EFFECT OF FRACTIONATION ON NUTRITIONAL VALUE OF WHEAT DISTILLERS GRAINS FOR RAINBOW TROUT (*ONCORHYNCHUS MYKISS*)

A Thesis Submitted to the College of

Graduate and Research

in Partial Fulfilment of the Requirements

for the Degree of Master of Science

In the Department of Animal and Poultry Science

University of Saskatchewan

Saskatoon

By

Felipe E. Reveco Urzúa

PERMISSSION TO USE STATEMENT

In presenting this thesis in partial fulfillment of the requirements for a Postgraduate degree from the University of Saskatchewan, I agree that the Libraries of this University may make it freely available for inspection. I further agree that permission for copying of this thesis in any manner, in whole or in part, for scholarly purposes may be granted by the professor or professors who supervised my thesis work or, in their absence, by the Head of the Department or the Dean of the College in which my thesis work was done. It is understood that any copying or publication or use of this thesis or parts thereof for financial gain shall not be allowed without my written permission. It is also understood that due recognition shall be given to me and to the University of Saskatchewan in any scholarly use which may be made of any materials in my thesis.

Requests for permission to copy or to make other use of material in this thesis in whole or part should be addressed to:

Head of the Department of Animal and Poultry Science
University of Saskatchewan
Saskatoon, Saskatchewan
S7N 5A8

ABSTRACT

In this study, the nutritional value of wheat distillers grains and the effect of further processing of these products on their nutritional value for rainbow trout were investigated in five experiments. In experiments 1 and 2, wheat distillers grains with solubles (WDDGS) was fractionated using grinding, sieving and elutriation sequentially. Apparent digestibility coefficients (ADC) of dry matter (DM), gross energy (GE), acid ether extract (AEE), ash and amino acids (AA) did not differ between the original WDDGS and the WDDGS protein concentrate ($P > 0.05$). However, the ADC of crude protein (CP) was significantly higher for WDDGS protein concentrate (88.0 %) than the original WDDGS (84.9 %) ($P < 0.05$). In experiments 3 and 4, the effect of aqueous fractionation on nutritional composition of wheat wet distillers grains (WWDG) from two local ethanol plants (plant 1 and plant 2) was evaluated. Aqueous fractionation increased levels of CP and GE in the processed WWDG from both plants. Fractionation significantly increased the ADC of DM, GE and AEE ($P < 0.05$). In contrast, protein digestibility was not influenced by the plant or the processing method ($P > 0.10$). In experiment 5, a 56 d growth trial was performed to determine the effect feeding the aqueous fractionated WWDG to rainbow trout on growth performance. Rainbow trout (n=22/ tank; body weight 136 g and 3 tanks/ treatment) were fed diets containing 0, 75, 150, 225 and 300 g kg^{-1} of the processed WWDG from plant 2. There were no significant linear or quadratic relationships between inclusion rate and specific growth rate (SGR), average daily gain (ADG) or feed to gain ratios (feed:gain). However, there was a significant negative linear relationship between inclusion rate and average daily feed intake (ADFI) ($P < 0.05$). The results of these studies suggest that both dry and aqueous fractionation are suitable methods to produce protein concentrates from wheat distillers grains but that the aqueous fractionation process was more effective in improving nutrient composition and increasing digestibility.

Key words: distillers grains, fractionation, digestibility, growth, trout.

ACKNOWLEDGEMENTS

I would like first to express my gratitude to my supervisor, Dr. Murray Drew for his support, guidance, and encouragement throughout the program. You were a great supervisor. I would also like to acknowledge my supervisory committee, Dr. Andrew Van Kessel, Dr. Martin Reaney, Dr. Bernard Laarveld and Dr. Gordon Zello for serving as my external examiner.

I am grateful to Agriculture Development Fund of Saskatchewan Agriculture, Feed Opportunities from the Biofuels Industries and Evonik industries for providing funding support for this project. I am also thankful to Dollie Hantelman Agricultural Scholarship that awarded my work.

Special thanks to the Prairie Aquaculture Research Center staff: Stephanie, Kyla, Janelle, Sabrina, Graeme, Atul, Bonita and Ravindra. As well as research scientists and laboratory staff of the Department of Animal and Poultry Science for their help in many senses that made possible to accomplish this experience: Zhiyuan Niu, Pam, Bryan, Angela, Charlotte, Dr. Colleen Christensen and Amanda.

I would like to dedicate this thesis to my parents and brother for their support, understanding and encouragement to pursue my goals. It is also dedicated to my friends (Family) in Canada and around the world: Ari, Waldo, Jose, Carlos, Paty, Sonia, Jerome, Lee-Anne, Diana, Joe, Ana, Zafer, Manu, Isa, Alex, Michelle, Manena, Vicky, Pato, Yuby, Fernanda and Beto.

TABLE OF CONTENTS

LIST OF TABLES

LIST OF FIGURES

LIST OF ABBREVIATIONS

ANF	Anti-nutritional factors
AA	Amino acids
ADC	Apparent digestibility coefficients
ADF	Acid detergent fibre
ADFI	Average daily feed intake
ADG	Average daily gain
ADIN	Acid-detergent insoluble nitrogen
AEE	Acid ether extract
AID	Apparent ileal digestibility
BPM	Bacterial protein meal
CDS	Condensed distillers solubles
CGF	Corn gluten feed
CGM	Corn gluten meal
CM	Canola meal
CP	Crude protein
DDG	Distillers dried grains
DDGS	Distillers dried grains with solubles
DE	Digestible energy
DM	Dry matter
E-Mill	Enzymatic milling
EAA	Essential amino acids
FEM	Feather meal
GE	Gross energy
HUFA	Highly unsaturated fatty acids
MBM	Meat and bone meal
MWM	Meal-worm meal
NDF	Neutral detergent fibre
NEAA	Non-essential amino acid
NSP	Non-starch polysaccharides

PBM	Poultry by-product meal
PUFA	Polyunsaturated fatty acids
QG	Quick germ
QGQF	Quick germ and quick fibre
SAA	Sulphur amino acids
SCP	Single cell proteins
TS	Thin stillage
U.S.	The United states
WDDGS	Wheat distillers grains with solubles
WDG	Wet distillers grains
WDGS	Wet distillers grains with solubles
WWDG	Wheat wet distillers grains

1. GENERAL INTRODUCTION

Aquaculture production is growing rapidly around the world. The global aquaculture production is increasing at a rate of 7 percent per year (FAO 2009). This is associated with a concomitant growth in aquafeed production and the specialized protein and oil ingredients used in their manufacture (FAO 2006; Gatlin et al. 2007).

Fishmeal has been the most important protein source in aquafeeds for finfish (Drew et al. 2007). Global fishmeal production is around 6.5 million tonnes annually and has remained essentially static over the last 20 years (New and Wijkström 2002). However, the world aquaculture feed industry uses around 5.6 million tonnes of fishmeal per annum (Rahnema et al. 2005) and the demand this product by aquafeed production is expected to exceed the supply of available fishmeal in the next decade (Gatlin et al. 2007). Based on these trends, expansion of the world aquaculture industry will be restricted due to increasing scarcity and the resulting increased cost of fishmeal. Furthermore, there are increasing concerns regarding the ethics of using food-grade fishery resources for animal feeding instead of using them for human consumption (Tacon et al. 2006). The aquaculture industry is therefore dependent on the development of alternative protein sources to replace fishmeal in aquafeeds (New and Wijkström 2002; Drew et al. 2007).

Fishmeal is the "gold standard" of protein sources in aquatic feeds for several reasons. For example, its amino acid balance is ideal for most carnivorous fish species. Moreover, it also contains unidentified growth factors missing from other protein sources (New and Wijkström 2002; Drew et al. 2007). Lastly, fishmeal is highly palatable, digestible and a rich source of essential fatty acids, energy and minerals (Webster et al. 1992; Webster et al. 1995). Traditionally, fishmeal is made from whole fish, such as herring and menhaden with 72 and 64 % protein content, respectively (Rahnema et al. 2005). Thus, replacing fishmeal with other alternative protein sources is a difficult challenge for fish nutritionists.

One solution to problem of replacing fishmeal as a protein source is using plant protein sources due to their availability and low prices compared to fishmeal and other animal protein sources. However, the use of plant proteins has been met with only limited success due to their inferior nutritional value compared to fishmeal and the presence of some anti-nutritional factors (ANF). Nonetheless, recent findings suggest using further processing methods such as dehulling

(Thiessen et al. 2003a), aqueous extraction (Kaushik et al. 1995; Mwachireya et al. 1999), extrusion (Thiessen et al. 2003a; Booth and Allan 2004), supplementation of AA (Cheng et al. 2003; Aksnes et al. 2008) and the use of enzymes (Cheng and Hardy 2004; Drew et al. 2005) will enhance the nutritional value of plant proteins in aquaculture. Plant proteins are less expensive than animal products and thus, it may be possible to improve their nutritional quality through processing and still produce feed products that are economically viable. However, much research remains to be done to identify the best plant proteins and methods for improving their nutritional value.

Distillers grains are a co-product from the ethanol industry and are a potential alternative protein source for aquafeed due to their increasing availability, competitive pricing and low levels of ANF compared to current alternative plant protein sources (e.g., soybean meal). Distillers grains have a high protein content but limited value for aquaculture due to their relatively high fibre content. Therefore, distillers grains require further processing to remove the fibre, thus, increasing their nutritional value for aquaculture. Based on this information or lack thereof, this project focused on increasing the nutritional value of wheat distillers grains by dry and aqueous fractionation methods and evaluating the effect of these fractionation processes on digestibility of nutrient components of wheat distillers grains in rainbow trout.

2. LITERATURE REVIEW

2.1 Nutritional Requirements of Salmonids

2.1.1 Protein and Amino Acids

Protein is the most costly and largest component in the diets of carnivorous fish. Proteins function is to supply adequate amounts of required AA to guarantee healthy growth processes and cell renewal (Friedman 1996; Garcia et al. 2000). In fish, protein is also used as an important energy source (Cowey 1992; Cowey and Cho 1993; NRC 1993).. It is suggested that diet formulation should meet essential amino acid (EAA) requirements due to AA that are not used for tissues synthesis or are provided in excess may be preferred as energy sources over carbohydrates and fat (Cowey 1992; Cowey and Cho 1993; Trushenski et al. 2006). Because of this, protein requirements should be expressed in terms of dietary energy (Cowey 1994). Protein requirements in fish diets range from 30 to 55 % of DM (Rahnema et al. 2005). The protein content of the diet must also meet the needs of fish for both essential and non-essential AA (NRC 1993).

In most studies, EAA requirements have been determined using dose-response (growth) curves (Cowey 1988). Some controversy has arisen concerning the best method to express the amino acid requirements of fish. Some researchers suggest EAA requirements are best expressed as a percentage of the diet since the dose-response relationship for EAA is linear for much of its length (Kim et al. 1991; NRC 1993). Others believe amino acid requirements should be expressed as a proportion of total dietary protein on a DM basis or in relation to the digestible energy (DE) content of the diet (Cowey and Cho 1993; Rodehutscord et al. 1997). However, it has been established that all fish examined have a requirement for 10 EAA; ariginine, histidine, isoleucine, leucine, lysine, methionine, threonine, tryptophan, phenylalanine and valine (Trushenski et al. 2006). While the exact requirements for EAA has not been determined in most fish species, the EAA requirements for rainbow trout have been recently reported by Encarnacao and Bureau (2009) (Table 2.1).

As previously mentioned, fishmeal has been traditionally used for fish feed formulation as the main protein source since its amino acid profile mimics the whole-body amino acid profile of the animal being fed (Trushenski et al. 2006). Due to fishmeal is a limited resource for carnivorous fish diets the use of protein as an energy source should be reduced by increasing the lipid (> 30 %) and energy (> 20 MJ kg^{-1}) contents of salmonid diets and reducing carbohydrate content (10 - 15 %) (Azevedo et al. 2004).

2.1.2 Lipids

Dietary lipids play an important role in almost every physiological process (Trushenski et al. 2006). Dietary lipids are the most economical sources of metabolic energy for all fish, particularly cold-water fish that have a restricted ability to use carbohydrates for energy (NRC 1993; Trushenski et al. 2006).

Dietary lipids are also a source of essential fatty acids. Some fatty acids are generally catabolised for energy while others are reserved for other purposes (Trushenski et al. 2006). Fish can neither synthesize linoleic (18:2n-6) nor α-linolenic (18:3n-3) acids *de novo*. These polyunsaturated fatty acids (PUFA) must therefore be supplied in the diet (NRC 1993; Trushenski et al. 2006). Marine fish have the capacity to produce highly unsaturated fatty acids (HUFA) such as eicosapentaenoate (EPA, 20:5n-3) and docosahexaenoate (DHA, 22:6n-3) from linolenic acid and arachidonate (ARA, 20:4n-6) from linoleic acid (Trushenski et al. 2006). However, marine fish are unable to produce HUFA from PUFA at a physiologically relevant rate (Trushenski et al. 2006). The essential fatty acid requirements of fish are thus related to their ability to modify PUFA metabolically and vary widely according to species (NRC 1993; Tocher 2003).

In fish, EPA, DHA and ARA have biochemical, cellular and physiological functions that can be divided into two categories: 1) maintaining cell membrane structure and function and 2) role as precursors of eicosanoids (Sargent et al. 1999). These compounds have several relevant functions within the body such as cardiovascular modulation, immunity and inflammatory response, renal and neural function and reproduction (Trushenski et al. 2006). The essential fatty acids requirements are easily supplied by fish oil due to its high content of HUFA, but the use of fish oil causes the same concern as fishmeal for future expansion of aquaculture industry due to

Table 2.1 Essential amino acid (EAA) requirement of rainbow trout as percentage of diet, g/MJ digestible energy (DE) and protein (g/16g N) as minimal protein levels based on sum of EAA plus non-essential amino acid (NEAA).

Amino acid	Requirement as percentage of diet	Requirement as g/MJ DE	Requirement as percentage of protein
Arginine	1.5	1.0	4.4
Histidine	0.7	0.5	1.6
Isoleucine	0.9	0.6	2.0
Leucine	1.4	0.9	3.6
Lysine	1.8	1.2	4.8
Methionine + Cysteine	1.0	0.7	3.3
Phenylalanine + Tyrosine	1.8	1.2	5.3
Threonine	0.8	0.5	2.0
Tryptophan	0.2	0.1	0.6
Valine	1.2	0.8	5.3
Sum EEA	11.3	7.5	-
NEAA	11.3	7.5	-
Total	22.6	15.1	-

NOTE: Dashes indicate data or information were not available

Adapted from Encarnacao and Bureau (2009)

its limited supply. Consequently, alternative plant lipid sources are being investigated as replacements for fish oil in aquafeeds.

2.1.3 Carbohydrate

Carbohydrates are poorly utilized by carnivorous fish due to evolutionary adaptation of digestive and metabolic processes (Shiau 1997; Lee et al. 2003). Therefore, carnivorous fish are considered to be glucose intolerant (Wilson 1994; Hemre et al. 2002). The utilization of carbohydrates is also affected by fish species (NRC 1993; Hua and Bureau 2009). Hemre et al. (2002) mentioned that cold-water carnivorous fish could have low capacity to digest raw starch compared to warm-water herbivorous or omnivorous fish. This poor utilization of carbohydrates is linked with low activity of α-amylase in the gastrointestinal tract and the complexity and level of inclusion of dietary carbohydrate (Storebakken et al. 1998a). For example, salmonids absorb glucose well, but are less efficient at utilizing dextrin or starch (NRC 1993). Further, raw starch is poorly digested by fish, but heat treatment of the starch by micronization or extrusion increases its digestibility (Drew et al. 2007). Although, enzymes and pathways for glucose metabolism have been found, the role of dietary carbohydrates providing the total energy requirement of fish remains unclear. For instance, if no carbohydrate is provided in the diet, protein and lipids are catabolized for energy with no resulting metabolic problems. Although, no dietary requirement for carbohydrate has been established for fish, they may be included in the diet as an inexpensive source of dietary energy while still regarding the maximum tolerable concentration by fish species and its role as a pellet binder in aquaculture feeds.

2.1.4 Fibre

Fibre is non-digestible plant material composed of complex carbohydrates such as cellulose, hemicellulose, lignin, pentosans and others. These components are not digested by fish due to the lack of the enzyme cellulase. Therefore, fibre does not have any positive effect on fish but it provides physical bulk to the feed (NRC 1993). Dietary fibre has been shown to increase gastric evacuation time, fecal output and reduce nutrient utilization (NRC 1993; Trushenski et al. 2006). Nevertheless, when fibre is used in carnivorous fish feeds as a binder improved fecal pellet stability is achieved (Trushenski et al. 2006). Most fish can tolerate up to 8 % of fibre content in

their diets (NRC 1993). Hence, alternative protein source should have low fibre content to limit the total fibre content of the diet.

2.1.5 Minerals

Minerals are essential for fish and have several key functions such as contributing to skeletal structure, participating in cellular electron transfer, regulation of physiological acid-base equilibrium, and osmoregulation (NRC 1993). Determination of mineral requirements is complicated as fish absorb appreciable quantities of some minerals not only from their diet but also from water through the gill epithelium (Cowey and Cho 1993). Dietary requirements have been reported for macro minerals (calcium, phosphorus, magnesium, sodium, potassium and chlorine) and for trace elements (iron, copper, zinc, manganese, selenium, iodine, fluorine and chromium) in fish nutrition (NRC 1993). However, quantitative dietary requirements have been reported for only nine minerals (calcium, phosphorus, magnesium, zinc, iron, copper, manganese, iodine and selenium) (NRC 1993; Lall 2007). Mineral deficiencies in fish include many different signs such as reduced bone mineralization, poor growth, anorexia, skeletal deformities, fin erosion, nephorocalcinosis, thyroid hyperplasia and muscular dystrophy (NRC 1993; Lall 2007). In addition, trace minerals are potentially lethal in amounts slightly above requirements, especially when they are present in water. Also, an excess of certain dietary minerals, particularly phosphorus can contribute to environmental eutrophication (Trushenski et al. 2006). Thus, our knowledge of the mineral nutrition of finfish must be improved for both nutritional and environmental reasons.

2.1.6 Vitamins

Vitamins are described as dietary organic compounds, required in trace amounts for normal growth, reproduction and health (NRC 1993). It has been established that fish require 11 water soluble and 4 fat soluble vitamins, but quantitative requirements have been determined for only a few species and are typically based on only one life phase (e.g. growth) (NRC 1993; Trushenski et al. 2006). In addition, every vitamin deficiency is associated with specific symptoms, including reduced growth, lethargy, anaemia, scoliosis, haemorrhages and mortality (NRC 1993). However, most symptoms of vitamin deficiency are present as a non-specific reduction in

growth performance. Vitamin requirements are influenced by size, age, environmental factors and nutrient interrelationships (NRC 1993). Therefore, more studies are needed to determine vitamin requirement of fish. In conclusion, potential replacements for fishmeal must possess certain characteristics that meet the nutritional requirements of fish as well as being widely available at an economical cost.

2.2 Nutritional Characteristics of Alternatives Protein Sources for Aquafeeds

Potential alternatives to fishmeal in aquafeeds must have qualities similar to fishmeal to be considered for formulation of cost-effective, practical aquafeeds. These qualities include high digestibility, low levels of carbohydrates and ANF, low nutrient variability, protein content equal to or greater than fishmeal, a good amino acid profile and finally, palatability.

The nutritional value of a feedstuff is based not only on its chemical composition but also on its digestibility (Gomes da Silva and Oliva-Teles 1998). Digestibility directly affects the potential availability of energy and nutrients for maintenance, growth and reproduction by the animal and also provides essential information for correct diet formulation (Cho 1993; Gomes da Silva and Oliva-Teles 1998). Digestibility and metabolizability have been used to express feedstuff values for fish (NRC 1993). The term digestibility involves the fraction of the nutrient or energy in the ingested ingredient that is not excreted in the feces while the metabolizable energy describes the fraction of digested energy not excreted in the urine and through the gills (NRC 1993). Nevertheless, DE is more appropriate since obtaining the metabolizable energy values for fish presents extreme difficulties due to energy and nutrient loss across the gill epithelium. The ADC of individual ingredients are additive and thus, the digestible nutrient content of a diet can be predicted from ADC both from a diet and from the sum of the ingredients in the diet along with amount of feed fed (Cho 1993). Consequently, most fish nutritionists prefer using digestibility coefficients to evaluate individual ingredient for fish diets.

ADC for ingredients are commonly calculated by an indirect measurement of the amount of nutrient ingested and excreted using non-digestible markers such as celite or chromic oxide (NRC 1993). This method compares the digestibility of a reference diet with that of a test diet, obtained by blending a proportion of reference diet that contains the marker, with a percentage of the test ingredient. This method assumes there are no interactions among dietary ingredients during digestion; an assumption that is not always true. For example, when ingredients possess

high levels of carbohydrates or when ANF are present, these components may reduce the digestibility of other ingredients (Gomes da Silva and Oliva-Teles 1998). In general, comparing different protein sources might consider digestible nutrient content and presence of ANF among others.

2.2.1 The Nutritional Value of Plant Proteins in Aquafeeds

Plant protein sources have the advantage of greater availability and more competitive prices compared to fishmeal (Oliva-Teles and Pereira 2002). However, the common plant protein sources used in aquafeeds such as soybean meal (SBM), canola meal (CM), peas and flax have a lower, protein content and poorer amino acid balance compared to fishmeal (Drew et al., 2007). Plant protein sources also have ANF that reduce feed intake and fish growth (Webster et al., 1995; Thiessen et al., 2003a; Thiessen 2003b; Drew et al., 2007). In addition, there are some difficulties in balancing the fatty acid composition of plant source diets. Hence, inclusion of supplemental AA and enzymes are generally used to improve the nutritional value of plant proteins based diets. Thus, the main drawbacks using plant proteins in fish diets are the presence of ANF, low palatability and unbalanced amino acid profile (Oliva-Teles and Pereira 2002).

Gatlin et al. (2007) described the nutritional properties that plant protein sources should possess to partially or completely replace fishmeal (Table 2.2). These nutrient content levels cannot be achieved when using many common plant protein sources thus the inclusion rate of plant proteins in aquafeeds has been restricted due mainly to the presence of ANF. Nevertheless, there are some available processing techniques (heat treatment, fractionation etc.) that are able to improve the nutritional value of plant proteins by destroying or reducing ANF.

2.2.1.1 Soybean Meal

SBM is the most widely used source of protein in salmonid feeds (Refstie et al. 2000; Drew et al. 2007). When added to extruded aquaculture diets SBM improves the physical characteristics of feed including breaking force, bulk density, durability, AA profile and competitive price (Webster et al. 1995; Refstie et al. 2000; Drew et al. 2007; Sorensen et al. 2009). Soybean products contain approximately 48 % CP (Hardy 2000). Therefore, soybean products are desirable plant ingredients for replacing fishmeal in salmonid diets. In spite of the positive

Table 2.2 Proximate and nutrient content (as-fed basis) of fishmeal, and targeted ranges in alternative feed ingredients derived from grains and oilseeds.

Category/nutrient (%)	Fishmeal (menhaden)	Target range for alternative ingredients
Crude protein	65 - 72	48 - 80
Crude lipid	5 - 8	2 - 20
Fibre	< 2	< 6
Ash	7 - 15	4 - 8
Starch	< 1	< 20
Non-soluble carbohydrates	None	< 8
Arginine	3.8	> 3.0
Lysine	4.7	> 3.5
Methionine	1.8	> 1.5
Threonine	2.5	> 2.2

Adapted from Gatlin et al. (2007)

nutritional attributes of SBM there have been problems feeding SBM in salmonid diets at inclusion levels above 200-300 g kg^{-1}. Above these levels, SBM decreases growth and feed intake and causes morphological changes in the intestinal epithelium that are described as inflammatory responses (Rumsey et al. 1994; Refstie et al. 2000; Krogdahl et al. 2003; Barrows et al. 2007; Drew et al. 2007).

SBM contains many ANF that may affect the digestive process (Refstie et al. 2000). These may be divided into 2 categories: heat-labile and heat-stable secondary compounds (Refstie et al. 2000; Drew et al. 2007). Heat-labile secondary compounds consist of protease inhibitors ("Kunitz type" and "Bowman-Birk type"), phytates, goitrogens, antivitamins and lectins (Dabrowski et al. 1989; Rumsey et al. 1994; Hardy 2000; Francis et al. 2001; Drew et al. 2007). Heat-stable secondary compounds include non-starch polysaccharides (NSP), saponins, phytoestrogens, lysinoalanine, antigenic proteins and some phenolic compounds (Dabrowski et al. 1989; Hardy 2000; Francis et al. 2001; Barrows et al. 2007; Drew et al. 2007). Heat processing of SBM can eliminate or decrease its heat-labile constituents (Barrows et al. 2007; Drew et al. 2007). Consequently, extrusion cooking of SBM increased growth performance, and digestibility of DM and GE in rainbow trout as well as palatability and pellet stability (Cheng and Hardy 2003; Barrows et al. 2007). Heat treatments must be performed carefully due to the risk to decrease protein degradability and AA availability (Francis et al. 2001). In contrast, heat stable ANF must be physically removed from soybean by fractionation. Fractionation of soybean can potentially produce two protein concentrates; the first one is named soy protein concentrate (SPC) that contains around 700 g kg^{-1} CP and the second one is called soy protein isolate with about 900 g kg^{-1} CP (Drew et al. 2007). Both products have decreased levels of NSP, fibre and saponin content compared to SBM, and thus, high nutritional value to fish (Drew et al. 2007). In conclusion, further processing of soybean is fundamental to improve its nutritional value in aquatic feeds.

Protease inhibitors are well-known ANF in many plants that may occur in aquatic feeds. Trypsin inhibitors in commercial soybean products range from 2 - 6 mg g^{-1} (Francis et al. 2001). However, tilapia, rainbow trout, channel catfish, salmon and seabream are able to compensate for the presence of trypsin inhibitors at levels below 5 mg/ g by increasing trypsin production (Francis et al. 2001).

Phytate is widespread in plant seeds and is the storage form of phosphorus in plants (Francis et al. 2001; Drew et al. 2007). It cannot be metabolized by fish, thus phytate phosphorus is unavailable to them. Phytate is also capable of chelating di- and trivalent mineral ions and consequentially it decreases their availability (Duffus and Duffus 1991; Drew et al. 2007). SBM contains around 10 - 15 g kg^{-1} phytate (Francis et al. 2001). High levels of phytate in salmonid diets reduces feed and protein utilization and disrupts thyroid function (Kissil et al. 2000). Moreover, inclusion of phytate in diets decreased growth in rainbow trout (Spinelli 1983), juvenile Chinook salmon (Richardson et al. 1985) and common carp (Hossain and Jauncey 1993). Francis et al. (2001) reported salmonids are able to tolerate dietary levels of phytate of 5 - 6 g kg^{-1}. Therefore, it was recommend to maintain phytate content below 5 g kg^{-1} in salmonid diets. Supplementation of diets with the enzyme phytase increased the ADC of P, Ca, Mg, Zn and protein (Storebakken et al. 1998b; Francis et al. 2001; Riche and Garling 2004; Drew et al. 2007; Sorensen et al. 2009). Thus, adding phytase to fish diets is highly recommended to improve availability of P and reduce the eutrophication caused by fish farm effluents.

