Chemical Composition and Standardized Ileal Amino Acid Digestibility of Manitoba Soybean Meal in Broiler Chickens

A Thesis

By

Arantxa Asun Pinar

Submitted to the Faculty of Graduate Studies of the University of Manitoba

In partial fulfillment of the requirements for the degree of

MASTER OF SCIENCE

Department of Animal Science

University of Manitoba

Abstract

This study aimed to evaluate the chemical composition and digestibility of expeller soybean meal (SBM) derived from Manitoba-grown soybean as a potential protein source for broiler chickens. The utilization of locally produced Manitoba SBM could offer a cost-effective and sustainable alternative to imported soybean meal. The standardized ileal amino acid digestibility (SIAAD) of Manitoba SBM was assessed through a digestibility study using a nitrogen-free diet (NFD). Three samples of Manitoba SBM and one sample of SBM from Ontario-grown seeds were analyzed and incorporated into diets as the sole source of nitrogen. Day-old Ross 308 chickens (n=320) were allocated to 64 cages with 5 birds per cage and fed a starter diet for 14 days. On day 15, the birds were randomly assigned to one of the five treatments, with 10 replicate cages per treatment, and stayed on trial for 5 days. The crude protein (CP) concentration of the Manitoba SBM samples ranged from 40.4-41.5% and the Ontario sample had a CP concentration of 47.9% on dry matter basis. The fat content of Manitoba and Ontario samples ranged from 10.6-11.3% and 9%, respectively. The total non-starch polysaccharides in the Manitoba and Ontario samples were 17.4-18% and 15.5%, respectively. Raffinose-family oligosaccharides were present at levels of 6.5- 8.7% in the Manitoba SBM samples and 6.2% in Ontario sample. The SIAAD of Manitoba SBM was similar to the control, except for histidine, methionine, and cysteine ($P < 0.05$) which were lower in two Manitoba samples. The mean standardized ileal CP digestibility of Manitoba SBM was 78.1%, compared to 81.1% for Ontario samples, and the difference was not statistically significant. The overall SIAAD values of Manitoba SBM were found to be lower than those reported in the literature for expeller-pressed SBM, explained by increased

antinutritional factor content and potential differences in processing techniques. In conclusion, Manitoba SBM holds promise as a viable protein ingredient for poultry nutrition, however further studies are necessary to understand its full potential.

Keywords: Manitoba soybean meal, standardized ileal digestibility of amino acids, nitrogen-free diet, antinutritional factors, broiler chickens.

Acknowledgements

I would like to express my gratitude to my supervisor Dr. Chengbo Yang, for his support and expertise throughout the research process. I am also very grateful to the members of my thesis committee, Dr. Anna Rogiewicz and Dr. James House, for their valuable insights, constructive feedback, and academic contributions that have greatly enhanced the quality of this work.

I want to thank my family for their love, encouragement, and belief in my abilities. To my mom Almoré Pinar, and my husband Jorge Flores, whose constant support and understanding have been sources of motivation and strength throughout this academic journey. I would like to acknowledge my teammates and friends who have provided stimulating discussions, collaboration, and moral support. Their comradeship and encouragement have made this research experience all the better. I am indebted to the University of Manitoba for providing an outstanding academic environment, access to resources, and opportunities for intellectual growth.

Finally, I am grateful to the funding entities, Manitoba Pulse and Soybean Growers and the Canadian Agricultural Partnership from Agriculture and Agri-food Canada for their financial support, which made this research possible.

Foreword

This thesis represents the culmination of extensive research in poultry nutrition, and I am delighted to present its findings and insights. I am grateful for the opportunity to share that a portion of this research was previously presented at the Animal Nutrition Conference of Canada 2023 in Montreal and as an oral presentation at the Manitoba Sustainable Protein Research Symposium 2023 in Winnipeg.

1. ASUN PINAR, A., Rogiewicz, A., Neijat, M., House, J., Yang, C. 2023. Standardized Ileal Digestibility of Limiting Amino Acids of Manitoba Soybean Meal in Broiler Chickens (poster). 2023. Animal Nutrition Conference of Canada, Montreal, May 9-11.
2. ASUN PINAR, A., Rogiewicz, A., Neijat, M., House, J., Yang, C. 2023. Manitoba-grown Soybean Meal as Protein Source in Broiler Chickens (oral presentation). 2023. Manitoba Sustainable Protein Research Symposium, June 20-21.

I extend my appreciation to the organizers of both events for facilitating the dissemination and discussion of my research. Constructive conversations from colleagues and experts have played a significant role in refining and enhancing the content of this thesis.

I hope that this thesis will contribute to the existing knowledge in poultry nutrition and inspire further exploration in the future.

Table of Contents

List of Figures

List of Tables

Abbreviations

AA	Amino acid
AID	Apparent ileal digestibility
AME	Apparent metabolizable energy
ANF	Antinutritional factor
CP	Crude protein
DM	Dry matter
Ha	Hectares
IAA_{end}	Endogenous ileal amino acid
MB	Manitoba
ME	Metabolizable energy
MPSG	Manitoba Pulse Soybean & Growers
NDF	Neutral detergent fibre
NFD	Nitrogen-free diet
NSP	Non-starch polysaccharide
ON	Ontario

PBS	Phosphate-buffered saline
SBM	Soybean meal
SEM	Standard error of means
SIAAD	Standardized ileal amino acid digestibility
SID	Standardized ileal digestibility
TID	True ileal digestibility

1. General Introduction

Poultry meat is widely recognized as one of the most consumed animal products globally, providing a reliable and affordable source of protein for a growing population. With the world's population projected to reach nine billion by 2050, there is an imminent need to produce 60% more food to meet the escalating demand (Hendriks *et al*., 2019). In Canada, chicken has become the preferred protein choice, renowned for its lean, nutritious, and readily available qualities, leading to an increase in consumption. Manitoba reached 34.4 kg of chicken meat consumed in 2020, increasing every year and it is currently at its highest in history (Manitoba Agriculture, 2022a).

Manitoba is a prominent agricultural region, playing a vital role in Canada's soybean production, accounting for over a fifth of the country's soybean output. Despite this, Manitoba primarily exports its soybean yield to Asia and the United States rather than utilizing it as a local animal feed ingredient. This study focuses on the concept of self-sustainability within Manitoba's poultry production, acknowledging that the province produces sufficient poultry meat to meet its population's needs. However, reliance on imported ingredients for animal feed remains an established practice.

Soybean meal, derived from soybean seeds through oil extraction processes, represents a valuable protein-rich feed ingredient commonly utilized in poultry nutrition. It possesses an optimal crude protein content ranging from 40 - 49%, and a well-balanced amino acid profile with methionine being the first limiting amino acid in poultry diets. Despite its numerous

qualities, soybean meal also contains antinutritional factors that require proper heat treatment for optimal utilization.

Considering the increasing global demand for poultry meat, projected to account for 41% of all meat protein by 2030, and the projected population growth to nine billion by 2050 (OECD/FAO, 2021), Manitoba faces the challenge of expanding poultry meat production to meet the surging demand. In this context, Manitoba-grown soybeans hold significant potential as a locally sourced, high-quality protein alternative for poultry production. By utilizing locally grown soybean meal, transportation costs can be reduced, supporting the provincial economy, and enhancing self-sustainability in poultry feed production.

This study aims to shed light on the chemical composition and digestibility of Manitoba soybean meal, offering insights into its amino acid digestibility and its potential as a primary protein ingredient in broiler chicken diets.

2. Literature Review

2.1 The Soybean

The soybean (*Glycine max* L.) is a plant of the Fabaceae family, also called legumes. Soybeans are among the most important plants globally for both human and animal nutrition. They have been cultivated in Asia for more than 3000 years, and there has been an increased interest in soybean throughout the world in the last century, with soybean occupating close to 6% of all cultivable land (Goldsmith, 2008) with 371.6 million tonnes produced globally in 2021 (FAO, 2023).

The domestication of the soybean occurred sometime around 1700-1100 BC in China during the Shang Dynasty. By the 16th century soybeans had become a staple in East Asia but it was not until the late 18th century when Europe began to grow them as a result of ongoing trade with China, however, they were only grown for scientific purposes (Kinney & Clemente, 2010). In 1804, the term *soybean* was first coined by Dr. James Mease and later in that century the soybean was first introduced to the Americas, starting in Illinois, then spreading throughout the rest of the Corn Belt of the United States (Hymowitz, 2008).

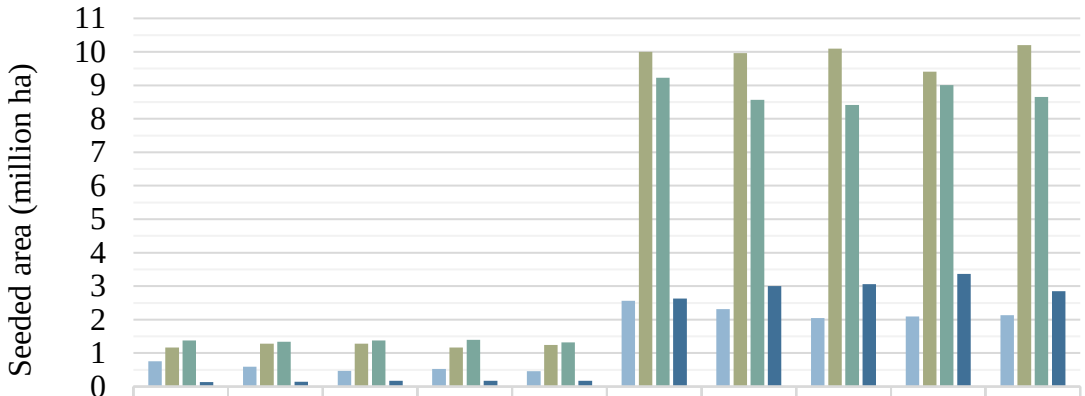
Many discoveries regarding legumes and soybeans were made throughout the 19th and 20th centuries, such as the discovery by Hellriegal and Wilfarth of nitrogen fixation by legumes, the finding by Osborne and Mendel of the increased nutrient quality of soybeans when heat-treated, or the identification by Garner and Allard of photoperiodism in soybeans (Hymowitz,

2008) with which scientists could then understand the relationship between daylight and plant growth.

Concurrently with the introduction of the soybean to the United States, they were also being introduced to both Argentina and Brazil (Kinney & Clemente, 2010). In the beginning soybeans were only grown for forage, but near the middle of the 20th century, soybeans began to be processed into soybean meal to be used in the animal feed industry, while the oil was kept for use in the human food industry (Kinney & Clemente, 2010).

Soybean production in Canada began in the 1920s in southwestern Ontario, but higher volume production began only after the mid-1970s (Cloutier, 2017). In spite of the beginning of a sharp decline in the number of farms across Canada after the sixties, Statistics Canada reports that soybean cultivation increased from 1.6% of all farms in 1961 to 13.2% by 2011. Genetic improvements together with years of research allowed for the success of the soybean in Canada, increasing from 0.08 million hectares (ha) planted in 1961, to 2.13 million hectares in 2022 (Statistics Canada, 2023a). As of 2022, soybean ranks as the fourth largest seeded area and the fourth most productive crop in Canada, after wheat, canola and barley (Figure 1).

Figure 1. Side to Side Comparison of Seeded Areas (in million hectares) of some of the Most Productive Crops (wheat, canola, barley, and soybean) in Manitoba and Canada from 2018 to 2022[a].



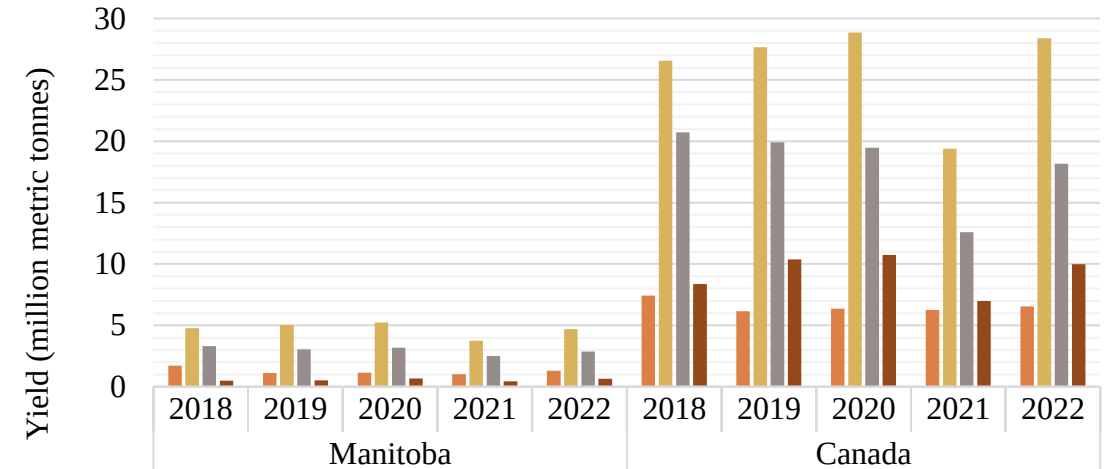
	Manitoba					Canada				
	2018	2019	2020	2021	2022	2018	2019	2020	2021	2022
Soybean	0.76	0.59	0.47	0.53	0.46	2.56	2.31	2.05	2.09	2.13
Wheat	1.17	1.28	1.28	1.17	1.24	10	9.96	10.1	9.41	10.2
Canola	1.38	1.34	1.38	1.4	1.32	9.23	8.57	8.41	9.01	8.65
Barley	0.13	0.14	0.17	0.17	0.17	2.63	3	3.06	3.37	2.85

[a] Adapted from Statistics Canada. Table 32-10-0359-01. Estimated areas, yield, production, average farm price, and total farm value of principal field crops, in metric and imperial units.

Canada is the seventh largest producer of soybeans in the world with a yearly production of over 6 million tonnes. Most of Canada's soybean yield comes from Ontario with a production of 3.99 million tonnes in 2022, followed by Manitoba (1.31 million tonnes) and Quebec (1.12 million tonnes) (Statistic Canada, 2023).

In Manitoba, soybean holds the third place for largest seeded area and third place for largest production, after canola and wheat (Figure 2).

Figure 2. Side to Side Comparison of the Production (in million metric tonnes) of some of the Most Productive Crops (wheat, canola, barley, and soybean) in Manitoba and Canada from 2018 to 2022[a].



	Manitoba					Canada				
	2018	2019	2020	2021	2022	2018	2019	2020	2021	2022
Soybean	1.73	1.12	1.16	1.01	1.31	7.42	6.15	6.36	6.27	6.54
Wheat	4.78	5.02	5.24	3.75	4.7	26.57	27.65	28.87	19.39	28.38
Canola	3.32	3.06	3.19	2.51	2.88	20.72	19.91	19.48	12.59	18.17
Barley	0.5	0.53	0.69	0.44	0.66	8.38	10.38	10.74	6.98	9.99

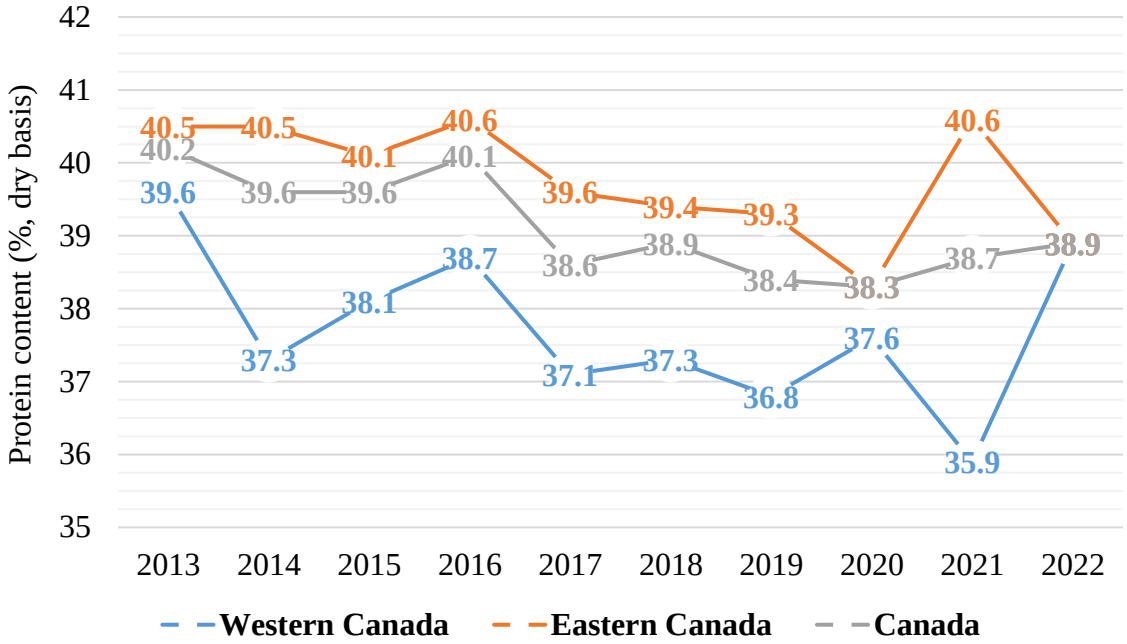
[a] Adapted from Statistics Canada. Table 32-10-0359-01. Estimated areas, yield, production, average farm price, and total farm value of principal field crops, in metric and imperial units.

