NUTRITIONAL EVALUATION OF NEW REDUCED-OLIGOSACCHARIDE SOYBEAN MEAL AND CANOLA MEALS PRODUCED FROM NEW VARIETIES OF CANOLA SEEDS FOR POULTRY

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Abstract

Reduced-Oligosaccharide Soybean Meal

There is currently much interest in developing new genetically modified soybean meal (SBM) that contains reduced oligosaccharide carbohydrates to improve its digestibility in poultry. The nutritional value of a new reduced-oligosaccharide soybean meal (SBM-RO) and conventional SBM (SBM-CV) were evaluated and compared in four experiments. The first experiment was a true metabolizable energy (TME_n) assay with conventional roosters. The second experiment was a precision-fed cecectomized rooster assay that was conducted to determine TME_n and amino acid digestibility. The third experiment was a standardized ileal amino acid digestibility (SIAAD) assay, in which broiler chicks were fed semi-purified diets containing 20% protein (from only the test ingredient) for 17–21 d of age and ileal digesta were collected on Day 21. The fourth experiment was a growth performance trial (8 to 21 d of age) where broiler chicks were fed corn-SBM diets (3.068 ME_n/g DM, adequate in all AA) containing 38.84% SBM-RO or SBM-CV. The protein content (100% DM basis) of the SBM-CV and SBM-RO was 51.85% and 54.75%, respectively. The gross energy of the two SBM was similar. The TME_n values in both conventional roosters and cecectomized roosters were significantly higher ($P < 0.05$) for SBM-RO than for SBM-CV (difference was approximately 200 kcal/kg DM). Amino acid digestibility in cecectomized roosters was not different between SBM-CV and SBM-RO, with the exception of Trp, Ala, Asp and Cys (SBM-RO > SBM-CV, $P < 0.05$). No significant differences between the SBMs were found for AA digestibility in the SIAAD assay. In the growth performance trial (Experiment 4), the corn-SBM diet containing SBM-RO yielded significantly higher feed efficiency than the diet

containing SBM-CV ($P < 0.0001$). The results indicated that the SBM-RO contains higher ME than the SBM-CV and that digestibility of AA in SBM-RO is similar or slightly higher than SBM-CV.

Canola meals produced from new varieties of canola seeds

The development of the double low (low erucic acid and low glucosinolates) cultivars of canola has resulted in increased usages of canola meal (CM) in poultry diets. If the CM had more digestible energy, more protein and less glucosinolates, it would likely be more competitive in the world market. This current study evaluated the nutritional value of 7 new genetically modified CM (Test CM), and compared them with conventional CM samples (Conv CM) and soybean meals (SBM). Three experiments were conducted. The first included Conv CM1, Test CM1 (laboratory-processed) and SBM1. The second experiment included Test CM 2 to 6 (laboratory-processed), Conv CM2 and SBM2. The third experiment included Conv CM3-HT, Conv CM4-LT and Test CM7, which were commercially-processed. For each experiment, a precision-fed rooster assay with conventional or cecectomized roosters was conducted to determine TME_n or amino acid (AA) digestibility. Analyzed nutritional composition of the CM samples indicated increases in CP and AA for all Test CMs (49.41 to 50.58% on a dry matter basis) compared to Conv CMs (40.73 to 43.01%). All the Test CMs also contained lower amounts of neutral detergent fiber and acid detergent fiber. When the TME_n values of Test CMs were compared to conventional CM, 6 of the 7 Test CMs had significantly higher values than the conventional CM samples ($P < 0.05$), but all were lower than SBMs ($P < 0.05$). For AA digestibility, the Test CMs had higher digestibility

coefficients than Conv CM in Experiment 1 and 2 ($P < 0.05$), and higher concentrations of digestible AA in all 3 experiments. The results of this study indicated that nutritional value of the genetically modified CMs was greater than Conv CM for poultry.

To my father, mother, and my dear fiancé

Thank you for always believing in me and for being there for me anytime.

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Chapter 1

Introduction and literature review

Part 1. Soybean Meal

Introduction

In today's livestock industry, it is important to determine the diet that will minimize feed costs yet optimize the efficiency of animal production. In the poultry industry, producers largely rely on soybean meal (SBM) as a quality protein source. Conventional SBM (44% crude protein) and the de-hulled SBM (47-48% crude protein) are the most commonly used SBMs. However, because of the low digestible oligosaccharides and non-starch polysaccharides in the soybean meal, it provides a lower metabolizable energy (ME) value than the prediction from its gross energy (GE) in poultry. Several attempts have been made to improve the nutritional value of SBM, including ethanol extraction, adding exogenous enzymes, and developing genetically modified SBM lines. Among the latter, genetic improvements in reduction or elimination of oligosaccharide carbohydrates are the most direct way and the most important economical traits. Since the nutritional characteristics of these new SBMs vary from company to company depending on the genetic line, geographic location, and specific techniques used to reduce oligosaccharides, it is necessary to determine the nutritional values of each individual SBM. This information will be important in the future for poultry producers to find the best source of SBM for poultry diets.

Literature Review

Solvent-extracted SBM, which is produced when the defatted soybean flakes are ground, is a valuable feed ingredient for livestock (Baker et al., 2011). It has been the dominant protein source in U.S. livestock and poultry rations since the 1950s (Smith, 1997), constituting more than two-thirds of the protein feeds given to livestock (USITC, 2000). Approximately one-third of the soybeans produced in the U.S. are exported as SBM or soybean oil (Cromwell, 2000). In poultry diets, SBM is extensively used; of the total SBM sold in the U. S., more than 50% is used by the poultry industry. Soybean meal dominates the market because of its high protein and energy content, its excellent amino acid quality and composition, as well as its high availability of amino acids (Stein et al., 2008). The standard de-hulled SBM contains 90% dry matter, 48.5% protein, 1.0% fat, and 3.9% crude fiber (NRC, 1994); whereas in comparison, canola meal only has an average of 38% protein and 11% fiber, and cottonseed meal has 41% of protein and greater than 12% fiber, for example. Broiler chicks require a great amount of protein and amino acids, thus a protein source high in amino acids is in demand (Waldroup, 2002). Soybean meal has high protein quality, and is an especially good source of lysine and tryptophan. Baker (2000) reported that the digestible lysine concentration in SBM exceeds the required amount of lysine for chicks. Although methionine is the first limiting amino acid in SBM, there are methionine supplements produced by chemical synthesis readily available in the market, making the formulation of diets based on corn-SBM with methionine supplementation very simple and economical (Waldroup, 2002). While poultry are

unable to synthesize arginine, SBM is a good source of arginine (2.97% digestible amino acid) and SBM can provide enough arginine to meet the chicks' requirement. Also, due to its low fiber content, SBM is able to provide a metabolizable energy (ME) level of 2,711 kcal/kg on a dry matter basis for poultry, which is 11 to 25% greater than that of other oilseed meals (Stein et al., 2008). At the same time, SBM contains considerable amounts of potassium, magnesium, copper, iron, and most of the water soluble B vitamins. Therefore, compared with other commonly used oilseed meals, SBM has higher protein and energy concentration, and lower fiber content, which allows for the formulation of a higher energy diet with a better feed to gain ratio (Smith, 1997). With increased knowledge about soybean meal and its efficacy as a feed ingredient, use of SBM will continue to increase in all sectors of animal agriculture (Bruce et al., 2006).

Soybean meal is a good energy source for poultry, yet it has a lower metabolizable energy (ME) value than the prediction from its gross energy (GE) based on proximate analysis for poultry (McGinnis, 1983). As calculated by Dale (2000), SBM contains approximately 10% more GE than corn; however, it contains only about 72% of the ME of corn. This is due to the low digestibility of the carbohydrates in SBM, which can potentially lead to a large energy loss and also a possible dilution of energy and other nutrients in the diet (Stein et al., 2008).

Soybean meal contains 25-32% carbohydrates. Much of this fraction is present as non-starch polysaccharides and oligosaccharides, primarily sucrose, raffinose and stachyose (Waldroup, 2002). These oligosaccharides account for approximately 11.28%

of the dry matter of SBM (Coon et al., 1988). Kawamura and Tada (1967) found that the oligosaccharide content varies with the soybean variety; the average content of the commonly used six varieties was 6.2% sucrose, 1.4% raffinose, and 5.2% stachyose on a dry matter basis. The alpha-1, 6 linkages of these oligosaccharides cannot be broken down by endogenous enzymes in the small intestine of poultry due to the absence of alpha-1, 6-galactosidase (Gitzelmann and Auricchio, 1965). Therefore, these oligosaccharides cannot be absorbed. It has also been shown that a high level of raffinose (> 6.7%) may decrease the hydrogen production in rats, resulting in osmotic catharsis; this can cause evacuation of part of the raffinose before its fermentation and microbial hydrolysis are completed, and thus decrease its digestibility (Wagner et al., 1976). Wiggins (1984) noted that when the presence of these oligosaccharides is high in the digestive tract, they may cause fluid retention and increase the digesta flow rate, leading to an adverse effect on the absorption and utilization of nutrients. The nitrogen-corrected true metabolizable energy (TME_n) of soy protein concentrate was significantly decreased by stachyose and raffinose inclusion in a dose dependent manner (Leske et al., 1993a). Leske et al. (1991) also suggested that the raffinose family of oligosaccharides can cause shortened transit time, which leads to a reduced fiber fermentation and thus a lower than expected TME_n value. Moreover, Waldroup (2002) pointed out that the excreted carbohydrates from broilers fed SBM are very sticky, which created wet litter that may adhere to poultry feet and potentially lead to feet and leg disorders.