Some SBM proteins are antigenic and may cause allergic-type reactions in the gut of salmonid fish. For example, glycinin and beta conglycinin are allergens in several animals and resistant to digestion in the intestinal tract (Francis et al. 2001; Drew et al. 2007). Antigenic proteins are able to cause intestinal mucosal lesions, abnormalities in the villi and abnormal movement of digesta through the gut (Rumsey et al. 1994; Francis et al. 2001). The persistence of these proteins may explain why heat treated soybean still negatively affects growth performance of salmonid fish (Francis et al. 2001). Rumsey et al. (1994) found rainbow trout fed diets high in levels of glycinin and beta conglicinin exhibited poor growth compared with a fishmeal basal diet and a SPC diet. However, rainbow trout fed the SPC diet showed poorer growth than the reference diet. Therefore, it was suggested the antigenic proteins still remain in SPC. In conclusion, SBM is commonly used in animal diets but its ANF reduce the growth performance in salmonids. Therefore, there is a need to develop processing methods to reduce its ANF and improve its nutritional quality.

2.2.1.2 Canola/Rapeseed Meal

Canola refers to Canadian varieties of rapeseed low in erucic acid (< 2 % in the oil fraction) and aliphatic glucosinolates (< 30 μmoles/g of air-dried, oil-free meal) (Higgs et al. 1995; Thiessen

2004; Klein-Hessling 2007). Canola ranks second behind soybeans in global production of protein from oil cakes and meals with approximately 46 million tonnes in 2004 (Mwachireya et al. 1999; Klein-Hessling 2007). CM is the residual product from solvent extraction of canola oil and is extensively available around the world (Thiessen 2004; Klein-Hessling 2007). CM has an amino acid balance similar to fishmeal while its cost on a per unit of protein basis is lower than SBM (Higgs et al. 1995). However, there are some potential problems associated with the use of CM in farmed salmon diets. As with SBM, CM contains ANF such as phytic acid, glucosinolates, tannins, phytates, phenolic compounds and fibre that may affect the nutritional value of CM in salmonid diets.

Several processing methods have been developed to improve the nutritional value of canola. Mansour et al. (1993) demonstrated heat processing reduced ANF in rapeseed products. Glucosinolates were reduced from 47 to 94 %, phytic acid from 9 to 43 % and tannic acid from 41 to 67 % after toasting and autoclaving treatments. Thiessen et al. (2003a) reported that sieving traditional CM using a 60 mesh screen reduced fibre, increased CP content and produced a final product called "CM fines". The latter had 429 CP g kg^{-1} and ADC of 0.72 for DM and 0.79 for GE in rainbow trout. Aqueous extraction of protein may also be used to remove ANF and produce a high quality canola protein concentrate with 724 CP g kg^{-1} (Thiessen 2004). This had an ADC of 0.8 % for DM, 0.89 for CP and 0.86 for GE in rainbow trout (Thiessen 2004). In general, fractionation of canola products significantly improves the nutritional quality of canola protein in aquaculture feeds.

2.2.1.3 Lupins

Generally, three lupin species (*Lupinus albus*, *Lupinus angustifolius* and *Lupinus luteus*) are produced and used as feed ingredients (Hawkins et al. 2008) with Australia producing around 80 % of the world production (Smith et al. 2007). Lupin seeds contain high protein (350-500 g kg^{-1} DM basis) and lipid content (80 - 100 g kg^{-1}) (Drew et al. 2007). They do not have lectins or protease inhibitors but they do contain some heat stable secondary compounds such as NSP, saponins, protein antigens, phytoestrogens and quinolizidine alkaloid that reduce its nutritional value (Aniszewski 1993; Friedman 1996; Francis et al. 2001). Lupins are useful as a replacement for fishmeal in fish diets (Carter et al. 2004; Hawkins and Glencross 2004). Traditionally, the lupin hulls are removed to reduce the fibre content and the resultant lupin kernel meals may be

used in fish feeds (Glencross et al. 2008; Hawkins et al. 2008). As with SBM, high levels (> 50 %) of lupin kernel meal reduces growth in rainbow trout (Glencross et al. 2004). New varieties of lupin seed containing lowered content of alkaloids and devoid of the highly toxic alkaloid anagyrine are being developed (Aniszewski 1993). Carter et al. (2004) evaluated the effect of protein concentration on the nutritional value of lupin kernel meal and found the ADC of nitrogen of unprocessed lupin kernel meal and digestibility of lupin kernel meal were both higher than 95 % in rainbow trout. However, digestibility of organic matter and GE was low 64 % and 70 %, respectively. It was suggested this could have been due to high levels of NSP in the meal. Aqueous fractionation of lupin kernel meal produced a lupin protein concentrate with a protein 690 g kg^{-1} CP and a lupin protein isolate with 810 g kg^{-1} CP (Carter et al. 2004). Digestibility of organic matter and GE of lupin protein concentrate and lupin protein isolate ingredients were significantly higher than lupin meal. However, there were no significant differences in digestibility of CP among lupin products. It can be concluded high protein lupin products have exceptional potential as alternative protein sources in aquafeed.

2.2.1.4 Peas

Field peas rank fourth in the world production of grain legumes (Drew et al. 2007). Peas contain an average 250 g kg^{-1} CP, 500 g kg^{-1} starch and they are also high in lysine but low in lipid 14 g kg^{-1} (Burel et al. 2000; Drew et al. 2007). Peas contain heat-labile (e.g. trypsin and lectins) and heat-stable (phytic acid, saponins, antivitamins and the protein antigens legumin and vicilin) secondary compounds (Francis et al. 2001; Drew et al. 2007). However, peas have low levels of the secondary compounds compared to other grain legumes (Drew et al. 2007).

Processing techniques have been used to improve the nutritional qualities of peas including dehulling to remove indigestible xylans and cellulose and more complex methods such as air classification and heat treatments (e.g. extrusion and micronization) to decrease ANF (Gomes et al. 1995; Thiessen et al. 2003a; Booth and Allan 2004; Schulz et al. 2007).

The hull of the pea comprises 7 – 14 % of the seed weight and consists of mainly cellulose and xylan (Drew et al. 2007). Dehulling increased the digestibility of peas in silver perch around 13 % for DM, OM, GE and 9 % for CP (Booth and Allan 2004). Rainbow trout fed diets with dehulled pea seed meal contributing up to 20 % of the dietary protein content, had increased growth performance and feed intake compared to fishmeal alone (Teles et al. 1993).

Air classification produces a protein concentrate (560 g kg^{-1} CP) with lowered levels of ANF such as protease inhibitors and phytic acid (Owusu-Ansah and McCurdy 1991). Aqueous fractionation results in a purer protein fraction, called pea protein isolate (800-900 g kg^{-1} CP) with further reduction in levels of ANF (Schulz et al. 2007). Thiessen et al. (2003a) evaluated air classification of dehulled peas, with autoclaving and reported the ADC for CP increased from 0.91 in raw dehulled peas to 0.95. There were also improvements of ADC for fat (from 0.68 to 0.86) and for starch (from 0.25 to 0.66). Booth and Allan (2004) examined the effects of extrusion on the digestibility of peas in silver perch and found the ADC of DM, CP, GE increased from 0.56, 0.82, 0.56 in raw whole peas to 0.70, 0.84 and 0.71 in extruded whole peas, respectively. It was suggested the improvement in ADC of GE and DM was caused by an increase in starch digestibility (Drew et al. 2007). Burel et al. (2000) reported the ADC of starch in extruded peas was 0.83 in rainbow trout and 0.75 in turbot. In another study, the ADC of starch in extruded dehulled peas was 1.00 in rainbow trout (Thiessen et al. 2003a). Schulz et al. (2007) evaluated pea protein isolate on tilapia without supplementation of essential synthetic AA and found fishmeal can be replaced up to 30 % with isolated pea protein without significant effects on the growth performance of tilapia. In conclusion, processed peas may be included up to 30 % in fish diets without negative effect on growth performance.

2.2.2 The Nutritional Value of Animal Proteins in Aquafeeds

2.2.2.1 Rendered Animal Proteins

Rendered animal protein ingredients such as blood meal (BM), feather meal (FEM), meat and bone meal (MBM) and poultry by-product meal (PBM) have been investigated as protein sources in salmonid diets (El-Haroun et al. 2009). However, their inclusion in fish diets has been restricted due to highly variable nutrient content and digestibility (Bureau et al. 2000). There are several factors that affect their nutritional quality such as the composition and freshness of the raw materials as well as the heat treatment used for cooking and drying (Bureau et al. 1999). Overall, the source and processing conditions drastically affect amino acid availability in rendered products (Rawles et al. 2006). Renderers have begun to improve their manufacturing practises since the price of animal protein sources has increased but the nutrient composition of

these ingredients still fluctuates (Bureau et al. 1999). For example, FEM, PBM and MBM from various origins were tested in rainbow trout and significant differences in the ADC for protein and energy were observed between sources (Bureau et al. 1999).

In spite of the nutritional variability of animal protein ingredients, they have been used successfully in diets for various fish species. Chinook salmon fed diet with PBM without supplementation of AA up to 20 % did not show any differences in weight gain and feed efficiency from fish fed a diet without PBM (Fowler 1991). SGR and weight were not significantly different between chinook salmon fed a diet with 15 % FEM and fish fed a diet without FEM (Fowler 1990). The protein quality of FEM was improved by fermentation, and fermented FEM could replace fishmeal at 25 % in the diet of hybrid clarias catfish (Rakyuttithamkul and Arunlertaree 2006). A mixture of PBM and FEM at an inclusion level of 27 % was able to replace fishmeal in rainbow trout (Steffens 1994). MBM was tested in gilthead seabream and it was concluded MBM can replace fishmeal at 20 % without any negative effect on feed utilization parameters (Robaina et al. 1997). A blend of BM, PBM and MBM successfully replaced 67 % of the fishmeal DM in gibel carp diets (Wang et al. 2008a). In general, rendered animal protein ingredients at levels of 5 – 25 % have successfully replaced fishmeal in high fishmeal control diets (El-Haroun et al. 2009). However, the latter authors pointed out the diets used in many of these studies did not represent diets commonly used in the aquaculture industry. Therefore, they tested spray-dried BM, FEM, MBM and PBM in practical diets on growth, feed efficiency ratio and body composition of rainbow trout and concluded all these rendered animal protein ingredients have excellent nutritive value and can be used, individually or combined, in salmonid diets to replace fishmeal.

2.2.2.2 Worm and Larval Insect Proteins

Worms have a generally high protein content that makes them suitable alternative animal protein sources to fishmeal (Liew et al. 2001). Although, mealworm is an unconventional protein source for fish, it has proved its potential in aquaculture. Worm meal from the common earthworms (*Eisenia fetida*) supported high growth rates in rainbow trout at an inclusion level of 500 g kg^{-1} (Velasquez et al. 1991). Mealworm, which is the larvae of *Tenebrio molitor*, was demonstrated to be a potential protein source for the African catfish (Liew et al. 2001). The mealworm was ground into powder and the resulting product was called meal-worm meal (MWM). The

composition of MWM on DM basis was 57.6 % CP, 28.6 % lipid, 4.5 % ash and 6.9 % crude fibre. They reported that MWM could replace up to 40 % fishmeal in catfish diets without affecting growth performance and feed utilization efficiency. Some reduction in growth performance and feed and protein utilization was seen in catfish fed diet with high levels (> 60 %) of MWM. It is suggested this may be caused due to the presence of chitin. Chitin is a polymer of glucosamine that is part of shells or walls of invertebrates (e.g. worms), fungi and yeasts (Shiau and Yu 1999; Liew et al. 2001; Gopalakannan and Arul 2006). Since chitin is a NSP it is regarded as a fibre component (Shiau and Yu 1999; Liew et al. 2001). The presence of chitin as low as of 2 % in diets decreased DM digestibility and had negative effect on growth performance and feed utilization efficiency in tilapia (Shiau and Yu 1999). Chitin supplementation depressed the growth of common carp (*Cyprinus carpio*) compared to the fish fed diet without supplementation of chitin (Gopalakannan and Arul 2006). In contrast, no growth depression was reported in other species such as sea bream, Japanese eel and yellowtail when these fish were fed diets supplemented with 10 % chitin (Kono et al. 1987). However, this study was criticized because the reference diet of this study was a commercial eel diet and the amount and the source of fibre in the commercial diet was unknown (Shiau and Yu 1999).

Worms as live feeds have also been used for the feeding of larval fish when nutrient requirement are unknown or the use of formulated dry diets has been unsuccessful. It is known that high mortality rates are observed in some carnivorous fish larvae fed artificial feed since they refused artificial diets or lack some enzymes involved in the digestive process (Liew et al. 2001). Several species of worms such as live aquatic oligochaete, earthworms, tubificids, worm slices of *Allolobophora Ionga* and *Lumbricus terrestis* have been used for fish nutrition (Stafford and Tacon 1984; De Silva 1989; Velasquez et al. 1991; Knights 1996). In conclusion, both worms and larval insect proteins have proved to be viable alternative protein sources.

2.2.2.3 Single Cell Proteins

Historically, single cell proteins (SCP) from microalgae, bacteria, and yeast have been the least studied of all alternative protein sources (McLean and Craig 2007). They have a high content of proteins, B-vitamins, pigments, complex carbohydrates (e.g. glucans) and a low level of phosphorus (Oliva-Teles and Goncalves 2001; McLean and Craig 2007). SCP from yeasts have been extensively used in aquafeed (Oliva-Teles and Goncalves 2001). It is believed some yeasts

such as *Candida* sp. and *Saccharomyces cerevisae* have immunostimulatory properties due to their complex carbohydrate components and nucleic acid content (Anderson et al. 1995). SCP is deficient in one or more EAA but the supplementation of yeast-based diets with synthetic AA had positive effects on fish growth (Kiessling and Askbrandt 1993; Oliva-Teles and Goncalves 2001). Rumsey et al. (1991) reported growth and feed utilization were not depressed in rainbow trout fed diets containing up to 25 % of brewer's dried yeast. Nevertheless, palatability was a concern since fish fed diets with high levels of SCP (50 and 75 %) yeast refused to eat their feed.

There has recently been interest in using bacterial protein meal (BPM) produced by bacteria grown on natural gas as an alternative protein for fish feed (Storebakken et al. 2004; Aas et al. 2006a; Aas et al. 2006b; Overland et al. 2006; Aas et al. 2007; Mydland et al. 2008). SCP from the fermentation of *Methylococcus capsulatus* is the main component of BPM but *Methylococcus capsulatus*, *Alcaligenes acidovorans*, *Bacillus brevis* and *B. firmus* have also been investigated (Aas et al. 2006b; Overland et al. 2006; Aas et al. 2007). BPM contains approximately 96 % DM, 70 % CP, 10 % crude lipid, 7 % ash and 10 % nucleic acids (Aas et al. 2006a; Overland et al. 2006). BPM has a CP content and amino acid profile similar to fishmeal except for a low lysine content. Therefore, it has an adequate amino acid composition for farmed salmonids and other monogastric livestock (Storebakken et al. 2004; Overland et al. 2006; Mydland et al. 2008). The nutritional value of BPM as a potential replacer of fishmeal has been successfully evaluated in Atlantic salmon and rainbow trout (Storebakken et al. 2004; Aas et al. 2006a; Overland et al. 2006). BPM did not decrease growth and feed efficiency in rainbow trout and Atlantic salmon fed diets containing up to 36 % BPM (Aas et al. 2006a; Aas et al. 2006b). However, the digestibility of nitrogen, AA, lipid and GE of BPM are low compared to those of fishmeal reference diet in Atlantic salmon (Storebakken et al. 2004; Aas et al. 2006a). Besides the lower digestibility values, the high content of nucleic acids and copper in SCP is another concern (Rumsey et al. 1991; Aas et al. 2006a). Nucleic acids in SCP from bacteria and yeasts range from 8 to 16 % and 5 to 12 %, respectively (Oliva-Teles and Goncalves 2001). Nucleic acid nitrogen is mainly present in the form of ribonucleic acid and for example, it constitutes about 25 % of the nitrogen content in brewers yeasts (Rumsey et al. 1991). In most monogastric animals, high levels of dietary nucleic acids is toxic and may lead to elevated plasma concentrations of uric acid from nucleic acid degradation due to a restricted capacity for the excretion of uric acid (Rumsey et al. 1991; Aas et al. 2006a; Aas et al. 2006b). In spite of the

detrimental effect shown in other monogastrics, fish fed diets containing high dietary nucleic acid content were able to adapt by increasing the activity of the urate uricase (Rumsey et al. 1991; Andersen et al. 2006). Nucleic acid may also be involved in diet palatability, fish feeding behaviour, biosynthesis of NEAA and improved growth in early stages of development, immunity and disease resistance of fish (Li and Gatlin 2006).

Copper is an essential element for fish metabolism but when it is fed above requirement it may produce toxic effects (Hylland et al. 1999). Although, BPM is high in copper, it has not shown any apparent negative effect on fish gut health and growth compared to plant proteins such as SBM (Berge et al. 2005; Aas et al. 2006a; Aas et al. 2006b).

In general, SCP has the potential to be an appropriate replacement for fishmeal in aquafeeds.

2.3.1 Ethanol Manufacturing Process

Ethanol production involves the conversion of sugars and/ or starch extracted from crops in (e.g. corn, wheat, barley) and sugar beets into ethanol and carbon dioxide during the fermentation process. Where the feedstock is rich in starch enzymatic hydrolysis is required to convert it to simple sugars, before or during the fermentation process. Ethanol production that uses sugar cane includes four or five steps: milling, pressing, fermentation and distillation, plus dehydration in the case of alcohol blends. When grains are used in the ethanol production process, this process in general includes the following seven steps: milling, liquefaction, saccharification, fermentation, distillation, dehydration and denaturation (Solomon et al. 2007).

The ethanol process using grain, as a source of starch is primarily divided into two processes: dry grinding (Figure 2.1) and wet milling (Figure 2.2) (Rausch and Belyea 2006). In the dry grinding process, the entire grain kernel is ground into flour (or “meal”) and processed, usually without separation of the meal into its components parts. The ground grain is mixed with water and α-amylase enzyme, which is added to break down the starch into short chain dextrins. The mixture “slurry” is processed in a high temperature cooker then cooled. A second enzyme, glucoamylase is added when the slurry is being pumped to the fermentation tanks, where the slurry is called “mash”. The latter enzyme hydrolyzes the dextrins to form simple sugars. Then, yeast is added to the fermenter to metabolize the sugar and release ethanol and carbon dioxide. After, 50 - 60 hours the fermented mash, which is referred to as beer, is pumped into distillation

columns where ethanol is separated and the residue is called whole stillage. This comprises soluble and insoluble-non-fermentable solids from the grain (ICM Inc 2010) and added yeast as well as liquid from the water added in the process. The whole stillage is sent to a centrifuge or screen for separation into suspended solids that is called wet distillers grains (WDG) that contains 30 % solids and a soluble "thin stillage" (TS) that contains about 8 % solids. The TS is sent to through an evaporator where it is concentrated into syrup called condensed distillers solubles (CDS) containing 35 % solids content. Also, in some processes a proportion of TS is recycled as makeup water (called backset) at the cook tanks, reducing the amount of fresh water required in the process. CDS may be sold into the feed market or mixed with WDG to produce "wet distillers grains with solubles" (WDGS) containing 30 % solids content. In most cases, this mixture is dried to produce distillers dried grains with solubles (DDGS) that has a 90 % solid content. In some cases the CDS is not reapplied to the WDG; then, this product is called distillers dried grains (DDG). Nevertheless, the majority of distillers grains are sold as DDGS (Leytem et al. 2008a). Quantitatively, from 100 kg of corn, approximately 40 L of ethanol, 30 - 32 kg of DGGS and 32 kg of carbon dioxide are produced (Schingoethe 2006; Cardona and Sanchez 2007).

The wet milling process has been exhaustively reviewed by Rausch and Belyea (2006). Wet milling requires high quality (No. 2 or better) corn (Klopfenstein et al. 2007). In wet milling, the crop is fractionated into its primary components (germ, fibre, and starch). The purpose of this process is to separate and recover starch to produce starch products. The first step of this process is called steeping where the grain kernel is soaked or "steeped" in water and dilute sulphurous acid for 24 to 48 hours (Figure 2.2). This step facilitates the separation of the kernel components. Light steepwater (4 – 8 % solids) is the resulting product from steeping. This product is concentrated to produce heavy steepwater (35 – 40 % solids) by evaporation. Steepwater solids contain 45 - 50 % total protein mostly in form of AA. After steeping, fibre and germ fractions are separated by differences in particle size and density, respectively. The germ is removed by hydroclonic separators, pressed and dried. The residual fibre is removed using screens. The fibre is mixed with the heavy steepwater and dried together to produce corn gluten feed (CGF). The heavy steep water may also be sold as a feed ingredient. The remaining solid is further fractionated to recover protein (called gluten) and starch fraction by using centrifugal and

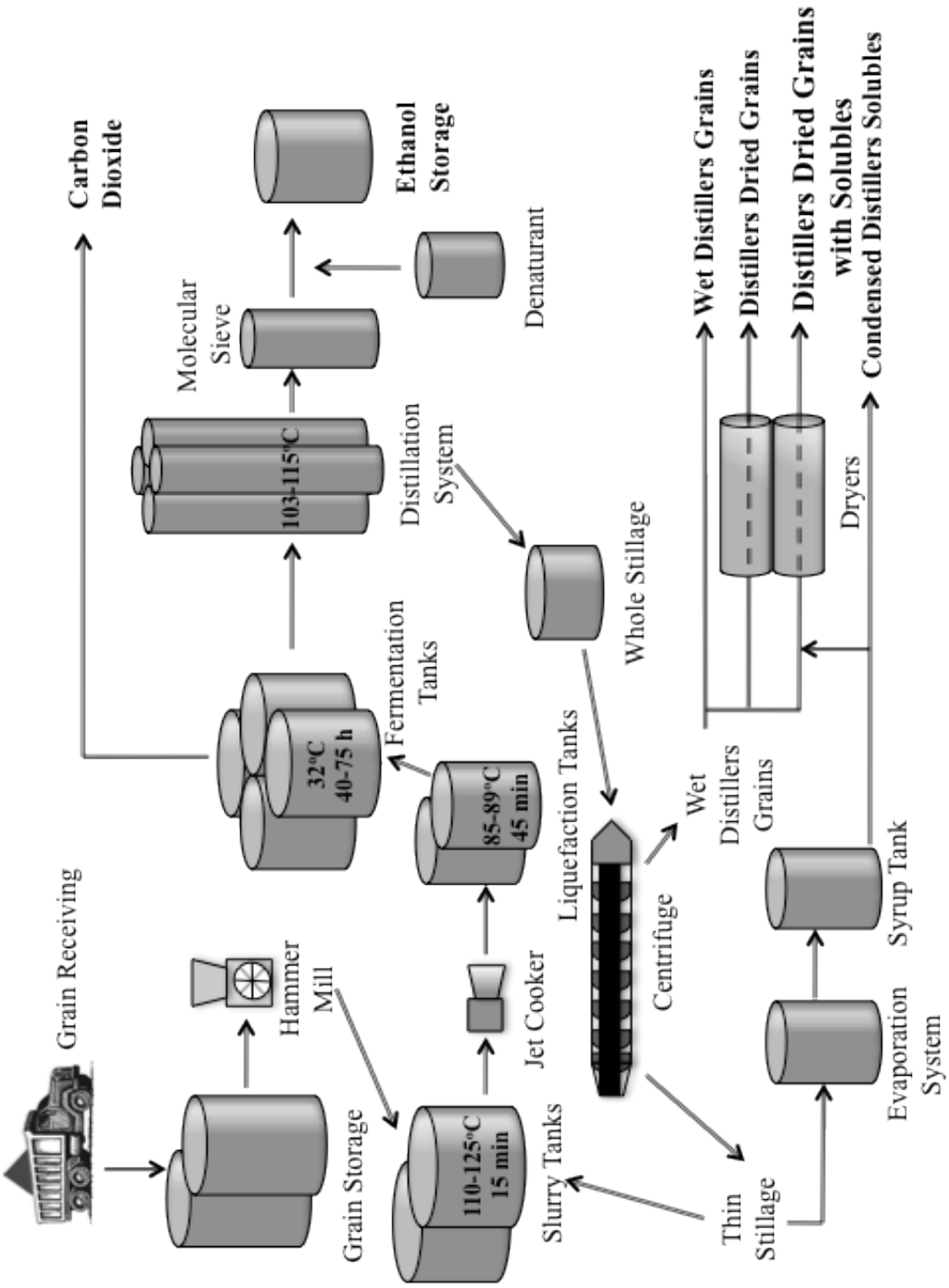


Figure 2.1 Typical dry grinding process of ethanol production. Adapted from ICM, Inc. 2010.

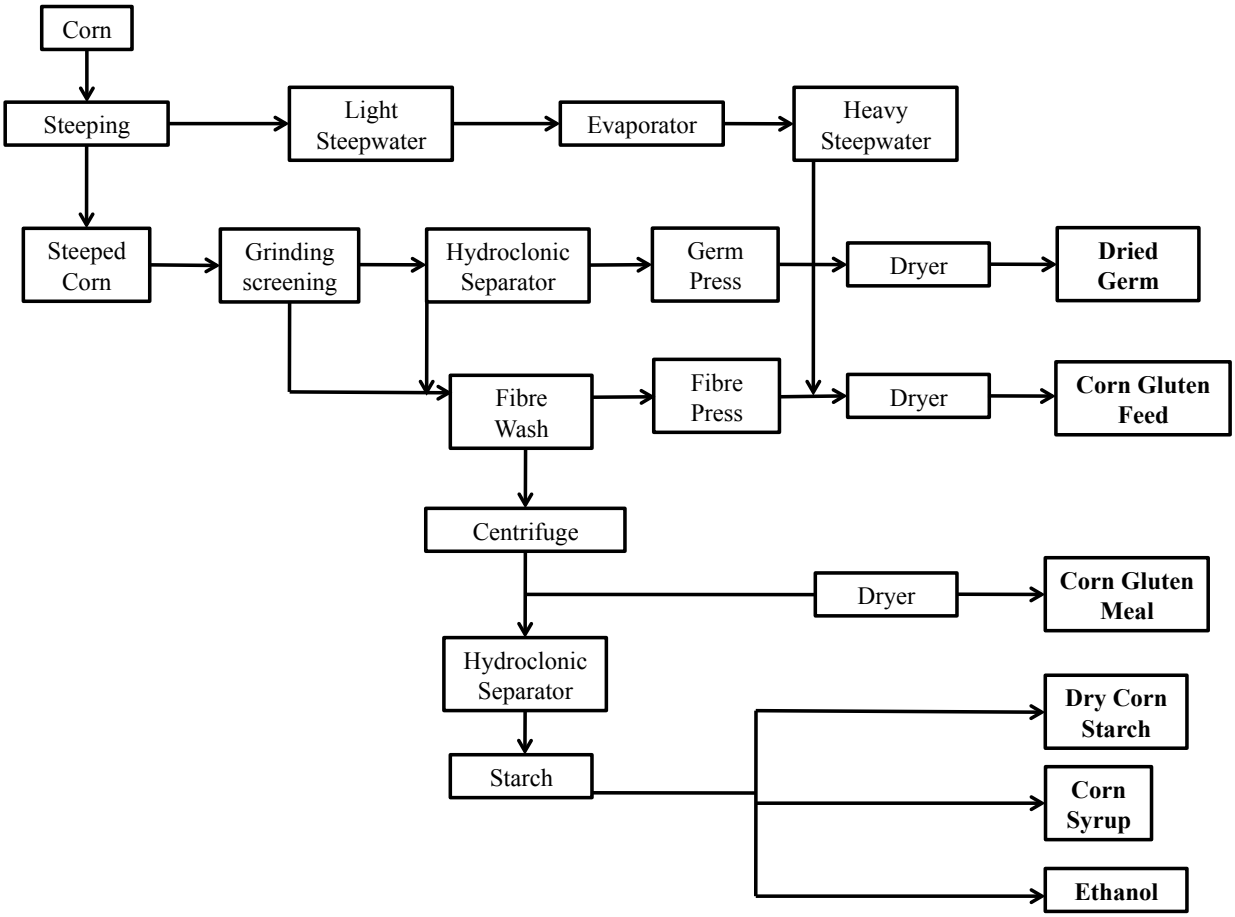


Figure 2.2 Typical corn wet milling process of ethanol production.

hydrocyclonic separators, respectively. The gluten component is centrifuged, filtered and dried to produce corn gluten meal (CGM), which contains high protein (65 – 67 % dry basis) and low fibre content. The CGM is sold as a feed ingredient for poultry and aquaculture industries. The starch and remaining water may be processed in one of three ways: dried and sold as dried starch, fermented into ethanol, or refined into corn syrup. For example, during of the wet milling processing of corn, 4.6 kg of CGM, and 24.0 kg of CGF are generated from 100 kg of corn (Cardona and Sanchez 2007). Ethanol plants in the United States (U.S.) have tended toward dry grind plants due to lower building costs (Rendleman and Shapouri 2007). The most important ethanol co-products are summarized in Table 2.3.

