EFFECT OF PROTEIN SOURCES ON EARLY TURKEY PERFORMANCE AND GASTROINTESTINAL TRACT DEVELOPMENT

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ABSTRACT

Nutrition during the early life of turkey poults has a long lasting impact on bird performance, as well as gastrointestinal tract (GIT) development. This research focused on understanding the impact of protein source provided in the feed on performance and GIT development. All statistical analysis was completed using Proc Mixed in SAS 9.3 and significant differences were set at $P \leq 0.05$, while trends were identified for $P \leq 0.10$. The first study (5 x 2 factorial arrangement) evaluated apparent metabolizable energy (AMEn) and apparent ileal amino acid digestibility (AIAAD) of five high protein feed ingredients (soybean meal, SBM; corn gluten meal, CGM; canola protein concentrate, CPC; fish meal, FM; and porcine meal, PM) in male broiler chickens at 5 and 21 d with 6 replications of 30 and 8 chicks, respectively. The AMEn was not affected by bird age for CPC, FM, CGM, and SBM, however, the d 5 value for PM was higher than the d 21 value. The response of AIAAD was variable and dependent on amino acid and protein source, but overall, there was an increase in AIAAD with increasing age, with the largest increase observed for CGM. These AMEn and AIAAD values were then used to formulate the diets for a second experiment. The diets for this experiment consisted of a high SBM control diet, and four additional diets with either CPC, FM, PM, or CGM replacing 25% of the protein that was supplied by SBM in the control diet. This experiment was set up as a completely randomized design with four pens of 23 turkey poults per protein source. Body weights, feed and water intake, and mortality were recorded on a weekly basis. At the same time, four pens of 21 poults per protein source were used to study the impact of these diets on GIT development and blood metabolic profiling. At hatch, placement, d 1, 2, 3, 5, 7, 14, and 21, intestinal tract and tissue weights were collected and recorded (2 poults per replication per time point). Ileal segments were collected at hatch, placement, d 1, 3, 5, and 7

from 2 poults per replicate pen of the SBM and PM diets to study the effect of diet on intestinal morphology, number of goblet cells, and transcript abundance for selected genes relating to barrier function and inflammation. Blood samples were also drawn at these time points for blood metabolite analysis. Data were analyzed as a 5x9 factorial for tissues weights, a 2x5 factorial for histology and gene expression, and a 5x5 factorial for blood analysis. Planned contrasts were used on the performance, tissue, and blood data to compare the SBM diet to the average of the remaining diets, the PM diet to the average of the remaining diets, and the addition of animal or vegetable proteins. Inclusion of an additional protein source increased body weight up to 14 d, in comparison to poults fed the SBM diet, but feed efficiency and water consumption were not affected. The effect of diet on tissue weights were small, with the exception of the pancreas weight, which were higher in the birds fed vegetable protein diets. Age had an effect on all tissue weights, which peaked between d 2 and 7, as well as on digestive tract morphology and gene expression. There was an increase in gene expression between placement and d 1, which could illustrate the importance of feed as an activator of barrier function and the immune system. The goblet cell counts revealed a greater proportion of neutral goblet cells in PM fed birds associated with accelerated mucus maturation. The research shows there are benefits to limiting the amount of SBM provided in early turkey feeds, especially prior to two weeks of age.

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LIST OF ABBREVIATIONS

AA	Amino acid(s)
AIAAD	Apparent ileal amino acid digestibility
AMEn	Nitrogen corrected apparent metabolizable energy
ANF	Anti-nutritional factor(s)
AnGap	Anion gap
BAI	Biogenic amine index
BEecf	Base excess in extracellular fluid
BM	Breast meat
BW	Body weight
CGM	Corn gluten meal
CPC	Canola protein concentrate
d	Day(s)
FM	Fish meal
g	gram(s)
GC	Goblet cell(s)
G:F	Gain:feed
GIT	Gastrointestinal tract
h	Hour(s)
H	Hatch
Hb	Hemoglobin
Hct	Hematocrit
IL	Interleukin
kg	Kilogram(s)
NS	Not significant
LW	Live weight
P	Placement
pCO_2	Partial pressure of carbon dioxide
PM	Porcine meal
SBM	Soybean meal
SEM	Standard error of the mean

TCO_2	Total carbon dioxide
VCR	Villus crypt ratio
wk	Week(s)

1.0 INTRODUCTION

The Canadian turkey industry is comprised of approximately 530 turkey farms nation-wide, which produced 171 million kg of eviscerated turkey meat in 2015 (Turkey Farmers of Canada, 2015). This industry has been showing positive growth every year for the past 35 years. As the Canadian turkey industry continues to grow, it is important to understand early nutrition of these birds, especially in terms of protein nutrition. This allows poultry nutritionists to formulate and provide high quality feed that will ensure the digestive tract develops and functions to its potential, as well as understanding how early nutrition impacts animal performance.

At hatch, turkey poults are protein deficient as protein is used as the preferential energy source during hatching (Uni and Ferket, 2004). Due to this deficiency, providing poults with protein immediately post-hatch is critical to their development as it supports the growth and maintenance of existing protein tissue growth (Firman and Boling, 1998). Currently in Canada, the most used protein source in poultry diets is soybean meal (SBM) due to its amino acid (AA) balance and relatively high energy level (Ravindran et al., 2014). Due to the high protein levels, SBM also plays a considerable role in the production of poultry products for the vegetable fed meat product markets. While an attractive feed ingredient economically, SBM contains anti-nutritional factors (ANF) that may contribute to a decrease in performance at high inclusion levels (Feng et al., 2007). Diluting the SBM content with a second high protein feed ingredient would decrease the intake of ANF, as well as potentially increasing the presence of bioactive compounds, providing biological benefits by effecting physiological functions (Muir et al., 2013).

During this protein deficient phase, the digestive tract is continuing to develop and mature. The development of the digestive tract is a very important process, occurring during incubation, as well as following hatch in turkey poults (Bohórquez et al., 2011). It is important because the gastrointestinal tract (GIT) plays a critical role in digestion, absorption, and protection within the body (Dibner et al., 1996). During the development of the GIT, there is an increase in surface area, due to increased villi height, crypt depth, and cell maturation, along with increased nutrient transporters and digestive enzyme secretion (Noy and Sklan, 1995).

The protection provided by the GIT stems from the development of the intestinal barrier, made up a single layer of epithelial cells and the mucus layer (Smith et al., 2000). The ability of this barrier to exclude contaminants while still allowing nutrients to be absorbed is dependent on the permeability of the cells, as well as the tightness of the junction proteins located between the epithelial cells (Feldman et al., 2005). Any disturbances to this barrier will increase the opportunity for translocation of contaminants (Smith et al., 2000), which may negatively impact bird health. A second component to this protection is the mucus layer, which covers the epithelium and protects from irritants, as well as helping the GIT resist bacterial translocation (Smirnov et al., 2005).

There is minimal research in the area of barrier function in turkeys, specifically in regards to the timing of development, as well as the impact of feeding different protein sources on this development. Therefore, the objectives of this study are to evaluate the feeding value, in terms of digestibility (at d 5 and 21), performance, and GIT development, of five different protein sources. A second objective of this research is to study the effect of reducing SBM levels in early turkey diets to improve poult performance.

2.0 LITERATURE REVIEW

The formulation and provision of high quality feed to turkey poults ensures the digestive tract develops and functions to its potential, as well as supporting animal performance. Understanding the timing of the development and the limitations to growth during the post-hatch period is necessary to optimize diet effects. This literature review will document work completed on morphological and barrier function development in the gastrointestinal tract (GIT), transition from yolk to exogenous feeds, and the high protein requirement in turkey poults.

2.1 Gastrointestinal tract

2.1.1 Morphological development of the gastrointestinal tract

The GIT plays a very important role in digestion and absorption of nutrients, and also protection of the body (Dibner et al., 1996). This is the first tissue that dietary nutrients, as well as any contaminants, encounter upon consumption by the poult (Dibner et al., 1996). The GIT is made up of the crop, proventriculus, gizzard, small intestine, colon, and caeca, with the major site of digestion and absorption being the small intestine. The main components of the small intestine (Figure 2.1) include enterocytes, crypts, villi, and microvilli (Garrett et al., 2010). Enterocytes make up the epithelial layer of the small intestine, are the site of nutrient adsorption, and are therefore responsible for the uptake of ions, water, sugars, peptides and amino acids (AA), and lipids (Leeson and Summers, 2001). Enterocytes differentiate and proliferate from the mucosal crypts, where the intestinal mucosa renews itself (Uni et al., 2000). As enterocytes proliferate from the crypts, they migrate up the villi and are eventually sloughed into the lumen at the villus tip (Uni et al., 2000). Villi and microvilli are responsible for increasing the

absorptive surface of the intestinal tract, allowing for rapid and efficient nutrient absorption (Uni et al., 2000).

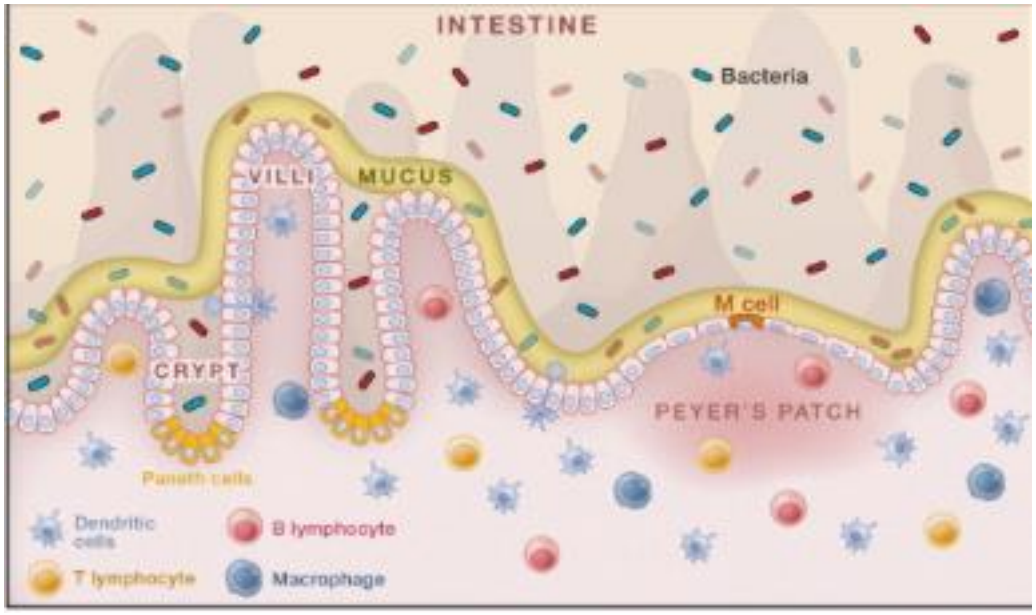

Figure 2.1 Structure of small intestine (Garrett et al., 2010). Reproduced with permission from Company of Biologists Ltd: Journal of Cell Science. 140:859-870 (Garrett et al.), copyright (2010).

During incubation, the swallowing of amniotic fluid helps initiate and support the development of the GIT (Bohórquez et al., 2011). Amniotic fluid, rich in proteins, AA, growth factors, and hormones, stimulates the division and proliferation of the intestinal epithelium and the consumption initiates the rapid growth and development of the GIT (Bohórquez et al., 2011). As the amniotic fluid volume is depleted at approximately 25 d of incubation in turkey embryos, a two-fold increase in the villus height can be observed, marking the beginning of a significant increase in surface area required for the absorption of nutrients from the yolk sac, as well as exogenous feed following hatch.

Early growth of the GIT includes the rapid increase in the villi length and overall surface area. The timing and extent of this development can be influenced by various factors, including feed restriction, the presence of dietary contaminants, and the use of antibiotics (Dibner et al.,

1996). This is important because it illustrates how adaptive and variable the timing of development is and suggests that feed composition, including the nature of protein sources, may impact this timing.

Significant changes in the intestinal morphology continue to occur following hatch, typically becoming functionally mature and effective in nutrient absorption by 12 d of age in poults (Bohórquez et al., 2011). Bohórquez et al. (2011) focused on the development of the small intestine in poults from 15 d of embryonic development up to 12 d post-hatch. These researchers found a two-fold increase in microvilli length around the time of hatch, contributing to a rapid increase in surface area in the intestinal tract, as well as a significant increase in crypt depth, which is required to sustain proliferation of enterocytes during rapid development. Immediately following hatch, the enterocytes are relatively non-polar and do not have a visible brush-border, but within approximately 24 h with feed access, they become polar and develop a distinct brush border (Geyra et al., 2001). The enterocytes then continue to mature and differentiate leading to further villi growth (Bohórquez et al., 2011). This growth increases the surface area within the small intestine, allowing for increased nutrient uptake.

Major morphological changes, including maturation and differentiation of enterocytes, as well as increased villi surface area and crypt depth, occur in the small intestine of the poult, both pre- and post-hatch. The crypt depth is an important part of the morphological development because it is responsible for the proliferation of enterocytes and replacement of damaged and old enterocytes. This is of importance in young poults because a high rate of proliferation is required to support the rapid growth of villi. At the same time, the secretion of bile acids, and pancreatic and brush border enzymes increases (Noy and Sklan, 1995; Uni et al., 1995). While these changes contribute to an increase in surface area and help prepare the digestive tract for

digestion and absorption of nutrients provided to the poult following the consumption of feed after hatch, they also indicate that a young bird is very unique and vulnerable immediately post-hatch.

2.1.2 Intestinal barrier development

The intestinal barrier, made up of extracellular and cellular components, provides the epithelium of the intestine with the ability to prevent foreign content from systemic encroachment (Nejdfors et al., 2000). Within the intestine, the barrier is made up of a single layer of selectively permeable epithelial cells (Smith et al., 2000). Following the swallowing of amniotic fluid, there is a gradual tightening of the spaces between the epithelial cells, as well as the development of mature goblet cells (Bohórquez et al., 2011). This is the beginning of the development of the intestinal barrier, as these changes are required to reduce the potential for bacteria, pathogens and toxins to pass through the enterocytes and become systemic. Mucin, produced and secreted by goblet cells, is a major component of the mucus layer, which covers the surface of the small intestine and contributes to the protection of the underlying epithelium from damage and infection (Azzam et al., 2011). Disturbances in the function of the intestinal barrier can lead to an increase in permeability and may allow for translocation of bacteria, toxins, and antigens (Smith et al., 2000), which impact bird health.

Protection of the intestinal tract is partially provided by the unstirred mucus layer synthesized by goblet cells (Smirnov et al., 2004). Goblet cells, arising from stem cells at the base of the crypt, synthesize and secrete polymeric mucin glycoproteins, leading to the creation of the mucus layer (Forder et al., 2007). The mucus layer covers the intestinal tract epithelium and has an effect on the protection and lubrication of the intestinal tract, as well as on the transportation of molecules from the intestinal lumen to the enterocytes (Smirnov et al., 2004). It

is continuously degraded, due to bacterial enzyme action and general wear, and renewed by the continued synthesis by the goblet cells (Smirnov et al., 2004). The presence of the mucus layer is important in intestinal barrier function because it resists bacterial translocation, as the pathogens must pass through this layer in order to access enterocytes and potentially damage connections between enterocytes, a next step in the translocation process (Smirnov et al., 2005). The mucus layer also protects enterocytes from irritants (Smirnov et al., 2004). Changes in the thickness of this layer have the potential to affect digestion and absorption of nutrients (Smirnov et al., 2004). A thickened mucus layer may result in impaired nutrient digestion, due to the distance the nutrients must move across the mucus layer. Alternatively, a thinner mucus layer allows for more efficient nutrient absorption, but also makes the GIT more susceptible to bacterial translocation. Goblet cells, as well as the mucus they create, can be subcategorized into neutral and acidic, or sulfated and nonsulfated (Smirnov et al., 2006). The nature of goblet cells affects the type of protection the mucus layer is able to provide, with acidic goblet cells producing mucin more able to protect against bacterial translocation because it is less susceptible to bacterial glycosidase (Uni et al., 2003). A high presence of acidic mucins during early small intestine development likely plays an important role in innate immunity, as the acquired immune system is not fully developed and functional at this point (Uni et al., 2003). Neutral goblet cells indicate a more mature gut, as previous research has shown a shift from acidic to neutral mucins following hatch (Uni et al., 2003).

The cellular components of the barrier include enterocytes, tight junctions, adherens junctions, gap junctions, and desmosomes. Tight junctions, adherens junctions, gap junctions, and desmosomes are all types of cell-to-cell junctions between the enterocytes (Figure 2.2) (Feldman et al., 2005). Tight junctions are seals between adjacent enterocytes within the wall of

the small intestine and they control passage of ions, water, and other molecules that move via paracellular pathways between these cells (Feldman et al., 2005). These junctions are important as they play a significant role in preventing macromolecule transmission across the intestinal wall (Zhang and Guo, 2009). Tight junctions are variable within the small intestine and, depending on their location, there are differences in electrical conductivity, ionic charge preference, and the level of permeability (Van Itallie et al., 2008). The main proteins involved in the structure of tight junctions are occludin and claudins (Zhang and Guo, 2009). Occludin plays a significant role in the trans-epithelial electrical barrier function (Feldman et al., 2005), while claudins influence charge selectivity of the tight junction (Van Itallie et al., 2008), as well as making up the backbone of the tight junction strands (Tsukita et al., 2001).

Adherens junctions, gap junctions, and desmosomes also play a role in regulating paracellular molecule passage, however, they differ from tight junctions in that there is more space between these junctions, while tight junctions have virtually no space between them

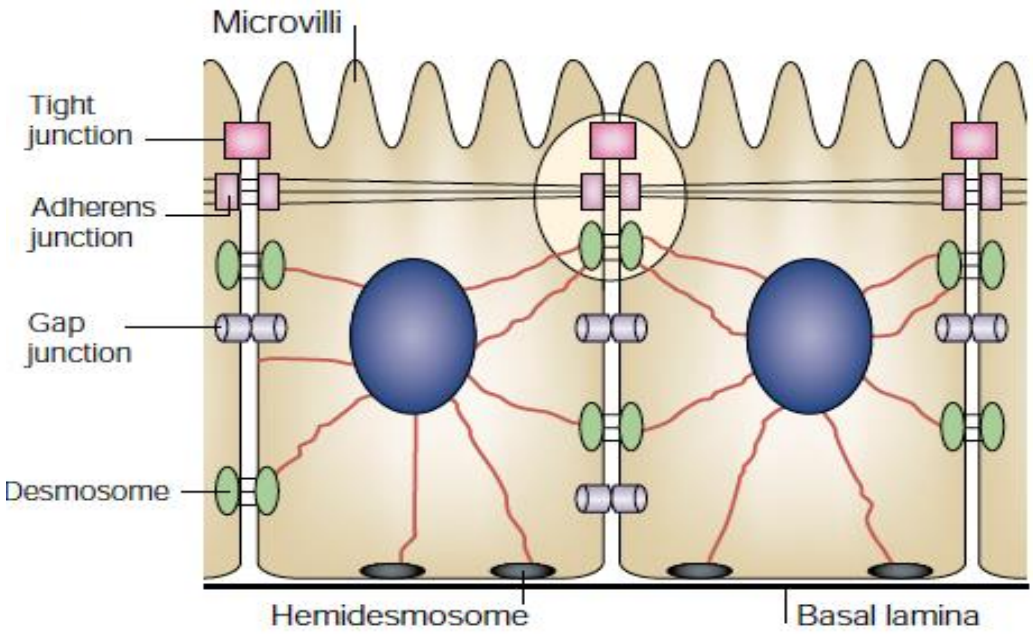

Figure 2.2 Cell-to-cell junctions between enterocytes (Tsukita et al., 2001). Reprinted by permission from Macmillan Publishers Ltd: Nature Reviews Molecular Cell Biology (Tsukita et al.), copyright (2001).

(Tsukita et al., 2001). Extracellular components of barrier function are variable as they depend on many factors, including the number of tight junctions strands, as well as the complexity of the junctions involved in regulating the para-cellular pathway between enterocytes (Tsukita et al., 2001).

2.2 Transition of poult nutrient supply from the yolk to exogenous feed

The yolk provides energy to the poult during incubation, mainly in the form of lipids (Noy and Sklan, 1998). Before hatch, the yolk lipids are transported directly into the circulating blood by endocytosis. After the yolk sac has been internalized prior to hatch, the yolk lipids are transported both directly into the bloodstream, as well as being transported through the yolk stalk into the small intestine (Noy and Sklan, 1998). These lipids are available to the poult for nutrition purposes until exogenous feed is provided.

At hatch, the yolk makes up approximately 10 to 12 percent of the body weight of the poult and it is used for maintenance energy during the first few days (Noy and Sklan, 1997). Following hatch, the poult must make the transition from yolk to exogenous nutrients made available in feed (Noy and Sklan, 1997). This transition is important in that yolk energy is supplied mainly in the form of lipids, while the energy derived from feed is largely is the form of carbohydrates (Noy and Sklan, 1997). Consumption of exogenous feed triggers an increased secretion of the enzymes required for digestion of carbohydrates and protein, and after these enzymes have reached sufficient levels to digest the nutrients being consumed, enzymes will be secreted at a relatively constant amount, relative to feed intake, as the bird grows (Sklan and Noy, 2000).

The transition from yolk to exogenous feed is a critical point in the development of the poult, as there are many functional and morphological changes happening in the digestive tract.

The increasing levels of enzyme secretion, as well as the effect of increasing surface area and digestive mechanisms in the small intestines create a highly effective and functional digestive tract, and these changes are important to consider when creating pre-starter diets for poults.

2.2.1 Enzyme secretion in the newly hatched poult

An important limitation to digestion is the levels of digestive enzymes being secreted by the poult (Sell, 1996). Following hatch, pancreatic secretion of lipase, trypsin, and amylase is relatively low and the secretion of these enzymes increases with age (Sklan and Noy, 2003). Although the secretion of these key enzymes increases with age, they increase at varying rates (Sklan and Noy, 2003). Sklan and Noy (2003) observed that the greatest increase was in amylase secretion, followed by trypsin, and finally lipase. Sulistiyanto et al. (1999) studied the utilization of fat, protein, and carbohydrates in the young chick in order to understand exactly how utilization of these nutrients changes as the bird ages. This research concluded that utilization of carbohydrates and protein increase as the bird ages. Lipase levels and subsequent fat utilization, on the other hand, remains stable during the first few days post-hatch. This agrees with the results found by Sklan and Noy (2003), as the lower utilization of carbohydrates and protein at hatch corresponds to the low levels of trypsin and amylase secretion. These results suggest that the protein and carbohydrates being fed to young birds are not effectively utilized until enzyme levels are being secreted at sufficient levels. Therefore, newly hatched poults are not able to digest and absorb all of the nutrients being provided to them due to the lack of enzymes and transport systems. Additionally, previous research indicates that the most limiting is protein digestion and AA absorption (Noy and Sklan, 1995; Noy and Sklan, 2001; Sulistiyanto et al., 1999), suggesting that the area of protein digestion requires the most attention when it comes to early turkey diets.

2.3 High protein requirement of the poult

The protein provided to poults in the pre-starter feed provides AA, which are the building blocks for protein within the body (Leeson and Summers, 2001). Protein requirements for turkey pre-starter diets are typically 28% crude protein (National Research Council, 1994; Leeson and Summers, 2005). Because protein is such an expensive component of the diet, it is important to optimize the level of protein being provided in the pre-starter diet (Forbes and Shariatmadari, 1994). It is not, however, as simple as providing a set level of crude protein and expecting it to meet the individual AA requirements of the poult. Protein sources vary in AA balance and digestibility, impacting the level of AA that are truly available to the poult (Firman, 1994).

In commercial turkey production, breast meat is the most valuable part of the bird, making the accretion of breast muscle a very important aspect of turkey production. Muscle fibre formation in breast meat is essentially complete when a bird hatches, and following hatch the breast muscle grows primarily by increasing the diameter and length of the muscle fibres, not by increasing the number of muscle fibres (Halevy et al., 2000). Satellite cells, precursor cells to muscle formation (Halevy et al., 2003), are capable of joining existing muscle fibres to increase their physical size (Halevy et al., 2000) and while these cells are rapidly proliferating immediately after hatch, the proliferation decreases dramatically as the bird begins to grow (Moore et al., 2005). Previous studies have demonstrated that delayed access to feed leads to decreased satellite cell proliferation and a negative impact on skeletal muscle growth (Halevy et al., 2000; Moore et al., 2005), while early access to feed stimulates satellite cell proliferation and skeletal muscle growth (Halevy et al., 2003). Additionally, access to high protein feed in the pre-starter diet has been shown to increase phosphorylation and activation of ribosomal protein S6 kinase, playing a significant role in muscle protein translation in broiler chicks (Everaert et

al., 2010). This is interesting because it indicates that feeding high protein diets as a pre-starter may lead to increased body weight gains and breast meat yield later in the production cycle, as the high protein activates the muscle protein translation mechanism.

The poult has a high protein requirement because of its rapid growth and development during the first few days following hatch. Not only is protein being used for maintenance of existing tissues, it is also being used to support the rapidly growing and developing GIT and satellite cells initiating the accretion of muscles (Firman and Boling, 1998). Another important aspect of the poult's high protein requirement is the type of protein being gained (Emmans, 1995). The AA composition of feathers is vastly different from that of muscle protein (Firman, 1994). This contributes to the high protein requirement because it means that there is an increased need for a wider variety of AA at this young age.

When comparing the recommended nutritional requirements and the growth rate provided by the genetics companies for chickens and turkeys, Ross 308 females have higher weights gains and eat more than the Hybrid Converter females (Aviagen, 2014; Hybrid, 2014). The feed conversion ratios in these documents also show that the Ross 308 females are more efficient than the Hybrid Converter hens. When this information is viewed in conjunction with the nutritional recommendations of 23% crude protein for a Ross 308 prestarter diet (Aviagen, 2014) and 27.5% crude protein for a Hybrid Converter prestarter diet (Hybrid, 2013), it suggests that chickens are able to use feed of lower nutritional value and still have higher weight gains than turkeys. This may be due to a variety of factors, including a difference in feathering time and the energy and AA required for feather growth, as well as a higher maintenance energy and AA requirement in turkeys due to a difference in body composition.

2.3.1 High protein feed ingredients

Due to the need for high protein in a turkey pre-starter diet, it is important to understand the nutritional value of high protein ingredients available for use in turkey feeds. When considering high protein concentrates for use in a turkey pre-starter feed, there are both vegetable and animal protein sources. Increased consumer preference for poultry meat products from flocks fed vegetable based diets is leading to the increased diet inclusion of vegetable protein sources, however, the ANF associated with many of these ingredients, coupled with lower protein quality (AA balance), may cause depressed performance in young turkeys.

