Norwegian University of Life Sciences
Faculty of Veterinary Medicine and Biosciences
Department of Animal and Aquacultural Sciences

Master Thesis 2014
60 credits

Rapeseed cake as a feed ingredient for Nile tilapia. Responses to replacing protein from soybean meal with rapeseed cake, and fine milling and autoclaving of the rapeseed cake

Fatemeh Nemati Shizari

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Master Thesis (60 credits)

In Feed Manufacturing Technology

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Abstract

This thesis consists of two mains parts: an introduction giving a literature review of key topics relevant for design and interpretation of the experiments carried out in this thesis, and an experimental part.

Recent reports from FAO have highlighted the need for increased utilization of more low cost material in food production industries. Aquaculture industry may play an increasingly important role in providing high quality food, and approximately half of the production costs are related to feed. It is, thus, becoming increasingly important to utilize non-food and low cost ingredients in fish feed. Nile tilapia is a major farmed fish species. Moreover, it has high capacity to tolerate a wide range of environmental stresses and the presence of antinutrients compounds in diet, making it an ideal target species for upgrading low-quality ingredients to high quality food.

Secondary products from rapeseed oil processing are highly abundant and represent an inexpensive source of protein. The major challenge for increased use is the presence of certain glucosinolate derivatives such as isothiocyanate and progoitrin, which previously have been reported to cause metabolic problems or reduce feed acceptability for fish. Secondary products of rapeseed also contain other antinutritional factors such as phytic acid and tannins that may represent metabolic challenges. Several of these factors can be reduced by relatively intense moist heating.

The overall aim of the research in this research was to find out if simple processing such as fine milling or moist heating influenced the nutritional value of rapeseed cake (RSC), a secondary product from rapeseed oil processing. Two experiments were carried out. The first experiment aimed at defining a dietary inclusion level to which Nile tilapias were sensitive to changes in nutritional quality of RSC. The second experiment was carried out to assess the effects of fine milling or the combination of fine milling and autoclaving of RSC.

The first experiment was designed on the base of a regression analysis to define the dose response of tilapia to inclusion of rapeseed cake (RSC) in diet. Five different isoenergetic and isonitrogenous, plant ingredient-based diets were produced with different level of inclusion of RSC. Crude protein from soybean meal (SBM) was gradually replaced by crude protein from RSC at 0, 25, 50, 75 and 100 % of replacement. The feeding trial was performed in two replicate tanks of Nile tilapia for each experimental diet. Each tank contained 20 tilapias with average weight of 19.9 g. Feeding was in excess, 3 times (40

min) per day. Feed was quantified on a daily basis for the first 3 weeks, and as a pooled value over the whole 6 week feeding period.

The findings from Exp.1 demonstrated a decline in feed intake and growth along with increasing the level of RSC in diet. A threshold effect was observed in the regression curve near of 50% replacement. A possible explanation for these results may be the presence of bitter component in RSC which caused poor palatability of RSC containing diet and reduces feed intake. The feed conversion ratio (FCR) (g DM intake (g gain)$^{-1}$) was almost close to 1 g g^{-1} for all levels, except for the groups feed 100% replacement of CP from RSC, where it was slightly elevated. No diet-related trends were observed apparent digestibility or retention of crude protein, mineral (Ca, P, Mg, Mn, Zn) absorption or concentration in blood plasma, energy content of whole body and energy and protein retention along with different inclusion of RSC in diet, indicating that protein from RSC and SBM had comparable availability, and that the effects of phytic acid from the two protein sources had comparable effects. Moreover, thyroid hormone (T4) in blood plasma was not markedly different for different treatments, indicating that glucosinolate derivatives may not have been a main factor in explaining the reductions in feed intake and growth. The lipid content of whole body composition decreased in treatments fed diet from 0% until 50% replacement and then increases up to 100% replacement of CP from RSC with CP from SBM. The same pattern was seen in content of DM which can be the result of lipid content of body.

It is assumed that presence of higher content of tannin in RSC on the base of feed intake pattern causes higher visceral fat deposition in fish after 50% replacement. The ration of liver weigh to body weight was increased from 0% until 50% replacement level and after that tended to reduce up to 100% replacement of CP from RSC with CP from SBM. Since the deposition of lipid in liver decreases by increasing content of tannin in diet, the pattern given from ratio of liver weight to body weight may be caused by this fact.

The aim of second experiment (Exp.2) was to assess whether fine milling and/or combination of fine milling and autoclaving the RSC applied in diet may affect the nutritional quality of feed for tilapia. Exp.2 was performed according to the results from Exp.1, on the 50% level of replacement of CP from SBM with CP from RSC in diet which causes more sensitivity in fish to nutritional quality of diet. This experiment was designed on the base of ANOVA analysis. A 3 weeks trail feeding tilapia was conducted with 3 different experimental diets. The RSC used in different experimental diets were 1mm ground (the same as Exp.1), milled to 0.5 mm of particle size, or milled to 0.5 mm and autoclaved for 10 min in 120^{o}C. Each diet fed to tilapia in 3 replicate tanks. Each tank contained 20 fish with the average weight of 37.3 gr. Feeding and monitoring of daily feed intake was the same as Exp.1.

Feed intake and gain were significantly ($P<0.05$) decreased by autoclaving the RSC. FCR was close to 1 g DM g^{-1} gain for all treatments ($P>0.05$). This may be due to autoclaving having a negative effect on the palatability of RSC due to production of glucosinolates breakdown products. A linear relationship was seen between feed intake and gain which may demonstrate that the main reason of growth depression is related to decrease of FI. The finding of increased glucose concentration in the diet with autoclaved RSC, probably originating from hydrolysis of glucosinolates, supports this hypothesis. A significant decrease ($P<0.05$) in content of DM and crude protein, and energy and nitrogen retention in whole body was seen in fish fed the diet containing autoclaved RSC.

It can, thus, be assumed that fine milling increased availability of components that produced in fine milled and autoclaved RSC may have negative effect on tilapias metabolism.

The content of whole body lipid, ash and energy did not show any significant difference ($P>0.05$) among different treatments. Also the content of minerals (except zinc) in blood plasma among different treatments fed different experimental diets was the same ($P>0.05$). However, the content of zinc in blood plasma of tilapia fed with diet containing fine milled and fine milled and autoclaved RSC tended ($0.05<P<0.10$) to be lower than the tilapia fed with diet containing 1mm ground RSC. The levels of T4 in blood plasma of all treatments were the same ($P>0.05$). It may prove that the certain level of inclusion of secondary compounds of RSC used in these diets may not have any goitrogenic effect on tilapias thyroid.

To conclude, this research show that presence of RSC in tilapia diet may reduce feed acceptability. However, it did not show different effects on metabolic function of fish than those caused by SBM. Fine milling did not affect the nutritional value of RSC, while autoclaving of RSC has negative effect on feed intake, energy utilization, and consequently on growth.

Contents

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Ås, 9-April-2014

List of abbreviations

µg =Micrigram
µM= Micro molar
µmol = Micromole
A= Autoclaving
AA =Amino acid
AD = Apparent digestibility
am = Before noon (Ante Meridiem)
ANF = Anti-nutritional factors
ANOVA= Analysis of variance
Ba = Barium
BW = Body weight
C22; n-9 = Erucic acid
Ca = Calcium
CFI = Cumulative feed intake
C-N= Carbon–nitrogen bond
CP = Crude protein
Cu=Cupper
DFI = Daily feed intake
DM = Dry matter
DNA= Deoxyribonucleic acid
EAA = Essential amino acids
Exp.1= Experiment 1
Exp.2= Experiment 2
FAO= Food and Agriculture Organization
FCR = Feed conversion ratio
Fe = Ferrous
FI= Feed intake
FW = Final average fish weight
G = relative centrifugal force
GC = Gas chromatography
GE = Gross energy
GIFT = *Genetically Improved Farmed Tilapia*
GIT = Gastrointestinal tract
GLS= Glucosinolate
GMO = Genetically modified organisms
HCl= Hydrochloric acid
hpi = Hours postinfection
HPLC = High-performance liquid chromatography
ICP = Inductively-coupled plasma
IHA = Institutt for husdyr- og akvakulturvitskap
ITC= Isothiocyanates
IU= International unit
IW = Initial average fish weight
kj= kilojoules
l = Liter
mBar = Millibar
Mg = Magnesium
mg = Milligram
MHz =Megahertz
min = Minute
MJ= Megajoules
mm = Millimeter
mM = Millimolar
Mn = Manganese
MPa = Megapascal
mRNA= Messenger ribonucleic acid
MS= Microsoft
N=S =C = Thiocyanate
NaHCO3= Sodium bicarbonate

NaHCO3= Sodium bicarbonate

ND= Not determined

NE = Net energy

ng =Nanogram

NH4CO3= Ammonium carbonate

NMBU = Norges miljø- og biovitenskapelige universitet (Norwegian University of Life Sciences)

NP = Net protein

NSP = Non-starch Polysaccharides

OZT= Oxazolidinethion

P = Phosphorus

pH= Scale of acidity

pm = After midday (Post Meridiem)

pM = Pico molar

R^2 =Determination coefficient

RF= Recovery factor

RIA = Radioimmunoassay

RNA= Ribonucleic acid

RPC =Rapeseed protein concentrate

RS =Rapeseed

RSC = Rapeseed cake

RSM = Rapeseed meal

s.e.m = Standard error of the mean

SAS= Statistical analysis system

SBM = Soybean meal

Sn = Tin (*stannum*)

Sr = *Strontium*

T3= Triiodothyronine

T4= Thyroxine

TEM = Transmission electron microscopy

US$= United States dollar

WG = Weight gain

Zn = Zinc

1. Introduction

Almost 200,000 people are added to world population every day and the ever increasing global food demand may not be satisfied through restricted available food recourses in the close future (Nellemann et al., 2009; Tilman et al., 2011). Shifting the land usage from agricultural purposes toward urban and industrial purposes, decreasing rural population and increasing urban population may also reduce the food production (Van Eetvelde and Antrop, 2004; Nellemann et al., 2009). Several other factors such as global warming by disruption of agriculture productivity especially in poor countries, changes in living standards and utilization of crops in biofuel industry are contributing to severity of this problem (Gibbs et al., 2008; Mendelsohn et al., 1994). Aquaculture is known as a fast growing sector in food production which can contribute to global food production by producing high quality source of protein (FAO, 2012). In the last decades, contribution of aquaculture to capture fisheries to provide food for human is increased (Figure 1). However by increasing costs of this industry such as price of energy and water it is necessary to keep the products price compatible with fishery productions (Nellemann et al., 2009; Tidwell and Allan, 2001). Approximately 50-70% of total fish production cost is dedicated to purchase of feed (Rana et al., 2009).

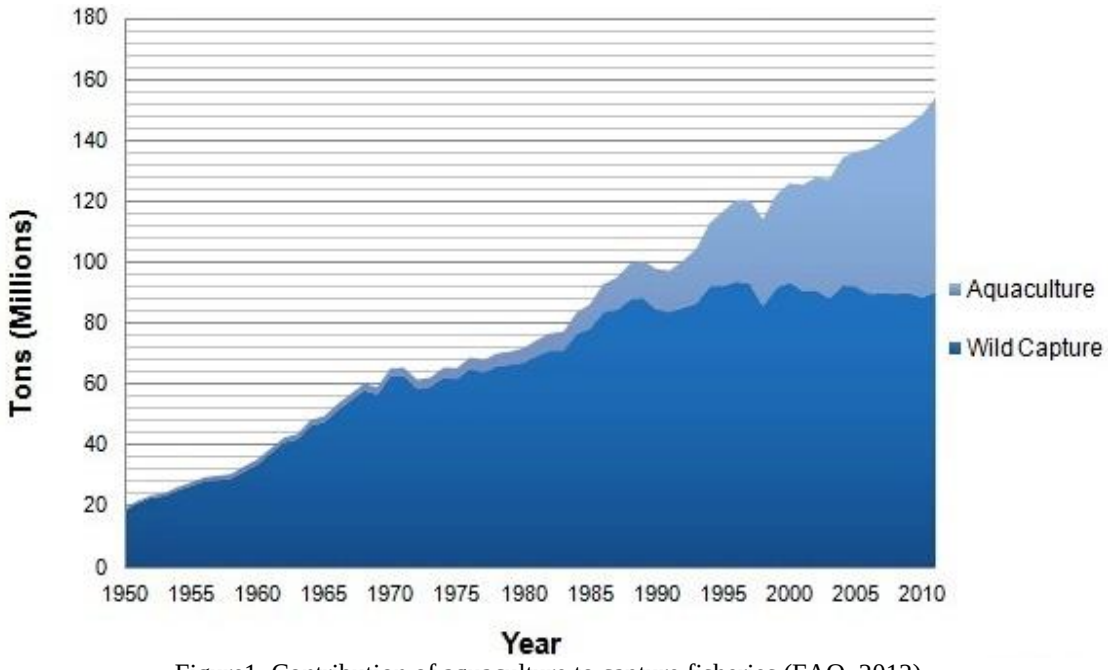

Figure1. Contribution of aquaculture to capture fisheries (FAO, 2012)

Fish feed consists a major part of the production costs. Protein is an expensive nutrient which previously was provided mainly from fish meal in feeds for carnivorous fish. There is a high interest to replace fish meal partly or completely with plant protein in fish feed. It has been proved in several investigations that this approach is successfully possible to be achieved even in carnivorous species (Salze et al., 2010; Zhou and Yue, 2010). Utilization of plant protein may be less challenging for omnivorous fish species (Hardy, 2010). Deficiency in essential amino acids was one of the challenges to use plant protein which may be possible to overcome to this problem by supplementation of them with necessary amino acids.

Soybean has a fair amino acid profile to be used in aqua feed. It is, however, highly useful in human food (van der Ingh et al., 1991). Replacing soy protein with a low price alternative without any negative impact on nutritional and physical quality of fish feed may reduce costs and helps to global food production. Understanding the best method of processing and utilization of low cost resources is necessary to utilize them in aqua feed (Guimarães et al., 2003). Numerous studies hare evaluated the possibility of utilization of cheaper protein resources such as industrial secondary products in aqua-feeds (Francis et al., 2001; Slawski et al., 2013; Collins et al., 2013).

Biofuel and cooking oil production industries demand huge amounts of oil seeds (FAO, 2012). The residues contain high amounts of protein and are valuable to be used as a protein source in fish feed. In addition to soybean, rapeseed in one of the main oil seeds which is used in these industries. Depending on oil extraction method, different secondary products are produced such as rapeseed meal (RSM) and rapeseed cake (RSC) which can be a cost-effective source of protein in a sufficient quantity to be applied as a sources of protein in fish feed (Hardy, 2010). Rapeseed secondary products are cheaper than soybean co-products. Between 2010 and 2011 the international price of soybean cake was 550 US$ per ton and for RSM this price was 279 US$ per ton (FAO, 2012).

Utilization secondary products of rapeseed are limited by the presence of anti-nutritional factors (ANF). Many investigations have been done to assess the best level of inclusion and method of processing to utilize secondary products of rapeseed in fish feed. According to previous studies use of RSM in tilapia feed is limited. Seneviratne et al. (2010) have reported that utilization of more than 30% unprocessed RSM results in growth depression in tilapia. However there are still many knowledge gaps about the function and composition of these ANF in different products. The optimal method of processing need to be clarified in details to be able to properly utilize these valuable materials in fish feed.

2. Literature review

2.1 Rapeseed

Rapeseed (*Brassica napus* L.) belongs to the Brassicaceae family (mustard or cabbage family). Rapeseed contains more than 40% oil. The production of oil per unit of land in rapeseed is higher than that from other crops. Whole rapeseed production in world in 2012 was reported around 62.6 million tones. China is the leading producer, followed by India. Germany and France are pioneers in biodiesel production from rapeseed oil and the two main rapeseed producers in Europe (FAO, 2013). Increasing biodiesel production will lead agriculture to increase rapeseed production in close future (Hoogeveen et al., 2009). RSC is one of the co-products of oil extraction processing after extracting approximately 70% of oil from seed (Leming and Lember, 2005; Spragg and Mailer, 2007). Utilization of RSC in fish feed can be beneficial due to local availability from small factories (Leming and Lember, 2005).

2.1.1 Nutritional properties of RSC

The most common method which results in RSC as a co-product is cold-press extraction. In comparison with other extraction methods such as solvent-extraction, cold-pressing leaves higher oil content in the residues (Woyengo et al., 2010). Also the content of amino acids especially lysine is higher than from expeller-pressed, due to lower processing temperatures result in lower Maillard reaction. Expeller-pressed RSC and RSM are produced after an oil extraction processing which seeds are heated by steam before oil extraction up to 110^{o}C. In this case the residual oil is lower and heating may negatively affects the amino acid content of co-products especially lysine (Seneviratne et al., 2010). However expeller-pressing may reduce the content of unwanted, heat labile compounds existing in intact rapeseed or what are producing during crashing and processing (Schöne et al., 2001; Newkirk and Classen, 2002). Different methods of extraction have been shown in Figure 2.

Some compounds which are removed from raw oil during oil extraction process such as gums, waxes and phospholipids are added back to RSC after processing. They can also affect the energy content of RSC and reduce dustiness (Booth and Gunstone, 2004).

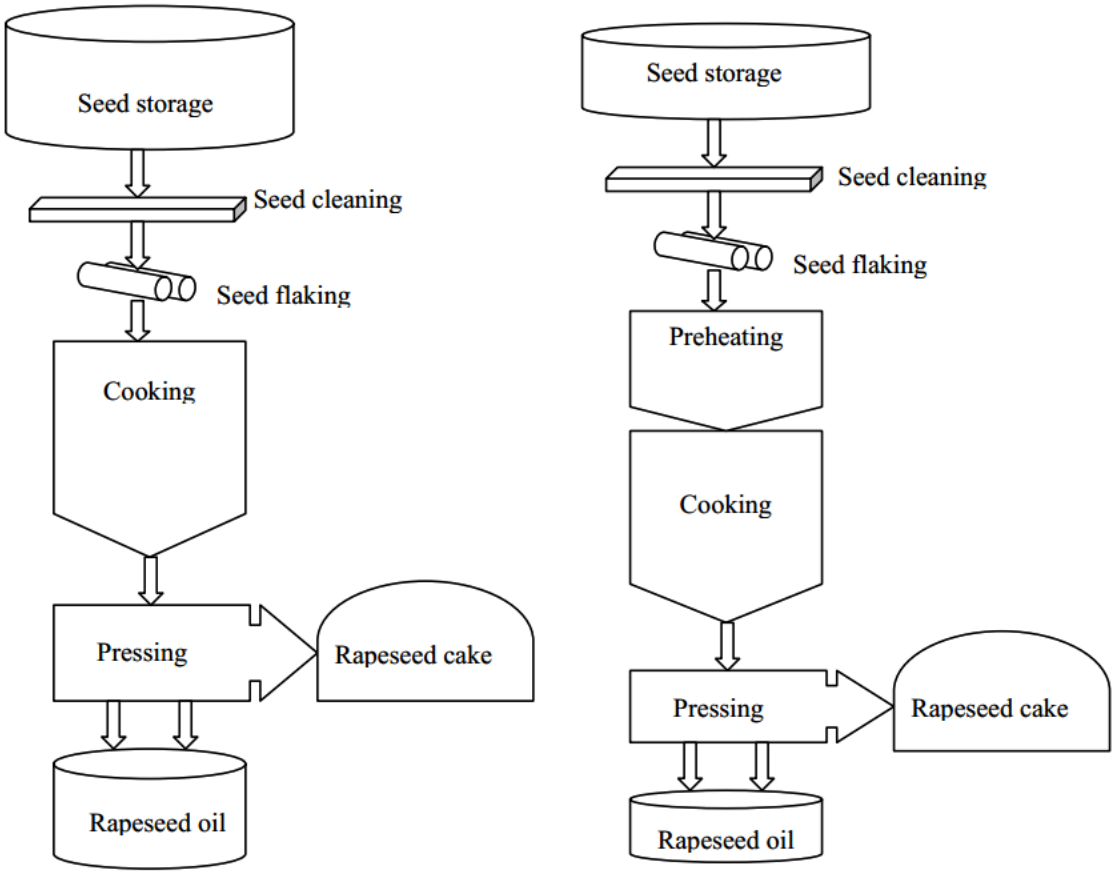

Figure 2: different oil extraction methods and production of RSC (Leming and Lember, 2005)

Rapeseed secondary products are low in lysine but contain more methionine than soybeans (Pastuszewska et al., 2000). Besides the composition of essential amino acids is sufficient to support a high biological value (Yang et al., 2014) making rapeseed an interesting alternative for soy protein in fish feed. However, in many countries RSC is utilized as a fertilizer or biomass (a source of carbon, hydrogen and oxygen) to produce energy (Özçimen and Karaosmanoğlu, 2004).