The relatively short growing season of the Canadian prairies and specially Manitoba poses a challenge to farmers growing soybeans for profit. Manitoba recorded its first significant soybean yield as recently as 2001, thanks to the plant breeding improvements which allowed for cold-resistant and early-maturing soybeans (Cloutier, 2017).

In Manitoba, the frost-free period ranges from 65 to 135 days, which is significantly shorter compared to other soybean-growing regions worldwide and even within Canada. For instance, in Ontario the frost-free period extends from 90 to 190 days (Ort *et al*., 2022). Due to this short length of time for plant growth in Manitoba, newer varieties of soybeans needed to be bred to generate a type of soybean that was cold resistant, high yielding, profitable, and able to grow within such a short period of time. This led to the production of short-season soybean cultivars, which are types of soybean that can reach maturity before the first frost of fall (Ort *et al.,* 2022). Along with the short growing season, Manitoba soybean producers also have to manage the risk of frost at the beginning and end of the season, cold soil temperatures at the time of seed planting, drought by the end of the season, and periods of excess moisture in the soil (Brar & Lawley, 2020).

In addition to these shortcomings, Western-Canada (Manitoba and Saskatchewan) soybeans have historically struggled with a low protein content compared to those grown in Eastern Canada (Ontario and Quebec) (Figure 3). Although a study by Lee & Choung (2010) concluded that the protein content of soybean depended mostly on the variety and genotype rather than the geographical location of where it was grown, new data from Ort *et al.* in 2022 on Manitoba and Ontario-grown soybeans suggested that the lower protein content in Manitoba soybeans could be due to lower mean temperatures and less precipitation compared to Ontario.

Figure 3. Protein Content (%, on DM basis) of Oilseed-type Soybeans from 2013 to 2022 in Canada[a]



[a] Adapted from the Canadian Grain Commission. Figure 3. Seed protein content (%, dry basis) of Canadian oilseed-type soybeans from 2006 to 2022.

Nowadays soybean seeds are grown around the world due to their high protein and oil content (Table 1). As previously mentioned, this crop has been widely used in Asia to make a range of products for human consumption such as soy sauce, soy oil, soy paste, miso, tofu, yuba and natto. In human nutrition, soybeans are also used as substitutes for animal proteins with products like soy milk, texturized soy protein and tempeh. The soybean is also a very important source of nutrition for animals in the form of soybean meal.

Table 1. Chemical Composition of Full Fat Extruded Soybean Seeds [a].

Parameters	as is basis, %	DM basis, %
Dry matter	88.1	88.1
Crude protein	34.8	39.5
Fat	17.9	20.3
Ash	5.2	5.9
Neutral Detergent Fibre (NDF)	11.0	12.5
Total sugars	7.7	8.7

[a] Adapted from Tables of Composition and Nutritional Value of Feed Materials. Sauvant *et al*. (2004).

With 39.5% of protein content on a dry basis, soybean seeds are a rich source of protein for both humans and animals alike. Of all soybeans produced globally, 77% are turned to soybean meal and fed to livestock (Ritchie & Roser, 2021), and 25% of this soybean meal is fed to poultry alone (Kinney & Clemente, 2010).

2.2 Soybean Processing

As an oilseed, soybeans are produced to obtain oil for human consumption or biofuels, and secondly to produce a protein-rich by-product used in animal nutrition. The two most common approaches to extract oil from the seeds are expelling and solvent extraction processes, although there are other methods used as well.

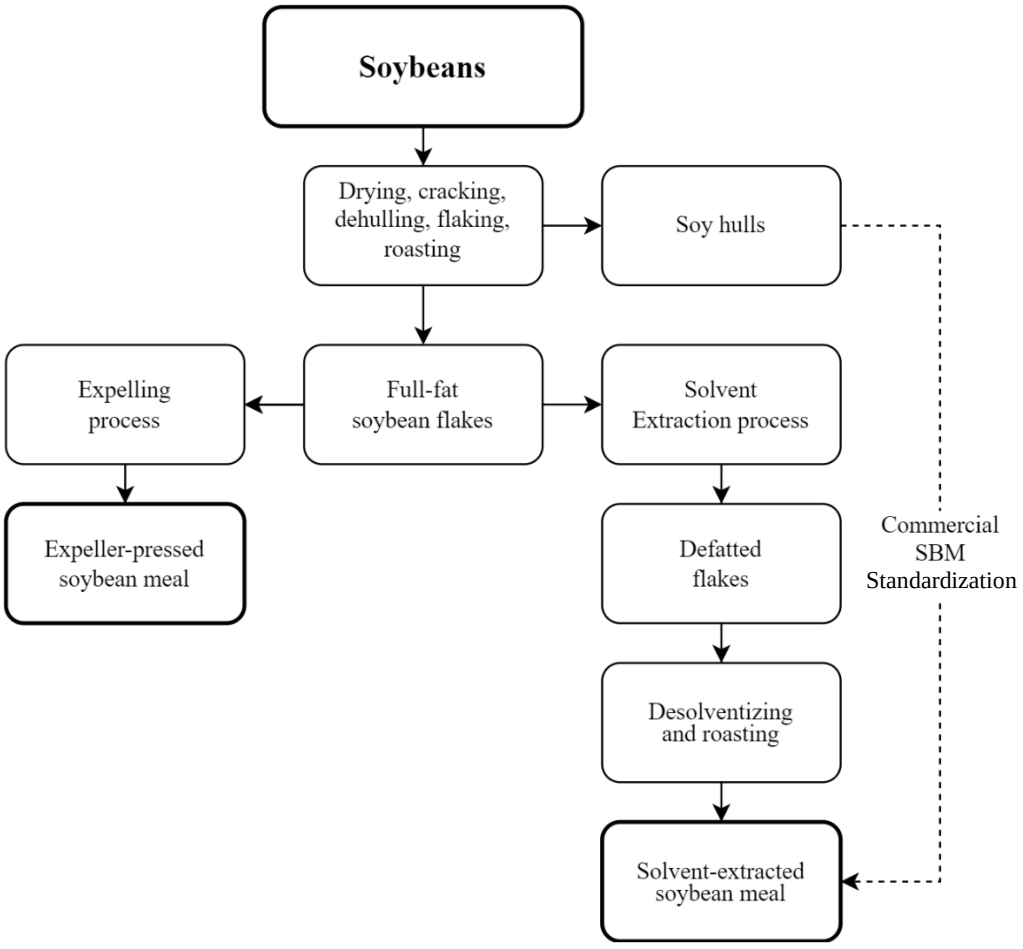
2.2.1 Expeller Pressing Method

The expeller pressing method consists of using a flight screw to press the oil from the seed during an extrusion process (Figure 4). With this procedure, 80-90% of the oil is extracted from the soybeans (Gaonkar & Rosentrater, 2019), making the resulting meal higher in oil and lower in protein concentration compared to other extraction methods. During the extrusion process heat is generated from the friction which reduces some antinutritional factors (ANF) (Gaonkar & Rosentrater, 2019), but the Maillard reaction caused by the high heat destroys amino acids and reduces their bioavailability (Žilić *et al*., 2012).

2.2.2 Solvent Extraction Method

The solvent extraction method is the most common method of obtaining oil from soybeans (The extraction process is depicted in Figure 4). Prior to the solvent process, seeds are dried, cracked, roasted and flaked, some manufacturers also dehull the seeds and may or may not add them back at the end of the process (Heuzé *et al.*, 2020), mostly to standardize the meals to the protein guarantees (Lusas, 2004). Commercial-grade hexane is the solvent typically used for the oil extraction process of soybeans (Lusas, 2004). In this process, the flaked seeds are mixed with the solvent in the extraction machine (called "the extractor"). After the flakes are drained from the solvent and exit the extractor, they are moved to another machine to get *desolventized*. \In this step, the remaining solvent is removed in a vacuum with heat added through steam injection. The heat applied in this process reduces the activity of ANFs (Lusas, 2004). This procedure is highly efficient and comes at a low cost (Sawada *et al*., 2014), allowing recovery of over 95% of the oil from the seed, making for a meal high in protein content (Gaonkar & Rosentrater, 2019).

Figure 4. Simplified Flowchart of the Process of Oil-Extraction from Soybeans[a].



[a] Adapted from Soybean Meal by Heuzé *et al*. (2020).

2.3 Soybean Meal

Soybean meal is the product obtained after the extraction of oil from the soybean seed by pressure and heat or a solvent process where its hull is commonly removed to improve nutrient digestibility (McDonald *et al.*, 2011). This processing leads to a high protein meal, which on average yields 77.2 kg of soybean meal per 100 kilograms of crushed soybean seeds

(Kinney & Clemente, 2010). Soybean meal has been considered one of the main sources of protein in the livestock industry, with 260.3 million tonnes produced globally in 2021 (SOPA, 2023). In that same year, the global soybean meal market was valued at US$59.2 billion and is projected to grow to US$94.2 billion by 2031 (AMR, 2023).

2.3.1 Soybean Meal in Animal Nutrition

This feedstuff is an excellent source of protein with 40-49% crude protein content in commercially available sources (Banaszkiewicz, 2011). One of the shortcomings of soybean meal is the presence of ANFs in its composition, such as trypsin inhibitors, lectins and saponins which may cause low digestibility and reduced growth in animals if the meal is not heat-treated (McDonald *et al.*, 2011).

Crude protein makes up between 18 and 23% of the total nutrients in the diet of a commercial broiler chicken (Aviagen, 2019, NRC, 1994), making soybean meal a very desirable protein source in chickens with 44-50% crude protein as-is, depending on the type of processing (Heuzé *et al.*, 2020) and a balanced amino acid profile, with the first limiting amino acid being methionine (Table 2). Although soybean meal is a poor source of B complex vitamins (McDonald *et al.*, 2011), it's usually the main protein ingredient in feed for monogastric animals because of the many qualities it has.

Table 2. Chemical Composition of Soybean meal, Type 46[a].

	SBM, Type 46[b] as-fed basis, %	**SBM, Type 46[b]** DM basis, %
Dry matter	87.6	87.6
Crude protein	43.3	49.4
Fat	1.7	1.9
Ash	6.5	7.4
Neutral Detergent Fibre (NDF)	12.4	14.2
Starch	0.0	0.0
Total sugars	8.5	9.7

[a] Adapted from Tables of Composition and Nutritional Value of Feed Materials. Sauvant *et al*. (2004).
[b] Commercial category, which should contain the crude protein amount (% as-fed basis) in their name.

2.3.2 Chemical Composition of Soybean Meal

Soybean meal has been regarded as a very complete source of protein for broiler chicken since it contains all the amino acids needed by poultry. However, methionine and cysteine are present in insufficient amounts (McDonald *et al.*, 2011). Considering that methionine is the first limiting amino acid (essential amino acid found in the smallest amount in relation to the requirements needed by the animal to synthesize protein), this can be an issue with the diets of high-performance broilers.

Commonly soybean seeds consist of 8% of hull, 90% cotyledon and 2% germ. After removing the hull, there is 40% protein, 30% carbohydrates, 20% oil, 6% water, and 4%

minerals. Meanwhile, after extracting the oil, about 48% protein, 35% carbohydrates, 10% water and <2% of other components are left. (Choct *et al*., 2010).

There are also a number of chemical components in the meal that can be detrimental in the digestibility of nutrients by poultry, for instance trypsin inhibitors, and some oligosaccharides and non-starch polysaccharides. High levels of SBM in the diet have been linked to an increase in pododermatitis (feet lesions) in chicks, as well as sticky droppings from high levels of potassium in the meal (NRC, 1994).

2.3.2.1 Carbohydrates

While soybean meal has traditionally been used as a protein ingredient in the diets of livestock, its carbohydrate content, which can reach as high as 40% of the meal (NRC, 2012), has been widely overlooked. The main types of carbohydrates in soybean meal are non-starch polysaccharides (NSP) and free sugars (monosaccharides, disaccharides and oligosaccharides) (Choct *et al*., 2010) with 20-30% and 10% concentration respectively (Macrae *et al*., 1993). These compounds need specific enzymes to be properly digested and monogastric animals lack them for the most part, therefore they need microbial degradation in the hindgut to be able to utilize their by-products (Choct *et al*., 2010). The concentration of NSP in soybean meal is known to vary depending on the geographical location of origin (Karr-Lilienthal *et al*., 2005), but genotype, processing of soybeans and type of analysis of the samples can also influence the results (Choct *et al.*, 2010).

For poultry, soy NSP are known to increase digesta viscosity causing decreased performance and wet droppings (McDonald, 2011). This effect is correlated with the amount of NSP added

in the diet (Choct & Annison, 1990). As the passage of the digesta through the gut of chickens is quite fast, the microbiota is limited in their capability to degrade all the NSP present in the digesta (Choct *et al.*, 2010). High NSP content is also known to cause gut diseases in poultry, such as necrotic enteritis (Kaldhusdal & Hofshagen, 1992). Moreover, NSP have the distinctive feature of binding and capturing other nutrients in the small intestine, therefore reducing their digestibility and absorption; the affected nutrients include lipids, proteins, starch, vitamins, and minerals that in turn form fibrous complexes in the lumen of the intestine (Wu, 2018). While large quantities of NSP become an antinutritional factor, adequate amounts are known to promote gut health by providing short-chain fatty acids via degradation throughout the small and large intestines, providing energy to the hindgut microbiota (Wu, 2018).

Oligosaccharides are carbohydrates made of 3 to 10 sugars. These polymers make up 5-6% DM of soybean meal (Liying *et al*., 2003), and they include compounds like stachyose, raffinose and verbascose. While oligosaccharides in chicks have been demonstrated to help with inhibiting pathogenic bacteria in the gut and improve immune status (Wang *et al*., 2003), stachyose and raffinose have shown negative effects in the gut of monogastrics due to their low digestibility making for soft digesta and loose stool (Wu, 2018). Considering the issues with the presence of NSP and oligosaccharides in soybean meal, it is important to be aware of means to mitigate such negative effects.

While endogenous enzymes which degrade these carbohydrates are not abundant in poultry, newly hatched chickens have increased activity of gastrointestinal carbohydrase enzymes in the apical membrane of their enterocytes. Combined with the grinding capabilities of the

gizzard, this allows them to eat corn-soybean meal diets right after hatch (Wu, 2018). Other ways to alleviate the negative effects of these polymers is the use of exogenous enzymes. A study by Meng & Slominski (2005), showed that soybean meal contained 136.7 mg/g of NSP (compared to 76.3 mg/g NSP for corn). Although supplementing with multi-carbohydrase did not affect growth performance significantly, the AME_n content was increased and the digestibility of NSP and protein was improved. Carbohydrases and proteases by themselves or in combination are commonly used as feed additives in the diets of poultry to benefit from the decrease of digesta viscosity caused by NSP and oligosaccharides found in soybean meal (Wu, 2018).

2.3.2.2 Proteins and Amino Acids

The protein content of commercial soybean meal is usually standardized to 44% or 48%, adding soy hulls to the SBM to achieve the standardized CP value (Elkin, 2002). The amino acid profile is excellent when combined with a grain like maize, making methionine commonly the sole limiting amino acid of this formulation (Leeson & Summers, 1997) as maize is generally deficient in lysine and SBM has a fair concentration of it (Dale, 1996).