2.3.2. Ethanol Industry in the United States and Brazil

Brazil and the U.S. have been interested in fuel ethanol (as a means of improving energy independence) since the OPEC oil embargo triggered an economic crises in the 1970s (Figure 2.3) (Renewable Fuel Association 2009). The two countries pursued different carbohydrate feedstocks for ethanol production. Brazil due to its climate was able to develop sugar cane-based ethanol production while in the U.S corn-based is possible. The U.S. and Brazil have been promoting the biofuel industry by stimulating both production and demand for fuel ethanol. All gasoline in Brazil must have at least 25 % ethanol and ethanol provides about 40 % of the total vehicle fuel used within the country (Solomon et al. 2007). The U.S. government subsidizes the ethanol industry through federal and state policies to increase production. The federal ethanol subsidy currently stands at 13.5 cents per liter. Minnesota state has a producer tax credit around 5.3 cents per liter; all gasoline sold in this state must have 10 % ethanol content (Solomon et al. 2007; Tyner 2008).

In addition to fuel, the U.S. ethanol industry has an important role in food and feed production due to the production of large amounts of ethanol co-products that provide high-quality feed for cattle, swine and poultry. During the 2007/2008 marketing year about 23 million tonnes of ethanol co-products were produced (Renewable Fuel Association 2009). This amount is equivalent to the combined annual amount of total feed consumed by the nation's four largest feedlot states (Texas, Kansas, Nebraska and Colorado) and to the 15 % of U.S. total feed use of corn, thus, decreasing the use of whole corn by the animal feed industry. The production for 2008/2009 was approximately 30 million metric tonnes including CGF and CGM. Therefore, the

Table 2.3 Potential co-products from grain ethanol industry.

Dry grinding process	Wet milling process
Condensed distillers solubles (CDS)	Corn gluten feed (CGF)
Wet distillers grains with solubles (WDGS)	Corn gluten meal (CGM)
Distillers dried grains (DDG)	Corn syrup
Distillers dried grains with solubles (DDGS)	Heavy steepwater
	Dried starch
	Dried germ

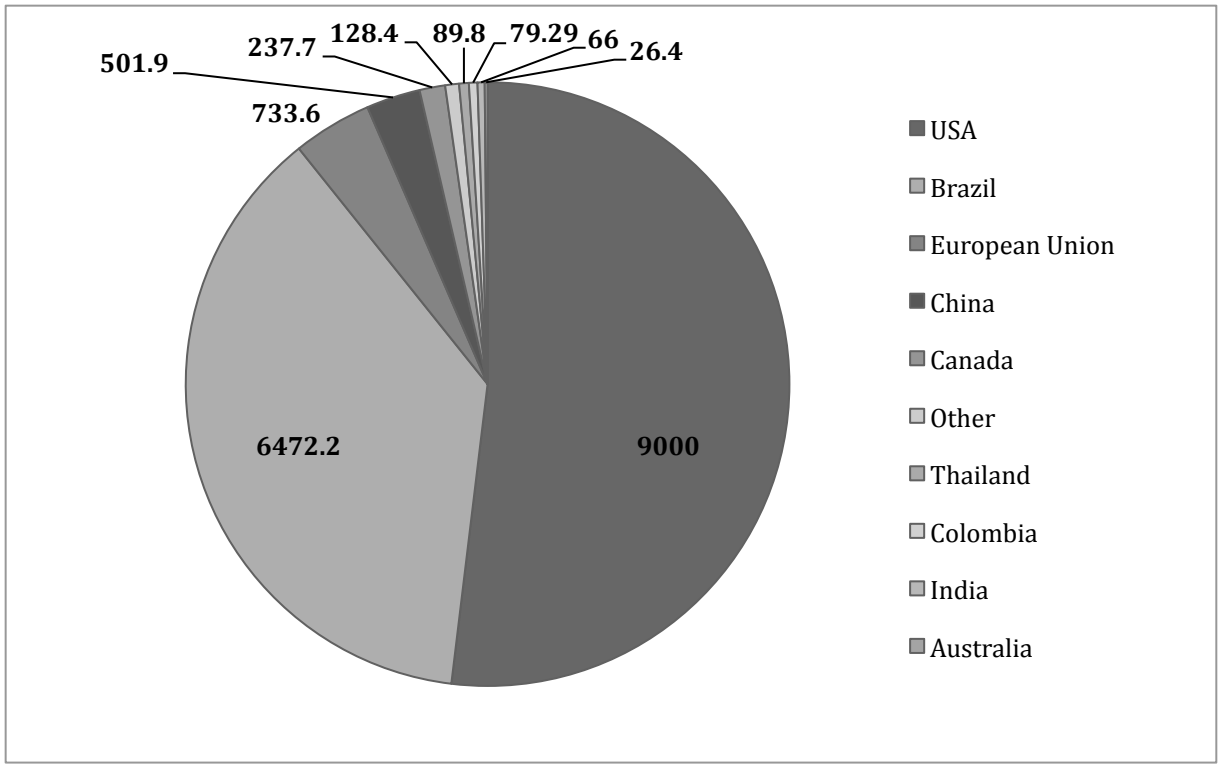


Figure 2.3 World fuel ethanol production in 2008 (Millions of U.S. gallons). Data from Renewable Fuel Association (2009).

U.S. ethanol industry will continue playing an important role in world biofuel production as well as world demand for food and feed. Sugar cane-based ethanol production in Brazil also produces valuable co-products that have application in feed and food markets (Table 2.4) (Cardona and Sanchez 2007).

2.3.3 Ethanol Industry in Canada

The Canadian ethanol industry is much smaller than the U.S. industry with 16 existing ethanol plants and 3 under construction. Canadian production of ethanol was approximately 1.5 billion litres in 2009 whereas in the U.S. there are 183 plants with a production capacity around 43.8 billion litres (Canadian Renewable Fuels Association 2009; Ethanol Producer Magazine 2009). In general, the ethanol industry in Canada is concentrated around Central Canada, mainly in Ontario with about 70 % of Canada's total ethanol production. As the U.S., central Canada ethanol production is corn-based whereas in Western Canada it is wheat and corn-based.

The Canadian federal government promotes the ethanol industry with the objective of having 5 % renewable fuel content in all gasoline and 2 % diesel fuel sold for transportation applications by 2010 and 2012, respectively (Canadian Renewable Fuels Association 2009). This would create a renewable fuel production of around 3.3 billion litres. Most provinces have mandated a minimum ethanol content in gasoline. For example, Saskatchewan has mandated a 5 % ethanol content by 2010. With the growth of the ethanol industry in Canada, there is an increasing amount of corn and WDDGS available for the feed market. Current production of corn DDGS is approximately 1.4 million metric tonnes and the volume of WDDGS is 350,000 tonnes. All DDGS available is traditionally used as livestock feed for ruminants (Widyaratne and Zijlstra 2007). However, there is an increasing interest in using this product in monogastric diets.

2.4 Chemical and Physical Characteristics of Distillers dried grains with solubles

Chemical and physical characteristics of DDGS have received great relative interest from nutritionists and researches of different areas such as animal science, feed and ethanol industry due to its properties as a feed ingredient in livestock, swine and poultry rations. It has been established that the chemical, physical and nutritional properties of DDGS depend on the type of

Table 2.4 Current and potential sugar-based ethanol co-products.

Co-product	Application
Yeast	Cattle feed supplement
Bagasse	Feedstock for production of animal feed, enzymes, AA, organic acids, pharmaceuticals, etc.
	Substrate for production of xylitol, single-cell protein, etc.
Fructose	Sweetener for food industry
Invertase	Enzyme used for the production of inverted sugar in food industry; analytical tests

Adapted from Cardona and Sanchez (2007)

grain used, the method of fermentation (batch vs. continue), addition of yeast cells in fermenters, duration of the fermentation process, drying process (e.g. temperature, duration) and the amount of solubles mixed back with WDG (Spiehs et al. 2002; Widyaratne and Zijlstra 2007; Liu 2009a). Also, variation in analytical test methods used to obtain nutrient composition of DDGS may influence the great variation observed among DDGS (Shurson 2005). It is likely that all these factors have a role in the variability associated with to the composition of DDGS. Table 2.5 and 2.6 summarize proximate analysis of corn and WDDGS, respectively, from several studies.

The nutrient content of corn DDGS has been extensively addressed, indicating that as a result of starch removal, corn DDGS contains approximately threefold the nutrient content of the unprocessed whole corn (Spiehs et al. 2002; Nuez and Yu 2009). In contrast, there is less information available about the nutrient composition of WDDGS. On the basis of the little data available, it is possible to speculate WDDGS composition as well as corn DDGS reflects the nutrient content of the original grain after removal of starch. Therefore, the content of CP, DM, neutral detergent fibre (NDF), acid detergent fibre (ADF), fat, ash, and other components in DDGS should be greater than the original wheat feedstock.

The data listed in Table 2.5 and 2.6 are good examples of the high variability in the composition of corn and WDDGS, respectively. For example, the CP content of WDDGS varied from 36.2 to 39.3 %; NDF from 41.4 to 49.9 %; ADF from 10.9 to 17.3 % and fat from 4.9 to 6.7 %. It has been suggested the lack of standardization of the compositional analysis procedures may have a role in this variability (Kim et al. 2008). In general, WDDGS is higher in protein but lower in fat than corn DDGS and both contain large amounts of NDF.

Physical characteristics, chemical composition and the interaction among them have been shown to be correlated to the nutritional value of DDGS in animal rations. For instance, lightness and yellowness of color of corn DDGS are positively correlated ($r^2 = 0.71$ and 0.74, respectively) with true lysine digestibility in poultry (Shurson and Noll 2005). A previous study showed a dark-colored corn DDGS sample had 0 % lysine apparent ileal digestibility (AID) for swine while “golden” corn DDGS had a lysine digestibility of 44 % (Whitney et al. 2000). Cromwell et al. (1993) evaluated the physical characteristics, chemical composition and nutritional value of corn DDGS in chicks and pigs. They observed the highest digestible lysine, arginine and cystine contents were found in the lightest-colored corn DDGS and the lowest contents in the darkest-

Table 2.5 Chemical composition of corn and corn distillers dried grains with solubles (g kg^{-1} dry matter basis).

Item	Corn[a]	Corn DDGS[c]		
		Nuez and Yu (2009)	Spiehs et al. (2002)	Schingoethe (2008)
DM	887.7	914.4	889.0	nd[c]
CP	101.3	320.1	302.0	301.0
NDF[a]	144.7	494.6	445.0	415.0
ADF[b]	36.6	146.8	162.0	161.0
Fat	45.9	165.3	109.0	107.0
Starch	634.1	43.8	nd[d]	nd[d]
Ash	17.3	43.2	58.4	52.0
Minerals				
Sulphur	1.2	7.2	4.7	4.4
Calcium	0.2	0.5	0.5	2.2
Phosphorus	2.9	7.7	8.9	8.3

[a] Neutral detergent fibre
[b] Acid detergent fibre
[c] Distillers dried grains with solubles
[d] Not done

Table 2.6 Chemical composition of wheat and wheat distillers dried grains with solubles (WDDGS) (g kg^{-1} dry matter basis).

Item	Wheat[a]	WDDGS		
		Nuez and Yu (2009)	Schingoethe (2008)	University of Saskatchewan (2010)
DM	895.2	937.6	nd[c]	909.0
CP	142.8	393.2	362.0	377.0
NDF[a]	172.2	480.7	414.0	499.0
ADF[b]	36.8	109.9	173.0	152.0
Fat	19.1	49.8	67.0	57.0
Starch	603.5	63.2	nd[c]	nd[c]
Ash	21.2	51.2	54.0	54.0
Minerals (g kg^{-1} dry matter)				
Sulphur	1.6	3.9	5.7	nd[c]
Calcium	0.7	1.8	3.0	nd[c]
Phosphorus	3.7	9.1	10.5	nd[c]

[a] Neutral detergent fibre
[b] Acid detergent fibre
[c] Not done

colored DDGS. Moreover, they observed that DDGS sources with dark color had higher acid-detergent insoluble nitrogen (ADIN) values and a burnt or smoky odour. Further, they found CP, lysine, cysteine and total sulphur amino acids (SAA) were highly correlated with feed efficiency and moderately correlated with growth rate. As well, the ADIN content was highly correlated with both gain and feed/gain. Thus, the wide range in color, odor, ADIN content, lysine and SAA digestibility especially among DDGS are related to heat damage during DDGS production in ethanol plants (Goering et al. 1973; Cromwell et al. 1993; Klopfenstein et al. 2007). In conclusion, lightness and yellowness appear to be good predictors of digestible lysine content of corn DDGS in swine and poultry. However, this correlation cannot be extrapolated to other grains (Shurson 2005).

2.5 Use of Ethanol Co-Products in Animal Rations

As previously mentioned, DDGS is the most available ethanol co-product on the market and is primarily used as a valuable feed primarily for beef and dairy cattle but also for swine and poultry (Robinson 2005; Jacob et al. 2008; Chevanan et al. 2009). In the U.S. feed market, it was estimated the ruminant animal segment consumed approximately 84 % of distillers grains in 2007, while swine consumed 11 % and poultry 5 % (Renewable Fuel Association 2009). On the basis of the data available, it was calculated 33 % DDGS was included in beef cattle diets, 20 % in dairy cattle diets, and 10 % in swine and poultry rations (Simpson et al. 2008).

2.5.1 Nutritional Value of Ethanol Co-Products for Ruminants

Most of studies on distillers grains as a feed ingredient have been performed on finishing cattle (Klopfenstein et al. 2007). DDGS has high NDF content which is readily digestible and this property allows DDGS partially replace forage, concentrates and grain in dairy and beef cattle rations (Schingoethe 2006; Hao et al. 2009). Moreover, DDGS can be a source of energy for lactation or growth without the ruminal acid load caused by rapidly fermented starchy components (Schingoethe 2006). However, DDGS may be very deficient in effective fibre to avoid milk fat depression due to the small particle size of the DDGS fibre (Schingoethe 2006). Feedlot diets that use more than 20 % (DM basis) corn DDGS, are used as an energy source for beef animals (Klopfenstein et al. 2007). WDDGS showed a reduction in acidosis problems

(Klopfenstein et al. 2007). No difference in carcass weight was observed in heifers fed diets containing up to 30 % corn DDGS compared to heifers fed no DDGS (Depenbusch et al. 2009). Carcass characteristics such as marbling score, longissimus muscle area and subcutaneous fat thickness over 12th rib remained similar between 0 to 60 % of inclusion level of corn DDGS but more than 75 % decreased its characteristics. Lastly, results from trained sensory panel evaluation did not show any significant differences in juiciness and off-flavour intensity among treatments (0 - 75 % DDGS). Beef flavour was greatest in heifers fed 45 and 60 % DDGS compared to heifers fed 0 % DDGS. Taken together, these observations indicate that DDGS is a good feed ingredient in ruminant diets.

2.5.2 Nutritional Value of Ethanol Co-Products for Swine

Stein and Shurson (2009) indicated the apparent total tract digestibility of dietary fibre is less than 50 % which reduces DM digestibility. Therefore, It has been suggested that the high fibre content of WDDGS may play a role in the low CP, AA, DM and energy digestibility observed in diets using this ingredient (Lan et al. 2008; Stein and Shurson 2009). Widyaratne and Zijlstra (2007) reported the AID of energy did not differ between corn DDGS (78.7 %) and WDDGS (77.4 %). Also, there was no significant difference in the AID of AA among DDGS samples, with the exception of threonine. The content of standardized ileal digestible AA was higher for WDDGS than for corn DDGS, except for lysine, methionine, leucine and threonine. They concluded that WDDGS showed a lower digestible nutrient content than corn DDGS for swine, which may have been due to AA damaged by heat processing, a higher xylose content and lower fat content (Widyaratne and Zijlstra 2007). The digestible phosphorus content determined in this study was similar between corn DDGS (55.5 %) and WDDGS (53 %). These results were in agreement with those reported by Stein and Shurson (2009). Both studies suggested DDGS has a low digestibility of lysine probably due to heat-damage during the drying process.

Several growth performance studies have described the effect of inclusion of DDGS in weanling, growing and reproducing swine (Spiehs et al. 2002; Widyaratne and Zijlstra 2007; Stein and Shurson 2009). Spiehs et al. (2002) proposed that feeding DDGS to swine will increase nitrogen excretion and thus, increase the requirement of metabolic energy for nitrogen removal, leaving less energy available to pigs for growth (Spiehs et al. 2002). Stein and Shurson (2009) summarized results from recent studies in which DDGS was fed to pigs. They concluded nursery

pigs from 2 to 3 weeks postweaning and finishing pigs can be fed diets containing up to 30 % DDGS without any detrimental effect on growth performance. Further, it was suggested lactating sow diets may contain up to 50 % DDGS without negative effect on sow or litter performance.

Widyaratne and Zijlstra (2007) also indicated DDGS is deficient in lysine, consequently DDGS may restrict protein accretion of pigs due to lysine being the first-limiting amino acid for swine (Widyaratne and Zijlstra 2007). However, supplementation with synthetic lysine can reduce this negative effect on growth (Spiehs et al. 2002). It was suggested that modifying the ethanol production process, dietary manipulations such as supplementation with crystalline AA and enzymes may improve the nutritional value of DDGS for pigs (Spiehs et al. 2002; Widyaratne and Zijlstra 2007; Lan et al. 2008).

2.5.3 Nutritional Value of Ethanol Co-Products for Poultry

Most studies have mainly determined the nutritional value of corn-based DDGS for poultry (Noll 2004; Lumpkins and Batal 2005; Parsons et al. 2006; Thacker and Widyaratne 2007; Youssef et al. 2008; Leytem et al. 2008a; Applegate et al. 2009). The majority concluded that the addition of DDGS in poultry diets is limited due to its high fibre content and variable digestible nutrient composition. Noll (2004), analyzed four samples of corn DDGS for proximate components and evaluated for AA digestibility using cecatomized roosters. A considerable variation in nutrient content was found between corn DDGS samples, especially for protein and fat content. There was also a large range in AA digestibility values, particularly the digestibility of lysine varied between 59 and 83 %. These values were in agreement with those reported by Parsons et al. 2006, using the precision-fed cecectomized rooster assay. Both studies suggested the great variation in lysine bioavailability could be due to differences in drying process between ethanol plants.

Several feeding trials have been conducted to test the inclusion level of DDGS in poultry diets. In one study, corn DDGS was included at two levels (0 and 15 %) and at two dietary energy levels (3200 and 3000 kcal/kg) in broiler feeds (Lumpkins et al. 2004). At 18 d of age, within the two levels of energy there was no difference in performance of chicks fed diets with 0 or 15 % DDGS. Other more recent experiment evaluated the effect of five levels of corn DDGS (from 0 to 50 %) on growth performance, dressing percentage and parts yield in broiler diets (Wang et al. 2008b). They reported that dietary inclusion of up to 20 % DDGS could be used in

broiler diets formulated on a digestible AA basis without reduction in body weight or performance. However, higher levels of DDGS could result in loss of dressing percentage or breast meat yield. It was also indicated that higher levels of DDGS (> 30 %) decreased the bulk density and pellet quality, decreasing energy density of DDGS diets.

Two inclusion levels of corn DDGS (0 and 15 %) were evaluated in laying hen diets for 25 to 43 weeks of age (Lumpkins et al. 2005). No differences were found in egg weight, yolk color, and exterior or interior egg quality. However, there was a significant decrease in the number of eggs produced through 35 weeks of age for hens fed 15 % corn DDGS. Subsequently, they suggested a maximum level of inclusion of 10 to 12 % corn DDGS in laying hens feeds and that inclusion should be in low-density diets.

In addition, Thacker and Widyaratne (2007) determined WDDGS may be included in poultry diets at a level of 15 % in broiler diets with no detrimental effects on performance. However, these diets were formulated on total AA basis. Youssef et al. (2008) reported feed intake, weight gain, excreta quality and digestibility of CP and DM were not significantly different between broiler fed diets containing up to 15 % DDGS (produced from wheat or barley) and broiler fed diets 0 % DDGS.

Based on the available published data, DDGS has only limited potential as a feedstuff in poultry diets. Nutritionists should consider amino acid digestibility, acceptable metabolizable energy value and phosphorus bioavailability, supplementary crystalline AA (e.g. lysine), formulation on a digestible AA basis, bulk density and pellet quality to formulate poultry diets that support optimum performance.

2.5.4 Nutritional Value of Ethanol Co-products for Aquaculture

The limited information published on the use of distillers grains suggests that the high fibre content and low amino acid availability of DDGS may be the main detractors of using DDGS as a feedstuff for fish. Fibre is known as an antinutritional factor for fish since it decreases feed consumption and nutrient digestibility (NRC 1993). A recent study reported the ADC for CP was 0.90, 0.66 for DM, 0.75 for GE, 1.00 for fat and 0.79 for ash in rainbow trout fed diets containing 30 % WDDGS (Randall and Drew 2010). Nile tilapia fed diets with 60 % corn DDGS without added lysine showed significantly lower final weight gain and feed efficiency ratio compared to control diet (Shelby et al. 2008). The addition of lysine to the 60 % DDGS diet

resulted in improved weight gains. In another experiment, tilapia fry fed 28 % protein diet with 82 % corn DDGS and supplemented with lysine and 32 % protein diet with 63 % corn DDGS without supplementation of lysine showed a low final weight gain compared to the control diet but the feed conversion ratio and protein efficiency ratio were not significant different from the control diet (Wu et al. 1997).

In spite of the results from these studies there has been success in feeding DDGS in fish diets. Corn DDGS has been described as a good protein source for channel catfish. In diets containing 40 % DDGS with no supplementary lysine, there were no adverse effects on growth and survival (Webster et al. 1992). Tilapia fed diets containing 30 % DDGS in combination with animal protein (e.g. fishmeal, MBM) had no significant effect on growth and feed utilization parameters compared to a control diet (Coyle et al. 2004). TS increased the feed intake of rainbow trout fed diets containing CM or air-classified pea protein over a four-day period, when it was included in levels of 3.3 and 3.9 % in diets (Thiessen et al. 2003b). A reduction in the palatability enhancing ability of TS was observed over a longer period. It was suggested this may be due to the fish becoming accustomed to the taste of TS or the AA involved in gustatory stimulation were damaged during feed preparation. Supplementation with commercial phytase (Ronezyme) showed to increase growth performance and feed utilization parameters in juvenile Nile tilapia fed diets containing DDGS replacing 50 % of SBM (Abo-State et al. 2009).

From the data available, it is speculated that omnivorous fish such as tilapia and catfish can tolerate high inclusion levels of DDGS with lysine supplementation with no detrimental effects on growth performance. Nevertheless, there is a lack of information about the nutritional value of WDDGS in salmonid diets. However, based on previous studies with plant proteins, both corn and WDDGS are low in protein and high in fibre to be viable aquafeed ingredients. Based on studies with soybeans, canola, lupins and peas, the value of DDGS might be improved by fractionation to produce a product with increased protein and decreased fibre content.

2.6 Fractionation

Fractionation is a separation method in which a material is divided into its constituent components. It separates chemical components based on attributes such as size, shape, mass density and dielectric properties, solubility and hydrophobicity (Hemery et al. 2007). It allows successive controlled recombination of these components to achieve the maximum content of a

specific nutrient or the specific market requests. Fractionation methods may be primarily classified into two types: aqueous and dry fractionation.

2.6.1 Aqueous Fractionation

Aqueous fractionation often involves several milling, extraction, filtration, precipitation and centrifugation, mechanical pressing (Russell et al. 1978). Other methods that affect protein properties and solubility including enzymatic hydrolysis (Celus et al. 2009), protein solvent extraction from cereal grains using various solvents systems (aqueous, alkaline, semi-alcoholic solvent systems (Vasanthan and Temelli 2008), precipitation by altering pH (Wu et al. 1977; Mwachireya et al. 1999; Classen et al. 2004; Hartmann and Koehler 2008) may also be incorporated in aqueous fractionation.

Satterlee et al. (1976) produced a protein concentrates from wheat and corn stillage by alkaline extraction. They examined the chemical, functional and nutritional characterization of these protein concentrates. The stillage was first separated into syrup and wet solids by centrifugation. The wet solids were extracted twice with sodium hydroxide solutions and centrifuged once after each extraction. Next, each of two alkaline extractions was adjusted to pH 4.0 and centrifuged to produce a precipitate and a supernatant. After that, the precipitate was washed sequentially with ethanol and water. The precipitate was freeze dried and called distillers protein concentrate. The best extraction yield conditions for wheat were at pH 12.2 at 23 ºC and pH 12.2 at 80 ºC for corn. The nutritional evaluation of both distillers protein concentrates showed they had similar protein quality to their corresponding start grain. Further, corn distillers protein concentrate had a protein efficiency ratio of 1.45 and a ADC of 0.78. The wheat distillers protein concentrates had a protein efficiency ratio of 1.26 and a ADC of 0.83.

Wu et al. (1985) reported the fractionation of the stillage resulting from fermentation of dry milled corn fractions, including corn grits, flour, degerminator meal and hominy feed. Each resulting stillage was passed through cheesecloth to produce “distillers grains” and TS. The latter was centrifuged to yield a supernatant called “stillage solubles” and a precipitate called “centrifuged solids”. The corn grits distillers grains had the highest protein content (68 %) among all protein-rich residues.

Cookman et al. (2009) extracted protein from corn distillers grains using aqueous ethanol, alkaline-ethanol, and aqueous enzyme solutions. Oil was extracted from distillers grains prior to

protein extraction. Subsequently, the defatted distillers grains was dried and with the residual material being a dried defatted distillers grains (DDDG). The protein was then extracted from the DDDG. The aqueous enzyme and alkaline-ethanol extractions were able to successfully extract protein from DDDG. It was determined that alkaline-ethanol extraction required milling, while the aqueous enzyme method did not need further processing of the DDDG.

Aqueous protein extraction is traditionally applied to co-products (residual defatted meal) of oilseed extraction processes. Many studies have been reported regarding protein extraction from mustard, canola, soybean, lupin, (Mwachireya et al. 1999; Maenz et al. 2004; Prapakornwiriya and Diosady 2004; Jung 2009). Prapakornwiriya and Diosady (2004) used alkaline conditions (pH 10 to 13) to extract protein from dehulled yellow mustard and microfiltration to concentrate and purify the protein extract. They found that around 90 % of the protein is extracted at pH 12.

Maenz et al. (2004) extracted canola protein from oil-extracted desolventized flakes from rapeseed or canola using aqueous solutions. The oil free flakes were mixed with the aqueous extraction media at 10 % to 50 % (w/v). The aqueous solution consisted of salt (NaCl or KCl) at pH 2 - 12. However, they suggested using only water as aqueous media. After that, the aqueous extract is dephytinized by phytase-enriched enzyme. The resultant extract is heat treated to induce curdling of protein contained in the extract and this precipitated protein is fractionated from the residual liquid by a solid-liquid separation. The final product is a dephytinized protein concentrate from canola seed called canola protein concentrate (CPC). CPC was evaluated for nutrient digestibility and its potential for replacing fishmeal protein in commercial-like diets in rainbow trout (Thiessen 2004). Results showed this product had high amino acid digestibility values (> 90 %) with the exception of threonine and phenylalanine. Results from the growth trial indicated CPC could replace up to 70 % of dietary fishmeal protein in commercial diets without any negative effect on rainbow trout performance.

