EFFECT OF SOME PROCESSES ON THE ANTINUTRITIONAL FACTORS OF CANOLA SEEDS AND ITS UTILIZATION FOR RED TILAPIA FISH DIETS

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ABSTRACT

Canola meal is an excellent source of protein (44.6%). The antinutritional factors; trypsin inhibitor, total polyphenol and phytic acid were determined to be 13, 0.7 and 5.1%, respectively. Soaking canola seeds in water and 0.1% citric acid (pH 4.94), resulted in gradual decline in all antinutritional factors examined during 12 h. These processes were effective in removing 12 -24% trypsin inhibitor, 7 -13% polyphenol and 18 -30% phytic acid. Soaking in 0.1% citric acid was effective in removing antinutritional factors except trypsin inhibitor. Heating was more effective than soaking in activating trypsin inhibitor but heating seed soaked for 12 h resulted in the removal of high amounts of antinutritional factors.

Two growth trials were conducted to assess the potential for incorporation canola seed meal (CM) as a partial or complete replacement for fish meal in diets of the red tilapia. Fish meal in the control diet was replaced proportionally (25, 50, 75 and 100%) by either raw canola seed meal (RCM) in the first experiment or heated canola seed meal (HCM) in the second experiment, either RCM or HCM incorporated in the experimental diets at rates of 10.23, 20.46, 30.69 and 40.92% in the diets 2, 3, 4 and 5 (for each experiment), respectively. Replacing fish meal by RCM at all replacing levels significantly adversed final body weight (BW), body length (BL), weight gain (WG), specific growth rate (SGR). The same trend was observed for feed utilization parameters whereas all replacing levels of fish meal by RCM significantly adversed feed conversion ration (FCR), decreased feed intake (FI) and decreased protein efficiency ratio (PER). Replacing fish meal by HCM up to 50% did not significantly affect all growth parameters (BW, BL, WG and SGR) and all feed utilization parameters (FI, FCR and PER) but the highest replacing levels (75 and 100%) significantly adversed all growth and feed utilization parameters.

Key words: *antinutritional factors, canola seeds, red tilapia fish.*

1. INTRODUCTION

Oil seeds are widely consumed as a source of edible oils and energy for human consumption. The remaining meals after oil extraction are rich in protein, dietary fibers and carbohydrates. Oil seed meals are consumed mainly for animal and poultry feeding (Abu-Shama, 1998). Canola seed (*Brassica napus*) contains about 40-45% oil and a good quality protein of up to 35% (Katiyar and Chamola, 2003). Breeding was successful to yield new varieties of canola seeds free of erucic and glucosinolate (Larsen and Sorensen, 1985). Canola meal is an important source of high quality plant protein for human consumption but the meals contain various antinutritional factors which elicit adverse nutritional effects such as phytic acid, phenolic compounds and trypsin inhibitor activity that play an important role in decreasing the nutritional value of oil seed meal foods (Barimalaa and Anoghal 1997; Bau *et al.* 1997).

Removal of undesirable components is essential to improve the nutritional quality of canola and effectively utilize their full potential as human food. It is widely accepted that simple and inexpensive processing techniques are an effective method of achieving desirable changes in the composition of seeds. Soaking could be one of the processes to remove soluble antinutritional factors which can be eliminated with the discarded soaking solution, but some metabolic reactions can take place during soaking and affect the content of some compounds (Vidal-Valverde *et al.* 1992). Vidal-Valverde *et al.* (1994) found that soaking lentil seed in $NaHCO_3$

did not seem as efficient as water in reducing the phytic acid content, whereas the citric acid solution was more efficient than water alone.

