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PARTIAL AND TOTAL REPLACEMENT OF SOYBEAN MEAL BY RAW AND HEAT TREATED LINSEED MEAL IN TILAPIA DIETS

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SUMMARY

The effect of replacing soybean meal protein (SBM) by raw linseed meal (RLSM), roasted linseed meal (ROLSM) and autoclaved linseed meal (ALSM) in tilapia diets on growth performance, feed utilization and body composition were evaluated in three separate experiments. SBM protein was replaced by RLSM, ROLSM or ALSM proteins in the 1st, 2nd and 3rd experiment, respectively at replacing levels of 0, 25, 75 and 100% for each experiment. The anti-nutritional factors, trypsin inhibitor, total cyanogenic, total polypenolic compounds and phytic acid in RLSM were determined to be 12.50 mg/g, 0.42 mg/g, 196 mg/100g and 2.90%, respectively. Roasting (140°C for 20 min) or autoclaving (121°C for 20 min) of RLSM totally destroyed cyanogenic glycosides and destroyed 54.80% and 80.40% of trypsin inhibitor, 66.40 and 74.10% of total polyphenolic compounds, 57.24% and 31.38% of phytic acid, respectively. Results of the first experiment indicated that, replacing 25% of SBM protein by RLSM protein in tilapia diets did not significantly affected body weight (BW) or body length (BL) however, the higher replacing levels, 50, 75 or 100% significantly ($P<0.001$) decreased BW and BL of Nile tilapia and the same trend was also observed for weight gain (WG), specific growth rate (SGR). The best feed conversion rate (FCR) was obtained when fish fed the RLSM25 diet.

Results of the second experiment showed that, the highest BW, WG and SGR values were recorded for fish group fed ROLSM25 diet (replacing level of 25%) however, the lowest ones were recorded for fish fed the ROLSM100 diet where SBM protein was completely replaced by ROLSM protein and the differences were significant ($P<0.001$). The best FCR was obtained for fish fed the diet ROLSM75. Similar results were obtained in the third experiment when SBM protein was replaced by ALSM protein.

From economic point of view, it was observed that replacing 25% of SBM by RLSM in tilapia diets reduced feed costs by 7.37% while replacing 75% of SBM by ROLSM or ALSM reduced feed costs by 18.83 and 17.58%, respectively without adverse effect on growth performance and feed utilization parameters.

***Keywords**: linseed meal, roasting,, autoclaving, soybean meal replacement, growth, feed utilization, tilapia.*

INTRODUCTION

Aquaculture has become the fastest-growing food production sector of the world, with an average annual increase of about 10% since 1984 compared with a 3% increase for livestock meat and a 1.6% increase for capture fisheries (FAO, 1997). To sustain such high rate of increase in aquaculture production; similar increase in the levels of fish feed production is required. The intensive use of soybean meal in poultry and fish feeds led to

increasing price of soybean meal with its unavailability. In 2003, Egypt imported one million ton of soybean in forms of seeds or meals (Osman and Sadek, 2004). In this context, research efforts have been directed to identify novel, alternative and economically viable plant protein sources for partially or completely replacing SBM in the fish feed. One of the possible alternatives plant protein source is linseed meal. Linseed (*Linum usitatissimum*) is cultivated for the production of fiber and oil. The average cultivated area of linseed in Egypt during the last years reached 21267 feddan with an average production of 711 kg seed/feddan as reported by the Ministry of Agriculture (2002). The resultant meal after oil extraction contains 33-45% protein with a good amino acid balance (Lee *et al.* 1991; Fahmy *et al.* 1996; El-Saidy and Gaber, 2001 and El-Kady *et al.* 2001 and Abdel-Fatah, 2004). Metabolizable energy (ME) in LSM was found to be 1700 Kcal/kg (Abbas *et al.* 1990). However due to the presence of several anti-nutritional factors such as cyanogenic glycosides, phytic acid, phenolics, trypsin inhibitor and mucilage limits the use of LSM in fish diets (Abdel-Hakim *et al.* 2003). Therefore, RLSM must be treated before incorporation in fish diets to reduce their contents of anti-nutritional substances and improve its nutritional value.

Flax seeds contains 5-8% mucilage (DeMiller, 1986 as cited by Fedeniuk and Biliaderis, 1994) and is a heterogenic polysaccharide that can be divided into acidic and neutral components (Cunnane *et al.* 1993 and Fedeniuk and Biliaderis, 1994). Mucilage also has a large capacity to bind to water and increases intestinal viscosity thus, reducing nutrient digestibility (Fedeniuk and Biliaderis, 1994). However, mucilage is heat stable and must be removed from the flax meal by other processing to eliminate its negative nutritional effects. Wanasundara and Shahidi (1997) found that the soaking of linseed in water or sodium bicarbonate solution reduced the amount of mucilage remaining in the seeds.

Phytic acid negatively affects the utilization of minerals which can be seen by its ability to bind up to 75% of all phosphorus (NRC, 1998). It can chelate di and trivalent metals including calcium, magnesium, zinc and iron into compounds that are less easily absorbed in the intestine. Phytic acid also has the ability to nonselectively bind to protein and inhibit activities of a number of digestive enzymes such as pepsin, trypsin and alpha-amylase (Liener, 1994). Negative effects were seen when rainbow trout was fed purified diets containing phytic acid. Phytic acid was included in the diets at levels typical of salmon diets (0.50%), leading to a reduction in growth and feed conversion between 8-10% over 150 day period (Spinelli *et al.* 1983).