Removal of oligosaccharides from SBM may increase fiber fermentation because of a slower transit time, and create a better cecal environment for the microbial hydrolyzation of carbohydrates, and thus improve the nutritional value of SBM for poultry (Coon et al., 1988). One approach to remove the sugars is through ethanol extraction. Coon et al. (1988) used an 80% ethanol extraction to reduce the oligosaccharide content from a 44% crude protein SBM. Conventional SBM and ethanol extracted SBM (EE-SBM) were precision-fed to Leghorn roosters. The result showed an increased TME_n in EE-SBM. In 1990, Coon et al. conducted a double 80% ethanol extraction of SBM. This extraction followed by a 30-min water wash, removed 97.5% of the total water-soluble carbohydrates in 44% CP SBM, reducing the sucrose, raffinose, and stachyose content to 0.13, 0.08, and 0.03% on a dry matter basis, respectively. When the EE-SBM was fed to Leghorn roosters, it had an increased TMEn value of 574 kcal/kg, along with an increased fiber fermentation, lengthened transit time, and increased cecal pH compared with conventional SBM (Coon et al., 1990). Leske et al, (1991, 1993b) also reported an improvement in non-starch polysaccharide fermentation and TME_n from the EE-SBM. Later, Leske and Coon (1999) determined digestibility in 21 adult Leghorn roosters fed ethanol-extracted SBM and the commercially used 47% CP SBM. Results indicated that the ethanol extraction procedure increased the dry matter digestibility of SBM by 11.2%, and increased the average amino acid digestibility from 88% to 91.6%. Also, the TMEn value of the control SBM was significantly increased by the extraction procedures ($P < 0.05$).

However, the nutritional value of EE-SBM for poultry remains controversial. In the experiments from Irish et al. (1995), when White Leghorn roosters were fed semi-purified diets containing SBM or ethanol-extracted SBM, no improvement in TME_n was observed. Similarly, when the two SBMs were fed to broiler chicks, there were no significant differences in weight gain, feed efficiency, or apparent protein digestibility. In another experiment, when compared to control SBM, a decrease in broiler performance and protein digestibility was observed when feeding EE-SBM in which 88% of the alpha-galactosides had been removed; compared with broilers fed 48% CP conventional SBM (Angel, 1988). These contradictory results may be related to specific experimental techniques, particularly the method of reducing the alpha-galactosides concentration. Although ethanol extraction resulted in better non-starch polysaccharide digestibility and TME_n of SBM (Coon et al., 1990; and Leske et al., 1991; 1993b), different results were reported from other studies. It is also possible that the ethanol extraction procedure may cause a simultaneous extraction of other nutrient components that can affect absorption and digestion of the feed.

Another attempt to minimize the adverse effects of SBM oligosaccharides is to add exogenous alpha-1, 6-galactosidase to the diet. Slominski et al. (1992) and Slominski (1994) found that when alpha-galactosidase was used together with invertase, it was much more effective in hydrolyzing raffinose and stachyose both *in vitro* and *in vivo* than alpha-galactosidase alone. In the *in vivo* study, cecectomized hens were fed a diet with the alpha-1, 6-galactosidase plus invertase supplementation. The supplementation hydrolyzed 88% of the alpha-galactosides of sucrose in the

gastrointestinal tract, which was significantly higher compared with only 35% hydrolysis from alpha-galactosidase alone. However, even though the alpha-1, 6 linkages were mostly removed by exogenous enzymes, no effect was found on non-starch polysaccharide fermentation in laying hens. Similarly, Veldman et al. (1993) found that adding the residue after evaporation of an 80% ethanol-extracted SBM significantly decreased the ileal digestibility of nutrients when fed to piglets, and the addition of alpha-galactosidase did not overcome the adverse effect. In addition, Angel et al. (1988) reported that the use of exogenous soybean alpha-galactosidase failed to produce any beneficial effects on the nutritional value of the SBM.

As previous research has shown that addition of exogenous enzymes is not effective, and using ethanol extraction does not provide a constant beneficial result, it is important to find another approach that is both consistently effective and economical to improve the nutritional value of soybean meal for poultry. During the last 15 to 20 years, considerable interest and activity arose in developing new genetically modified or genetically enhanced crops and feed ingredients that have increased nutritional value. Many companies have successfully developed genetic lines of soybeans that have greatly reduced oligosaccharide levels as well as an increased protein concentration compared with those in conventional soybeans (Parsons et al., 2000).

The nutritional value of low-oligosaccharide SBMs (LO-SBMs) varies from company to company depending on the genetic line, geographic location, specific

techniques used to reduce oligosaccharides, etc. One source of LO-SBM was reported to contain 7 to 9% more ME when fed to roosters (Parsons et al., 2000). In another source of LO-SBM, the concentrations of stachyose and raffinose were significantly reduced; however, when fed to growing pigs, the digestibility of amino acids in LO-SBM was not different from that in conventional SBM (Baker and Stein, 2009). Though there was no significant positive result from the LO-SBM, the results did indicate that there were no detrimental effects to amino acid digestibility of reducing the oligosaccharides (Baker and Stein, 2009). In more recent research (Baker et al., 2011), a precision-fed rooster assay was conducted to compare a LO-SBM (53.6% crude protein), a control SBM (47.5% CP) and a high-protein SBM (HP-SBM) (54.9%). The results failed to show any difference in the standardized digestibility of amino acids among the 3 sources of SBM, and the concentration of TME_n in the LO-SBM (2984 kcal/kg of DM) was not significantly different from the control SBM (2963 kcal/kg). This is inconsistent with the previous study, where the concentration of TME_n was greater in LO-SBM than in conventional SBM (Parsons et al., 2000). When evaluating growth performance using broiler chicks in the latter study, 3 diets that contained the same level of TMEn and CP were formulated from the same 3 sources of SBM, but the inclusion of LO-SBM and HP-SBM were only 32.60% and 31.21% compared to 38.21% of conventional SBM; this was due to the higher concentration of digestible amino acid in the two new SBM sources. There were no differences in body weight gain or feed efficiency among chicks fed the 3 SBM sources (Baker and Stein, 2009). Therefore, it was concluded that compared with

conventional SBM, HP-SBM and LO-SBM have a greater nutritional value in broiler chick diets, mainly due to their higher CP and digestible amino acid content. Further research will be conducted in this thesis to evaluate the nutritional value of SBM from a new reduced-oligosaccharide soybean variety developed at Purdue University.

Part 2. Canola Meal

Introduction

Canola meal is a commonly used vegetable protein source in poultry diets. As the prices of SBM in the U.S. have increased in recent years, canola meal is often an economically viable alternative to replace some of the SBM in poultry diets.

Historical use of conventional rapeseed meal has been limited in poultry diets due to the presence of anti-nutritional factors in rapeseed meal, primarily glucosinolates, which can lead to a reduced feed intake, a reduced growth rate, liver damage or other diseases in poultry. Therefore, new varieties of canola that are low in anti-nutritional factors have been developed. Another factor impacting the use of canola meal is the higher fiber concentration and lower ME_n in canola meal compared with SBM. It is important to test the nutritional value of new canola meals that are developed by genetic modification and to determine the maximum inclusion rate that can be used in broiler chicken diets without reducing performance.

Literature Review

Rapeseed is the fifth most important oilseed of commerce (Fenwick and Curtis, 1980). Rapeseed meal has been used as a protein source in poultry diets since the 1930's. There are different cultivars of rapeseed, such as the low erucic acid cultivar and the "double low" cultivar (low in erucic acid and glucosinolates). The name "canola" was adopted referring to all "double low" cultivars in North America. Canola meal is the product of crushing and oil extraction of canola seed (Bell, 1984).

The canola seed contains about 40%-50% oil by weight and yields a protein supplement containing about 38% protein on average (Fenwick and Curtis, 1980); therefore, it is a valuable source of energy and protein in poultry diets (Leeson et al., 1978; Shen et al., 1983; Salmon et al., 1988). Variation in crude protein content due to varietal differences was reported by Finlayson (1974) and, similarly, Goh et al. (1980) reported the crude protein content to vary from 362 to 400 g/ kg in different canola cultivars (as-is basis). The canola protein fraction has a high biological value (Campbell et al., 1981). Its amino acid balance is considered better when compared with many other plant sources, e.g. SBM (Clandinin et al., 1966); the former is richer in methionine, which is the first limiting amino acid in poultry, and the latter is richer in lysine. Therefore, there are advantages to using combinations of these two feedstuffs in poultry diets (Fenwick and Curtis, 1980). The minerals in canola meal can be well utilized according to several studies in which supplemental minerals did not increase performance (Thomas and Crissey, 1983; Summers and Robblee, 1985).

Although canola meal is an excellent feed ingredient for both ruminant and non-ruminant animals, several anti-nutritional factors are associated with canola meal, which can lead to serious negative effects on animal growth and reproduction. The major anti-nutritional factors include glucosinolates, tannins, erucic acid, sinapine, phytic acid, and mucilage. The latter can cause liver damage (Pearson, 1979), decrease performance and feed consumption (Kyriazakis and Emaans, 1992), and can have a negative effect on reproductive performance (Muenger, 1996).

