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EFFECTS OF FLAXSEED PROCESSING ON NUTRIENT UTILIZATION, FATTY ACID DEPOSITION, PERFORMANCE RESPONSE OF BROILERS, AND ON FLAXSEED HYDROGEN CYANIDE CONTENT

by

YINGRAN SHEN

A Thesis

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Department of Animal Science

McGill University

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Effects of Flaxseed Processing on Nutrient Utilization, Fatty Acid Deposition, Performance Response of Broilers, and on Flaxseed Hydrogen Cyanide Content

Yingran Shen

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NUTRITIVE VALUE IMPROVEMENT OF FLAXSEED BY ITS PROCESSING FOR BROILERS

Abstract

Master of Science Animal Science

Yingran Shen

Effects of Flaxseed Processing on Nutrient Utilization, Fatty Acid Deposition, Performance Response of Broilers, and on Flaxseed Hydrogen Cyanide Content

A series of experiments were carried out to study the effect of dietary enzyme inclusion or flaxseed processing on feeding value of flaxseed for broilers. The feed enzymes tested containing xylanase activities failed to produce any positive performance response when used in a 10% flaxseed diet with manufacturer recommended dosage for broilers (D 1 to 21).

Autoclaving of flaxseed at 16.5 kg/cm^2 and 120°C for 15 min slightly improved the performance of young broilers fed a 10% flaxseed diet. This improvement was not observed at lower temperature and shorter period of autoclaving, but was magnified at higher flaxseed level. Autoclaving, microwave roasting, pelleting significantly ($P<0.05$) reduced HCN content in flaxseed. The greatest HCN reduction was observed with repeated pelleting (54.9%) and microwave roasting (83.2%), from its 377 mg/kg of raw flaxseed.

When tested with roosters, flaxseed processing effectively increased ME values, dry matter, and ether extract utilization. The raw flaxseed TME and TMEn of 3343 and 3225 kcal/kg, respectively, was significantly ($P<0.01$) increased by repeated pelleting (44%) and microwave roasting (32%). It was the result of significant improvement ($P<0.05$) of EE utilization by the relevant processing. These improvements had a similar effect on total FA and linolenic acid utilization.

The processing method and flaxseed level had a very significant effect on deposition of total T-3 FAs in breast and thigh meat ($P<0.001$) of 40 days old broilers. The highest level of T-3 FAs in muscle lipids of 23.04% and 26.46% for breast and thigh, respectively, was achieved with the highest flaxseed level (14% in days 1 to 21 days, and 17% in days 22 – 40) and pellet-then-mash processing, which lead to low T-6/T-3 ratios of 0.81 and 0.80 in breast and thigh muscle lipid, respectively. The proper flaxseed processing allowed more flaxseed (up to 12%) to be included in broiler diets without obvious growth depression, while achieving the higher desired T-3 FAs deposition in meat.

Résumé

Maîtrise en Science Zootechnie

Yingran Shen

Les effets du conditionnement de la graine de lin sur l=utilisation des nutriments, le dépôt d=acide gras et le rendement des poulets de chair et sur le contenu en cyanure d=hydrogène de la graine de lin.

Des expériences furent réalisées pour étudier les effets de l'inclusion d'enzyme alimentaire ou du conditionnement de la graine de lin sur sa valeur alimentaire pour les poulets de chair. Les enzymes alimentaires essayées contenant principalement l'activité de la xylanase n'ont pas réussi à augmenter les performances zootechniques lorsqu'utilisées dans une ration ayant 10% de graine de lin avec la dose recommandée par le manufacturier pour les poulets de chair(agés de 1 a 21 jours).

Le conditionnement de la graine de lin à l'autoclave à 16.5 kg/cm et 120º C pour 15 min. a légèrement amélioré le rendement des jeunes poulets de chair nourris avec une ration de 10% de graine de lin. Cette amélioration n'a pas été observée à une température plus basse et pour une période de temps plus courte, mais a augmenté aves des niveaux plus élevés de graine de lin. Quant à la réduction en HCN, l'autoclave, le rôtissage aux micro-ondes et la mise en cube ont tous eu un effet significatif ($P<0.05$). La plus grande réduction en HCN fut observée avec la mise en cube répétée (54.9%) et le rôtissage aux micro-ondes(83.2%), la graine de lin non-conditionnée en contenait 377 mg/kg.

Quand quantifié sur des coq, le conditionnement de la graine de lin en augmenta les valeurs ME et l'utilisation de la MS et de l'EE. Le TME et TMEn de la graine de lin brute, 3343 et 3225 kcal/kg, respectivement, étaient très significativement ($P<0.01$) augmentés par la mise en cube répétée (44%) et le rôtissage aux micro-ondes (32%). C'était le résultat de l'amélioration significative de l'utilisation de l'EE ($P<0.05$) par le conditionnement approprié. Ces améliorations ont eu un effet similaire sur l'utilisation des acides gras totaux et l'acide linolénique.

Le conditionnement et la proportion en graine de lin ont eu un effet très significatif sur le dépôt des acides gras ω-3 totaux dans la viande de la poitrine et de la cuisse ($P<0.001$) des poulets de chair de 40 jours. Le taux le plus élevé d'acide gras ω-3 dans le gras des muscles soit 23.04% et 26.46% pour la poitrine et la cuisse respectivement, a été atteint avec la proportion la plus élevée en graine de lin (14% de 1 à 21 jours et 17% de 22 à 40 jours) et avec le conditionnement cubage puis broyage, qui a mené à un faible taux W6/W3 de 0.81 et 0.80 des lipides de la poitrine et de la cuisse, respectivement. Un conditionnement plus approprié de la graine de lin en a permis l'inclusion jusqu'à 12% dans les rations pour poulets de chair sans perturber la croissance, en obtenant dans la viande un dépôt élevé et désiré d'acide gras ω-3.

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ABBREVIATION KEYS

AD	Apparent digestibility
ANFs	Antinutritional factors
DM	Dry matter
DHA	Docosahexaenoic acid ($C_{22:6\omega3}$)
EPA	Eicosapentaenoic acid ($C_{20:5\omega3}$)
FA	Fatty acid
FS	Flaxseed
HCN	Hydrogen cyanide
LNA	Linolenic acid
MUFA	Total monounsaturated fatty acids
PUFA	Total polyunsaturated fatty acids
SAT	Total saturated fatty acids
SNSP	Soluble non-starch polysaccharides
TAAA	True amino acid availability

CONTRIBUTIONS OF AUTHORS

Yingran Shen

I, Yingran Shen, was the principal researcher for all the experimental work discussed in the papers listed in this thesis. I carried out all the feeding trials and data analysis relevant to the papers. Except for hydrogen cyanide analysis that was mainly conducted by Dingyuan Feng, the first author of "the influence of processing on flaxseed hydrogen cyanide content," I performed all the lab analysis discussed in this thesis. All papers listed in this thesis were written by me.

Dr. E.R. Chavez, the supervisor of my thesis, supervising all the experimental work. Substantial advices were given from him regarding experimental designing, feeding trial organizing, and paper drafting.

