Effect of yeast-fermentation of canola meal on digestibility, growth performance and hindgut inflammation of Nile tilapia *Oreochromis niloticus* and rainbow trout *Onchorynchus mykiss*

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1. Introduction

In the last three decades, aquaculture has expanded by almost 1200% with an average annual growth rate of 8.8% (FAO, 2012). The rapid expansion of aquaculture has resulted in increased demands for the primary fish feed raw ingredients - fish oil and fishmeal (Lunger et al., 2007). The sustainability of the aquaculture industry is limited due to the discrepancy between the supply and demand for fishmeal and fish oil. Therefore, future formulations of fish feed will require a wide range of alternative ingredients to replace these marine products. The use of plant sources of protein as fishmeal replacements in fish feed is currently a vital research area for the sustainability of the aquaculture industry.

Canola meal (CM), the by-product of canola crushing, is the primary high-protein ingredient in Western Canada, which is used as a protein supplement in feed rations for livestock. However, the use of canola/rapeseed meal in fish feed is limited due to its low nutrient digestibility and adverse effects on fish growth, even at low inclusion rates in rainbow trout (Meta-analysis, Collins et al., 2013), Japanese seabass (Cheng et al., 2010) and hybrid tilapia (Zhou and Yue, 2010). This is primarily due to the presence of antinutritional factors (ANFs), such as glucosinolates, tannins, phytic acid and indigestible carbohydrates (Francis et al., 2001; Bonnardeaux, 2007). Research on the improvement of the nutrient value of CM in fish diets has focused mainly on the fractionation of CM to create canola protein concentrate (CPC; Drew, 2004; Thiessen et al., 2004; Burr et al., 2013; Collins et al., 2013). However, the high cost of the fractionation process has confined the acceptance of CPC in aquafeed industry.

Currently, a concerted effort has been put towards to the improvement the nutritive value of animal feeds by using exogenous enzymes, such as a phytase, protease and/or carbohydrase. The use of protease to produce protein isolates from vegetable sources has been shown to effectively increase the protein yields to 90% for soybean meal and to 50% for rapeseed meal (Sari et al., 2013). Similarly, the protein content of canola meal was increased from 40 to 68% with the treatment of Viscozyme L (a commercially available carbohydrase enzyme mixture; Rodrigues et al., 2014). In aquafeeds, fishmeal has higher amount of phosphorus (P), whereas, vegetable ingredients generally contains lower level of P due to the major amount of P is bound as phytic acid (IP6; a heat-stable antinutrient). The presence of IP6 reduces the availability of P and other minerals (such as zinc, magnesium and calcium) for fish caused by the chelating

properties (Storebakken et al., 1998). Improved mineral utilization was found in Atlantic salmon fed phytase pre-treated diet (Denstadli et al., 2007).

Fermentation has traditionally been used to prepare foods in Asia (Lee, 1998; Lim et al., 2010). Microbial fermentation is one of the most promising approaches to reducing ANFs and improving the nutritional value of plant-based feed ingredients (Oboh and Akindahunsi, 2003; Bake et al., 2015). Fermentation also has the potential for growth enhancement and immunostimulants in aquaculture (El-Sayed, 2003; Bairagi et al., 204; Ramacandran and Ray 2007). Moreover, fermentation increases levels of protein and the availability of minerals (such as iron, calcium and magnesium) and vitamins (such as riboflavin, thiamine, niacin, B6 and B12) in some plant meals (Oboh and Akindahunsi, 2003; Oboh and Elusiyan, 2007; Bake et al., 2015). Several recent experiments have shown that yeast fermentation (*Saccharomyces cerevisiae*) of CM and/or CPC, overnight at room temperature, result in a product with improved nutrient value and reduced negative effects on the growth of Asian sea bass (up to 50 %, Plaipetch and Yakupitiyage, 2012; CPC, Safari et al., 2012). This has also been shown in black sea bream with *S. cerevisiae*-fermented soybean meal at levels up to 20 % (Zhou et al., 2011), Nile tilapia at levels up to 50 % (Plaipetch and Yakupitiyage, 2014).

The present study aims to examine the effects of feeding enzyme pre-treated and yeast-fermented CM on the nutrient digestibility and growth performance of Nile tilapia and rainbow trout.

2. Objectives

1) To determine the effect of extrusion combined with *C. utilis* fermentation of CM on the nutrient digestibility, growth performance and intestinal inflammation and function of Nile tilapia. Nile tilapia represents an omnivorous, warm-water species, which is reputed to be less sensitive to the effects of canola ANFs.
2) To determine the effect of extrusion combined with *C. utilis* fermentation of CM on the nutrient digestibility, growth performance and intestinal inflammation and function in rainbow trout of rainbow trout. Rainbow trout represents a carnivorous, cold-water species that is known to be sensitive to ANFs present in CM.

3. A Meta-Analysis of the Effects of Dietary Inclusion of Microbial Fermented Plant Products on Growth Rate of Fish

3.1. Abstract

The effects of feeding fermented plant-derived ingredients (FPIs) on the specific growth rate (SGR) of carnivorous, omnivorous and herbivorous species were quantified using a meta-analysis of published results. From 1176 studies identified, 29 studies were applied in the present meta-analysis. Hedeges' g was used to measure the standardized mean difference (effect size) between control (fishmeal control/unprocessed raw ingredient control) and experiment SGR. Linear and quadratic regressions were used to determine the relationship between SGR and dietary inclusion levels of FPIs. The results showed that replacing fishmeal (FM) with FPIs had significantly negative effect on SGR of carnivorous fish ($P < 0.05$), whereas, the effect was not significant on herbivorous and omnivorous fish ($P > 0.05$). By contrast, FPs showed significantly positive effect on SGR of herbivorous and omnivorous species when replacing the unprocessed raw ingredients (RI) in their diets ($P < 0.05$). The significant effect was not found in carnivorous fish ($P > 0.05$). There were no significant linear or quadratic effects on SGR with different inclusion levels of fermented products in all fish species ($P > 0.05$). The results suggest that fermented plant proteins could be used in diets for herbivorous and omnivorous fish without negative effects on growth of fish. However, it should be concerned when using fermented plant proteins as fishmeal replacer in carnivorous fish.

Abbreviations: ANFs, anti-nutritional factors; CI, confidence interval; FPIs, fermented plant-derived ingredients; FM, fishmeal; HG, Hedeges' g; RIs, unprocessed raw ingredients; SD, standard deviation; SEM, standard error of mean; SMD, standardized mean difference; TGC, thermal growth coefficient;

3.2.Introduction

In the last three decades, aquaculture has expanded by almost 1200% with an average annual growth rate of 8.8% (FAO, 2012). The rapid expansion of aquaculture has highlighted the necessity of finding a wide range of alternative ingredients to replace the marine origin raw materials - fishmeal (FM) and fish oil. Partially or totally replacing FM with plant sources of protein in fish feed is currently a vital research area for the sustainability of the aquaculture industry. However, the use of plant proteins is limited due to the unbalance amino acid profile, low protein content, poor palatability as well as the presence of anti-nutritional factors (ANFs). Production-related impacts when feeding plant proteins are observed in nutrient digestibility, growth performance, feed intake and feed conversion (Drew et al., 2005; Forster et al., 1999; Gao et al., 2011; Refstie et al., 1998; Torstensen et al., 2008). Additional influences of plant protein sources include detrimental impacts on gut histology and morphology, altered liver morphology and increased expression of inflammatory marker genes and shifts in intestinal microbial populations (Bakke-McKellep et al., 2000; Burrells et al., 1999; Desai et al., 2012; Krogdahl et al., 2003; Mansfield et al., 2010; Merrifield et al., 2009; Sørensen et al., 2011). The rapid advancement in feed processing techniques has improved the nutritional content and reduced the ANFs of the plant-based proteins, thus, improved the nutrient digestibility and utilization of plant proteins in fish (Bake et al., 2015). These processing techniques include fractionation (Drew, 2004; Thiessen et al., 2004; Burr et al., 2013), heat treatment (Arndt et al., 1999; Peres et al., 2003), enzyme supplementationm (Rodrigues, et al., 2014; Denstadli et al., 2007) and microbial fermentation (Ramacandran and Ray 2007; Lim et al., 2010; Plaipetch and Yakupitiyage, 2014; Trushenski et al., 2014).

Fermentation has traditionally been used to prepare foods in Asia (Lee, 1998; Lim et al., 2010). Microbial fermentation is one of the most promising approaches to reducing ANFs and improving the nutritional value of plant-based feed ingredients (Oboh and Akindahunsi, 2003; Bake et al., 2015). Fermentation also has the potential for growth enhancement and immunostimulants in aquaculture (El-Sayed, 2003; Bairagi et al., 204; Ramacandran and Ray 2007). Moreover, fermentation increases levels of protein and the availability of minerals (such as iron, calcium and magnesium) and vitamins (such as riboflavin, thiamine, niacin, B6 and B12) in some plant meals (Oboh and Akindahunsi, 2003; Oboh and Elusiyan, 2007; Bake et al., 2015).

Several meta-analysis studies have been performed on feeding plant-derived ingredients to fish. The effects of dietary inclusion of soybean products and canola/rapeseed in fish were reviewed by Sales (2009) and Enami (2011), respectively. Collins et al. (2013) investigated the effects of feeding six plant ingredients including pea meal, pea protein concentrate, soybean meal, soy protein concentrate, canola/rapeseed meal and canola/rapeseed protein concentrate on specific growth rate (SGR) in salmonid fish. Francis et al. (2001) reviewed the effects of ANFs in plant-based ingredients on fish. Hua and Bureau (2012) examined the effect of replacing fishmeal with plant protein ingredients on thermal growth coefficient (TGC) using meta-analysis and nutritional model simulation-based approaches. However, no previous reviews have been carried out to determine the use of fermented plant-derived ingredients (FPIs) in feeds for farmed fish.

Summative results from narrative review do not take measures of dispersion into account and consider all studies with equal weight. By contrast, meta-analysis, which provides a quantitative synthesis of data, can be used to evaluate and integrate results from a group of studies (Sales, 2009). The objectives of the present study were to: 1) conduct a meta-analysis of feeding FPIs to determine the relationships between dietary inclusion levels of FPIs on growth performance of different fish species; 2) compare the difference in growth performance between carnivorous species and herbivorous and omnivorous species when replacing FM or unprocessed plant raw ingredients (RIs) with FPIs. 3) perform a comprehensive comparison of all published results from the existing literature in order to present baseline values for future studies.

3.3. Materials and Methods

3.3.1 Search strategy and inclusion criteria

In July of 2015, a comprehensive literature search was conducted on the Internet using the search engines of WEB OF Science (1900-2015) and SCIRUS (1900-2015) with the following search terms: Topic (complete document) = (fermentation OR fermented) AND Topic (complete document) = (fish feed) AND Topic (complete document) = (growth OR SGR). Manual searches using Google Scholar supplemented the data base search strategy. To prevent selection bias, studies were screen based on the pre-specified inclusion criteria: 1) random allocation of animals; 2) growth study; 3) use of fish species; 4) used as protein source, not oil; 5) only test

ingredient was fermented, not the whole diet; 6) test ingredient should be plant-based; 7) presence of a control group not fed the test ingredient; 8) the experimental diets were formulated to contain the same amount of FM when comparing fermented test ingredients with unprocessed raw ingredients; 9) FPIs were used to replace either FM or the corresponding unprocessed raw ingredient (e.g. fermented canola meal vs. canola meal). 10) the experimental diets should be formulated to be iso-nitrogenous and iso-energetic; 11) SGR was either reported or could be calculated form the reported data; 12) written in English. Duplicate reports, reviews and conference proceedings were removed. The selected studies were separated according to fish species (carnivorous, omnivorous and herbivorous) and control ingredient (FM or RI). Due to the limited number of studies on omnivorous species, the studies of omnivorous and herbivorous species were combined together.

3.3.2. Data extraction

From each study, the following information was extracted: authors (&year), fish species, microbial species, sample size, test ingredient type and inclusion level. In addition, the growth indicator- specific growth rate (SGR) and a reported standard deviation (SD) were directly extracted or calculated. SGR from the entire experimental period was used even some studies reported several SGR from different time intervals throughout the trial. The equation for calculating SGR is as follows: $SGR = 100 * [\ln W_1 - \ln W_0] / D$, where W_0 and W_1 represent initial and final weights (means of each experimental unit); and D represent feeding days.

3.3.3. Statistical analysis

A random effect model of Review Manager (RevMan) [Computer program] (Version 5.3, Copenhagen: The Nordic Cochrane Centre, The Cochrane Collaboration, 2014) was used to conduct the meta-analyses. Standardized mean difference (SMD; effect size) between control and experimental SGR was measured using Hedeges' g (HG; Hedges and Olkin, 1985), with a 95% confidence interval (CI) and an alpha level of $P < 0.05$. HG was calculated using the following equation: $HG = (SGR_T - SGR_C)/SD_{pooled}$, where SGR_T and SGR_C represent the SGR of test-diet fed fish and control diet-fed fish, respectively; SD_{pooled} represent the pooled standard deviation (SD) for the two groups. A random-effects model was used to calculate summary

statistics in order to consider between-trial variability (true heterogeneity) and within-trial variability (sampling error) (Hedges and Vevea, 1998).

Linear Model procedure of SPSS (Version 22, SPSS IBM Inc. Armonk, NY, USA) was used to analyze the weighted linear and quadratic regression of incorporation of FPIs on SMD. Regressions were considered significant when $P < 0.05$. Only linear regression was reported for all studies as the P values and R^2 were almost identical for both linear and quadratic regressions. t-test was used for the comparison between herbivorous and omnivorous species and carnivorous species when using FPIs to replace either fishmeal or RIs in diets for fish.

3.4. Results

3.4.1 Study Description

Figure 3.1. summarizes the selection process for the present meta-analysis study. Of forty-five randomized, controlled trials, sixteen trials were excluded due to the following reasons: diets were not formulated to be iso-nitrogenous and iso-energetic (Li et al., 2010); six trial did not report measure of variation or indicate the reported values were SD or SEM (standard error of mean) (Alegbeleye and Olude, 2009; El-Sayed, 2003; Iluyemi et al., 2010; Ng et al., 2002; Olaniyi and Falaye, 2013; Ademola and Oyedokun, 2009); seven trial did not provide enough information for the calculation of SGR or the SD for the SGR (Barnes et al., 2015; Hassaan et al., 2015; Kim et al., 2009; Plaipeth and Yakupitiyage, 2010; Shamma et al., 2015; Shiu et al., 2015; Ubalua and Ezeronye, 2008); phytase was added to the test diets (Lim et al., 2010); Obasa et al. (2013) didn't use any microbial species in their fermentation process (only water soaking).