Citric acid is widely distributed in plant and animal tissues and is an intermediate in the Krebs cycle. It is odorless white solid and extensively used in medicine for many years without any adverse effects. Moreover, citric acid and its salts are widely used as chelators and acidulants in food industry. It is used as a synergist both primary antioxidant and oxygen scavengers at levels of 0.1-0.3% (Maga and Tu, 1994). Recently, in (1998) Han *et al.* reported that, addition of 1.5% citric acid to 10-15% wheat middling and 300u/kg microbial phytase in maize-soya diets for growing pigs gave a high gain to feed ratio. Besides, Drusch *et al.* (1995) concluded that the addition of citric acid improved Zn absorption from phytin complex in male rats. However, Tawfeek *et al.* (1994) in their study on New Zealand white (NZW) rabbits had given a basal diet alone or that diet supplemented with 0.5% citric acid, found that supplementing citric acid, increased digestibility of crude protein (CP), crude fiber and the percentage of nitrogen retained, while digestibility of ether extract, organic matter and total digestible nutrients was not affected. In addition, Niiyama *et al.* (1992) reported that citric acid prevented renal calcium accumulation in rats fed with diets containing citric acid (1.7 and 2.3%) and different protein sources. Many authors reported that soaking, cooking, microwave heating and germination improve quality of seeds because of the removal of some antinutritional factors (Barampama and Simard, 1994; Vidal-Valverde, *et al.* 1994; Abd El-Rahman and Abd El Aleem, 1996 and Haddad and Allaf, 2004). The purpose of the present investigation was to study the removal of antinutritional factors from canola seed meal by soaking followed by heating. The produced protein was isolated and evaluated using both raw and treated canola meal in red tilapia fish diets formulation.

2. MATERIALS AND METHODS

2.1.Sampling: Seeds of canola (*Brassica napus* L.) described as "double zero" because of the absence of erucic acid and low glucosinolate were used. Seeds were kindly supplied from the Oil Crops Research Section, Agricultural Research Center, Egypt.

2.2.Soaking treatment

Raw seeds were soaked at room in distilled water and 0.1% citric acid solution (pH 4.94) for 3, 6, 9 and 12 hours. The soaked seeds were drained, weighed and some are ground.

2.3.Heating treatment

The mature soaked and unsoaked seeds were heated at 100°C for 40 min and were left to cool at ambient temperature. Seeds were cleaned and ground. Hexane was used for the extraction of oil from ground seeds according to Vidal-Valverde *et al.* (1994).

2.4.Extraction of canola meal protein

Canola meal protein was extracted according to the method described by Klockeman *et al.* (1997). The extracted protein was precipitated at isoelectric point of the protein. The precipitate was washed by distilled water and centrifuged, then freeze dried (protein isolate).

2.5.Chemical analysis

Moisture, crude fat, total protein, crude fiber and ash contents were determined in canola meal 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 was determined as mentioned by Latta and Eskin (1980) and total phenols were calorimetrically 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 determination of amino acids in canola meal according to Moore *et al.* (1958). Cystine was microbiologically determined as described by Barton (1952). Tryptophan

was colorimetrically determined in the alkaline hydrolysate of samples adopting the method of Blouth *et al.* (1963).

2.6.Experimental diets

Ten experimental diets were formulated (Table 1) to replace 0, 25, 50, 75 or 100% of fish meal by raw canola meal (untreated), canola meal (RCM), or treated (soaking followed by heating) canola meal (SHCM) as a partial or total replacement of fish meal. All diets were formulated to be isonitrogenous (30% protein) and isocaloric (2600 kcal metabolizable energy/kg diet). In preparing the diets, dry ingredients were first ground to a small particle size. Ingredients were thoroughly mixed and then water was added to obtain 30% moisture level. Diets were passed through a mincer with a diameter of 2 mm and dried for 48 h.

2.7.Experimental system and animals

For each experiment, ten rectangular aquaria 100 × 40 × 50 cm (200 liter for each) were used in two replications. All aquaria were aerated with compressed air and each aquarium was stocked with 15 red tilapia fish obtained from Abbassa hatchery. Fish in the two experiments were given the pelleted diets (2 mm in dia.) at a daily rate of 4% and fed 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). Records of live BW (g) and BL (cm) of individual fish were measured initially and at the end of the experimental period for each aquarium. Growth parameters were measured by using the following equations:

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

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).