Dr. P.C. Laguë, one of my supervisor committee members, helped in designing of experiments and organizing of feeding trials. Substantial advices about my thesis drafting were also obtained from him.

Dr. Dingyuan Feng performed the lab analysis of hydrogen cyanide in flaxseed. He contributed the major part in designing and conducting the feeding trials discussed in sections V and section VI. Important advices were also obtained from him regarding the thesis drafting.

General Introduction

General Introduction

Flaxseed regains nutritionists' attention due to two main reasons: its relative nutritive value and its economic cost. Flaxseed has the attributes of an oilseed, that is, being high in oil and protein content. Its relative lower price and abundant supply in Canada, the biggest flax producer in the world with around one million tons yearly (Flax Council, 1999), make flaxseed a potential feed ingredient.

The other main consideration for flaxseed is its unique fatty acid (FA) composition. Flaxseed is the best non-marine source of the ω-3 FA, α-linolenic acid. The health properties related to ω-3 FAs make it desirable to incorporate flaxseed in animal feed, with the expectation that flaxseed can alter the FAs composition of animal tissue. By consuming T-3 FAs enriched animal products, one can obtain health benefits. One possible commodity to offer the unique properties of flaxseed for human health could be poultry.

Poultry is one of the major sources of meat for human. Broiler chickens have the advantage of being fast growers and efficient feed converters. Canada chicken farmers produced 798,507 tonnes of chicken meat in 1998. Over the past 10 years, per capita consumption of chicken meat has steadily increased. It reached 32.4 kg in 1998, an increase of 2.8% over the previous year, unlike per capita beef consumption, which has been slightly dropping yearly over the same period (Statistics Canada 1997-1998). Therefore, there are nutritional and economic benefits to explore the advantage of flaxseed in chicken diet.

However, the inclusion of flaxseed in animal diets, especially for chicken or other monogastric animals, is very often accompanied with depressed growth, when it is used at a level greater than 5 to 10%. The presence of antinutritional factors (ANFs) and the physical structure are the main limiting factors. Cyanogenic glycosides, linatine (a vitamin B_6 antagonist), and soluble nonstarch polysaccharides (SNSP) are the main ANFs. Monogastric animals are less tolerant to these ANFs and are therefore sensitive to dietary flaxseed inclusion.

By investigating the reports on flaxseed usage, we could notice that flaxseed was mostly used in a ground form, which is still in its raw form. For most oilseed crops, it is essential to properly process them prior to feeding. Their requirement may apply for

flaxseed to reach its maximum benefits. At the same time, adding dietary enzyme or vitamin B_6 might counteract the deleterious effect exerted by SNSP or linatine and achieve improved performance response.

The primary goals of this study, therefore, were to improve the feeding value of flaxseed for broiler through either processing, dietary feed enzymes inclusion, or extra vitamin B_6 addition. They included:

- Feeding trials to study the performance response of broilers (aged 1 to 21 days, fed 10% flaxseed) to the addition of various feed enzymes (Natugrain, Natugrain Blend, Allzyme A, Allzyme B, Avizyme^{TM} 1500, lipase, xylanase, arabinofuranosidase, and the combination of xylanase and arabinofuranosidase), to flaxseed autoclaved at different protocol, or to whole flaxseed.
- Feeding trials to study the performance and nutrient utilization of broilers (aged 1 to 21 days) fed different levels (0, 10, 12, 14%) of flaxseed, which was processed by pelleting, autoclaving, or whole seed only.
- Determination of hydrogen cyanide status in flaxseed as affected by various processing methods: pelleting, autoclaving, microwave roasting, and oven dry heating.
- Quantifying rooster TME and TMEn values, utilization of ether extract, dry matter, total and the main individual FA of flaxseed, as affected by pelleting, autoclaving, and microwave roasting.
- Analyzing the performance response, carcass cut-up percentage, and ω-3 FAs deposition in breast and thigh meat of broilers fed different levels (0, 12, or 14% in day 1 to 21, and 0, 15 17% in day 22 to 40) of flaxseed processed by "pelleted and regrinding."

Section I

Literature Review:

Flaxseed as a feed ingredient for chicken

Literature Review:

1. Potential of flaxseed as feed ingredient

1.1. Composition of flaxseed

Flaxseed (*Linum usitatissimum L.*) is an oilseed that consists of seed coat, embryo or germ, a thin endosperm and two cotyledons. Cotyledons make up 55% of the seed and are the storage tissue of flax oil, whereas the seed coat, endosperm and embryo axis take up the others (Dorrell 1970). Mazza and Biliaderis (1989) reported that flax hull is tough and fibrous, contains little protein and oil. There are four layers of flax hull. The outer one contains a mucilaginous carbohydrate material. Bhatty (1990) reported the proportion of flaxseed meal being 37.5% hull and 62.4% flour, while canola meal is 18.6 and 81.3% respectively.

Flax oil is in the form of triacylglycerols, which are present in discrete intracellular organelles or oil bodies. About 98% of these oil bodies are neutral lipids, while other are protein (1.3%), phospholipids (0.9%) and free FA (0.1%). About 90% of the phospholipids are phosphatidyl choline and phosphatidyl serine (Tzen et al. 1993).

1.1..1. Nutrient composition of flaxseed and linseed meal

Oil and protein are the main components of flaxseed. Flaxseeds grown in Canada are mainly brown varieties, which differ from yellow varieties that grow in some areas of United States. In general, Canadian flaxseed contains 24% protein, 41% oil, and 5% crude fiber (Bhatty 1997). Roth-Maier et al. (1998a) reported nutrition content of flaxseed grown in Germany: 21.0% crude protein, 46.5% crude fat, and 4.0% crude fibre. It is generally recognized that the brown flaxseed contains less protein, more crude fiber and neutral detergent fiber. Bell and Keith (1993) reported the neutral detergent fibre of 24% and 29%, and the crude fibre of 8.7% and 11.7% for linseed meal from yellow and brown seed, respectively.

1.1..2. Protein quality of flaxseed and linseed meal.

It is generally recognized that the digestibility of flaxseed protein by monogastric animals is lower than that of canola. Using broiler cockerels, Grossu et al. (1998) reported that flaxseed contained 22.5% digestible protein. The available protein is 7.1%, while there is 6.0% retained protein. The digestibility of essential amino acids is between 74 and 87%. Among them, lysine and methionine are 82 and 80%, respectively. Lee et al. (1995)

recorded similar values of between 71-89% for true amino acid availability (TAAA) by using mature roosters for flax protein of seed and meal. It was lower than that of canola products, which is 79 to 94%. Similar results had also been reported by Barbour and Sim (1991). These authors attributed the lower availability of these essential amino acids to the existence of ANFs and higher fibrous proportion. It is expected that young chicks may have even lower nutrient utilization, as they may be less tolerable to ANFs and fibrous proportion in flaxseed than older birds.

1.1..3. Energy value of flaxseed and linseed meal

Barbour and Sim (1991) recorded a lower TMEn value (3957 kcal/kg) for flaxseed than for canola seed, even though these two seeds have similar oil and other nutrients concentration. They attributed this lower ME value to the same reason as is for lower TAAA. The reported ME value of flaxseed and flax products are shown in Table 1.1.