Glucosinolates have received the most attention, as ingestion of substantial amounts is deleterious to animal health and production. Similar to other anti-nutritional factors, glucosinolates are known to reduce feed intake (Hill, 1991), induce iodine deficiency (Burel et al., 2000), cause hypertrophy of liver, kidney and thyroid (Mandiki et al., 1999) and cause higher levels of mortality (CSWRI, 2002). Glucosinolates are biologically inactive molecules, but their degradation products are biologically active and responsible for the biological effects described above. For instance, isothiocyanates are responsible for bitterness which results in a decreasing feed intake (Van Doorn et al., 1998; Mithen et al., 2000), whereas thiocyanates, thiourea and oxazolidithione may disrupt iodine availability to the thyroid, thus affecting thyroid function (Wallig et al., 2002).

In general, deleterious effects of glucosinolates are greater in non-ruminant animals compared to ruminants (Burel et al., 2000). In poultry, high glucosinolate ingestion can lower feed intake, impair growth and increase mortality (McNeill et al., 2004; Campbell and Smith, 1979). Fenwick and Curtis (1980) reported that

glucosinolate intake problems appear to be more severe in laying hens and turkeys than in broilers. The latter may be due to the fact that broilers are reared for 6–8 weeks only; thus, this short period of feeding probably was not enough to produce deleterious effects that were as severe as in laying hens and turkeys that are fed longer. However, a glucosinolate content above 8.0 μmol/g diet was found to induce a severe growth depression even in broilers. In contrast, Marangos et al. (1974) and Lesson et al. (1987) reported a dietary glucosinolates tolerance of up to 11.6 μmol/g by broilers. In the experiment of Mawson et al. (1994), the growth depression effect of dietary glucosinolates initiated between 2 and 4 μmol/g diet, but the effect was limited; when level of glucosinolates increased to 6–10 μmol/g there was a sharp reduction in growth, and when above 10 μmol/g, growth was severely affected. Up to 10% raw canola seeds can be used in diets for broiler chickens, layers, and turkeys (Leeson et al., 1978; Salmon et al., 1988; Ajuyah et al., 1991). Although the concentration of glucosinolates has been reduced to low levels in canola meal (<30 μmol/g), there is evidence that the glucosinolates in canola meal can induce thyroid impairment (Ochetim et al., 1980; Christison and Laarveld, 1981). In addition, glucosinolates reduce diet palatability for pigs and poultry. Bjerg et al. (1987) concluded that several kinds of glucosinolates in rapeseed caused anti-nutritional or other toxic effects even when ingested at levels similar to those of canola meals. Levels below 2.5 μmol/g of diet were recommended. Therefore, further reduction in glucosinolates through plant breeding is both possible and desirable (Bell, 1985).

Canola meal also contains high levels of crude fiber (12%) and phytate (3.1%), which significantly limit nutrient utilization of canola meal (Baidoo and Aheme, 1985; Fan et al., 1996). Canola meal contains about 240 g/kg NDF, 190 g/kg ADF and 130 g/kg crude fiber, which is about three times that of soybean meal (Bell and Keith, 1991). This is due to the high hull content of canola seed. Mustafa et al. (1997) reported that high fiber canola meal exhibits lower total tract digestibility of CP and gross energy and a lower digestible energy content compared with SBM.

To improve the nutritive quality of canola meals, several approaches have been ued. Heat and mechanical treatments (e.g., flaking, steam pelleting, and extrusion) of canola seeds have proven beneficial in improving the feeding value of canola seeds (Shen et al., 1983; Salmon et al., 1988). Woyengo et al. (2010) fed broiler chicks expeller-extracted canola meal, and results indicated that the expeller-extracted canola meal had higher standardized ileal digestible amino acid and AME_n contents than solvent-extracted canola meal. Hence, expeller-extracted canola meal may be a better source of protein and energy for broiler chicks. Meng et al. (2006) reported that carbohydrase enzyme supplementation of a canola meal diet resulted in improved feed nutritional value, with a significant increase in apparent total tract digestibility of DM, fat, non-starch polysaccharide (NSP), and AMEn content of the canola seed diet. The positive effect of carbohydrase enzyme supplementation was likely due to enhanced energy use as a result of the reduced oil-encapsulating effect of the cell walls. Barrett et al. (1998) reported that alkaline heating of canola meals could reduce toxicity for chicks. In their experiment, chicks fed untreated canola meals gained less weight than

those fed a SBM diet; whereas the weight gains of the chicks fed the alkaline heated meals were not significantly different from those of chicks fed the SBM diet. The nutritive value of canola meal can also be improved by removing or reducing the hull content. Dehulling of canola/rapeseed meal increase DE and digestible CP contents and improve the protein quality (Sarwar et al., 1981). Bell (1993) evaluated the nutritive value of dehulled canola meal for growing pigs, which resulted in an improved CP and reduced fiber content. However, no improvement in pig performance was observed compared with the original canola meal. While most attempts of improving canola meal quality are focused on processing, several companies are also working on genetically modifying canola meals or developing new varieties of canola to beneficially change the composition of canola seeds and canola meal. Dow Agro Science LLC. has developed new varieties of canola that have lower concentrations of glucosinolates and dietary fiber and higher concentrations of CP and amino acids than conventional varieties of canola. The canola meal that is produced after crushing of these new varieties is, therefore, expected to have improved feeding value compared with traditional or conventional canola meal. A series of digestibility experiments will be conducted to determine if the canola meal from the new varieties has improved nutritional value, primarily higher ME_n and digestible amino acid levels.

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Chapter 2

Nutritional evaluation of new reduced oligosaccharide soybean meal in poultry

Introduction

Soybean meal (SBM) has been a dominant protein source in the U.S. poultry feed industry since the 1950s (Smith, 1997). It is extensively used because of its high protein and energy content, its excellent amino acid (AA) quality and composition, as well as its high availability of AA (Stein et al., 2008). De-hulled SBM is the most commonly used SBM. It is an especially good source of lysine and tryptophan. Also, due to its low fiber content, SBM provides a metabolizable energy (ME) level of 2,711 kcal/kg on a dry matter basis for poultry, which is 11 to 25% greater than that of other oilseed meals (Stein et al., 2008). However, SBM has a lower metabolizable energy (ME) value than the prediction from its gross energy (GE) for poultry. The latter is partially due to the low digestibility of the oligosaccharides in SBM, which can potentially lead to a large energy loss, and also a possible dilution of energy and other nutrients in the diet (Stein et al., 2008). The oligosaccharides account for approximately 11.28% of the dry matter of SBM (Coon et al., 1988), the alpha-1, 6 linkages of these oligosaccharides cannot be broken down by endogenous enzymes in the small intestine of poultry due to the absence of alpha-1, 6-galactosidase (Gitzelmann and Auricchio, 1965), and therefore are unable to pass through the intestinal wall. If the concentration of these oligosaccharides is high in the digestive tract, they may cause fluid retention and increase the digesta flow rate, leading to an

adverse effect on the absorption and utilization of nutrients (Wiggins, 1984). Leske et al. (1991) suggested that the oligosaccharides can cause shortened transit time, which leads to a reduced fiber digestion and thus a lower than expected TME_n value. The TME_n of soy protein concentrate was reported to be significantly decreased by stachyose and raffinose inclusion in a dose dependent manner (Leske et al., 1993).

Removal of the oligosaccharides from SBM may increase the fiber fermentation because of a slower transit time and create a better cecal environment for the microbial hydrolyzation, and thus improve the nutritional value of the SBM for poultry (Coon et al., 1988). Many processing approaches have been studied in order to improve the nutritional value of SBM, including ethanol extraction, adding exogenous enzymes, and developing genetically modified SBM lines, etc. Among the latter, genetic improvements in reduction or elimination of oligosaccharides are the most effective way. Since the nutritional characteristics of these new low-oligosaccharide SBMs (LO-SBMs) varies from company to company depending on the genetic line, geographic location, and specific technique used to reduce oligosaccharides, it is important to determine the nutritional values of each individual SBM. The present study was conducted to determine the TME_n value, AA digestibility, and growth performance response of a new reduced-oligosaccharide SBM in poultry.

Materials and Methods

Nutrient analysis

Four experiments were conducted to compare the nutritional value of the reduced-oligosaccharide soybean mean (SBM-RO) with that of conventional soybean meal (SBM-CV). Two sources of SBM (SBM-CV and SBM-RO) were used in these experiments. These SBMs were obtained from United Soybean Board. All experimental protocols were reviewed and approved by the Institutional Animal Care and Use Committee at the University of Illinois. The two sources of SBM were analyzed for gross energy (GE) using bomb calorimetry (Model 6300, Parr Instruments, Miline, IL) and crude protein and dry matter using the AOAC (1995) procedures. Amino acid (AA) profile, sugar content and fiber content were analyzed.

TME_n assay with conventional roosters (Experiment 1)

TME_n in the two sources of SBM were determined using a precision-fed rooster assay with conventional Single Comb White Leghorn roosters. Forty intact roosters were housed individually in 22.5 x 36 cm cages with raised wire floors in an environmentally controlled room. A 16-h light: 8-h dark cycle was provided, and water was accessible at all times. The roosters were fasted for 26 h to empty the gastrointestinal tract of all dietary residues. Then, 20 conventional roosters were tube-fed 30 grams of SBM-CV and another 20 were tube-fed 30 g of the SBM-RO. The roosters were then returned to individual cages, and all excreta (feces + urine) were collected for 48 h. The excreta and feed samples were freeze-dried, ground, and

analyzed for gross energy and nitrogen using bomb calorimetry (Model 6300, Parr Instrument). The TME_n value in each source of SBM was calculated as described by Parsons et al. (1982).