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

Ingredients	Diets				
	SHCM0	SHCM25	SHCM50	SHCM75	SHCM100
Fish meal	20	15	10	5	0
Yellow corn	31	28	27	27	25
Soybean meal	32	32	32	33	34
Canola meal*	0	9.3	18.6	27.9	35.5
Wheat bran	10.5	10.2	6.9	1.6	0
Vegetable oil	3	2	2	2	2
Vit. & Min. Mixture[1]	3.5	3.5	3.5	3.5	3.5
Sum	100	100	100	100	100
Proximate analysis	*(determined on dry matter basis)*				
Moisture	6.15	7.24	6.37	6.54	6.14
Crude protein (CP)	31.00	30.18	29.97	30.27	29.31
Ether extract (EE)	4.28	4.12	5.62	5.62	6.12
Crude fiber (CF)	7.72	8.02	10.99	10.32	11.25
Ash	10.00	10.21	10.17	11.54	11.33
NFE[2]	47.00	47.47	43.25	42.25	41.99
ME[3] (Kcal/kg diet)	2648.0	2620.4	2600.8	2650.0	2600.2
P/E ratio[4]	117.1	115.2	115.2	114.2	112.7

* Raw canola meal for the first experiment and treated canola meal for the second experiment

[1] 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.

[2] Nitrogen free extract (NFE) =100-(CP+EE+CF+Ash)

[3] Metabolizable energy was calculated from ingredients based on NRC (1993) values for tilapia.

[4] Protein to energy ratio in mg protein/kcal ME.

2.8. 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 canola meal (Table 2) exhibited high crude protein (44.6) which confirms that canola meal is an excellent source of protein (El-Morsi *et al.* 2000 ; Abbas, 2004).

The antinutritive factor trypsin inhibitor, phytic acid and total phenolic compounds in the canola meal were 0.13%, 5.1% and 0.7%, respectively. These data are in agreement with those of El-Morsi *et al.* (2000).

Table (2): Chemical composition of canola meal (g/100 g dry weight basis).

Moisture (%)	Protein (%)	Fat (%)	Ash (%)	Fiber (%)	Phytic acid (%)	Total phenoles (%)	Trypsin inhibitor (%)
8.3	44.6	1.6	7.6	7.1	5.1	0.7	0.13

3.1.Extraction of canola meal protein isolate

These experiments were carried out to detect the proper pH values required for canola meal protein extraction. Results are presented in Figure (1). The maximum protein extraction was achieved at pH 10.0. On the other hand, 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).

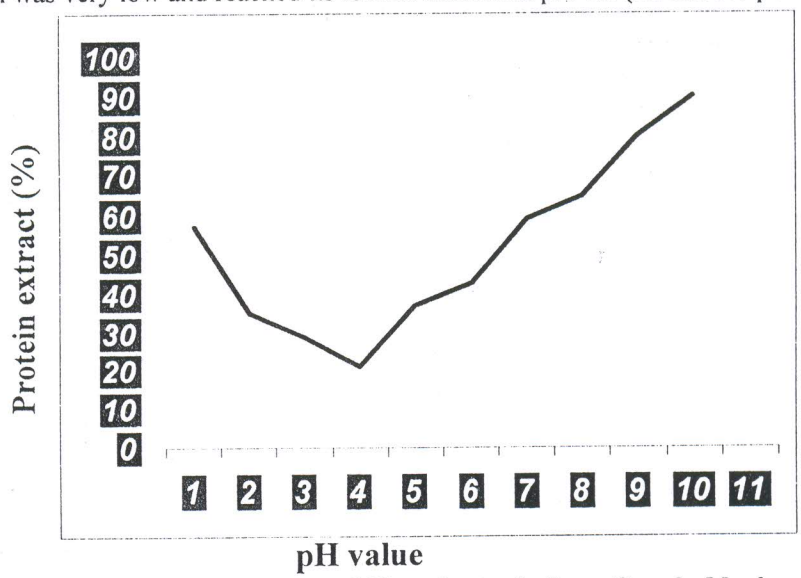

Fig. (1) : Effect of pH on extractability of protein from Canola Meal

3.2.Amino acids of canola meal and protein isolate

Results of amino acids analysis of canola meal and protein isolate are presented in Table (3). The provisional amino acids scoring pattern proposed by FAO/WHO (1973) qualified and ideal protein as one in which 36% of the total essential amino acids. Canola meal protein isolate had higher essentiai amino acids than the proposed 36% for an ideal protein. In general, the profile of essential amino acids of canola protein isolate was similar or even better than soy protein isolate and could supply the requirements of all age groups (El-Morsi *et al.* 2000).