Table 1.1. The energy values of flaxseed, flaxseed meal, and canola seed in broiler

Kcal/kg	ME	TME	TMEn
Flaxseed[a]	4779		
Flaxseed[b]		4156	3957
Flaxseed[c]		3960	3750
Flaxseed oil[c]		8610	8280
Canola seed[b]		4623	4487
Canola seed[c]		4730	4560
Canola oil[c]		8610	8460
Flaxseed meal[b]		2320	2050
Canola meal[b]		2049	1964

a: Grossu et al 1998; b: Barbour and Sim 1991; c: Lee et al. 1995.

Lower energy value for other flax product, flaxseed meal and flaxseed oil, was not observed. The same group of researchers (Barbour and Sim 1991, Lee et al. 1995) reported similar ME values between canola meal and linseed meal, by using single Comb White Leghorn roosters. Depending on the production method used, there may be less ANFs for some linseed meal than for others. If that is the case, the limiting factors for the low ANFs linseed meal will then be low amino acid availability and the adverse effect of high fibre.

1.2. Composition and availability of flaxseed oil and its effect on fatty acids deposition in chicken

1.2..1. Composition of flaxseed oil

The unique feature of flaxseed is its high α-linolenic acid content. Grossu et al (1998) reported that flaxseed oil contains 44.9% α-linolenic acid. Bhatty (1997) reported the variation for ∀-linolenic acid was in a range of 46-71%, while oleic acid of 14-60%, and linoleic acid from 3-21%. The total polyunsaturated FAs in flaxseed oil are between 62 to 73%, as shown in data reported by these authors. Polyunsaturated FAs are divided into two families; one being ω-3 FA and the other, ω-6 FAs, depending on the position of first double bond from methyl end of carbon chains. Their metabolism and physiological roles in animal and human have drawn extensive interest.

1.2..2. Utilization of flaxseed oil

The lower ME values for flaxseed may not be related to its high content of polyunsaturated FAs. There has been extensive research indicating unsaturated FAs could be better utilized (Ketels and De Groote 1989, Leeson and Attech 1995, Blanch et al. 1996, and Vila and Esteve-Garcia 1996). Lee et al. (1995) reported the TME value of flax oil (8610 kcal/kg) was the same as canola oil (8610 kcal/kg), while the TMEn of flax oils is slightly lower (8280 vs 8460 kcal/kg).

Olomu and Baracos (1991) studied the influence of dietary flaxseed oil on the performance, muscle protein deposition and FA composition of broiler chicks. When flax oil is used, not flaxseed, up to 4.5% flax oil caused neither any difference as compared to diets of same amount of animal tallow in weight gain, feed consumption or feed efficiency, nor the muscle proportion of *Extensor digitorum communis* and *Sartorius* muscles. As flaxseed normally contains 37% oil, 12% flaxseed will provide around 4.5% oil. It could then be inferred that it is not the oil in flaxseed that causes the performance reduction, but other components. Olomu and Baracos (1991) concluded that there is no known deleterious factors in flaxseed oil.

1.2..3. The effect of dietary FAs on FAs deposition in tissue

There has been quite extensive research to study the effect of dietary FAs on animal product FAs profiles. The general conclusion from these researches is that tissue FA profile is affected by dietary FA composition. Studies with broilers supported this

conclusion (Pinchasov and Nir 1992, Iji 1993), suggesting that the FA contents of broiler chicken meat could be manipulated to suit market demand through the feeding of the appropriate lipid sources or blends.

Ajuyah et al. (1993) studied the effect of 15% mash flaxseed on FAs deposition in white and dark meat of broilers. They observed that dietary inclusion of flaxseed for 42 days reduced the monounsaturated FAs (MUFA), increased the PUFA both in white and dark meats, and in fractions of triacylglyceride phopholipids, as compared to the corn-soybean diet. The increase in total ω-3 FAs was much greater than that of total ω-6 FAs.

1.3. ω-3 FAs and health.

T3-FAs draw intensive interest beyond the understanding of their essentiality for cell membrane development and maintenance. Galli and Simopoulos (1988) suggested that Eicosanoids, transformed from eicosapentaenoic acid ($C_{20:5\omega3}$) and arachidonic acid ($C_{20:4\omega6}$), is involved in regulation of blood platelets, blood vessels and leukocytes in preventing cardiovascular disease. In contrast, ω-6 FAs, especially arachidonic acid, have been linked to the production of metabolites that lead to constrict blood vessels and aggregate platelet that are involved in the formation of arterial thrombosis (Holub 1995). Leaf and Weber (1988) stated that ω3-FAs, including α-linolenic acid (LNA), eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA, $C_{22:6\omega3}$), reduce the risk of coronary heart disease. As cited by Roth-Maier et al. (1998a), it is based on the mechanism that eicosanoids derived from eicosapentaenoic acid, the ω-3 FA, have anti-inflammatory and anti-thrombotic properties, while the eicosanoids derived from arachidonic acid, an ω-6 FA, have pro-thrombotic effects. The dietary recommendation for fat consumption had been made to increase the ω-3 proportion and reduce the ω-6 proportion. With this expectation, flaxseed would not be omitted, due to its features of low cost and high ω3-FAs content. It is a popular practice for some people to regularly consume a certain amount of flaxseed in believing that ω-3 FAs in flax oil will prevent cardiovascular disease (Allman et al. 1995).

A higher ω6 to ω3 FAs ratio leads to a high level of arachidonic acid production and inhibits the synthesis of the ω3 FAs family and restricts the conversion of $C_{18:3\omega3}$ to longer carbon chain ω3 FAs. For the prevention of cardiovascular disease, it is generally

recommended we consume diets that have a ω6 to ω3 FAs ratio of not more than 4 to1, while North American adults consume diets that range from 10 and even 50 to1. It is obvious therefore that animal products with low ω6-to-ω3 FAs ratio could have health benefits (Galli and Simopoulos 1988).

1.4. Dosage and period dependent of ω-3 FA deposition

As previously mentioned, the FAs composition in tissue is generally reflected by the FAs profile of the dietary source. Ajuyah et al. (1990) reported a proportional change of the FAs profile in white and dark meat of broiler chicks as affected by the change of dietary oil sources. The incorporation of full-fat flax seeds or flax oil plus flax meal into broiler diets at 10 or 20% resulted in elevated deposition of ω-3 FAs, in an interaction pattern between source and level. Higher inclusion rate lead to more ω-3 FAs deposition.

Bond et al. (1997) also demonstrated the dose response of FA profile in chicks' erythrocyte membranes. Broilers were fed diets containing 10, 20, 30% flaxseed. The total saturated FAs in erythrocyte membranes were reduced with the increase of flaxseed inclusion in the diet, as well as the increase in the ratio of ω-3 FAs to ω-6 FAs. Increased levels of linolenic acid and its metabolite eicosapentaenoate (20:5ω3) were responsible for the change. The reduction of saturated FAs is mainly the result of lower level of myristate (14:0) and palmitate (16:0). With the increase of flaxseed in the diet, reduction of saturated FA reaches their minimum when the flaxseed level is 20%. Considering that flaxseed contains 40% oil, it means that 8% of flax oil will have the maximum effect in reducing saturated FAs in erythrocyte membrane.