TME_n and AA digestibility assay with cecectomized roosters (Experiment 2)

In Experiment 2, 40 cecectomized Single Comb White Leghorn roosters were used to determine the TME_n and AA digestibility of the 2 sources of SBM using a precision-fed rooster assay. The roosters were housed individually in raised-wire cages (22.5 x 36 cm) in an environmentally controlled room. Cecectomy was performed according to the procedures described by Parsons (1985). Water was accessible at all times. After 26 hours of feed withdrawal to empty the gastrointestinal tract of all dietary residues, 20 roosters were tube-fed 30 grams of SBM-CV and another 20 were tube-fed 30 grams of the SBM-RO. The roosters were then returned to individual cages, and all excreta (feces + urine) were collected for 48 h. The excreta and feed samples were freeze-dried, weighed, ground, and analyzed for gross energy and nitrogen using bomb calorimetry (Model 6300, Parr Instrument, Moline, IL). The TME_n value in each source of SBM was calculated as described by Parsons et al. (1982). The excreta and feed samples were also analyzed for AAs at the Agricultural Experiment Station Laboratory, University of Missouri-Columbia and AA digestibility coefficients were calculated. Basal endogenous AAs was corrected using roosters that had been fasted for 48 hours.

Standardized ileal amino acid digestibility experiment (SIAAD) (Experiment 3)

A SIAAD assay was conducted using semi-purified diets that contained 20% protein from only SBM-CV or SBM-RO. It is hypothesized that the SBM-RO has higher AA digestibility than the SBM-CV. A total of 100 commercial broiler chicks were obtained from a hatchery and fed a nutritionally complete corn-SBM starter diet until 17 days of age. This diet was formulated to meet all of the NRC (1994) nutrient requirements. On day 18 after hatching, all chicks were weighed, wing banded, and assigned to treatment groups so that their initial weights were similar among the groups. From 18 to 21 days of age, ten replicate groups of 5 chicks were fed each of two semi purified diets containing 20% protein from either SBM-CV or SBM-RO as the only source of protein (Table 2.1). Feed and water were provided for ad libitium consumption. At the conclusion of the experiment (day 21), all chicks were weighed and euthanized using CO_2 gas. The ileal digesta samples were collected, freeze-dried and analyzed for AA and chromium. Chromic oxide was used as a digesta marker to calculate AA digestibility. Standardized AA digestibility was calculated using the AA excretion of chicks fed an N-free diet as the basal endogenous correction.

Broiler growth performance (Experiment 4)

The fourth experiment was a growth performance trial using diets that contained equal amounts of SBM-CV and SBM-RO. A total of 160 commercial broiler chicks were fed a nutritionally complete corn-SBM starter diet for 7 d. This diet was formulated to meet all NRC (1994) nutrient requirements. On d 7 post-hatch,

all chicks were weighed, wing banded, and assigned to treatment groups so that their initial weights were similar among the groups. Chicks were randomly allotted to 2 diets in a completely randomized design with 5 chicks per group and 16 replicate groups per diet. Chicks were housed in battery cages with raised wire floors in an environmentally controlled room. Water was provided at all times. From 7 to 20 days of age, chicks were fed each of two diets containing 38.84% of SBM-CV or SBM-RO (Table 2.2). Each diet was formulated to contain 2,985 kcal of TME_n/kg, 24% CP, 1.10% Met + Cys, and 1.40% Lys. At the conclusion of the experiment (d 20), all chicks were weighed, and data for body weight gain, feed intake, and feed efficiency were calculated.

Statistical analysis

Data for TME_n, AA digestibility and growth performance were analyzed by ANOVA using the SAS system (SAS Institute, 1990) for complete randomized designs. Statistical significance of differences among individual treatment means were assessed using the least significant difference test. Each individual rooster was the experimental unit for all TME_n and AA digestibility calculations, and a P-value of 0.05 was used to assess differences among means.

Results and Discussion

Nutrient composition

Nutrient compositions of the SBM-CV and SBM-RO are presented in Table 2.3. The crude protein (CP) content was increased from 51.85% for SBM-CV to 54.75% for SBM-RO on a DM-basis. The concentration of Lys was 3.30 and 3.46% (DM-basis) in SBM-CV and SBM-RO, respectively. The concentration of sucrose was 8.02% (DM-basis) in SBM-CV, but it was much higher in SBM-RO (15.73%, DM-basis). The concentration of stachyose and raffinose were 5.16 and 1.01% (DM-basis) in SBM-CV, but were decreased greatly to 0.40 and 0.35% (DM-basis) in SBM-RO, respectively. Although the CP and carbohydrate levels varied, the gross energy of the two SBM was similar. As expected based on the higher CP level, SBM-RO had higher levels of almost all AA in comparison to SBM-CV.

The decrease in the concentrations of stachyose and raffinose in SBM-RO was expected because SBM-RO was produced from a selected variety that has low concentrations of oligosaccharides. The increased CP and AA concentrations in SBM-RO compared with SBM-CV concurs with previous data for another reduced oligosaccharide SBM (Baker and Stein, 2009; Baker et al., 2011). From the compositional data, it seems likely that genetic modification of reducing oligosaccharides in soybeans may result in slight increase in CP and sucrose. This is consistent with what Parsons et al. reported in 2000.

TME_n values

The TME_n of SBM-RO was significantly greater ($P < 0.05$) than SBM-CV both in cecectomized roosters and conventional roosters (Table 2.4). The difference was approximately 200 kcal/kg DM. The latter difference in TME_n values for SBM-CV and SBM-RO concur with previously published values (Parsons et al., 2000). The increased TME_n for SBM-RO can be attributed mainly to the reduction of the concentration of raffinose and stachyose, which cannot be digested in the small intestine due to a lack of endogenous α-(1,6)-galactosidase in poultry. Part of the increase may also be due to the increased level of CP and sucrose in SBM-RO, which displace poorly digestible ADF and NDF (Baker et al., 2011) and are highly digested by chickens (Sibbald, 1986). Therefore, the poorly digested oligosaccharides are replaced by more highly digestible nutrients.

The TME_n values were higher for conventional roosters than for cecectomized roosters for both SBM. Coon et al. (1990) reported that the digestibility of raffinose and stachyose based on excreta collection was significantly higher than ileal digestibility in roosters. This indicates that due to the absence of galactosidase in the small intestine of poultry, most of the raffinose and stachyose are fermented by bacteria in the ceca and colon. The latter may partially explain the difference in TME_n between cecectomized and conventional roosters for SBM-CV. However, there was also a large difference in TME_n between bird types for SBM-RO indicating that other carbohydrates in the SBMs were digested or fermented in the ceca of conventional roosters.

Amino acid digestibility

The AA digestibility coefficients in cecectomized roosters are shown in Table 2.5. No differences were observed between the two sources of SBM for digestibility of any indispensable AA, with the exception of Trp (SBM-RO > SBM-CV, P = 0.006). For dispensable AA, there were significant differences in the digestibility of only Ala, Asp and Cys, with the SBM-RO having higher digestibility (P < 0.05) than that of SBM-CV. No significant differences between the SBMs were found for AA digestibility in the SIAAD assay (Table 2.6).

The results indicate that digestibility of AA in SBM-RO is similar or slightly higher than in SBM-CV. Similarly, Baker and Stein (2009) have reported a lack of difference among three different sources of SBM (SBM-High Protein, SBM-Low Oligosaccharides and SBM-CV) in the standardized ileal digestibility of AA for pigs. These observations suggest that the AA digestibility coefficient values of SBM-CV can be used when formulating diets for broiler chicks if SBM-RO is used. Consequently, as AA in both SBMs can be absorbed to the same degree, less SBM is needed to add into the diet if SBM-RO is used instead of SBM-CV because SBM-RO contains higher levels of digestible AA.

Broiler Growth Performance

No significant difference was observed between treatments in body weight gain of the chicks (P > 0.05) (Table 2.7). However, the feed intake for the SBM-RO treatment was significantly lower than in the SBM-CV group (701.9 and 724.0g,

respectively, P = 0.005). Therefore, the diet containing SBM-RO yielded significantly higher feed efficiency than the diet containing SBM-CV (P < 0.0001). These results are not in agreement with Baker et al. (2011), who reported no differences in growth performance between chicks fed diets containing a SBM-LO and SBM-CV. Irish et al. (1995) reported that broiler chickens fed a diet containing an ethanol-extracted reduced oligosaccharide SBM had significantly poorer weight gains and feed efficiencies than chicks fed SBM-CV, possibly because the diet containing the ethanol-extracted SBM was less palatable due to the higher maize starch content. In the current study, the genetically modified SBM-RO did not adversely impact the feed intake or the weight gain of broiler chicks as was observed earlier for ethanol-extracted SBM. In addition, the improved feed efficiency obtained with the SBM-RO in the current study indicates that this SBM has increased ME_n, which is an agreement with the rooster TME_n results.

