EVALUATION OF CANOLA MEAL AS AN ALTERNATIVE PLANT PROTEIN SOURCE IN

NURSERY PIG DIETS

by

JULIUS JEROME GENERAO ELE

(Under the Direction of C. Robert Dove)

ABSTRACT

The objective of this study was to determine what level of canola meal can replace soybean meal as a plant protein source for nursery pigs. Two experiments on weanling pigs were performed to evaluate growth performance, thyroid hormone status and digestibility of the diets with varying levels of canola meal in the diet. Results indicate that canola meal is an acceptable partial soybean meal replacement at no greater than 13% in nursery pig diets.

INDEX WORDS: Nursery pigs, Performance, Canola meal, Thyroid hormone, Digestibility

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DEDICATION

This is for my family for their unwavering love, support and encouragement.

Ma, Ate Hide, Ate Lara, Kuya Gerry, Kuya Ted, Mark;

My aunts and uncles;

My cousins;

My brothers-in law;

My nieces and nephews;

My friends in Mindanao;

And to

Jane and Jewel.

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CHAPTER 1

INTRODUCTION

Canola meal is considered one of the alternative protein sources for soybean meal in the swine industry (Shelton et al., 2001). A minor hindrance seen by swine producers for maximizing its increased use in countries outside Canada, Australia and Europe is the antinutritional factors and fiber that the meal contains. These are considered to be factors in reducing the animal's growth potential. However, a recent study has shown that solvent extracted canola meal can be included in diets of early weaned pigs at 25% with only 3% dietary soybean meal, 30% for growing and finishing pigs and 20.2% for lactating sows as the sole source of plant protein without adversely affecting the performance of these different swine stages (King et al., 2001). These results indicate that canola meal is an effective plant protein source.

The cost of canola meal is cheaper than soybean meal (Feedstuffs, 2004). This would significantly reduce total costs for swine production. It can be concluded that canola meal is a cost-effective substitute for soybean meal employing equal animal performance.

The values of the nutrients contained in canola meal are highly comparable to soybean meal (Table 2.1). But canola meal would be deemed inferior, among certain nutrients, of the two protein sources. With this consideration, a major issue is the nutrient composition in constructing an efficient and properly formulated diet. Castell (1980) stated that a deficiency in protein quality is corrected through the addition of supplemental amino acids. Hence, if canola meal and other feed ingredients are insufficient in lysine, then adequate nutrients should be supplemented to meet the required level needed by the animal.

Failure for the desired performance of animals fed with canola meal is due to insufficient knowledge on the nutrient digestibility of canola meal as a feed protein source. Assessment of the proper use of canola meal and any feed ingredient includes factors that are limiting in a meal and considers proper resolutions to attain a well-balanced feed that meets the feedstuffs maximum nutritional potential for good animal performance.

Considering the ideals of proper nutrition, canola meal compared with its rapeseed meal predecessor is exceptionally better in nutrient composition. Moreover, the anti-nutritional factors present in the meal are greatly reduced, rendering it a satisfactory alternate feedstuff for soybean meal and an effective source of protein for pigs.

CHAPTER 2

LITERATURE REVIEW

Brief History of Canola Meal

Canola was the name chosen when low erucic acid rapeseed oil was developed in Canada (Bell, 1993). The seed has less than 2% erucic acid and less than 30 µmol of alkenyl glucosinolates per gram of oil-free dry matter (Bell, 1993). Before the name of canola was employed, it originated from rapeseed belonging to the genus *Brassica* (Bell, 1982).

The distribution of rapeseed (*Brassica campestris*) is of three major origins including Asiatic, Mediterranean and West European (Bell, 1982). Bell (1982) stated that the first rape widely grown in Canada was a forage rape (*Brassica napus*). In 1936, the introduction of oilseed rape (*B. campestris*) began. A Polish immigrant, Fred Solvonik, received *B. campestris* seed from a contact from his country, which became the seed source used for research tests. The original interest in canola was due to its high erucic acid employed as an engine lubricant, but after World War II, interest in animal use, oil for man and plant breeding, evolved (Bell, 1982).

In 1944, Canada's first rapeseed breeding was initiated by W. J. White and the breakthrough began in 1967 when another scientist from Poland discovered a very low glucosinolate variety named Bronowski (*B. napus*) that he brought with him (Bell, 1982). Furthermore, this became the source of Canada's first two "double low" rapeseed varieties, low in glucosinolate and low in erucic acid. In 1974, Stefansson named his first "double low" rapeseed variety Tower. In 1977, the second "double low" variety of rapeseed developed by R. K. Downey was named Candle (Bell, 1982). Inclusion levels increased with the characteristic changeover to canola meal from rapeseed meal where glucosinolate and erucic acid levels of

rapeseed are 110-150 µmoles and 45%, respectively, while canola has 10-16 µmoles glucosinolate and less than 1% to negligible or undetectable amounts of erucic acid (Ackman, 1983). The differences with old and new varieties of rapeseed were greatly significant that a new generation for the oil and meal began using the new name "canola" (Bell, 1982).

Levels of glucosinolates have been reduced ten-fold for canola meal compared to rapeseed meal (Naczk et al., 1998; Mawson et al., 1995a). The high temperature employed in processing greatly reduces glucosinolate levels and increases palatability of the meal. It has also been found that the induction of direct steam reduces the levels of the compound up to 50% (Mawson et al., 1995a).

The development of low glucosinolate cultivars of rapeseed have resulted in major improvements in the meal (Bell, 1993).

Canola Meal Nutrient Composition and Digestibility Levels

Crude Protein. Feeding values of oilseeds such as canola meal are based on their protein content (Tkachuk, 1969). Crude protein content of canola meal ranges from 34.5 to 39% on a dry matter basis (Singnam and Lawrence, 1979; Clanindin and Robblee, 1981; Corino et al., 1991). Moreover, levels as high as 40 - 41.85% CP have been reported (Naczk et al., 1998; Schone et al., 1997; Keith and Bell, 1991; Bell and Keith, 1991). Canola has a good amino acid profile making it a good quality meal (Table 2.2). However, canola meal has a low lysine level (Etienne and Dourmad, 1994) compared with the soybean meal, indicating the need to supplement lysine (Summers et al., 1989). Supplementing lysine to double low glucosinolate rapeseed meal improves growth performance (Bourdon and Aumaitre, 1990). The reason for reduced lysine levels is due to the heating and extrusion in seed processing to lower the myrosinase activity and thiocyanate levels of the meal as well as increasing the risk of lowering lysine availability of the meal (Ochetim et al., 1980a).

Results for digestibility studies performed for starter and growing to finishing pigs gave variable results. One study found that nitrogen digestibility of canola meal when fed at 16.4% to 65.8% of the diet to 9-kg pigs was within 69.8% to 77.7% (McIntosh and Aherne, 1985). These results showed that nitrogen digestibility increases with increasing levels of canola meal. While ileal and total digestibility for protein in another experiment with 41.0% dietary inclusion of full fat canola for 8.9 kg pigs was 60.1% and 74.5%, respectively (Fan et al., 1995). The results for this experiment showed that canola meal had a significantly lower total digestibility compared with soybean meal. But the ileal digestibility of canola meal was not significantly different from soybean meal.

Canola meal when fed at 40% to growing finishing pigs weighing 45 kg had an ileal and fecal protein digestibility of 66.5% and 80.0%, respectively (Imbeah and Sauer, 1991), while feeding a dietary inclusion of 50% of a high fat, low glucosinolate rapeseed meal to 86-kg pigs gave 68.5% protein digestibility (Schone et al., 1996). However, when canola was fed at a lower dietary inclusion rate of 15% and 30%, protein digestibility of canola meal was at 73.1% and 75.8% for 45 kg-pigs (Bell and Keith, 1989). These results are similar when canola meal was fed at higher levels. Similar levels of substitution for heavier pigs from different cultivars resulted in a range of 79.0% to 81.9% (Bell et al., 1998), which has a better digestibility result than the other studies mentioned.

Energy and Fiber. The digestible energy level of double zero rapeseed meal or canola meal is 2900 kcal/kg and is generally lower by about 18% compared with soybean meal (Siljander-Rasi et al., 1996; Patience and Thacker, 1989). In a review by Clandinin and Robblee (1981), if level of fiber present in the meal is reduced, available energy will be increased. The neutral detergent fiber (NDF) value of the meal is within 21.54 to 28.4% (Bell, 1993; Slominski and Campbell, 1990), while it is only 7.1% for soybean meal (Bell, 1993). Since canola meal has about 3 to 4 times the fiber content of soybean meal, this may be the reason why energy is lower in canola meal compared with soybean meal. This is in agreement with the previous

statement that fiber lowers energy availability. However, not all NDF values are undigested since some fiber bound in *Brassica* meals may be partially digested by pigs (Bell et al., 1998). The reason for partial digestion of fibers in canola meal is attributed to genetic differences in the chemical components of NDF among cultivars of canola meal (Bell, 1998). Bell and Keith (1988a) found that the effect of dietary level of CP on apparent digestibility is due to the changing proportions of metabolic fecal protein (MFP) and undigested dietary CP. The results of their study showed that MFP is influenced by fiber content, and that fiber reduces protein digestibility. They also found that % NDF for fiber indication is highly correlated to MFP than % acid detergent fiber (ADF) and % crude fiber (CF). The high correlation of NDF to MFP makes it a good parameter in measuring fiber relationship to protein (Bell and Keith, 1989; Bell and Keith, 1988a).