The current research is focused on understanding the feeding value of three vegetable protein ingredients (soybean meal, IFN # 5-04-612; canola protein concentrate, no IFN; corn gluten meal, IFN # 5-02-900) and two animal protein ingredients (fish meal, IFN # 5-01-974; porcine meal, IFN # 5-00-385).

2.3.1.1 Vegetable proteins

Vegetable protein ingredients are used extensively in poultry diets, however, care must be taken to ensure that anti-nutritional factors (ANF) such as protease inhibitors, phytic acid, and oligosaccharides are not having a negative effect on performance. It is important to understand ingredients individually, identify their potential ANF, and understand how these ingredients work when fed in combination.

2.3.1.1.1 Soybean meal

The most used protein source in poultry diets worldwide is SBM because it has an AA profile that closely matches poultry requirements and has a relatively high protein and energy content, as well as being an economically attractive ingredient (Ravindran et al., 2014). Briefly, SBM is created by cracking the soybeans to loosen the hulls for removal, followed by flaking

using a roller mill, solvent extraction to remove the oil, and finally heating to destroy ANF. This ingredient typically has approximately 45 to 48% crude protein and a metabolizable energy value of 2320 kcal/kg (National Research Council, 1994). While SBM is a high quality ingredient, there are concerns with high levels of its use in turkey pre-starter diets. These concerns relate to the potential of ANF (protease inhibitors, lectins, oligosaccharides, phytic acid, and allergens) and excess potassium to reach dietary levels that are detrimental to poultry performance (Feng et al., 2007)

The ANF of highest concern in commercial turkey production are Kunitz and Bowman-Birk protease inhibitors, which inhibit protein digestion by binding to trypsin and chymotrypsin within the digestive tract. This causes impaired protein digestion, leading to reduced feed efficiency and body weight gains. Protease inhibitors can also lead to pancreas hypertrophy, due to an overstimulation of the pancreas to secrete proteases (Herkelman et al., 1993). SBM also contains lectins, which bind to the digestive wall tract and lead to reduced nutrient digestibility (Fasina et al., 2003). Because these compounds are able to bind to carbohydrates, they are able to disrupt and damage the intestinal membrane (Fasina et al., 2003). The ability of these compounds to damage the intestinal membrane is of significance in early turkey nutrition as it causes reduced digestibility. It also affects the development of the intestinal barrier and may allow harmful toxins and pathogens to move across the intestine. Protease inhibitors and lectins are both heat labile and considered to be effectively removed during SBM processing.

Oligosaccharides, including raffinose and stachyose, found in SBM, are carbohydrates that cannot be digested by poultry due to a lack of the enzyme, alpha-galactosidase (Chen et al., 2013). This allows the undigested carbohydrates to move into the lower gut, where they are available for bacterial fermentation. Whether this is a positive or negative impact is difficult to

determine and may vary depending on many factors. On one hand, bacterial fermentation can lead to the creation of gases and discomfort with the bird, but on the other hand they may serve as prebiotics within the lower gut, allowing for increased beneficial bacterial colonization.

Phytic acid, the storage form of phosphorus, causes issues within the poultry digestive tract because of its ability to bind to protein, starch, and minerals, such as calcium, magnesium, zinc, and iron (Pallauf and Rimbach, 1997; Karr-Lilienthal et al., 2005; Cowieson et al., 2006). Once bound, nutrients are not available for absorption by the bird, and proteins, such as proteases, have reduced functionality, leading to decreased animal performance.

SBM also contains glycinin and beta-conglycinin, which are antigenic proteins (Liu et al., 2007). These proteins cause an allergic response in the epithelium of animal digestive tracts. Although this has not yet been demonstrated in poultry, it may be a concern in pre-starter diets containing SBM meal as the only protein concentrate.

A final concern when using SBM is its high level of potassium. This leads to increased moisture output in the excreta and wet litter in the barn (Youssef et al., 2011). This is concerning for poultry production as high litter moisture levels can lead to footpad dermatitis and increased barn ammonia levels, reducing bird performance and welfare.

Both under and over processing of SBM can affect its quality. Under processing SBM will result in increased levels of ANF. Nutritionists rely on processing to destroy heat sensitive negative components of SBM, but if adequate temperatures are not reached during processing, these components will be present in the meal and will have subsequent negative effects on the birds (Aburto et al., 1998). On the other hand, over processing SBM will decrease the nutritional value of SBM, as overheating has a negative impact on protein, and can reduce AA content and digestibility (Aburto et al., 1998)

In most poultry diets, the levels of SBM are not high and as a result concerns regarding any residual ANF and under or over processing are typically minimal. There are of more concern in early turkey nutrition because many turkey pre-starter diets contain between 40 and 50% SBM in order to reach the bird's protein requirement. When feeding levels that high, it is possible that the residual ANF levels will be high enough in the feed to have a measurable negative impact on the birds. Because of the ANF in SBM and concerns associated with high levels of SBM use in early turkey diets, it is of interest to investigate alternative high protein concentrates to replace a proportion of SBM.

2.3.1.1.2 Corn gluten meal

Corn gluten meal (CGM) is a byproduct of wet milling corn for the production of high fructose corn syrup (Leeson and Summers, 2001). This process removes the majority of the starch in the corn, leading to an increased concentration of protein, and creating an ingredient containing approximately 60% crude protein and a metabolizable energy value of approximately 3720 kcal/kg (Leeson and Summers, 2001). The use of CGM may be limited due to a severely imbalanced AA profile because of a low lysine level (Peter et al., 2000). The low lysine levels may be overcome by synthetic lysine supplementation (Leeson and Summers, 2001). A previous study in chickens found that CGM fed at lower levels in a commercial diet is beneficial due to its high protein, xanthophyll, and methionine levels (Peter et al., 2000).

2.3.1.1.3 Canola protein concentrate

Canola protein concentrate (CPC) is a novel ingredient in turkey nutrition. This product is created by putting canola meal in solution and extracting the solubilized protein. This permits concentration of the protein as a result of fibre removal and also offers a liquid environment for

phytase activity and dephytinization of the final meal. The final product is a high protein (approximately 65%) and low anti-nutritional factor feed ingredient. Previous studies using CPC as a feed ingredient have been mainly focused on fish, weanling pigs and broiler chickens (Drew et al., 2007; Thiessen et al., 2004; Thacker and Petri, 2011). These studies all concluded that replacing a significant level of protein in the diet with CPC does not compromise performance. Further research is required on CPC to understand if it is a viable feed ingredient for turkey pre-starter diets. As of the writing of this thesis, CPC is an experimental feed product and not available commercially.

2.3.1.2 Animal proteins

Animal protein sources naturally contain biogenic amines, which are biologically active compounds synthesized from AA (Smith et al., 2000). These compounds are typically created in feed ingredients due to the action of microorganisms (Smith et al., 2000). Mammalian cells contain specific types of biogenic amine, called polyamines, including putrescine, spermidine, and spermine (Smith et al., 2000). These polyamines are synthesized from ornithine and methionine and are essential for cell growth, as well as being correlated with anabolic processes and hormone-like properties within the cell (Smith et al., 1996). Previous research has found that dietary polyamines are beneficial to growth and intestinal development at low levels, but will become toxic to the animal past a critical level (Smith et al., 2000). Polyamines are low molecular weight with a positive charge and the strength of this charge impacts how toxic that polyamine is to the animal, with a stronger charge increasing the toxicity and decreasing the growth promoting potential of that compound (Smith et al., 2000). Bioactive compounds obtained from synthesis within the body, or from the consumption of animal protein, appears to have a positive impact on poultry production at low levels (Michiels et al., 2012). Polyamines

also have the potential to support growth, particularly when intestinal integrity is compromised, which may be of importance immediately post-hatch when rapid growth and development is taking place and the birds are particularly susceptible to pathogens and bacteria (Smith et al., 2000). This is likely also true during times of disease challenge.

Research that establishes the threshold levels of biogenic amines that cause negative effects in poultry is not available. Guidelines are available for maximum histamine levels acceptable in fish for human consumption, with the CFIA and the European Union stating a maximum of 100 mg/100kg of fish (Karovicova and Kohajdova, 2003; CFIA, 2014). Additionally, the Biogenic Amine Index (BAI) has been established as a measure of biogenic amine contamination (Karovicova and Kohajdova, 2003). The BAI is calculated as (mg/kg histamine + mg/kg putrescine + mg/kg cadaverine)/(1 + mg/kg spermine + mg/kg spermidine), with a resulting value greater than 10 indicating a loss of quality of meat meal products (Karovicova and Kohajdova, 2003). It is important to view these thresholds as an indication only, as they are based on guidelines for human safety and consumption and not threshold levels in animal feed.

2.3.1.2.1 Fish meal

Fish meal (FM) is made up of harvested small fish, as well as leftovers from the fish industry, including trimmings, fins, skeletons, skin, and viscera (Kim and Mendis, 2006). It is a high sodium, calcium, and phosphorus feed ingredient, with a good AA balance for poultry production (Leeson and Summers, 2001). It typically has a crude protein of approximately 60% and a metabolizable energy value of approximately 3190 kcal/kg (National Research Council, 1994). There are concerns associated with feeding FM to birds due to gizzard erosion, caused by gizzerosine and histamine that may be found in the FM (Leeson and Summers, 2005).

Gizzerosine is created by a reaction between lysine and histidine or histamine, and once ingested, leads to erosion of the gizzard lining in a bird (Tišljar et al., 2002). This happens because gizzerosine acts on the histamine receptors in the proventriculus, leading to increased secretion of gastric acid, which then erodes the gizzard lining. Processing of FM can affect the level of gizzerosine, as a result of variable histamine content. Histamine is water soluble, and during the cooking and pressing stage of processing, a high loss of histamine reduces the potential for gizzerosine production (Köse et al., 2003). If the meal is not properly processed, however, the risk for gizzerosine production is increased and may lead to gizzard erosion in the birds following ingestion. A second concern associated with feeding FM is wet litter concerns leading to footpad dermatitis, due to high sodium levels (Murakami et al., 2000). This is typically controlled by limiting the inclusion level of FM in the feed. Traditionally, poultry fed FM have very good performance due to high palatability and digestibility (Hossain et al., 2013). This high performance may also be due to the polyamines present in FM, as research has indicated that high levels of compounds such as histamine, putrescine, and cadaverine are found in FM (Meat and Livestock Australia, 2001). As noted previously, high levels of polyamines can also cause a toxic effect on the bird.

2.3.1.2.2 Porcine meal

Porcine meal (PM) is a high calcium and phosphorus animal based protein concentrate (Leeson and Summers, 2005). Processing of PM is important, as the carcasses of the pig must be ground, the fat removed, and the product is dried to create PM. During these processes, there is the potential for heat to cause denaturation of protein and AA, leading to decreased nutritional value of the final product. Polyamines are present in this feed ingredient, however, at lower levels than FM (Kalač and Krausová, 2005). There is a potential for PM to have a beneficial

effect on performance and gastrointestinal health and function, however, very little research can be found on this feed ingredient for poultry. Previous research focused on the effect of meat meal in poultry diets typically does not specify the type of meat product the meat meal comes from, be it ruminant, porcine, or a blend, making it difficult to interpret the results. This has led to a gap in knowledge of the effect of feeding PM to poults. To the author's knowledge, research has not been completed on PM derived from Western Canada.

2.4 Conclusions

Early turkey nutrition is an often-overlooked area of research due to the expensive nature of this research, as well as the assumption that broiler chicken data can be applied to turkeys. This is concerning due to the vast differences in lengths of production cycles and the much higher protein levels required in turkey diets. Although a good understanding of turkey GIT development is in place, more research is required to fully understand the development of the digestive tract of the poult in order to accurately supply the poult's nutritional requirements in the first three wks post-hatch. Research is limited on the effect of different feed ingredients on poult GIT development and skeletal muscle accretion. Understanding ingredient effects would enhance the probability of formulating a turkey pre-starter feed that provides the poult with the required nutrients for maximum growth and development.

As consumer demand is moving towards a vegetable-fed meat product, the feed industry is being forced to examine the role vegetable protein concentrates play in feed formulation. Due to the pressure to use these ingredients, it is important to understand the effect all-vegetable poultry rations have on production parameters, as well as early growth and development. As the feed industry tends to depend on SBM as a well-balanced and well-suited protein source for poultry production, it is leading to high SBM rations, which may be causing depressed poult

growth and development. Understanding how other vegetable protein sources complement SBM is necessary to move forward in producing the product consumers are demanding. Additionally, studying how animal source proteins complement SBM is also of significance, as eliminating these ingredients from poultry rations entirely may lead to reduced performance, and the removal of polyamines may reduce to the integrity of the intestinal wall and the ability of the bird to digest nutrients and ward off infection.

The first objective of this research is to utilize AA digestibility and AMEn to determine the feeding value of high protein feed ingredients in turkey poults at 5 and 21 d of age to accurately formulate diets for the second part of the research. The second objective is to use these values to formulate pre-starter diets for turkey poults and study the effect of protein source on production parameters and GIT development.

3.0 THE EFFECT OF PROTEIN SOURCES ON PERFORMANCE CHARACTERISTICS OF TURKEYS IN THE FIRST THREE WEEKS

3.1 Abstract

The effect of nutrition during the early life of turkey poults has a long lasting impact on bird performance. This study assessed the digestibility of five high protein feed ingredients (soybean meal, SBM; corn gluten meal, CGM; canola protein concentrate, CPC; fish meal, FM; and porcine meal, PM) in broiler chickens, as well as their use in turkey pre-starter diets fed to 21 d of age. The first experiment (5 x 2 factorial arrangement) determined nitrogen corrected apparent metabolizable energy (AMEn) and apparent ileal amino acid digestibility (AIAAD) of each ingredient in broiler chickens at 5 and 21 d of age, using six replications of 30 and 8 chicks, respectively. In the second experiment (completely randomized design), four replication pens, each containing 23 d-old poults were randomly assigned to one of five dietary treatments. The diets were formulated based on the AMEn and AIAAD values derived in Experiment 1, and consisted of a high SBM control diet, and four additional diets with either CPC, FM, PM or CGM replacing 25% of the protein supplied by SBM in the control diet. Statistical analysis was completed using Proc Mixed in SAS 9.3. Planned contrasts were used to compare treatments in the second experiment. Trends were identified at $P \leq 0.10$ and significant differences identified at $P \leq 0.05$. Bird age did not affect CPC, FM, CGM, and SBM AMEn, but the 5 d value for PM was higher than the value obtained at 21 d. AIAAD increased with increasing age for most amino acids (AA), but the response was AA and protein source dependent. The largest average increase in AIAAD between 5 and 21 d of age was observed for CGM. Inclusion of CPC, FM, PM, or CGM increased body weight up to 14 d, in comparison to poults fed the SBM diet, but feed efficiency and water consumption were not affected. Terminal ileum digesta moisture values were higher for birds fed SBM when compared to those fed PM. These results

demonstrate that combining SBM with CPC, FM, PM, or CGM improves poult performance during the first 14 of age in comparison to feeding SBM alone.

3.2 Introduction

Turkey poults are severely protein depleted at hatch, as protein is preferentially used as an energy source during the hatching process. This is because gluconeogenesis from protein does not require oxygen, which is limited during the transition from chorioallantois to pulmonary respiration (Uni and Ferket, 2004). Additionally, protein digestion is impaired in young birds because of the transition from yolk sac nutrients, which are predominantly lipids, to a high protein exogenous feed (Noy and Sklan, 1997; Sklan, 2001). The transition from yolk to feed is compromised because of the immature state of the digestive tract in young poults, and therefore there is a reduced ability to digest and absorb protein. The ability to use the protein and amino acids (AA) being provided at a young age is important as these nutrients are required for basic body function, as well as muscle cell proliferation and subsequent meat production (Firman and Boling, 1998; Halevy et al., 2000; Halevy et al., 2003; Moore et al., 2005). Due to the protein depletion and decreased ability of the poult to digest protein, it is essential to provide the birds with high levels of quality protein in the diet following hatch to meet the poult's amino acid requirement.

Currently, the most used protein source in poultry diets is soybean meal (SBM) because it's nutritional profile (energy, AA level and balance) is complimentary to that of cereal grains and the poult's requirements. The market for products from vegetable fed animals has increased the level of SBM used in turkey diets. Despite the positive nutritional profile of SBM, when used at high levels as the sole protein source in practical pre-starter diets, the effect of anti-nutritional factors (ANF) may be underestimated. These ANF include protease inhibitors, which impair protein digestion (Borchers et al., 1948), lectins, which affect carbohydrate digestion, as well as disrupting and causing damage to the intestinal wall (Fasina et al., 2003), phytic acid,

which interferes with mineral and protein absorption (Cowieson et al., 2004). Oligosaccharides, which cause fluid retention, increased passage rate, and subsequent poor nutrient absorption when in high enough concentrations within the digestive tract (Stein et al., 2008). Although industrial processing reduces the level of protease inhibitors and lectins (Kumar, 1992), the high inclusion rates of SBM may result in diet levels that have adverse effects. A final nutrient in SBM that may have an ANF effect is potassium, as previous research has indicated that the high levels of potassium in SBM are associated with increased water intake, leading to increased excreta moisture (Youssef et al., 2011).

Reducing the SBM levels in pre-starter feeds by including other protein sources may be advantageous in terms of production, by diluting the level of ANF present in the feed. Other protein sources might also provide beneficial effects related to their nutritional properties and potentially bioactive compounds. Bioactive compounds provide biological benefits to the bird by affecting physiological functions (Muir et al., 2013) and they may be contributed by both vegetable and animal protein sources. Animal tissue cells contain a very specific type of bioactive compounds called polyamines (Smith et al., 2000). Polyamines are synthesized from ornithine and methionine and are essential for cell growth (Smith et al., 1996). The presence of these compounds in the feed due to the addition of animal protein concentrates may be beneficial to the performance of poults.

Based on the rationale that high levels of dietary SBM in turkey pre-starter diets have detrimental effects on turkey poults and that rendered meat products might provide beneficial effects, performance was compared for poults fed a diet containing SBM as the sole protein source to diets where 25% of the protein provided by SBM was replaced by one of two plant based protein sources (corn gluten meal, CGM; canola protein concentrate, CPC) or one of two

rendered products (fish meal, FM; porcine meal, PM). To increase the accuracy of diet formulation and provide additional digestibility data on these ingredients, the feeding value (apparent ileal amino acid digestibility, AIAAD; and nitrogen corrected apparent metabolizable energy, AMEn) of the protein sources was determined using 5 and 21 d-old broiler chicks. The hypotheses for this research were that protein source digestibility increases with age, that reducing SBM in pre-starter diets by including other protein sources would improve turkey performance, and that PM and FM will outperform CPC and CGM as SBM replacements.

3.3 Materials and methods

All experimental procedures were approved by the Animal Care Committee at the University of Saskatchewan and birds were cared for according to the Canadian Council of Animal Care (1993).

3.3.1 Digestibility assay

3.3.1.1 Birds and housing

Male Ross 308 x 308 broiler chicks were obtained from a commercial hatchery at d of hatch (Lilydale, Wynyard, Canada) and used to determine AMEn and AIAAD on 5 and 21 d of age. For 5 d AMEn and AIAAD, 1,080 birds were randomly assigned in groups of 15 to one of 72 Jamesway battery brooder cages (50 cm wide x 85 cm long x 25 cm high) at d of hatch (d 0). Each cage was randomly assigned to one of 6 treatments, with 12 replication cages per treatment. An additional 288 birds used for d 21 digestibility were placed in a floor pen until random allocation to one of 72 battery cages (4 birds per cage) on d 5. Room temperature was initially set at 32°C and then gradually decreased by 3°C every wk. Birds were exposed to 20 h of light

(20 lux) and 4 h of dark for the duration of the experiment. Feed and water were supplied *ad libitum* throughout the experiment. Cages were checked daily for mortality.

3.3.1.2 Dietary treatments

The d 5 digestibility chicks were fed experimental diets from d 0 until 5. The d 21 digestibility chicks were fed a commercial starter diet from d 0 until 15, after which they were switched to the experimental diets. Six treatment diets were fed in this experiment (Table 3.1) and all protein concentrates were analyzed for AA and mineral content prior to diet formulations (Table 3.2). Protein sources used were: SBM, CGM, CPC, FM, and PM. CPC is an experimental ingredient created by solubilizing canola meal in water and treating it with an enzyme to remove phytate. Protein sources were included at 40% of the diet in exchange for corn, SBM, and canola oil in the basal diet. Celite was added at 1.5% of the diet as an indigestible marker. Diets were fed as a mash.

3.3.1.3 Data collection

Body weight gain and feed consumption were measured on a cage basis from d 0 to 5 for d 5 digestibility and d 15 to 21 for d 21 digestibility. Excreta was collected four times over 48 h on plastic sheets placed under the cages beginning on d 3 for d 5 digestibility birds and on d 19 for d 21 digestibility birds. The samples were dried in a forced air oven at 55°C, pooled on a cage basis, and ground (1.0 mm screen) using a Retsch grinder (Hann, Germany). Following grinding and prior to chemical analysis, samples from two cages were pooled to create a total of six replications per treatment for analysis. The samples were analyzed in duplicate for dry matter (AOAC, 2006), crude protein (AOAC, 2006; N x 6.25), acid insoluble ash (Vogtmann et al., 1975), and gross energy (AOAC, 2006). Analyzed values were used to calculate AMEn at d 5 and 21 using the following equations (Scott et al., 1998):

Table 3.1 Composition of test diets for apparent ileal amino acid digestibility and nitrogen corrected apparent metabolizable energy determination.

	Diets					
	Basal	**SBM**	**CGM**	**CPC**	**FM**	**PM**
Ingredients (%)						
Corn	80.41	46.41	46.41	46.41	46.41	46.41
Soybean meal	13.31	47.68	7.68	7.68	7.68	7.68
Canola oil	0.88	0.51	0.51	0.51	0.51	0.51
CGM	-	-	40.00	-	-	-
CPC	-	-	-	40.00	-	-
FM	-	-	-	-	40.00	-
PM	-	-	-	-	-	40.00
Dicalcium phosphate	1.63	1.63	1.63	1.63	1.63	1.63
Limestone	1.35	1.35	1.35	1.35	1.35	1.35
Celite	1.50	1.50	1.50	1.50	1.50	1.50
Vitamin/mineral premix[1]	0.50	0.50	0.50	0.50	0.50	0.50
Sodium chloride	0.32	0.32	0.32	0.32	0.32	0.32
Choline chloride	0.10	0.10	0.10	0.10	0.10	0.10
Calculated nutrient composition (%)						
ME (kcal/kg)	3075	2703	3263	2917	2903	3263
Crude protein	14.00	27.32	34.80	32.52	32.80	28.60
Calcium	1.05	1.05	1.05	1.14	1.57	2.48
Chloride	0.22	0.22	0.22	0.22	0.22	0.22
Available phosphorous	0.56	0.55	0.43	0.65	1.14	1.62
Potassium	0.51	1.09	0.43	0.40	0.64	0.43
Sodium	0.17	0.23	0.16	0.25	0.75	0.16

[1] Supplied per kg of diet: 11,000 IU vitamin A; 2,200 IU vitamin D_3; 300 IU vitamin E; 2.0 mg menadione; 1.5 mg thiamine; 6.0 mg riboflavin; 60.0 mg niacin; 4.0 mg pyridoxine; 0.02 mg vitamin B_{12}; 10 mg pantothenic acid; 0.6 mg folic acid; 0.15 mg biotin; 80 mg iron; 80 mg zinc; 80 mg manganese; 10 mg copper; 0.8 mg iodine; 0.3 mg selenium.

SBM=soybean meal; CGM=corn gluten meal; CPC=canola protein concentrate; FM=fish meal; PM=porcine meal.

Table 3.2 Analyzed nutrient content (% as-is) of protein concentrates on as-is basis.

	SBM	CGM	CPC	FM	PM
Crude Protein[1]	47.30	66.00	60.30	61.20	49.40
Alanine	1.99	5.19	2.82	3.88	3.69
Arginine	3.79	2.33	4.11	4.64	3.91
Cysteine	0.74	1.06	1.18	0.56	0.48
Glycine	1.80	1.66	3.32	4.07	6.28
Histidine	1.51	1.18	1.17	1.71	1.42
Isoleucine	2.21	2.45	2.91	2.84	1.79
Leucine	3.73	10.40	5.28	4.99	3.65
Lysine	2.98	1.03	3.41	5.68	3.52
Methionine	0.63	1.47	1.27	1.64	0.90
Phenylalanine	2.48	4.07	3.26	2.88	2.15
Proline	2.46	5.53	3.53	2.97	4.05
Serine	2.54	3.44	3.09	2.71	2.22
Threonine	1.88	2.19	2.38	2.68	1.67
Tyrosine	2.01	3.61	2.14	2.39	1.51
Valine	2.20	2.76	3.41	3.21	2.34
Minerals					
Calcium	0.38	0.14	0.97	3.81	6.43
Total phosphorus	0.54	0.28	1.06	2.25	3.92
Sodium	0.19	0.07	1.08	1.50	0.47
Potassium	2.27	0.26	0.40	0.88	0.53

[1]Crude protein=Nx6.25.

SBM=soybean meal; CGM=corn gluten meal; CPC=canola protein concentrate; FM=fish meal; PM=porcine meal.

