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Research Article

Efficacy of various processed canola meals to replace fish meal in Nile tilapia *Oreochromis niloticus* diet: Growth performance, digestive enzymes, immune parameters, and liver antioxidative status

Mohammadi M.[1]; Imani A.[1*]; Farhangi M.[2]; Gharaei A.[3]; Hafezieh M.[4]

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Abstract

Replacing fish meal by convenient and economic sources is necessary for sustainable development of aquaculture industry. The present study was designated to explore the effect of different processed canola meals as fish meal replacement on growth performance, digestive enzymes activity, immune parameters, and liver antioxidative status of Nile tilapia *Oreochromis niloticus*. Four isonitrogenous and isoenergetic diets were used with, (1) no canola meal (control), (2) 25% non-processed canola meal (CM), (3) 25% dephytinized (using 2000 IU/kg phytase) ammonia methanol solution treated canola meal (CPM), and (4) electron beam irradiated (15 kGy) ammonia methanol solution treated canola meal (ECM). Juvenile male Nile tilapia with an average body weight of 3.5 g±0.1 (n=17 fish per tank) were fed the experimental diets three times a day until apparent satiation for 36 days. Results revealed that the fish fed the control diet showed the highest daily growth coefficient (DGC) (p<0.05). CPM group significantly showed greater final weight and DGC in comparison to other treatments (p<0.05). CRM included diet showed lower FE and PER (p<0.05). In addition, those fish fed control diet with no canola meal content showed significantly higher feed intake, followed by CPM contained diet (663.7±9.2 g and 477±20.2 g, respectively, p<0.05). Regression analysis also revealed a statistically significant relationship between total feed intake and DGC. Our results also indicated a significant quadratic relationship between dietary glucosinolate content and DGC implying that as dietary glucosinolate content increased total feed intake and subsequently DGC decreased. Regarding dietary protein digestibility (ADCp), there were no consistent results. Regarding digestive enzymes activity, mucosal innate immunity, liver antioxidant enzyme activities and liver tissue malondialdehyde content (MDA) content, there were no significant differences among experimental groups (p>0.05). It is concluded that dietary ANTs contents mainly glucosinolate affected juvenile Nile tilapia growth performance and nutritional indices mainly via reducing feed intake following feeding on diets contained differently processed or crude canola meal.

Keywords: Ammonia methanol solution, Phytase, Electron bean irradiation, Antinutrients, Canola, Nile tilapia

1-Department of Fisheries, Faculty of Agriculture and Natural Resources, Urmia University, Urmia, Iran
2-Department of Fisheries, Faculty of Natural Resources, University of Tehran, Karaj, Iran
3-Department of Fisheries, Hamoun International Wetland Research Institute and Faculty of Natural Resource, University of Zabol, Zabol, Iran
4-Iranian Fisheries Sciences Research Institute, Agricultural Research, Education and Extension Organization (AREEO), Tehran, Iran
*Corresponding author's Email: a.imani@urmia.ac.ir

Introduction

Sustainable intensive aquaculture relies on feed industry. Such development requires introducing fish meal alternatives. Nowadays, there is an increasing trend for using oilseed cakes for aquaculture feed production (Kotecka-Majchrzak *et al*., 2020). Canola meal as an oilseed by-product is a suitable fish meal substitute for different animals (Mwachireya *et al*., 1999; Toghyani *et al*., 2014; Brand *et al*., 2020; Hernández *et al*., 2020; Sekali *et al*., 2020; Sajedkhanian *et al*., 2021) however, deleterious effects of antinutritional factors (ANTs), including glucosinolate, phytic acid, phenolic compounds, tannins, non-starch polysaccharides (NSPs) limit its dietary inclusion (Lim *et al*., 2008).

The most important ANTs of canola include glucosinolate, phytic acid, phenolic compounds like tannin and NSPs (Shahidi, 1997). Residues of glucosinolate derivatives are toxic and cause abnormalities in carp and tilapia at dietary levels of 3.3 and 2.5 g/kg, respectively (Francis *et al*., 2001). Its bitter taste and pungent odor reduces feed intake and therefore growth performance by fish (Shahidi, 1997; Lim *et al*., 2008). Another consequential ANTs of plant protein sources is their phytic acid content (Maga, 1982). Phytate would decrease the availability of various nutrients, such as amino acids, proteins, lipids and some minerals mainly, Ca^{++}, Mg^{++}, Fe^{++}, Zn^{++}, Cu^{++} and Mn^{++} (Maga, 1982; Pallauf and Rimbach, 1997; Rabia *et al*., 2017). Phenolic compounds may also affect animal feed intake due to their unpleasant taste. Certain minerals including Zn^{++} might also be rendered unavailable in presence of such phenolic compounds. Glycation of their breakdown products with nutrients including amino acids, especially in the course of thermal processing of such ingredients, renders the nutrients indigestible (Shahidi, 1997). NSPs also exert adverse effects on intestinal nutrient digestion/absorption and gut microbiota with subsequent poor animal growth performance (Bederska-Łojewska *et al*., 2017). Therefore, with respect to animal species of concern and cost-effectiveness of methods various processing operations have been proposed for reducing /removing of ANTs from plant protein sources such as canola meal (CM).