The energy and fiber digestibility results for experiments for starter pigs and grower pigs have been documented. The energy digestibility of canola meal when fed as the sole source of plant protein at 16.4%, 32.9%, 49.3% and 65.8% canola meal in the diet for 9-kg pigs was at 87.3%, 83.1%, 80.0% and 76.1%, while NDF digestibility was at 58.8%, 64.9%, 63.1% and 61.1%, respectively (McIntosh and Aherne, 1985). The results for energy digestibility in this experiment significantly decreased as canola meal in the diet is increased. The pigs responded inconsistently to canola meal in the diet for NDF digestibility.

While feeding 50% of high fat low glucosinolate rapeseed meal to growing pigs at 86 kg gave 37.9% apparent digestibility for NDF (Schone et al., 1996). The energy digestibility for 15% and 30% dietary canola meal was at 77.9% and 76.5% for 45-kg pigs (Bell and Keith, 1989). It would appear that there is a trend for energy digestibility to decrease as dietary canola increases. The same inclusion levels (15% and 30%) for heavier pigs from different cultivars of canola resulted in a range of 76.4% to 80.8% energy digestibility, while NDF was at 46% to 58% digestible (Bell et al., 1998). But from the results illustrated, the NDF digestibility results seemed to vary.

Ether extract. The ether extract level of canola meal is 3.59% compared with 0.7% for soybean meal (Bell, 1993), while some results have soybean meal to be about 33% lower in ether extract (Siljander-Rasi et al., 1996). The reason for high ether extract levels in canola meal is the addition of gum back to the meal during oil refining. The gum consists of glycolipids, phospholipids and variable amounts of fatty acids, sterols and triglycerides that should be able to increase energy levels in canola meal (Clandinin and Robblee, 1981).

Ether extract digestibility for pigs at 9-kg was highest for pigs fed 65.8% canola meal at 90.3% and was lowest at 16.4% canola meal with only 72.1% digestibility (McIntosh and Aherne, 1985). On the other hand, feeding 50% of high fat low glucosinolate rapeseed meal to 45-kg pigs had a crude fat digestibility of 78.4% (Schone et al., 1996).

Minerals and Vitamins. Ash levels are generally higher for canola meal compared with soybean meal. The ash level for canola meal is 7.18% to 8.4% (Bell and Keith, 1988a; Bell and Keith, 1987) compared with 5.8% for soybean meal (Siljander-Rasi et al., 1996). Available Ca, Mn, P, Zn, Se and Mg are higher in canola meal than soybean meal, while Cu and vitamin K are lower (NRC, 1998). Compared with soybean meal, canola meal is also higher in vitamin E, riboflavin, niacin, pyridoxine, thiamin and choline but soybean meal has higher panthothenic acid and folacin (NRC, 1998).

Apparent digestibility of ash for 9-kg pigs was at 52.5% for pigs fed with 16.4% canola meal and 51.1% for those receiving 65.8% canola meal, while the 32.9% and 49.3% canola meal had lower apparent digestibility at 47.7% and 48.7%, respectively (McIntosh and Aherne, 1985). These values for ash digestibility of canola meal and soybean meal are not statistically different.

Variation for nutrient content of canola may be due to the fertility of the soil, environmental factors, cultivars used and processing conditions (Bell and Keith, 1991).

Action of Anti-nutritional Factors

Experimental results for the action of anti-nutritional factors are conflicting and not easily understood (Etienne and Dourmad, 1994). Anti-nutritional element effects are rapid, involve numerous parameters, and are completely disrupted when intake is stopped (Etienne and Dourmad, 1994). Also, presence of anti-nutritional factors influences diet acceptability and palatability, which is dictated by odor and flavor as well as longer term intake (Mawson et al., 1993). Canola meal has a few anti-nutritional factors that are considered minor problems and known to cause reductions in animal performance. But response of species to anti-nutritional factors is influenced by age, animal genetics and growth (Mawson et al., 1993).

Anti-nutritional Factors in Canola Meal

Glucosinolates. Glucosinolate decomposition to toxic compounds, such as nitriles, thiocyanates and oxazolidinethione (Belzille and Bell, 1966) may occur during processing of canola meal or in the gastrointestinal tract of the animal (Rowan et al., 1991). The toxic compounds derived from glucosinolates depend on the action of the enzyme known as myrosinase (or thioglucosidases) found in the gastrointestinal tract (Oginski et al., 1965). The naturally occurring glucosinolates in rapeseed and canola meal include gluconapin, glucobrassicanapin (Rowan et al., 1991), thiocyanate compounds, nitriles and progoitrin (Slominski et al., 1988; Etienne and Dourmad, 1994; Belzile and Bell, 1966). The breakdown of glucosinolates by myrosinase is influenced by pH, vitamin C, type of concentration, buffer employed (Oginski et al., 1965), nature of the compound and temperature (Etienne and Dourmad, 1994).

Glucosinolates are known to contribute to smell and flavor (Mawson et al., 1993). Reduced appetite occurs due to the mustard odor, lachrymatory or tear-producing effects, garlic-like and horse radish-like taste features and bitterness (Mawson et al., 1993) of glucosinolates reduced appetite occurs (Etienne and Dourmad, 1994). However, progoitrin, a non-volatile and water soluble compound, is not the actual factor of bitterness for *Brassica* but

only the potential factor since myrosinase actually produces the hydrolysis products responsible for bitterness (Fenwick et al., 1983). Oginski et al. (1965) found that progoitrin compounds are broken down by myrosinases to form goitrin. They also found that aside from goitrin, sulfur compounds are produced. These sulfur compounds are often associated with strong and distinct flavors such as bitterness (Mawson et al., 1993). The bitter taste affects palatability and may have detrimental effects on feed acceptability for animals (Mawson et al., 1993)

After 18 days of feeding in rats, the glucosinolate content in rapeseed is the identified factor for inducing anorexia (Sharpe et al., 1975).

Erucic acid. The erucic acid present in canola meal in is 1% to negligible or undetectable amounts (Ackman, 1983). However, the potential presence of erucic acid is seen as a hindrance for the use of canola for swine production since producers still consider rapeseed's and canola's nutritional and anti-nutritional factors to be the same. High amounts of erucic acid are known to affect hematological parameters of pigs. Ingestion of high erucic acid in oils of rapeseed and canola causes decreased red blood cell count, lower hemoglobin, lower packed cell volume and higher bleeding times compared with soybean oil in newborn piglets, and these effects are due to the high amounts of 18:1 n-9 fatty acid and lack of dietary saturated fatty acids when feeding canola oil (Kramer et al., 1998).

Phenolic compounds. Tannins are the main phenolic compounds present in canola attributing to an astringent, unpleasant and bitter taste. Tannins are also known to complex with protein, inhibit lipase and lipoxygenase action and inhibit iron absorption (Naczk et al., 1998). Also these compounds contribute to sense of taste and flavor especially bitterness (Mawson et al., 1995a). Other compounds that contribute to bitterness in *Brassica* sp. include saponins and sinapine esters (Fenwick et al., 1983).

Phytic acid. Phytic acid is the major source of total phosphorus for plants accounting up to 88.1%, this compound found in canola meal results in the binding of mono and divalent metal ions of phosphorus, rendering phosphorus unavailable hence decreasing bioavailability (Naczk

et al., 1998). The other minerals that can bind to phytic acid include Ca, Mn, Cu and Zn. However, in a study among broilers, the phosphorus content was efficiently utilized although canola meal had higher phytic acid (Summers and Leeson, 1985). The total phosphorus in canola meal is 1.22%, of which 0.53% is phytate bound (Bell, 1993). On the other hand, soybean meal has 0.66% total phosphorus and 0.38% is phytate bound (Bell, 1993).

Hulls. Hulls present in canola meal are also considered a hindrance in using canola meal (Naczk et al., 1998). Hulls are comprised mainly of fiber and remain in the meal representing 30% of the meal weight (Bell, 1993). In addition, tannins bind to them (Clandinin and Robblee, 1981).

Effects of Feeding Canola Meal on the Growth Performance of Pigs

Early weaned and starter pigs. Pigs weighing 10 kg to 20 kg were fed 0, 5%, 10%, 15% or 20% canola meal and results were similar for daily gain and feed intake of pigs fed up 10% canola meal. This study also showed that below 10% of canola meal in the diet had better feed to gain ratio against those fed with higher amounts of canola meal (Anderson and Van Lunen, 1984). The levels of canola meal in the diet fed by King et al., (2001) were increased and doubled in their experiment compared to Anderson and Van Lunen's (1984) acceptable level of canola meal inclusion. When 6.27 kg-pigs were fed with 5%, 7.5%, 10%, 15%, 20% and 25% of solvent extracted canola meal, results showed that no differences in feed intake when canola meal was fed up 25% from day 20 to day 62 (King et al., 2001). However, between 20 and 41 days of age and the whole experiment, feed efficiency increased as the level of canola increased due to low glucosinloate levels (supported by thyroid weights) and underestimation of the nutritive value (particularly energy) of canola meal. Also, the level of soybean meal only decreased from 5% to 3.6% from the same study (King et al., 2001). Moreover, similar weight and age of pigs were used by Baidoo et al. (1987), this time substituting soybean meal with 25%, 50%, 75% and 100% canola meal with 8.8%, 17.6%, 26.5% and 35.3% canola meal

inclusion in the diet. Their results revealed that as canola is increased, the average daily intake and gain is decreased.