3.3.Effect of soaking and heating on antinutritional factors

Soaking canola seeds in water and 0.1% citric acid (pH, 4.94) caused a gradual decline in all antinutritional factors examined during 12 hr soaking period (Figure 2). This processing method is effective in removing 12 - 24% of trypsin inhibitor, 7 - 13% of polyphenol and 18 - 30% phytic acid. Soaking in 0.1 citric acid was more effective than water in removing antinutritional factors except trypsin inhibitor. The results presented in (Figure 3) indicated that heating was more effective than soaking in activating trypsin inhibitor but heating seed soaked for 12 h resulted in the removal of high amounts of antinutritional factors. Similar observation was reported by Abd El-Aleem (2000).

Table (3): Amino acids of canola seed meal and protein isolate (g/100 g protein).

Amino acids	Canola meal	Protein isolate
Essential Amino acids		
Lys.	5.79	5.90
Leu.	6.30	6.50
Isoleu.	3.40	3.50
Cys.	0.82	0.93
Met.	1.46	1.52
Phe.	4.40	4.40
Tyr.	2.99	2.90
Thr.	4.92	5.19
Val.	4.96	4.84
Try.	1.20	1.30
Total E.A.A.	36.24	36.98
N. E. A. A..		
His.	2.90	2.70
Arg.	6.80	7.10
Asp.	8.50	7.60
Glu.	16.90	15.50
Ser.	4.50	3.80
Pro.	5.90	6.70
Gly.	5.40	4.60
Ala.	4.80	4.40
T.N.E.A.A..	55.70	52.40
T.A.A.	91.94	89.38

3.4.Effect of processes on in-vitro protein digestibility

The results in Figure (4) show that the *in vitro* protein digestibility of the unprocessed canola seed was 74.5%. Soaking the seeds for 12 h in water and citric acid solution increased the digestibility by 2-3%. On the other hand, soaking in citric acid and heating gave the highest the digestibility by 2-3%. On the other hand, soaking in citric acid and heating gave the highest digestibility index (86.7). The increase in protein digestibility after soaking and heating could be partially attributed to the protein denaturation which improve protein susceptibility to enzyme attack. Furthermore, inactivation of trypsin inhibitor would certainly improve protein digestibility (Youssef *et al.* 1985).

3.5.Growth trials

3.5.1. First experiment

The data presented in Table (4) show that the final body weight (BW) and body length (BL) averaged 35.61 to 50.70 g and 12.11 to 15.13 cm with significant ($P<0.001$) differences between fish groups for BW and BL, respectively. Values of final BW, BL, weight gain (WG) and specific growth rate (SGR) after 90 days were inversely related to the level of RCM in the tested diets.