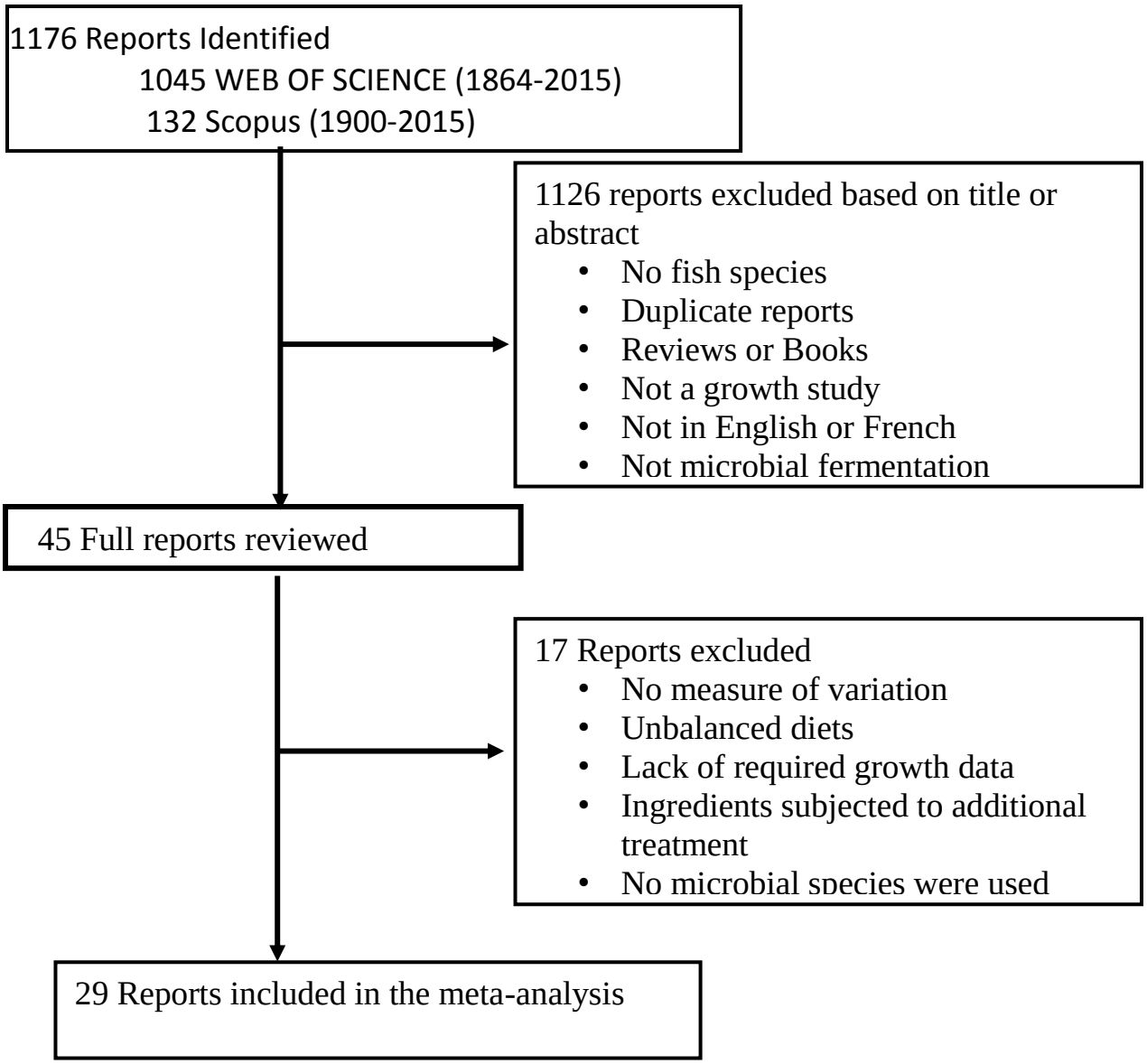


Figure 3.1. Flow diagram of study selection process.

The inclusion criteria were met by 29 trials, which were reported between1999 and 2015. The sample sizes of these trials varied between two to four experiment units per treatment with a total of 834 experiment units. These trials were separated into four groups according to the control ingredient (fishmeal vs. unprocessed raw materials) and fish species (carnivorous vs. herbivorous & omnivorous). The dietary inclusion levels of FPIs in herbivorous and omnivorous species ranged from 100-800 g/kg for FM control group and 30-656 g/kg for RI control group); and the inclusion levels for carnivorous species varied between 22-521 g/kg and 30-521 g/kg for FM control group and RI control group, respectively. Forest plot (**Figure 3.2 and 3.3**) demonstrate the pooled effect of replacing FM or RIs with FPIs in different fish species.

3.4.2. Fishmeal replacement in herbivorous and omnivorous fish

Thirty-nine data points from ten studies were included in the analysis. The effect size (**Figure 3.2a**) for the comparison between FRIs and FM control in herbivorous and omnivorous fish varied from -17.13 (426.7 g/kg) to 13.39 (400 g/kg). The overall mean was 0.41 (95% CI: -0.1 to 0.92; $P = 0.11$; **Table 3.1**). The weighted linear and quadratic regressions were not significant ($P > 0.05$; **Figure 3.4 a**).

3.4.3. Fishmeal replacement in carnivorous fish

Forty-five data points from thirteen studies were included in the analysis. The effect size (**Figure 3.2b**) for the comparison between FPIs and FM in carnivorous species ranged between -8.79 (360 g/kg) and 4.39 (87 g/kg). The overall mean was - 0.54 (95% CI: -0.87 to 0.21; $P = 0.001$; **Table 3.1**). The weighted linear and quadratic regressions were not significant ($P > 0.05$; **Figure 3.4b**).

3.4.4. Unprocessed raw material replacement in herbivorous and omnivorous fish

Forty data points from ten studies were included in the analysis. The effect size (**Figure 3.2c**) for the comparison between FPIs and RI control in herbivorous and omnivorous species ranged between -1.15 (228 g/kg) and 24.7 (400 g/kg). The overall mean was 0.32 (95% CI: -0.

28 to 0.92; *P* = 0.001; **Table 3.1**). The weighted linear and quadratic regressions were not significant (*P* > 0.05; **Figure 3.4c**).

3.4.5. Unprocessed raw ingredient replacement in carnivorous fish

Thirteen data points from five studies were included in the analysis. This was the smallest data set among the four groups of studies. Effect size (**Figure 3.2 d)** for the comparison between FPIs and RI control in carnivorous species ranged between -0.09 (250g/kg) and 0.64 (462.5 g/kg). The overall mean was 0.93 (95% CI: 9.38 to 1.48; *P* = 0.30; Table 9). The weighted linear and quadratic regressions were not significant (*P* > 0.05; **Figure 3.4d**).

3.4.6. Fishmeal replacement in all fish species

The overall effect size for the comparison between FPIs and FM in all fish species was -0.19 (95% CI: -0.49 to 0.11; *P* = 0.22) (**Figure 3.3a**; Table 9). The weighted linear and quadratic regressions were not significant (*P* > 0.05; **Figure 3.4e**). The effect of replacing FM with FPIs on SGR was significantly different between herbivorous and omnivorous fish and carnivorous fish (P < 0.05; **Table 3.9**).

3.4.7. Unprocessed raw ingredient replacement in all fish species

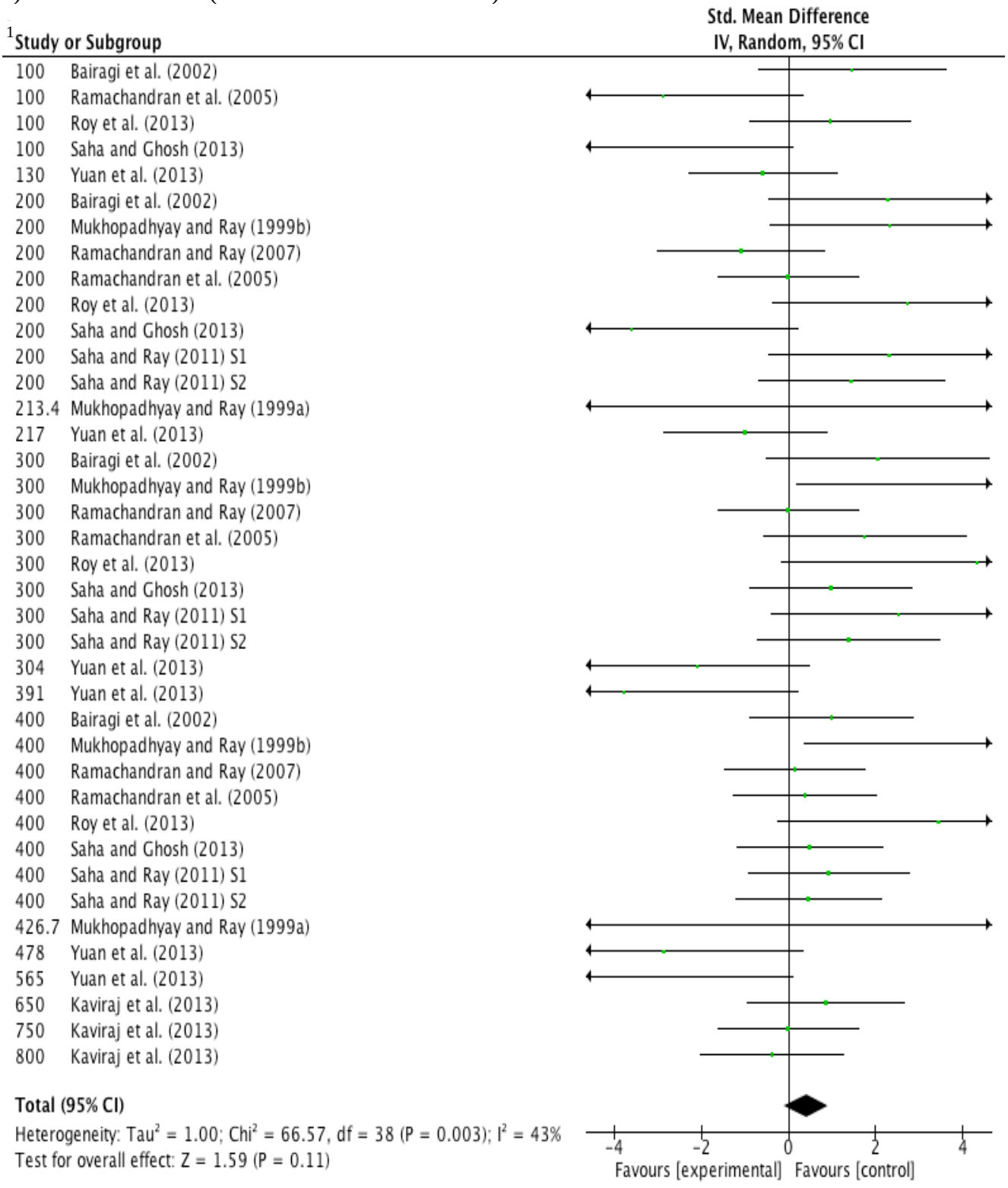
The overall effect size for the comparison between FPIs and RI control in all fish species was 0.70 (95% CI: 0.28 to 1.13; *P* = 0.001; **Figure 3.3b; Table 3.1**). The weighted linear and quadratic regressions were not significant (*P* > 0.05; **Figure 4f**). There was no significant difference between herbivorous and omnivorous fish and carnivorous fish when replacing RIs with FPIs (P > 0.05; **Table 3.1**).

3.5. Conclusion

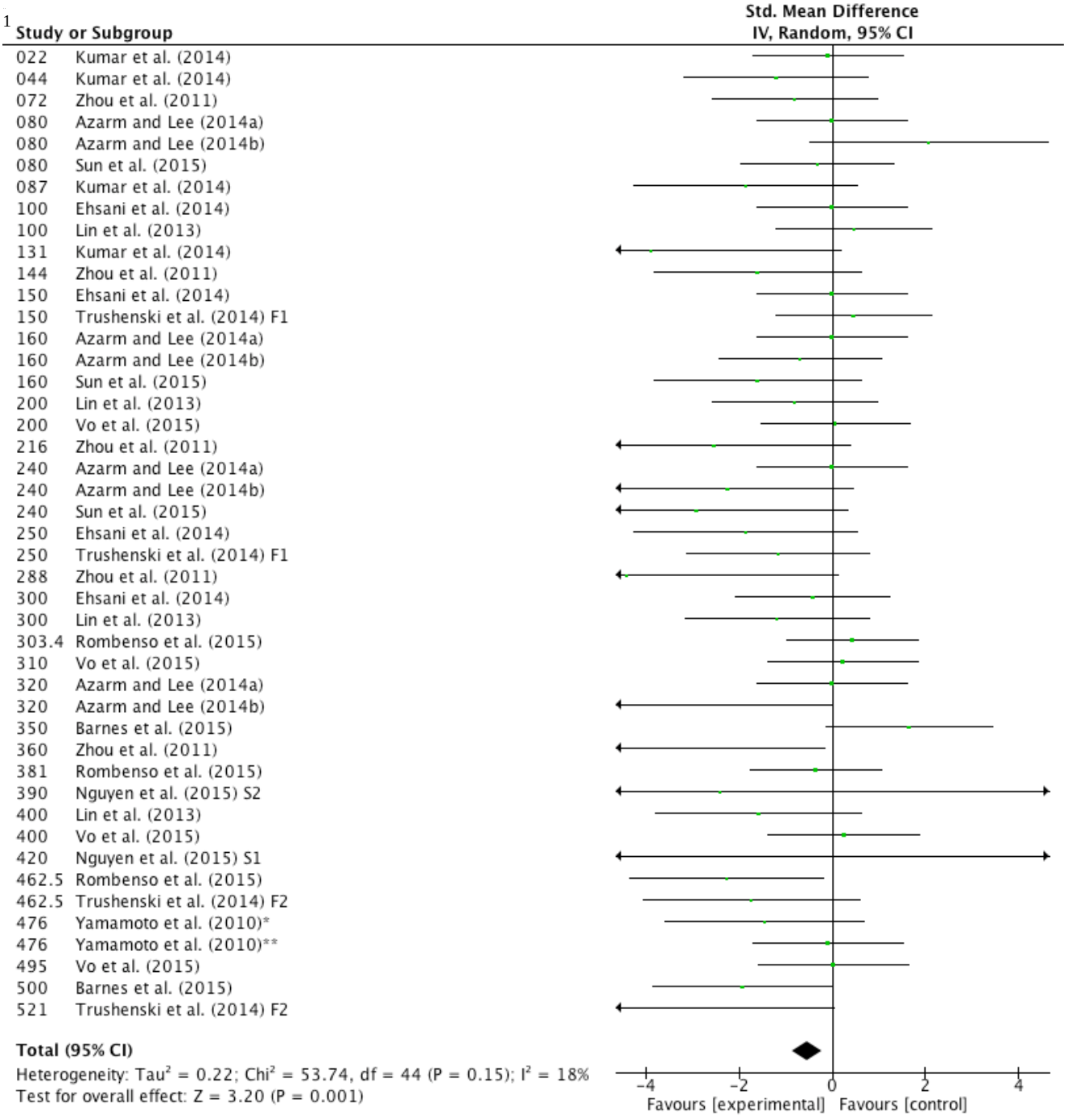
According to the results obtained from the existing literature, the meta-analysis indicated that fermented plant-derived proteins could be incorporated in diets for herbivorous and omnivorous fish without compromising growth of fish. However, the use of fermented plant-derived proteins as

fishmeal replacement in diets for carnivorous fish should be done with caution. In conclusion, fermented plant proteins have the potential to be fishmeal alternatives in fish diets. A future recommendation would be to investigate the possible approaches for further increasing the efficiency of microbial fermentation process, such as using exogenous enzyme to breakdown fibre component of plant-based products.