AMEn/g feed=gross energy/g feed – gross energy excreta/g diet – {8.22 kcal/g x g nitrogen/g diet – [g nitrogen/g excreta x (% acid insoluble ash in diet/% acid insoluble ash in excreta)]}

AMEn/g ingredient=AMEn/g basal diet - {(AMEn/g basal diet – AMEn/g test diet)/% test ingredient in test diet)}

On d 5 and 21, the birds were euthanized by cervical dislocation and the contents were collected from the distal 50% of the ileum, following the removal of 1 cm adjacent to the ileo-caecal junction. The ileal contents were gently squeezed into collection vials, pooled on a cage basis, immediately frozen at -20°C and then freeze-dried. Following freeze-drying, the samples were ground using a mortar and pestle and analyzed for dry matter, crude protein, AA content (AOAC, 2006), and acid insoluble ash. Apparent ileal amino acid digestibility coefficients (AIAADC) were calculated for d 5 and 21 collections using the following equation (Ten Doeschate et al., 1993):

AIAADC (%) = 1 – [(acid insoluble ash in diet (%)/acid insoluble ash in digesta (%)) x (nutrient in digesta (%)/nutrient in diet (%))]

3.3.2 Production trial

3.3.2.1 Birds and housing

Hybrid Converter turkey eggs (1,500) were obtained from a commercial hatchery (Lilydale, Edmonton) and hatched at the University of Saskatchewan Poultry Centre. At 11 d of incubation, the eggs were candled and eggs with no evidence of living embryos were removed. On d 25 of incubation, the eggs were transferred from the incubator into hatching baskets and placed in the hatchers. Beginning at 26 d +10 h of incubation and ending at 28 d + 4 h, the hatchers were checked every 6 h for hatched poults. Poults ready for pull, defined as completely

emerged from the eggshell and greater than 80% dry, were removed from the hatchers and placed in cardboard poult boxes until the time of placement. Poult vent temperature was monitored periodically from hatch removal to placement to confirm the temperatures were approximately 40°C. All poults were individually wing banded and wing bands were recorded at d of hatch to allow for tracking of hatch time. Twenty-four h following 28 d incubation, 460 poults were randomly assigned to one of 20 pens (4.5 m^2), while ensuring that the proportion of poults from each hatch window was the same in all pens. Shavings were added to the pens at a minimum depth of 7.5 cm and a heat lamp was provided in each pen for the first 7 d. Room temperature was set at 32°C at bird placement and decreased 0.5°C each d. Light was provided for 23 h at an intensity of 20 lux for the first seven d, after which birds received 18 h of light per d at an intensity of 10 lux. Water was provided *ad libitum* to the poults in 4 l plastic fount drinkers. One drinker was provided in each pen for the first 7 d, after which a second drinker of the same style was added for the remainder of the trial. Pens were checked for mortality and bird morbidity twice daily, and dead and culled poults were removed and sent for necropsy to determine cause of death or illness (Prairie Diagnostic Services, Western College of Veterinary Medicine, University of Saskatchewan). Feed was provided *ad libitum* in one tube feeder (36 cm diameter) per pen for the duration of the trial. In addition, supplemental feed was provided in flip-top plastic feeders (10.16 cm x 50.80 cm x 7.62 cm) for the first 7 d.

3.3.2.2 Dietary treatments

Each pen was randomly assigned one of five treatment diets (Table 3.3) for the duration of the trial. A basal wheat-SBM diet was formulated to meet Hybrid requirements (Hybrid, 2013). Because the digestible AA requirements for turkey poults are based on digestibility values derived from older animals, the 21 d determined digestibility values were used in feed

Table 3.3 Composition of test diets for the turkey production trial.

	Diets				
	SBM	CGM	CPC	FM	PM
Ingredients (%)					
SBM	46.97	35.23	35.23	34.69	35.23
Wheat	40.62	44.78	45.73	48.30	47.31
CPC	-	-	8.27	-	-
FM	-	-	-	8.00	-
PM	-	-	-	-	9.59
CGM	-	8.74	-	-	-
Canola Oil	4.23	2.8	3.11	2.52	2.20
Monophosphate Dicalcium	2.74	2.77	2.35	1.66	0.98
Limestone	2.13	2.18	2.19	1.9	1.36
DL-methionine	0.47	0.35	0.38	0.4	0.46
Salt	0.39	0.38	0.16	0.11	0.28
Threonine	0.06	0.05	0.06	0.05	0.1
Lysine HCl	0.02	0.35	0.15	0.01	0.13
Celite	1.50	1.50	1.50	1.50	1.50
Ameri-Bond 2X[1]	0.50	0.50	0.50	0.50	0.50
Vitamin/mineral premix[2]	0.23	0.23	0.23	0.23	0.23
Choline chloride	0.10	0.10	0.10	0.10	0.10
Endofeed W[3]	0.03	0.03	0.03	0.03	0.03
Calculated nutrient composition (%)					
AME (kcal/kg)	2850	2850	2850	2850	2850
Crude protein	27.7	28.6	28.6	28.5	28.8
Crude fat	5.40	4.20	4. 40	4.50	4.10
Calcium	1.40	1.40	1.40	1.40	1.40
Chloride	0.29	0.28	0.18	0.17	0.31
Available phosphorus	0.75	0.75	0.75	0.75	0.75
Potassium	1.12	0.93	0.94	0.98	0.96
Sodium	0.18	0.18	0.18	0.19	0.18
Digestible arginine	1.85	1.68	1.79	1.81	1.81
Digestible lysine	1.62	1.62	1.62	1.62	1.62
Digestible methionine	0.78	0.74	0.74	0.79	0.79
Digestible Met & Cys	1.05	1.05	1.05	1.05	1.05
Digestible threonine	0.96	0.96	1.17	0.96	0.96
Dietary electrolyte balance (mEQ/kg)	340.3	286.1	284.4	288.9	277.2

[1] Ameri-Bond 2X, Borregaard LignoTech Feed Additives, New Jersey, USA.

[2] Supplied per kg of diet: 14,885 IU vitamin A; 5460 IU vitamin D_3; 100 IU vitamin E; 3.65 mg menadione; 4.15 mg thiamine; 26.78 mg riboflavin; 124.5 mg niacin; 6.75 mg pyridoxine; 0.04 mg vitamin B_{12}; 26.50 mg pantothenic acid; 2.65 mg folic acid; 0.51 mg biotin; 80.05 mg iron; 120.07 mg zinc; 120.08 mg manganese; 18.76 mg copper; 2.10 mg iodine; and 0.30 mg selenium.

[3] Endofeed W, GNC Bioferm Inc, Box 6, Bradwell, SK, Canada. This enzyme contains a minimum of 700 units per gram of beta-glucanase and a minimum of 2,250 units per gram of xylanase.

SBM=soybean meal; CGM=corn gluten meal; CPC=canola protein concentrate; FM=fish meal; PM=porcine meal.

formulation. The decision to use d 21 values for the first 3 wks was made in order to create a practical, commercially applicable diet. The remaining four diets were formulated by calculating the percentage of protein being supplied by the SBM in the basal diet and mathematically substituting one of four alternative ingredients to replace 25% of the protein SBM was contributing. The alternative ingredients were CGM, CPC, FM, and PM. All diets were analyzed for the following key nutrients: moisture, AOAC 930.15; crude protein (N x 6.25), AOAC 990.03; calcium, AOAC 985.01; phosphorus, AOAC 985.01; potassium, AOAC 985.01; sodium, AOAC 985.01; and chloride, AOAC 985.01 (AOAC, 2006). All diets were formulated on a digestible AA basis and met the minimum values in the Hybrid requirements (Hybrid, 2013). Analyzed values were similar to calculated values shown in Table 3.3. An exception was the sodium value in the CPC diet, which was analyzed at 0.15%, which is 0.03% below the expected value. Diets were fed as a crumble.

3.3.2.3 Data collection

Feed intake (pen basis) was measured weekly for the duration of the trial. All water added to the drinkers was weighed and all drinkers were weighed every 24 h to determine daily water intake. Water consumption was evaporation corrected on a daily basis using four additional drinkers placed outside of the pens throughout the barn. The evaporation values were an average of 130 g for wk 1 and 100 g for wk 2 and 3. Birds were group weighed on a pen basis on d 0, 7, 14, and 21. At the end of the trial, a sample of 30 birds per treatment (10 birds from three replication pens) was used to determine individual sexed body weight and breast meat yield. These data were used exclusively for the meat yield calculations to ensure that differences in sex ratios were not skewing the results. Contents of the terminal ileum were also taken to determine moisture and osmolarity. Moisture in the content of the terminal ileum was

determined by weighing immediately after collection, drying at 55°C for 48 h in a forced air oven, and then weighing again; these values were used to calculate percent moisture. For testing osmolarity, approximately 5 g of ileal content were transferred to a microfuge tube and centrifuged at 15,000 RPM for 7 min in a Beckman Microfuge E (Beckman Coulter, Inc., Mississauga, ON, Canada). The supernatant was removed and microfuged again for an additional 5 min to ensure no particulates remained in the sample. The supernatant was poured into a new tube and osmolality was measured in duplicate using an osmometer (Advanced Model 3250 Single-Sample Osmometer, Advanced Instruments Inc., Norwood, MA, USA). Finally, the remainder of the birds were sexed at the conclusion of the trial to confirm that the gender proportions were similar in each pen.

3.3.2 Statistical analysis

All data were analyzed using Proc Mixed in SAS 9.3 (SAS, 2011). Differences were considered significant when $P \leq 0.05$ and trends were identified when $P \leq 0.10$. When significant differences were observed, mean separation was completed using the Tukey method.

Data for AMEn and AIAAD were analyzed as a 5 (five protein sources) x 2 (d of age) factorial arrangement to examine main effects (diet and age), as well as their interactions. Data were checked for normality prior to analysis. The experimental model statement for this analysis was:

$$Y = \mu + T + D + T*D + e$$

where, μ is the overall mean

T is the treatment (fixed effect)

D is the age (fixed effect)

T*D is the interaction between treatment and age (fixed effect), and

e is the error term.

Data for the production trial were analyzed as a one-way analysis of variance, with 5 treatments and 4 replications, in a completely randomized design, with diet as the main effect. Data were transformed using (log+1) prior to analysis when normality assumptions were not met. *A priori* contrasts were also used to compare the SBM control diet to the average of CGM, CPC, FM, and PM (Contrast #1), PM to the average of SBM, CGM, CPC, and FM (Contrast #2), and the addition of vegetable (CGM and CPC) and animal protein (FM and PM) (Contrast #3). The model used for analysis was:

$$Y = \mu + T + e$$

where μ is the overall mean

T is the treatment (fixed effect), and

e is the error term.

A one-way analysis of variance, as above, was also used to determine the effect of hatch window on final body weights, with the treatment being seven 4-hour hatch windows.

3.4 Results

3.4.1 Apparent metabolizable energy with nitrogen correction and apparent ileal amino acid digestibility

With the exception of PM, age did not affect protein source AMEn; the AMEn of PM was lower on d 21 than d 5 (Table 3.4). Overall, there was a significant effect of treatment and age on digestibility of all AA, with significant main effect interactions for all AA except cysteine

Table 3.4 Effect of age on nitrogen corrected apparent metabolizable energy (kcal/kg) of protein sources[1] as determined in broiler chickens.

	Age			Pooled
Treatment	5	21	*P*-Value	SEM
SBM	2415	2368	NS[2]	35.8
CGM	3745	3726	NS	30.3
CPC	2553	2424	NS	43.8
FM	3069	2951	NS	45.5
PM	2723	2550	0.0043	36.7

[1] Means of 6 replications; pooled excreta of 30 (5 d) or 8 (21 d) birds per replicate.
[2] NS=*P*>0.05.
SBM=soybean meal; CGM=corn gluten meal; CPC=canola protein concentrate; FM=fish meal; PM=porcine meal; SEM=standard error of the mean.

Table 3.5 Effect of protein source and age on apparent ileal amino acid digestibility coefficients in broiler chickens.

	Treatment[1]					Day of age[2]		Interaction	Pooled
	SBM	CGM	CPC	FM	PM	5	21	*P*-Value	SEM
Alanine	0.82^{ab}	0.84^{a}	0.80^{bc}	0.81^{ab}	0.77^{c}	0.77^{b}	0.84^{a}	0.0342	0.007
Arginine	0.89^{a}	0.87^{ab}	0.86^{b}	0.82^{c}	0.78^{d}	0.82^{b}	0.87^{a}	0.0022	0.007
Cysteine	0.48^{c}	0.78^{a}	0.77^{a}	0.55^{b}	0.39^{d}	0.55^{b}	0.64^{a}	NS[3]	0.022
Glycine	0.81^{a}	0.80^{a}	0.77^{ab}	0.77^{b}	0.73^{c}	0.75^{b}	0.80^{a}	0.0029	0.006
Histidine	0.87^{a}	0.84^{a}	0.84^{a}	0.77^{b}	0.70^{c}	0.78^{b}	0.83^{a}	0.0025	0.010
Isoleucine	0.84^{a}	0.83^{a}	0.80^{b}	0.77^{b}	0.70^{c}	0.75^{b}	0.83^{a}	0.0009	0.010
Leucine	0.82^{b}	0.86^{a}	0.82^{b}	0.79^{b}	0.74^{c}	0.76^{b}	0.85^{a}	0.0002	0.009
Lysine	0.87^{a}	0.86^{a}	0.83^{b}	0.76^{c}	0.72^{d}	0.78^{b}	0.83^{a}	NS	0.009
Methionine	0.88^{b}	0.88^{b}	0.88^{b}	0.98^{a}	0.74^{c}	0.84^{b}	0.90^{a}	<.0001	0.011
Phenylalanine	0.84^{a}	0.85^{a}	0.82^{ab}	0.79^{b}	0.74^{c}	0.77^{b}	0.85^{a}	0.0033	0.009
Proline	0.82^{ab}	0.85^{a}	0.80^{b}	0.76^{c}	0.69^{d}	0.75^{b}	0.82^{a}	0.0004	0.010
Serine	0.83^{a}	0.14^{e}	0.77^{b}	0.71^{c}	0.62^{d}	0.55^{b}	0.67^{a}	<.0001	0.034
Threonine	0.79^{ab}	0.82^{a}	0.77^{bc}	0.75^{c}	0.66^{d}	0.72^{b}	0.80^{a}	0.0125	0.010
Tyrosine	0.86^{a}	0.88^{a}	0.88^{a}	0.80^{b}	0.74^{c}	0.79^{b}	0.87^{a}	0.0160	0.009
Valine	0.82^{a}	0.83^{a}	0.65^{d}	0.76^{b}	0.70^{c}	0.72^{b}	0.79^{a}	0.0011	0.011
Average	0.82	0.80	0.80	0.77	0.70	0.74	0.81		

[1] Means of 6 replications; pooled ileal samples from 30 (5 d) or 8 (21 d) birds per replicate.
[2]Means of 30 replications; pooled ileal samples from 30 (5d) or 8 (21 d) birds per replicate.
[3] NS=*P*>0.05.
[a-d] Means within the same row and main effect with no common superscript differ significantly ($P \leq 0.05$).
SBM=soybean meal; CGM=corn gluten meal; CPC=canola protein concentrate; FM=fish meal; PM=porcine meal; SEM=standard error of the mean.

and lysine (Table 3.5). The ingredient with the highest average digestibility coefficient was SBM (0.82), followed by CGM and CPC (0.80), FM (0.77), and PM (0.70). Overall, AA digestibility increased 7% from d 5 to 21. Digestibility of all evaluated AA in SBM, CGM, and FM increased from d 5 to 21 (Table 3.6), with an average increase of 8.17, 19.27, and 7.75% respectively (Table 3.7). All AA increased from d 5 to 21 in CPC, except cysteine and serine, with an overall average increase of 4.22%. In PM, AIAAD increased for all AA except for alanine, arginine, glycine, histidine, and proline, with an overall average increase of 9.14%. The largest increase in average AIAAD from d 5 to 21 was observed for CGM, while the smallest increase was observed for CPC.

3.4.2 Production trial

3.4.2.1 Performance data

Hatch time affected d 21 body weight, with body weight increasing with increasing incubation time (Figure 3.1). With one exception, there was no effect of diet on production parameters when assessed by analysis of variance. Mortality corrected gain:feed in wk 2 was the exception with poults fed CGM being more efficient than those fed CPC (Table 3.8). There was a trend for an effect of diet on several aspects of growth, however. Body weight at 14 d was highest for PM (P=0.0645) and similarly weight gain was highest for PM and lowest for SBM during wk 1 (P=0.0752 and wk 2 (P=0.0547). Feed consumption also tended to be highest for PM and lowest for SBM during wk 2 (P=0.0564), as well as when feed consumption for the duration of the trial was analyzed (P=0.0889). Mortality corrected gain:feed had a tendency to be improved in CGM as compared to CPC when analyzed for the duration of the trial (P=0.0748). There was no effect of treatment on mortality.

Table 3.6 Effect of protein source on amino acid content and apparent ileal amino acid digestibility coefficients[1] in 5 and 21 d old broiler chickens.

	SBM			CGM			CPC			FM			PM		
	%	5 d	21 d	%	5 d	21 d	%	5 d	21 d	%	5 d	21d	%	5 d	21 d
Crude Protein[2]	47.3			66.0			60.3			61.2			49.4		
Alanine	1.99	0.77^{b}	0.86^{a}	5.19	0.78^{b}	0.89^{a}	2.82	0.77^{b}	0.82^{a}	3.88	0.79^{b}	0.84^{a}	3.69	0.75	0.79
Arginine	3.79	0.87^{b}	0.91^{a}	2.33	0.82^{b}	0.93^{a}	4.11	0.84^{b}	0.87^{a}	4.64	0.80^{b}	0.84^{a}	3.91	0.76	0.80
Cysteine	0.74	0.45^{b}	0.52^{a}	1.06	0.72^{b}	0.84^{a}	1.18	0.76	0.79	0.56	0.50^{b}	0.61^{a}	0.48	0.33^{b}	0.45^{a}
Glycine	1.80	0.77^{b}	0.84^{a}	1.66	0.76^{b}	0.85^{a}	3.32	0.76^{b}	0.79^{a}	4.07	0.75^{b}	0.79^{a}	6.28	0.73	0.73
Histidine	1.51	0.84^{b}	0.90^{a}	1.18	0.79^{b}	0.90^{a}	1.17	0.84^{b}	0.85^{a}	1.71	0.75^{b}	0.80^{a}	1.42	0.68	0.72
Isoleucine	2.21	0.81^{b}	0.88^{a}	2.45	0.76^{b}	0.91^{a}	2.91	0.77^{b}	0.82^{a}	2.84	0.74^{b}	0.80^{a}	1.79	0.67^{b}	0.74^{a}
Leucine	3.73	0.78^{b}	0.86^{a}	10.4	0.78^{b}	0.94^{a}	5.28	0.80^{b}	0.84^{a}	4.99	0.76^{b}	0.83^{a}	3.65	0.70^{b}	0.77^{a}
Lysine	2.98	0.84^{b}	0.90^{a}	1.03	0.83^{b}	0.90^{a}	3.41	0.81^{b}	0.84^{a}	5.68	0.74^{b}	0.78^{a}	3.52	0.70^{b}	0.75^{a}
Methionine	0.63	0.84^{b}	0.92^{a}	1.47	0.82^{b}	0.94^{a}	1.27	0.86^{b}	0.89^{a}	1.64	0.97^{b}	0.98^{a}	0.90	0.71^{b}	0.78^{a}
Phenylalanine	2.48	0.80^{b}	0.87^{a}	4.07	0.78^{b}	0.93^{a}	3.26	0.80^{b}	0.85^{a}	2.88	0.75^{b}	0.83^{a}	2.15	0.70^{b}	0.78^{a}
Proline	2.46	0.78^{b}	0.86^{a}	5.53	0.78^{b}	0.91^{a}	3.53	0.78^{b}	0.82^{a}	2.97	0.72^{b}	0.79^{a}	4.05	0.68	0.70
Serine	2.54	0.80^{b}	0.86^{a}	3.44	-0.02^{b}	0.31^{a}	3.09	0.76	0.79	2.71	0.67^{b}	0.75^{a}	2.22	0.57^{b}	0.67^{a}
Threonine	1.88	0.76^{b}	0.83^{a}	2.19	0.75^{b}	0.88^{a}	2.38	0.75^{b}	0.79^{a}	2.68	0.71^{b}	0.78^{a}	1.67	0.61^{b}	0.70^{a}
Tyrosine	2.01	0.84^{b}	0.89^{a}	3.61	0.82^{b}	0.93^{a}	2.14	0.86^{b}	0.90^{a}	2.39	0.76^{b}	0.83^{a}	1.51	0.70^{b}	0.78^{a}
Valine	2.20	0.79^{b}	0.86^{a}	2.76	0.77^{b}	0.90^{a}	3.41	0.64^{b}	0.66^{a}	3.21	0.73^{b}	0.79^{a}	2.34	0.67^{b}	0.74^{a}
Average		0.78	0.85		0.73	0.90		0.79	0.82		0.74	0.80		0.66	0.73

[1] Means of 6 replications; pooled ileal samples from 30 (5 d) or 8 (21 d) birds per replicate.
[2] Nitrogen levels were determined using a Leco Protein Analyzer, from which crude protein was calculated (Crude protein=%N x 6.25).
[a,b] Means within the same row and protein source with no common superscript differ significantly ($P \leq 0.05$).
SBM= soybean meal; CGM=corn gluten meal; CPC=canola protein concentrate; FM=fish meal; PM=porcine meal.

Table 3.7 Percentage increase[1] of apparent ileal amino acid digestibility coefficients[2] of protein sources from 5 to 21 d.

	Treatment				
	SBM	CGM	CPC	FM	PM
Alanine	10.47	12.34	6.10	5.95	5.06
Arginine	4.40	11.83	3.45	4.76	5.00
Cysteine	13.46	14.29	3.80	18.03	26.67
Glycine	8.33	10.59	3.80	5.06	0.00
Histidine	6.67	12.22	1.18	6.25	5.56
Isoleucine	7.95	16.48	6.10	7.50	9.46
Leucine	9.30	17.02	4.76	8.43	9.09
Lysine	6.67	7.78	3.57	5.13	6.67
Methionine	8.70	12.77	3.37	1.02	8.97
Phenylalanine	8.05	16.13	5.88	9.64	10.26
Proline	9.30	14.29	4.88	8.86	2.86
Serine	6.98	102.30	3.80	10.67	14.92
Threonine	8.43	14.77	5.06	8.97	12.86
Tyrosine	5.62	11.83	4.44	8.43	10.26
Valine	8.14	14.44	3.03	7.59	9.46
Average	8.17	19.27[3]	4.22	7.75	9.14

[1] Percentage increase calculated as: (d 21 digestibility – d 5 digestibility)/d 21 digestibility *100.

[2] Means of 6 replications; pooled ileal samples from 30 (5 d) or 8 (21 d) birds per replicate.

[3] Average percentage increase of apparent ileal amino acid digestibility coefficients of CGM is 13.34%, when serine is removed from the calculation.

CPC=canola protein concentrate; FM=fish meal; PM=porcine meal; CGM=corn gluten meal; SBM=soybean Meal; SEM=standard error of the mean.

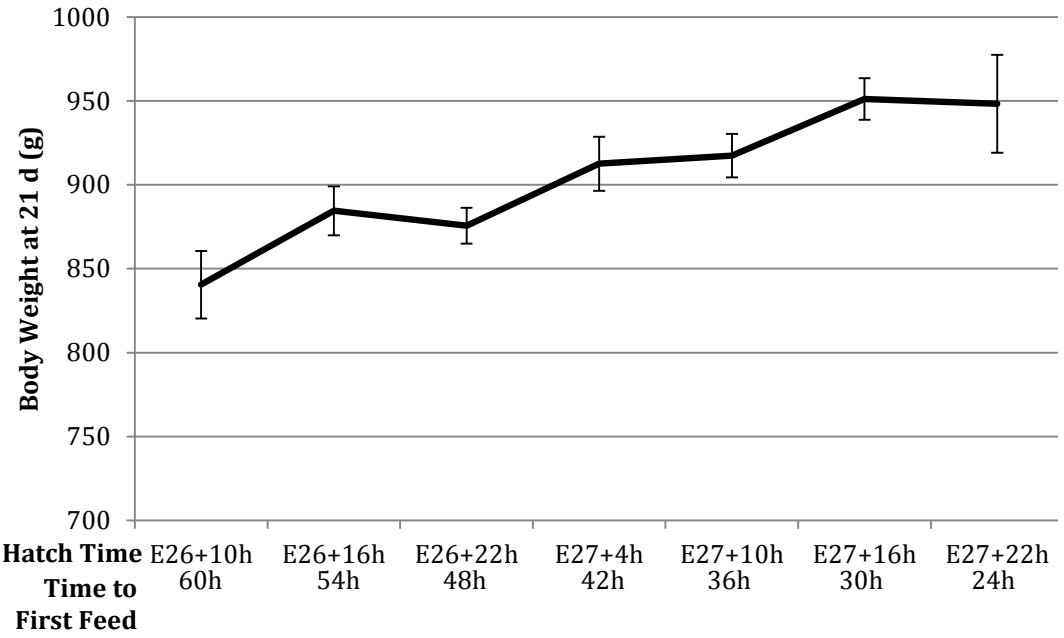

Figure 3.1 Effect of hatch time of turkey poults on day 21 body weight. E*n*=d and h of incubation at hatch pull.

Table 3.8 Effect of treatment on production parameters in turkey poults from day 0 to 21[1].

	Treatment						*A priori* contrast *P*-values		
	SBM	CGM	CPC	FM	PM	SEM	Contrast #1	Contrast #2	Contrast #3
Body weight (g)									
Wk 0	60.7	60.9	60.7	60.3	60.2	0.0002	NS	NS	NS[2]
Wk 1	177.3	183.0	183.3	181.5	186.0	0.0011	0.0197	NS	NS
Wk 2	419.4	432.9	431.7	424.1	443.5	0.0029	0.0433	0.0173	NS
Wk 3	801.9	814.2	814.6	811.2	843.1	0.0052	NS	0.0125	NS
Average gain (g)									
Wk 1	116.6	122.2	122.7	121.2	125.8	0.0011	0.0141	0.0396	NS
Wk 2	242.1	249.8	248.3	242.6	257.5	0.0019	NS	0.0112	NS
Wk 3	382.5	381.3	382.9	387.1	399.6	0.0029	NS	0.0328	NS
Wk 1 – 3	741.3	753.3	753.9	751.0	782.9	0.0052	NS	0.0113	NS
Feed consumption (g)									
Wk 1	128.7	130.0	135.7	133.6	136.7	0.0012	NS	NS	NS
Wk 2	304.6	311.5	322.3	303.9	324.6	0.0003	NS	0.0421	NS
Wk 3	532.4	530.4	556.2	540.1	551.9	0.0042	NS	NS	NS
Wk 1 – 3	965.7	971.9	1014.2	977.7	1013.3	0.0076	NS	NS	NS
Mortality corrected gain:feed ratio (g:g)									
Wk 1	0.9034	0.9437	0.9036	0.9293	0.9192	0.0083	NS	NS	NS
Wk 2	0.7946[ab]	0.8022[a]	0.7708[b]	0.7988[ab]	0.7985[ab]	0.0037	NS	NS	NS
Wk 3	0.7183	0.7191	0.6889	0.7179	0.7241	0.0055	NS	NS	NS
Wk 1 – 3	0.7670	0.7757	0.7436	0.7730	0.7745	0.0049	NS	NS	NS
Mortality (%)									
Wk 1	3.3	3.3	6.5	6.5	6.5	1.0685	NS	NS	NS
Wk 2	0.0	0.0	0.0	0.0	2.2	0.4348	NS	NS	NS
Wk 3	0.0	0.0	0.0	0.0	0.0	0.0000	--	--	--
Wk 1 – 3	3.3	3.3	6.5	6.5	8.7	1.3110	NS	NS	NS

[1] Means of 4 replications of 23 birds per replicate.

[2] NS=$P>0.05$.

[a,b] Means within the same row with no common superscript differ significantly ($P\leq0.05$).