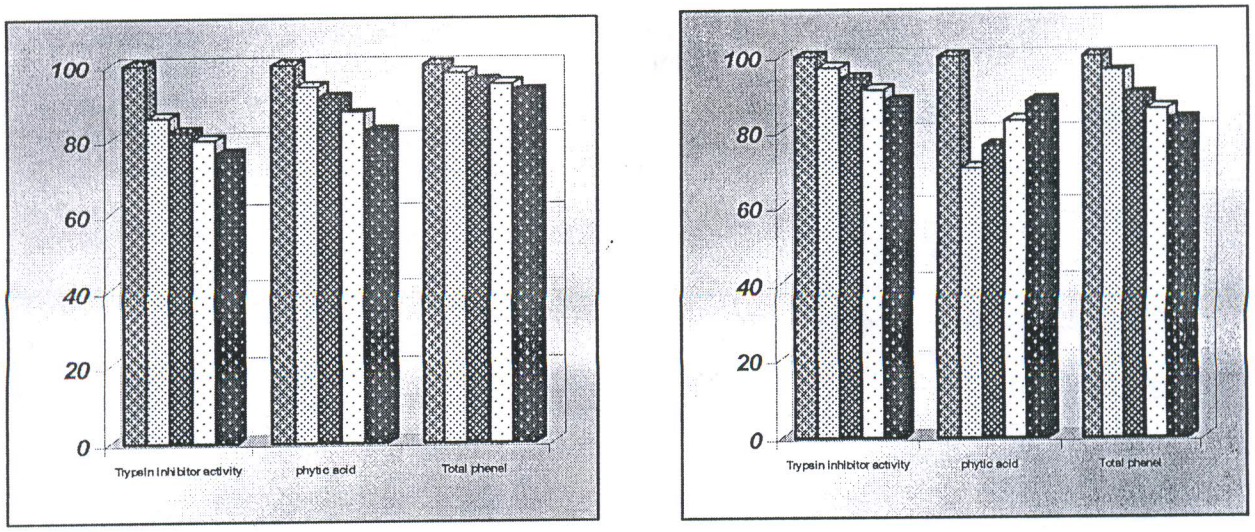

Fig (2) Effect of soaking on antinutritional factors:

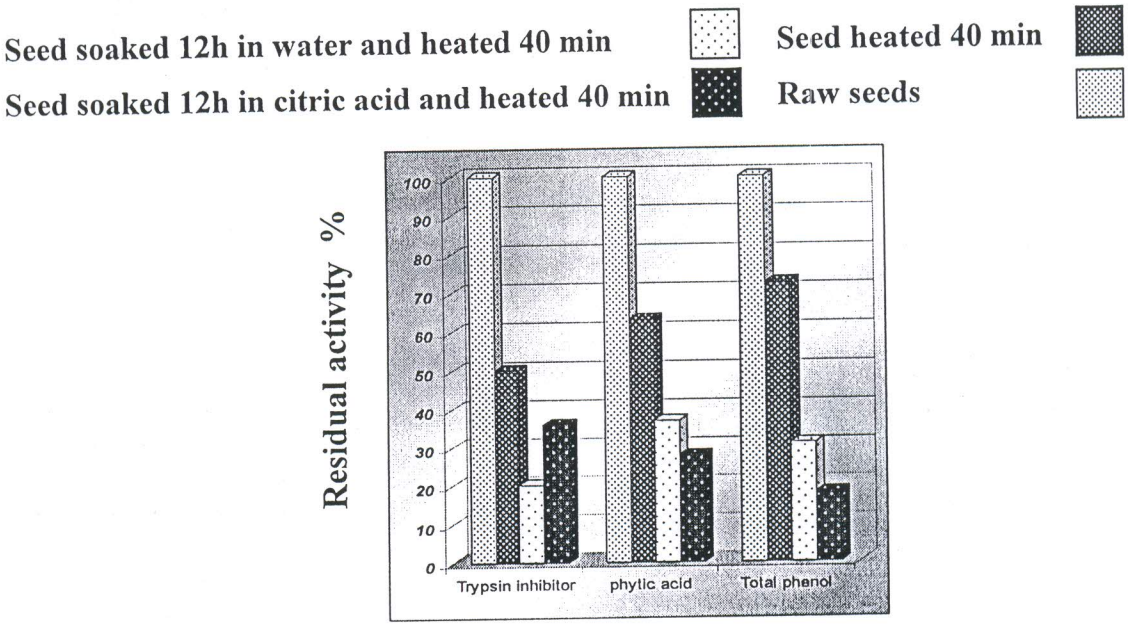

Fig (3) Effect of heating on antinutritional factors:

Accordingly, the best growth performance among the fish fed diets occurred in fish group fed the control fish meal-based diet (RCM0). Feed utilization parameters, feed intake (FI), feed conversion ratio (FCR) and protein efficiency ratio (PER) were parallel to growth performance parameters. Incorporation of RCM in tilapia diet, irrespective of its level (9.30, 18.60, 27.90 and 35.50%), as fish meal substitute (25, 50, 75 and 100%) led to significant reduction in all growth parameters (BW, BL,

WG and SGR) and feed utilization parameters (FI, FCR and PER). Studies of Davies *et al.* (1990) indicated a practical inclusion limit of 15% in tilapia diet. Higher growth and feed efficiency were obtained in young Yellowtail fish with diet containing 10% of RCM and further increase up to 20 or 30% had a negative effect on growth parameters (Shimeno *et al.* 1993). In the same trend, Webster *et al.* (1997) found that, incorporation of RCM in channel catfish (*Ictalurus punctatus*) diets up to 12% did not significantly affect BW, FCR and PER. However, the higher incorporation levels (24 or 48%) significantly adversed these parameters.