Toxicity of cyanogenic glycosides 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 linseed meal by boiling in water, dry and wet autoclaving; acid treatment followed by autoclaving and alkanol treatments (Wanasundara and Shahidi, 1997 and El-Kady *et al.* 2001). The use of methanol is efficient in removing of glucosinolate and phenolic compounds (Abu-Shama, 1998 and Taha 2003) from rapeseed and sunflower meals.

There is little information available on the use of LSM as a plant protein source in aquatic animals feeds, therefore the present study was carried out to evaluate the effect of soaking or autoclaving of LSM on removing anti-nutritional factors and studying the possibility of replacing SBM by each of raw, roasted or autoclaved linseed meal in Nile tilapia diets.

MATERIALS AND METHODS

Processing of LSM and diets preparation:

Defatted linseed meal (variety Vaiking) was prepared from pressed seed meal obtained from Shobra melles, Zefta, Egypt. LSM was divided into 3 parts, each part was subjected to the following treatments: the first one was served as RLSM without any treatment, the second and the third parts were soaked in water (1:3 w/v) for 5 hrs with intermittent stirring and an hourly change of water to remove mucilage. The residual meal was separated by vacuum filtration. After vacuum filtration, the second part was roasted (140°C for 20 min) while the third part was autoclaved (121°C at 15 psi for 20 min maintaining a meal-water ratio of 1:5 w/v). Three separate experiments were conducted in the present study to evaluate each of RLSM, ROLSM and ALSM as substitute ingredients for SBM in tilapia diets at levels of, 0, 25, 50, 75 and 100%.

Fifteen experimental diets (five diets for each experiment) were formulated (Table 1 a, b and c) to replace 0, 25, 50, 75 or 100% of SBM protein by each of RLSM, ROLSM or ALSM proteins. All diets were formulated to be isonitrogenous (30% protein) and isocaloric (2600 Kcal ME/kg diet). In preparing the diets, dry ingredients were first ground to a small particle size. Ingredients were thoroughly mixed and water was added to obtain a 30% moisture content. Diets were passed through a mincer with diameter of 2 mm and were sun dried for 48 hrs to obtain dry pellets.

Experimental procedure:

For each experiment ten (2 replicates for each treatment) rectangular aquaria 100 × 50 × 40 cm (200 liter for each) were used. All experimental aquaria were aerated with compressed air and each aquarium was stocked with 25 Nile tilapia fish. The average body weights (BW) were nearly similar and ranged between 2.76 - 2.91; 2.74 - 2.89 and 2.75 - 2.88 g for the 1st, 2nd and 3rd experiments, respectively. Fish in the three experiments were given the pelleted diets (2 mm in diameter) at a daily rate of 10% (during the 1st month), then gradually reduced to 7% (2nd month), 4% of total biomass (3rd month) and fish were fed 6 days/week (twice daily at 9.00 am and 3.00 pm). The amount of feed was bi-weekly adjusted according to the changes in body weight throughout the experimental period (90 days). About one half of water volume in each aquarium was daily replaced by aerated fresh water after cleaning and removing the accumulated excreta. Water temperature, pH and dissolved oxygen were measured daily at 2.00 pm while ammonia was weekly determined before water change. Water temperature averaged 27.7±0.4, dissolved oxygen 5.9±0.8 mg/l, total ammonia 0.18±0.7 and pH 8.5±0.2 during the three experimental periods, these averages were within the acceptable limits for fish growth and health (Boyd, 1979).

Records of live BW (g) and BL (cm) of individual fish were measured at the start and the end of the two experimental periods for each aquarium. Growth performance 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)

At the end of each experiment, four fish were chosen at random from each aquarium and subjected to the body composition of whole fish body.

Chemical analysis:

Dry matter (DM), ether extract (EE), crude protein (CP), crude fiber (CF) and ash contents of LSM, diets and fish were determined according to the methods described in AOAC (1990): DM after drying in an oven at 105°C until constant weight; ash content by incineration in a muffle furnace at 600°C for 12 h; CP (N × 6.25) by the kjeldhal method after acid digestion; EE by petroleum ether (60-80°C) extraction. The trypsin inhibitor activity was measured as described by Hamerstrand *et al.* (1981). Phytic acid content in raw and treated linseed meal samples was estimated colorimetrically using Wade reagent (Latta and Eskin, 1980). Total phenols were colorimetrically determined in the ethanolic extracts by using the Folin Denis reagent as described by Gutfinger (1981). Amino acid analyzer (Model 121) was used for determination of amino acids in SBM and LSM as described by Moore *et al.* (1958).

Statistical analysis:

The statistical analysis of data was carried out by applying the computer program, SAS (1996) by adopting the following model:-

$$Y_{ij} = \mu + \alpha_i + E_{ij}$$

Where, Y_{ij} = the observation on the ij^{th} fish eaten the i^{th} diet; μ = overall mean, α_i = the effect of j^{th} diet and E_{ijk} = random error.

RESULTS AND DISCUSSION

Results of feeding Nile tilapia, *Oreochromis niloticus* on the different experimental diets containing the different levels of RLSM, ROLSM or ALSM during the feeding trials are going to be discussed under three points: a) chemical analysis of linseed meal , b) effect of roasting or autoclaving of LSM on the reduction of anti-nutritional factors and c) effect of replacing SBM by RLSM, ROLSM or ALSM on growth performance, feed utilization and body composition of whole body of Nile tilapia.

a) Chemical analysis of LSM:

Chemical analysis and amino acid composition of SBM and LSM used in the present study are shown in Table (2). SBM had higher contents of CP and NFE than those of LSM, whereas the reverse was true for EE. As described in the same table, LSM contains 90.30, 36.90, 5.40, 6.14, 8.90 and 42.66% for DM, CP, EE, CF and NFE, respectively and these values are relatively similar to those obtained for roasted or autoclaved LSM (Table 2). Abbas *et al.* (1990) found that, LSM contained 33.57, 5.88, 10.62 and 6.40% CP, EE, CF and ash, respectively. Also, Abdel-Fatah (2004) reported that, LSM contained 32.49, 4.07, 8.67 and

8.67% for CP, EE, CF and ash, respectively. Chemical analysis of LSM varied largely due to differences between the cultivars, environmental and soil conditions in different geographical locations and also due to its oil extraction method. Generally, the high protein content of LSM is makes it to be an excellent source of plant protein in fish diets.