New fractionation processes were developed to recover nonfermentable (germ, endosperm fibre and pericarp fibre) components of corn before fermentation in dry grinding ethanol process. These processes include quick germ (QG) (Singh and Eckhoff 1996), quick germ and quick fibre (QGQF) (Singh et al. 1999), and enzymatic milling (E-Mill) (Singh et al. 2005). The QG process involves soaking whole corn in water for 12 h at 60 ^{o}C prior to degermination. The germ is removed by germ hydrocyclones. The rest of the crop is ground and

conventional processed for ethanol production. This method provides ethanol production with a new valuable co-product (corn oil) and, an increasing capacity of the plant (Singh et al. 1999). The QGQF is a modification of the QG process. The modifications consisted of addition of 3 ml of enzyme (α-amylase, *Bacillus amyloliquefaciens*, 1,4-α-D-glucan glucanohydrolase, 9000-85-5, MFCD000813119), incubation of the slurry for 4 h after soaking and coarse grinding of the corn kernels. After germ recovery, corn coarse fibre is recovered by flotation using hydrocyclones. This recovered fibre is called quick fibre. Thus, this process adds a new co-product (quick fibre) to ethanol industry. The quick fibre can be further processed to produce other valuable co-products such as corn fibre oil and corn fibre gum. The E-Mill method is an improvement of the previous two processes and also allows removal of endosperm fibre. This method includes soaking of corn kernel in water for 12 h. After soaking, the material is coarse ground and incubated with starch-degrading enzymes (55 ^{o}C and pH 5.0) for 2 h and proteolytic enzymes (45 ^{o}C and pH 5.0) for 2 h. These enzymes increase specific gravity of the slurry and facilitate the separation of individual corn components. After incubation, germ and pericarp fibre are recovered as was previously mentioned and endosperm fibre can be removed by screening either prior to fermentation or after fermentation (Singh and Eckhoff 1996; Singh et al. 1999; Singh et al. 2005; Wang et al. 2005). The comparison of these methods with the conventional dry grinding process for DDGS composition showed these new processes reduced the fibre content from 11 to 2 % in the DDGS and increased the protein content from 28 to 58 % in the DDGS (Singh et al. 2005).

2.6.2 Dry Fractionation

Dry fractionation has some advantages over a conventional wet fractionation process such as it does not require solvent extraction and recovery (Liu et al. 2009). Various methods of dry fractionation have been described including: (1) size reduction including dehulling, pearling, grinding and milling (Yeung and Vasanthan 2001; Izydorczyk et al. 2003; Thiessen et al. 2003a; Vasanthan and Temelli 2008), (2) sieving (Wu and Stringfellow 1986; Thiessen et al. 2003a; Liu 2009a), (3) air classification (Singh et al. 2002), (4) and combinations of these methods such as grinding and sieving (Wu and Stringfellow 1982; Thiessen et al. 2003a; Randall and Drew 2010), grinding, sieving and air classification (Wu and Nichols 2005), sieving followed by air classification (e.g. elutriation or winnowing) (Thiessen et al. 2003a; Srinivasan et al. 2005b;

Srinivasan et al. 2008; Yadav et al. 2008; Liu 2009b), combination of dehulling, pearling, milling and sieving (Liu et al. 2009), and dehulling and subsequent air classification (Thiessen et al. 2003a). Dehulling which can grind or rip the seed hull from the seed enables the removal of indigestible xylans and cellulose (Thiessen 2004). Pearling consists of gradual removal of outer layers (i.e. pericarp, testa, aleurona, and subaleurona layers) of grain tissue by abrasion without cracking the grain (Yeung and Vasanthan 2001; Vasanthan and Temelli 2008). Milling is a fractionation method that disintegrates grains into fine particles (Liu et al. 2009). Roller milling is a simple method that allows grains to pass through sifters for fractionation but gives several products of greatly variable composition (Izydorczyk et al. 2003; Liu et al. 2009).

Sieving (also known as screening) is the term applied to the separation of a mixture of various sizes of particles into several fractions by passing over a screen (Rosentrater et al. 2006). Sieve screens are made of metal bars, perforated plates or cylinders and woven cloth or fabrics (Rosentrater et al. 2006).

Air classification separates on the basis of shape, density, mass, size features and projected area in the direction of flow (Srinivasan et al. 2005b; Liu et al. 2009). Particles are passed through an air stream and the force of the air flow carries low density particles further than high density particles (Rosentrater et al. 2006). Elutriation and winnowing are both types of air classification. Thus, elutriation separates particles using an upward-flowing stream of air in a vertical pipe, therefore the opposing effects of gravitational force and air drag are used (Srinivasan et al. 2005b; Rosentrater et al. 2006). In contrast, winnowing uses a horizontal or slanted stream of air (Liu 2009b). In general, the dry fractionation starts with reduction of particle size by grinding or milling and continues with sieving and/or air classification.

Simple sieving was used to fractionate corn DDG and DDGS using four U.S. standard screens (20, 35, 50 and 80), resulting in fine fractions with elevated protein and reduced fibre contents compared to the initial material (Wu and Stringfellow 1986). The high protein DDG fraction had a 49 % protein content and 10 % crude fibre content while the initial material (unsieved DDG) had 26% protein content and 17 % crude fibre content. For corn DDGS, the protein contents varied between 29.5 and 36.8 % and NDF contents ranged from 40.8 to 18.1 %.

In addition, sieving was used to evaluate the effect of particle sizes on physical and chemical characteristics of corn DDGS (Liu 2009a). As the particle size decreased from 2.36 to 0.11 mm in DDGS fractions, protein content of DDGS increased. It was concluded that finer

fractions of DDGS had higher protein content than coarser fractions. The oil content followed a decreasing trend as the particle size decreased, ranging from 11.0 to 12.2 %. Regarding, surface color of sieved fractions of DDGS it was concluded that smaller size particles were comparatively lighter, less red but more yellow.

Wu and Stringfellow (1982) used dry milling and sieving for fractionation of corn DDG and DDGS with different moisture contents ranging between 5 and 31 %. The materials were pin milled using 9000, 14,000 and 18,000 rpm and then screened on several screens. The best yield for corn DDG was obtained at 21 % moisture and pin-milling at 14,000 rpm. The result was a high protein fraction (50 % protein content).

Randall and Drew (2010) fractionated WDDGS using milling and sieving. The material was passed through a hammer mill equipped with a 3-mm screen. After milling, six sieve sizes (20, 30, 40, 50, 60 and 80) were used to fractionate the ground material into seven fractions (with particle distribution ranging from > 841 to < 177 μm). Sieving increased protein content by 6 % and reduced NDF by 5.6 % and ADF by 2 %. They determined the ADC of CP, GE, acid ether extract, ash and DM of each sieved WDDGS fraction and the original unsieved WDDGS in rainbow trout. The results showed grinding followed by sieving improved the ADC of GE, acid ether extract and DM of the two WDDGS fractions with the lowest particle sizes while the ADC of CP remained relatively constant at 90 %. These techniques produced fractions with high protein content and ADC compared to the initial material. It was suggested that more advance fractionation techniques such as air classification could be more effective to produce enriched protein DDGS fractions.

Singh et al. (2002) used an air classification technique to remove fibre from corn DDGS. This method consisted of placing the material on a 20 mesh screen (850 μm) and aspirating with an air jet at a pressure of 2.8 atm. The results showed that there were moderate increases in oil and protein contents and reduction of NDF values compared to the conventional DDGS. The increase in oil content ranged from 0.2 to 1.9 % and between 0.4 and 1.4 % for protein. It was concluded air classification alone had limited success in fibre removal from DDGS.

Most recent experiments have described the effect of the combination of two or more dry fractionation methods such as pearling, milling, sieving and air classification. Srinivasan et al. (2005a) reported that sieving corn DDGS using four screens (869, 582, 447 and 234 μm) and subsequent elutriation increased protein and fat content while reduced fibre content. This process

was shown to be more efficient than sieving or elutriation alone in removing fibre from corn DDGS. This process creates two value-added products: an enriched protein product (heavier fraction) and a product with high fibre content (lighter fraction). They indicated the high protein product can be used in nonruminant rations at higher levels of inclusion and the fibre fraction can be used in ruminant diets, production of corn fibre gum and corn fibre oil. A high-protein corn DDGS produced as described by Srinivasan et al. (2005b) was tested in cecectomized roosters to estimate amino acid digestibility (Parsons et al. 2006). The results of this study indicated the process had little or no effect on amino acid digestibility DDGS. Recently, Liu (2009b) indicated sieving followed by winnowing is effective as a dry fractionation process since it resulted in a fraction with 31.2 % higher protein content than original DDGS sample.

Dehulling peas resulted in a dehulled pea with a higher protein content than raw peas but did not improve the digestibility of peas in rainbow trout (Thiessen et al. 2003a). Subsequent air classification with autoclaving of dehulled peas produced a pea product with higher protein and fat content. This pea protein concentrate had higher ADCs for CP, fat, starch, GE and DM in rainbow trout than. Nevertheless, these differences in digestibility between dehulled peas and air-classified peas had no effect on growth and feed utilization. These results suggested further processing of peas is needed to decrease ANF and improve the nutritional value as a feed ingredient.

Thiessen et al. (2003a) used a 60 mesh screen to remove course fibre particles from CM. The final product was called CM fines and consisted of 43 % protein, 54 % fat and 14 % starch. This product was tested to replace SBM in rainbow trout diets through a feeding trial. The results of this study showed that 20 % of CM fines can be included in rainbow trout diets for replacement of SBM without detrimental effects on performance.

In conclusion, aqueous and dry fractionation may be used to produce value-added feed ingredients with enhanced protein content and other nutrients of particular interest by removing fibre, contaminants in the outer layers (mycotoxins, pesticides residues, etc.) and reducing ANF. Therefore, aqueous and dry fractionation have been the most commonly used methods for improving the nutritional quality of common pulses and grains in human food and animal feed.

2.7 Hypothesis

There is a pressing need to develop new plant-based proteins for use in diets fed to salmonid fish. WDDGS is promising due to its low cost per unit of protein. However, WDDGS is too low in CP content and too high in fibre content for use in salmonid diets. Dry and aqueous fractionations have been successfully used to improve the nutrient value of crops such as canola and peas in salmonids. However, aqueous fractionation is more effective than dry fractionation in concentrating protein. Based on these observations, it was hypothesized:

1) Dry fractionation using sieving and elutriation will remove fibre and increase the protein content and nutrient digestibility of WDDGS in rainbow trout.
2) Aqueous fractionation of wheat distillers grains will be more effective than dry fractionation in the removal of fibre and increasing the protein content and nutrient digestibility of wheat distillers grains in rainbow trout.

3. EFFECT OF FRACTIONATION ON THE NUTRITIONAL VALUE OF WHEAT DISTILLERS GRAINS IN RAINBOW TROUT (*ONCORHYNCHUS MYKISS*)

3.1 Introduction

Global aquaculture has increased rapidly over the past decade at annual growth rate of 7 % (FAO 2009). It produced 1 million tonnes per year in the early 1950s and this has grown to 51.7 million tonnes in 2006 (FAO 2009). In 2009, aquaculture is predicted to supply 47 % of the total fish available for human consumption (Naylor et al. 2009). In recent years, the finfish and crustacean aquaculture sectors have showed the fastest growth rate per year (8.5 %) with a production of 37 million tonnes in 2006 (Tacon and Metian 2008). Thus, this sector corresponds to 56.3 % of total global aquaculture production. However, future growth of this sector may be constrained due to its reliance on marine feed ingredients such as fishmeal and fish oil (Tacon et al. 2006; Tacon and Metian 2008). It is estimated that 68 % of global fishmeal production is used for aquafeed and over 50 % of this is consumed by carp and salmon (Tacon and Metian 2008; FAO 2009). Although, the use of fishmeal by aquaculture has increased, its inclusion rate in aquafeed has decreased from 45 to 30 % for salmonid fish (Tacon and Metian 2008; Naylor et al. 2009). The main reasons for this decreasing trend in inclusion levels of fishmeal in aquafeed have been the static fishmeal production for the last two decades with an annual average of 5 - 7 million tonnes of fishmeal. This and the decreasing of the market availability of fishmeal from capture fisheries, environmental protection policies and increasing global fishmeal price (Gatlin et al. 2007; Tacon and Metian 2008; Naylor et al. 2009) have reduced the use of this product in salmonid diets. Therefore, the main challenge for aquaculture nutritionists is to find alternative protein sources to replace fishmeal in aquafeeds to maintain the long term sustainability of aquaculture industry. Fishmeal is highly palatable, a rich source of energy and has an essential amino acid profile that closely meets the requirement of fish (NRC 1993). In general, this challenge will be more feasibly solved for herbivorous and omnivorous fish since other available feed ingredients in the market can be used as protein sources. However, this is a more complicate task for carnivorous fish due to their high dietary protein requirement (NRC 1993; Tacon 2004). Potential

replacements for fishmeal must meet certain characteristics such as ready availability, competitive pricing, palatability, ease of handling, shipping and storage and of course high protein quality (Naylor et al. 2009).

Recently, animal and plant proteins have been investigated as alternative protein sources. The inclusion levels of animal co-products in aquafeed have been restricted due to their high levels of phosphorus which can lead to eutrophication, high variability in nutritional composition and poor digestibility (Bureau et al. 1999; Bureau et al. 2000; Barrows and Gaylord 2007). Plant proteins were also evaluated as replacements of fishmeal in salmonid feeds (Mwachireya et al. 1999; Opstvedt et al. 2003; Thiessen et al. 2003a; Thiessen et al. 2004; Drew et al. 2005; Aslaksen et al. 2007). Most plant proteins have detrimental effects on the growth performance of salmonids due to the presence of ANF and poor palatability (Thiessen et al. 2003a; Barrows and Gaylord 2007). Therefore, the complete replacement of fishmeal by plant proteins has remained infeasible. Recently, further processing of plant proteins using dehulling (Thiessen et al. 2003a; Booth and Allan 2004), heat treatment (Thiessen et al. 2003a; Booth and Allan 2004), aqueous extraction (Mwachireya et al. 1999; Maenz et al. 2004) and other methods has shown to greatly increase the nutritional value of plant proteins for aquaculture. This approach may lead to the development of improved plant protein sources for salmonid fish.

The recent expansion of the ethanol industry in North America has resulted in the availability of a promising protein source for animal feeds: DDGS. This product has ready availability, relatively high protein content, low starch content, and fewer ANF than SBM which is the most common alternative protein source in fish feed. The nutritional value of corn-based DDGS in fish diets has been documented (Webster et al. 1991; Webster et al. 1992; Wu et al. 1996; Wu et al. 1997; Shelby et al. 2008). However, most of these studies have been conducted using omnivorous and herbivorous fish species (e.g., carp and tilapia) because they are more tolerant to high fibre content in feed than carnivorous fish species. Thus, the main drawback of using DDGS in salmonid feeds is its high fibre level. Recently, several studies have shown that further processing of corn DDGS is able to increase protein and decrease fibre content and thus, its nutritional value for animal feed (Srinivasan et al. 2005a; Rausch and Belyea 2006; Yadav et al. 2008; Liu 2009b). Such processing methods have not been investigated using WDDGS and the nutritional value of WDDGS as protein sources in salmonid feeds has not been investigated. Based on these observations, we hypothesized that the fractionation of WDDGS to remove fibre

and create a protein concentrate would result in an ingredient with high protein quality for use in rainbow trout diets. Therefore, dry and aqueous fractionation of distillers grains were performed and the effect of these fractionation methods on nutritional value of distillers grains were evaluated in rainbow trout (Figure 3.1).

3.2 Materials and Methods

In 2008, WDDGS was supplied by NorAmera BioEnergy (Weyburn, SK, Canada) and the wheat variety was unknown. During May 2009, WWDG was collected by two ethanol plants: Pound-Maker (Lanigan, SK, Canada) and Terra Grain Fuels (Belle Plaine, SK, Canada) (Table 3.1). The wheat varieties used at Pound-Maker are Canadian Prairie Spring Wheat, Fall Rye, Durum, Triticale, Winter Wheat and soft white wheat (AC Andrew) where as the wheat variety used at Terra Grain Fuels is AC Andrew. These two last plants were named as plant 1 and plant 2, respectively, and the two WWDG were called unprocessed WWDG for purposes of this study. All experiments were carried out at the Prairie Aquaculture Centre, Department of Animal and Poultry Science, University of Saskatchewan (Saskatoon, SK, Canada).

3.2.1 Dry Fractionation Process

The dry fractionation of WDDGS was performed to increase protein content and nutritional value of WDDGS in rainbow trout. The dry fractionation of WDDGS involved grinding, sieving and elutriating successively (Figure 3.2). WDDGS was first milled using a hammer mill equipped with a 3-mm screen (Model Y60, Buhler manufacturing, Winnipeg, MB) and then, sieved using a Tyler Industrial Ro-Tap® testing sieve shaker (Mentor, Ohio) equipped with six selected screens (20 M, 30 T, 40 T, 50 T, 60 M and 80 M) and a pan. The sieve screens were fitted into the sieve shaker in descending mesh size and batches of 500 g of WDDGS were sieved by shaking for 12 min. After sieving the weight of each resultant fraction was obtained and recorded to determine the material retained (%) on every screen. Each sieve fraction was referred according to the sieve number in which was retained (Nos. 20, 30, 40, 50, 60 and 80) and pan (Figure 3.2). The initial WDDGS without sieving is referred as original WDDGS.

An elutriator was built to further fractionate the 20, 30, 40, 50, 60 and 80 sieved fractions (Figure 3.3). The design of the elutriator used in this study was based on elutriation apparatus

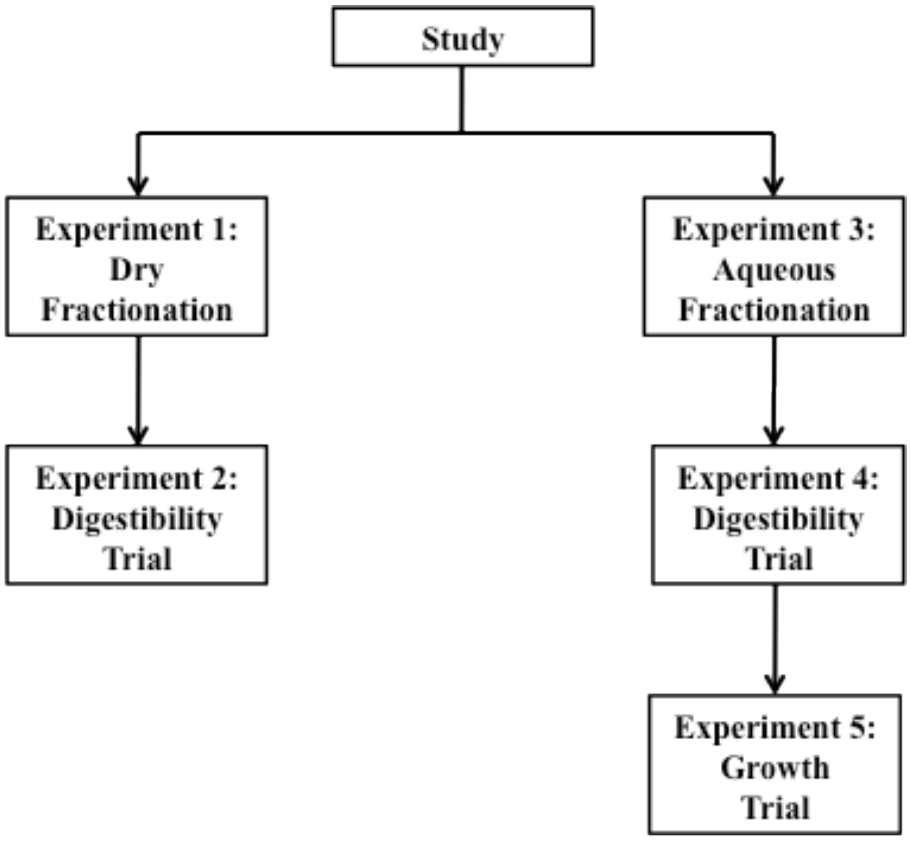


Figure 3.1 Outline of the study.

Table 3.1 Nutrient compositions (g kg^{-1}) of unprocessed wheat wet distillers grains (U).

Parameter	Plant 1	Plant 2
	U	U
Dry matter	949.4	881.7
Crude protein	249.5	431.8
Gross energy (MJ kg^{-1})	21.2	20.1
Acid ether extract	120.6	74.1
Ash	53.3	134.2
NDF[1]	238.1	555.0
ADF[2]	90.3	202.6
Essential amino acids		
Arginine	14.5	19.3
Histidine	5.9	8.8
Isoleucine	8.7	15.5
Leucine	16.4	28.6
Lysine	9.5	10.0
Phenylalanine	10.4	19.4
Threonine	8.3	12.9
Valine	12.0	18.9
Methionine	4.1	6.9

[1] Neutral detergent fibre.
[2] Acid detergent fibre

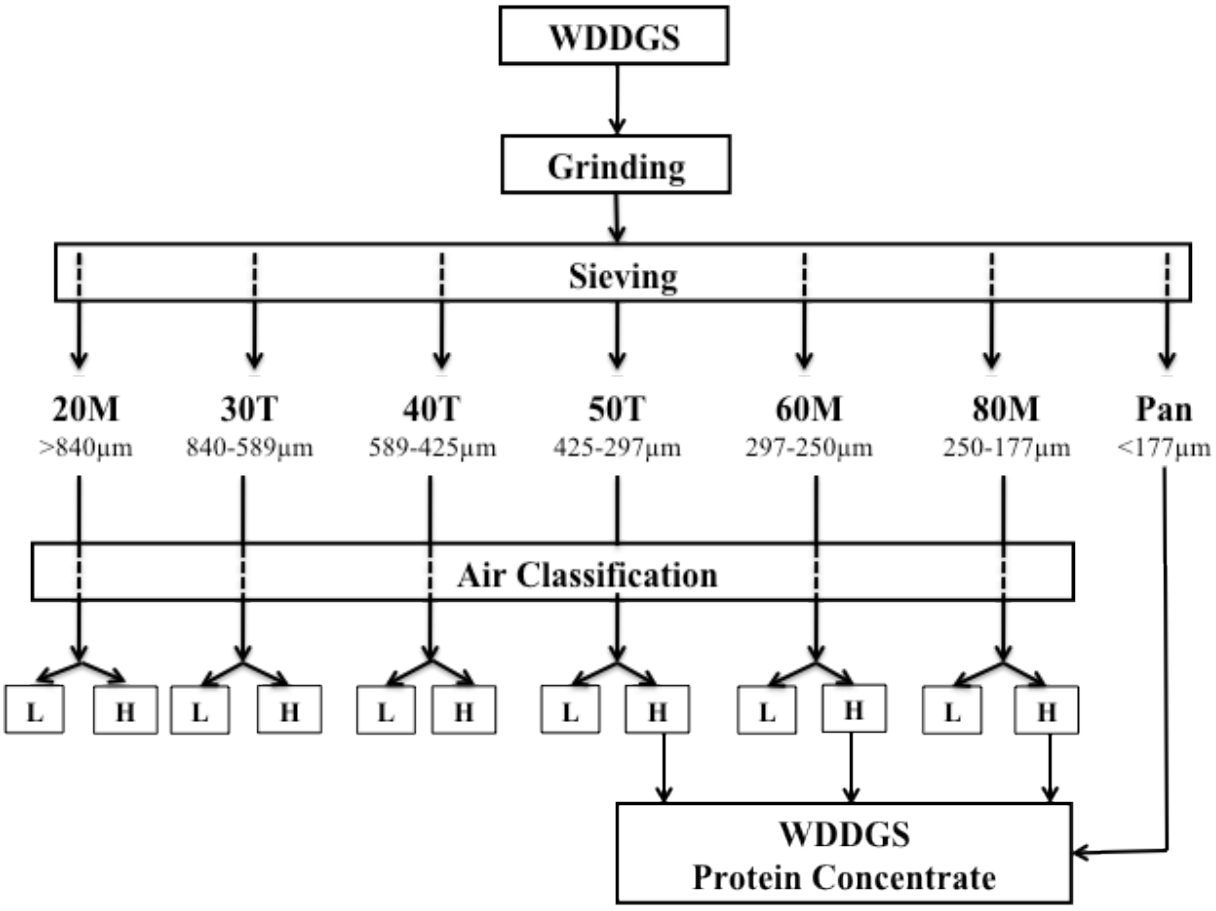


Figure 3.2 Outline of the dry fractionation process used to produce the wheat distillers dried grains with solubles (WDDGS) protein concentrate. Lower density sub-fraction is abbreviated as L while higher density sub-fraction is abbreviated as H.

developed by Srinivasan et al. (2005a). The elutriator consisted of a vertical tube, an air blower for supplying air into the vertical tube through 2 air-lines (main and secondary air-line, respectively), three valves for manipulating the airflow, one feeder for supplying the WDDGS into the vertical tube and two containers located on top and at bottom of the vertical tube to collect the low density material carried up by the air flow and the high density material that settled at the bottom. Material collected on top was called lower density sub-fraction and material collected at bottom was referred as higher density sub-fraction. The feeder was located on the secondary air-line, and just after a constricted section that produced a Venturi effect for the introduction of material into the elutriation tube. The internal diameter of the vertical tube was 6.5 cm and the distance from the material inlet to the air inlet was 52 cm. The distance from the lower density sub-fraction container to the material inlet was 126 cm. The lower density sub-fraction container (29 X 26 X 41 cm) was a box of transparent plastic material with a hole (6.9 cm diameter) in the centre of the bottom plate to insert the container onto the vertical tube. The distance of the higher density sub-fraction container to the air inlet was 27 cm. The higher density sub-fraction container was a cap container of transparent plastic material. During elutriation, the open top was covered up to 95 % of its area using a plastic lid. The air velocity was measured in two points by inserting a hotwire anemometer (Model 8382, TSI Velocicalc, St. Paul, MN) into the measuring point in the main air-line and using a turbo meter anemometer (Davis Instruments, Hayward, CA, USA) onto the vertical tube. The air stream in the elutriator was supplied by a blower (Model: BL1200-CA, Black & Decker). Elutriation was conducted at different air velocities depending on WDDGS sieve fraction features such as density, shape and particle size, and with the objective to achieve adequate protein content in each higher density sub-fraction. The elutriation process was repeated three times for each selected air velocity.

The final WDDGS protein concentrate was produced by mixing the pan fraction and higher density sub-fractions of the 50, 60 and 80 sieve fractions in the following proportion 637.4, 45.2, 229.5 and 87.8 g kg^{-1}, respectively.

