NUTRITIONAL VALUE IMPROVEMENT OF FLAX MEAL AND ITS UTILIZATION FOR CARP FISH DIETS

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ABSTRACT

The antinutritive factors; trypsin inhibitor, total cyanogenic, total polypenol and phytic acid of the flax meal (36.9% protein) were determined.

Roasting and autoclaving for 20 min inactivated trypsin inhibitor to 80.4 and 54.8%, respectively. The cyanogenic glycosides were totally destroyed after 20 min of the treatment. Autoclaving seems more effective on destruction of total phenolic compounds compared with roasting and solvent treatments but less effective on destruction of phytic acid compared with roasting.

Roasted, autoclaved and protein isolate gave the higher digestibility index 84.80, 85.20 and 89.19%, respectively than solvent treatments.

Replacing 25% of soybean meal (SBM) by raw flax meal (RFM) in carp diets did not significantly affect body weight (BW) or body length (BL) of the fish. However, the higher replacing levels of 50, 75 and 100% significantly decreased BW and BL. The same trend was observed for weight gain (WG) and specific growth rate (SGR). The best results were obtained when fish were fed the RFM25 diet. Increasing replacing level of SBM by RFM from 50 to 100% (with an increment of 25%) in the common carp, diets did not significantly affect the feed conversion ratio (FCR). The best protein efficiency ratio (PER) was scored when 50% of SBM protein was replaced by RFM and the worst was recorded with RFM25 diet.

The highest BW, WG and SGR values were recorded for the group fed roasted flax meal (ROFM75) diet, however, the lower ones were reported for fish fed the ROFM100 diet. The best FCR was obtained when fish were fed the ROFM75. The PER decreased gradually with increasing replacing level of SBM by RFM up to 75%, while the complete replacement increased PER.

Key words: *antinutritive factors, carp fish, diets, flax meal.*

1. INTRODUCTION

Flax (*Linum usitatissimum*) is an important oilseed crop overall the world. The average cultivated area of flax seed in Egypt during 1999-2003 reached 148866 feddans with an average production of 644 kg seed/feddan as reported by the Ministry of Agriculture and Land Reclamation. The resultant meal after oil extraction contains 36-45% protein. Thus, flax meal is considered an important source of protein (Fahmy *et al.*, 1996; Tolba, 1999; El-Kady *et al.*, 2001; El-Sweify *et al.*, 2003). The presence of several antinutritional factors such as cyanogenic-glycosides, phytic acid, phenolics, trypsin inhibitor and mucilage limits the use of flax meal in feed and food formulations (Oomah *et al.*, 1996).

Toxicity of cyanogenic glycosides (Fig. 1) is due to the release of hydrogen cyanide by the action of β-glycosidase, which acts as a potent respiratory inhibitor by complexing with metalloporphyin-containing enzymes (Poulton, 1989). Therefore attempts have been made to remove the cyanogenic compounds of flaxseed meal by boiling in water, dry and wet autoclaving, acid treatment followed by autoclaving and alcohol treatments (Madhusudan and Singh, 1985; Wanasundara and Shahidi, 1997 and El-Kady *et al.*, 2001).

The use of methanol–ammonia-water/ hexane was efficient in the removal of glucosinolate (Shahidi and Gabon, 1990) and phenolic compounds (Abu-Shama 1998 and Taha, 2003), from rapeseed and sunflower. In addition, the procedures were tested to remove cyanogenic glycosides from flax seed effectively (Wanasundara *et al.* 1993). Umoren *et al.* (1998) found that autoclaving completely eliminated trypsin inhibitor, hemagglutinin and hydrogen cyanide in cowpea. Vijayakumari *et al.* (1998) studied the effect of soaking, cooking and autoclaving on the levels of total free phenols, tannins, phytic acid and protein digestibility in seeds of *Vigna sinenssis* . Autoclaving seemed to be the most efficient for reducing the content of the antinutrients except phytic acid and improving protein digestibility.