Amino acids profile of SBM and LSM (Table 2) showed that, SBM displayed better amino acid profile as SBM had higher levels of all amino acids compared to LSM. Abbas *et al.* (1991) reported that, the first limiting amino acid in LSM was found to be lysine followed by methionine. Barbour and Sim (1991) indicated that glutamic acid, serine, valine, phenylalanine, isoleucine, leucine and methionine contents of RLSM were significantly lower than those of SBM.

b): Effect of different treatments on the removal of anti-nutritional factors from linseed meal:

The anti-nutritional factor trypsin inhibitor, total cyanogenic, total polypenolic compounds and phytic acid in the RLSM were determined to be 12.50 mg/g, 0.42 mg/g, 196 mg/100g and 2.90%, respectively. Similar results were obtained by El-Kady *et al.* (2001). The effect of different treatments of RLSM on it anti-nutritional factors content is presented in Table (3). As described in this table, roasting (140°C) or autoclaving (121°C) of RLSM for 20 min destroyed 54.80% and 80.40% of trypsin inhibitor content, 66.40 and 74.10% of polyphenolic compounds and 57.24 and 31.38% of phytic acid, respectively. The cyanogenic glycosides were completely destroyed after 20 min of roasting or autoclaving. It seems that, autoclaving is more effective in destruction of trypsin inhibitor; total polyphenolic compounds but had less effect on destruction of phytic acid compared to roasting treatment. Similar observation was also reported by Vijayakumariet *et al.* (1998). Burel *et al.* (2000 b) indicated that, heat treatment of rapeseed meal decreased the total glucosinolate level from 40 to 26 µ mol/g. Laz (2001) found that, heat treatment under pressure completely destroyed trypsin inhibitor from soybean meal. In the same trend Satoh *et al.* (1998) evaluated three rapeseed protein products, commercial rapeseed meal, low temperature extruded rapeseed meal (90°C) and high temperature extruded rapeseed meal (150°C) as partial replacement of herring meal by a rate of 15 or 30%. They found that the phytic acid content of commercial rapeseed meal was reduced by about 10% and 30% from the original level by extrusion cooking at low (90°C) and high (150°C) temperature, respectively. Siddhuraju and Becker (2003) found that, soaking of raw mucuna seed meal in various solutions followed by autoclaving was more effective in destroying the heat labile anti-nutrients such as trypsin (93-94%) and chemotrypsin (100%) inhibitors and lectin activity (100%) than heat-stable anti-nutrients, total phenolic (50 - 64.4%), tannins (50 - 83%) and phytates (46.3-65.3%) compared with the raw samples.

c): Effect of replacing SBM by RLSM, ROLSM or ALSM on growth performance and feed utilization of Nile tilapia:

The Effect of replacing SBM by RLSM, ROLSM and ALSM in tilapia diets on growth performance, feed utilization and body composition of fish were evaluated in three separate experiments at a replacing levels of 0, 25, 75 and 100% for each experiment.

First experiment:

Data presented in Table (4) showed that, the initial BW and BL were nearly similar and ranged between 2.76 and 2.91 g for BW and 5.40 and 5.63 cm for BL with insignificant differences between fish groups for both traits. Final BW and BL averaged 20.12 to 29.30 g and 10.43 to 12.24 cm with significant differences between fish groups for BW ($P<0.001$) and BL ($P<0.01$), respectively. From the results obtained, it is observed that, replacing 25% of SBM protein by RLSM protein in tilapia diets had no significant effect on BW or BL however, the higher replacing levels, 50, 75 or 100% significantly ($P<0.001$) decreased BW and BL of Nile tilapia and the same trend was also observed for WG and SGR. The reduction in BW, BL, WG and SGR increased with increasing RLSM level in tilapia diets from 25 to 100%. These results revealed the possibility of replacing only 25% of the high price SBM by the low price RLSM in tilapia diets, whereas, increasing the level of RLSM in the experimental diets above this level (25%) significantly ($P<0.001$) decreased BW, BL, WG and SGR of Nile tilapia, *O. niloticus*. Borgeson (2005) indicated that, flax seed had negative apparent digestibility coefficients for crude protein, gross energy and dry matter which may have been caused by high levels of mucilage resulting in increased intestinal content. The low consumption of the flax diet couples with high gut viscosity may have increased endogenous nutrient losses in the gut of the fish resulting in the negative digestibility coefficients observed. These results are in accordance with those reported by Abdel-Hakim *et al.* (2003) who reported that, replacing 50% of SBM by RLSM significantly decreased BW, WG and SGR of Nile tilapia, *O. niloticus*. The same findings were reported by Keembiyehetty and de Silva (1993) when Nile tilapia reared on diets containing various levels of black gram (*Phaseolus mungo*) seed. In this connection, Abdul-Aziz *et al.* (1999) successfully fed Nile tilapia on diets containing RLSM up to 50% as a substitute of SBM without adverse effect on BW or WG, despite a significant lower SGR compared to the control fish. In another study, El-Saidy and Gaber (2001) revealed that, fish meal could be replaced by RLSM up to 75% without adverse effect on BW, WG and SGR of Nile tilapia, *O. niloticus*.