Physical, chemical, biochemical and biotechnological procedures are used with concern to ANTs contents of plant sourced feedstuffs, including soaking, germination, fermentation, irradiation and enzymes hydrolyses with some success regarding economic issues and their availability (Cao *et al*., 2008; Ebrahimi *et al*. 2009; Taghinejad-Roudbaneh *et al*. 2010; Anwar *et al*., 2015). Although, various processing procedures, such as milling, grinding, irradiation and methanol-ammonia solution reflux are proved to be effective in decreasing ANTs content of the plant ingredients, they are not successfully effective on some ANTs or even result in higher ANTs content in the final product (Mwachireya *et al*., 1999; Taghinejad *et al*., 2009; Shawrang *et al*.,

2011; Kumar *et al*., 2012; Anwar *et al*., 2015). Meanwhile, it has recently been shown that some plant metabolites might have beneficial effects on animal cell functions like acting as natural antioxidative agents (Wu *et al*., 2019; Mohammadi *et al*., 2020). According to the literature, in most studies conducted to process plant protein resources only one processing method was used (Naczk and Shahidi, 1989; Shahidi and Naczk, 1990; Ebrahimi *et al*., 2009; Taghinejad *et al*., 2009 and Taghinejad-Roudbaneh *et al*., 2010; Shawrang *et al*., 2011; Anwar *et al*., 2015) and little attention is paid on comparing various combinations of different processing methods to decrease/remove ANTs contents of plant ingredients. The present study presents a set of processing techniques to address ANTs contents of CM to introduce a suitable plant protein product for Nile tilapia, *O. niloticus*, diet formulation.

Material and methods

CM processing

To find a more appropriate processing method of canola meal for Nile tilapia nutrition, three processing procedures including refluxing with methanol-ammonia solution, electron beam irradiation and phytase hydrolysis were used. In this regard, double zero CM, purchased from Golbahar Sepahan Co., Known as KVSGS Co. (Isfahan, Iran), was subjected to combinations of various processing methods (Table 1).

Table 1: Proximate composition and ANTs content of CM, CPM and CRM used in the present study.

	CM [a]	CPM [b]	CRM [c]
Crude protein (%)	36.80	37.50	36.70
Crude lipid (%)	2.50	0.11	1.75
Ash (%)	7.04	8.11	7.68
NFE (%) [d]	35.65	32.94	32.27
Gross energy (Kcal/g)	3.77	3.48	3.56
Glucosinolate (µmol/g)	0.94	0.16	0.62
Phytic acid (%)	4.68	2.43	0.80
Phenolic compounds (%)	2.82	0.91	0.76
Tannin (%)	0.64	0.09	0.08
Viscosity (cP)	1.49	1.57	1.78

a: non-processed canola meal.
b: dephytinized canola meal subjected to the ammonia methanol solution.
c: irradiated canola meal subjected to the ammonia methanol solution.
d: Nitrogen-free extract = dry matter – (crude protein + crude lipid + ash + fiber).

Phytase treatment

In accordance with Cain and Garling (1995), CM was subjected to phytase (Agranco Co. USA) hydrolysis. The enzyme (2000 IU/kg) was dissolved in citrate buffer (pH=4.6, 0.2 M) and mixed with CM at 1:1 ratio of (w/v). The mixture was incubated at 55°C for 6 h based on the enzyme manufacturer's recommendation. Finally, the product was oven dried at 60°C to a constant weight.

Electron beam irradiation

Polyethylene bags containing CM were exposed to 15 kGy electron beam (Rhodotron accelerator model TT-200, IBA Co., Belgium) at room temperature with 3 mA beam of 10 MeV electrons. Dose accuracy was estimated using cellulose triacetate thin films (Bahraini *et al*., 2017).

Methanol-ammonia solution treatment

DCM and ECM were subjected to methanol-ammonia solution, containing 10:5:85% (v:v:v) ammonium hydroxide, water and absolute methanol, at ratio of 10:1 (w:w) for 2 h under continuous stirring at room temperature for three times. The resultant product was finally oven dried at 60°C to a constant weight (Shahidi, 1990).