Several studies have been done to investigate nutritional value of rapeseed secondary products. The majority of these studies have evaluated the feasibility of utilization of RSM in animal feed (Moset et al., 2012; Luo et al., 2012). However, few experiments have been assessed the nutritional values of cold-press RSC and practical methods to remove its ANF to be feasible to apply in fish feed.

Secondary toxic metabolites in plants may have a defensive function to protect plant from environmental stresses such as pests attack or being eaten by herbivorous animals (Bennett and Wallsgrove, 1994).

2.1.2 Anti-nutritional factors

Rapeseed secondary products that may have negative impacts on animal's growth and health these effects are ascribed to ANF. The intolerance level of fish to these secondary products is varying among species. There is a limitation for presence of ANF in feed to prevent negative effects on fish performance such as growth rate, feed intake (FI) or metabolic problems such as hyperthyroidism (Gatlin et al., 2007). Certain ANF contained in rapeseed secondary products may also affect nutrient availability, as illustrated by the impact of isothiocyanate on lysine bioavailability (Nakamura et al., 2009) (Figure 3).

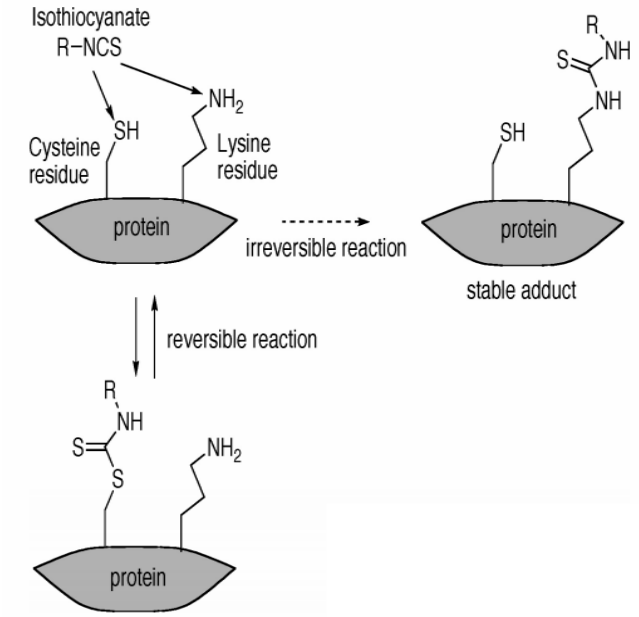

Figure 3: Reaction between isothiocyanate and lysine (Nakamura et al., 2009)

Main ANF in RSC are glucosinolates, erucic acid, phytic acid, sinapinic acid, tannins, indigestible carbohydrates, lipoxygenase, lectins, urease, trypsin inhibitors, flavonoids and estrogenic compounds (Francis et al., 2001).

Each compound plays specific role in the biology of the plants and is accumulated in specific tissues. For example indolics glucosinolate derivatives have antifungal effect (Bednarek et al., 2009) and flavonoids, sinapates and other phenolics are known as a responsible for protecting plant from ultraviolet-B stress (Li et al., 2010). Tannins are other phenolic polymers existing in rapeseed secondary products. They can decrease energy and protein digestibility by binding nutritive and form

indigestible complexes in feed (Enami, 2011). Table 1 shows a comparison between phenolic acids in rapeseed and some other oilseeds

Table 1: Total content of phenolic acids in some oilseed products

Oilseed product	(g kg-1 dry basis)
Soybean flour	0.23
Cottonseed flour	0.57
Peanut flour	0.63
Rapeseed canola flour	6.4-12.8
Canola meal	15.4-18.4
Soybean meal	4.6

Developed from Kozlowska et al., 1991 and Naczk et al., 1986

Sinapine mostly exists in seeds embryo and affects the palatability by giving bitter taste to feed and reduces the FI (Solá-Oriol et al., 2011).

Glucosinolates and erucic acid are responsible for majority of negative effects of RSC on fish metabolisms (Slawski et al., 2011a,b). Phytates are not toxic compounds but by forming indigestible chelates with cations may reduce minerals bioavailability (Maenz, 2001).

2.1.3 Non-starch Polysaccharides

Non-starch Polysaccharides (NSP) is another category of unwanted compounds in RSC. They are complex compounds often consisting of combination of hexoses and pentoses monomers with non digestible linkages such as β-(1-3) and β-(1-4). Classification of NSP was previously on the base of extraction and isolation methods or deference's on solubility and pH of soluble which was used for extraction (Neukom, 1976). Another classification which is mainly on the base of molecular structure which has been done by Butler and Bailey (1973) includes cellulose, non-cellulosic polymers and pectic polysaccharides (Table 2).

Rapeseed contains a wide range of NSP including cellulose, pectic polysaccharides (i.e., rhamnogalacturonans) and a several non-cellulosic polysaccharides such as xylans, xyloglucans, arabinans, arabinogalactans and galactomannans (Slominski and Campbell, 1990; BachKnudsen, 1997). Especially pectic polysaccharides and those which are not bond to cell wall may increase the viscosity of their solutions (Sinha et al., 2011). Rapeseed secondary products contain higher level of

NSP in comparison with soybean meal (SBM) (46% in Canola meal and 19% in SBM) (Kocher, 2002).

Table 2: Classification of NSP (Sinha et al., 2011)

Category	Subcategory	Monomeric residue	linkage	source
Cellulose	Cellulose	Glucose	β-(1-4)	Most cereals and legumes
Non-cellulotic polymers	Arabinoxylan	Arabinose and xylose	β-(1-4) - linked xylose units	Wheat, rye. Barley, oat, rice
	Mixed- linked β-glucan	Glucose	β-(1-3) and β-(1-4)	Barley, oat
	Mannans	Mannose	β-(1-4)	Coffee seed
	Galactomannans	Galactose and mannans	β-(1-4)- linked mannan chain with α-(1-6)-linked galactosyl side groups	Locust bean gum and guar gum
	Glucomannans	Glucose and mannans	β-(1-4)- linked mannan chain interspersed glucose n the mian chain	Sugar-beet pulp
Pectic polysaccharides	Arabinan	Arabinose	α-(1-5)	Cereal co-products
	Galactan	Galactose	β-(1-4)	Sugar-beet pulp
	Arabinogalactan (type I)	Arabinose and galactose	β-(1-4) galactan backbone substituted with 5- linked and terminal arabinose	Grain legumes
	Arabinogalactan (type II)	Arabinose and galactose	β-(1-3,6)- linked galactose polymers associated with 3- or 5- linked arabinose	Rapeseed cotyledon

High content of fiber including NSP such as lignin with associated polyphenols and glycoproteins is known as one of the reasons for metabolic problem in poultry which receives rapeseed secondary products in their diets (Khajali and Slominski, 2012).

Digestion and absorption of lipid and protein in gastrointestinal tract (GIT) of fish may be affected by NSP (Refstie et al., 1999; Sinha et al., 2011). Also inclusion of NSP in fish diet may affect the passage rate in GIT and availability of nutrients (Storebakken et al., 1999; Storebakken and Austreng, 1987).

An experiment with tilapia demonstrated that the negative effect of increasing viscosity on growth performance is not only because of decreasing nutrient digestion but it may be also due to differences in mineral absorption and excretion of sodium (Leenhouwers et al., 2007). A comparison between tilapia and other fish such as catfish or salmonids demonstrated that tilapia is more resistant to viscose dietary ingredients. It may be due to its feeding habits since tilapia is more herbivorous than those other species (Amirkolaie et al., 2005).

2.1.3.1 Erucic acid

Erucic acid is a mono-unsaturated fatty acid (C22:1, n-9) present in rapeseed. Extracted oil with high percentage of erucic acid is used for non-food purposes such as carburant and lubricants. High percentage of erucic acid in feed and food is associated with health problems. This fatty acid is responsible for fat deposits in heart muscle. In salmon, erucic acid from the feed may be accumulated in the body lipid (Nath et al., 2009).

2.1.3.2 Phytic acid

In most of the seeds and cereals phosphorus is mainly (60–90%) stored in the form of phytate which contains 3 to 4 % of rapeseeds weight (Uppström and Svensson, 1980). Phytates may reduce amino acid bioavailability by formation of indigestible compounds with proteins. Also by affecting aminopeptidases through chelation of cationic minerals such as Zn^{2+}, Mg^{2+}, Ca^{2+} and Fe^{2+} (Storebakken et al., 1998) that are both important cofactors and contribute to mineralization of hard tissues. The function of several digestive enzymes such as α-amylase, trypsin, tyrosinase and pepsin may be affected by presence of phytic acid in dietary ingredients (El-Batal and Abdel-Karem, 2001). Factors such as the phosphorous content of soil may affect the content of phytate in rapeseed (Khattab et al., 2010).

2.1.3.3 Glucosinolates

2.1.3.3.1 Molecular structure

Glucosinolates are organic sulfur containing compounds. The general structure of these molecules consists of a β-glucose, a sulfonated oxime group and a side chain which is bond to the central carbon and normally is an amino acid. In order to side chain, glucosinolates are categorized into three groups: aliphatic, aromatic and indolylic. The amino acids belonging to the aliphatic group are methionine, leucine, alanine, isoleucine and valine while phenylalanine and tyrosine are aromatic and tryptophane belongs to the indolyl group (Halkier et al., 2006) (Figure 4).

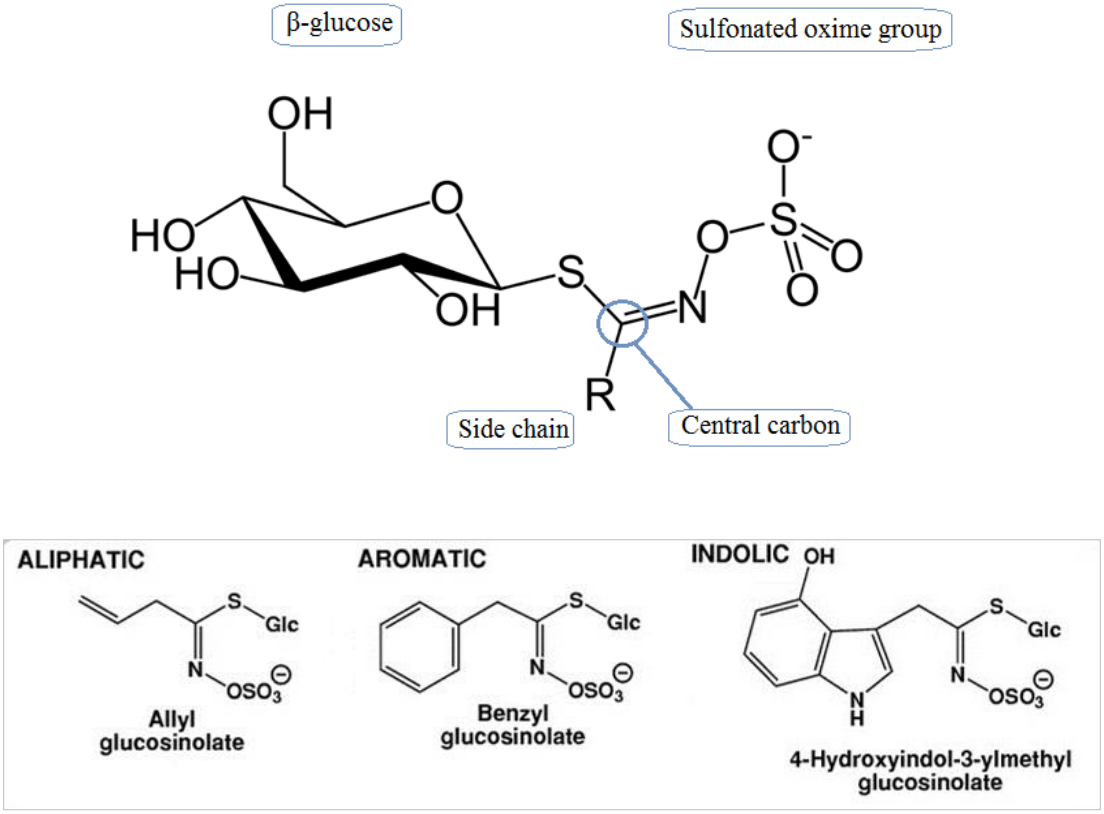

Figure 4: Structure of glucosinolates (Textor and Gershenzon, 2009)

The majority of intact glucosinolates in rapeseed are consisting of progoitrin, epiprogoitrin, gluconapoleiferin, gluconapin, 4-hydroyglucobrassicin, glucobrassicanapin, glucobrassicin, and gluconasturtiin. Glucosinolates are varying in content and distribution among different varieties of

rapeseed and environmental situation (Millán et al., 2009). For example Indian rapeseed mainly contains gluconapin (Tyagi, 2002) while in European varieties progoitrin, 4-hydroxyglucobrassicin and gluconapin are dominant (Mabon et al., 2000; Leming et al., 2004).

2.1.3.3.2 Metabolism in plants tissues

Biosynthesis of glucosinolates in plant has three stages: 1) side-chain elongation of amino acids, 2) development of the core structure and 3) side-chain modifications. The concentrations of glucosinolates in the plant organs are different. Higher glucosinolate concentration is found in reproductive organs such as seeds and flowers (Brown et al., 2003).

Glucosinolates may be localized in aqueous vacuoles in cells. In case of mechanical damages glucosinolates are released and hydrolyzed in the cytoplasm by an enzyme called myrosinase (Koroleva et al., 2010). Another hypothesis for the location of glucosinolate and myrosinase in plant cells is intracellular or intercellular localization. The first hypothesis explains that glucosinolate and myrosinase are localized in the same cell but in separated organelles or vacuoles. During extraction or in case of tissue damage, glucosinolate and myrosinase will be released, and glucosinolate hydrolysis starts (Kissen et al., 2009). The second hypothesis addresses the possibility of localization of myrosinase and glucosinolate in different cells in the plants body (Figure 5) which has been reported by Bridges et al. (2002). During the crushing and pressing stages in the oil extraction process, the majority of glucosinolates are realized may being released and hydrolyzed by myrosinase. Therefore secondary products of rapeseed contain glucosinolates hydrolysis products. Rapeseed varieties with very low, low, moderate and high glucosinolate content contain respectively 5, 9, 14 and 26 µmol glucosinolate per gram seed. Rapeseed secondary products with approximately 10 µmol glucosinolate or less per gram seed are applicable in animal feed (Jensen et al., 2010).

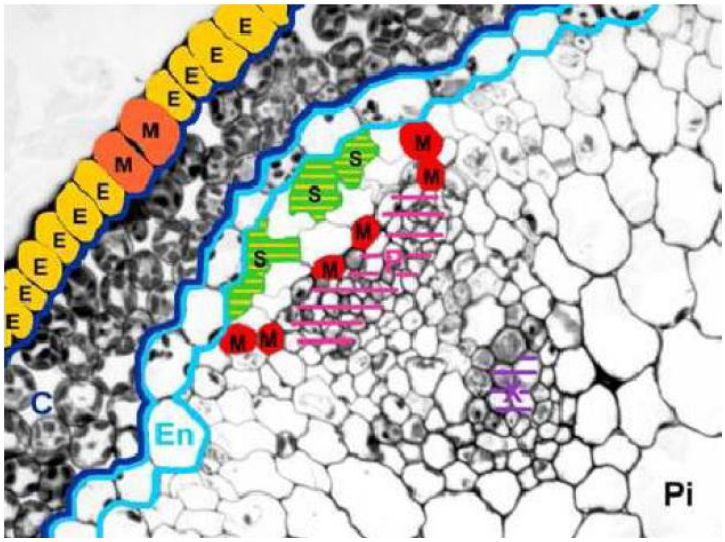

Figure 5: S: glucosinolates containing cells; M: myrosinase-expressing phloem cells and guard cells respectively; E: epidermal cells; S: cellular co-localization of glucosinolates and ESP (Kissen et al., 2009)

2.1.3.3.3 Effect of oil extraction methods on glucosinolate content

Oil extraction methods can affect the content of glucosinolates in rapeseed co-products such as RSM and RSC. The level of toxins on dehulled extraction and expeller extraction co-products is lower than solvent extraction co-products (Bourdon and Aumaitre, 1990; Glencross et al., 2004) (Table 3).

Table 3: Effect of extraction on glucosinolates content of rapeseed secondary products.

Extraction process	Rapeseed type	Total GLS(µmol/g)	ITC(mg/g)	OZT (mg/g)
Solvent extracted	RSM 0	166	3.5	9.2
	RSM 00	38	1.3	2.4
	Canola meal	3.62	ND	ND
Dehulled extracted	RSM 0	151	4.7	11.5
	RSM 00	30	0.8	1.6
Expeller extracted	RSM 00	36	1.3	3.5
	Canola meal	1.1	ND	ND

RSM: rapeseed meal, GLS: glucosinolate, ITC: isothiocyanates, OZT: oxazolidinethion, ND: not determined, (Tripathi and Mishra, 2007)

2.1.3.4 Glucosinolate hydrolysis products

Glucosinolates may break down into variety of compounds which may have toxic effect on animal fed by glucosinolate containing diet. Hydrolysis of glucosinolate results in unstable intermediate

compounds. These compounds through further reactions convert into isothiocyanates, nitriles, thiocyanates, indoles and oxazolidinethiones (Kleinwächter and Selmar, 2004).

Several external factors may affect end products of glucosinolate hydrolysis. In low pH, production of nitriles is dominant and by increasing pH, isothiocyanate production increases. Various types of glucosinolates result in different derivatives. For example sinigrin hydrolysis results in more allyl cynaid but in gluconapine hydrolysis production of butenyl cyanide is dominant. Furthermore, toxicity of each compound differs from the other. By increasing the percentage of the C-N or N=C=S groups in hydrolysis products the toxicity of glucosinolate derivatives increases (Wittstock et al., 2003) (Figure 6).