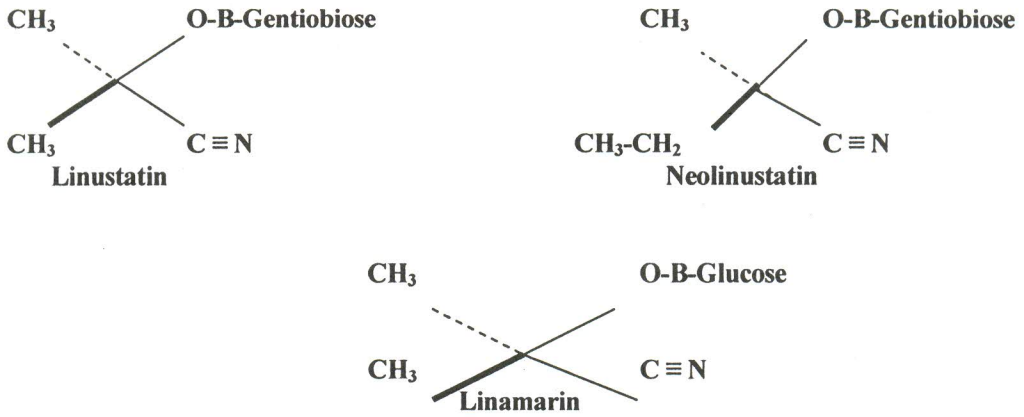

Fig . (1): Possible cyanogenic glycosides of flaxseed

Studies have shown that flax seed meal can improve milk production, carcass grade and the finish and appearance of the products (Bhatty and Cherdkiatgumchai, 1990). Incorporation of flax seed meal in the diets of rats at a level of 40% had no adverse effects on their growth rate.

Flax mucilage is a heterogenic polysaccharide and contributes largely to the soluble fiber fraction of flaxseed, which is suggested to have a hypoglycaemic effect in humans (Cunnane *et al.*, 1993).Wanasundara and Shahidi (1997) found that soaking of seeds in water or sodium bicarbonate solution reduced the amount of mucilage remaining in the seeds. Also, mucilage isolated from the flax consists of two polysaccharide components, acidic and neutral at a ratio of 2:1. The acidic contains L-rhamnose, L-fucose; L-galactose and D-galactouronic acid while the neutral contains L-arabinose, D-xylose and D-galactose.The purpose of the present investigation was to study the removal of antinutritional factors from flax seed meal using solvents, soaking followed by heating. The produced protein isolate was evaluated and both raw and treated flax meals were used in carp fish diets formulation.

2. MATERIALS AND METHODS

2.1.Sampling

Meal of flax seed variety Vaiking was obtained from Shobra melles, Zefta, Egypt. Removal of cyanogenic glycosides and mucilage was employed as follows :

1- Cold hexane extract of the residual oil was prepared by blending sample with hexane in a warring blender for 2 min at low speed 2000 rpm. The meal was separated by vacuum. filtration according to Dipak *et al.* (1986).
2- Two-phase solvent extraction system consisting of alcohol ammonia-water and hexane was employed for extracting oil and detoxification of flax seed meal. Alcohols used are methanol, ethanol and isopropanol. The meal was separated by vacuum filtration according to Jahitha and Shahidi (1994).

3- Meal was soaked in water (1:3 w/v) for 5 hr with intermittent stirring and an hourly change of water to facilitate the mucilage dispersal and removal. The residual meal was heated by using autoclaving (121°C) or roasting (140°C) for 20 min according to Madhusudan and Singh (1985).

2.2.Extraction of flaxseed meal protein

Flaxseed meal protein was extracted according to the method of Dipak *et al.* (1986). The extracted protein was precipitated at isoelectric point of the protein. The precipitate was washed by distilled water and centrifuged, then dried using freeze dried (protein isolate)

2.3.Chemical analysis

Moisture, crude fat, total protein, crude fiber, HCN and ash content were determined in flaxseed meal by-products according to the methods described in A.O.A.C. (1995). Trypsin inhibitor activity (TIA) was measured as described by Hamerstrand *et al.* (1981) modified with respect to the initiation of (TIA) assay, i.e. trypsin was added to the inhibitor-substrate mixture (Stauffer, 1993). Phytic acid content (Latta and Eskin, 1980), total phenols were colorimetrically determined as described by Gutfinger (1981). The digestibility of protein *in vitro* was carried out as described by Ford and Salter (1966). Amino acid analyzer (Model 121) was used for the determination of amino acids in flax seed meal as described by Moore *et al.* (1958). Cystine was microbiologically determined as described by Barton (1952). Tryptophan was colorimetrically determined in the alkaline hydrolysate of samples according to the method of Blouth *et al.* (1963).

