	3.10 ± 0.49	2.91 ± 0.13	1.07 ± 0.08
Soy	6.78 ± 0.38	3.15 ± 0.19	3.01 ± 0.28	1.05 ± 0.08
Casein	6.86 ± 0.67	3.16 ± 0.11	3.04 ± 0.13	1.04 ± 0.15

SPI = Sesame protein isolates. FPI = Flaxseed protein isolates. CPI = Canola protein isolates.

Table 5: Renal and hepatic functioning tests of experimental rats.

Diets	Urea (mg/dL)	Creatinine (mg/dL)	Alanine aminotransferase (U/L)	Aspartate aminotransferase (U/L)	Alkaline phosphatase (U/L)
SPI	15.25 ± 0.78	0.32 ± 0.02	40.08 ± 2.69	78.14 ± 4.64	146.12 ± 7.98
FPI	14.98 ± 1.02	0.30 ± 0.02	38.92 ± 2.90	76.48 ± 9.92	144.66 ± 10.90
CPI	14.87 ± 0.78	0.31 ± 0.02	39.36 ± 2.35	77.57 ± 5.74	146.22 ± 6.91
Soy	15.35 ± 0.62	0.33 ± 0.01	38.95 ± 4.81	77.56 ± 3.38	144.75 ± 13.75
Casein	14.85 ± 0.70	0.32 ± 0.03	40.10 ± 2.64	78.45 ± 5.65	145.84 ± 5.52

SPI = Sesame protein isolates. FPI = Flaxseed protein isolates. CPI = Canola protein isolates.

exhibit 54% net protein utilization. Likewise, Li et al. [34] expounded that diets containing partially defatted flaxseed flour showed 54.65% NPU. One of their peers observed 58.4% NPU for extruded flaxseed meal [21]. Moreover, Mariotti and Gardner [37] explicated that net protein utilization values for soy proteins ranged from 71 to 78%. In the present study, FPI-based diet showed the lowest NPU value owing to reduced absorption and increased excretion of consumed nitrogen in the body. Moreover, higher NPU value designates the existence of high quantity of essential amino acids.

The current outcomes are supported by the findings of Ismail et al. [38] who explicated serum total proteins as 6.86 g/dL in male albino mice treated with sesame oil. Moreover, the serum albumin was recorded as 4.30 g/dL. Likewise, one of the scientific studies reported albumins and globulins as 3.67 and 27.17 g/dL, respectively, in rats fed with diet containing genetically modified flaxseeds. Moreover, the value for total protein was observed as 58.83 g/dL [39].

In another research trial, Khan and Khan [40] studied the hepatoprotective effect of ethanolic extract of sesame seeds in rats suffering from liver damage. They inferred that the serum albumin increased from 4.00 to 4.18 g/dL with increasing concentration of sesame extract. Similarly, serum total protein increased from 6.95 to 7.03 g/dL. Likewise, Enyenihi et al. [41] reported that the albumin level increased from 2.2 to 4.2 g/dL with varying levels of sesame seed meal in rabbit diets. The values for serum globulin were observed in the range of 1.3 to 2.0 g/dL whilst the total protein varied from 5.5 to 6.1 g/dL in various diets. According to Passi et al. [42], the values for serum albumin, globulin, and total protein for control diet were 3.42, 3.25, and 6.13 g/dL, respectively. They also studied the effect of sesame seed addition in the diet of lambs and elucidated an increase in these traits in experimental diets as compared to control. The values reported for albumin, globulin, and total protein were 3.69, 4.08, and 7.77 g/dL, respectively, for diet containing 4% sesame seeds. Likewise, for diet having 8% sesame seeds, the values for these traits were recorded as 3.78, 4.19, and 7.97 g/dL, correspondingly.

Reference [43] treated female rats with sesame oil and deduced plasma urea and creatinine as 26.90 and 0.84 mg/dL, correspondingly. Earlier, Hemdan and Abdulmaguid [44] studied the renal protective effect of a mixture of seeds (flaxseed, purslane, and pumpkin) on hypercholesterolemic rats and reported the values for urea and creatinine in control group as 24.05 and 1.01 mg/dL, respectively. Furthermore, the group fed with flaxseed/pumpkin mixture showed increased levels of urea (29.97 mg/dL) and creatinine (1.19 mg/dL) as compared to control.

In another scientific exploration, it was explicated that urea nitrogen level decreased from 46.603 to 38.109 mg/dL with increasing level of flaxseed in the diet of rats suffering from liver damage. Moreover, the creatinine level also decreased from 1.376 to 0.972 mg/dL El-Hashash et al. [45]. Likewise, Campos et al. [46] studied the benefits of defatted flaxseed supplemented bread in normal and type 2 diabetic subjects. They observed creatinine levels in normal and diabetic subjects fed with supplemented bread as 0.791 mg/dL and 0.86 mg/dL, correspondingly.

According to Hemdan and Abdulmaguid [44], the blood urea level of weanling rabbits increased from 2.5 to 5.8 mmol/L with increasing levels of sesame seed meal. Furthermore, the creatinine level varied from 44.0 to 59.0 mmol/L for diets with different levels of sesame seed meal. Likewise, El-Enzi et al. [22] elucidated the values for urea and creatinine in control diet as 26.4 and 0.94 mg/dL, respectively. They further explicated that the urea and creatinine levels decreased with increasing levels of sesame seeds in the experimental diet of lambs.

The activity of these enzymes (ALT, AST, and ALP) determines the hepatic functioning. In a research trial, Rezaeipour et al. [47] evaluated liver functioning of Japanese quails by providing sesame meal based diets. They revealed that the values for ALT ranged from 24.03 to 25.94 IU/L whilst the values for AST and ALP varied from 247.3 to 274.7

and 1278 to 1360 IU/L, respectively. In another experimental trial, El-Enzi et al. [22] assessed the effect of sesame seeds supplementation on the growth of lambs and reported ALT (18.1 to 18.7 IU/L), AST (33.9 to 35.6 IU/L), and ALP (43.5 to 45.7 IU/L) for the experimental diets. Likewise, El-Hashash et al. [45] observed the effect of flaxseed on the liver enzymes of rats suffering from liver damage and illustrated that the AST and ALT reduced with increase in flaxseed in the experimental diets. The values for these traits decreased from 186.137 to 175.535 and 92.892 to 83.971 U/L in respective manner. Likewise, [43] observed the activities of ALT and AST in female rats treated with sesame oil as 21.2 and 31.5 U/L, respectively. Similarly, [36] documented ALT (27.4 U/L), AST (48.61 U/L), and ALP (46.01 U/L) for male albino mice treated with sesame oil.

5. Conclusion

The outcomes of growth study parameters as well as nitrogen balance study parameters indicated that the protein isolates from defatted oilseeds specifically sesame protein isolates have a health promoting effect and can substantially increase the protein content in living body. Likewise, the safety assessment studies clearly indicated that these protein isolates bear no adverse health effects, hence, can be potentially utilized to uplift the protein levels. Moreover, these protein isolates can play an imperative role in combating the dilemma of protein energy malnutrition especially in developing economies of the world.

6. Disclosure

A preprint is available in the HEC repository (https://prr.hec.gov.pk/jspui/handle/123456789/8378). The researchers intended to get the result of their research study published in a peer reviewed journal, and therefore, they have decided to submit it to the journal.

Data Availability

The data that support the findings of the study are available from the corresponding author upon request.

Ethical Approval

The study was approved by the Directorate of Advanced Studies of the University of Agriculture, Faisalabad, Pakistan after departmental scrutiny.

Conflicts of Interest

The authors declare that they have no conflicts of interest.

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