SBM=soybean meal; CGM=corn gluten meal; CPC=canola protein concentrate; FM=fish meal; PM=porcine meal; SEM=standard error of the mean.

Contrast #1= SBM versus the average of CGM, CPC, FM, and PM; Contrast #2=PM versus the average of SBM, CGM, CPC, and FM; Contrast #3=Vegetable (CGM and CPC) versus animal (FM and PM) addition to SBM.

A priori contrast #1 (SBM vs average of CGM, CPC, FM and PM) showed that poults fed SBM had lower body weights at d 7 and 14 (177.3 and 419.4 g, respectively) and lower gains during wk 1 (116.6 g) as compared to the average of birds fed the remaining treatments (183.5, 433.1, and 123.0 g). Poults fed SBM tended to grow more slowly during wk 2 in comparison to birds in other treatments (P=0.0972). Contrast #2 (PM versus the average of SBM, CGM, CPC, and FM) showed that birds fed PM had heavier body weights at d 14 and 21 (443.5 and 843.1 g, respectively) than the average of remaining treatments (427.0 and 810.5 g, respectively), with a trend for increased body weights for poults fed PM at d 7 (P=0.0631). Increased gains were observed for the PM treatment during wk 1, 2, and 3 (125.8, 257.5, and 399.6 g, respectively) than the average of the other treatments (120.7, 245.7, and 749.9 g, respectively). The PM treatment also resulted in increased feed consumption during wk 2 (324.6 vs. 310.6 g), with a trend for increased consumption during wk 1 (P=0.0979).

Body weight, body weight gain, feed consumption, mortality corrected gain:feed, and mortality were not affected by feeding a vegetable (CGM and CPC) vs an animal (FM and PM) protein source (Contrast #3). There was a trend for vegetable protein treatments to produce increased gain during wk 3 of the trial (P=0.0868).

Breast meat yield as a percentage of live weight was not affected by treatment, however, there was a significant effect of gender, with females having increased yield (Table 3.9). *A priori* contrasts did not show a significant effect of dietary treatment on proportional breast meat yield.

3.4.2.2 Water consumption and terminal ileum digesta moisture content and osmolarity

Dietary treatment only affected evaporation corrected water consumption for wk 2, with poults fed CPC drinking more than those fed FM (Table 3.10); all other treatments were intermediate and not

Table 3.9 Effect of protein source on breast meat yield at 21 days of age[1].

	Treatment[1]						Sex			Pooled	*A priori* contrast *P*-values		
	SBM	CGM	CPC	FM	PM	P-Value	Male	Female	P-Value[3]	SEM	Contrast #1	Contrast #2	Contrast #3
Total BM (g)	147.0	146.1	148.7	147.2	153.4	NS[2]	152.8	144.1	0.0005	1.23	NS	0.0470	NS
P. maj. (g)	118.6	118.0	120.4	119.3	124.5	NS	124.0	116.4	0.0003	1.03	NS	0.0356	NS
P. min. (g)	28.4	28.1	28.3	27.8	28.9	NS	28.8	27.8	0.0251	0.23	NS	NS	NS
BM as % LW	16.6	16.4	16.8	16.6	16.7	NS	16.4	16.9	0.0001	0.06	NS	NS	NS
P. maj. as % LW	13.4	13.2	13.6	13.5	13.6	NS	13.3	13.6	0.0025	0.05	NS	NS	NS
P. min. as % LW	3.2	3.2	3.2	3.1	3.2	NS	3.1	3.2	<.0001	0.02	NS	NS	NS

[1] Means of 3 replications of 10 birds per replicate.
[2] NS=*P*>0.05.
[3] Interaction *P*-value was not significant (*P*>0.05) for all parameters.
BM=breast meat; P. maj.= pectoralis major; P. min.=pectoralis minor; LW=live weight; SBM=soybean meal; CGM=corn gluten meal; CPC=canola protein concentrate; FM=fish meal; PM=porcine meal; SEM=standard error of the mean.
Contrast #1= SBM versus the average of CGM, CPC, FM, and PM; Contrast #2=PM versus the average of SBM, CGM, CPC, and FM; Contrast #3=Vegetable (CGM and CPC) versus animal (FM and PM) addition to SBM.

Table 3.10 Effect of treatment on water consumption in turkey poults from day 0 to 21[1].

	Treatment					Pooled	*A priori* contrast *P*-values		
	SBM	CGM	CPC	FM	PM	SEM	Contrast #1	Contrast #2	Contrast #3
Evaporation corrected water consumption (g/bird)									
Wk 1	432.7	444.9	467.0	438.2	449.0	6.91	NS	NS	NS
Wk 2	777.7^{ab}	741.9^{ab}	945.7^{a}	684.3^{b}	780.2^{ab}	33.20	NS	NS	0.0204
Wk 3	1142.6	1232.5	1370.9	1175.1	1205.9	29.05	NS	NS	0.0407
Water:feed (g:g)									
Wk 1	3.39	3.44	3.45	3.36	3.33	0.046	NS	NS	NS
Wk 2	2.60^{b}	2.43^{c}	2.89^{a}	2.50^{ab}	2.42^{c}	0.059	NS	0.0006	0.0008
Wk 3	2.12^{b}	2.31^{ab}	2.55^{a}	2.20^{b}	2.24^{ab}	0.052	0.0912	NS	0.0152
Mortality corrected water:gain (g:g)									
Wk 1	3.75	3.65	3.81	3.43	3.63	0.058	NS	NS	NS
Wk 2	3.29^{b}	3.00^{c}	3.82^{a}	3.03^{c}	3.02^{c}	0.101	0.0672	0.0003	0.0001
Wk 3	2.92^{b}	3.22^{ab}	3.72^{a}	3.17^{ab}	3.09^{b}	0.098	NS	NS	0.0311
Terminal ileum digesta moisture at day 21 (%)									
	81.1^{a}	79.7^{ab}	81.0^{ab}	79.7^{ab}	78.8^{b}	0.28	0.0409	0.0143	0.0458
Terminal ileum digesta osmolarity at day 21 (mOsm)									
	411.1	389.6	388.6	396.3	394.3	4.59	NS	NS	NS

[1] Means of 4 replications of 23 birds per replicate.

[2] NS=P>0.10.

Significance set at P≤0.05 and trends identified at 0.05<P≤0.10.

[a-c] Means within the same row with no common superscript differ significantly (P ≤0.05).

SBM=soybean meal; CGM=corn gluten meal; CPC=canola protein concentrate; FM=fish meal; PM=porcine meal; SEM=standard error of the mean.

Contrast #1= SBM versus the average of CGM, CPC, FM, and PM; Contrast #2=PM versus the average of SBM, CGM, CPC, and FM; Contrast #3=Vegetable (CGM and CPC) versus animal (FM and PM) addition to SBM.

different from the extreme values. Water:feed ratio was affected by treatment for wk 2 with the CPC treatment resulting in a higher ratio compared to all treatments except FM. Further, the water:feed ratio was higher for SBM than the values recorded for CGM and PM. For wk 3, the CPC treatment resulted in a higher water:feed ratio compared to SBM and FM, while CGM and PM values were intermediate. For wk 2, CPC had the highest water:gain ratio, followed by SBM, and CGM, FM, and PM treatments resulted in the lowest values. For wk 3, CPC had the highest water:gain ratio, but it was only significantly higher than the value for the PM treatment. Terminal ileum digesta moisture content at d 21 was higher in SBM as compared to PM, while CGM, CPC and FM values were intermediate and not different than SBM and PM treatments. Terminal ileum digesta osmolarity at d 21 was not affected by treatment.

A priori contrast #1 revealed poults fed SBM had increased ileal digesta moisture at d 21 (81.1%) as compared to the other four treatments (79.8%). Identified trends were a decreased water:feed ratio during wk 3 (P=0.0912) and an increased mortality corrected water:gain ratio during wk 2 (P=0.0672) for the SBM treatment. No difference was observed between SBM and the remaining treatments for evaporation corrected water consumption, water:feed during wk 1 and 2, mortality corrected water:gain ratio during wk 1 and 3, and terminal ileum digesta osmolarity at d 21.

Contrast #2 showed that PM had a lower water:feed ratio (2.42 g:g) during wk 2, mortality corrected water:gain ratio in wk 2 (3.02 g:g), and terminal ileum digesta moisture at d 21 (78.8%), than the other four treatments (2.61 g:g, 3.29 g:g, and 80.4%, respectively). No difference was identified between PM and the remaining treatments for evaporation corrected water consumption, water:feed ratio during wk 1 and 3, mortality corrected water:gain ratio during wk 1 and 3, and terminal ileum digesta osmolarity at d 21.

Contrast #3 revealed no difference between vegetable and animal protein for all water consumption parameters during the first wk, as well as in terminal ileum digesta osmolarity at d 21. There was an effect observed during the second and third wk of the trial. Evaporation corrected water consumption was increased during wk 2 and 3 in vegetable protein diets (843.8 g and 1301.7 g, respectively) when compared to animal protein diets (732.3 g and 1190.5 g, respectively). The same effect was observed in water:feed ratio during wk 2 and 3 with 2.66 and 2.43 g:g, respectively for vegetable protein diets and 2.46 and 2.22 g:g, respectively for animal protein diets. This effect was also seen for mortality corrected water:gain ratio during wk 2 and 3 with 3.41 and 3.47 g:g, respectively, for vegetable protein diets and 3.03 and 3.13 g:g for animal protein diets. Finally, birds consuming vegetable protein diets had higher terminal digesta moisture at d 21 (80.4%) than birds consuming animal protein diets (79.3%).

3.5 Discussion

3.5.1 Apparent metabolizable energy with nitrogen correction and apparent ileal amino acid digestibility

The lack of effect of age on AMEn observed in this study agrees with previous research (Lopez and Leeson, 2008), where there was no effect of age on the AME or AMEn of SBM. Other research, however, has noted an increase in AMEn with increasing age (Batal and Parsons, 2002), while still others have observed a decrease in AMEn with increasing age (Cho, 2011). When AMEn is observed in broiler chicks over time, from d 2 to 21, AMEn is high following hatch, followed by a decrease to d 6, and a subsequent increase to d 21 (Thomas et al., 2008). This may explain why there was no effect on AMEn in the current study, as AMEn values at d 5 and 21 may be similar due to the timing of the decrease and subsequent increase of AMEn. Research on the effect of age on AMEn is quite varied in terms of results, as it is influenced by

many factors, such as chronological age and when feed is offered to the poults, making it difficult to compare results to previously conducted studies.

The AMEn values were similar to values found in the literature for FM, CGM, and SBM (National Research Council, 1994). As there is very little previous research examining PM and CPC in poultry, the AMEn values cannot be compared to previous literature. It can be noted, however, that PM and CPC have greater AMEn values than the values for meat meal and canola meal (approximately 600 and 500 kcal/kg higher, respectively) reported in National Research Council (1994).

The increase in AIAAD with increasing age, as well as the variability observed in this increase between protein sources, as well as AA, supports previous research studying the effect of age on AA digestibility (Huang et al., 2005; Adedokun et al., 2008; Rynsburger, 2009; Cho, 2011). This increase in digestibility is likely attributed to the development of the gastrointestinal tract (GIT). Enzymes required to digest proteins, nutrient transporters and other aspects of digestive physiology increase rapidly after the bird first consumes feed, leading to an increasing ability to hydrolyze diet proteins and absorb resulting AA (Sklan and Noy, 2000). At the same time, the GIT is rapidly growing and developing physically to be able to effectively utilize the nutrients being provided (Dibner et al., 1996). The combination of a more mature and functional GIT and the rise in digestive enzymes works together to increase the ability of the bird to digest protein and therefore contribute to the increase in AA digestibility observed between d 5 and 21.

The digestibility coefficients in this study for FM, CGM, and SBM compare very closely to those values found in the literature, as well as reference values (Degussa, 2005; Huang et al., 2005; Ravindran and Morel, 2006). As this is one of the first studies to look at the effect of age on AIAAD in CPC and PM, it is difficult to compare these values to previous work, however, the

values can be compared to those of canola meal and high protein meat and bone meal. Although CPC is an experimental product, when the CPC digestibility values are compared to canola meal values, the results are similar (Huang et al., 2005) and when PM is compared to meat and bone meal values, the digestibility values for meat and bone meal tend to be higher than those found for PM (Degussa, 2005; Huang et al., 2005).

The largest increase in AIAAD from d 5 to 21 was observed in CGM. This is an interesting observation because of the unique nature of CGM proteins. These unique properties, including low solubility in some solutions, are likely due to the presence of zein protein in corn (Shukla and Cheryan, 2001). Zein is difficult to solubilize in water, likely because it has a high proportion of non-polar AA and is lacking basic and acidic AA (Shukla and Cheryan, 2001). This may help explain the poor AIAAD observed at d 5. Because this protein is difficult to solubilize, it may also be difficult to digest by the immature digestive tract. Another response of interest in CGM is the negative digestibility value of serine at d 5. This response may be because serine is part of mucin and biliary acids (Cowieson et al., 2004; Horn et al., 2009), however, the same negative digestibility is not seen in other AA that make up mucin and biliary acids, such as cysteine, glycine, proline, and threonine. This result does not agree with what previous researchers have found and there is no obvious reason for why this difference exists. A study by Kim et al. (2012) found serine digestibility in CGM to be between 85.3 and 92.4%, depending on the assay used, which is much higher than what was found in this research. Because of the negative digestibility value observed for serine at d 5, the overall increase in AA digestibility in CGM is high at 19.27%. The increase, however, is still high when the serine digestibility is removed, with an overall increase in AA digestibility of 13.34%, remaining the highest increase when the 5 test ingredients are compared.

The results of the AMEn and AIAAD research indicate the importance of formulating diets with a good understanding of the ingredients being used. It is important to remember that these results are based on only one sample of these ingredients and the results could be impacted by processing and storage times. The results suggest that SBM and CPC are good ingredients for early turkey diets, due to high AIAAD at 5 d. On the other hand, the samples of CGM, FM, and PM used for this experiment indicate that these protein sources may need to be limited in early turkey diets due to low AIAAD at 5 d. In terms of AMEn, CGM provides the highest AMEn of the five ingredients. Finding the balance between AIAAD and AMEn in terms of evaluating ingredients for early turkey nutrition can be challenging. Choosing the ingredients with the correct balance is important as energy and AA are the major components of formulation for commercial poultry diets. Because the poult has such a high protein requirement at the beginning of its life, it may be valuable to choose ingredients that have high AIAAD digestibility. This would point to using ingredients such as SBM and CPC in early turkey diets, however, the feeding value of these ingredient only offers theoretical values and results obtained from growth performance studies are also very valuable. Choosing ingredients that are highly digestible in the young turkey is an advantage. The high digestibility means the poults can use those ingredients for early growth and development, while minimizing the fermentation of non-digestible protein by the digestive tract microbiota, as large fractions of undigested protein are associated with poorer performance and impaired GIT morphology (Qaisrani et al., 2014).

3.5.2 Production trial

3.5.2.1 Performance data

The lower body weights observed during the first two wks, as well as the trend for lower feed consumption during the first wk and decreased average gains from d 0 to 14 for birds fed

SBM may indicate an influence of ANFs, such as protease inhibitors, lectins, oligosaccharides, and/or mineral imbalances. This suggests the first two wks are a crucial time, after which the poults may be better able to cope with ANFs and have an increased ability to digest the nutrients being provided in the feed. This may be due to increased enzyme secretion, as well as the increased surface area of the GIT for digestion and absorption of nutrients. The lack of difference between animal and vegetable protein sources for body weight, average gain, feed consumption, and mortality corrected G:F indicates that regardless of the protein source being provided, supplementing a high SBM diet with a second protein source is beneficial to production parameters in turkeys. The lack of protein source effect agrees with Vieira and Lima (2005) who did not observe a difference in performance between broilers fed all vegetable diets or diets containing meat meal.

The depressed performance observed with feeding high SBM levels could be attributed to the dietary concentration of ANF, such as protease inhibitors, lectins, oligosaccharides, phytate, and potentially allergens. It has been demonstrated that protease inhibitors reduce performance by inhibiting protein digestion (Borcher et al., 1948), however, these compounds are heat labile and are reduced during processing. There is minimal research into the critical level of protease inhibitors that will negatively affect poultry performance, so it is difficult to conclude if residual levels of these compounds remaining after heat processing would be enough to have such an effect on performance. Lectins have the ability to bind carbohydrates within the digestive tract of the bird (Fasina et al., 2003). In addition to this, they can also bind to the enterocytes in the GIT leading to the disruption and damage of the intestinal membrane. This leads to impairment of nutrient digestibility, as the carbohydrates are less available for digestion and the GIT is damaged and not functioning correctly. They are, however, heat labile compounds and their

levels within SBM should be reduced due to heat application during processing. Because energy is not likely to be the limiting factor to performance in turkey pre-starter diets, it is unlikely that any residual levels of lectins will have caused the depressed performance observed in this research. Phytates can also lead to impaired digestion and absorption of minerals (including phosphorus, calcium, magnesium, zinc, and iron) starch and protein by forming complexes that cannot be digested and absorbed (Selle et al., 2000). Phytates are not heat labile compounds and would be present in the diet, however, because there was no performance differences when animal or vegetable protein sources were added to the SBM, this would indicate that the phytate is not what was depressing performance of poults fed the SBM diet. Although the majority of these ANF are considered to be effectively removed during SBM processing, there are still residual levels present, which are highly dependent on the SBM processing temperature (Fasina et al., 2003). With a lower processing temperature, the effect of these ANF may be underestimated and this may be contributing to the decreased performance observed in this study.

Performance could also be affected by imbalances in minerals or protein within the diet. Mineral imbalances should not be an issue, as all diets were balanced to the same calcium, phosphorus, sodium, and chloride levels. The most notable mineral difference was a higher potassium level for the SBM diet. The SBM diet contained 1.12% potassium, while the remaining diets ranged from 0.93 to 0.98% potassium. The dietary electrolyte balance was also calculated for all diets (Table 3.3). The electrolyte balance was higher in the SBM diet, due to the higher potassium levels in that diet, while the remaining diets had similar electrolyte balances. This higher potassium level in the SBM feed may have caused the poults to reduce their feed consumption, as they had the lowest overall consumption, which may have contributed

to the reduced growth. This is, however, in disagreement with previous research conducted in broiler chickens (Borges, 2003; Ahmad, 2009), which concluded that increasing dietary electrolyte balancing results in increasing feed intake. The research concluded that an increase in the sodium levels in the diets is what is responsible for the increased the dietary electrolyte balance, as well as causing the resulting increase in feed intake (Ahmad, 2009). This is different from the current study where an increase in potassium led to the increased dietary electrolyte balance and a reduced feed intake was observed. A second dietary factor that may have affected performance is a protein imbalance, however, all diets were balanced to very similar digestible arginine, lysine, methionine, methionine and cysteine, and threonine levels (Table 3.3), so this should not have caused the negative impact on growth that was observed in the SBM diet.

3.5.2.2 Water consumption and terminal ileum digesta moisture content

The highest weekly and overall (2783.6 mL) water consumption was seen for poults fed the CPC treatment. This treatment effect is difficult to explain, particularly because all diets were balanced to the same minimum mineral content. Sodium, calcium, phosphorus, and potassium were similar in the CPC, CGM, FM, and PM treatments, yet water consumption was much higher in the CPC treatment. Sodium is typically viewed as the nutrient that causes high water consumption, however, sodium was not higher in the CPC diet and on an ingredient level, CPC was not the ingredient with the highest sodium level, as FM had a sodium level of 1.50%, while the level of sodium in CPC was 1.08%. This indicates that there is another component of the diet that was not assessed, but may cause the birds on the CPC treatment to have a higher water intake.

Water consumption of poults on the SBM diet was not higher than birds fed PM, which was interesting to note as the digesta moisture was significantly higher in SBM than PM. This

suggests that another factor, other than water consumption influenced digesta moisture, such as protein level, electrolyte balance, or non-digestible fractions of the diet (Francesch and Brufau, 2004). The effect of age on the water:feed and water:gain ratio is interesting, as SBM has relatively high values in the first two wks, but in wk 3, it has the lowest values. This indicates that there something in SBM that causes increased water consumption in the first two wks, but after that time, poults appear to adapt. This could be due to a variety of factors, including changes in microbiota of the GIT as they adapt to the feed or the effect of oligosaccharides within the GIT. This may also indicate that d 21 was too late to look at the digesta moisture and osmolarity, and that a larger effect would have been found if these samples were also collected on d 7 and 14. The increased digesta moisture in the SBM treatment group may be attributed to the presence of oligosaccharides in the diet, as these compounds tend to be hydroscopic and lead to water retention in the digestive tract (Graham et al., 2002). The potassium level may also have influenced the digesta moisture level, as it was higher in the SBM (1.12%) than any of the other diets (0.93 to 0.98%).

When looking at the response variables between animal and vegetable protein sources, the diets containing vegetable protein had increased water consumption, water:feed, water:gain, and terminal ileum digesta moisture at day 21. The elevated water consumption and excreta moisture in the vegetable fed diets was also observed by Vieira and Lima (2005). This response may, however, be skewed by the fact that the CPC values were so high.

3.6 Conclusions

Determining digestibility and energy levels and using this information to determine which ingredient fits best into early turkey diets is a crucial first step when formulating these diets. The feeding value results from this study indicate that ingredients such as SBM and CPC

are valuable in terms of providing highly digestible protein to poults. This contradicts the results from the performance trial, however, which indicate that SBM at very high levels can reduce early poult growth. Feeding high levels of SBM leads to decreased performance and has other effects, including increased water:gain, feed:gain, and digesta moisture that may have negative consequences. These effects appear to last for the first 2 wks only and can be mitigated with the addition of a second protein source within the diet. Choosing the partner protein source for SBM does not appear to be highly important, as all protein sources tested in this research had a positive impact on performance. The highly digestible SBM is still the base of the diet and poultry nutritionists can make up for any lacking AA with synthetic AA, however, this can only be accomplished if the digestibility of the feed ingredients are taken into account. Due to the importance of the first two wks to ensure the poults have a good start, it is important to understand the effect of supplementing SBM with an alternative protein source to reduce the negative performance impact observed in this study. The best performance was observed with PM was added to SBM. This is an interesting result because PM was one of the more poorly digestible ingredients tested, so the increased performance is likely do to other attributes of PM.

Formulating early turkey diets based on accurate digestibility values and understanding exactly what is being provided to the poult in the feed will have a positive effect on performance. Using this strategy gives the formulator the opportunity to strategically choose ingredients which the poult is able to use efficiency. Overall, combining SBM with an alternative protein source, and particularly combining SBM with PM, has a positive effect of poult performance in the first two wks and is recommended to avoid potential effects from residual ANF in the SBM, as well as to help reduce the digesta moisture.

4.0 THE EFFECT OF PROTEIN SOURCES ON HISTOLOGICAL AND BARRIER FUNCTION DEVELOPMENT IN THE GASTROINTESTINAL TRACT OF TURKEYS IN THE FIRST THREE WEEKS

4.1 Abstract

Early nutrition in turkey poults impacts gastrointestinal tract (GIT) development and has a lasting effect on bird performance. This study investigated the impact of replacing 25% of the soybean meal (SMB) protein in a SBM based starter diet with corn gluten meal (CGM), canola protein concentrate (CPC), fish meal (FM), or porcine meal (PM) on turkey GIT development and blood metabolic profiles. Twenty-one poults were randomly assigned to each of 4 replications for 5 treatment diets. Intestinal segments and other tissues were sampled and weighed at hatch (H), placement (P), and d 1, 2, 3, 5, 7, 14, and 21 (2 poults per replication per time). Samples were collected from the ileum for histology, goblet cell (GC) counts, and analysis of claudins (CLDN-1, -5, and -10), interleukins (IL-1, -6, and -8), and mucin (Muc2) transcript abundance from the SBM and PM treatment groups at H, P, and d 1, 3, and 7. Blood samples for metabolic profiling were collected from all treatments at H, P, and d 1, 3, and 7. Data were analyzed as a 5x9, 2x5, or 5x5 factorial arrangement, respectively. Planned contrasts were used to compare the control to the average of the remaining 4 diets, PM to the average of the remaining 4 diets, and the addition of animal or vegetable proteins. Differences were considered significant when $P \leq 0.05$ and trends were identified at $P \leq 0.10$. Protein source effects on proportional tissue weights were minor, with one exception being pancreas weights, which were heavier in poults fed vegetable protein diets. With exception of the heart, all proportional tissue weights increased to peak values between 2 and 7 d, followed by a gradual decrease. Morphology of the GIT and gene expression of all genes were affected only by age, with all parameters increasing with increasing age. Gene expression dramatically increased between P

and d 1, reflecting either a major ontogenic event or a response to feed as an activator of barrier function and the immune system. GC counts revealed a greater proportion of neutral GC in PM fed birds, which may signify an earlier maturing mucus barrier. All blood metabolic profile variables were affected by age, whereas treatment only affected blood potassium and hemoglobin levels. Dietary protein source had minor effects on GIT morphology and gene expression, tissue size, and blood metabolic profile, but the data demonstrate a major GIT maturation event in barrier function within 24 h of placement and coinciding with initiation of feed intake.

4.2 Introduction

The digestive system plays a significant role in digestion and absorption of nutrients, as well as providing protection to the bird against pathogens, toxins, and bacteria (Dibner et al., 1996). Many factors, including the physical size, surface area, and permeability of the components of the gastrointestinal tract (GIT), as well as the rate and extent of growth and development, influence the efficiency of this system.

Immediately post-hatch, the GIT rapidly increases in length and its surface area increases due to an increase of villi length (Dibner et al., 1996). The increase in surface area, in addition to the maturation of the enteroctyes, contributes to the development of a functional GIT and an increase in nutrient uptake. Previous research indicates that the GIT becomes functionally mature and effective at digestion and nutrient absorption by 12 d of age in poults (Bohórquez et al., 2011). A second important aspect of digestive tract maturation after hatch is the development of barrier function. Barrier function is often associated with a grouping of mechanisms, including those designed to prevent damage to the intestinal epithelium layer, as well as control of the paracellular translocation of larger molecular weight molecules and pathogens. There are several components present within the GIT, which play a role in barrier function and are important for protection of the epithelium, including mucins, tight junction proteins (e.g. claudins), and innate and adaptive immune responses affected by interleukin synthesis (ILs).

Mucins are glycoproteins produced by goblet cells (GC) that form a mucus layer associated with the epithelial layer (Smirnov et al., 2004) and play an important role in the protection of the epithelium against damage and infection (Azzam et al., 2011). The mucus layer aids in preventing bacterial translocation by increasing the distance bacteria must move to be in

contact with the GIT wall. The nature of the GC themselves are also important as this impacts their ability to protect the GIT (Uni et al., 2003). Acidic mucin producing GC are able to protect the GIT from bacterial translocation, which is important immediately following hatch and before feed access. Neutral mucin producing GC are associated with a more mature GIT, as previous research has demonstrated that there is a shift from acidic to neutral GC following hatch (Uni et al., 2003).