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Tables

Table 2.1 Composition of diets containing conventional soybean meal (SBM-CV) or reduced oligosaccharide soybean meal (SBM-RO) used in the chick standardized ileal amino acid digestibility trial (as-fed basis)

Item	Diet	
	SBM-CV	SBM-RO
	(%)	
SBM-CV	42.30	---
SBM-RO	---	40.20
Cornstarch	26.60	27.65
Dextrose	26.60	27.65
Dicalcium Phosphate	1.00	1.00
Limestone	1.30	1.30
Vitamin Premix [1]	0.20	0.20
Mineral Premix [2]	0.15	0.15
Sodium Chloride	0.35	0.35
NaHCO3	0.30	0.30
K2CO3	0.50	0.50
Choline Chloride	0.30	0.30
Chromic Oxide	0.40	0.40
Calculated analysis		
Protein	20.0	20.0
ME, kcal/kg	2,929	2,952
Ca	1.0	1.0
Available P	0.5	0.5
Lys	1.3	1.3
Met	0.3	0.3
Cys	0.3	0.3
Thr	0.8	0.8

[1] Provided per kilogram of complete diet: retinyl acetate, 4,400 IO; cholecalciferol, 25 IU; DL-α-tocopheryl acetate, 11 IU; vitamin B12, 0.01 mg; riboflavin, 4.41 mg; D-pantothenic acid, 10 mg; niacin, 22 mg; and menadione sodium bisulfite, 2.33 mg.

[2] Provided per kilogram of complete diet: manganese, 75 mg from MnSO4·H2O; iron, 75 mg from FeSO4·H2O; zinc, 75 mg from ZnO; copper, 5 mg from CuO4·5H2O; iodine, 0.75 mg from ethylene diamine dihydroiodide; and selenium, 0.1 mg rom Na2SeO3.

Table 2.2 Composition of diets containing conventional soybean meal (SBM-CV) or reduced oligosaccharide soybean meal (SBM-RO) used in the chick growth performance trial (as-fed basis)

Item	Diet	
	SBM-CV	SBM-RO
	(%)	
Corn	55.64	55.77
SBM	38.84	38.84
Soybean Oil	1.00	1.00
Limestone	1.20	1.20
Dical	1.60	1.60
Sodium Chloride	0.40	0.40
DL-Met	0.32	0.30
L-Lys HCl	0.11	0.03
Thr	0.07	0.04
Mineral Mix [1]	0.15	0.15
Vitamin Mix [2]	0.20	0.20
Choline Chloride	0.10	0.10
OptiPhos phytase [3]	0.025	0.025
BMD 30 [4]	0.042	0.042
Chromic Oxide	0.30	0.30
Calculated analysis		
Protein	23.9	24.0
ME, kcal/kg	2,985	2,985
Ca	0.9	0.9
Available P	0.4	0.4
Lys	1.4	1.4
Met	0.7	0.7
Cys	0.4	0.4
Thr	1.0	1.0

[1] Provided per kilogram of complete diet: manganese, 75 mg from MnSO4·H2O; iron, 75 mg from FeSO4·H2O; zinc, 75 mg from ZnO; copper, 5 mg from CuO4·5H2O; iodine, 0.75 mg from ethylene diamine dihydroiodide; and selenium, 0.1 mg rom Na2SeO3.

[2] Provided per kilogram of complete diet: retinyl acetate, 4,400 IO; cholecalciferol, 25 IU; DL-α-tocopheryl acetate, 11 IU; vitamin B12, 0.01 mg; riboflavin, 4.41 mg; D-pantothenic acid, 10 mg; niacin, 22 mg; and menadione sodium bisulfite, 2.33 mg.

[3] OptiPhos phytase supplied 500 FTU of phytase per kg of diet.

[4] Bacitracin Methylene Disalicylate is supplied in concentrations of 30 grams of BMD per pound of diet.

Table 2.3 Analyzed energy and nutrient composition of soybean meal produced from conventional (SBM-CV) or reduced-oligosaccharide (SBM-RO) varieties of soybeans (100% dry matter basis)

	Ingredient	
Item	SBM-CV	SBM-RO
Gross energy (kcal/kg)	4643	4690
CP (%)	51.85	54.75
Ether extract (%)	1.20	0.95
Sucrose (%)	8.02	15.73
Stachyose (%)	5.16	0.40
Raffinose (%)	1.01	0.35
Trypsin inhibitor (TIU/g)	2959	2160
Urease pH change (Units)	0.00	0.03
KOH Solubility (%)	71.36	74.13
Protein dispersibility index (PDI) (%)	20.12	25.23
Indispensable amino acids (%)		
Arg	3.72	4.14
His	1.38	1.42
Ile	2.42	2.44
Leu	4.00	4.09
Lys	3.30	3.46
Met	0.70	0.72
Phe	2.61	2.68
Thr	1.97	2.03
Trp	0.70	0.72
Val	2.56	2.64
Dispensable amino acids (%)		
Ala	2.18	2.27
Asp	5.73	6.03
Cys	0.83	0.86
Glu	9.07	9.35
Pro	2.81	2.90
Ser	2.19	2.28
Tyr	1.89	1.89

Table 2.4 True metabolizable energy (TME_n) of conventional soybean meal (SBM-CV) and reduced-oligosaccharide soybean meal (SBM-RO) in cecectomized and conventional roosters [1]

Item	Cecectomized TME_n	Conventional TME_n
	(kcal/g DM)	
SBM-CV	2.560 [b]	2.775 [b]
SBM-RO	2.755 [a]	3.003 [a]
Pooled-SEM	0.029	0.018

[a,b] Means within a column with no common superscript letters are different ($P<0.05$),

[1] Data are means of 20 roosters per treatment.

Table 2.5 Standardized amino acid digestibility coefficients (%) for conventional soybean meal (SBM-CV) and reduced-oligosaccharide soybean meal (SBM-RO) in cecectomized roosters [1]

	Ingredient			
Amino acid	SBM-CV	SBM-RO	SEM	P-value
Indispensable amino acids				
Arg	90.3	91.2	0.63	0.292
His	90.4	90.8	0.46	0.549
Ile	90.8	91.8	0.48	0.133
Leu	90.6	91.3	0.47	0.292
Lys	89.0	88.9	0.61	0.834
Met	91.9	92.3	0.50	0.646
Phe	91.2	91.6	0.47	0.549
Thr	87.1	88.1	0.56	0.248
Trp	96.4 [b]	97.2 [a]	0.18	0.006
Val	87.2	88.3	0.64	0.233
Dispensable amino acids				
Ala	86.8 [b]	89.2 [a]	0.57	0.006
Asp	89.2 [b]	90.5 [a]	0.40	0.028
Cys	81.5 [b]	85.0 [a]	1.03	0.025
Glu	92.3	93.0	0.38	0.230
Pro	90.0	91.0	0.56	0.206
Ser	88.7	89.7	0.57	0.217
Tyr	90.5	91.4	0.45	0.132

[a,b] Means within a row with no common superscript letters are different ($P<0.05$),
[1] Data are means of 20 roosters per treatment.

Table 2.6 Standardized ileal digestibility coefficients of amino acids (%) for conventional soybean meal (SBM-CV) and reduced-oligosaccharide soybean meal (SBM-RO) fed to 18-21 day-old broiler chicks [1]

	Ingredient			
Item	SBM-CV	SBM-LO	SEM	*P*-value
Indispensable amino acid				
Arg	90.1	89.3	1.04	0.591
His	99.2	97.8	1.14	0.412
Ile	85.1	84.0	1.27	0.438
Leu	85.2	83.3	1.34	0.333
Lys	86.0	84.5	1.69	0.553
Met	85.7	83.3	2.14	0.444
Phe	86.5	84.5	1.15	0.232
Thr	82.5	79.8	1.56	0.231
Trp	84.2	84.6	1.24	0.793
Val	83.2	81.6	1.48	0.470
Dispensable amino acid				
Ala	84.2	82.7	1.54	0.489
Asp	84.9	83.4	1.03	0.319
Cys	81.3	79.3	1.48	0.344
Glu	90.0	87.6	0.98	0.171
Gly	82.3	81.3	1.44	0.621
Pro	86.8	85.1	1.03	0.267
Ser	86.9	83.5	1.39	0.102
Tyr	86.6	84.5	1.27	0.255

[1] Data are means of 10 replicate groups of 5 chicks per treatment.

Table 2.7 Growth performance from d 7 to 20 post-hatch of broiler chicks fed diets containing SBM-CV or SBM-RO [1]

	Diet			
Item	SBM-CV	SBM-RO	Pooled SEM	*P*-value
BW gain (g)	557 [a]	566 [a]	5.4	0.218
Feed intake (g)	724 [a]	702 [b]	5.1	0.005
Gain: Feed (g/kg)	769 [b]	807 [a]	5.1	< 0.0001

[a,b] Means within a row with no common superscript letters are different (P< 0.05),

[1] Values are means of 16 replicate groups of 5 broiler chicks per treatment.

Chapter 3

Nutritional evaluation of canola meals produced from new varieties of canola seeds in poultry

Introduction

Canola is the registered name for a cultivar of rapeseed, which is defined as *Brassica napus* and *Brassica campestris* containing less than 2% erucic acid in the oil and less than 30 μmol/g glucosinolates in the air-dried, oil-free meal (Newkirk, 1990). Canola meal is a commonly used vegetable protein source in poultry diets and is an economically viable alternative to replace some of the SBM in poultry diets (Mushtaq et al., 2007). Historically, the use of CM has been limited in poultry diets due to the presence of anti-nutritional factors, primarily glucosinolates. Ingestion of glucosinolates can lead to a reduced feed intake, a reduced growth rate, liver damage or other diseases in poultry. Although the concentration of glucosinolates has already been reduced to low levels in CM, there is evidence that the conversion from rapeseed meal to CM has reduced, but not eliminated, the liver hemorrhage problem (Ochetim et al., 1980; Christison and Laarveld, 1981, Campbell and Slominski, 1991). Bjerg et al. (1987) concluded that several kinds of glucosinolates in rapeseed caused anti-nutritional or other toxic effects even when ingested at levels similar to those of canola meals. Therefore, further reduction in glucosinolates through plant breeding is desired (Bell, 1993). Canola meal also contains higher fiber, which is largely responsible for a lower ME_n compared with SBM. If CM had more digestible energy,

more protein and less glucosinolates, it could be more competitive in the world market (Bell, 1993). Therefore, there is much interest in developing new varieties of canola to beneficially change the composition of canola and CM. Dow Agro Science LLC. has developed new varieties of canola that have lower concentrations of glucosinolates and dietary fiber, and higher CP and AAs than conventional varieties of canola. The CM that is produced after crushing of these new varieties is, therefore, expected to have improved feeding value compared to traditional or conventional CM. A series of digestibility experiments were conducted to determine if the CM from the new varieties has improved nutritional value, primarily higher ME_n and digestible AA levels.