2.4.Growth experiments

Nine experimental diets were formulated (Table, 1) to contain 0, 25, 50, 75 or 100% RFM or ROFM as a partial or total replacement of SBM in separate two experiments. For each experiment, ten (2 replicates for each treatment) rectangular aquaria 100 × 40 × 50 cm (200 liter for each) were prepared. Each aquarium was stocked with 20 carp fish that obtained from Abbassa hatchery. Fish in the two experiments were given the pelleted diets (3 mm in diameter) at a daily rate of 4% of total biomass during the experimental period 6 day/week (twice daily at 9.00 am and 3.00 pm) and the amount of feed was bi-weekly adjusted according to the changes in body weight throughout the experimental period (90 days).

Table (1): Composition and proximate analysis of the experimental diets (Exps. 1 and 2).

Ingredients	Diets				
	FM0	FM25	FM50	FM75	FM100
Fish meal	22	22	22	22	22
Yellow corn	35	35	35	35	35
Soybean meal	28	21	14	7	0
Flax meal	0	8	16	24	32
Wheat bran	7	6	5	5	4
Corn oil	4	4	4	3	3
Vit. & Min. Mixture[2]	3	3	3	3	3
Di-calcium phosphate	0.3	0.3	0.3	0.3	0.3
Ascorbic acid	0.2	0.2	0.2	0.2	0.2
Cr_2O_3	0.5	0.5	0.5	0.5	0.5
Sum	100	100	100	100	100
Proximate analysis (determined on dry matter basis)					
Crude protein (CP)	31.25	31.12	30.99	30.97	30.84
Ether extract (EE)	7.45	7.32	6.95	7.83	6.96
Crude fiber (CF)	7.54	7.99	9.23	9.76	9.68
Ash	10.03	9.61	9.88	9.76	8.91
NFE[3]	43.73	43.96	42.95	41.68	43.61
ME (Kcal/kg diet)[4]	2887	2897	2908	2749	2860
P/E ratio	108.3	107.4	106.6	108.7	107.8

[1] Raw flax meal for the first experiment or roasting flax meal for the second experiment.
[2] Vitamin & mineral mixture/kg premix : Vitamin D_3, 0.8 million IU; A, 4.8 million IU; E, 4 g; K, 0.8 g; B1, 0.4 g; Riboflavin, 1.6 g; B6, 0.6 g, B12, 4 mg; Pantothenic acid, 4 g; Nicotinic acid, 8 g; Folic acid, 0.4 g Biotin,20 mg , Mn, 22 g; Zn, 22 g; Fe, 12 g; Cu, 4 g; I, 0.4 g, Selenium, 0.4 g and Co, 4.8 mg.
[3] Nitrogen free extract (NFE) =100-(CP+EE+CF+Ash)
[4] Based on kilocalorie values of 4.50 g^{-1} protein, 8.51 g^{-1} lipid and 3.49 g^{-1} NFE (Jauncy, 1982).

Records of live body weight (g) and body length (cm) of individual fish were measured initially and at the end of the experiment for each aquarium. Growth parameters were measured by using the following equations:

$$\text{Specific growth rate (SGR)} = \frac{\mathbf{LnW2 - LnW1}}{\mathbf{t}} \mathbf{x}\ 100$$

where:- Ln, the natural log, W1, initial fish weight; W2, the final fish weight in "grams" and, t, period in days, weight gain (WG), final weight (g) initial weight (g), feed conversion ratio (FCR), feed ingested (g)/weight gain (g), protein efficiency ratio (PER), weight gain (g)/protein ingested (g)

At the end of the experiment, four fishs were chosen at random and subjected to the proximate analysis of whole fish body.

2.5.Statistical analysis

The statistical analysis of the data was carried out applying the computer program (SAS, 1996).

3. RESULTS AND DISCUSSION

The chemical analysis of flax meal (Table 2) showed high crude protein (36.9) which confirms the view that flax meal by-products are excellent source of protein (Tolba, 1999; El-Kady *et al.* 2001).

The antinutritive factor trypsin inhibitor, total cyanogenic, total phenolic compounds and phytic acid in the flax meal were 2.5 mg/g, 0.42 mg/g, 196 mg/100g and 2.9%, respectively. These data are in agreement which those of El-Kady *et al.* (2001).

Table (2): Chemical composition of flax meal (on dry weight basis).