The protein quality of a soybean meal is affected by many factors, such as bean variety, origin, and storage, as well as the characteristics of processing such as moisture, temperature and time to achieve appropriate cooking conditions (Barros-Dourado *et al.*, 2011).

There are different methods to assess protein quality *in-vitro*. These techniques include but are not limited to: trypsin inhibitor analyses, urease activity (urease index), and protein solubility in potassium hydroxide (KOH) (Barros-Dourado *et al.*, 2011). The urease index

assessment relies on the urease enzyme in the soybean meal, which is not relevant in poultry nutrition but is a thermolabile compound like some ANF in the meal (e.g. trypsin inhibitors). As this assay is less complex than trypsin inhibitor analyses, the urease index is commonly used as an indirect marker for ANF (Soybeans: Quality control, n. d.). The KOH protein solubility analysis in soybean meal is inversely related to the level of heat treatment; for raw SBM flour the solubility value is near 100%, while for over-cooked meals the solubility can be as low as 40-30% (Soybeans: Quality control, n. d.). Protein solubility below 59% results in a linear drop in feed efficiency in broiler chicks (Parsons *et al.*, 1991) and Araba & Dale (1990) found that when the protein solubility is below 70%, the SBM is considered over processed and reflects a considerable decrease in lysine availability. There is no specific number for protein solubility, but an acceptable range for growing chicks is 78-84% protein solubility, and 84-89% could be potentially used for laying hens and older broiler chickens as they are less susceptible to ANF (Soybeans: Quality control, n. d.).

Protein is digested into dipeptides and amino acids to facilitate digestion by animals. In poultry, the crop, proventriculus, gizzard, pancreas and small intestine take part in the protein digestion and absorption process (Wu, 2018). The gut pH plays an important role in the digestion of proteins. In the proventriculus (glandular stomach of birds), HCl and pepsin are produced, combined with a low pH of 1.8, the denaturation of dietary proteins begins (Dozier & Hess, 2011). The pancreas supplies important enzymes to the small intestine: trypsin, chymotrypsin, proelastase, and carboxypeptidase, breaking down polypeptides into oligopeptides (Alpers, 1994), which are then absorbed into the enterocytes by AA and protein transporters (Wu, 2018).

As previously mentioned, soybean meal has an ideal AA profile for poultry nutrition, as shown in Table 3. Methionine is the limiting AA, but combined with maize, which has a concentration of 1.7 g/kg (Sauvant *et al.*, 2004), the diet for broilers can be balanced with the supplementation of commercial AA.

Table 3. Amino acid Composition of Soybean Meal Type 46[a] and Broiler Chicken Total Amino Acid Requirements in Grower Phase (g/kg, as-fed basis)[b]

Amino acid	**SBM, Type 46**	**Broiler Chicken Grower Requirements (g/kg)**
Indispensable Amino Acids		
Arginine	32.0	13.7
Histidine	11.5	3.2
Isoleucine	19.9	8.9
Leucine	31.9	14.2
Lysine	26.6	12.9
Met + Cys	12.7	9.9
Phenylalanine	21.7	6.5
Phe + Tyr	36.3	12.2
Threonine	17.0	8.8
Tryptophan	5.6	2.1
Valine	20.8	10.0
Arginine	32.0	13.7
Dispensable Amino Acids		
Alanine	19.0	-
Aspartic acid	49.0	-
Cysteine	6.5	-

Table 3. Amino acid Composition of Soybean Meal Type 46[a] and Broiler Ross 308 Total Amino Acid Requirements in Grower Phase (g/kg, as-fed basis)[b] (*Continued*)

Amino acid	SBM, Type 46	Broiler Chicken Grower Requirements (g/kg)
Glutamic acid	77.1	-
Glycine	18.1	-
Proline	21.6	-
Serine	21.8	-
Tyrosine	14.6	-

[a] Adapted from Tables of Composition and Nutritional Value of Feed Materials by Sauvant *et al.* (2004).
[b] 11-24 Days, as-hatched. Target weight 2.5-3.0 kg. Ross 308 Requirements, Aviagen (2019), NRC (1994).

2.3.2.3 Lipids

As an oilseed, lipids in the soybean seed are one of the main reasons this crop was developed over the years. Soy fats are present in the cotyledon at a concentration of about 20% (Choct *et al.*, 2010) and the main fatty acids found in the soybean are palmitic, stearic, oleic, linoleic and linolenic fatty acids (Gerde & White, 2008).

Considering soybean meal is the product of the extraction of oil from the seed, the oil content is often quite low for solvent-extracted meal with about 1.5% of fat in the meal (Heuzé *et al*., 2020), or a much higher 10-20% for expeller-pressed soybean meals (Gaonkar & Rosentrater, 2019). Chicken diets usually contain 5%, 6% and 8% of as-fed oil content for starter, grower and finisher diets respectively (Wu, 2018). The main purpose of adding oil to a diet is to increase palatability, to add lubricant or improve pelleting in feed manufacturing and to reduce dust in feed production, all this while also packing energy in the animal diet.

2.3.2.4 Antinutritional Factors

Antinutritional factors are phytochemicals in the feed ingredients that negatively affect either the feed intake or feed utilization by the animal (Fuller, 2004). These compounds are made by plants as a defense mechanism against pathogens or predators (McDonald *et al*., 2011).

The soybean ANFs can be divided into two categories, thermolabile (protease inhibitors, lectins, goitrogens, and antivitamins) and thermostable (saponins, tannins, flatulence factors and phytate) (Wu, 2018; Liener, 1994). These categories are important to soybean meal as the beans have to undergo processing that includes different levels of heating, which may or may not inactivate thermolabile ANF.

Protease inhibitors are compounds that impair the proteolytic activity of enzymes. In soybeans there are two types of this ANF: the first targets trypsin and the second targets chymotrypsin and trypsin at different active sites in the enzymes (Liener, 1994). The mode of action of these ANFs relies on an "enzyme-substrate" complex, whereas, unlike a normal complex, they do not result in products and enzymes but stay mostly bound together, inhibiting further activity of the enzyme (Liener, 1994).

As thermolabile compounds, they are inactivated with heat, but the duration, time, temperature and moisture of the samples will cause the results to vary (Vagadia *et al*., 2017). Although roasting is the most commonly used method to inactivate trypsin inhibitors, it only deactivates up to 85% of these ANFs (Vagadia *et al*., 2017). Other methods within heat treatment are boiling, autoclaving and microwaving. These types of processing are highly efficient and standardized and while they also decrease some NSP, there are disadvantages

such as a decrease in lysine, tryptophan and sulfur-containing amino acids, mineral losses, and a reduction in B vitamins (Avilés-Gaxiola *et al*., 2017).

Extrusion is another common processing method to reduce protease inhibitors. There are several studied methods that reduce these compounds, such as ultrasound, instant controlled pressure drop, infrared radiation, and gamma radiation, among others (Avilés-Gaxiola *et al*., 2017). Another way to reduce protease inhibitors is by pre-treating the soybean with exogenous enzymes. A meta-analysis by Cowieson & Roos (2014) reported an increase of 2.7 to 5.4% of ileal amino acid digestibility with the addition of exogenous proteases. The authors reached the conclusion that proteases are the most effective when the digestibility of the amino acids in the diets is low. They discovered that for every 10% decrease of ileal amino acid digestibility, the efficacy of exogenous proteases double (Cowieson & Roos, 2014).

Although lectins have been given lesser attention as an ANF of soybeans, their effect can be quite detrimental to the growth performance of monogastrics. These compounds are proteins (also regarded as hemagglutinins) that bind to the lining of epithelial cells in the small intestine, reducing nutrient absorption by the gut (McDonald *et al*., 2011). The presence of lectins has also shown to decrease the immune response in animals, suggesting that lectins encourage colonization by coliforms and that 50% of the growth-retardation effect of raw soybean meal is attributed to these compounds (McDonald *et al*., 2011). Similarly to trypsin inhibitors, heat treatment destroys the hemagglutinins; however it is important to note that adding moisture to the heat process greatly increases the inactivation of these compounds as opposed to using dry heat (Liener, 1994).

The goitrogenic factors in soybeans have not yet been determined (Liener, 1994), however it has long been reported that soybeans that have not been heat-treated possess the ability to either disrupt the uptake of iodine by the thyroid, increase loss of thyroxine in the feces or induce iodine deficiency in animals (Liener, 1994). However, the effects of these ANFs can be removed with heat treatment.

Raw soybean meal contains thermolabile antivitamins such as lipoxygenase, which oxidizes and destroys carotene (Vitamin A), it has also been suggested that the increase in α-tocopherol (Vitamin E) requirements in chicks following the feeding of soybean meal could be due to the presence of α-tocopherol oxidase in the meal. There have also been reports of an increase of vitamin B_{12} requirements when soybean meal is added to the diet, but the causing agent of this effect is still unknown (Liener, 1994).

Saponins are phytochemicals characterized by hemolytic and foaming properties (Liener, 1994). High concentrations are known to add a bitter taste and astringency to feed (Liener, 1994). Although most saponins are considered an antinutritional factor in general, soybean saponins (soyasapogenols) are mostly benign in nature, even when fed at three times the concentration of soybeans (Gestetner *et al*., 1968).

Tannins are polyphenolic compounds known to form complexes with proteins under specific pH conditions (McDonald *et al*., 2011), damage the intestinal mucosa, as well as disrupt the absorption of iron, glucose and vitamin B_{12} (Liener, 1994). Tannin content is dependent on the color of the coat of the seed: lighter colored seeds carry a lower concentration of these compounds, explaining the lower levels of tannins in soybeans and making it a minor concern as an ANF in soybean meal when the hull has been removed (Liener, 1994).

"Flatulence factors", named for their flatulence effect in human nutrition, include low molecular weight oligosaccharides like raffinose and stachyose. As mentioned earlier in section 2.3.2.1 Carbohydrates, these oligosaccharides are not digested in the small intestine and they enter the large intestine to be broken down by the resident microbiota, thus generating gases like CO_2, H_2 and methane. Although the response to these compounds varies greatly from person to person, symptoms like nausea, diarrhea, abdominal rumbling, and passing gases may occur. (Liener, 1994). On the other hand, the concern about these ANFs in animal nutrition, especially for poultry, lies in the fact that high contents of α-galactose-containing oligosaccharides (stachyose and raffinose) in the diet produce an osmotic effect in the gut and a coating of nutrients, which could explain the decrease in absorption (Choct *et al.*, 2010).

A broiler chick study by Baker *et al*. (2011) reported better amino acid digestibility in soybean meal with a low content of oligosaccharides in comparison with conventional SBM. A more recent study in pigs developed a prediction model to estimate the apparent ileal digestibility (AID) and standardized ileal digestibility (SID) of amino acids based on stachyose and raffinose content, with R^2 of over 70% for some amino acids (Li *et al*., 2017), suggesting that some of the reduction in amino acid digestibility is due to the negative effects of these oligosaccharides. For this type of ANF, exogenous enzymes have been used as a method to mitigate their negative effects.

Most carbohydrate-hydrolyzing enzymes work in two ways: the first is by preventing the encapsulation of nutrients so they are readily available to be broken down by endogenous enzymes, and the second is by decreasing the digesta viscosity (Cowan *et al*., 2002). The

positive effect of carbohydrases has shown an increase in AME_n (Cowan *et al*., 2002), N, DM, and P (Cowieson & Adeola, 2005) digestibilities in broiler chickens.

Phytate (phytic acid) is a cyclic compound containing six phosphate groups. In soybeans, 40-60% of the phosphorus is tied to a phytate molecule (Liener, 1994), making this element unavailable to the animals. Phytate is also known to chelate calcium, magnesium, zinc, and iron, forming compounds that are difficult to absorb while also decreasing the effectiveness of endogenous enzymes (Liener, 1994). The most common way to break down phytate in animal diets is by adding exogenous enzymes (phytases). Studies have shown that the addition of phytase increases growth performance, but careful attention must be paid to levels of P, Ca and dietary electrolyte balance as it could be disrupted due to the surge in P from the broken down phytate (Selle & Ravindran, 2006). A study by Cowieson & Adeola (2005) in broiler chicks showed an additive effect of supplementing a marginally low-nutrient diet with multi-carbohydrases (xylanase and amylase) with phytase in the digestibility of digestible energy, N, DM, Ca, and P, suggesting a benefit in adding phytase to increase the nutrient digestibility of lower cost diets. There have also been studies showing improved trace mineral utilization by broiler chickens after the supplementation with phytase (Schöner & Hoppe, 2002).

2.4 Protein Digestibility

The concentration of nutrients in feed ingredients is important when it comes to choosing the feedstuffs to be used to formulate a diet, but it is essential to know how digestible these

ingredients are, especially for proteins which are the building blocks of animal growth. Digestibility is defined as the portion of nutrients in the ingested diet that is absorbed by the animal. Since it does not measure the nutrients in the feces, it can be measured with Equation [1]; where AA_{diet} is the amino acid present in the formulated diet and $AA_{excreta}$ is the amount of AA found in the feces (Lemme *et al.*, 2004). The digestibility of a feedstuff is not constant; it can vary by the method of feeding or by animal factors like age, sex, or physiological stage (Fuller, 2004).

Eq. 1

$$\text{Digestibility (\%)} = \left(\frac{AA_{diet} - AA_{excreta}}{AA_{diet}}\right) \times 100$$

Although a protein ingredient has a balanced AA profile, if the peptides cannot be hydrolyzed by the endogenous enzymes, then the gut will not be able to utilize the resources (Wu, 2018). In light of this, it is crucial to undertake biological studies alongside chemical analyses in order to assess the *in vivo* nutritive value of AA in a given feed ingredient (Wu, 2018).

2.4.1 Ileal Amino Acid Digestibility

There are several studied methods for analyzing amino acid digestibility *in vivo*, older studies used excreta AA digestibility using precision feeding assay developed by Sibbald (1979), however the disadvantages of this method lie in the fact that the excreta AA include both fecal and urinary AA. Additionally, this method ignores the effect of hindgut microbiota on protein digestion and their contribution to fecal AA profiles (Lemme *et al*., 2004). These

limitations of the precision feeding assay were successfully addressed by employing caecectomized roosters (Parsons, 1986). The method used nowadays stems from Ravindran & Bryden (1999) where the test ingredient is formulated along an indigestible marker with a nearly 100% recovery rate (e.g. chromium oxide, titanium dioxide). Then, digesta is collected from the ileum of humanely euthanized birds, from the vitelline diverticulum (Meckel's diverticulum) to a few centimetres proximal to the ileocecal junction (Lemme *et al*., 2004). Equation 2 is used to calculate the apparent ileal digestibility coefficients, with M_{diet} being the marker in the diet and $M_{digesta}$ the marker recovered from the digesta (Lemme *et al*., 2004; Adeola *et al*., 2016)

Eq. 2

$$\text{AID } (\%) = 100 - \left(\frac{M_{diet} - AA_{digesta}}{M_{digesta} \times AA_{diet}}\right) \times 100$$

Given that a marker is used in the diet, the quantification of the feed intake is not necessary for the measurement of digestibility and the feed can be offered *ad libitum* (Lemme *et al*., 2004). This method usually uses the test ingredient as the sole source of AA, but it is known that the results are an underestimation of the *true* digestibility, as due to the nature of the test diet there are endogenous AA excreted into the lumen and not reabsorbed (Lemme *et al*., 2004; Adeola *et al*., 2016). In light of this, it is increasingly important to account for the endogenous AA losses to obtain reliable digestibility results.

2.4.1.1 Apparent, True and Standardized Ileal Digestibilities

Depending on the *in vivo* method to measure digestibility, the coefficients may yield results for apparent, standardized or true ileal digestibility. The term "apparent ileal digestibility" refers to the percentage of a nutrient that is digested and absorbed by the animal, this value considers the endogenous losses (e.g. enzymes, mucin) (McDonald *et al*., 2011; Lemme *et al.*, 2004), (see Equation 2). "True ileal digestibility" (TID) and "standardized ileal digestibility" (SID) are similar in nature, where in the TID the *total* endogenous losses of amino acids are corrected for (see Equation 3) (Adeola *et al.*, 2016), whereas the SID takes into account a correction for *basal* endogenous losses (see Equation 4) (Adeola *et al*., 2016).