3.2.2 Aqueous Fractionation Process

The aqueous fractionation of WWDG was performed due to we hypothesized that this method would be more effective than dry fractionation in the removal of fibre and increasing the protein content and nutritional value of wheat distillers in rainbow trout. The WWDG was first blended

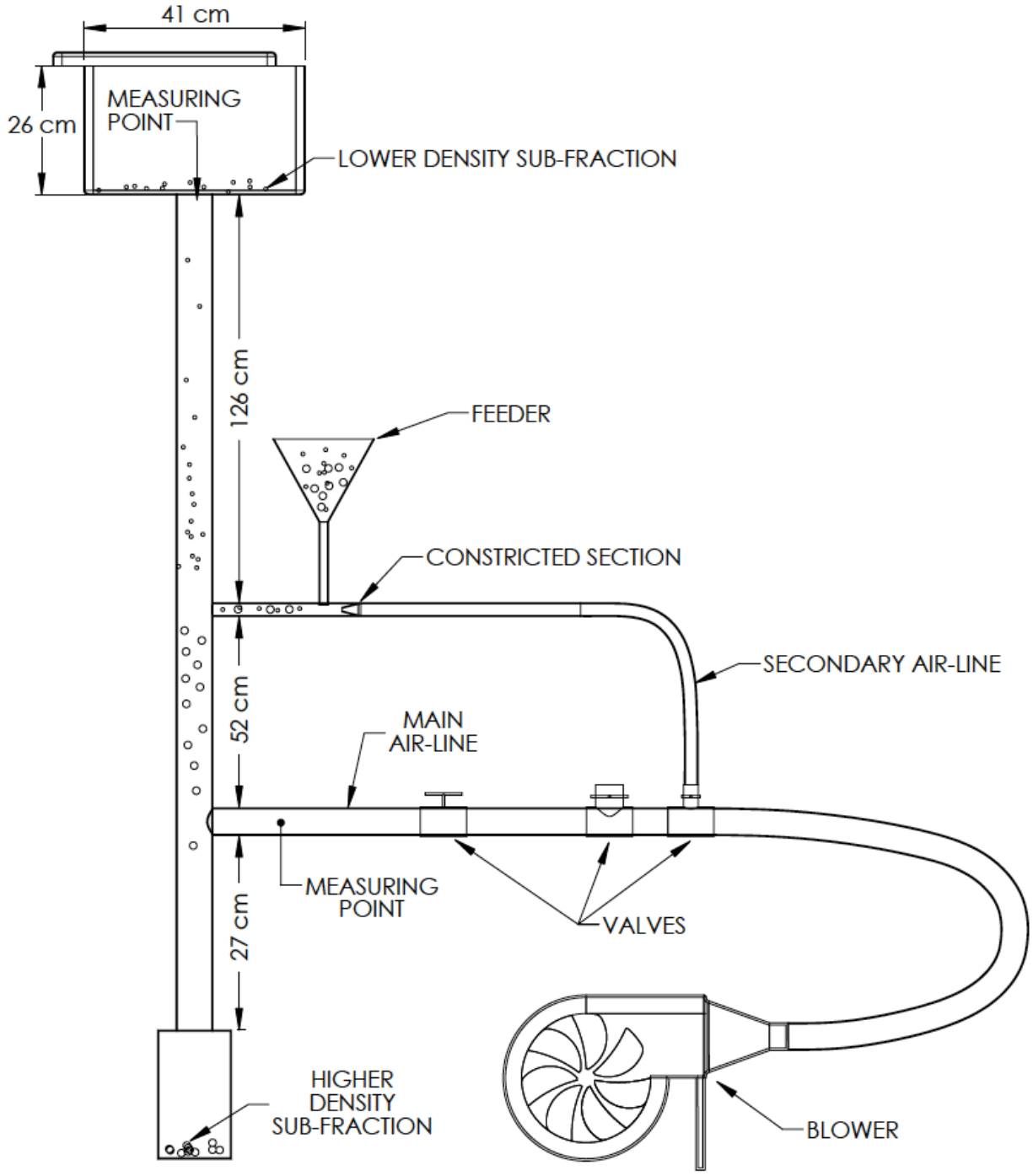


Figure 3.3 Schematic drawing of the elutriator.

with tap water at room temperature and at a weight to volume ratio (w:v) of 1:5 and stirred for 60 min. This 1:5 (w:v) ratio was the best ratio obtained to perform the aqueous fractionation (See Appendix A). This 1:5 (w:v) ratio was the resulting best ratio to perform aqueous fractionation The material obtained after blending was screened with an electrostatic filter (Web products, Inc., Kansas, KS) (Figure 3.4). The resultant slurry was autoclaved to produce a supernatant and a precipitate (121 ºC, 30 min). The supernatant was removed by siphoning and then, the precipitate was collected and dried in a forced air oven (55 ºC, overnight). Finally, the dried material was ground using a Retsch Mill (Model ZM100, Haan, Germany) equipped with a 1.0 mm screen and was named processed WWDG.

3.2.3 Digestibility Experiments

Two digestibility trials were conducted to assess the effect of dry and aqueous fractionation of distillers grains on digestibility of nutrients in rainbow trout (*Oncorhynchus mykiss*) (Figure 3.1). Rainbow trout (female triploid) were acquired from the Cangro Processors Ltd. (Lucky Lake, SK, Canada) and maintained according to the guidelines established by Canadian Council on Animal Care (2005). The fish were housed in 120 L tanks in an indoor, semi-closed recirculating system using biological filtration. Photoperiod was 14 h light / 10 h dark cycle. The water was maintained at 15 ± 2 °C and daily, dissolved oxygen, pH and temperature were observed and recorded. Chlorine, nitrate, nitrite and ammonia were monitored on a weekly basis. The first digestibility trial used a total of 126 fish (236 g) randomly assigned to 9 tanks (14 fish / tank). The second digestibility trial utilized a total of 105 fish (298 g) randomly assigned to 15 tanks (7 fish / tank). Three replicates were used for each diet. Fish were fed by hand twice daily to visual satiation.

Six test ingredients were evaluated in the two digestibility experiments; the first trial evaluated the original WDDGS and the corresponding WDDGS protein concentrate produced by dry fractionation (Table 3.7) while the second trial evaluated the processed WWDG produced and their corresponding start material from plants 1 and 2 (Table 3.10). A fishmeal reference diet (Table 3.2) was formulated according to Bureau and Cho (1994). The experimental diets consisted of 700 g kg^{-1} of the reference diet and 300 g kg^{-1} of test ingredient (as is basis). All diets contained celite (10 g kg^{-1}) as the indigestible marker. All ingredients were mixed using a Hobart mixer (Model L-800, Ohio, U.S.). The resulting dough was pelleted and cold extruded

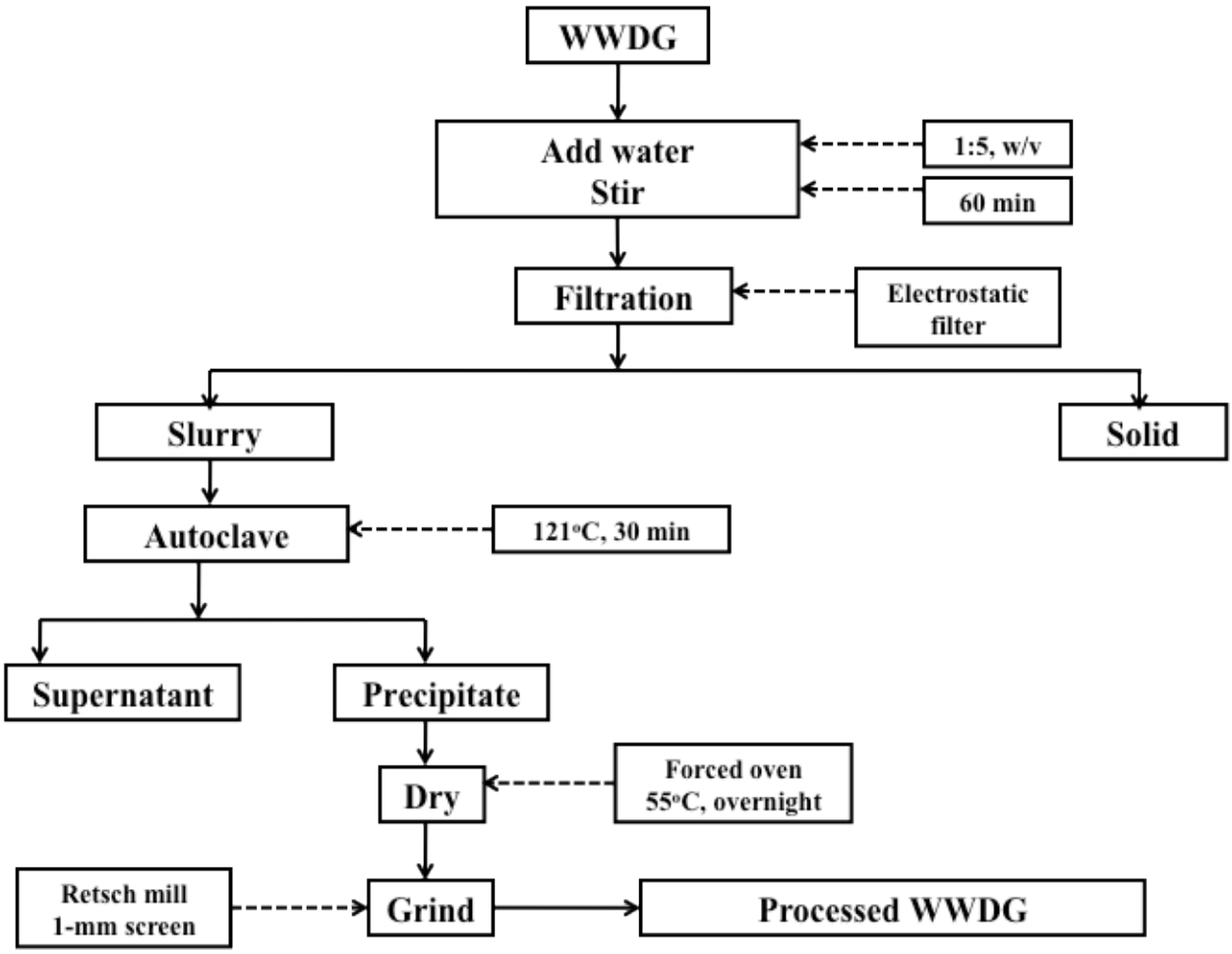


Figure 3.4 Outline of the aqueous fractionation processes used to produce the processed wheat wet distillers grains (WWDG).

using a Hobart food grinder (Model 4822, Ohio, U.S.) equipped with a 5-mm die. After extrusion, the diets were dried in a forced air oven (55°C, 12 h), chopped by hand and screened to obtain a suitable pellet size. The diets were stored at –20°C prior to feeding. Feed samples were taken for chemical analyses.

The fish were adapted to their diets for one week before feces collection. The fecal material was collected overnight for each tank using a settling column which separates feces from the effluent water as described by Hajen et al. (1993). After the feces collection, the fecal material was centrifuged (Beckman, model J6-MC, Palo Alto, CA, USA; 3000 x *g*, 15 min, 4ºC), frozen and freeze dried.

The ADC (%) of the reference and experimental diets were determined as follows (Cho et al. 1982):

$$ADC = 1 - (F/D \times Di/Fi)$$

Where:

F = % nutrient in the feces (DM basis)

D = % nutrient in the diet (DM basis)

Di = % indicator (AIA) in the diet (DM basis)

Fi = % indicator (AIA) in the feces (DM basis)

The ADC of the experimental ingredients were calculated as follows (Sugiura et al. 1998):

$$ADC_I = ADC_T + ((1-s)\, D_R/s\, D_I)\, (ADC_T - ADC_R)$$

Where:

ADC_I = Apparent digestibility coefficient of test ingredient

ADC_T = Apparent digestibility coefficient of test diet

ADC_R = Apparent digestibility coefficient of the reference diet

D_R = % nutrient (or kJ/g gross energy) of the reference diet mash (DM basis)

D_I = % nutrient (or kJ/g gross energy) of the test ingredient (DM basis)

s = Proportion of test ingredient in test diet mash (DM basis)

3.2.4 Growth Experiment

A growth experiment was performed to determine the effect of inclusion rate of the processed WWDG on the growth of rainbow trout. Results from the second digestibility trial showed the

Table 3.2 Composition of reference diet used in digestibility experiments.

Ingredient	Inclusion (g kg^{-1})
Fishmeal[1]	300
Soybean meal	170
Corn gluten meal	130
Wheat flour	280
Vitamin mineral premix[2]	10
Celite[3]	10
Fish oil[4]	100
Total	1000

[1] South American Aquagrade; EWOS Canada Ltd.
[2] The vitamin/mineral premix was a commercial premix.
(EWOS; closed formulation) formulated to meet the requirements of juvenile rainbow trout.
[3] Celite 545, <125μm; Celite Corporation, World Minerals Co., Lompoc, CA, USA.
[4] Mixed variety fish oil; EWOS Canada Ltd.

processed WWDG product from plant 2 had the highest digestibility values (Table 3.10). In addition, plant 2 showed high recovery of protein and yield compared to plant 1 (See Appendix B Figures A.1 and A.2). Thus, this product was selected to be use in the growth experiment. This product was called WWDG protein concentrate. s. Five dietary treatments containing 0, 75, 150, 225 and 300 g kg^{-1} of the WWDG protein concentrate were formulated and all diets contained the same level of DE and digestible CP (Table 3.3 and Table 3.4) and met or exceeded the nutrient requirements of rainbow trout (NRC 1993). All diets were also balanced for EEA according to Mambrini and Guillaume (1999). These diets were extruded, dried, chopped, screened and stored as previously described.

The growth trial lasted 56 days. Fish were weighed twice, at the start and at the end of the experiment. Feed intake was recorded on daily basis. Growth was assessed by calculating ADG; ADFI; SGR ([ln final weight – ln initial weight] / time (days) x 100) and feed:gain ratios (feed intake / wet weight gain) were determined on a per fish basis.

3.2.5 Chemical Analysis

Feces, feed ingredients and feed samples were ground through a 1-mm screen using a Retsch Mill (Model ZM 100, Haan, Germany) prior to chemical analyses. All analyses were conducted in duplicate and included the following procedures: Moisture content by drying at 135ºC in an air-flow type oven for two hours; ash (Association of Official Analytical Chemist (AOAC) 1990, method no. 942.05); GE using an Parr adiabatic oxygen bomb calorimeter (Model 1281, Parr Instrument Co., Moline, IL, USA); the nitrogen content was determined by combustion method (AOAC 1995, method no. 990.03) using a Leco protein/N analyzer (Model FP-528, Leco Corp., St. Joseph, MI, USA). Crude protein content was determined by multiplying nitrogen content by 6.25; acid insoluble ash; AEE (AOAC 1995, method no. 954.02). AA analysis was determined using chromatographic analysis at Evonik Industries, Essen, Germany. All sieved fractions and elutriated sub-fractions were analyzed for content of moisture, protein and NDF. Also, sieve fractions were also analyzed for ash and GE. NDF value was determined using an Ankom fibre analyzer (model ANKOM^{200}, Ankom Technology, Fairport, NY). Sodium sulfite and alpha-amylase were used in the NDF procedure. All feed ingredients used for the two digestibility trials

Table 3.3 Ingredient composition of fishmeal control and wheat wet distillers grains protein concentrate based experimental diets used for the growth trial.

Ingredient (g kg^{-1})	Experimental Diets				
	0 g kg^{-1}	75 g kg^{-1}	150 g kg^{-1}	225 g kg^{-1}	300 g kg^{-1}
Wheat wet distillers grains protein concentrate	0.0	75.0	150.0	225.0	300.0
Fishmeal	400.0	355.4	310.9	266.3	221.8
Meat and bone meal	304.0	249.7	195.5	141.3	87.1
Fish oil	120.5	127.4	134.3	141.2	148.1
Wheat flour	100.0	100.0	100.0	100.0	100.0
Solkafloc	57.8	73.9	90.1	106.3	122.5
Choline chloride	4.0	4.0	4.0	4.0	4.0
L-Lysine	1.9	2.8	3.7	4.6	5.5
DL-Methionine	1.8	1.5	1.3	1.0	0.8
Vitamin mineral premix	10	10	10	10	10

Table 3.4 Calculated digestible nutrient composition of fishmeal control and wheat wet distillers grains protein concentrate based experimental diets used for the growth trial.

Digestible Nutrient (g kg^{-1})	Experimental Diets				
	0 g kg^{-1}	75 g kg^{-1}	150 g kg^{-1}	225 g kg^{-1}	300 g kg^{-1}
Phosphorus	24.6	21.0	17.3	13.7	10.0
Digestible energy (MJ kg^{-1})	17.58	17.58	17.59	17.59	17.59
Crude protein	386.2	386.2	386.2	386.2	386.2
Methionine	9.9	10.1	10.3	10.5	10.7
Cysteine	3.4	4.0	4.7	5.3	5.9
Methionine and Cysteine	15.2	15.2	15.2	15.2	15.2
Lysine	29.2	28.1	27.0	25.8	24.7
Threonine	16.1	15.7	15.4	15.0	14.6
Arginine	25.7	24.7	23.7	22.7	21.7
Isoleucine	15.7	31.6	15.9	16.0	16.1
Valine	20.7	41.2	20.5	20.4	20.3

were measured for NDF as previously described and for ADF in sequence. The NDF and ADF were not adjusted for ash.

3.2.6 Statistical Analysis

The first digestibility experiment was designed and analyzed as a completely randomized design using the General Linear Models procedure of SAS (v.9.1.3, SAS Institute inc., Cary, NC, USA) according to the following model:

$$y_{ij} = \mu + t_i + \varepsilon_{ij}$$

Where:

y = the dependent variable

μ = the overall mean

t = the effect of the i^{th} ingredient

ε = the residual error.

The second digestibility experiment was designed and analysed as a 2 X 2 factorial design with 2 ethanol plants (plant 1 and plant 2) and 2 levels of ingredient processing (unprocessed and processed) by the Mixed Models procedure of SAS (v.9.1.3, SAS Institute inc., Cary, NC, USA) using the following model:

$$y_{ijk} = \mu + \alpha_i + \beta_j + (\alpha\beta)_{ij} + \varepsilon_{ijk}$$

Where:

y = the dependent variable

μ = the overall mean

α = the effect of the ethanol plant

β = the effect of ingredient processing

$\alpha\beta$ = the effect of interaction between ethanol plant and ingredient processing

ε = the residual error.

Differences between means of treatments were calculated using the Ryan-Einot-Gabriel-Welsch F test and the level of significance was set at $P < 0.05$, while tendencies were set at $0.10 > P \geq 0.05$. For the growth experiment linear and quadratic regression models were fitted to the data and regressions were considered significant when $P < 0.05$. The inclusion rates that resulted in growth performances that were 95 and 90% of the control diet (100%) were calculated.

3.3 Results

3.3.1 Dry Fractionation Process

The CP content increased from 39.8 % in the original WDDGS to 44.9 % in the pan fraction (Table 3.5) while the NDF content decreased from 34.8 % to 24.2 %. As a result of sieving, there was a total increase of 5.1 % CP and a total reduction of 10.6 % NDF. The effect of reduction of particle size on GE and moisture content was small, showing only an increase of 128 kcal kg^{-1} and 1.5 % of moisture in the pan fraction compared to the original WDDGS. The ash content did not show a general pattern as the particle size decreased. The pan fraction had the highest CP, moisture and GE contents and the lowest NDF content and therefore, it was decided not to elutriate the pan fraction.

Elutriation produced changes in chemical composition between the start material (sieve fractions) and the corresponding higher density and lower density sub-fractions (Table 3.6). There were also differences in chemical composition between higher density and lower density sub-fractions from the same WDDGS sieve fraction. Protein was concentrated in all higher density sub-fractions while NDF was concentrated in all lower density sub-fractions. All higher density sub-fractions had higher CP content and lower NDF content than their corresponding start WDDGS sieve fraction. In contrast, all lower density sub-fractions had reduced CP content and increased NDF value compared to the corresponding start DDGS sieve fraction except for the lower density sub-fraction from 50 sieve fraction. Chemical analyses of WDDGS protein concentrate revealed it was 60.6 g kg^{-1} higher in CP and 74.5 and 23.0 g kg^{-1} lower in NDF and ADF, respectively, than the original WDDGS (Table 3.7). As well, WDDGS protein concentrate had higher levels of AA and slightly lower level of AEE, DM and ash compared to the start material.

3.3.2 Effect of Dry Fractionation on Digestibility

The effect of dry fractionation of WDDGS on nutrient digestibility of WDDGS is presented in Table 3.8. Dry fractionation had significant effect on the digestibility of CP ($P < 0.05$) but had not significant effect on DM, GE, AEE, ash and individual AA digestibility. Thus, the ADC of CP for WDDGS protein concentrate was 88 % and for the original WDDGS

Table 3.5 Chemical composition (% dry matter basis) and percent of material retained on each screen.

Sieve Fraction	µm Openings	Moisture (%)	Crude Protein	Sd[1]	NDF[2]	Sd[1]	Ash	Gross Energy (MJ kg^{-1})	Screen Retention (%)	Sd[1]
Original material		6.6	39.8		34.8		6.1	21.2	-	
20	>840	7.6	39.8	1.02	31.2	1.11	6.2	21.1	41.7	5.08
30	840-589	8.1	37.6	0.47	34.4	0.62	6.0	21.3	22.0	0.45
40	589-425	7.7	37.9	0.24	37.5	0.17	6.0	21.3	10.3	0.97
50	425-297	7.7	39.3	0.23	30.9	0.09	6.2	21.2	9.8	1.19
60	297-250	7.7	41.0	0.08	31.8	0.34	6.1	20.2	3.3	0.54
80	250-177	7.4	41.9	0.11	31.3	0.08	6.3	21.2	4.8	0.84
Pan	<177	8.2	44.9	0.13	24.2	2.31	6.2	21.7	7.8	1.17

[1] Standard deviation.
[2] Neutral detergent fibre

Table 3.6 Chemical composition (% dry matter basis) and yield for original material, sieve fractions and elutriation sub-fractions of wheat distillers grains with soluble.

Sieve Fraction	Velocity (m/s)		Crude protein			NDF[1]			Yield (%)		
	Point										
	1	2	H[2]	Std[3]	L[4]	Std[3]	H[2]	Std[3]	H[2]	Std[3]	L[4]	Std[3]
Original material			39.8				34.8					
20	0*		39.8				31.2		—		—	
	5.75	3.7	40.1	1.16	33.5	0.36	30.2	0.36	39.8	0.94	89.6	0.32	10.3	0.32
30	0*		37.6				34.4		—		—			
	5.0	3.1	40.2	0.27	34.6	0.72	32.5	0.86	40.8	1.65	69.1	4.26	30.8	4.26
40	0*		37.9				37.5		—		—		—	
	4.33	2.9	41.5	0.74	35.4	0.71	32.4	1.55	39.4	1.08	42.7	2.58	57.2	2.58
50	0*		39.3				30.9							
	4.14	2.9	43.3	0.68	38.6	1.13	25.5	1.58	31.9	4.31	14.6	3.20	85.3	3.20
60	0*		41.0				31.8		—		—		—	
	2.76	2.1	44.8	0.71	39.2	0.75	28.4	2.97	33.3	0.73	32.5	0.36	67.4	0.36
80	0*		41.9				31.3		—		—		—	
	1.74	1.5	44.8	1.11	39.1	1.92	28.0	1.00	34.8	2.18	63.6	5.89	36.3	5.89

* Values at 0 m/s means initial material.
[1] Neutral detergent fibre.
[2] Higher density sub-fraction.
[3] Standard deviation.
[4] Lower density sub-fraction.

Table 3.7 Nutrient composition (g kg^{-1}) of original wheat distillers dried grains with solubles (WDDGS) and the WDDGS protein concentrate produced by the dry fractionation process.

Parameter	WDDGS	WDDGS –PC[1]
Dry matter	944.7	942.5
Crude protein	394.0	454.6
Gross energy (MJ kg^{-1})	20.5	20.7
Acid ether extract	79.4	78.9
Ash	58.6	49.2
NDF[2]	334.9	260.4
ADF[3]	116.2	93.2
Essential amino acids		
Arginine	17.6	18.8
Histidine	8.5	9.5
Isoleucine	14.2	16.4
Leucine	26.4	30.2
Lysine	8.5	9.5
Phenylalanine	17.7	20.7
Threonine	11.6	13.3
Valine	17.1	19.5
Methionine	6.2	7.1

[1] Wheat distillers dried grains with solubles protein concentrate
[2] Neutral detergent fibre.
[3] Acid detergent fibre.

Table 3.8 Apparent digestibility coefficients (%) of wheat distillers dried grains with solubles (WDDGS) ingredients for rainbow trout.

Parameter	WDDGS	WDDGS-PC[1]	S.E.M.[2]	*P*-value
Dry matter	70.4	71.8	2.08	0.780
Crude protein	84.9[b]	88.0[a]	0.83	0.043
Gross energy	77.3	74.4	1.08	0.210
Acid ether extract	75.0	74.9	3.01	0.991
Ash	69.5	76.1	4.49	0.520
Essential amino acids				
Arginine	88.4	89.3	0.38	0.293
Histidine	83.3	85.1	0.82	0.325
Isoleucine	79.4	81.8	1.12	0.340
Leucine	83.0	84.0	0.71	0.574
Lysine	80.3	80.8	1.98	0.921
Phenylalanine	89.1	89.3	0.58	0.886
Threonine	82.9	84.5	0.69	0.313
Valine	83.2	85.0	0.63	0.158
Methionine	81.9	83.7	1.05	0.450

[1] Wheat distillers dried grains with solubles protein concentrate.
[2] Standard error of the mean.
[a-b] Different letters denote significant differences within a row ($P < 0.05$).

was 84.9 %. The digestible nutrient content of WDDGS ingredients is listed in Table 3.9. The level of digestible CP and individual AA was significantly increased ($P < 0.05$) in the WDDGS protein concentrate compared to the original WDDGS with the exception of lysine ($P > 0.1$). There were not significant differences in digestible DM, energy, AEE and ash ($P > 0.1$).

3.3.3 Aqueous Fractionation Process

The protein content of the processed WWDG from plant 2 was 685.1 g kg^{-1} protein and for the processed WWDG from plant 1 was 522.2 g kg^{-1}. The level of DM, CP, GE, AEE and all individual AA increased while the level of ash decreased in the processed WWDG from plant 2 compared to its corresponding start material (Table 3.10). In plant 1, the level of DM, CP and all individual AA increased but the level of GE, AEE and ash decreased in the processed WWDG compared to its corresponding start material (Table 3.10). NDF and ADF contents of the processed WWDG from plant 2 were markedly decreased by the aqueous fractionation process. NDF was 555 g kg^{-1} in the start material and 166.5 g kg^{-1} in the processed WDG and ADF went from 202.6 to 72.1 g kg^{-1}. In contrast, WWDG from plant 1 showed similar values for NDF and ADF between the unprocessed material and the corresponding processed material.

Regarding the recovery of protein and the yield of the process, plant 2 showed high recovery of protein and yield compared to plant 1 (See Appendix B Figures A.1 and A.2).

3.3.4 Effect of Aqueous Fractionation on Digestibility

Digestibility of CP and AA was not influenced both by the plant and the processing method ($P > 0.1$) with the exception of arginine and histidine digestibility, which was both significantly reduced ($P < 0.05$) (Table 3.11). The ADC of DM (60.2 - 81.0 %), GE (68.8 - 84.4 %), AEE (75.7 - 84.2 %) and ash (37.8 - 57.4 %) for plant 1 were significantly lower compared to plant 2 ($P < 0.05$). The ADC of protein was similar between unprocessed WWDG and processed WWDG with 84.5 and 86.1 %, respectively ($P > 0.05$). The ADC of DM (64.6 - 76.6 %), GE (70.4 - 84.4 %), AEE (72.9 - 87.0 %) was lower in unprocessed WWDG than in processed WWDG ($P < 0.05$). Unprocessed WWDG had a significant high ash digestibility (60.3 %) compared to processed WWDG (35.0 %) ($P < 0.05$). There were significant interactions between plant and processing for the ADC of GE and histidine ($P < 0.05$) (Table 3.12). In the plant 1,

Table 3.9 Digestible nutrient content (g kg^{-1} DM) of wheat distillers dried grains with solubles (WDDGS) ingredients for rainbow trout.