Moisture (%)	Fat (%)	Protein (%)	Ash (%)	Fiber (%)	Trypsin inhibitor (mg/g)	Total cyanogenic (mg/g)	Total phenolic compounds (mg/100 g)	Phytic acid (%)
9.7	5.4	36.9	6.14	8.9	2.5	0.42	196	2.9

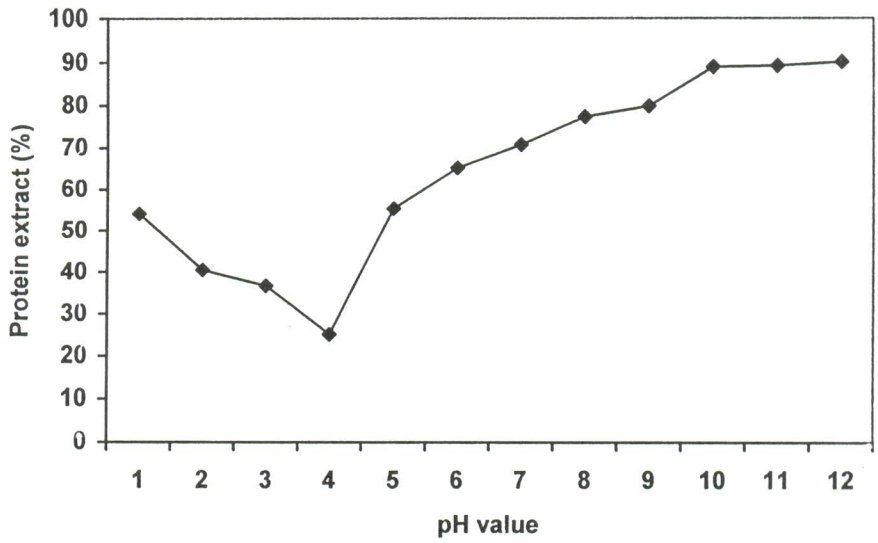

Fig. (2): Effect of pH on the extractability of protein from flax seed meal by-products.

3.1.Extraction of flax meal protein isolate

3.1.1.Effect of pH of the extracting solvent on protein

The results illustrated in Figure (2) demonstrate that the maximum protein extraction was achieved at pH 10. On the acidic pH range, the percentage of the extracted protein was very low and reached its lowest amount at pH 4.0 (isoelectric point).

3.2.Chemical composition of flax meal protein isolate

The data in Table (3) show the chemical composition of flax meal protein isolate. It is clear that protein isolate has high protein content (90.1%), low ash (2.1) and fiber (0.97%) with free trypsine inhibitor activity and cyanoginic glycosides, but it contains little amounts of total phenolic compounds (23.2 mg/g) and phytic acid (0.24). Similar results were obtained by El-Kady *et al.* (2001) and Taha and Mohamed (2003).

Table (3): Chemical composition of flax meal protein isolate (on dry weight basis).

Moisture (%)	Fat (%)	Protein (%)	Ash (%)	Fiber (%)	Trypsin inhibitor (mg/g)	Total cyanogenic (mg/g)	Total phenolic compounds (mg/100 g)	Phytic acid (%)
8.4	0.0	90.1	2.10	0.97	0.0	0.0	23.2	0.24

3.3.Effect of flax treatments on the removal of antinutritional factors

The effect of different treatments of flax meal are presented in Table (4) which indicated that roasting and autoclaving of flax meal for 20 min inactivated trypsin inhibitor to 80.4% and 54.8%, respectively.

The cyanogenic glycosides were totally destroyed after 20 min autoclaving and roasting. As shown in Fig (3) autoclaving seems more effective on the destruction of total phenolic compounds compared with roasting and solvent treatments but less effective on the destruction of phytic acid compared with roasting. Similar observation was reported by Vijayakumari *et al.* (1998).

3.4.Effect of different treatments on flax meal *In vitro* protein digestability

The results in Fig. (4) show that the digestability index for flax meal before treatments was 61% while after treatment with different organic solvents protein the index was increased. On the other hand, roasting, autoclaving and protein isolate gave the higher digestability index than the other treatments 84.8%, 85.2% and 89.2%, respectively. This increment was due to the decrease of antinutritional matters from flax meal.

Table (4): Effect of different treatments on the removal of antinutritional factors from flax meal.