Another study involving young pigs was performed by McIntosh et al. (1986) in which pigs weighing about 6 kg were fed canola meal substituted for soybean meal from one fourth to 100% replacement at 0, 9%, 18%, 27% and 36% of canola meal in the diet. This research showed that for every 1% increase in dietary canola meal, average daily intake decreased by 4 g and average daily gain decreased by 2 g. However, McKinnon and Bowland (1977) did a study with pigs about 3 wk of age at 5.3 kg using the Tower variety of canola, rapeseed and soybean meal. No significant difference was found when 11.9% of canola meal was fed as a replacement for 50% of the dietary soybean meal. However, using 25.3% of canola meal as total replacement for soybean meal revealed a significant decrease in gain and intake. A similar study in which starter pigs, averaging 8.41 kg and 3 to 4 wk of age, were fed low a glucosinolate, full fat rapeseed meal replacing full fat soybean at 0, 50% and 100% with 20% and 40% of low glucosinolate rapeseed meal revealed a significant decrease in intake, gain and feed conversion with low glucosinolate full fat rapeseed meal inclusion (Gill and Taylor, 1989).

In a preference study for starter pigs weighing 7.4 kg and about 5 wk old of age, pigs were given a choice among 0, 5%, 10%, 15% and 20% canola meal with the level of soybean meal decreasing from 26.5% to 11.5%. This study showed that pigs preferred soybean meal over the different canola meal diets and were able to detect canola meal at a level as low as 5% consuming 2.5 to 7 times more control diet (Baidoo et al., 1986).

Growing-finishing pigs. In a study by Baidoo and Aherne (1987) using 20.3-kg pigs, soybean meal in the diet was replaced with 25%, 50%, 75% and 100% of 4.6%, 9.1%, 13.1% and 19.6% canola meal. Data from this study showed that average daily feed intake and feed to

gain ratio was not affected. However, in the same study, canola meal replacing soybean meal greater than 75% showed a significantly lower gain.

In another study, pigs weighing 23 kg and 57 kg were used in which 0, 50%, or 100% of the dietary soybean meal was replaced with canola meal at 5% and 10% for grower and 2.2% and 4.0% for the finisher diets. Pigs fed these diets revealed no differences for gain, intake or feed efficiency (Bell and Keith, 1987). Bell and Keith (1988b) carried out another study with the same weight of pigs and revealed that use of soybean meal resulted in a greater average daily gain than those fed canola meal. This same study showed that canola meal fed pigs supplemented with lysine was not significantly different from those fed with soybean meal. These two studies concluded that lysine supplementation for growing and finishing stages improved daily gains. Conversely, in another experiment by Bell and Keith (1988c) with the same weight group (23 kg until finishing at 102 kg) found that pigs fed soybean meal performed better than those fed with canola meal, but lysine addition seemed to be of no significant value for overall growing-finishing period performance. Also, Bell et al. (1991) evaluated canola meal with two different canola varieties, Tobin and VLG, at 18.42% and 20.90%, respectively, to completely replace soybean meal. Their study using pigs with an initial weight of 23 kg up to 57 kg showed that soybean meal was better in terms of daily gain and daily intake compared with the two canola varieties.

Another study that used pigs weighing 25 kg to 97 kg and fed by combining 12.4% and 6.1% canola meal with 14.1% and 28.3% pea screenings, respectively, showed that animal gain and intake for canola meal resulted in no significant difference from those fed soybean meal and showed an increased growth rate as the canola meal and pea screenings increased (Castell and Cliplef, 1993). This study stated that canola and pea screenings were complementary protein sources for their barley based diet.

A study consisting of two experiments in which canola was fed to pigs at 23 kg with canola levels of 0, 5%, 10%, 15% and 20% as substitute for Australian sweet lupins resulted in no significant difference up to 20% dietary canola meal (Mullan et al., 2000). However, with the same study, and in another experiment using the same levels of canola, a linear decline with feed to gain ratio as canola levels increased was revealed.

Shelton et al. (2001) evaluated different animal and plant protein sources including 31.98% soybean meal and 48.84% canola meal inclusion in the diet for 30 kg to 114 kg pigs. Their results revealed that average daily gain was better for soybean meal on the grower and overall period. The same study also revealed that gain to feed was different in the grower and early finisher period but no differences were observed for average daily feed intake in all stages (grower, finisher and late finisher including overall performance). Also, no growth parameter differences at the late finisher period were observed in this experiment.

The Thyroid and Canola Meal

Thyroid reaction has been one of the main points of study for canola meal. Moreover, measurement of the thyroid hormone levels when canola meal is fed seems to be a necessity because of the small amount of glucosinlates in canola meal.

Thyroid and thyroid hormones. The thyroid gland is associated with iodine (Taurog and Chaikoff, 1948) and the production of thyroid hormones. The thyroid hormones account for most of the iodine needed in the body and with the accumulation of iodide in several other tissues including the salivary glands, gastric mucosa and lactating mammary gland (Spitzweg, 1998). Moreover, this fundamental property is the initial step in the production of thyroid hormones (Spitzweg, 1998).

The thyroid and thyroid hormones are influenced by plane of nutrition and both macronutrition and micronutrition can alter metabolism, secretion, synthesis and function of thyroid hormones (Danforth and Burger, 1989).

The thyroid releasing hormone (TRH) is the first hypothalamic peptide that was isolated and is distributed in the central nervous system, gastrointestinal tract, pancreas, testes and placenta (Danforth and Burger, 1989). TRH is the major stimulator and triiodothyronine (T_3) is the major inhibitor of thyroid stimulating hormone (TSH) into the peripheral circulation. It is important to note that pituitary and intracerebral conversions of T_3 are dependent on the conversion of T_4 (thyroxine) to T_3 through the enzyme 5'-deiodinase (Danforth and Burger, 1989).

 T_3 and T_4 action. The thyroid gland produces 10 times more T_4 than T_3 rendering the name prohormone for T_4 (Danforth and Burger, 1989). Also, only small amounts of T_3 are secreted from the thyroid gland when nutritional conditions are normal. The levels of T_4 are not easily affected, but fall slightly, during starvation or caloric restriction unlike T_3 , which decreases rapidly within 24 hours of fasting and remains 40-50% below normal after three days when carbohydrate levels are restricted (Danforth and Burger, 1989). Moreover, T_3 amplifies the stimulatory effect of carbohydrate on fatty acid synthesis and transcription of some key enzymes in rat liver (Danforth and Burger, 1989).

Therefore, thyroid hormones are important components of metabolic adaptations associated with changes in food intake (Danforth and Burger, 1989). In addition to the metabolic roles associated to food intake, thyroid hormones are important in regulation of animal growth and development, and are thought to regulate protein turnover and concentrations of structural cellular enzymes, membranes and organelles (Danforth and Burger, 1989).

Effects of Canola Meal and its action on Thyroid and Triiodothyronine (T_3) and Thyroxine (T_4).

General effects. Thiocyanate such as tapazole (TAP) at 0.075% and potassium thiocyanate at 0.5% compounds when fed to pigs weighing 9.5 kg to 12.4 kg increased thyroid weight and reduced protein plasma iodine (Sihombing et al., 1974). These compounds have similar actions to canola and rapeseed meal glucosinolate hydrolysis products. Moreover, glucosinolates are considered to be iodine antagonists (Schone et al., 2001).

As early as 1948, thyroid enlargement (Astwood et al., 1949) and goitrogenecity effects has been observed as a result of feeding rapeseed meal (Bell, 1993). Rapeseed meal contains 10 times the level of glucosinolates compared with canola meal (Naczk et al., 1998; Mawson et al., 1995a). Substitution of rapeseed meal with canola meal reduces but does not eliminate glucosinolates to counteract the mechanism of the thyroid. The goitrogenic effect is dependent on the species, type of glucosinolates, feeding period and total consumption of glucosinolates (Mawson et al., 1993). Furthermore, in a review by Rundgren (1983), the thyroid gland is enlarged, when canola meal is fed, although to a smaller extent compared with rapeseed meal diets.

Thyroid function is disrupted when uptake of glucosinolate occurs, reducing levels of T_3 and T_4 , which lead to reduced hormone secretion and lower circulating levels. Reduced plasma bound iodine results in a hypothyroid state (Mawson et al., 1993).

The anti-thyroidic properties are due to (1) thiocyanates and iodine competition and (2) goitrin increased iodine transfer and inhibition of synthesis and release of T_3 and T_4 . Aside from this action, the distinction of glucosinolate effects should be considered between the indirect effect (exemplified by the result of their action on the thyroid and its hormones reducing intake and performance of animals) and the direct effect (result of their effect on diet acceptability and palatability) (Mawson et al., 1993).

Effects on sows and newborn piglets. When diets containing 20% rapeseed or canola were fed to pregnant sows, thyroid hypertrophy, increased thyroid weight and low iodine in plasma in newborn pigs were observed. Glucosinolates compete with iodine to pass from the blood supply to the mammary gland, lowering T₄ levels and decreasing body weight of fetuses (Etienne and Dourmad, 1994). Similarly, increased thyroid weights and decreased T₃ and T₄ levels occurred when 17-kg pigs were fed diets with 17.5% Tower variety of rapeseed meal (Grandhi et al., 1976). Moreover, similar effects for thyroid weight and the T₃ and T₄ levels were seen for the piglets and sows used when rapeseed was mixed with potato meal (Schone et al., 1986).

Effects on early-weaned and starter pigs. Feeding young pigs (5.3 kg) with 14.1% and 31.3% Tower variety decreased both T₃ and T₄, as well as increased thyroid weight (McKinnon and Bowland, 1979). Similarly, Bowland (1975) did an earlier study on 7-kg pigs fed with 9.5% and 19.5% canola meal and revealed decreased T₄ levels and protein-bound iodine. Moreover, T₄ and protein-bound iodine decreased when 7-kg pigs were fed with 20% and 24% of the same meal (Ochetim et al., 1980b). However, Christison and Laarveld (1981) fed 15% canola meal and rapeseed meal, and found that T₄ was not significantly different due to dietary treatments for 13-kg pigs. The same study on the other hand revealed that T₃ was higher for canola meal compared with soybean meal.