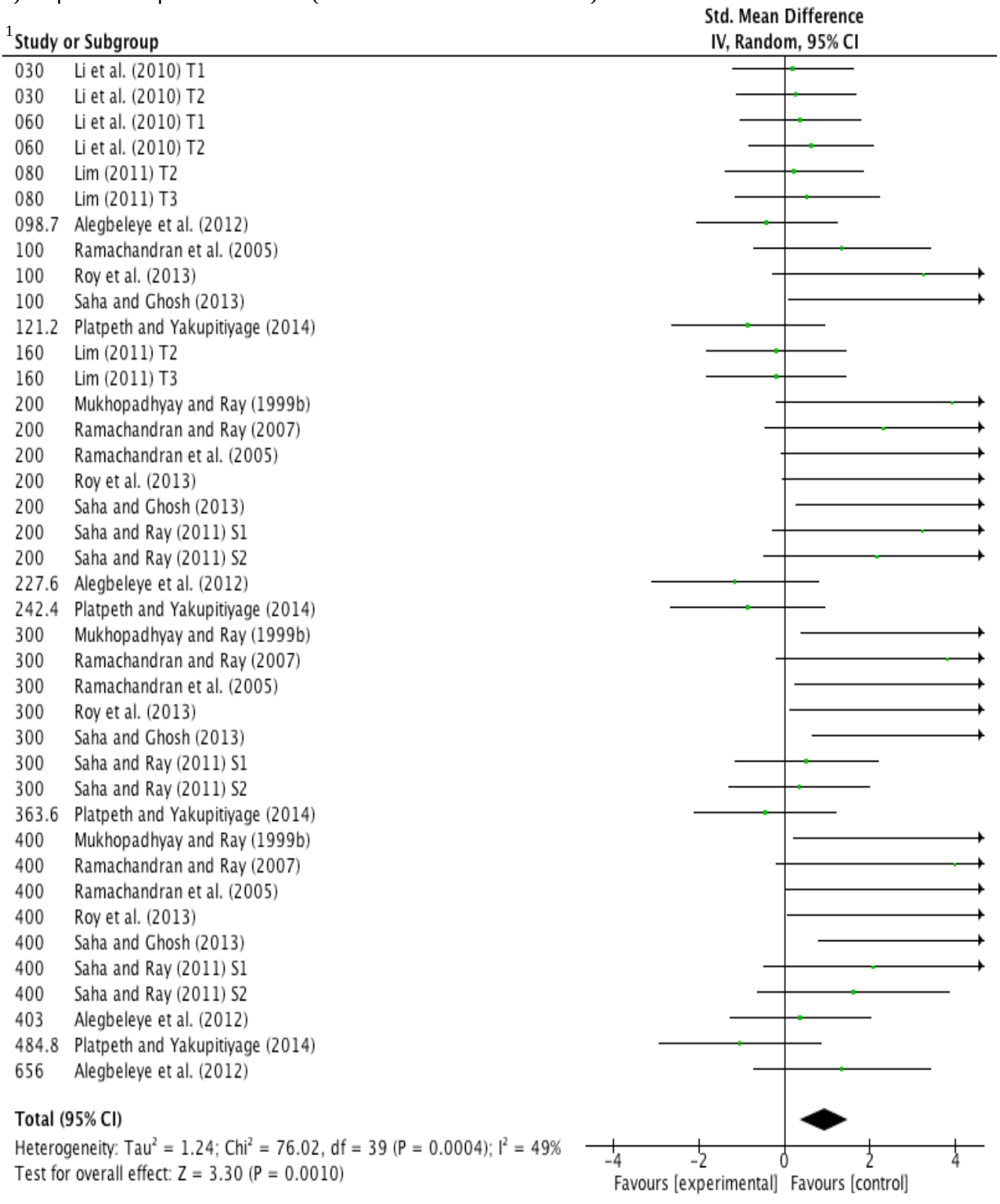
a) Fishmeal control (Herbivorous & Omnivorous)



b) Fishmeal Control (Carnivorous)



c) Unprocessed product control (Herbivorous & Omnivorous)



d) Unprocessed product control (Carnivorous)

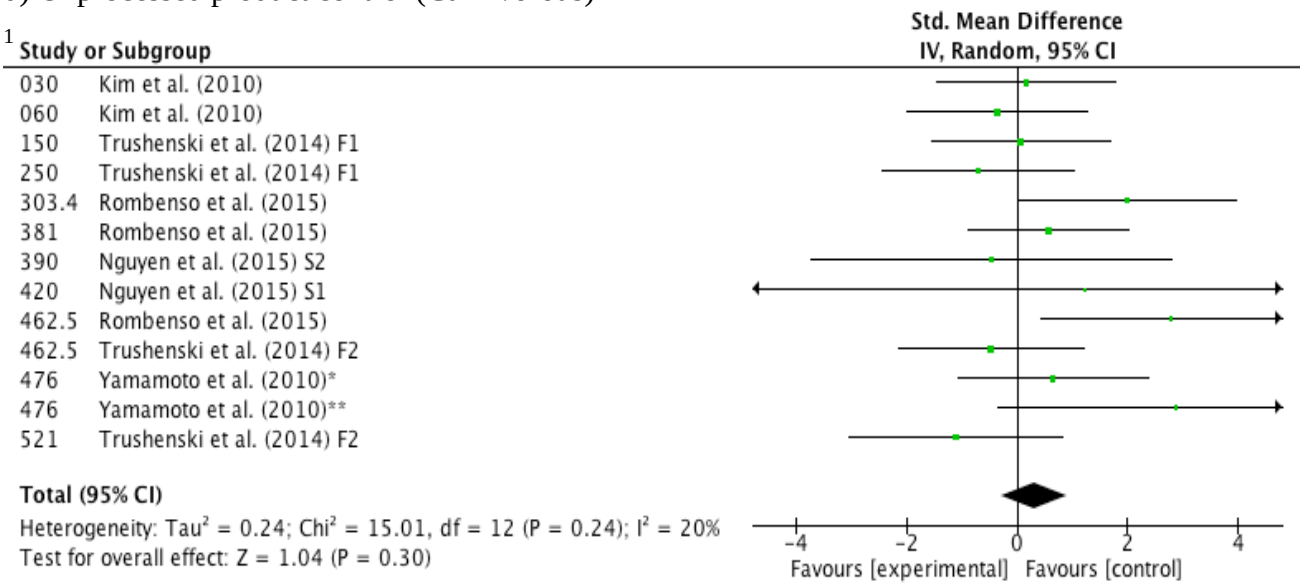
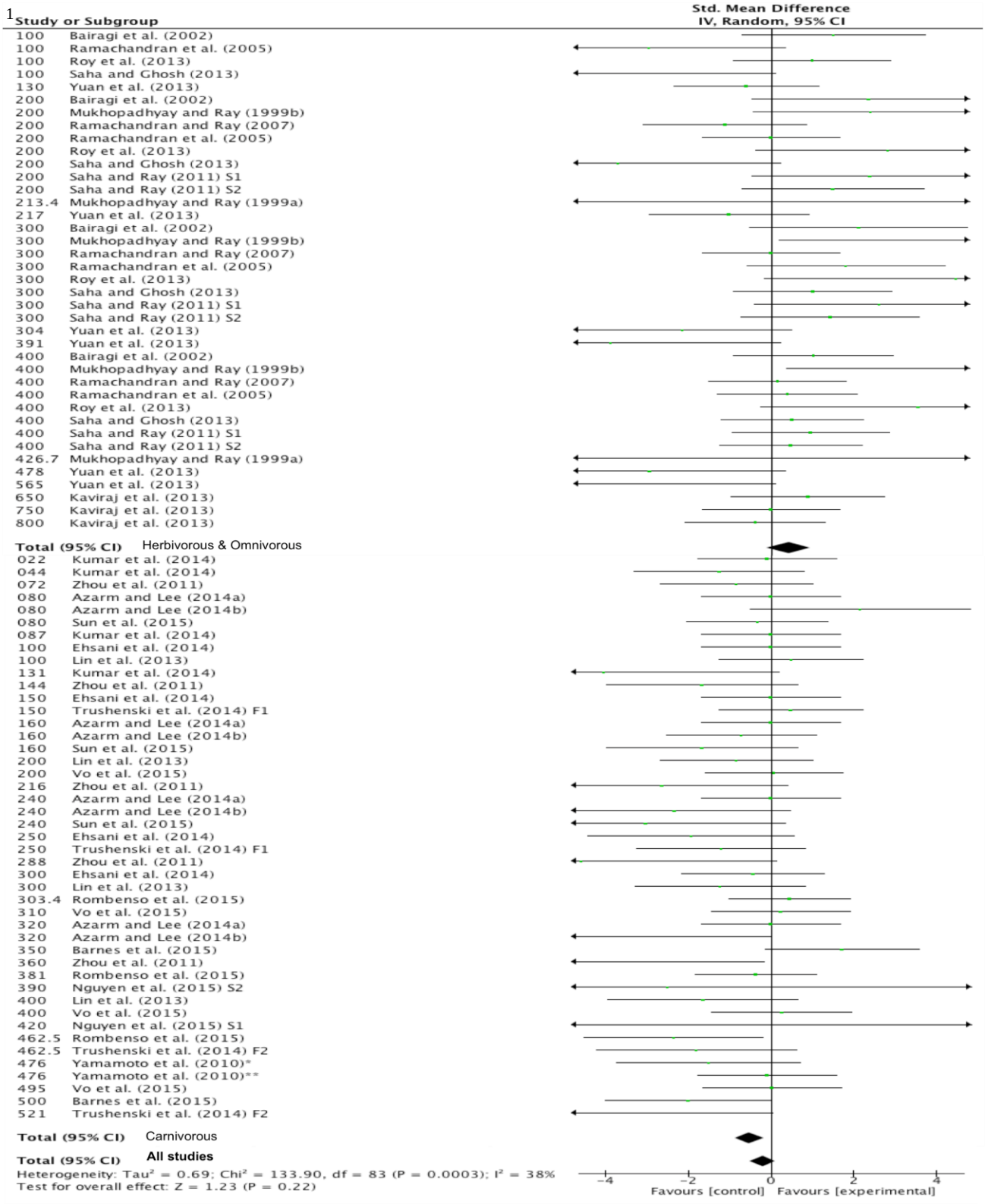


Figure 3.1. Forest plots of treatment effect sizes (Hedges' g) by dietary inclusion levels for four data sets: a) 39 studies of fermented ingredient versus fishmeal control in herbivorous and omnivorous fish; b) 45 studies of fermented ingredient versus fishmeal control in carnivorous fish; c) 40 studies of fermented ingredient versus unprocessed ingredient control in herbivorous and omnivorous fish; and d) 13 studies of fermented ingredient versus unprocessed ingredient control in carnivorous fish.

Notes: [1] number in front of each study stands for dietary concentration of fermented ingredient (g/kg); S1, S2: different bacterial species used in fermentation process; F1, F2: different fish species; *, **: different fermentation methods; T1: rapeseed meal; T2: cottonseed meal; T3: soybean meal.

a) Fishmeal control



b) Unprocessed ingredient control

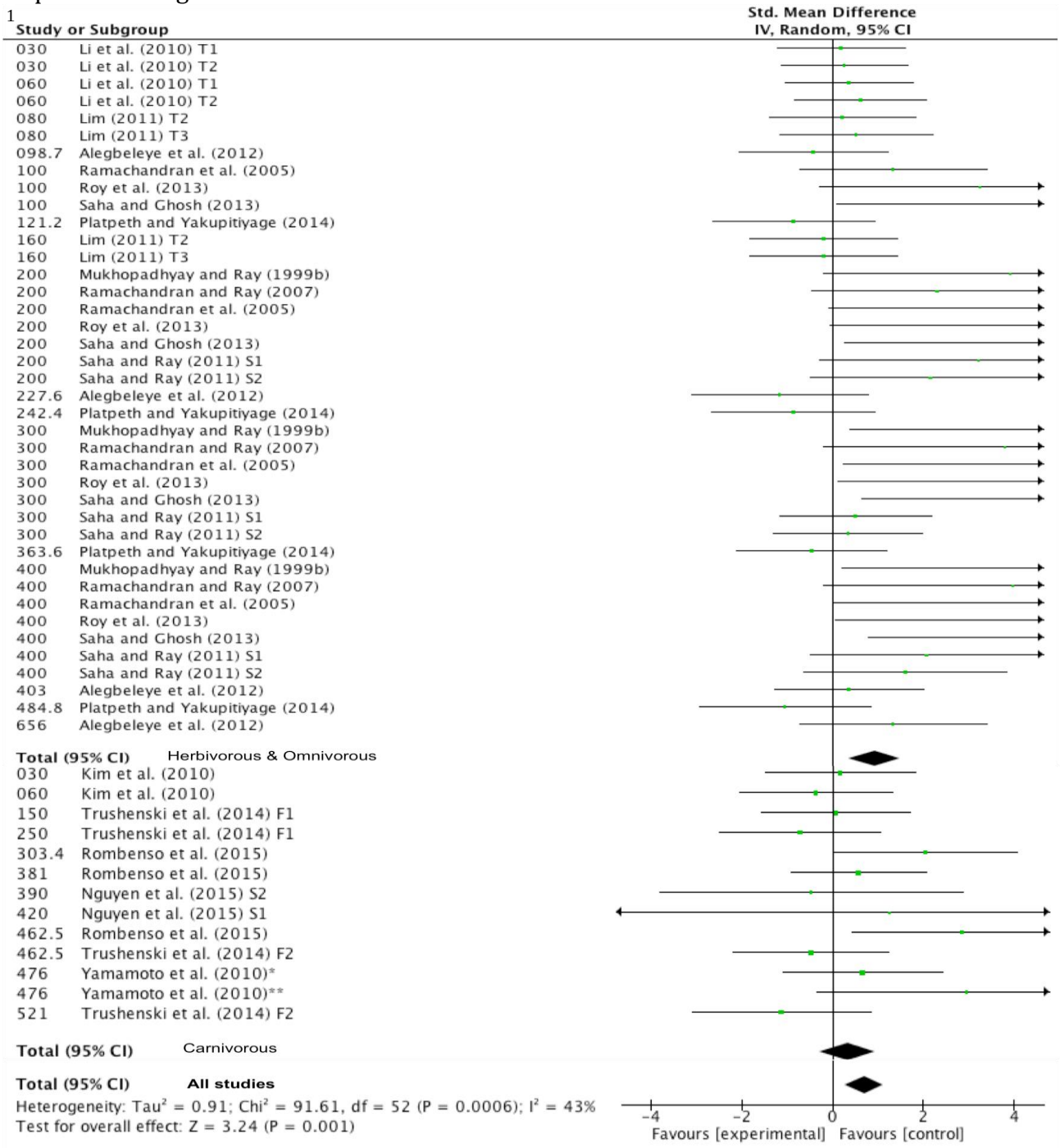
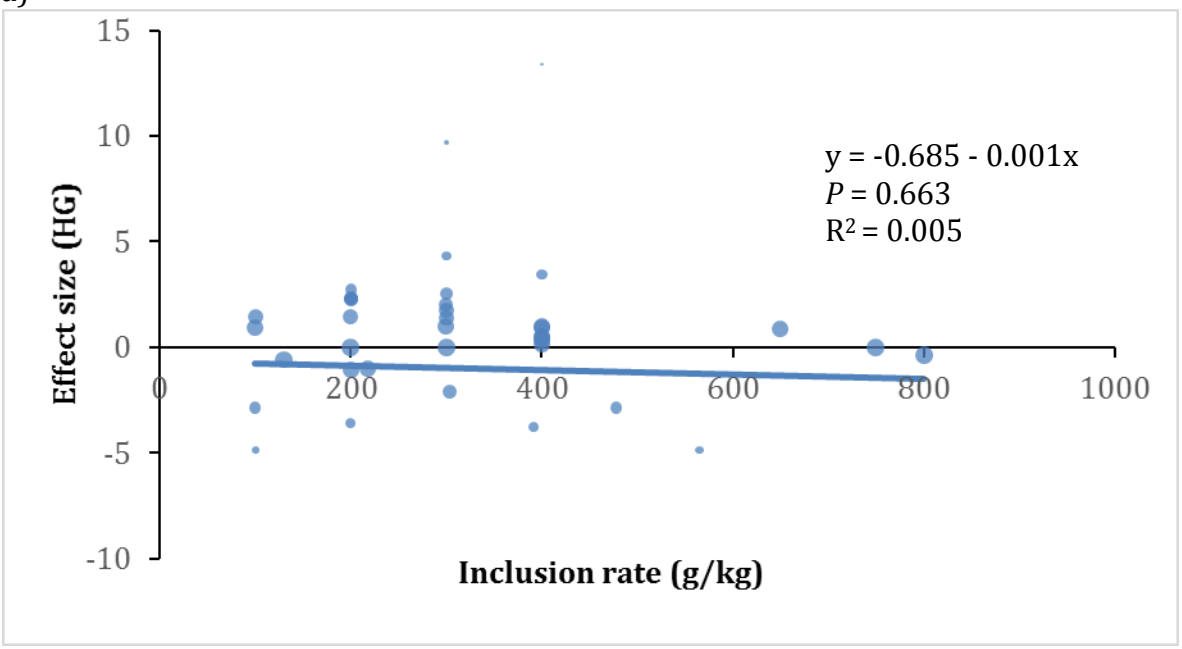


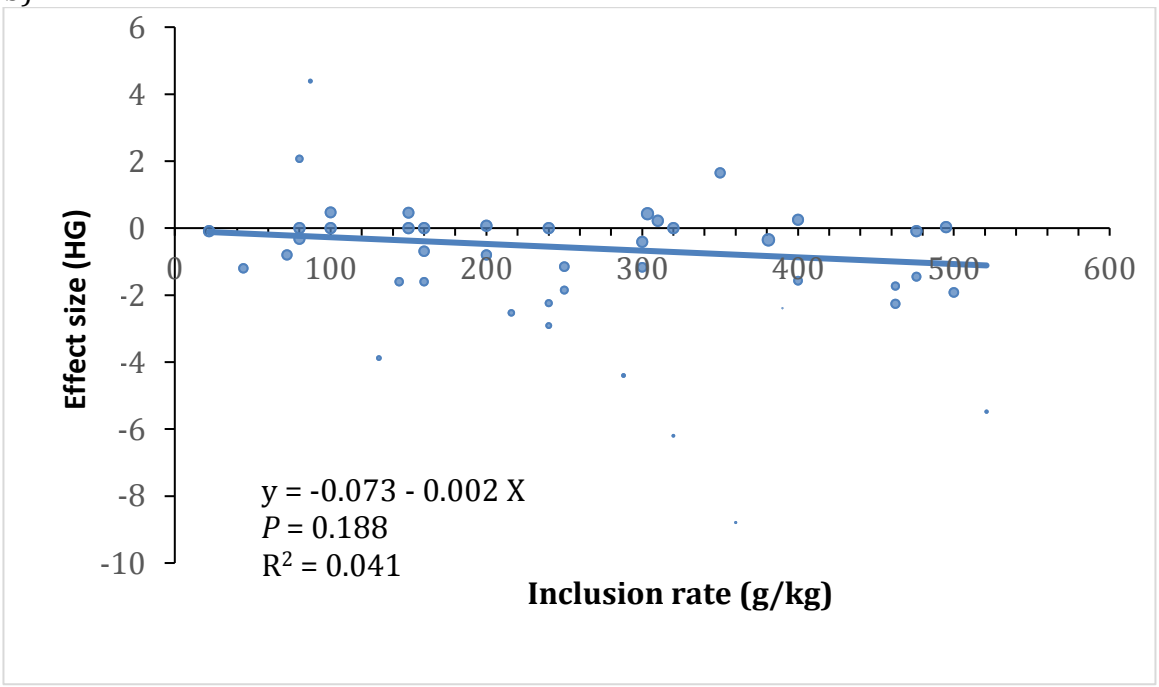
Figure 3.2. Forest plots of treatment effect sizes (Hedges' g) by dietary inclusion levels for two data sets: a) 84 studies of fermented ingredient versus fishmeal control in all fish species; and b) 53 studies of fermented ingredient versus unprocessed ingredient control in all fish species.