Dosage and period response were also studied by Olomu and Baracos (1991) they used 0, 1.5, or 4.5% flax oil and monitored the FA composition in lipid of *Sartorius* muscle after 7 or 21 days of feeding. Increased amount of ω-3 FAs was recorded, at the expense of ω-6 FAs. Feeding flaxseed oil to chicks for a prolong period depressed the concentration of $C_{20:2\omega6}$, $C_{20:3\omega6}$, $C_{20:4\omega6}$, and $C_{22:4\omega6}$, possibly the result of competition of $C_{18:3\omega3}$ with $C_{18:2\omega6}$ for)-6-desaturase.

Considerable research efforts have been carried out to enrich animal products with ω-3 FAs in monogastric animals by using flax product in the diets. However, when they show a sensitive and proportional response to dietary flaxseed in the tissue FA profile proportional

to dietary dose and period basis, the high dietary flax inclusion diminishes their performance.

2. Limitation of flax products in animal feed

2.1. Anti-nutritional factors in flaxseed

The recognition of anti-nutritional factor (ANFs) in flaxseed products has long been associated with the use of linseed meal. Linseed meal (over 5% in a chick diet) would depress the growth of chicks, unless it was treated with water (Kratzer 1946, 1947). Batterham et al. (1991) stated that the presence of ANFs could be part of the reasons that pigs fed a linseed meal diet had depressed growth, lower feed intake, and decreased the weight of kidney, spleen, and pancreas but not liver. Nevertheless, the addition of pyridoxine at 2.5 mg/kg did not alleviate the possible linatine depressing effect on weight gain. They concluded that linseed meal was inferior to soybean meal as protein source. The probable reason, even though not established, could be the presence of ANFs.

The effectiveness of processing could support the argument of the presence of ANFs in linseed meal. Madhusudhan et al. (1986) reported that raw linseed meal significantly reduced chick-growth response and caused histo-pathological changes, while water-boiled linseed meal at a level of 13%, which was 50% protein replacement of expeller groundnut cake, had the same growth rate as compared to a yellow-ground peanut diet.

2.1..1. Cyanogenic glycosides

Cyanogenic glycosides, protection for plants and their toxicity

Cyanogenic glycosides are glycoside of aldehyde or ketone cyanohydrin. They yield glucose, acetone and HCN on hydrolysis. Over 1000 species of plant are known to produce hydrogen cyanide. Cyanogenic glycosides are not toxic as such, but only through their release of HCN, which occurs when they have been broken down either after ingestion or as a result of plant cell damage before ingestion (Bell 1981).

The toxicity of linamarin in cassava is well documented (Tewe and Iyayi 1989). It is an inhibitor of cytochrome oxidase and consequently a cellular respiration inhibitor. Its absorption is very rapid, so is the toxic effect. The minimum lethal dose to man is quoted at between 0.5 to 4 mg/kg body weight (Bell 1981, Duffus and Duffus 1991). There may be species sensitivity across the animal kingdom.

The forms and metabolism of cyanogenic glycoside in flaxseed

There are four forms of cyanogenic glycosides in flaxseed: linamarin, linustatin, lotaustralin and neolinustatin. Linustatin and neolinstatin are diglycosides, while linamarin and lotaustralin are monoglycosides. Seigler (1981) explained the metabolism of cyanogenic glycosides. The linamarin and linustatin are derived from valine, while isoleucine is the precursor for lotaustralin and neolinastatin. The first step is the oxygenation of amine nitrogen of the precursor amino acid to form the corresponding aldoxime. Dehydration of aldoxime yields a nitrile whose stereospecific oxygenation produces cyanohydrin. The last step is the glycosylation of cyanohydrin catalyzed by UDP-glucose-ketone cyanohydrin β-glucosyl transferase, yielding cyanogenic glycosides (Hahlbrock and Conn 1970).

Flaxseed contains a very low level of linamarin, but a considerable amount of the diglycosides linustatin and neolinustatin (Mazza and Oomah 1995). Young flax contains mainly the monoglycosides, linamarin and lotaustralin, which could be over 90% of the total cyanogenic glycosides, whereas older flax contains about 30% of the diglycosides linustatin and neolinustatin.

The enzymatic degradation of cyanogenic glycosides is under the action of one or more β-glycosidase. It undergoes cleavage first in glycoside bond. The intermediate may decompose either spontaneously, or enzymatically to yield HCN and an aldehyde or ketone, by the action of ∀-hydroxynitrile lyase (Mazza and Oomah 1995). Cyanogenic glycosides could also be hydrolysed in the gut, as the gut microflora could produce β-glycosidase (Majak et al 1990). Catabolism of HCN in the animal body, as explained by Mazza and Oomah (1995), occurs by the enzyme rhodonase (thiosulfate sulfurtransferase). This enzyme is present in liver, kidney and thyroid tissue. The compensatory enlargement of these organs may be expected, if the animal consumes a considerable amount of cyanogenic glycosides. The metabolized product of cyanide is thiocyanate, which is much less toxic, even though it causes thyroid hypertrophy. Most animals can produce HCN detoxification enzymes.

Content of cyanogenic glycoside in flaxseed products

The reported content of hydrogen cyanide in flaxseed varies, due to cultivar, difference in detection methods, or as the expression in different compounds. Rosling (1993) reported

that the cyanide content varied from 4 to 12 mmol/kg (104 to 312 mg/kg). Bhatty (1993) reported the average HCN content of seven western Canadian grown flaxseed cultivars being 7.9 to 9.8 ug/100 g seed by using a colorimetric method (barbituric acid-pyridine reaction). He concluded that the HCN content was largely affected by environments (location and season), and to a small extent, by cultivars. Chadha et al. (1995) detected 124-196 mg of cyanide per kg flaxseed using HPLC system.

When expressed as cyanogenic glycosides, Wanasundara et al. (1993) detected 4420 mg/kg linustatin, 1900 mg/kg neolinustatin, and 410 mg/kg total HCN equivalent (de-fatted basis). As cited by Mazza and Oomah (1995), a range of 2180-5380 mg/kg and 730-4540 mg/kg of linustatin and neolinustatin, respectively, were reported for 48 varieties grown at one German location; 2130-3520 and 910-2030 mg/kg for linustatin and neolinustatin respectively, was found for 10 Canadian varieties. As can be seen, the linustatin makes up 54-76% of total cyanogenic glycosides.

Removal of cyanogenic glycosides from flaxseed products

Selective processing can significantly reduce the content of cyanogenic glycoside. If methanol is used in flaxseed extraction system, it can reduce the cyanogenic glycosides content, as they are soluble in such a system (Mazza and Oomah 1995). An extraction system of hexane/methanol/water removed cyanogenic glycosides by 56, 80 and over 90% by 1, 2 and 3 times extraction, respectively. Soaking linseed meal by water with four times its weight reduces one half of its cyanogenic glycoside, while autoclaving under 10.5 kg/cm^2 for 15 minutes caused maximum reduction from 85 to 12 ppm HCN for linseed-cake (Deshmukh et al. 1982).