Effects on growing and finishing pigs. Busato et al. (1991) fed "double zero" rapeseed meal to 37-kg pigs at 5%, 15% and 20% showed that there were no differences with thyroid weight compared with pigs fed soybean meal. The same study showed that T₃ and T₄ results were lower compared with control but were not significantly different. Also, feeding 31.5-kg pigs 5%, 10% and 15% low glucosinolates rapeseed press cake revealed that T₄ levels were decreased and thyroid weight were increased (Schone et al., 1997). Similarly, Corino et al., (1991) fed low glucosinolate rapeseed meal at 5%, 10% and 15% and found increased thyroid weight and decreased T₄ levels.

Pigs weighing 30-35 kg fed with 5% to 10% double zero rapeseed presscake meal showed that the thyroid and epithelial thyroidal DNA increased and T_3 and T_4 levels decreased with increasing levels of the meal, and that marked hypothyroidism was evident (Spiegel et al., 1993). A different result was observed by Bell et al. (1988) when 4% and 9.37% canola meal was included in diets for 23-kg and 57-kg pigs, respectively. Compared with soybean meal, no differences in T_3 and T_4 levels were observed in pigs fed canola meal.

Effects of Canola Meal on Other Species

The source of trimethylamine (TMA) in "fishy eggs" is sinapine (Clandinin and Robblee, 1981). Organisms present in ceca of hens are capable of converting the choline moiety of sinapine to TMA (Clandinin and Robblee, 1981). In reviews by Bell (1993) and Mawson (1995b), it was reported that certain strain of hens that lay brown eggs are susceptible to production of fishy taint or "fishy eggs" when they lack the liver enzyme TMA oxidase. Affected birds are unable to oxidize TMA to odorless TMA-oxide, hence, TMA is deposited in their eggs (Clandinin and Robblee, 1981). This creates problems when high yields of choline occur following breakdown of sinapine in the gut resulting in an increased TMA production, which is passed to the developing egg. Bell (1993) stated even 0.1% sinapine causes this fishy egg smell and canola meal has 0.6 to 1.8% sinapine.

In a study by Summers et al. (1989), the inclusion of 40% and 43% canola meal in a corn basal diet compared with 31% inclusion of soybean meal in a corn basal diet for chickens had a significantly lower average gain. Even though gain for canola meal basal diets when supplemented with essential amino acids improved, gain was still higher for birds fed with soybean basal diet. It was suggested that excess levels of methionine (methionine in canola meal and supplemental DL-methionine) might have caused toxicity, although this conclusion needs further verification (Summers et al., 1989). Also, with the same study, feed to gain was higher for the soybean basal diet, but no difference for average intake was observed. However,

when canola meal was fed at 25% as a partial protein source, no significant difference was observed on all growth parameters (Summers et al., 1992).

Leeson et al. (1987) found no significant differences on gain, feed intake, feed to gain ratio, digestibility of nutrients and bone ash and minerals for broilers fed 0, 9.47%, 18.95%, 28.42% and 37.89% canola meal inclusion when replacing 0 to 100% of the dietary soybean meal. Similarly, layer performance when fed 0, 6.32%, 12.64% and 25.28% canola meal as partial to full substitution to soybean meal revealed no significant differences in feed intake, egg production, egg weight and egg shell deformation (Leeson et al., 1987).

Dairy cattle fed with a low glucosinolate, low erucic acid rapeseed or high glucosinolate, low erucic acid rapeseed meal or soybean meal revealed no significant differences among serum amino acid, blood chemistry and hematological parameters (Laarveld and Christensen, 1976). Similarly, for the same study, no indication of hypothyroidism or reduced intake was observed. Iwaarson et al. (1973) showed that when high glucosinolate rapeseed was fed to growing bulls, chemical analyses of blood revealed no significant differences, but thyroid weight was increased compared with soybean meal. In this study, all animals were euthyroid and the animals fed rapeseed meal had better weight gain, carcass weight and a thicker layer of fat (Iwaarson et al., 1973).

Summary

Canola meal is a good plant protein source for animal diets because of its comparable nutritional characteristics to soybean meal. However, some feed users have expressed biased opinions and misconceptions on the toxicity and palatability of canola meal. Palatability of rapeseed meal is generally lower because of its high erucic acid and high glucosinolate levels. But, this concern is not valid for canola meal which is a superior and a better cultivar wherein both the anti-nutritional element levels, if not negligible, are greatly reduced.

Failure for the optimum performance of animals fed with canola meal is due to lack of familiarity with the nutrient composition and digestibility of canola meal as a feed protein source. The need to supplement energy and lysine for canola meal is a necessity since the meal is relatively low in these two nutrients, thus supplementation will bring the meal's potential benefits to a maximum.

Effects of canola meal on pigs seemed to be varied. Sows, and especially their fetuses and newborn piglets, are affected by low amounts of canola meal in their diet. However, nursery and starter pigs, as well as, grower and finisher pigs, respond inconsistently to canola meal in the diet. The nature of different responses on growth and thyroid hormone levels when feeding canola meal to animals could be due to the very small amounts of anti-nutrients, fiber content, different cultivars, processing of the meal and environmental factors.

Generally, pig responses are better if canola meal is given as a partial plant protein source substitute initiating a synergistic action and complementing other feed ingredients in the diet.

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Table 2.1. Chemical Composition of Canola Meal and Soybean Meal ^a.

Component	Canola Meal	Soybean Meal
Dry Matter, %	90	90
Crude Protein, %	35.6	47.5
Ether Extract, %	3.5	3.0
Neutral Detergent Fiber, % Digestible Energy, kcal/kg	21.2 2885	8.9 3685
Ash ^b , %	8.4	7.67
Minorala		
Minerals Phosphorus, %	1.01	0.69
Calcium, %	0.63	0.34
Potassium, %	1.22	2.14
Magnesium, %	0.51	0.30
Sulfur, %	0.85	0.44
Sodium, %	0.07	-
Copper, mg/g	6.0	20
Iron, mg/g	142	176
Manganese, mg/g	49	36
Selenium, mg/kg	1.10	0.27
Zinc, mg/kg	69	55
Vitamins, mg/kg_		
Vitamin E	14.5	2.3
Panthothenic Acid	9.5	15.0
Niacin	160	22
Choline Riboflavin	6700 5.8	2731 3.1
Biotin	0.98	0.26
Folacin	0.83	1.37
Pyridoxine	7.2	6.4
Thiamin	5.2	3.2

^a NRC (1998). ^b Bell et al. (1998) for soybean meal and Bell and Keith (1987) for canola meal.

Table 2. 2. Amino Acid Composition of Canola Meal and Soybean Meal ^a.

Amino Acids, %	Canola Meal	Soybean Meal	
Arginine	2.21	3.48	
Histidine	0.96	1.28	
Isoleucine	1.43	2.16	
Leucine	2.58	3.66	
Lysine	2.08	3.02	
Methionine	0.74	0.67	
Cysteine	0.91	0.74	
Phenylalanine	1.43	2.39	
Tyrosine	1.13	1.82	
Threonine	1.59	1.85	
Tryptophan	0.45	0.65	
Valine	1.82	2.27	

^aNRC (1998).

CHAPTER 3

EVALUATION OF CANOLA MEAL AS AN ALTERNATIVE PLANT PROTEIN SOURCE IN NURSERY PIG DIETS ¹

¹Ele, J.J.G., C. R. Dove and M. J. Azain. To be submitted to the *Journal of Animal Science*.

Abstract

Two experiments using 400 nursery pigs were conducted to evaluate canola meal (CM) in nursery diets on growth performance, thyroid hormone status and digestibility of nutrients. Experiment 1 used 384 nursery pigs (6.16 kg at 18 d) in three trials to determine the effects of CM as a replacement for dietary soybean meal (SBM). The levels of CM used to replace SBM were at 33%, 66% and 100% of CM at post weaning, for phase I (d 0 to d 11) and phase II (d 11 to 35) diets. During phase I, pigs fed 33% or 66% CM were not significantly different (p>0.10) from control. Pigs fed 100% CM gained less than other treatments (p<0.001). Gain to feed had a trial by treatment interaction due to low response of trial 3 for phase I. However, the trend for the 33% and 100% CM substitution are the same. For phase II, significant differences (p<0.01) for gain and feed intake (p<0.01) were seen. The 33% CM was numerically higher but was not significantly different from control at d 21 to d 35. As CM increased beyond 33%, gain and feed intake decreased. However, no significant difference (p>0.10) was observed for feed efficiency. No thyroid hormone differences were observed among treatments. The levels of triiodothyronine (T_3) and thyroxine (T_4) were similar in all treatments. In Exp. 2, using 16 barrows (8.83 kg at 37 d) to determine nutrient utilization, digestibility parameters showed statistical differences in energy, CP and nitrogen, ash, DM (p<0.001) and NDF (p<0.05) among treatments. The control and 33% CM were significantly better in nutrient utilization of energy, CP and nitrogen, ash and DM except for NDF. NDF digestibility in 33% CM was significantly different (p<0.05) from 66% CM but was not significantly different (p>0.10) from the control and 100% CM. In conclusion, these studies demonstrate that canola meal can partially replace soybean meal in nursery pig diets if used at no greater than 13% in the diet.