Notes: [1]: number in front of each study stands for dietary concentration of fermented product (g/kg); S1, S2: different bacterial species used in fermentation process; F1, F2: different fish species; *, **: different fermentation methods; T1: rapeseed meal; T2: cottonseed meal; T3: soybean meal.

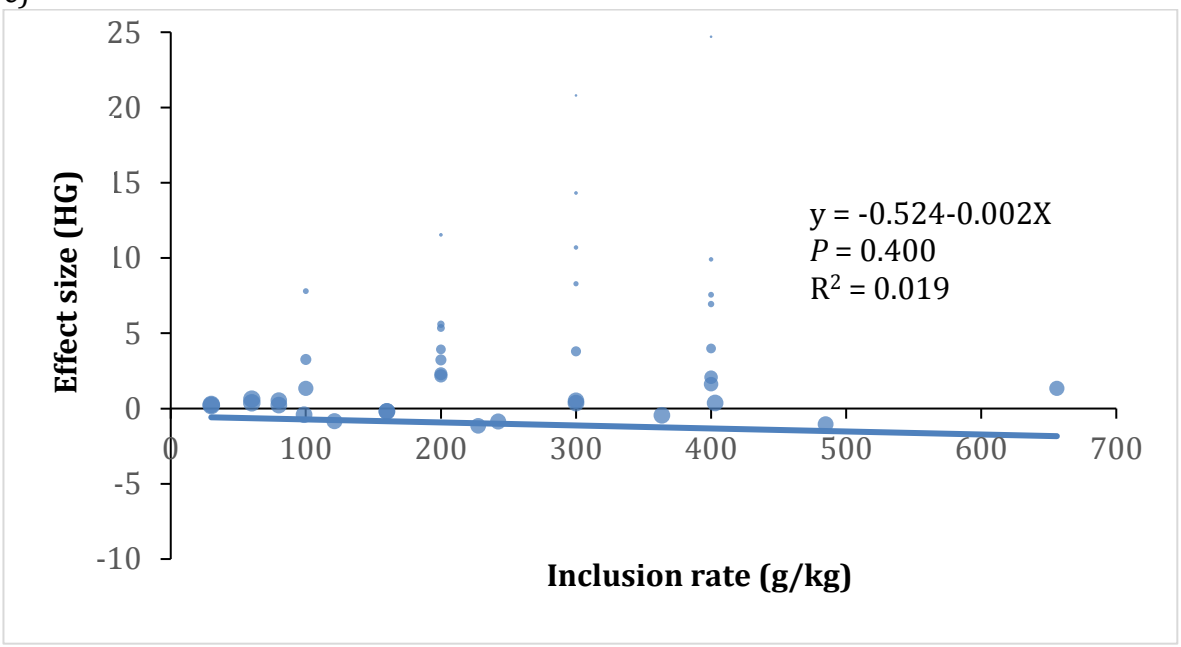
a)


15
10
5
0
-5
-10
0
200
400
600
800
1000
Effect size (HG)
Inclusion rate (g/kg)
y = -0.685 - 0.001x
P = 0.663
R² = 0.005

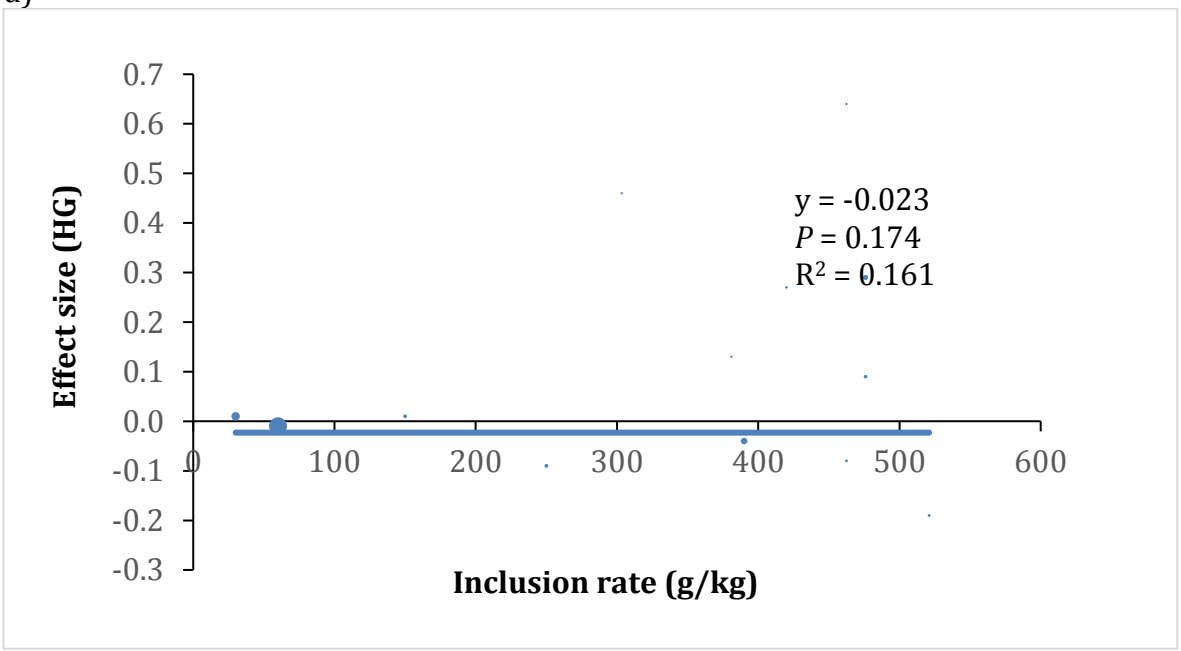
b)


6
4
2
0
-2
-4
-6
-8
-10
0
100
200
300
400
500
600
Effect size (HG)
Inclusion rate (g/kg)
y = -0.073 - 0.002 X
P = 0.188
R² = 0.041

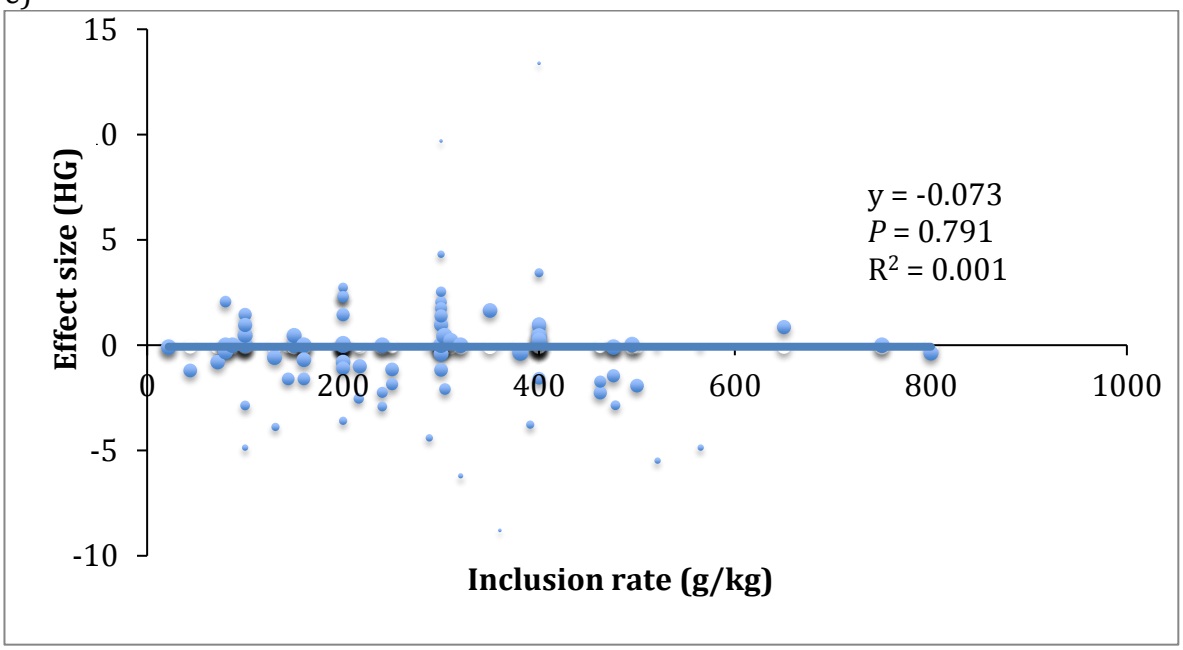
c)


25
20
15
10
5
0
-5
-10
0
100
200
300
400
500
600
700
Effect size (HG)
Inclusion rate (g/kg)
y = -0.524-0.002X
P = 0.400
R2 = 0.019

d)


0.7
0.6
0.5
0.4
0.3
0.2
0.1
0.0
-0.1
-0.2
-0.3
0
100
200
300
400
500
600
Effect size (HG)
Inclusion rate (g/kg)
y = -0.023
P = 0.174
R2 = 0.161

e)



f)

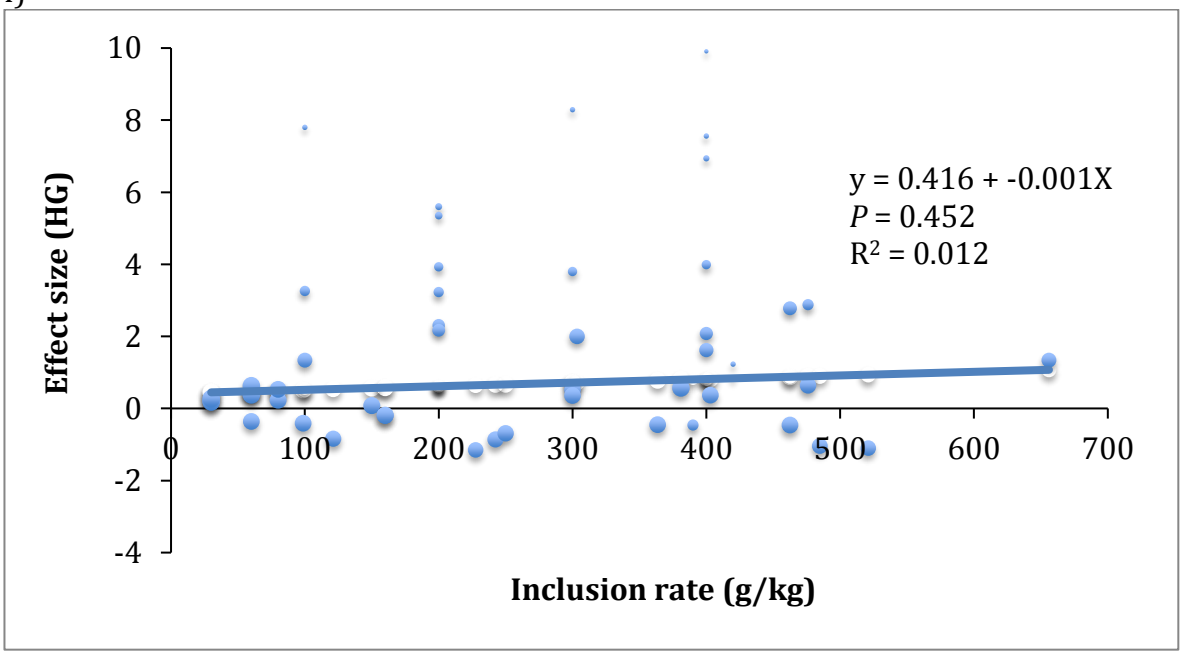


Figure 3.3. Weighted regression of effect size (HG) on dietary replacement of fishmeal or unprocessed ingredients with fermented ingredients on specific growth rate (SGR): a) replacement of fishmeal in herbivorous and omnivorous fish; b) replacement of fishmeal in carnivorous fish; c) replacement of unprocessed ingredient in herbivorous and omnivorous fish; d) replacement of unprocessed ingredient in carnivorous fish; e) replacement of fishmeal in all fish species; f) replacement of unprocessed ingredient in all fish species

Table 3.1. The overall effect size (HG) and 95% confidence interval (CI) and comparison between carnivorous species and herbivorous and omnivorous species for specific growth rate (SGR) when replacing fishmeal or unprocessed ingredients with fermented ingredients

		Overall Effect size (CI)	No. of Studies	*P*-value	t-test *P*-value
Fishmeal Control	Herbivorous & Omnivorous	0.41 (-0.1, 0.92)	39	0.11	0.002
	Carnivorous	-0.54 (-0.87, 0.21)	45	0.001	
	Overall	-0.19 (-0.49, 0.11)	84	0.22	
Unprocessed Ingredient Control	Herbivorous & Omnivorous	0.32 (-0.28,0.92)	40	0.001	0.232
	Carnivorous	0.93 (0.38, 1.48)	13	0.30	
	Overall	0.70 (0.28, 1.13)	53	0.001	

4. Canola meal processing method development

CM (obtained from Cargill feed mill, North Battleford, Saskatchewan, Canada) was hammer milled to a particle size of 1 and 0.5 mm. 1g carbohydrase (Viscozyme L, Novozyme) per kg of CM was added to CM and well mixed. The hydrolysis process was performed at 45 ^{o}C, pH 5.5 for 6 hours with stirring for 2 min every 15 mins. The carbohydrase pretreated CM was inoculated with *Candida utilis* of 10% v/w (10^7 CFU/ml). Distilled water was added to increase the moisture content of CM to 65% (Rodriguez-Leon et al., 2008). The inoculant and CM was mixed thoroughly. 0.5% $NH_4H_2PO_4$ was added as additional nitrogen source. CM fermentation was conducted in a 50 lb meat mixer with continuous agitation using a four flat-bladed impeller. In addition, air was pumped into the mixer throughout the fermentation process for 3 days. At the end of the fermentation period, the fermented CM was heated at 60 ^{o}C for 2 hours in order to inactivate the yeast. After that, the fermented CM was dried at 60 ^{o}C in a forced air drying oven. Samples was taken and ground for chemical analyses, including CP, Gross Energy, Lipid and amino acids.

4.1. Results—Carbohydrase with *C. utilis* fermentation of CM

- This trial was used to investigate if smaller particle size and/or carbohydrates pretreatment could enhance the fermentation process (**Table 4.1**)
- In all treatments, CP increased from 37% to 40%.
- Canola meal ground to 0.5mm without enzyme had the highest total amino acid (36.05%) content among all the treatments; 3% higher than the unprocessed CM.
- CM ground to 0.5 mm particle size and fermented with *C. utilis* without enzyme had:
    - lower levels of phytate, phytate phosphorus, sinapine and glucosinolates CM compared to unprocessed CM (**Table 4.2**)
    - Crude protein levels that were significantly higher that unprocessed CM
    - Higher levels of NSP and tannins than unprocessed CM; due to yeast biomass
- Based on these results, we used CM ground to 0.5 mm particle size, fermented with *C. utilis* without enzyme for all subsequent fish experiments

Table 4.1. The crude protein and amino acid compositions of CM after *C.utilis* fermentation with or without the pretreatment of carbohydrase.