Results of Table (4) also showed that, feed intake (FI) of *O. niloticus* insignificantly changed until the replacing level of SBM protein by RLSM protein reached 50%, after this level (50%) FI was significantly ($P<0.01$) decreased. These results may be due to the presence of different anti-nutritional substances in RLSM that limit consumption of diets containing RLSM. Also, RLSM is not as palatable as SBM or corn gluten meal (Schaible, 1970) and these results suggested that RLSM has anorectic effect for Nile tilapia. These results disagreed with those obtained by El-Saidy and Gaber (2001) who found that, replacement of fish meal by RLSM up to 75% did not significantly affected FI in Nile tilapia, however Abdel-Hakim *et al.* (2003) found that replacing of SBM by RLSM significantly decreased FI by Nile tilapia. Values of FCR ranged from 1.95 to 2.67. The best rate was obtained when fish fed the RLSM25 diet (25% of SBM was replaced by RLSM) where only 1.95 kg of feed was required to produce one kilogram of live fish weight. Increasing the replacing level of SBM by RLSM from 50 to 100% (with an increment of 25%) in *O. niloticus* diets did not significantly ($P<0.05$) affect the FCR. The best (1.71) protein efficiency ratio (PER) was obtained by fish fed the diet RLSM25 and the

worst PER (1.20) was obtained with fish fed the diet RLSM50. These results disagreed those obtained by Abdul-Aziz *et al.* (1999) who found that replacing 25 or 50% of SBM by RLSM significantly adversed FCR and PER values compared to control fish group. Also, Abel-Hakim *et al.* (2003) reported that replacing 50 of SBM by RLSM significantly adversed FCR and PER values of Nile tilapia, however El-Saidy and Gaber (2001) showed that, replacement of fish meal by RLSM up to 75% did not significantly affected FCR and PER.

Results of body composition of fish body (Table 4) showed that, the graded increase of RLSM in the experimental diets decreased DM and EE contents while ash content showed an opposite trend, the differences were almost significant compared to control fish group. Protein content of tilapia fish lie in three clusters, the first one includes fish groups fed the diets RLSM0 and RLSM25 and the second one include fish fed the diets RLSM50 and RLSM75 and the third one includes fish fed the diet RLSM100, the differences between the first and the third clusters were significant while those between the second cluster and each of the first and the third clusters were not significant. These results indicated that, replacing SBM by RLSM from 0 up to 75% had no significant effect on protein content of whole fish body while the complete replacement significantly decreased protein content of tilapia fish. These results are partial in agreement with those obtained by Abdul-Aziz *et al.* (1999). They found that replacing SBM by RLSM up to 50% in Nile tilapia diets did not significantly affected the percentages of CP or DM but it significantly decreased EE content of whole fish.

Results of the present experiment indicated that RLSM has a lower potential as a SBM substitute in tilapia diets and these results may be attributed to the high content of anti-nutritional factor (Table 3) and the lake of some essential amino acid content of RLSM compared to SBM (Table 2). Similar results were reported by Abbas *et al.* (1991) and Barbour and Sim (1991).

Second experiment:

The growth performance and other related parameters for Nile tilapia fed the experimental diets are presented in Table (5). As shown in this table, the initial BW and BL averaged 2.74 and 2.89 g for BW and 5.32 and 5.47 cm for BL with no significant differences between fish groups for both traits. Final BW and BL averaged 25.87 to 33.27 g and 11.65 to 13.09 cm, respectively with significant ($P<0.001$) differences between fish groups for BW and BL. The highest BW (33.27 g) was recorded for fish group fed the diet ROLSM25 while the lowest BW and BL values were recorded for fish fed the diet ROLSM100 where SBM protein was completely replaced by ROLSM protein and the same trend was also observed for WG and SGR values.

As described in Table (5), the higher FI was recorded for fish fed the ROLSM25 diet followed in a decreasing order by fish fed diets ROLSM0, ROLSM50, ROLSM75 and ROLSM100, respectively with significant differences between fish groups fed the experimental diets. The best FCR was recorded by fish fed the diet ROLSM75. PER increased ($P<0.05$) gradually with increasing the replacing level of SBM by RLSM up to 75% and the complete replacement did not significantly affected PER compared to control group (RLSM0).

Results obtained revealed the possibility of replacing up to 75% of SBM by ROLSM in tilapia diets without any adverse effect on growth performance parameters (BW, BL, WG

and SGR) of Nile tilapia, but the complete replacement significantly adversed all these growth parameters. The high potential of ROLSM as SBM substitute in tilapia diets may be due to heat treatment of LSM which completely destroyed cyanogenic glycosides and different proportions of the other anti-nutritional factors (54.8, 66.4 and 57.24% of trypsin inhibitor, total polyphenolic compounds and phytic acid, respectively), as shown in Table (3). Trypsin inhibitors in raw or inadequately heated SBM adversely affected growth of trout (Sandholm *et al.* 1976), carp (Viola *et al.* 1983), and channel catfish (Robinson *et al.* 1981 and Wilson and Poe, 1985). Heat treatment of SBM improves growth in fish and destroys protease inhibitors (Viola *et al.* 1983; Robinson, 1984; Balogun and Ologhobo, 1989 and Wee and Shu, 1989). Burel *et al.* (2000 b) demonstrated that, incorporation of raw rapeseed meal as a substitute of fish meal at the level of 30% in turbot (*Psetta maxima*) diets significantly decreased BW, WG and FI while, incorporation of heated rapeseed meal in the diets by the same level (30%) did not lead to significant decrease in growth performance and they concluded that, preliminary heat treatment of rapeseed meal is necessary in order to improve its nutritional quality.

