Total and Partial Substitution of the Local Grass Pea Seed *Lathyrus sativa* Processed by Different Methods by Soy Bean Meal *Glycine max* in Small Common Carp *Cyprinus carpio* L. Diets

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Abstract

This experiment was carried out in the laboratories of fish and animal resource center-agricultural research directorate from 1/3-30/6/2015 to study the possibility of improving the nutritional value of local grass pea seeds *Lathyrus sativa* (GPS) by degradation of anti-nutritional factor and using it as protein source for common carp *Cyprinus carpio* L. diets. Three different treatments for GPS meal, fermented, germinated and soaking (GPS) were used as partial or total replacement of soybean meal (SBM) for practical diets of common carp *Cyprinus carpoi* L. ration. Thirteen experimental diets were formulated, the diets 1, 2 and 3 were used crud GPS without any treatment at the substitute ratio 33%, 66% and 100% of (SBM), the diets 4, 5 and 6 contained fermented GPS at the same substitute ratio. The diets 7, 8 and 9 were germinated GPS at the same substitute ratio. The diets 10, 11 and 12 were soaking GPS at the same substitute ratio and diet 13 for control without GPS. The results showed no significant differences between control treatment (T 13) without GPS and soaking at substitution ratio 33% and 66% (T 10 and T 11), which were significantly differed ($P \leq 0.05$) with other treatments. Thereby, it is recommended soaking GPS and at substitute 33% and 66% of SBM for common carp diets. [DOI: 10.22401/JNUS.20.3.16]

Keywords: grass pea seed, *Lathyrus sativa*, *Cyprinus carpio* L., soy bean meal.

Introduction

The nutrition and the type of the food in the fish breeding were the main causes of increasing the cost and production diets in fish breeding farms in Iraq. There were many attempts to reduce the cost of the fish diets by reducing the protein ratio in the diet or replacement of one ingredient by using unconventional food, including the byproduct of the food industry and other agricultural crops [1]. The attention of researchers have tended to use unconventional alternatives feed with a protein suitable content and replace the traditional feed sources in the diets of fish, particularly protein sources [2], these unconventional alternatives feed need to studies and evaluation for the purpose of access to the possibility of substitution in whole or in part, the meals like soybean meal occupies first ranked among imported plant protein sources which use in fish diets, and local unconventional alternatives sunflower meal, legume and grass pea seed (GPS) *Lathyrus sativus* L. which is one of the important legumes grows in Bangladesh, Egypt and North of Iraq, with the possibility to use as a plant protein source (27-33%) [3]. However, the (GPS) contain many anti-nutritional substances, which hinder free nutritional utilization in mono gastric animals like fish and poultry which called enzymes inhibitors like (tannin protease and amylase, saponins, non-starch polysaccharides and phytates) [4]. The seeds of (GPS) contains an acidic neurotoxic amino acid 3-N-oxaly-L-2,3-diaminopropionic acid (β-ODAP) [5]. These anti-nutritional factors require reducing different processing methods such as autoclaving, extrusion, fermentation and germination prior to inclusion in fish rations [6 and 7]. Animal protein concentrate (APC) and soy bean meal (SBM) *Glycine max* were considered as an important protein ingredients in fish rations in Iraq. However, the cost of (APC) and (SBM) have soared so high recently that it is becoming uneconomical to use in fish feeds. Soybean meal was used widely as a source of vegetarian protein for animal feeds, and the price of most other protein meals and grain legumes are set

relative to this commodity [3]. The present study was designed to evaluate raw, fermented, germinated and soakage local (GPS) meal as total and partial substitution for (SBM) in diets of common carp fingerlings based on its effect on growth indicia, food conversion rate (FCR), food efficiency ratio (FER), protein protective value (PPV) and apparent digestible coefficient (ADC).

Materials and Methods

The experiment were conducted in laboratories of fish and animal resource center, Baghdad, IRAQ.