2.1..2. Linatine

Linatine is another main ANFs in flaxseed. Kratzer et al. (1954) reported that chicks reared on linseed meal diets developed vitamin B6 deficiency symptoms which were overcome when 20 ppm of pyridoxine was added to the diet, or by injection. Klosterman et al. (1967) revealed the toxic effect of flaxseed extract. They dissolved flaxseed extract into physiological saline solution and injected this mixture intraperitoneally into chicks. The birds experienced mild to severe vitamin B6 deficiency symptoms and death at higher dosage. The poor growth and the deficiency symptoms were alleviated by administration of pyridoxine. The authors suggested the vitamin B6 antagonist to be linatine.

2.1..3. Water-soluble nonstarch polysaccharides

One of the features of flaxseed is its high content of soluble non-starch polysaccharides (SNSP), or mucilage. Its presence in chicken gut could lead to antinutritional effects. As any other SNSP, it may reduce the enzymatic action on other nutrients and increase fermentation in the gut.

Oomah et al. (1995) performed an in-depth analysis of over one hundred samples of flaxseed from over the world. They collected the flaxseeds from twelve geographical regions and included oil, fiber, to determine their composition of water-soluble polysaccharides. It was found that flaxseed contains about 3.6 to 8% water-soluble polysaccharides. The neutral monosacchrides of the water-soluble polysaccharides fraction are mainly glucose, xylose, galactose, and rhamnose. Glucose ranged from 21-40% and is the major monosaccharide. The Rhamnose to xylose ratio in flaxseed is an indicator of the viscous flaxseed gum (Fedeniuk and Biliaderis 1994).

For comparison, the SNSP content of some ingredients other than flaxseed cited by Smits and Annison (1996) is between 2.4 to 13.9% for grains and oilseeds. Among them, wheat, rye, barley chickpeas, lupins, navy beans, soyabean meal, and rapeseed meal have SNSP of 2.4, 4.6, 4.5, 3.3, 4.0, 5.7, 13.9, and 11.3%, respectively.

2.1..4. Phenolic acid

Phenolic acids may form insoluble complexes with essential minerals, protein, and carbohydrate in feedstuffs and lower their nutritional value (Naczk and Shahidi 1997). Varga and Diosady (1994) reported the polyphenols content in flaxseed to be 4.41g/kg. The total phenolic acid of linseed meal was about 0.22%, as cited by Wanasundara and Shahidi (1994). Oomah et al. (1995) reported 8-10 g/kg of total, 5 g/kg of esterified, and 3-5g/kg etherified phenolic acids. They are affected mostly by season.

2.1..5. Trypsin inhibitor

Lab-prepared raw linseed meal contained 42-51 units of trypsin inhibitor activity, while commercially obtained samples contained 14-37 units, as reported by Bhatty (1993). For comparison, raw rapeseed and soybean meals contain 99 and 1650 units, respectively.

2.1..6. Phytate

From 8 Canadian grown flaxseed cultivars, 2.3-3.3% of phytic acid in flaxseed meal was found in the meal by Oomah et al. (1996), which is higher than soy, peanut and sesame meal that normally range around 1.5% (Erdman 1979). The content of phytic acid in extraction resultant linseed meal was 2.4-3.2% (Wanasundara and Shahidi 1994). Phytate is known to reduce mineral bioavailability and may react with protein and starch to reduce their utilization. It may be beneficial to supplement phytase for monogastric animals, if a high dietary inclusion rate of flax product is the case, as its phytate content is relatively high.

2.2. Peroxidation

Flaxseed oil is traditionally considered not edible, due to its tendency for peroxidation. Instead, the conventional use of flaxseed oil is in the oleochemical industry. Flaxseed oil still does not have the status of "Generally Recognized as Safe" by the Food and Drug Administration of the United States (Carter 1993). Based on the desire to reduce the peroxidation potential, low ∀-linolenic cultivars were introduced. The content of ∀-linolenic acid in the new mutant was reported lower than 3%, while the concomitant increase of linoleic acid ranges between 50.7 and 67.3% (Bhatty 1997).

3. Utilization of flaxseed for chicken

3.1. Increasing flaxseed feeding value by processing

3.1..1. Microwave roasting

Microwave processing has been extensively used for the material and food industries. Microwaves lie between radiowave frequencies and infrared frequencies in the electromagnetic radiation spectrum. The interaction between microwave and material generates heat that is used for material processing, which is achieved through either polarization or conduction processes. Polarization involves the short-range displacement of charge through the formation and rotation of electric dipoles (Clark 1996). The advantage of microwave processing is that the materials processed interact with the "cold" microwaves, instead of radiant heat of conventional furnaces. The material itself generates the heat and this generation is fast and volumetric. That results in better product uniformity throughput, and less wasteful heating (Clark 1996). It is unlike the conventional heat where

the processed materials receive heat from the outer layer. Microwave is credited with high efficiency, because of the high penetrating power (Decareau 1985).

Microwave processing is used for enzyme inactivation in food. Devece et al. (1999) studied enzyme inactivation for mushroom blanching. Mushroom browning is affected by the presence of polyphenoloxidase activity. Microwave processing leads to the inactivation of the enzyme and shortens the processing time. Vetrimani et al. (1992) showed that microwave ovens were effective in inactivating the enzymes lipase and lipoxygenase in cereal bran and germ and in soybean. Hajela et al. (1998) reported the effect of inactivation of trypsin-chymotrypsin inhibitor of Blackgram (Phaseolus mungo L.) by microwave heating, as compared to traditional heating methods. They found that microwave heating decreased the trypsin/chymotrypsin inhibitor in a manner similar to that of traditional heating methods.

3.1..2. Heating

Heat is involved in many food processing. Kozlowska (1989) reported positive results in reducing HCN content by heat treatment. He demonstrated that the HCN content of yellow flaxseed was decreased from 208 mg to 10 mg/kg with an increased temperature (120°C) and roasting time.

It is expected that poisoning by cyanogenic glycosides will be greatly diminished if the endogenous enzymes are inactivated by heat treatment. Nonetheless, Kratzer et al. (1954) observed no benefit by dry heat processing of linseed meal. They compared their result with other processing that showed positive growth response. They thought that treatments like autoclaving or water soaking acted on chemical destruction of growth inhibitors, rather than the effects from heat, microbiological or enzymatic actions. Mazza and Oomah (1995) also observed when flaxseed is processed to produce the meal, there is no reduction of cyanogenic glycosides but an increase by 25%. That may explain why cyanogenic compounds are intact and not affected by the deactivation of intracellular enzymes. Besides, intestinal microorganisms may produce linamarase activity, as demonstrated by Majak and Cheng (1987) and the cyanogenic glycoside after heat processing could still by degraded to produce HCN in their intestine.

The ineffectiveness of heat processing was also reported by Lee et al. (1991) who employed 85°C for 30 min. for flaxseed roasting. No positive response on performance,

protein retention, and ME value are observed for chicks fed 10% processed flaxseed, as compared to those fed raw flaxseed.