Determination of ANTs contents of processed CM

Phytate

Phytate content of processed CMs was determined according to Makkar *et al.* (2007) by extracting with 3% trichloroacetic acid solution. After centrifuging at 3000 rpm, the supernatant kept in boiling water bath by rapid addition of 36 mM $FeCl_3$ solution. The product was then centrifuged at room temperature and the supernatant was discarded. The remnant was washed twice using 3% trichloroacetic acid solution and finally with distilled water. Afterwards, the precipitate was dissolved in 3 mL 1.5 M NaOH solutions and filtered through Whatman paper filter No.2. The solids remained on the filter were dissolved in hot 40 ml 3.2 N nitric acid solution and finally, 20 mL 1.5 M KSCN was add. The absorbance was recorded at 480 nm and phytate content was reported as percentage of dry matter.

Glucosinolate

As explained in Rossetto *et al.* (2013), CM samples were homogenized with 70% methanol solution and trifluoroacetic acid. The extracts were kept in 70°C warm water bath for 30 min and centrifuged at 8000 rpm. The supernatant was oven dried at 40°C for about 72 h until complete evaporation of the solvent. The precipitant was dissolved in HEPES-KOH at pH 7.0 and incubated with 0.12 U thioglucosidase solutions at 37°C for 24 h. The enzyme activity was terminated by 5 µl 18 mmol/l perchloric acid solution. Total glucosinolate content was finally calculated using moles of glucose released.

Phenolic compounds and Tannin

Total phenolic content of samples was determined as described by Makkar *et al*. (2007). In brief, defatted and dried CM samples were mixed with 70% aqueous acetone solution and exposed to 300 W ultrasonic waves at room temperature for 20 min. After centrifuging at 3000 rpm at 4°C, the supernatant was removed and kept on ice. Then, it was diluted by distilled water and mixed with 250 µmol Folin-Ciocalteu reagent (1N) and 1.25 mL sodium carbonate at darkness. After 40 min, the absorbance was read at 725 nm.

Tannin was extracted by mixing samples with 100 mg polypyrrolidone solution for 15 min at 4°C. Then the mixture was centrifuged at 3000 rpm. Finally, tannin content of samples was calculated according to Makkar *et al*. (2007).

Acid and neutral detergent fiber

According to Van-Soest *et al*. (1991), neutral detergent fiber was measured based on washing CM samples by neutral solution. The residue including hemicelluloses, cellulose and lignin was measured and reported as NDF. The acid detergent fiber content of CM samples was determined via subjecting the samples acid solution.

Experimental diets and fish husbandry

Fish husbandry

All male fingerlings of Nile tilapia with an initial weight 3.5±0.1 g were obtained from National Research Center of Saline Water Aquatics (Yazd province, Iran). Fish were randomly stocked in 150 l polyethylene tanks (17 fish per tank) containing aerated underground brackish water in a flow-through system (5±0.48 l/min). Optimum environmental requirements of fish including photoperiod 12 L: 12 D, pH=7.7±0.06, DO=5.77±0.33 ppm, temperature=29.2±0.22°C, salinity= 8±0.2 ppt, total ammonia nitrogen=0.014±0.002 ppm were provided (Hassaan *et al*., 2019). Fish were hand-fed to apparent satiation three times a day (08:00, 12:00 and 16:00 h) and uneaten feed was collected 20 min after feeding for calculating total feed intake according to (Mohammadi *et al*., 2020).

Experimental diets

Four isonitrogenous and isoenergetic experimental diets including control diet (C: canola free) and three diets with 25% of dietary fish meal replacement by CM, CPM and CRM were formulated (Table 2). In brief, all grinded and finely sieved ingredients were weighted to the nearest one gram and thoroughly mixed carefully according to the experimental diets formulation. Then, oil was added and the dough was thoroughly mixed. Finally, cold distilled water was added until stiff dough yielded. The dough was grinded using kitchen meat grinder and the strands were dried at 60°C, then crumbled into appropriate size and kept at 4°C until use. According to Lee *et al*. (2002) chromic oxide was used as digestibility marker at a rate of 0.5 g/100 g dried feed to determine ADCp of experimental diets at the last two weeks of the trial. Proximate composition of ingredients and experimental diets were analyzed according to AOAC (2005). Samples were oven dried at 60°C to constant weight. Auto Kjeldahl system (Behr, Germany) was used for determining crude protein content of samples. Soxhlet system (Behr TRS300, Germany) was used for determining crude lipid content.

Table 2: Ingredients and proximate composition of the experimental diets.