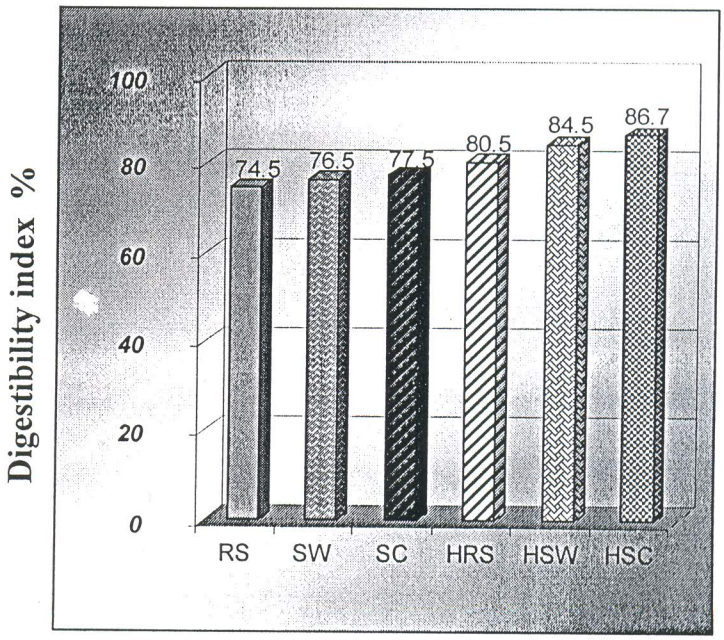

Fig (4) Effect of processing on protein digestibility

RS, Raw seed; **SW**, seed soaked in water (12 h); **SC**, seed soaked in citric acid (12h); **HRS**, Heated raw seed; **HSW**, Heated soaked in water; **HSC**, Heated soaked in citric acid

3.5.2.Second experiment

The growth and other related parameters for red tilapia fed on the experimental diets are presented in Table (5). Final BW and BL ranged from 35.12 to 51.30 g and 12.90 to 15.12 cm with significant ($P<0.001$) differences between fish groups for BW and BL, respectively.

The higher BW or BL was recorded for fish fed the diet SHCM25, however, the lower one was observed for fish group fed the diet SHCM100 where fish meal was totally replaced by SHCM. Similar trend was observed in respect to WG and SGR. The trend for feed utilization paralleled that for growth whereas the diet SHCM25 showed the highest FI and the best FCR values and the diet SHCM100 showed the worst values for FI and FCR.

The results of growth and feed utilization (Table 5) indicate that substitution of 25 or 50% of fish meal in the control diet by SHCM did not significantly affect growth performance parameters (BW, BL, WG and SGR) and also feed efficiency parameters (FI, FCR and PER). However, the higher replacing levels (75 or 100%) significantly reduced all growth and feed efficiency parameters and this probably because of the reductions in the diet palatability and the presence of low levels of anti-nutritional factors. The depression in growth performance (at the high incorporation levels of SHCM, 75 and 100%) was also due to a decrease in feed intake.

Table (4): Growth performance, feed utilization and proximate analysis of red tilapia as affected by replacing FM by RCM.

	No.	Diets					±SE	Prob.
		RCSM0	RCSM25	RCSM50	RCSM75	RCSM100		
Growth performance								
Body weight (g)								
Initial	30	12.30	12.29	12.70	12.93	12.00	0.11	0.8755
Final	30	50.70 a	44.90 b	40.30 c	37.18 cd	35.61 d	0.45	0.0011
Body length (cm)								
Initial	30	8.12	8.00	7.96	8.54	8.20	0.12	0.9584
Final	30	15.13 a	14.00 b	14.10 b	13.12 c	12.11 c	0.10	0.0021
Weight gain (g/fish)	2	38.10 a	32.61 b	27.60 c	24.25 d	23.61 d	0.78	0.0012
Specific growth rate	2	1.57 a	1.43 b	1.28 c	1.17 d	1.21 e	0.11	0.0024
Feed utilization								
FI (g/fish)	2	66.13 a	57.12 b	55.54 b	53.11 bc	50.76 c	1.14	0.0001
FCR	2	1.72 e	1.75 d	2.01 c	2.19 b	2.15 a	0.07	0.0001
PER	2	1.94 a	1.90 b	1.66 c	1.52 d	1.55 e	0.12	0.0011

Table (5): Growth performance, feed utilization and proximate analysis of red tilapia as affected by replacing FM by SHCM.