In addition, excessive heating may reduce the nitrogen digestibility while inactivating some toxic compounds (Young 1982). Oomah and Mazza (1998) demonstrated that protein solubility of processed linseed meal (44.9%)was lower than that of untreated meal (68.3%,), but the protein digestibility increased from 36.1 to 53.2 g/kg (acid-corrected pepsin digestible protein). Heating reduced total phenolic content from 13.14 g/kg in seed to 8.8 g/kg in meal.

3.1..3. Extraction

Crushing and extraction of flaxseed are the main processing procedures to obtain oil and meal. The solvents used in extraction may affect the oil content while ANFs may remain in the meal, due to their difference in solubility. Varga and Diosady (1994) reported the effect of two-phase extraction that included methanol solution with 10% water and 2.5-5% ammonia, or 10% water and .08% NaOH. They obtained higher protein flaxseed meal (40-47%) while cyanogenic glycosides were reduced by 90-100%. Total polyphenols were also reduced by about 20%.

Wanasundara and Shahidi (1994) found that the solvent extraction of flaxseed by methanol-ammonia-water could effectively reduce phenolic acids, condensed tannins soluble sugars, and cyanogenic glycosides. The content of condensed tannins is approximately 136mg/100g in flaxseed. The two-phase extraction system reduced the tannin content by 26-74%. When ammonia is included in the extraction system, the removal of tannin is increased to 74%. Phytic acid in resultant meals was not affected by the two-phase solvent-extraction.

3.1..4. Autoclaving

Various reports showed that autoclaving is effective in lowering ANFs level and improving nutritive values for oilseeds and beans. Prolonged autoclaving for 2 hours lowered phytate content in isolated soybean protein by 70% (De Boland et al. 1975). Autoclaving was effective in improving the nutritive value for high tannin sorghum, but not for low tannin sorghum (Price et al. 1978). Regarding rapeseed processing, autoclaving at 120°C for 30 min. reduced 3-butenyl isothiocyanate and 4-pentenyl isothiocyanate by 98 to 100%. These compounds carry antithyroid activity (Nakaya 1980). Nwokolo (1987)

demonstrated that the nutritional value of African breadfruit, which depressed the growth of chick when used without autoclaving, was improved by autoclaving. Among those, protein digestibility was improved from 50.3% to 82.7%, while ME, from 8.9 to 11.9 MJ/kg. The poor growth rate and high mortality when fed 20 or 30% unautoclaved breadfruit seeds, was not experienced among chicks fed autoclaved seeds. Autoclaving was also able to lower trypsin and chymotrypsin inhibitor activities in karanja (Pongamia glabra) oil seed residue by 83.35 and 54.86%, respectively, as reported by Rattansi and Dikshit (1997).

When related to linseed meal processing, autoclaving was able to alleviate some adverse effect of ANFs. Kratzer et al. (1954) stated that autoclaving seemed to be as effective as water treatment in deleting the growth depression effect of raw linseed meal for chicks. Deshmukh et al. (1982) reported that autoclaving reduced cyanogenic glycoside content from 85 ppm to 12 ppm and significantly improved feed efficiency and weight gain in chicks. Mandokhot and Singh (1983) revealed that the wet autoclaving is superior to dry autoclaving in improving the linseed meal for chick performance. The diet used was based on corn and ground peanut meal. The linseed meal was used to replace peanut meal (25% protein). The autoclaving was performed for 15 minutes. Both dry and wet autoclaving reduced the HCN from 47-49 mg/100 g to negligible amounts. Compared to the dry autoclaving, wet autoclaving improved chick performance by 10%, and was the same performance as the control group.

Autoclaving has the disadvantage of lowering the essential amino acids content. Venkatesan and Rege (1968) reported that autoclaving might lead to devaluation of some essential amino acids for some oilseed meal. A similar finding was reported by Nath et al. (1981) who revealed lower available lysine content of Guar meal as a result of autoclaving.

3.1..5. Water soaking

Water soaking had long been shown to bear a positive effect on increasing the feeding value of linseed meal. MacGregor and McGwnis (1948) tested the effect of water soaking processing. They used 3 parts of water and 1 part of linseed meal and had them soaked for 18 hrs at room temperature (28°C). The inclusion of 10, 20, or 30% water-treated linseed meal did not depress growth, while 4.5% untreated linseed meal did. Kratzer (1954) reported that chicks fed water soaked linseed meal did not experience vitamin B6

deficiency. Chadha et al. (1995) found that, after boiling flaxseed or flaxseed homogenate, cyanide content was reduced to a very low level (3.5 mg/kg) for whole seed and 1.2 mg/kg for the homogenate. They explained that the enzyme responsible for releasing cyanide was inactivated during boiling, since their raw flaxseed contained cyanide at 124-196 mg/kg.

Some of the disadvantages of these procedures are the loss of nutrients during processing and the decrease in nutrient availability. Water boiling reduced available lysine content by 30%, even though the solubility increased by 38% in vitro (Madhusudhan and Singh 1985). Flaxseed water soaking would be industrially impractical, as the outer layer mucilage could easily form a sticky mash with water.

3.1..6. Pelleting

General benefit of pelleting

Two main advantages of using pelleted feed are efficient feed management and nutrition improvement. It simplifies feed handling and reduces feed waste. Another advantage includes increased feed consumption. As a rule, pelleting improves primarily the energy efficiency and sometimes the nitrogen efficiency. Nevertheless, these results are not always obtained since they largely depend on the method of pelleting. Pelleting under steam gave a significant improvement on the above-mentioned efficiency (Calet 1965).

The improved nutrition of pelleted feed could be attributed to the benefit of pressure and higher temperature during pelleting. Calet (1965) pointed out two predominant effects. Firstly, pelleting increases the density of the feed and breaks down the cells of the starch grains. Secondly, wet heat can likewise make the nutrients other than starch more available. Also, these two effects are complementary because pelleting is not truly effective unless the effects of pressure and steam are combined. Feed is normally pelleted at 65-80°C (Decksheimer 2000), and is preconditioned with steam, which, in itself, could be considered another processing. The temperature of the pellet can go to 90°C or higher during the passage of feed through the die. Heat does not cause any chemical modification of the grain, but the joint action of heat and pressure could result in modifying the structure of the feed.

Boggs et al. (1960) reported an improved effect of pelleting on the nutritive value for some oilseed. The improvement in weight gain and feed efficiency were observed not only for the cottonseed containing diet, but also the soybean diet after the diets were pelleted.

There are factors like age, sex and strain that will influence the effectiveness of pelleted feed. For younger birds, starter feed is better used if in the form of crumble, even though not all the results support this practice. In cockerels, growth is improved by 12-15% when pellets rather than mash are fed (Heywang and Morgan 1944).

Fat and pelleting

Pelleting can increase the availability of the oil in the seed by increasing its absorbability. Pressure and heat during pelleting break the cell wall and liberate the oil (Carew et al. 1959). Carew and Nesheim (1962) found that pelleting ground soybeans improved oil availability in the beans. The absorbability of soybean oil in the ground soybeans was raised from 73% (unpelleted) to 91% (whole diet contained 61% ground soybean pelleted then reground). The best results for performance and fat absorbability were found with diets that contained ground soybean that had been heated and then pelleted. However, in general, soybean seeds treated in this way do not improve growth (Calet 1965).