Eq. 3

$$\text{TID}\ (\%) = \text{AID}\ + \left(\frac{\text{IAA}_{\text{end}}}{\text{AA}_{\text{diet}}}\right) \times\ 100$$

Eq. 4

$$\text{SID}\ (\%) = \text{AID}\ + \left(\frac{\text{Basal IAA}_{\text{end}}}{\text{AA}_{\text{diet}}}\right) \times\ 100$$

Where IAA_{end} stands for the ileal AA endogenous losses (Adeola *et al*., 2016). In a recent review study by Ravindran (2021), the author suggests to use the term "true" and "standardized" ileal digestibility interchangeably to avoid confusion to the end users, considering that both methods take into account the basal endogenous amino acid losses.

2.4.1.2 Endogenous Amino Acid Losses

Endogenous AA losses are the normal losses of AA due to metabolic processes. The sources include: saliva, bile, gastrointestinal secretions, gut epithelial cells and possibly gut bacteria

(Wu, 2018). Different methods can be used to measure the IAA_{end}. These include a nitrogen-free diet, a fasting method, and a highly digestible protein diet, among others (Adeola *et al*., 2016). The aforementioned analyses are both expensive and difficult to carry out, therefore the SID is the preferred method nowadays. For this method, we need to estimate the Basal IAA_{end} (Equation 5) (Adeola *et al*., 2016).

Eq. 5

$$\text{Basal } IAA_{end} = AA_{ileal} \times \left(\frac{M_{diet}}{M_{ileal}}\right)$$

There are concerns regarding the use of nitrogen-free diets for estimating endogenous AA losses in animals, particularly in relation to their physiological status. This arises from the understanding that when there is a deficiency of AA, the body resorts to the degradation of its own proteins to sustain essential bodily functions (Wu, 2018; Adeola *et al*., 2016).

2.5 Broiler Chicken Consumption

Poultry is one of the most consumed meats globally, along with pork (Hendriks *et al.*, 2019). The OECD and FAO predict that by 2030 poultry meat will represent 41% of all the protein from meat sources (OECD/FAO, 2021). It is also predicted that the world population will increase to 9 billion by 2050 and 60% more food will be needed as a result (Hendriks *et al.*, 2019).

2.5.1 Chicken Consumption in Canada and Manitoba

In Canada, chicken is the number one protein choice. It is considered by Canadians to be a lean, healthy, and nutrient dense source of protein, its consumption increases every year, and it is currently at its highest level in history (Manitoba Agriculture, 2022a). In 2020, poultry consumption accounted for 34.4 kg per person, up by 2.8 kg from 2015 (Manitoba Agriculture, 2022a). Manitoba is currently self-sufficient in poultry meat and egg production (Manitoba Agriculture, 2022b).

2.5.2 The Role of Soybeans in Manitoba's Economy

Soybeans are one of the main sources of protein for livestock, and about 25% of all soybean meal is used as feed in poultry production (Kinney & Clemente, 2010). In 2022, Manitoba produced 21.5% of all soybeans in Canada, and soybeans were the third largest seeded area in Manitoba that year, following canola and wheat (Statistics Canada, 2023a). However, Manitoba exports about 90% of all soybeans produced to the US, Japan, Singapore, and Malaysia, and crushes merely 10% of its soybean production in three crushing plants in the province (MB Agriculture, 2022c).

2.6 Exploring Manitoba Soybean Meal for Broiler Chickens

While soybean meal is commonly used as a protein ingredient in poultry diets due to its high protein content and balanced amino acid profile, there is limited information available specifically on the nutritive value of Manitoba-grown soybean meal and its suitability as a protein source in broiler nutrition.

Considering the higher reported protein values associated with the eastern variety of soybean meal, particularly that from Ontario, our study hypothesized that the amino acid digestibility of Manitoba soybean meal would be comparable to that of Ontario. This hypothesis suggests that Manitoba soybean meal exhibits a similar level of nutritive performance to Ontario soybean meal, positioning it as a potential alternative in terms of amino acid availability and overall nutritional quality for broiler chickens.

The gap identified in this study revolves around the need for a better understanding of the digestibility and protein quality of Manitoba-grown soybean meal, as well as the identification and mitigation of factors that may affect its digestibility. By addressing these gaps, we can provide valuable guidance to Manitoba poultry producers on the use of locally grown soybean meal as a cost-effective and sustainable protein source for broiler nutrition.

3. Thesis Hypothesis and Objectives

3.1 Hypothesis

Soybean meal from Manitoba-grown soybeans has equivalent chemical composition, nutritive value, and standardized ileal amino acid digestibility in broiler chickens compared to the eastern (Ontario) variety.

3.2 Objectives

1. To characterize the chemical composition and nutritive value of soybean meal from Manitoba-grown soybeans.
2. To determine the standardized ileal amino acid digestibility of Manitoba soybean meal in broiler chickens.

4. Evaluation of the Chemical Composition and Nutrient Digestibility of Manitoba Soybean Meal in Broiler Chickens

4.1 Introduction

Soybean meal (SBM) is a widely recognized and commonly used protein ingredient in poultry diets due to its balanced amino acid profile and high protein content. The availability and quality of SBM can vary depending on factors such as the oil extraction process, geographic origin, and the antinutritional factors present. In this study, we aimed to evaluate the suitability of Manitoba soybean meal as a protein source for broiler chickens, focusing on its amino acid digestibility and potential contribution to local poultry production.

The use of locally sourced ingredients in animal feed formulations offers numerous advantages, including reduced reliance on imported feed ingredients, cost savings, and support for local agricultural economies. Manitoba is a major soybean-producing region in Canada and has the potential to provide a significant portion of the protein requirements for the poultry industry within the province. However, a comprehensive assessment of the nutritional value and digestibility of Manitoba soybean meal is essential for determining its suitability and optimal utilization in broiler diets.

The objective of this study was to evaluate the amino acid digestibility of expeller soybean meal derived from seeds grown in Manitoba and compare it with the control counterpart from seeds grown in Ontario and other SBM varieties reported in the literature. Specifically, research focused on the standardized ileal amino acid digestibility (SIAAD) of Manitoba

SBM, which reflects the proportion of amino acids available for absorption by the birds' digestive system.

Understanding the digestibility characteristics of Manitoba soybean meal is crucial for formulating diets that meet the nutritional requirements of broiler chickens. Furthermore, it allows us to identify any potential limitations or factors that may affect the utilization of this protein source, enabling us to provide more precise recommendations to local poultry producers.

In this study, the chemical composition was analyzed and a digestibility study was conducted to assess the SIAAD of Manitoba soybean meal. The results of this study will contribute to our understanding of the protein quality and digestibility of Manitoba SBM and aid in optimizing its utilization in broiler chicken nutrition. The aim of this work is to provide a thorough assessment of Manitoba soybean meal as a protein source for broiler chickens, considering its amino acid digestibility and potential implications for local poultry production. By describing the digestibility characteristics of Manitoba SBM and comparing it with literature findings, we can offer informed recommendations to optimize its utilization and enhance the sustainability of the poultry industry in the region.

4.2 Materials and Methods

This study consisted of two parts: a chemical analysis of the soybean meal samples and a subsequent nutrient digestibility animal trial to determine the standardized ileal digestibility of the soybean meals.

The digestibility study was reviewed and approved by the Animal Care Committee from the University of Manitoba (Winnipeg, Canada) with the protocol #F22-007. The experiment took place in the Small Animal Research Facility in the Animal Science department of the University of Manitoba. The birds were cared for as stated in the standard operational procedures of the facility which are in accordance with the Canadian Council for Animal Care Guidelines (CCAC, 2009).

4.2.1 Ingredients

Three (3) expeller-pressed Manitoba soybean meal samples (named *A*, *B*, and *C*) were sourced from a local soybean crushing plant (Delmar Commodities, Winkler, MB, Canada) and one (1) expeller-pressed soybean meal sample from Ontario (named *D*) was sourced from a crushing plant in Cambridge, Ontario (Grand Valley Fortifiers, Cambridge, ON, Canada) to serve as a positive control in the chemical analyses and digestibility study. The conditions of the processing of these soybean meals were not disclosed, but were informed that hulls were included.

4.2.2 Digestibility Study Design

This type of study consists of feeding test ingredients as the sole source of the nutrient of interest to a group of animals for a short period of time. In our study the indicator method was followed, which means an indigestible marker was added to the test diets to quantify the digestibility of the nutrients. To measure the digestibility of these nutrients, it was decided to take into consideration the basal endogenous losses of the nutrients to obtain a standardized ileal digestibility coefficient. For this, a nitrogen-free diet (NFD) was included in the study to better measure the digestibility of the soybean meals.

The study was designed as a completely randomized design with 4 treatments (test diets) and 10 replicates (replicate cages) each, where the experimental unit was the cage. In this design, the treatments were randomly assigned to the replicates to ensure fairness and minimize biases. Additionally, an NFD was included as a *treatment* for practical purposes, however, it is important to note that the results of the NFD are not intended for statistical analysis like the other treatments. The NFD consisted of 24 replicates as according to Dr. Rogiewicz (personal communication, 2022), the nature of an NFD leads to very low digesta contents in the ileum of birds. To account for this, a higher number of replicates was used to allow for randomly pooling digesta samples from different cages if necessary, after sample collection.

4.2.3 Birds and Housing

A total of 320 day-old as-hatched Ross 308 broiler chickens were purchased from a local hatchery (Dunn-Rite Food Products Ltd, Winnipeg, MB, Canada) and placed on battery cages (“Poultry Super Brooder” by Alternative Designs Manufacturing & Supply, Inc., Siloam

Springs, United States) (Figure 5). The chickens were distributed in 64 pens with 5 birds in each pen. Each test diet had 10 replicate cages with the NFD having 24 replicate cages.

The light program was set as per the standard of the breed, as well as the temperature, which was set to 30°C for the room and 32°C for the heated area in the cages for day 1. The temperature was gradually decreased according to the standard, reaching 23°C in the room and 24°C in the heated area of the cage by day 19. Water was supplied through a nipple drinker system and was offered ad libitum. For the first week chicks were manually encouraged to drink water and to find the feed on sheets of paper on the grid of their pens with some feed on top (Figure 6); feed was dispensed in feed troughs at the same time. After the first week, feed was only dispensed on the manually-filled feed troughs with ad libitum availability. The health of the birds was checked twice daily by visual inspection throughout the experiment. Birds were also weighed weekly at random to ensure weight gain according to the breed.

Figure 5. Poultry Super Brooder. Each unit had 16 pens. Four of these units were used to house all 320 chicks.

4.2.4 Diets

During the initial 14-day period of the animal study, the birds were provided with a commercial starter diet (Table 4) to facilitate gut adaptation. This diet was formulated as a standard corn-soybean meal diet that met or exceeded the nutritional requirements of the breed (Aviagen, 2019).

Figure 6. Bird access to feed**.** During the first week, birds had access to feed from a sheet of paper on their cage to encourage feed consumption (left), as well as from the feed trough (right).

After the adaptation period, the test diets were given to the animals for five days. The test diets were formulated with the Manitoba and Ontario soybean meals as the only source of nitrogen in the diet, as presented in Table 4. The diets were formulated to meet or exceed the dietary requirements of Ross 308 (Aviagen, 2019). For all test diets, the vitamin premix was mixed by hand to avoid the inclusion of methionine in the formulation. Sucrose and cornstarch were chosen as the complimentary sources of energy in the test diets to specifically avoid the inclusion of any other ingredients containing nitrogen. Similarly, the

NFD was also formulated using sucrose-cornstarch as the foundation. Additionally, soy oil was added to the NFD as a binding agent and cellulose was incorporated as a source of fiber to add bulk and provide a thicker consistency to the digesta. This formulation aligns with the recommendations proposed by Adeola *et al*. (2016) on the measurement of basal endogenous losses in poultry.

Table 4. Composition of diets (%, as-fed basis) used in the experiment.

		Manitoba			Ontario	
Ingredients	**Starter**	**A**	**B**	**C**	**D**	**N-free**
Corn	55.9	-	-	-	-	-
Cornstarch	-	10	10.25	9.77	12.05	20
Sucrose	-	30.44	30.47	29.55	34.78	63.85
Soy oil	5.2	-	-	-	-	5
Fishmeal	3	-	-	-	-	-
Soybean meal	31.35	55	54.72	56.15	48.55	-
Cellulose	-	-	-	-	-	5
Calcium carbonate	1.35	0.94	0.94	0.93	0.92	0.95
Di-calcium phosphate	1.1	1.82	1.82	1.8	1.9	2.4
Mineral premix	0.5	0.5	0.5	0.5	0.5	0.5
Vitamin premix	1	1	1	1	1	1
L-lysine	0.26	-	-	-	-	-
DL-Methionine	0.17	-	-	-	-	-
Threonine	0.17	-	-	-	-	-
Potassium carbonate[a]	-	-	-	-	-	0.6
Potassium chloride[a]	-	-	-	-	-	0.1
Magnesium oxide	-	-	-	-	-	0.3
Titanium dioxide	-	0.3	0.3	0.3	0.3	0.3
Calculated composition						
CP	23	21.5	21.5	21.5	21.5	-
ME, kcal/kg	3050	3100	3100	3100	3100	3100
Methionine	0.56	0.51	0.51	0.51	0.51	-
Lysine	1.28	1.29	1.29	1.29	1.29	-

Table 4. Composition of diets (%, as-fed basis) used in the experiment. (*Continued*)

Ingredients	Starter	Manitoba		Ontario		N-free
		A	B	C	D	
Analyzed composition						
DM	91.47	95.57	95.39	95.38	94.87	97.32
CP	23.05	20.45	21.25	22.05	22.1	0.17
NDF	8.68	6.62	6.34	7.36	5.48	5.20
Methionine	-	0.83	0.73	0.72	0.87	0.00
Lysine	-	2.56	2.60	2.54	2.90	0.00

[a] $(Na^+ + K^+) - Cl^-$ milliequivalency value is 104.

Potassium carbonate and potassium chloride were added to the NFD to establish an electrolyte balance between Na, K, and Cl. These additives were chosen to achieve and maintain the proper ratio of electrolytes necessary for optimal physiological functioning of the birds (NRC, 1994). In order to assess the digestibility of nutrients, titanium dioxide was incorporated into all treatments as an indigestible marker at an inclusion rate of 0.3% across all treatments.

On day 15 of the study, all 64 pens were assigned to one of the experimental diets randomly, allocating birds by uniform mean weight between pens. The birds had *ad libitum* access to the test feed and water throughout the trial period as well as the rest of the experiment. On day 19 of the study, all birds were euthanized by CO_2 asphyxiation to collect ileal digesta.

4.3 Sample Preparation and Chemical Analyses

Birds were euthanized in a CO_2 chamber as a group per pen to avoid mixing birds from different treatments. Ileal digesta was collected from the birds immediately after culling. The ileal contents were obtained by gently flushing with a phosphate-buffered saline (PBS) solution as the pH and ionic composition of PBS closely resembles physiological conditions, making it a suitable choice for maintaining the integrity of the tissue and minimizing any potential alterations in the samples during the flushing procedure (AAT Bioquest, 2023). Digesta was collected from terminal part of the ileum, specifically from the Meckel's diverticulum until 2 cm above the caecal junction and then pooled per cage in a plastic bag. Digesta samples were frozen immediately at -20°C and then freeze dried and ground to analyze for dry matter, nitrogen, amino acid, and titanium content.

To address the limited amount of ileal contents in birds fed the NFD, the digesta of all 24 bags were randomly combined and pooled into 10 bags after freeze-drying and prior to grinding. This pooling approach ensured a sufficient quantity of sample for conducting the necessary analyses.

Prior to formulating the diets, representative samples of all soybean meals were taken and analyzed for dry matter (DM), crude protein (CP), ash, amino acids (AA), fat, neutral detergent fibre (NDF), non-starch polysaccharides (NSP), sugars, and phytate phosphorus. The test diets and digesta were analyzed for DM, CP, AAs, and titanium dioxide after being freeze-dried. All analyses were in duplicates.