Ingredients (% dry matter)	Dietary treatments			
	C [a]	CM [a]	CPM [c]	CRM [d]
Fish meal [e]	29.1	16.80	16.60	16.90
Soybean meal [f]	12.00	12.00	12.00	12.00
Meat meal [g]	5.00	5.00	5.00	5.00
Corn gluten [h]	10.00	10.00	10.00	10.00
CM	-	25.00	-	-
CPM	-	-	25.00	-
CRM	-	-	-	25.00
Corn starch [i]	32.30	13.00	16.90	17.20
Fish oil [a]	-	1.50	1.50	1.50
Canola oil [j]	1.45	1.47	2.80	2.30
Molasses [k]	5.00	5.00	5.00	5.00
Vitamin premix [l]	2.00	2.00	2.00	2.00
Mineral premix [m]	1.00	1.00	1.00	1.00
Amino acids premix [n]	0.20	0.20	0.20	0.20
Choline chloride [o]	0.50	0.50	0.50	0.50
Vitamin C [p]	0.10	0.10	0.10	0.10
Threonine [q]	0.25	0.25	0.25	0.25
Methionine [q]	0.25	0.25	0.25	0.25
Lysine [q]	0.70	0.70	0.70	0.70
Chromic oxide [r]	0.50	0.50	0.50	0.50
Crude protein (%)	38.00	38.00	38.00	38.00
Ether extract (%)	8.40	8.60	9.40	9.30
NFE (%)	41.80	36.90	35.10	35.10
Ash (%)	6.99	7.76	7.86	7.49
Gross energy (kcal/g)	4.66	4.47	4.47	4.47
Glucosinolate (µmol/g)[s]	-	23.50	3.88	15.50
Phytic acid (%)[s]	-	1.17	0.61	0.20
Phenolic compounds (%)[s]	-	0.71	0.23	0.19
Tannin (%)[s]	-	0.16	0.02	0.02

a: control group
b: non-processed canola meal
c: dephytinized canola meal subjected to ammonia methanol solution
d: irradiated canola meal subjected to ammonia methanol solution
e: Parskilka Mazandaran, Iran.
f: Behpak Industrial Co., Behshahr, Mazandaran, Iran
g: Eris Trade Co., Yazd, Iran
h: Mahshad Yazd Co., Yazd, Iran
i: Mahshad Yazd Co., Yazd, Iran
j: Golbahar Sepahan Co., Isfahan, Iran
k: Eris Trade Co., Mashhad, Iran
l: Composition of vitamin premix (IU, g or mg/kg): A (3600000 IU), D_3 (8000000 IU), E (14.4 g), K_3 (800 mg), B_1 (7 g), B_2 (2.64 g), Niacin (11.8 g), Calcium pantothenate (3.92 g), B_6 (0.4 g), Biotin (40 mg), Choline chloride (100000 mg), Aras Bazar Pharmaceutical Co., Mazandaran, Iran.
m: Composition of mineral premix (g/kg): Mn (39.68 g), Fe (20 g), Zn (33.88 g), Co (4 g), I (0.39 g), Se (0.08 g). Aras Bazar Pharmaceutical Co., Mazandaran, Iran.
n: Composition of amino acid premix (mg/l): L-aspartic 3600; L-glycine 2400; L-lysine 3100; L-histidine 800; L-arginine 2100; L-isoleucine 1900; L-leucine 3200; L-tyrosine 1200; L-phenylalanine 1900; L-alanine 2500; L-cysteine 600; L-valine 2800; DL-methionine 700; L-threonine 2100; L-serine 2700; L-glutamic 6200; L-proline 2100, Kimiafaam Co., Tehran, Iran
o: Eris Trade Co., Mashhad, Iran
p: Kimiafaam Co., Tehran, Iran
q: Eris Trade Co., Mashhad, Iran
r: Merck Co., Germany
s: calculated just based on canola content

Gross energy and ash content of samples were also quantified by colorimetric bomb (PARR, 1261) and muffle furnace (Fanazmagostar FM2P) at 550°C, respectively. According to Austreng *et al*. (2000) chromium oxide contents of combusted feed and faeces samples were determined using phosphoric acid digestion.

Growth and nutritional indices

At the end of the experiment, fish were fasted for 24 h. and the growth indices, feed and protein performance were evaluated using the following formulae (Dossou *et al*., 2018b; Prabu *et al*., 2020):

Total feed intake (g) = total feed fed by fish during the experimental period; FE = body weight gain (g)/feed intake (g); DGC (%day^{-1}) =100×($W_f^{1/3}$ - $W_i^{1/3}$)/t, PER=(W_f - W_i)/(total feed intake×crude protein)×100. Where W_f and W_i were final and initial body weights (g), respectively and t was experimental period in days.