		Diets					±SE	Prob.
		HCSM0	HCSM25	HCSM50	HCSM75	HCSM100		
Growth performance								
Body weight (g)								
Initial	30	12.21	12.30	12.81	11.91	12.13	1.85	0.9887
Final	30	51.30 a	51.80 a	48.70 a	40.00 b	35.12 b	2.66	0.0040
Body length (cm)								
Initial	30	8.30	8.13	8.15	8.50	8.22	0.80	0.8878
Final	30	15.12 a	15.54 a	14.33 a	13.90 b	12.91 b	1.21	0.0014
Weight gain (g/fish)	2	39.09 a	39.50 a	35.89 a	28.09 b	22.99 b	0.21	0.0001
Specific growth rate	2	1.60 a	1.60 a	1.48 a	1.34 b	1.18 c	0.24	0.0001
Feed utilization								
FI (g/fish)	2	66.25 a	67.23 a	62.31 a	58.72 b	50.46 b	1.42	0.0016
FCR	2	1.69 b	1.70 b	1.74 b	2.09 a	2.19 a	0.17	0.0066
PER	2	1.97 a	1.96 a	1.92 a	1.59 b	1.52 b	0.34	0.0064

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

A lower feed intake of the SHCM-based diets is attributed to the presence of sinapine or tannins, which affect the palatability of the diet. The same was previously found in rainbow trout (Hilton and Slinger, 1986).

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تأثير بعض المعاملات على العوامل المضادة للتغذية لبذور الكانولا واستخدامها فى تغذية أسماك البلطى الأحمر

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ملخص

أجريت هذه الدراسة لتقدير كفاءة استبدال مسحوق السمك جزئياً وكلياً بكسب بذور الكانولا في علائق اسماك البلطي .

دلت نتائج التحليل الكيميائى لكسب الكانولا احتوائه على ٤٤,٦% بروتين خام ، وتم تقدير العوامل المضادة للتغذية مثل مثبط التربسين وحمض الفيتك والفينولات الكلية فكانت ٠,١٣% ، ٥,١% ، ٠,٧% على الترتيب.

وجد أن نقع بذور الكانولا فى الماء أو محلول ٠,١% حمض ستريك نشأ عنه تناقص تدريجى فى جميع العوامل المضادة للتغذية وذلك أثناء عملية النقع التى استغرقت ١٢ ساعة. كان النقع فى حمض الستريك أكثر تأثيراً فى تناقص جميع العوامل المضادة للتغذية فيما عدا مثبط التربسين.

ولقد دلت النتائج أن التسخين كان أكثر تأثيراً من النقع فى تقليل نشاط مثبط التربسين كما أن النقع لمدة ١٢ ساعة ثم التسخين قد أزال كميات عالية من العوامل المضادة للتغذية عند مقارنتها بالبذور التى تم تسخينها بدون نقع.

أظهرت تجربة التغذية الأولى لأسماك البلطى أن احلال مسحوق السمك بكسب الكانولا الخام بجميع نسب الإحلال أدى إلى انخفاض صفات النمو (طول ووزن الجسم ــ الزيادة فى وزن الجسم ــ معدل النمو) كما لوحظ نفس الاتجاه بالنسبة للكفاءة الغذائية للعليقة.

وقد أظهرت نتائج التجربة الثانية أن احلال مسحوق السمك بكسب بذور الكانولا المعامل حرارياً حتى ٥٠% لم يؤثر معنوياً على صفات النمو والاستفادة من الغذاء أما نسب الاحلال الأعلى ٧٥ و ١٠٠ % فقد أثرت تأثيراً سلبياً على صفات النمو والاستفادة من الغذاء.