Processing of Grass Pea Seed (GPS)

The local GPS required for the trial was obtained from a local market in Baghdad, and then divided into lots that were processed as follows:

a. Soaking: The GPS were soaked by maintaining in the water for 24 hour, then changing the water and soaking again in the water for another 24 hour.
b. Fermentation: Finely Ground GPS was passed through a fine mesh sieve to ensure homogeneity. GPS was fermented by an enzyme produced by the bacterium *Bacillus* sp., isolated from the intestine of common carp. The selected bacterium was grown in shakein bottles in 4% tryptone soya broth (Hi-Media) for seed culture. After 24 hrs of growth at 37^{o} C. The average viable count was about 10^{7} cells ml^{-1} of broth. This was used as bacterial seed for seed meal fermentation. A portion of sieved GPS meal was moistened with 50% w/v liquid basal medium containing (g l_1): KH_2PO_4; Na_2HPO_4; $MgSO_4$; $7H_2O$, 0.2; $CaCl_2$, 0.001; $FeSO_4$ $7H_2O$, 0.004 and autoclaved for sterilization. The sterilized seed meal was fermented with Bacillus culture at the rate of 10^{8} bacterial cells/g of dried seed meal for 10 days at $37^{o}\pm$ 2 C in an incubator.
c. Germination: The dry GPS were germinated in wet smooth weft piece and for 72 hour and dried by oven at 65 C^{o} for 24 hour.

Experimental fish and maintenance conditions

Common carp *C. carpio* L. fingerlings obtained from a local fish dealer were acclimatized in rectangle metallic tanks at the laboratory conditions for 5 days and fed with a mixture of commercial diets and 5% protein concentrated. The fishes were sterilized by saline solution (3%) for 3 minutes to get rid of parasite and bacterial infection. The feeding trial was conducted in glass aquarium and acclimated for 10 days (including breeding system, diets formulate and the time of food intake). Fingerlings (18.7±0.82g) were randomly distributed in 26 glass aquarium at the rate of 8 fish per glass aquarium, three replicates for each experimental diet. Each glass aquarium was supplied with air pump water from a deep tube well with continuous aeration. Fish were fed twice daily at a fixed feeding rate of 3% body weight per day for 90 days. The quantity of feed given was every 15th day after weighing the fish. To determine the feed consumption, any leftover feed was collected 6 hrs after each feeding and weighed after oven drying. Water of the aquarium was partially changed to approximately 50% per day for water to exclude chloride a temperature of the laboratory. Daylight-balanced by fluorescent discharge lamps maintained at 12 hrs light/12 hrs dark photoperiod for 90 days of feeding trial.

Formulated diets

The ingredients were ground individually by grinder and mixed together for homogenized. Diets were formulated in 13 treatments, T1, T2 and T3 includes raw GPS at substitution levels of 33%, 66% and 100% of SBM respectively, T4, T5 and T6 includes soaking GPS at the same substitution levels, T7, T8 and T9 have fermentation GPS at the same substitution levels, T10, T11 and T12 germination GPS at the same substitution levels, at last, T13 was formulated without any GPS as control diet Table (2).

Digestibility Experiment

The digestibility experiment was conducted separately in glass aquarium. Chrome Oxide Cr_2O_3 was added at 1% to the ingredients and formulated to pellets. Fishes were fed at the same program of the nutrition experiment with

incessant feces samples were collected and dried then the mixture of all replicates was assembled [8] and [9]. The standard curve was conducted to estimate the concentration of Cr_2O_3 according to [10].

Chemical analyses and data collection

Samples of experimental diets were analyzed including protein% for GPS and SBM Table (1), fecal (for ADC APD), body composition (parameter of PPV) and all chemical composition for experimental diet samples were analyzed. Moisture, crude protein, ether extract, crude fiber and ash determine according to the official methods of analysis (Association of Official Analytical Chemists)[11]. The Nitrogen Free Extract (NFE) was determined by using the eqution:
NFE= 100 – (CP% - EE% - Ash% - CF%) (Maynard *et al*., 1979) Table (3). Water quality parameters (O_2, pH and water temperature) were monitored following the methods outlined by APHA [12].

Table (1)
Effect of Different Processes of GPS on protein ratio compared with SBM.

N	The Processing of GPS	Protein%	Soybean meal%
1	Raw GPS	29.31	43.25
2	Cooking for 30 minute until boiling	27.63	
3	Roaster for 15 minute	27.38	
4	Autoclaving (Sterilization and Temperature 115c, Press 1.5 bar for 15 minute	27.31	
5	Germination for 72h	30.94	
6	Fermentation	33.31	

Table (2)
Diets components of experimental diets (Dry Matter basis %).