Introduction

Protein feed sources are becoming limited and expensive for animal use, increasing the need to identify alternative protein sources (Brand et al., 1999). One of the alternative plant protein sources identified is canola meal (CM) (Shelton, 2001). Canola meal is a protein supplement that contains most B-complex vitamins and essential nutrients but available energy and lysine are limiting factors (Bell, 1993). Canola meal can be used as the sole source of protein for finishing pigs (Clandinin and Robblee, 1981). However, feeding CM to younger pigs has been an issue. The palatability of CM (Baidoo et al., 1986), the presence of glucosinolates (McKinnon and Bowland, 1977; Ochetim et al., 1980; McIntosh et al., 1986) and high fiber (McIntosh and Aherne, 1985) have led to reduced feed intake, consequently leading to poor performance. The amount of CM inclusion for starter pigs has been varied. Recommendations have stated that CM can be fed as high as 25% for pigs weighing 6 kg (King et al., 2001) and as low as 5% for 7-kg pigs (Baidoo et al., 1987).

The low digestibility of CM has been due to fiber (Bell and Keith, 1989), sinapine and glucosinolates (Schone et al., 1996). Aside from reduced digestibility, the glucosinolates in CM are also related to an increase in thyroid weight (Bourdon and Aumaitre, 1990). The effects of CM on triiodothyronine (T₃) and thyroxine (T₄) are conflicting. Some state that T₃ and T₄ tend to decrease with CM addition (McKinnon and Bowland, 1979; Christison and Laarveld, 1981) while others claim that both thyroid hormones are unaffected by CM supplementation (Bell et al., 1988).

The present study was performed to (1) evaluate growth performance and determine acceptable levels of inclusion of CM, (2) determine the effect of CM on thyroid hormones (T_3 and T_4) and (3) determine the digestibility of the diets with varying levels of canola meal on nursery pigs.

Materials and Methods

General. Protocols for the experiment were approved by the University of Georgia Institutional Animal Care and Use Committee. Canola meal used in both studies was obtained from one source (Cal-Maine Feed Mill, Gainesville, GA). Canola meal and mixed diets were analyzed for dry matter, ash, CP, NDF, nitrogen and energy. Moisture and ash content were performed. Nitrogen content was determined using the nitrogen analyzer (LECO FP-528, LECO Corp., St. Joseph, MI) for the calculation of CP. Gross energy determination was performed on a bomb calorimeter (Parr 1261, Parr 1563, Parr Instrument Co., Moline IL). In addition, digestible energy was calculated (Adeola, 2001) and NDF (ANKOM, 220, Fairport, NY) were calculated. The experimental diets for phase I and phase II (Table 3.2 and 3.3) used were variations of the University of Georgia starter diets. The levels of canola meal used for the two experiments were at 0, 33%, 66% and 100% substitution of soybean meal on a CP basis in the nursery pig diets for both phases of the experiment. Animal protein source amounts were maintained for both phases. Corn levels were decreased as fat was increased to maintain similar energy composition among treatments. Lysine levels in the diet were increased as the level of fat increased to maintain similar ratio of energy to lysine. The diets for the phase I were in pellet form while those in phase II were in meal form.

Experiment 1. The experiment was conducted at the University of Georgia Swine Center. A total of 384-pigs (PIC C42 x 280) were used for three trials. Each trial consisted of 128 pigs. Average weaning age of the trials was approximately 18 d with an initial average weight of 6.16 kg raised up to a final average weight of 19.6 kg at 53 d of age. Pigs were housed in an environmentally controlled, totally slatted nursery with pen dimensions of 1.22 m x 2.84 m and with an average temperature of 27.8°C. Pigs were alloted on bases of sex, ancestry and weaning weight to one of the four experimental diets. All three trials were distributed to 16 pens with 8 pigs per pen, with an equal male to female ratio within each replication. Pigs were fed with the phase I diet from d 0 to d 11. At d 11, pig body weights were determined and the

feeders were emptied to measure the feed intake. From d 11 to d 35 phase II diets were fed. The same procedures for measuring body weights and feed intake was done for d 21 and d 35.

At d 35, all pigs from trial 2 and trial 3 were bled through the intra-orbital sinus to collect blood samples (Huhn et al., 1969). Collected blood samples were centrifuged and blood serum was removed and was kept frozen until analyzed. Blood samples were analyzed for triiodothyronine (T_3) and thyroxine (T_4) (RIA¹²⁵I, MP Biomedicals, Inc; NY).

Experiment 2. The digestibility trial was conducted at the Large Animal Research Unit of the University of Georgia. A total 16 barrows (PIC C42 x 280), weighing an average of 8.83 kg at 37 d raised up to a final average weight of 25.2 kg at 69 d of age, were individually housed in stainless steel metabolism crates measuring 0.71 m H x 0.59 m W x 0.81 m D. The four treatments were similar to the phase II treatment diets except for the addition of 0.10% chromic oxide used that was used as a digestibility marker (Table 3.4). Diets were fed in meal form at a level approximately 4% of body weight in two equal amounts at 0800 h and 1600 h. Water was supplied ad libitum from a low pressure nipple drinker. Pigs were allotted to one of the four dietary treatments following a replicated 4 x 4 Latin Square Design. A 12-hour lighting system was followed starting at 0700h and ending at 1900h. Temperature was maintained at 27.2°C. The 32-day study consisted of four periods with 8 days per period. Pigs were allowed to adjust to the diet for the first 5 days and feed refused after each feeding was taken to avoid contamination of feces. Feces were collected twice daily and weighed for the next 3 days of each experimental period. Fecal samples were kept frozen until subjected to analysis. At the end of each collection period, fecal samples were combined for each individual pig for the 3-day collection period. Fecal samples were thoroughly mixed then freeze dried (Labconco, Kansas, MO) and finely ground and kept in tightly covered small plastic bottles to avoid moisture entry. Crude Protein, DM, NDF, energy, ash and nitrogen were analyzed for both treatment diets and feces using procedures mentioned previously. Fecal and treatment diet samples were digested with a sodium molybdate-perchloric acid mixture until color change was evident. Chromic oxide

content was determined using a UV spectrophotometer through assessment of sample absorbance (UV160U, Shimadzu Corp., Columbia, MD) (Fenton and Fenton, 1979).

Statistical Analysis. Data were analyzed using the PROC-MIXED (Generalized Least Square) of SAS (SAS Institute, Inc., Cary, NC). Experiment 1 was analyzed by randomized block design with four treatments using pen as the experimental unit. Experiment 2 was a 4 x 4 Latin Square Design using individual pig as the experimental unit. Differences were considered significant at p<0.05. Treatment differences were separated by differences of least square means.

Results

Canola Meal Analysis. Chemical analyses of CM and SBM are presented in Table 3.1. All values are reported in dry matter basis. CM had 89.42% DM, which is similar to SBM. Energy results revealed 4501 kcal/kg for CM which is 4% less than SBM. Ash for CM was higher by almost 7% at 8.74%, while NDF of CM is 2.46 times that of SBM. Crude protein of CM was at 35.52% which is only 72% that of SBM.

Experiment 1. The effects of CM in phase I and phase II diets on performance of nursery pigs are presented in Table 3.5. Daily gain from d 0 to d 11 (phase I) was similar among pigs fed 33% and 66% CM. However, pigs fed 100% CM had a significantly decreased gain (p<0.001) compared with the control and 33% CM. Moreover, at d 11 to d 21, daily gain showed a significant difference (p<0.01) among treatments. During this time period, daily gain was highest for pigs fed on control diet and continually decreased as the CM was increased. From d 21 to d 35, daily gain values were significantly lower (p<0.01) from control for pigs fed all diets containing CM. During phase II (d 11 to d 35) control, 33% and 66% CM substitution was significantly different (p<0.01) from full SBM replacement (100% CM). Overall, d 0 to d 35 highest gain was obtained by 33% CM and was significantly different (p<0.001) only from 100%

CM. Trial by treatment interactions were seen in d 11 to d 21 and overall performance (d 0 to d 35) due to different pig response within trials (Appendix A.4).

For feed intake, only the treatment with 100% CM substitution showed a trend and was lower than the other levels of CM substitution for d 0 to d 11 (p=0.10). Intake was highest in control fed pigs and gradually decreased as CM level increased. For d 11 to d 21, significant differences (p<0.01) were observed with the control having the highest feed intake, with intake gradually decreasing with increasing CM inclusion. The lowest value for intake was obtained at 100% CM substitution. For d 21 to d 35, treatment with 33% CM level of substitution was significantly higher than the control (p<0.01) as well as the 66% and 100% CM substitutions. Moreover, 100% CM substitution was lowest among the treatments. For the whole phase II (d 11 to d 35), no significant difference was observed for the control and 33% level of substitution but the 33% CM was significantly higher from the 66% CM. Also, there was no significant difference observed for the control and 66% CM. However, the control and 33% CM substitution were significantly different (p<0.01) from 100% CM. Finally, the overall performance (d 0 to d 35) revealed that a significant reduction (p<0.01) in intake was found when the 100% CM level of substitution to SBM was fed. The other treatments were not significantly different from each other. However, d 11 to d 21 showed a trial by treatment interaction. This is due to the different response of the trials (Appendix A.5).

For d 0 to d 11, a significant difference (p<0.001) was observed for gain to feed ratio. There were no differences for control and 33% CM substitution. However, 33% CM was significantly different from the 66% CM substitution and 100% CM substitution. Moreover, the lowest value of gain to feed ratio was obtained by 100% CM substitution. No differences (p>0.10) were observed for feed efficiency during phase II or the over the 35-d experiment. Trial by treatment interaction was observed for phase I due to low intake values of trial 3. However, careful examination shows that although there were low values of intake compared with the first

two trials, a similar response was seen in that with increasing CM inclusion, gain decreases (Appendix A.6).