Beneath the mucus layer and between the enterocytes are tight junction proteins. These proteins affect cell-to-cell junctions that control the paracellular movement of ions, water, and other molecules to move via paracellular pathways (Feldman et al., 2005). Claudins, which are tight junction proteins, influence the charge selectivity of tight junctions (Van Itallie et al., 2008), as well as the permeability of the paracellular pathway across the epithelium (Amasheh et al., 2011). Finally, ILs are signaling molecules that play a role in innate and adaptive immunity against pathogens (Hong et al., 2006). While the demonstration of these barrier mechanisms has been well documented in chickens (Rothwell et al., 2004; Smirnov et al., 2004; Feldman et al., 2005; Smirnov et al., 2005; Hong et al., 2006; Smirnov et al., 2006), research is lacking in turkeys.

The development of barrier function begins following the swallowing of amniotic fluid prior to hatch (Bohórquez et al., 2011). This begins the process as there is gradual tightening of the spaces between the epithelial cells and mature GC begin to develop. Understanding the development and timing of this barrier is important because any disturbances or damage allow for the translocation of bacteria, toxins, and antigens, and will ultimately impact bird health and growth (Smith et al., 2000).

Previous research has shown multiple factors may influence the timing and extent of development of the GIT, including the presence or absence of feed, dietary contaminants, and antibiotics (Dibner et al., 1996), as these all impact the size and development of the GIT and its morphology. Another dietary effect may be the specific nutrients and compounds being provided in the feed. Smith et al. (2000) indicated that the development of the GIT may be influenced by the nature of the protein source. These authors focused on the ability of low levels of polyamines found in animal protein concentrates to influence intestinal development (Smith et al., 2000), as these compounds are correlated with anabolic processes and possess hormone-like properties (Smith et al., 1996). Additional research has suggested that polyamines have a greater impact when intestinal integrity is compromised (Smith et al., 2000), which may indicate that a larger impact might also be seen in the immature digestive tract following hatch in poultry species. This indicates that investigation of the effect of dietary protein source, specifically animal or vegetable, on GIT development is of practical and scientific interest.

The objective of this study was to investigate the effect of feeding different protein sources on GIT development, specifically the effect on morphological and barrier function development. In addition, the study aimed to understand the specific timing of morphological and barrier function development within the small intestine of the turkey poult. The final objective was to study the effect of protein source and age on blood metabolic profiling. It was hypothesized that the nature of dietary protein would influence tissue weights and GIT maturation in turkey poults.

4.3 Materials and methods:

Birds were cared for according to the Canadian Council of Animal Care (1993) guidelines and the Animal Care Committee at the University of Saskatchewan approved all

experimental procedures. This trial was run concurrently with the trial studying the effect of protein source on performance in the previous chapter, using a different group of birds.

4.3.1 Birds and housing:

Hybrid Converter eggs (1,500) were obtained from a commercial hatchery and set in incubators at the University of Saskatchewan Poultry Centre. The eggs were candled at 11 d of incubation and any eggs without a viable embryo were removed from the incubators. Eggs were transferred to hatchers at 25 d of incubation and hatch removal began at 26 d +10 h of incubation. Hatched poults were removed at 6 h intervals, ending at 28 d + 4 h of incubation, and were defined as ready to remove when they were completely emerged from the eggshell and 80% dried. Twenty-four h after the final hatch pull, the poults were randomly allocated at consistent proportions to 20 pens ($4.5m^2$). The pens were bedded with shavings (7.5 cm depth) and heat lamps were provided for the first 7 d. A standard temperature curve was used, with an initial room temperature of 32°C at placement and then decreasing 0.5°C each d for the remainder of the experiment. Daylength was 23 h from placement to 7 d (20 lux) and 18 h (10 lux) thereafter Birds were provided with *ad libitum* feed and water throughout the trial, with 1 tube feed (36 cm diameter) and 1 bell drinker in each pen. For the first 7 d, supplemental feed and water were also provided in a plastic flip top tray (10.16 cm wide x 50.80 cm long x 7.62 cm high) and a 4 L fount drinker. Pens were checked for mortality twice daily and dead and cull birds were sent for necropsy to determine cause of death and/or morbidity (Prairie Diagnostic Services, Western College of Veterinary Medicine, University of Saskatchewan).

4.3.2 Dietary treatments

Each pen was randomly assigned to one of 5 treatment diets (Table 3.3), creating 4 replications per treatment. The basal diet was a wheat-soybean meal (SBM) based diet

formulated to meet the nutrient requirements of the poults. The remaining 4 treatment diets were formulated by mathematically replacing 25% of the protein being supplied by SBM in the basal diet with one of 4 alternative ingredients. The four alternative ingredients were: corn gluten meal (CGM), canola protein concentrate (CPC), fish meal (FM), and porcine meal (PM). All treatment diets were formulated to meet Hybrid nutrient recommendations and were sent to an external lab for verification of crude protein, calcium, phosphorus, sodium, potassium, and magnesium levels.

4.3.3 Data collection

4.3.3.1 Animal data

Eight birds per treatment were euthanized by cervical dislocation immediately following hatch, at placement, and 8 birds per treatment were euthanized on d 1, 2, 3, 5, 7, 14, and 21. On each collection day, two birds were randomly selected from each pen and individually weighed. Prior to euthanasia at hatch, placement, and d 1, 3, and 7, blood samples were drawn from the jugular vein of each bird and metabolic profiling was completed using EC8+ cartridges in an i-STAT blood analyzer (Abbott Laboratories, Abbott Park, IL). The poults were then euthanized by cervical dislocation and the liver, heart, pancreas, yolk sac, and GIT were immediately removed. Full weights were recorded for the proventriculus, gizzard, duodenum, jejunum, ileum, and caeca, as well as lengths of the duodenum, jejunum, ileum and caeca. Intestinal tract segments were then emptied by gently rolling the segments, after which empty weights were recorded. The weights of the liver, pancreas, and yolk sac, as well as poult gender were recorded.

4.3.3.2 Histology and gene expression

Following euthanasia of the birds on at hatch, placement, and d 1, 3, and 7, a 2 cm segment was removed from the midpoint of the ileum of birds from the SBM and PM treatments and immediately placed in 10% neutral buffered formalin for preservation. The samples were dehydrated, cleared, embedded in paraffin, cut into cross-sections, and fixed on slides. One slide was stained with hematoxylin and eosin to study GIT architecture, while a second was stained with Alcian Blue/Periodic acid-Schiff for differentiation of goblet cells (Osho et al., 2016).

Villi length and width, as well as crypt depth, were measured by an observer blinded to treatment and reported as the mean of 8 – 10 well-oriented and representative villi and crypts per bird. Images were taken using a Olympus BX41 microscope (Center Valley, Pennsylvania, USA) with a Olympus DP71 camera. The software utilized for image capture was DP Manager/DP Controller (Olympus). Image-Pro Plus 7.0 (Media Cybernetics Inc, Rockville, Maryland, USA) was used for subsequent measurement of captured images. The villi length was defined as the measurement from the tip of the villus to the villus crypt junction, villi width was measured at the half height of the villus, and crypt depth was defined as the depth of the invagination between adjacent villi. The surface area of the villi was calculated based on height and width, and the villus to crypt ratio (VCR) was determined using the villi length and crypt depth measurements.

Goblet cell (GC) were counted around the perimeter of the villi and crypts from 8 – 10 well-oriented villi and crypts per bird and reported as the mean. GC were categorized into three types: acidic mucin producing (appearing blue on the slide), neutral mucin producing (appearing magenta), and mixed (appearing purple) (Osho et al., 2016).

A second 2 cm segment of the mid ileum was removed, immediately snap frozen in liquid nitrogen, and subsequently stored at -80°C. Total RNA was extracted from 30 – 40 mg of ground ileal tissue using TRI Reagent Solution (Applied Biosytems). The amount of RNA was quantified by measuring absorbance at 260/280 nm using a spectrophotometer (NanoDROP 2000 Spectrophotometer, ThermoFisher Scientific, Mississauga, ON, Canada) and 1 μg/μl of RNA was used to generate cDNA using the High-Capacity cDNA Reverse Transcription Kit according to the manufacturer's protocol (Applied Biosystems). Because turkey genes are not well sequenced, primers were chosen based on chicken sequences. Primers (Table 4.1) were obtained for chicken GAPDH, claudin-1, claudin-5, IL-8, and Muc2, whereas primers for IL-1 (NCBI Accession # NM_204524), IL-6 (NCBI Accession # NM_204628), and claudin-10 (NCBI Accession # NM_00127769.1) were designed using Primer3 in PrimerBLAST (NCBI).

The transcript abundance of claudin-1 and claudin-5 were used as an indication of the tightness of the gut barrier, as these claudins are involved in the tightening the space between the epithelial cells, while claudin-10 was used as an indication of tight junction proteins involved in mediating the permeability of the gut barrier (Amasheh et al., 2011). These claudins were chosen not only to observe the effect of diet on GIT barrier function, but also to observe the effect of age on the GIT barrier development. IL-1 and IL-6 are pro-inflammatory cytokines, while IL-8 is a chemokine, part of the innate immune system response (Kogut, 2010). These interleukins were chosen as an indication of irritation to the GIT, the presence of bacterial translocation, and whether there may be an allergic response in the poults fed the SBM diet. Muc2 transcript abundance was studied as an indication of the nature of the mucus layer within the GIT (Cheled-Shoval et al., 2011).

Table 4.1 Primers used for qPCR reactions.

Target	Ori[1]	Sequence (5' – 3')	Size (bp)	Origin
GAPDH	f[2]	GTGAAAGTCGGAGTCAACGGA	101	Cheled-Shoval et al., 2011
	r[3]	AAGGGATCATTGATGGCCAC		
Claudin-1	f	TGGAGGATGACCAGGTGAAGA	115	Shao et al., 2013
	r	CGAGCCACTCTGTTGCCATA		
Claudin-5	f	CAGAAGCGGGAGATAGGGG	128	Seo et al., 2010
	r	TACTTGACGGGGAAGGAGGT		
Claudin-10	f	CAACGTGGAAAAAGCTGCAAGA	250	Designed with Primer3 (NCBI)
	r	ATAATGGAGCCGCATCTGTGA		
IL-1	f	AGGTGACGGTGCGGAAGG	210	Designed with Primer3 (NCBI)
	r	AGCGGGTGTAGCGGAAGG		
IL-6	f	GAGAGGTTGGGCTGGAGGAG	175	Designed with Primer3 (NCBI)
	r	TTGGGCAGGTTGAGGTTGTTC		
IL-8	f	ATGAACGGCAAGCTTGGAGCT	312	Khatri and Sharma, 2006
	r	TCACAGTGGTGCATCAGAATTGA		
Muc2	f	CCTGTGCAGACCAAGCAGAAA	100	Cheled-Shoval et al., 2011
	r	CCTCTGTTTTTCAGCAAAGAACAC		

[1]Orientation.
[2]Foward.
[3]Reverse.

GAPDH was used as a housekeeping gene to normalize data as its expression is not typically affected by treatment or age. Each PCR reaction included 1 µl of template cDNA, 1µL of 10 µM forward primer, 1µL of 10 µM reverse primer, 10 µL of SsoFastTM EvaGreen Supermix (BioRad, Laboratories (Canada) Ltd., Mississauga, ON, Canada), and 7µL of Nuclease-Free Water (Ambion, Life Technologies Inc., Burlington, ON, Canada). The PCR reaction conditions for all primers, except claudin-10, were 1 x 65°C for 5 s, 40 x (95°C for 5 s, 60°C for 5 s for annealing), followed by a melt curve analysis from 65°C to 95°C in 0.5°C increments for 5 s each. PCR conditions for claudin-10 were the same except the annealing temperature used was 63°C. A CFX96 Real-Time System with a C1000 Thermal Cycler (BioRad) was used for realtime analysis product amplification and BioRad CFX manager software (version 3.1, BioRad) was used for transcript quantification. Melt curve analysis was completed to ensure single product amplification and products were sequenced for verification. The mRNA abundance was calculated based on a standard curve prepared by serial tenfold dilutions of a cDNA pool of all samples. mRNA abundance of all genes of interest were then normalized to the level of GAPDH in each sample.

4.3.4 Statistics

Statistical analysis was completed using SAS 9.3 (SAS,2011). Because only 8 birds were sacrificed at time of hatch and placement, the data for these two times points was replicated and each treatment was applied, as this was considered the baseline data for the treatment effects. Tissue and blood data were analyzed using a 5 (dietary treatments) x 9 (ages) factorial, with 4 replications per dietary treatment, in a completely randomized design. Histology and gene expression data were analyzed using a 2 (dietary treatments) x 5 (ages) factorial. Blood data were analyzed using a 5 (dietary treatments) x 5 (ages) factorial. Data were transformed (log+1)

when the data was not normally distributed. Differences were considered significant when $P \leq 0.05$ and trends identified when $P \leq 0.10$. Mean separation was completed using the Tukey test when significant differences were observed. *A priori* contrasts were conducted on tissue and blood data to allow comparison of the SBM control diet to the average of CGM, CPC, FM, and PM (Contrast #1), PM to the average of SBM, CGM, CPC, and FM (Contrast #2), and the addition of vegetable (CGM and CPC) and animal protein (FM and PM) (Contrast #3). These contrasts were chosen because SBM was the protein source that was focused on in this study, PM had the highest growth in the previous chapter, and because the effect of animal and vegetable protein was of interest in terms of growth and development. The model used for analysis was:

$$Y = \mu + T + D + T*D + e$$

where, μ is the overall mean

T is the treatment (fixed effect)

D is the age (fixed effect)

T*D is the interaction between treatment and age (fixed effect), and

e is the error term.

4.4 Results

4.4.1 Tissue weights as a percentage of live weight

While the statistics were run as a factorial, the main effects have been displayed separately in Table 4.2 (protein source effect) and 4.3 (age effect) due to space constraints. The interactions of this model were only significant for the following variables: pancreas (P=0.0047), empty gizzard (P=0.0252), and full ileum (P=0.0064). Pancreas weight (Figure 4.1) as a proportion of live weight for CPC peaked at d 5, while the remaining treatments peaked at d 7.

Table 4.2 Effect of protein source on tissue measurements in proportion to live weight (%, cm/g) in turkey poults from hatch to 21 days of age[1].

	SBM	CGM	CPC	FM	PM	*P*-Value	SEM
Heart	0.61[b]	0.66[a]	0.62[ab]	0.65[ab]	0.64[ab]	0.0136	0.005
Liver	3.28[b]	3.57[a]	3.40[ab]	3.39[ab]	3.47[ab]	0.0025	0.009
Pancreas	0.34[bc]	0.36[ab]	0.38[a]	0.33[c]	0.34[bc]	<0.0001	0.004
Yolk sac	1.29	1.65	1.23	1.39	1.23	NS[2]	0.035
Full proventriculus	0.76	0.75	0.74	0.75	0.75	NS	0.005
Empty proventriculus	0.72	0.73	0.71	0.72	0.73	NS	0.005
Full gizzard	6.09	6.15	6.10	6.19	6.09	NS	0.012
Empty gizzard	3.62[b]	3.86[a]	3.66[ab]	3.83[ab]	3.78[ab]	0.0126	0.011
Full duodenum	1.45	1.46	1.49	1.47	1.48	NS	0.007
Empty duodenum	0.66	0.66	0.70	0.67	0.75	NS	0.010
Duodenum length[3]	0.08	0.08	0.08	0.08	0.08	NS	0.001
Full jejunum	2.49	2.36	2.50	2.34	2.43	0.0846	0.011
Empty jejunum	1.31	1.29	1.38	1.33	1.38	0.0833	0.009
Jejunum length[3]	0.15	0.15	0.16	0.15	0.15	0.0851	0.002
Full ileum	2.18[ab]	1.97[c]	2.24[a]	2.04[bc]	2.10[abc]	0.0005	0.012
Empty ileum	1.21[a]	1.11[b]	1.19[a]	1.13[ab]	1.16[ab]	0.0019	0.007
Ileum length[3]	0.17[ab]	0.16[b]	0.18[a]	0.17[ab]	0.17[ab]	0.0483	0.002
Full caeca	1.24[ab]	1.23[ab]	1.20[b]	1.34[ab]	1.38[a]	0.0439	0.160
Empty caeca	0.74	0.70	0.69	0.71	0.71	NS	0.008
Caeca length[3]	0.12	0.11	0.12	0.12	0.12	NS	0.002
Full small intestine	5.18[ab]	4.93[b]	5.27[a]	4.99[ab]	5.10[a]	0.0145	0.067
Empty small intestine	2.83[ab]	2.75[b]	2.90[a]	2.80[ab]	2.92[a]	0.0127	0.040
Body weight (g)	219.3	223.0	215.0	223.0	229.4	NS	3.804

[1]Means of 4 replications of 2 birds at 9 time points (n=72).
[2]NS=$P>0.10$.
[3]Length: cm/g.
[a,b] Means within the same row with no common superscript differ significantly ($P\leq0.05$).
SBM=soybean meal; CGM=corn gluten meal; CPC=canola protein concentrate; FM=fish meal; PM=porcine meal.
Statistical analysis was conducted as a factorial design, but due to space constraints, the data have been split into two tables (Table 4.2 and 4.3).

Table 4.3 Effect of age on tissue measurements as a proportion of live weight (%, cm/g) in turkey poults from hatch to 21 days of age[1].

	Day										
	H	P	1	2	3	5	7	14	21	*P*-Value	SEM
Heart	0.59^{de}	0.67^{b}	0.54^{e}	0.59^{de}	0.58^{de}	0.66^{bc}	0.74^{a}	0.74^{a}	0.61^{cd}	<.0001	0.007
Liver	2.49^{f}	2.78^{e}	3.59^{bc}	3.63^{b}	3.67^{ab}	3.90^{a}	3.37^{c}	3.12^{d}	2.68^{ef}	<.0001	0.012
Pancreas	0.14^{h}	0.19^{g}	0.24^{f}	0.29^{e}	0.35^{cd}	0.41^{ab}	0.44^{a}	0.37^{bc}	0.33^{d}	<.0001	0.006
Yolk sac	12.11^{a}	9.29^{b}	4.84^{c}	2.68^{d}	1.61^{e}	0.31^{f}	0.05^{fg}	0.01^{g}	0.01^{g}	<.0001	0.047
Full proventriculus	0.51^{f}	0.60^{e}	0.70^{d}	0.81^{c}	0.85^{bc}	0.92^{a}	0.87^{ab}	0.60^{e}	0.48^{f}	<.0001	0.006
Empty proventriculus	0.49^{e}	0.57^{d}	0.68^{c}	0.80^{b}	0.83^{ab}	0.87^{a}	0.83^{ab}	0.59^{d}	0.47^{e}	<.0001	0.006
Full gizzard	3.42^{e}	4.18^{d}	6.67^{ab}	6.83^{a}	6.84^{a}	6.82^{a}	6.17^{b}	5.03^{c}	4.49^{d}	<.0001	0.017
Empty gizzard	3.14^{d}	3.92^{bc}	4.04^{abc}	4.26^{a}	4.15^{ab}	4.04^{abc}	3.78^{c}	3.14^{d}	2.82^{e}	<.0001	0.015
Full duodenum	0.56^{g}	0.70^{f}	1.13^{e}	1.38^{c}	1.69^{b}	2.00^{a}	1.74^{b}	1.23^{d}	1.09^{e}	<.0001	0.009
Empty duodenum	0.50^{d}	0.53^{cd}	0.53^{cd}	0.64^{b}	1.04^{a}	1.10^{a}	0.62^{bc}	0.48^{de}	0.40^{e}	<.0001	0.014
Duodenum length[2]	0.10^{b}	0.13^{a}	0.11^{b}	0.12^{ab}	0.11^{b}	0.09^{c}	0.07^{d}	0.04^{e}	0.03^{f}	<.0001	0.002
Full jejunum	0.59^{g}	0.77^{f}	1.73^{e}	2.23^{c}	2.94^{b}	3.33^{a}	2.71^{b}	2.03^{cd}	1.96^{d}	<.0001	0.014
Empty jejunum	0.53^{h}	0.64^{g}	0.90^{f}	1.14^{de}	1.67^{b}	1.87^{a}	1.49^{c}	1.23^{d}	1.05^{e}	<.0001	0.013
Jejunum length[2]	0.17^{c}	0.22^{a}	0.19^{b}	0.21^{a}	0.22^{a}	0.18^{bc}	0.15^{d}	0.08^{e}	0.05^{f}	<.0001	0.003
Full ileum	0.52^{f}	0.70^{e}	1.60^{d}	2.10^{c}	2.65^{b}	2.90^{a}	2.28^{c}	1.69^{d}	1.53^{d}	<.0001	0.016
Empty ileum	0.45^{h}	0.62^{g}	0.85^{f}	1.11^{d}	1.41^{b}	1.52^{a}	1.27^{c}	1.02^{d}	0.93^{e}	<.0001	0.009
Ileum length[2]	0.19^{c}	0.24^{a}	0.21^{b}	0.23^{a}	0.24^{a}	0.20^{bc}	0.16^{d}	0.09^{e}	0.06^{f}	<.0001	0.003
Full caeca	0.78^{f}	0.92^{ef}	1.02^{de}	1.25^{bc}	1.88^{a}	1.45^{b}	1.09^{cde}	1.09^{cde}	1.15^{cd}	<.0001	0.021
Empty caeca	0.46^{d}	0.62^{c}	0.65^{c}	0.74^{ab}	0.81^{a}	0.75^{ab}	0.65^{c}	0.68^{bc}	0.68^{bc}	<.0001	0.011
Caeca length[2]	0.16^{b}	0.19^{a}	0.15^{b}	0.16^{b}	0.17^{b}	0.13^{c}	0.10^{d}	0.06^{e}	0.04^{f}	<.0001	0.002
Full small intestine	1.67^{h}	2.17^{h}	4.46^{f}	5.71^{d}	7.28^{b}	8.23^{a}	6.74^{c}	5.00^{e}	4.58^{f}	<.0001	0.089
Empty small intestine[2]	1.48^{g}	1.80^{f}	2.29^{e}	2.89^{d}	4.12^{b}	4.49^{a}	3.38^{c}	2.73^{d}	2.37^{e}	<.0001	0.053
Small intestine length, cm/g	45.87^{c}	58.41^{a}	55.90^{b}	55.90^{a}	57.03^{a}	47.35^{bc}	38.28^{d}	20.19^{e}	13.24^{f}	<.0001	0.822
Body weight	65.6^{fg}	62.3^{g}	76.3^{fg}	87.4^{ef}	100.1^{e}	138.5^{d}	183.5^{c}	438.0^{b}	845.5^{a}	<.0001	5.104

[1]Means of 4 replications of 2 birds per time point.

[2]Length: cm/g.

[a-g] Means within the same row with no common superscript differ significantly ($P \leq 0.05$).

H=Hatch; P=Placement.

Statistical analysis was conducted as a factorial design, but due to space constraints, the data have been split into two tables (Table 4.2 and 4.3).

Following the peak, all treatments decreased, however, the decrease in CPC was more gradual and did not decrease to the extent of the other treatments until d 21. Empty gizzard weights (Figure 4.2) were similar among treatments for most d, with the exception of d 3 and 21. On d 3, CPC had the heaviest gizzard, followed by FM, PM, CGM, and finally SBM. On d 21, SBM had the heaviest gizzard, followed by CPC, PM, CGM, and FM. The response of full ileum weight (Figure 4.3) as a proportion of live weight to age and dietary treatments was similar on all d, except for d 7 and 21. On d 7, CPC had the greatest weight, followed by PM, CGM, SBM, and finally FM. Full ileum weights at d 21 were greatest for CPC, intermediate for SBM, and least for CGM, FM, and PM.

While the interactions were significant for the pancreas, empty gizzard, and full ileum as a proportion of live weight, there appears to be little biological effect, therefore the main effects are also shown and discussed for this research. Dietary treatment significantly affected the proportional heart, liver, pancreas, empty gizzard, full ileum, empty ileum, and full caeca weights, as well as the full and empty weights of the small intestine as a whole (Table 4.2). The heart, liver, and empty gizzard weights all demonstrated the same effect, with CGM being heavier than SBM. The pancreas proportional weight was heavier for CPC as compared to FM and PM. Proportional full ileum weight was heavier in CPC than in FM and CGM, while proportional ileum length and the full and empty weights of the small intestine as a whole were heavier in CPC than CGM. The proportional empty ileum weight was heavier in SBM and CPC than in CGM. Finally, the proportional full caeca weight was heavier in PM than CPC.

All tissue weights as a proportion of live weight were significantly affected by age (Table 4.3). Ileum and jejunum length peaked at placement, as well as d 3. Duodenum and caeca length increased from hatch to placement, after which they decreased. Full gizzard, full caeca,

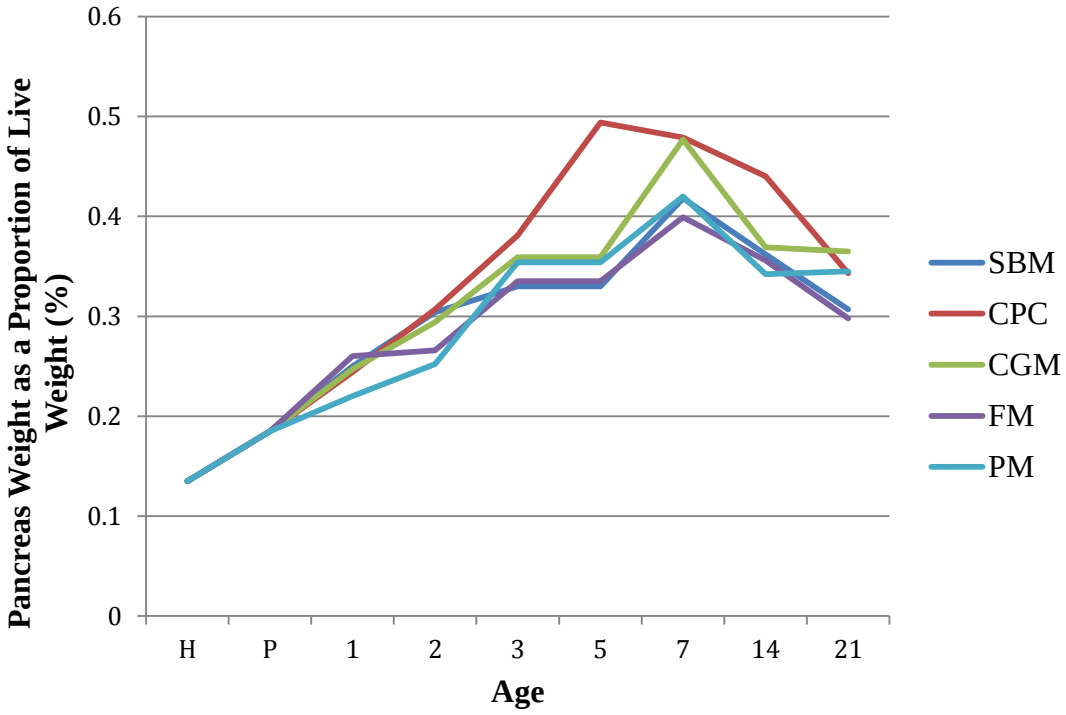

Figure 4.1 Effect of the interaction between dietary treatment and age on pancreas weight as a proportion of live weights in turkey poults from hatch to 21 days of age. Means of 4 replications of 8 birds per time point.
SBM=soybean meal; CGM=corn gluten meal; CPC=canola protein concentrate; FM=fish meal; PM=porcine meal; H=hatch; P=placement.