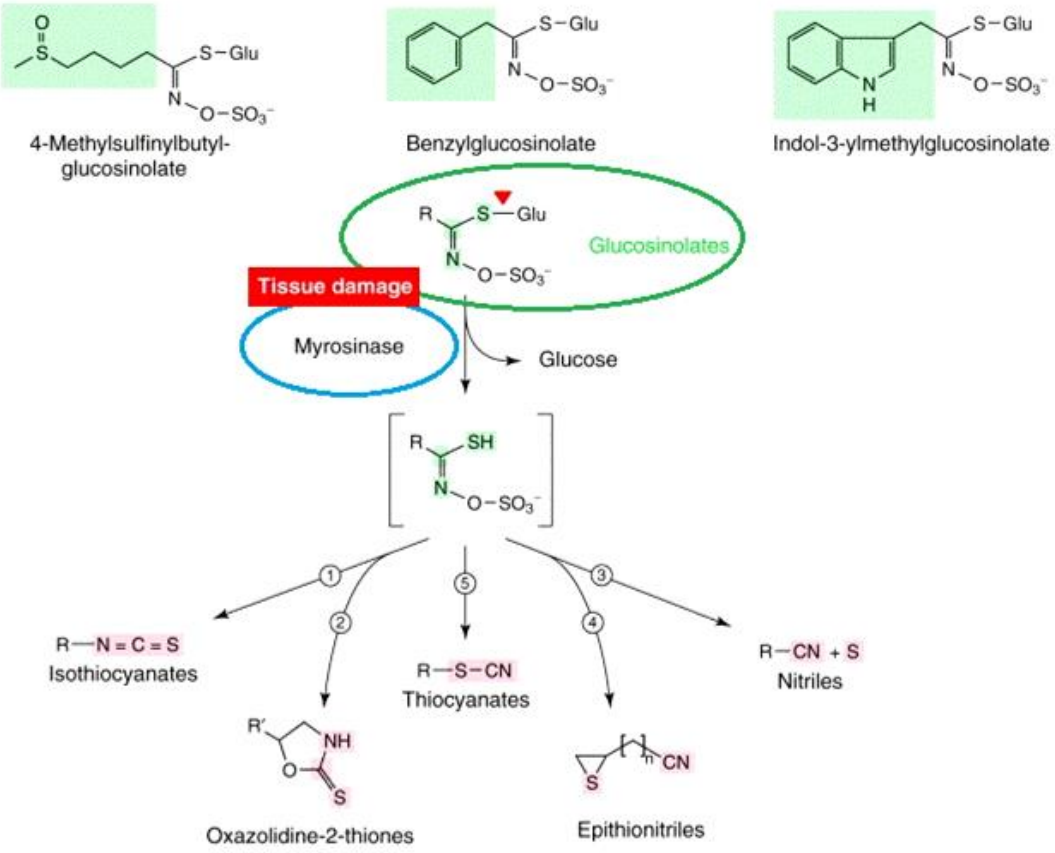

Figure 6: Enzymatic breakdown of glucosinolates (Wittstock and Halkier, 2002)

2.1.3.4.1 Isothiocyanate and nitrile production:

Glucosinolates hydrolysis in neutral pH normally results in isothiocyanates but in lower pH main product of reaction is nitrile. The spicy hot taste of rapeseed is due to presence of isothiocyanates, which may reduce FI (Wittstock and Halkier, 2002). Isothiocyanates have been used in food industry because of its strong antimicrobial effects. The main group in this category which is intensively investigated is allyl isothiocyanate (Obaidat and Frank, 2009).

2.1.3.4.2 Thiocyanate production:

Three compounds of derivatives from glucosinolate hydrolysis are categorized in this group: allyl-, benzyl- and 4-(methylthio) butyl-glucosinolates.

2.1.3.4.3 Epithioalkanes:

Hydrolysis of alkenyl glucosinolates by presence of epithiospecifier protein by affecting the enzyme myrosinase functions results in epithioalkanes (Verkerk and Dekker, 2009).

2.1.4 Myrosinase

The enzyme myrosinase (β-thioglucosidase, EC3.2.1.147) is located in protein-accumulating cells which call myrosin cells (Kissen et al., 2009). In case of tissue damage myrosinase realizes from vacuoles (Figure 7) and reacts with glucosinolate.

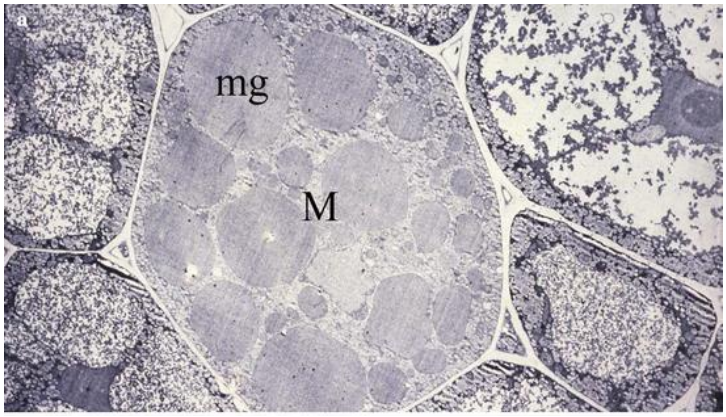

Figure 7: TEM picture of an idioblastic myrosin cell (M) of R. sativus surrounded by ground tissue cells; mg: one of the myrosin grains (Kissen et al., 2009)

Moisture level, temperature and pH may affect myrosinases activity (Plaipetch and Yakupitiyage, 2013). Hydrolysis of thioglucoside bonds results in one molecule of glucose and thiohydroxamate-O-sulfonate which is an unstable compound (Choubdar et al., 2010). Rearrangement of unstable compound results in production of glucosinolates hydrolysis products and elementary sulphur (Vig et al., 2009). Not only plants but also fungi and bacteria can produce myrosinase-like to hydrolyze glucosinolate. Production of myrosinase from GIT microflora (endogenous myrosinase) may affect nutritive value of glucosinolate containing feeds (Kiebooms et al., 2012 a,b).

2.1.4.1 Molecular structure of myrosinase

The structure of myrosinase molecules consists of glycopeptides (such as thiol groups), disulphids and salt bridges and a zinc atom between subunits (Rask et al., 2000; Kumar et al., 2011) (Figure 8). It can be connected to other proteins and form a high molecular weight compounds (Rask et al., 2000).

Myrosinase has more activity in seeds and seedlings (Bones, 1990). Development stages of mature rape plant almost 2 to 5 % of cells are producing and storing myrosinase (Andréasson and Jørgensen, 2003).

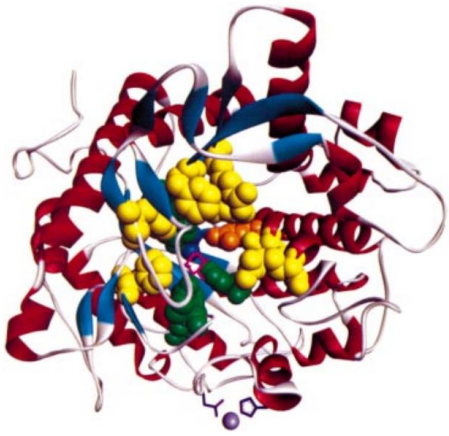

Figure 8: Schematic of myrosinase subunit based on the crystal structure. The Zn^{+2} ion is shown in purple (Rask et al., 2000)

2.1.4.2 Myrosinase properties

Myrosinase is very pH and temperature sensitive. The heat stability and optimal pH for maximum activity of myrosinase differs between different sources. The pH for optimal enzyme activity in

mustard and rapeseed is reported between 4.5 and 4.9 (Ludikhuyze et al., 2000) and optimal temperature is 60°C (Yen and Wei, 1993).

Pressure may affect the activation energy of myrosinase. The enzyme is more stable in the pressure below 200 MPa and by increasing temperature and pressure; enzymes stability may decrease (van Eylen et al., 2007).

2.1.4.3 Endogenous myrosinase

The endogenous enzymes may hydrolyze the glucosinolate content of diet and affect the nutritive value. The main products of endogenous myrosinases are 5-vinyl-1,3-oxazolidine-2-thione (5-VOT) and the thiocyanate ions. These compounds may affect the thyroid glands function and cause metabolic problems (Mawson et al., 1994; Gutzweiler, 1996, Kiebooms et al., 2012a).

Enzymes with myrosinase-like activity have been detected in different species of fungus such as fungi *Aspergillus sydowi* and *Aspergillus niger* (Ohtsuru et al., 1973, Rakariyatham et al., 2006). Also intestinal bacteria such as *Enterobacter cloacae* and *Faracolobactrum aerogenoides* have shown hydrolysis activity on glucosinolates (Tani et al., 1974; Oginsky et al., 1965; Aires et al., 2009).

2.1.4.4 Myrosinase inhibitors

Enzyme inhibitors are molecules which reduce or inhibit enzymes activity by binding the active site or non-catalytic site of enzyme. Non-covalent myrosinase inhibitors such as acarbose and nojirimycin (Li et al., 2005; Kim et al., 1999) may reduce or inhibit the function of myrosinase by affecting the active site. On the other hand some glycosides such as isothiocyanate, epoxides and α-halocarbonyls have reactivation function on the enzyme (Marshall et al., 1981).

The most effective compound which may inhibits myrosinase activity is 2-fluoro-2-deoxy-glucotropaeolin which makes a covalent glucosyl-enzyme intermediate and deactivates the active site of enzyme (Lefoix et al., 2002; Cerniauskaite et al., 2009).

2.1.4.5 Effects of ascorbic acid and ions on myrosinase activity

It has been shown that ascorbic acid is able to modulate the function of myrosinase. Accumulation of ascorbic acid in high density may inhibit myrosinase function. It can compete with substrate and connect to the enzyme irreversibly (Andersson et al., 2009). The effect of ascorbic acid on

degradation of different glucosinolates is varying. It may increase the degradation of sinigrin decrease the speed of indole glucosinolates hydrolysis such as glucobrassicin (indol-3-ylmethyl glucosinolate) and neoglucobrassicin (1-methoxyindol-3-ylmethyl glucosinolate) (Tsuruo and Hata, 1968).

Metal ions may affect the hydrolysis products. For example, presence of ferrous ions may increase nitriles production (Kong et al., 2012). It has been reported that Sn^{+2}, Sr^{+2} and Ba^{+2} have been strongly activated cauliflower seedling myrosinases while Fe^{+3}, Fe^{+2}, Zn^{+2} and Cu^{+2} have been deactivated myrosinases or reduced enzyme's activity (Prakash and Gupta, 2012). Also ferrous ions may affect epithiospecifier proteins (ESP) activity (Williams et al., 2010).

2.1.4.6 Epithiospecifier proteins

Epithiospecifier proteins are small proteins which can regulate myrosinases function by attaching at the non-catalytic site of enzyme. Increasing epithionitriles production, decreasing isothiocyanates formation and regulate the nitrile formation are some of the functions of ESP. Ions, temperature and pH may affect the function of ESP (Williams et al., 2010). Presence of ESP results in rearrangement of double bond of isothiocyanate through myrosinase and production of epithionitrile (Rodman, 1981). Since ESP are heat labile, short term heat treatment may affects their function (Mathusheki et al., 2006). Epithiospecifier proteins are located at different cells from myrosinase containing cells and at the same cells with glucosinolates (Koroleva et al., 2000; Kissen et al., 2009) (Figure 5).

2.1.5 Metabolic effects of dietary glucosinolates derivatives

Intact glucosinolates don't have negative effect on animal performance; however, glucosinolates hydrolysis products may cause metabolic problems in animal fed by diet containing these compounds. Goitrogenecity, mutagenecity, hepatotoxicity and nephrotoxicity of glucosinolates hydrolysis products have been reported by many investigations (Burel et al., 2000a; Tripathi et al., 2001b; Wallig et al., 2002; Tanii et al., 2004). The level of toxicity depends on the type and accumulation of glucosinolates derivatives (Wittstock and Halkier, 2002).

Glucosinolates hydrolysis products may affect palatability of feed. Bitter taste of sinigrin and progoitrin reduce FI and causes weak growth performance and production in animal fed by glucosinolate containing feed (Traka et al., 2009). Hydrolysis of progoitrin through myrosinase or heat treatment increases the bitterness of derivatives more than sinigrin (van Doorn et al., 1998). The effect of gluconapin on FI depends on its quantity in feed. It may reduce growth performance by decreasing FI (Tripathi et al., 2001a,b).

Goitrogenecity of glucosinolate derivatives is mainly due to production of thiouracil which may decrease production of thyroxin (T4) and triiodothyronine (T3) (Courtheyn et al., 2002). Thiocyanates, thiourea and oxazolidithione by reducing iodine availability for thyroid may affect its function and cause hyperthyroidism (Wallig et al., 2002) (Figure 9).

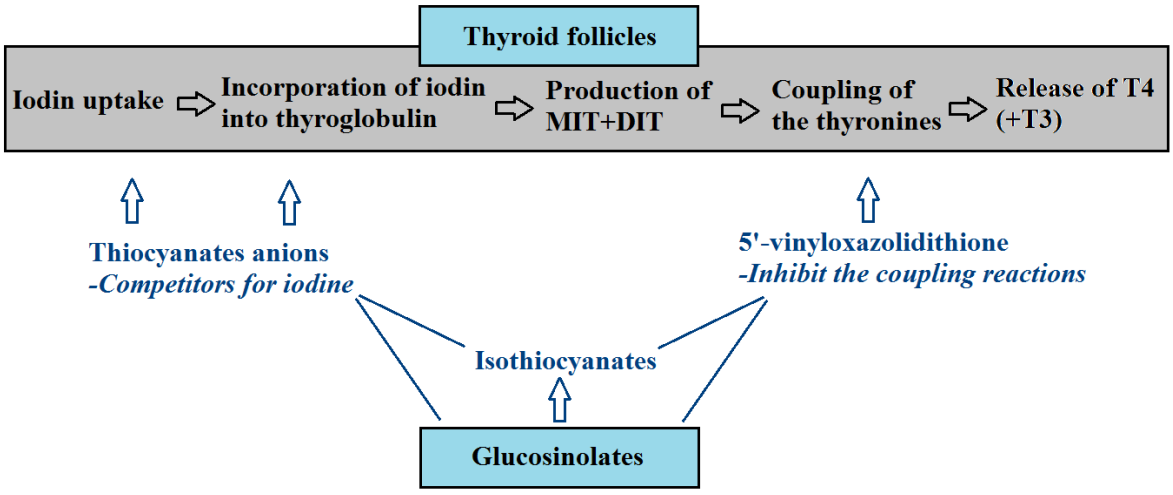

Figure 9: Mechanism of glucosinolates effect on thyroid (developed from Burel et al., 2001)

2.1.6 Effect of glucosinolate on fish metabolism

Tolerance to glucosinolates hydrolysis products differs between different fish species. Inclusion of toxic compounds beyond tolerance point of fish may cause metabolic problems. For example snapper (*Pagrus auratus*) can tolerate 2.2–21.8 mol glucosinolate per kg fish body weight per day but exceeding this level results in negative effects on growth or thyroid performance (Glencross et al., 2004; Burel et al., 2000a).

2.1.6.1 Thyroid responses

Feeding tilapia by glucosinolates containing diet may affect thyroid functions. It has been reported that in comparison with fish fed by glucosinolate free diet, the thyroid follicles had significant taller epithelial cells in fish fed by experimental diet the level of T3 and T4 in blood was significantly higher than fish fed with glucosinolate free diet (Gatlin et al., 2007; Zhou and Yue, 2010).

Growth and FI depression, increasing mortality and liver and kidney damages have been monitored in fish fed by glucosinolate containing diet (Van Etten and Tookey, 1983; Campbell and Schöne, 1998; Tan et al., 2013).

2.1.6.2 Immunological responses

Environmental and nutritional stresses on fish may cause physiological impairment which directly affects its immune system (Black and Pickering, 1998). An inappropriate protein source in diet may cause nutritional stress in tilapia (Watanabe, 2002). For example presence of ANF in main protein source of diet not only reduces growth rate but also affects immune system and reduces disease resistance in tilapia due to disruption of immune response (Vazzana et al., 2002).

There is always a normal level of pathogens in environment which healthy fish is resistance against them. By weakening immune system by environmental stresses pathogens may cause acute disease in fish population. It is very important to reduce stress in industrial units which have a high density of fish in tanks or pools (Bly et al., 1996).

Garcia and Villarroel (2009) have been demonstrated that feeding frequency may affect immune response in tilapia. However, no significant difference was reported between different protein sources. They have assessed the level of plasma cortisol as an index for stress measurement (Table 4). They have discussed that tilapia is resistance against environmental stresses. However their experiment was designed for a short duration and in longer duration bacteria may have enough time to inter into macrophages. Different factors such as the number of bacteria in the macrophages, the number of white blood cells and mortality may monitor the status of immune system of fish body.

Intensive large scale aquaculture demands preparing optimal condition. Disease may easily transmit between fish in high density. Increasing mortality and feed conversion ratio (FCR) in large scales causes a huge financial loss for producers. An optimal diet may keep the immune system on a proper condition, prevents poor growth performance, eventual diseases and high mortality.

Streptococcosis is one of the challenges in tilapia farming which manly causes by *Streptococcus*, *Lactococcus* and *Vagococcus* bacteria. The percent of infectivity and mortality for this disease is high. It causes many economic losses in many countries on several fish species especially in warm water aquaculture such as tilapia (Bowser et al., 1998; Ye et al., 2011; Chen et al., 2012). Immunization of fish through vaccination is used when the risk of disease is high. Passive immunization may be used in many cases in intensive large scale aquaculture.

Table 4: Weight gain, plasma cortisol and phagocytosis results of the four feed type and the two feeding frequencies (Garcia and Villarroel, 2009).

Feed type and feeding frequency	**Weight gain**	**Plasma cortisol (ng m l^{-1})**	**Phagocytosis[a]**		
			0 hpi	4 hpi	24 hpi
Soy-2	2 3.07	20.47	5.23	6.44	6.35
Soy-8	2.61	54.8	5.50	6.46	4.68
Sun-2	3.09	24.7	5.28	6.53	6.22
Sun-8	2.46	30.5	5.39	6.37	4.55
Pea-2	3.03	35.2	5.29	6.57	6.26
Pea-8	2.64	38.6	5.53	6.35	3.94
Glu-2	2.71	ND[b]	5.47	6.46	6.02
Glu-8	2.59	16.6	5.42	6.46	4.49

[a] Phagocytosis: log n bacteria recovered from macrophages at 0, 4 and 24 h postinfection (hpi). [b] ND: not determined

2.1.6.3 Vaccination and immunization

Vaccination is one of the solutions to reduce mortality. Different methods have been used to produce vaccines. Killed and modified live vaccines are popular in aquaculture and provide a long term immunization against disease (Garcia et al., 2008).

To prevent streptococcosis, several vaccines with different formulations have been developed. Eldar et al. (1997) reported that formalin-killed *Streptococcus iniae* vaccine has protective effects on tilapia (Pridgeon and Klesius, 2011).

Immunization of tilapia through toxoid-enriched bacterin has been resulted at different levels of protection on different fish size. Immunization of 25gr tilapia resulted in 95.3% survival rate and for 100gr tilapia the survival rate was reported between 84.2 to 94.7 % (Romalde et al., 1996). Evans et al. (2004) founded that different vaccine dosage may result in different survival rate in tilapia.