Processing of GPS	Treatment	Substitution ratio% for soy meal	Ingredient%							
			Animal Protein concentration	Soy bean meal	GPS	Yellow Corn	Local Barley	Wheat bran	Vit	Salt
Raw GPS Without Processing	T1	33	10	16.75	8.25	15	22	25	2	1
	T2	66	10	8.25	16.75	15	22	25	2	1
	T3	100	10	0	25	15	22	25	2	1
Soaking GPS	T4	33	10	16.75	8.25	15	22	25	2	1
	T5	66	10	8.25	16.75	15	22	25	2	1
	T6	100	10	0	25	15	22	25	2	1
Fermentation GPS	T7	33	10	16.75	8.25	15	22	25	2	1
	T8	66	10	8.25	16.75	15	22	25	2	1
	T9	100	10	0	25	15	22	25	2	1
Germination GPS	T10	33	10	16.75	8.25	15	22	25	2	1
	T11	66	10	8.25	16.75	15	22	25	2	1
	T12	100	10	0	25	15	22	25	2	1
Control	T13	0	10	25	0	15	22	25	2	1

Table (3)
Chemical composition for experimental diets (calculated by dry matter basis %).

Processing of GPS	Treatment	Substitution ratio% for SBM	Nutrition constituents for diets						** Metabolic Energy KJ/Kg
			Moisture	Crud Protein	Ether Extract	Fiber	Ash	NFE *	
Raw GPS Without Processing	T1	33%	6.01	27.58	6.27	5.85	7.62	47.67	1386.39
	T2	66%	6.89	27.18	6.08	6.12	6.09	47.64	1372.09
	T3	100%	6.24	26.79	6.22	7.02	6.43	47.30	1364.76
Soaking GPS	T4	33%	6.04	26.63	5.95	7.22	6.31	47.85	1360.29
	T5	66%	6.92	26.44	5.91	6.92	6.54	47.27	1347.38
	T6	100%	6.91	27.11	6.11	6.10	6.51	47.26	1366.54
Fermentation GPS	T7	33%	6.98	27.61	6.20	6.00	5.96	47.25	1378.81
	T8	66%	7.08	27.09	6.24	6.23	6.11	47.25	1370.38
	T9	100%	7.09	27.36	5.88	5.86	6.24	47.57	1367.80
Germination GPS	T10	33%	6.89	27.71	6.03	5.90	6.26	47.21	1374.45
	T11	66%	7.00	26.91	5.81	6.07	6.33	47.88	1361.28
	T12	100%	7.99	26.98	6.09	6.79	7.59	50.65	1410.20
Control	T13	0	7.97	27.81	6.31	7.12	7.42	50.79	1435.11
Raw GPS			8.21	29.31	2.3	8.3	3.6	48.28	
Soaking GPS			9.75	29.31	2.3	8.3	3.6	46.74	
Fermentation GPS			8.57	30.94	1.9	7.8	3.1	47.69	
Germination GPS			7.95	33.31	1.7	7.5	2.9	46.64	

Nitrogen Free Extract, ** Smith [13] : ME (MJ) = protein × 18.8 + fat × 33.5 + NFE × 13.8

Studied Parameters

Weight Gain (WG)g/fish=Final Weight (FW)-Initial Weight (IW)

Daily Weight Gain (g/fish/day)

$$= \frac{\text{Final Weight (FW) (g/fish) - Initial Weight (IW) (g/fish)}}{\text{number of days}} \quad [14]$$

(RGR) Relative Growth Rate

$$= \frac{\text{Final Weight (FW) (g/fish) -Initial Weight (IW) (g/fish)}}{\text{Initial Weight (IW) (g/fish)}} \times 100 \quad [14]$$

(FCR) Feed Conversion Rate

$$= \frac{\text{Food Intake (g/fish)}}{\text{Weight Gain (g/fish)}} \quad [14]$$

(FER)% Feed Efficiency Ratio

$$= \frac{\text{Weight Gain (g/fish)}}{\text{Food Intake (g/fish}} \quad [15]$$

(P. P.V) Protein Productive Value

$$= \frac{\text{Body protein in end experiment - Body protein in Initial experiment}}{\text{Protein intake (g/fish)}} \quad [16]$$