Results of body composition of fish showed that, incorporation of ROLSM in tilapia diets as a replacement of SBM up to 75% did not significantly affected DM, CP and ash contents however the complete replacement of SBM by ROLSM significantly decreased DM and CP and increased ash content of whole fish body. Compared to control group, all replacing levels of SBM by ROLSM significantly ($P<0.001$) decreased fat content of tilapia fish. Burel *et al.* (2000 b) found that, incorporation of heated rapeseed meal up to 46% in turbot, *Pesetta maxima* diets as a fish meal substitute did not significantly altered protein or energy content of fish bodies.

Third experiment:

Data in Table (6) showed that, the initial BW and BL averaged 2.75 to 2.88 g for BW and 5.38 to 5.55 cm for BL with insignificant differences between fish groups for both traits. Final BW and BL ranged from 25.12 to 33.34 g and 11.44 to 13.39 cm, respectively with significant ($P<0.001$) differences between fish groups for BW and BL. The highest BW (33.34 g) was shown by fish fed ALSM25 diet; whereas the lowest BW (25.12 g) was achieved by fish fed the diet ALSM100 (SBM was completely replaced by ALSM). Compared to control group, increasing the replacing levels of SBM by ALSM by rates of 25, 50 or 75% did not significantly adversed the final BW or BL but the complete replacement of SBM by ALSM (100%) significantly decreased BW and BL values. Similarly, the same trend was observed for WG and SGR.

The higher FI was recorded for fish fed the control diet (ALSM0) followed in a decreasing order by ALSM50, ALSM25, ALSM100 and ALSM75, respectively with significant differences ($P<0.05$) between fish groups fed the experimental diets (Table 6). The best FCR was obtained when fish fed the ALSM75 diet and the same trend was observed for PER as ALSM75 diet showed the higher (2.02) PER value.

The high potential of ALSM as SBM substitute in tilapia diets may be due to the heat under pressure treatment which resulted in complete destruction of cyanogenic glycosides and different proportions of the other anti-nutritional factors (80.40, 74.10 and 31.38% of trypsin inhibitor, total polyphenolic compounds and phytic acid, respectively). Laz (2001) reported that heat under pressure completely destroyed trypsin inhibitor of soybean meal.

Trypsin inhibitor considered the main factor affecting protein digestibility causing hypertrophy and reduction of availability of amino acids, vitamins and minerals. Also, it may inhibit growth, depress metabolizable energy and fat depression (Liener, 1980 and Kadam *et al.* 1987). Burel *et al.* (2000 a) found that heat-treated rapeseed meal (pressure-cooking) in freshwater-grown rainbow trout and seawater-grown turbot improved the digestibility of rapeseed meal and feed utilization efficiency. In this connection, Abdel-Hakim *et al.* (2003) found that, replacing 50% of SBM by RLSM significantly adversed growth performance and feed utilization of Nile tilapia and they recommended that, linseed must be treated before incorporation in tilapia diets to reduce the anti-nutritional factors of linseed.

Results of body composition of fish (Table 6) showed that, partial or complete replacement of SBM by ALSM did not significantly affected DM, while partial replacement up to 50% did not significantly affected CP and ash contents. However, the higher replacing levels (75 or 100%) significantly decreased CP and increased ash content of whole fish. Compared to control, the graded increase of ALSM in tilapia diets significantly decreased fat content of tilapia fish. El-Saidy and Gaber (2001) reported that, the highest value of protein content was obtained with fish fed 75% LSM (as substitute of fish meal) and the lowest one was obtained by fish fed 100% LSM.

Economical efficiency:

The current investigation highlights the potential of using LSM for partial or complete replacement of SBM in Nile tilapia diets. Generally, results of the present study showed the possibility of replacing SBM by RLSM up to 25% (1st experiment) and SBM by ROLSM or ALSM up to 75% (2nd and 3rd experiments) with no adverse effect on growth performance and feed utilization. Feeding costs in fish production is about 50% of the total production costs (Collins and Delmendo, 1979). All other costs in the present study are constant, therefore, the feeding costs required to produce one kg gain in weight could be used to compare the different experimental treatments. The calculated figures showed that, the cost of one ton feed mixture was reduced in all replacing levels of SBM by RLSM, ROLSM or ALSM (Tables 7 and 8). Replacing 25% of SBM by RLSM could be reduce feeding costs by 7.37% while replacing 75% of SBM by ROLSM or ALSM reduced feeding costs by 18.83 and 17.58, respectively.

With regard to RLSM, results in Table (7) showed that, replacing SBM by RLSM up to 25% reduced the feeding costs/kg weight gain but the higher replacing level (50%) seemed to be not economic. However, replacing SBM by ROLSM or ALSM up to 100% economically reduced the feeding costs/kg weight gain.

Conclusion:

In conclusion, results of the present study indicated that roasting or autoclaving of LSM is necessary for improving the nutritional value of this feed ingredient and it could be incorporated up to 75% as a SBM substitute in tilapia diets instead of 25% for RLSM. It is therefore worth to recommend treating LSM with heat (roasting or autoclaving) before incorporation in fish diets to reduce their anti-nutritional contents.