Samples	*1 NE	1 E	**0.5 NE	0.5E	1 Original	0.5 Original
DM (%)	94.843	93.545	95.474	92.638	89.401	90.166
CP (%DM)	40.578	39.708	40.529	40.221	36.241	37.437
AA (%; DM basis)						
Taurine	0.08	0.08	0.07	0.10	0.11	0.12
Hydroxyproline	0.27	0.26	0.28	0.26	0.29	0.29
Aspartic Acid	2.65	2.61	2.67	2.62	2.47	2.49
Threonine	1.64	1.60	1.66	1.61	1.50	1.52
Serine	1.45	1.42	1.47	1.42	1.36	1.38
Glutamic Acid	6.46	6.22	6.58	6.25	5.97	6.00
Proline	2.42	2.37	2.44	2.40	2.19	2.19
Lanthionine	0.00	0.00	0.00	0.00	0.00	0.00
Glycine	1.94	1.88	1.96	1.88	1.76	1.76
Alanine	1.84	1.78	1.86	1.79	1.65	1.65
Cysteine	0.90	0.88	0.87	0.84	0.84	0.85
Valine	2.08	2.02	2.08	2.02	1.85	1.86
Methionine	0.82	0.74	0.77	0.73	0.70	0.77
Isoleucine	1.60	1.57	1.62	1.56	1.44	1.44
Leucine	2.92	2.85	2.95	2.88	2.66	2.66
Tyrosine	1.07	1.05	1.08	1.06	0.99	1.01
Phenylalanine	1.62	1.59	1.62	1.59	1.46	1.47
Hydroxylysine	0.15	0.12	0.16	0.12	0.11	0.11
Ornithine	0.06	0.07	0.05	0.07	0.02	0.02
Lysine	2.15	1.82	2.21	1.89	2.01	2.03
Histidine	1.03	1.00	1.04	1.00	0.95	0.96
Arginine	2.17	2.09	2.19	2.10	2.02	2.06
Tryptophan	0.42	0.43	0.42	0.42	0.42	0.40
Total (AA)	**35.74**	**34.45**	**36.05**	**34.61**	**32.77**	**33.04**

* **1**: 1mm; ** 0.5: 0.5mm; NE: without enzyme; E: with enzyme; Original: unprocessed CM

Table 4.2. Antinutrients of 0.5 mm CM fermented with *C. utilis* at 30 ºC for 3 days.

	CP (%)	Phytic Acid (PA) %	Phytate P (PAP) %	Sinapine (%)	Tannins (%)	NSP (%)	Glucosinolates (%)
CM	40.59	2.23	0.58	0.009	3.58	18	1.67
FCM	46.18	0.62	0.16	0.002	4.28	20.8	0.17

5. Effect of Yeast-Fermentation of Canola Meal on Digestibility and Growth Performance of Nile Tilapia and Rainbow Trout

5.1. Abstract

Canola meal (CM) was fermented with *Candida utilis* and evaluated as an ingredient in diets for Nile tilapia and rainbow trout. Fermentation process resulted in increased amino acid content and decreased glucosinolates and phytate. Fermented canola meal (FCM) had significantly higher crude protein digestibility in rainbow trout, whereas no significant difference was found on nutrient digestibility in Nile tilapia. Subsequently, two eight-week feeding trials were conducted to examine the impact of feeding various levels of CM or FCM on the growth performance of Nile tilapia and rainbow trout. In the Nile tilapia study, regression analysis revealed that CM inclusion rate had a significantly negative linear and quadratic relationship with final fish weight (FW) and average daily gain (ADG). By contrast, no significant regression trends were observed between FCM inclusion rates and grow performance, indicating that FCM could be included up to 600 g kg^{-1} without compromising Nile tilapia growth. Although the rainbow trout study showed the inclusion of CM or FCM had significantly negative linear effects on FW, ADG, specific growth rate (SGR) and feed conversion ratio (FCR), the comparison of regression slopes indicated fermentation process significantly improved the growth performance of rainbow trout compared with unprocessed CM.

5.2. Introduction

Canola meal (CM) / rapeseed meal is the second ranked plant protein meal behind soybeans in the global production and trade (Suarez et al., 2009). The cost of CM protein is approximately half that of fishmeal per unit protein basis, and the protein efficiency ratio of CM protein is high (3.29; Sarwar et al., 1984). The main chemical components of CM include protein, carbohydrates [simple sugars, sucrose, oligosaccharides, starch and nonstarch polysaccharides (NSP)], lignin with associated polyphenols, glycoproteins, fat and ash (Khajali and Slominski, 2012). Although CM/rapeseed meal has the best amino acid balance among the currently available commercial vegetable protein sources (Friedman, 1996; Ghodsvali et al., 2005; Salunkhe et al., 1992), the ANFs in CM such as glucosinolates, phytic acid, tannins, sinapine and insoluble and soluble NSP limit the use of CM in aquafeeds (Francis et al., 2001; Collins et al., 2013).

A novel approach to reducing ANFs and improving the nutritional value of plant-proteins, particularly CM, is a low cost, minimal effort procedure of yeast-fermentation (Oboh and Akindahunsi, 2003). Yeast-fermentation process requiring only hammer milling and retention of CM and yeast in a stirred vessel. Saccharomyces cerevisiae is a versatile species of yeast that is readily available, cheap and has GRAS status (generally recognized as safe) for use in both animal and human diets (Hertrampf and piedad-Pascual, 2000). This yeast has been used in brewing, baking and wine making for centuries and has the ability to grow in aerobic conditions and convert sugars to ethanol and carbon dioxide. *S. cerevisiae* is rich in protein (~50%), low in fat and contains a mix of vitamins (B-complex) and minerals (selenium and chromium), enzymes, amino acids and fatty acids (Sontakke, 2012). Fermentation with *S. cerevisiae* has been shown to decrease phytate via degradation of phytic acid (Greiner and Konietzny, 2006) and increase levels of protein and minerals in plant meals (Oboh and Akindahunsi, 2003; Oboh and Elusiyan, 2007). In addition, fermentation of canola meal for more than 8 hours can effectively degrade glucosinaolates by activating the endogenous canola myrosinase (Youngs and Wetter, 1996). Several recent experiments have shown that *S. cerevisiae* fermentation of CM and/or CPC, overnight at room temperature, results in a product with improved nutrient value and reduced negative effects on the growth of Asian sea bass (up to 50 %, Plaipetch and Yakupitiyage, 2012; CPC, Safari et al., 2012). This has also been shown in Nile tilapia fed with *S. cerevisiae* fermented soybean meal at levels up to 50 % (Plaipetch and

Yakupitiyage, 2014) and with bacteria-fermented (*Lactobacillus acidophilus*) sesame seed meal in rohu (*Labeo rohita*, carp family), at levels up to 400 g kg-1 (Mukhopadhyay and Ray, 1999).

Candida utilis is also an industrially important yeast, which have been used as nutritional supplements in animal feeds for over six decades (Bekatorou et al., 2006). It contains over 50% of protein and is rich in minerals and B-complex vitamins (Bekatorou et al., 2006). In contrast with *S. cerevisiae*, C. utilis has relatively higher concentrations of essential amino acids, such as lysine, threonine and valine (Shay and Wegner, 1985; Bekatorou et al., 2006). *C. utilis* is capable of metabolize a wide range of carbon and nitrogen sources, including pentose sugars, organic acids, alcohols, urea, ammonia salts, pyrimidine and amino acids (Shay and Wegner, 1985; Buerth et al., 2011). By contrast, S. cerevisiae is glucose sensitive and cannot metabolize pentose (Boze et al., 1992). Furthermore, under aerobic condition, *C. utilis* is in favor of biomass production, as it does not produce ethanol, which inhibits the growth of other yeast, such as S. cerevisiae (de Deken, 1966; Boze et al., 1992). Zhou et al. (2011) showed that *C. utilis*-fermented soybean meal could be used to replace fishmeal at levels up to 20% without compromising the growth performance of black sea bream. Moreover, a recent study by Grammes et al. (2013) showed that the addition of *C. utilis* could reduce intestinal inflammation in soymeal fed - Atlantic salmon, however, the beneficial effect was not found when *S. Cerevisiae* was fed. We therefore hypothesized that *C. utilis* fermentation of CM could lower levels of ANFs and improve the growth performance of fish fed canola meal-containing aquafeeds.

5.3. Materials and Methods

5.3.1. Candida utilis culture production

Candida utilis (ATCC® 9950™) used in this study was maintained in sterile 80% (v/v) glycerol solution at -80^{o}C. For flask cultures, *Candida utilis* was reactivated on YGC plate agar (Sigma-Aldrich Co. LLC.) and incubated at 30^{o}C for 2-3 days. Two loopfuls of colonies were transferred with a platinum needle into a 250ml conical flask containing 100 ml YPD liquid medium (Sigma-Aldrich Co. LLC.). The culture was incubated on a horizontal shaker at 120 rpm at 30^{o}C for 15 hours. After that, 10 ml of the culture was inoculated into 3 of 250ml YPD liquid

medium in a 500ml conical flask. The cells were harvested after 10 hours incubation (at the end of exponential growth phase) with mechanical shaking at 120 rpm at 30^{o}C.

5.3.2. Canola meal fermentation

CM (obtained from Cargill feed mill, North Battleford, Saskatchewan, Canada) was hammer milled to a particle size of 0.5 mm. Distilled water was added to increase the moisture content of CM to 65% (Rodriguez-Leon et al., 2008), and then the CM was inoculated with *Candida utilis* of 10% v/w (107 CFU/ml). The inoculant and CM was mixed thoroughly. 0.5% NH4H2PO4 was added as additional nitrogen source. CM fermentation was conducted in a 50 lb meat mixer with continuous agitation using a four flat-bladed impeller. In addition, air was pumped into the mixer throughout the fermentation process for 3 days. At the end of the fermentation period, the fermented CM was heated at 60 ^{o}C for 2 hours in order to inactivate the yeast. After that, the fermented CM was dried at 60 ^{o}C in a forced air drying oven.

5.3.3. Fish management

Nile tilapia (Nobleford, AB) and triploid female rainbow trout (Wild West Steelhead, Lucky Lake, SK) was housed in a recirculating aquaculture system filtered via biological filtration at the Prairie Aquaculture Research Centre (University of Saskatchewan, Saskatoon, SK). Water temperature was maintained at 23 ± 1°C for Nile tilapia and 12 ± 1°C for rainbow trout. Photoperiod was a 14 h light/10 h dark cycle. Dissolved oxygen, pH and water temperature were measured daily and chlorine, nitrate, nitrite and ammonia were monitored on a weekly basis or more often if deemed necessary. All fish were maintained in accordance with the guidelines established by the Canadian Council on Animal Care (CCAC, 2005). For all the digestibility and growth trials, three replicates per dietary treatment were used and all fish were hand-fed twice a day to visual satiation.

5.3.4. Digestibility trials

5.3.4.1 Diets

Two digestibility trials were conducted to investigate the digestibility of CM and FCM in Nile tilapia and rainbow trout. The reference diet (**Table 5.1**) was formulated according to Bureau and Cho (1994). Celite (Celite co. World Minerals Co., Lompoc, CA, USA) was added to the reference diet at 10g kg^{-1} as a non-absorbable inert marker for indirect measurement of digestibility. The experimental diets contained 700 g kg^{-1} reference diet and 300 g kg^{-1} test ingredients. All ingredients were well mixed in a Hobart legacy floor mixer (Hobart Corporation, Troy, OH) and were then cold extruded using a 4822 Hobart Food Grinder (Hobart Corporation, Troy, OH) with a 5 mm die. The resulting pellets were dried in a forced air oven (55°C, 12 h), chopped and screened to obtain the appropriate pellet size. The diets were kept at -20°C prior to use.

Table 5.1 Composition of the reference diet used in digestibility trials for Nile tilapia and rainbow trout (g kg^{-1}).

Ingredient	Inclusion (g kg^{-1})
Fishmeal[1]	300.00
Soybean meal	170.00
Corn gluten meal	130.00
Wheat flour	280.00
A-DHA	0.00
Vitamin mineral premix[2]	10.00
Celite[3]	10.00
Fish oil[4]	100.00

[1]South American Aquagrade; EWOS Canada Ltd.

[2]The vitamin/mineral premix was a commercial premix (EWOS FISH-STR VIT PX, Surrey, BC; closed formulation)

[3]Celite 545, <125μm; Celite Corporation, World Minerals Co., Lompoc, CA, USA.

[4]Mixed variety fish oil; EWOS Canada Ltd.

5.3.4.2. Digestibility trial design

All fish were housed in 360 L tanks. In digestibility trial 1, a total of 207 Nile tilapia with average initial weight of 450g were randomly allocated to 9 tanks (23 fish/tank). Digestibility trial 2 used 180 triploid female rainbow trout with 20 fish per tank (260g initial

weight). The fish were adapted to the respective diets for 7 days, and then followed by 3-week fecal collection using a settling column (Hajen et al., 1993). The collected feces were centrifuged at 3000 rpm for 15 minutes at 4^{o}C (Beckman Coulter J6-MC Centrifuge, Mississauga, ON), frozen and freeze-dried prior to analysis. Apparent digestibility coefficients (ADC) of the reference and test diets as well as ADC of test ingredients (ADCI) were estimated using the equations of Cho et al. (1982) and Sugiura et al. (1998) as described by Bureau and Cho (1999).

5.3.5. Growth trials

5.3.5.1. Diets

Two 56-day growth trials were performed to evaluate of effects of increasing inclusion rates of CM and FCM on the growth performance of Nile tilapia and rainbow trout. In growth trial 1, seven experimental diets containing 0, 200, 400 and 600 g kg^{-1} CM and FCM were tested in Nile tilapia. The 0 and 400 g kg^{-1} diets were formulated using Concept 4 (CFC Tech Services, Inc. Pierz, MN, USA), which were then mixed in relative proportions to obtain the formulations of the rest diets. All diets contained 321 g kg^{-1} digestible crude protein and 15.2 MJ kg^{-1} digestible energy (**Table 5.9**). In growth trial 2, seven iso-nitrogenous (386.2 g kg^{-1} digestible crude protein) and iso-energetic (17.6 MJ kg^{-1}) experimental diets (**Table 5.10**) containing 0, 100, 200, 300 g kg^{-1} CM and FCM were formulated and tested in rainbow trout. The 0 and 200 g kg^{-1} diets were formulated using Concept 4, and then used to calculate the formulations of the 100 and 300 g kg^{-1} diets. All diets were formulated based on digestible nutrients obtained from digestibility trial 1 & 2. All diets were balanced for digestible essential amino acids according to NRC (2011). All diets contained same level of fish meal in order to better compare the effects of test ingredients on fish growth performance (Collins et al., 2012). The diets were processed using the same procedures as described for the digestibility trials.

5.3.5.2.Growth trial design

Growth trial 1 was conducted in twenty-four 350 L tanks with 13 fish per tank (average initial weight of 580g). For growth trial 2, twenty-four 120 L tanks were used, with 20 fish per tank (average initial weight of 202 g). The diets were were randomly assigned to tanks with three replicates per treatment. Feed consumption for each tank was recorded weekly. Each tank of fish

were weighed on day 0 and 56. Fish growth performance was assessed using final weight (FW), average daily gain (ADG), average daily feed intake (ADFI), specific growth rate (SGR = [ln final weight – ln initial weight] / time (days) x 100), feed conversion rate (FCR = feed intake (as fed) / wet weight gain).

5.3.6. Chemical analysis

Experimental diets, test ingredients and fecal samples were analyzed for moisture (AOAC 930.15), dry matter (100-moisture), crude protein (AOAC 990.03), gross energy (1281 bomb calorimeter, Parr Adiabatic Calorimeter, Model 1200, Moline, Illinois), crude fat (ACCS AM 5-04) and ash ACAC 923.03). Amino acid profile of all samples were analyzed by acid hydrolysis using HPLC (Central testing lab, Winnipeg, MB, Canada). Acid insoluble ash (APAC 920.08) was only performance on digestibility trial diets.