Residual / Treatments	Trypsin inhibitor (%)	Cyanogenic glycosides (%)	Phytic acid (%)	Total phenolic compounds (%)
Solvents:				
Hexane	100	100	100	100
Methanol	89.4	13	84.38	56.8
Ethanol	85.1	45	80.2	81.9
Isopropanol	84.8	31	83.4	86.3
Heating:				
Roasting	45.2	0.0	42.69	33.6
Autoclaving	19.6	0.0	68.75	25.9

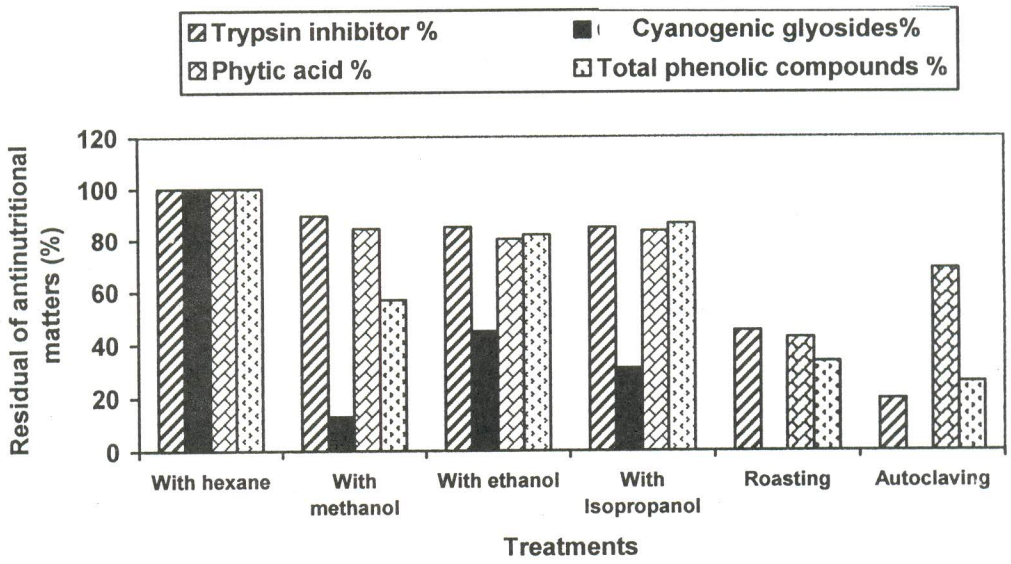

Fig. (3): Effect of different flax treatments on the removal of antinutritional factors

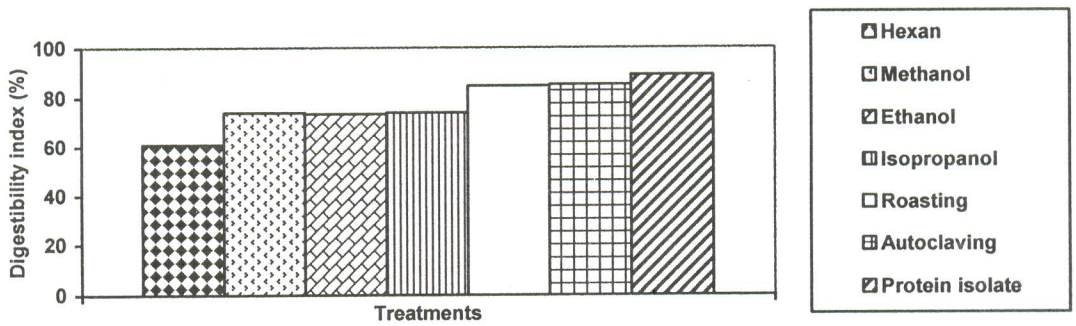

Fig. (4): Effect of different treatments on the protein digestibility index of flax meal

3.5.Amino acid profile of flax meal and protein isolate

The results of amino acid analysis of flax meal and protein isolate are presented in Table (5). The provisional amino acid scored pattern proposed by FAO/WHO (1973) qualified an ideal protein as one in which 36% of the total residues are essential amino acids. The flax meal protein isolate had higher essential amino acid contents than the proposed 36% for an ideal protein.

The data in Table (5) indicate that flax meal protein isolate contains high levels of Arg, Asp, Glu and Leu. but low amount of Lys compared with soybean protein. Consequently, it may be considered as a potential source of high quality plant protein for incorporation into food products (Madhusudan and Singh, 1983).