Enzyme activities

Fish were not fed at the last day of the experiment. Three fish from each treatment were randomly sampled, anesthetized using 200 ppm clove powder (Naderi *et al*., 2012). An abdominal incision was made and the intestine was dissected out. The intestinal samples were opened, washed by cold normal saline solution after removing visceral fat and stored at -80°C. The tissues were homogenized using cold 50 mM tris-HCl buffer (pH=7.5). The homogenates were centrifuged (1000 rpm) at 4°C and the supernatants were stored at -80°C until analyses. Alkaline protease activity was determined using Azocasein 2% in Tris-HCl buffer (pH=7.5) as substrate (Garcia-Carreno and Haard, 1993). Nitrophenyl myristate and starch were used as substrate for lipase and α-Amylase determination respectively (Bernfeld, 1955; Iijima *et al*., 1998). The activity of all enzymes was reported as U/g tissue. Each unit of enzyme's activity was defined as µmol of substrate hydrolyzed per minute (Jenabi Haghparast *et al*., 2019).

Mucosal innate immunity

Three fish from each treatment were randomly taken and kept in polyethylene plastic bags containing 10 mL 50 mM sodium chloride solution for 2 min. The secreted mucus was centrifuged at 4°C. The supernatant was stored at -80°C until analyses (Ross *et al*., 2000). Lysozyme, total protease and total immunoglobulin content of samples were determined based on Cuesta *et al*. (2004), Palaksha *et al*. (2008) and Jenabi Haghparast *et al*. (2019), respectively. Mucosa alkaline phosphatase activity was also calorimetrically determined following DGKC (Deutsche Gesellschaft für Klinische Chemie) procedure (Pars-Azmun Co., Tehran, Iran). Total soluble protein content was quantified according to Bradford (1976).

Liver antioxidative enzymes activity and MDA content

Three fish were randomly taken from each treatment. Liver tissues were

dissected out after anesthetizing the fish. Samples were individually frozen and kept at -80°C. Samples were homogenized in phosphate buffer (0.050 M, pH=7.4) at the rate of 10% (w/v) and centrifuged at 12000 rpm, 4°C for 15 min. The supernatant was stored at -80°C until analyses. SOD, CAT and GPx were spectrophotometrically measured at 560, 240 and 340 nm, respectively (Paglia and Valentine, 1967; Aebi, 1984; Kakkar *et al*., 1984, respectively). Reaction of supernatant with thiobarbituric acid solution at high temperature was used to quantify liver tissue MDA content according to Draper and Hadley (1990).

Data analyses

IBM SPSS statistics software version 22.0 (IBM Crop, Armonk, NY, USA) was used for statistical analyses. Before conducting one-way ANOVA, normality of data, distribution and homogeneity of variances were investigated by Shapiro-Wilk's and Levene's tests, respectively. Tukey's HSD test was employed for *post hoc* analysis. Differences were considered statistically significant at $p<0.05$% and the results were reported as mean±standard deviation. Relationships between total feed intake (g) and dietary glucosinolate (µmol/g) content with daily growth coefficient (DGC) were also assessed using polynomial regression analyses.

Results

Growth and nutritional indices and protein digestibility

There was no mortality during the experiment. The diets containing different CM sources significantly affected final weight and DGC ($p<0.05$). The higher final weight and DGC were observed in control diet (27.2±0.97 and 4.23±0.06, respectively). CPM group significantly showed greater final weight and DGC, 21.4±2 and 3.57±0.22, respectively in comparison to other treatments ($p<0.05$). Feed and protein utilization were estimated using FE and PER, respectively. In this regard, CRM included diet showed lower FE and PER. Those fish fed control diet with no canola meal content showed significantly higher feed intake, followed by CPM diet, 663.7±9.2 g and 477±20.2 g, respectively. Regression analysis also revealed a statistically significant relationship between total feed intake and DGC (Fig. 1). Our results also indicated a significant quadratic relationship between dietary glucosinolate content and DGC (Fig. 2), implying that dietary glucosinolate content increased total feed intake and subsequently DGC decreased. Regarding dietary protein digestibility (ADCp), there were no consistent results and the lowest ADCp was observed in fish fed control diet (72.1±1.6) and the highest value was belonged to CM included diet (86.0±1.3) (Table 3, $p<0.05$).

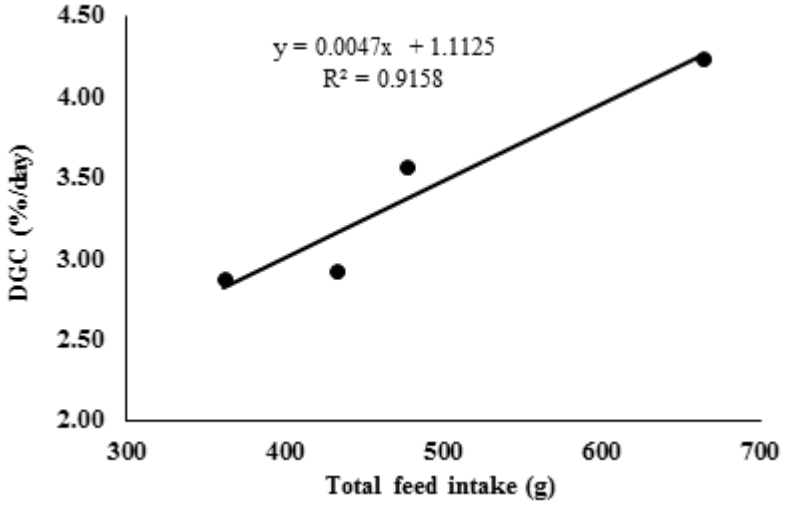

Figure 1: Linear relationship between total feed intake (g) and DGC (%/day).