2.1.6.4 Hepatic responses

Utilization of rapeseed secondary products in fish feed may cause liver damages and changes in enzymes status due to presence of ANF (Vilhelmsson et al., 2004). However few investigations have been done to assess hepatic damages in tilapia.

Since liver has important functions on nitrogen metabolism it is crucial to investigate several pathways related to protein metabolism which are taking place in liver tissue. Several genes, enzymes and metabolic pathways in liver may affect through ANF existing in rapeseed secondary products (Vilhelmsson et al., 2004).

Lin et al. (2010) found that inclusion of 50% RSM in diet reduces growth performance significantly in tilapia fed in comparison with fish fed with diet containing SBM or cotton seed meal. They also report that RSM causes hepatic damages and changes hepatic factors status. The study showed that hepatopancreas, glutamate-oxaloacetate transaminase, glutamate-pyruvate transaminase and superoxide dismutase in liver have been reduced due to negative effect of RSM's ANF.

2.1.7 Molecular analysis

Nowadays, it is possible to observe the function of ANF on several organs in fish body on the molecular level. Differences between macromolecules such as proteins, nucleic acids and metabolites are possible to assess through several analytical methods such as proteomic and genomic assessment.

Proteomic analysis results in a quantitative description of protein expression (such as enzymes, receptor, or membrane channels). It is possible to detect changes in protein expression after affecting by ANF or environmental stresses (Figure 10). Proteomic analysis provides this opportunity to assess differences in gene expression after affecting by diseases and environmental stresses (Karim et al., 2011).

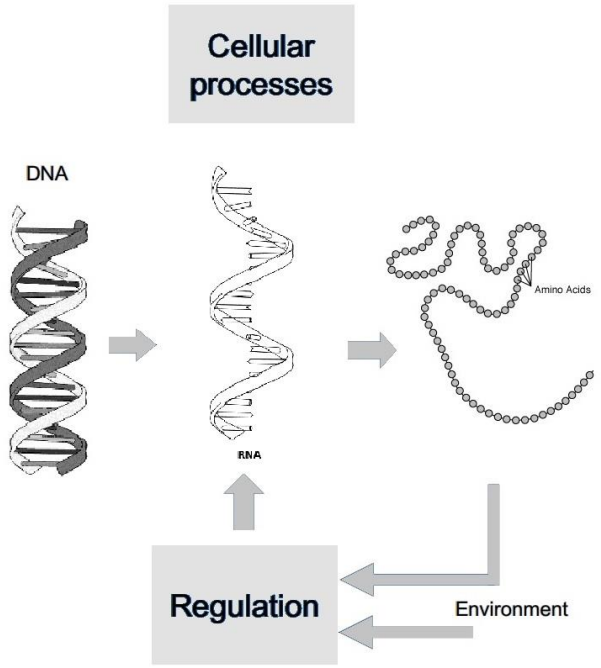

Figure 10: Schematic of cellular regulation on protein synthesis

2.1.7.1 Proteomic analysis vs. genomic analysis:

Genomic and transcriptomics technologies are good instruments to investigate gene expression. Investigation of cellular regulation of mRNA expression from DNA may give valuable data from metabolic status of fish. However, mRNA abundance doesn't translate directly to the protein. Also it degrades and disappears in cell in a short time and results from genomic analysis may affect by degradation of mRNA. Measurement of protein abundance is directly related to its function (Anderson and Anderson, 1998; Pradet-Balade et al., 2001a,b). A proteomic approach which investigates the effect of ANF on fish performance may give a more clear view from metabolic status and changes than genomic.

Metabolomic approach is also another way to assess the entire metabolisms in organisms. The expression of genes and the function of proteins and the interaction between them result in different metabolites. Metabolomic analysis gives opportunity to determine the sum of all metabolites (other substances than DNA, RNA or protein) in a biological system: organism, organ, tissue or cell (Müller and Kersten, 2003) (Figure 11). Studying the effect of toxic compounds such as glucosinolates on several metabolites especially in target tissues like liver and thyroid may helps better understanding of function of anti-nutrients in nutritional science.

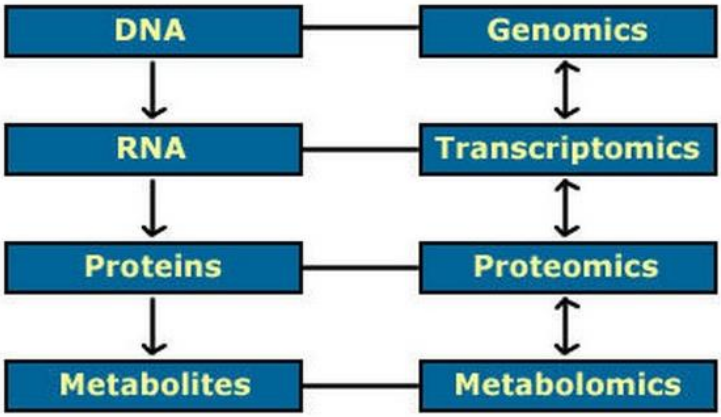

Figure 11: Genes, proteins, and molecular machines (Isaaaa, 2014)

It has been reported that majority of glucosinolate are responsible for metabolic problems (Ahlin et al., 1993). Several processing methods have been tested to reduce content of glucosinolates and their derivatives in rapeseed secondary products. A proper processing may decrease content of toxic compounds and improve health and growth performance of fish without negative effect on protein and other nutrients bioavailability (Seneviratne et al., 2011; Plaipetch and Yakupitiyage, 2013).

2.2 Different varieties of rapeseed

Conventional plant breeding achieves significant successes in manipulating chemical composition of rapeseed. The toxic compounds have been reduced by the introduction of genetically modified organisms (GMO).

Several varieties of rapeseed are available which according to the content of unwanted compounds are destined for different use (edible or non-food). The variety which is called “zero” contains lower erucic acid in comparison with traditional varieties. In “double-zero” variety the content of both erucic acid and glucosinolates are reduced (Thompson, 1983). The Canadian variety which is called “Canola” is the most common variety to use in the world and contains lower amount of erucic acid (2%) and glucosinolates than traditional varieties (Augustine et al., 2013). The content of glucosinolate, erucic acid and fibers is reduced in variety which is called “triple-low”, “triple-zero” or “Candle”. Lower fiber is due to presence of thinner hull which gives yellow color to seeds in this variety. However, the content of unwanted compounds still causes growth and FI depression and metabolic problems in monogastric animals (Burel et al., 2001).

2.3 Effect of processing on ANF

It is possible to overcome the limitation for applying rapeseed secondary products as a protein source in fish diet by reducing the content of ANF through processing. Several processing techniques such as steam stripping, solvent extraction (Das and Singhal, 2005), toasting (Newkirk and Classen, 2002), yeast fermentation (Plaipetch and Yakupitiyage, 2013), alkaline treatment ($NaHCO_3$ and NH_4HCO_3) (Barrett, et al., 1998) and acid treatments (HCl) (Tripathy et al., 2001b) may reduce or eliminate ANF from rapeseed and decrease the level of toxicity.

There are some disadvantages for many of investigated methods such as reducing amino acids bioavailability and hygienic issues (Plaipetch and Yakupitiyage, 2013). So it is very crucial to define a proper and feasible method of processing to improve nutrition value of rapeseed secondary products. Some of the chemical and physical treatments which have been applied to overcome toxicity of unwanted compounds are discussed below:

2.3.1 Protein concentration

Fractionating and concentrating protein from rapeseed secondary products improves the nutritional value and reduces unwanted compounds. It has been demonstrated that canola protein isolate is more digestible than fish meal protein (Slawski et al., 2013).

Disadvantage of this method is high costs of processing and expensive products.

2.3.2 Water and metal ions treatment

It has been demonstrated that soaking RSM in copper sulphate solution may reduce toxicity of glucosinolate derivatives especially by affecting isothiocyanates production (Das and Singhal, 2005). It may leads hydrolysis reaction toward production of non-toxic and volatile compounds. The other hypothesis for this function is rearrangement of structure of toxic compounds into allylamine or thiourea. Growth and thyroid function are improved in monogastric animals (broilers and pig) fed by copper sulphate treated RSM in comparison with feed containing untreated RSM (Rouzaud et al., 2003; Das and Singhal, 2005).

Disadvantage of this method is related to removing added water and hygienic issues during processing.

2.3.3 Microwaving

Preconditioning rapeseed through microwaving at 2450-MHz for 2.5 min reduced glucosinolates hydrolysis due to deactivation of myrosinase (Aumaitre et al., 1989). This method reduced growth depression and goitrogenic effects on mice and pig fed by rapeseed secondary products (Tu et al., 2012).

Disadvantages of this method is that the glucosinolate remains intact may be hydrolyzed by endogenous myrosinase and in monogastric animals with longer intestine, toxins may be absorbed and cause metabolic problems (Rouzaud et al., 2003). Also glucosinolates in secondary products of rapeseed are hydrolyzed during oil processing and myrosinase deactivation may not help to reduce toxicity.

2.3.4 Fermentation

There are two different methods of fermentation for rapeseed secondary products: Wet fermentation and solid state fermentation.

Plaipetch and Yakupitiyage (2013) demonstrated that utilization of yeast fermented RSM in tilapia feed doesn't have any negative effect on thyroid and growth performance. However in previous studies it has been reported that fermentation of RSM through *S. cerevisiae* cannot eliminate glucosinolate since yeast doesn't produce myrosinase (Chen and Halkier, 1999). Another investigation it demonstrated that yeast fermented canola contains soluble toxic compounds from glucosinolate hydrolysis which may affect thyroid function and growth performance (McCully et al., 2008).

In wet fermentations such as yeast fermentation, hygienic issues, removing added water and drying the products after fermentation is still a problem for applying this method in commercial production (Plaipetch and Yakupitiyage, 2013).

Solid state fermentation is processing with microorganism without presence of free liquid. Different microorganisms such as *Rhizopus oligosporus* and *Aspergillus sp* have been used to detoxify rapeseeds ANF. These microorganisms may utilize toxic compound as a source of energy and convert them to the non-toxic compounds (Rakariyatham and Sakorn, 2002). However, production of unknown toxic compounds through aerobic fermentation may reduce nutritive value of rapeseed secondary products.

2.3.5 Heat treatment

Non-enzymatic degradation of glucosinolates hydrolysis products or deactivation of myrosinase by heating may improve nutritive quality of rapeseed secondary products. Different heat treatment methods such as toasting and cooking have been resulted in lower glucosinolates toxic derivatives in comparison with non-heated material (Leming et al., 2004). Conditioning factors such as time and moisture may affect the results of treatment. Glencross et al. (2004) demonstrated that utilization of toasted RSM improves growth performance in fish (*Pagrus auratus*). Toasting the RSM in 105 to 110^{o}C may reduce content of glucosinolates up to 40% (Figure 12).

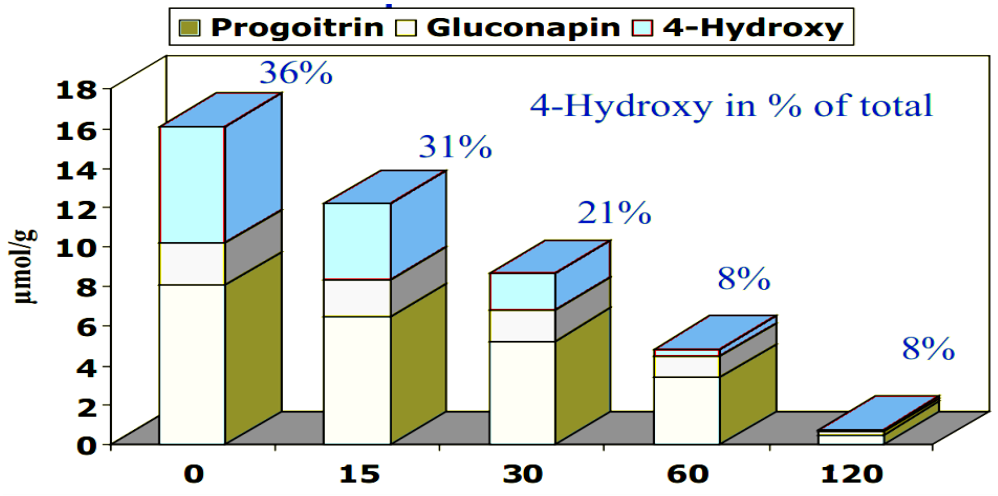

Figure 12: Effect of toasting (107^{o}C, min) on glucosinolate content of RSM (Holst-Jensen et al., 2009)

However, heating has some disadvantages on protein content of rapeseed secondary products. Heat treatment reduces lysine bioavailability through Maillard reaction and decreases protein solubility (Figure 13). It has been demonstrated that content of glucosinolates affects reduction of lysine bioavailability by heat treatment. Optimization of processing factors such as moisture and time may reduce negative effects of heat treatment on protein value (Holst-Jensen et al., 2009).

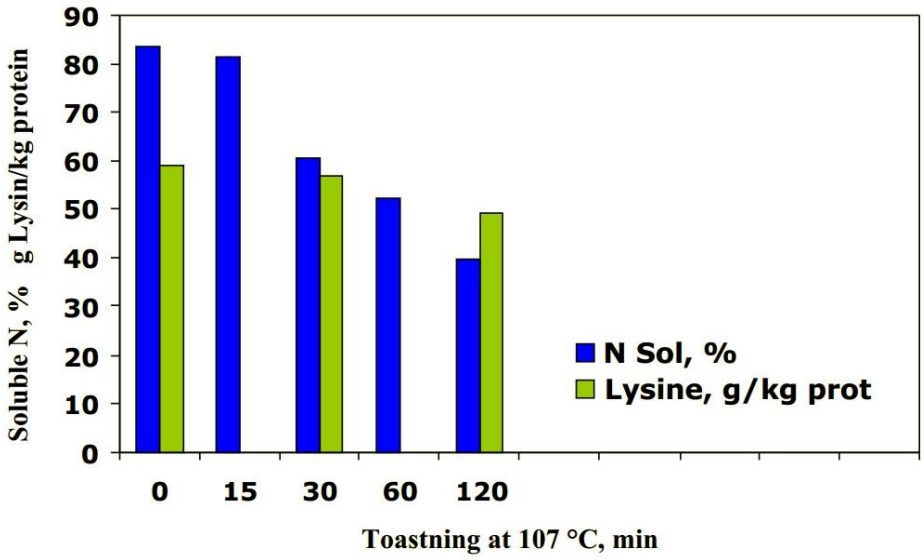

Figure 13: effect of heat treatment on protein solubility (Holst-Jensen et al., 2009)

Schöne et al. (2001) have demonstrated that moist-heat treatment affects glucosinolate content of rapeseed and RSC. They have report that heat treatment may improve nutritional quality of RSC by decreasing glucosinolate content of RSC (Table 5).

The pressure and moisture level may affect result from moist-heat treatment. Different methods have been used for heat treatment by application of moisture and/or pressure. Application of steam is a common method to add moisture and temperature to the feed ingredients.

2.3.6 Extrusion

Extrusion processing is a combination of applying high temperature (120-130^{o}C), high pressure (20-30 bar) and high content of moisture (25-30%) in a short time (Barrows et al., 2007). Extrusion is widely applied in fish feed production intensively. It applies heat, pressure and moisture, and can improve nutritive quality of some of the feed ingredients (Burel et al., 2000b). Typically, the ANF content of plant protein sources may decreases during extrusion processing. However, extrusion may affect chemical composition and bioavailability of amino acids negatively. By optimization of extruder parameters such as retention time and screw configuration it may be possible to reduce negative effects and improve nutrient quality of plant protein sources (Zarkadas and Wiseman 2005; Romarheim et al., 2005).

Table 5: Effect of moist-heat treatment on glucosinolate content of rapeseed and RSC (Schöne et al., 1997)

Experiment	**1-RS[a] untreated**	**2- RPC[b] untreated**	**3- RS[a] soaked[c] and dried**	**4- RPC[b] soaked[c] and dried**
Gluconapin	4.0	3.1	0.4	ND
Glucobrassicanapin	1.3	0.6	0.1	ND
Progoitrin	10.8	7.3	1.2	0.1
Pronapoleiferin	0.2	0.2	ND	ND
4-Hydroxy-glucobrassicin	2.9	4.7	0.2	0.2
Glucobrassicin	0.1	0.3	ND	ND
Neoglucobrassicin	0.1	0.1	ND	ND
Others [d]	0.5	2.2	0.2	ND
Total	19.9	18.5	2.1	0.3

ND, not detectable (<0.1 mmol/kg DM).[a] Winter cultivar 'Madora' (one batch).[b] Winter cultivar 'Falcon' (one batch).[c] Crushed RS or RPC (1 kg) was soaked with 1 litre water in a feed mixer and the mash dried to constant weight at 60°C. [d] Glucoraphanin, glucoallysin, gluconasturtiin and undetectable glucosinolates

2.4 Utilization of rapeseed secondary products in fish feed

Global production of RSM and cake has the second rank behind SBM. On the base of Crude protein (CP), RSM and RSC have lower price than soybean. In comparison with soybean, the amino acid profile in rapeseed has higher value (Sarwar et al., 1984) and feasibility of rapeseed secondary products utilization in fish feed as a source of protein has been proved in previous studies (Plaipetch and Yakupitiyage, 2013).

The harmful level of inclusion of glucosinolate differs between species. Juvenile rainbow trout are more sensitive than Chinook salmon to presence of glucosinolate in diet. The total glucosinolate level of 158µg/g or more have deleterious effects on salmonids performance (Hilton and Slinger, 1986). However, tilapia is more resistant to high content of glucosinolate in diet in comparison with salmonids since it is an omnivorous fish and more adapted to plants ANF (Hardy, 2010).

2.5 Tilapia

Tilapia (*Oreochromis spp*) is a common name for wide groups of fresh water fish belonging to cichlid fish species from the family Cichlidae. Some species have been commercialized and cultured

intensively in lakes, ponds and tanks. One of the common commercial species is Nile tilapia (*Oreochromis niloticus*) which is important in food production sectors in almost 100 countries (Lim and Webster, 2006). Annual production of Nile tilapia is rapidly increasing. Production of tilapia between years 2005 - 2009 increases from 1.82 to 2.79 million tons. China is the top producer of tilapia in the world (FAO, 2010). Figures 14 and 15 represent a global status of tilapia production in recent years.