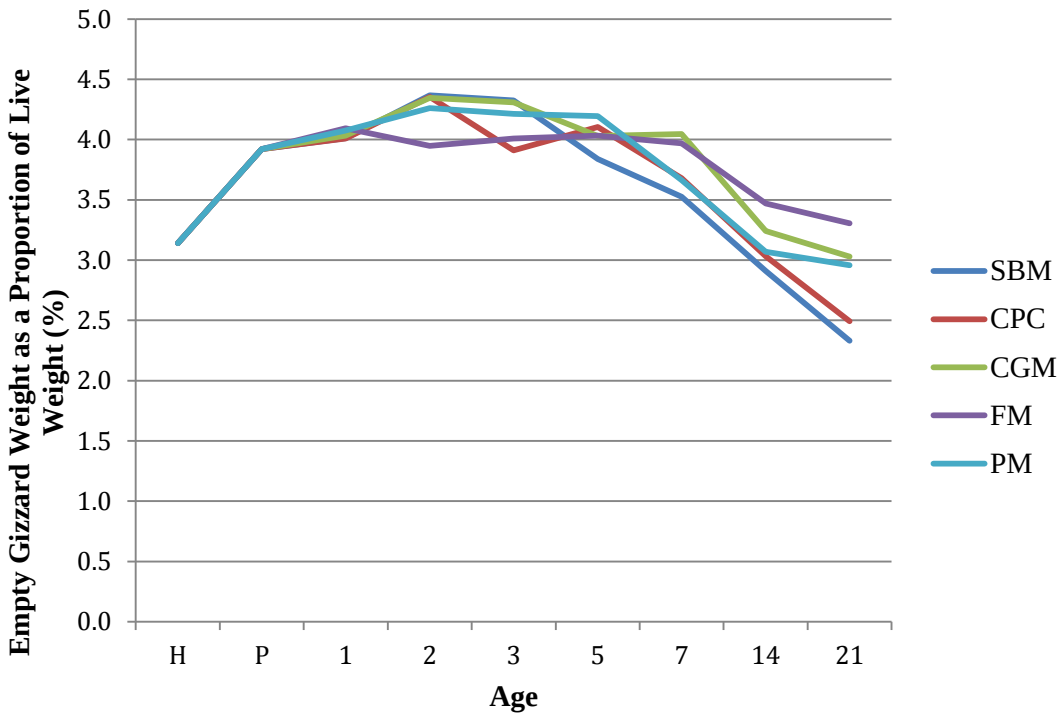

Figure 4.2 Effect of the interaction between dietary treatment and age on empty gizzard weight as a proportion of live weights in turkey poults from hatch to 21 days of age.
Means of 4 replications of 8 birds per time point.
SBM=soybean meal; CGM=corn gluten meal; CPC=canola protein concentrate; FM=fish meal; PM=porcine meal; H=hatch; P=placement.

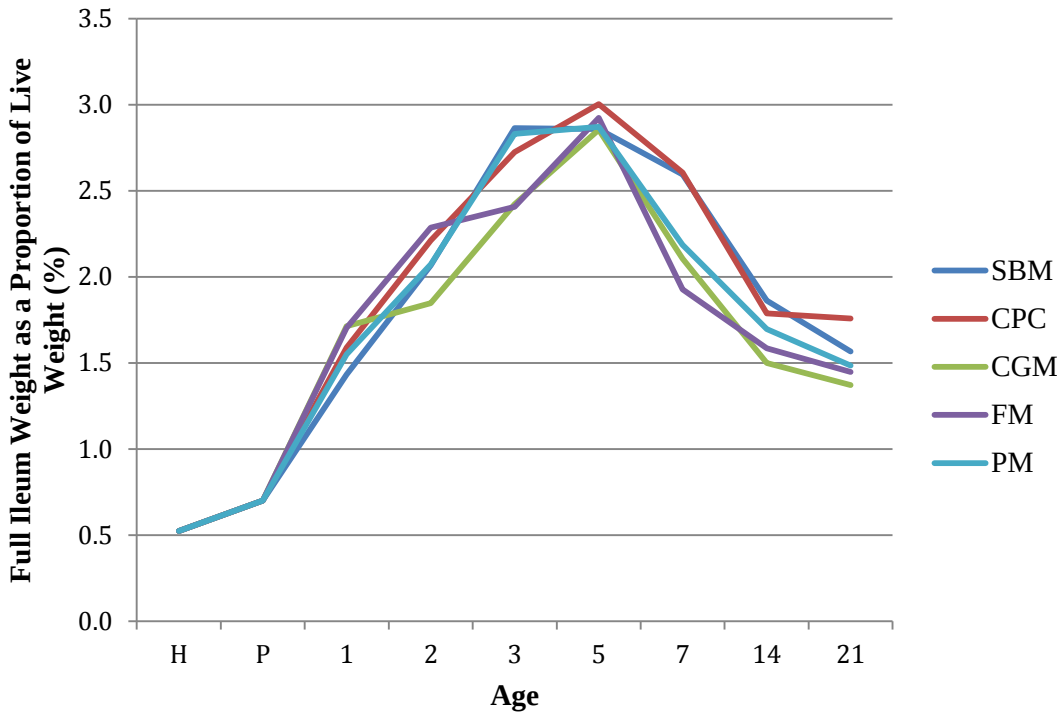

Figure 4.3 Effect of the interaction between dietary treatment and age on full ileum weight as a proportion of live weights in turkey poults from hatch to 21 days of age. Means of 4 replications of 8 birds per time point.
SBM=soybean meal; CGM=corn gluten meal; CPC=canola protein concentrate; FM=fish meal; PM=porcine meal; H=hatch; P=placement.

and empty caeca increased with increasing age up to d 3, after which they decreased. Heart, liver, full and empty proventriculus, full and empty duodenum, full and empty jejunum, and full and empty ileum followed the same trend, but the peak was at d 5. Pancreas weight increased with increasing age up to d 7 after which, they decreased. Yolk sac weight decreased with increasing age.

No significantly differences were noted for *a priori* contrast #1 or 2 (Table 4.4). A trend was noted for contrast #2 with the full caeca weights being increased for PM (1.38%) when compared to the other dietary treatments (1.25%). Contrast #3 was significant for proportional pancreas weight, with vegetable protein treatments (0.37%) having an increased weight than the animal protein treatments (0.34%), and full caeca weight, with vegetable protein treatments (1.22%) having a lower weight than the animal protein treatments (1.36%).

4.4.2 Blood metabolic profiling

Analysis of blood showed an effect of dietary treatment on potassium and hemoglobin levels. Potassium levels were the greatest in SBM, intermediate for CGM and CPC and lowest in FM and PM treatments. Hemoglobin levels were higher in SBM and CPC than the other three treatments (Table 4.5). *A priori c*ontrast #2 revealed that PM had significantly lower pCO_2 (34.3 mmol/L) than the other treatments (36.3 mmol/L), while a significant p-value for contrast #3 revealed that pCO_2 was higher in vegetable (36.3 mmol/L) than animal protein treatments (35.3 mmol/L) (Table 4.6).

Age had a significant effect on all of the parameters measured. Sodium and chloride levels increased between hatch and placement, after which lower values (not different than at hatch) were found for days 1, 3 and 7. Hematocrit, and hemoglobin values followed the same pattern, but the increase between hatch and placement was not significant. Glucose increased

Table 4.4 Contrast p-values for tissue size in proportion to live weight in turkey poults.

	Contrast		
Parameter	1	2	3
Heart	NS[1]	NS	NS
Liver	NS	NS	NS
Pancreas	NS	NS	0.0231
Yolk sac	NS	NS	NS
Full proventriculus	NS	NS	NS
Empty proventriculus	NS	NS	NS
Full gizzard	NS	NS	NS
Empty gizzard	NS	NS	NS
Full duodenum	NS	NS	NS
Empty duodenum	NS	NS	NS
Duodenum length[2]	NS	NS	NS
Full jejenum	NS	NS	NS
Empty jejenum	NS	NS	NS
Jejenum length[2]	NS	NS	NS
Full ileum	NS	NS	NS
Empty ileum	NS	NS	NS
Ileum length[2]	NS	NS	NS
Full caeca	NS	0.0950	0.0339
Empty caeca	NS	NS	NS
Caeca length[2]	NS	NS	NS
Full SI	NS	NS	NS
Empty SI	NS	NS	NS
SI length[2]	NS	NS	NS

[1]NS=*P*>0.10.

[2]Length: cm/g.

Contrast #1= Soybean meal (SBM) versus the average of corn gluten meal (CGM), canola protein concentrate (CPC), fish meal (FM), and porcine meal (PM); Contrast #2=PM versus the average of SBM, CGM, CPC, and FM; Contrast #3=Vegetable (CGM and CPC) versus animal (FM and PM) addition to SBM.

Table 4.5 Effect of dietary protein source on the blood metabolic profile of turkey poults[1,2].

	Treatment						Day						Pooled
Parameter	SBM	CGM	CPC	FM	PM	*P*-Value	H	P	1	3	7	*P*-Value	SEM
Na (mmol/L)	136	135	134	135	134	NS[3]	135^{c}	149^{a}	133^{c}	134^{c}	138^{b}	<.0001	0.5
K (mmol/L)	4.7^{a}	4.5^{ab}	4.5^{ab}	4.3^{b}	4.3^{b}	0.0380	3.2^{c}	3.1^{c}	3.9^{b}	4.6^{a}	4.7^{a}	<.0001	0.06
Cl (mmol/L)	104	104	102	103	103	NS	107^{b}	127^{a}	102^{c}	102^{c}	105^{b}	<.0001	0.6
TCO_2 (mmol/L)	25	25	26	26	25	NS	21^{b}	15^{c}	25^{a}	26^{a}	25^{a}	<.0001	0.3
Glucose (mmol/L)	15.7	15.5	15.8	15.2	15.8	NS	11.7^{c}	12.8^{c}	16.4^{a}	14.9^{b}	15.5^{ab}	<.0001	0.21
Hct (%PCV)	21	20	21	20	20	NS	23^{b}	26^{a}	22^{b}	19^{c}	20^{c}	<.0001	0.3
pH	7.418	7.415	7.441	7.45	7.445	NS	7.387^{b}	7.272^{c}	7.426^{ab}	7.443^{a}	7.432^{a}	<.0001	0.0058
pCO_2 (mmol/L)	36.4	36.4	36.2	36.2	34.3	NS	33.1^{ab}	31.3^{b}	35.6^{a}	35.6^{a}	35.9^{a}	0.0160	0.29
HCO_3 (mmol/L)	23.8	23.4	24.6	24.6	23.8	NS	20.0^{b}	14.5^{c}	23.7^{ab}	24.5^{a}	24.0^{a}	<.0001	0.33
BEecf (mmol/L)	-0.7	-1.1	0.5	0.6	-0.3	NS	-5.0^{b}	-12.4^{c}	-0.7^{a}	0.4^{a}	-0.4^{a}	<.0001	0.41
AnGap (mmol/L)	12.5	12.6	12.5	12.2	11.4	NS	11.7^{b}	11.0^{b}	11.2^{b}	12.1^{ab}	13.4^{a}	0.0020	0.23
Hb (mmol/L)	4.5^{a}	4.1^{b}	4.5^{a}	4.2^{b}	4.1^{b}	0.0265	4.9^{ab}	5.5^{a}	4.7^{b}	4.0^{c}	4.2^{c}	<.0001	0.06

[1]Means of 4 replications of 2 birds per treatment at each time point (n=40 for treatment; n=8 for day).
[2]Interaction *P*-values are not displayed, as all are not significant (*P*>0.05).
[3]NS=*P*>0.05.
[a-c] Means within the same row and main effect with no common superscript differ significantly (*P*≤0.05).
SBM=Soybean meal; CGM=Corn gluten meal; CPC=Canola protein concentrate; FM=Fish meal; PM=Porcine meal; H=Hatch; P=Placement; SEM=Standard error of the mean; TCO_2=Total carbon dioxide; Hct=Hematocrit; pCO_2=Partial pressure of carbon dioxide; BEecf=Base excess in extracellular fluid; AnGap=Anion gap; Hb=Hemoglobin.

Table 4.6 Contrast p-values for blood parameters in turkey poults.

	Contrast		
Parameter	1	2	3
Na (mmol/L)	NS[1]	NS	NS
K (mmol/L)	NS	NS	NS
Cl (mmol/L)	NS	NS	NS
TCO_2 (mmol/L)	NS	NS	NS
Glucose (mmol/L)	NS	NS	NS
Hct (%PCV)	NS	NS	NS
pH	NS	NS	NS
pCO_2 (mmol/L)	NS	0.0155	0.0159
HCO_3 (mmol/L)	NS	NS	NS
BEecf (mmol/L)	NS	NS	NS
AnGap (mmol/L)	NS	NS	NS
Hb (mmol/L)	NS	NS	NS

[1]NS=*P*>0.05.

TCO_2: Total carbon dioxide; Hct=Hematocrit; pCO_2=Partial pressure of carbon dioxide; BEecf=Base excess in extracellular fluid; AnGap=Anion gap; Hb=Hemoglobin.

Contrast #1= Soybean meal (SBM) versus the average of corn gluten meal (CGM), canola protein concentrate (CPC), fish meal (FM) supplemented diets, and porcine meal (PM); Contrast #2=PM supplemented diet versus the average of other dietary treatments; Contrast #3=Vegetable (CGM and CPC) versus animal (FM and PM) protein addition to SBM.

from hatch and peaked at d 1, followed by a decrease on d 3 and an intermediate value on d 5. Potassium increased with days on feed, reaching a plateau at d 3. Total carbon dioxide and HCO_3 levels decreased from hatch to placement, and then increased after feeding to values that were either higher than the hatch values. Blood pH decreased from hatch to placement, after which it increased to levels similar to those found at hatch. Base excess in extracellular fluid decreased from hatch to placement, followed by an increase and stabilization at d 1 and continuing to d 7. Partial pressure of carbon dioxide (pCO_2) increased following access to feed, while anion gap increased with increasing age. There was no significant effect of interactions noted.

4.4.3 Histology and gene expression

Histological and gene expression data were collected from the ileum of poults fed diets that resulted in the most rapid (PM) and slow (SBM) growth in the previous chapter (Chapter 3). There was no effect of diet on villus length and width, crypt depth, and villus:crypt ratio (VCR) (Table 4.7). Age affected villus length, with an increase from hatch to placement, followed by a decrease on d 1 and a steady increase from there until length at d 7 was similar to that at placement. Villus width remained stable from hatch to placement, after which an increase was seen with increasing age. Crypt depth was only affected by age on d 3, when an increase in crypt depth was observed. Finally, VCR was higher at placement and d 7 than other ages. Interactions between diet and age were not significant.

Diet had an effect on the proportion of neutral and acidic mucin producing GC in the villi (Table 4.8). Total neutral mucin producing GC were increased in PM, and the proportional data illustrated a higher proportion of neutral mucin producing GC in PM and acidic mucin producing

Table 4.7 Effect of protein source and age on gut architecture in the ileum of turkey poults from 0 – 7 days[1].

	Treatment			Day						Interaction	Pooled
Parameter	SBM	PM	*P*-Value	H	P	1	3	7	*P*-Value	*P*-Value	SEM
Villus Length (μm)	364	355	NS[2]	337[b]	419[a]	248[c]	382[ab]	410[a]	<.0001	NS	23.3
Villus Width (μm)	104	104	NS	67[d]	72[d]	84[c]	101[b]	126[a]	<.0001	NS	2.8
Crypt Depth (μm)	67	65	NS	65[b]	66[b]	57[b]	79[a]	63[b]	<.0001	NS	1.4
VCR[3]	5.2	5.2	NS	5.2[b]	6.4[a]	4.3[b]	4.8[b]	6.6[a]	<.0001	NS	0.15

[1]Means of 4 replications of 2 birds per treatment at each time point (n=40 for treatment; n=8 for day).
[2]NS=*P*>0.05.
[3]Villus:crypt ratio.
[a,b] Means within the same row with no common superscript differ significantly ($P \leq 0.05$).
SBM=Soybean meal; PM=Porcine meal; H=Hatch; P=Placement; SEM=Standard error of the mean.

Table 4.8 Effect of protein source and bird age on goblet cells in the turkey ileum[1].

	Treatment			Day						Interaction	Pooled
Goblet Cells	SBM	PM	*P*-Value	H	P	1	3	7	*P*-Value	*P*-Value	SEM
Villus – Total (goblet cells per villus)											
Acidic	23.1	21.0	0.0507	18.4^{bc}	22.7^{b}	14.1^{c}	21.4^{b}	34.0^{a}	<0.0001	NS[2]	1.86
Neutral	2.2^{b}	3.0^{a}	0.0008	1.8^{c}	3.1^{ab}	1.8^{c}	2.2^{bc}	4.1^{a}	<0.0001	0.0057	0.35
Mixed	5.9	5.8	NS	3.3^{d}	5.2^{bc}	4.0^{cd}	6.2^{b}	10.5^{a}	<0.0001	NS	0.56
Crypt – Total (goblet cells per crypt)											
Acidic	11.3	10.9	NS	8.6^{c}	10.5^{b}	9.8^{bc}	12.4^{a}	14.0^{a}	<0.0001	0.0154	0.61
Neutral	1.0	1.3	NS	1.3	1.1	1.0	1.1	1.2	NS	NS	0.21
Mixed	4.9	5.1	NS	2.7^{b}	2.8^{b}	3.4^{b}	5.5^{a}	6.0^{a}	<0.0001	NS	0.41
Villus – Length corrected (goblet cell/µm villus length)[3]											
Acidic	11.0	10.4	NS	9.2^{b}	8.6^{b}	8.9^{b}	9.4^{b}	13.8^{a}	0.0001	NS	0.45
Neutral	1.0^{b}	1.4^{a}	0.0007	0.9^{b}	1.2^{ab}	1.1^{b}	1.0^{b}	1.8^{a}	0.0008	0.0179	0.09
Mixed	3.1	3.4	NS	1.7^{c}	2.0^{bc}	2.5^{b}	2.7^{b}	4.7^{a}	<0.0001	NS	0.18
Crypt – Depth corrected (goblet cell/µm crypt depth)[3]											
Acidic	23.6	23.3	NS	20.2^{b}	22.3^{ab}	23.1^{ab}	21.8^{ab}	25.3^{a}	0.0159	NS	0.56
Neutral	2.2	2.6	NS	3.2	2.4	2.4	2.0	2.0	NS	NS	0.20
Mixed	8.0	8.4	NS	6.4^{b}	6.1^{b}	8.1^{ab}	9.8^{a}	11.1^{a}	<0.0001	NS	0.42
Proportion of goblet cells/villus (% of total goblet cells/villus)											
Acidic	72.6^{a}	68.8^{b}	0.0495	78.0^{a}	73.2^{ab}	70.9^{b}	71.4^{b}	69.4^{b}	0.0004	0.0062	0.83
Neutral	6.3^{b}	10.4^{a}	0.0029	7.8	10.1	8.8	7.5	8.8	NS	NS	0.49
Mixed	21.1	20.8	NS	14.1^{c}	16.7^{bc}	20.3^{ab}	21.1^{a}	21.9^{a}	<0.0001	NS	0.63
Proportion of goblet cells/crypt (% of total goblet cells/crypt)											
Acidic	68.1	65.5	NS	68.0	72.5	68.7	65.1	66.3	NS	NS	1.01
Neutral	5.3	7.1	NS	10.8^{a}	7.9^{ab}	6.9^{ab}	5.8^{b}	5.6^{b}	0.0028	NS	0.51
Mixed	26.6	27.4	NS	21.3^{b}	19.5^{b}	24.4^{ab}	29.1^{a}	28.0^{a}	<0.0001	NS	0.93

[1]Means of 4 replications of 2 birds per treatment at each time point (n=40 for treatment; n=8 for day).
[2]NS=P>0.05.
[3]Values are number of goblet cell/µm^2 x 10^3.
[a-d] Means within the same row and main effect with no common superscript differ significantly ($P \leq 0.05$).
SBM=Soybean meal; PM=Porcine meal; H=Hatch; P=Placement; SEM=Standard error of the mean.

GC in SBM. There was also a trend (P=0.0507) for higher numbers of total acidic neutral producing GC in the villi of SBM fed poults.

Age had an effect on total acidic and mixed mucin producing GC in the villi, total neutral and mixed mucin producing GC in the crypt, acidic and mixed mucin producing GC/μm of villus, acidic and mixed mucin producing GC/μm of crypt, and the proportion of neutral and mixed mucin producing GC in the crypt. Total acidic, neutral, and mixed mucin producing GC in the villi, as well as total acidic and mixed mucin producing GC in the crypt followed the same trend. There was an increase from hatch to placement, followed by a decrease to d 1, after which there was an increase with increasing age to d 7. Acidic, neutral, and mixed mucin producing GC/μm of villus increased with increasing age from hatch to d 7.

The interaction of diet and age was significant for total neutral mucin producing GC in the villi (Figure 4.4), total acidic mucin producing GC in the crypts (Figure 4.5), neutral mucin producing GC/μm of villus (Figure 4.6), and the proportion of acidic mucin producing GC in the villi (Figure 4.7). Total neutral mucin producing GC in the villi were similar for both treatments at all time points, except at d 7 when PM was higher than SBM. Total acidic mucin producing GC in the crypt was similar for SBM and PM at d 1, after which SBM had a drastic increase on d 3 while PM remained stable. By d 7, PM had increased to the same level as SBM. Neutral mucin producing GC/ μm of villus was increased in SBM at d 1 and similar for SBM and PM at d 5, after which PM had increased levels as compared to the SBM diet. The proportion of acidic mucin producing GC in the villi was similar for SBM and PM at d 1 and d 3, after which PM had lower acidic mucin producing GC than SBM.

An increase in the housekeeping gene (GAPDH) from placement to d 1 made it difficult to interpret the normalized gene expression values. For this reason, transcript abundance for

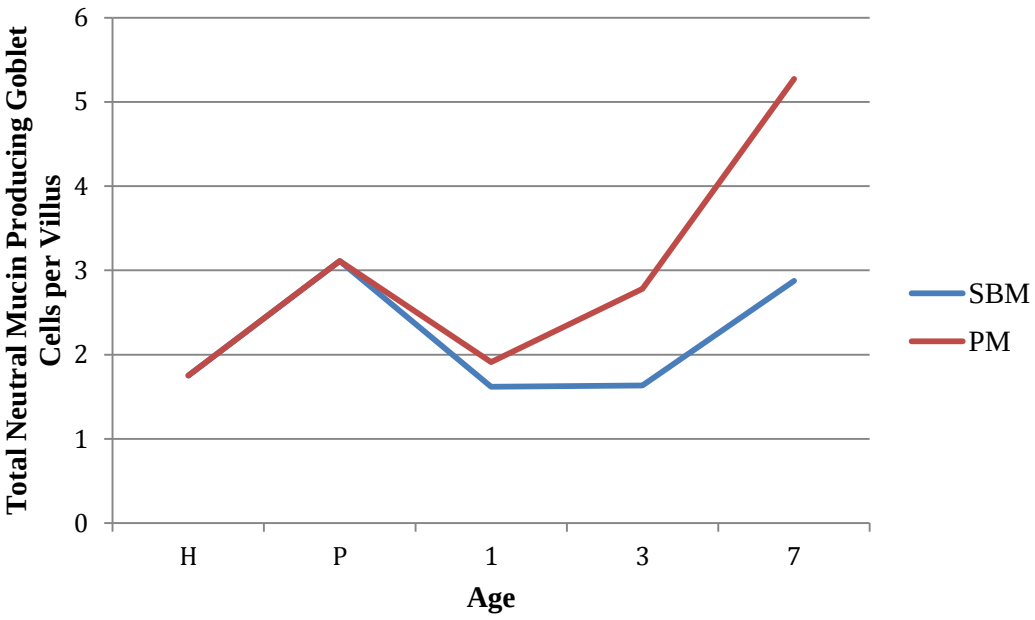

Figure 4.4 Effect of the interaction of treatment and age on total neutral mucin producing goblet cells per villus in turkey poults from hatch to 7 days of age. Means of 4 replications of 2 birds per time point.
SBM=soybean meal; PM=porcine meal; H=hatch; P=placement.

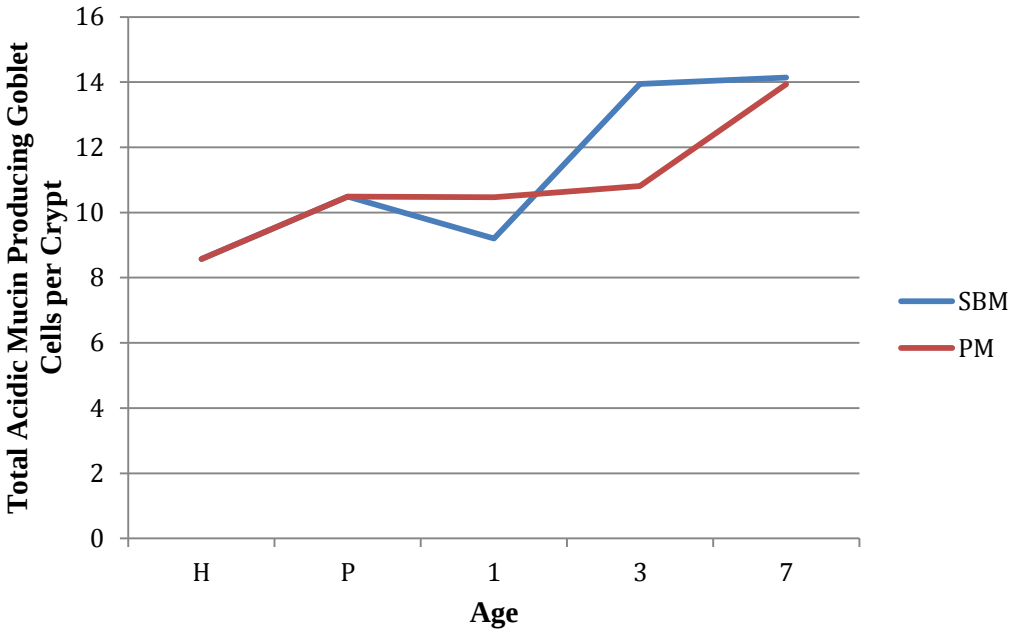

Figure 4.5 Effect of the interaction of treatment and age on total acidic mucin producing goblet cells per crypt in turkey poults from hatch to 7 days of age. Means of 4 replications of 2 birds per time point.
SBM=soybean meal; PM=porcine meal; H=hatch; P=placement.

