EXPELLER/COLD-PRESSED CANOLA AS A VALUABLE FEED INGREDIENT FOR POULTRY

by

Tetiana Sessingnong

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Department of Animal Science
University of Manitoba
Winnipeg, Manitoba
Canada. R3T 2N2

ABSTRACT

The utilization of the expeller/cold-pressing method for producing canola oil from its seeds is becoming increasingly popular. Apart from its relatively high protein content, expeller/cold-pressed canola (ECPC) retains residual oil, making it a valuable protein and energy source for poultry. Unlike the conventional pre-press solvent extraction process, cold pressing does not apply heat or moisture to canola seeds. As a result, the use of different processing technology leads to variation in the chemical composition and nutritive values of ECPC. Therefore, the primary objective of this research was to determine the detailed chemical composition and nutritive profile of twenty-two ECPC samples collected from canola seed expelling facilities in Western Canada. On a dry matter (DM) basis, ECPC contains on average 16.7% of ether extract (EE), 36.5% of crude protein (CP), 24.1% of neutral detergent fibre (NDF), 31.8% of total dietary fibre (TDF), 8.04% of sugars, 6.1% of ash, 1.0% of total P, 0.5% of non-phytate P, and 7.85 µmol/g of glucosinolates (GLS). Variations were observed in the chemical composition of ECPC, with particular differences in the EE and GLS contents, which ranged from 8.5 to 24.1%, and from 4.8 to 15.7 µmol/g DM, respectively. These discrepancies can be attributed to differences in the employed processing methods. Furthermore, the content of other chemical components in ECPC showed quantitative differences, which were dependent on the varying fat content.

The second objective of this study was to determine the nitrogen-corrected apparent metabolizable energy (AMEn) and the standardized ileal amino acid digestibility (SIAAD) of ECPC for broiler chickens. Additionally, the aim was to develop a prediction equation for the AMEn content of ECPC for broiler chickens based on the analyzed nutrient content. Five ECPC samples sourced from different processing plants were subjected to a broiler chicken study to determine the AMEn and SIAAD. One-day-old Ross 308 broiler chickens were fed a pre-experimental starter diet from 1 to 14 d of age. On day 14, birds were allocated to 6 treatments with 6 replicate pens of 6 birds each and fed the basal diet or test diets that contained 70% of the basal diet and 30% of the test ECPC. On day 19, excreta samples were collected for AMEn determination. On day 21, birds were euthanized, and ileal digesta samples were collected for SIAAD determination. The AMEn averaged 2386 kcal/kg but ranged from 2128 to 2604 kcal/kg, on a DM basis. The diverse processing conditions and residual oil levels contributed to variation in the AMEn among ECPC samples from different processing plants ($P<0.05$). Prediction equations for energy availability demonstrated a strong relationship between the fat, CP, ash, and NDF and the AMEn content (R^2 = 0.94-0.99; $P<0.05$). The SIAAD of Lys, Met, Arg, and Thr averaged 85.2%, 93.5%, 88.1%, and 80.4%, respectively, and their standardized ileal digestible content averaged 1.61, 0.45, 1.57, and 1.17% on a DM basis, respectively. There were significant differences between plants in the standardized ileal digestibility of all amino acids except for Thr. Moreover, the significant variations were observed in the standardized ileal digestible content of all amino acids.

The third objective of this research was to evaluate the impact of ECPC as a dietary component for the growth performance of broiler chickens. The results of this study showed that there were no significant differences in the growth performance of broilers fed ECPC compared to the control treatment when diets were formulated based on digestible amino acid and metabolizable energy contents. Additionally, the developed prediction equations were successfully validated in growth performance study.

In conclusion, it is important to consider the differences in processing methods and the chemical composition of ECPC when formulating diets for broiler chickens. Having a comprehensive understanding of the relationship between the chemical composition and nutritive values of ECPC can effectively address this concern.

Keywords: Expeller/cold-pressed canola; chemical composition; broiler; AMEn; amino acids; growth performance

DEDICATION

I dedicate this thesis to God Almighty, who is Alpha and Omega, who is, and who was and who is to come!

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Manuscript III.

Sessingnong, T., Rogiewicz, A., Barthet, V., and B.A. Slominski. Evaluation of growth performance of broiler chickens fed diets containing expeller/cold pressed canola.

Sessingnong T. is the first and presenting author of all manuscripts. She was instrumental in study design, conducted experiments, performed analytical procedures, statistical analyses, and interpretation, and wrote the manuscripts. **Rogiewicz, A.** planned the experiments, trained Sessingnong T. in analytical procedures, and supervised her in research activities. **Rogiewicz, A.** is the corresponding author, contributing to reviewing, editing, and proofreading of manuscripts. **Barthet, V.** made contribution in the design of the analytical work and experiments. **Slominski, B. A.** made a substantial contribution in the conception and design of the research, interpretation of data and editing of manuscripts. He acquired funding for this research. Authors are representing the Department of Animal Science, University of Manitoba.

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LIST OF ABBREVIATIONS

AA	Amino acid
ADF	Acid Detergent Fibre
AME	Apparent Metabolizable Energy
AME_n	Nitrogen-corrected AME
ANOVA	Analysis of variance
Ala	Alanine
AOAC	Association of Official Analytical Chemists
Arg	Arginine
Asp	Aspartic acid
BWG	Body weight gain
°C	Celsius degrees
CCAC	Canadian Council on Animal Care
CM	Canola meal
CO_2	Carbone dioxide
CP	Crude protein
Cr_2O_3	Chromium (III) oxide
Cys	Cysteine
DM	Dry matter
EE	Ether Extract
ECPC	Expeller/cold-pressed canola
FCR	Feed conversion ratio
FI	Feed intake
G	Gram
GE	Gross energy
GLM	Generalized linear model
GLS	Glucosinolates
Glu	Glutamine

Gly	Glycine
HCL	Hydrochloric acid
His	Histidine
IEL	Ileal endogenous losses
Ile	Isoleucine
Kcal	Kilocalorie
Kg	Kilogram
Leu	Leucine
Lys	Lysine
MB	Manitoba
ME	Metabolizable energy
MT	Metric ton
NaOH	Sodium hydroxide
NDF	Neutral detergent fibre
NDICP	Neutral detergent insoluble crude protein
NSP	Non-starch polysaccharides
NRC	National Research Council
Phe	Phenylalanine
Pro	Proline
R^2	Coefficient of determination
SAS	Statistical Analysis System
SBM	Soybean meal
Ser	Serine
SIAAD	Standardized ileal amino acid digestibility
SID	Standardized ileal digestibility
SK	Saskatchewan
TDF	Total dietary fibre
Thr	Threonine

Tyr	Tyrosine
Val	Valine

CHAPTER 1

1.0 GENERAL INTRODUCTION

Canola was developed by Canadian plant breeders through the intensive genetic selection of *Brassica napus* and *Brassica rapa* (formerly *campestris*) species to obtain seeds with low erucic acid content (<2%) in the oil and a glucosinolate content below <30 µmol/g oil-free meal (Khajali and Slominski, 2012). It is a main crop in Western Canada and is significant for the Canadian food, feed, and biofuel industries. The total Canadian canola seed production in 2022 was 18.2 million metric tons, covering the equivalent of 8.66 million hectares at a yield of 2114kg/hectares The Canola Council of Canada's strategic plan is for 26 million tonnes of canola production on the same acreage by 2025 (Canola Council of Canada, 2019).

Canola seed contains around 45% oil and 20% crude protein. The oil extracted from the seeds is rich in mono- and poly-unsaturated fatty acids and contains the lowest amount of saturated fatty acids when compared to other sources of fatty acids for human consumption. Canola oil is one of the most versatile and healthy cooking oils due to its favorable flavor, texture, and physicochemical properties. Oil extracted from canola seed is also used as a biofuel. After further processing, the remaining part of the seed can be utilized in the form of a meal as a valuable feed ingredient for livestock. The seed processing methods and conditions, i.e., temperature and moisture, have a significant impact on the nutritive value of the meal affecting not only the fat, crude protein, amino acids, and dietary fibre contents but also the quality of the protein (Khajali and Slominski, 2012; Adewole et al. 2016).

The canola seed crushing industry utilizes three extraction methods to separate oil from seeds. Most commonly, canola seeds are processed using pre-press hexane extraction. Canola seeds are pre-conditioned at about 75-78°C and flaked to facilitate an initial expeller extraction. It results in a seed cake with 20% oil content. It is followed by the hexane extraction, desolventizing and toasting of the cake at temperatures of 100 to 115°C. The second method of processing canola seeds is the expeller oil extraction, where the seeds are conditioned to optimize the oil extraction performed by mechanical pressing. The temperature can reach 135°C during the short period of seed pressing. The canola seeds cold pressing method, though less commonly utilized, is becoming more popular. In this process, no additional heat or moisture is applied. Although the canola seeds are not pre-conditioned some heat is generated as an effect of the mechanical friction during expelling reaching temperatures around 65°C or higher. In two latter processes, the cake can be double pressed to increase efficiency oil extraction. Expelling processing is used mainly to produce biofuel by smaller seed-crushing facilities (Spragg and Mailer, 2007). Although some differences in the pre-press solvent extraction processing conditions between crushing plants occurs, the technology is well established and significantly improved towards efficient oil extraction and production of the high-quality canola meal. Much wider variation in expelling and cold press processing technology exists between canola seed crushers, which is mainly reflected in the amount of the residual oil in the meal.

As stated earlier, each method used to extract the oil results in a product of different nutritional value. The oil residue in the expeller-extracted canola meal varies between 8-15% while solvent-extracted has less than 5% of oil content (Spragg and Mailer, 2007). The crude protein and amino acid contents also vary between solvent- and expeller- canola meals (NRC, 1994). Consequently, the metabolizable energy and amino acids digestibility also differs between the various canola meals as a function of the processing extraction (Woyengo et al., 2010).

The extraction process also has an impact on anti-nutritional factors. The temperature conditions used during processing may lead to the degradation of GLS (Campbell and Slominski, 1990). Higher temperatures lead to a decrease in the total amount of GLS in the meal (Toghyani et al., 2014; Adewole et al., 2016). The reduction of GLS is desirable for nutritional reasons. However, excessive temperatures applied during processing can cause a Maillard reaction between amino acids and reducing sugars, leading to reduced digestibility of lysine and other amino acids (Nursten, 2005). Furthermore, increasing the temperature may also result in higher levels of neutral detergent insoluble crude protein (NDICP) in the final meal (Toghyani et al., 2014; Adewole et al., 2016).

The objective of this study was to evaluate and understand the utilization and application of expeller/cold pressed canola from different processing plants in Western Canada as a component in broiler chicken diets. The full nutritional profile of expeller/cold pressed canola material was determined prior conducting the evaluation of apparent metabolizable energy (AMEn) and standardized ileal amino acid digestibility (SIAAD). Subsequently, a final test was conducted to evaluate the growth performance of broiler chicken when expeller/cold pressed canola was included in their diets.

CHAPTER 2

2.0 LITERATURE REVIEW

2.1 HISTORY OF CANOLA SEED DEVELOPMENT AND ITS UTILIZATION

Canola is a type of rapeseed that was developed in Canada in the 1970s through the efforts of Canadian plant breeders. The word "canola" is derived from "Canadian oil, low acid," which reflects the Canadian origin and the low level of erucic acid in the oil (Bell, 1982; Khachatourians et al., 2001). Early record of rapeseed production in Canada comes from 1927, when Fred Solvoniuk, a Polish farmer in Saskatchewan, first planted a small number of seeds obtained from Poland. The development of canola began in the early 1950s when Canadian scientists began searching for a rapeseed variety with improved nutritional quality that could be grown in the cooler climate. At the time, rapeseed was primarily used for industrial purposes because the high erucic acid content made it unsuitable for human consumption (Bell, 1982).

Canadian plant breeders developed a new variety of rapeseed by crossbreeding various varieties and selectively breeding for low erucic acid with high oil content and low glucosinolates content. In 1970s, Canadian seed that contained low erucic acid in the oil and low GLS level in the meal was distinguished from the other rapeseed that was on the market at that time. Dr. J. M. Bell in his publication “From rapeseed to canola: a brief history of research for superior meal and edible oil” (1982) acknowledged a Polish scientist who visited the Agriculture Canada research station in Saskatoon and brought with him a sample of the low-glucosinolate canola cultivar Bronowski, which was then used by renown Canadian breeders Dr. K. Downey and Dr. B. Stefansson to develop low-glucosinolate canola variety Tower. To this day, Bronowski is considered the only genetic source of low glucosinolate canola. It was important to brand this “product” and change the name to reflect a more forward-thinking image because vigorous breeding programs changed the attributes of the rapeseed (Stefansson, 1974; Khachatourians et al., 2001).

The term "canola" was first registered as a trademark in 1978 and officially recognized as the name for the newly developed variety of rapeseed belonging to the *Cruciferae* (*Brassicaceae*, mustard) family in the genus Brassica. As Canada rose to become the top exporter in the world, the industry eventually came to refer to it as the "Cinderella" crop of Canada. The word Canola represents the strict quality standard of seeds defining it as a seed containing less than 2% of erucic acid in the oil and less than 30 µmol of GLS per gram in an air-dried defatted meal (Khachatourians et al., 2001).

Most of the Canola seeding areas in Canada are distributed within the Prairie Provinces (Figure 2.1) such as Manitoba, Saskatchewan, and Alberta (Statistics Canada, 2022)

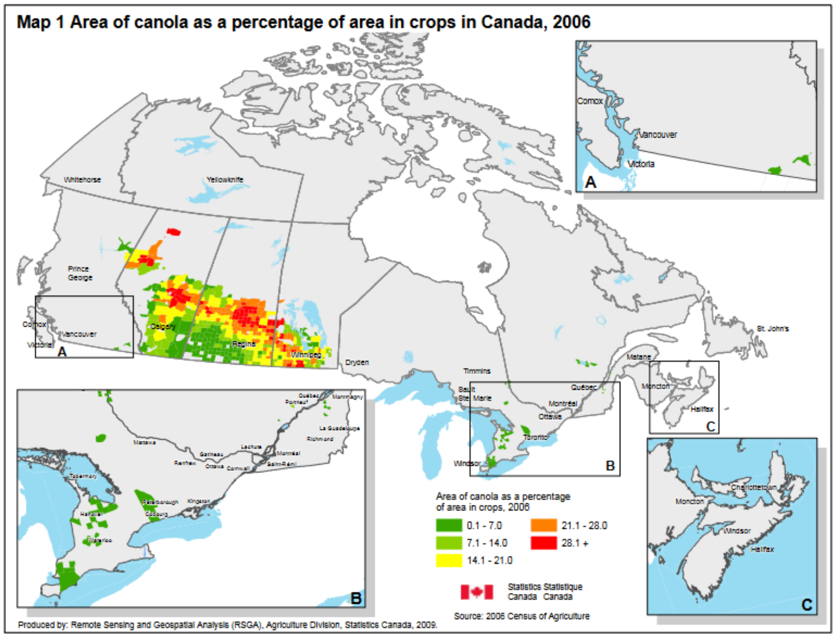


Figure 2.1. Area of canola as a percentage of area in crops in Canada, 2006 (adapted from Statistics Canada, 2022)

In Canada, the canola production sector generates approximately 20 million metric tonnes of canola annually (Canadian Canola Growers Association, 2023) It is anticipated that the production of canola seeds will increase reaching 26 million metric tonnes by 2025. Around 10% of the oil extracted through conventional solvent extraction is allocated for biofuel production. Currently (2023), there are fourteen Canadian plants using the conventional pre-press solvent extraction technology representing a processing capacity (crushing and refining) of 11 million metric tonnes of canola seeds per year. With a planned addition of five new canola processing plants to the industry, the processing capacity is set to increase by 60% adding another 6.7 million metric tonnes (Canadian Canola Growers Association, 2023) of crushing capacity. With the anticipated growth in canola seed availability, processors utilizing expeller press extraction and cold pressing technology will potentially make significant contribution to the biofuel production.

2.2 PROCESSING METHODS OF CANOLA SEED

After harvesting, canola seeds are preprocessed prior the oil is extracted depending on the processing method. The meal that is left over from the seeds after the processing is used as a feed ingredient in the animal nutrition (Canola Council of Canada, 2009).

Majority of Canadian canola oil comes from the prepress solvent extraction process. Smaller portion of Canadian canola seeds undergo processing that does not involve solvent extraction; it includes expeller press extraction, and cold pressing.

2.2.1 Conventional Pre-press Solvent Extraction of Canola Seeds

The solvent extraction of canola seed is a widely used method for extracting oil from canola seeds. Prior to canola oil extraction, seed cleaning is necessary to remove any impurities or foreign matter, such as dirt, stones, chaff, or other plant debris. Subsequently, seed conditioning is performed to adjust the moisture content to facilitate the extraction process. The conditioned seeds are then processed into flakes using mechanical rollers to increase the surface area available for extraction. The portion of the oil is expelled at this step. Subsequently, the canola pressed cake is subjected to solvent extraction with hexane, food-grade solvent, to remove the oil from the pressed cake. The remaining solids (meal), are then separated from the solvent and desolventized to remove any residual solvent, typically using heat and steam. The solvent is recovered from the meal and recycled. The crude oil obtained from the solvent extraction process is then refined to remove impurities and improve its quality. This process may involve degumming, neutralization, bleaching, and deodorization. Heat and moisture are applied during the seed processing steps. The temperature during the expelling, desolventizing and toasting can reach around 100-120℃ (Spragg and Mailer, 2007). The heat treatment, it includes a combination of temperature and length of the exposure to heat can affect the nutritive value of the protein final product (Khajali and Slominski, 2012; Adewole et al. 2016).

2.2.2 Expeller Pressing of Canola Seeds

Expelling is an alternative method for extracting oil from canola seed that does not involve the use of solvents. In this process, canola seed is cleaned and subsequently preheated to reduce viscosity of the oil to facilitate the mechanical oil extraction. The seed are then crushed using mechanical rollers or a screw press to expel the oil. Seed can be pressed once to produce a cake, but this product can also be subjected to the second round of oil expelling. This processing steps are the same as in the pre-pressed solvent extraction, except that the meal after expelling is not processed further. Expelling is a less efficient method for oil extraction compared to solvent extraction, and typically results in lower oil extraction yields. Consequently, the resultant expeller pressed canola typically contains higher amount of residual oil, therefore can provide more energy than the solvent-extracted meal (Unger, 2011). In this process, the heat is applied prior the expelling but also can be generated by the mechanical friction during the pressing, it can exceed 135℃ for a short time.

2.2.3 Expeller Cold Pressing of Canola Seeds

Canola seeds can be crushed using expelling method without an extra heat and moisture application. The cold pressing is gaining more popularity because it can be performed on a small scale, and it is simpler and less expensive than the other methods. The cold pressing involves the mechanical press to crush the canola seeds at low temperatures, typically around 60-70°C, to extract the oil (Spragg and Mailer, 2007). The final product, known as expeller cold-pressed canola, may contain a relatively higher amount of residual oil as the extraction process is less efficient compared to other methods. The oil content usually varies between products and can be present at 8 to around 20% (Leming and Lember, 2005; Spragg and Mailer, 2007; Seneviratne et al., 2011; Grageola et al., 2013; Kasprzak et al., 2016; Woyengo et al., 2016; Lee and Woyengo, 2018; Agyekum and Woyengo, 2022;).

Determination of chemical composition and nutritive value of expeller/cold-pressed canola produced in Western Canada was a main objective of this research.

2.3 PRODUCTION OF EXPELLER/COLD-PRESSED CANOLA (ECPC)

With the development of new technologies that are more efficient and approaches in the agronomic field, it is projected that by the year 2025, Canada will reach the production goal of 26 million tonnes of canola seed with a harvest of 1.3 tonnes per acre of land (Canola Council of Canada, 2019). According to Renewable Fuel Regulations (2022) in Canada, it is required to have 2% of biodiesel in the total amount of diesel. Canola oil, can be utilized because canola seed is recognized as a sustainable raw material for the production of biofuel (Davis and Long, 2015). The oilseed such as canola seems to be a sustainable raw material source in the production of biofuel

(Davis and Long, 2015). Some small-scale on-farm operations use their own equipment to extract oil from canola seed and produce an expeller/cold-pressed canola (ECPC). As per 2016 census, 370 Hutterite colonies live in Canada, predominantly in Alberta (16,935), Manitoba (11,275), and Saskatchewan (6,250), and most of them are farmers that produce various crops, including canola (The Canadian Encyclopedia, 2019). Canola seed can be crushed locally to provide an oil and maintain the sustainability of the colonies. Samples of ECPC for this research were collected from small and medium size crushing plants from across Western Canada. The study did not include information on specific conditions used during the seed expelling process.

2.4 EXPELLER/COLD PRESSED CANOLA IN THE FEED INDUSTRY

Due to absence of a widely accepted nomenclature for expeller/cold-pressed canola, it is often mistaken for both expeller-pressed canola and conventional canola meal, causing a potential confusion. Across studies, the expeller/cold-pressed canola is presented with different names. For example, Seneviratne et al. (2011), Grageola et al. (2013) and Lee and Woyengo, (2018) referred to it as “cold-pressed canola cake” while Kasprzak et al. (2016) called it “a cold-pressed double-low rapeseed cake”. Leming and Lember (2005) called it “cold-pressed canola”, while Agyekum and Woyengo, (2022) used name “an expeller/cold-pressed canola meal” and as presented in the Australian study, Spragg and Mailer (2007) used name such as “cold-pressed canola meal”. Also, within the feed industry, a standardized term for expeller/cold-pressed canola is yet to be established.

2.5 FACTORS INFLUENCING THE VARIABILITY IN THE NUTRITIVE VALUE OF EXPELLER/COLD-PRESSED CANOLA

The variability in the nutritive value of the expeller/cold-pressed canola can be caused by many factors such as the seed processing conditions, different years of harvesting seeds type, weather conditions, hulls removal, etc. (Bell and Keith, 1991; Bell, 1993; Khajali and Slominski, 2012; Adewole et al., 2016; Adewole et al., 2017; Chew, 2020;).

First, variety of the rapeseed and method of processing may influence content of amino acids (AA) and CP in solvent extracted double-low rapeseed and cold-pressed double-low rapeseed cake (Kasprzak et al., 2016). Their research has shown that in the double-low rapeseed meal that was solvent-extracted in milder conditions together with suitably selected cultivar of the rapeseed has resulted in improved digestibility values of AA and CP in comparison with cold-pressed double-low rapeseed cake.