5.3.7. Statistical analysis

ADC of diets and test ingredients were analyzed as a completely randomized design (CRD) using the General Linear Model procedure of SPSS (Version 23, SPSS Inc., Chicago, IL, USA).The Ryan-Einot-Gabriel-Welsh F test was used to determine differences between means of treatments, with the accepted level of significancee at $P < 0.05$. Linear and quadratic regression models were calculated for the growth performance parameters on test ingredient inclusion rate using the Regression procedure of SPSS (Version 24, SPSS Inc., Chicago, IL, USA). Regressions were considered significant when $P < 0.05$. The student's t-test was used to determine whether the two slopes of linear regressions for CM and FCM were different, with significance being attributed to $P < 0.05$.

5.4.Results

5.4.5. Digestibility

The proximate and amino acid composition of CM, FCM and experimental diets are reported in **Table 5.2**. Fermentation increased the CP level from 40.6% to 46.2%. The total and individual amino acid all increased after fermentation.

The ADC of diets and test ingredients for Nile tilapia are shown in **Table 5.3** and **Table 5.4**. All three diets had high digestibility in Nile tilapia, with >95% for CP and >92% for GE. There's no significant difference between ADC of CM and FCM ($P > 0.5$). **Table 5.5** shows in Nile tilapia, fermented canola meal had higher digestible protein (400 vs. 358 g kg^{-1}) and gross energy (14.45 vs. 14.0 MJ kg^{-1}).

For rainbow trout, the ADC of CP and GE were significantly lower in fish fed CM diets than in those fed control diet (**Table 5.6**). Fish fed FCM diet also had significantly lower ADC of CP compared with control diet-fed fish (89% vs 91%), but no significantly difference was found on ADC of GE (**Table 5.6**). **Table 5.7** shows that FCM had significantly higher digestibility of CP (85.6 vs 84.2%) and fat (99.7 vs 89.8%). Fermentation increased the digestible CP from 333 to 390 g kg^{-1} (**Table 5.8**).

5.4.6. Growth performance

Over the two 8-week growth trials, mortality was 3.5% for Nile tilapia and 2.5% for rainbow trout, respectively. The Nile tilapia study revealed that CM inclusion had a significantly negative linear relationship with FW and ADG, but for ADFI, SGR and FCR, both linear and quadratic regressions were not significant (**Table 5.13**). No significant linear or quadratic relationships were found between FCM inclusion rate and FW, ADG, ADFI, SGR and FCR (**Table 5.13**). Although the increasing inclusion of either CM or FCM showed a significantly negative effect on FW, ADG, SGR and FCR in rainbow trout (**Table 5.14**), the comparison of regression slopes indicated significant difference between the CM and FCM groups on FW, ADG, SGR and FCR. The inclusion of CM or FCM had no linear or quadratic relationship with ADFI in rainbow trout (**Table 5.14**),

Table 5.2. Proximate and amino acid composition (g kg^{-1} dry matter) of the test ingredients (CM and FCM) and experiment diets used in Nile tilapia and rainbow trout digestibility trials.

Component	CM	FCM	Control Diet	CM Diet	FCM Diet
Proximate composition (g kg^{-1} dry matter)					
Dry matter	898.5	932.3	944.0	958.5	942.1
Crude protein	405.9	461.8	439.8	434.0	450.2
Gross energy (MJ kg^{-1})	17.88	19.06	20.21	20.7	19.97
Crude Fat	15.5	8.5	138.2	110.7	103.9
Ash	68.4	79.3	96.8	96.8	90.21
Acid insoluble ash	-	-	1.9	0.6	5.7
Amino acids (g kg^{-1} dry matter)					
Alanine	14.7	17.0	14.8	15.3	13.6
Arginine	23.7	24.9	17.2	17.2	17.1
Asparagine	29.8	33.2	24.9	25.5	23.9
Cysteine	5.3	5.5	1.9	3.0	2.5
Glutamic Acid	65.8	72.3	50	52.5	49.5
Glycine	17.9	20.2	15.2	16.6	14.4
Histidine	11.1	12.3	7.1	9.3	7.4
Isoleucine	12.8	14.6	10.7	8.5	10.6
Leucine	25.4	29.0	24.0	23.1	22.6
Lysine	21.5	23.2	17.2	16.0	16.6
Methionine	4.3	4.5	5.5	5.7	4.4
Phenylalanine	15.4	17.5	13.1	12.5	12.8
Proline	28.6	30.0	20.6	23.5	20.4
Serine	18.0	19.9	13.5	15.2	13.4
Threonine	16.0	17.9	10.9	11.6	11.2
Tyrosine	10.2	11.7	9.3	8.9	8.9
Valine	16.7	19.0	12.8	11.1	13.0
Total AA	337.2	372.7	268.7	275.5	262.3

Table 5.3. Apparent digestibility coefficients (ADC, %) of dry matter, crude protein (N × 6.25), gross energy, lipid, ash and amino acids in Nile tilapia fed three different diets.

	Control	CM Diet	FCM Diet	SEM	P-Value
Proximate Component					
Dry matter	98.0	91.1	89.5	2.80	0.48
Crude Protein	98.9	95.9	95.1	1.29	0.50
Gross energy	98.8	93.2	92.2	2.15	0.47
Crude Fat	99.8	99.2	99.8	0.24	0.57
Ash	91.6	79.3	76.3	6.22	0.63
Amino acids					
Alanine	98.4	98.0	93.8	1.07	0.16
Arginine	98.9	98.4	95.9	0.73	0.21
Asparagine	98.5	98.0	94.5	0.98	0.19
Cysteine	99.7	99.7	98.8	0.22	0.18
Glutamic Acid	99.1	98.8	96.4	0.64	0.16
Glycine	97.9	98.1	92.2	1.35	0.11
Histidine	97.2	95.9	89.3	1.93	0.22
Isoleucine	98.7	97.2	94.7	1.03	0.33
Leucine	98.9	98.0	95.4	0.85	0.24
Lysine	98.8	97.8	94.8	0.96	0.23
Methionine	98.5	98.4	94.8	0.90	0.16
Phenylalanine	98.7	97.9	95.0	0.92	0.23
Proline	98.7	98.3	94.3	1.00	0.13
Serine	98.7	97.9	94.3	1.01	0.17
Threonine	98.3	97.2	93.3	1.23	0.22
Tyrosine	98.7	98.0	95.4	0.85	0.26
Valine	98.7	97.0	94.4	1.10	0.31

[ab] Means in the same row with different superscripts are significantly different ($P < 0.05$)

SEM=Standard error of the mean

Table 5.4. Apparent digestibility coefficients (ADC, %) of dry matter, crude protein (N × 6.25), gross energy, lipid, ash and amino acids for canola meal and fermented canola meal in Nile tilapia.

	CM	FCM	SEM	P-Value
Proximate Component				
Dry matter	74.3	69.5	13.26	0.88
Crude Protein	88.2	86.5	6.23	0.91
Gross energy	78.1	75.2	10.71	0.91
Lipid	86.7	99.0	8.04	0.51
Ash	38.4	32.5	3.77	0.95
Amino acids				
Alanine	97.2	84.6	4.73	0.21
Arginine	97.6	91.1	2.70	0.27
Asparagine	97.1	87.4	3.88	0.25
Cysteine	99.6	98.1	0.56	0.22
Glutamic Acid	98.2	92.0	2.39	0.22
Glycine	98.4	82.1	5.44	0.15
Histidine	94.1	78.7	6.53	0.28
Isoleucine	94.2	88.0	4.01	0.50
Leucine	96.1	88.7	3.57	0.35
Lysine	95.8	87.9	3.7	0.34
Methionine	98.0	84.2	5.05	0.19
Phenylalanine	96.2	88.4	3.62	0.33
Proline	97.6	87.2	3.69	0.18
Serine	96.6	87.5	3.69	0.25
Threonine	95.5	86.1	4.25	0.31
Tyrosine	96.3	89.1	3.53	0.36
Valine	94.2	87.6	4.06	0.48

[ab] Means in the same row with different superscripts are significantly different ($P < 0.05$)

SEM=Standard error of the mean

Table 5.5. Digestible nutrients (g kg^{-1}) of CM and FCM for Nile tilapia.

Component	DM	CP	DE (MJ/kg)	Crude Fat	Ash
CM	667.2	358.2	14.83	1.34	2.63
FCM	647.6	399.7	14.26	0.84	2.58

Table 5.6. Apparent digestibility coefficients (ADC, %) of dry matter, crude protein (N × 6.25), gross energy, lipid, ash and amino acids in rainbow trout fed three different diets.

	Control Diet	CM Diet	FCM Diet	SEM	*P*-Value
Proximate Component					
Dry matter	66.2	65.5	66.5	0.36	0.58
Crude Protein	91.1[a]	88.5[b]	89.2[b]	0.40	<0.01
Gross energy	76.1[a]	73.6[b]	74.4[ab]	0.45	<0.05
Crude Fat	98.2[ab]	97.6[b]	99.2[a]	0.28	<0.05
Ash	27.6[b]	27.5[b]	35.0[a]	1.59	<0.05
Amino acids					
Alanine	93.9	92.7	92.5	0.30	0.14
Arginine	95.5	94.6	94.9	0.25	0.38
Asparagine	93.6	92.8	92.9	0.20	0.28
Cysteine	94.0	93.9	93.5	0.29	0.83
Glutamic Acid	96.3	95.8	95.3	0.20	0.07
Glycine	89.4	89.7	90.0	0.43	0.89
Histidine	89.3[a]	85.3[b]	83.9[b]	0.90	<0.01
Isoleucine	95.6[a]	93.3[b]	93.9[b]	0.39	<0.01
Leucine	96.1[a]	94.1[b]	94.1[b]	0.36	<0.01
Lysine	95.2[a]	93.7[b]	93.9[b]	0.27	<0.05
Methionine	95.4	93.6	94.7	0.72	0.65
Phenylalanine	95.6[a]	94.0[b]	93.7[b]	0.36	<0.05
Proline	94.0	93.1	92.2	0.35	0.09
Serine	94.4[a]	92.8[b]	92.4[b]	0.34	<0.05
Threonine	93.8[a]	91.8[b]	91.9[b]	0.37	<0.01
Tyrosine	95.9	94.4	94.8	0.33	0.12
Valine	94.8[a]	92.7[b]	92.5[b]	0.42	<0.05

[ab] Means in the same row with different superscripts are significantly different ($P < 0.05$)

SEM=Standard error of the mean

Table 5.7. Apparent digestibility coefficients (ADC, %) of dry matter, crude protein (N × 6.25), gross energy, lipid, ash and amino acids for canola meal and fermented canola meal in rainbow trout.

	CM	FCM	SEM	*P*-Value
Proximate Component				
Dry matter	64.0	67.4	1.15	0.14
Crude Protein	82.2[b]	84.5[a]	0.64	<0.05
Gross energy	67.2	70.3	1.02	0.14
Crude Fat	89.8[b]	99.7[a]	2.71	<0.05
Ash	27.2[b]	56.3[a]	6.71	<0.01
Amino acids				
Alanine	88.7	88.0	1.32	0.81
Arginine	92.4	93.8	0.97	0.55
Asparagine	90.6	90.8	0.78	0.91
Cysteine	93.8	92.7	1.22	0.70
Glutamic Acid	94.3	92.5	0.71	0.26
Glycine	90.5	91.5	2.04	0.85
Histidine	76.8	72.9	2.01	0.40
Isoleucine	86.9	89.1	0.98	0.30
Leucine	87.6	87.7	0.80	0.98
Lysine	90.3	90.9	0.63	0.71
Methionine	86.7	92.1	4.98	0.65
Phenylalanine	88.9	88.1	1.10	0.75
Proline	90.7	87.6	1.32	0.28
Serine	88.3	87.5	0.77	0.68
Threonine	87.2	88.0	0.71	0.61
Tyrosine	89.2	91.2	1.43	0.53
Valine	87.6	87.3	0.87	0.88

[ab] Means in the same row with different superscripts are significantly different ($P < 0.05$)

SEM=Standard error of the mean

Table 5.8. Digestible nutrients (g kg^{-1}) of CM and FCM for rainbow trout.

Component	DM	CP	DE (MJ/kg)	Crude Fat	Ash
CM	574.7	333.4	12.76	13.9	18.6
FCM	628.6	390.3	13.55	9.80	44.6

Table 5.9. Composition of the diets used in growth trial for Nile tilapia (g kg-1).

	Diets						
Ingredient	Control	20CM	40CM	60CM	20FCM	40FCM	60FCM
Fish meal[1]	50.0	50.0	50.0	50.0	50.0	50.0	50.0
Corn gluten meal	250.0	234.6	118.0	0	234.0	130.0	38.8
Soy protein concentrate	36.9	78.2	62.7	53.4	46.8	17.4	10.0
Poultry by-product meal	320.0	100.0	80.0	50.0	131.2	100.0	20.0
Wheat flour[2]	160.0	160.0	160.0	160.0	160.0	160.0	160.0
DL-methionine[3]	3.1	2.6	2.6	2.6	3.0	3.0	3.0
Vitamin mineral premix[4]	10.0	10.0	10.0	10.0	10.0	10.0	10.0
Alpha-cellulose[5]	100.0	94.6	46.7	4.0	95.0	59.6	38.2
Fish oil[6]	70.0	70.0	70.0	70.0	70.0	70.0	70.0
Canola meal	0.0	200.0	400.0	600.0	-	-	-
Fermented canola meal	-	-	-	-	200.0	400.0	600.0
Digestible crude protein	321.0	321.0	321.0	320.4	321.0	321.0	321.0
Digestible energy (MJ/kg)	15.3	15.2	15.2	15.2	15.3	15.2	15.1

[1]South American Aquagrade; EWOS Canada Ltd.

[2]Robin Hood All-Purpose Flour; Robin Hood Multifoods Corporation, Markham, ON, Canada.

[3]DL-methionine, feed grade. Degussa Corporation, Theodore, AL, USA.

[4]The vitamin/mineral premix was a commercial premix (EWOS FISH-STR VIT PX, Surrey, BC; closed formulation)

[6]Mixed variety fish oil; EWOS Canada Ltd.

Table 5.10. Composition of the diets used in growth trial for rainbow trout (g kg-1).

	Diets						
Ingredient	Control	10CM	20CM	30CM	10FCM	20FCM	30FCM
Fish meal	200.6	200.6	200.6	200.6	200.6	200.6	200.6
Corn gluten meal	123.6	107.5	91.4	75.3	122.7	121.7	120.8
Soy protein concentrate	98.7	93.8	88.9	84.0	78.0	57.3	36.6
Poultry by-product meal	300.0	242.2	184.1	126.6	241.5	183.0	124.5
Wheat flour	80	80	80	80	80	80	80
DL-methionine	1.1	0.9	0.7	0.5	0.75	0.4	0.05
L-lysine	5.0	4.5	4.0	3.5	4.5	4.0	3.5
Vitamin mineral premix[2]	10.0	10.0	10.0	10.0	10.0	10.0	10.0
Alpha-cellulose	60.0	45.0	30.0	15.0	45.0	30.0	15.0
Fish oil	121.0	107.5	91.4	75.3	122.7	121.7	120.8
Canola meal	0.0	100.0	200.0	300.0	-	-	-
Fermented canola meal	-	-	-	-	100.0	200.0	300.0
Digestible crude protein	321.0	321.0	321.0	320.4	321.0	321.0	321.0
Digestible energy (MJ/kg)	15.3	15.2	15.2	15.2	15.3	15.2	15.1

[1]South American Aquagrade; EWOS Canada Ltd.

[2]Robin Hood All-Purpose Flour; Robin Hood Multifoods Corporation, Markham, ON, Canada.

[3]DL-methionine, feed grade. Degussa Corporation, Theodore, AL, USA.

[4]The vitamin/mineral premix was a commercial premix (EWOS FISH-STR VIT PX, Surrey, BC; closed formulation)

[6]Mixed variety fish oil; EWOS Canada Ltd.

Table 5.11. Analyzed proximate and amino acid composition of experimental diets used in growth trial for Nile tilapia (g/kg).