Parameter	WDDGS	WDDGS-PC[1]	S.E.M.[2]	*P*-value
Dry matter	665.6	677.0	1.95	0.806
Crude protein	334.7[b]	398.4[a]	1.43	< 0.01
Energy (MJ kg^{-1})	15.8	15.4	49.68	0.301
Acid ether extract	59.6	59.1	0.23	0.934
Ash	40.7	37.4	0.22	0.522
Essential amino acids				
Arginine	15.5[b]	16.8[a]	0.02	< 0.01
Histidine	7.0[b]	8.0[a]	0.02	< 0.01
Isoleucine	11.2[b]	13.3[a]	0.04	< 0.01
Leucine	21.8[b]	25.4[a]	0.08	< 0.01
Lysine	6.8	7.6	0.02	0.111
Phenylalanine	15.7[b]	18.4[a]	0.06	< 0.01
Threonine	9.6[b]	11.2[a]	0.03	< 0.01
Valine	14.2[b]	16.5[a]	0.05	< 0.01
Methionine	5.1[b]	5.9[a]	0.01	< 0.01

[1] Wheat distillers dried grains with solubles protein concentrate.
[2] Standard error of the mean.
[a-b] Different letters denote significant differences within a row ($P < 0.05$).

Table 3.10 Nutrient compositions (g kg^{-1}) of unprocessed wheat wet distillers grains (U) and processed wheat wet distillers grains (P) used in the second digestibility trial.

Parameter	Plant 1		Plant 2	
	U	P	U	P
Dry matter	949.4	952.3	881.7	956.6
Crude protein	249.5	522.2	431.8	685.1
Gross energy (MJ kg^{-1})	21.2	21.1	20.1	23.7
Acid ether extract	120.6	89.7	74.1	116.7
Ash	53.3	50.1	134.2	45.3
NDF[1]	238.1	249.7	555.0	166.5
ADF[2]	90.3	97.9	202.6	72.1
Essential amino acids				
Arginine	14.5	21.2	19.3	30.1
Histidine	5.9	10.6	8.8	14.3
Isoleucine	8.7	20.5	15.5	29.6
Leucine	16.4	37.1	28.6	52.7
Lysine	9.5	13.9	10.0	18.0
Phenylalanine	10.4	24.3	19.4	31.4
Threonine	8.3	16.4	12.9	22.0
Valine	12.0	24.2	18.9	34.9
Methionine	4.1	9.8	6.9	14.1

[1] Neutral detergent fibre.
[2] Acid detergent fibre.

Table 3.11 Effect of plant and processing on apparent digestibility coefficients (%) of macronutrients and essential amino acids in rainbow trout.

Parameter	Plant			Processing			*P-value*	
	Plant 1	Plant 2	S.E.M.[1]	U[2]	P[3]	S.E.M.[1]	Plant	Processing
Dry matter	60.2[b]	81.0[a]	1.05	64.6[b]	76.6[a]	1.05	<0.01	<0.01
Crude protein	85.0	85.6	1.54	84.5	86.1	1.54	0.793	0.492
Gross energy	68.8[b]	84.4[a]	0.69	70.4[b]	84.4[a]	0.69	<0.01	<0.01
Acid ether extract	75.7[b]	84.2[a]	1.58	72.9[b]	87.0[a]	1.58	< 0.01	< 0.01
Ash	37.8[b]	57.4[a]	5.77	60.3[a]	35.0[b]	5.77	0.042	0.014
Essential amino acids								
Arginine	89.7	88.5	0.75	90.9[a]	87.3[b]	0.75	0.281	0.009
Histidine	77.6	82.7	1.87	86.8[a]	73.5[b]	1.87	0.091	< 0.01
Isoleucine	84.6	81.3	1.07	84.1	81.9	1.07	0.063	0.185
Leucine	85.2	83.4	1.14	85.6	83.0	1.14	0.283	0.140
Lysine	80.2	84.1	1.36	83.5	80.9	1.36	0.076	0.213
Phenylalanine	86.9	89.1	0.81	88.6	87.4	0.81	0.090	0.343
Threonine	81.3	83.6	0.99	83.2	81.7	0.99	0.138	0.321
Valine	84.9	84.2	0.99	85.3	83.7	0.99	0.614	0.292
Methionine	84.8	82.7	1.16	84.9	82.6	1.16	0.225	0.198

[1] Standard error of the mean.
[2] Unprocessed wheat wet distillers grains.
[3] Processed wheat wet distillers grains.
[a-b] Different letter denote significant differences within a row ($P < 0.05$)

ADC of GE was 59.2 and 78.4 %, in the unprocessed and processed WWDG, respectively. In the plant 2, the ADC of GE was 81.5 and 87.3 %, in the unprocessed and processed WWDG, respectively. ADC of GE was low both in the unprocessed (59.3 %) and in the processed WWDG (78.4 %) from plant 1 compared to the unprocessed (81.5 %) and processed WWDG (87.4 %) from plant 2. In the plant 1, ADC of histidine was 87.7 and 67.6 %, in the unprocessed and processed WWDG, respectively while in plant 2, the ADC of histidine was 86.0 and 79.5 %, in the unprocessed and processed WWDG, respectively. There were no significant interactions between plant and processing on CP, AEE and ash digestibility ($P > 0.10$).

Digestible nutrient content of DM, CP and AA was significant affected by plant ($P < 0.05$) but digestible AEE was not affected ($P > 0.10$) (Table 3.13). Plant 2 had high digestible content of DM, CP, energy, ash and all AA compared to plant 1 ($P < 0.05$). Processing significantly increased digestible nutrient ($P < 0.05$) but dramatically reduced digestible ash ($P < 0.05$) (Table 3.13). Significant interactions between plant and processing were found on digestible content of AEE, ash and all AA except isoleucine, which presented tendency ($P = 0.098$) (Table 3.13). The interaction between plant and processing had no effect on digestible content of DM, protein and energy ($P > 0.10$).

3.3.5 Growth Trial

The growth performance of rainbow trout is shown in Table 3.14. There were no significant linear or quadratic relationships between inclusion rate and SGR, ADG or feed:gain ratios (Table 3.15). However, there was a significant negative linear relationship between inclusion rate and ADFI (Table 3.15 and Figure 3.5). ADFI was reduced to 95 % of the controls at 8.7 % inclusion rate and 90 % of controls at 15.8 % inclusion rate.

3.4 Discussion

Depression of growth performance due to the inclusion of plant proteins in salmonid feeds is caused by several factors including the quality of protein, the type and amount of ANF, fibre level and palatability among others (Thiessen et al. 2003; Drew 2007). Most plant proteins including SBM (Refstie et al. 1998; Refstie et al. 2000; Krogdahl et al. 2003), rapeseed/canola (Higgs et al. 1995; Thiessen et al. 2004) and pea (Burel et al. 2000; Thiessen et al. 2003a) have

Table 3.12 Effect of interaction between plant and processing on apparent digestibility coefficients (%) of macronutrients and essential amino acids in rainbow trout.

Parameter	Plant 1		Plant 2		S.E.M.[3]	*P-value*
	U[1]	P[2]	U[1]	P[2]		
Dry matter	52.8	67.8	76.6	85.4	1.49	0.072
Crude protein	84.7	85.3	84.4	86.9	2.18	0.671
Gross energy	59.3[b]	78.4[a]	81.5[x]	87.4[y]	0.97	0.0001
Acid ether extract	67.9	83.6	78.0	90.5	2.23	0.483
Ash	53.4	22.4	67.3	47.7	8.16	0.506
Essential amino acids						
Arginine	90.9	86.1	91.0	88.5	1.06	0.326
Histidine	87.8[a]	67.6[b]	86.0[x]	79.5[y]	2.64	0.032
Isoleucine	85.2	84.2	83.1	79.7	1.52	0.460
Leucine	86.9	83.6	84.4	82.4	1.61	0.690
Lysine	82.8	77.7	84.2	84.1	1.92	0.219
Phenylalanine	87.5	86.3	89.7	88.6	1.14	0.983
Threonine	82.2	80.6	84.4	83.0	1.41	0.948
Valine	85.4	84.5	85.3	83.1	1.41	0.657
Methionine	86.6	83.2	83.3	82.1	1.64	0.508

[1] Unprocessed wheat wet distillers grains.
[2] Processed wheat wet distillers grains.
[3] Standard error of the mean.
[a-b, x-y] Different letter denote significant differences within plants ($P < 0.05$).

Table 3.13 Effect of plant and processing on digestible nutrient content (g kg^{-1} DM) of macronutrients and essential amino acids in rainbow trout.

Parameter	Plant			Processing			*P*-value		
	Plant 1	Plant 2	S.E.M.[1]	U[2]	P[3]	S.E.M.[1]	Plant	Processing	Interaction
Dry matter	573.3[b]	746.2[a]	0.98	588.2[b]	731.4[a]	0.98	<0.01	<0.01	0.925
Crude protein	328.5[b]	479.8[a]	0.81	287.8[b]	520.4[a]	0.81	<0.01	<0.01	0.892
Energy (MJ kg^{-1})	14.5[b]	18.5[a]	34.57	14.5[b]	18.6[a]	34.57	<0.01	<0.01	0.455
Acid ether extract	78.4	81.6	0.15	69.8[b]	90.2[a]	0.15	0.174	<0.01	<0.01
Ash	19.8[b]	55.9[a]	0.32	59.3[a]	16.4[b]	0.32	<0.01	<0.01	< 0.01
Essential amino acids									
Arginine	15.7[b]	22.0[a]	0.01	15.3[b]	22.4[a]	0.015	<0.01	<0.01	<0.01
Histidine	6.1[b]	9.5[a]	0.01	6.3[b]	9.2[a]	0.019	<0.01	<0.01	0.012
Isoleucine	12.3[b]	18.2[a]	0.01	10.1[b]	20.4[a]	0.016	<0.01	<0.01	0.098
Leucine	22.6[b]	33.7[a]	0.03	19.2[b]	37.2[a]	0.030	<0.01	<0.01	<0.01
Lysine	9.3[b]	11.7[a]	0.01	8.1[b]	12.9[a]	0.015	<0.01	<0.01	<0.01
Phenylalanine	15.0[b]	22.5[a]	0.01	13.2[b]	24.3[a]	0.013	<0.01	<0.01	0.006
Threonine	10.0[b]	14.6[a]	0.01	8.8[b]	15.7[a]	0.012	<0.01	<0.01	0.026
Valine	15.3[b]	22.5[a]	0.01	13.2[b]	24.7[a]	0.018	<0.01	<0.01	< 0.01
Methionine	5.8[b]	8.6[a]	0.007	4.6[b]	9.8[a]	0.007	<0.01	<0.01	< 0.01

[1] Standard error of the mean.
[2] Unprocessed wheat wet distillers grains.
[3] Processed wheat wet distillers grains.
[a-b] Different letter denote significant differences within a row ($P < 0.05$).

Table 3.14 Growth performance of rainbow trout fed various levels of diets containing wheat wet distillers grains protein concentrate over a 56 days.

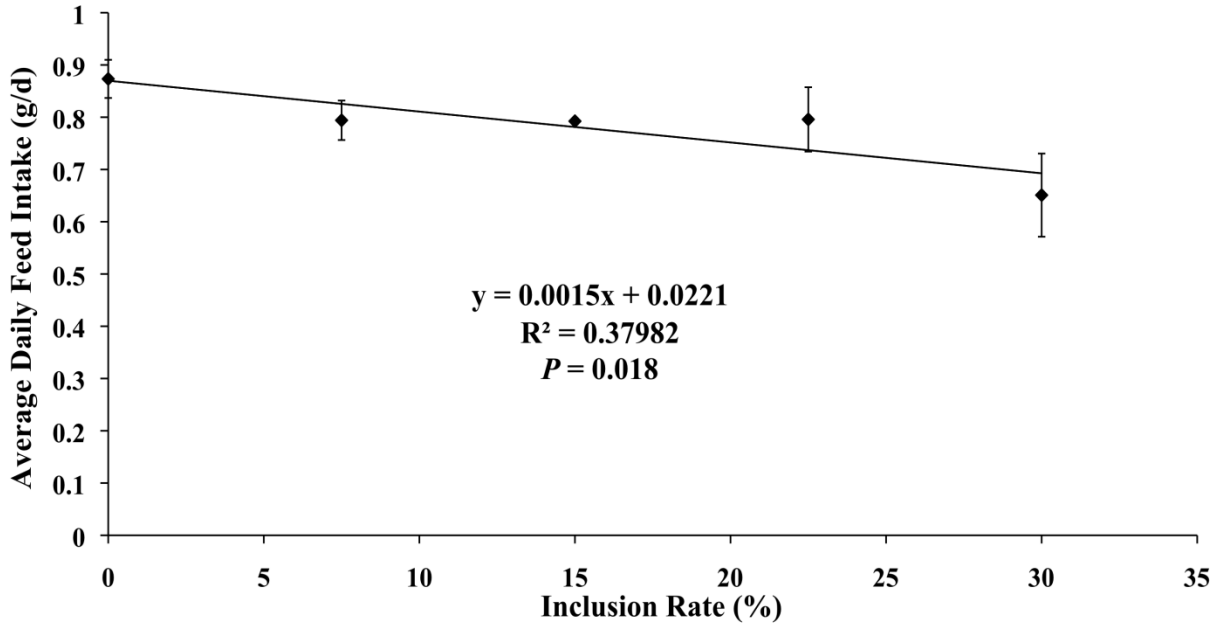
Inclusion Level ($g\ kg^{-1}$)	Average Daily Gain ($g\ d^{-1}$)	Specific Growth Rate (%)	Feed to Gain Ratio ($g\ d^{-1}$)	Average Daily Feed Intake ($g\ d^{-1}$)
0	0.95	0.67	0.94	0.87
75	0.74	0.52	1.12	0.79
150	0.68	0.45	1.21	0.79
225	0.74	0.53	1.06	0.79
300	0.76	0.49	1.06	0.65
Stdev	0.20	0.16	0.23	0.09

[1] Standard deviation.

Table 3.15 Linear and quadratic regression parameters of the relation between wheat wet distillers grains protein concentrate inclusion rate and average weight gain, specific growth rate, feed to gain ratio and average daily feed intake of rainbow trout during the growth trial.

Parameter	Unstandardized Coefficients				
	Constant	Inclusion	Inclusion^2	r^2	*P*-value
Average Daily Gain					
Linear	0.855	-0.005		0.081	0.305
Quadratic	0.934	-0.026	0.001	0.204	0.255
Specific Growth Rate					
Linear	0.598	-0.005		0.102	0.245
Quadratic	0.653	-0.019	5×10^{-6}	0.195	0.272
Feed to Gain Ratio					
Linear	1.04	0.003		0.019	0.636
Quadratic	0.957	0.026	-8×10^{-6}	0.127	0.474
Average Daily Feed Intake					
Linear	0.866	-0.005		0.383	0.018
Quadratic	0.853	-0.002	-8×10^{-6}	0.402	0.059

Figure 3.5 Linear regression of average daily feed intake on inclusion rate of wheat wet distillers grains protein concentrate produced by aqueous fractionation.



significant levels of ANF which negative affect intestinal structure, nutrient digestibility andgrowth performance of carnivorous fish. Wheat and WDDGS have only one known antinutritional factor for fish: soluble fibre from xylans and β-glucans. These are the major constituent of the fibre of wheat and are present at high levels in WDDGS due to the removal of starch during fermentation and the subsequent concentration of fibre in the DDGS (Mathlouthi et al. 2002; Mathlouthi et al. 2003; Widyaratne and Zijlstra 2007; Widyaratne et al. 2009). In general, the detrimental effect of NSP on the absorption of nutrient and the growth of salmonids is related to increasing viscosity of the digesta and increasing water content in the feces (Refstie et al. 1998; Storebakken et al. 1998a; Storebakken et al. 1998b; Refstie et al. 1999; Storebakken et al. 1999). It is also indicated the negative effect of NSP might be due to binding of bile acids, obstructing action of digestive enzymes and thus, affecting the capacity for maximum feed intake (Refstie et al. 1999; Francis et al. 2001).

Processing of plant proteins has shown to decreased the levels of ANF in plant proteins. Procedures such as dehulling, grinding, sieving, air classification, aqueous extraction and heat treatment have been commonly used (Mwachireya et al. 1999; Thiessen et al. 2003a; Booth and Allan 2004; Barrows et al. 2007; Randall and Drew 2010). In this study, several processing methods were employed to modify the nutrient composition of wheat distillers grains. The two fractions with the smallest particle size had increased levels of CP and decreased levels of xylans and β-glucans as represented by the NDF value in WDDGS. These finding were in general in agreement with those reported for sieving of wheat and corn DDGS (Wu and Stringfellow 1986; Srinivasan et al. 2005a; Liu 2009b; Randall and Drew 2010). Thus, protein is more concentrated in fine particles after grinding DDGS using a hammer mill. Ash and GE content did not differ due to differences in particle size. These observations are consistent with the findings of Randall and Drew 2010. However, Wu and Stringfellow (1986) and Liu (2008) reported ash content increased as the particle size decreased when corn DDGS was sieved. Overall, sieving was effective to produce a pan fraction with high CP and low NDF contents compared to the original WDDGS. Further reduction of NDF and consequently, increase of CP content in WDDGS was achieved with elutriation. Protein was higher in all higher density sub-fractions while NDF was higher in all lower density sub-fractions compared to their corresponding start material. This was consistent with the earlier findings of Liu (2009a) and Srinivasan et al. (2005a).

The digestibility of CP may be affected by high NDF intake and heat damage during the fermentation or drying processes of WDDGS, resulting in reduced CP digestibility (Goering et al. 1973; Hurrell et al. 1976; Widyaratne and Zijlstra 2007). The digestibility of CP for WDDGS (84.9 %) obtained in this study was lower than those reported by Randall and Drew (2010) for WDDGS (90.0 %). However, the latter authors utilized WDDGS supplied by another ethanol plant. Thus, the digestibility of WDDGS may be markedly affected by plant to plant and within plant variations due to different bio-ethanol processing methods. This may be partially confirmed by the lower level of moisture content and higher NDF content of the WDDGS used in this study compared to that used by Randall and Drew (2010) (5.5 % compared to 10.5 % and 26 % compared to 21.5 %, respectively). The increased protein digestibility was not matched by increased digestibility of AA. This might be explained due to protein content of the samples was calculated by multiplying nitrogen content by 6.25 while AA content was determined using a chromatographic analysis which detects and calculates the specific amount of individual AA. Therefore, the protein is referred as CP.

In addition, the AA digestibility values obtained in the fish fed WDDGS protein concentrate are in agreement with earlier findings in other monogastric species (Parsons et al. 2006). The latter authors noted removal of fibre from corn DDGS by a combination of sieving and elutriation had non-significant effects on the digestibility of AA in cecectomized roosters.

In the present study, the removal of fibre from WDDGS did not result in an increase of DM and GE digestibility. It is known that the digestibility of energy and DM is inversely correlated by fibre content (Mwachireya et al. 1999; Borgeson et al. 2006; Widyaratne and Zijlstra 2007). Cromwell et al., (1993) indicated ADF appeared to be more related to the nutritional value of DDGS than NDF concentrations. In contrast with the findings of the present study, Randall and Drew (2010) observed that sieving significantly increased DM and GE digestibility. Although, in both studies the fractionated methods used were able to reduce ADF content in a similar range (2.2 - 2.3 %), the WDDGS protein concentrate used in this study had higher levels of ADF compared to that reported by Randall and Drew (2010). Fish fed the WDDGS protein concentrate diet did not improve AEE digestibility. Several studies have reported fibre decreases fat digestibility by disturbing emulsification and lipolysis in the intestine of fish (Refstie et al. 1999). The present data support these findings and confirm that fibre plays an important role in fat, DM and GE digestibility in rainbow trout. Thus, it is possible that the

dry fractionation process used in the current study did not remove sufficient fibre from the WDDGS to affect AEE, DM and GE digestibility. Thus, the WDDGS might require further processing to become a suitable ingredient for salmonid diets.

The chemical variation between WWDG from plant 1 and WWDG from plant 2 suggested that the bio-ethanol processing methods used in these ethanol plants were different. For example, the type of grain, method of fermentation (batch or continue), addition of yeats, distillation, amount of solubles added back and drying process would affect the chemical profile of distillers grains. As was mentioned, both ethanol plants used different feedstock. Moreover, plant 1 does not add solubles back and this might explain the low CP content of WWDG from this plant compared to the WWDG from plant 2. Furthermore, the low CP content could also have been due to the fact that the soluble fraction of WWDG was draining out when the WWDG from plant 1 was collected.

Aqueous fractionation of WWDG resulted in more substantial increases in protein content than were achieved using dry fractionation. Furthermore, the WDDGS from two plants with markedly different production systems were tested to determine the robustness of the aqueous fractionation method. There were no significant interactions between plant and processing on digestibility content of DM, CP, AEE and ash. Therefore, it can be concluded that the aqueous process used in this study is independent of plant, DM, protein, AEE and ash contents in the start material. It should also be mentioned that processing resulted in numerically low ADC of AA. A negative effect of the Maillard reaction produced during this process on digestibility of AA might be the logical explanation. However, this is not supported by the fact that this aqueous fractionation was performed in a liquid medium which is not known as a factor that predisposes the production of Maillard products.

There were differences in ash digestibility between WWDG products. These differences are not easy to explain due to fish are able to obtain considerable amounts of minerals from the environmental water. However, it can be suggested the difference in ash content between products might play a role in the resulting ash digestibility. Therefore, it could be proposed to analyze the WWDG products for mineral composition.

SBM and CGM are currently widely used in aquaculture due to their relatively high CP content and high CP digestibility (Gatlin et al. 2007). Soybean meal has about 48 % CP while CGM has approximately 60 % CP (Hardy 2000). However, their nutritional value in salmonid

diets is affected due to SBM has significant levels of ANF which impair the growth and performance of fish while CGM might impart yellow pigmentation to fish flesh if it is included in high proportion of diet (Hardy 2000; Francis et al. 2001; Gatlin et al. 2007). In this study, the processed WWDG showed lower CP and AA digestibility than values reported for SBM and CGM in rainbow trout (Cheng and Hardy 2003; Thiessen et al. 2004). Despite these lower digestibility values, the levels of digestible CP in the processed WWDG were higher than those of SBM. Furthermore, WDDGS is among the cheapest sources of protein available for animal feeds at present. Thus on a per tonne basis and protein basis, WDDGS has a significant price advantage over CGM and SBM (Table 3.16).

It should be mentioned it was not possible to keep constant all ingredients in experimental diets due to the WWDG protein concentrate was high in CP content. As a consequence, the digestible phosphorus content varied in these diets. However, this does meet the requirement for rainbow trout. Growth performance of rainbow trout revealed inclusion of 300 g kg^{-1} WWDG protein concentrate did not negatively affect SGR, ADG and feed:gain ratios. However, the significant negative linear relationship between inclusion rate and ADFI indicated that ADFI was reduced to 95 % of the controls at 8.7 % inclusion rate and 90 % of controls at 15.8 % inclusion rate. It is difficult to identify which component (s) or fraction (s) in the WWDG affected ADFI on the growth experiment. It is likely that the reduction in ADFI may have been due to residual levels of soluble fibre or NSP in the WWDG protein concentrate. However, the soluble NSP content was not determined in this study. The xylanase and β-glucanase enzymes have shown to be able to hydrolyze arabinoxylan and β-glucan that are present in NSP, respectively (Mathlouthi et al. 2002; Widyaratne et al. 2009). Therefore, the antinutritional properties of NSP might be attenuated by the supplementation of xylanase and β-glucanase to the WWDG protein concentrate-based diet and thus, allows the use of higher inclusion levels of this product.

3.5 Conclusions

Information on the nutritional value of wheat distillers grains in salmonids is scarce. Thus, the information collected from this study contributes to expanding knowledge concerning effects of further processing of distillers grains on its nutritional value in rainbow trout. Aqueous

Table 3.16 Average price of available protein (% dry basis) for selected feedstuffs.

	Unit	Wheat DDGS	Corn Gluten meal	Soybean Meal
Price range	US$/ton	180[1]	530-590[2]	255-340[2]
Average	US$/ton	180	560	595
Protein content	% DM[3]	37.7[4]	60.0[5]	48.0[5]
Protein price (ave.)	US$/kg protein	0.47	0.93	1.23

[1] C. Christensen, Feed Innovation Institute USASK, personal communication, 2010)
[2] University of Missouri 2010
[3] Dry matter
[4] University of Saskatchewan 2010
[5] Hardy 2000

fractionation produced a high WWDG product relative to the original WDG, WDDGS or WDDGS protein concentrate. Inclusion of 300 g kg^{-1} WWDG protein concentrate in rainbow trout diets resulted in not negative effect on growth performance. As a result, WWDG protein concentrate appears to be feasible protein source for rainbow trout diets. In general, WWDG protein concentrate has a great potential to enter into aquafeeds as an alternative protein source. This product provides a cheap source of protein and is therefore economically feasible to be considered for formulation of cost-effective diets. The commercial production of aqueous extracted WWDG protein concentrate would be a cheap source of AA for the replacement of fishmeal in aquafeeds. This will help to the sustainability of aquaculture industry in the current environment where fishmeal has become scarce and expensive. Regarding the impact of this research on the bio-ethanol industry, it can be indicated that this aqueous fractionation process would provide two more valuable co-products; the WWDG protein concentrate and a high fibre - low protein product. The WWDG protein concentrate might also enter to other markets for feed products for other species including dogs, cats, pigs and poultry. The high fibre – low protein product might be used as feedstock for ruminants. Nevertheless, further studies such as protein mapping, carbohydrate profiling and the effect of supplementary xylanase and β-glucanase enzymes on WWDG nutritional value are required. The effect of WWDG on intestinal morphology, gene expression and bacterial populations in salmonids is also required as the addition of NSPs present in WDDGS might alter susceptibility to enteric disease.

Wheat distillers grains are a readily available source of AA for many monogastric species but the high levels of soluble NSPs have limited their use. The low cost fractionation methods described in these studies may contribute to more widespread the use of these products and thus the economical viability of aquaculture and agriculture productions systems throughout the world.

4. REFERENCES

Aas, T., Grisdale-Helland, B., Terjesen, B. and Helland, S. 2006a. Improved growth and nutrient utilisation in Atlantic salmon (*Salmo salar*) fed diets containing a bacterial protein meal. Aquaculture 259(1/4):365-376.

Aas, T., Hatlen, B., Grisdale-Helland, B., Terjesen, B., Bakke-McKellep, A. and Helland, S. 2006b. Effects of diets containing a bacterial protein meal on growth and feed utilisation in rainbow trout (*Oncorhynchus mykiss*). Aquaculture 261(1):357-368.

Aas, T., Hatlen, B., Grisdale-Helland, B., Terjesen, B., Penn, M. and Bakke-McKellep, A. 2007. Feed intake, growth and nutrient utilization in Atlantic halibut (*Hippoglossus hippoglossus*) fed diets containing a bacterial protein meal. Aquaculture Research 38(4):351-360.

Abo-State, H. A., Tahoun, A. M. and Hammouda, Y. A. 2009. Effect of replacement of soybean meal by DDGS combined with commercial phytase on Nile Tilapia. (*Oreochromis niloticus*) Fingerlings growth performance and feed utilization. American-Eurasain Journal of Agricultural & Environmental Sciences 5(4):473-479.

Aksnes, A., Mundheim, H., Toppe, J. and Albrektsen, S. 2008. The effect of dietary hydroxyproline supplementation on salmon (*Salmo salar* L.) fed high plant protein diets. Aquaculture 275(1-4):242-249.

Andersen, Ø., Aas, T. S., Skugor, S., Takle, H., van Nes, S., Grisdale-Helland, B., Helland, S. J. and Terjesen, B. F. 2006. Purine-induced expression of urate oxidase and enzyme activity in Atlantic salmon (*Salmo salar*). Cloning of urate oxidase liver cDNA from three teleost species and the African lungfish *Protopterus annectens*. The FEBS Journal 273:2839-2850.