Another important factors that can influence the quality of canola are weather conditions. For example, when canola seeds are exposed to frost before maturity, this may result in higher levels of chlorophyll content in the oil and can shorten its shelf life; also protein may become damaged and content of the oil reduced, therefore the quality of the meal will be reduced (Unger, 2011). High moisture during harvesting may lead to bin heating, which can cause protein damage in the meal. High moisture of the seeds (above 10%) seems to impact the majority of the processing equipment (Unger, 2011). Dickson, (2014) investigated how weather conditions impact canola quality. This research demonstrated that high precipitation led to reduced oil content and increased protein level, when compared to samples that were exposed to low precipitation (Dickson, 2014)

The next significant factor is the dehulling of canola seeds. It was reported by that dehulling increased the digestible energy (DE) value of CM and the crude protein (CP) content in swine trial (De Lange et al., 1998)

Finally, processing conditions, one of the most important factors that can influence the quality of the meal. In the study investigating the effect of processing conditions and chemical composition on apparent metabolizable energy corrected for nitrogen (AMEn) of expeller-extracted canola meal exposed to different temperatures (90, 95 and 100°C) during conditioning stage, Toghyani et al. (2014) reported that conditioning seeds at 90°C led to highest AMEn utilization. In the same study, the effect of screw torque on the energy utilization in poultry was investigated. The highest energy utilization occurred when expeller-extracted canola were subjected to higher screw torque during processing when compared with low screw torque ($P<0.001$) (Toghyani et al., 2014).

2.6 EFFECT OF PROCESSING ON EXPELLER/COLD-PRESSED CANOLA QUALITY

The quality of the expeller/cold-pressed canola (ECPC) could be affected during processing. The ECPC product is produced by mechanical pressing and the temperature during extraction usually does not exceed 60°C. However, some producers may increase the speed of the screw torque, which may increase the temperature in the meal. Others may increase the duration of the processing time. If the temperature during processing exceeds the acceptable level, the quality of the meal might be affected, especially of some amino acids such as lysine (temperature sensitive). The findings of the study conducted by Theodoridou and Yu (2013) suggested that the processing method had a significant effect on the chemical composition of yellow-seeded (*Brassica juncea*) and brown-seeded (*B. napus*) canola meal and brown seeded (*B. napus*) canola press cake. Canola press cake had the highest amount of oil content (14.7%) compared to yellow-seeded (2.3%) and brown-seeded (4.0%) canola meals. However, the protein level was the lowest (34.6%) in the canola press cake compared to yellow-seeded (47.0%) and brown-seeded (40.0%) canola meals. Such notable differences in the oil and protein content can probably be attributed to the fact that canola press cake does not include the solvent extraction step during the processing (Theodoridou and Yu, 2013).

Lysine is sensitive to heat treatment. When feeds are exposed to higher temperatures, a Maillard reaction occurs, resulting in the damage of lysine not available for animal digestion (Hendriks, 2018). This reaction leads to a reduction of the nutritive value of feeds (Slominski, 2018). The intensity of the Maillard reaction is influenced by the exposure time and degree of heat: when the processing temperature reaches over 100°C, the impact of this reaction becomes more noticeable (Ruan et al., 2018). Moreover, toasting also affects the lysine content. For instance, Salazar-Villanea et al. (2017) observed a linear decrease ($P<0.001$) in the lysine content of double-low rapeseed meal (RSM) with an increase in toasting time from 60 minutes to 120 minutes.

Adewole et al. (2017) investigated the impact of source and pelleting on the nutritive value of CM. Variations were observed in the heat-sensitive components, including lysine, GLS, neutral detergent insoluble crude protein (NDICP), and dietary fibre fractions among different processing plants. Total lysine content in CM ranged from 1.66% to 2.45%, GLS from 2 µmol/g to 5.9 µmol/g, NDICP from 2.5% to 6.8%, NDF from 24.9 % to 32.4%, and total dietary fibre from 32.5% to 39.3%. These variations were attributed to differences in processing conditions among the processing plants. The results suggest that source and pelleting techniques significantly affect the nutritive value of CM and should be taken into consideration when formulating diets for livestock.

Adewole et al. (2017) also investigated the correlations between nutrient content in canola meal (CM) and the implications for heat damage of protein. The study reported positive correlations between total dietary fibre content and NDICP ($R^2=0.79$), GLS and lysine ($R^2=0.56$), while negative correlations were observed between total dietary fibre and lysine ($R^2=0.64$), and between lysine and NDICP levels ($R^2=0.48$). Heat sensitivity of GLS may indirectly indicate heat damage to protein, particularly lysine. When GLS level was reduced, NDICP level likely increased, therefore GLS levels could indirectly indicated protein heat damage (Adewole et al., 2017). In a study conducted by Newkirk et al. (2003) to assess the impact of heat treatment on canola meals, toasted and non-toasted meals from various processing facilities were compared. The results indicated a significant difference ($P<0.001$) in the average level of lysine between toasted and non-toasted meals, 2.35% for non-toasted meal and 2.16% for toasted meal. In addition, the apparent ileal amino acid digestibility of lysine in broilers chickens was lower (76.5%) for toasted canola meal than for non-toasted CM (89.7%). The heat treatment during processing may have an adverse impact on the nutritional quality of canola meal, specifically on the lysine content and apparent ileal amino acid digestibility.

2.7 EXPELLER/COLD-PRESSED CANOLA CHEMICAL COMPOSITION

The chemical composition of cold-pressed canola is presented in Tables 2.1, and 2.2 and compared to solvent-extracted canola and conventional soybean meal.

Expeller/cold-pressed canola is a good source of CP for the poultry diet; however, the level of CP (Table 2.1) is not consistent across reported studies. Such inconsistency in the results could be related to differences in the processing conditions. For instance, the protein level reported by Grageola et al., (2013) was 28.1% DM, however, Seneviratne et al., (2011) reported value of 40.4% DM. The total amino acid content of cold-pressed canola derived as an average value (31.61% DM, SD=4.77), based on data from research by Seneviratne et al. (2019), Lee and Woyengo (2018), Grageola et al. (2013), and Agyekum and Woyengo (2022), was lower in comparison to CM (35.48% DM) as reported by Adewole et al. (2016), and also to SBM (46% DM) according to findings by Abdel-Raheem et. al. (2023). However, some AA in cold-pressed canola derived as an average value from the studies reported by Seneviratne et al. (2019), Lee and Woyengo (2018), Grageola et al. (2013), and Agyekum and Woyengo (2022) were higher than in CM (Adewole et al.,2016). Isoleucine was 1.76% DM, Methionine 0.63% DM, Cystine 0.89% DM, Threonine 1.40% DM, Valine 1.93% DM, and Glycine 1.83% DM compared to 1.19% DM, 0.59% DM, 0.72% DM, 1.35% DM, 1.85% DM, and 1.56% DM in CM, respectively.

Similarly, to the crude protein content, the ether extract levels also vary greatly among expeller/cold-pressed canola (Table 2.1). The level of ether extract is significantly higher in expeller/cold-pressed canola than that of solvent-extracted canola. Expeller/cold-pressed canola meal had a higher content of oil than solvent-extracted canola meal and conventional soybean meal (Leming and Lember, 2005; Spragg and Mailer, 2007; Seneviratne et al., 2011; Grageola et al., 2013; Kasprzak et al., 2016; Woyengo et al., 2016; Lee and Woyengo, 2018; Agyekum and Woyengo, 2022). In expeller/cold-pressed canola meals, the minimum ether extract level was 13.8% (DM basis) and the maximum 23.1% (DM basis) (Spragg and Mailer, 2007; Woyengo et al., 2016). The solvent-extracted canola meal was 3.51% (DM basis) (Adewole et al., 2016) and the soybean meal was 2.5% (DM basis) (Rogiewicz and Slominski, 2019).

The average values of ADF (acid detergent fiber) and NDF (neutral detergent fiber) calculated from reported studies by Leming and Lember (2005), Spragg and Mailer (2007), Seneviratne et al. (2011), Grageola et al. (2013), Kasprzak et al. (2016), Woyengo et al. (2016), Lee and Woyengo (2018), and Agyekum and Woyengo (2022) averaged 15.4% DM (SD=3.04) and 22.9% DM (SD=5.35) in expeller/cold-pressed canola. However, Adewole et al. (2016) reported higher values of ADF and NDF in CM, which were 18.6% DM and 29.4% DM, respectively due to much lower oil content of CM.

Ash content in expeller/cold-pressed canola was reported as an average of 6.1% DM (SD=1.02), calculated from studies by Leming and Lember (2005), Spragg and Mailer (2007), Seneviratne et al. (2011), Grageola et al. (2013), Kasprzak et al. (2016), Woyengo et al. (2016), Lee and Woyengo (2018), and Agyekum and Woyengo (2022), while the ash content of solvent-extracted canola meal was 7.5% DM (Adewole et al, 2016). This difference might be due to the diluting effect of residual fat in expeller/cold-pressed canola.

Spragg and Mailer (2007) and Grageola et al. (2013) reported comparable calcium levels 0.5% on as-is basis (around 0.56% on DM basis) in both expeller/cold-pressed canola and solvent-extracted CM. In contrast, Leming and Lember (2005) and Seneviratne et al. (2011) observed higher calcium levels, with values of 0.84% and 1.0% on DM basis in expeller/cold-pressed canola, and solvent-extracted CM, respectively. Such discrepancy might be due to different environmental plant growing conditions and would be unlikely related to seed processing. The average value of total phosphorus in expeller/cold-pressed canola, as published in three different studies (Leming and Lember, 2005; Spragg and Mailer, 2007; Seneviratne et al., 2011), was 1.25% DM.

Glucosinolates are considered as anti-nutritional factors in brassica species and still a concern for canola seeds. Through extensive breeding, researchers have successfully developed new rapeseed varieties containing less than 30 µmol/g of GLS in the meal, these varieties achieved the canola quality standard (Khachatourians et al., 2001). Spragg and Mailer, (2007) reported a very low level of GLS (1.73 µmol/g on as-is basis) in solvent-extracted canola meal. Glucosinolates are sensitive to heat and moisture, therefore their level could be reduced through exposure to heat (Adewole et al., 2017). The multiple heat-intensive solvent-extraction processing steps may explain the low presence of these chemical compounds (Spragg and Mailer, 2007) in conventional commercial canola meal. In contrast, during the cold processing of canola seeds, where temperatures generally remain below 60℃, the GLS tend to be higher (Spragg Mailer, 2007). Lee and Woyengo, (2018) reported nearly15 µmol/g on DM basis of GLS in cold-pressed canola cake and low glucosinolate cold-pressed rapeseed cake. Kasprzak et al. (2016) reported 17.5 µmol/g on DM basis of GLS. However, other researchers have reported lower amounts of GLS. Specifically, Seneviratne et al., (2011) and Grageola et al., (2013) reported 4.24 µmol/g on DM basis) and 5.63 µmol/g on as-is basis), in expeller/cold-pressed canola.

Table 2.1. Chemical composition of expeller/cold-pressed canola, solvent-extracted canola meal and soybean meal, (% DM)

Item	Expeller Cold Pressed Canola[1-8]		Pre-Press Solvent Extracted Canola Meal[9]	Soybean Meal[10]
	Average	Range		
Crude Protein (N x 6.25)	33.8	28.1-40.4	41.7	50.2
Ether Extract	18.0	13.8-23.1	3.51	2.5
Acid Detergent Fibre	15.4	12.5-19.7	18.6	9.2
Neutral Detergent Fibre	22.9	16.8-29.1	29.4	14.4
Ash	6.1	4.9-7.1	7.5	7.0
Phosphorus	1.2	0.9-1.5	1.1	0.7
Total Glucosinolates, µmol/g	10.7	4.2-17.5	4.6	NA[11]

[1]Leming and Lember, 2005; [2]Spragg and Mailer, 2007; [3]Seneviratne et al., 2011; [4]Grageola et al., 2013; [5]Kasprzak et al., 2016; [6]Woyengo et al., 2016; [7]Lee and Woyengo, 2018; [8]Agyekum and Woyengo, 2022; [9]Adewole et al,. 2016; [10] Rogiewicz and Slominski, 2019; [11]not applicable

Table 2.2. Amino acids content of cold-pressed and solvent-extracted canola (% DM)

Item	Expeller Cold Pressed Canola[1-4]		Pre-Press Solvent Extracted Canola Meal[5]	Soybean Meal[6]
	Average	Range		
Indispensable amino acids				
Arginine	2.00	1.6-2.4	2.46	3.45
Histidine	0.84	0.6-1.0	1.24	1.24
Isoleucine	1.76	1.2-2.6	1.19	2.25
Leucine	2.56	2.0-2.9	2.71	3.78
Lysine	1.93	1.6-2.3	2.18	3.1
Methionine	0.63	0.5-0.7	0.59	0.67
Phenylalanine	1.45	1.1-1.7	1.53	2.47
Threonine	1.40	1.2-1.6	1.35	1.82
Valine	1.93	1.4-2.3	1.85	2.35
Dispensable amino acids				
Alanine	1.54	1.3.-1.8	2.06	2.17
Aspartic acid	2.56	2.0-2.8	2.78	5.40
Cystine	0.89	0.6-1.0	0.72	0.68
Glutamic acid	6.26	4.4-7.8	7.31	8.55
Glycine	1.83	1.4-2.1	1.56	2.03
Proline	1.88	1.1-2.4	3.06	2.37
Serine	1.19	0.9-1.4	1.95	2.11
Tyrosine	0.93	0.8-1.1	0.95	1.72
Total AA	31.61		35.48	46.16

[1] Seneviratne et al., 2011; [2]Grageola et al., 2013; [3]Lee and Woyengo, 2018; [4]Agyekum and Woyengo, 2022; [5]Adewole et al., 2017; [6]Abdel-Raheem et al., 2023

2.8 NUTRITIVE VALUE OF EXPELLER/COLD-PRESSED CANOLA FOR POULTRY

There is limited available data in the published literature about the utilization of apparent metabolizable energy corrected for nitrogen (AME_n) for broiler chickens from expeller/cold-pressed canola. Agyekum and Woyengo, (2022) reported an AMEn value of 1,994 kcal/kg (DM basis) in expeller/cold-pressed canola meal with 16.6% of oil content. In another broiler chicken study, Smulikowska et al. (2006) reported AME_n value of 2,772 kcal/kg (DM basis) in low-glucosinolates rapeseed expeller cake pressed at 60℃ with an ether extract of 22.2%. Additionally, research conducted in Poland determined that the AMEn value of dark-seeded low-glucosinolate rapeseed cake was 3,022 kcal/kg (Czerwinski et al, 2012). To compare, Adewole et al. (2017) reported the AME_n value of 1,798 kcal/kg (DM basis) for pre-press solvent-extracted canola meal.

There have been only a few studies conducted on the standardized ileal digestibility of amino acids (SID) in expeller/cold-pressed canola for broiler chickens. The reported values of SID of amino acids are presented in Table 2.3. In studies conducted by Agyekum and Woyengo (2022) and Kasprzak et al. (2016), the SID of indispensable and dispensable amino acids in expeller/cold-pressed canola were higher than those of conventional, solvent- extracted CM (Park et al., 2019).

Table 2.3. Comparison of standardized ileal amino acids digestibility (%) in expeller/cold-pressed canola and solvent-extracted canola meal for broiler chickens[1]

Amino Acid	Expeller Cold Pressed Canola	Solvent Extracted Canola Meal
Indispensable amino acids		
Arginine	90.9	87.1
Histidine	88.6	61.0
Isoleucine	85.6	79.5
Leucine	86.2	83.1
Lysine	83.7	78.9
Methionine	91.1	87.5
Phenylalanine	87.4	83.1
Threonine	81.1	75.1
Tryptophan	94.5	-
Valine	83.0	77.1
Dispensable amino acids		
Alanine	90.7	83.4
Aspartic acid	90.1	76.5
Cystine	82.3	72.0
Glutamic acid	94.3	88.2
Glycine	88.9	79.6
Proline	84.0	76.4
Serine	85.3	76.6
Tyrosine	84.0	87.0

[1] Average values for broiler chickens calculated from Kasprzak et al., 2016; Adewole et al., 2017; Rogiewicz and Slominski, 2019; Agyekum and Woyengo, 2022

2.9 MEANS TO IMPROVING THE NUTRITIONAL QUALITY OF BYPRODUCTS OF CANOLA PROCESSING

2.9.1 Fermentation

Fermentation processing represents a promising method to enhance the quality of by-products derived from canola processing. This process involves the introduction of microorganisms such as yeast and bacteria into the canola by-products, allowing them to proliferate (Dunford, 2012). The advantage of fermentation goes beyond improving the nutritional value of canola by-products. It also reduces the presence of anti-nutrients such GLS and phytate (Alhomodi et al, 2021). It has been reported that enzymatic fermentation of double-low rapeseed cake (Goodarzi Boroojeni et al., 2022) or canola meal (Niu et al., 2023) effectively reduced phytic acid and insoluble non-starch polysaccharide NSP contents, resulting in improved feed conversion ratio for broiler chickens. Another study conducted by Xu et al. (2012) demonstrated some positive impact of fermentation on the quality of rapeseed meal. By incorporating up to 10% of fermented rapeseed meal in the diet, broiler chicken growth performance, phosphorus and calcium levels, as well as intestinal morphology were improved (Xu et al., 2012). Drazbo et al. (2019) carried out an investigation where they included 15% of fermented canola cake in turkey diets. The findings indicated that fermentation of canola cake led to the reduction in GLS and phytate P contents. Moreover, substituting raw canola cake with fermented canola cake in the diets resulted in an increase in the final body weight of turkeys. Ashayerizadeh et al., (2017) showed that the inclusion of fermented canola meal in broiler chicken diets led to reduced *Salmonella typhimurium* colonization, decreased stress levels, and improved growth performance.

2.9.2. Utilization of the Exogenous Enzyme Technology

Over the past three decades, enzyme technology has advanced considerably in terms of cost-effectiveness and customization of activity to specific substrate (Choct, 2006). Enzymes capable of degrading the cell wall matrix, especially insoluble components, can facilitate the release of nutrients encapsulated within the cell walls or embedded in the wall structure itself, thus allowing for easier access by digestive enzymes (Choct, 2006). The inclusion of enzymes such as phytase, protease, and carbohydrases is an effective approach to enhance the utilization of P, protein and energy in poultry diets (Khajali and Slominski, 2012). Enzyme supplementation in poultry diets improves productivity through multiple mechanisms. These include the release of P trough phytate hydrolysis, enhanced availability of energy and amino acids by reducing the nutrient encapsulation effect of cell walls, increased efficiency of hindgut fermentation through solubilization of cell wall polysaccharides, improved availability of amino acids trough hydrolysis of carbohydrate-protein bonds, and elimination of antinutritive properties, particularly NSP, trough enzymatic degradation. This comprehensive approach has the potential to improve gut health and promote intestinal maturation in young chickens (Slominski, 2011).

A broiler chicken study conducted by Radfar et al. (2016) investigated the effect of multi-carbohydrase supplementation on CM. The researchers found that enzyme addition resulted in the increased AMEn value for *B. napus* CM from 1,865 to 1,984 kcal/kg, resulting in improved WG and FCR in growing birds. Similarly, Kozlowski et al. (2018) reported that addition of 200 g/kg of multi-carbohydrase to the CM improved FCR in young turkeys and increased the production of short chain fatty acids (SCFA), which were beneficial for gut health of turkeys. However, an earlier study conducted by Meng and Slominski (2005) did not observe any significant effects of the growth performance of broiler chickens fed CM with enzyme supplementation.

In a recent study conducted by Niu et al. (2022), the researchers investigated the effect of various multi-carbohydrase preparations on CM NSP degradation. The *in vitro* studies revealed that a combination of two enzyme preparations that expressed pectinase activities and were fortified with xylanase activity resulted in depolymerization of around 49% of NSP present in CM. Additionally, the same enzyme blend was used in *in vivo* study with broiler chickens, it was reported that the BWG and the coefficient of apparent total tract NSP digestibility were improved (Niu et al., 2022).

Feeding enzymes to monogastric animals offers various advantages beyond increased growth performance and improved feed conversion. Enzyme supplementation also helps mitigate environmental problems by reducing nutrient excretion. Additionally, applying enzymes in animal diets provides greater accuracy and flexibility in the preparation of cost-effective feeds and improved animal welfare (Choct, 2006).

2.10 INCLUSION LEVELS OF EXPELLER/COLD-PRESSED CANOLA IN POULTRY DIETS

Canola meal is a valuable source of protein that could be used effectively at 15-20% in broiler chicken, turkey, and laying hen diets, providing that the diets are formulated based on digestible AA and available energy contents without adverse effect on animal health and growth (Gopinger et al., 2014: Ariybi et al., 2018; Rogiewicz and Slominski, 2019).

To the best of our knowledge, only a limited number of growth performance experiments have been performed with broiler chickens to determine the optimal level of expeller/cold-pressed canola in their diets. Based on the study by Thacker and Petri (2009), it could be assumed that the safe inclusion rate of expeller/cold-pressed canola was likely similar to solvent- and expeller-extracted canola meals. The body weight gain and feed intake for broiler chickens fed double-low cold-pressed rapeseed cake and conventional CM were found to be comparable (Thacker and Petri, 2009). According to Canola Council of Canada (2019), recommended inclusion levels of CM in broiler chicken diets are 20% during the starter phase, 30% during the grower phase, and up of 40% during the finisher phase. However, some studies reported that with increased amount of CM in the broiler chicken’s diet, the growth performance was decreased and feed intake was reduced (Woyengo et al., 2011; Gorski et al., 2017).

The effect of different inclusion levels of CM on growth performance of broiler chickens has also been studied by Ariyibi et al. (2018), where CM was incrementally included in the Pre-starter diets from 0 to15 %, in Starter diets from 0 to 18 %, in Grower 1 diets from 0 to 25 %, and Grower 2 diets from 0 to 30 % of CM. Diets were balanced for SID AA contents and AME. The results showed that although dietary fibre and GLS contents of diets differed substantially with increased levels of CM, growth performance was not significantly (P>0.05) affected by CM inclusion levels. These results showed the benefit of formulating diet on available energy and digestible AA content. Such approach was not utilized in research by Woyengo et al. (2011) or Gorski et al. (2017), therefore broiler's growth performance results were negatively affected by high inclusion of CM in the diet. In another study, where the determined SID values of amino acids and the AMEn contents of CM were used for diets formulation, no significant difference in feed conversion ratio (FCR) between the control and the diet containing 15% of CM (Rad-Spice et al., 2018) were found, which confirms that it is worthwhile to invest effort in determining the energy and amino acids digestibility of feed ingredients in order to fully realize their nutritional potential.

Woyengo et al., (2011). conducted a project in which a high level of expeller-extracted canola meal (EECM) was fed to broiler chickens. The inclusion of EECM from 0 to 40% resulted in the linear decrease in the feed intake and BW gain (P<0.001) by 4.8 and 6.0 g/21-d period for each 1% increment in EECM, respectively. They demonstrated that high inclusion level of expeller-extracted canola into broiler chicken's diet resulted in less effective growth performance, and they suggested that it could be attributed to increasing concentration of GLS, which could affect the liver physiology and therefore growth performance. Such high inclusion of EECM in broiler chicken diet seems not to be practical, however their research demonstrated the possible effects of antinutritional factors that can negatively affect the growth of broiler chickens. The impact of the high inclusion of double-low cold-pressed rapeseed cake (RPC) in the broiler diets on their growth performance has been investigated by Thacker and Petri (2009). Broiler chicks were fed the diets containing 5, 10, and 15 % of RPC and control diets with the same amount of conventional CM for 21 days. The authors reported that there was no significant difference in BWG and feed intake between broilers fed RPC and those fed the conventional CM.