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Table (1 a): Composition and chemical analysis of the first experimental diets (RLSM)

Feed ingredients	Experimental diets				
	RLSM0	RLSM25	RLSM50	RLSM75	RLSM100
Fish meal (65%)	16	16	16	16	16
Yellow corn	28	28	25	22	22
Soybean meal (40%)	40	30	20	10	0
Raw linseed meal	0	11.4	22.8	34.2	45.6
Wheat bran	10.5	9.1	9.7	9.8	7.9
Vegetable oil	2	2	3	4.5	5
Vit. & Min. mixture[1]	3.5	3.5	3.5	3.5	3.5
Sum	100	100	100	100	100
Chemical analysis	*(determined on dry matter basis)*				
Moisture	6.12	6.98	7.10	6.54	6.00
Crude protein (CP)	31.12	30.06	31.20	30.50	30.35
Ether extract (EE)	4.45	4.32	4.45	4.83	4.96
Crude fiber (CF)	9.01	9.05	9.00	10.23	10.17
Ash	10.63	10.61	10.40	10.76	10.91
NFE[2]	44.39	45.96	44.95	43.68	43.61
ME[3] (Kcal/kg diet)	2627.8	2608.4	2616.9	2610.0	2629.6
P/E ratio[4]	118.4	115.2	119.2	116.9	115.4

Table (1 b): Composition and chemical analysis of the second experimental diets (ROLSM)

Feed ingredients	Experimental diets				
	ROLSM0	ROLSM25	ROLSM50	ROLSM75	ROLSM100
Fish meal (65%)	16	16	16	16	16
Yellow corn	28	28	25	22	22
Soybean meal (40%)	40	30	20	10	0
Roasted linseed meal	0	11.4	22.8	34.2	45.6
Wheat bran	10.5	9.1	9.7	9.8	7.9
Vegetable oil	2	2	3	4.5	5
Vit. & Min. mixture[1]	3.5	3.5	3.5	3.5	3.5
Sum	100	100	100	100	100
Chemical analysis	*(determined on dry matter basis)*				
Moisture	5.87	6.14	6.10	5.44	5.87
Crude protein (CP)	30.20	29.99	30.09	29.74	30.04
Ether extract (EE)	4.62	4.54	4.35	4.62	5.01
Crude fiber (CF)	9.64	10.06	9.83	10.79	10.51
Ash	10.72	10.45	10.78	9.87	10.74
NFE[2]	44.82	44.96	44.95	44.98	43.70
ME (Kcal/kg diet)[3]	2620.0	2600.1	2610.7	2591.0	2607.0
P/E ratio[4]	115.3	115.3	115.3	114.8	115.2

Table (1 c): Composition and chemical analysis of the third experimental diets (ALSM).

Feed ingredients	Experimental diets				
	ALSM0	**ALSM25**	**ALSM50**	**ALSM75**	**ALSM100**
Fish meal (65%)	16	16	16	16	16
Yellow corn	28	28	25	22	22
Soybean meal (40%)	40	30	20	10	0
Autoclaved linseed meal	0	11.4	22.8	34.2	45.6
Wheat bran	10.5	9.1	9.7	9.8	7.9
Vegetable oil	2	2	3	4.5	5
Vit. & Min. mixture[1]	3.5	3.5	3.5	3.5	3.5
Sum	100	100	100	100	100
Chemical analysis	*(determined on dry matter basis)*				
Moisture	7.44	6.55	6.12	7.15	5.89
Crude protein (CP)	31.18	31.06	30.01	30.80	29.91
Ether extract (EE)	4.25	4.24	4.24	4.32	4.77
Crude fiber (CF)	9.93	10.23	10.00	9.99	10.44
Ash	10.25	10.54	9.97	10.22	10.18
NFE[2]	45.39	43.93	45.78	44.67	44.70
ME (Kcal/kg diet)[3]	2627.0	2609.4	2618.0	2620.0	2592.0
P/E ratio[4]	118.7	119.0	114.6	117.6	115.4

[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.

Table (2): Chemical composition and amino acid composition of SBM and LSM

Item	Chemical composition (%), on dry weight basis					
	DM	CP	EE	Ash	CF	NFE
SBM	91.00	40.21	2.31	6.16	6.50	44.82
RLSM	90.30	36.90	5.40	6.14	8.90	42.66
ROLSM	91.00	36.30	5.51	6.81	8.55	42.83
ALSM	90.00	35.59	5.59	7.00	8.99	42.83

Amino acids profile (g/100 g protein)

Amino acid	SBM	RLSM
Lysine	1.60	1.23
Leucine	2.60	1.71
Isoleucine	2.40	1.32
Cystine	0.50	0.36
Methionine	0.71	0.48
Phenylalanine	2.40	1.77
Tyrosine	1.11	1.02
Threonine	1.35	1.14
Valine	1.89	1.68
Histidine	1.20	0.78
Arginine	3.08	2.82
Asparatic acid	3.17	3.15
Glutamic acid	5.17	4.15
Serine	1.92	1.56
Proline	1.36	1.32
Glycine	2.15	1.35
Alanine	1.90	1.29

Table (3): Effect of roasting and autoclaving on anti-nutritional factors content of linseed meal (calculated on DM basis).

Item	Trypsin inhibitor mg/g	Total cyanogenic mg/g	Total polyphenolic mg/100g	Phytic acid %
RLSM	12.50	0.42	196	2.90
ROLSM				
Anti-nutritional content	5.65	0.00	65.86	1.24
Reduction %	54.80	100	66.40	57.24
ALSM				
Anti-nutritional content	2.45	100	50.76	1.99
Reduction %	80.40	100	74.10	31.38

Table (4): Growth performance, feed utilization and body composition of Nile tilapia as affected by replacing SBM by RLSM.