%(ADC) Coefficient Apparent Digestible

$$= \frac{Cr_2O_3 \text{ in food}}{Cr_2O_3 \text{ in faces}} \times 100) - 100 \quad [9]$$

Apparent Protein Digestible (APD)

$$= 100 - \left(\frac{Cr_2O_3 \text{ in food}}{Cr_2O_3 \text{ in faces}} \times \frac{\text{Protein ratio in feces}}{\text{Protein ratio in food}} \times 100 \right) \quad [17]$$

Experimental design statistical analysis

The complete randomized design (CRD) was used to study experimental parameters, the significant was tested between means according to Duncan's multiple range test at significant level $P \leq 0.05$ [18]. The officinal statistic program (Statistical Analysis System) was used for data analysis [19].

Results and Discussion

The water temperature, dissolved O_2 and pH during the experiment trial were 26.8 – 29.6C^o, 6.6-7.5 mg/L and 7.3-7.8 respectively which were suitable for fish performance [20 and 21]. Results of weight gain (WG) and daily weight gain (DWG) showed no significant differences between control diet (T_{13} without GPS) and the T_4 (33% soaking GPS), T_7 (33% fermentation GPS), T_{10} and T_{11} (33% and 66% germination GPS) which were 22.815, 19.78, 18.77, 21.475 and 19.615 g/fish respectively for WG, 0.2535, 0.2195, 0.208, 0.238, 0.2195 and 0.2535 g/fish/day respectively for DWG (Table 3). RGR, WG and DWG showed significant decrease $P \leq 0.05$ for all treatment of the raw GPS (T_1, T_2 and T_3) and the substitution levels 66% and 100% (T_5, T_6, T_8, T_9 at soaked and fermented and T_{12}) compared with T_{13} (Table 3).

Table (4) shows no significant differences between control diet (T13) and T4, T5 (33 and 66% GPS soaking) in FCR, T7 (33% GPS fermentation), T10 and T11 (33% and 66% GPS germination) which were 3.77, 4.13, 4.24, 4.34, 4.29 and 4.42 respectively Table (4). Results for FER% were concordant with the results of FCR Table (3). Statistical analysis of PPV showed no significant differences between control diet (T13) and T4, T7 and T10, and the ADC% showed no significant differences between control diet (T13) and T4, T5,T7, and T10. The results of APD% showed no significant differences between control diet (T13) and T4, T7 and T10.

The results of this study demonstrate the suitability of soaking, fermented and germination GPS instead of protein source in formulated diets for common carp. It is evident from this investigation that soaking and germination of GPS could be Integrated up to 33% and 66% in the diet. Performance of fish in the rations containing similar levels of unfermented GPS was inferior to those reared on fermented ones. The results of the present study also indicated that bacterial fermentation improves the nutritive value of GPS [22]. Nutritionally, it is tasty and protein-rich [6], but the presence of a variety of anti-nutritional factors hinders its free nutritional utilization [5]. Tannin, phytic acid and β-ODAP could be significantly reduced in grass pea fermented with *Bacillus sp*. which isolated from common carp intestine. These particular bacterial strains have considerable extra cellular amylolytic, cellulolytic, proteolytic and lipolytic activities [23].

In comparison with WG, DWG and RGR for SBM, neither processed nor unprocessed could substitute the protein source supplied by SBM, the decrease was found to be more effective when increasing the substitution level (66% and 100%) specially in T_1, T2 and T_3. The reason for this phenomenon may be the negative effect of trypsin inhibitor [24] or the effect of the poison β-ODAP which blocked the digestive enzymes and pause the benefit of nutritional substitution [5]. Also, the initially low protein level of GPS compared with SBM Table (1), as well as the non sufficient processes (soaking, fermentation and germination) may consider as another impact on decreasing growth parameter particularly in the high ration of substitution. These results agree with [25] and [4]. On the other hand, soaking, fermentation and germination processes may due to some extent to inhibit the anti-nutritional substance. Yan *at al*., [26] illustrate that some inhibitors like tannin have unpalatable taste, phytic acid has the affinity to bind Ca. Mg, Zn, and Fe ions to make un digestible complex, and other inhibiters (trypsine and chmotrypsine inhibiter) which were found naturally in the feedstuff. However, processing legume seed specially by fermentation could increase the growth performance of fish after the destruction of poison compound (β-ODAP) [5]. The PPV which is an important parameter to evaluate the protein in diets and protein nutrition given to fish which occasionally called Efficiency of Protein Utilization (EPU) [27], observed a positive effect of all processing (soaking, fermentation and germination). The value of PPV was improved Table (5), it seems that the process has no effect with the substitution ratio