CHAPTER 3

3.0 HYPOTHESIS AND OBJECTIVES

The thesis aimed to test the following hypotheses:

1. There might be variations in the physical characteristics and chemical composition of expeller/cold-pressed canola (ECPC) samples obtained from different producers across Western Canada.

2. The apparent metabolizable energy (AMEn) values for broiler chickens may vary across expeller/cold-pressed canola (ECPC) samples from different processing plants.

3. The standardized ileal amino acids digestibility (SIAAD) of expeller/cold-pressed canola (ECPC) for broiler chickens is expected to vary across different processing plants.

4. The apparent metabolizable energy (AMEn) content will be accurately estimated using the prediction equations developed based on the content of commonly measurable nutrients.

5. Broiler chickens fed diets with ECPC will exhibit comparable growth performance to broilers fed the conventional diet.

The overall objective of this research was to develop a thorough nutrient profile of expeller/cold-pressed canola (ECPC), aiming to identify its best possible applications and to optimize nutrient utilization in the formulation of poultry diets.

The research objectives were as follows:

1. To determine the detailed chemical composition of ECPC samples obtained from canola seed crushing plants in Western Canada.

2. To determine the apparent metabolizable energy (AMEn) content of ECPC for broiler chickens.

3. To determine the standardized ileal digestibility of amino acids in ECPC for broiler chickens.

4. To develop a prediction equation for AMEn value of ECPC for broiler chickens from analyzed nutrient content.

5. To investigate the impact of including ECPC as a dietary component on growth performance of broiler chickens.

CHAPTER 4

4.0 MANUSCRIPT I

CHEMICAL COMPOSITION OF EXPELLER/COLD-PRESSED CANOLA

4.1 ABSTRACT

Twenty-two samples of expeller/cold pressed canola (ECPC) were collected from eight processing plants in Western Canada in 2020 and 2021. The study aimed to determine the chemical composition, physical characteristics variations for ECPC among different processing plants. Samples were subjected to chemical analysis to determine their chemical profile, and visually inspected to assess their physical form and color. On average, ECPC samples contained, on DM basis 16.8% of ether extract, 36.5% of crude protein (CP: Nx6.25), 24.1 % of neutral detergent fibre (NDF), 20.5% of acid detergent fibre (ADF), 31.8% of total dietary fibre (TDF), 18.8% non-starch polysaccharides (NSP), 8.04% of carbohydrates, 6.09% of ash, 1.03% of total phosphorus, 0.47% of non-phytate phosphorus and glucosinolates (GLS) at 7.85µmol/g. Variations (P<0.05) between processing plants were observed for DM, NSP, glycoproteins, sucrose, oligosaccharides, GLS, ash, total phosphorus, and non-phytate phosphorus. Significant variations were observed in the physical form and color of ECPC, with Plant 4 exhibiting the most noticeable difference in color, appearing as a dark brown shade. Chemical analysis revealed that sample from this plant also had the highest levels of glycoproteins and the lowest levels of GLS compared to the other six processing plants. It is recognized that elevated glycoproteins level and reduced GLS content are indicative of heat damage of protein in ECPC. In conclusion, the chemical composition of ECPC and physical appearance and color displayed variations across processing plants. These differences in both chemical composition and physical appearance are likely attributed to variances in the processing conditions employed by the producers.

Keywords: Expeller/cold-pressed Canola; chemical composition

4.2 INTRODUCTION

Canola (low-glucosinolate rapeseed) is a popular crop grown around the world for the production of edible oil. Canada alone is home to 25% of the world's canola production (Canola Council of Canada, 2023). As the oil is usually used for human consumption, the residual coproduct following crushing of the seeds is effectively utilized by the animal feed industry. The most popular methods of processing canola seeds are pre-press solvent extraction and expeller extraction that produces the conventional CM and expeller extracted canola meal, respectively (Spragg and Mailer, 2007). However, as the demand for biofuels increases due to the need for renewable energy, the expeller/cold press process is gaining popularity (Pratte, 2020). This method is utilized by the small-scale industry or in a small on-farm operations by using the expeller cold press equipment with low heat involvement (60°C) during processing. This results in the meal with higher residual oil content than that of conventional pre-press solvent-extracted CM (Grageola et al., 2013). Several studies in our laboratory evaluated the chemical composition of conventional CM (Campbell and Slominski., 1990; Meng and Slominski 2005; Adewole et al., 2016; Radfar et al., 2017; Rad-Spice et al., 2018; Niu et al., 2022). For example, in a study conducted by Adewole et al. (2016) variation in the chemical profile of conventional CM between processing plants in Western Canada was observed, and it was suggested that the variation was caused by differences in processing conditions between the crushing plants. Data from a few existing studies that included partial chemical composition of ECPC suggest that there is a lot of variation in the chemical composition of the expeller/cold-pressed canola (Leming and Lember, 2005; Spragg and Mailer, 2007; Seneviratne et al., 2011; Grageola et al., 2013; Kasprzak et al., 2016; Woyengo et al., 2016; Lee and Woyengo, 2018; Agyekum and Woyengo, 2022). However, there is a limited information on the thorough chemical profile of expeller/cold press canola processed in Western Canada. Therefore, the objective of this study was: To evaluate the physical appearance and to determine the chemical composition of ECPC samples from different Western Canadian processing plants.

4.3 MATERIALS AND METHODS

4.3.1 Expeller/Cold-Pressed Canola Sample Collection

Twenty-two samples of expeller/cold-pressed canola samples were collected from eight different canola seed crushing plants across Western Canada. Samples were provided by six producers from Alberta (Pleasant Valley Oil Mills, Millford Hutterite Colony, Prairie Home Hutterite Colony, High-Oil Meal, Miltow Hutterite Colony, and Mountain View Hutterite Colony) and by one crusher each from Manitoba and Saskatchewan (M&C Commodities, Bio Meal Milligan Biofuels, respectively). For data presenting purpose plant numbers were assigned randomly. The samples were collected in the spring of 2020 and 2021. The distribution of samples from each plant was as follow: two samples from Plant 1, five samples from each of Plant 2 and3, four samples from Plant 4, two samples from each of Plant 5 and 6, and single samples from Plants 7 and 8. Additionally, a sample of expeller-extracted canola meal (EECM) was received from the large-scale expeller crushing facility (Viterra, MB) and used as a reference.

4.3.2 Sample Preparation and Chemical Analysis

Samples were received in different physical forms, i.e., pellets, flakes, and crumbs. All samples were ground to pass through 1 mm sieve and subjected to the evaluation of their chemical composition.

The samples were analyzed in duplicate for the content of dry matter (**DM**), crude protein (**CP**), amino acids (**AA**), ether extract (**EE**), ash, total phosphorus, phytate phosphorus, glucosinolates (**GLS**) and sugars, i.e., sucrose, simple sugars, oligosaccharides. The fibre was analyzed in form of neutral detergent fibre (**NDF**), acid detergent fibre (**ADF**), and total dietary fibre (**TDF**) with its components, i.e., non-starch polysaccharides (**NSP**), lignin and polyphenols and glycoproteins (**NDICP** neutral detergent insoluble crude protein). Total dietary fibre was calculated according to Slominski and Campbell (1994).

Crude protein (N x 6.25) was analyzed with the combustion method (AOAC 990.03) using a LECO FP828 nitrogen analyzer (LECO Corp., St. Joseph, MO). Dry matter was determined according to AOAC Method (945.15) by drying 0.5g of samples at 104℃ overnight. Hexane extraction (Method 920.39, AOAC 2005) in Ankom Extraction System (Macedon, NY, USA) was used to determine the EE. Ash and total phosphorus were determined following the AOAC (2005) method 942.05 and 965.17, respectively. Phytate phosphorus content was measured according to the method published by Haug and Lantzsch (1983). Non-phytate phosphorus was calculated by subtraction of the phytate phosphorus from the total phosphorus content. The ANCOM Fiber Analyzer (Ankom Technology, Macedon, NY, USA) was used to determine ADF and NDF with AOAC procedures 973.18 and 2002.04, respectively. Simple sugars (glucose and fructose), sucrose, and raffinose family oligosaccharides (raffinose and stachyose) were measured by gas-liquid chromatography using 2% OV-7 column and Varian 430GC (Agilent Technology, Canada) as described by Slominski et al. (1994).

Non-starch polysaccharides were determined by gas-liquid chromatography (component sugars) using SP-2340 column and Varian CP-3380 GC (Agilent Technology, Canada) and by colorimetry (uronic acids) using Biochrom Ultrospec 50 (Biochrom Ltd., UK) according to the procedure described by Englyst and Cummings (1984), Englyst and Cummings (1988) with some modifications (Slominski and Campbell, 1990). In brief, a 100-mg sample was treated with dimethylsulphoxide and incubated overnight at 45°C with a solution of starch-degrading enzymes composed of amylase and amyloglucosidase (Sigma, St. Louis, MO). Ethanol was then added, the mixture left for 1 h, centrifuged and the supernatant discarded. The dry residue was dissolved in 1 mL of 12M H2SO4 and incubated for 1 h at 35°C. Six mL of water and 5 mL of myo-inositol (internal standard) solution were then added, and the mixture was boiled for 2 h. One mL of the hydrolysate was then taken and neutralized with 12 M ammonium hydroxide, reduced with sodium borohydride, and acetylated with acetic anhydride in the presence of 1-methylimidazole.

Total dietary fibre was determined by a combination of NDF and neutral detergent soluble NSP measurements to address the solubility of NSP in the neutral detergent solution that causes a loss of NSP on NDF assay. Total dietary fibre was calculated as the sum of NDF and ND-soluble NSP (Slominski et al., 1994). Neutral detergent soluble NSP were calculated as total sample NSP minus NSP present in the NDF residue. Neutral detergent insoluble crude protein (NDICP, glycoprotein) represented the amount of crude protein present in the NDF residue. The value for lignin and associated polyphenols was calculated by difference [NDF – (NSP +NDICP)].

Glucosinolate analysis was performed as described by Slominski and Campbell (1987). Briefly, 300 mg of canola samples were weighed into 15 mL centrifuge tubes. Two milliliters of methanol, 1.0 mL of sinigrin (internal standards, 0.5 mM), and 0.1 mL of lead–barium acetate were added to the tubes, extracted for 3 h at room temperature, and then centrifuged for 10 min at

3000 RPM. Two mL of supernatant were transferred to a DEAE-Sephadex column, which was washed successively with 1 mL of each of 67% methanol, water, and pyridine acetate. Purified sulfatase solution was then added to the column, and the contents were incubated at room temperature overnight. The resulting desulfated GLS were eluted with 4 mL of 60% methanol and dried. The dry residue was trimethylsilylated by adding 0.2 mL of a mixture of anhydrous acetone/N, O-bis (trimethylsilyl) acetamide (BSA)/trimethylchlorosilane (TMCS)/1-methylimidazole (2:1:0.1:0.05 v/v) and incubated for 30 min at room temperature. The trimethylsilyl derivatives of desulfoGLS were separated by gas−liquid chromatography using a glass column packed with 2% OV-7 and Varian 430GC (Agilent Technology, Canada).

4.3.3 Statistical Analysis

The nutritional content of ECPC was evaluated in One-way-ANOVA. The statistical difference between averages was considered 95%. The GLM procedure of SAS 9.4 was used to perform the analysis. Tukey test was used for pairwise comparison when the treatment differed in $P<0.05$.

4.4 RESULTS

4.4.1 Chemical and Nutritive Composition of ECPC

The mean and the range values of ECPC samples from the eight processing plants, along with the values of expeller extracted canola meal (ECPC) and canola meal (CM) are presented in Table 4.1 and 4.2. These tables also show the chemical composition of EECM and CM, serving as references. The data in the Table 4.1 indicate that the mean value of DM in ECPC samples was 92.65%, and EE was 16.75% on DM. On a DM basis, the average CP content was 36.50%, GLS accounted for 7.85µmol/g, ash was 6.09%, and the total, phytate and non-phytate P accounted for 1.03%, 0.56%, 0.47%, respectively.

Table 4.1. Chemical composition of expeller/cold-pressed canola, expeller extracted canola meal and pre-press solvent-extracted canola meal (%, DM)

Item	Expeller/cold-pressed canola, ECPC[1]			EECM[2]	CM[3]
	Mean, n=22	Min	Max		
Dry Matter	92.65	90.96	95.89	93.69	91.50
Ether Extract	16.75	8.49	24.09	12.86	3.20
Crude Protein (N x 6.25)	36.50	30.63	42.69	39.32	42.90
Carbohydrates					
Simple sugars[4]	0.87	0.25	1.53	0.08	0.46
Sucrose	5.18	3.37	8.0	6.53	7.29
Oligosaccharides [5]	1.99	1.24	2.76	2.47	3.30
Dietary Fibre	31.81	28.14	37.32	31.38	36.4
Ash	6.09	5.13	6.81	7.68	7.20
Total Phosphorus	1.03	0.90	1.27	1.03	1.20
Phytate P	0.56	0.35	0.73	0.60	0.65
Non-Phytate P	0.47	0.36	0.67	0.43	0.55
GLS, µmol/g[6]	7.85	4.86	15.69	6.81	1.44

[1] n=22 Expeller/cold-pressed canola samples from 8 processing plants.

[2] Expeller extracted canola meal used as a reference.

[3] Conventional solvent extracted canola meal used as a reference.

[4] Includes fructose and glucose.

[5] Raffinose Family Oligosaccharides- includes raffinose and stachyose.

[6] Includes gluconapin, glucobrassicanapin, progoitrin, gluconapoleiferin, glucobrassicin, and 4-hydroxyglucobrassicin.

On a DM basis the average content of simple sugars, sucrose, and oligosaccharides (raffinose and stachyose) was 0.87%, 5.18%, 1.99%, respectively (Table 4.2.). The fiber analyses showed that, on a DM basis, the average ECPC total dietary fiber content was 31.81%, with NSP, lignin and polyphenols, and glycoproteins (NDICP) on average accounted for 18.80%, 9.02%, 3.98%, respectively. The average content of NDF and ADF were 24.06% and 20.48% DM, respectively. The results of the chemical composition of ECPC showed that they were within range when compared to EECM and CM, except EE, GLS, and NSP in CM. The EE and GLS in CM were lower than in ECPC, however NSP was higher in CM than in ECPC.

Table 4.2. Dietary fibre components of expeller/cold-pressed canola samples from different processing plants, expeller extracted canola meal, and pre-press solvent-extracted canola meal (%, DM)

Item	Expeller/cold-pressed canola, ECPC[1]			EECM[2]	CM[3]
	Mean, n=22	Min	Max		
Dietary Fibre	31.81	28.14	37.32	31.38	36.40
Non-Starch Polysaccharides	18.80	16.58	21.57	19.82	23.58
Lignin and polyphenols	9.02	6.98	10.99	8.77	7.78
Glycoproteins (NDICP)[4]	3.98	2.23	7.63	2.79	5.03
NSP component sugars					
arabinose	3.75	3.31	4.29	4.03	4.69
xylose	1.30	0.96	1.55	1.47	1.55
mannose	0.28	0.21	0.50	0.31	0.25
galactose	1.19	0.95	1.39	1.37	1.36
glucose	6.13	5.37	6.87	6.33	7.24
uronic acids	6.15	5.20	7.50	6.30	8.50
Neutral Detergent Fibre	24.06	21.16	29.94	21.96	25.29
Acid Detergent Fibre	20.48	17.13	26.03	18.88	

[1] n=22 Expeller/cold-pressed canola samples from 8 processing plants.

[2] Expeller extracted canola meal used as a reference.

[3] Conventional solvent extracted canola meal used as a reference.

[4] Neutral Detergent-Insoluble Crude Protein

The ECPC were classified based on their respective plant of origin. The number of ECPC samples collected varied across the crushing plants. Out of eight crushing plants, statistical analyzes were performed on samples from six plants, as only single samples were obtained from Plants 7 and 8. The chemical composition of ECPC samples from the six processing plants, is presented in Tables 4.3, 4.4, and 4.5. The significant variation ($P<0.05$) between processing plants was observed in the content of DM, GLS, ash, sucrose, oligosaccharides, total and non-phytate P, NSP and NDICP. Although neither CP nor EE varied significantly, the wide range of GLS (6.3-15.3μmol/g DM), glycoproteins (2.3-6.0% DM), NSP (17.6-21.1% DM), and DM (91.6-95.1%) contents reflected the differences in the processing conditions.

Furthermore, there was no significand difference in NDF, ADF, TDF, lignin and polyphenols, simple sugars, phytate P, xylose, and galactose between processing plants. Although the above chemical components did not differ statistically, they differed numerically. Because the ECPC samples varied within the processing plants themselves, they were not statistically different, which can be particularly noted in the fat content.

Table 4.3. Chemical composition of expeller/cold-pressed canola from six processing plants, (%, on a DM basis)

Item	Expeller/cold-pressed canola, Plant[1]						*P* value	SEM
	1	2	3	4	5	6		
Dry Matter	92.79^{ab}	91.59^{b}	91.60^{b}	95.14^{a}	92.93^{ab}	92.29^{b}	0.0009	0.67
Ether Extract	14.49	20.10	18.27	14.92	12.70	15.28	0.0614	2.15
Crude Protein (N x 6.25)	36.08	34.54	36.48	37.22	38.49	36.75	0.6638	2.11
Carbohydrates								
Simple sugars[2]	0.26	0.88	0.84	1.08	0.45	1.52	0.1521	0.57
Sucrose	7.10^{a}	4.90^{ab}	5.22^{ab}	3.69^{c}	7.05^{a}	4.11^{bc}	0.0040	0.63
Oligosaccharides [3]	2.65^{a}	1.97^{ab}	2.06^{ab}	1.32^{c}	2.47^{ab}	1.73^{bc}	0.0006	0.19
Dietary Fibre	32.05	30.51	30.24	34.83	33.14	33.88	0.1461	1.86
Ash	6.40^{a}	5.56^{b}	5.96^{ab}	6.28^{a}	6.25^{a}	6.29^{a}	0.0015	0.16
Total Phosphorus	1.16^{a}	0.96^{c}	1.01^{bc}	0.98^{bc}	1.08^{ab}	1.02^{bc}	0.0070	0.03
Phytate P	0.54	0.56	0.56	0.49	0.60	0.56	0.2733	0.04
Non-Phytate P	0.62^{a}	0.40^{bc}	0.44^{c}	0.49^{b}	0.48^{bc}	0.46^{bc}	0.0001	0.03
GLS, µmol/g[4]	15.26^{a}	6.89^{b}	7.18^{b}	6.29^{b}	8.92^{b}	7.77^{b}	0.0001	0.88

[a–c] Means followed by different letters within rows are significantly different ($P < 0.05$)

[1] Plant 1, n=2; Plant 2, n=5; Plant 3, n=5; Plant 4, n=4; Plant 5, n=2; Plant 6, n=2;

[2] Includes fructose and glucose.

[3] Raffinose Family Oligosaccharides, galactooligosaccharides- includes raffinose and stachyose.

[4] Includes gluconapin, glucobrassicanapin, progoitrin, gluconapoleiferin, glucobrassicin, and 4-hydroxyglucobrassicin.

[a,b,c,] Within a row, means without common superscript differ ($P < 0.05$).

Among six processing plants, Plant 4 exhibited the highest DM content in its ECPC, whereas Plants 2, 3, and 6 had significantly lower DM content ($P<0.05$) than Plant 4. The average DM content across all processing plants was 92.72%.

Although there was no statistical difference in EE content among the six processing plants, there were numerical variations. Specifically, Plant 2 had the highest fat content, while Plant 5 had the lowest. The overall average fat content of the ECPC from all six processing plants was 15.96%.

Despite the absence of statistically significant differences in CP content among the six processing plants, numerical variations were observed. Plant 5 had the highest CP content among the six Plants, while Plant 2 had the lowest. The overall average CP content of the ECPC from the six processing plants was 36.59% DM. ECPC from Plant 1 showed the highest ash content, whereas ECPC from Plant 2 had significantly lower ash content ($P<0.05$). The ash content averaged 6.12% DM. It could correspond with the highest content of P in Plant 1, whereas ECPC from Plants 2, 3, 4, and 6 were significantly lower ($P<0.05$) in total P. The total P content averaged 1.03% DM. The phytate P content in ECPC samples among six plants was similar, but the non-phytate P content seemed to be affected by the total P, thus the significant differences between Plant 1 and Plants 2, 3, 4, and 6 were also observed ($P<0.05$).

The glucosinolates content in all samples was relatively high and averaged 8.72µmol/g DM. ECPC from Plant 1 contained the highest GLS level when compared to these from Plants 2, 3, 4, 5 and 6 ($P<0.05$).

In terms of sugar content, ECPC from Plant 1 and 5 exhibited the highest sucrose content, while ECPC from Plants 4 and 6 had significantly lower sucrose content ($P<0.05$). The overall average sucrose content of the ECPC from the six processing plants was 5.35% DM., regarding the simple sugars content, there were no statistically significant differences among the ECPC from all processing plants. Samples collected from Plant 6 had the highest content of simple sugars, while those from Plant 1 had the lowest content. The overall average simple sugars content of ECPC was 0.84% on a DM basis. Regarding the oligosaccharides content, ECPC from Plant 1 displayed the highest levels, whereas ECPC from Plants 4 and 6 showed significantly lower oligosaccharides content ($P<0.05$) compared to Plant 1. The overall average oligosaccharides content of the ECPC from the six processing plants was 2.03% DM.

There were no statistically significant differences in NDF content among samples from the six processing plants, but they differed numerically. ECPC from Plant 4 had the highest NDF content, whereas from Plant 1 had the lowest. The average NDF content of the ECPC from the six processing plants was 24.31% on a DM basis. The average ADF content of the ECPC from the six processing plants was 20.80% on a DM basis. ECPC from Plant 6 had the highest ADF content while from Plant 5 the lowest, but no significant difference in ADF content among the six processing plants was observed. Similarly, the overall TDF in ECPC content was 32.44% on a DM basis, but it did not differ between plants.

On average the NSP content was 19.39%, with the significant difference between Plant 1 (21% on a DM basis) and Plant 2 and 3 (both 17.1% DM). Statistical differences in the profile of NSP, were observed in the contents of arabinose, mannose, glucose, and uronic acids ($P<0.05$), however the differences were reflected in the total NSP values. The lignin and polyphenols levels were not different among processing plants and averaged 9.16% on a DM basis. The samples collected from the six processing plants were statistically different for glycoprotein or NDICP content ($P<0.05$) between Plant 4 (5.98% on a DM basis) and Plant 1 (2.27% on a DM basis). On average NDICP accounted for 3.88% on a DM basis.