Materials and Methods

Nutritional analysis

A total of 7 test sources of CM samples, 4 sources of conventional CM samples, and 2 soybean meal (SBM) samples were used in this study. The 7 test CM samples were labeled Test CM 1-7, and conventional CMs were labeled Conv CM 1-4. The Test CM 1, 2, 3, 4, 5 and 6 were laboratory-processed, the rest of the CMs, including the 4 conventional CMs, were commercially-processed. There were 3 experiments. The first experiment included Conv CM1, Test CM1, and a control soybean meal (SBM1). The second experiment included Test CM 2, 3, 4, 5, 6, Conv CM2 and SBM2. The third experiment included Conv CM3-HT, Conv CM4-LT, and Test CM7. The HT refers to processing at a high temperature and LT refers to a reduced

processing temperature. All samples of CM and SBM were analyzed for concentration of gross energy (GE), dry matter (DM), crude protein (CP), carbohydrates, ether extract, fiber, AA profile, and minerals. Carbohydrates in the samples were characterized by analyzing oligosaccharides, crude fiber, and neutral and acid detergent fiber. The concentration of phytate, and phytate bound P were measured as well. Dry matter, GE, CP, Ether extract and ash were analyzed at the University of Illinois. All other analyses were conducted at University of Missouri-Columbia. All data are on a 100% DM basis.

TME_n and amino acid digestibility assays (Experiments 1-3)

A series of precision-fed rooster assays were conducted to determine the TME_n and standardized AA digestibility coefficients for the CM and SBM samples. Roosters were housed individually in 22.5 x 36 cm cages with raised wire floors in an environmentally controlled room. A 16-h light: 8-h dark cycle was provided, and water was accessible at all times. Cecectomy was performed according to the procedures described by Parsons (1985). The roosters were fasted for 26 hours to empty the gastrointestinal tract of all dietary residues. Then, roosters were tube-fed 30 grams of a CM or SBM. The roosters were then returned to individual cages, and all excreta (feces and urine) were collected for 48 h. The excreta and feed samples were freeze-dried, ground, and analyzed as needed for gross energy at the University of Illinois using bomb calorimetry (Model 6300, Parr Instrument Co., Moline, IL). Nitrogen and AAs were analyzed at the University of Missouri-Columbia. In

Experiment 1, the TME_n of the 2 CMs and SBM1 were determined in conventional roosters, 10 roosters per sample. Amino acid digestibility was determined in cecectomized roosters, 10 roosters per sample. In Experiment 2, both TME_n and AA digestibility were determined in cecectomized roosters, 5 roosters per sample. In Experiment 3, the TME_n of the 3 CMs were determined in conventional roosters, 10 roosters per sample. Amino acid digestibility was determined in cecectomized roosters, 4 roosters per sample. The TME_n values and standardized AA digestibility coefficients for each source of CM and SBM were calculated as described by Parsons et al. (1982).

Statistical analysis

Data for TME_n and AA digestibility were analyzed by ANOVA using the SAS system (SAS Institute, 1990). The significance of differences among individual treatments was assessed using the least significant difference test. The individual rooster was the experimental unit for all calculations, and a P-value of 0.05 was used to assess differences among treatment means.

Results and Discussion

Nutrient composition

As expected, the CP and AA content of the Test CM samples increased (approximately by 18% and 20%, respectively), whereas the neutral detergent fiber (NDF) and acid detergent fiber (ADF) content decreased by up to 60% compared with

Conv CM samples (Table 3.1, 3.2, and 3.3). The gross energy (GE) values of Test CMs were generally similar or slightly lower than that of Conv CMs. The ether extract content was consistently lower in Test CMs. Both SBM samples contained higher CP and AA concentrations than the CMs. Test CM 2-6 contained significantly higher stachyose and raffinose levels than Test CM 1 and 7. This could depend on the original content in canola seed (effect of breed, soil condition, or weather during harvesting), the processing (temperature, moisture, heating time, and type of solution), and other nutrients composition remaining in the meal that could affect oligosaccharide content by the ratio.

The results indicating that Conv CMs contained less GE, less protein and more fiber than de-hulled SBM concur with what Bell and Keith (1991) reported. The Test CMs had an enhanced nutrient profile compared with Conv CMs, except that the GE values were similar or lower in Test CMs than in Conv CMs. This may be due to the decreased fat content in Test CMs that leads to decreased energy content. The average GE value of CMs (Conv CMs and Test CMs) from current study is approximately 300 kcal/kg higher than previously reported value (4455 kcal/kg) (Bell and Keith, 1991); this is possibly due to genetic selection over the past decade on canola seeds or difference in efficiency of oil extraction from the seeds.

TME_n and amino acid digestibility in Experiment 1

The TME_n values of the samples from Experiment 1 are presented in Table 3.4. The Test CM1 had an increase of 179 kcal/kg DM in TME_n in comparison with Conv

CM1. The difference was significant at P< 0.066. The SBM1 contained a significantly higher TME_n level than the CMs (P < 0.05). The AA digestibility coefficients of all AAs, except Cys, in Test CM1 were significantly higher (P< 0.05) than in Conv.CM1 (Table 3.5). When compared with SBM1, the digestibility coefficients of 4 indispensable AAs (Leu, Lys, Met, and Trp) and 2 dispensable AAs (Ala and Glu) in Test CM1 were not statistically different. The digestibility values of the other AAs were higher in SBM1 (P < 0.05).

The TME_n values of canola meal (low glucosinolates) in roosters were reported to range from 2.054 to 2.271 kcal/g DM (Sibbald, 1986), which are comparable to TME_n value in Conv CM1 from the current study. The factors that may influence the TME_n content include the increased CP content and decreased fiber content. The GE of the meal is largely determined by the relative proportion of protein and carbohydrate. Protein is much more digestible than the carbohydrate (high in fiber) in CM; this enhances the ME values of the meal (Bell, 1993). In addition, the Test CM1 yielded higher digestibility values for almost all AAs than Conv CM1 (P < 0.05). Thus, the test CM1 not only contained higher levels of CP and AAs, but the digestibility of the CP and AA were also higher, which probably accounts for a large portion of the increased TME_n.

Insoluble dietary fiber can impede total tract digestibility of dietary nitrogen (Shi and Noblet, 1993; LeGoff and Noblet, 2001) and ether extract (LeGoff and Noblet, 2001). Therefore, the decreased fiber content in Test CM1 may have also contributed to the enhanced energy utilization by decreasing its negative effects. The partial

replacement of the fiber and galacto-oligosaccharides (raffinose and stachyose) in Test CM1 by more useful sources of ME such as protein accounts for a part of the observed improvement (Bell, 1993). However, selection for fiber has not been a priority for ongoing breeding programs in the past (Mailer, et al., 2008). The result of the current study showed that further selection for reduced fiber would be both desirable and effective in improving CM quality.

TME_n and amino acid digestibility in Experiment 2

All Test CMs (Test CM 2-6) contained significantly higher TME_n (2.347 to 2.635 kcal/g DM) than Conv CM2 (2.000 kcal/g DM) ($P < 0.05$), but the values were not as high as that of SBM2 (2.913 kcal/g DM) (Table 3.6). The increased TME_n in Test CM 2-6 is primarily due the increased protein content and decreased fiber content as discussed above. The stachyose and raffinose levels in Test CM 2-6 were much higher than in Test CM1, which theoretically would lead to a decreased TME_n because of their low digestibility and the possible anti-nutritional effects of these oligosaccharides. Given that TME_n in Experiment 2 were determined in cecectomized roosters, the values should again be expected to be lower than TME_n in Experiment 1 which was determined in conventional roosters. However, the TME_n levels of Test CM 2-6 were generally not lower than Test CM1. This lack of difference may be due to the Test CM 2-6 containing higher levels of sucrose that is highly digestible by poultry.

All the Test CMs in Experiment 2 had similar or a slightly higher AA digestibility than Conv CM2 (ranged from 77.4 to 90.2%), with several of the differences being significant ($P < 0.05$) (Table 3.7). The SBM2 contained higher AA digestibility values (ranged from 89.2 to 98.0 %). This agrees with NRC (1994). However, the digestibility values of all indispensable AAs and most dispensable AAs in Test CM3, 5, and 6 were not significantly different from SBM2.

The results from this study indicate that genetic modification can yield CMs that have AA digestibilities not different from SBM. The standardized ileal digestibility of AA of genetically modified CMs for poultry has not been researched or published. However, results from this study ndicated that genetic modification of canola can improve the AA profile and AA digestibility in poultry.