	Diets						
Ingredient	Control	20CM	40CM	60CM	20FCM	40FCM	60FCM
Proximate component (g kg^{-1} dry matter)							
Dry matter	940.6	927.1	927.6	930.6	945.0	935.0	932.6
Crude Protein	471.7	397.8	401.9	374.5	421.6	410.3	384.4
Gross energy (MJ kg^{-1})	22.2	22.0	22.1	21.7	22.2	22.0	21.6
Crude Fat	132.6	107.1	108.1	106.1	107.5	105.7	88.6
Ash	75.4	59.8	59.2	78.9	60.3	67.4	67.5
Amino acids (g kg^{-1} dry matter)							
Alanine	32.7	26.8	26.4	18.5	26.8	24.3	20.0
Arginine	25.3	22.1	21.1	22.4	21.4	22.9	22.3
Asparagine	34.9	30.8	30.0	29.0	30.2	31.0	30.5
Cysteine	4.4	4.2	5.7	5.3	4.4	5.1	5.0
Glutamic Acid	77.6	76.3	76.6	66.1	74.3	74.7	71.7
Glycine	32.7	21.8	21.6	21.8	23.1	23.1	20.1
Histidine	11.9	10.9	10.6	11.1	10.9	11.4	11.5
Isoleucine	16.8	15.7	15.9	14.8	14.7	15.7	15.7
Leucine	43.9	40.9	41.2	26.3	38.9	36.4	29.8
Lysine	20.0	17.4	17.6	21.0	17.2	19.2	20.5
Methionine	12.1	9.6	12.4	8.7	9.8	10.9	8.9
Phenylalanine	21.0	19.3	19.4	14.9	18.4	18.1	16.8
Proline	32.5	28.1	31.7	22.2	29.4	27.3	24.7
Serine	21.9	20.1	19.1	16.3	20.0	19.5	17.8
Threonine	16.8	15.4	16.2	15.5	15.0	16.0	16.0
Tyrosine	14.9	13.4	13.4	10.4	12.9	12.6	11.0
Valine	19.8	18.5	19.1	18.8	17.8	19.1	19.2

Table 5.12. Analyzed proximate and amino acid composition of experimental diets used in growth trial for rainbow trout (g/kg).

	Diets						
Ingredient	Control	10CM	20CM	30CM	10FCM	20FCM	30FCM
Proximate component (g kg^{-1} dry matter)							
Dry matter	965.6	943.2	956.1	965.2	954.6	93.12	965.6
Crude Protein	536.0	533.1	490.5	494.9	522.4	514.1	502.0
Crude Fiber	65.5	62.9	80.8	78.5	71.1	72.3	80.0
Gross energy (MJ kg^{-1})	23.3	22.8	22.5	22.4	22.9	22.6	22.4
Crude Fat	185.1	169.3	164.7	153.6	176.0	164.1	155.3
Ash	97.6	100.0	97.1	90.8	96.8	97.4	92.6
Amino acids (g kg^{-1} dry matter)							
Alanine	36.3	35.9	31.7	29.1	34.5	34.9	32.3
Arginine	31.7	30.3	29.4	28.1	29.9	29.2	29.6
Asparagine	43.4	43.5	42.3	40.1	43.5	42.6	39.9
Cysteine	3.2	3.6	1.5	2.2	2.6	3.1	3.3
Glutamic Acid	80.0	80.4	80.2	77.2	82.8	84.4	81.8
Glycine	39.3	39.3	35.5	31.9	37.0	36.8	32.9
Histidine	2.2	15.2	14.2	12.5	12.7	14.8	14.1
Isoleucine	18.9	19.4	20.4	17.6	18.8	19.6	18.6
Leucine	41.5	40.7	41.0	36.2	42.1	43.1	40.9
Lysine	32.7	33.1	31.8	30.0	32.5	31.3	30.5
Methionine	11.5	13.4	12.0	9.1	11.2	12.2	10.5
Phenylalanine	21.2	22.7	20.6	19.4	21.6	21.7	20.7
Proline	32.2	29.8	29.8	28.9	33.1	34.6	31.5
Serine	23.0	23.1	22.5	21.6	23.6	23.7	22.7
Threonine	19.6	19.8	19.6	18.9	20.2	20.3	20.0
Tyrosine	15.5	16.5	15.8	13.8	16.1	15.6	15.6
Valine	22.5	23.2	20.8	21.2	22.5	23.5	24.5

Table 5.13. Linear and quadratic regression parameters for the relation between growth parameters and inclusion levels of canola meal and fermented canola meal in Nile tilapia.

		Unstandardized coefficients				
		Constant	Inclusion	Inclusion^2	r^2	*P* - Values
Growth Parameters for CM						
Final Weight	Linear	829.584	-1.580		0.596	<0.01
	Quadratic	837.767	-2.807	0.020	0.628	<0.05
ADG	Linear	4.106	-0.021		0.376	<0.05
	Quadratic	4.216	-0.038	0.000	0.396	0.10
ADFI	Linear	7.199	-0.009		0.196	0.15
	Quadratic	7.292	-0.023	0.000	0.237	0.30
SGR	Linear	0.580	-0.002		0.294	0.07
	Quadratic	0.592	-0.004	2.862×10^{-5}	0.307	0.19
FCR	Linear	1.826	0.009		0.258	0.09
	Quadratic	1.806	0.012	-4.894×10^{-5}	0.261	0.26
Growth Parameters for FCM						
Final Weight	Linear	822.348	-0.769		0.398	0.07
	Quadratic	832.381	-2.274	0.025	0.399	0.10
ADG	Linear	4.063	-0.007		0.078	0.38
	Quadratic	4.157	-0.021	0.000	0.109	0.59
ADFI	Linear	7.272	-0.007		0.152	0.21
	Quadratic	7.248	-0.004	-5.909×10^{-5}	0.156	0.47
SGR	Linear	0.578	-0.001		0.027	0.61
	Quadratic	0.585	-0.002	1.882×10^{-5}	0.039	0.84
FCR	Linear	1.797	0.001		0.110	0.74
	Quadratic	1.804	0.000	1.790×10^{-5}	0.130	0.94

Table 5.14. Linear and quadratic regression parameters for the relation between growth parameters and inclusion levels of canola meal and fermented canola meal in rainbow trout.

		Unstandardized coefficients				
		Constant	Inclusion	Inclusion^2	r^2	*P* - Values
Growth Parameters for CM						
Final Weight	Linear	406.287	-3.031		0.723	<0.01
	Quadratic	420.343	-6.967	0.141	0.872	<0.01
ADG	Linear	3.641	-0.055		0.708	<0.01
	Quadratic	3.903	-0.128	0.003	0.865	<0.01
ADFI	Linear	2.986	-0.002		0.030	0.87
	Quadratic	2.923	0.017	-0.001	0.043	0.82
SGR	Linear	1.240	-0.014		0.725	<0.01
	Quadratic	1.306	-0.033	0.001	0.871	<0.01
FCR	Linear	0.835	0.018		0.701	<0.01
	Quadratic	0.770	0.036	-0.001	0.793	<0.01
Growth Parameters for FCM						
Final Weight	Linear	408.409	-1.327		0.585	<0.05
	Quadratic	404.820	0.162	-0.049	0.629	<0.05
ADG	Linear	3.663	-0.024		0.575	<0.05
	Quadratic	3.588	0.008	-0.001	0.635	<0.05
ADFI	Linear	3.299	-0.004		0.027	0.61
	Quadratic	3.303	-0.005	3.987×10^{-5}	0.028	0.88
SGR	Linear	1.246	-0.006		0.563	<0.05
	Quadratic	1.222	0.004	0.000	0.650	<0.05
FCR	Linear	0.886	0.007		0.540	<0.05
	Quadratic	0.921	-0.008	0.000	0.703	<0.05

6. Proposed timelines

Timeline	**April – Dec 2017**			
Month	April-June	July-August	September	December
Tasks	Gene expression	Histology Work	Permission to Write Meeting	Defence

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8. Appendix- Additional growth performance data

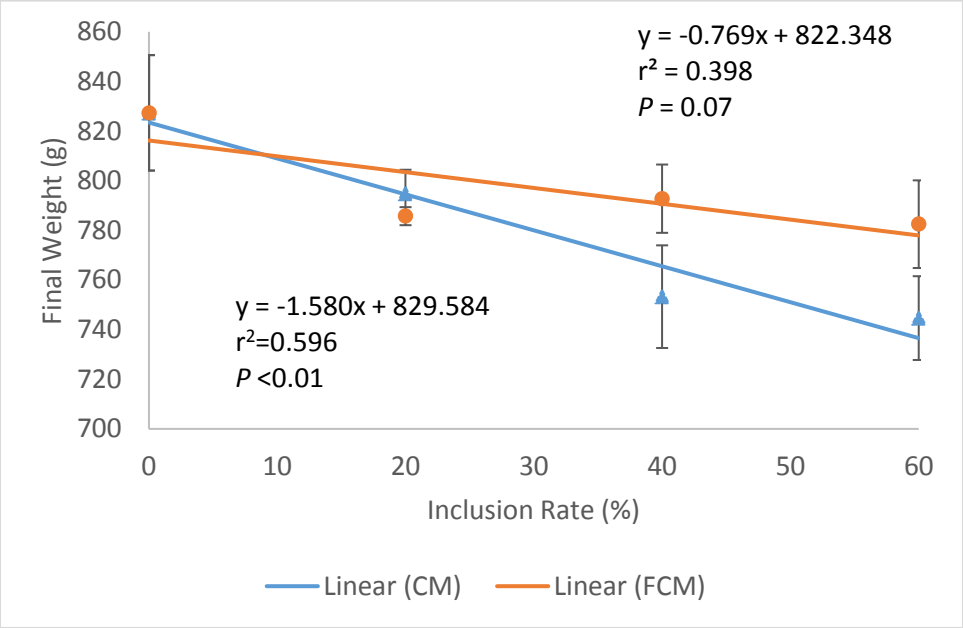

Figure 5.1. Regression analysis of ingredient inclusion level in Nile tilapia on Final weight (g) ±SEM.

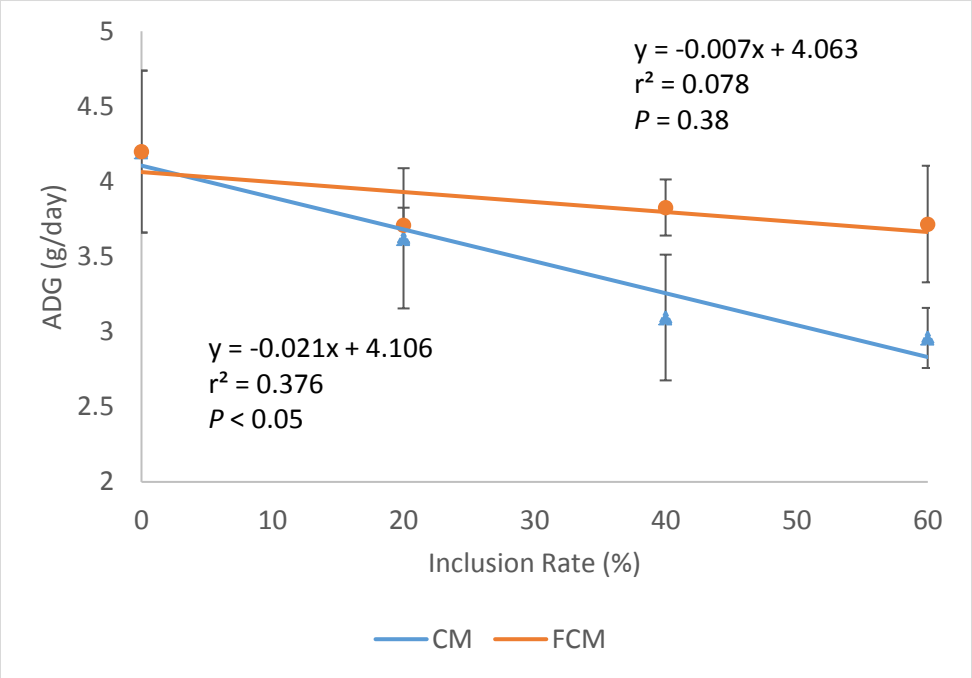

Figure 5.2. Regression analysis of ingredient inclusion level in Nile tilapia on ADG (g/day) ±SEM.

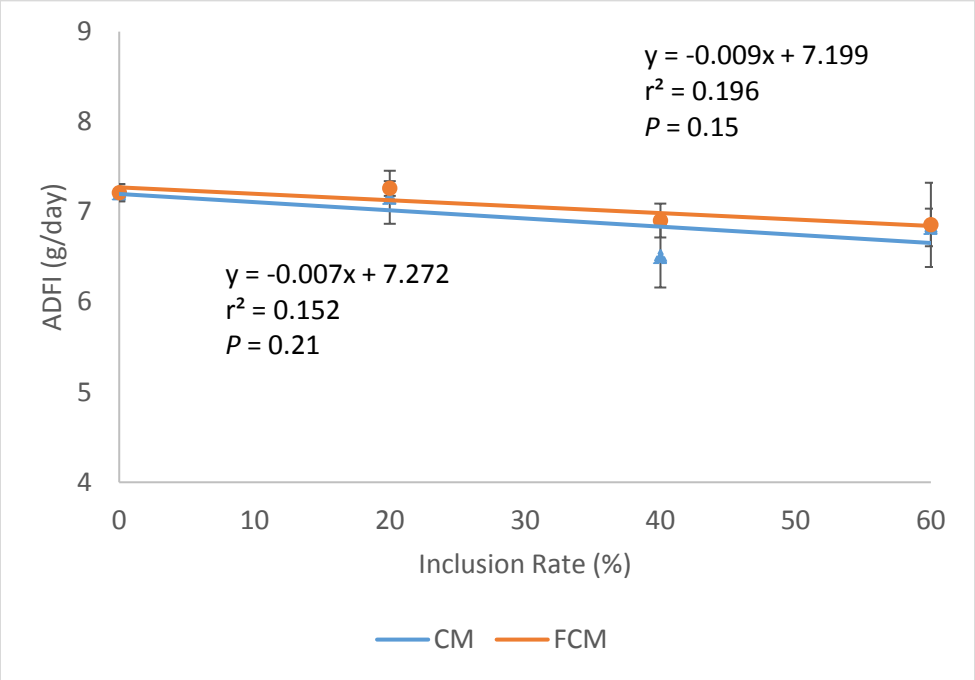

Figure 5.3. Regression analysis of ingredient inclusion level in Nile tilapia on ADFI (g/day) ±SEM.

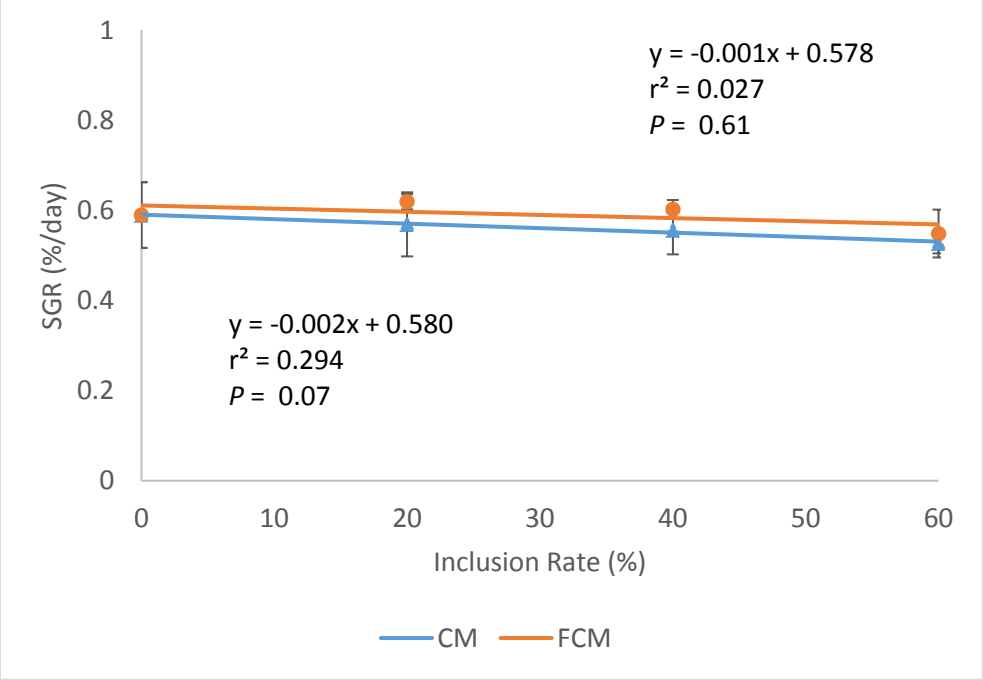

Figure 5.4. Regression analysis of ingredient inclusion level in Nile tilapia on SGR (%/day) ±SEM.