The effect of CM inclusion on T_3 and T_4 is presented in Table 3.6. The results for T_3 and T_4 revealed no significant differences among treatments. The T_3 values, although not significantly different, were highest for the 100% CM followed by the 66% CM. Moreover, lowest T_3 was obtained in 33% CM and the control had a higher amount compared with 33% CM by less than 7 units. The T_4 levels, although not significantly different, showed that the levels of CM with 33%, 66% and 100% replacement for SBM were higher than the control. Results for T_4 seemed to show that T_4 is increasing with increasing CM levels.

Experiment 2. Digestibility of CM in phase II diets is presented in Table 3.7. All the digestibility results are presented on a DM basis. The performance parameter results revealed no significant difference (p>0.10) in average daily gain, feed intake and gain to feed across treatments in the digestibility trial. All the values for gain, intake and gain to feed, although not significantly different across treatments, were generally highest in the 33% CM. The nutrient utilization for CP and nitrogen results were similar for control and 33% CM but significantly lower (p<0.001) for 66% CM and 100% CM. The energy digestibility was similar for the control and the 33% CM but was significantly lower (p<0.001) from the 66% CM and the 100% CM. Ash and DM results were not significantly different among 66% CM and 100% CM, but these levels were significantly lower for ash (p<0.001) than the control and 33% CM. The digestibility of NDF is significantly different (p<0.05) only between 33% CM and 66% CM. Also, the NDF digestibility of the control, 33% CM and the 100% CM were not significantly different (p>0.10) from each other. However, NDF digestibility was highest for 33% CM than NDF digestibility of the control.

Overall results were highest for the control, although not significantly different from 33% CM, than the 66% CM and 100% CM in nutrient utilization parameters including CP, energy, DM, ash and nitrogen except for NDF. The digestibility results for CP, energy, DM, ash and

nitrogen of the diets were decreased when above 33% CM replacement for SBM was fed to the pigs.

Discussion

Canola Meal Analysis. The nutrient composition of canola meal was similar to previously reported values. Dry matter value for the CM used in this experiment is within 1% to 4% of values previously reported (Bell et al., 1991; Bell et al., 1988). Ash and CP results had a negligible difference to those reported by Bell and Keith (1987) and Bell et al. (1991). Gross energy value was higher by 86 kcal/ kg in this experiment compared with results by Bell et al. (1998). Finally, NDF values are slightly higher in this experiment compared with those reported by Bell et al. (1991). The same authors also showed that NDF values for two varieties of CM had increased in fiber value by 11% to 12% in less than a year span. However, the result in this experiment is less than 1% lower than NDF value of CM reported by Slominski and Campbell (1990). Soybean meal has 48% CP, 7.1% NDF and 90% DM (Bell, 1993) and 4129 kcal/kg gross energy and 7.67% ash (Bell et al., 1998). The results for SBM analysis used in these experiments were similar to these values reported. Dry matter and CP were within 1% to 4% of the reported values. Energy, ash and NDF values of SBM used in these experiments were 12%, 21% and 33% less, respectively, than values in the literature. Fiber value was greater in CM than in SBM, which agrees with the analyzed results in these experiments and the literature values reported. Energy, ash and DM were comparable for CM and SBM both for the results of the analysis and those reported in literature. Crude protein is greater for SBM than CM. These chemical analyses results deemed to make CM comparable to SBM.

Slight differences in nutrient composition of CM can be due to differences in soil fertility, cultivar composition, processing of canola seed and environmental factors during seed development (Bell and Keith, 1991).

Experiment 1. Pig performance when SBM was replaced with CM in the diet was similar to the work of McKinnon and Bowland (1977), wherein partial replacement had no significant difference from the control treatment. However, unlike McKinnon and Bowland's (1977) results for growth performance, inclusion of 100% CM replacement (full replacement of SBM with CM), for this experiment was significantly decreased from the control treatment. However, the highest percentage of CM used by McKinnon and Bowland (1977) when substituting for SBM was 25.3% (full or 100% replacement for SBM in the diet). This value is slightly higher than the 22.9% of CM used in the 66% CM substitution diet in this experiment. While the full replacement for the two phases in this experiment are 27% to 37% higher. Decreased feed intake and gain above 66% CM substitution for SBM observed in this experiment, except for d 11 to d 21, is similar to Gill and Taylor (1989). Also, the levels of 20% and 40% CM in the diet used for their experiment is almost equal to the values used in this study. Gill and Taylor (1989) suggested that tolerance may be achieved at lower rates of inclusion, and that addition within tolerable amounts would reduce detrimental effects on intake and palatability.

Furthermore, glucosinolates (Mawson et al., 1993; Gill and Taylor, 1989; Baidoo et al., 1986), thiocyanate (Christison and Laarveld, 1981), sinapine and tannins (Corino et al., 1991; Naczk et al., 1998), phytic acid (Bell, 1993) and fiber (Baidoo et al., 1986; Baidoo and Aherne, 1987) alone or in combination (Baidoo et al., 1986) and bitterness (Spiegel and Blum, 1993; Mawson et al., 1993) may have contributed to the reduced feed intake in this experiment except for d 11 to d 21.

Only the first 11 d of feed intake and gain results agree with McIntosh et al. (1986) and Baidoo et al. (1987) in that as levels of CM increase, gain and intake decrease. Similarly, Bowland (1975) stated that the first 3 weeks might be expected to be the most critical period for a 10-week test period.

Interestingly, even though it is not statistically different, the pigs fed 33% CM replacement outperformed the control pigs starting at 21 d until 35 d. This could be related to

complementary action of nutrients present in CM and SBM. This synergistic action is similar to the CM and pea screenings diet response of growing pigs by Castell and Cliplef (1993).

The trial by treatment interaction on gain to feed on d 0 to d 11 is due to reduced gain and low feed intake in trial 3, but the trend for the 33% CM and 100% CM substitution for SBM are the same. On the other hand, trial by treatment interaction for gain and feed intake at d 11 to d 21 is mainly due to the different response of treatment 4 on trial 2, thereby indicating no significant differences among treatments. This trial by treatment interaction at d 11 to d 21 was carried out the responses for gain in d 0 to d 35, causing trial by treatment interaction as well.

One possible reason for this different response of treatment with 100% CM substituting SBM in trial 2 may be due to the gastrointestinal reaction and its action on intact glucosinolates (Slominski et al., 1988). These authors stated that different responses to the level of intact glucosinolate breakdown depend on the gastrointestinal tract action. Mawson et al., (1993) found that glucosinolates affect palatability and intake due to its bitterness. These glucosinolates would occur as progoitrin, which is not the actual factor of bitterness (Fenwick et al., 1983). The actual indicators of bitterness are the products produced when myrosinase acts on progoitrin (Fenwick et al., 1983). These products include oxazolidinethione (Fenwick et al., 1983) and sulfur compounds (Mawson et al., 1993), which are associated with strong and distinctive flavors (Mawson et al., 1993). More likely, in this experiment, the small levels of progoitrin present in CM were hydrolyzed by the intestinal tract to a small extent, making the feed more palatable and increasing feed intake in pigs fed with 100% CM substitution to SBM.

Secondly, the nutrient composition of CM especially its energy utilization component may be underestimated (King et al., 2001). In a study by King et al. (2001), weaned pigs fed with 25% CM diet had significantly better (p<0.05) feed conversion compared with the control pigs. Moreover, their feed intake result for 25% CM inclusion was comparable to the control, and growth rate was higher than the control although not statistically different. These results

were similar to trial 2 for this experiment. Thirdly, the fiber in CM is associated with reduced digestibility, although fiber bound CP in CM is partially digestible (Bell et al., 1998). The partial digestibility of protein-bound NDF may be proven by the result of NDF digestibility in this study.

T₃ and T₄ responses are very similar to results of Christison and Laarveld (1981) and McKinnon and Bowland (1979) when early weaned and starter pigs were fed CM as the partial or full replacement with soybean meal. Their thyroid hormone responses to CM were slightly higher T₃ levels, which were not significantly different from SBM, similar to this experiment. However, earlier work of Bowland (1975) stated that full replacement of SBM with CM caused significantly lower T₄ values. The T₄ levels for this experiment fall within the T₄ range reviewed by McKinnon and Bowland (1979), although 100% CM level for SBM substitution was 9% higher. Schone et al. (1997a) stated that T₃ is derived from T₄ and that elevated T₃ concentrations occur when deficiencies of iodine and anti-thyroid agents are present. Although less than 30 µmol of alkenyl glucosinolates per gram of oil-free CM are present in CM (Bell, 1993), these are still classified as iodine antagonists (Schone et al., 2001). Since treatment diets were similar among treatments, iodine deficiency for slightly increased T₃ for 66% and 100% CM to substitute SBM seemed not to be the case. Danforth and Burger (1989) stated that micronutrition can alter synthesis, function and secretion of thyroid hormones (Danforth and Burger, 1989). Hence, micronutrition and anti-thyroid agents may explain the slightly higher levels of the T₃ for 66% and 100% CM substitution for SBM in this experiment.

Thyroid hormones are inadequate parameters for anti-thyroidal effects of CM, since results of different experiments in feeding CM seemed to be inconsistent and confusing, due differences in dietary levels, sources and processing of CM including environmental conditions (Christison and Laarveld, 1981). This seemed to be the case for this experiment. In addition, a study by Dauncey et al. (1983), accounting other possibilities, suggests that even eating a meal and energy intake may induce increased T₃ and T₄. Lastly, Schone et al. (1997b) stated that in

case small amounts of anti-thyroid compounds are present, the minute quantity of iodine found in dietary grain and other plant ingredients is capable of suppressing hypothyroidism.