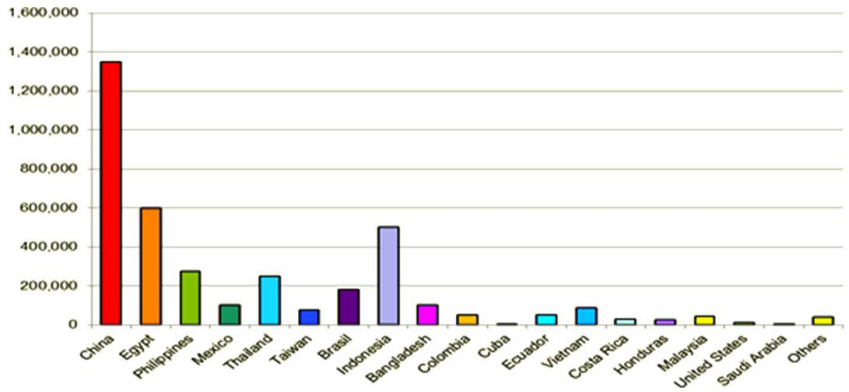

Figure 14: Global aquaculture production of tilapia (Fitzsimmons et al., 2011)

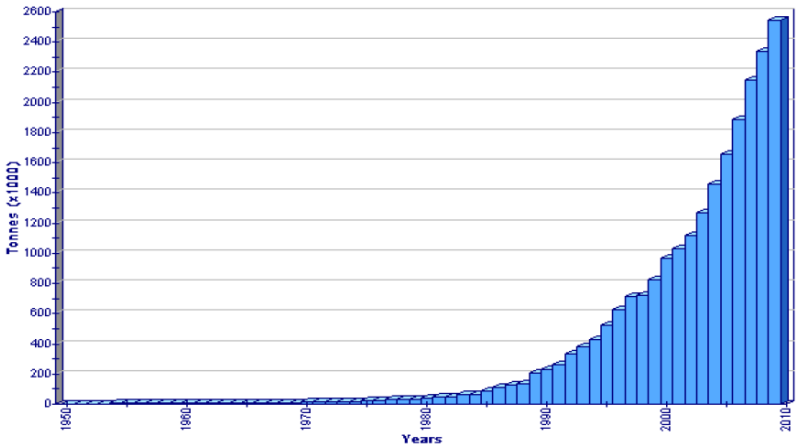

Figure 15: Global production of Nile tilapia (FAO, 2012)

2.5.1 Advantages of tilapia farming

Tilapia has unique features which make it a good option to produce in many countries especially in developing countries (Table 6). It is an omnivorous fish which can utilize wide range of low quality and cost-effective raw materials such as industrial by-products efficiently. It has high growth rate and

short generation interval. Tilapia is well resistance to disease and environmental stresses such as temperature changes, water quality and level of dissolved oxygen. Tilapia is able to take commercial feed immediately after yolk-sac absorption. In natural environments, it does not need too much supplementary feed. It is also good candidate for culturing at variety of production systems either and geographic regions. It has good marketing opportunity all over the world (El-Sayed, 2006).

Table 6: The comparison of major farmed fish (Fitzsimmons, 2011)

Species	**Geography**	**Consumer**	**Fish meal**	**Systems**	**Fresh water or marine**
Salmon	Regional	Global	Moderate	Cage	Requires both
Carp	Global	Regional	Minimal	Pond and cage	Fresh water
Catfish	Global	Global	Minimal	Pond and cage	Fresh water
Tunas	Regional	Global	High	Cage	Marine
Sea bass, cobia, snapper	Global	Global	High	Recirculation systems, cage	Marine
Tilapia	**Global**	**Global**	**Minimal**	**Cages, ponds, raceways, recirculation systems**	**Either**

2.5.2 Optimal feed requirements of tilapia

Commercial pellets are produced at different sizes from powdered feed for fry up to bigger pellets for larger fish. The optimal size of feed for each stage of tilapia life has been shown in Table 7 (Riche and Garling, 2003).

Optimum feeding rate depends on fish body weight and the optimum feeding interval depends on feed composition and energy content of feed. Fish size, age and environmental conditions such as culture method and water temperature may affect these parameters (Riche and Garling, 2003).

Table 7: Suggested standard pellet sizes used for feeding tilapia from hatching to market size (Riche and Donald, 2003)

Size of fish (grams)	Optimal feed size
0-3	"00" or "0" (powder)
3-10	1 mm
10-25	2 mm
50-40	3 mm
40-100	3/32 Inch
Larger than 100	1/8 Inch

Tilapia is able to use plant protein intensively. Feasibility of utilization of protein sources such as soybean secondary products (Yi and Hualin, 2011), lupins (Abdel-Moneim and Yones, 2010), maize gluten meal, and cottonseed meal (Rinchard et al., 2002) have been demonstrated in previous studies.

Optimal protein requirement of Nile tilapia is estimated from 32 to 50% and for larger tilapia 25 to 30% (El-Saidy et al., 2005; Nguyen, et al., 2009; Abdel-Tawwab et al., 2010).

Dietary lipid requirements also have been estimated from 5 to 12%. The optimal level of lipid in feed is 6% in dry matter (DM) (Lim et al., 2011).

Tilapia can utilize starch efficiently from 22 to 46% dietary starch while 22% is considered as optimum level for juvenile tilapia (Wang et al., 2005). However a recent study at NMBU-IHA (Storebakken, personal communication) on tilapia suggested that optimal ratio of protein to starch in tilapia feed is 1:1 and the best level of inclusion of nutritive are 30% protein, 30% starch and 6% lipid.

The sinking rate which is regulating by steam added to barrel in extruder during feed processing is an important factor. Feeding behavior of tilapia shows that the utilization of sinking pellet is more efficient than floating pellet. However, features and feeding behaviors may differ between tilapia species (Tidwell et al., 2010).

3. Aims and response criteria

Two experiments were conducted to investigate the nutritional value of RSC in comparison with SBM in diets for Nile tilapia.

The aim of the first experiment was to define a level of dietary RSC that ensured that the fish were sensitive to changes in nutritional quality of RSC. The aim of the second experiment was to find out if

fine milling or a combination of fine milling and autoclaving affected the nutritional value of the RSC when used in feed for tilapias at the sensitive level of replacement defined in experiment 1 (Exp.1).

The key response criteria chosen to assess sensitivity to RSC as a source of dietary protein were:

FI to assess the acceptability of RSC as a feed ingredient for Nile tilapia, and to find out if different doses or treatments of the RSC gave different feed intake patterns.

Growth and body composition, apparent protein digestibility, net utilization of dietary DM, nitrogen and energy for growth to assess the nutritional value of RSC as a source of dietary protein and energy.

- Thyroid function (Plasma T4 levels) to indicate if myrosinase-induced metabolites of glucosinolates represented a physiological challenge to Nile tilapia.

- Uptake and utilization of cationic mineral elements to evaluate if the high concentration of phytic acid in the RSC limited mineralization of the fish.

In addition to the research presented in this thesis, samples have been taken to investigate:

- If replacing SBM with RSC affects the responsiveness to vaccination against *Streptococcus spp.* In Nile tilapia. This work is done in collaboration with Professor Øystein Evensen at NMBU, and is planned to be published jointly with his group.
- If the replacing SBM with RSC affects to composition of stable isotopes of carbon and nitrogen in muscle (slow turnover tissue) and liver (fast turnover tissue) in Nile tilapia, and if this commonly used ecological method to assess sources of FI in ecological studies is useful in controlled nutritional studies. This work is done in collaboration with the ecology group at University of Juväskulä in Finland, and is planned to be published jointly with them.

4. Materials and methods

4.1 Experiment 1: Definition of intolerance level to RSC) in juvenile tilapia

4.1.1 Diets composition and formulation

Five experimental diets were produced in order to replace CP from SBM with CP from RSC. CP from RSC and/or SBM accounted for 53% of CP in the diet (Table 8). The levels of replacements were 0, 25, 50, 75, and 100%. The calculated composition of the diets, and ratios between nutrients and energy is presented in Table 8. The diets were composed according to Table 9.

Table 8. Calculated chemical composition, and distribution of gross energy (GE) from energy-bearing nutrients, and added process water

Feed code	**0%**	**25%**	**50%**	**75%**	**100%**
Planned composition, kg DM^{-1}					
Dry matter (DM), g	883	884	885	886	888
Crude protein (CP), g	319	319	319	319	319
Fat, g	70	70	70	70	70
Starch, g	313	313	313	313	313
Ratio CP from (SBM+RSC)/Total, %	53	53	53	53	53
Ratio CP from (RSC)/ (SBM+RSC), %	0	0.25	0.50	0.75	1.00
In feed[1]					
GE from protein, kJ g^{-1}	5.42	5.42	5.41	5.42	5.42
GE from starch, kJ g^{-1}	5.32	5.32	5.33	5.32	5.32
GE from lipid, kJ g^{-1}	2.60	2.58	2.58	2.60	2.61
GE from nutrients, kJ g^{-1}	13.34	13.32	13.33	13339	13.34
CP/GE g/kJ	**24**	**24**	**24**	**24**	**24**
Added water during processing, g kg^{-1}	300	350	400	450	500

[1]Assuming 17 kJ g^{-1} CP or starch and 37 kJ g^{-1} lipid.

The diet formulation is presented in Table 9. Main ingredients used in this experiment were SBM, RSC, corn gluten meal and pre-gelatinized potato starch. The SBM was diluted by cellulose to achieve the same level of protein as found in the RSC, in order to simplify formulation of the diets. Rapeseed oil was the main source of dietary lipid. All diets were supplemented with minerals and

vitamins to cover the requirements (NRC, 1993), and essential amino acids to match the whole body amino acid profile of Nile tilapia (Gao, 2011). Sodium alginate was used as a binder and yttrium oxide as inert marker for digestibility measurement.

Table 9. Formulation of the experimental diets

Feed code	**0%**	**25%**	**50%**	**75%**	**100%**
Ingredient, g kg^{-1}					
SBM[a]	334	251	167	84	0
RSC[b]	0	116	230	345	460
Corn gluten meal[c]	207	208	209	210	210
Gelatinized potato starch[d]	286	284	283	281	280
Cellulose[e]	73	55	37	18	0
Rapeseed oil[f]	49.0	36.0	24.0	12.0	0.0
Threonine[g]	0.7	0.3	0.0	0.0	0.0
Methionine[h]	4.7	4.4	4.1	3.7	3.4
Phenylalanine[i]	0.4	0.7	1.0	1.2	1.5
Taurine[j]	1.5	1.5	1.5	1.5	1.5
Lysine[k]	3.3	3.4	3.5	3.5	3.5
Tryptophane[l]	0.0	0.1	0.2	0.2	0.3
Mono calcium phosphate[m]	10.0	10.0	10.0	10.0	10.0
Premix[n]	10.0	10.0	10.0	10.0	10.0
Yttrium oxide	0.1	0.1	0.1	0.1	0.1
Vit-C 35%[p]	0.1	0.1	0.1	0.1	0.1
Sodium alginate[q]	20.0	20.0	20.0	20.0	20.0
Total	1000	1000	1000	1000	1000

[a]Soybean meal, Denosoy, Denofa, Fredrikstad, Norway. [b]Rapeseed cake (Expeller-pressed presscake from "double low" winter rapeseed, Mestilla Rapeexpeller "00", UAB Sekargas IR Co., Klaipeda, Lithuania, supplied by Felleskjøpet Rogaland & Agder, Stavanger, Norway; origin of material not further specified by the supplier). [c]Maize gluten, Cargill 13864. [d]Gelatinized potato starch, Culinar, LYGel F60. [e]Alpha-Cel™ C100, International Fibre Europe NV, Belgium. [f]Food grade Eldorado, Oslo, Norway. [h]Adisseo Brasil Nutricao Animal Ltda, Sao Paulo, Brazil. [j]Taurine-JP8, Qianjiang Yongan Pharmaceutical Co., Ltd., Hubei, China. [n]Contents per kg: Vitamin A 2500.0 IU; Vitamin D_3 2400.0 IU; Vitamin E 0.2 IU; Vitamin K_3 40.0 mg; Thiamine 15.0 mg; Riboflavin 25.0 mg; d-Ca-Pantothenate 40.0 mg; Niacin 150.0 mg; Biotin 3.0 mg; Cyanocobalamine 20.0 g; Folic acid 5.0 mg; Pyridoxine 15.0 mg; Vitamin C: 0.098 g (Stay-C 35, ascorbic acid phosphate, DSM Nutritional Products, Basel, Switzerland); Cu: 12.0 mg; Zn: 90.0 mg; Mn: 35.0 mg; I: 2.0 mg; Se: 0.2 mg; Cd = 3.0 g; Pb = 28.0 g; total Ca: 0.915 g; total K 1.38 g; total Na 0.001 g; total Cl 1.252 g; Trouw Nutrition, LA Putten, The Netherlands. [o]Metal Rare Earth Limited, Jiaxing, China. [p]Stay-C 35, ascorbic acid phosphate, DSM Nutritional Products, Basel, Switzerland.

4.1.2 Diet production

SBM, RSC and corn gluten meal were ground by a laboratory mill (Model ZM 100, Retsch Technology GmbH., Haan, Germany) with 1mm screen size. All ingredients were mixed in a spiral dough mixer (Moretti Forni Grain, Italy) for 15 min before processing. Water (heated to 80^{o}C) and rapeseed oil were added to the ingredients during mixing.

Additional process water increased along with increasing RSC inclusion in diet. In diet with 0% replacement, the additional water was 30% of the weight of dry mix of ingredient. By each 25% replacement of CP from SBM by RSC, 5% more water was added to dry mix of ingredients to achieve proper lubrication during processing (Table 8).

All diets were mixed and passed 3 times through a pasta extruder (P55DV, Italgy, Carasco, Italy) at NMBU. The temperature of the die was 45^{o}C. During the two first passings (conditioning), a 4-mm die was used. During the last passing, diets were shaped during 2.5 mm and 4 mm dies. The knife speed was adjusted to produce short (3 mm) and long (4 mm) pellets shaped through the 4-mm dies, in order to have optimal pellet size as the tilapias grew larger. Pellets were dried at 54^{o}C until the moisture content was approximately 10%.

A 100 g representative sample from each diet was taken for chemical composition analysis.

4.1.3 Fish keeping facilities

The experiment was conceded at the fish laboratory of NMBU, between August 2013 and January 2014. The Nile tilapias were hatched at the fish laboratory and fed on a commercial diet (Aller Aqua, Denmark) until the individual body weight was approximately 20 g. The broodstock was GIFT tilapia (Eknath et al., 1993) originating from the 12th generation of selection for rapid growth by Genomar AS (Oslo, Norway). The experiment lasted for 6 weeks.

Fish were distributed randomly to 10 tanks (size: 70×50×50 cm) with 20 fish per tank and approximately equal biomass for all tanks (398±2g (mean±s.e.m.), with a mean fish weight of 19.9 g).

The average of water temperature during experiment was 27^{o}C. Water flow for all tanks kept approximately equal and the average was 150 l min^{-1}. Dissolved oxygen was approximately 7.5 mg^{-1} which was measured daily online by oxygenmeter (Oxyguard Commander, DO probe, Farum,

Denmark) and ammonia which was 0.7 mg l^{-1} (Merck 114752, Spectroquant NOVA 60) once per each 3 weeks.

4.1.4 Feeding and FI assessment

Recovery of dietary DM in feed collected after passing through the system was determined as described by Helland et al. (1996) for each diet and each tank, receiving the same feeds as being used in the experiment.

Fish fed each of the five diets in two replicate tanks. An electrically driven band feeder was allocated for each tank. Fish were fed 3 times per day at 9 am, 1 am and 5 pm, each meal lasting 40 min. The fish were fed approximately 30% in excess of appetite. The 2.5 mm pellets were fed during the 2 first weeks, 4 mm short pellets were fed during the next 3 weeks, and the 4 mm long pellets were applied during the last week.

The cylindrical tanks with conically shaped bottoms were constructed to allow FI estimation according to Helland et al. (1996). Uneaten feed was collected by strainers under the water outlet during and for 15 min after each feeding. Then, the daily dietary DM intake was assessed by drying the daily collection of uneaten feed overnight at 105^{o}C and correcting for DM recovery.

4.1.5 Sampling and sample preparation

An anesthetic agent (MS-222, 0.1 g l^{-1} water, buffered with $NaHCO_3$, 0.1 g l^{-1} water, Western Chemical Inc. Washington USA) was used before weighing and sampling. Fish were weighed by tank at 0 and 6 weeks.

Faeces for digestibility assessment were collected on the last day of feeding, from the last 10 cm of the distal intestine by dissection from 5 fish per each tank. All collected faces were pooled by tank, stored at -20^{o}C and subsequently freeze-dried (Beta 1-6, LMC-2, Christ, Osterode, Germany) at -56^{o}C and 25 mBar for 96 hours. Freeze-dried faces were homogenized with a pestle and mortar.

Five fish were randomly taken from each tank and the individual body weight liver weights were measured. Sampling for liver weight was done simultaneously with faces collection, in fish that were not starved.

Blood sample was taken from caudal vein of 5 fed fish per tank, using heparinized syringes. The blood samples were centrifuged within 20 min after collection for 10 min at 3000*G to separate plasma. One m1 of plasma from each fish was pipetted into each of two pooled samples per tank and stored at -20^{o}C. One sample was used for analysis and one sample kept as backup.

Two samples, each containing three fish were taken randomly at the beginning of the experiment for whole body composition analysis. One sample of five fish was taken from each tank after 6 weeks of feeding. Fish were starved for 24 hours before each sampling. The samples were ground in a laboratory blender. Representative samples were taken and kept in freezer at -20^{o}C and subsequently freeze-dried. Freeze-dried samples were ground along with CO_2 ice in order to efficiently grind the tough skin of the tilapias along with the other body components.

4.2 Experiment 2: Fine milling and autoclaving of RSC

The experiment was planned and analyzed according to a model for one-way analysis of variance. On the base of results from Exp.1, 50% replacement level seemed sensitive to nutritive quality of the diet. So the diet formulation for Exp.2 was based on the diet formulation with equal contribution of CP from RSC and SBM (Table 9).

Three diets were produced in the second experiment, all with the same formulation (50%, Table 9), except for the treatment of the RSC. One diet utilized RSC ground through a 1 mm screen (D 1.0), the same as in Exp1. In the second diet (D 0.5) the RSC was ground through a 0.5 mm screen. For diet 3, the RSC was ground to 0.5mm and autoclaved for 10 min in 120^{o}C and 0.11 MPa (HiClave HV-50, HMC Europe, GmbH, Tüssling, Germany) (D 0.5+A). All facilities and the other material which were used in Exp.2 for diet production were the same as Exp.1. Samples from each diet were taken for nutrients analysis.

The 3 diets were each fed to 3 replicate groups of tilapia. Each tank contained 20 fish to similar weight and age. The average biomass in each tank was (747.5±7.5 g (mean±s.e.m)). The experiment lasted for 3 weeks. Feeding, FI assessment and sampling were the same as in Experiment 1.

4.2.1 Chemical analysis

All diet samples were ground with a pestle and mortar and analyzed for DM (105^{o}C), CP (Kjeldahl, 1883), lipid (HCl hydrolysis followed by diethyl ether extraction (Commission dir.98/64/EC)), starch (McCleary et al., 1994), ash (550^{o}C, overnight), yttrium and mineral elements (Ca, P, Mg, Mn, Zn) by

ICP and gross energy (Bomb calorimeter Parr 1281, Moline, Illinois, USA), Amino acids (except tryptophan) were analyzed according to EC (98/64). Tryptophan was analyzed according to EC (2000/45). In addition, the diets from Exp.2 were analyzed for free glucose content, by the same colorimetric reaction employed to quantify glucose in the starch analysis.

Freeze dried whole body samples were analyzed for DM, protein, ash, mineral elements (to be reported when the results are published) and gross energy, by the same methods as used for diets. Lipid analysis for samples from freeze-dried initial and final whole body was done without HCl hydrolysis. Freeze dried faeces were analyzed for nitrogen by Dumas method, yttrium and mineral elements (Ca, P, Mg, Mn, Zn) by ICP.