Table 4.4. Dietary fibre components of expeller/cold-pressed canola samples from different processing plants (%, on a DM basis)

Item	Expeller/cold-pressed canola, Plant [1]						*P* value	SEM
	1	2	3	4	5	6		
Dietary Fibre	32.05	30.51	30.24	34.83	33.14	33.88	0.1461	1.86
Non-Starch Polysaccharides	21.07^{a}	17.60^{c}	17.61^{bc}	19.44^{abc}	20.62^{a}	20.03^{ab}	0.0005	0.63
Lignin and polyphenols	8.71	9.10	8.86	9.42	9.45	9.45	0.9244	0.74
Glycoproteins (NDICP)[2]	2.27^{b}	3.81^{ab}	3.77^{ab}	5.98^{a}	3.07^{ab}	4.40^{ab}	0.0223	0.80
NSP component sugars								
arabinose	4.29^{a}	3.60^{c}	3.63^{c}	3.70^{bc}	4.11^{ab}	3.81^{abc}	0.0016	0.12
xylose	1.36	1.24	1.28	1.29	1.53	1.35	0.4806	0.12
mannose	0.28^{ab}	0.24^{b}	0.27^{ab}	0.28^{ab}	0.29^{ab}	0.41^{a}	0.0371	0.03
galactose	1.27	1.10	1.15	1.25	1.25	1.32	0.1127	0.07
glucose	6.66^{a}	5.82^{ab}	5.76^{b}	6.52^{ab}	6.24^{ab}	6.55^{ab}	0.0051	0.22
uronic acids	7.21^{a}	5.60^{b}	5.52^{b}	6.39^{a}	7.19^{a}	6.59^{a}	0.0001	0.25
Neutral Detergent Fibre	22.60	23.59	23.39	26.31	23.86	26.12	0.3521	1.71
Acid Detergent Fibre	20.80	19.87	19.46	22.92	19.44	22.32	0.1815	1.49

[1] Plant 1, n=2; Plant 2, n=5; Plant 3, n=5; Plant 4, n=4; Plant 5, n=2; Plant 6, n=2.

[2] Neutral Detergent-Insoluble Crude Protein

$^{a\text{–}c}$ Means followed by different letters within rows are significantly different ($P < 0.05$)

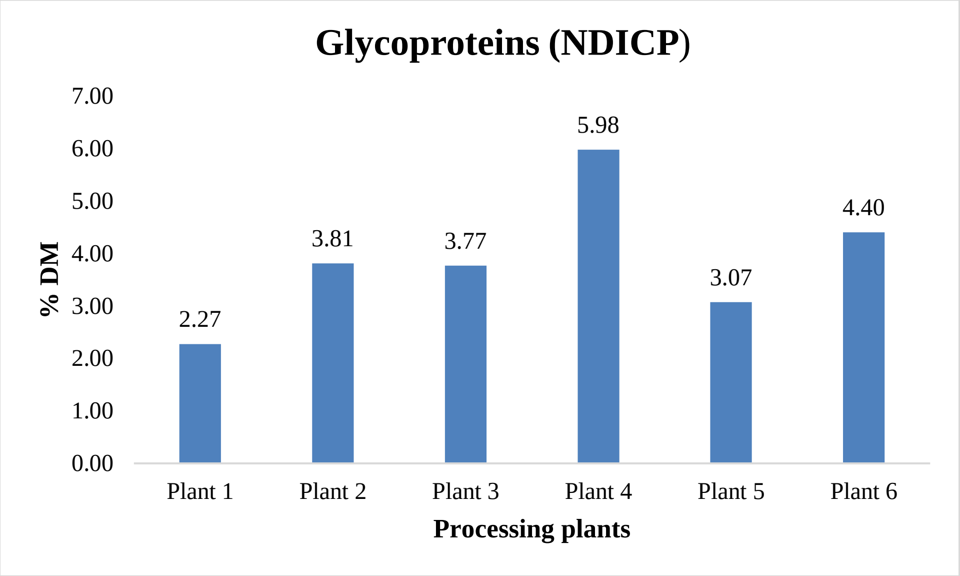
Table 4.5. Variations within the processing plants in values of dry matter, ether extract, crude protein, dietary fiber, ash, and glucosinolates in expeller/cold-pressed canola samples (%, on a DM basis)

Item	Expeller/cold-pressed canola, Plant [1]						*P* value	SEM
	1	2	3	4	5	6		
Dry matter	92.79^{ab}	91.59^{b}	91.60^{b}	95.14^{a}	92.93^{ab}	92.29^{b}	0.0009	0.67
SD[2]	0.23	0.32	0.39	1.08	2.79	0.44		
Ether extract	14.49	20.10	18.27	14.92	12.70	15.28	0.0614	2.15
SD	0.35	2.95	2.44	3.14	5.94	2.56		
Crude protein	36.08	34.54	36.48	37.22	38.49	36.75	0.6638	2.11
SD	0.04	3.17	3.20	1.63	5.94	0.15		
Dietary fiber	32.05	30.51	30.24	34.83	33.14	33.88	0.1461	1.86
SD	0.78	3.64	1.68	1.65	0.61	4.86		
Ash	6.40^{a}	5.56^{b}	5.96^{ab}	6.28^{a}	6.25^{a}	6.29^{a}	0.0015	0.16
SD	0.02	0.33	0.15	0.14	0.28	0.26		
Glucosinolates	15.26^{a}	6.89^{b}	7.18^{b}	6.29^{b}	8.92^{b}	7.77^{b}	0.0001	0.88
SD	0.61	1.25	0.59	1.66	1.09	2.02		

[1] Plant 1, n=2; Plant 2, n=5; Plant 3, n=5; Plant 4, n=4; Plant 5, n=2; Plant 6, n=2.

[2] Standard deviation

[a–c] Means followed by different letters within rows are significantly different ($P < 0.05$)



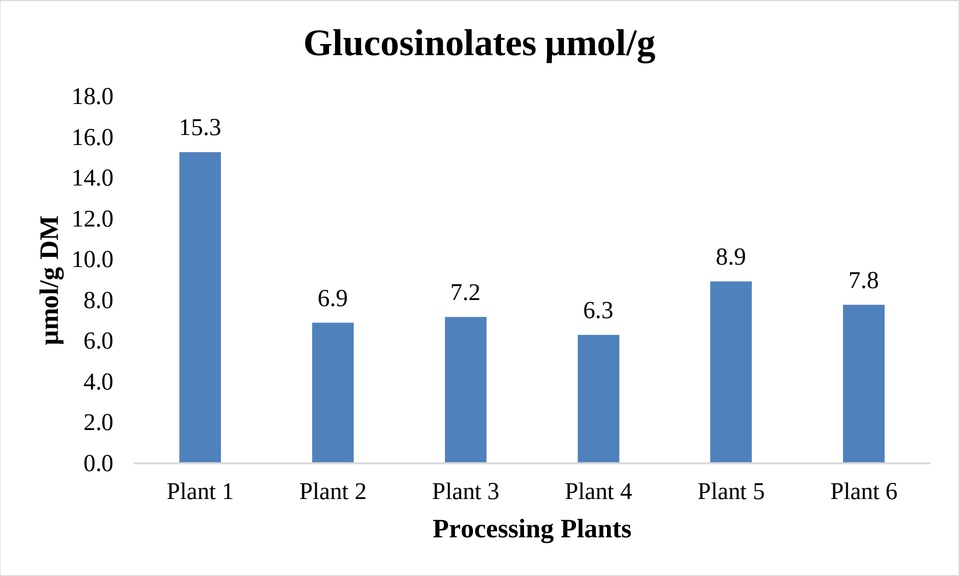


Figure 4.1. Relationship between glucosinolates (GLS) and glycoproteins contents in expeller/cold- pressed canola from six processing plants in Western Canada

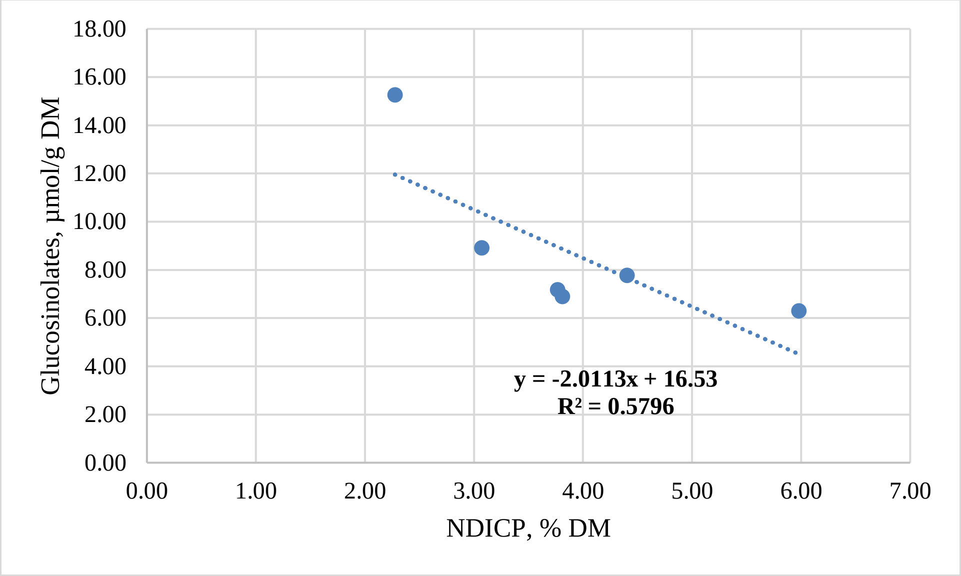


Figure 4.2. Negative relationship between glucosinolates (GLS) and neutral detergent insoluble crude protein (NDICP) contents in expeller/cold-pressed canola among 6 processing plants

4.4.2 Physical Form and Color of ECPC Samples

Figure 4.3 displays the physical appearance and color of the ECPC samples. The visual characteristics can be closely related to the chemical composition of the material. There were distinct differences in both physical form and the color among samples. The ECPC samples from Plant 1 and 7 were received in the form of meal, exhibiting an olive-green hue. Interestingly, the ECPC from Plant 1 contained the highest levels of GLS, while displaying the lowest level of glycoproteins. This observation suggested that the processing of ECPC in Plant 1 might not involve heat in the process. On the other hand, the ECPC from Plant 2 appeared as both a meal and a cake, with a light brown color. Comparative analysis revealed that this ECPC had the highest EE content, but the lowest protein content when compared to the samples from Plants 1, 3, 4, 5, and 6.

Figure 4.3. Physical form and color of expeller/cold-pressed canola (ECPC) and expeller extracted canola meal (EECM) material (in as-received form)

The ECPC obtained from Plant 3 was received as both a meal and a crumb, shared a similar color with the ECPC from Plant 2. As for the ECPC from Plant 4, it was observed as a meal and crumbs, varying in size. A notable distinction of this particular ECPC was its darker brown color, along with the highest levels of DM, NDF, glycoproteins, while showing the lowest content of GLS among the samples from all plants. The ECPC samples sourced from Plant 5 were in the form of the light flakes of various sizes, bearing a color similar to ECPC from Plant 1. The ECPC from Plant 5 had the lowest fat content and the highest crude protein content when compared to other plants. Lastly, the ECPC from Plant 6 arrived in the form of meal and smaller crumbs, showcasing a distinct color combination of gray and green.

4.5 DISCUSSION

The expeller/cold pressing processing method employed for canola seed distinguishes itself from conventional pre-press solvent extraction and expeller extraction techniques. Cold pressing eliminates the need for heat, moisture and the solvent typically used in conventional canola oil extraction process. Expeller extraction, on the other hand, is a one of the steps of the conventional meal extraction, except for the absence of solvent. Although the equipment used in cold expeller processing may vary among processing plants, particularly in smaller production, these presses generally consist of a screw or auger that rotates in a cylindrical chamber to crush and press the canola seed. The length of the screw press can vary from machine to machine. The duration that the meal is in the expeller press depends on several factors, including the throughput capacity of the machine and the specific parameters chosen by the operator. With the longer screw press, the meal stays in the expeller longer and therefore the temperature inside the expeller can increase due to the mechanical friction created during this process. This variability between processing equipment can lead to differences in the chemical composition of ECPC (Spragg and Mailer, 2007). The application of heat can negatively impact protein quality and its availability for animal utilization (Khajali Slominski, 2012; Adewole et al., 2016). Furthermore, during processing, the presence of amino acids and reducing sugars in ECPC, combined with the heat generated, may trigger a Maillard reaction (Theodoridou Yu, 2013).

It is important to recognize that the quality of the seeds used for crushing also may play a significant role in the variability of ECPC samples. The processing plants may receive batches of seeds that vary in quality. Seeds that are off-grade or screenings yield less oil and would have different amount of other nutrients, affecting the overall chemical composition of ECPC.

4.5.1 Chemical Composition of ECPC from Different Processing Plants

The variation in DM content of ECPC observed between different processing plants may be attributed to differences in processing conditions employed by each plant.

Regarding the EE content of ECPC, it aligned with the findings of Seneviratne et al. (2011) and Lee and Woyengo (2018), who reported values of 16.6% and 16.0% on a DM basis, respectively. However, Grageola et al. (2013) and Woyengo et al. (2016) reported higher EE values, 22% and 23.1% on a DM basis, respectively. This difference could be possibly attributed to variations in processing. Expectably, the EE values in conventional CM reported by Adewole at al. (2016) and Niu et al. (2022) were much lower than in ECPC evaluated in this study. This discrepancy could be explained by the fact that conventional CM involves solvent extraction, which removes almost all oil leaving a low residual fat in the meal. The ether extract (EE) content in conventional canola meal (CM) primarily reflects the amount of soap stock reintroduced to the meal during the oil refining process, rather than indicating incomplete oil extraction (Rahmani, 2017). The variations in the levels of EE of ECPC observed among processing plants could be attributed to differences in processing methods, aligning with previous study that indicated similar variations in EE content of CM across different processing plants (Adewole et al., 2016; Rahmani, 2017).

The CP content of ECPC in this study was consistent with earlier findings reported by Agyekum and Woyengo, (2022), who reported a CP content of 35.9% on a DM basis. However, other authors who analyzed chemical composition of ECPC have reported both higher and lower values of CP content compared to the values in this study (Seneviratne et al., 2011; Lee and Woyengo, 2018; Grageola et al., 2013; Woyengo et al., 2016). It is worth noting that the CP in conventional CM reported by Adewole et al. (2016) was higher than in the current study. The variation in CP content of ECPC across different reported studies may be attributed to differences in the oil content of ECPC. The oil can dilute the protein content in the meal. Moreover, the observed differences in CP content of ECPC among processing plants aligned with results reported by Adewole et al. (2016), who also found variation in CP content across CM producing plants. These variations could be attributed to differences in processing techniques employed by each processing plants and the growing conditions of canola (Vera et al., 2007; Adewole et al. 2016).

The variation in the carbohydrates content could be an effect of the growing condition and the seed maturation because sucrose and oligosaccharides content of ECPC varied between processing plants. Simple sugar content in ECPC did not differ statistically It is possible that, during the production of oil as a biofuel, off-grade or underdeveloped seeds are utilized, which could lead to higher sucrose content in the resulting ECPC.

Variations in ash content were observed among different processing plants in the current study, potentially linked to the variation in EE content in ECPC. The average ash content was determined to be 6.1% on a DM basis, which is in agreement with earlier studies reporting values of 6.5% and 6.9 % on a DM basis, as well as similar values of EE in ECPC as observed in the current study (Lee and Woyengo, 2018; Seneviratne et al., 2011). However, the ash content of conventional CM reported by Adewole et al. (2016), Radfar et al. (2017), and Rad-Spice et al. (2018) was higher than the values observed in the current study, with values of 7.5%, 8.3% and 8.5% on a DM basis, respectively.

The average concentration of total P content observed in this study was consistent with the findings reported by Grageola et al. (2013) and Spragg and Mailer, (2007), who reported total P content of 0.98% and 0.91% on a DM basis, respectively, in expeller cold pressed canola. However, other studies have reported slightly higher total P content in expeller cold pressed canola and CM, ranging from 1.1 to 1.4% on a DM basis (Leming and Lember, 2005; Seneviratne et al., 2011; Adewole et al., 2016; Radfar et al., 2017; Niu et al., 2022). While no differences were observed in total P content among processing plants for ECPC, there were variations in the content of phytate P and non-phytate P in ECPC between processing plants. These differences could be attributed to variations in growing conditions or soil type where the canola seeds were cultivated (Bell and Keith, 1991; Adewole et al., 2016; Canola Council of Canada, 2023).

Statistical analysis revealed significant variation ($P<0.05$) in the total GLS content among processing plants, with values ranging from 4.9 to 15.7 µmol/g DM. These values were substantially lower than the maximum acceptable threshold of 30 µmol/g of GLS in canola meal according to the canola definition. ECPC from Plant 1 exhibited the highest GLS content and lowest NDICP content. Samples from Plant 1 presented a canola meal form with olive green color. In contrast, Plant 4 had the lowest GLS content in ECPC and highest NDICP content. It was in the form of roasted crumbs with dark brown color, suggesting possible overheating during processing by this specifying processor (Figure 4.1, 4.2). In the process of cold-pressing extraction, the usual approach involves gently pressing canola seeds. This is done to control friction and avoid raising temperatures excessively. However, the expeller screw presses might be different in length and operates at a various speed setup, therefore it might result in generating different heat level and will expose the expelled material to heat at various duration. A study conducted by Seneviratne et al. (2011) demonstrated that higher screw speed led to increased EE content in cold-pressed canola cake due to reduced crushing time. Such processing differences could contribute to chemical composition variations in ECPC within and between processing plants. The observed results could be attributed to the heat sensitivity of GLS. When subjected to heat treatment, their concentration decreases, indicating potential protein damage, which may contribute to the increase in TDF (Campbell and Slominski, 1990). These findings aligned with the earlier study conducted by Adewole et al. (2016), who also observed similar trends. The average GLS value observed in this study was 7.9 µmol/g DM, which was higher than the values reported for expeller cold pressed canola (6.1 µmol/g), and in CM 4.9 µmol/g and 1.7 µmol/g reported by Grageola et al. (2013), Adewole et al. (2016), and Niu et al. (2022), respectively. However, other studies have reported higher GLS values in ECPC, such as 14.9 µmol/g and 17.5 µmol/g on a DM basis, respectively (Kasprzak et al., 2016; Lee and Woyengo, 2018).

Statistical analysis revealed no significant differences in NDF, ADF, TDF, and lignin with polyphenols among ECPC producers. However, there were numerical variations observed. In contrast, significant differences (<0.05) were found in the contents of NSP and NDICP among processing plants. When compared to the reference EECM sample, the differences were not substantial. These findings suggest the possibility that Maillard reactions may have influenced the results. Surprisingly, this study discovered that that some ECPC were likely subjected to excessive heat, possibly caused by mechanical friction in the expeller screw press, resulting in Maillard reactions, which in turn caused the increased levels of NDF, and NDICP, contributing to the higher

TDF content. These results aligned with those of Adewole et al. (2016), which also reported variations in dietary fibre fractions in CM between different processing plants. Another reasonable explanation could be the quality of the seeds used by the processing plants. Smaller seeds may increase dietary fibre content, while smaller embryo cell size could increase cell-wall polysaccharides, consequently increasing the fibre content in the meal (Slominski, 1997).

4.5.2. Physical Appearance and Color of ECPC Samples

The difference in physical appearance and color of the ECPC samples among processing plants could be attributed to the different levels of residual oil in the ECPC, and possible heat damage which may occurred somehow during processing. Interesting to note that some of the ECPC samples were darker in color and appeared to be roasted. A possible explanation for this could be that somehow heat was released during processing, which triggered the Maillard reactions (van Soest Mason, 1991). Another possible explanation of the difference in color among ECPC could be related to the different levels of the residual oil in the ECPC among processing plants.

4.6 CONCLUSIONS

This study has identified the variations in the chemical composition and physical form and color of ECPC samples between different processing plants in Western Canada. The variations were observed in the contents of DM, CP, sucrose, oligosaccharides, ash, phytate and non-phytate phosphorus, GLS, NDICP, and NSP and some of its component sugars. Given the varying forms in which the ECPC samples were received, an important measure involved grinding them to ensure uniform nutrient distribution. However, this preparatory step introduces an additional cost factor.

It is possible that the high NDICP with correspondingly low GLS content observed in ECPC from some processing plants was caused by overheating. The overheating might arise from increased mechanical friction, particularly linked to longer screw press, and extended duration of the material exposed to generated heat. These conditions can facilitate the formation of Maillard reaction products, ultimately impacting the nutrient composition of the ECPC.

CHAPTER 5

5.0 MANUSCRIPT II

DETERMINATION OF APPARENT METABOLIZABLE ENERGY AND STANDARDIZED ILEAL DIGESTIBLE AMINO ACID CONTENT IN EXPELLER/COLD PRESSED CANOLA FOR BROILER CHICKENS

5.1 ABSTRACT

Two studies were conducted to investigate the impact of different sources of expeller/cold-pressed canola (ECPC) from five processing plants in Western Canada and one expeller extracted canola meal (EECM) on the apparent metabolizable energy corrected for ileal endogenous losses of nitrogen (AMEn) and standardized ileal amino acid digestibility content (SIAAD) contents. A total of 252 birds were housed, with six birds per cage, and divided into seven dietary treatments. Each treatment was replicated six times, and diets were randomly assigned. From day 1 to 14, the birds were fed a conventional corn/soybean starter basal diet. From the day 14 to 19 (AMEn study) and from day 14 to 21 (SIAAD study), the birds were fed test diet that comprising 70% of the basal diet and 30% of the tested ECPC samples. An indigestible marker, chromium oxide, was added at a rate 0.3% in each experimental diet. On day 19, excreta samples were collected for AMEn determination, and on day 21, birds were euthanized by CO_2 asphyxiation, and digesta samples were collected from the terminal ileum for SID AA analysis. The AMEn and standardized ileal digestible AA content of ECPC samples varied between processing plants ($P<0.05$). However, when comparing AMEn and SIAAD (Lys, Arg, Met, Thr, and Cys) of ECPC with those of the EECM, no significant differences were observed. The AMEn content ranged from 2128 to 2604 kcal/kg DM, with an average value of 2386 kcal/kg DM. The average standardized ileal digestible contents of Arg, Lys, Met, and Thr were 1.57%, 1.61%, 0.45%, and 1.17%, respectively.

In conclusion, variations were observed in AMEn and standardized ileal digestible AA contents of ECPC among different processing plants. Regression equations were developed to predict AMEn content from simple measures of EE, CP, ash, and NDF.

Keywords: expeller/cold-pressed canola; AMEn; amino acid digestibility; broiler chickens

5.2 INTRODUCTION

Expeller/cold-pressed canola production is growing in response to the global shift toward more sustainable energy and food production practices. This production method, particularly suitable for small-scale on-farm operations, involves mechanical pressing of canola seeds for oil extraction. Expeller/cold-pressed canola (ECPC) is a co-product obtained from such process. Unlike conventional methods involving solvent extraction, expeller/cold pressing minimizes heat exposure; thereby preserving the quality of the meal but resulting in a higher residual oil content (Spragg and Mailer, 2007). This process ensures that the meal retains its nutritional quality and offers a higher energy content, making it a valuable source of amino acids and energy for poultry. Due to its high levels of methionine and cysteine, meal can be used in poultry diets as an excellent supplement to other sources of protein (Barthet and Daun, 2011).