100% and has a little effect on the substitution ratio 66%, It is evident that the reduced PPV% of fish fed raw grass pea meal diets may due to the effects of anti-nutritional factors [28]. The PPV of all treatments decreased when the substitution ratio increased Table (5). This may explain the unbalance of essential amino acids in the treatment ration which caused a shortage in the nutrition requirement of the fish when fed on GPS compared with SBM [29].

Table (5) revealed that raw GPS treatments (T_1, T_2 and T_3) exhibited the lowest ADC% and APD% within the other treatments. In contrast, control (T13) showed the highest ADC% and APD% insignificant difference in T_4, T_5, T_7 and T_{10} for ADC% and T_4, T_7 and T_{10} for APD%. The presence of anti-nutritional factors may influence the digestibility of various nutrients in the diet and give erroneous results [30]. However, the apparent digestibility values for protein were higher in the group of fish fed diet T4, T7, T10 and T13, containing 33% GPS soaking, fermentation, germination and control respectively. Results are accordance with [31]. In general, the results showed improvement of all treatments with GPS (soaking, fermentation and germination) compared with the treatments of raw GPS (T_1, T_2 and T_3). Therefore, we can use germination GPS (the best one) at substitution ratio% for soy meal 33% and 66%. In addition, local GPS in Iraq was cheaper than the widely used SBM, thus, the study suggest replacing 66% at the SBM in fish diets by germinated GPS.

Table (4)
Effect of different levels of GPS processing on growth indicia.

Ingredient			Studied Parameters (Growth indicia)				
Different Processing of GPS	**Treatments**	**Substitution ratio% for soy meal**	**IW**	**FW**	**WG**	**DWG**	**RGR**
Raw GPS Without Processing	T1	33	37.110 ± 1.32 a	40.785 ± 1.45 ef	4.145 ± 0.325 ef	0.0461 ±0.003 e	10.205 ± 1.165 d
	T2	66	36.485 ± 0.375 a	39.23 ± 0.29 ef	2.745 ± 0.85 ef	0.0305 ±0.009 e	7.00 ± 0.270 d
	T3	100	36.305 ± 4.655 a	36.91 ± 4.58 f	0.605 ± 0.075 f	0.007 ±0.001 e	1.690 ± 0.410 d
Soaking GPS	T4	33	38.225 ± 0.225 a	58.005 ±1.885 abc	19.78 ± 1.66 abc	0.2195 ± 0.185 abc	51.725 ± 4.045 abc
	T5	66	35.675 ± 0.195 a	52.845 ± 2.72 bcd	17.365 ± 2.725 bcd	0.193 ± 0.030 bcd	48.708 ± 7.888 abc
	T6	100	36.460 ± 1.13 a	49.665 ± 2.81 cd	13.195 ± 1.685 cd	0.146 ± 0.019 cd	36.064 ± 3.501 bc
Fermentation GPS	T7	33	36.545 ± 0.165 a	55.315 ± 5.295 abc	18.770 ± 5.13 abc	0.208 ± 0.057 abc	51.49 ±13.86 abc
	T8	66	36.380 ± 0.10 a	50.155 ± 0.655 cd	13.775 0.555 cd	0.1525 ±0.006 cd	37.875 ± 1.428 bc
	T9	100	35.905 ± 0.65 a	47.455 ± 1.625 de	11.55 ± 1.69 de	0.128 ± 0.019 d	32.153 ± 4.763 c
Germination GPS	T10	33	37.64 ± 1.435 a	59.120 ± 2.10 ab	21.475 ± 3.535 ab	0.238 ± 0.039 ab	57.485 ± 11.56 ab
	T11	66	36.87 ± 1.62 a	56.35 ± 0.41 abc	19.615 ± 1.345 abc	0.2195 ±0.009 abc	53.089 ±5.619 abc
	T12	100	36.30 ± 0.79 a	50.545 ± 2.925 bcd	14.245 ± 2.135 bcd	0.1580 ± 0.024 bcd	39.130 ±5.027 bc
11 Control	T13	0	36.91 ± 0.23 a	59.725 ± 1.525 a	22.815 ± 1.755 a	0.2535 ±0.195 a	61.845 ±5.135 a