Dry matter was analyzed following the AOAC (1997) method 925.09 by oven drying a 1g sample at 105°C overnight. The nitrogen content was measured using an N analyzer (Model CNS-2000, LECO Corp., St. Joseph, MO) and estimation of crude protein was obtained by multiplying the N values by the conversion factor 6.25. Ash was determined by combusting samples at 600°C for 12 hours in a muffle furnace. Amino acids were analyzed using modified versions of AOAC (1997) method 982.30 for regular amino acids, AOAC (1997) method 994.12 for oxidized amino acids, and ISO 13904:2005 for tryptophan. Fat was analyzed after hexane extraction with an ANKOM Extractor (Macedon, NY, USA), following the manufacturer's instructions which are in accordance with AOCS (2017) Am 5-04. The NDF was measured using an ANKOM Fibre Analyzer, following the manufacturer's instructions with the filter bag method. Phytate P was assessed using the technique described by Haug & Lantzsch (1983). Simple sugars, sucrose and oligosaccharides, were analyzed using gas-liquid chromatography following the methodology outlined by Slominski *et al*. (2004). The determination of NSP involved both gas-liquid chromatography and colorimetry, following the procedure described by Englyst & Cummings (1984; 1988) with modifications as specified by Slominski *et al*. (2006). Titanium dioxide was determined using Inductively Coupled Plasma Optical Emission spectroscopy using an internal method CTL-MICPSOP by the Central Testing Laboratory (Winnipeg, Canada).

4.4 Statistical Analysis

The apparent ileal digestibility (AID) of crude protein and amino acids was calculated using the following equation:

Eq. 6

$$\text{Apparent ileal Digestibility (\%)} = 100 - \left[100 \times \left(\frac{\text{TiO}_{2\text{ diet}}\ g/kg \times \text{Nutrient}_{\text{digesta}}\ g/kg}{\text{TiO}_{2\text{digesta}}\ g/kg \times \text{Nutrient}_{\text{diet}}\ g/kg}\right)\right]$$

For the measurement of the standardized ileal digestibility of crude protein the following equation was used, as stated by Adeola *et al*., (2016):

Eq. 7

$$\text{Standardized ileal Digestibility (\%)} = \text{AID} + \left(\frac{\text{Basal}_{\text{end}}\ g/kg}{\text{Nutrient}_{\text{diet}}\ g/kg}\right) \times 100$$

Where in equation [2] $\text{Basal}_{\text{end}}$ is the mean of the basal endogenous losses for the nutrients, taken from Adedokun *et al*., (2007) as displayed in the equation below.

Eq. 8

$$\text{Basal}_{\text{end}}\ (g/kg) = \text{Nutrient}_{digesta}\ g/kg \times \left(\frac{\text{TiO}_{2\,diet}\ g/kg}{\text{TiO}_{2\,digesta}\ g/kg}\right)$$

All statistical analyses were carried out using one-way ANOVA in R Studio (RStudio: Integrated Development for R. RStudio, PBC, Boston, MA), in datasets where all ANOVA assumptions were met. To compare treatment means, the post-hoc Tukey's test was used with

the function Tukey.HSD from the Agricolae package (de Mendiburi, 2021). In the case of non-normality of certain datasets, there was no removal of outliers or transformation of the data, instead the non-parametric Kruskal-Wallis test was used to assess statistical differences between means. The Bonferroni correction post-hoc test for multiple comparisons was then used to assess differences between treatments in this type of data. The statistical significance value for this thesis is $P < 0.05$.

4.5 Results and Discussion

The chemical composition of the soybean meals is presented on Table 5. The dry matter content of all MB soybean meals was comparable, suggesting that the processing conditions of these samples were similar. Considering the nature of the processing of these samples (expeller-pressed), the oil content of the SBM was considerably higher compared to the common 1% of solvent-extracted SBM (NRC, 1994).

Although a higher concentration of oil in the meal is expected for this type of processing, the mean oil content of 10.9% on a DM basis for Manitoba samples was higher than in the other expeller SBM reported (5.9%, Ishler & Varga, 2008; 8.6%, Quality Roasting, 2021; 9.9-10.5%, Opapeju *et al*., 2006; 3.5%, Johnson & Smith, 2017; 8.7% Powell *et al*., 2011; 6.5%, 7.6% Minoy *et al*., 2023; 6.0% McNess, 2021; 5.43% Woodworth *et al*., 2001; 4.6% Sakkas *et al*., 2019), and also higher than the Ontario control.

Table 5. Chemical Composition of Manitoba and Ontario Soybean Meals (g/kg).

Component	Manitoba						Ontario	
	A		B		C		D	
	As-is	DM	As-is	DM	As-is	DM	As-is	DM
Dry Matter	944.8		945.5		946.0		924.2	
Fat	103.9	110	100.6	106.4	107.3	113.4	83.4	90.2
Crude Protein	391	413.8	393	415.7	383	404.9	443	479.3
NDF	79.5	84.1	89.7	94.9	96.1	101.6	91.2	98.7
Total NSP	167.6	177.4	164.6	174.1	170.9	180.7	143.2	154.9
Rhamnose	0	0	0	0	0	0	0	0
Arabinose	23.9	25.3	22.6	23.9	23.6	24.9	19.3	20.9
Xylose	13.4	14.2	12.7	13.4	14.7	15.5	12.4	13.4
Mannose	7.3	7.7	7.1	7.5	7.7	8.1	6.9	7.5
Galactose	48.2	51	45.8	48.4	46.7	49.4	34.5	37.3
Glucose	43.3	45.8	42.8	45.3	47.4	50.1	39.4	42.6
Uronic acids	31.5	33.3	33.7	35.6	30.7	32.5	30.7	33.2
Sugars	207.6	219.7	159	168.2	216	228.3	148.5	160.7
Sucrose	116.1	122.9	88.3	93.4	120.9	127.8	81.6	88.3
Simple sugars[1]	11.8	12.5	8.7	9.2	12.3	13	9.5	10.3
Raffinose Family Oligosaccharides [2]	79.8	84.5	62.1	65.7	82.8	87.5	57.4	62.1
Ash	47.1	49.9	52.1	55.1	51.1	54	53.6	58
Phytate Phosphorus	2.7	2.9	2.7	2.9	2.3	2.4	2.7	2.9
Total Indispensable AA	180.8	191.4	183.7	194.3	177	187.1	208.6	225.7
Arginine	26.5	28	27.9	29.5	25.9	27.4	32.7	35.4
Histidine	14.3	15.1	12.9	13.6	13.2	14	16.1	17.4

Table 5. Chemical Composition of Manitoba and Ontario Soybean Meals (g/kg). (*Continued*).

Component	Manitoba						Ontario	
	A		B		C		D	
	As-is	DM	As-is	DM	As-is	DM	As-is	DM
Isoleucine	18	19.1	18.6	19.7	17.8	18.8	20.6	22.3
Leucine	30	31.8	30.9	32.7	30.3	32	35.2	38.1
Lysine	25.6	27.1	26	27.5	25.4	26.8	29	31.4
Methionine	8.3	8.8	7.3	7.7	7.2	7.6	8.7	9.4
Phenylalanine	19	20.1	20	21.2	18.7	19.8	22.4	24.2
Threonine	15.5	16.4	16	16.9	15.4	16.3	17.4	18.8
Tryptophan	4.9	5.2	5	5.3	4.7	5	5.4	5.8
Valine	18.7	19.8	19.1	20.2	18.4	19.5	21.1	22.8
Total Dispensable AA	213.5	226	217.2	229.7	213.1	225.3	253.4	274.2
Alanine	17.4	18.4	17.7	18.7	17.5	18.5	19.7	21.3
Aspartic Acid	46.3	49	46.9	49.6	46.8	49.5	55.5	60.1
Cysteine	8.3	8.8	7.4	7.8	7.5	7.9	8.8	9.5
Glutamic Acid	72.1	76.3	73.6	77.8	72.3	76.4	88.5	95.8
Glycine	16.5	17.5	16.9	17.9	16.6	17.5	18.8	20.3
Proline	19.2	20.3	20	21.2	19.3	20.4	22.9	24.8
Serine	19.9	21.1	20.5	21.7	20.1	21.2	23.2	25.1
Tyrosine	13.8	14.6	14.2	15	13	13.7	16	17.3

[1] includes glucose and fructose.
[2] includes raffinose and stachyose.

A study by Karr-Lilienthal *et al*., (2006) analyzed 7 samples from commercial expeller-processing plants in the US, and their fat content values ranged from 4.9-11.3% on DM basis. Heuzé *et al*. (2020) categorized expeller soybean meal with a fat content of 9.5% on DM basis as "high oil", however, it is worth noting that there is a lack of standardized values or categories in published literature or commercial formulations concerning the oil content of expeller SBM.

The crude protein content of Manitoba SBM in this study showed a difference of more than 5% below that of Ontario. Historically, Western-Canada soybeans have faced challenges with protein content compared to those grown in Eastern Canada. However, a study conducted by Lee & Choung (2011) found that soybean seeds from different geographical locations exhibiting a wide range of CP values (37% to 46%) had no statistically significant difference in CP content, suggesting that the protein content of soybeans is primarily influenced by the variety and genotype rather than the geographical location of cultivation. In contrast, a more recent study by Ravindran *et al*. (2014) did find variations in crude protein contents among samples from different geographical locations, but these differences were attributed to temperature and variety, rather than geographical variability. Notably, the findings from Ort *et al*. (2022) indicate that lower protein content in Manitoba soybeans may be associated with lower mean temperatures and reduced precipitation when compared to Ontario soybeans.

In a report from 2019 by Manitoba Pulse & Soybean Growers (MPSG), 88 herbicide-tolerant *Eastern Manitoba* soybean varieties were assessed for CP content in 5 locations in MB (Arborg, Carman, Morris, Portage la Prairie and Ste. Adolphe), resulting in a 41.4% average

CP across samples (38.1% min., 44.7% max.). In a similar 2019 report by MPSG, the CP content of 63 samples of herbicide-tolerant *Western Manitoba* soybean varieties in 2 MB locations (Dauphin and Hamiota) was assessed, obtaining a mean CP content of 41.3% (38.1% min., 44.3% max.). In a hypothetical scenario, if we consider that the typical oil content of soybean seeds is 20% and the test meal from our study contained approximately 10% fat, if the soybean seeds from MPSG were processed into soybean meal using a similar expelling process, the estimated average CP content would be about 45.6%. This value remains higher than the CP contents observed in the present work.

With a crude protein content of 41.1% on a DM basis, Manitoba SBM is also relatively lower than the findings reported in the literature and commercial formulations of expeller SBM (44%, Opapeju *et al*., 2006; 48%, Ishler & Varga, 2008; 42% Woodworth *et al*., 2001; 49%, McNess, 2021; 42%, Johnson & Smith, 2017; 48%, Powell *et al*., 2011; 50%, Sakkas *et al*., 2019; 45-52%, Karr-Lilienthal *et al*., 2006). Considering the higher oil content present in the tested meals, it is understandable to observe this difference in protein content. For a better comparison, we can analyze the data on an 'oil-corrected' basis, where all the oil is removed from the meal. When applying this adjustment and comparing the protein concentrations, SBM A, B, and C from this study would exhibit CP concentrations of approximately 46%, 46%, and 45%, respectively. While these adjusted CP concentrations bring them closer to the protein values reported in the literature and commercial formulations for expeller SBM, they still remain comparatively lower when considering the varying fat composition of the published values, which ranged from 3.5% to 11.3%.

Neutral detergent fibre values on a DM basis for all soybean meals in this study are lower than the expeller SBM in published results (12.5%, Ishler & Varga (2008); 13.8%, NRC, 2012; 14.5% Quality Roasting, 2021) and within range of the content of the NDF in conventional solvent-extracted SBM in the literature on a DM basis (7.4-18.9%, Banaszkiewicz, 2011; 7.6% Baker *et al*., 2011; 7.9%, Zduńczyk *et al*., 2020; 5.2-12.8% Edwards *et al*., 2000; 8.5-14.3% Ravindran *et al*., 2014; 12.0-19.4%, Li *et al*., 2017; 11.1-13.0, Grieshop *et al*., 2003). It is worth noting that numerous published studies and commercial products on expeller SBM utilize alternative measurements of fibre, including crude fibre or total dietary fibre values, instead of NDF. (Sakkas *et al*., 2019; Powell *et al*., 2011; Minoy *et al*., 2023; Woodworth *et al*., 2001; Johnson & Smith, 2017; McNess, 2021).

The explanation for a lower NDF value in this study in comparison to other expeller SBM could be due to a difference in variety, considering that soy hulls are included in its composition.

In Table 6, the amino acid composition of Manitoba SBM is presented relative to CP. The analysis reveals that over 50% of the amino acids in Manitoba SBM are dispensable amino acids, however the relative contents of indispensable amino acids are comparable to or higher than those reported for expeller and solvent extracted soybean meals in previous studies (Elkin, 2002; Ravindran *et al*., 2014; Stein *et al*., 2013). This table serves the purpose of providing a standardized and proportional representation of amino acids within the protein component. Although the absolute content of amino acids is lower in the Manitoba samples, consistent with its lower CP composition, there are notable increases in the relative presence of methionine and lysine, exhibiting increases of up to 1.2% when compared to the literature.

This subtle yet significant improvement emphasizes the quality of Manitoba soybean meal in terms of indispensable amino acids.

Table 6. Total Amino Acid Content (g/100g CP) in Manitoba and Ontario Soybean Meal Samples.

Component	Manitoba			Ontario
	A	**B**	**C**	**D**
Total Indispensable AA	46.24	46.82	46.13	47.1
Arginine	6.78	7.12	6.74	7.38
Histidine	3.66	3.28	3.43	3.63
Isoleucine	4.60	4.75	4.64	4.65
Leucine	7.68	7.87	7.89	7.95
Lysine	6.54	6.63	6.62	6.56
Methionine	2.13	1.85	1.88	1.96
Phenylalanine	4.85	5.10	4.88	5.06
Threonine	3.96	4.08	4.02	3.93
Tryptophan	1.26	1.28	1.23	1.22
Valine	4.78	4.86	4.80	4.76
Total Dispensable AA	54.63	55.32	55.55	57.27
Alanine	4.44	4.52	4.56	4.45
Aspartic Acid	11.85	11.94	12.20	12.55
Cysteine	2.13	1.88	1.96	1.99
Glutamic Acid	18.45	18.74	18.83	20.01
Glycine	4.21	4.30	4.33	4.25
Proline	4.92	5.09	5.04	5.18
Serine	5.09	5.23	5.24	5.24
Tyrosine	3.54	3.62	3.39	3.60

Additionally, with regard to dispensable amino acids, Manitoba SBM exhibits relative contents that are equal to or higher than those reported in the referenced studies, particularly

displaying a 0.61% increase in cysteine content (Elkin, 2002), indicating a high-quality composition in terms of indispensable as well as dispensable amino acids for broiler chickens, despite the lower crude protein content. Furthermore, the SBM samples from Manitoba exhibit a comparable proportion of both indispensable and dispensable amino acids to that of the control, providing support for the hypothesis proposed in this study.

Upon arrival, the birds displayed variation in weight and size, which we addressed when allocating them into the cages. Throughout the entire 19-day experiment, the birds remained in good health without any signs of illness, and no mortalities were reported. Birds consumed the starter diet well and gained weight as per the standard of the breed (Aviagen, 2019). Despite the test diets having a powdery texture (attributed mostly to the high inclusion of cornstarch) and a whiter color compared to the starter diet, the birds exhibited no difficulties in consuming them. The results for the basal endogenous AA flow are presented in Table 7.

The concentration and profile of Basal IAA_{end} in poultry can vary significantly depending on various factors. Therefore, it is advisable to include an NFD in amino acid studies (Adeola *et al*., 2016). It is typically recommended that AA digestibility studies are beconducted at a minimum age of 15 days, as there is documented variation in Basal IAA_{end} levels before this age, which stabilizes between days 15 and 21 (Adeola *et al*., 2016). In our study, the sampling was conducted when the birds were 19 days old, aligning with the recommended age range indicated in the current literature.

Table 7. Basal Ileal Endogenous Amino Acid Flow (g/kg DM).