Blood plasma samples were analyzed for free thyroid hormone (T4) by RIA (dog assay, method developed from: Lindstedt et al., 1994; Hay et al., 1991), and plasma minerals (inorganic phosphate, Ca, Mg, Mn, Zn) by ICP.

Analyses on blood plasma were done by NMBU Central Laboratory in Oslo. ICP analyses on feeds, feces and whole bodies, and Dumas analysis were done by Eurofins, Moss, Norway. Dietary glucose and tryptophan were analyzed by Master Lab, Putten, The Netherlands. Bomb calorimetry and free glucose in the diets from Exp.2 were done by NMBU-IHA. The rest of the analyses were performed by Skretting ARC, Stavanger, Norway.

4.3 Calculations and statistical analyses

Daily feed intake (g DM) was calculated as: DFI =DM of daily feed (g) – (DM of daily uneaten feed (g)/recovery factor, RF), where the RF was defined as Dietary DM recovered from outlet water/Dietary DM supplied in a tank without fish.

Cumulative feed intake (CFI) was calculated as the sum of DFI over the feeding period.

Weight gain (WG) was calculated as: Final average fish weight (FW, g) – Initial average fish weight (IW, g).

Feed Conversion Ratio (FCR) for the whole feeding period was calculated as: FCR= CFI/WG.

Daily feed intake on Day i, in percent of estimated fish weight ($PDFI_n$) was calculated as:100* DFI_i / (FW_{i-1}+DFI_{i-1} / FCR).

Protein or energy retention (%) was calculated as: 100*(R in gain/R in CFI), where R in gain was calculated as ($IW*R_{initial}$) – ($FW*R_{final}$), with R denoting the concentration of energy (kJ) or CP (g) in whole fish body and R in CFI was the concentration in DM.

Apparent nitrogen or mineral digestibilities (AD, %) were calculated as: 100 – ((100*Yttrium in feed (mg kg^{-1}) / Yttrium in feces (mg kg^{-1}))*(R in feces (kg^{-1}) / R in feed (kg^{-1}))), where R is the concentration of a nitrogen or a mineral (g kg^{-1}).

Exp.1 was statistically analyzed by linear or 2nd degree polynomial regression analysis in MS EXCEL, depending on which model gave the highest R^2. Only results with $R^2 \geq 0.35$ were presented as graphs. If the R^2 values were <0.35, the results were presented as means±s.e.m. of all observations (n=9). Exp.2 was analyzed by one-way analysis of variance by the General Linear Models procedure in SAS (SAS, 1991). Significant ($P<0.05$) differences were ranked by P-diff under LSmeans in SAS, and were indicated by different superscript letters.

5. Results

5.1 Experiment 1: Definition of intolerance level to RSC in juvenile tilapia

5.1.1 Chemical analysis of experimental diets

The composition of all diets used in Exp.1 is shown in Table 10. Different additional water during processing resulted in difference in content of moisture in different diets. The content of CP, lipid, ash, minerals and essential amino acids in all diets were almost the same and didn't show notable differences by increasing the inclusion of RSC in diet formulation. Also no notable differences were observed between expected composition from formulation and the analyzed diets.

Table 10. Analyzed composition of the diets used in Exp.1

	Dietary ratio CP from RS:CP from RSC+SBM, %				
	0	**25**	**50**	**75**	**100**
Dry matter (DM), g kg^{-1}	886	889	869	934	958
In DM, kg^{-1}					
Crude protein, g	326	322	317	328	313
Lipid, g	78	76	73	74	70
Starch, g	345	307	334	322	306
Ash, g	54	54	54	52	52
Calcium%	8.5	8.7	8.2	7.7	7.7
Phosphorus%	9.5	9.6	9.7	9.7	9.9
Magnesium%	1.9	2.1	2.4	2.5	2
Manganese, mg	74	82	80	75	74
Zinc, mg	311	316	318	324	312
Yttrium, mg	67	81	62	55	89
Energy , MJ	19.2	19.2	19.4	19.5	19.4
Essential amino acids, g					
Arginine	17	16	15	15	14
Histidine	7	7	7	7	7
Isoleucine	13	13	13	12	12
Leucine	35	35	35	34	33
Lysine	15	15	15	14	14
Methionine	10	10	10	10	10
Phenylalanine	17	16	18	16	15
Threonine	12	12	11	11	11
Valine	14	14	14	14	14
Tryptophan	3	3	3	3	3
Semi EAA					
Tyrosine	12	11	12	11	10
Cyst(e)ine	5	5	6	6	6
Total AA	317	311	311	302	293

5.1.2 Feed intake

The calculated CFI intake shows a gradual reduction along with increasing the level of replacement of CP from SBM with RSC up to 50% and after this point tended to be constant. Thereafter, the regression shows a flat pattern up to 100% replacement of CP (Figure 16). This relationship was best explained by a second degree polynomial regression (CFI = $0.003RSC^2$ - 0.4169RSC + 57.76, R^2 = 0.59).

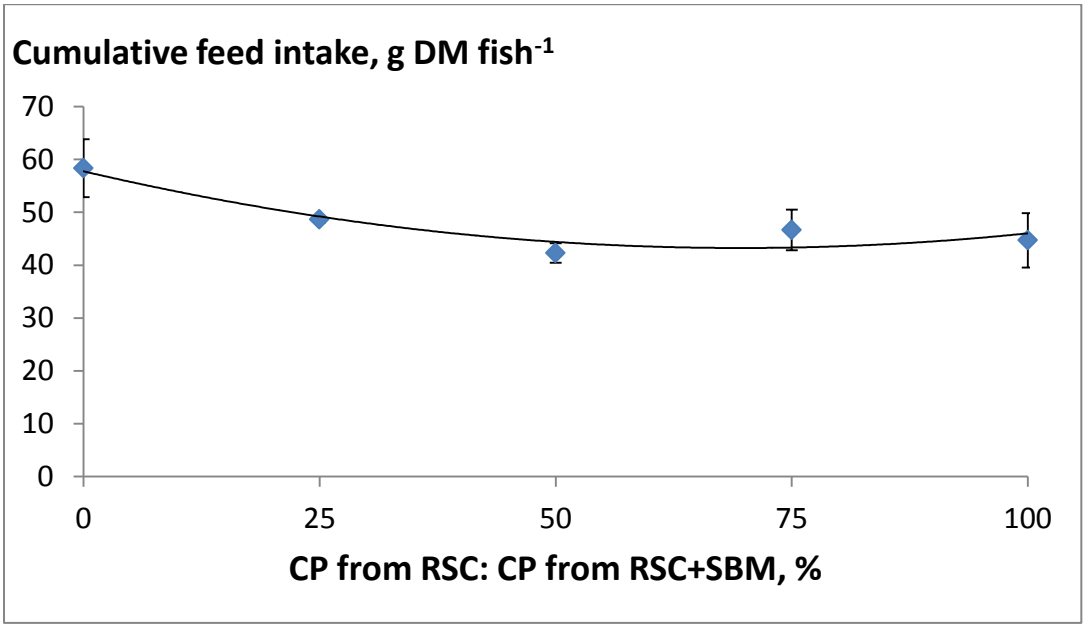

Figure 16. CFI intake over 6 weeks of feeding of the tilapias, (mean ± s.e.m. of 2 tanks)

5.1.3 Weight gain and survival

By increasing the inclusion of RSC in diet weight gain tended to decrease sharply up to 50% replacement, followed by a flat response for higher inclusion levels (Figure 17). This relationship was best explained by a second degree polynomial regression (Gain = $0.0019RSC^2$ - 0.3382RSC + 55.986, R^2 = 0.63).

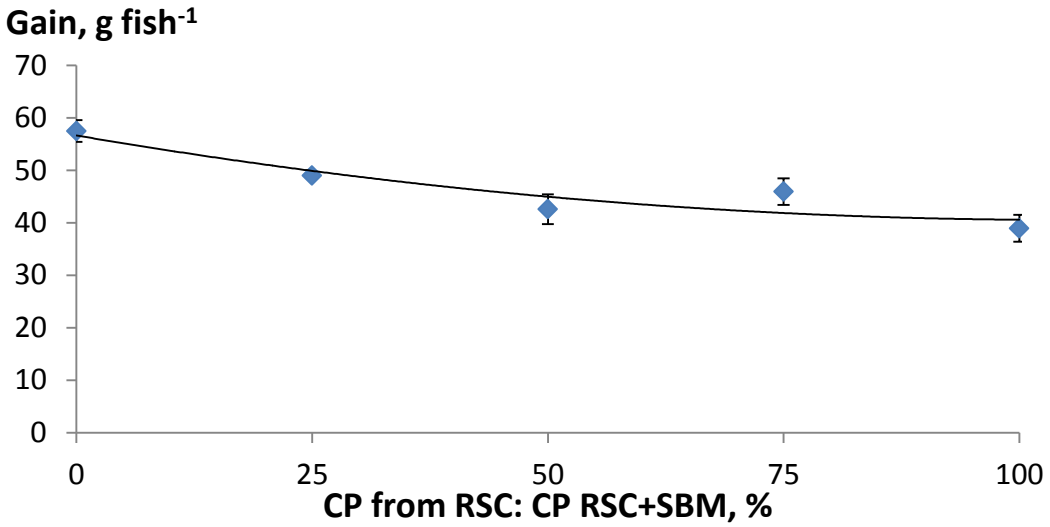

Figure 17. Weight gain (mean ± s.e.m. of 2 tanks) of the tilapias during 6 weeks feeding, (mean ± s.e.m. of 2 tanks). Mean start weight: 398±2g (n=10 tanks).

Five fish (2.5%) died during the experiment in treatments fed by 0%, 25% and 100% replacement of CP from SBM with RSC. Mortality was not systematically related to the different diets.

5.1.4 Weight gain of the tilapias in relation to feed intake

The relation between weight gain and CFI followed a 2nd degree polynomial pattern (CFI= 0.0254 gain^2 – 1.539 gain + 63.323, R^2 = 0.84) (Figure 18).

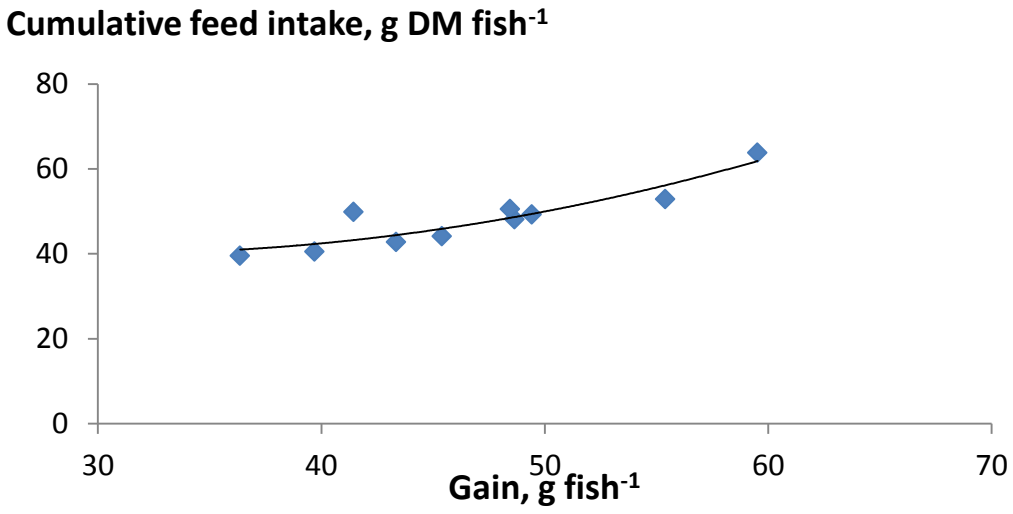

Figure 18. Weight gain of the tilapias in relation to CFI

5.1.5 Feed conversion ratio

The calculated FCR values for all treatments were almost the same and ranged from 0.9 to 1.2. By increasing the level of inclusion of RSC in diet the regression line is almost flat up to 75% replacement of CP from SBM with RSC and after that the FCR value increases mildly for 100%. However no significant difference was seen between FCR for diet with 100% replacement and the other treatments. The best explanation for this ratio was a second degree polynomial regression (FCR = 4E-05RSC ^{2} - 0.0025RSC + 1.021, R^2 = 0.63) (Figure 19).

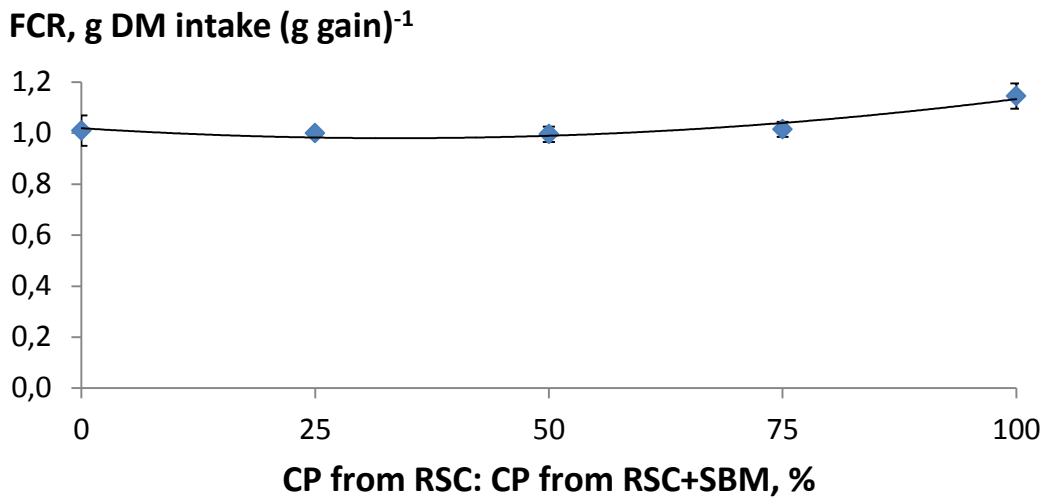

Figure 19. Feed conversion ratio (FCR, g DM intake (g gain)$^{-1}$ of the tilapias, (mean ± s.e.m. of 2 tanks)

5.1.6 Daily feed intake in percent of estimated body weight

Daily feed intake in percent of estimated daily body weight during the first three weeks of feeding is presented in Figure 20. Three different DFI patterns were observed. Tilapias fed the diet without RSC rapidly approached DFI of 4%, and had no decline in DFI after pellet size was increased at day 6. All dietary treatments with RSC showed higher variability in DFI than the fish fed the diet without RSC. The fish fed diets with 25 to 75% replacement levels approached 4% DFI at the same rate as the ones fed the diet without RSC. Their DFI, however, declined after this. Tilapias fed the diet with 100% replacement had lower initial DFI (2% of BW) than the other groups (3%), and used 3 weeks to approach 4% DFI.

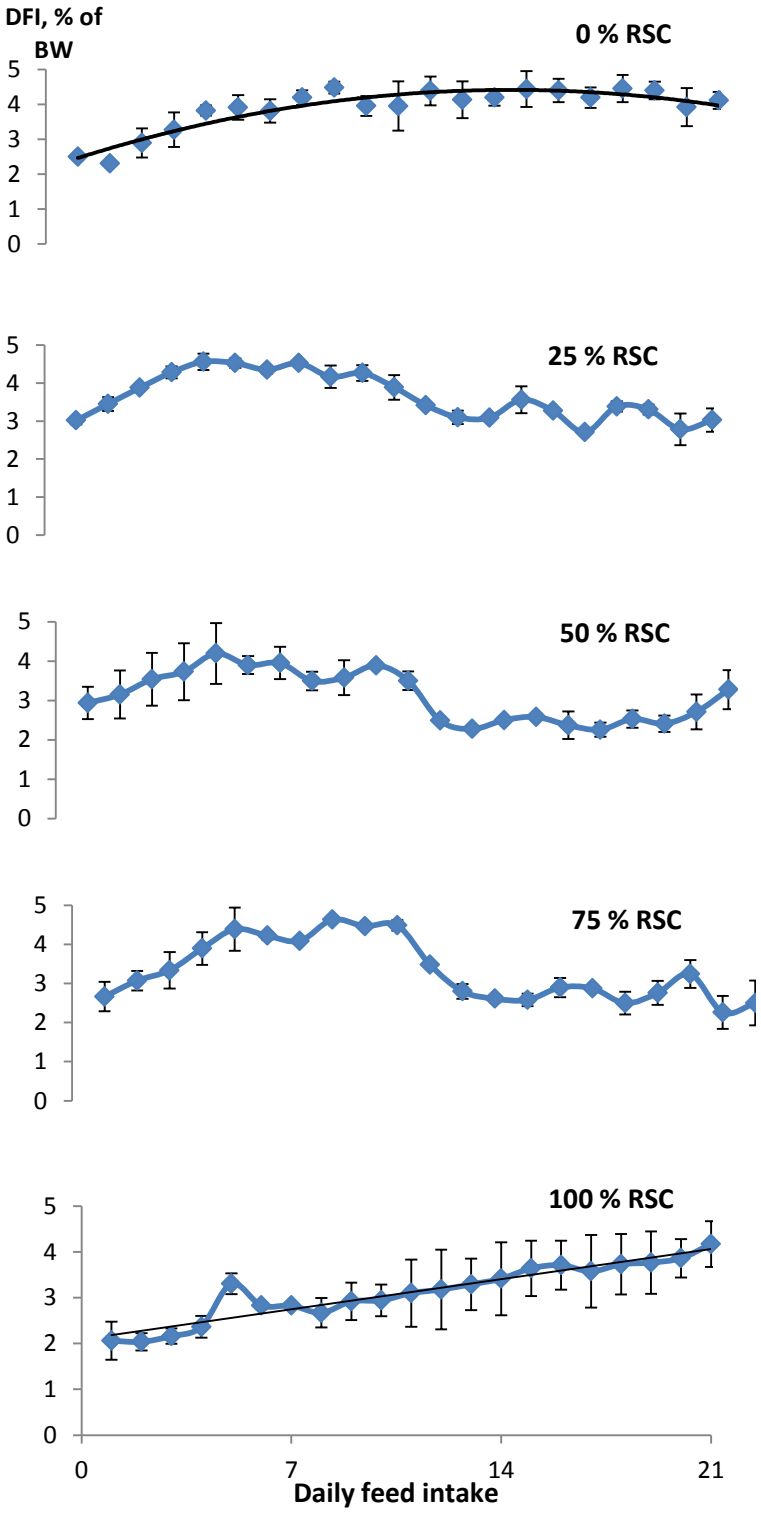

Figure 20. Daily feed intake (DFI, g DM) in percent of estimated body weight (BW, g) of the tilapias during the first 3 weeks of feeding, (mean ± s.e.m. of 2 tanks).

5.1.7 Liver weight to body weight

Liver in percent of whole body weight is presented in Figure 21. The ratio on liver weight to body weight increased from 0% replacement up to 50% replacement and after this point tended to reduce up to 100% replacement. (Liver weight percent = - 0.0289 RSC^2 + 0.0324 RSC + 0.0328, R^2 = 0.38).