The data presented in Table (6) show that the initial body weight (BW) and body length (BL) were nearly similar and ranged between 7.79 and 8.02 g for BW, 7.50 and 7.68 cm for BL with insignificant differences between fish groups for both traits. Final BW and BL averaged 30.15 to 39.15 g and 10.40 to 12.30 cm with significant differences between fish groups for BW ($P<0.001$) and BL ($P<0.01$), respectively. Replacing 25% of SBM by RFM in carp diets did not

significantly affect BW or BL, however, the higher replacing levels of 50, 75 or 100% significantly (P<0.001) decreased BW and BL of common carp. The same trend was observed

Table (5): Amino acids profiles of flax meal and flax meal protein isolate (g/100 g protein).

Amino acids	Flax meal	Protein isolate
Essential Amino acids		
Lys	4.1	4.3
Leu	5.7	6.1
Isoleu	4.4	4.6
Cys	1.2	1.4
Met	1.6	1.7
Phe	5.9	5.7
Tyr	3.4	3.3
Thr	3.8	4.0
Val	5.6	5.7
Total E.A.A	35.7	36.9
N. E. A. A.		
His	2.6	2.5
Arg	9.4	9.8
Asp	10.5	10.3
Glu	17.2	16.1
Ser	5.2	5.3
Pro	4.4	4.5
Gly	4.5	4.3
Ala	4.3	4.27
T.N.E.A.A.	58.1	57.07
T.A.A.	93.80	93.97

for weight gain (WG), specific growth rate (SGR). These results revealed the possibility of replacing 25% of the high cost SBM by the low cost RFM in carp diets but increasing the level of RSM in the experimental diets above this level (25%) significantly (P<0.001) decreased BW, BL, WG and SGR of common carp, *C. carpio*.

Feed intake (FI) of *C. carpio* was insignificantly changed until the replacing level of SBM by RFM reached 50%, thereafter, replacing level (50%) FI significantly decreased. Feed conversion ratio (FCR) ranged from 2.43 to 3.15 (Table, 7). The best rate was obtained when fish were fed the control (RFM0) diet where only 1.95 kg of feed was required to produce one kilogram of live fish. Increasing replacing level of SBM by RFM from 50 to100% (with an increment of 25%) in *C. carpio,* diets did not significantly affect the FCR. The best protein efficiency ratio (PER) was obtained when 25% of SBM protein was replaced by RFM and the worst PER was obtained with fish fed RFM2100 diet.

Table (6): Growth performance, feed utilization and proximate analysis of common carp as affected by replacing SBM by RFM.

Tested parameter	No.	Diets					
		RFM0	RFM25	RFM50	RFM75	RFM100	SE
Body weight (g)							
Initial	40	7.80	8.01	7.81	7.79	8.02	0.20
Final	40	39.15 a	39.00 a	32.40 b	31.00 b	30.15 c	0.32
Body length (cm)							
Initial	40	7.50	7.60	7.44	7.50	7.68	0.05
Final	40	12.30 a	12.10 a	11.60 ab	11.8 ab	10.40 b	0.11
Weight gain (g/fish)	2	31.35 a	30.99 a	24.59 b	23.21 b	22.13 b	0.80
Specific growth rate	2	1.80 a	1.76 a	1.58 b	1.54 b	1.47 b	0.15
FI (g/fish)	2	80.50 a	75.37 a	75.60 a	70.00 b	69.80 b	2.01
FCR	2	2.57 b	2.43 b	3.07 a	3.02 a	3.15 a	0.76
PER	2	1.30 a	1.37 a	1.08 b	1.11 b	1.06 b	0.03

Means with the different letters in each row for each trait are significantly different (P<0.05).
FI: Feed intake. FCR: Feed conversion ratio PER: Protein efficiency ratio

The data presented in Table (7) show that the initial BW and BL were nearly similar and averaged 7.66 to 8.14 g and 7.65 to 7.98 cm, respectively with insignificant differences between fish groups for both traits. Final BW and BL ranged between 30.73 to 40.10 g and 11.90 to 12.30 cm with significant (P<0.0001) differences between fish groups. The highest BW was recorded for the group fed ROFM75 diet (replacing level of 75%). The lowest BW was recorded for fish fed the ROFM100 diet where SBM was totally replaced by ROFM and the differences in BW and BL between fish groups fed the experimental diets were significant (P<0.001). This trend was obtained for WG and SGR. These results revealed the possibility of replacing 75% of the high cost SBM by the low cost ROFM in carp diets without any adverse effect on growth parameters (BW, BL, WG and SGR), but the complete replacement of SBM by RFM in carp diets significantly adversely affected all these growth parameters.