Accurate determination of the apparent metabolizable energy corrected for nitrogen (AMEn) and standardized ileal amino acid digestibility (SIAAD) in ECPC is crucial for the feed industry, enabling the formulation of nutritionally balanced poultry diets. Despite its significance, there is limited literature available on AMEn and SID of amino acids in ECPC for poultry (Agyekum and Woyengo, 2022; Kasprzak et al., 2016). The objectives of this study were to determine the AMEn and SIAAD of ECPC for broiler chickens and to develop prediction equations for AMEn content of ECPC for broiler chickens based on its analyzed nutrient content.

This research addresses the critical need for comprehensive nutritional information to support optimal diet formulations in poultry production.

5.3 MATERIALS AND METHODS

5.3.1 Expeller/Cold-Pressed Canola Samples

The ECPC samples were supplied by eight crushing facilities across Western Canada. Based on the chemical composition of twenty-two ECPC samples, five ECPC samples differing in the nutritive content were selected from different processing facilities (i.e., ECPC A, B, C, D and E) to evaluate the apparent metabolizable energy content (AME_n) and standardized ileal digestible AA contents for growing chickens. One expeller extracted canola meal (EECM) was used as a reference sample in both studies. The ECPC test products used in this study were received in a variety of forms including meal, crumbs, flakes, and cake. To ensure the consistency, the ECPC test materials were ground through a 2 mm sieve in order to standardize the particle size and proper mixing with the basal diet. Accordingly, the broiler chickens were fed the test material in the same form. Tables 5.1 and 5.2 shows the chemical composition of 5 selected ECPC samples and EECM.

Table 5.1. Chemical composition of Expeller/Cold-Pressed Canola (ECPC) test ingredients used in nitrogen-corrected apparent metabolizable energy (AMEn) and standardized ileal amino acid digestibility (SIAAD) assays (%, on a DM basis)

Item	ECPC					EECM[1]
	A	B	C	D	E	
Dry Matter	94.90	95.15	91.98	92.11	91.93	93.69
Ether Extract	8.49	12.89	13.47	16.01	17.72	12.86
Crude Protein (N x 6.25)	42.69	39.11	36.86	38.64	35.89	39.32
Carbohydrates						
Simple sugars[2]	0.61	1.08	1.51	0.94	1.38	0.08
Sucrose	8.00	3.37	3.71	5.00	3.62	6.53
Oligosaccharides[3]	2.19	1.28	1.45	2.01	1.24	2.47
Dietary Fibre	32.70	37.12	37.32	32.55	36.92	31.38
Ash	6.45	6.36	6.47	6.20	5.83	7.69
Total Phosphorus	1.11	1.03	1.04	1.06	0.97	1.03
Phytate P	0.64	0.55	0.58	0.60	0.58	0.60
Non-Phytate P	0.47	0.48	0.46	0.46	0.38	0.43
GLS, µmol/g[4]	9.69	6.29	6.34	7.37	5.01	6.81

[1] Expeller Extracted Canola Meal used as a reference.

[2] Includes fructose and glucose.

[3] Raffinose Family Oligosaccharides or galactooligosaccharides- includes raffinose and stachyose.

[4] Includes gluconapin, glucobrassicanapin, progoitrin, gluconapoleiferin, gluconasturtin, glucobrassicin, and 4-hydroxyglucobrassicin.

Table 5.2. Dietary fibre components of Expeller/Cold-Pressed Canola (ECPC) test ingredients used in nitrogen-corrected apparent metabolizable energy (AMEn) and standardized ileal amino acid digestibility (SIAAD) assays (%, on a DM basis)

Item	Expeller/cold-pressed canola ECPC					EECM[1]
	A	B	C	D	E	
Dietary Fibre	32.70	37.12	37.32	32.55	36.92	31.38
Non-Starch Polysaccharides	20.44	18.84	20.75	19.10	19.48	19.82
Lignin and polyphenols	9.27	10.65	10.86	8.49	10.99	8.77
Glycoproteins (NDICP)[2]	2.99	7.63	5.70	4.96	6.44	2.79
NSP component sugars						
arabinose	4.25	3.42	3.86	3.93	3.79	4.03
xylose	1.51	1.44	1.49d	1.49	1.35	1.47
mannose	0.30	0.32	0.31	0.31	0.30	0.31
galactose	1.30	1.31	1.39	1.34	1.30	1.37
glucose	6.20	6.27	6.84	6.24	6.37	6.33
uronic acids	6.89	6.08	6.85	5.80	6.38	6.30
Neutral Detergent Fibre	23.08	27.88	29.26	24.85	29.94	21.96
Acid Detergent Fibre	18.35	26.04	25.04	19.50	22.81	18.88

[1] Expeller Extracted Canola Meal used as a reference.

[2] Neutral Detergent-Insoluble Crude Protein

5.3.2 Experimental Diets

During the 14-day pre-experimental period, the birds were fed the basal starter mash diet. On day 1, the birds were individually weighed and then allocated to the assigned cages (with six birds per cage) based on their body weight (BW). Using a completely randomized design, each of the seven test diets was assigned to six replicate cages. The experimental diets, consisting of 70% of the basal diet and 30% of the tested ECPCs, were fed from day 14 to 19 for the apparent metabolizable energy corrected for nitrogen (AMEn) study, and from day 14 to 21 for the standardized ileal amino acid digestibility (SIAAD) study. The corn/CBM basal diet was formulated to meet the nutrient requirements for broiler chickens as specified for Ross 308

(Aviagen, 2019). An indigestible marker, chromium oxide, was added at a rate 0.3% in each experimental diet. The composition of the basal diet is presented in Table 5.3.

Table 5.3. Composition of basal diet used in nitrogen-corrected apparent metabolizable energy (AMEn) and standardized ileal amino acid digestibility assays (%, on as-fed basis)

Ingredient	Basal diet
Corn	54.50
Soybean meal	36.89
Vegetable oil	3.36
Calcium Carbonate (Limestone)	1.54
Biofos	1.30
L-lysine	0.25
DL-Methionine	0.20
Threonine	0.16
Mineral Premix [a]	0.50
Vitamin Premix [b]	1.00
Cr_2O_3	0.30
	100.0
Calculated composition	
Metabolizable energy (Kcal/kg)	3050
Crude protein	22.47
Calcium	0.95
Available phosphorus	0.45
Digestible Lysine	1.28
Digestible Methionine	0.52
Digestible Methionine + cysteine	0.87
Digestible Threonine	0.86
Digestible Arginine	1.28
Analyzed composition (%)	
Crude protein	23.00
AMEn	2863

[a] Provided per kg of diet: 70 mg Mn, 80 mg Zn, 80 mg Fe, 10 mg Cu, 0.3 mg Se, 0.5 mg I, 337 g Na.

[b] Provided per kg of diet: 8250 IU vitamin A, 3000 IU vitamin D3, 30 IU vitamin E, 0.13 mg vitamin B12, 2 mg vitamin K3, 6 mg riboflavin, 40.3 mg niacin, 1301 mg choline, 4 mg folic acid, 0.25 mg biotin.

5.3.3 Birds and Housing

Two-hundred fifty-two 1-day-old as-hatched Ross 308 broiler chickens were purchased from a local hatchery (Carlton Hatchery, Grunthal, Manitoba, Canada). The study was carried out at the Small Animal Research Facility of the Department of Animal Science, University of Manitoba, Canada. Birds were raised in the electrically heated battery housing system (Super Brooders, Alternative Design Manufacturing and Supply, Inc., Siloam Springs, AR, USA) under a controlled environment. The temperature was monitored, daily recorded, and adjusted in response to the comfort of the birds. Birds had free access to water and feed. All husbandry activities involving broiler chickens were conducted in compliance with the guidelines by the Animal Care Council of Canada (2009) and approved protocols by the Animal Care Committee of the University of Manitoba.

5.3.4 Sample Collection and Chemical Analysis

On day 19 of the AMEn study, excreta samples were collected and immediately frozen at -20℃ before undergoing freeze-drying. Duplicate samples of diets and excreta were analyzed for chromium, nitrogen (N), and gross energy (GE). Diets and excreta samples were analyzed for chromium content after the samples were ashed at 600℃ for 12 hours in a muffle oven, using inductively coupled plasma atomic emission spectroscopy (ICP-AES Vista, Varian, USA) following the AOAC (2005, method 985.01) guidelines. The DM content of ECPC and diet samples was analyzed following AOAC 2005 method 930.15, involving the overnight drying of 5.0 g of the sample in an oven at 104℃. Nitrogen analysis was conducted for diets and excreta using the combustion method (AOAC 990.03) with a LECO FP828 nitrogen analyzer (LECO Corp., St. Joseph, MO). Gross energy was measured in diet and excreta samples using a Parr adiabatic oxygen bomb calorimeter (Parr Instrument Co., Moline, IL).

On day 21 of the SIAAD study, the birds were humanely euthanized using CO2 asphyxiation. Digesta were collected from the ileum, specifically from Meckel's diverticulum to a point 4 cm proximal to the ileocecal junction. The digesta was obtained by gently squeezing the contents into sample bags. To ensure representative samples, the digesta from the birds in each cage were pooled, immediately frozen, and subjected to freeze-drying. The dried ileal digesta were stored in tightly sealed plastic bags at room temperature before undergoing chemical analysis. In parallel, duplicate ECPC samples were analyzed for the content of crude protein (CP), ether extract (EE), neutral detergent fibre (NDF), acid detergent fibre (ADF), total phosphorus, phytate phosphorus, ash, sucrose, simple sugars, oligosaccharides, non-starch polysaccharides (NSP), amino acids (AA) and GLS (GLS). Total Dietary Fibre was calculated as described by Slominski and Campbell (1994).

The expeller/cold-pressed canola samples, the test diets, and the digesta samples were finely ground using an IKA Analytical Mill (IKA A11 Basic). Duplicate samples of diets and ileal digesta were analyzed for dry matter (DM), chromium, nitrogen (N), and total amino acids. To determine the DM content, ECPC and diet samples were dried in an oven at 104℃ overnight, following AOAC 2005 method 930.15, using 5.0 g of the sample. Chromium content in diets and digesta samples was analyzed according to the AOAC (2005, method 985.01) guidelines. The ECPC samples, diets, and excreta were analyzed for N using a combustion method, as described earlier. The amino acid content in diets, ileal digesta, and ECPC samples was determined in a single using Method 994.12 (AOAC, 1990) and the procedure outlined by Mills et al. (1989). Briefly, 100 mg of the sample was digested in 4 ml of 6M HCL *in vacuo* for 24 h at 110℃. The mixture was neutralized with 4 mL of 6.25M NaOH and cooled to room temperature. The sodium citrate buffer solution (pH 2.2) was used to dilute the neutralized solution and bring to 50ml volume. Methionine and cysteine were oxidized with performic acid before acid hydrolysis. Hydrolysates were then subjected to analysis using AA analyzer S4300 (Sykam GmbH, Eresing, Germany).

Ether extract in ECPC samples was determined by hexane extraction (Method 920.39, AOAC 2005) in Ankom Extraction System (Macedon, NY, USA). Ash and total phosphorus were determined following the AOAC (2005) Method 942.05 and 965.17, respectively. Phytate phosphorus content was measured according to the method published by Haug and Lantzsch (1983). Non-phytate phosphorus was calculated by subtraction of the phytate phosphorus from the total phosphorus content. Acid Detergent Fibre (ADF) and Neutral Detergent Fibre (NDF) were determined using the ANCOM Fibre Analyzer (Ankom Technology, Macedon, NY, USA) and AOAC procedures 973.18 and 2002.04, respectively. The NDF residue was collected and analyzed for N and NSP. Simple sugars (glucose and fructose) sucrose, and raffinose family oligosaccharides (raffinose and stachyose) were measured by gas-liquid chromatography using 2% OV-7 column and Varian 430GC (Agilent Technology, Canada) as described by Slominski et al. (1994). Non-starch polysaccharides were determined by gas-liquid chromatography (component sugars) using SP-2340 column and Varian CP-3380 GC (Agilent Technology, Canada) and by colorimetry (uronic acids) using Biochrom Ultrospec 50 (Biochrom Ltd., UK) according to the procedure described by Englyst and Cummings (1984) and Englyst and Cummings (1988) with modifications (Slominski and Campbell, 1990). In brief, a 100-mg sample was treated with dimethylsulphoxide and incubated overnight at 45°C with a solution of starch-degrading enzymes composed of amylase and amyloglucosidase (Sigma, St. Louis, MO). Ethanol was then added, the mixture left for 1 h, centrifuged and the supernatant discarded. The dry residue was dissolved in 1 mL of 12M sulfuric acid (H_2SO_4) and incubated for 1 h at 35°C. Six mL of water and 5 mL of myo-inositol (internal standard) solution were then added, and the mixture was boiled for 2 h. One mL of the hydrolysate was then taken and neutralized with 12 M ammonium hydroxide, reduced with sodium borohydride, and acetylated with acetic anhydride in the presence of 1-methylimidazole.

Total dietary fibre (TDF) was determined by a combination of NDF and neutral detergent soluble NSP measurements to address the high solubility of NSP in the neutral detergent solution that causes a loss of NSP on NDF assay. Total dietary fibre was calculated as the sum of NDF and ND-soluble NSP (Slominski et al., 1994). Neutral detergent soluble NSP were calculated as total sample NSP minus NSP present in the NDF residue. Neutral detergent insoluble crude protein (NDICP, glycoprotein) represented the amount of crude protein present in the NDF residue. The value for lignin and associated polyphenols was calculated by difference [NDF – (NSP +NDICP)].

Glucosinolate analysis was performed as described by Slominski and Campbell (1987). Briefly, 300 mg of canola meal samples were weighed into 15 mL centrifuge tubes. Two milliliters of methanol, 1.0 mL of sinigrin (internal standards, 0.5 mM), and 0.1 mL of lead–barium acetate were added to the tubes, extracted for 3 h at room temperature, and then centrifuged for 10 min at 3000 RPM. Two mL of supernatant were transferred to a DEAE-Sephadex column, which was washed successively with 1 mL of each of 67% methanol, water, and pyridine acetate. Purified sulfatase solution was then added to the column, and the contents were incubated at room temperature overnight. The resulting desulfated GLS were eluted with 4 mL of 60% methanol and dried. The dry residue was trimethylsilylated by adding 0.2 mL of a mixture of anhydrous acetone/N,O-bis (trimethylsilyl) acetamide (BSA)/trimethylchlorosilane (TMCS)/1-methylimidazole (2:1:0.1:0.05 v/v) and incubated for 30 min at room temperature. The trimethylsilyl derivatives of desulfoGLS were separated by gas−liquid chromatography using a glass column packed with 2% OV-7 and Varian 430GC (Agilent Technology, Canada).

5.3.5 Calculation

The Apparent Metabolizable Energy corrected for nitrogen (N) (AME_n) value was calculated using the formulas below.

- $\mathrm{ME_{basal\ diet}} = \mathrm{GE_{Diet}} - \left(\frac{\mathrm{GE_{Excreta}}}{\mathrm{g_{\ Diet}}} + \mathrm{N_{Correction}}\right)$
- $\mathrm{ME_{test\ diet}} = \mathrm{GE_{Diet}} - \left(\frac{\mathrm{GE_{Excreta}}}{\mathrm{g_{\ Diet}}} + \mathrm{N_{Correction}}\right)$

Where,

ME (Kcal/kg) – Metabolizable energy content of the diet;

$\mathrm{GE_{Diet}}$ – Gross energy of the diet (Kcal/kg);

$\mathrm{GE_{Excreta}}$- Gross energy of excreta (Kcal/kg);

$\mathrm{N_{Correction}}$=$\mathrm{N_{retained}}$ x 8.22

Calculation of retained nitrogen:

- $\mathrm{N_{retained}} = \mathrm{N_{Diet}} - (\mathrm{N_{Excreta}} \times \mathrm{Cr_{Diet}} \div \mathrm{Cr_{Excreta}})$

Where,

$\mathrm{N_{Diet}}$ (%) - N content of the diet;

$\mathrm{N_{Excreta}}$ (%) - N content of the excreta;

$\mathrm{Cr_{Diet}}$ (mg/g) - Chromium oxide concentration in the diet;

$\mathrm{Cr_{Excreta}}$ (mg/g) - Chromium oxide concentration in the excreta.

The AME_n of the test ingredient was calculated as shown in the following formula:

- $\mathrm{AME_{n\ test\ ingredient}} = \mathrm{ME_{Basal\ diet}} - \left[\left((\mathrm{ME_{Basal\ diet}} - \mathrm{ME_{Test\ diet}}) \div 0.3\right)\right]$

The SIAAD value was calculated using the formulas below.

$$\mathrm{AIAAD\%} = (1 - \frac{\mathrm{Cr2O3\%_{diet}} \div \mathrm{Cr2O3\%_{ileal\ digesta}}}{\mathrm{AA\%_{diet}} \div \mathrm{AA\%_{ileal\ digesta}}}) \times 100$$

$$\mathrm{SIAAD}\ _{\mathrm{diet}} = \mathrm{AIAAD} + (\mathrm{basal\ IAA_{end}} \div \mathrm{AA_{diet}}) \times 100$$

Where, basal $\mathrm{IAA_{end}}$ refers to basal ileal endogenous AA loss, which were determined previously with nitrogen-free diet in our laboratory (Table 5.4).

$$\mathrm{SIAAD}_{ingredients\ (\%)} = \frac{\mathrm{SIAAD_{test\ diet}} \times \mathrm{AA_{test\ diet}} - 0.7 \times \mathrm{SIAAD_{basal\ diet}} \times \mathrm{AA_{basal\ diet}}}{0.3 \times \mathrm{AA_{ingrediets}}}$$

To calculate the amount of standardized digestible AA present, the standardized digestibility values were multiplied by the respective AA content.

Table 5.4. Ileal endogenous losses in broiler chickens determined in earlier studies in our laboratory[1]

Amino acid	% of DM intake	mg/kg of DM intake
Indispensable AA		
Arginine	0.015	150
Histidine	0.008	80
Isoleucine	0.012	120
Leucine	0.021	210
Lysine	0.010	100
Methionine	0.005	50
Cysteine	0.014	140
Phenylalanine	0.012	120
Threonine	0.035	350
Valine	0.018	180
Dispensable AA		
Alanine	0.017	170
Aspartic acid	0.031	310
Glutamic acid	0.040	400
Glycine	0.020	200
Proline	0.027	270
Serine	0.031	310
Tyrosine	0.098	980

[1] Ileal endogenous amino acid losses values are from birds fed N-free diet. It was composed of cornstarch and sucrose (50/50 weight/weight), cellulose, vegetable oil, calcium carbonate, di-calcium phosphate, potassium carbonate, mineral and vitamin premix, NaCl, chromium oxide as a marker. Electrolytes were balanced. Birds were fed the N-free diet for 72 hours.

5.3.6 Statistical Analysis

This experiment was Completely Randomized design, PROC GLM of SAS 9.4 was used for mean comparison, Tukey's test was used for the adjustment of multiple comparisons, the P-value<0.05 was set significant. The t-test was used to compare the means of AMEn, Lys, Arg, Meth, Thr, and Cys in ECPC and EECM. The PROC REG procedure was used to determine prediction equations of AMEn in ECPC. All variables were assessed, and a model simplification process was performed, considering all the variables, ultimately resulting in the identification of the best model.

5.4 RESULTS

The analyzed chemical composition of the ECPC samples is presented in Tables 5.1 and 5.2. The chemical composition of the ECPC samples used in both studies varied, with EE content ranging from 8.49 to 17.72% DM, CP from 35.89 to 42.69% DM, NDF from 23.08 to 29.94% DM, ADF from 18.35 to 26.04% DM, TDF from 32.55 to 37.32% DM, NSP from 18.84 to 20. 75% DM, lignin and polyphenols from 8.49 to 10.99% DM, NDICP from 2.99 to 7.63% DM, sucrose from 3.37 to 8.00% DM, simple sugars from 0.61 to 1.51% DM, GLS from 5.09 to 9.69 µmol/g, ash from 5.83 to 6.47% DM and total P from 0.97 to 1.11% DM. This illustrates that samples with distinct chemical properties were selected for the AMEn and SIAAD evaluation, thereby enabling a more comprehensive understanding of the relationship between the chemical composition and the nutritive value of ECPC.

5.4.1 Nitrogen-Corrected Apparent Metabolizable Energy (AMEn)

As shown in Table 5.5, significant differences ($P = 0.0021$) were observed in the AMEn content of ECPC samples. The AMEn content ranged from 2128 to 2604 kcal/kg DM, with an average of 2386 kcal/kg DM. Notably, ECPC A exhibited a significantly lower ($P<0.05$) AMEn content of 2128 kcal/kg compared to the other samples.

Table 5.5. Apparent metabolizable energy content (AMEn) of expeller/cold-pressed canola for broiler chickens (Kcal/kg, DM)

Item	AMEn
ECPC A	2128^{b}
ECPC B	2328^{ab}
ECPC C	2326^{ab}
ECPC D	2545^{a}
ECPC E	2604^{a}
Mean	2386
SEM[1]	75.78
P-value	0.0021

[1] Standard error of the mean.

[2] P-value of different crushing plants.

[a–b] Means followed by different letters within column are significantly different ($P < 0.05$)

Table 5.6 provides a comparison between the AMEn values of ECPC and EECM samples. When ECPC samples were compared to the EECM reference sample, no statistical differences were observed in AMEn contents.

Table 5.6. Comparison of apparent metabolizable energy (AMEn) of the expeller/cold-pressed canola (ECPC) with expeller extracted canola meal (EECM) in apparent metabolizable energy (kcal/kg, on a DM basis)

Item	AMEn
ECPC	2386
EECM	2444
SEM[1]	101.4
P-value[2]	0.4733

[1] Standard error of the mean.

[2] P-value of different crushing plants.

The relationships between AMEn and potential metabolizable energy contributors, i.e., EE, NDF, TDF, CP, and sucrose is illustrated in Figures 5.1, 5.2, 5.3, 5.4, and 5.5, respectively. As presented in Figure 5.1, results confirmed that EE and AMEn are strongly positively correlated (R^2=0.97, $P<0.05$). Some positive correlation was observed between AMEn and NDF (Figure 5.2), however it was not strong and not significant (R^2=0.25, P=0.39). No correlation was observed between AMEn and TDF (Figure 5.3). Crude protein was strongly but not significantly negatively correlated with AMEn (Figure 5.4), while sucrose was also negatively correlated with AMEn, but the relationship was not strong (R^2=0.37, P=0.27).