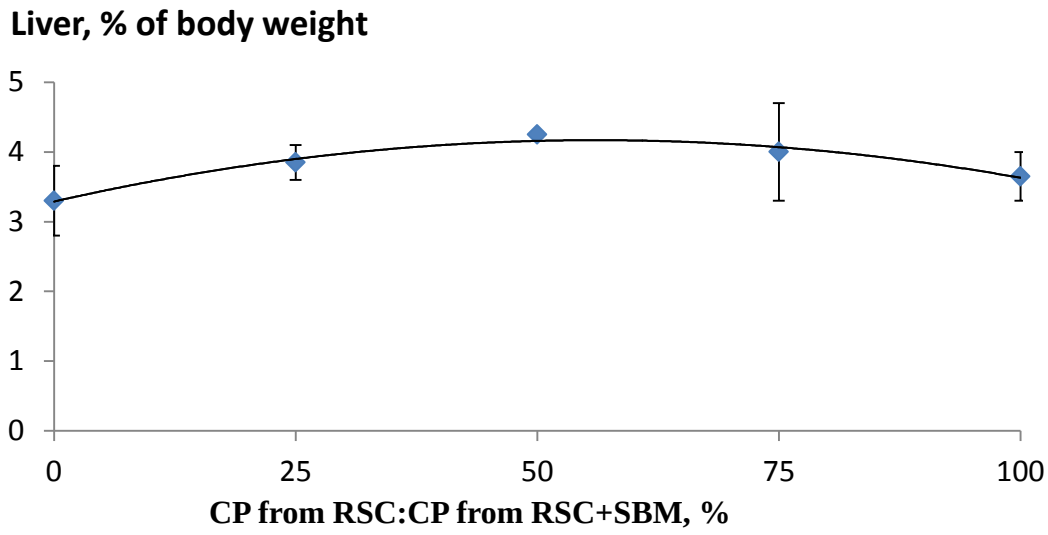

Figure 21. Liver, % of body weight, mean ± s.e.m. of 2 tanks

5.1.8 Blood plasma minerals and thyroid hormone concentrations

None of the plasma mineral compositions were significantly (P>0.05 by regression analysis) affected by the dietary ratio of CP from rapeseed vs that from soy:

Inorganic phosphate: Regression R^2=0.075; mean±s.e.m., n=10 (5 diets, 2 replicates): 2.69±0.09 mM.

Calcium: R^2=0.14, 3.54±0.11 mM.

Magnesium: R^2=0.073, 1.25±0.01 mM.

Zinc: R^2=0.003, 644±9 µM.

A similar lack of significant effects of dietary ratio between dietary RSC and SBM was seen for free T4 (thyroid hormone) in blood plasma: R^2=0.034, 19.7±1.7 pM.

5.2 Whole body compositions

Whole body protein did not follow any specific pattern related to the dietary treatments (R^2=0.34 for a 2nd degree polynomial regression). The protein concentration in tilapias at the beginning of the experiment (g kg^{-1}, mean±s.e.m., n=2), crude protein: 144±4.7. The mean concentration of CP in the fish at the end of the experiment was 146±1 g kg^{-1} (n=9 tanks).

There was a tendency that whole body lipid followed a 2nd degree polynomial development in response to dietary RSC inclusion (Figure 22): Whole body lipid, g kg^{-1}= 0.005 RSM^2 - 0.548 RSM + 119.13, R^2 = 0.36. The initial whole body lipid concentration in the fish was 94.0±1.6 g kg^{-1}.

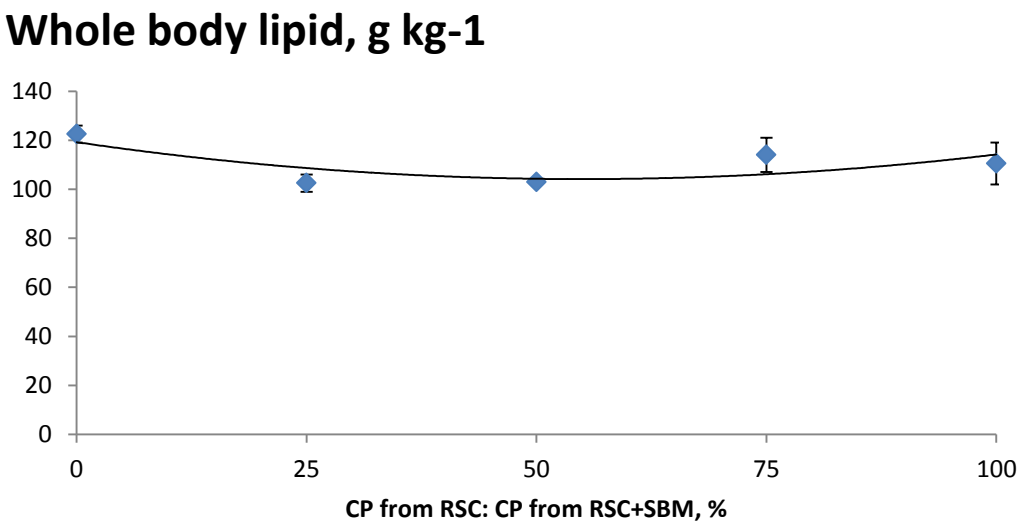

Figure 22: Whole body lipid concentration in the tilapias after 6 weeks of feeding (g kg^{-1}, mean±s.e.m., n=2).

The same pattern became more evident for whole body energy concentration (Figure 23): Whole body energy, kJ kg^{-1}= 0.0002 RSC^2 – 0.0284 RSC + 8.5057, R^2 = 0.53. The initial whole body energy concentration was 6.23±0.02 kJ.

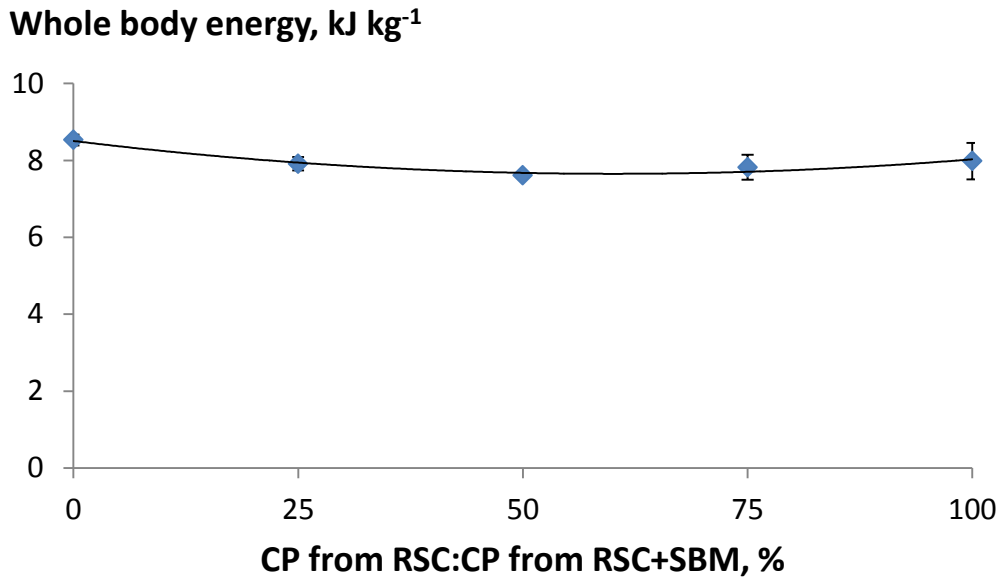

Figure 23: Whole body energy in the tilapias after 6 weeks of feeding (kJ kg^{-1}, mean±s.e.m., n=2).

The whole body ash concentration (g kg^{-1}) declined with increasing concentration of RSC in the diet (Figure 24; Whole body ash, g kg^{-1} = 0.0003 RSC^2 – 0.0614 RSC + 39.522, R^2 = 0.45). The initial value was 32.7±1.1 g kg^{-1}.

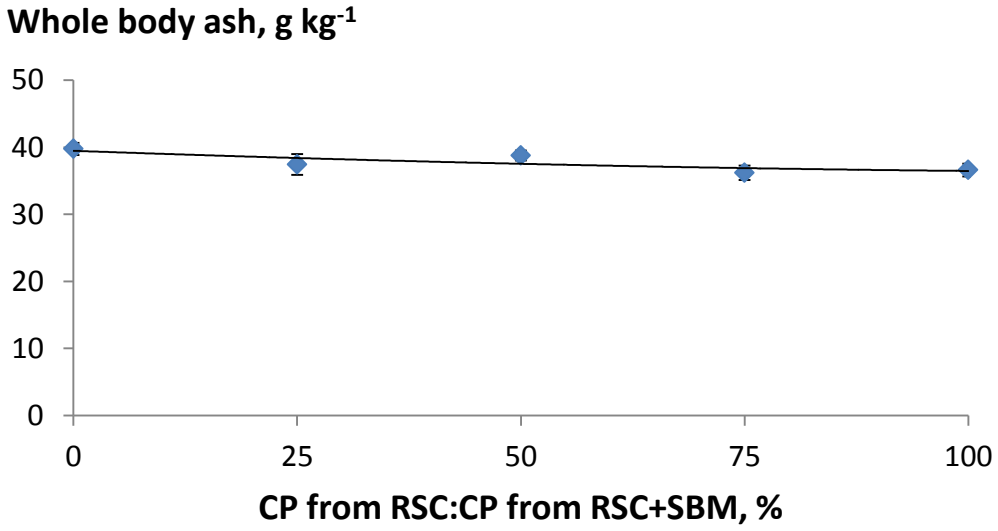

Figure 24: Whole body ash in the tilapias after 6 weeks of feeding (g kg^{-1}, mean±s.e.m., n=2).

5.2.1 Nutrient digestibilities and retentions

Neither the apparent digestibility (AD) of crude protein nor the apparent absorption (AAb) of mineral elements were notably affected by dietary RSC inclusion. The R^2 values from linear functions and mean±s.e.m. (n=9) were as follows:

Nutrient	**R^2**	**AD or AA (%, mean±s.e.m.)**
Crude protein	0.15	73.4±1.8
Calcium	0.16	1.8±7.9
Phosphorous	0.01	51.4±3.6
Magnesium	0.14	0.45±0.06
Manganese	0.14	99.6±0.1
Zinc	0.0	100±0

The same lack of effect of dietary inclusion of RSC was observed for retention of crude protein intake: R^2=0.27, mean±s.e.m.=44.3±0.8%. The energy retention (Figure 25) tended to follow a 2nd degree polynomial pattern (Energy retention, % = 0.0005 RSC^2 – 0.1239 RSC + 46.109, R^2 = 0.39).

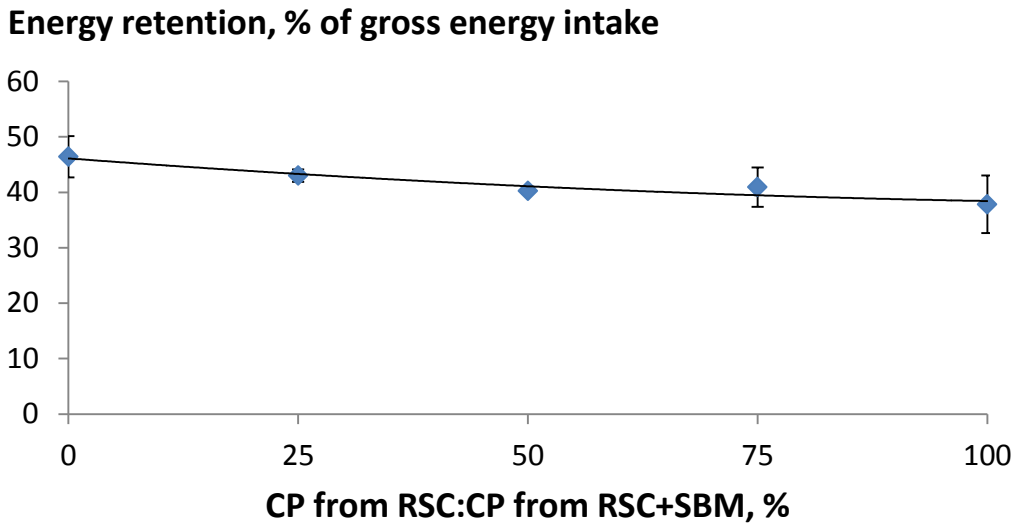

Figure 25: Energy retention (% of GE intake) during 6 weeks of feeding (g kg^{-1}, mean±s.e.m., n=2).

5.3 Experiment 2: Effect of fine milling and steam treatment on nutritional value of RSC.

5.3.1 Chemical analysis of experimental diets

The composition of all 3 different diets used in Exp.2 is shown in Table 11. No significant difference was seen among the content of CP, lipid, ash, minerals and essential amino acids in all three diets. The composition of diet with 50% of replacement of CP from SBM to CP from RSC in Exp.1 did not have any significant difference in the level of inclusion of nutrients with diet D 1.0 in Exp.2. The main difference between the expected composition from formulation and the diet analyzes was that the concentration of starch declined with increasing dietary concentration of RSC. The content of glucose in Table 11 is measured for different diets in Exp.2. Content of CP, starch, ash, lipid, minerals, energy, essential amino acids and glucose content of experimental diet are shown in Table 11.

5.3.2 Survival, feed intake, weight gain and feed conversion

No fish died during the 3 week experiment.

CFI, weight gain and FCR for tilapias fed diets with equal amounts of CP from SBM and RSC, with the RSC milled through 1.0 mm dies (D 1.0), 0.5 mm dies (D 0.5), or milled through 0.5 mm dies and autoclaved (D 0.5+A) are presented in Table 12.

CFI and gain in tilapia fed the D 0.5+A was significantly ($P<0.05$) lower than the other groups. No significant difference was seen for FRC among the dietary treatments. The percent of energy retention in fish fed by different experimental diets did not indicate significant difference.

5.3.3 Daily feed intake in percent of estimated body weight

Daily feed intake in percent of estimated body weight during the three weeks trail is presented in Figure 27. No notable difference in DFI pattern was observed among the three dietary treatments. The drop in DFI in treatments fed with D 0.5+A is sharper than the other treatments. All three dietary treatments reached 4% DM intake at one week, and maintained their DFI at this level for the two subsequent weeks.

Table 11: Analyzed composition of the diets used in Exp.2

Diet	**D 1.0**	**D 0.5**	**D 0.5+A**
Composition, kg-1			
Dry matter (DM), g	919	782	824
In DM			
Crude protein, g	316	314	324
Lipid, g	728	719	749
Ash, g	53	54	54
Calcium, g	8.1	8.2	8.2
Phosphorus, g	9.8	9.9	9.9
Magnesium, g	2.3	2.4	2.3
Manganese, mg	76.4	79.0	77.0
Zinc, mg	311	320	313
Yttrium, mg	53	53	59
Energy, MJ	20.0	20.2	19.5
Free glucose, g	3.1	4.1	4.5
Amino acids, g			
EAA			
Arginine	16	15	16
Histidine	8	8	8
Isoleucine	13	13	13
Leucine	36	36	38
Lysine	15	15	15
Methionine	10	10	10
Phenylalanine	17	17	17
Threonine	12	12	12
Valine	14	15	15
Tryptophan	30	30	32
Semi EAA			
Cyst(e)in	60	60	60
Tyrosine	12	11	13
Total AA	316	318	325

[1]Essential amino acids

Table 12. Cumulative feed intake (CFI), weight gain and feed conversion

Diet	**D 1.0**	**D 0.5**	**D 0.5+A**	**Pooled s.e.m**	**P<F[1]**
Feed intake (CFI), g DM fish^{-1}	46.1^{b}	43.9^{b}	39.3^{a}	1.1	0.013
Start weight, g fish^{-1}	37.4	37.21	37.45	0.5	0.20
Gain, g fish^{-1}	44.5^{b}	43.1^{b}	37.5^{a}	1.2	0.0075
FCR, g DM intake (g gain)$^{-1}$	1.04	1.02	1.06	0.01	0.37

[1]Different superscript letters^{a,b} indicate significant (P<0.05) differences, ranked by P-diff in the LSmeans procedure in SAS (1991).

The relation between weight gain and CFI followed a linear pattern: Gain = -1.0096 FI – 2.00; R^2 = 0.87 (Figure 26).

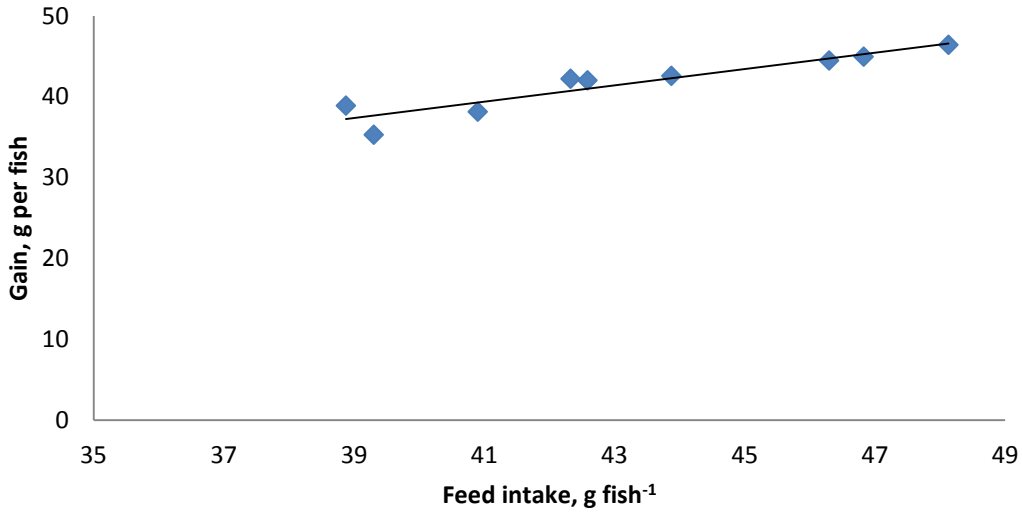

Figure 26. Linear regression of CFI on weight gain

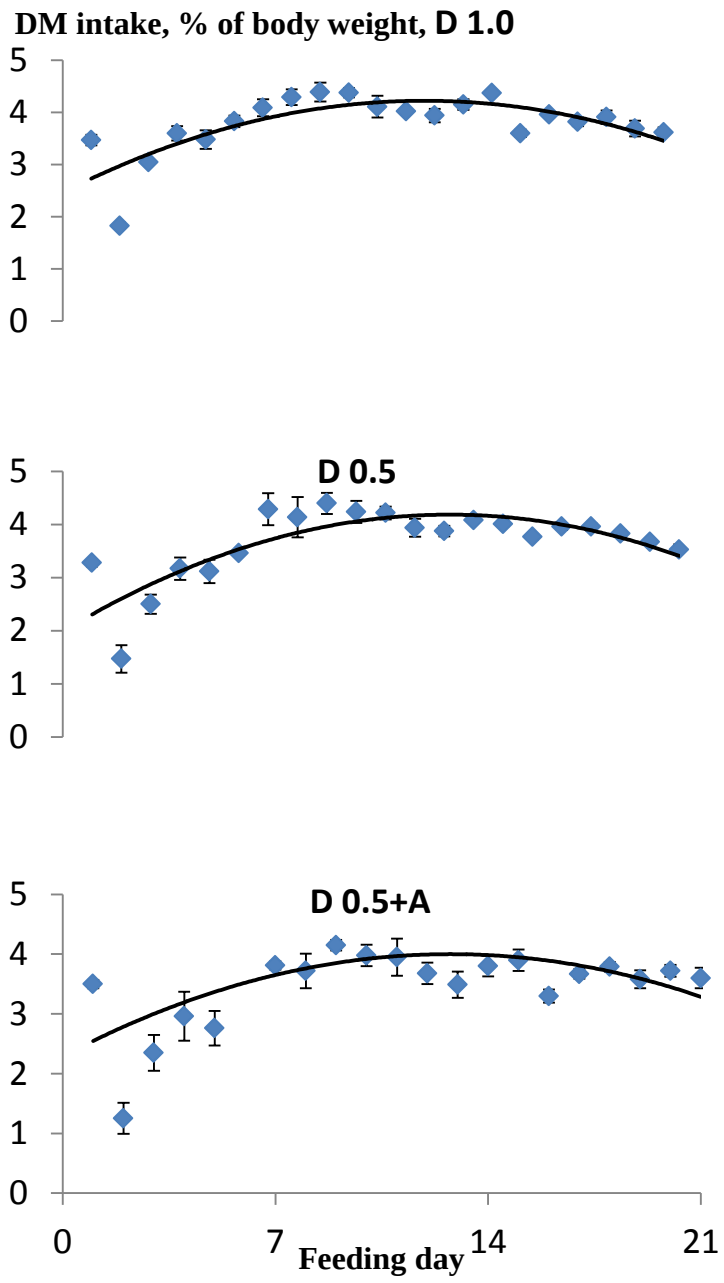

Figure 27. Daily dietary DM intake, % of estimated biomass (mean±s.e.m., 3 replicates per diet)

5.4 Whole body composition, apparent protein digestibility, and retentions of crude protein and energy

Whole body chemical composition and retention of protein and energy of the tilapias is presented in Table 13. The content of DM and CP in tilapia fed the D 0.5+A was significantly lower than that in the two other treatments. Also the retentions of energy crude protein into growth were significantly lower for the fish fed the diet with autoclaved RSC than for those who received D 0.5 or D 1.0.