Experiment 2. The results for the digestibility of nutrients indicate that the control and 33% CM level of substitution to SBM were better than the 66% and 100% substitution diets. The digestibility for DM and energy was similar to the work of McIntosh and Aherne (1985) and Bell and Keith (1989) in that exceeding the amount of CM in the diet above 16.4% reduces the digestibility. However, nitrogen utilization in this experiment was opposite to the results of McIntosh and Aherne (1985) in that as the level of CM is increased nitrogen utilization also increased. Results for CP in this experiment were comparable to the work of Bell and Keith (1989), who found that as CM increased, digestibility decreased. Likewise, CP results agree with the results of Stein et al. (1999), that SBM as the sole supplement of protein had better digestibility compared with use of CM as a source. Also, the results obtained for CM digestibility for CP in this experiment were higher than those reported by Fan and Sauer (1995).

NDF digestibility varied from treatment to treatment. This may partially be related to findings of Slominski and Campbell (1990), who concluded that NDF gives a better total fiber estimate since some of the non-starch polysaccharides present in CM may be partially digested by the microbial action in the lower gut (Bell, 1993). Similarly, Bell et al. (1998) emphasized that some protein-bound fiber for *Brassica* meals may be partially digested by pigs.

Results for ash digestibility were different from those of McIntosh and Aherne (1985) since results were not linearly dropping as CM increased. A possible reason for decreased digestibility of ash or minerals above the 33% CM is phytic acid content. Bell (1993) and Naczk et al. (1998) stated that phytic acid which stores phosphorus in the canola seed, can bind to other minerals such as Ca, Mn, Cu and Zn. If minerals bind to phytic acid, their bioavailability is reduced (Bell, 1993; Naczk et al., 1998).

Lenis et al. (1996) found that fiber is capable of reducing nutrient digestibility due to diminished absorption of nutrients. Moreover, these authors determined that addition of purified

NDF (from wheat bran) or fibrous product in growing pigs increased the flow of DM and nitrogen, thereby reducing digestibility. Furthermore, Lenis et al. (1996) stated that digestibility of amino acids is reduced from 2% to as high as 18%. The results for the CP and nitrogen digestibility of CM in this experiment confirm the findings of Lenis et al. (1996) that increased fiber causes decreased digestibility. Results of the canola meal digestibility diets and canola meal experimental diets in phase I and phase II in these experiments indicate this increase in diet NDF as CM is increased (Appendix A.1 and A.2). Likewise, this is further substantiated by Moeser et al. (2002) through use of corn grain with 6.7% NDF compared with 3.4% NDF of degermed and dehulled corn. Their results equated to significant increase in DM, energy and nitrogen digestibility by 13%, 15% and 7%, respectively. Furthermore, Imbeah and Sauer (1991) evaluated CM rate of passage compared with SBM and found that CM had a faster rate of passage. These results were attributed to fiber contributing to a lower digestibility of protein.

Implications

Conclusions of these studies suggest that CM can be used as a partial replacement for SBM for nursery pigs. Incorporation of CM should be fed at less than 13% of total diets of weanling pigs. Decreased palatability and increased fiber in CM causes decreased feed intake and lower digestibility of nutrients. The use of CM as an alternate plant protein source at an appropriate level has the potential to complement other feed ingredients and helps in maximizing pig performance.

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Table 3.1. Chemical Analysis of Canola Meal and Soybean Meal (dry matter basis).

	Canola Meal	Soybean Meal
Dry Matter, %	89.42	89.07
Ash, %	8.74	6.09
CP, %	35.52	50.09
Gross Energy, kcal/kg	4501	4648
NDF, %	29.82	12.12

Table 3. 2. Composition of Canola Meal Nursery Pig Diets (Exp.1- Phase I).

Treatment	Control	33%	66%	100%
Experiment 1				
Phase I				
Ingredient, %				
Corn	42.19	35.61	29.05	22.48
Soybean Meal	18.75	12.50	6.25	-
Canola Meal	-	11.45	22.90	34.35
Whey	27.50	27.50	27.50	27.50
Fish Meal	3.00	3.00	3.00	3.00
Fat	1.00	2.50	4.00	5.50
SD Blood Cells	5.00	5.00	5.00	5.00
Dicalcium Phos	1.10	1.05	0.95	0.90
Limestone	0.55	0.45	0.40	0.30
Zinc Oxide	0.37	0.37	0.37	0.37
Vitamin Premix ^a	0.25	0.25	0.25	0.25
Mineral Premix b	0.15	0.15	0.15	0.15
Methionine	0.09	0.09	0.09	0.09
Lysine	0.05	0.08	0.09	0.11
Calculated Composition				
CP, %	21.11	21.02	20.89	20.79
Lysine, %	1.40	1.41	1.40	1.40
Threonine, %	0.99	1.00	1.02	1.04
Tryptophan, %	0.25	0.25	0.25	0.25
Methionine, %	0.45	0.45	0.45	0.45
Ca, %	1.15	1.15	1.16	1.16
Total P, %	0.83	0.87	0.90	0.95
Digestible P, %	0.60	0.61	0.60	0.60
ME, kcal/kg	3331	3320	3309	3298

^a The vitamin premix provided the following per kg of complete diet: 11000 IU of vitamin A, 1650 IU of vitamin D, 44 IU of vitamin E, 44 ug of vitamin B_{12} , 4.4 mg of menadione, 55 mg of niacin, 33 mg of panthothenic acid, and 9.9 mg of riboflavin. ^b The mineral premix provided the following per kg of complete diet: 16.5 of copper, 165 mg of iron, 39.6 mg of manganese, 165 mg of zinc, 0.3 mg of iodine and 0.3 mg of selenium.

Table 3. 3. Composition of Canola Meal Nursery Pig Diets (Exp.1 - Phase II).

Treatment	Control	33%	66%	100%
Experiment 1				
Phase II Ingredient, %	58.57 21.75 - 10.00 3.00 1.00 2.50 1.74 0.59 0.25 0.25 0.15 0.11	51.13 14.50 13.29 10.00 3.00 2.50 2.50 1.65 0.49 0.25 0.25 0.15 0.11	43.77 7.25 26.57 10.00 3.00 4.00 2.50 1.55 0.39 0.25 0.25 0.15 0.11 0.21	36.41 - 39.85 10.00 3.00 5.50 2.50 1.47 0.28 0.25 0.25 0.15 0.11 0.23
Calculated Composition CP, % Lysine, % Threonine, % Tryptophan, % Methionine, % Ca, % Total P, % Digestible P, % ME, kcal/kg	20.39 1.31 0.86 0.23 0.46 1.00 0.84 0.57 3335	20.29 1.31 0.87 0.23 0.47 1.00 0.89 0.56 3311	20.19 1.32 0.89 0.23 0.47 1.00 0.93 0.56 3288	20.08 1.31 0.91 0.23 0.48 1.00 0.98 0.56 3264

a,b See Table 3.2

Table 3. 4. Composition of Canola Meal Nursery Pig Diets (Exp.2).

Treatment	Control	33%	66%	100%
Phase II				
Ingredient, %				
Corn	58.47	51.08	43.73	36.37
Soybean Meal	21.71	14.49	7.24	-
Canola Meal	-	13.28	26.54	39.81
Whey	9.98	9.99	9.99	9.99
Fish Meal	2.99	2.99	2.99	2.99
Fat	1.00	2.49	3.99	5.49
SD Blood Cells	2.49	2.49	2.49	2.49
Dicalcium Phos	1.74	1.64	1.54	1.47
Limestone	0.59	0.49	0.39	0.28
Zinc Oxide	0.25	0.25	0.25	0.25
Vitamin Premix ^a	0.25	0.25	0.25	0.25
Mineral Premix b	0.15	0.15	0.15	0.15
Methionine	0.11	0.11	0.11	0.11
Lysine	0.16	0.18	0.21	0.23
Chromic Oxide ^c	0.10	0.10	0.10	0.10
Calculated Composition				
CP, %	20.39	20.29	20.19	20.08
Lysine, %	1.31	1.31	1.32	1.31
Threonine, %	0.86	0.87	0.89	0.91
Tryptophan, %	0.23	0.23	0.23	0.23
Methionine, %	0.46	0.47	0.47	0.48
Ca, %	1.00	1.00	1.00	1.00
Total P, %	0.84	0.89	0.93	0.98
Digestible P, %	0.57	0.56	0.56	0.56
ME, kcal/kg	3335	3311	3288	3264

a,b See Table 3.2

^c Treatment diets used in Exp.1- phase II are identical to Exp. 2 except that chromic oxide is used as a marker to determine digestibility.