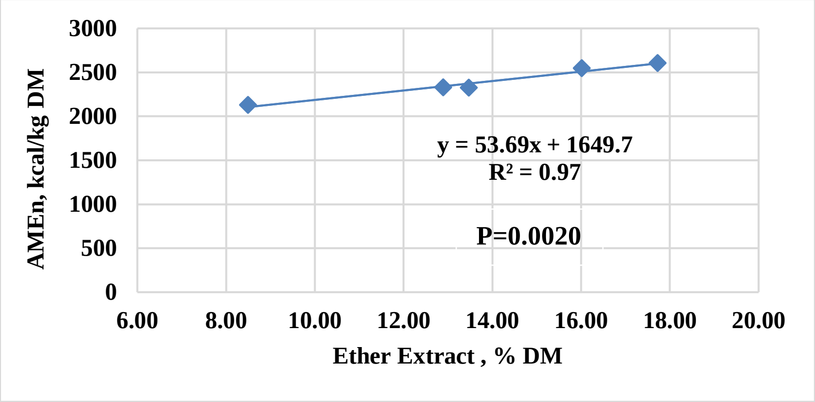


Figure 5.1. Relationship between apparent metabolizable energy (AMEn) and ether extract content of expeller/cold-pressed canola

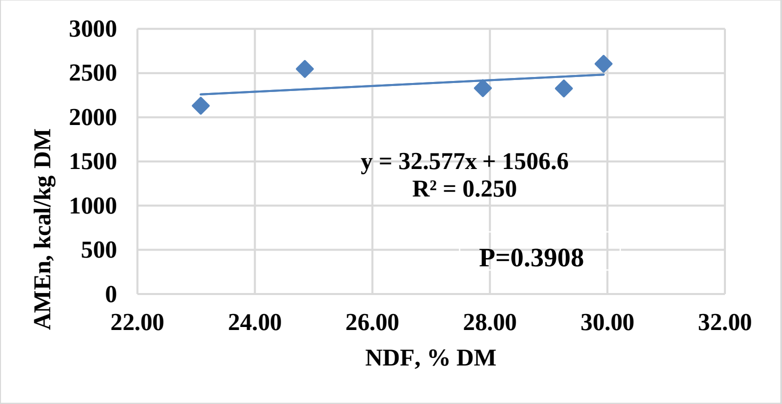


Figure 5.2. Relationship between apparent metabolizable energy (AMEn) and neutral detergent fiber content of expeller/cold-pressed canola

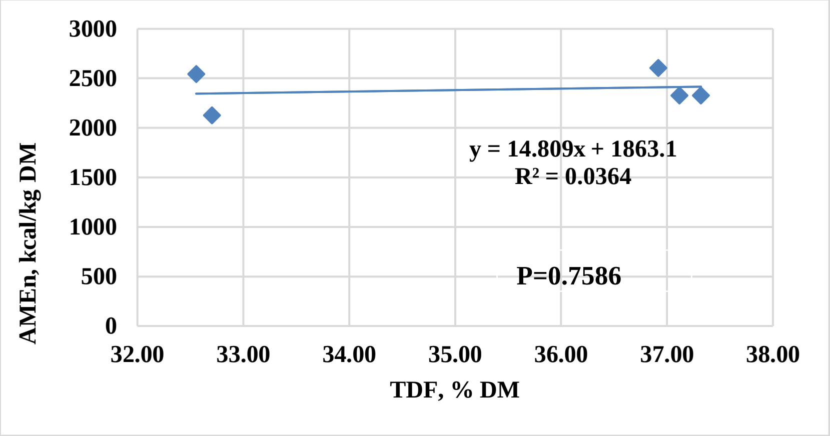


Figure 5.3. Relationship between apparent metabolizable energy (AMEn) and total dietary fiber content of expeller/cold-pressed canola

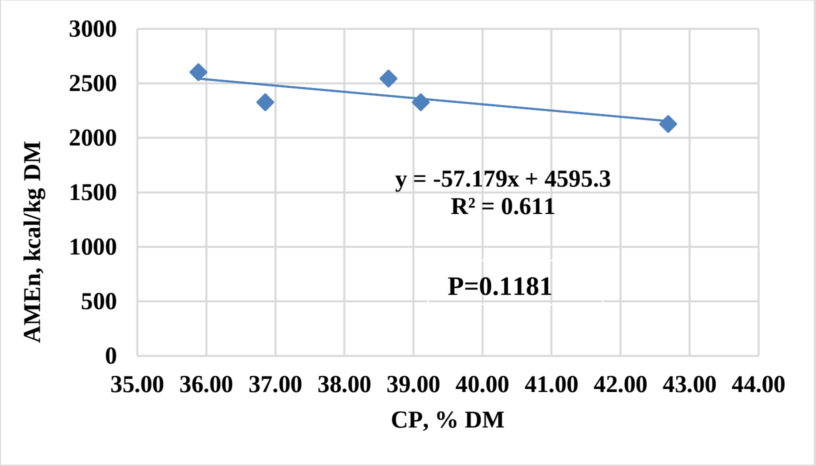


Figure 5.4. Relationship between apparent metabolizable energy (AMEn) and crude protein content of expeller/cold-pressed canola

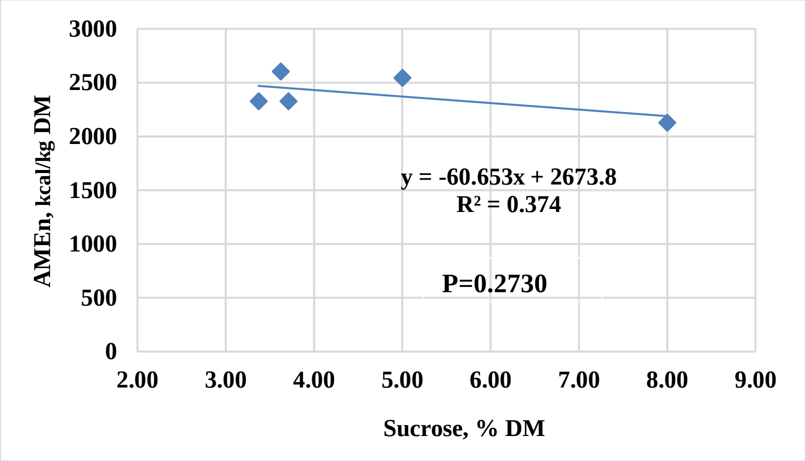


Figure 5.5. Relationship between apparent metabolizable energy (AMEn) and sucrose content of expeller/cold-pressed canola

The linear regression equations that were developed to predict AMEn values using the proximate measurements of EE, CP, Ash, NDF, and ADF are presented in Table 5.7. The regression equations, including those based on the contents of EE and CP, EE, EE, CP, and ash, and NDF, EE, and CP were found to be statistically significant and demonstrated excellent R^2 values ranging from 0.9379 to 0.9999.

Table.5.7. Prediction equation for estimating the apparent metabolizable energy (AMEn) contents of expeller/cold-pressed canola for broiler chickens from using the EE, CP, ash and NDF, ADF values (n=5)

	Equation	SEM[1]	Adj R-Sq[2]	R^2	P value[3]
1	AMEn=406.92+68.89EE+26.00CP	4.77	0.9979	0.9989	0.0011
2	AMEn=1620.47+47.19EE	7.01	0.9172	0.9379	0.0067
3	AMEn=655.38+65.67EE+25.70CP-33.67Ash	13.37	0.9996	0.9999	0.0128
4	AMEn=395.24+0.18NDF+68.95EE+26.17CP	11.07	0.9957	0.9989	0.0416
5	AMEn=2021.58+42.12EE-57.60Ash	203.44	0.8816	0.9408	0.0592
6	AMEn=1877.80+54.49EE-18.38NDF+5.38ADF	61.61	0.8640	0.9660	0.2334
7	AMEn=1946.18+50.90EE – 11.15NDF-15.86Ash	248.83	0.8547	0.9637	0.2412

[1] Standard error of the mean.

[2] Adjusted R square.

[3] P-value for prediction equation.

As is presented in Table 5.8, the analyzed AMEn values of five ECPC samples closely aligned with the predicted values obtained from developed prediction equations.

Table 5.8. Comparison apparent metabolizable energy (AMEn) analyzed vs predicted (kcal/kg, on as-is basis)

Sample ID	Analyzed	Predicted			
		Equation 1[1]	Equation 2[2]	Equation 3[3]	Equation 4[4]
ECPC A	2019.2	2015.6	2000.9	2019.8	2015.2
ECPC B	2214.8	2219.2	2199.2	2213.3	2219.4
ECPC C	2139.1	2141.8	2205.1	2139.9	2141.5
ECPC D	2344.3	2348.3	2316.5	2346.4	2347.7
ECPC E	2393.4	2386.9	2389.2	2392.6	2386.8

[1] In equation 1 contents of EE and CP were used to predict AMEn: AMEn=406.92+68.89EE+26.00CP

[2] In equation 2 contents of EE were used to predict AMEn: AMEn =1620.47+47.19EE

[3] In equation 3 contents of EE, CP, and ash were used to predict AMEn: AMEn=655.38+65.67EE+25.70CP-33.67Ash

[3] In equation 4 contents of NDF, EE, and CP were used to predict AMEn: AMEn=395.24+0.18NDF+68.95EE+26.17CP

5.4.2 Standardized Ileal Amino Acid Digestibility (SIAAD) Value, and Total and Digestible AA contents of ECPC.

The results of the total analyzed amino acid content in the five ECPC are shown in Table 5.9. Due to analysis of single samples, statistical evaluation was not performed. However, numerical differences in AA content were observed among ECPC samples, which align with the corresponding CP content. It is a clear indication of the correlation between CP and amino acids, as they are the building blocks of the protein. The ECPC A exhibited the highest total content of both the indispensable and dispensable amino acids, while ECPC E had the lowest total AA content, except for Val, which was the lowest in ECPC B.

Table 5.9. Crude protein and total amino acids (AA) content in expeller/cold-pressed canola (ECPC) for broiler chickens (%, on a DM basis)

Item	Expeller/cold-pressed canola (ECPC)				
	A	B	C	D	E
Crude Protein (N x 6.25)	42.69	39.11	36.86	38.64	35.89
Indispensable AA					
Arginine	2.45	1.58	1.72	1.76	1.34
Histidine	1.13	0.84	0.86	0.87	0.66
Isoleucine	1.83	1.25	1.35	1.36	1.12
Leucine	3.06	2.48	2.47	2.51	2.02
Lysine	2.53	1.62	1.83	1.98	1.48
Methionine	0.53	0.49	0.47	0.51	0.39
Cysteine	0.97	0.85	0.78	0.89	0.66
Phenylalanine	1.76	1.47	1.43	1.43	1.14
Threonine	1.65	1.48	1.45	1.45	1.21
Valine	2.28	1.59	1.72	1.73	1.64
Dispensable AA					
Alanine	1.83	1.60	1.55	1.60	1.28
Aspartic acid	3.22	2.52	2.52	2.60	2.13
Glutamic acid	8.16	6.48	6.36	6.72	5.20
Glycine	2.29	1.84	1.83	1.83	1.48
Proline	2.68	2.22	2.17	2.22	1.77
Serine	1.56	1.54	1.45	1.47	1.19
Tyrosine	1.12	0.92	0.92	0.90	0.86
Total AA	39.05	30.76	30.88	31.84	25.60

The SID of AA in ECPC fed to broiler chickens is shown in Table 5.10. There were differences ($P<0.05$) among ECPC samples, except for threonine. Among evaluated samples, ECPC E displayed the highest ($P<0.05$) digestibility values for His, Ile, Leu, Phe, Val, Gly, Pro, and Tyr. On the other hand, ECPC A exhibited the highest digestibility values for Arg, Cys, Asp, and Glu. Serine demonstrated the highest digestibility in ECPC C, while Lys, Met, and Ala in ECPC D were digested most efficiently. Interestingly, ECPC B had the lowest digestibility for all AA, except for Ser, which was the lowest for ECPC E.

Table 5.10. Standardized ileal digestibility of amino acids in expeller/cold-pressed canola fed to broiler chickens (%)

Amino Acid	Expeller/cold-pressed canola					Mean	SEM[1]	P-value[2]
	A	B	C	D	E			
Indispensable								
Arginine	94.08^{a}	81.09^{b}	85.96^{ab}	89.03^{ab}	90.55^{ab}	88.14	2.45	0.0119
Histidine	84.87^{a}	68.22^{b}	77.61^{a}	81.46^{a}	85.15^{a}	79.46	2.15	0.0001
Isoleucine	76.92^{a}	59.00^{b}	69.71^{ab}	76.85^{a}	81.89^{a}	72.87	2.98	0.0001
Leucine	82.94^{a}	73.05^{b}	80.17^{ab}	83.55^{a}	84.96^{a}	80.93	2.36	0.0119
Lysine	85.94^{bc}	78.76^{c}	81.71^{bc}	91.75^{a}	87.70^{ab}	85.17	2.15	0.0024
Methionine	94.96^{a}	85.31^{b}	94.35^{a}	97.18^{a}	95.72^{a}	93.50	2.01	0.0027
Cysteine	87.44^{a}	67.62^{c}	73.57^{bc}	82.78^{ab}	81.90^{ab}	78.66	2.35	0.0001
Phenylalanine	85.05^{ab}	76.27^{b}	84.86^{ab}	86.86^{a}	89.09^{a}	84.43	2.24	0.0056
Threonine	82.24	77.75	82.47	80.85	78.85	80.43	2.36	0.5539
Valine	91.14^{a}	85.09^{b}	91.15^{a}	89.49^{ab}	93.52^{a}	90.08	1.19	0.0020
Dispensable								
Alanine	83.54^{ab}	75.62^{b}	83.15^{ab}	87.39^{a}	86.78^{ab}	83.30	2.11	0.0045
Aspartic acid	86.87^{a}	72.61^{b}	77.78^{ab}	84.33^{a}	83.53ab	81.02	2.71	0.0069
Glutamic acid	90.90^{a}	78.82^{b}	84.35^{ab}	88.24^{a}	86.62^{a}	85.79	1.59	0.0002
Glycine	74.43^{ab}	66.00^{b}	76.70^{a}	81.48^{a}	82.57^{a}	76.23	2.07	0.0001
Proline	75.44^{a}	64.53^{b}	71.89^{ab}	75.15^{a}	75.95^{a}	72.59	2.11	0.0037
Serine	88.00^{ab}	89.88^{ab}	92.48^{a}	87.00^{ab}	80.34^{b}	87.54	2.64	0.0395
Tyrosine	89.56^{ab}	83.36^{b}	93.02^{a}	89.46^{ab}	97.80^{a}	90.64	2.28	0.0091

[1] Standard error of the mean.

[2] P-value of different crushing plants.

[a–b] Means followed by different letters within columns are significantly different ($P < 0.05$)

The comparison of the standardized ileal digestibility value of Lys, Arg, Met, Thr, and Cys in five ECPC samples from different processing plants with EECM used as a reference is presented in Table 5.11. There was no significant difference between ECPC and EECM in the above AA.

Table.5.11. Comparison of the expeller/cold-pressed canola (ECPC) with expeller extracted canola meal (EECM) in standardized ileal digestibility of lysine, arginine, methionine, threonine, and cysteine (%, on a DM basis)

Amino Acid	ECPC	EECM	SEM[1]	P value
Lysine	85.17	89.50	2.93	0.2708
Arginine	88.14	94.82	2.7	0.0791
Methionine	93.50	99.70	2.99	0.1090
Threonine	80.43	82.56	1.28	0.4337
Cysteine	78.66	84.25	6.67	0.5370

[1] Standard error of the mean.

Standardized ileal digestible content of AA is presented in Table 5.12. Significant differences were observed among samples obtained from different processors in the content of digestible AA. The ECPC A exhibited the highest content of digestible AA for most of the AA, except for Ser, which was the highest in the ECPC B. Conversely, ECPC E had the lowest content of digestible AA for most of the AA, except for Ile, Lys, Val, Gly, and Tyr, which was lowest in ECPC B.

Table 5.12. Digestible amino acid content of expeller/cold-pressed canola (%, on a DM basis)

Amino Acid	Expeller/cold-pressed canola					Mean	SEM[1]	P-value
	A	B	C	D	E			
Indispensable AA								
Arginine	2.31^{a}	1.28^{c}	1.48^{b}	1.57^{b}	1.22^{c}	1.57	0.04	0.0001
Histidine	0.96^{a}	0.58^{c}	0.66^{b}	0.71^{b}	0.56^{c}	0.69	0.02	0.0001
Isoleucine	1.40^{a}	0.74^{c}	0.94^{b}	1.05^{b}	0.92^{b}	1.01	0.04	0.0001
Leucine	2.54^{a}	1.81^{cd}	1.98^{bc}	2.10^{b}	1.72^{d}	2.03	0.06	0.0001
Lysine	2.18^{a}	1.28^{d}	1.49^{c}	1.82^{b}	1.30^{d}	1.61	0.04	0.0001
Methionine	0.50^{a}	0.42^{bc}	0.44^{b}	0.50^{a}	0.38^{c}	0.45	0.01	0.0001
Cysteine	0.85^{a}	0.57^{c}	0.58^{c}	0.73^{b}	0.54^{c}	0.65	0.02	0.0001
Phenylalanine	1.50^{a}	1.12^{bc}	1.21^{b}	1.24^{b}	1.02^{c}	1.22	0.03	0.0001
Threonine	1.36^{a}	1.15^{b}	1.20^{b}	1.18^{b}	0.95^{c}	1.17	0.04	0.0001
Valine	2.08^{a}	1.35^{c}	1.57^{b}	1.55^{b}	1.54^{b}	1.62	0.02	0.0001
Dispensable AA								
Alanine	1.52^{a}	1.21^{cd}	1.29^{bc}	1.39^{ab}	1.12^{d}	1.31	0.03	0.0001
Aspartic acid	2.80^{a}	1.83^{c}	1.96^{bc}	2.20^{b}	1.78^{c}	2.11	0.07	0.0001
Glutamic acid	7.42^{a}	5.11^{c}	5.36^{c}	5.93^{b}	4.51^{d}	5.67	0.11	0.0001
Glycine	1.70^{a}	1.22^{c}	1.40^{b}	1.49^{b}	1.23^{c}	1.41	0.04	0.0001
Proline	2.02^{a}	$1.43\mathrm{d}^{c}$	1.56^{bc}	1.67^{b}	1.34^{d}	1.60	0.05	0.0001
Serine	1.37^{a}	1.38^{a}	1.34^{a}	1.28^{a}	0.95^{b}	1.27	0.04	0.0001
Tyrosine	1.01^{a}	0.76^{b}	0.85^{b}	0.80^{b}	0.84^{b}	0.85	0.02	0.0001
Total AA	33.51	23.23	25.33	27.20	21.91	-	-	-

[1] Standard error of the mean.

[a–b] Means followed by different letters within columns are significantly different ($P < 0.05$)

Figure 5.6 illustrates the relationship between digestible Lys and GLS, as well as digestible Lys and NDICP in the ECPC samples obtained from different processing plants. To assess the correlation between the digestible Lys and GLS, and between digestible Lys and NDICP, a linear regression analysis was conducted. The analysis revealed a significant positive correlation (R^2=0.89, P=0.015) between digestible Lys and GLS. This indicated that an increase in GLS levels coincided with an increase in digestible Lys content. A significant negative correlation was observed between digestible Lys and NDICP (R^2=0.93, P=0.008). This suggested that as the level of NDICP rose the level of digestible Lys decreased. These results indicate that higher NDICP levels were associated with lower levels of digestible Lys, while higher GLS levels, which indicated mild heat treatment, were related to the impact on the digestible Lys content, thus better quality of CP. Figure 5.6 also displays images of ECPC. The ECPC B and E exhibited the lowest contents of digestible Lys and GLS, while displaying the highest NDICP content. In addition, the images in Figure 5.6, shows the toasted, dark-brown color in both ECPC samples. Figures 5.7 and 5.8 show the negative relationship between GLS and NDICP with (R^2=0.74, P=0.061) in ECPC among 5 processing plants.

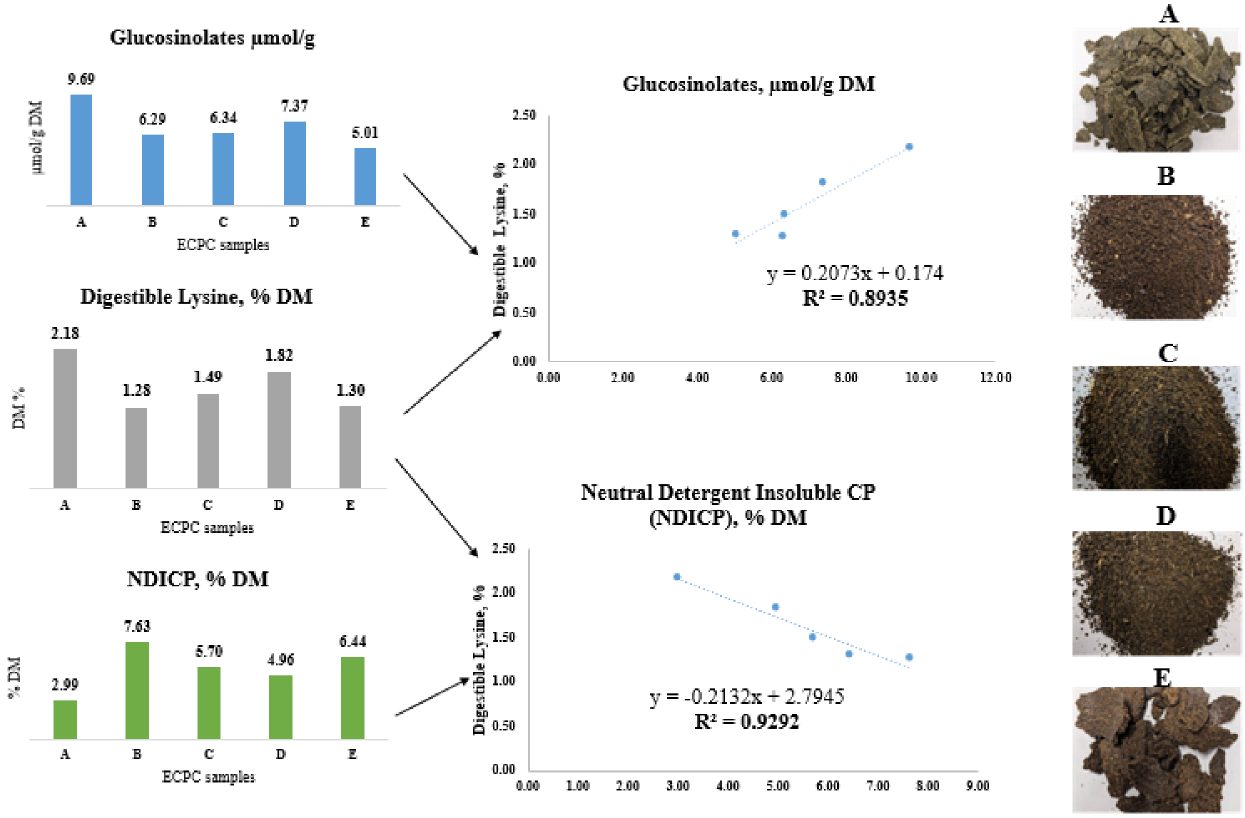


Figure 5.6. Relationship between the digestible lysine and glucosinolates (GLS) content, and between digestible lysine and neutral detergent insoluble crude protein (NDICP) in expeller/cold-pressed (ECPC) samples from different processing plants