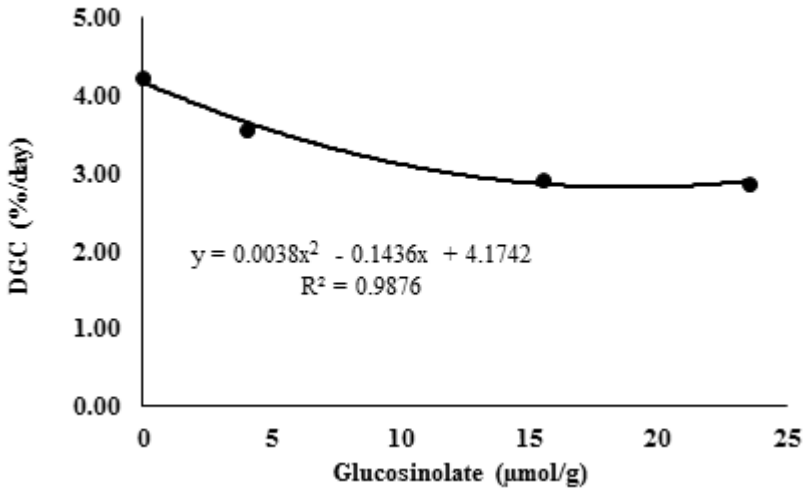

Figure 2: Quadratic relationship between dietary glucosinolate content (µmol/g) and DGC (%/day).

Table 3: Growth and feed performance of experimental groups at the end of the trial (mean ± SD, n=3).

Treatment	Wi (g)	Wf (g)	DGC (%day^{-1})	Total feed intake (g)	PER	FE	ADC_p (%)
C**	3.54 ± 0.09^{a*}	27.2 ± 0.98 ^{c}	4.23 ± 0.06^{c}	663.7 ± 9.2 ^{d}	1.69 ± 0.07^{b}	0.60 ± 0.02 ^{a}	72.15 ± 1.59^{a}
CM	3.49 ± 0.05^{a}	15.9 ± 0.56 ^{a}	2.88 ± 0.10^{a}	361.5 ± 4.2 ^{a}	1.64 ± 0.06 ^{ab}	0.59 ± 0.02 ^{a}	86.01 ± 1.34^{c}
CPM	3.56 ± 0.11^{a}	21.5 ± 2.05 ^{b}	3.57 ± 0.22^{b}	477.0± 20.2^{c}	1.78 ± 0.12 ^{b}	0.63 ± 0.04^{a}	76.88± 0.85^{b}
CRM	3.66 ± 0.04^{a}	16.9 ± 1.09^{a}	2.92 ± 0.15^{a}	433.2± 16.5^{b}	1.45 ± 0.07^{a}	0.52 ± 0.02^{b}	80.17± 1.79^{b}

* Values with different subscripts in each column denote statistically significant differences at $p>0.05$.

** C control diet, CM diet containing non-processed canola meal, CPM diet containing dephytinized canola meal subjected to ammonia methanol solution and CRM diet containing irradiated canola meal subjected to ammonia methanol solution.

Digestive enzymes activity

There were no significant difference regarding various intestinal digestive enzymes activity, i.e. alkaline protease, lipase and amylase, among experimental groups fed diets containing various canola meal sources (Table 4; $p>0.05$).

Table 4: Digestive enzymes activity of experimental groups at the end of the trial (mean ± SD, n=3).

Treatment	Amylase (U/g)	Lipase (U/g)	Alkaline Protease (U/g)
C	58.70 ± 8.21 [a]	2.28 ± 0.20 [a]	14.50 ± 1.68 [a]
CM	54.10 ± 3.06 [a]	2.42 ± 0.10 [a]	14.10 ± 1.73 [a]
CPM	51.50 ± 5.98 [a]	2.03 ± 0.01 [a]	13.20 ± 1.34 [a]
CRM	52.90 ± 2.82 [a]	2.23 ± 0.11 [a]	13.50 ± 1.00 [a]

* Values with different subscripts in each column denote statistically significant differences at P>0.05.