TME_n and amino acid digestibility in Experiment 3

The TME_n of Test CM7 from Experiment 3 (2.524 kcal/g DM) was not significantly different from the TME_n of Conv CM3-HT at $P<0.05$, but was significantly higher than that of Conv CM4-LT (Table 3.8). The insignificant difference between Test CM7 and Conv CM3-HT was possibly due to the genetic improvement was not large enough to compensate for the higher GE and fat content of Conv CM3-HT compared with Test CM7 (GE of 4.846 and 4.794 kcal/g DM and fat of 4.13 and 3.68%, respectively).

No difference was found in AA digestibility values among the 3 samples in Experiment 3 ($P > 0.05$) (Table 3.9). These three samples were all

commercially-processed. Although there were no differences in digestibility values, the Test CM7 contained higher levels of digestible AA because of the higher CP and analyzed AA levels. There was no significant effect of processing temperature on the digestibility of AA in the Conv CM3-HT and Conv CM4-LT.

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Tables

Table 3.1 Analyzed energy and nutrient composition of a conventional canola meal (Conv CM1), test canola meal (Test CM1) and soybean meal (SBM1) (100% dry matter basis) in Experiment 1

	Ingredient [1]		
Item (%)	Conv CM1	Test CM1	SBM1
DM	90.29	92.30	89.64
Gross energy (kcal/kg)	4707	4710	4751
CP	41.46	49.44	53.56
Ether extract	4.16	3.14	2.62
Sucrose	7.28	4.63	7.95
Stachyose	1.72	0.31	6.31
Raffinose	0.79	0.21	1.32
Crude fiber	8.89	8.32	4.53
NDF	32.66	20.69	9.75
ADF	21.18	15.32	6.70
Calcium	0.74	0.83	0.41
Phosphorus	1.04	1.50	0.67
Non-phytate P	0.28	0.30	0.24
Indispensable amino acids (%)			
Arg	2.28	2.77	3.34
His	1.02	1.25	1.18
Ile	1.55	1.76	2.14
Leu	2.71	3.21	3.55
Lys	2.13	2.71	2.92
Met	0.75	0.89	0.61
Phe	1.56	1.85	2.37
Thr	1.63	1.89	1.74
Trp	0.48	0.63	0.80
Val	2.02	2.30	2.25
Dispensable amino acids (%)			
Ala	1.71	2.00	1.96
Asp	2.70	3.34	5.09
Cys	0.91	1.16	0.61
Glu	6.43	8.17	8.12
Pro	2.16	2.67	2.23
Ser	1.50	1.85	1.93
Tyr	1.14	1.31	1.74

[1] The Test CM1 was laboratory-processed and the Conv CM1 and SBM1 were commercially-processed.

Table 3.2 Analyzed energy and nutrient composition of 5 test canola meals, one conventional canola meal (Conv CM2), and soybean meal (SBM2) (100% dry matter basis) in Experiment 2

Item (%)	Ingredient [1]						
	Test CM2	Test CM3	Test CM4	Test CM5	Test CM6	Conv CM2	SBM2
DM	92.39	91.52	90.7	91.8	91.94	88.89	88.81
Gross energy (kcal/kg)	4789	4790	4828	4732	4739	4825	4830
CP	50.29	50.05	50.58	49.41	49.46	43.01	55.71
Ether extract	3.19	3.43	2.68	2.58	3.05	4.27	1.52
Sucrose	7.61	5.91	8.51	8.34	7.73	8.11	7.32
Stachyose	3.24	2.75	2.68	2.42	2.14	2.17	5.46
Raffinose	0.74	0.63	0.54	0.59	0.63	0.61	1.24
Crude fiber	9.25	10.09	6.95	9.52	9.59	9.38	3.51
NDF	20.23	21.22	22.49	19.53	19.96	32.98	10.61
ADF	15.63	16.38	17.61	14.6	14.57	18.96	5.26
Calcium	0.75	0.74	0.97	0.84	0.76	0.80	0.41
Phosphorus	1.39	1.50	1.49	1.49	1.42	1.14	0.75
Non-phytate P	0.25	0.22	0.32	0.32	0.35	0.30	0.25
Indispensable amino acids (%)							
Arg	3.04	2.98	3.09	2.8	2.98	2.49	3.83
His	1.29	1.26	1.26	1.29	1.31	1.15	1.37
Ile	1.93	1.86	1.95	1.88	1.87	1.79	2.56
Leu	3.47	3.39	3.43	3.21	3.38	3.01	4.13
Lys	2.78	2.69	2.67	2.81	2.91	2.31	3.32
Met	0.95	0.91	0.88	0.93	0.96	0.85	0.88
Phe	1.98	1.93	1.96	1.82	1.94	1.71	2.75
Thr	2.10	2.01	1.98	1.88	2.08	1.78	2.00
Trp	0.65	0.68	0.66	0.61	0.59	0.53	0.80
Val	2.45	2.36	2.46	2.36	2.37	2.24	2.64
Dispensable amino acids (%)							
Ala	2.09	2.03	2.05	1.96	2.1	1.86	2.29
Asp	3.69	3.54	3.56	3.08	3.37	2.96	6.01
Cys	1.14	1.10	1.10	1.22	1.25	0.99	0.69
Glu	8.51	8.28	8.35	8.50	8.68	7.24	9.42
Pro	2.98	2.90	2.87	2.86	2.96	2.67	2.72
Ser	1.88	1.82	1.75	1.76	2.00	1.53	2.17
Tyr	1.37	1.35	1.38	1.22	1.37	1.21	1.93

[1] The 5 test canola meals were laboratory-processed and the conventional canola meal (Conv CM2) and soybean meal (SBM2) were commercially-processed samples.

Table 3.3 Analyzed energy and nutrient composition of 3 commercially- processed canola meals (100% dry matter basis) in Experiment 3 [1]

	Ingredient		
Item (%)	Conv CM3-HT	Conv CM4-LT	Test CM7
DM	88.44	90.35	90.22
Gross energy (kcal/kg)	4846	4799	4794
CP	40.73	40.94	49.57
Ether extract	4.13	3.60	3.68
Sucrose	7.58	7.84	7.58
Stachyose	1.19	1.14	0.35
Raffinose	0.57	0.60	0.14
Crude fiber	8.93	8.00	9.72
NDF	31.73	29.86	23.07
ADF	21.44	21.25	15.34
Calcium	0.73	0.72	0.89
Phosphorus	1.20	1.23	1.59
Non-phytate P	0.27	0.30	0.32
Indispensable amino acids (%)			
Arg	2.32	2.36	2.77
His	1.06	1.08	1.26
Ile	1.67	1.69	1.90
Leu	2.78	2.81	3.25
Lys	2.27	2.32	2.67
Met	0.78	0.80	0.92
Phe	1.56	1.59	1.83
Thr	1.61	1.63	1.87
Trp	0.51	0.52	0.68
Val	2.09	2.13	2.43
Dispensable amino acids (%)			
Ala	1.73	1.75	2.02
Asp	2.73	2.76	3.42
Cys	0.89	0.93	1.16
Glu	6.45	6.49	7.84
Pro	2.46	2.45	2.90
Ser	1.31	1.33	1.61
Tyr	1.12	1.13	1.24

[1] The conventional meals were processed at either a high (HT) or low (LT) temperature.

Table 3.4 True metabolizable energy (TME_n) of a conventional canola meal (Conv CM1), Test CM1, and soybean meal (SBM1) in conventional precision-fed roosters in Experiment 1 [1]

Item [2]	Gross Energy as-is(kcal/g)	Dry Matter (%)	TME_n [3] (kcal/g DM)
Conv CM1	4.250	90.3	2.275 [b]
Test CM1	4.347	92.3	2.454 [b]
SBM1	4.258	89.6	2.938 [a]
Pooled SEM			0.065

[a,b] Means within a column with no common superscript letters are different ($P<0.05$).
[1] Data are means of 10 roosters per treatment.
[2] The Test CM1 was laboratory-processed and the Conv CM1 and SBM1 were commercially-processed.
[3] TME_n of Conv CM1 and Test CM1 are not different at $P<0.05$ but are different at $P= 0.066$.

Table 3.5 Standardized ileal amino acid digestibility coefficients (%) of conventional canola meal (Conv CM1), test canola meal(Test CM1) and soybean meal (SBM1) in cecectomized roosters in Experiment 1 [1]

	Ingredient [2]			
Amino acid	Conv CM1	Test CM 1	SBM1	Pooled-SEM
Indispensable amino acids				
Arg	87.6 [c]	90.2 [b]	92.7 [a]	0.77
His	84.5 [c]	88.7 [b]	90.6 [a]	0.64
Ile	81.5 [c]	85.7 [b]	91.2 [a]	0.88
Leu	84.0 [b]	88.1 [a]	90.2 [a]	0.92
Lys	75.2 [b]	85.3 [a]	88.7 [a]	1.20
Met	88.9 [b]	91.6 [a]	92.0 [a]	0.78
Phe	84.5 [c]	88.4 [b]	91.9 [a]	0.89
Thr	77.6 [c]	81.2 [b]	86.8 [a]	1.11
Trp	96.7 [b]	99.3 [a]	96.2 [b]	0.40
Val	78.6 [c]	82.9 [b]	87.8 [a]	1.01
Dispensable amino acids				
Ala	81.1 [b]	86.3 [a]	87.2 [a]	1.22
Asp	80.0 [c]	86.8 [b]	90.4 [a]	0.86
Cys	78.7 [b]	80.5 [b]	86.5 [a]	1.28
Glu	88.8 [b]	92.0 [a]	93.2 [a]	0.55
Pro	79.0 [c]	84.3 [b]	90.2 [a]	0.95
Ser	76.7 [c]	82.4 [b]	88.7 [a]	1.37
Tyr	80.7 [c]	84.0 [b]	91.4 [a]	1.04

[a-c] Means within a row with no common superscript letters are different ($P<0.05$).
[1] Data are means of 10 roosters per treatment.
[2] The Test CM1 was laboratory-processed and the Conv CM1 and SBM1 were commercially-processed.