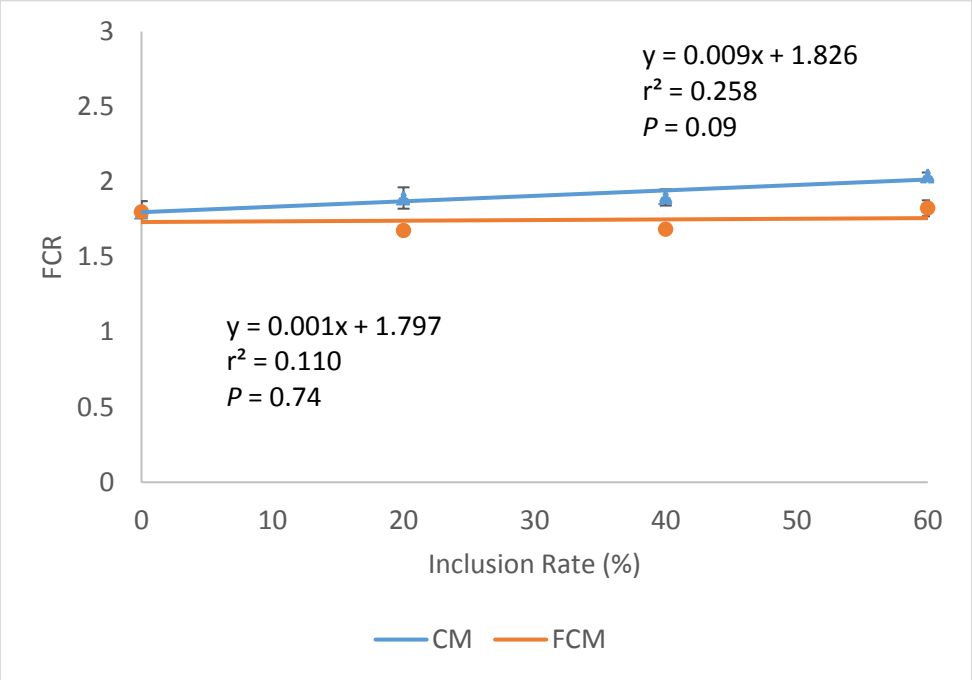

Figure 5.5. Regression analysis of ingredient inclusion level in Nile tilapia on FCR±SEM.

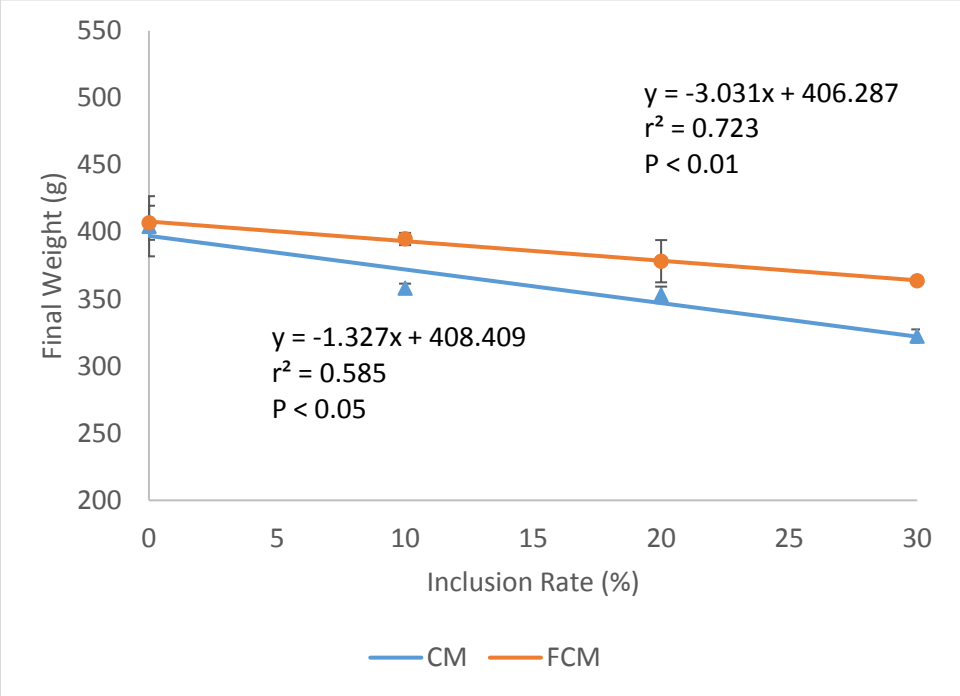

Figure 5.6. Regression analysis of ingredient inclusion level in rainbow trout on Final weight (g) ±SEM

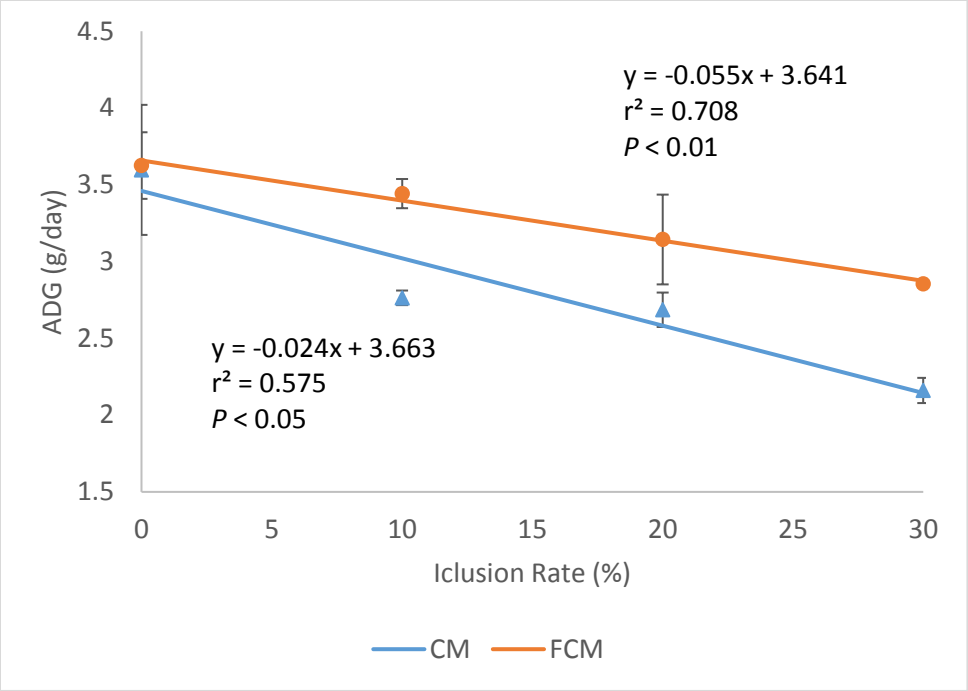

Figure 5.7. Regression analysis of ingredient inclusion level in rainbow trout on ADG (g/day) ±SEM.

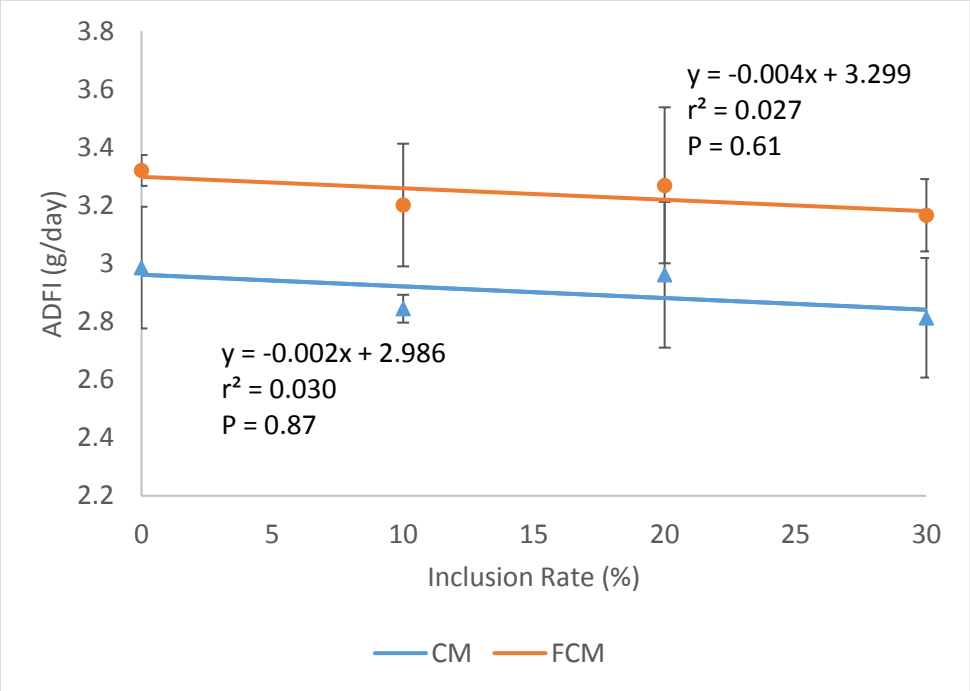

Figure 5.8. Regression analysis of ingredient inclusion level in rainbow trout on ADFI (g/fish/day) ±SEM.

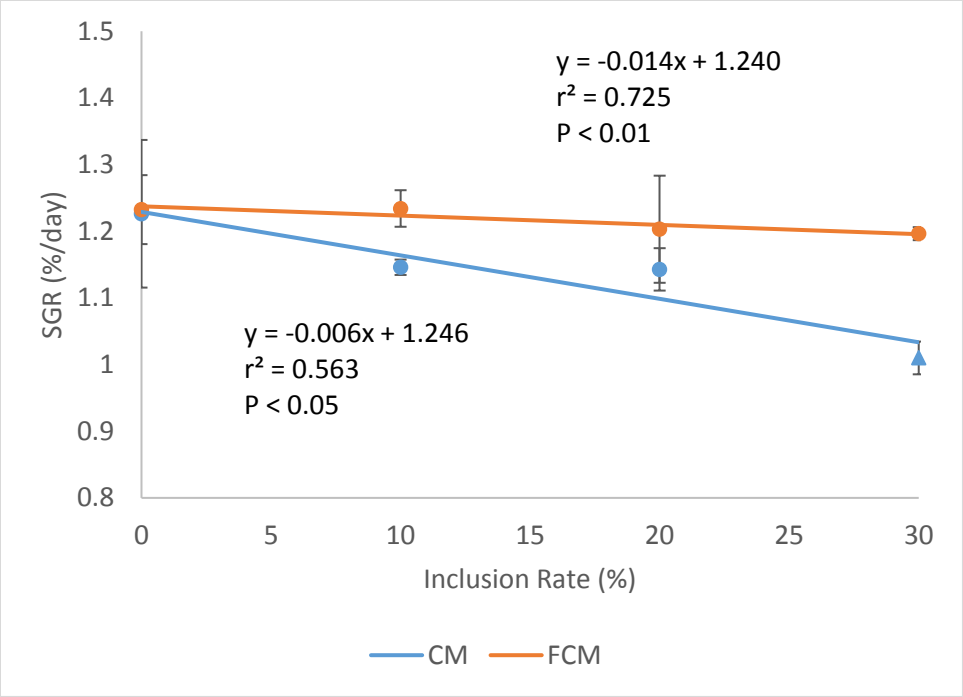

Figure 5.9. Regression analysis of ingredient inclusion level in rainbow trout on SGR (%/day) ±SEM.

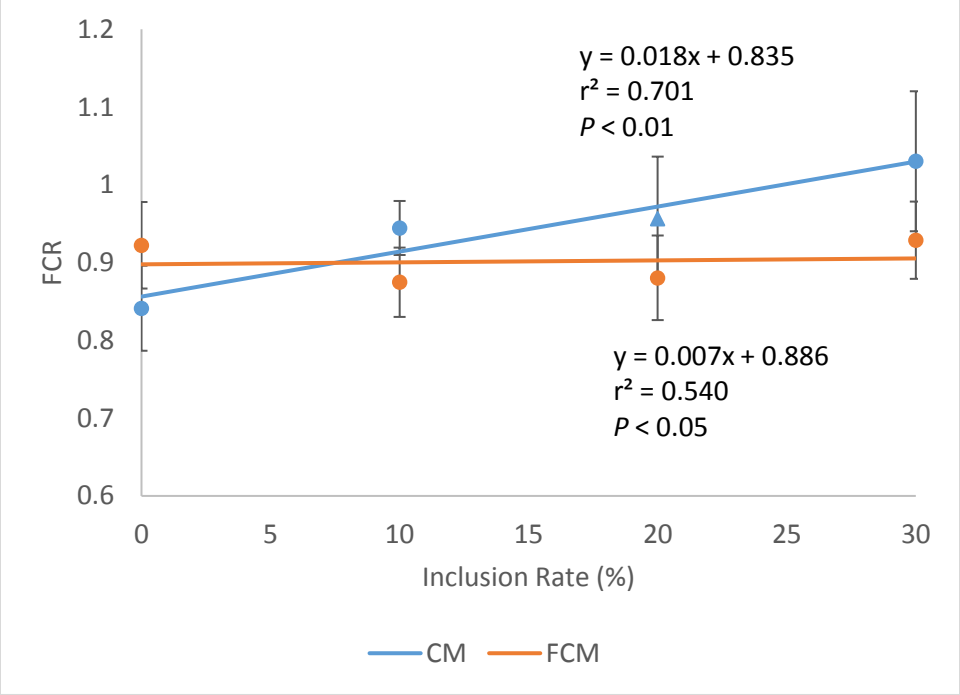

Figure 5.10. Regression analysis of ingredient inclusion level in rainbow trout on FCR±SEM.

Table 5.15. Growth performance data of Nile tilapia fed seven different diets. Data are presented as mean (n=3).

	Diets								
	Control	20CM	40CM	60CM	20FCM	40FCM	60FCM	SEM	P-value
Initial Weight (g)	600.98	591.94	580.00	578.98	577.95	578.46	574.36	3.600	0.46
Final Weight (g)	827.35^{a}	794.75^{ab}	753.28^{ab}	744.64^{b}	785.71^{ab}	792.76^{ab}	782.49^{ab}	8.235	<0.05
ADG (g)	4.20	3.62	3.09	2.96	3.71	3.83	3.72	0.145	0.28
ADFI (g/fish/day)	7.21	7.16	6.51	6.83	7.26	6.90	6.86	0.102	0.48
SGR (% /day)	0.59	0.53	0.47	0.45	0.55	0.56	0.55	0.191	0.42
FCR (feed /weight gain)	1.80	2.04	2.17	2.33	1.82	1.80	1.87	0.071	0.30

^{abc} Values with different superscripts within rows are significantly different (P <0.05)
SEM = Standard error of the mean
ADG = Average daily gain
ADFI = Average daily feed intake
SGR = Specific growth rate
FCR = Feed conversion ratio

Table 5.16. Growth performance data of rainbow trout fed seven different diets. Data are presented as mean (n=3).

	Diets								
	Control	10CM	20CM	30CM	10FCM	20FCM	30FCM	SEM	*P*-value
Initial Weight (g)	202.83	203.50	202.67	201.50	202.00	202.17	208.83	0.302	0.42
Final Weight (g)	404.28^{a}	358.27^{abc}	353.09^{bc}	322.49^{c}	394.78^{ab}	378.22^{ab}	363.65^{abc}	6.548	< 0.01
ADG (g/day)	3.60^{a}	2.76^{abc}	2.69^{bc}	2.16^{c}	3.44^{ab}	3.14^{abc}	2.85^{abc}	0.117	< 0.01
ADFI (g/fish/d)	2.99	2.84	3.21	2.81	3.20	3.27	3.17	0.067	0.44
SGR (% /day)	1.23^{a}	1.01^{abc}	0.99^{bc}	0.84^{c}	1.20^{ab}	1.12^{abcd}	1.03^{abcd}	0.031	<0.01
FCR (g feed / g gain)	0.84^{a}	1.03^{ab}	1.2^{ab}	1.31^{b}	0.93^{ab}	1.04^{ab}	1.11^{ab}	0.038	<0.01
HSI (%)	1.23	1.22	1.27	1.12	1.10	1.22	1.19	0.021	0.52

^{abc} Values with different superscripts within rows are significantly different (P <0.05)

SEM = Standard error of the mean
ADG = Average daily gain
ADFI = Average daily feed intake
SGR = Specific growth rate
FCR = Feed conversion ratio
HSI = Hepatosomatic index