The highest FI was recorded for fish fed ROFM25 followed in a descending order by ROFM0, ROFM50, ROFM75 and ROFM100, respectively. The best FCR was obtained when fish were fed the ROFM75 diet (75% of SBM replaced by ROFM). PER decreased gradually with increasing replacing level of SBM by ROFM up to 75% but the complete replacement increased PER.

Table (7): Growth performance, feed utilization and proximate analysis of common carp as affected replacing SBM by ROFM.

Tested parameter	No.	Diets					
		ROFM0	ROFM25	ROFM50	ROFM75	ROFM100	SE
Body weight (g)							
Initial	50	7.93	7.70	7.98	8.14	7.66	0.30
Final	50	38.10 a	37.18 a	36.78 a	40.10 a	30.73 b	1.50
Body length (cm)							
Initial	50	7.84	7.65	7.98	7.88	7.67	1.33
Final	50	12.15	12.00	12.11	12.30	11.90	1.13
Weight gain (g/fish)	2	30.17 a	29.48 a	28.80 a	31.96 a	23.07 b	2.15
Specific growth rate	2	1.74 a	1.75 a	1.69 a	1.77 a	1.54 b	0.03
FI (g/fish)	2	74.60	77.21	70.60	68.30	66.80	2.10
FCR	2	2.47 c	2.61 b	2.45 c	2.14 d	2.90 a	0.16
PER	2	1.35 b	1.27 c	1.36 b	1.56 a	1.15 d	0.03

Means with the different letters in each row for each trait are significantly different (P<0.05).

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تحسين القيمة الغذائية لكسب الكتان واستخدامه فى تغذية أسماك المبروك

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ملخص

دلت نتائج التحليل الكيميائى لكسب الكتان صنف فيكنج على احتوائه على ٣٦,٩% بروتين . تم تقدير العوامل المضادة للتغذية مثل مثبط التربسين والجليكوسيدات السيانوجينية وحمض الفيتيك والفينولات الكلية فكانت ٢,٥ مجم/جم، ٠,٤٢ مجم/جم، ٢,٩%، ١٩٦ مجم/١٠٠ جم على الترتيب.

أظهرت النتائج أن معاملة كسب الكتان بالتحميص وكذلك التسخين بالبخار فى الأوتوكلاف لمدة ٢٠ دقيقة أدى إلى التخلص من معظم مضادات التغذية مقارنة بالمذيبات، كما أدت هذه المعاملات إلى التخلص كلياً من جليكوسيدات السيانوجين وقد أظهرت النتائج كذلك أن طريقة التسخين فى الأوتوكلاف كانت أكثركفاءة من التحميص فى التحلص من كل المركبات السامة الموجودة فى كسب الكتان فيما عدا حمض الفيتك.

وباستخدام كسب الكتان فى تغذية أسماك المبروك وجد أن إحلال ٢٥% من كسب فول الصويا بكسب الكتان الخام فى علائق أسماك المبروك العادى لم يؤثر معنوياً على وزن وطول الجسم أما نسب الإحلال الأكبر ٥٠ , ٧٥ , ١٠٠% فقد أدت إلى خفض وزن وطول الجسم معنوياً لأسماك المبروك لوحظ هذا الإتجاه بالنسبة لمقدار الزيادة فى وزن الجسم ومعدل النمو. كما وجد أن زيادة نسبة الإحلال من ٥٠ إلى ١٠٠ % لم تؤثر معنوياً على معدل تحويل الغذاء كما وجد أن كفاءة تحويل البروتين قد وصلت إلى أقصى قيمها عندما كانت نسبة الإحلال ٥٠%.

أظهرت تجربة النمو الثانية أن أكبر قيم لوزن وطول الجسم قد سجلت للأسماك التى تغذت على العليقة التى كانت نسبة الإحلال بها ٧٥% كما أعطت الأسماك التى تغذت على العليقة التى تمت فيها عملية الإحلال بنسبة ١٠٠% أقل قيم لوزن وطول الجسم. كما أن نسبة الإحلال ٧٥% أعطت أفضل لمعدل تحويل الغذاء. ومن ذلك يستنتج أنه يمكن إحلال كسب فول الصويا بكسب الكتان الخام بنسبة تصل إلى ٢٥% فقط كما يمكن زيادة هذه النسبة إلى ٧٥% فى حالة إستخدام كسب الكتان المعامل بالتحميص.