Item	No.	Experimental diets						
		RLSM0	RLSM25	RLSM50	RLSM75	RLSM100	±SE	*Prob.*
Growth performance								
BW (g)								
Initial	50	2.76	2.91	2.80	2.79	2.79	0.07	0.5987
Final	50	29.16 a	29.30 a	22.23 b	20.98 b	20.12 b	0.56	0.0001
BL (cm)								
Initial	50	5.40	5.51	5.44	5.48	5.63	0.05	0.0324
Final	50	12.24 a	12.09 a	11.57 b	10.99 c	10.43 c	0.14	0.0011
WG (g/fish)	2*	26.38 a	26.39 a	19.43 b	18.19 b	17.32 b	0.73	0.0020
SGR	2*	2.61 a	2.57 a	2.30 b	2.24 b	2.19 b	0.06	0.0238
Feed utilization								
FI (g/fish)	2*	55.20 a	51.36 a	51.83 a	45.04 b	45.60 b	1.14	0.0119
FCR	2*	2.09 b	1.95 c	2.67 a	2.48 ab	2.63 a	0.11	0.0317
PER	2*	1.54 b	1.71 a	1.20 c	1.32 c	1.25 c	0.05	0.0373
Body composition of whole fish (%)								
DM	8	29.34 a	28.11 ab	27.56 b	27.76 b	27.90 b	0.32	0.0170
CP	8	68.60 a	68.24 a	66.62 ab	66.54 ab	65.88 b	0.57	0.0310
EE	8	18.98 a	17.55 ab	16.55 b	16.33 b	16.20 b	0.52	0.0158
Ash	8	11.98 b	12.16 b	12.51 b	13.87 b	15.22 a	0.30	0.0001

Means followed by the different letters in each row for each trait are significantly different (P<0.05).
* average of two aquaria (2 replicates)

Table (5): Growth performance, feed utilization and body composition of Nile tilapia as affected by replacing SBM by ROLSM.

Item	No.	Experimental diets						
		ROLSM0	ROLSM25	ROLSM50	ROLSM75	ROLSM100	±SE	*Prob.*
Growth performance								
BW (g)								
Initial	50	2.77	2.83	2.74	2.89	2.80	0.10	0.8602
Final	50	29.59 b	33.27 a	30.32 b	29.78 b	25.87 c	0.86	0.0001
BL (cm)								
Initial	50	5.38	5.32	5.35	5.47	5.45	0.05	0.2659
Final	50	12.21 b	13.09 a	12.25 b	12.39 b	11.65 c	0.06	0.0001
WG (g/fish)	2*	26.82 a	29.94 a	27.57 a	26.89 a	23.08 b	0.83	0.0105
SGR	2*	2.63 ab	2.71 a	2.67 a	2.59 ab	2.48 b	0.04	0.0989
Feed utilization								
FI (g/fish)	2*	54.66 a	57.20 a	50.40 ab	48.24 b	46.80 b	1.23	0.0155
FCR	2*	2.04 a	1.91 ab	1.83 b	1.79 b	2.03 a	0.05	0.0477
PER	2*	1.6 b	1.75 ab	1.82 a	1.87 a	1.64 b	0.02	0.0327
Body composition of whole fish (%)								
DM	8	29.55 a	29.27 a	28.50 ab	28.14 ab	27.76 b	0.39	0.0518
CP	8	68.85 a	67.45 a	67.19 a	66.91 a	64.44 b	0.78	0.0054
EE	8	21.05 a	19.98 b	19.89 b	18.98 b	16.28 c	0.44	0.0001
Ash	8	10.83 b	12.14 b	12.32 b	12.60 b	15.52 a	0.06	0.0001

Means followed by the different letters in each row for each trait are significantly different (P<0.05).
* average of two aquaria (2 replicates)

Table (6): Growth performance, feed utilization and body composition of Nile tilapia as affected by replacing SBM by ALSM.

Item	No.	Experimental diets						
		ALSM0	ALSM25	ALSM50	ALSM75	ALSM100	±SE	*Prob.*
Growth performance								
BW (g)								
Initial	50	2.78	2.84	2.75	2.88	2.81	0.09	0.8448
Final	50	29.43 b	33.34 a	31.10 ab	29.46 b	25.12 c	0.89	0.0001
BL (cm)								
Initial	50	5.39	5.39	5.38	5.49	5.55	0.06	0.1149
Final	50	12.24 ab	13.39 a	12.68 ab	12.18 ab	11.44 b	0.16	0.0001
WG (g/fish)	2*	26.65 ab	30.50 a	28.35 a	26.58 ab	22.31 b	1.41	0.0516
SGR	2*	2.62 ab	2.74 a	2.70 a	2.59 ab	2.44 b	0.06	0.1039
Feed utilization								
FI (g/fish)	2*	55.14 a	52.08 a	53.16 a	42.68 b	50.42 a	1.52	0.0233
FCR	2*	2.07 ab	1.71 bc	1.88 b	1.61 c	2.26 a	0.11	0.0553
PER	2*	1.55 c	1.89 ab	1.78 b	2.02 a	1.48 a	0.04	0.0420
Body composition of whole fish (%)								
DM	8	27.76	27.03	26.53	25.70	25.35	0.38	0.1150
CP	8	68.89 a	68.60 a	68.00 a	64.82 b	64.64 b	0.53	0.1147
EE	8	20.11 a	18.50 b	18.44 b	16.58 c	16.55 c	0.55	0.1812
Ash	8	10.13 b	10.87 b	11.15 b	15.16 a	15.22 a	0.27	0.0030

Means followed by the different letters in each row for each trait are significantly different (P<0.05).
* average of two aquaria (2 replicates)

Table (7): Feed costs (LE) for producing one kg weight gain by fish fed the experimental diets.