The means which have similar number in the same column no significant differences between at probability level ($P \leq 0.05$) (Mean±standard deviation).

Table (4)
Effect of different levels of GPS processing on FI, FCR, FER, PPV%, ADC% and APD%).

Ingredient			Studied Parameters (FI, FCR, FER, PPV%, ADC% and APD%)					
Different Processing of GPS	**Treatments**	**Substitution ratio% for soy meal**	**FI**	**FCR**	**FER%**	**PPV%**	**ADC%**	**APD%**
Raw GPS Without Processing	T1	33	40.815 ± 1.04 c	9.885 ± 0.525 c	10.145 ± 0.535 cde	40.60 ± 1.03 h	34.49 ± 0.65 g	31.17 ± 0.90 g
	T2	66	34.54 ± 1.08 cd	12.58 ± 0.005 c	7.95 ± 0.00 de	42.385 ± 3.835 h	32.68 ± 0.565 gh	30.38 ± 0.17 g
	T3	100	28.825 ± 0.345 d	48.46 ± 6.58 d	2.105 ± 0.285 e	33.615 ± 1.205 i	29.77 ± 0.332 h	28.12 ± 0.45 g
Soaking GPS	T4	33	81.57 ± 2.83 ab	4.135 ± 0.205 a	24.25 ± 11.50 ab	72.365 1.175 ab	62.955 ± 0.215 abc	69.19 ± 1.22 ab
	T5	66	80.635 ± 1.55 ab	4.245 ± 0.155 a	21.47 ± 2.96 ab	66.59 ± 0.52 def	65.18 ± 1.10 ab	65.265 ± 0.32 bc
	T6	100	79.21 ± 4.46 b	6.055 ± 0.435 b	16.58 ± 1.19 cd	62.395 ± 0.385 fg	40.97 ± 0.865 f	50.58 ± 0.70 f
Fermentation GPS	T7	33	81.57 ± 4.61 ab	4.34 ± 1.82 ab	23.11 ± 5.29 ab	71.70 ± 0.870 abc	61.12 ± 0.15 ab	69.085 ± 0.175 ab
	T8	66	82.06 ± 0.160 ab	5.96 ± 0.25 b	18.29 ± 0.79 bcd	63.145 ± 1.065 fg	55.84 ± 0.435 de	61.18 ± 1.17 ce
	T9	100	79.48 ± 2.22 b	7.005 ± 0.835 c	14.47 ± 1.72 cde	60.735 ± 0.515 g	59.28 ± 0.81 cd	62.21 ± 0.64 cde
Germination GPS	T10	33	89.625 ± 1.115 a	4.29 ± 0.760 a	24.01 ± 4.24 ab	70.776 ± 0.736 abc	63.74 ± 3.30 ab	74.485 ± 1.395 ab
	T11	66	85.57 ± 4.135 ab	4.42 ± 0.49 ab	22.885 ± 2.52 ab	67.845 ± 0.67 cde	56.71 ± 2.50 de	64.13 ± 3.09 cde
	T12	100	79.915 ± 3.485 b	5.7 ± 0.61 b	17.735 ± 1.895 cd	64.155 ± 0.915 efg	54.635 ± 0.855 e	61.47 ± 1.0 de
Control	T13	0	85.595 ± 2.535 ab	3.77 ± 0.17 a	26.615 ± 1.265 a	76.535 ± 0.725 a	67.585 ± 1.665 a	79.42 ± 0.960 a

The means which have similar number in the same column no significant different between at probability level (P≤0.05) (Mean±standard deviation).

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