** C control diet, CM diet containing non-processed canola meal, CPM diet containing dephytinized canola meal subjected to ammonia methanol solution and CRM diet containing irradiated canola meal subjected to ammonia methanol solution.

Mucosal innate immunity

The results revealed that total immunoglobulin, lysozyme, alkaline protease and alkaline phosphatase activities did not exhibit any significant difference among experimental diets (Table 5; $p>0.05$). Meanwhile, higher alkaline protease and phosphatase activity and lower immunoglobulin content were observed in CRM group.

Table 5: Mucosal innate immunity of experimental groups at the end of the trial (mean ± SD, n=3).

Treatment	Lysozyme (U/ml/min)	Alkaline phosphatase (U/mL)	Alkaline protease (U/ml)	Immunoglobulin (U/mL)
C	18.000 ± 4.24 [a]	0.048 ± 0.010 [a]	0.067 ± 0.003 [a]	0.050 ± 0.006 [a]
CM	21.500 ± 6.36 [a]	0.046 ± 0.012 [a]	0.066 ± 0.040 [a]	0.048 ± 0.001 [a]
CPM	15.500 ± 3.54 [a]	0.046 ± 0.013 [a]	0.074 ± 0.013 [a]	0.046 ± 0.002 [a]
CRM	21.000 ± 2.83 [a]	0.051 ± 0.010 [a]	0.079 ± 0.025 [a]	0.045 ± 0.001 [a]

* Values with different subscripts in each column denote statistically significant differences at P>0.05.

** C control diet, CM diet containing non-processed canola meal, CPM diet containing dephytinized canola meal subjected to ammonia methanol solution and CRM diet containing irradiated canola meal subjected to ammonia methanol solution.

Liver antioxidative enzymes activity and MDA content

Despite replacing non-processed and differently processed canola meals, liver antioxidative enzymes activity, including SOD, GPx and CAT were similar among various experimental groups (Table 6; $p>0.05$). However, higher SOD, GPx and CAT activity was observed in CPM group (0.235±0.054, 0.903±0.109 and 0.395±0.104, respectively) and those fish fed on diet contained non-processed canola meal had lower activities (0.216±0.040, 0.803±0.146 and 0.370±0.056, respectively; Table 6). It was also found that there were no significant differences among experimental groups regarding MDA content of liver tissue ($p>0.05$).

Table 6: Liver antioxidant enzymes activity and MDA content of experimental groups at the end of the trial (mean ± SD, n=3).

	SOD (U/mg)	GPx (U/mg)	CAT (U/mg)	MDA (µmol/g)
C	0.228 ± 0.049 [a]	0.856 ± 0.234 [a]	0.363 ± 0.110 [a]	0.401 ± 0.159 [a]
CM	0.216 ± 0.040 [a]	0.803 ± 0.146 [a]	0.370 ± 0.056 [a]	0.399 ± 0.154 [a]
CPM	0.235 ± 0.054 [a]	0.903 ± 0.109 [a]	0.395 ± 0.104 [a]	0.363 ± 0.063 [a]
CRM	0.222 ± 0.027 [a]	0.813 ± 0.175 [a]	0.357 ± 0.061 [a]	0.356 ± 0.086 [a]

* Values with different subscripts in each column denote statistically significant differences at P>0.05.

** C control diet, CM diet containing non-processed canola meal, CPM diet containing dephytinized canola meal subjected to ammonia methanol solution and CRM diet containing irradiated canola meal subjected to ammonia methanol solution.

Discussion

Necessity of finding suitable alternative dietary protein and oil sources and limitation of ANTs content of plant protein resources (Mohammadi *et al*., 2020) resulted in considerable scientific research on efficacy of different processing procedures to improve quality of plant proteins for feed formulation of various fish and shrimp species. However, in most studies the efficacy of a single processing method has been investigated (Jannathulla *et al*., 2019; Hernández *et al*., 2020).

Including 25% dietary canola meal with different qualities of various ANT contents was based on using different plant protein sources at the recommended level of 25% of diets for different fish species, including tilapia. It has been shown that subtle reduction was observed in different growth indices, mainly SGR, due to partial dietary fish meal substitution by roasted (20%), yeast-fermented (24.5%) or ammonia-methanol solution refluxed dephytinized canola meal (25%) in diets of Japanese sea bass, *Lateolabrax japonicus*, Asian sea bass, *Lates calcarifer*, or Nile tilapia, *Oreochromis niloticus*, respectively (Cheng *et al*., 2010; Plaipetch and Yakupitiyage, 2012; Mohammadi *et al*., 2020). However, our results revealed a considerable decrease in growth performance of fish received diets including differently processed canola meal to the extent that CPM group showed significantly lower DGC in comparison to control group while its ANTs content was considerably decreased in comparison to other canola meal contained diets (3.57±0.22 vs. 4.23±0.06). In terms of DGC, similar results have been previously reported in tilapia and red-tailed catfish when received plant protein included diet (Deng *et al*., 2017; Zhang *et al*., 2020). According to Table 1, glucosinolate contents of CM, CRM and CPM diets (µmol/100 g diet) were 23.5, 15.5 and 4, respectively, implying that it might undesirably affect growth performance of the fish (Francis *et al*., 2001). Also, as illustrated in Figures 1 and 2 and Table 2, decreased feed intake due to dietary contents of ANTs might result in poor fish growth performance. Similarly, lower feed intake following feeding on different experimental diets including processed and non-processed canola meal has been reported in Nile tilapia