Table 3. 5. Effect of Canola Meal in Phase I and Phase II Diets on Nursery Pig Performance.¹

Treatment ²	Control	33%	66%	100%	SEM	Р	Trl x Trt
Gain, g/d							
Wean-Day 11	212 ^a	210 ^a	198 ^{ab}	178 ^b	7.14	0.001	NS
Day 11-Day 21	359 ^a	330 ^{ab}	314 ^{bc}	298°	10.72	0.01	0.02
Day 21-Day 35	525 ^a	575⁵	554 ^b	522 ^a	9.64	0.01	NS
Day 11-Day 35	456 ^a	473 ^a	454 ^a	429 ^b	7.49	0.01	NS
Day 0-Day 35	379 ^{ab}	390 ^a	373 ^b	350°	5.65	0.001	0.03
Feed Intake, g/d							
Wean-Day 11	300 ^a	293 ^a	295 ^a	275 ^b	7.02	0.10	NS
Day 11-Day 21	589 ^a	555 ^{bc}	532 ^{cd}	507 ^d	13.77	0.01	0.03
Day 21-Day 35	853 ^b	896 ^a	862 ^b	822 ^c	12.68	0.01	NS
Day 11-Day 35	743 ^{ab}	754 ^a	724 ^b	691°	11.81	0.01	NS
Day 0-Day 35	603 ^a	610 ^a	589 ^a	560 ^b	9.17	0.01	NS
Gain:Feed, g/kg							
Wean-Day 11	693 ^{ab}	702 ^a	663 ^b	606 ^c	13.66	0.001	0.01
Day 11-Day 21	607	593	591	586	13.77	NS	NS
Day 21-Day 35	618	642	643	639	8.57	NS	NS
Day 11-Day 35	616	628	628	623	6.26	NS	NS
Day 0-Day 35	630	641	634	624	5.54	NS	NS

¹Results represent least square means for a total of 12 pens per treatment (with 8 pigs per pen). ²Means within a row lacking a common superscript letter differ at respective p-level.

Table 3. 6. Effect of Canola Meal Inclusion on Triiodothyronine (T3) and Thyroxine (T4) Levels. 1

Treatment ²	Control	33%	66%	100%	SEM	Р	Trl x Trt
T3, ng/dl	136.72	129.35	141.51	146.42	6.52	NS	NS
T4, μg/dl	4.33	4.66	4.56	4.93	0.20	NS	NS

¹Results represent least square means for a total of 8 pens per treatment (with 8 pigs per pen)-Trial 2 and 3. ²Means within a row lacking a common superscript letter differ at respective p-level.

Table 3. 7. Digestibility of Canola Meal in Phase II Diets. 1

Treatment ²	Control	33%	66%	100%	SEM	Р
Gain, g/d Intake, g/d Gain:Feed, g/kg	583 857 670	590 877 670	580 863 664	571 854 670	19.21 18.62 13.42	NS NS NS
Dry Matter,% CP,% Nitrogen,% Energy, kcal/kg Ash NDF	86.04 ^a 83.58 ^a 82.61 ^a 86.23 ^a 56.56 ^a 40.65 ^{ab}	85.24 ^a 82.55 ^a 81.49 ^a 85.79 ^a 53.49 ^a 42.76 ^b	80.99 ^b 80.13 ^b 79.00 ^b 81.85 ^b 45.54 ^b 36.07 ^a	79.36 ^b 79.25 ^b 78.00 ^b 81.51 ^b 42.61 ^b 41.79 ^{ab}	0.43 0.65 0.67 0.49 0.98 1.99	0.001 0.001 0.001 0.001 0.001 0.05

¹Results represent least square means for a total of 16 barrows of 4 pigs per treatment. ²Means within a row lacking a common superscript letter differ at respective p-level.

CHAPTER 4

CONCLUSIONS

Canola meal (CM) was evaluated in a series of studies in nursery pigs based on growth performance, thyroid hormone levels and nutrient digestibility. The first experiment consisted of two phases. In phase I, pigs had reduced feed intake and gain as CM was added to the diet. During phase II, pigs fed with 33% CM replacement for soybean meal (SBM) had a highly comparable performance in terms of feed intake and gain to the pigs fed with SBM. Pigs fed 33% CM replacement for SBM at d 21 to d 35 had higher performance results than the control treatment, although these were deemed not to be significant. Also as the level of substitution for SBM with CM increased beyond 33% CM, reductions in feed intake and gain were observed. However, gain to feed results in general did not reveal any significant differences among treatments. The performance results at d 21 to d 35 verified that partial substitution at a certain level is beneficial as shown by higher performance results. But when pigs at d 0 to d 11 were fed with CM, reduced performance was evident. These results have been verified by some authors that younger pigs are easily affected by even the smallest amount of anti-thyroid compounds present. However, results for the thyroid hormone levels in this study showed no significant differences among treatments. These results indicate that the level of anti-thyroid elements were not detrimental to the nursery pigs.

Digestibility experiment results revealed that pigs had reduced nutrient utilization when fed with greater than 13% CM in the diet. Dry matter, CP, energy, ash, NDF and nitrogen results were lower for those pigs fed with 66% and 100% CM as a replacement for SBM. Chemical analysis was done to all the diets. These results showed that as CM was increased the fiber

level increased. This suggests that fiber is a contributing factor to decreased digestibility for CM diets, and could also be the reason for reduced performance in the first study conducted.

Results of these studies showed that CM can be given only as a partial SBM replacement at no greater than 13% in the diet. Evidence from this study also showed that partial inclusion of CM in the diet has proven to complement other sources, thereby maximizing pig performance. Canola meal may be inferior in some aspects, but correct usage and proper understanding of its nutritional qualities has been proven to be of benefit. Compared with other feed sources, cost of CM can reduce the cost of swine production. Thus, CM can be used as a partial plant protein source for nursery pigs.

APPENDIX A TABLES

Table A. 1. Chemical Analysis of Canola Meal Digestibility Diets (dry matter basis).

Treatment	Control	33%	66%	100%
Dry Matter, %	89.17	89.36	89.60	89.60
CP, % Energy, kcal/kg	20.53 4323	20.54 4372	22.73 4443	22.60 4521
Ash, %	5.10	5.35	5.63	6.31
NDF, %	11.35	12.27	14.93	17.19
Nitrogen, %	2.91	2.93	3.25	3.24

Table A. 2. Analyzed Composition of Canola Meal Experimental Phase I and Phase II Diets (as fed basis).

Treatment	Control	33%	66%	100%
Phase I				
Dry Matter, % CP, % Energy, kcal/kg Ash, % NDF, %	90.55 19.59 3867 5.78 9.31	90.58 21.75 3880 6.26 11.13	90.39 20.92 3950 6.72 12.21	91.19 21.20 4061 6.93 14.58
Phase II				
Dry Matter, % CP, % Energy, kcal/kg Ash, % NDF, %	90.37 18.65 3834 5.60 14.84	90.52 18.07 3963 5.63 16.26	90.77 20.26 4001 5.86 20.56	90.89 20.58 4048 6.51 21.44

Table A. 3. Amino Acid Analysis of Canola Meal (dry matter basis)*.

	Percent (%)	
L-Aspartic Acid	2.72	
L-Threonine	1.52	
L-Serine	1.54	
L-Glutamic Acid	6.24	
L-Proline	2.14	
L-Glycine	1.70	
L-Alanine	1.56	
L-Valine	1.50	
L-Methionine	0.59	
L-Isoleucine	1.26	
L-Leucine	2.50	
L-Tyrosine	0.79	
L-Phenyalanine	0.01	
L-Histidine	0.97	
L-Lysine	1.87	
L-Arginine	0.09	
Ammonia	0.04	

^{*}Amino acids were analyzed at UGA Experimental Station, Tifton, GA.

Table A. 4. Gain of Pigs Fed Phase I and Phase II Canola Meal Diets Under Different Trials.

Treatment	Control	33%	66%	100%
d 0 – d 11, g/d Trial 1 Trial 2 Trial 3	266 252 119	275 226 129	234 229 130	230 234 69
d 11- d 21, g/d Trial 1 Trial 2 Trial 3	420 296 360	380 282 328	334 292 315	316 319 258
d 21-d 35, g/d Trial 1 Trial 2 Trial 3	484 536 556	544 596 586	542 553 567	506 546 515
d 11- d 35, g/d Trial 1 Trial 2 Trial 3	457 436 474	476 465 479	456 444 462	427 452 408
d 0- d 35, g/d Trial 1 Trial 2 Trial 3	379 378 363	413 390 369	386 377 358	365 383 301

Table A. 5. Feed Intake of Pigs Fed Phase I and Phase II Canola Meal Diets Under Different Trials.

Treatment	Control	33%	66%	100%
d 0 – d 11, g/d Trial 1 Trial 2 Trial 3	357 299 243	375 276 229	348 299 237	342 297 187
d 11- d 21, g/d Trial 1 Trial 2 Trial 3	669 525 572	616 522 529	565 522 508	530 551 442
d 21-d 35, g/d Trial 1 Trial 2 Trial 3	834 870 854	867 945 877	853 904 828	793 908 765
d 11- d 35, g/d Trial 1 Trial 2 Trial 3	765 726 737	762 769 732	733 745 695	683 759 630
d 0- d 35, g/d Trial 1 Trial 2 Trial 3	637 592 582	641 614 574	612 605 551	576 614 491

Table A. 6. Gain to Feed of Pigs Fed Phase I and Phase II Canola Meal Diets Under Different Trials.

Treatment	Control	33%	66%	100%	
d 0 – d 11, g/kg					
Trial 1	745	731	674	672	
Trial 2	842	813	766	784	
Trial 3	490	562	548	360	
d 11- d 21, g/kg					
Trial 1	628	617	587	595	
Trial 2	563	540	562	579	
Trial 3	629	622	622	585	
d 21-d 35, g/kg					
Trial 1	581	628	637	639	
Trial 2	618	632	613	603	
Trial 3	656	667	680	674	
d 11- d 35, g/kg					
Trial 1	598	624	622	625	
Trial 2	601	606	598	596	
Trial 3	648	654	663	648	
d 0- d 35, g/kg					
Trial 1	624	644	631	634	
Trial 2	640	636	624	624	
Trial 3	627	642	647	615	