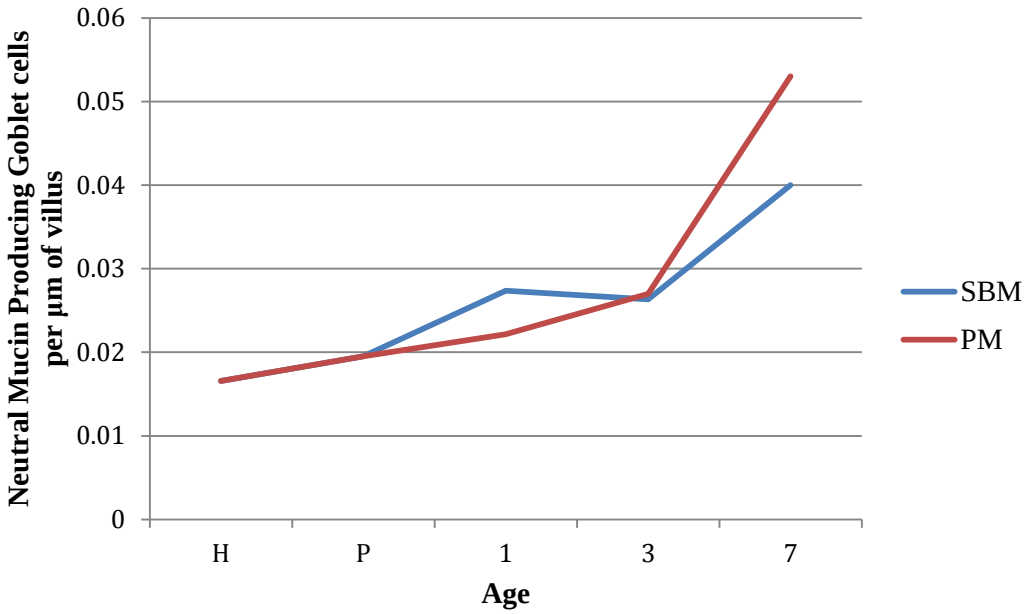

Figure 4.6 Effect of the interaction of treatment and age on neutral mucin producing goblet cells per µm of villus in turkey poults from hatch to 7 days of age.
Means of 4 replications of 2 birds per time point.
SBM=soybean meal; PM=porcine meal; H=hatch; P=placement.

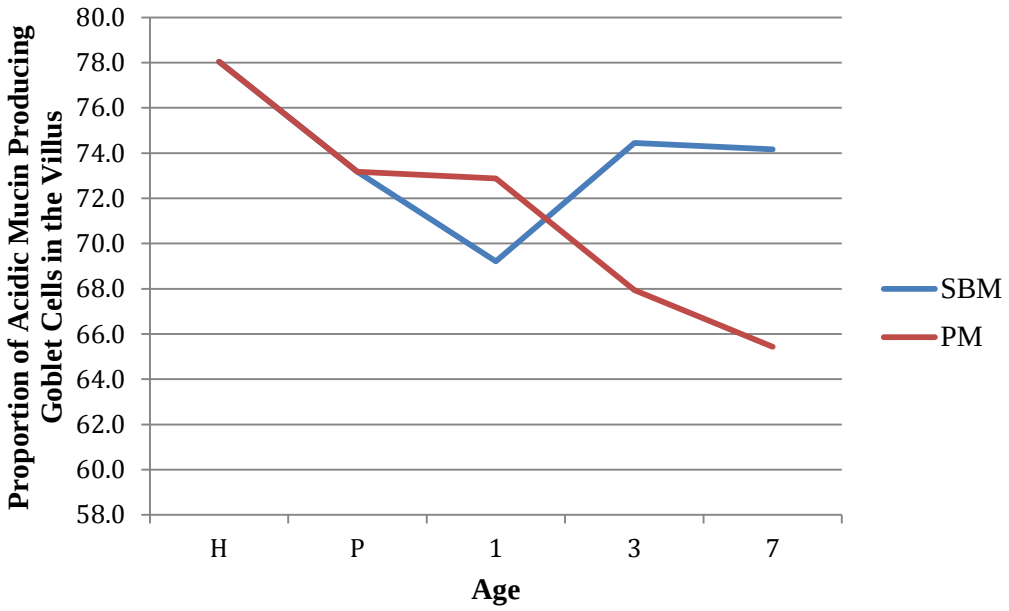

Figure 4.7 Effect of the interaction of treatment and age on proportion of acidic mucin producing goblet cells in the villus in turkey poults from hatch to 7 days of age. Means of 4 replications of 2 birds per time point.
SBM=soybean meal; PM=porcine meal; H=hatch; P=placement.

genes of interest were normalized to transcript abundance for GAPDH and mg of RNA used to prepare cDNA. The normalized data have been further split and analyzed as two blocks of time, before feed access and after feed access (Table 4.9 and 4.10).

Age did not have an effect on GAPDH-normalized gene expression between hatch and placement (Table 4.9). Dietary treatment did not have an effect on GADPH-normalized gene expression of any genes, however, there was a trend (P=0.0573) for PM to have increased expression of claudin 10 (Table 4.10). Age only affected normalized gene expression for IL 6, IL 8, and Muc2. Expression of IL 6 was low on d 1, and higher and equal on d 3 and 7. Expression of IL 8 was similar from d 1 to d 3, after which there was a decrease in GAPDH-normalized expression. Expression of Muc2 was lower and not different on d 1 and 3, and both were lower than on d 7. There was a trend (P=0.0858) for age to have an effect on claudin 1 expression, with d 3 values being higher than d 1 and 7. There were no interactions identified for GAPDH-normalized gene expression.

There was no effect of diet on transcript abundance when normalized per mg of RNA (Table 4.11). There was a trend (P=0.0526) for SBM fed poults to have higher expression of IL 1 than the PM fed poults. Gene expression per mg of RNA was significantly affected by age for all genes of interest, except for IL 8. Expression of all genes except IL 1, 6 and 8 increased from placement to d 1. A similar trend was seen for IL 1, but the difference was not significant. Claudin 1 expression remained stable from hatch to placement, followed by an increase, peaking at d 3, and then decreasing to an intermediate level at d 7. Claudin 5 expression peaked at d 1, after which the expression decreased to an intermediate level. Claudin 10, IL 1, IL 6, and Muc2 expression remained stable from hatch to placement, after which there was an increase in expression with increasing age. Differences in IL 8 expression approached significance (P =

Table 4.9 Effect of age on GAPDH-normalized[1] gene expression in the ileum of turkeys at hatch and placement[2].

Gene	Hatch	Placement	*P*-Value	SEM
Claudin 1	1.17	1.20	NS[3]	0.253
Claudin 5	51.40	5.87	NS	12.520
Claudin 10	1.42	0.52	NS	0.251
IL 1	1.76	2.15	NS	0.572
IL 6[4]	0.04	0.01	NS	0.009
IL 8	20.92	0.67	NS	9.429
Muc2	0.78	1.38	NS	0.186

[1]Normalized by dividing transcript abundance for gene of interest by transcript abundance for GAPDH.
[2]Means of 8 birds per time point.
[3]NS=$P>0.05$.
[4]IL 6 shown as expression x 10^3.

Table 4.10 Effect of diet and age on GAPDH-normalized[1] gene expression in the ileum of turkeys from day 1 – 7[2].

	Treatment			Day				Interaction	Pooled
Parameter	SBM	PM	*P*-Value	1	3	7	*P*-Value	*P*-Value	SEM
Claudin 1	1.10	0.92	NS[3]	0.74	1.81	0.49	NS	NS	0.200
Claudin 5	0.58	0.93	NS	0.86	0.80	0.59	NS	NS	3.448
Claudin 10	0.44	0.74	NS	0.42	0.56	0.79	NS	NS	0.089
IL 1	0.99	0.99	NS	0.54	1.16	1.27	NS	NS	0.187
IL 6	0.43	0.85	NS	0.0001[b]	0.95[a]	0.96[a]	0.05	NS	0.142
IL 8	0.51	0.39	NS	0.31[ab]	0.83[a]	0.21[b]	0.02	NS	2.400
Muc2	0.80	0.91	NS	0.65[b]	0.65[b]	1.27[a]	0.0001	NS	0.072

[1]Normalized by dividing transcript abundance for gene of interest by transcript abundance for GAPDH.
[2]Means of 4 replications of 2 birds per replicate (n=24 for treatment effect; n=8 for age effect).
[3]NS= $P>0.05$.
[a-b] Means within the same row with no common superscript differ significantly ($P\leq 0.05$).
SBM=Soybean meal; PM=Porcine meal; H=Hatch; P=Placement; SEM=Standard error of the mean.

Table 4.11 Effect of age on gene expression normalized per mg of RNA in the ileum of turkeys from day 1 – 7[1].

	Treatment			Day						Interaction	Pooled
Parameter	SBM	PM	*P*-Value	H	P	1	3	7	*P*-Value	*P*-Value	SEM
GAPDH	15125	13376	NS	1409[a]	1232[b]	24263[a]	23770[a]	20579[a]	<0.0001	NS	1433.9
Claudin 1	11577	8600	NS	1560[c]	1498[c]	17482[ab]	21258[a]	8646[bc]	<0.0001	NS	1398.4
Claudin 5	861	776	NS	5980[b]	6312[b]	20169[a]	10024[b]	10988[b]	<0.0001	NS	804.6
Claudin 10	6781	7275	NS	1941[c]	648[c]	10120[b]	7791[b]	14640[a]	<0.0001	NS	740.6
IL 1	13777	9054	0.0526	1376[d]	2680[cd]	12235[bc]	17301[ab]	23485[a]	<0.0001	NS	1509.7
IL 6	6413	5021	NS	0.06[c]	0.02[c]	1.3[c]	11254[b]	17331[a]	<0.0001	NS	989.8
IL 8	11047	9953	NS	1781	649	7182	58456	3633	0.0711	NS	7727.3
Muc2	11828	10079	NS	1104[c]	1598[c]	14929[b]	13293[b]	23846[a]	<0.0001	NS	1126.0

[1]Means of 4 replications of 2 birds per time point (n=40 for treatment effect; n=8 for age effect).
[2]NS= $P>0.05$; trends identified with $0.10>P>0.05$.
[a-d] Means within the same row with no common superscript differ significantly ($P\leq0.05$).
H=Hatch; P=Placement; SEM=Standard error of the mean.

0.0711) with values tending to decrease from hatch to placement, and then increase with a peak 3 d after poult access to feed. No interactions were found between diet and age.

4.5 Discussion

There were numerous treatment effects on tissue weights observed in this study, however, this research is not able to establish the mechanisms that caused the changes. The increased pancreas weight observed in CPC may be related to the increased water intake observed in the previous chapter. The duodenum is responsible for the secretion of cholecystokinin, which is released in response to partially digested fats and proteins, and the pancreas is responsible for the secretion of secretin and vasoactive intestinal peptide, which are released in response to acid in the duodenum and regulates the pancreatic juice secretion (Denbow, 2015). This response indicates that there is a component of CPC that was not analyzed that is either stimulating increased production of one or more of these hormones or stimulating the need for more bicarbonate release due to increased acid production. It is interesting to note that the small intestine was also the largest in the CPC fed birds. This may indicate a feedback mechanism is present that is increasing small intestine size to compensate for the factor causing increased water consumption. These observations do not appear to be due to amino acid digestibility, as they were determined in the previous chapter and used to formulate these experimental diets. Feeding CGM resulted in heavier heart, liver, and empty gizzard weights, when compared to values for poults fed SBM. There are no explainable reasons for these treatment effects.

The results of empty weights of the small intestine segments show a changing effect as the digesta moves from the beginning to the end of the small intestine. No effect of diet was observed on the empty weight of the duodenum, followed by a trend for the empty jejunum to be the largest for CPC and PM fed poults. Poults fed SBM and CPC had the largest empty ileum.

These treatments had the highest terminal ileum digesta moisture and the CPC treatment resulted in higher water intake than other diets. Possibly the ileum enlarges so as to aid in the removal of moisture from the digesta when there is more water than can be recovered in the caeca and colon of the bird. The increased weight of the empty ileum in SBM fed birds may also relate to the presence of undigested components, such as oligosaccharides, which are available for fermentation in the lower GIT (Choct et al., 1996).

The lack of significant contrasts for the majority of the tissues indicates that there is not a consistent effect of the nature of protein, either animal or vegetable, on these variables. There are many factors that could explain this effect, including variability within the categories of protein sources. The variation observed between the rendered products may also be due to the presence and variable nature of natural and bioactive compounds in these ingredients. For example, PM and FM may vary in polyamine content, which may be influenced by the source of the protein (for example, pork or fish), storage time, transportation, and processing and handling methods (Eliassen et al., 2002). This may indicate that while the PM had a level of polyamines that supported improved growth and development, FM may have had either too low or too high of a concentration of polyamines to see the same effect, as high levels can have a toxic effect (Smith et al., 2000). This effect may also simply be because there is no difference between adding animal or vegetable source proteins and any protein source addition to reduce the level of SBM will have a positive effect on performance.

Differences in the anatomy of the small intestine in response to feeding SBM and PM were restricted to changes in the nature of goblet cells. The increased neutral mucin producing GC observed in villi of poults fed PM, combined with the trend (P=0.0507) for increased acidic mucin producing GC in the SBM fed poults, may indicate an earlier maturing gut when PM is

fed. Acidic mucin producing GC are believed to protect against bacterial translocation and are, therefore, an important part of the innate defense barrier in an immature gut before the acquired immune system is functional (Deplancke and Gaskins, 2001). Previous research in chicks has indicated that the ratio of neutral to acidic mucin production increases following hatch (Uni et al., 2003). Acceleration in the shift in this proportion may indicate that some component of PM is decreasing the time required for the GIT to mature. This may be due to the hormone-like properties of the biogenic amines found in PM, as it has been observed that these compounds have a significant impact on GIT development particularly when intestinal integrity is compromised (Smith et al., 2000).

Treatment effects on blood metabolites were restricted to blood potassium and hemoglobin levels. The effect of higher blood potassium in SBM fed poults when compared to the FM and PM fed poults is not surprising, as SBM has a higher potassium content. The effect of treatment on hemoglobin levels (mmol/L) is likely of little importance, as this hemoglobin is calculated as hematocrit x 0.34, and the hematocrit level was not significant.

There were many effects of age on the measured data in this study and these data will add to the existing knowledge base of the effect of age on tissue weights, GIT development, and blood metabolic profiling. The tissue weights as a percentage of live weight increased with increasing poult age. The increase observed between hatch and placement appears to be due to the lower poult weight at placement. This demonstrates that dehydration is taking place when the poults do not have access to water or feed. Additionally, the combination of decreasing pH and bicarbonate observed in the blood metabolic profiling, along with increased sodium and chloride levels and reduced pH at placement also indicate that the poults were dehydrated and likely acidodic at the time of placement. This is due to the lack of access to feed and water that is

typical between hatch and placement in the poultry industry. This contributes to delayed GIT development and maturity, and may have a negative impact on growth (Vierira and Moran, 1999; Uni et al., 2003; Careghi et al., 2005; Potturri et al., 2005). These data highlight the importance of providing feed and water to the birds as soon as possible. The poults did recover well from the dehydration, as after 24 hours on feed, the blood metabolites stabilize and homeostasis was maintained for the rest of the sampling time points.

Gut architecture observations of villus width support previous literature, which found increasing width with increasing age in poults (Bohórquez et al., 2011) and chicks (Potturri et al., 2005; Sklan and Noy, 2003). Villus length, however, did not follow the same trend, as there was a significant decrease at d 1, which is in contrast to previous literature (Bohórquez et al., 2011; Sklan and Noy, 2003). Although not as dramatic as the current study, Potturri et al. (2005) and Applegate et al. (2005) also observed a slight decrease in villus height after 24 h on feed in poults. The decrease in villus height after 24 h on feed is not well understood, but may simply be due to the abrasion of feed as it moves through the fragile, rapidly developing digestive tract for the first time or a thinner mucus layer as the mucus layer was still developing at this point. Similarly, crypt depth increased significantly at d 3, which is in contrast to previous work in poults that observed consistent crypt depth during this time frame (Bohóquez et al., 2011; Potturri et al., 2005). Apart from the significant increase at d 3, the crypt depth remained constant. The consistent crypt depth agrees with results reported by Uni et al. (1999), where they did not observe an increase in crypt depth in the ileum of turkey poults until 6 d posthatch.

A high level of variability was observed between samples at hatch and placement in GAPDH-normalized gene expression data, making it difficult to note differences. This variability is possibly due to differences in breeder hen age, egg size, and hatch timing, as this

impacts the development of the poult (Applegate et al., 1999). It may also be due to the influence egg size and hatch timing have on the microbiota present within the GIT. Crhanova et al. (2011) investigated the microbiota present in natural colonization of the chicken GIT and observed more variability in young birds presumably due to variable initial colonization by bacteria present in the hatcher; variability in microbiota decreased with age. Due to the importance of bacteria on the development of the immune system, this could explain the variability observed in the genes of interest at hatch and placement.

The increase in expression per mg of RNA of all genes of interest, except IL 6 and 8, from placement to d 1 indicates a major development event at this time. Given the time between placement and d 1 coincides with the first introduction to feed, the expression profile could indicate the importance of feed, and possibly bacteria, as an activator of the immune system and barrier function. The increased expression of GAPDH per mg of RNA from placement to d 1 makes use of GADPH as a normalization gene for comparison of transcript abundance before and after d 1 impossible. Increased transcript abundance of GAPDH suggests that the developmental event between placement and d 1 may occur broadly across the genome, including shifts in energy metabolism. The observed increase of expression following feed access is consistent with increased entry of bacteria into the GIT, after which the expression begins to plateau as the bacterial colonization becomes more stable. Germfree animals also demonstrate the importance of bacteria for the creation of the mucus layer, as germfree rodents have fewer and smaller GC with a thin mucus layer (Szentkuti et al., 1990; Forder et al., 2007). There are few studies focused on turkey poults that have been conducted on the specific timing of when barrier function and the immune system become functional on a gene expression level, particularly focusing immediately after hatch. These data point to a lack of effect of the nature

of protein, either vegetable or animal, on the development of barrier function and immune function, as there was no treatment effects noted on gene expression, normalized or not.

4.6 Conclusions

The results of this study indicate that the most significant effect on GIT morphology, gene expression, and tissue growth is age. While there were slight effects observed between treatments, such as a potentially earlier GIT maturation when fed PM, the most notable changes were between placement and d 1. While age had a logical and predictable effect on development following feed access, the differences observed between placement and d 1 were much more exaggerated. This points to the importance of understanding how the post hatch environment, exposure to bacteria and the introduction of feed impact the development of the poult's digestive tract. It is clear that this is an important part of the initiation of the immune system and the maturation of the GIT to become effective in protecting the poult against pathogens.

5.0 OVERALL DISCUSSION

5.1 Reducing soybean meal to improve poult performance

High levels of soybean meal (SBM) in the diet had a negative impact on the performance of poults, however, this effect only lasted for the first two wks in the current research. It is unlikely that this response was due to the presence of phytates, as there was no difference between performance when animal or vegetable protein was added. It is also unlikely that the response was due to oligosaccharides, lectins, as energy is not typically the limiting factor in turkey prestarter diets, or typsin inhibitors, as there was no effect seen on the pancreas size. It is interesting that this effect only lasted for the first two wks, however, this could be due to having only four replications per treatment. An increased number of replications may have allowed for this effect to be established for the duration of the trial. The results of this part of the study indicate that it is important to restrict SBM in early turkey diets to avoid the negative impact on growth performance. This performance depression can be overcome regardless of what additional protein source is being added to dilute the SBM.

There was a lack of effect of adding animal derived protein to the diets, with the exception of water consumption and terminal ileum moisture at d 21. This response was not what was expected because animal derived protein has a reputation for improved growth and performance (Smith et al., 2000). The differences noted in the growth response between fish meal (FM) and porcine meal (PM) indicate that there is more to this response than just the fact that they are derived from animal protein. This difference in performance between FM and PM cannot be attributed to digestibility and energy levels, as this was accounted for in formulation. This difference could be due to another component of animal protein, such as biogenic amines. Previous research indicates that the level of biogenic amines can be affected by many variables,

including quality, storage, transportation, and processing (Eliassen et al., 2002). Previous research has also indicated that there is a fine line between when biogenic amines are beneficial and when they become toxic (Smith et al., 2000; Michiels et al., 2012). Further research on where that line is would be very beneficial. The increased performance observed in PM may have been due to a synergistic relationship of PM with SBM, however the protein levels and digestible AA levels were similar in the PM and FM diets.

5.2 Age-appropriate amino acid digestibility values

This study determined the effect of age on apparent ileal amino acid digestibility (AIAAD) at d 5 and 21. The results showed that there was an increase in AIAAD from d 5 to 21, however, the increase was different for different protein sources and different amino acids (AA). While the results for AIAAD at d 5 and 21 were determined, only the d 21 values were used for final formulation. This is because it was difficult to balance the diets when using the d 5 values. In addition, the AA requirements used to formulate diets are based on older bird digestibility measurements. Increasing the difficulty of using young bird values is the lack of knowledge of the physiological status of the poults used for testing. The hatch time, as well as time to placement and access to feed, all have an influence on gastrointestinal tract (GIT) development. Changes in GIT development, and hence digestibility, can markedly affect the results of digestibility experiments. Because of the rapid changes taking place in the poults in the days following hatch (Sulistiyanto et al. 1999; Bohórquez et al., 2011), it is important to understand exactly when the birds hatched. Variation in terms of hatch time and transport time to the farm will have an effect on how physiologically old the birds are when they arrive on farm. This variation makes it difficult to use these age specific digestibility values as any more than an indication of which ingredients will cause issues. The ingredients identified with the

biggest digestibility increase are the most difficult to use because that large increase is taking place very early in life, but when the levels are limited in finished feeds, these ingredients should not pose any significant problems. This approach gives nutritionists the ability to limit ingredients that may not be suitable for young birds, but not to exclude them. It is important from the perspective of a commercial feed nutritionist to find the balance between lower quality, cost-effective ingredients and inclusion levels that will not be harmful to the poult's early growth. Most AIAAD work is completed using birds with a mature GIT. Data collected from studies such as the current one gives nutritionists clues on the difficulties that may be encountered formulating with digestible AA for young birds, as well as giving them an understanding of why it is important to take digestibility values for young birds into account while formulating practical diets.

5.3 Gastrointestinal tract development and age

The GIT is a very important component of the bird, as it is vital for many processes, including digestion, absorption, barrier function, and immune status (Dibner et al., 1996). Access to feed had a dramatic effect on the poult's development, in terms of performance and GIT development, as indicated by gene expression and histology. This research illustrated that the tissue weights as a proportion of body weight all peak between 3 and 7 d of age. These results emphasize the needs to get birds on feed as quickly as possible, as feed appears to be a very important activator of growth, as well as the development of the immune system in the poult. The results indicate that the poults that hatched the earliest were 100 g lighter at 21 d than the poults that hatched the latest. This further demonstrates that getting birds on feed as early as possible, as well as tightening the hatch window, will have a positive impact on body weights. It is important to note that these results were collected in a well-controlled, clean environment and

may have been more exaggerated if there was a high pathogen load introduced to the eggs throughout incubation and hatching. More research is required to understand why there is such an effect of early hatch time on body weights.

The effect of feed on GIT development was demonstrated in the gene expression results, as the expression of all genes increased between placement and d 1, or the first 24 h on feed. These gene expression results are based on the ileum, however, the jejunum may have been a better and more important indicator as this is the major site of AA absorption. The decreased villus height observed after the birds were on feed for 24 h is an interesting result. This result may simply have been due to chance or abrasion as feed moves down the GIT for the first time, however, other researchers have seen a similar trend. This may indicate that there is something meaningful happening at this time point and further investigation is required. Using further histological measures to compare digestive tract growth, and potential deterioration due to lack of feed access, on birds based on hatch time would be an interesting next step. Further research to expand the scope of the histology and gene expression results to study all regions of the small intestine, as well as to further investigate the effect of ingredients on GIT maturity would provide useful information for the feeding and management of turkey poults. Additionally, further investigation into bacterial translocation prior to feed access and its effect on the development of the GIT and immune system would be valuable. This is important because of the implications it has on the poult development, as poults are very vulnerable after hatch. They are highly susceptible to bacteria because their immune system is not well established and barrier function is not complete. This points to the importance of using strategies to minimize the risk of bacterial translocation including emphasizing sanitation at the breeder farm, during transport to the hatchery, in the hatcher, and during transport to the farm where feed access is provided. It

also indicates the importance of a short, well-timed hatch window to ensure that the poults are not spending any unnecessary time in the hatcher, where they are exposed to any bacteria present on the egg shells.

5.4 Blood metabolic profiling

There was a clear effect of treatment on the blood potassium level, with SBM fed poults having the highest blood potassium level. The results of the blood metabolic parameters contribute to existing data, which has clinical applications. These data may be useful for assessing nutrition and husbandry programs, as well as giving insight in a disease situation. Further research is required to summarize the existing data and create standards for normal ranges for each parameter in different species.

5.5 Lessons learned

This research included an additional experiment that has not been included in this thesis. This trial was based on feeding semi-purified diets to determine AIAAD in turkey poults and was based on research completed by Adedokun et al. (2007, 2008). The diets were formulated so that 100 percent of the protein supplied in the diet was being provided by the test ingredient. Standardization values for this trial were to be derived from Adedokun et al. (2008). Once the poults were in the barn and on test feed, it became evident that the poults would not eat this feed. Poults on some treatments lost weight when on the treatment diets. In addition to the lack of feed consumption, there were issues with heating in the barn and temperatures dropped well below the set-points on two different occasions. While the trial was completed, the ileal contents collected were not enough to perform lab analysis on. In early life in particular, these birds are growing and developing physiological systems. By not giving these poults the ability to eat balanced protein, there is an evident impact on their growth and development. This brings into

question the validity of experiments done when the birds are not in a normal physiological state because they are being provided deficient diets.