Component	N-Free Diet	Siriwan *et al.*, 1994	Adeola *et al.*, 2016
Indispensable AA	2.48	-	-
Arginine	0.26	0.25	0.39
Histidine	0.04	0.14	0.18
Isoleucine	0.27	0.2	0.37
Leucine	0.42	0.47	0.56
Lysine	0.39	0.24	0.39
Methionine	0.05	0.07	0.11
Phenylalanine	0.21	0.29	0.37
Threonine	0.41	0.54	0.6
Tryptophan	0.09	-	0.09
Valine	0.34	0.42	0.51
Dispensable AA	2.87	-	3.34
Alanine	0.29	0.27	0.39
Aspartic Acid	0.59	0.61	0.73
Cysteine	0.12	-	0.41
Glutamic Acid	0.72	0.29	0.98
Glycine	0.27	0.74	0.47
Proline	0.32	-	0.5
Serine	0.40	0.51	0.56
Tyrosine	0.16	0.32	0.3

The basal endogenous AA flow values obtained in this study demonstrate a favorable trend, with lower losses observed compared to the published studies. This positive outcome may be attributed to the use of titanium dioxide as a marker, which has been reported to result in lower measured losses (Favero *et al*., 2014). While the factors influencing endogenous AA losses are not yet fully understood, it is notable that the reduced losses observed in our study could potentially be associated with the feed intake (Kong *et al*., 2014). Lower feed intake leads to reduced AA intake, which can have unfavourable effects on the physiological state of the birds in the experiments, potentially resulting in increased basal IAA_{end} levels. While

direct measurements of feed intake were not taken due to the ad libitum access to feed, it is worth noting that daily observations revealed normal consumption of the test diets by all birds, indicating a satisfactory intake level.

An additional factor that may contribute to the lower values observed in our study compared to the literature is the use of sucrose instead of dextrose in the formulation. The proposed method for an NFD by Adeola *et al*. (2016), which is commonly followed by researchers, involves a cornstarch-dextrose diet with a ratio of 200.5:640 g/kg for broilers. In our study, we used a similar ratio (200:638.5 g/kg) but substituted dextrose with sucrose for its ease of access from retailers.

Dextrose (glucose) is a monosaccharide that is readily digestible, while sucrose, a disaccharide composed of glucose and fructose, requires enzymatic digestion in the small intestine. However, the studied NFD exhibited an apparent ileal digestibility coefficient of 0.84 for DM. This indicates that despite the more complex nature of cornstarch compared to sucrose, both components are highly digestible, which could account for the observed low yield in digesta collection. The DM digestibility value of 0.84 aligns with the findings reported in the study by Kong *et al*. (2014).

Furthermore, the basal IAA_{end} levels could also be influenced by an increase in intestinal mucosal glycoproteins such as mucins. These proteins contain approximately 30% threonine, and studies in rats have shown that the dietary requirement for this amino acid increases during periods of stress (Faure *et al*., 2005). The threonine concentration in the studied basal IAA_{end} was found to be lower than that reported in the literature review by Adeola *et al*. (2016) and also lower than the findings of Siriwan *et al*. (1994). This suggests a potentially

lower content of mucins in the gut, prompting the exploration of an alternative approach with the use of sucrose in an NFD in future research to validate these decreased values of basal IAA_{end}.

The apparent and standardized ileal digestibility coefficients are presented in Table 8. The apparent DM digestibility of the Manitoba samples was found to be in the range of 62-65%, which is similar to the control group. However, there is limited research available on the apparent ileal digestibility of DM in expeller soybean meal (SBM) for poultry.

In a study conducted by Sakkas *et al*. (2019), different types of SBM processing methods were assessed. The study reported DM ileal digestibility values ranging from 70% to 72% for expeller SBM with hulls. Interestingly, the study did not find a significant difference in DM digestibility between the various processing methods investigated. The present study's DM digestibility results appear to be notably low.

It is possible that the presence of fiber and carbohydrate fractions in the studied SBM contributes to this lower digestibility. However, the exact values for these components were not reported in the referenced studies, making it difficult to do a precise comparison.

The apparent CP digestibility for the Manitoba samples was determined to be 74%, which is 3% lower than the control group. Although there are limited published studies on the apparent ileal digestibility of CP or AA in expeller soybean meal (SBM) for poultry, solvent-extracted SBM has been extensively studied. In Bandegan *et al*., (2010), the AID of CP values ranged from 82-89% in expeller SBM samples.

Table 8. Apparent and Standardized ileal digestibility (%) of CP and AA of Manitoba and Ontario SBM in broiler chicks.

Component	Apparent					Standardized				
	A	**B**	**C**	**D**	**SEM**	**A**	**B**	**C**	**D**	**SEM**
CP	74.7	74.6	74.4	77.3	0.71	78.6	78.6	77.2	81.1	0.72
DM	63.0	62.4	65.5	63.2	0.63					
Indispensable AA										
Arginine	89.6	89.5	89.1	88.9	0.47	91.4	91.3	90.9	90.5	0.47
Histidine	78.9[a]	79.0[a]	84.4[b]	85.3[b]	0.71	79.5[a]	79.6[a]	84.8[b]	85.7[b]	0.70
Isoleucine	78.5	80.6	80.9	78.7	0.68	81.2	83.3	83.5	81.4	0.67
Leucine	77.5	80.5	79.7	80.7	0.57	80.1	83.0	82.1	83.1	0.57
Lysine	80.7	83.0	82.6	80.6	0.68	83.5	85.7	85.4	83.5	0.68
Methionine	76.9[a]	79.4[a]	85.7[b]	85.9[b]	1.09	78.4[a]	81.0[ab]	86.7[bc]	86.9[c]	1.06
Phenylalanine	81.6	83.8	83.2	83.3	0.48	83.6	85.8	85.0	85.2	0.48
Threonine	68.9	72.0	72.9	68.2	0.90	73.8	76.7	77.5	73.1	0.89
Tryptophan	71.0	75.4	71.5	73.8	0.73	74.2	78.4	74.7	76.9	0.73
Valine	75.6	78.0	78.2	75.6	0.76	79.0	81.1	81.4	78.9	0.75
Dispensable AA										
Alanine	75.5	78.5	77.3	76.9	0.61	78.6	81.5	80.2	80.0	0.61
Aspartic acid	79.2	80.5	80.1	79.9	0.48	81.5	82.7	82.4	82.2	0.48
Cysteine	53.1[a]	55.5[a]	72.5[b]	70.9[b]	1.70	57.0[a]	59.4[a]	74.9[b]	73.4[b]	1.60
Glutamic acid	84.3	86.1	85.3	85.7	0.42	86.1	87.9	87.0	87.5	0.42
Glycine	70.6	73.3	72.6	73.4	0.65	73.5	76.1	75.5	76.3	0.64
Proline	76.8	78.7	77.9	78.2	0.52	79.9	81.6	80.8	81.0	0.52
Serine	74.3	77.4	76.7	77.5	0.58	78.0	80.9	80.2	80.9	0.58
Tyrosine	80.2	82.6	84.9	84.4	0.80	82.9	85.2	87.3	86.8	0.80

[a, b, c] Mean values with different superscript letters indicate significant difference ($p < 0.05$).

A, B, C: Manitoba soybean meal samples, D: Ontario soybean meal sample.

In a study by Hemetsberger *et al*. (2021) on solvent-extracted SBM, soybean meals with varying levels of heat treatment exhibited DM ileal digestibilities of 69-71%, which are lower than other values reported and closer to the results in the present study, explained by the different varieties of SBM used. Interestingly, the use of a low heat-treating process in a heat-stable variety of SBM not only increased nitrogen retention but also improved the apparent digestibility of cysteine compared to other treatments. These findings further support the use of this technique to enhance the digestibility of soybean meals, particularly those with low protein content.

Bovera *et al*. (2016) reported DM ileal digestibilities of 88% and CP ileal digestibilities of 87% for a solvent-extracted commercial source of soybean meal with hulls in broiler chickens. However, the absence of fibre analysis in their study makes it challenging to determine the precise reason behind the observed DM results. Another study by Stefanello *et al*. (2016) analyzed two samples of soybean meal from Brazil, obtaining ileal digestibilities of 67% and 82% for DM and CP, respectively. Notably, in this study, the authors added exogenous proteases to one of their test diets, resulting in an increased crude protein digestibility to 85%.

Another study conducted at the University of Alberta by Oryschak *et al*. in 2012 reported an apparent ileal digestibility of 79% for crude protein in commercial soybean meal. However, the study did not specify the extraction process used to obtain the oil from the seeds. These studies collectively demonstrate that the digestibility of dry matter and crude protein can vary depending on the type of soybean meal, the extraction process employed for oil removal, and the antinutritional profile of the residual meal.

As for the apparent ileal digestibility of AA, for most AA there are no statistically significant differences with the exception of histidine, methionine, and cysteine. Overall, the AID of AA is quite low when compared to the mean values for AA digestibility reported by Bangedan *et al*., (2010) where all AA were higher than the ones in the present study, with methionine being the indispensable AA with the most variation in comparison. As for dispensable AA, cysteine was significantly different, with Diet A having an AID 19% lower than and Diet C and 17% lower than the control. As for studies regarding solvent-extracted SBM, the results from the present study are consistently lower than the reports of Ravindran *et al*. (1999) and Kong & Adeola (2013) with only arginine having a comparable AID.

Cysteine digestibility is low in comparison to the rest of the AA in both studies (72% and 68%, respectively), moreover the results in the present study have a wide variation of 53.1-72.6%, with very low AID in diets A and B which could be due to the relatively low content of this amino acid in the composition of the SBM (Kong & Adeola, 2013).

Threonine AID is also very low in comparison, about 10% lower which could be explained by the loss of mucin in the gut, which is not easily broken down by enzymes and could potentially influence the lower digestibility of threonine (Scawen and Allen, 1977). Methionine AID also had a very high variation among samples (8.8%). This AA was 13% lower than the values from Bangedan *et al*. (2010) and Ravindran et al. (1999) and 15% lower than with the results from Kong & Adeola (2014). In a study by Leung & Kiarie (2020), SBM were analyzed by source and by being conventional or organic, SID of methionine in organic Ontario showed similar results to our control SBM from Ontario and a difference of 1% in

digestibility compared to Diet C, suggesting that a difference in SBM variety may affect methionine digestibility.

The high variation among Manitoba SBM samples and low AID of methionine in comparison to other expeller and conventional SBM is important to note, as this is the first limiting amino acid in conventional poultry diets and its reduced digestibility directly impairs broiler performance (Sekiz *et al*., 1975). The reason for these differences is largely unknown. Considering that the NSP and sugar content that could impair AA digestibility are seemingly the same across Manitoba SBM samples, it can only be hypothesized that given the nature of the processing of the SBM, considerable trypsin inhibitor concentration may be present, affecting the AID.

In terms of SID, the differences between the AID and SID ranged from 0.4-4.9%, with a mean value of 2.6%, reinforcing the recommendation by Adeola *et al*. (2016) to include an NFD to estimate the SID. The AA that had the most variation was threonine, which again could be explained by the loss of mucin in the gut (Scawen & Allen, 1977). Again, histidine, methionine, and cysteine are the only amino acids expressing a significantly different SID, with very low SID digestibility values for cysteine in diet A, which does not coincide with lower AA concentration in the SBM or high ANF, leaving this value largely unexplained.

Arginine was the AA with the highest AID and SID, comparable with the reports by Ravindran *et al*. (1999, 2014); Bangedan *et al*. (2010); Kong & Adeola (2016), with all other AA having lower SID with a range of about 5-16% for indispensable AA and 5-20% for dispensable AA. Cysteine also often has the lowest reported SID value in the literature

withabout 63-89% (Ravindran *et al*., 2014; Kong & Adeola, 2014; Karr-Lilienthal *et al*., 2006; Leung & Kiarie, 2020), which is consistent with the lower value presented in this study.

There are few studies reporting low SIAAD in SBM, as this protein ingredient is known to be very well digested by broiler chickens. Expeller-pressed SBM studies are few and far in between as well, especially with regard to broiler chicken nutrition, let alone with low SIAAD. Karr-Lilienthal *et al*. (2006) reported low true AA digestibilities in expeller SBM that were heat-treated at different temperatures. From their results, the SIAAD from the present study resemble those of SBM treated to 135-150°C, with a much higher digestibility reported at 160°C in that study, giving further credit to the explanation that the low SID values in this study could be due an improper heat-treatment, leading to an increased concentration of thermolabile antinutritional factors, negatively impacting the digestibility of Manitoba SBM.

Another factor affecting the SIAAD could be a higher concentration of raffinose-family oligosaccharides. Karr-Lilienthal *et al*. (2005) reported stachyose, raffinose and verbascose values for different maturity groups of US soybean meals, showing a significant difference in their maturity groups. In the present study the raffinose-family oligosaccharides is higher by about 2% than those in the cited study, however studies regarding variety of oligosaccharides in soybean varieties used in Manitoba are lacking.

A study by Barua *et al*. (2020) reported that the form of the feed influences its SID, as mashed diets had higher SID of AA in SBM samples. In the present study, the form of the diet was mashed, which aligns with the recommendation by Barua *et al*. (2020) but may affect future

diet formulations with Manitoba SBM, as it is a common practice in the industry to use pellet form to feed broilers and this could lower the SID of AA.

Table 9. Digestible Amino Acids of Manitoba Soybean Meal in Broiler Chickens (g/kg, DM basis).

Amino Acids	Manitoba			Ontario
	A	B	C	D
Indispensable AA				
Arginine	25.6	26.9	24.9	32.0
Histidine	12.0	10.8	11.9	14.9
Isoleucine	15.5	16.4	15.7	18.2
Leucine	25.5	27.1	26.3	31.7
Lysine	22.6	23.6	22.9	26.2
Methionine	6.9	6.2	6.6	8.2
Phenylalanine	16.8	18.2	16.8	20.6
Threonine	12.1	13.0	12.6	13.7
Tryptophan	3.9	4.2	3.7	4.5
Valine	15.6	16.4	15.9	18.0
Dispensable AA				
Alanine	14.5	15.2	14.8	17.0
Aspartic Acid	39.9	41.0	40.8	49.4
Cysteine	5.0	4.6	5.9	7.0
Glutamic Acid	65.7	68.4	66.5	83.8
Glycine	12.9	13.6	13.2	15.5
Proline	16.2	17.3	16.5	20.1
Serine	16.5	17.6	17.0	20.3
Tyrosine	12.1	12.8	12.0	15.0

Table 9 displays the digestible amino acid profiles of Manitoba and Ontario soybean meals. As expected, Manitoba SBM demonstrates a slightly lower digestible amino acid content than Ontario, mainly because the initial amino acid composition of the eastern soybean meal

was higher. Nonetheless, these values offer significant insights to incorporate Manitoba soybean meals in poultry diets by Manitoba chicken producers.

4.6 Conclusion

The main interesting characteristic of Manitoba soybean meal compared to typical solvent-extracted SBM is their possess higher oil content, which could positively impact diet formulation for broiler chickens, become a source of energy for the bird as well as a binding agent for the diet. Although Manitoba soybean meals have lower protein content, their amino acid composition is comparable to the control and some expeller SBM in the literature. This suggests that despite the lower protein content, Manitoba SBM can still provide valuable amino acids to birds at an adequate concentration. Most standardized ileal digestibility coefficients for amino acids were found to be comparable to control values but lower than most published values. The presence of NSP and carbohydrates in SBM could potentially contribute to the lower digestibility. These antinutritional factors, may limit the nutrient availability of SBM, therefore it is crucial to consider these components and evaluate strategies to enhance the digestibility of Manitoba SBM in poultry diets. Further research is guaranteed to optimize the utilisation of Manitoba SBM in broiler nutrition, considering it contains significant amounts of crude protein in its composition. In conclusion, Manitoba SBM shows promise as a potential feed ingredient, and efforts should be made to unlock its full potential in broiler diets.

5. General Discussion

Manitoba Agriculture (2021) reported that the province imported approximately 250.5 thousand tonnes of soybean meal in 2021. Furthermore, according to Manitoba Agriculture (2023), the province spent $164 million USD in 2022 on soybean meal imports from the United States. Considering that soybeans typically contain about 20% oil, Manitoba's production of 1.3 million tonnes of soybeans in 2022 suggests a potential yield of over 1 million tonnes of soybean meal. This calculated amount significantly surpasses the quantity currently imported. Consequently, Manitoba has the capacity to achieve self-sufficiency in this protein ingredient for animal production, producing a considerably larger quantity than its current imports.