Harmful factors overcome by pelleting

Some growth promoting effect of pelleting had been noted by various researchers. The adverse effect associated with the use of linseed meal could partly be overcome by pelleting, as revealed by Nikolaiczuk (1950). Chicks fed a diet containing 10% granular linseed meal, whose texture ranged from 0.5 to 2 mm in diameter, had about the same response as those fed water treated linseed meal. The coarse and medium textures were superior to the fine texture. Lindblad et al (1955) noticed that, while the addition of 50 percent barley to corn in a mash diet is detrimental, it is not the case when pelleted. The deleterious effect of high cellulose was overcome by pelleting. Bolton (1960) stated that the growth-promoting effect of pelleting might be due to increased palatability, increased density of food, the destruction of some growth inhibitor or some combination of these three. There may be separate effect of heat, pressure, and steam during pelleting, but heat alone is not effective in destroying some toxic elements, particularly in linseed meal.

3.1..7. Extrusion

Extrusion is another kind of thermal processing where shear forces are increased during the extrusion. Cellular disruption is expected during this process. Marsman et al. (1997) found that extrusion significantly improved feed conversion and apparent ileal digestibility

of crude protein and nonstarch polysaccharides of soybean meal (SBM) for broiler chicks. As compared with toasting, they found that extrusion increases crude protein digestion from 82.2 to 87.5%, and nonstarch polysaccharides from 11.4 to 26.7%. Extrusion of SBM at the highest shear level caused a significant increase in the water holding capacity, chyme viscosity, and concentration of soluble nonstarch polysaccharides in the chyme, compared with extrusion at lower shear levels. The increase in chyme viscosity did not affect growth performance nor did it influence apparent ileal nutrient digestibilities. The extrusion of full fat soybean is a regular soybean processing now.

3.2. Exogenous enzyme inclusion

There has been positive response in nutrient utilization and performance improvement for broiler by dietary enzyme inclusion. By using a multi-enzyme complex, Avizyme^{TM} that contains 100 u/g of ∃-glucanase, 300 u/g xylanase and 800 u/g protease, Villamide et al. (1997) observed a significant interaction between barley cultivars and enzyme supplement for energy utilization. For barley or wheat type diet, Esteve-Garcia et al. (1997) noticed improved feed efficiency and reduced intestinal viscosity and vent pasting for broilers, when either ∃-glucanase or xylanase was added to the diet.

An interaction between fat and xylanase addition was also reported by Däniche et al. (1997) for rye-type diets. Fat digestibility of young broilers was improved by xylanase in both tallow and soy-oil type diets, but to a greater extend for the tallow diet. Protein digestibility and AMEn values were significantly improved by xylanase, but only for the tallow diet. Enzyme supplement affected the ileal digestibility of nitrogen and amino acid for both fats, but was generally more profound for the tallow diet. Xylanase increases the deposition of fat-soluble vitamin A, E, and the digestibility of insoluble pentosan. An opposite effect for soluble pentosan was noted.

Langhont et al. (1997) also recorded an interaction between fat type (soy oil and animal blend fat) and addition of endo-xylanase for chicks based on wheat and rye diets. Only feed conversion was improved for the soy oil diet. For chick fed animal blend fat, endo-xylanase improved weight gain by 9.5%, feed conversion by 6%. Digestibilities of organic matter, crude fat, crude fiber, and neutral detergent fiber were not affected for soy-oil diet, whereas for the animal blend fat diet, the improvement was significant ($P<0.05$).

3.3.Using whole raw flaxseed

The advantaged of using whole flaxseed over ground seed is that long chain PUFAs will have a protection layer against peroxidation. The storage of feed products may require less critical conditions, not like ground flaxseed diets that may require special attention. Some studies were conducted to evaluate the response of poultry to whole flaxseed, but most of these studies were focused on the hen, in believing that hen can digest whole seed better than broiler (Aymond and Elswyk 1995, Roth-Maier et al. 1998b).

4. Flaxseed in poultry feeding

4.1. Hen

It is generally thought that hens fed a flaxseed containing-diet can enrich egg with T-FAs, while being more tolerant to the ANFs than broilers. Aymond and Elswyk (1995) used two levels of flaxseed, 5 and 15%, either ground or whole seed, to test the laying hen's production performance and response of FA in yolk for 5 wks. All flaxseed treatments increased total ω-3 FAs ($C_{18:3}$, $C_{20:5}$, $C_{22:6}$), but not the ω-6 FAs ($C_{20:5}$, $C_{22:6}$). Seed form did not influence ω-3 FAs deposition at the 5% level of flaxseed. But when the flaxseed level was increased to 15%, the ground flaxseed group had higher proportions of ω-3 FAs in yolk lipids than the whole seed group at the same level. Birds that received diets containing 15% flaxseed, either in ground or in whole form, consumed less feed. Egg production generally decreased with extended period of flaxseed feeding. It was concluded that ground flaxseed was superior over whole seed when the dietary flaxseed level is over 5%. Even though high level of ground flaxseed promotes greater ω-3 FAs deposition in yolk, its practicality is limited due to the depressed production.

FAs profile in egg yolk from hens fed different levels of flaxseed was discussed by Eder et al. (1998). They used 3-flaxseed inclusion rates, 5, 10 or 15%. Increasing flaxseed in the diet promoted higher level of ω-3 linolenic acid deposition, at the expense of saturated and monounsaturated FAs. Highly unsaturated polyunsaturated FAs such as EPA and DHA were slightly elevated but remained at a low level. The FA composition of yolk lipids was slightly more affected by dietary ground flaxseed than by whole flaxseed. Levels of ω-3 linolenic acid in yolk lipids caused by ground flaxseed at 5, 10, or 15%

dietary inclusion were 3.6, 8.0, and 11.7% versus 2.8, 6.3, and 10.4% for whole flaxseed, respectively. Dietary flaxseed markedly reduced the ratio between ω-6 and ω-3 polyunsaturated FAs and increased PUFA/SAT ratio and the double bond index of egg yolk FAs. Feeding diets with 15% flaxseed increased the concentration of egg yolk total lipids slightly.

Laying hens showed no adverse production response when diets contained up to 10% flaxseed (Eder et al. 1998), but not when flaxseed was 20% in the diet. At this level, both ground and whole seed diets depressed egg mass and rate of production to a similar scale, 10 to 11%. The whole seed group showed a further depression in feed consumption of 17%, whereas the ground seed group depression was 9%, as compared to control diet.

4.2. Broiler

4.2..1. Performance and carcass quality

Ajuyah et al. (1990) found that flaxseed levels over 10% caused significant depressed growth and carcass yield. Similar results were reported from the same laboratory (Ajuyah et al. 1993) when broilers were fed diets with 15% flaxseed (mash). The authors observed that birds gained significantly less, and had a poorer feed conversion rate. The flax diets caused a reduction in live weight of about 17%. The poor growth rate could be the consequence of the toxic substances present in raw flaxseed and the physical barrier of whole seed to fat digestion, as reasoned by Mandokhot and Singh (1983). However, all these researchers found no difference in carcass cut-ups percentage among dietary treatments.