5.5 Conclusions

Overall, the current study shows there are benefits to limiting the amount of SBM being offered in early turkey feeds, as a positive growth response was observed. The feed samples used for this research were representative of the Western Canadian feed industry, therefore the AMEn and AIAAD values contribute to the data available for commercial poultry nutritionists, with the exception of CPC, as it is not a commercially available feed ingredient at this time. The effect of diet on GIT development and maturation was difficult to assess, however, the results indicate that there may be an effect on the maturation time of the GIT, as increased neutral goblet cells were observed in the PM fed poults. Further research is required to understand more specific effects of diet on GIT development, as well as to study the effect of disease introduction on both performance and GIT development could provide more insight into the current research.

6.0 REFERENCES

Aburto, A., M. Vazquez, and N.M. Dale. 1998. Strategies for utilizing over processed soybean meal: I. Amino acid supplementation, choline content, and metabolizable energy. J. Appl. Poult. Res. 7:189-195.

Adedokun, S.A., O. Adeola, C.M. Parson, M.S. Lilburn, and T.J. Applegate. 2008. Standardized ileal amino acid digestibility of plant feedstuffs in broiler chickens and turkey poults using a nitrogen-free or casein diet. Poult. Sci. 87:2535-2548.

Adedokun, S.A., C.M. Parsons, M.S. Lilburn, O. Adeola, and T.J. Applegate. 2007. Standardized ileal amino acid digestibility of meat and bone meal from different sources in broiler chicks and turkey poults with a nitrogen-free or casein diet. Poult. Sci. 86:2598-2607.

Ahmad, T., T. Mushtaq, M.A. Khan, M.E. Babar, M. Yousaf, Z.U. Hasan, and Z. Kamran. 2009. Influence of varying dietary electrolyte balance on broiler performance under tropical summer conditions. J. Anim. Physio. An. N. 93:613-621.

Amasheh, S., M. Fromm, and D. Gunzel. 2011. Claudins of intestine and nephron – a correlation of molecular tight junction structure and barrier function. Acta. Physiol. 201:133-140.

AOAC. 2006. Official Methods of Analysis. 18th ed. Assoc. Off. Anal. Chem., Gaithersburg, MD.

Applegate, T.J., J.J. Dibner, M.L., Kitchell, Z. Uni, and M.S. Lilburn. 1999. Effect of turkey (*Meleagridis gallopavo*) breeder hen age and egg size on poult development. 2. Intestinal villus growth, enterocyte migration and proliferation of the turkey poult. Comp. Biochem. Physiol. B. Biochem. Mol. Biol. 124:381-389.

Applegate, T.J., D.M. Karcher, and M.S. Lilburn. 2005. Comparative development of the small intestine in the turkey poult and Pekin duckling. Poult. Sci. 84:426-431.

Aviagen. 2014. Ross 308 Broiler: Performance Objectives. Accessed July 2015. http://en.aviagen.com/assets/Tech_Center/Ross_Broiler/Ross-308-Broiler-PO-2014-EN.pdf.

Azzam, M.M.M., X.T. Zou, X.Y. Dong, and P. Xie. 2011. Effect of supplemental L-threonine on mucin 2 gene expression and intestine mucosal immune and digestive enzymes activities of laying hens in environments with high temperature and humidity. Poult. Sci. 90:2251-2256.

Batal, A.B. and C.M. Parsons. 2002. Effects of age on development of digestive organs and performance of chicks fed a corn-soybean meal versus a crystalline amino acid diet. Poult. Sci. 81:1338-1341.

Bohórquez, D.V., N.E. Bohórquez, and P.R. Ferket. 2011. Ultrastructural development of the small intestinal mucosa in the embryo and turkey poult: A light and electron microscopy study. Poult. Sci. 90:842-855.

Borchers, R., C.W. Ackerson, and F.E. Mussehl. 1948. Trypsin inhibitor VI. Effect of various heating periods on the growth promoting value of soybean oil meal for chicks. Poult. Sci. 27:601-604.

Borges, S.A., A.V. Fischer da Silva, J. Ariki, D.M. Hooge, and K.R. Cummings. 2003. Dietary electrolyte balance for broiler chickens exposed to thermoneutral or heat-stress environments. Poult. Sci. 82:42-435.

Canadian Council on Animal Care. 1993. Guide to the care and use of experimental animals. Vol 1. 2nd ed. Olfert, E.D., B.M. Cross, and A.A. McWilliam. Canadian Council on Animal Care. Ottawa, ON, Canada.

Careghi, C., K. Tona, O. Onagbesan, J. Buyse, E. Decuypere, and V. Bruggeman. 2005. The effects of the spread of hatch and interaction with delayed access after hatch on broiler performance until seven days of age. Poult. Sci. 84:1314-1320.

CFIA. 2014. Canadian guidelines for chemical contaminants and toxins in fish and fish products. Accessed November 2016. http://www.inspection.gc.ca/DAM/DAM-food-aliments/STAGING/text-texte/fish_man_standardsmethods_appendix3_1406403090196_eng.pdf.

Cheled-Shoval, S.L., E. Amit-Romach, M. Barbakov, and Z. Uni. 2011. The effect of in ovo administration of manna oligosaccharide on small intestine development during the pre- and posthatch periods in chickens. Poult. Sci. 90:2301-2310.

Chen, L., R.L. Madl, P.V. Vadlani, L. Li, and W. Wang. 2013. Value-added products from soybean: removal of anti-nutritional factors via bioprocessing. Pages 161-179 in Soybean – Bio-Active Compounds. H.A. El-Shemy, ed. InTech, Croatia.

Cho, M. 2011. The Impact of Diet Energy and Amino Acid Content on the Feed Intake and Performance of Broiler Chickens. Master's Thesis. University of Saskatchewan, Saskatoon, Canada.

Choct, M., R.J. Hughes, J. Wang, M.R. Bedford, A.J. Morgan, and G. Annison. 1996. Increased small intestinal fermentation is partly responsible for the anti-nutritive activity of non-starch polysaccharides in chickens. Br. Poult. Sci. 37:609-621.

Cowieson, A.J., T. Acamovic, and M.R. Bedford. 2004. The effects of phytase and phytic acid on the loss of endogenous amino acids and minerals from broiler chickens. Br. Poult. Sci. 45:101-108.

Cowieson, A.J., T. Acamovic, and M.R. Bedford. 2006. Phytic acid and phytase: Implications for protein utilization by poultry. Poult. Sci. 85:878-885.

Crhanova, M., H. Hradecka, M. Faldynova, M. Matulova, H. Havlickova, F. Sisak, and I. Rychlik. 2011. Immune response of chicken gut to natural colonization by gut microflora and to *Salmonella enterica* serovar enteritidis infection. Infect. Immun. 79:2755-2763.

Degussa. 2005. AminoDat 4.0. Evonik Degussa. Accessed June 2014. http://animal-nutrition.evonik.com/product/feed-additives/en/services/analytical-services/aminodat/Pages/default.aspx.

Denbow, D.M. 2015. Gastorintestinal Anatomy and Physiology. Pages 337-366 in Sturkie's Avian Physiology. 6th ed. C.G. Scanes, ed. Saint Louis, MO, USA.

Deplancke, B. and H.R. Gaskins. 2001. Microbial modulation of the innate defense: goblet cells and the intestinal mucus layer. Am. J. Clin. Nutr. 73:11315-11415.

Dibner, J.J., M.L. Kitchell, C.A. Atwell, and F.J. Ivey. 1996. The effect of dietary ingredients and age on the microscopic structure of the gastrointestinal tract in poultry. J. Appl. Poult. Res. 5:70-77.

Drew, M.D., A.E. Ogunkoya, D.M. Janz, and A.G. Van Kessel. 2007. Dietary influence of replacing fish meal and oil with canola protein concentrate and vegetable oils on growth performance, fatty acid composition, and organochlorine residues in rainbow trout (*Oncorhynchus mykiss*). Aquaculture 267:260-268.

Eliassen, K.A., R. Reistad, U. Risoen, and H.F. Ronning. 2002. Dietary polyamines. Food Chem. 78:273-280.

Emmans, G.C. 1995. Problems in modelling the growth of poultry. World Poultry Sci. J. 51:77-89.

Everaert, N., Q. Swennen, S.M. Coustard, H. Willemsen, C. Careghi, J. Buyse, V. Bruggeman, E. Decuypere, and S. Tesseraud. 2010. The effect of the protein level in a pre-starter diet on the post-hatch performance and activation of ribosomal protein S6 kinase in muscle of neonatal broilers. Br. J. Nutr. 103:206-211.

Fasina, Y.O., H.L. Classen, J.D. Garlich, H.E. Swaisgood, and D.A. Clare. 2003. Investigating the possibility of monitoring lectin levels in commercial soybean meals intended for poultry feeding using steam-heated soybean meal as a model. Poult. Sci. 82:648-656.

Feldman, G.J., J.M. Mullin, and M.P. Ryan. 2005. Occludin: structure, function and regulation. Adv. Drug Deliv. Rev. 57:883-917.

Feng, J., X.Liu, Z.R. Xu, Y.Z. Wang, and J.X. Liu. 2007. Effects of *Aspergillus oryzae 3.042* fermented soybean meal on growth performance and plasma biochemical parameters in broilers. Anim. Feed. Sci. Technol. 134:235-242.

Firman, J.D. 1994. Turkey growth modelling: metabolic approach. J. Appl. Poult. Res. 3:373-378.

Firman, J.D. and S.D. Boling. 1998. Lysine: Ideal protein in turkeys. Poult. Sci. 77:105-110.

Forbes, J.M. and F. Shariatmadari. 1994. Diet selection for protein by poultry. World Poultry Sci. J. 50:7-24.

Forder, R.E.A., G.S. Howarth, D.R. Tivey, and R.J. Hughes. 2007. Bacterial modulation of small intestinal goblet cells and mucin composition during early posthatch development of poultry. Poult. Sci. 86:2396-2403.

Francesh, M. and J. Brufau. 2004. Nutritional factors affecting excreta/litter moisture and quality. Worlds Poult. Sci. J. 60:64-75.

Garrett, W.S., J.I. Gordon, and L.H. Gilmcher. 2010. Homestatis and inflammation in the intestine. J. Cell. Sci. 140:859-870.

Geyra, A., Z. Uni, and D. Sklan. 2001. Enterocyte dynamics and mucosal development in the posthatch chick. Poult. Sci. 80:776-782.

Graham, K.K., M.S. Kerley, J.D. Firman, and G.L. Allee. 2002. The effect of enzyme treatment of soybean meal on oligosaccharide disappearance and chick growth performance. Poult. Sci. 81:1014-1019.

Halevy, O., A. Geyra, M. Barak, Z. Uni, and D. Sklan. 2000. Early posthatch starvation decreases satellite cell proliferation and skeletal muscle growth in chicks. J. Nutr. 130:858-864.

Halevy, O., Y. Nadel, M. Barak, I. Rozenbloim, and D. Sklan. 2003. Early posthatch feeding stimulates satellite cell proliferation and skeletal muscle growth in turkey poults. J. Nutr. 133:1107-1108.

Herkelman, K.L., G.L. Cromwell, A.H. Cantor, T.S. Stahly, and T.W. Pfeiffer. 1993. Effects of heat treatment on the nutritional value of conventional and low trypsin inhibitor soybeans for chicks. Poult. Sci. 72:1359-1369.

Hong, Y.H., H.S. Lillehoj, E.P. Lillehoj, and S.H. Lee. 2006. Changes in immune-related gene expression and intestinal lymphocyte subpopulations following *Eimieria maxima* infection of chickens. Vet. Immul. Immunop. 114:259-272.

Horn, N.L., S.S. Donkin, T.J. Applegate, and O. Adeola. 2009. Intestinal mucin dynamics: Response of broiler chicks and White Pekin ducklings to dietary threonine. 88:1906-1914.

Hossain, M.A., A.F. Islam, and P.A. Iji. 2013. Growth responses, excreta quality, nutrient digestibility, bone development and meat yield traits of broiler chickens fed vegetable or animal protein diets. S. Afr. J. Anim. Sci. 43:208-218.

Huang, K.H., V. Ravindra, X. Li, and W.L. Bryden. 2005. Influence of age on the apparent ileal amino acid digestibility of feed ingredients for broiler chickens. Brit. Poult. Sci. 46:236-245.

Hybrid. 2013. Commercial Nutrient Guidelines Calculator. Accessed July 2015. http://resources.hybridturkeys.com/nutrition/commercial-guidelines.

Hybrid. 2014. Performance Goals Converter Commercial Females. Accessed July 2015. http://www.hybridturkeys.com/~/media/8D8C5076C16B4D8B87B6303C3E21FC4C.pdf.

Kalač, P. and P. Krausová. 2005. A review of dietary polyamines: Formation, implications for growth and health and occurrence in foods. Food Chem. 90:219-230.

Karovicova, J. and Z. Kohajdova. 2003. Biogenic amines in food. Chem. Pap. 59:70-79.

Karr-Lilienthal, L.K., P.L. Utterback, C. Martinez Amerzcua, C.M. Parsons, N.R. Merchen, and G.C. Fahey, Jr. 2005. Relative bioavailability of phosphorus and true amino acid digestibility by poultry as affected by soybean extraction time and use of low-phytate soybeans. Poult. Sci. 84:1555-1561.

Khatri, M. and J.M. Sharma. 2005. Infectious bursal disease virus infection induces macrophage activation via p38 MAPK and NF-B pathways. Virus Res. 118:70-77.

Kim, S. and E. Mendis. 2006. Bioactive compounds from marine processing byproducts – a review. Food Res. Int. 39:383-393.

Kim, E.J., P.L. Utterback, and C.M. Parsons. 2012. Comparison of amino acid digestibility coefficients for corn, corn gluten meal, and corn distillers dried grains with solubles among 3 different bioassays. Poult. Sci. 91:3141-3147.

Kogut, M.H. 2010. Cytokines and prevention of infectious diseases in poultry: A review. Avian Pathol. 29:395-404.

Köse, S. P. Quantick, and G. Hall. 2003. Changes in the levels of histamine during processing and storage of fish meal. Anim. Feed Sci. Tech. 107:161-172.

Kumar, R. 1992.Anti-nutritional factors, the potential risks of toxicity and methods to alleviate them. Pages145-160 in Legume Trees and other Fodder Trees as Protein Sources for Livestock. FOA Animal Production and Health Paper.

Leeson S. and J.D. Summers, 2001. Scott's Nutrition of the Chicken. 4th rev. ed. University Books, Canada.

Leeson S. and J.D. Summers. 2005. Commercial Poultry Nutrition. 3rd rev. ed. University Books, Canada.

Liu, X., J. Feng, Z. Xu, Y. Lu, and Y. Liu. 2007. The effects of fermented soybean meal on growth performance and immune characteristics in weaned piglets. Turk. J. Vet. Anim. Sci. 31:341-345.

Lopez, G. and S. Leeson. 2008. Assessment of the nitrogen correction factor in evaluating metabolizable energy of corn and soybean meal in diets for broilers. Poult. Sci. 87:298-306.

Meat and Livestock Australia. 2001. Biogenic amines in meat meal. Accessed September 2016. http://www.meatupdate.csiro.au/infosheets/Biogenic%20Amines%20in%20Meat%20Meal.pdf.

Michiels, J., L. Maertens, J. Buyse, A. Lemme, M. Rademacher, N.A. Dierick, and S. De Smet. 2012. Supplementation of guanidinoacetic acid to broiler diets: Effects on performance, carcass characteristics, meat quality, and energy metabolism. Poult. Sci. 91:402-412.

Moore, D.T., P.R. Ferket, and P.E. Mozdziak. 2005. The effect of early nutrition on satellite cell dynamics in the young turkey. Poult. Sci. 84:748-756.

Muir, W.I., G.W. Lynch, P. Williamson, and A.J. Cowieson. 2013. The oral administration of meat and bone meal-derived protein fractions improved the performance of young broiler chicks. Anim. Prod. Sci. 53:369-377.

Murakami, A.E., E.A. Saleh, S.E. Watkins, and P.W. Waldroup. 2000. Sodium source and level in broiler diets with and without high levels of animal protein. J. Appl. Poult. Res. 9:53-61.

National Research Council. 1994. Nutrient Requirements of Poultry. 9th rev. ed. Natl. Acad. Press, Washington, DC.

Nejdfors, P., M. Ekelund, B. Jeppsson, and B.R. Weström. 2000. Mucosal in vitro permeability in the intestinal tract of the pig, the rat, and man: Species- and region-related differences. Scand. J. Gastroentero. 35:501-507.

Noy, Y., and D. Sklan. 1995. Digestion and absorption in the young chick. Poult. Sci. 74:366-373.

Noy, Y. and D. Sklan. 1997. Posthatch development in poultry. J. Appl. Poult. Res. 6:344-354.

Noy, Y. and D. Sklan. 1998. Yolk utilisation in the newly hatched poult. Br. Poult. Sci. 39:446-451.

Noy, Y. and D. Sklan. 2001. Yolk and exogenous feed utilization in the posthatch chick. Poult. Sci. 80:1490-1495.

Osho, S.O., T. Wang, N.L. Horn, and O. Adeola. 2016. Comparison of goblet cell staining methods in jejunal mucosa of turkey poults. Poult. Sci. 0:1-4.

Pallauf, J. and G. Rimbach. 1997. Nutritional significance of phytic acid and phytate. Arch. Tierernahr. 50:301-319.

Peter, C.M., Y. Han, S.D. Boling-Frankenbach, C.M. Parsons, and D.H. Baker. 2000. Limiting order of amino acids and the effects of phytase on protein quality in corn gluten meal fed to young chicks. J. Anim. Sci. 78:2150-2156.

Potturi, P.V.L., J.A. Patterson, and T.J. Applegate. 2005. Effects of delayed placement on intestinal characteristics in turkey poults. Poult Sci. 84:816-824.

Qaisrani, S.N., P.C.A. Moquet, M.M. van Krimpen, R.P. Kwakkel, M.W.A. Verstegen, and W.H. Hendriks. 2014. Protein source and dietary structure influence growth performance, gut morphology, and hindgut fermentation characteristics in broilers. Poult. Sci. 93:3053-3064.

Ravindran, V., M.R. Abdollahi, and S.M. Bootwalla. 2014. Nutrient analysis, metabolizable energy, and digestible amino acids of soybean meals of different origins for broilers. Poult. Sci. 93:2567-2577.

Ravindran, V. and P.C.H. Morel. 2006. Ileal amino acid digestibility of some novel dietary protein sources for growing chickens. J. Sci. Food Agric. 86:2603-2608.

Rothwell, L., J.R. Young, R. Zoorob, C.A. Whittaker, P. Hesketh, A. Archer, A.L. Smith, and P. Kaiser. 2004. Cloning and characterization of chicken IL-10 and its role in the immune response to *Eimeria maxima*. J. Immunol. 173:2675-2682.

Rynsburger, J.M. 2009. Physiological and Nutritional Factors Affecting Protein Digestion in Broiler Chickens. Master's Thesis. University of Saskatchewan, Saskatoon, Canada.

SAS Institute. 2011. SAS User's Guide. Version 9.3 ed. SAS Institute, Inc., Cary, NC.

Scott, T.A., F.G. Silversides, H.L. Classen, M.L. Swift, and M.R. Bedford, 1998. Comparison of sample source (excreta or ileal digesta) and age of broiler chick on measurement of apparent digestible energy of wheat and barley. Poult. Sci. 77:456-463.

Sell, J.L. 1996. Physiological limitations and potential for improvement in gastrointestinal tract function of poultry. J. Appl. Poult. Res. 5:96-101.

Selle, P.H., V. Ravindran, A. Caldwell, and W.L. Bryden. 2000. Phytate and phytase: consequences for protein utilization. Nutr. Res. Rev. 13:255-278.

Seo, H.W, D. Rengaraj, J.W. Choi, S.E. Ahn, Y.S. Song, G. Song, and J.Y. Han. 2010. Claudin 10 is a glandular epithelial marker in the chicken model as human epithelial ovarian cancer. Int. J. Gynecol. Cancer. 20:1465-1473.

Shao, Y., Y. Guo, and Z. Wang. 2013. β-1,3/1,6-glucan alleviated intestinal mucosal barrier impairment of broiler chickens challenged with *Salmonella enterica* serovar Typhimurium. Poult. Sci. 92:1764-1773.

Shukla, R. and M. Cheryan. 2001. Zein: the industrial protein from corn. Ind. Crop. Prod. 13:171-192.

Sklan, D. 2001. Development of the digestive tract of poultry. World Poultry Sci. J. 57:415-428.

Sklan, D. and Y. Noy. 2000. Hydrolysis and absorption in the small intestines of posthatch chicks. Poult. Sci. 79:1306-1310.

Sklan, D. and Y. Noy. 2003. Crude protein and essential amino acid requirements in chicks during the first wk posthatch. Br. Poult. Sci. 44:266-274.

Smith, T.K., J.L. Mogridge, and M.G. Sousadias. 1996. Growth-promoting potential and toxicity of spermidine, a polyamine and biogenic amine found in foods and feedstuffs. J. Agric. Food Chem. 44:518-521.

Smith, T.K., M. Tapia-Salazar, L. Cruz-Suarez, and D. Ricque-Marie. 2000. Feed-borne biogenic amines: natural toxicants or growth promoters? In Avances en Nutricion Acuicola V. Memorias del V Simposium Internacional de Nutricion Acuicola. L.E. Cruz-Suarez, D. Ricque-Marie, M. Tapia-Salazar, M.A. Olvera-Novao, and R. Civera-Cerecedo, eds. November 19 – 22, 2000. Merida, Yucatan, Mexico.

Smirnov, A., D. Sklan, and Z. Uni. 2004. Mucin dynamics in the chick small intestine are altered by starvation. J. Nutr. 134:736-742.

Smirnov, A., R. Perez, E. Amit-Romach, D. Sklan, and Z. Uni. 2005. Mucin Dynamics and Microbial Populations in Chicken Small Intestine Are Changed by Dietary Probiotic and Antibiotic Growth Promoter Supplementation. J. Nutr. 135:187-192.

Smirnov, A., E. Tako, P.R. Ferket, and Z. Uni. 2006. Mucin gene expression and mucin content in the chicken intestinal goblet cells are affected by in ovo feeding of carbohydrates. Poult. Sci. 88:669-673.

Stein, H.H., L.L. Berger, J.K. Drackley, G.C. Fahey Jr., D C. Hernot, and C M. Parsons. 2008. Nutritional properties and feeding values of soybeans and their co-products. Pages 613–660 in Soybeans, Chemistry, Production, Processing, and Utilization. L. A. Johnson, P. J. White, and R. Galloway, ed. AOCS Press, Urbana, IL.

Sulistiyanto, B., Y. Akiba, and K. Sato. 1999. Energy utilization of carbohydrate, fat and protein sources in newly hatched broiler chicks. Br. Poultry Sci. 40:653-659.

Szentkuti, L., H. Riedesel, M. Enss, K. Gaerner, and W. von Engelhardt. 1990. Pre-epithelial mucus layer in the colon of conventional and germ-free rats. Histochem. J. 22:491-497.

Ten Doeschate, R.A.H.M, C.W. Scheele, V.V.A.M. Schreurs, and J.D. Van Der Klis. 1993. Digestibility studies in broiler chickens: Influence of genotype, age, sex and method of determination. Br. Poult. Sci. 34:131-146.

Thacker, P.A. and D. Petri. 2011. Nutritional evaluation of canola protein concentrate for broiler chickens. Asian Australas. J. Anim. Sci. 24:1607-1614.

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

Thomas, D.V., V. Ravindran, and G. Ravindran. 2008. Nutrient digestibility and energy utilisation of diets based on wheat, sorghum or maize by the newly hatched broiler chick. Br. Poult. Sci. 49:429-435.

Tišljar, M., Ž. Grabarević, B. Artuković, Z. Šimec, P. Džaja, ð. Vranešic, A. Bauer, M. Tudja, V. Herak-Perković, P. Juntes, and M. Pogačnik. 2002. Gizzerosine-induced histopathological lesions in broiler chicks. Br. Poult. Sci. 43:86-93.

Tsukita, S., M. Furuse, and M. Itoh. 2001. Multifunctional strands in tight junctions. Nature Rev. Mol. Cell. Biol. 2:285-293.

Turkey Farmers of Canada. 2015. Canadian Turkey Stats 1974 – 2015. Accessed September 2016. https://www.turkeyfarmersofcanada.ca/industry-information/industry-statistics/.

Uni, Z. and R.P. Ferket. 2004. Methods for early nutrition and their potential. World Poultry Sci. J. 60:101-111.

Uni, Z., A. Geyra, H. Ben-Hur, and D. Sklan. 2000. Small intestinal development in the young chick: Crypt formation and enterocyte proliferation and migration. Br. Poult. Sci. 41:544-551.

Uni, Z., Y. Noy, and D. Sklan. 1995. Posthatch changes in morphology and function of the small intestines in heavy- and light-strain chicks. Poult. Sci. 74:1622-1629.

Uni, Z., Y. Noy, and D. Sklan. 1999. Posthatch development of small intestinal function in the poult. Poult. Sci. 78:215-222.

Uni, Z., A. Smirnov, and D. Sklan. 2003. Pre- and post-hatch development of goblet cells in the broiler small intestine: effect of delayed access to feed. Poult. Sci. 82:320-327

Van Itallie, C.M., J. Holmes, A. Bridges, J.L. Gookin, M.R. Coccaro, W. Proctor, O.R. Colegio, and J.M. Anderson. 2008. The density of small tight junction pores varies among cell types and is increased by expression of claudin-2. J. Cell. Sci. 121:298-305.

Vieira, S.L. and E.T. Moran Jr. 1999. Effects of delayed placement and used litter on broiler yields. J. Appl. Poult. Res. 8:75-81.

Vierira, S.L. and I.L. Lima. 2005. Live performance, water intake and excreta characteristics of broilers fed all vegetable diets based on corn and soybean meal. Int. J. Poult. Sci. 4:365-368.

Vogtmann, H., P. Friter, and A.L. Prabuck, 1975. A new method of determining metabolizability of energy and digestibility of fatty acids in broiler diets. Br. Poult. Sci. 67:641-646.

Youssef, I.M.I., A. Beineke, K. Rohn, and J. Kamphues. 2011. Effects of litter quality (moisture, ammonia, uric acid) on development and severity of foot pad dermatitis in Growing Turkeys. Avian Dis. 55:51-58.

Zhang, B. and Y. Guo. 2009. Supplemental zinc reduced intestinal permeability by enhancing occluding and zonula occludens protein-1 (ZO-1) expression in weaning piglets. Br. J. Nutr. 102:687-693.