However, the protein content of the soybean meal imported from the US is much higher than the Manitoba SBM results obtained in this study, as commercial SBM has a range of 47-49% crude protein content (Heuzé *et al*., 2020) and there are reports from processing plants in the US with over 55% CP on a DM basis (Grieshop *et al*., 2003). The protein content of Manitoba soybean meal is lower than the protein content reported in other studies and commercial formulations of expeller soybean meal as well. This difference in protein content can be explained by the higher oil content present in Manitoba samples. However, after adjusting for the oil content, the protein concentrations of Manitoba soybean meals align more closely with the values reported in the literature, although they still remain lower.

The amino acid composition of Manitoba soybean meal is comparable to or higher than expeller and solvent-extracted soybean meals reported in previous studies. Although the absolute content of amino acids is lower in Manitoba samples due to their lower protein content, there are notable increases in the relative presence of methionine and lysine, indicating a high-quality composition in terms of indispensable amino acids.

Although Manitoba is self-sufficient in poultry production (Manitoba Agriculture, 2022b), its yearly production is relatively low, to the extent that Statistics Canada does not have a separate category to measure it and groups it with Saskatchewan. In 2022, Manitoba and Saskatchewan collectively produced 101 tonnes of eviscerated chicken, while Ontario and Québec accounted for approximately 60% of Canada's chicken production, producing a combined total of 815 tonnes (Statistics Canada, 2023b). Despite Manitoba's comparatively lower chicken meat production due to the Canadian Supply Management System (Manitoba Chicken Producers, 2023), it is crucial to recognize the potential benefits of producing soybean meal locally instead of relying on imports from the US, especially considering Manitoba’s substantial soybean production.

Importing soybean meal involves certain environmental considerations. Firstly, the transportation of soybean meal from the US to Manitoba implicates long-distance shipping, which contributes to greenhouse gas emissions and energy consumption. On the other hand, producing soybean meal locally can offer several environmental advantages. By sourcing soybeans from local farms, the transportation distance and associated emissions can be significantly reduced. Moreover, supporting local soybean production and processing industries contributes to regional economic growth and employment opportunities. By

strengthening the local agricultural sector, the community can benefit from increased resilience, reduced dependency on external sources, and enhanced food security.

The soybean meal market has been subject to various disruptions in recent years, including the COVID-19 pandemic and the trade conflict between the US and China in 2018-2020 (Unveren & Luckstead, 2020; Barichello, 2020). The COVID-19 pandemic caused significant disruptions in global supply chains, affecting the availability and prices of agricultural commodities, including soybean (Barichello, 2021). Importing soybean meal from the US became more challenging and expensive due to disruptions in transportation and trade restrictions because of the trade war between the US and China which resulted in the imposition of tariffs on US soybean imports by China (Singh & Roca, 2022). This trade conflict led to a decline in trade between the two countries and increased price volatility in the global soybean meal market (Tortajada & Zhang, 2022). By investing in local production of soybean meal, Manitoba can enhance its resilience and reduce its dependence on international markets. Producing soybean meal locally would help mitigate the risks associated with global trade tensions, ensure a stable supply of feed ingredients for the poultry sector, and promote economic independence and food security within the region.

In this study, it's important to acknowledge certain limitations. The sample size for analysis was limited, which makes it challenging to draw broad conclusions about Manitoba soybean meal, given the wide range of soybean varieties grown across the province. Additionally, a significant limitation stems from the absence of detailed information about processing conditions and a comprehensive profile of antinutritional factors. Having this crucial

information could have led to a better understanding of whether the processing conditions were optimal or not.

While there may be challenges associated with low protein digestibility and the presence of antinutritional factors, these limitations can be addressed through further research in the areas pointed out in this study. Future studies should focus on improving amino acid digestibility, reducing antinutritional factors, and exploring appropriate heat processing methods to maximize the nutritional value of Manitoba SBM. Despite these challenges, Manitoba SBM holds great promise due to its high protein content, availability, and the potential for local production. Further research and investment in this area are crucial to fully unlock the potential of Manitoba SBM and address the shortcomings observed, ultimately benefiting the poultry sector and the overall agricultural landscape.

6. General Conclusion

Expeller Manitoba soybean meal demonstrated a favorable amino acid profile, comparable to other expeller and solvent-extracted soybean meals. Despite having a lower absolute crude protein content, it contains optimal concentrations of indispensable amino acids necessary for broiler nutrition. This indicates that it can be a valuable protein source for broiler chickens. When considering Manitoba soybean meal as a protein source, it is crucial to consider its SIAAD. The SIAAD reflects the amount of amino acids that are actually available for absorption by the chicken's digestive system.

In the present study, the reported results indicate a considerably lower digestibility of amino acids in Manitoba SBM compared to what is described in the literature. The SIAAD of Manitoba SBM was found to be not significantly different from the control group in the study. However, when comparing to Ontario values outside this research and the broader literature, the SIAAD of Manitoba SBM was lower. This suggests that the control group in the study may not have been processed in an optimal setting for achieving the highest digestibility of that soybean meal, as demonstrated by the cited literature.

This study sheds light on the chemical composition of Manitoba SBM, providing insights into the digestibility of its amino acids and the potential use of this meal as a primary protein ingredient in locally produced broiler chicken diets. Utilizing a local ingredient like Manitoba soybean meal can reduce the expenses associated with importing soybean meal while contributing to the province's economy.

In this study, Manitoba soybean meal was comparable to eastern varieties, with similar AID of dry matter, crude protein and SIAAD. To better comprehend the lower digestibilities observed in this study compared to the literature, further analyses are required. It is suggested that improper heat treatment of the tested SBM may have led to an ingredient with a high concentration of thermolabile ANF. However, additional research is necessary to draw definitive conclusions.

For future digestibility studies on Manitoba SBM, the addition of exogenous enzymes such as multi-carbohydrases and proteases is recommended. This inclusion can assess the extent to which these additives enhance Manitoba protein digestibility. Existing literature reviews indicate the beneficial effects of these enzymes on protein digestibility when incorporated into formulations. This approach would lead to a better understanding of the protein quality of Manitoba soybean meal in poultry nutrition and enable researchers to provide better guidance to local poultry producers regarding the use of locally grown SBM.

This study highlights the potential of Manitoba SBM as an excellent protein source for commercial poultry nutrition. However, further analyses and studies are required to fully unlock its true potential.

7. References

AAT Bioquest (2023). PBS (Phosphate Buffered Saline). Preparation and Recipe. AAT Bioquest. Retrieved June 3, from https://www.aatbio.com/resources/buffer-preparations-and-recipes/pbs-phosphate-buffered-saline.

Adeola, O., Xue, P. C., Cowieson, A. J., Ajuwon, K. M. (2016). Basal endogenous losses of amino acids in protein nutrition research for swine and poultry. Animal Feed Science and Technology, 221: 274–283. doi.org/10.1016/j.anifeedsci.2016.06.004

Allied Market Research (2023). Soybean Meal Market Research, 2031. Retrieved on May 30 2023, from https://www.alliedmarketresearch.com/soybean-meal-market

Alpers, D. H. (1994). Digestion and absorption of carbohydrates and proteins. In: L. R. Johnson, D. H. Alpers, J. Christensen, Jacobson, and J. H. Walsh (Ed.), Physiology of the Gastrointestinal Tract. (pp. 1723-1749). Raven Press, New York, NY.

AOAC. (1997). Official Methods of Analysis of AOAC International. AOAC International.

AOCS. (2017). Am 5-04: Determination of moisture and volatile matter content. In Official Methods and Recommended Practices of the AOCS (7th ed., pp. 1-2). AOCS Press.

Araba M. & Dale N. M. (1990). Evaluation of protein solubility as an indicator of overprocessing of soybean meal. Poultry Science. 69:76.

Aviagen. (2019). Broiler Nutrition Specifications, Ross 308. Aviagen. http://en.aviagen.com/brands/ross/products/ross-308

Avilés-Gaxiola, S., Chuck-Hernández, C., Serna-Saldívar, S. (2017). Inactivation Methods of Trypsin Inhibitor in Legumes: A Review. Journal of Food Science. 83:17-29

Bagadia, B. H., Vanga, S. K., Raghavan, V. (2017). Inactivation methods of soybean trypsin inhibitor – A review. Trends in Food Science & Technology. 64: 115-125.

Baker, K. M, Utterback, P. L., Parsons, C. M., Stein, H. H. (2011). Nutritional value of soybean meal produced from conventional, high-protein, or low-oligosaccharide varieties of soybeans and fed to broiler chicks. Poultry Science 90: 390–395

Banaszkiewicz, T. (2011). Nutritional Value of Soybean Meal. In H. El-Shemy. Soybean and Nutrition. (pp. 1-20). Intechopen. https://doi.org/10.5772/23306

Bandegan, A., Kiarie, E., Payne, R. L., Crow, G. H., Guenter, W., Nyachoti, C. M. (2010). Standardized ileal amino acid digestibility in dry-extruded expelled soybean meal, extruded canola seed-pea, feather meal, and poultry by-product meal for broiler chickens. Poultry Science. 89(12): 2626-2633.

Barichello, R. (2020). The COVID-19 pandemic: Anticipating its effects on Canada's agricultural trade. Canadian Journal of Agricultural Economics/Revue canadienne d'agroeconomie, 68(2), 219-224.

Barichello, R. (2021). Revisiting the effects of the COVID-19 pandemic on Canada's agricultural trade: The surprising case of an agricultural export boom. Canadian Journal of Agricultural Economics/Revue canadienne d'agroeconomie, 69(2), 251-260.

Barros-Dourado, L. R., Fonseca-Pascoal, L. A., Kazue-Sakomura, N., Perazzo-Costa, F. G. (2011) Soybeans (Glycine max) and Soybean Products in Poultry and Swine Nutrition. In: D. Krezhova, Recent Trends for Enhancing the Diversity and Quality of Soybean Products (pp. 175-190). IntechOpen.

Barua, M., Abdollahi, M. R., Zaefarian, F., Wester, T. J., Girish, C. K., Ravindran, V. (2020). Standardized ileal amino acid digestibility of protein sources for broiler chickens is influenced by the feed form. Poultry Science. 99(12): 6925-6934.

Bovera, F., Loponte, R., Marono, S., Piccolo, G., Parisi, G., Iaconisi, V., Gasco, L., Nizza A. (2016). Use of Tenebrio molitor larvae meal as protein source in broiler diet: Effect on growth performance, nutrient digestibility, and carcass and meat traits. Journal of Animal Science. 94(2): 639-647.

Brar, N & Lawley, I. (2020). Short-season soybean yield and protein unresponsive to starter nitrogen fertilizer. Agronomy Journal. 116: 5012-5023

Canadian Council on Animal Care (2009). CCAC guidelines on: the care and use of farm animals in research, teaching and testing.

Canadian Grain Commission. (2023). Quality of Canadian oilseed-type soybeans 2022. Retrieved April 25, 2023, from https://grainscanada.gc.ca/en/grain-research/export-quality/oilseeds/soybean-oil/2022/pdf/quality-report-soybean-oilseed-type-2022.pdf

Choct, M. & G. Annison. (1990). Anti-nutritive activity of wheat pentosans in broiler diets. Poultry Science. 31: 811-821.

Choct, M., Dersjant-Li, Y., McLeish, J., Peisker, M. (2010). Soy Oligosaccharides and Soluble Non-starch Polysaccharides: A Review of Digestion, Nutritive and Anti-nutritive Effects in Pigs and Poultry. Asian-Australasian Journal of Animal Sciences. 23(10): 1386-1398.

Cloutier, J. (2017). VISTA on the Agri-food Industry and the Farm Community Soy Story: A Short History of Glycine max in Canada. Statistics Canada. https://www150.statcan.gc.ca/n1/pub/21-004-x/2017001/article/14779-eng.htm

Cowan, W. D., Petterson, D. R., Rasmussen, P. B. (2002). The Influence of lipase, a-galactosidase or multi-component pectinase enzymes on energy and amino acid availability in feedstuffs. In J.M. McNab and K.N. Boorman (Ed.). Poultry Feedstuffs: Supply, Composition and Nutritive Value. (pp. 337-344). CAB International.

Cowieson, A. J. & Adeola, O. (2005). Carbohydrases, protease, and phytase have an additive beneficial effect in nutritionally marginal diets for broiler chicks. Poultry science. 84(12): 1860-1867.

Cowieson, A. J. & Roos, F. F. (2014). Bioefficacy of a mono-component protease in the diets of pigs and poultry: a meta-analysis of effect on ileal amino acid digestibility. Journal of Applied Animal Nutrition. (2) 13: 1-8.

Dale, N. (1996) Variation in feed ingredient quality: oilseed meals. Animal Feed Science and Technology. 59: 129–135.

De Mendiburu, F. (2021). Agricolae: Statistical Procedures for Agricultural. R package version 1.3-5. <https://CRAN.R-project.org/package=agricolae>.

Dozier, W. A. & Hess, J. B. (2011). Soybean Meal Quality and Analytical Techniques. In El-Shemy, H. (Ed.). Soybean and Nutrition. (pp. 111-124). IntechOpen.

Edwards 3rd, H. M., Douglas, M. W., Parsons, C. M., Baker, D. H. (2000). Protein and energy evaluation of soybean meals processed from genetically modified high-protein soybeans. Poultry Science. 79(4): 525-527.

Elkin, R. G. (2002). Nutritional components of feedstuffs: a qualitative chemical appraisal of protein. In: J.M. McNab and K.N. Boorman (Ed.), Poultry Feedstuffs: Supply, Composition and Nutritive Value (pp. 57-86). CAB International.

Englyst, H. N. & J. H. Cummings. (1988). Improved method for the determination of dietary fiber as non-starch polysaccharides in plant foods. Journal - Association of Official Analytical Chemists. 71: 808-814.

Englyst, H. N., & J. H. Cummings. (1984). Simplified method for the measurement of total non-starch polysaccharides by gas-liquid chromatography of constituent sugars as aditol acetates. Analyst. 109: 937-942.

FAO. (2023). Crops and livestock products. Retrieved May 26, 2023, from https://www.fao.org/faostat/en/#data/QCL

Faure, M., Moënnoz, D., Montigon, F., Mettraux, C., Breuille, D., Ballevre, O. (2005). Dietary threonine restriction specifically reduces intestinal mucin synthesis in rats. The Journal of Nutrition. 135(3): 486-491.

Favero, A., Ragland, D., Vieira, S.L., Owusu-Asiedu, A., Adeola, O., (2014). Digestibility marker and ileal amino acid digestibility in phytase-supplemented soybean or canola meals for growing pigs. Journal of Animal Science. 92: 5583–5592.

Fuller, M. F. (2004). The Encyclopedia of Farm Animal Nutrition. Cambridge, MA, USA. CABI Publishing

Gaonkar, V. & Rosentrater, K. (2019). Soybean. Z. In Z. Pan. R. Zhang, S. Zicari, Integrated Processing Technologies for Food and Agricultural By-Products. (pp. 73-104). Elsevier Inc. https://doi.org/10.1016/B978-0-12-814138-0.00004-6

Gerde, J. A. & White, P. J. (2008). Lipids. In Johnson, L., White, P., Galloway, R. (Ed.) Soybeans: Chemistry, Production, Processing, and Utilization. (pp. 193-227).

Gestetner, B., Birk, Y., Tencer, Y. (1968). Soybean saponins. Fate of ingested soybean saponins and the physiological aspect of their hemolytic activity. Journal of Agricultural and Food Chemistry, 16(6): 1031-1035.

Goldsmith, P. D. (2008). Economics of Soybean Production, Marketing, and Utilization. In L. Johnson, P. White, R. Galloway. Soybeans: Chemistry, Production, Processing, and Utilization. (pp. 117-150). Elsevier Inc. https://doi.org/10.1016/C2015-0-02416-9

Grieshop, C. M., Kadzere, C. T., Clapper, G. M., Flickinger, E. A., Bauer, L. L., Frazier, R. L., Fahey, G. C. (2003). Chemical and nutritional characteristics of United States soybeans and soybean meals. Journal of Agricultural and Food Chemistry. 51(26): 7684-7691.

Haug, W., & Lantzsch, H. J. (1983). Sensitive method for the rapid determination of phytate in cereals and cereal products. Journal of the Science of Food and Agriculture. 34(12): 1423-1426.