Table 13. Liver to body weight (BW) ratio, whole body composition, protein digestibility, energy and protein retentions

Diet	**D 1.0**	**D 0.5**	**D 0.5+A**	**Pooled s.e.m**	**P<F**
Liver BW^{-1}, %	2.7	2.3	2.8	0.1	0.33
Body composition[2], kg^{-1}					
Dry matter (DM), g	291[b]	287[b]	267[a]	4.3	0.013
Crude protein (CP), g	145[b]	143[b]	138[a]	1.1	0.0063
Lipid, g	99	97	88	2.8	0.24
Ash, g	34	34	33	0.2	0.19
Energy, MJ	8.2	8.0	7.9	1.1	0.9
Apparent CP digestibility, %	75.0	76.4	69.6	2.0	0.41
Retention, % of intake					
CP[3]	43.2[b]	42.8[b]	38.1[a]	0.9	0.0019
Energy	44.1[b]	45.0[b]	38.0[a]	1.3	0.026

[1]Different superscript letters[a,b] indicate significant (P<0.05) differences, ranked by P-diff in the LSmeans procedure in SAS (SAS, 1991). [2]Initial values (mean±s.e.m., n=2, kg^{-1}): DM, 250±3 g; CP, 147±2 g; Lipid, 65±0.2 g; Ash, 34.6±0.2 g; Energy, 6.7±0.1 MJ ; [3]Only 2 replicates per diet due to limited amounts of feces sample.

5.4.1 Apparent mineral absorption and blood plasma analysis

Calcium displayed negative values for apparent absorption in diet D 0.5+A, which tended (P=0.073) to differ from the other treatments. No significant differences in apparent absorption of P, Mg, Mn and Zn was observed. The dietary content of Mn and Zn was completely absorbed for all diets.

The level of minerals (Ca, Mg, Zn and inorganic P) in blood plasma of tilapia fed by different experimental diets have no significant difference (P>0.05). There was, however, a trend (0.05<P≤0.10) towards the concentration of zinc being lower in the plasma of tilapias fed the two

diets with RSC ground at 0.5 mm than in the fish fed a diet with RSC ground by 1.0 mm dies. No significant difference was observed for thyroid hormone (Table 14).

Table 14. Plasma thyroid hormone (T4), apparent mineral absorptions, and plasma mineral concentrations

Diet	D 1.0	D 0.5	D 0.5+A	Pooled s.e.m	P<F
Free plasma T4, pM	23.7	22.7	15.0	2.9	0.48
Apparent mineral absorption, %					
Ca	21.6	3.7	-9.5	5.9	0.073
P	61.6	56.7	51.4	2.3	0.20
Mg	59.2	60.3	55.4	2.2	0.69
Mn	99.9	99.9	99.9	0.0	1.0
Zn	99.9	99.9	99.9	0.0	1.0
Plasma minerals					
Ca, mM	3.4	3.3	3.1	0.08	0.30
Inorganic P, mM	3.4	2.5	2.4	0.3	0.27
Mg, mM	1.4	1.3	1.3	0.03	0.16
Zn, µM	590	539	530	12.3	0.071

6. Discussion

6.1 Experiment 1:

6.1.1 Diet formulation and processing

By increasing RSC inclusion in the feed formulation, viscosity of the mixed ingredients was increased during feed processing. This in term necessitated increased water addition in order to achieve satisfactory technical quality of the pellets. The increased viscosity probably is due to differences between content of soluble NSP such as pectic polysaccharides between RSC and SBM (Thakur et al., 1997; Bach-Knudsen, 2001). It can be also realized by difference between water binding properties of proteins in SBM and RSC. Moreover, the added oil to diet composition was decreased along with increasing the level of RSC.

6.1.2 Nutritional responses to replacing CP from SBM with CP from RSC

The relationship between CFI and weight gain is demonstrated with a second degree polynomial regression. However, this pattern was strongly driven by one point (59.5, 63.9), one of the observations from the treatment fed RSC free diet. By removing this observation, the regression line will turn to a linear pattern with the following equation: FI = 0.6809 gain + 15.521, R^2 = 0.69.

This apparently linear relationship between CFI and weight gain demonstrated that FI was the main factor to explain differences in growth of the tilapias. In the first experiment, CFI and growth are decreasing along with increasing inclusion of RSC in diet up to 50% replacement (23% RSC in diet) and after this level remained constant. It can be assumed that fish may adjust the unwanted compounds entry to the body with livers capacity to detoxify them up to 50%. After 50% replacement, energy requirements of body may not allow fish to reduce CFI since the ratio of DP (digestible protein) to DE (digestible energy) is kept almost the same in all diets. By increasing the level of toxic compounds entry to body, tilapia may need more energy and protein secretion (enzyme) to detoxify and overcome anti-nutritional effects of them. It may increase maintenance energy requirements in tilapia in comparison with those are receiving lower percent of RSC from diet and may affect FI after 50% replacement. It can be assumed that the threshold effect in CFI at 50% replacement may be due to increasing energy requirements for maintenance. Bitterness of certain glucosinolates hydrolysis products such as goitrin is known responsible for poor palatability and FI depression (van Doorn et al., 1998; Mithen et al., 2000).

Presence of toxic components in diet which can negatively affect metabolism may cause a steady increase in FCR value (Zhou and Yue, 2010). However, no significant difference was seen in FCR among different treatments. However, this value tended to increase mildly after 75% replacement non- significantly. A constant FCR was seen in the experiment performed by Luo et al. (2012) along with increasing the level of RSM in diet.

The low and similar FCR along with steady decline in CFI may indicate that the content of glucosinolates and their hydrolysis products in RSC used in this experiment did not have any toxic effect on tilapias metabolism up to 75% replacement or tilapia were resistant to these toxins and could modulate or detoxify the compounds entered to the body. Furthermore, it is interesting to note that no significant difference in mortality or abnormal behavior of fish was observed in any treatments of this study. The slight threshold effect in FCR after 75% may represent that utilization of more than 460 g per kg feed (with equal ANF with RSC used in this experiment) may negatively affect tilapias metabolism and FCR.

No difference in digestibility of protein and minerals among several experimental groups indicates that, in comparison with SBM, the applied RSC does not negatively affect digestibility of protein and uptake of minerals in Tilapia, compared to what was the case with SBM. Thus, no significant negative effect from phytic acid and fiber in the RSC was observed in comparison with SBM.

The regression analysis of the content of DM in whole body demonstrated a decline from 0% to 50% replacement and then an increase up to 100% replacement. It can be caused by the content of lipid in body which demonstrated the same pattern as DM. There was no clear relationship (R^2=0.099) for the linear regression of whole body weight on whole body lipid content, when combining observations from fish in both Exp.1 and 2. The R^2 values became even lower when this analysis was done within experiment. It has been reported that presence of condensed tannin in diet increases the visceral fat deposition in tilapia (Aiura and Carvalho, 2007). Since the level of condensed tannin and phenolic compounds in RSC is higher than SBM (Naczk et al., 1998), tilapia may reduce the tannin intake by declining FI. However, after reaching the threshold level for CFI at 50% replacement, no further reduction in the CFI to body weight ratio was observed. Thus, referring to the literature review and the Table 1, the content of tannin entering to the body may have been higher than the capacity of tilapia to tolerate. It may cause an increase in the level of visceral fat deposition in tilapia.

Aiura and Carvalho (2007) demonstrated that deposition of lipid in other parts of the fish body is decreasing by presence of high content of condensed tannin in tilapia diet. Moreover, Pinto et al.

(2001) demonstrated that condensed tannin may reduce fat deposition in the liver of piauçu fish. As a result of the findings from this experiment and previous reports, in may be assumed that the pattern of regression line for the percent of liver weight to body weight (an increase from 0% to 50% replacement and then a decline up to 100% replacement) can be explained by the difference in deposition of lipid in liver and whole body.

Three different patterns were seen from estimated daily ratio of DFI to biomass for different treatments. Apart from diet with 100% replacement, the feed intake first approached 4% of BW, and then gradually decreased. The feed intake level at 4% of BW a day was similar to the findings of Riche and Garling (2003) who recommended that tilapia (from 20 g BW) should be fed 4% a day. At 100% replacement, the fish used 3 full weeks to reach 4% DFI.

One reason for the declining DFI and increased variability between replicate groups in the fish fed the diets from 25 to 75% replacement may have been stress in connection with changing pellet size after day 6. The results may also indicate that the presence of RSC in diet may causes higher sensitivity to change in feed particle size, with negative effects on FI and growth performance if tilapia. Tilapia can efficiently utilize small pellets relative to their body weight in comparison with other commonly cultured species such as salmon, trout or catfish (Riche and Garling, 2003). Thus, it is recommended to keep the pellet size as constant as possible during further experiments.

Contrary to expectations from some other monogastric animals due to the higher concentration of phytic acid in RSC than in SBM (Francis et al., 2001; Al-Kaiesy et al., 2003), this study did not indicate decrease in uptake of cationic elements in tilapia. Results from this experiment show that unwanted components such as phytic acid, fiber and tannins, could not change nutrients absorption and protein and energy retention in tilapia by replacing CP from SBM with CP from RSC.

It is argued that, thus, constant protein and energy retention, mineral absorption and FCR may be explained by: 1) equal level of available energy provided by nutrients (protein, fat and starch); 2) application of well balanced diet supplemented with minerals and essential amino acids; 3) equalization of fiber level in all diets by utilization of cellulose in SBM containing diets. This result also may be explained by the fact that the ratio of NP/NE was almost constant in all experimental diets.

Previous studies have hypothesized that glucosinolates may have negative effect on thyroid function in salmonids and cod (Gatlin et al., 2007; Hossain and Jauncey, 1988). However, the findings of the

current study did not demonstrate any significant difference among the level of T4 from different treatments. As mentioned in the literature review there is a difference in capability of intolerance to glucosinolates among different animals (Tripathi and Mishra, 2007). A possible explanation for this might be that tilapia has higher capability to detoxify or tolerate goitrogenic glucosinolates. However, T4 detection assays were performed according to kit instructions modified for dog blood plasma and may not be fully homologous with tilapia T4. Limited information is found in the literature about the thyroid glands and the effect of glucosinolates on thyroid function and hormones in tilapia (Geven et al., 2007).

In the current study, comparing liver weight with body weight indicates that liver size, and then possibly liver function was affected by dietary RSC. These results agree with the findings of other studies, which have been demonstrated hyperplasia and hyperactivity of liver to detoxify ANF (Burel et al., 2000a; Tripathi et al., 2001; Mabon et al., 2000). No previous experiment has been analyzing the effect of rapeseeds containing diets on liver in tilapia.

Hence, it could conceivably be hypothesized that in 50% replacement of CP from SBM with RSC, tilapia is sensitive to changes in nutritional quality of the feed. The second experiment was, thus, conducted with 50% replacement of CP from SBM with CP from RSC.

6.2 Experiment 2:

6.2.1 Nutritional responses to pretreatment of RSC

The results demonstrated that the combination of fine milling and autoclaving RSC has significantly negative effect on CFI and consequently weight gain in tilapia. The first hypothesis is that combination of fine milling and autoclaving RSC may decreases the palatability of diet dramatically since it could provide proper situation for hydrolysis of intact remaining glucosinolates in RSC to more bitter components. Since one of the products from hydrolysis reaction is free glucose, the results from glucose analysis may proves that further hydrolysis reactions may accrued in fine milled and autoclaved RSC. In presence of some intact progoitrin which is non-bitter glucosinolates in rapeseeds secondary products, high temperature processing causes progoitrins degradation and production of goitrin which is extremely bitter compound (Heaney and Fenwick, 1980) which may have negative effect on palatability and FI. However, glucose enhancement can be the result of other unknown reactions in RSC.

A part from poor palatability, negative effect of heating on content of protein may change the ratio of NP/NE in diet containing fine milled and autoclaved RSC. Since very few water vas absorbed by RSC during autoclaving process it can be assumed as a dry heating process which may damage protein. Furthermore, decreasing lysine bioavailability by heat treatment may also contribute to this effect (Morken et al., 2012; Singh et al., 2007). It has been calculated that 99% of reduction in energy retention is caused by depression of protein retention. Results from protein retention and energy retention can prove this fact that the content of protein and energy of diet were not efficiently utilized by fish in diet containing fine milled and autoclaved RSC.

Although not many similar experiments can be found in the literature to assess the effect of fine milling and/or autoclaving rapeseeds secondary products on tilapia; but, there are some reports available from utilization of heat treated RSM in chicken and broilers which resulted in different responses. In the assessment performed by Chamani et al. (2009) autoclaving RSM could improve biological performance of chicken. However, Khan et al. (1998) observed that although autoclaving RSM may decrease the content of allylisothiocyanate but no significant difference was reported in CFI and growth performance. As mentioned in the literature review, content of glucosinolates in different rapeseed secondary product defers with the other. Moreover, the response of different animals to presence of glucosinolates hydrolysis products in diet differs with the other species.

A strong linear relationship between weight gain and CFI with lack of significant difference between FCR in different treatments may represent that: 1) FI is the main factor which can affect weight gain, 2) presence of glucosinolates hydrolysis products on a certain level in tilapia diet may not have any toxic effect on fish metabolisms or tilapia is able to tolerate a certain level of toxins and detoxify them. No similar experiments have been done previously to assess the variation of FCR value before and after fine milling and autoclaving processing in tilapia.

The pattern of DFI and biomass at different treatments was almost the same. The ratio of DFI to biomass increased up to almost 4% and after that gradually decreased. An environmental stress (tank cleaning and diet changing) decreased the DFI at the beginning of experiment dramatically. It has been demonstrated that fish fed by diet containing fine milled and autoclaved RSC were more sensitive to environmental stresses. In treatment fed with diet containing fine milled and autoclaved RSC physical stresses caused more dramatic decrease in DFI in comparison with the other treatments.

Digestibility of protein in diet D 0.5+A tended to be lower than that from the two other diets. It has been argued in literature that overheating during processing can decreases digestibility of protein due to formation of strong hydrogen or S=S bonds (Opstvedt et al., 1984), or Maillard reaction.

Apparent absorption of measured minerals, except Ca, were similar in all three diets, while that of calcium ($AD_{Ca} \leq 0$) tended to be lower in D 0.5 than D 1.0. It is also affected by fine milling and autoclaving of RSC inclusion of diet. Presence of RSC in Exp.1 didn't cause any negative effect on Ca digestibility. The possible reason for negative digestibility of Ca in treatment fed D 0.5+A and D 0.5 may be the secretion of Ca from colon walls into the faeces. Calcitonin is the hormone responsible for regulation of Ca metabolism in body. In mammals the main source of calcitonin is parafollicular or C cells in the thyroid gland. It is also detected in fish species. Probably the function of glucosinolates on thyroid cells is not limited to the level of T4 secretion. Since the glucose assay showed an increase in content of glucose in fed D 0.5+A and D 0.5 in comparison with D 1.0, the calcium regulation may be affected by production of certain glucosinolates hydrolysis products. Tilapia is able to uptake Ca from water and excretes it from large intestine and gills.

The lack of significant difference among diets in plasma mineral (P, Ca, Mg and Zn) levels support the absorption data and the indication that RSC and SBM did not affect utilization of minerals differently.

The complete absorption of dietary Mn and Zn may show that the probably phytic acid content of diets in both experiment 1 and 2 could not have any negative effect of minerals digestibility.

Although it was assumed that autoclaving may reduce the content of goitrogenic glucosinolates in rapeseeds secondary products (Khan et al., 1998) but the results of this study did not show any significant increase among free T4 in blood plasma taken from different treatments in this experiment. However, it has been demonstrated in Exp.1 that presence of RSC in diet does not affect the level of free T4 in juvenile Nile tilapia. It may be assumed that after adaptation, tilapia is highly resistant to tolerate goitrogens in RSC.

Although the Exp.1 and Exp.2 were performed at different times, but, all the experimental situations were kept constant and no significant difference was seen between treatments fed with diet 50% in Exp.1 and diet 1mm in Exp.2 in protein and energy retentions and mineral absorption.

6.3 Conclusion and suggestions for further work

The present study was designed to determine the critical level of replacement of CP from SBM with CP from RSC. The next step was to find out whether fine milling and combination of fine milling and autoclaving can affect nutritional value of RSC.

The present investigation has argued that presence of RSC in tilapia diet may reduce feed acceptability. However, it did not show different effects on metabolic function of fish than those caused by SBM. Fine milling did not affect the nutritional value of RSC, while autoclaving of RSC has negative effect on diet palatability and utilization.

It would be interesting to assess the effects of utilization of appetizers in combination with supplementation with essential amino acids and minerals in diet containing certain level of rapeseed secondary products in further studies.

The current research was not specifically designed to evaluate effects of glucosinolates and their hydrolysis products. Further research is needed to determine how important these products are for performance of the tilapias, and which level of inclusion of these compounds in different secondary products of rapeseed are tolerable in diets for tilapia.

The current investigation was limited by time. However, feeding trials with longer duration is recommended to assess long-term effects of secondary products of rapeseed on fish health, performance and physiology.

The results and information from this investigation can be used to develop a targeted investigation on improvement of nutritional quality of rapeseed secondary products as a protein rich non-food ingredient in diets of monogastric animals. This research may have a higher focus on diet acceptability and lower focus on glucosinolates and their hydrolysis products than traditionally anticipated in research with secondary products of rapeseed in feed for fish.

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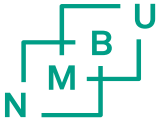

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