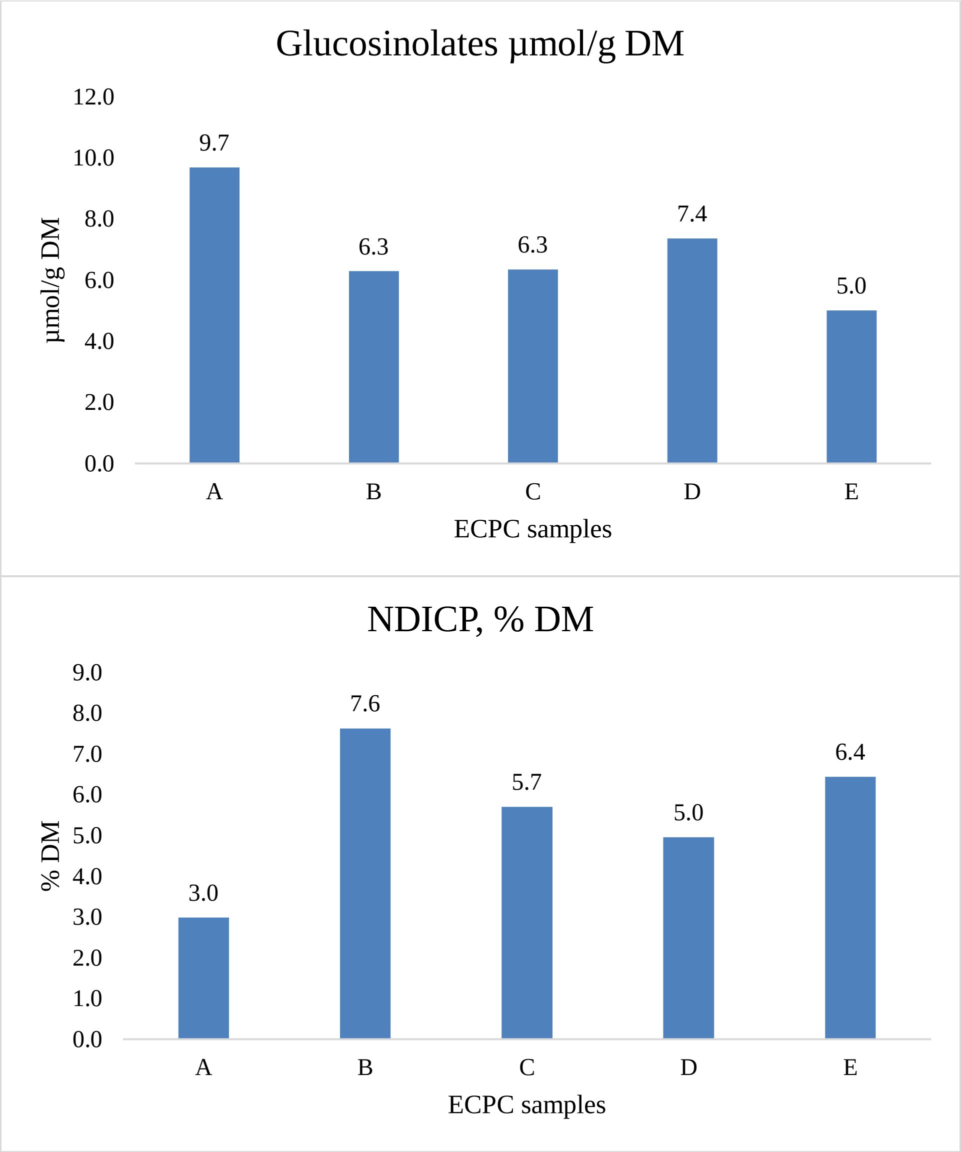


Figure 5.7. Relationship between glucosinolates (GLS) and neutral detergent insoluble crude protein (NDICP) contents in expeller cold pressed canola from five processing plants in Western Canada

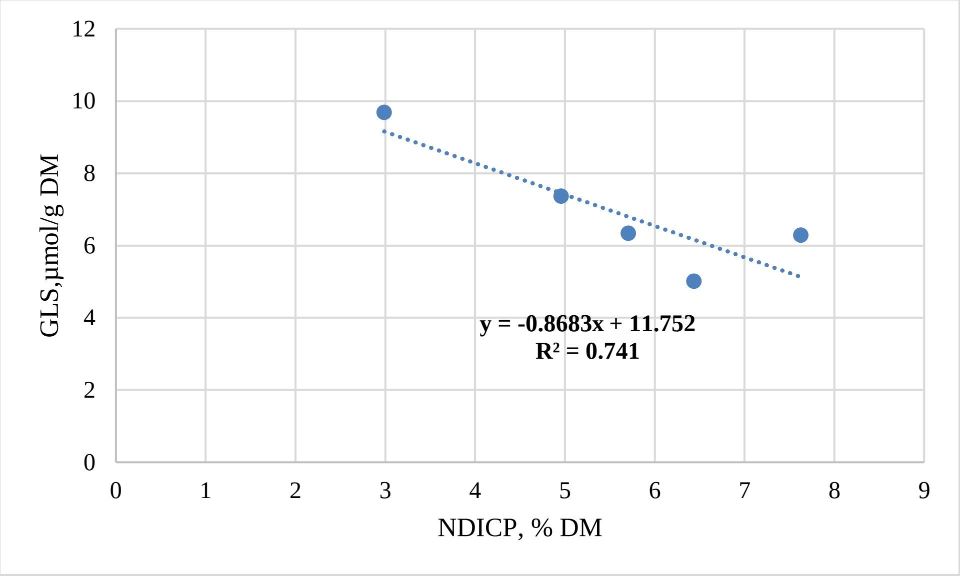


Figure 5.8. Negative relationship between glucosinolates (GLS) and neutral detergent insoluble crude protein (NDICP) contents of ECPC among 5 processing plants

The varying residual fat content in ECPC samples could potentially mask the fiber component content due to the dilution effect. To address this, alongside moisture correction, the results were standardized and calculated on a fat-free basis. Table 5.13 shows the comparison of NDICP, % DM vs. NDICP on moisture- and fat-free basis, digestible Lys, % DM vs. digestible Lys on moisture and fat-free basis, and GLS, % DM vs. GLS on moisture- and fat-free basis between ECPC samples. There were significant differences ($P<0.05$) among samples in NDICP, digestible Lys, and GLS content of ECPC on both DM and on moisture- and fat-free basis (Table 5.13). The differences in the NDICP content observed between ECPC samples on DM basis and fat-free basis were consistent. On DM basis, the digestible Lys contents in ECPC C and E were significantly different, however, when standardized for moisture- and fat content, no significant differences were found. Similarly, the GLS content in ECPC A and D differed on a DM basis, but not when additionally standardized for fat content.

Table 5.13. Comparison of digestible lysine, neutral detergent insoluble crude protein (NDICP), glucosinolates (GLS) on DM vs. digestible lysine, neutral detergent insoluble crude protein (NDICP), and glucosinolates (GLS) on DM fat free basis

Item	NDICP DM	NDICP fat free DM	Digestible Lys DM	Digestible Lys fat free	GLS DM	GLS fat free DM
ECPC A	2.99^{e}	3.27^{e}	2.18^{a}	2.38^{a}	9.69^{a}	10.59^{a}
ECPC B	7.63^{a}	8.75^{a}	1.28^{d}	1.46^{d}	6.29^{bc}	7.22^{bc}
ECPC C	5.70^{c}	6.59^{c}	1.49^{c}	1.72^{c}	6.34^{bc}	7.33^{bc}
ECPC D	4.96^{d}	5.90^{d}	1.82^{b}	2.17^{b}	7.37^{b}	8.77^{ab}
ECPC E	6.44^{b}	7.82^{b}	1.30^{d}	1.58^{cd}	5.01^{c}	6.09^{c}
SEM[1]	0.00801	0.01084	0.01069	0.01373	0.28799	0.34869
P-value	<0.0001	<0.0001	<0.0001	<0.0001	0.0028	0.0045

[1] Standard error of the mean.

$^{a–d}$ Means followed by different letters within columns are significantly different ($P < 0.05$)

5.5 DISCUSSION

5.5.1 Apparent Metabolizable Energy (AMEn) Values

Differences in chemical composition may account for the variation in AMEn content among ECPC samples found in this study. In a 2017 study, Adewole et al. found that AMEn content varied in CM samples, and this was attributed to differences in CM chemical composition. The residual oil and protein are two major components that affect the content of metabolizable energy in oil seed (Khajali and Slominski, 2012). In this study, the strong positive correlation between EE and AMEn suggested that EE was a significant contributor to the metabolizable energy value of the ECPC as an increase in EE content in ECPC was associated with a higher AMEn value. On the other hand, CP demonstrated negative correlation with AMEn, although it was not statistically significant. The observed correlation between CP and AMEn value may be attributed to the interconnected relationship between CP and EE levels. It is likely that CP could be concentrated when EE content was low or diluted when EE content was high. The influence of NDF on AMEn suggested that carbohydrates present in ECPC do not strongly contribute to its metabolizable energy. Despite the sucrose content (ranging from 3.4% to 8.0 % on a DM basis), among the evaluated ECPC samples, it did not show relevance to the metabolizable energy contents. Moreover, no significant correlation was found between TDF and AMEn, indicating that TDF might not be a reliable predictor of AMEn. These findings led to the selection of nutrients for the development of prediction equations based on their correlations with AMEn. This study aimed to explore how simple and proximate measurements, like, EE, CP, ash, and NDF content could be utilized to predict the metabolizable energy of ECPC.

Several regression equations were developed to predict AMEn value in ECPC, and these with the R^2 value of 0.99 and P value less than 0.05 were considered as satisfactory. Based on the statistical analyses conducted, the equation utilizing EE and CP appeared as the most optimal model for predicting AMEn. It should be noted that this equation was comparable to the one where EE, CP, and ash and NDF, EE, and CP were used. However, the primary advantage of the first equation was its ability to accurately predict AMEn using only two nutrient measurements, making it a simpler model. As well these results suggested that the contents of EE, CP, ash, and NDF could be used to accurately predict AMEn in ECPC, although the equation was based on a limited sample size. This information may be utilized by nutritionists in the formulation of animal diets, ensuring that the animals are receiving the adequate amount of energy to maintain their nutritional requirements without having to conduct lengthy and expensive laboratory analysis.

The AMEn value of EECM, as reported in this study, was consistent with the values reported by Toghyani et al. (2007). The AMEn content of EECM did not show any significant difference compared to the averaged value of ECPC evaluated in this study. This similarity in AMEn values could be attributed to the comparable chemical composition of ECPC and EECM. The average value of AMEn value obtained in this study was 2386 kcal/kg on a DM basis, which was higher than the value reported earlier by Agyekum and Woyengo (2022) in their study with broiler chickens, where they reported 1994 kcal/kg DM of AMEn in cold-pressed canola. As expected, the AMEn values of ECPC in this study were higher than those observed in conventional CM, as indicated in previous research conducted by Adewole et al. (2017), Rad-Spice et al. (2018), and Radfar et al. (2017) with values of 1789, 1879, and 1876 kcal/kg DM, respectively.

5.5.2 Standardized Ileal Amino Acid Digestibility (SIAAD) of ECPC

In this study, variations were observed in the SID of the total AA, and in the digestible AA content among ECPC samples. These findings aligned with the variations reported by Adewole et al. (2017) in conventional, solvent extracted CM. The variability in chemical composition between ECPC samples could be attributed to differences in processing methods employed by the plants, particularly affecting the heat-sensitive components like GLS, lysine, and NDICP. The lower total AA content observed in ECPC B and E compared to ECPC A, C, and D suggested variations in seed processing conditions resulting in residual oil content of the samples, which could dilute the nutrient components, including AA. The total level of digestible AA in ECPC was found to be slightly higher (by 2.24%) than previously reported levels in CM (Adewole et al., 2017; Rad-Spice., 2018). While digestible Arg and Met levels were lower in ECPC compared to CM, digestible His, Ile, Leu, Lys, Cys, Phe, Thr, Val, Asp, and Gly were higher in ECPC (Adewole et al., 2017; Rad-Spice., 2018). Digestible levels of Ala, Glu, Pro, Ser, and Tyr in ECPC were found to be similar to those reported by Adewole et al. (2017) and Rad-Spice et al. (2018) in CM.

The results of digestible Lys content in ECPC samples obtained from different processing plants revealed that ECPC A had the highest digestible Lys content among all samples. This was further reflected in the content of NDICP, which was the lowest in this sample, and GLS, which was the highest. On the contrary, ECPC B and E had the lowest content of digestible Lys, the highest content of NDICP and the lowest content of GLS. Visual inspection of the samples also revealed that ECPC A was green in color, indicating that a milder processing method was likely used. ECPC B and E were darker brown in color and appeared to be roasted, which could be an indication of overheating during processing that likely triggered the Maillard reaction. This reaction could have caused some of the digestible Lys to be converted into fiber type components, resulting in higher NDICP levels in ECPC B and E (Hendriks, 2018; Slominski, 2018; Adewole et al., 2017). It is also possible that the level of EE in the ECPC samples may have affected the color of the samples and make it appeared darker. The varying residual fat content in ECPC samples did not affect contents of NDICP, GLS and digestible Lys, which was demonstrated when results were standardized and calculated on a fat-free basis. The lack of statistical differences between ECPC and EECM in the digestible content of Lys, Arg, Met, Thr, and Cys, may indicate that expeller processing was a milder oil extraction method that may be able to preserve the quality of the meal.

5.5 CONCLUSIONS

Expeller/cold-pressed canola showed potential as a superior source of metabolizable energy for broiler chickens compared to conventional CM. However, the variation in apparent metabolizable energy AMEn values of ECPC could pose challenges in formulating poultry diets. To address that, the AMEn values of ECPC could be accurately predicted using regression equations based on simple analytical measurements of ether extract and crude protein.

Furthermore, significant variations were observed in the standardized ileal amino acid digestibility and digestible amino acid content of ECPC obtained from different processing plants. The lack of standardized seed processing methods may be the primary cause of variability in the nutritive value, and the chemical and physical characteristics of ECPC. It is important to address these variations to ensure consistent nutritional value and quality of ECPC as a feed ingredient for poultry.

Given the different forms in which the ECPC samples were received, a critical step was to grind them to achieve a consistent nutrient distribution within the broiler's diets. Grinding ECPC to a finer particle size might enhance nutrient digestibility. It is worth noting, however, that this preparatory step introduces an additional cost element into the diet formulation process.

CHAPTER 6

6.0 MANUSCRIPT III

EVALUATION OF GROWTH PERFORMANCE OF BROILER CHICKENS FED DIETS CONTAINING EXPELLER/ COLD PRESSED CANOLA

6.1 ABSTRACT

The detailed chemical composition and nutritive value of Expeller/cold-pressed canola (ECPC), including standardized ileal amino acids contents and AMEn values, were utilized in formulating diets for broiler chickens. The growth performance study was conducted in a three-week study using as-hatched Ross 308 broilers to investigate the effect of three different ECPC products (ECPC1, ECPC2 and ECPC3). The birds were assigned into four dietary treatments, with each consisting of seven replicate cages, each housing six birds. Throughout the study, the birds were fed starter (4-10 days), grower (10-21days) and finisher (21-28 days) diets. Diets included a Control corn-SBM-wheat-CM-based, as well as three diets containing 100 g/kg, 125 g/kg and 150 g/kg of ECPC, respectively, in each phase. The iso-nitrous and iso-energetic diets were formulated to meet the breeder's recommendation. Body weight gain (BWG) and feed intake (FI) of birds were monitored weekly and feed conversion ratio (FCR) was calculated accordingly. The AMEn value for ECPC2 was not evaluated in earlier study, thus, previously developed prediction equations were employed to estimate this value for formulating the test diet. A completely randomized design was used, and the data were analyzed using the GLM procedure of SAS. The results showed that the growth performance of broiler chickens was not significantly affected by the inclusion of ECPC in the diets, indicating that ECPC could be used as an alternative feed ingredient at each phase of broiler chicken growth without compromising their performance.

Furthermore, the prediction equation developed in earlier study for AMEn estimation was validated, demonstrating its reliability for calculating AMEn based on the relatively simple analytical measurements.

In conclusion, ECPC can be effectively incorporated into broiler chicken nutrition at inclusion levels of 100-150 g/kg when diets are formulated based on digestible amino acid and metabolizable energy contents.

Keywords: expeller/cold-pressed canola; growth performance; broiler chickens

6.2 INTRODUCTION

Canada is the largest exporter of canola to many countries worldwide. The increasing global demand to reduce CO_2 emissions provides an opportunity to use canola seeds to produce biodiesel (Raboanatahiry et al., 2021). The utilization of canola seed in Canada in the biofuel sector is significant in the global market. The biofuel sector consumed an estimated three million tonnes of canola seed in 2020, which accounts for approximately 10% of Canada's overall production from that year (Pratte, 2020). Following the processing of the canola seed, the resulting co-product, such as the expeller/cold pressed canola, can be used as a valuable feed ingredient for poultry as a protein and amino acid source for the feed industry. It can partially substitute SBM and, depending on availability and opportunity, can mitigate feed costs for poultry producers. This becomes particularly relevant as the cost of feed ingredients for poultry increases due to concerns such as climate change, military conflicts, geopolitical issues, pandemics, etc. Monogastric animals can be fed the residual meal from canola seed processing, thus minimise the waste and facilitate the recycling of by-products. This allows for conversion of protein that is not-edible for human in form of the meal into animal protein, ultimately contributing to the food production. It is important to ensure accurate formulation of diets in order to provide balanced nutrients that meet the nutritional requirement of the animal. It is necessary to determine the metabolizable energy and the levels of digestible amino acids available from ECPC, which should be used in the diet formulation for broiler chickens.

Expeller/cold pressed canola is produced using mechanical or screw pressing methods. This technology involves minimal heat and no moisture during processing and does not involve solvent extraction. As a result, the product generally retains its quality and has higher residual oil content compared to conventional CM (Spragg and Mailer, 2007). Consequently, the meal exhibits higher energy content and can serve as a valuable source of amino acids and energy for poultry. However, due to variations in processing methods employed by different producers, ECPC may exhibit variability in the contents of NDF, TDF, EE, GLS, AA, and AMEn. These variations can potentially impact growth performance of broiler chickens. The objectives of this study were to utilize the analyzed chemical composition, AMEn and digestible AA contents to formulate precise diets for broiler chickens and to investigate the growth performance of broiler chickens when fed diets containing 100 g/kg, 125 g/kg and 150 g/kg of expeller/cold pressed canola during the starter, grower and finisher phases of their growth. Secondly, the study aimed to validate a previously developed prediction equation for estimating the apparent metabolizable energy content of one of the ECPC product based on the relationship observed between estimated AMEn and broiler chicken growth performance.

6.3 MATERIALS AND METHODS

All research methods and procedures involving animals were conducted according to the guidelines of the Canadian Council on Animal Care. The animal care protocol for this study was approved by the Animal Care Committee of the University of Manitoba (F20 - 001/1/2, AC11558).

6.3.1. Materials

Expeller/Cold-Pressed Canola (ECPC) were collected from three canola processing plants in Western Canada. All samples were subjected to determination of the chemical composition following the analytical methodology as described in Chapter 4 (4.3.2.). The ECPC test materials used in this study were received as meal, crumbs, and cake. Therefore, the test ECPC materials were ground with a hammer mill and passed through a 2 mm sieve to standardize the particle size and allow proper mixing with the basal diet. Accordingly, the broiler chickens were fed the test material in the same form. The AMEn and SID of amino acids in ECPC 1 and ECPC 3 for broiler chicken were determined in study presented in Chapter 5, and values were used for precise diet formulation. The ECPC1 would be an equivalent of ECPC C and ECPC3 would be the sample ECPC E that have been analysed in earlier study. The AMEn value for ECPC2 was not evaluated in earlier study; thus, previously developed prediction equations were employed to estimate this value (Table 6.1).

Table. 6.1. The apparent metabolizable energy contents (AMEn) value calculated for diet formulation with expeller/cold-pressed canola 2 (ECPC2) for broiler chickens using prediction equations developed in the previous apparent metabolizable energy (AMEn) study (kcal/kg, on as is basis).

	Predicted				
	Equation 1[1]	Equation 2[2]	Equation 3[3]	Equation 4[4]	Mean
ECPC2	2112.1	2143.8	2107.9	2111.7	2118.9

[1] Equation 1 $AMEn=406.92+68.89EE+26.00CP$

[2] Equation 2 $AMEn=1620.47+47.19EE$

[3] Equation 3 $AMEn=655.38+65.67EE+25.70CP-33.67Ash$

[4] Equation 4 $AMEn=395.24+0.18NDF+68.95EE+26.17CP$

Standardized digestible content of Lys, Meth, Cyst, Thr and Arg in ECPC2 was calculated based on the average SID of amino acids in ECPC determined in earlier study. The chemical composition and nutritive value of ECPC used in the study is presented in table 6.2.

Table 6.2. Chemical composition of expeller/cold-pressed canola (ECPC) samples used in growth performance study (%, as-is basis)

Item	ECPC1	ECPC2	ECPC3
Dry Matter	91.98	93.61	91.93
AMEn, kcal/kg	2139	2119[1]	2393
Ether Extract	12.39	11.09	16.29
Crude Protein (N x 6.25)	33.90	36.20	32.99
Standardized Ileal Digestible[2]			
Lysine	1.37	1.50	1.19
Methionine	0.41	0.42	0.35
Cystine	0.53	0.61	0.50
Threonine	1.10	1.09	0.87
Arginine	1.36	1.47	1.12
Carbohydrates	6.14	6.32	5.75
Total Dietary Fibre	34.33	33.27	33.94
Non-Starch Polysaccharides (NSP)	19.09	18.01	17.91
Lignin and polyphenols	9.99	9.73	10.10
Glycoproteins (NDICP)[3]	5.25	5.53	5.92
Neutral Detergent Fibre	26.91	24.76	27.52
Acid Detergent Fibre	23.03	22.24	20.97
Ash	5.95	6.12	5.36
Total Phosphorus	0.96	0.86	0.89
Phytate P	0.54	0.33	0.54
Non-Phytate P	0.42	0.53	0.35
GLS, µmol/g[4]	5.84	8.20	4.61

[1] Calculated using developed prediction equation.

[2] Determined in ECPC 1 and ECPC 3; calculated in ECPC 2

[3] Neutral Detergent-Insoluble Crude Protein

[4] Includes gluconapin, glucobrassicanapin, progoitrin, gluconapoleiferin, gluconasturtin, glucobrassicin, and 4-hydroxyglucobrassicin.

6.3.2 Birds and Housing

As-hatched Ross 308 broiler chickens were used to evaluate effect of inclusion of ECPC products in the starter, grower, and finisher diets on their growth performance. The study was carried out at the Small Animal Research Facility of the Department of Animal Science, University of Manitoba, Canada. One hundred sixty-eight one-day-old chicks were provided by a local hatchery and raised in electrically heated battery housing system (Super Brooders, Alternative Design Manufacturing and Supply, Inc., Siloam Springs, AR, USA) under a controlled environment. The temperature was monitored, daily recorded, and adjusted in response to the comfort of the birds. Birds had free access to water and feed.