Anderson, D. P., Siwicki, A. K. and Rumsey, G. L. 1995. Injection or immersion delivery of selected immunostimulants to trout demonstrate enhancement of nonspecific defense mechanisms and protective immunity. In: Shariff, M., Arthur, J. R. and Subasinghe, R. P. (Eds.), Diseases in Asian aquaculture: II, Fish Health Section. Asian Fisheries Society. Manila, Philippines, 1995. pp. 413-426.

Aniszewski, T. 1993. Nutritive quality of the alkaloid-poor Washington lupin (*Lupinus polyphyllus*. Lindl var SF/TA) as a potential protein crop. Journal of the Science of Food and Agriculture 61(4):409-421.

Applegate, T., Troche, C., Jiang, Z. and Johnson, T. 2009. The nutritional value of high-protein corn distillers dried grains for broiler chickens and its effect on nutrient excretion. Poultry Science 88(2):354-359.

Aslaksen, M., Kraugerud, O., Penn, M., Svihus, B., Denstadli, V. and Jorgensen, H. 2007. Screening of nutrient digestibilities and intestinal pathologies in Atlantic salmon, *Salmo salar*, fed diets with legumes, oilseeds, or cereals. Aquaculture 272(1/4):541-555.

Azevedo, P. A., Leeson, S., Cho, C. Y. and Bureau, D. P. 2004. Growth, nitrogen and energy utilization of juveniles from four salmonid species: diet, species and size effects. Aquaculture 234(1-4):393-414.

Barrows, F. and Gaylord, T. 2007. Changing technologies, ingredients and formulations to replace fish meal in salmonid diets. In: Lyons T. P., Jacques K. A. and Horver J. M. (Eds.), Nutritional biotechnology in the feed and food industries: Proceedings of Alltech's

23rd Annual Symposium. The new energy crisis: food, feed or fuel?. Lexington, Kentucky, USA, pp. 307-315.

Barrows, F., Stone, D. and Hardy, R. 2007. The effects of extrusion conditions on the nutritional value of soybean meal for rainbow trout (*Oncorhynchus mykiss*). Aquaculture 265(1/4):244-252.

Berge, G., Baeverfjord, G., Skrede, A. and Storebakken, T. 2005. Bacterial protein grown on natural gas as protein source in diets for Atlantic salmon, *Salmo salar*, in saltwater. Aquaculture 244(1/4):233-240.

Booth, M. and Allan, G. 2004. Effects of extrusion processing on digestibility of peas, lupins, canola meal and soybean meal in silver perch *Bidyanus bidyanus* (Mitchell) diets. Aquaculture Research 35(10):981-991.

Borgeson, T. L., Racz, V. J., Wilkie, D. C., White, L. J. and Drew, M. D. 2006. Effect of replacing fishmeal and oil with simple or complex mixtures of vegetable ingredients in diets fed to Nile tilapia (*Oreochromis niloticus*). Aquaculture Nutrition 12(2):141-149.

Bureau, D. P., Harris, A. M. and Cho, C. Y. 1999. Apparent digestibility of rendered animal protein ingredients for rainbow trout (*Oncorhynchus mykiss*). Aquaculture 180(3-4):345-358.

Bureau, D. P., Harris, A. M., Bevan, D. J., Simmons, L. A., Azevedo, P. A. and Cho, C. Y. 2000. Feather meals and meat and bone meals from different origins as protein sources in rainbow trout (*Oncorhynchus mykiss*) diets. Aquaculture 181(3-4):281-291.

Burel, C., Boujard, T., Tulli, F. and Kaushik, S. 2000. Digestibility of extruded peas, extruded lupin, and rapeseed meal in rainbow trout (*Oncorhynchus mykiss*) and turbot (*Psetta maxima*). Aquaculture 188(3/4):285-298.

Canadian Renewable Fuels Association. 2009. Industry Information. Available at http://greenfuels.org/lists.php#ethProd. Accessed on September 23, 2009.

Cardona, C. A. and Sanchez, O. J. 2007. Fuel ethanol production: Process design trends and integration opportunities. Bioresource Technology 98(12):2415-2457.

Carter, C., Duijster, N., Evans, D., Dods, K., McCafferty, P. and Glencross, B. 2004. A comparison of the digestibility of a range of lupin and soybean protein products when fed to either Atlantic salmon (*Salmo salar*) or rainbow trout (*Oncorhynchus mykiss*). Aquaculture 237(1-4):333-346.

Celus, I., Brijs, K. and Delcour, J. 2009. Fractionation and characterization of brewers' spent grain protein hydrolysates. Journal of Agricultural and Food Chemistry 57(12):5563-5570.

Cheng, Z. and Hardy, R. 2004. Effects of microbial phytase supplementation in corn distiller's dried grain with solubles on nutrient digestibility and growth performance of rainbow trout, *Oncorhynchus mykiss*. Journal of Applied Aquaculture 15(3/4):83-100.

Cheng, Z. J. and Hardy, R. W. 2003. Effects of extrusion processing of feed ingredients on apparent digestibility coefficients of nutrients for rainbow trout (*Oncorhynchus mykiss*). Aquaculture Nutrition 9(2):77-83.

Cheng, Z. J., Hardy, R. W. and Usry, J. L. 2003. Effects of lysine supplementation in plant protein-based diets on the performance of rainbow trout (*Oncorhynchus mykiss*) and apparent digestibility coefficients of nutrients. Aquaculture 215(1-4):255-265.

Chevanan, N., Muthukumarappan, K. and Rosentrater, K. 2009. Extrusion studies of aquaculture feed using distillers dried grains with solubles and whey. Food and Bioprocess Technology 2(2):177-185.

Cho, C. 1993. Digestibility of feedstuffs as a major factor in aquaculture waste management. In: In: Kaushik, S. J. and Luquent P. (Eds.), Fish Nutrition in Practice: Proceeding of IVth International Symposium on Fish Nutrition and Feeding, Biarritz, France, 1991. pp. 365-374.

Cho, C. Y., Slinger, S. J. and Bayley, H. S. 1982. Bioenergetics of salmonid fishes: energy intake, expenditure and productivity. Comparative Biochemistry and Physiology 73B(1):25-41.

Classen, H. L., Newkirk, R. W. and Maenz, D. D. 2004. Effects of conventional and novel processing on the feed value of canola meal for poultry. In: Scott T (Ed), Proceedings of the 16th Australian Poultry Science Symposium Sydney, New South Wales, Australia, 2004.

Cowey, C. 1994. Amino acid requirements of fish: a critical appraisal of present values. Aquaculture 124(1/4):1-11.

Cowey, C. B. 1988. The Nutrition of Fish: The Developing Scene. Nutrition Research Reviews 1(01):255-280.

Cowey, C. B. 1992. Nutrition: estimating requirements of rainbow trout. Aquaculture 100(1-3):177-189.

Cowey, C. B. and Cho, C. Y. 1993. Nutritional requirements of fish. In: Westertep K R (Ed), Fish and Nutrition: Proceedings of the Nutrition Society, 52(3):417-426.

Coyle, S. D., Mengel, G. J., Tidwell, J. H. and Webster, C. D. 2004. Evaluation of growth, feed utilization, and economics of hybrid tilapia, *Oreochromis niloticus* x *Oreochromis aureus*, fed diets containing different protein sources in combination with distillers dried grains with solubles. Aquaculture Research 35(4):365-370.

Cromwell, G. L., Herkelman, K. L. and Stahly, T. S. 1993. Physical, chemical, and nutritional characteristics of distillers dried grains with solubles for chicks and pigs. Journal of Animal Science 71(3):679-868.

Dabrowski, K., Poczyczynski, P., Kock, G. and Berger, B. 1989. Effect of partially or totally replacing fish meal protein by soybean meal protein on growth, food utilization and proteolytic enzyme activities in rainbow trout (*Salmo gairdneri*). New in vivo test for exocrine pancreatic secretion. Aquaculture 77(1):29-49.

De Silva, K. 1989. Cheap sources of alternate feed for the farming of snakehead fish (Teleostei: *Channidae*) in Sri Lanka. In: Aquacultural research in Asia: management techniques and nutrition: Proceedings of the Asian seminar on aquaculture organized by IFS, Malang, Indonesia, 1988. pp. 241-253.

Depenbusch, B., Coleman, C., Higgins, J. and Drouillard, J. 2009. Effects of increasing levels of dried corn distillers grains with solubles on growth performance, carcass characteristics, and meat quality of yearling heifers. Journal of Animal Science 87(8):2653-2663.

Drew, M. D., Racz, V. J., Gauthier, R. and Thiessen, D. L. 2005. Effect of adding protease to coextruded flax:pea or canola:pea products on nutrient digestibility and growth performance of rainbow trout (*Oncorhynchus mykiss*). Animal Feed Science and Technology 119(1-2):117-128.

Drew, M. D., Borgeson, T. L. and Thiessen, D. L. 2007. A review of processing of feed ingredients to enhance diet digestibility in finfish. Animal Feed Science and Technology 138(2):118-136.

Duffus, C. M. and Duffus, J. H. 1991. In: D'Mello, F.J.P., Duffus, C.M., Duffus, J.H. ŽEds.., Toxic Substances in Crop Plants. The Royal Society of Chemistry, Thomas Graham House, Science Park, Cambridge CB4 4WF, Cambridge:1-21.

El-Haroun, E. R., Azevedo, P. A. and Bureau, D. P. 2009. High dietary incorporation levels of rendered animal protein ingredients on performance of rainbow trout *Oncorhynchus mykiss* (Walbaum, 1972). Aquaculture 290(3-4):269-274.

Ethanol Producer Magazine. 2009. Plant list. Available at http://www.ethanolproducer.com/plant-list.jsp?view=production&country=USA. Accessed on September 23, 2009.

FAO. 2006. State of World Aquaculture. FAO Fisheries Technical Paper No 500 FAO, Rome 145 pp

FAO. 2009. (Food and Agriculture Organization of the United Nations) The State of World Fisheries and Aquaculture 2008. Rome, Italy. 176 p.

Fowler, L. G. 1990. Feather meal as a dietary protein source during parr-smolt transformation in fall chinook salmon. Aquaculture 89(3-4):301-314.

Fowler, L. G. 1991. Poultry by-product meal as a dietary protein source in fall chinook salmon diets. Aquaculture 99(3-4):309-321.

Francis, G., Makkar, H. P. S. and Becker, K. 2001. Antinutritional factors present in plant-derived alternate fish feed ingredients and their effects in fish. Aquaculture 199(3-4):197-227.

Friedman, M. 1996. Nutritional Value of Proteins from Different Food Sources. A Review. Journal of Agricultural and Food Chemistry 44(1):6-29.

Garcia, G., Higuera, M. and Sanz, A. 2000. Protein nutrition in fish: protein/energy ratio and alternative protein sources to fish meal. Journal of Physiology and Biochemistry 56(3):275-282.

Gatlin, D. M., Barrows, F. T., Brown, P., Dabrowski, K., Gaylord, T. G., Hardy, R. W., Herman, E., Hu, G., Krogdahl, A., Nelson, R., Overturf, K., Rust, M., Sealey, W., Skonberg, D., J Souza, E., Stone, D., Wilson, R. and Wurtele, E. 2007. Expanding the utilization of sustainable plant products in aquafeeds: a review. Aquaculture Research 38(6):551-579.

Glencross, B., Evans, D., Hawkins, W. and Jones, B. 2004. Evaluation of dietary inclusion of yellow lupin (*Lupinus luteus*) kernel meal on the growth, feed utilisation and tissue histology of rainbow trout (*Oncorhynchus mykiss*). Aquaculture 235(1-4):411-422.

Glencross, B., Hawkins, W., Evans, D., Rutherford, N., McCafferty, P., Dods, K. and Sipsas, S. 2008. Assessing the implications of variability in the digestible protein and energy value of lupin kernel meals when fed to rainbow trout, *Oncorhynchus mykiss*. Aquaculture 277(3-4):251-262.

Goering, H., Van Soest, P. and Hemken, R. 1973. Relative susceptibility of forages to heat damage as affected by moisture, temperature, and pH. Journal of Dairy Science 56(1):137-143.

Gomes da Silva, J. and Oliva-Teles, A. 1998. Apparent digestibility coefficients of feedstuffs in seabass (*Dicentrarchus labrax*) juveniles. Aquatic Living Resources 11(3):187-191.

Gomes, E. F., Rema, P. and Kaushik, S. J. 1995. Replacement of fish meal by plant proteins in the diet of rainbow trout (*Oncorhynchus mykiss*): digestibility and growth performance. Aquaculture 130(2-3):177-186.

Gopalakannan, A. and Arul, V. 2006. Immunomodulatory effects of dietary intake of chitin, chitosan and levamisole on the immune system of Cyprinus carpio and control of Aeromonas hydrophila infection in ponds. Aquaculture 255(1-4):179-187.

Hajen, W. E., Beames, R. M., Higgs, D. A. and Dosanjh, B. S. 1993. Digestibility of various feedstuffs by post-juvenile chinook salmon (Oncorhynchus tshawytscha) in sea water. 1. Validation of technique. Aquaculture 112(4):321-332.

Hao, X., Benke, M., Gibb, D., Stronks, A., Travis, G. and McAllister, T. 2009. Effects of dried distillers' grains with solubles (wheat-based) in feedlot cattle diets on feces and manure composition. Journal of Environmental Quality 38(4):1709-1718.

Hardy, R. W. 2000. New developments in aquatic feed ingredients, and potential of enzyme supplements. In: Cruz-Suarez, L E, Ricque-Marie, D, Tapia-Salazar, M, Olvera-Novoa, M A and Civera-Cerecedo, R (Eds), Avances en Nutricion Acuicola V: Memorias de lV Simposium Internacional de Nutricion Acuicola Merida, Mexico, 2000 pp 216-226.

Hartmann, S. and Koehler, P. 2008. Fractionation of cereal flour by sedimentation in non-aqueous systems. I. Development of the method and chemical characterisation of the fractions. Journal of cereal science 47(3):576-586.

Hawkins, W. and Glencross, B. 2004. A comparison of the digestibility of lupin (*Lupinus sp.*) kernel meals as dietary protein resources when fed to either, rainbow trout, *Oncorhynchus mykiss* or red seabream, *Pagrus auratus*. Aquaculture nutrition 10(2):65-73.

Hawkins, W., Evans, D., Rutherford, N., Dods, K., McCafferty, P. and Glencross, B. 2008. Evaluation of the influence of Lupinus angustifolius kernel meal on dietary nutrient and energy utilization efficiency by rainbow trout (*Oncorhynchus mykiss*). Aquaculture nutrition 14(2):129-138.

Hemery, Y., Rouau, X., Lullien-Pellerin, V., Barron, C. and Abecassis, J. 2007. Dry processes to develop wheat fractions and products with enhanced nutritional quality. Journal of Cereal Science 46(3):327-347.

Hemre, G.-I., Mommsen, T. P. and Krogdahl, Å. 2002. Carbohydrates in fish nutrition: effects on growth, glucose metabolism and hepatic enzymes. Aquaculture Nutrition 8(3):175-194.

Higgs, D., Dosanjh, B., Prendergast, A., Beames, R., Hardy, R. and Riley, W. 1995. Use of rapeseed/canola protein products in finfish diets. In: Lim, C.E., Sessa, D.J. (Eds.), Nutrition and Utilization Technology in Aquaculture. AOCS Press, Champaign, USA. 1995. pp. 130–156.

Hossain, M. and Jauncey, K. 1993. The effects of varying dietary phytic acid, calcium and magnesium levels on the nutrition of common carp, *Cyprinus carpio*. In: Kaushik, S. J. and Luquent P. (Eds.), Fish Nutrition in Practice: Proceeding of IVth International Symposium on Fish Nutrition and Feeding, Biarritz, France, 1991. pp. 705–715.

Hua, K. and Bureau, D. P. 2009. A mathematical model to explain variations in estimates of starch digestibility and predict digestible starch content of salmonid fish feeds. Aquaculture 294(3-4):282-287.

Hurrell, R. F., Carpenter, K. J., Sinclair, W. J., Otterburn, M. S. and Asquith, R. S. 1976. Mechanisms of heat damage in proteins. British Journal of Nutrition 35(03):383-395.

Hylland, K., Bonga, S., Maage, A. and Berntssen, M. 1999. Toxic levels of dietary copper in Atlantic salmon (*Salmo salar* L.) parr. Aquatic Toxicology 46(2):87-99.

ICM Inc. 2010. Ethanol Production. Available at: http://www.icminc.com/ethanol/production_process. Accessed on April 28, 2010. .

Izydorczyk, M., Dexter, J., Desjardins, R., Rossnagel, B., Lagasse, S. and Hatcher, D. 2003. Roller milling of Canadian hull-less barley: optimization of roller milling conditions and composition of mill streams. Cereal Chemistry 80(6):637-644.

Jacob, M. E., Fox, J. T., Narayanan, S. K., Drouillard, J. S., Renter, D. G. and Nagaraja, T. G. 2008. Effects of feeding wet corn distillers grains with solubles with or without monensin and tylosin on the prevalence and antimicrobial susceptibilities of fecal foodborne pathogenic and commensal bacteria in feedlot cattle. Journal of Animal Science 86(5):1182-1190.

Jung, S. 2009. Aqueous extraction of oil and protein from soybean and lupin: a comparative study. Journal of food processing and preservation 33(4):547-559.

Kaushik, S. J., Cravedi, J. P., Lalles, J. P., Sumpter, J., Fauconneau, B. and Laroche, M. 1995. Partial or total replacement of fish meal by soybean protein on growth, protein utilization, potential estrogenic or antigenic effects, cholesterolemia and flesh quality in rainbow trout, Oncorhynchus mykiss. Aquaculture 133(3-4):257-274.

Kiessling, A. and Askbrandt, S. 1993. Nutritive value of two bacterial strains of single-cell protein for rainbow trout (*Oncorhynchus mykiss*). Aquaculture 109(2):119-130.

Kim, K., Kayes, T. B. and Amundson, C. H. 1991. Purified diet development and re-evaluation of the dietary protein requirement of fingerling rainbow trout (*Oncorhynchus mykiss*). Aquaculture 96(57-67).

Kim, Y., Mosier, N., Hendrickson, R., Ezeji, T., Blaschek, H. and Dien, B. 2008. Composition of corn dry-grind ethanol by-products: DDGS, wet cake, and thin stillage. Bioresource Technology 99(12):5165-5176.

Kissil, G. W., Lupatsch, I., Higgs, D. A. and Hardy, R. W. 2000. Dietary substitution of soy and rapeseed protein concentrates for fish meal, and their effects on growth and nutrient utilization in gilthead seabream (*Sparus aurata* L.). Aquaculture Research 31(7):595-601.

Klein-Hessling, H. 2007. Canola: an ingredient with opportunity. World Poultry 23(10):22-23.

Klopfenstein, T., Erickson, G. and Bremer, V. 2007. Feeding corn milling byproducts to feedlot cattle. The Veterinary Clinics of North America: Food Animal Practice 23(2):223-245.

Knights, B. 1996. Studies of feeding stimulation in young eels, *Anguilla anguilla* L., before and after first-feeding using a novel rapid-screening method. Aquaculture Research 27(5):379-385.

Kono, M., Matsui, T. and Shimizu, C. 1987. Effect of chitin, chitosan and cellulose as diet supplements on the growth of cultured fish. Nippon Suisan Gakkaishi 53:125-129.

Krogdahl, A., Bakke-McKellep, A. and Baeverfjord, G. 2003. Effects of graded levels of standard soybean meal on intestinal structure, mucosal enzyme activities, and pancreatic response in Atlantic salmon (*Salmo salar* L.). Aquaculture Nutrition 9(6):361-371.

Lall, S. 2007. Comparative mineral nutrition of fish: sources and requirements. In: Lyons T. P., Jacques K. A. and Horver J. M. (Eds.), Nutritional biotechnology in the feed and food industries: Proceedings of Alltech's 23rd Annual Symposium. The new energy crisis: food, feed or fuel?. Lexington, Kentucky, USA, pp. 387-391.

Lan, Y., Opapeju, F. and Nyachoti, C. 2008. True ileal protein and amino acid digestibilities in wheat dried distillers' grains with solubles fed to finishing pigs. Animal Feed Science and Technology 140(1/2):155-163.

Lee, S.-M., Kim, K.-D. and Lall, S. P. 2003. Utilization of glucose, maltose, dextrin and cellulose by juvenile flounder (*Paralichthys olivaceus*). Aquaculture 221:427-438.

Leytem, A., Kwanyuen, P. and Thacker, P. 2008a. Nutrient excretion, phosphorus characterization, and phosphorus solubility in excreta from broiler chicks fed diets containing graded levels of wheat distillers grains with solubles. Poultry Science 87(12):2505-2511.

Li, P. and Gatlin, D. 2006. Nucleotide nutrition in fish: current knowledge and future applications. Aquaculture 251(2/4):141-152.

Liew, F., Ang, L., Wong, K. and Ng, W. 2001. Potential of mealworm (*Tenebrio molitor*) as an alternative protein source in practical diets for African catfish, *Clarias gariepinus*. Aquaculture Research 32:273-280.

Liu, K. 2009a. Effects of particle size distribution, compositional and color properties of ground corn on quality of distillers dried grains with solubles (DDGS). Bioresource Technology 100(19):4433-4440.

Liu, K. 2009b. Fractionation of distillers dried grains with solubles (DDGS) by sieving and winnowing. Bioresource technology 100(24):6559-6569.

Liu, K., Barrows, F. and Obert, D. 2009. Dry fractionation methods to produce barley meals varying in protein, beta-glucan, and starch contents. Journal of Food Science 74(6).

Lumpkins, B., Batal, A. and Dale, N. 2004. Evaluation of distillers dried grains with solubles as a feed ingredient for broilers. Poultry Science 83(11):1891-1896.

Lumpkins, B., Batal, A. and Dale, N. 2005. Use of distillers dried grains plus solubles in laying hen diets. The Journal of Applied Poultry Research 14(1):25-31.

Lumpkins, B. S. and Batal, A. B. 2005. The bioavailability of lysine and phosphorus in distillers dried grains with solubles. Poultry Science 84(4):581-586.

Maenz, D. D., Newkirk, R. W., Classen, H. L. and Tyler, R. T. 2004. Fractionation and processing of oil seed meal. United States Patent No. 6,800,308.

Mambrini, M. and Guillaume, J. 1999. Nutrition protéique. In: Guillaume, J., Kaushik, S.J., Bergot, P. & Métailler, R. (Eds) Nutrition et alimentation des poissons et des crustacés. Collections Du Labo au Terrain. Éditions INRA, Paris, France.

Mansour, E. H., Dworschak , E., Lugasi, A., Gaal, Ö., Barna, E. and Gergely, A. 1993. Effect of processing on the antinutritive factors and nutritive value of rapeseed products. Food chemistry 47(3):247-252.

Mathlouthi, N., Saulnier, L., Quemener, B. and Larbier, M. 2002. Xylanase, B-Glucanase, and Other Side Enzymatic Activities Have Greater Effects on the Viscosity of Several Feedstuffs than Xylanase and B-Glucanase Used Alone or in Combination. Journal of agricultural and food chemistry 50(18):5121-5127.

Mathlouthi, N., Juin, H. and Larbier, M. 2003. Effect of xylanase and beta-glucanase supplementation of wheat- or wheat- and barley-based diets on the performance of male turkeys. British poultry science 44(2):291-298.

McLean, E. and Craig, S. 2007. Designing organic aquaculture systems: can we integrate microbial products and by-products?. In: Lyons T. P., Jacques K. A. and Horver J. M. (Eds.), Nutritional biotechnology in the feed and food industries: Proceedings of Alltech's 23rd Annual Symposium. The new energy crisis: food, feed or fuel?. Lexington, Kentucky, USA, pp. 317-25.

Mwachireya, S. A., Beames, R. M., Higgs, D. A. and Dosanjh, B. S. 1999. Digestibility of canola protein products derived from the physical, enzymatic and chemical processing of

commercial canola meal in rainbow trout (*Oncorhynchus mykiss*) (Walbaum) held in fresh water. Aquaculture Nutrition 5(2):73-82.

Mydland, L., Froyland, J. and Skrede, A. 2008. Composition of individual nucleobases in diets containing different products from bacterial biomass grown on natural gas, and digestibility in mink (*Mustela vison*). Journal of Animal Physiology and Animal Nutrition 92(1):1-8.

Naylor, R., Hardy, R., Bureau, D., Chiu, A., Elliott, M. and Farrell, A. 2009. Feeding aquaculture in an era of finite resources. Proceedings of the National Academy of Sciences of the United States of America 106(36):15103-15110.

New, M. B. and Wijkström, U. N. 2002. Use of Fishmeal and Fish Oil in Aquafeeds: Further Thoughts on the Fishmeal Trap. FAO Fisheries Circular. No. 975. Rome, Italy. 61 p.

Noll, S. 2004. DDGS in Poultry Diets - Does It Make Sense? In: Proceedings of Midwest Poultry Federation Convention. St. Paul, USA, 2004. pp. 6-10.

NRC. 1993. (National Research Council) Nutrient Requirements of Fish. National Academy Press, Washington, DC, USA.

Nuez, W. G. and Yu, P. 2009. Nutrient variation and availability of wheat DDGS, corn DDGS and blend DDGS from bioethanol plants. Journal of the Science of Food and Agriculture 89:1754-1761.

Oliva-Teles, A. and Goncalves, P. 2001. Partial replacement of fishmeal by brewers yeast (*Saccaromyces cerevisae*) in diets for sea bass (*Dicentrarchus labrax*) juveniles. Aquaculture 202(3-4):269-278.

Oliva-Teles, A. and Pereira, T. 2002. Preliminary evaluation of pea seed meal in diets for gilthead sea bream (*Sparus aurata*) juveniles. Aquaculture Research 33(14):1183-1189.

Opstvedt, J., Aksnes, A., Hope, B. and Pike, I. H. 2003. Efficiency of feed utilization in Atlantic salmon (Salmo salar L.) fed diets with increasing substitution of fish meal with vegetable proteins. Aquaculture 221(1-4):365-379.

Overland, M., Romarheim, O., Hovin, M., Storebakken, T. and Skrede, A. 2006. Apparent total tract digestibility of unprocessed and extruded diets containing basic and autolyzed bacterial protein meal grown on natural gas in mink and rainbow trout. Animal Feed Science and Technology 129(3/4):237-251.

Owusu-Ansah, Y. J. and McCurdy, S. M. 1991. Pea proteins; a review of chemistry, technology of production, and utilization. Food Reviews International 7:103-134.

Parsons, C., Martinez, C., Singh, V., Srinivasan, R. and Noll, S. 2006. Nutritional value of conventional and modified DDGS for poultry. In: Proceedings Multi-State Poultry Nutrition Feeding Conference. Indianapolis, USA, 2004.

Prapakornwiriya, N. and Diosady, L. 2004. Isolation of yellow mustard proteins by a microfil tration-based process. International Journal of Applied Science and Engineering 2(2):127-135.

Rahnema, S., Borton, R. and Shaw, E. 2005. Determination of the effects of fish vs plant vs meat protein-based diets on the growth and health of rainbow trout. Journal of Applied Animal Research 27(2):77-80.

Rakyuttithamkul, E. and Arunlertaree, C. 2006. Utilization of fermented feather meal as a replacement of fish meal in the diet of hybrid clarias catfish. Kasetsart Journal, Natural Sciences 40(2):436-448.

Randall, K. M. and Drew, M. D. 2010. Fractionation of wheat distiller's dried grains and solubles using sieving increases digestible nutrient content in rainbow trout. Animal Feed Science and Technology. (in press).

Rausch, K. and Belyea, R. 2006. The future of coproducts from corn processing. Applied Biochemistry and Biotechnology 128(1):47-86.