Hemetsberger, F., Hauser, T., Domig, K. J., Kneifel, W., Schedle, K. (2021). Interaction of soybean varieties and heat treatments and its effect on growth performance and nutrient digestibility in broiler chickens. Animals. 11(9): 2668.

Hendriks, W. H., Verstegen, M. W., Babinszky, L. (Eds.). (2019). Poultry and pig nutrition: Challenges of the 21st century. Wageningen Academic Publishers.

Heuzé V., Tran G., Kaushik, S. 2020. Soybean meal. Feedipedia. Retrieved September 9, 2022, from https://www.feedipedia.org/node/674

Hymowitz, T. 2008. The History of the Soybean. In L. Johnson, P. White, R. Galloway. Soybeans: Chemistry, Production, Processing, and Utilization. (pp. 1-31). Elsevier Inc.

Ishler, V., & Varga, G. (2008). Soybeans and soybean byproducts for dairy cattle. Dairy and Animal Sciences, 1, 13.

ISO. (2005). ISO 13904:2005 - Animal feeding stuffs: Determination of tryptophan content. ISO. https://www.iso.org/obp/ui/#iso:std:iso:13904:ed-1:v1:en

Johnson, L. & Smith, K. (2017). Soybean Processing Fact Sheet. Retrieved on June 3 from https://www.soymeal.org/wp-content/uploads/2018/04/soybean_processing.pdf.

Kaldhusdal, M., & Hofshagen, M. (1992). Barley inclusion and avoparcin supplementation in broiler diets: 2. clinical, pathological, and bacteriological findings in a mild form of necrotic enteritis. Poultry Science, 71(7), 1145-1153.

Karr-Lilienthal, L. K., Bauer, L. L., Utterback, P. L., Zinn, K. E., Frazier, R. L., Parsons, C. M., Fahey, G. C. (2006). Chemical composition and nutritional quality of soybean meals prepared by extruder/expeller processing for use in poultry diets. Journal of Agricultural and Food Chemistry. 54(21): 8108-8114.

Karr-Lilienthal, L. K., Kadzere, C. T., Grieshop, C. M., Fahey Jr, G. C. (2005). Chemical and nutritional properties of soybean carbohydrates as related to nonruminants: A review. Livestock Production Science. 97(1): 1-12.

Kinney, A. & Clemente, T. (2011). Soybeans. In N. Halford, A. Karp. Energy Crops. RSC Publishing. (pp. 148-164).

Kong, C., & Adeola, O. (2014). Evaluation of amino acid and energy utilization in feedstuff for swine and poultry diets. Asian-Australasian Journal of Animal Sciences. 27(7): 917.

Kong, C., Ragland, D., Adeola, O. (2014). Ileal endogenous amino acid flow response to nitrogen-free diets with differing ratios of corn starch to dextrose in pigs. Asian-Australasian Journal of Animal Sciences. 27(8): 1124.

Lee, J. H., & Choung, M. G. (2011). Comparison of nutritional components in soybean varieties with different geographical origins. Journal of the Korean Society for Applied Biological Chemistry. 54: 254-263.

Leeson, S. & Summers, J.D. (1997) Commercial Poultry Nutrition, 2nd Ed. University Books, Guelph, Canada, 355 pp.

Lemme, A., Ravindran, V., Bryden, W.L. (2004). Ileal digestibility of amino acids in feed ingredients for broilers. Poultry Science. 60: 423-437 DOI: 10.1079/WPS200426

Leung, H., & Kiarie, E. G. (2020). Standardized ileal digestibility of amino acids and apparent metabolizable energy in corn and soybean meal for organic broiler chicken production in Ontario. Canadian Journal of Animal Science, 100(3), 447-454.

Li, Z., Wang, Q., Xie, F., Liu, D., Li, Y., Lyu, Z., Lai, C. (2017). Oligosaccharides are a key factor in prediction of amino acid digestibility in soybean meal of different origins when fed to growing pigs. Asian-Australasian Journal of Animal Sciences, 30(12), 1724.

Liener, I. E. (1994). Implications of Antinutritional Components in Soybean Foods. Critical Reviews in Food Science and Nutrition, 34(l):31-67 (1994)

Liying, Z., Li, D., Qiao, S., Johnson, E. W., Li, B., Thacker, P. A., Han, I. K. (2003). Effects of stachyose on performance, diarrhoea incidence and intestinal bacteria in weanling pigs. Archives of Animal Nutrition, 57(1), 1-10.

Lusas, E. (2004). Soybean Processing and Utilization. In R. Boerma, J. Specht Soybeans: Improvement, Production, and Uses (3rd Ed.). (pp. 949-1045). American Society of Agronomy-Crop Science Society of America-Soil Science Society of America.

Macrae, R., R. K. Robinson, M. J. Sadler. (1993). Soya beans. In: R. Macrae, R. K. Robinson and, M. J. Sadler (Ed.). Encyclopaedia of Food Science, Food Technology and Nutrition. (pp. 4215-4218) Academic Press; London.

Manitoba Agriculture (2021). Manitoba Soybean Opportunity. Retrieved July 3, 2023 from https://www.manitoba.ca/agriculture/protein/pubs/soybean-opportunity-factsheet-accessible.pdf

Manitoba Agriculture (2022a). The Manitoba Advantage in Chicken Protein. Manitoba Agriculture. Retrieved September 9, 2022 from https://www.gov.mb.ca/agriculture/protein/protein-supply/chicken.html

Manitoba Agriculture (2022b). Commercial Poultry. Manitoba Agriculture. Retrieved on September 9, 2022, from https://www.gov.mb.ca/agriculture/livestock/production/poultry/

Manitoba Agriculture (2022c). Grains and Oilseeds Market Price: Current Manitoba Markets Weekly. Manitoba Agriculture. Retrieved September 9, 2022, from https://www.gov.mb.ca/agriculture/markets-and-statistics/crop-statistics/grains-oilseeds-market-prices-current-year.html

Manitoba Agriculture (2023). Manitoba Trade with the U.S. Retrieved July 3, 2023 from https://www.gov.mb.ca/agriculture/markets-and-statistics/trade-statistics/pubs/us-trade-profile.pdf

Manitoba Chicken Producers (2023). Canada's Chicken Supply Management. Retrieved August 10, 2023, from https://manitobachicken.ca/supply-management/

McDonald, P., Edwards, R., Greenhalgh, J., Morgan, C., Sinclair, L., Wilkinson, R., Greenhalgh, M., Wilkinson, M. (2011). Animal Nutrition (7th Ed). Pearson Canada.

McNess (2021). Extruded/Expelled Soybean Meal, Product Profile. Retrieved on June 3 from https://www.mcness.com/wp-content/uploads/2021/08/EE-Soybean-Meal-Product-Guide-EESB-PG-SS-D1521.pdf.

Meng, X. & Slominski, B. A. (2005). Nutritive values of corn, soybean meal, canola meal, and peas for broiler chickens as affected by a multicarbohydrase preparation of cell wall degrading enzymes. Poultry science. 84(8): 1242-1251.

Minoy, C., Su, A. L., Stein, H. (2023). Is there a difference in nutritional value between conventional SBE and Photoseed SBE when fed to growing pigs?. Retrieved on June 3 from https://www.nationalhogfarmer.com/nutrition/evaluation-soybean-expellers-fed-growing-pigs

NRC (1994). Nutrient requirements of domestic animals. Nutrient Requirements of Poultry (9th Ed.) National Academy Press, Washington, DC.

NRC (2012). Nutrient requirements of swine. 11th ed. National Academy Press, Washington, DC.

OECD/FAO (2021). OECD-FAO Agricultural Outlook 2021-2030. OECD Publishing. Retrieved September 9, 2022, from https://doi.org/10.1787/19428846-en

Opapeju, F. O., Golian, A., Nyachoti, C. M., Campbell, L. D. (2006). Amino acid digestibility in dry extruded-expelled soybean meal fed to pigs and poultry. Journal of animal science, 84(5), 1130-1137.

Ort, N. W. W., Morrison, M. J., Cober, E. R., McAndrew, D., Lawley, Y. E. (2022). A comparison of soybean maturity groups for phenology, seed yield, and seed quality components between eastern Ontario and southern Manitoba. Canadian Journal of Plant Science. 102(4): 812-822.

Oryschak, M., Korver, D., Beltranena, E. (2012). Nutrient digestibility in Canadian grown pulse crops compared to soybean meal for growing broilers at 15- and 29-d of age. Retrieved December 21, 2022, from https://www1.agric.gov.ab.ca/$Department/deptdocs.nsf/all/lr14763/$FILE/nutr-dig-in-pulse-crops-vs-soybean-meal-for-growing-broilers.pdf

Parsons, C. M. (1986). Determination of digestible and available amino acids in meat meal using conventional and caecectomized cockerels or chick growth assays. British Journal Nutrition 56: 227-240.

Parsons, C. M., Hashimoto, K., Wedekind, K. J., Baker, D. H. (1991). Soybean protein solubility in potassium hydroxide: an in vitro test of in vivo protein quality. Journal of Animal Science. 69: 2918-2924.

Powell, S., Naranjo, V. D., Lauzon, D., Bidner, T. D., Southern, L. L., Parsons, C. M. (2011). Evaluation of an expeller-extruded soybean meal for broilers. Journal of Applied Poultry Research. 20(3): 353-360.

Quality Roasting. (2021). Exceller Meal ® Product Specification Sheet. Retrieved on June 3, from https://www.qualityroasting.com/wp-content/uploads/2021/04/Exceller-Meal-Spec_2021.pdf.

Ravindran, V. & Bryden, W.L. (1999) Amino acid availability in poultry – In vitro and in vivo measurements. Australian Journal of Agricultural Research 50, 889–908.

Ravindran, V., Abdollahi, M. R., Bootwalla, S. M. (2014). Nutrient analysis, metabolizable energy, and digestible amino acids of soybean meals of different origins for broilers. Poultry Science. 93: 2567–2577 http://dx.doi.org/ 10.3382/ps.2014-04068

Ravindran. V. (2021). Progress in ileal endogenous amino acid flow research in poultry. Journal of Animal Science and Biotechnology. https://doi.org/10.1186/s40104-020-00526-2

Ritchie, H. & Roser, M. (2021). "Forests and Deforestation". OurWorldInData.org. Retrieved September 9, 2022, from https://ourworldindata.org/forests-and-deforestation

Roberts, M. (2021). 2021 Grains, oilseeds, and pulses outlook update: Tight stocks and uncertain demand. FCC. Retrieved September 9, 2022, from https://www.fcc-fac.ca/en/knowledge/economics/2021-grains-oilseeds-pulses-outlook-update-stocks-demand.html

Sakkas, P., Royer, E., Smith, S., Oikeh, I., Kyriazakis, I. (2019). Combining alternative processing methods for European soybeans to be used in broiler diets. Animal Feed Science and Technology. 253: 45-55.

Sauvant, D., Perez, J. M., Tran, G. (2004). Tables of composition and nutritional value of feed materials: pigs, poultry, cattle, sheep, goats, rabbits, horses and fish. Wageningen Academic Publishers.

Sawada, M., Venancio, L., Toda, T., Rodrigues, E. (2014). Effects of different alcoholic extraction conditions on soybean oil yield, fatty acid composition and protein solubility of defatted meal. Food Research International. 62: 662–670.

Scawen, M. & Allen, A. (1977). The action of proteolytic enzymes on the glycoprotein from pig gastric mucus. Biochemical Journal, 163(2), 363-368.

Schöner, F. J. & Hoppe, P. P. (2002). The effects of phytase in poultry nutrition. In: J.M. McNab and K.N. Boorman (Ed.), Poultry Feedstuffs: Supply, Composition and Nutritive Value (pp. 363-373). CAB International.

Sekiz, S. S., Scott, M. L., Nesheim, M. C. (1975). The effect of methionine deficiency on body weight, food and energy utilization in the chick. Poultry Science. 54(4): 1184-1188.

Selle, P. H. & Ravindran, V. (2006). Microbial phytase in poultry nutrition. Animal Feed Science and Technology. 135: 1–41.

Sibbald, I.R. (1979). A bioassay for available amino acids and true metabolizable energy in feedingstuffs. Poultry Science. 58: 668-75

Singh, V. & Roca, E. D. (2022). China’s geopolitical risk and international financial markets: evidence from Canada. Applied Economics, 54(34), 3953-3971.

Siriwan, P., Bryden, W. L., Annison, E. F. (1994). Use of guanidinated dietary protein to measure losses of endogenous amino acids in poultry. British Journal of Nutrition, 71(4), 515-529.

Slominski, B. A., D. Boros, L. D. Campbell, W. Guenter, O. Jones. (2004). Wheat by-products in poultry nutrition. Part 1. Chemical and nutritive composition of wheat screenings, bakery by-products and wheat mill run. Canadian Journal of Animal Science. 84: 421-428.

Slominski, B. A., Meng, X., Campbell, L. D., Guenter, W., Jones, O., (2006). The use of enzyme technology for improved energy utilization from full-fat oilseeds. Part II: Flaxseed. Poultry Science. 85: 1031-1037.

Soybeans: Quality Control. (n.d.). Retrieved May 29, 2023 from University of Giorgia. https://poultry.caes.uga.edu/extension/poultry-nutrition/soybeans/quality-control.html.

Statistics Canada. (2023a). Estimated areas, yield, production, average farm price and total farm value of principal field crops, in metric and imperial units. Statistics Canada. Retrieved April 25, 2023, from https://doi.org/10.25318/3210035901-eng

Statistics Canada (2023b). Poultry Production Report: Chicken, Year 2022. Retrieved July 3, 2023 from https://agriculture.canada.ca/en/sector/animal-industry/poultry-and-egg-market-information/production#poultry

Stefanello, C., Vieira, S., Rios, H, Sorbara, J. (2016). Energy and nutrient utilisation of broilers fed soybean meal from two different Brazilian production areas with an exogenous protease. Animal Feed Science and Technology 221: 267–273p.

Stein, H. H., J. A. Roth, K. M. Sotak, O. J. Rojas. (2013). Nutritional value of soy products fed to pigs. Swine Focus #004. https://nutrition.ansci.illinois.edu/sites/default/files/SwineFocus004.pdf

The Soybean Processors Association of India. (2023). World Soymeal Production. Retrieved May 26, 2023, from https://www.sopa.org/statistics/world-soymeal-production/

Tortajada, C. & Zhang, H. (2022). Policies and Politics: Effects on US-China Soybean Trade. Georgetown Journal of International Affairs. Retrieved July 3, 2023 from https://gjia.georgetown.edu/2022/10/26/policies-and-politics-effects-on-us-china-soybean-trade/

Unveren, H., & Luckstead, J. (2020). Comprehensive Broiler Supply Chain Model with Vertical and Horizontal Linkages: Impact of US–China Trade War and USMCA. Journal of Agricultural and Applied Economics, 52(3), 368-384.

Vagadia, B. H., Vanga, S. K., Raghavan, V. (2017). Inactivation methods of soybean trypsin inhibitor–A review. Trends in Food Science & Technology, 64, 115-125.

Wang, X. W., Du, Y. G., Bai, X. F., Li, S. G. (2003). The effect of oligochitosan on broiler gut flora, microvilli density, immune function and growth performance. Acta Zoonutrimenta Sinica. 15(4): 32-35.

Woodworth, J. C., Tokach, M. D., Goodband, R. D., Nelssen, J. L., O'Quinn, P. R., Knabe, D. A., Said, N. W. (2001). Apparent ileal digestibility of amino acids and the digestible and metabolizable energy content of dry extruded-expelled soybean meal and its effects on growth performance of pigs. Journal of Animal Science. 79(5): 1280-1287.

Wu, G. (2018). Principles of Animal Nutrition. Boca Raton, FL, USA. CRC Press.

Zduńczyk, Z., Jankowski, J., Mikulski, D., Zduńczyk, P., Juśkiewicz, J., Slominski, B. A. (2020). The effect of NSP-degrading enzymes on gut physiology and growth performance of turkeys fed soybean meal and peas-based diets. Animal Feed Science and Technology. 263: 114448.

Žilić, S., Bozović, I., Hadži-Tašković Šukalović, V. (2012). Thermal inactivation of soybean bioactive proteins. International Journal of Food Engineering. 8(4).