6.3.3 Experimental Diets

Birds were fed the Control starter diet throughout the 4-day pre-experimental period to allow for yolk sac absorption. On day 4, chicks were randomly assigned into four dietary treatments, with each consisting of seven replicate cages, each housing six birds. Throughout the study, the birds were fed starter (4-10 days), grower (10-21days) and finisher (21-28 days) diets. Diets included a Control corn-SBM-wheat-CM-based, as well as three diets containing 100 g/kg, 125 g/kg and 150 g/kg of ECPC product, respectively, in each phase. Three ECPC products, i.e., ECPC1, ECPC2, and ECPC3 were evaluated. The iso-nitrous and iso-energetic diets were formulated to meet the breeder’s recommendation. Diets were formulated to have similar NSP level. The composition of the starter, grower, and finisher diets for broiler chickens are shown in Tables 6.3, 6.4, and 6.5, respectively.

Table 6.3. Composition of experimental starter diets (%, as-fed basis)

Ingredient (%)	Starter (4-14 d of age)			
	Control	ECPC1	ECPC2	ECPC3
Corn	44.73	43.95	44.68	44.17
Soybean meal	29.73	29.10	28.50	29.27
Wheat	5.00	5.00	5.00	5.00
Canola meal	8.20	0.00	0.00	0.00
ECPC1	0.00	10.00	0.00	0.00
ECPC2	0.00	0.00	10.00	0.00
ECPC3	0.00	0.00	0.00	10.00
Vegetable oil	6.75	6.41	6.29	6.00
Calcium carbonate	1.34	1.32	1.34	1.32
Mono calcium phosphate	1.57	1.53	1.51	1.55
L-Lysine	0.36	0.38	0.38	0.38
DL-Methionine	0.32	0.31	0.30	0.31
Threonine	0.20	0.20	0.20	0.20
Mineral premix[1]	0.50	0.50	0.50	0.50
Vitamin premix[2]	1.00	1.00	1.00	1.00
Cr2O3	0.30	0.30	0.30	0.30
Total	100	100	100	100
Calculated composition (%)				
Crude Protein	23.00	23.04	23.03	23.04
ME, kcal/kg	3002	3000	3001	3000
Non-Starch Polysaccharides (NSP)	9.94	9.95	9.79	9.88
Calcium	0.95	0.95	0.95	0.95
Total phosphorus	0.75	0.74	0.72	0.74
Available phosphorus	0.50	0.50	0.50	0.50
Digestible Lysine	1.32	1.32	1.32	1.32
Digestible Methionine	0.63	0.62	0.62	0.62
Digestible Methionine + cysteine	1.00	1.00	1.00	1.00
Digestible Cysteine	0.31	0.32	0.32	0.31
Digestible Threonine	0.88	0.88	0.88	0.88
Digestible Arginine	1.34	1.31	1.31	1.31
Analyzed composition				
Crude protein (Nx6.25)	23.2	22.7	22.1	22.6

[1] Provided per kg of diet: 70 mg Mn (as manganese oxide), 80 mg Zn (as zinc oxide), 80 mg Fe (as ferrous sulphate), 10 mg Cu (as copper sulphate), 0.3 mg Se (as sodium selenite), 0.5 mg Iodine (as calcium iodate), 337 g Na (as sodium chloride). [2] Provided per kilogram of diet: 8250 IU vitamin A, 3000 IU vitamin D3, 30 IU vitamin E, 0.13 mg vitamin B12, 2 mg vitamin K3, 6 mg riboflavin, 11 mg pantothenic acid, 40.3 mg niacin, 1301 mg choline, 4 mg folic acid, 0.25 mg biotin, 4 mg pyridoxine, 4 mg thiamine, 125 mg antioxidants (Endox, Kemin), 11 mg virginiamycin, 99 mg monensin sodium.

Table 6.4. Composition of experimental grower diets (%, as-fed basis)

Ingredient (%)	Grower (14-21 d of age)			
	Control	ECPC1	ECPC2	ECPC3
Corn	47.25	45.45	46.46	45.80
Soybean meal	25.34	23.36	22.56	23.50
Wheat	7.50	7.50	7.50	7.50
Canola meal	8.50	0.00	0.00	0.00
ECPC1	0.00	12.50	0.00	0.00
ECPC2	0.00	0.00	12.50	12.50
ECPC3	0.00	0.00	0.00	10.00
Vegetable oil	6.68	6.52	6.34	6.00
Calcium carbonate	0.99	0.95	0.98	0.95
Mono calcium phosphate	1.23	1.18	1.13	1.20
L-Lysine	0.30	0.33	0.33	0.34
DL-Methionine	0.26	0.25	0.24	0.25
Threonine	0.15	0.16	0.16	0.16
Mineral premix[1]	0.50	0.50	0.50	0.50
Vitamin premix[2]	1.00	1.00	1.00	1.00
Cr2O3	0.30	0.30	0.30	0.30
Total	100	100	100	100
Calculated composition (%)				
Crude Protein	21.51	21.51	21.50	21.50
ME, kcal/kg	3050	3050	3050	3050
Non-Starch Polysaccharides (NSP)	9.77	9.93	9.73	9.83
Calcium	0.75	0.75	0.75	0.75
Total phosphorus	0.67	0.67	0.64	0.66
Available phosphorus	0.42	0.42	0.42	0.42
Digestible Lysine	1.18	1.18	1.18	1.18
Digestible Methionine	0.56	0.55	0.55	0.55
Digestible Methionine + cysteine	0.92	0.92	0.92	0.92
Digestible Cysteine	0.30	0.31	0.31	0.31
Digestible Threonine	0.79	0.79	0.79	0.79
Digestible Arginine	1.23	1.19	1.19	1.18
Analyzed composition				
Crude protein (Nx6.25)	21.50	22.00	21.9	20.3

[1]Provided per kg of diet: 70 mg Mn (as manganese oxide), 80 mg Zn (as zinc oxide), 80 mg Fe (as ferrous sulphate), 10 mg Cu (as copper sulphate), 0.3 mg Se (as sodium selenite), 0.5 mg Iodine (as calcium iodate), 337 g Na (as sodium chloride). [2]Provided per kilogram of diet: 8250 IU vitamin A, 3000 IU vitamin D3, 30 IU vitamin E, 0.13 mg vitamin B12, 2 mg vitamin K3, 6 mg riboflavin, 11 mg pantothenic acid, 40.3 mg niacin, 1301 mg choline, 4 mg folic acid, 0.25 mg biotin, 4 mg pyridoxine, 4 mg thiamine, 125 mg antioxidants (Endox, Kemin), 11 mg virginiamycin, 99 mg monensin sodium.

Table 6.5. Composition of experimental finisher diets (%, as-fed basis)

Ingredient (%)	Finisher (21-28 d of age)			
	Control	ECPC1	ECPC2	ECPC3
Corn	50.00	48.00	49.15	48.50
Soybean meal	18.30	16.12	15.20	16.28
Wheat	10.00	10.00	10.00	10.00
Canola meal	10.50	0.00	0.00	0.00
ECPC1	0.00	15.00	0.00	0.00
ECPC2	0.00	0.00	15.00	0.00
ECPC3	0.00	0.00	0.00	15.00
Vegetable oil	6.84	6.59	6.40	5.91
Calcium carbonate	0.88	0.82	0.87	0.81
Mono calcium phosphate	0.97	0.92	0.84	0.94
L-Lysine	0.34	0.38	0.38	0.39
DL-Methionine	0.23	0.22	0.21	0.22
Threonine	0.14	0.15	0.15	0.15
Mineral premix[1]	0.50	0.50	0.50	0.50
Vitamin premix[2]	1.00	1.00	1.00	1.00
Cr2O3	0.30	0.30	0.30	0.30
Total	100	100	100	100
Calculated composition (%)				
Crude Protein	19.51	19.50	19.50	19.50
ME, kcal/kg	3102	3101	3103	3099
Non-Starch Polysaccharides (NSP)	9.60	9.76	9.52	9.64
Calcium	0.65	0.65	0.65	0.65
Total phosphorus	0.62	0.61	0.57	0.60
Available phosphorus	0.36	0.36	0.36	0.36
Digestible Lysine	1.08	1.08	1.08	1.08
Digestible Methionine	0.51	0.50	0.50	0.50
Digestible Methionine + cysteine	0.86	0.86	0.86	0.86
Digestible Cysteine	0.29	0.30	0.30	0.30
Digestible Threonine	0.72	0.72	0.72	0.72
Digestible Arginine	1.07	1.02	1.02	1.01
Analyzed composition				
Crude protein (Nx6.25)	19.5	19.7	19	18.5

[1] Provided per kg of diet: 70 mg Mn (as manganese oxide), 80 mg Zn (as zinc oxide), 80 mg Fe (as ferrous sulphate), 10 mg Cu (as copper sulphate), 0.3 mg Se (as sodium selenite), 0.5 mg Iodine (as calcium iodate), 337 g Na (as sodium chloride). [2] Provided per kilogram of diet: 8250 IU vitamin A, 3000 IU vitamin D3, 30 IU vitamin E, 0.13 mg vitamin B12, 2 mg vitamin K3, 6 mg riboflavin, 11 mg pantothenic acid, 40.3 mg niacin, 1301 mg choline, 4 mg folic acid, 0.25 mg biotin, 4 mg pyridoxine, 4 mg thiamine, 125 mg antioxidants (Endox, Kemin), 11 mg virginiamycin, 99 mg monensin sodium

6.3.4 Performance Data

Body weight and feed consumption were recorded weekly. Average body weight gain (BWG), feed intake (FI), and feed conversion ratio (FCR) were used to determine performance and were calculated on a cage basis with each cage representing one replicate.

6.3.5 Statistical Analysis

Four treatments were considered as independent variables. The dependent variables were the body weigh gain, feed intake and feed conversation ratio. The experimental unit was the cages. The experiment was structured in one-way-ANOVA. The data were analysed using the GLM procedure of SAS STUDIO (SAS on demand) considering $P<0.05$ significant. The mean comparisons were conducted using Tukey's test post hoc with $P<0.05$ considered significant.

6.4 RESULTS

Growth performance of broiler chickens fed starter (4-14 days), grower (14-21 days), and finisher (21-28 days) diets containing three different ECPC products is presented in Table 6.6. ECPC was included in the diet at relatively high level, i.e., 100 g/kg in starter, 125 g/kg in grower, and 150 g/kg in finisher diets and tested against the Control, conventional broiler diet. The Control diets included CM at 82, 85, and 105 g/kg in starter, grower, and finisher, respectively to balance for the content of NSP. Although numerical, but not statistically significant differences were observed in the growth performance parameters, i.e., BWG, FI and FCR throughout each phase and overall growth period.

Table 6.6. Growth performance of broiler chickens fed starter, grower, and finisher diets containing three different expeller/cold-pressed canola (ECPC) products

Phase		Treatment					
		Control[1]	ECPC1	ECPC2	ECPC3	SEM[2]	P-value[3]
Starter, 4-14 d	BWG, g/bird	329.3	329.9	312.5	330.9	10.63	0.4945
	FI, g/bird	377.5	386.7	371.9	375.1	9.39	0.6229
	FCR, g/g	1.15	1.18	1.19	1.13	0.027	0.3907
Grower, 14-21 d	BWG, g/bird	437.2	425.6	430.5	417.5	20	0.8929
	FI, g/bird	556.5	558.5	548	542.5	15.46	0.8419
	FCR, g/g	1.28	1.32	1.28	1.3	0.34	0.7567
Finisher, 21-28 d	BWG, g/bird	548.5	490.8	512.7	496.8	25.26	0.2653
	FI, g/bird	732.4	693.6	716.4	694.4	28.13	0.6354
	FCR, g/g	1.34	1.42	1.41	1.41	0.059	0.6533
Overall, 4-28 d	BWG, g/bird	1315	1246.4	1255.6	1245.1	41.44	0.4748
	FI, g/bird	1666.3	1638.7	1636.4	1611.9	44.41	0.8246
	FCR, g/g	1.27	1.32	1.31	1.29	0.021	0.2672

[1] The composition of the control diet is in Tables 6.3, 6.4, and 6.5.

[2] Standard error of the mean; Number of observations contributing to each mean=7

[3] Overall *P*-value of four experimental diets.

The ECPC products were included at 100, 125, and 150 g/kg in starter, grower, and finisher, respectively.

6.5 DISCUSSION

In the previous AMEn study (Chapter 5) equations were developed to predict AMEn value in ECPC from the simple measurement of EE, CP, NDF, and ash contents. In this study, these equations were applied to predict energy value of ECPC2, since this particular product was not included in the AMEn assay. The similarity in BWG, FI, and FCR between ECPC2 treatment and the other treatments including the control treatment, suggests the accuracy of the calculated AMEn value. The equations developed from the AMEn study could be used by the feed industry to calculate AMEn values of ECPC when formulating poultry diets instead of the more time consuming and costly AMEn trials with broilers.

The expeller/cold pressed canola is an alternative feed ingredient for poultry; therefore, it was important to explore its impact on the growth performance of broiler chickens. Results of the study suggested that ECPC had a similar effect on broiler growth performance as the conventional solvent-extracted CM, indicating its potential as a valuable protein and digestible AA source in poultry diets. As the intake of diets containing ECPC was not affected, it indicated that the palatability of the diets was not compromised, and birds accepted and consumed the feed willingly. This finding aligned with previous research conducted by Thacker and Petri (2009), where diets containing different levels of cold press cake (50g/kg, 100g/kg, and 15g/kg) showed no significant difference in the growth performance of broiler chickens (0-21 days) compared to the control treatment that contained conventional CM. Consequently, these results supported the inclusion of ECPC in poultry diets. Gao et al. (2020) also reported consistent results with the current study by using 15% inclusion level of double-low rapeseed cake in broilers diet, showing no adverse effects on BWG, FI and FCR). However, Woyengo et al. (2011) observed decreased BWG of broiler chickens (0-21 days study) when expeller extracted canola meal (EECM) was included at a 400g/kg. This decrease was attributed to reduced feed intake, increased liver metabolic activities, and an observed increase in liver size. It could also indicate that inclusion of EECM at the level higher than 300g/kg may not be recommended due to the potential palatability problems.

6.6 CONCLUSIONS

In conclusion, the consistent growth performance observed across all treatment groups throughout the experiment indicated the potential of ECPC as a valuable feed ingredient for poultry. The importance of precise formulation of diets based on the metabolizable energy and standardized ileal amino acids contents must be emphasized.

Furthermore, the developed prediction equations were employed to calculate the AMEn value of one ECPC used in the present study (ECPC2). Given that the growth performance of broilers fed ECPC2 was comparable to those fed conventional diet and other two ECPC products, it could be concluded that these equations offer a reliable tool for the estimation of the metabolizable energy value of ECPC using proximate analysis.

CHAPTER 7

7.0 GENERAL DISCUSSION

Nutritionists have recognized the economic challenges associated with animal feed production. This prompted them to seek out alternative feed ingredients that offer cost-effectiveness while meeting the animal nutrient requirements. Recycling agricultural by-products as animal feed serves the dual purpose of reducing organic matter waste in landfills while providing valuable source of nutrients for livestock. Poultry producers are aiming to optimize production efficiency while providing safe, high-quality products to consumers. A main aspect of achieving this goal is implementing a cost-effective feeding strategy, which includes careful consideration of the nutritive composition and value of alternative feedstuffs that are available at a lower price. Formulating diets based on metabolizable energy and digestible AA values is crucial to ensure effective digestion and absorption by poultry. Additionally, understanding the risks related to the presence of antinutritional factors is important, as they can interfere with nutrients digestion and absorption, therefore negatively impacting poultry health and performance.

Expeller/cold pressed canola (ECPC) is a co-product obtained from the cold pressing of canola seeds for the extraction of oil for the food or biofuel industries. Unlike solvent-extracted CM, ECPC contains higher levels of residual oil, energy, and digestible AA due to its minimal exposure to high temperatures during processing. The utilization of ECPC as a feed ingredient in poultry diets is becoming increasingly popular due to its favourable nutritional value and low environmental impact. Numerous studies have been conducted on the evaluation of CM and expeller-extracted canola meal (EECM) in broiler chicken diets (Newkirk et al., 2003; Meng and Slominski, 2005; Woyengo et al., 2009; Toghyani et al., 2014; Adewole et al., 2016; Adewole et al., 2017; Radfar et al., 2017; Rad-Spice et al.,2018; Niu et al., 2022). However, limited research exists on evaluation of ECPC for broilers that would include analyses of its chemical composition, determination of nitrogen-corrected metabolizable energy and standardized ileal digestible amino acid content (Leming and Lember, 2005; Spragg and Mailer, 2007; Seneviratne et al., 2011; Grageola et al., 2013; Kasprzak et al., 2016; Woyengo et al., 2016; Lee and Woyengo, 2018; Agyekum and Woyengo, 2022). It is important to determine the nutritional value of ECPC and explore any potential variations between processing plants. This understanding can contribute to optimizing its inclusion in poultry diets and further explore its potential as a valuable feed ingredient.

In this thesis, samples of ECPC were collected from eight processing plants located across Western Canada. The research was not specifically designed to evaluate the technical aspects of seeds processing and its impact on the nutritive value of ECPC, as data related to the processing conditions were unavailable. However, studies by Adewole et al. (2016) have indicated a relationship between processing conditions and ingredient quality. Due to the absence of a standardized seed crushing technology among processing plants and the utilization of various equipment and techniques, it was anticipated that these factors could influence the residual oil content, leading to variation in the quality of ECPC. Therefore, it was hypothesized that there would be variations in the chemical composition of ECPC among different processing plants, as well as in its metabolizable energy and digestible AA values for poultry. Additionally, it was hypostatized that by evaluating the relationship between chemical components and nutritive value of ECPC, it will be possible to develop equations to predict its metabolizable energy value while employing basic analyses such as ether extract, crude protein, neutral detergent fibre and other relevant parameters.

The chemical composition and physical appearance of ECPC samples demonstrated variation among processing plants, indicating the impact of different processing techniques employed during the oil extraction process. In this study, the ECPC samples contained on an average on a DM basis of 16.8% ether extract, 36.5% crude protein (N x 6.25), 31.8% total dietary fibre, 18.8% non-starch polysaccharides, 6.1% ash, 1.03% total phosphorus and 7.85µmol/g of glucosinolates. The ether extract content in ECPC samples ranged widely, varying from 8.5% to 24.1% on a DM basis. The differences in the content of other chemical components of ECPC were mainly quantitative, as they depended on the variable fat content present in the samples.

Manuscript I presented results that revealed both statistically significant and numerical differences in the chemical composition of ECPC across various processing plants. These variations were primarily observed in the content of ether extract, crude protein, dietary fiber and its components, glucosinolates, ash, and total phosphorus. These results suggested that differences in processing conditions played an important role in the observed variation in the chemical composition of ECPC. This was similar to results of study conducted by Adewole et al. (2016), which showed comparable results for conventional CM. Moreover, the study established a correlation between GLS and neutral detergent insoluble crude protein. Additionally, the physical appearance of the samples, with some showing signs of roasting, provided evidence that certain samples may have undergone overheating possibly caused by the mechanical friction in the expeller during processing resulting in a Maillard reaction.

The results presented in Manuscript II of this thesis showed significant variations in the nitrogen-corrected metabolizable energy (AMEn) value of ECPC for broilers chickens across different processing plants. On a DM basis, the mean value of AMEn was 2386 kcal/kg, ranging from a minimum of 2128 kcal/kg to a maximum of 2604 kcal/kg. The primary component influencing AMEn value was the ether extract content of ECPC, as residual oil serves as an energy source for birds. To accurately predict AMEn value, prediction equations were developed based on simple measurements of crude protein, ether extract, neutral detergent fibre, and ash. These equations demonstrated satisfactory accuracy in estimating the AMEn value of the ECPC. Additionally, no statistical difference was observed between ECPC and expeller extracted canola meal, which was used as the reference product in this study. This suggested that expeller processing could maintain a similarly good-quality resulting meal as cold pressing. Differences were also observed in the content of digestible amino acids of ECPC across processing plants, indicating the potential influence of differences in processing conditions. This effect was particularly evident for the heat-sensitive lysine, as reflected in the relationship between lysine and glucosinolates and lysine and neutral detergent insoluble crude protein. This variation was further shown in ECPC B and E, which displayed brown roasted appearance, highest neutral detergent insoluble crude protein and lowest lysine levels. These observations suggested possible heat damage that could be due to mechanical forces, which could increase the temperature in the expeller and lead to the occurrence of Maillard reactions.

The results of the growth performance study discussed in Manuscript III of this thesis, demonstrated that there were no significant differences in growth performance among broilers fed diet containing ECPC compared to the control treatment. This indicates that ECPC can be effectively incorporated into broiler diets, provided that the diets are formulated using accurate values of metabolizable energy and digestible amino acids. Additionally, the developed equations for predicting AMEn values were utilized to calculate the AMEn value of ECPC2 and subsequently used in formulating the broilers diets. The results revealed that there were no significant differences in growth performance among birds fed diet containing ECPC2 compared to other diets. This suggested that the prediction equations developed in this study are valuable tools that can be utilized by nutritionists to accurately estimate the AMEn value of ECPC.

Overall, the results demonstrated the practical applicability of ECPC in broiler chicken nutrition at every phase of their growth and emphasized the importance of precise diet formulation based on the accurate AMEn values and digestible amino acids contents. The developed prediction equation provided resources for poultry nutritionists to support an effective incorporation of ECPC into broiler chicken diet formulation.

CHAPTER 8

8.0 CONCLUSION AND FUTURE STUDIES

8.1 Conclusions

1. Visible variations in physical appearance were observed in ECPC samples, and this was reflected in significant differences in the contents of DM, sucrose, oligosaccharides, ash, total P, non-phytate P, GLS, NSP and NDICP between processing plants.
2. The variation of ECPC samples in physical forms required grinding for uniform particle size and consistent nutrient distribution in the broiler diets, therefore it may contribute to extra costs for the feed industry.
3. The significant variations were found in the levels of AMEn and SID AA among canola cold pressing processing plants for broiler chickens.
4. The overall average of determined and predicted AMEn values of ECPC for broiler chickens were 2222 and 2119 kcal/kg on as is basis, respectively.
5. The average levels of digestible AA such as Lysine, Arginine, Methionine, Threonine, and Cystine were 1.61%, 1.57%, 0.45%, 1.17%, and 0.65% on a DM basis, respectively.
6. Prediction equations based on simple measurements of CP and EE were found to be accurate in estimating the AMEn value of ECPC.
7. No statistical differences were observed between ECPC and EECM in their value of AMEn and contents of SID levels of Lysine, Arginine, Methionine, Threonine, and Cystine.
8. There were no significant differences in growth performance parameters, i.e., FI, BWG and FCR between broilers fed diets containing ECPC and those in the control treatment.

8.2 Future Studies

1. To conduct studies to determine the metabolizable energy and SIAAD of ECPC for laying hens and turkeys to provide a comprehensive understanding of the efficacy of ECPC in poultry nutrition.
2. To further explore the different inclusion levels of ECPC on performance of broiler chickens, turkeys, and laying hens.

CHAPTER 9

9.0 LITERATURE CITED

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