Table 3.6 True metabolizable energy (TME_n) of 5 test canola meals, one conventional canola meal (Conv CM2), and soybean meal (SBM2) in cecectomized precision-fed roosters in Experiment 2 [1]

Ingredient [2]	Gross Energy as-is (kcal/g)	Dry Matter (%)	TME_n (kcal/g DM)
Test CM2 - laboratory	4.425	92.4	2.353 [c]
Test CM3 - laboratory	4.384	91.5	2.635 [b]
Test CM4 - laboratory	4.379	90.7	2.347 [c]
Test CM5 - laboratory	4.344	91.8	2.460 [bc]
Test CM6 - laboratory	4.357	91.9	2.611 [b]
Conv CM2 - commercial	4.289	91.9	2.000 [d]
SBM2	4.289	88.8	2.913 [a]
Pooled-SEM			0.085

[a-d] Means within a column with no common superscript letters are different ($P < 0.05$).

[1] Data are means of 5 roosters per treatment.

[2] The 5 test canola meals were laboratory-processed and the conventional canola meal (Conv CM2) and soybean meal (SBM2) were commercially-processed samples.

Table 3.7 Standardized amino acid digestibility coefficients (%) of 5 test canola meals, one conventional canola meal (Conv CM2), and soybean meal (SBM2) in cecectomized roosters in Experiment 2 [1]

	Ingredient [2]							
Amino acid	Test CM2	Test CM3	Test CM4	Test CM5	Test CM6	Conv CM2	SBM2	Pooled-SEM
Indispensable amino acids								
Arg	91.8 [abc]	94.1 [ab]	90.9 [bc]	92.7 [abc]	93.1 [abc]	90.1 [c]	95.2 [a]	1.17
His	87.5 [ab]	90.1 [a]	86.0 [ab]	89.2 [ab]	89.5 [a]	84.5 [b]	90.7 [a]	1.63
Ile	86.5 [bc]	90.0 [ab]	86.0 [bc]	88.9 [bc]	89.8 [b]	85.2 [c]	93.9 [a]	1.38
Leu	89.9 [abc]	92.8 [a]	87.9 [bc]	91.4 [ab]	92.6 [a]	87.2 [c]	93.3 [a]	1.24
Lys	86.0 [ab]	87.9 [a]	83.5 [b]	87.2 [ab]	86.6 [ab]	77.4 [c]	89.5 [a]	1.44
Met	92.4 [ab]	94.3 [a]	90.7 [b]	93.7 [a]	94.8 [a]	90.4 [b]	94.8 [a]	0.96
Phe	89.3 [bc]	92.1 [ab]	87.7 [c]	90.4 [abc]	91.9 [ab]	86.9 [c]	93.9 [a]	1.28
Thr	85.6 [bcd]	87.2 [abc]	83.2 [cd]	86.2 [abc]	88.3 [ab]	80.9 [d]	91.0 [a]	1.62
Trp	85.5 [ab]	99.5 [a]	85.0 [c]	98.2 [ab]	99.5 [a]	84.0 [bc]	98.0 [abc]	1.39
Val	84.1 [bc]	86.4 [ab]	82.4 [bc]	85.9 [bc]	86.5 [ab]	81.6 [c]	90.5 [a]	1.51
Dispensable amino acids								
Ala	88.4 [ab]	91.2 [a]	86.6 [bc]	89.5 [ab]	91.1 [a]	84.4 [c]	90.5 [ab]	1.35
Asp	88.9 [a]	91.7 [a]	87.6 [a]	89.0 [a]	90.8 [a]	81.6 [b]	90.6 [a]	1.57
Cys	83.6 [ab]	89.0 [a]	80.0 [b]	86.1 [a]	87.0 [a]	79.4 [b]	89.2 [a]	2.03
Glu	92.9 [abc]	94.5 [a]	91.4 [bc]	93.8 [ab]	94.3 [a]	90.2 [c]	94.4 [a]	0.97
Pro	86.0 [bcd]	88.8 [bc]	86.6 [cd]	87.7 [bc]	89.9 [ab]	82.7 [d]	94.3 [a]	1.59
Ser	86.8 [bc]	88.9 [ab]	83.3 [cd]	87.3 [abc]	90.1 [ab]	80.6 [d]	92.2 [a]	1.81
Tyr	85.5 [bc]	89.0 [b]	85.0 [bc]	85.8 [bc]	88.3 [b]	84.0 [c]	93.5 [a]	1.39

[a-d] Means within a row with no common superscript letters are different ($P<0.05$).

[1] Data are means of 5 roosters per treatment.

[2] Test CM2 – Test CM6 are laboratory-processed canola meals, Conv CM2 and SBM2 are commercially-processed samples.

Table 3.8 True metabolizable energy (TME_n) of 3 commercially-processed canola meals in conventional precision-fed roosters in Experiment 3 [1]

Canola meal [2]	Gross Energy as-is(kcal/g)	Dry Matter (%)	TME_n (kcal/g DM)
Conv CM3-HT	4.285	88.4	2.373 [ab]
Conv CM4-LT	4.336	90.4	2.320 [b]
Test CM7	4.326	90.2	2.524 [a]
Pooled-SEM			0.061

[1] Data are means of 10 roosters per treatment.

[2] The conventional meals were processed at either a high (HT) or low (LT) temperature.

Table 3.9 Standardized ileal amino acid digestibility coefficients (%) of 3 commercially-processed canola meals in cecectomized roosters in Experiment 3 [1]

Amino acid	Ingredient [2]			Pooled-SEM
	Conv CM3-HT	Conv CM4-LT	Test CM7	
Indispensable amino acids				
Arg	89.6	90.1	88.3	2.44
His	84.7	85.4	84.5	2.46
Ile	83.2	83.9	83.9	2.81
Leu	85.2	86.0	86.1	2.89
Lys	76.6	78.9	80.4	2.67
Met	88.1	88.8	90.0	1.72
Phe	84.1	85.0	85.3	2.83
Thr	79.3	80.0	80.3	3.45
Trp	98.9	97.9	97.5	1.01
Val	79.1	80.1	80.6	3.15
Dispensable amino acids				
Ala	82.7	83.9	84.9	2.95
Asp	82.8	83.0	85.1	2.55
Cys	79.8	81.8	79.1	3.84
Glu	89.9	90.4	89.0	2.26
Pro	81.8	82.7	82.8	3.03
Ser	77.9	80.9	81.6	3.98
Tyr	80.7	81.5	81.5	3.17

[1] Data are means of 4 roosters per treatment.
[2] The conventional meals were processed at either a high (HT) or low (LT) temperature.

Chapter 4

General Summary

Reduced-oligosaccharide soybean meal

The nutritional value of a new reduced-oligosaccharide soybean meal (SBM-RO) and conventional SBM (SBM-CV) were evaluated and compared in four experiments. The protein content (100% DM basis) of the SBM-CV and SBM-RO was 51.85% and 54.75%, respectively. The gross energy of the two SBM was similar. The TME_n values in both conventional roosters and cecectomized roosters were significantly higher for SBM-RO than for SBM-CV ($P < 0.05$) (difference was approximately 200 kcal/kg DM). Amino acid digestibility in cecectomized roosters was not different between SBM-CV and SBM-RO, with the exception of Trp, Ala, Asp and Cys (SBM-RO > SBM-CV, $P < 0.05$). No significant differences between the SBMs were observed for AA digestibility in the SIAAD assay. In the growth performance trial (Experiment 4), the corn-SBM diet containing SBM-RO yielded significantly higher feed efficiency than the diet containing SBM-CV ($P < 0.0001$). Results indicated that SBM-RO contains higher ME than SBM-CV and that digestibility of most AAs in SBM-RO is not different from that of SBM-CV. Consequently, because SBM-RO contains higher concentrations of AA than SBM-CV, SBM-RO contains higher levels of digestible AA than SBM-CV. Thus, as AA in both SBMs can be absorbed to the same degree, less SBM is needed in the diet for broiler chicks to meet digestible AA requirement if SBM-RO is used instead of SBM-CV.

The higher ME and digestible AA in SBM-RO clearly indicate that it is a nutritionally superior SBM and also of greater economic value.

Canola meals produced from new varieties of canola seeds

The nutritional value of 7 new genetically modified CM (Test CM) was evaluated and compared with conventional CM samples (Conv CM) and soybean meals (SBM). Results indicated that there were increases in CP and AA for all Test CMs (49.41 to 50.58%, DM-basis) compared with Conv CMs (40.73 to 43.01%). All the Test CMs also contained lower amounts of fiber as measured by neutral detergent fiber and acid detergent fiber. When TME_n values of Test CMs were compared with conventional CM, 6 of the 7 Test CMs had significantly higher values than the conventional CM samples ($P < 0.05$), but all were lower than SBM ($P < 0.05$). For AA digestibility, the Test CMs had higher digestibility values than Conv CM in Experiment 1 and 2 ($P < 0.05$), and higher concentrations of digestible AA in all 3 experiments. Results of this study indicate that genetic modification of canola can increase TME_n and digestible AA in CM for poultry. Also, further selection for reduced fiber is both desirable and effective in improving the nutritional value of CM for poultry.