(Nemati Shirazi, 2014; Mohammadi *et al.,* 2015, 2020).

Except for CRM, there were no significant differences regarding FE and PER among other experimental groups. Although, the highest DGC was observed in those fish received diet devoid of canola meal, the CPM showed better FE and PER implying acceptable protein quality of the processed canola meal. The same results are shown in Nile tilapia (Soares *et al*., 2001; Luo *et al*., 2012) and Spotted rosed snapper, *Lutjanus guttatus*, (Hernández *et al*., 2020). However, it was not in agreement with ADCp in comparison to CM and CRM. One possible explanation of higher ADCp in those fish received canola meal is that they had lower feed intake. Therefore, fish utilized dietary protein more efficiently than control group which consumed two times more feed in comparison to CM group with significantly higher ADCp. Meanwhile, thorough understanding the underlying mechanism requires more research.

We estimated that the best processed canola meal in our study, i.e. CPM, would cost ca. 1200 $/Mton, while the international price for fishmeal is around 1500 $/Mton. Therefore, regarding the feed efficiency (FE) of fish received CPM included diet, using 25% CPM in juvenile Nile tilapia diet not only would result in acceptable growth performance at the end of the growth period, but also considerable reduction in final fish price would be achieved.

It is revealed that dietary fish meal substitution by differently processed canola meal did not affect intestinal digestive enzymes activity. However, in previous studies on replacing fish meal by plant protein sources digestive enzymes activity, including alkaline protease, lipase and amylase changed. For instance, dietary inclusion of sunflower meal in juvenile Nile tilapia diet resulted in lower trypsin, chymotrypsin, lipase and amylase activity (Hassaan *et al*., 2019). Meanwhile, similar to our results Cheng *et al*. (2010) and Dossou *et al*. (2018a, b) witnessed that there were no differences among fish received fermented rapeseed meal (Nile tilapia) or roasted canola meal (Japanese sea bass) and those fish fed on diet contained fish meal in this regard.

Body surface mucus is considered the first defense line in fish (Salinas *et al*., 2011). Various indices including mucosal alkaline phosphatase, alkaline proteases and lysozyme activity or immunoglobulin content are involved in initial wound healing and mucosal antibacterial properties (Zhu *et al*., 2013). Our results indicated that dietary inclusion of differently processed and crude canola meal instead of fish meal did not significantly affect mucosal innate immunity of juvenile Nile tilapia. Similarly, Dossou *et al*. (2018a, b) reported that diets containing fermented canola meal did affect immune responses in red seabream, *Pagrus major*. Meanwhile, it is discussed that dietary ANTs content beyond tolerance threshold of fish could undesirably affect fish immune-competence similar to results reported on fry yellowfin seabream, *Acanthopagrus latus*,

following replacing dietary fish meal by plant protein (Abbasi *et al*., 2020).

Superoxide dismutase (SOD) catalyzes superoxide radicals (O_2^-) dismutation to molecular oxygen and hydrogen peroxide (Borgstahl and Oberley-Deegan, 2018), which in turn is further converted to water and ordinary molecular oxygen by GPx and CAT (Bai *et al*., 2019). In addition, tissue content of malondialdehyde (MDA) as an indicator of lipid peroxidation is investigated especially when fish were fed on diet contained plant protein sources (Morales and Munné-Bosch, 2019). In the present study various dietary treatments did not affect these markers of oxidative stress in fish liver samples. Similar to our results, SOD and GPx activity of yellow catfish, *Tachysurus fulvidraco*, were not affected following feeding on diets contained soybean meal (Jiang *et al*., 2018). Wang *et al*. (2017) also found that MDA content and CAT and GPx activity of yellow croaker, *Larimichthys crocea*, did not differ in response to feeding diets with graded levels of soybean meal found.

In conclusion, juvenile Nile tilapia showed lower growth performance and nutritional indices mainly due to reduced feed intake following feeding on diets contained differently processed or crude canola meal. In addition, various mucosal innate immunity and liver anti-oxidative indices did not differ among experimental groups.

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