Experimental diets	Costs (L.E)/ ton	Relative to control %	Decrease in feed cost (%)	FCR	Feed costs* (L.E)/kg weight gain	Relative to control %
			RLSM			
RLSM0	2750.0	100	0.00	2.09	5.75	100
RLSM25	2547.2	92.63	7.37	1.95	4.97	86.43
RLSM50	2364.9	86.00	14.00	2.67	6.31	109.74
RLSM75	2198.1	79.93	20.07	2.48	5.45	94.78
RLSM100	2010.8	73.12	26.88	2.63	5.29	92.00
			ROLSM			
ROLSM0	2750.0	100	0.00	2.04	5.61	100
ROLSM25	2558.6	93.04	6.96	1.91	4.89	87.17
ROLSM50	2387.7	86.83	13.17	1.83	4.37	77.90
ROLSM75	2232.3	81.17	18.83	1.79	4.00	71.30
ROLSM100	2056.4	74.78	25.22	2.03	4.17	74.33
			ALSM			
ALSM0	2750.0	100	0.00	2.07	5.69	100
ALSM25	2570.0	93.45	6.55	1.71	4.39	77.15
ALSM50	2410.5	87.65	12.35	1.88	4.53	79.61
ALSM75	2266.5	82.42	17.58	1.61	3.65	64.15
ALSM100	2102.0	76.44	23.56	2.26	4.75	83.48

* Feed costs/kg weight gain = FCR × costs of kg feed.

Table (8): Local market price (LE/ton) for feed ingredients used in formulating the experimental diets.

Ingredients	Price (L.E.) / ton
Fish meal	4972
Yellow corn	1250
Soybean meal	2700
RLSM	700
ROLSM	800
ALSM	900
Wheat bran	900
Corn oil	4000
Vit. & Min. mixture	10000

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الإحلال الجزئى والكلى لكسب فول الصويا بكسب الكتان الخام والمعامل حرارياً فى علائق أسماك البلطى

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فى هذه الدراسة تم إختبار تأثير إحلال كسب فول الصويا بكسب الكتان الخام والمعامل حرارياً أو المعامل بالأوتوكلاف (حرارة تحت ضغط) فى علائق أسماك البلطى على صفات النمو والإستفادة من الغذاء وكذلك التحليل الكيماوى لأجسام الأسماك وذلك بإجراء ثلاث تجارب منفصلة حيث تم إحلال كسب فول الصويا بكسب الكتان الخام أو كسب الكتان المعامل حراريا (تحميص) أو كسب الكتان المعامل بالأوتوكلاف (حرارة تحت ضغط) وكانت نسب الإحلال فى التجارب الثلاثة صفر، ٢٥ ، ٥٠ ، ٧٥ ، ١٠٠%.

وقد أظهرت نتائج التحليل الكيميائى أن كسب الكتان الخام يحتوى على العديد من المركبات السامة مثل مثبط إنزيم التربسين، مركبات السيانوجنيك والفينولات الكلية وحمض الفيتيك بنسب وصلت إلى 12.50 مجم/جم ، ٠.٤٢ مجم/جم ، ١٩٦ مجم/١٠٠ جم ، 2.90% لهذه المركبات على التوالى. وقد أدت المعاملة الحرارية لمدة ٢٠ دقيقة سواء بالتحميص أو المعاملة بالأوتوكلاف إلى التخلص كلياً من مركبات السيانوجنيك وخفض باقى هذه المركبات بنسبة 54.80 ، 80.40% بالنسبة لمثبط التربسين ، 66.40 ، 74.10 % من الفينولات الكلية و٥٧.٢٤، ٣١.٣٨% من حمض الفيتيك على التوالى.

وقد أظهرت نتائج التجربة الأولى أن إحلال ٢٥% من بروتين كسب فول الصويا ببروتين كسب الكتان الخام لم يؤثر معنوياً على وزن وطول جسم أسماك البلطى أما نسب الإحلال الأعلى (٥٠ ، ٧٥ ، ١٠٠%) فقد أدت إلى خفض معنوى فى وزن وطول جسم أسماك البلطى وقد لوحظ نفس الإتجاه بالنسبة لباقى صفات النمو مثل الزيادة فى وزن الجسم ومعدل النمو كما أظهرت النتائج أن أفضل معدل لتحويل الغذاء قد ظهر عند نسبة الإحلال ٢٥%.

أظهرت نتائج التجربة الثانية أن أكبر قيم لوزن وطول الجسم والزيادة فى وزن الجسم وكذلك معدل النمو قد سجلت للأسماك التى تغذت على العليقة التى كانت نسبة إحلال بروتين كسب فول الصويا ببروتين كسب الكتان المحمص بنسبة ٢٥%، كما أعطت الأسماك التى تغذت على العليقة التى تمت فيها عملية الإحلال بنسبة ١٠٠% أقل قيم لهذه المقاييس. كما أظهرت النتائج أن نسبة الإحلال ٧٥% قد أعطت أفضل قيم لمعدل تحويل الغذاء. وقد تشابهت نتائج التجربة الثالثة مع نتائج التجربة الثانية عند إحلال كسب فول الصويا بكسب الكتان المعامل بالأوتوكلاف.

من الناحية الإقتصادية وجد أن إحلال ٢٥% من بروتين كسب فول الصويا ببروتين كسب الكتان الخام أدى إلى تقليل تكاليف التغذية بنسبة ٧.٣٧% فى حين أن إحلال ٧٥% من بروتين كسب فول الصويا ببروتين كسب الكتان المعامل حراريا (التحميص) أو المعامل حرارياً تحت ضغط (الأوتوكلاف) قد أدى إلى تقليل تكاليف التغذية بنسب وصلت إلى١٨.٨٣% ، ١٧.٥٨% على التوالى دون أن يؤثر ذلك على صفات النموو الكفاءه الغذائية للغذاء.