Rawles, S. D., Riche, M., Gaylord, T. G., Webb, J., Freeman, D. W. and Davis, M. 2006. Evaluation of poultry by-product meal in commercial diets for hybrid striped bass (Morone chrysops x M. saxatilis) in recirculated tank production. Aquaculture 259(1-4):377-389.

Refstie, S., Storebakken, T. and Roem, A. 1998. Feed consumption and conversion in Atlantic salmon (*Salmo salar*) fed diets with fish meal, extracted soybean meal or soybean meal with reduced content of oligosaccharides, trypsin inhibitors, lectins and soya antigens. Aquaculture 162(3/4):301-312.

Refstie, S., Svihus, B., Shearer, K. and Storebakken, T. 1999. Nutrient digestibility in Atlantic salmon and broiler chickens related to viscosity and non-starch polysaccharide content in different soyabean products. Animal Feed Science and Technology 79(4):331-345.

Refstie, S., Korsoen, J., Storebakken, T., Baeverfjord, G., Lein, I. and Roem, A. 2000. Differing nutritional responses to dietary soybean meal in rainbow trout (*Oncorhynchus mykiss*) and Atlantic salmon (*Salmo salar*). Aquaculture 190(1/2):49-63.

Rendleman, M. and Shapouri, H. 2007. New Technologies in Ethanol Production. USDA Agricultural Economic Report No 842.

Renewable Fuel Association. 2009. Statistics. Available at http://wwwethanolrfaorg/industry/statistics/#E Accesed on September 16, 2009.

Richardson, N. L., Higgs, D. A., Beames, R. M. and McBride, J. R. 1985. Influence of dietary calcium, phosphorous, zinc and sodium phytate level on cataract incidence, growth and histopathology in juvenile chinook salmon (*Oncorhynchus tshawytscha*). The Journal of Nutrition 115:553-567.

Riche, M. and Garling, D. 2004. Effect of phytic acid on growth and nitrogen retention in tilapia *Oreochromis niloticus* L. Aquaculture Nutrition 10(6):389-400.

Robaina, L., Moyano, F. J., Izquierdo, M. S., Socorro, J., Vergara, J. M. and Montero, D. 1997. Corn gluten and meat and bone meals as protein sources in diets for gilthead seabream (*Sparus aurata*): Nutritional and histological implications. Aquaculture 157(3-4):347-359.

Robinson, P. H. 2005. Ethanol industry co-products: milling process, nutrient content, and variation. Abstract presented at the ASAS/ADSA Midwestern Meeting, Des Moines, USA. Journal of Animal Science 83 (Suppl. 2):49.

Rodehutscord, M., Becker, A., Pack, M. and Pfeffer, E. 1997. Response of rainbow trout (*Oncorhynchus mykiss*) to supplements of individual essential amino acids in a semipurified diet, including an estimate of the maintenance requirement for essential amino acids. The Journal of Nutrition 127(6):1166-1175.

Rosentrater, K., Subramanian, D. and Krishnan, P. 2006. Fractionation techniques to concentrate nutrient streams in distillers grains. In: Proceeding of ASABE Annual International Meeting, Portland, Oregon, USA, 2006.

Rumsey, G. L., Kinsella, J. E., Shetty, K. J. and Hughes, S. G. 1991. Effect of high dietary concentrations of brewer's dried yeast on growth performance and liver uricase in

rainbow trout (*Oncorhynchus mykiss*). Animal Feed Science and Technology 33(3-4):177-183.

Rumsey, G. L., Siwicki, A. K., Anderson, D. P. and Bowser, P. R. 1994. Effect of soybean protein on serological response, non-specific defense mechanisms, growth, and protein utilization in rainbow trout. Veterinary Immunology and Immunopathology 41(3-4):323-339.

Russell, J. R., Hurst, J. P., Jorgensen, N. A. and Barrington, G. P. 1978. Wet Plant Fractionation: Utilization of Pressed Alfalfa Silage. Journal of Animal Science 46(1):278-287.

Sargent, J., Bell, G., McEvoy, L., Tocher, D. and Estevez, A. 1999. Recent developments in the essential fatty acid nutrition of fish. Aquaculture 177(1-4):191-199.

Schingoethe, D. 2006. Utilization of DDGS by cattle. In: Proceeding of 27th Western Nutrition Conference, Winnipeg, Canada, 2006. pp. 61-74.

Schulz, C., Wickert, M., Kijora, C., Ogunji, J. and Rennert, B. 2007. Evaluation of pea protein isolate as alternative protein source in diets for juvenile tilapia (*Oreochromis niloticus*). Aquaculture Research 38(5):537-545.

Shelby, R., Lim, C., Yildrim-Aksoy, M. and Klesius, P. 2008. Effect of distillers dried grains with solubles-incorporated diets on growth, immune function and disease resistance in Nile tilapia (*Oreochromis niloticus* L.). Aquaculture Research 39(12):1351-1353.

Shiau, S. and Yu, Y. 1999. Dietary supplementation of chitin and chitosan depresses growth in tilapia, *Oreochromis niloticus* x *O. aureus*. Aquaculture 179(1/4):439-446.

Shiau, S. Y. 1997. Utilization of carbohydrates in warmwater fish with-particular reference to tilapia, *Oreochromis niloticus* X *O. aureus*. Aquaculture 151:79-96.

Shurson, G. 2005. Issues and opportunities related to the production and marketing of ethanol by-products. USDA Agricultural Outlook Forum, Arlington, USA, 2005. pp. 1-8.

Shurson, J. and Noll, S. 2005. Feed and Alternatives uses for DDGS. Paper Presented at Energy from Agriculture Conference. St. Louis, USA, 2005.

Simpson, T., Sharpley, A., Howarth, R., Paerl, H. and Mankin, K. 2008. The new gold rush: fueling ethanol production while protecting water quality. Journal of Environmental Quality 37(2):318-324.

Singh, V. and Eckhoff, S. R. 1996. Effect of soak time, soak temperature and lactic acid on germ recovery parameters. Cereal Chemistry 73:716-720.

Singh, V., Moreau, R. A., Doner, L. W., Eckhoff, S. R. and B., H. H. 1999. Recovery of fiber in the corn drygrind ethanol process: a feedstock for valuable coproducts. Cereal Chemistry 76:868-872.

Singh, V., Moreau, R. A., Hicks, K. B., Belyea, R. L. and Staff, C. H. 2002. Removal of fiber from distillers dried grains with solubles (DDGS) to increase value. Transactions of the ASAE 45(2):389-392.

Singh, V., Johnston, D. B., Naidu, K., Rausch, K. D., Belyea, R. L. and E., T. M. 2005. Comparison of modified dry-grind corn processes for fermentation characteristics and DDGS Composition. Cereal Chemistry 82:187-190.

Smith, D. M., Tabrett, S. J., Glencross, B. D., Irvin, S. J. and Barclay, M. C. 2007. Digestibility of lupin kernel meals in feeds for the black tiger shrimp, *Penaeus monodon*. Aquaculture 264(1-4):353-362.

Solomon, B. D., Barnes, J. R. and Halvorsen, K. E. 2007. Grain and cellulosic ethanol: History, economics, and energy policy. Biomass and Bioenergy 31(6):416-425.

Sorensen, M., Stjepanovic, N., Romarheim, O., Krekling, T. and Storebakken, T. 2009. Soybean meal improves the physical quality of extruded fish feed. Animal Feed Science and Technology 149(1/2):149-161.

Spiehs, M., Whitney, M. and Shurson, G. 2002. Nutrient database for distiller's dried grains with solubles produced from new ethanol plants in Minnesota and South Dakota. Journal of Animal Science 80(10):2639-2645.

Spinelli, J., Houle, C.R., Wekell, J.C. 1983. The effect of phytates on the growth of rainbow trout (*Salmo gairdneri)* fed purified diets containing varying quantities of calcium and magnesium. Aquaculture 30:71-83.

Srinivasan, R., Moreau, R. A., Rausch, K. D., Belyea, R. L., Tumbleson, M. E. and Singh, V. 2005a. A new process for removing fiber from distillers dried grains with solubles. Paper presented at ASAE Annual International Meeting. Tampa, FL, USA, 2005. .

Srinivasan, R., Moreau, R. A., Rausch, K. D., Belyea, R. L., Tumbleson, M. E. and Singh, V. 2005b. Separation of fiber from distillers dried grains with solubles (DDGS) using sieving and elutriation. Cereal Chemistry 82(5):528-533.

Srinivasan, R., Moreau, R., Parsons, C., Lane, J. and Singh, V. 2008. Separation of fiber from distillers dried grains (DDG) using sieving and elutriation. Biomass and Bioenergy 32(5):468-472.

Stafford, E. A. and Tacon, A. G. J. 1984. Nutritive value of the earthworm, *Dendrodrilus subrubicundus*, grown on domestic sewage, in trout diets. Agricultural Wastes 9(4):249-266.

Steffens, W. 1994. Replacing fish meal with poultry by-product meal in diets for rainbow trout, *Oncorhynchus mykiss*. Aquaculture 124(1-4):27-34.

Stein, H. and Shurson, G. 2009. The use and application of distillers dried grains with solubles in swine diets. Journal of Animal Science 87(4):1292-1303.

Storebakken, T., Kvien, I., Shearer, K., Grisdale-Helland, B., Helland, S. and Berge, G. 1998a. The apparent digestibility of diets containing fish meal, soybean meal or bacterial meal fed to Atlantic salmon (*Salmo salar*): evaluation of different faecal collection methods. Aquaculture 169(3/4):195-210.

Storebakken, T., Shearer, K. and Roem, A. 1998b. Availability of protein, phosphorus and other elements in fish meal, soy-protein concentrate and phytase-treated soy-protein-concentration-based diets to Atlantic salmon, *Salmo salar*. Aquaculture 161(1/4):365-379.

Storebakken, T., Kvien, I., Shearer, K., Grisdale-Helland, B. and Helland, S. 1999. Estimation of gastrointestinal evacuation rate in Atlantic salmon (*Salmo salar*) using inert markers and collection of faeces by sieving: evacuation of diets with fish meal, soybean meal or bacterial meal. Aquaculture 172(3/4):291-299.

Storebakken, T., Baeverfjord, G., Skrede, A., Olli, J. and Berge, G. 2004. Bacterial protein grown on natural gas in diets for Atlantic salmon, (*Salmo salar*), in freshwater. Aquaculture 241(1/4):413-425.

Sugiura, S. H., Dong, F. M., Rathbone, C. K. and Hardy, R. W. 1998. Apparent protein digestibility and mineral availabilities in various feed ingredients for salmonid feeds. Aquaculture 159(3-4):177-202.

Tacon, A. 2004. Use of fish meal and fish oil in aquaculture: a global perspective. Aquatic Resources Culture and Development 1(1):3-14.

Tacon, A. G. J., Hasan, M. R. and Subasinghe, R. P. 2006. Use of fishery resources as feed inputs for aquaculture development: trends and policy implications. FAO Fisheries Circular. No. 1018. Rome, Italy. 99p.

Tacon, A. G. J. and Metian, M. 2008. Global overview on the use of fish meal and fish oil in industrially compounded aquafeeds: Trends and future prospects. Aquaculture 285(1-4):146-158.

Teles, A., Gomes, E., Rema, P. and Gouveia, A. 1993. Effect of cooking/expansion of three legume seeds on growth and food utilization by rainbow trout. Fish Nutrition in Practice: 4th International Symposium on Fish Nutrition and Feeding, Biarritz, France, 24-27 June, 1991:933-938.

Thacker, P. A. and Widyaratne, G. P. 2007. Nutritional value of diets containing graded levels of wheat distillers grains with solubles fed to broiler chicks. Journal of the Science of Food and Agriculture 87(7):1386-1390.

Thiessen, D. L., Campbell, G. L. and Adelizi, P. D. 2003a. Digestibility and growth performance of juvenile rainbow trout (*Oncorhynchus mykiss*) fed with pea and canola products. Aquaculture Nutrition 9(2):67-75.

Thiessen, D. L., Campbell, G. L. and Tyler, R. T. 2003b. Utilization of thin distillers' solubles as a palatability enhancer in rainbow trout (*Oncorhynchus mykiss*) diets containing canola meal or air-classified pea protein. Aquaculture Nutrition 9(1):1-10.

Thiessen, D. L. 2004. Optimization of Feed Peas, Canola and Flaxseed for Aquafeeds; the Canadian Prairie Perspective. In: Cruz-Suarez, L. E., Ricque-Marie, D., Nieto Lopez, M. G., Villareal, D., Scholz, U. y Gonzalez, M. (Eds.), Avances en Nutricion Acuicola VII. Memorias del VII Simposium Internacional de Nutricion Acuicola. Hermosillo, Mexico, 2004. pp. 259-277.

Thiessen, D. L., Maenz, D. D., Newkirk, R. W., Classen, H. L. and Drew, M. D. 2004. Replacement of fishmeal by canola protein concentrate in diets fed to rainbow trout (*Oncorhynchus mykiss*). Aquaculture Nutrition 10(6):379-388.

Tocher, D. R. 2003. Metabolism and functions of lipids and fatty acids in teleost fish. Reviews in Fisheries Science 11:107-184.

Trushenski, J., Kasper, C. and Kohler, C. 2006. Challenges and opportunities in finfish nutrition. North American Journal of Aquaculture 68(2):122-140.

Tyner, W. 2008. The US ethanol and biofuels boom: its origins, current status, and future prospects. Bioscience 58(7/8):646-653.

Vasanthan, T. and Temelli, F. 2008. Grain fractionation technologies for cereal beta-glucan concentration. Food Research International 41(9):876-881.

Velasquez, L., Ibanez, I., Herrera, C. and Oyarzun, M. 1991. A note on the nutritional evaluation of worm meal (*Eisenia fetida*) in diets for rainbow trout. Animal Production 53(1):119-122.

Wang, P., Singh, V., Xu, L., Johnston, D. B., Rausch, K. D. and Tumbleson, M. E. 2005. Comparison of enzymatic (E-mill) and conventional dry grind corn processes using a granular starch hydrolyzing enzyme. Cereal Chemistry 82:734-738.

Wang, Y., Luo, Z., Zhao, M., Xiong, B., Qian, X. and Hu, M. 2008a. Evaluation of rendered animal protein ingredients for replacement of fish meal in practical diets for gibel carp, *Carassius auratus gibelio* (Bloch). Aquaculture Research 39(14):1475-1482.

Wang, Z., Cerrate, S., Coto, C., Yan, F. and Waldroup, P. 2008b. Evaluation of high levels of distillers dried grains with solubles (DDGS) in broiler diets. International Journal of Poultry Science 7(10):990-996.

Webster, C., Tidwell, J. and Yancey, D. 1991. Evaluation of distillers' grains with solubles as a protein source in diets for channel catfish. Aquaculture 96(2):179-190.

Webster, C., Tidwell, J., Goodgame, L., Yancey, D. and Mackey, L. 1992. Use of soybean meal and distillers grains with solubles as partial or total replacement of fish meal in diets for channel catfish, *Ictalurus punctatus*. Aquaculture 106(3/4):301-309.

Webster, C., Tidwell, J., Goodgame-Tiu, L. and Yancey, D. 1995. Evaluation of distillers grains with solubles as an alternative plant protein in aquaculture diets. In: Lim, C., Sessa, D.J. (Eds.), Nutrient Utility and Techniques in Aquaculture: AOCS. Illinois, USA, 1995. pp. 189-198.

Whitney, M., Spiehs, M., Shurson, G. and Baidoo, S. 2000. Apparent ileal amino acid digestibilitiesof corn distiller's dried grains with solubles produced from new ethanol plants in Minnesota and South Dakota. Journal of Animal Science 78 (Suppl. 1):185.

Widyaratne, G. and Zijlstra, R. 2007. Nutritional value of wheat and corn distiller's dried grain with solubles: digestibility and digestible contents of energy, amino acids and phosphorus, nutrient excretion and growth performance of grower-finisher pigs. Canadian Journal of Animal Science 87(1):103-114.

Widyaratne, G., Patience, J. and Zijlstra, R. 2009. Effect of xylanase supplementation of diets containing wheat distiller's dried grains with solubles on energy, amino acid and phosphorus digestibility and growth performance of grower-finisher pigs. Canadian Journal of Animal Science 89(1):91-95.

Wilson, R. P. 1994. Utilization of dietary carbohydrate by fish. Aquaculture 124(1-4):67-80.

Wu, Y., Sexson, K., Cluskey, J. and Inglett, G. 1977. Protein isolate from high-protein oats: preparation, composition and properties. Journal of Food Science 42(5):1383-1386.

Wu, Y. and Stringfellow, A. 1982. Corn distillers'dried grains with solubles and corn distillers' dried grains: Dry fractionation and composition. Journal of Food Science(47):1155-1157.

Wu, Y. and Stringfellow, A. 1986. Simple dry fractionation of corn distillers' dried grains and corn distillers' dried grains with solubles. Cereal Chemistry 63(1):60-61.

Wu, Y., Rosati, R. and Brown, P. 1996. Effect of diets containing various levels of protein and ethanol coproducts from corn on growth of tilapia fry. Journal of Agricultural and Food Chemistry 44(6):1491-1493.

Wu, Y., Rosati, R. and Brown, P. 1997. Use of corn-derived ethanol coproducts and synthetic lysine and tryptophan for growth of tilapia (*Oreochromis niloticus*) fry. Journal of Agricultural and Food Chemistry 45(6):2174-2177.

Wu, Y. and Nichols, N. 2005. Fine grinding and air classification of field pea. Cereal Chemistry 82(3):341-344.

Yadav, M., Belyea, R., Rausch, K., Pruiett, L., Johnston, D. and Srinivasan, R. 2008. Fiber separation from distillers dried grains with solubles using a larger elutriation apparatus and use of fiber as a feedstock for corn fiber gum production. Biological Engineering 1(1):39-49.

Yeung, J. and Vasanthan, T. 2001. Pearling of Hull-less Barley: Product Composition and Gel Color of Pearled Barley Flours As Affected by the Degree of Pearling. Journal of Agricultural and Food Chemistry 49(1):331-335.

Youssef, I., Westfahl, C., Sunder, A., Liebert, F. and Kamphues, J. 2008. Evaluation of dried distillers' grains with solubles (DDGS) as a protein source for broilers. Archives of Animal Nutrition 62(5):404-414.

5. APPENDICES

5.1 Appendix A

This experiment was conducted to find out the weight volume ratio which supports the best protein extraction then, it can be used to perform the aqueous fractionation of WWDG.

The WWDG used in this experiment was collected from plant 2. The WWDG was first blended with tap water at room temperature and at different weight to volume ratios (1:5, 1:6 and 1:7) to determine the best ratio to produce the WWDG protein concentrate. Then, the material was stirred for 60 min. The material obtained after blending was screened with an electrostatic filter (Web products, Inc., Kansas, KS). The resultant slurry was autoclaved to produce a supernatant and a precipitate (121 ºC, 30 min). The supernatant was removed by siphoning and then, the precipitate was collected and analyzed in duplicate for nitrogen content was determined by combustion method (AOAC 1995, method no. 990.03) using a Leco protein/N analyzer (Model FP-528, Leco Corp., St. Joseph, MI, USA). Crude protein content was calculated by multiplying nitrogen content by 6.25.

It was observed that the 1:5 (w:v) ratio had the highest crude protein yield (Table A.1). Therefore, it was concluded from this experiment that the 1:5 (w:v) ratio was the best ratio to perform aqueous fractionation of WWDG.

Table A.1 Effect of solid to solvent ratio on protein extraction from wheat wet distillers grains (WWDG) from plant 2.

Solid:solvent ratio (w:v)	Initial material (g)	Precipitate (g as is)	Moisture (%)	Crude Protein (% DM)	Dry Matter (%)	Total Crude Protein (g DM)
1:5	100	62.7	89.5	76.2	10.5	5.0
1:6	100	61.3	90.0	76.6	10.0	4.7
1:7	100	50.1	89.2	77.2	10.8	4.2

5.1 Appendix B

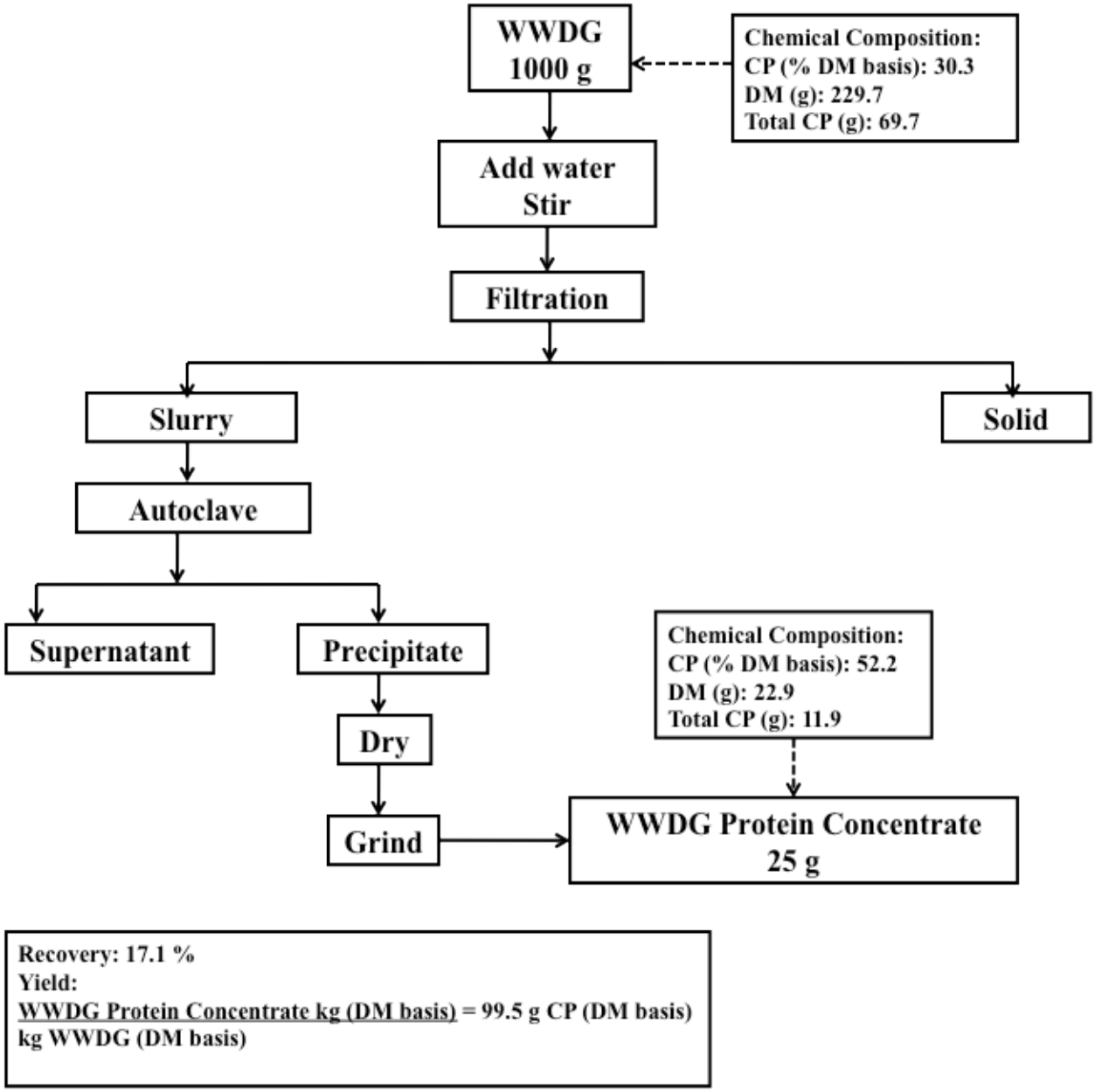


Figure A.1 Recovery and yield of aqueous fractionation of wheat wet distillers grains (WWDG) from plant 1.

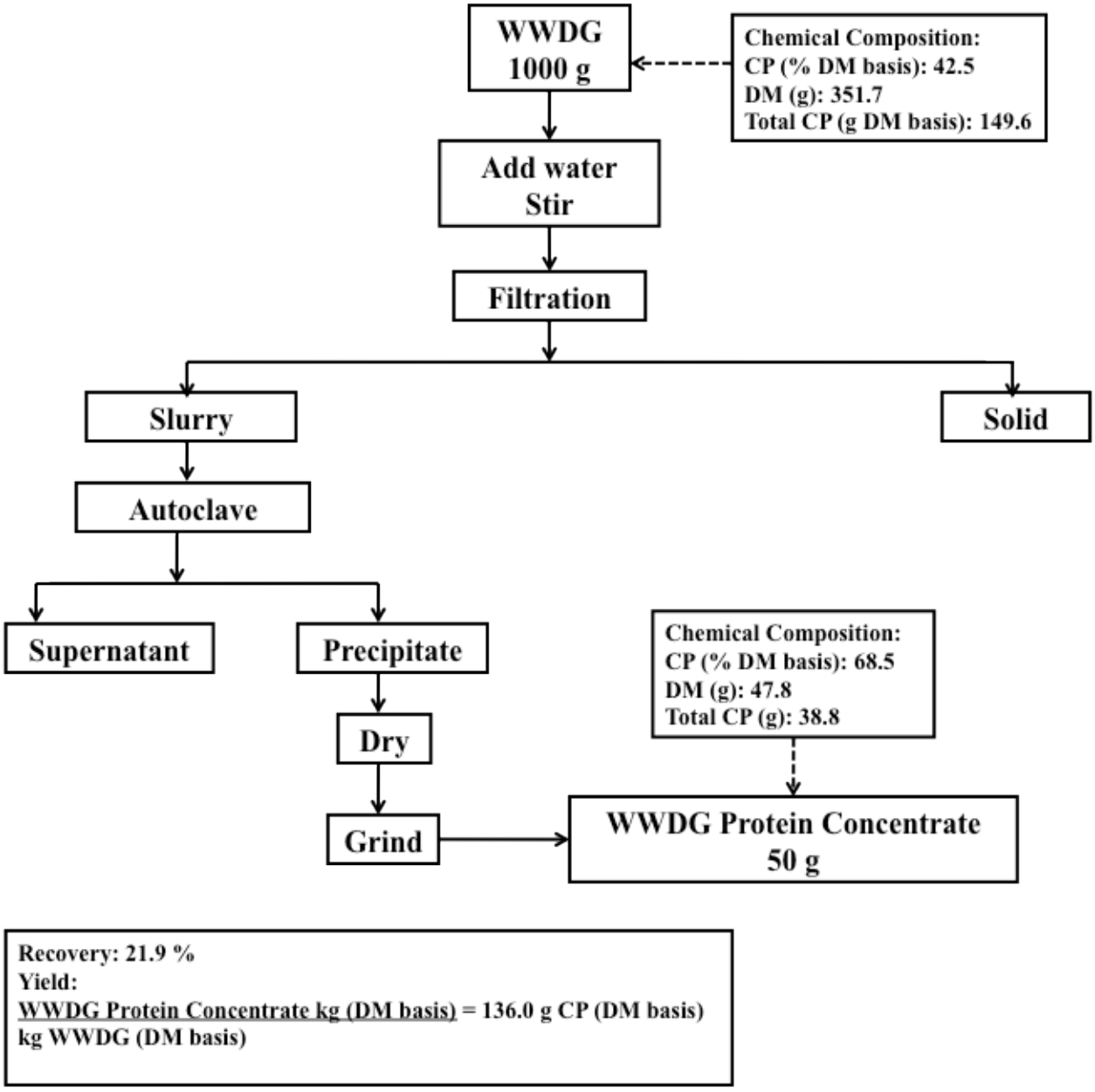


Figure A.2 Recovery and yield of aqueous fractionation of wheat wet distillers grains (WWDG) from plant 2.