Dietary flaxseed can also exert its effects on carcass composition. Ajuyah et al. (1990) reported that the addition of 10 or 20% of either flaxseed or flaxseed meal decreased significantly the tissue lipid content both in white and dark meats. The lipid content in white and dark meat of broiler was 12.0 and 20.2 g/kg tissue and 13.6 and 24.6 g/kg tissue for birds fed 10% and 20% flaxseed diets, respectively. They suggested that dietary flaxseed product could affect total carcass fat.

Zollisch et al. (1997) reported that young chicks utilize unsaturated FAs better when fed diets containing 3.5% fat from different sources. Different fat sources caused no difference in proportions of leg, breast meat and abdominal fat, nor in the fat content of abdominal fat. Thus, growth performance of broilers can be improved by the incorporation

of polyunsaturated FAs at levels higher than generally recommended without negative effects on quantitive carcass characteristics. Ochrimenko et al. (1997) also reported that the performance of broilers was affected by feeding flaxseed in the diet. In their 1st experiment, 10% flaxseed in the ration resulted in decreased growth. Feed intake and weight gain in the 2nd experiment were affected by as low as 2% flaxseed in the diet.

Similar growth depression with dietary flaxseed was reported by other researchers. Bond et al. (1997) incorporated 10, 20 and 30% flaxseed in broiler diets and observed that growth of the birds was reduced with increasing levels of flaxseed. Roth-Maier et al. (1998a) showed that as low as 5% ground or whole flaxseed slightly reduced the body weight and feed conversion rate; at 7.5%, they observed a further increase in these negative effects, even though they had provided grit *ad libitum*. Whole seed diet slightly caused an increase in the feed to gain ratio. Feed intake was not affected by flaxseed. Based on their experimentation, they recommended that broiler rations should not contain more than 5% flaxseed in order to avoid growth depression and poor feed conversion.

Higher proportion of linolenic acid in meat tissue may affect its market quality. Ochrimenko et al. (1997) demonstrated decreased stability of carcass fat against oxygen by increased amounts of flaxseed in the diet. Consequently the taste of meat changed after storage. Leeson and Summers (1997) also stated that flaxseed may be responsible for discontent taste in broiler meat, in which linolenic acd is responsible for the fish oil smell.

Regarding flax oil, Olomu and Baracos (1991) reported that 4.5% flax oil did not cause performance difference, nor the lipid and protein content of *extensor digitorum communis* and Sartorius muscles. Both Olomu and Baracos (1991) and Lee et al. (1995) reported that there is no known deleterious effect of flax oil for birds. The restored diet from flax oil and meal did not lower ME value, while flaxseed did.

Based on these reports, it is evident that broilers have a limited tolerance to flaxseed. When used in higher amounts, mostly over 10%, raw flaxseed causes growth depression. However flax oil does not cause adverse growth nor alter carcass characteristics, when used at levels up to 4.5% in broiler diets.

4.2..2. FA deposition

The main interest in feeding flax to broilers in recent years is to increase T-3 FAs deposition in meat products. Broilers respond sensitively to dietary inclusion of flaxseed.

Zollisch et al. (1997) reported that the FAs profile in edible parts (meat) and in abdominal fat changed with flaxseed levels. Increased amount of dietary flaxseed caused the content of oleic and linoleic acid to decrease slightly, while the linolenic acid increased significantly.

Using 5 or 7.5% whole or ground flaxseed, Roth-Maier et al. (1998a) reported that response of tissue polyunsaturated FAs to flaxseed intake is dose dependent, whereas the whole flaxseed had only a moderate effect in this regard. Feeding ground flaxseed increased LNA and DHA, slightly decreased EPA, and markedly reduced the ratio between ω-6 and ω-3 FA's. There are fewer changes in birds fed whole flaxseed than in those fed ground seed.

Ajuyah et al. (1991a) reported the effect of 10 or 20% full-fat flaxseed or canola seed on FA profile of white and dark meat of broilers fed over a 6-weeks period, as shown in Tables 1.2. Source and level of full-fat oil seed significantly modulate the FAs in tissue.

Table 1.2. Effect of full-fat flaxseed on the FA composition of lipid of white and dark meat

FA, %	Full-fat flaxseed					
	0%	10%	20%	0%	10%	20%
	In white meat lipid, %			In dark meat lipid, %		
$C_{16:0}$	18.1	18.0	19.1	18.4	15.0	13:4
$C_{18:0}$	12.5	11.0	12.4	10.9	11.0	14.4
$C_{18:1\omega9}$	33.5	28.9	19.0	37.4	28.6	21.1
$C_{18:2\omega6}$	18.4	20.6	23.8	18.5	22.1	26.9
$C_{20:4\omega6}$	8.0	4.8	3.4	5.7	4.9	3.4
$C_{18:3\omega3}$	1.2	4.1	7.0	1.2	6.9	10.3
$C_{20:5\omega3}$	0.8	2.1	3.6	0.5	1.3	2.2
$C_{22:5\omega3}$	2.0	3.1	4.7	1.5	2.7	2.9
$C_{22:6\omega3}$	2.5	4.8	3.9	2.3	4.7	2.8
SAT	30.8	29.3	31.7	29.6	26.3	28.0
MUFA	35.6	30.3	19.9	40.1	30.5	22.5
ω-3	6.4	14.1	19.2	5.4	15.6	18.2
ω-6	27.1	26.3	29.2	24.8	27.6	31.3
ω-6/ω-3	4.3	1.9	1.5	4.6	1.8	1.7

Source: Ajujah et al. 1991a.

5. Conclusion

Flaxseed can be effectively used in enriching ω-3 FAs in chicken egg or meat, while playing a role as an alternative feed ingredient. The production performance of these flaxseed-fed animals is very often reduced, even more so with high amount of flaxseed. Most results reported on these topics, either in hens or in broilers, were obtained with diets that used ground or whole raw flaxseed. While it is well established in the feed industry that many feed ingredients (oilseed) must be properly processed prior to feeding to birds, it is important to evaluate various processing protocols for flaxseed in order to enhance its value as a feedstuff. Another approach to increase its feeding value is to try to reduce the deleterious influence of ANFs through the addition of relevant dietary enzymes and vitamin B6.

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Section II

Effect of Dietary Enzymes Supplementation and Flaxseed Autoclaving on the Performance Response of Broilers Fed Flaxseed

Connecting Text for Section II

Research efforts related to the nutritive value of flax products had pointed to the existence of antinutritional factors in linseed meal, the defatted product of flaxseed. Overcoming the antinutritional effect of these factors had been the key step in improving its feeding value. However, knowledge about the nutritional value of flaxseed and its use in animal feed are limited. Better understanding could benefit the increased interest of T-3 fatty acid, as well as in developing flaxseed as an alternative ingredient. Like any oilseed, proper processing or treatment may improve utilization of flaxseed, especially for monogastric animal, like broilers. The following experiments were conducted to screen the effect of feed enzyme addition, or flaxseed processing as autoclaving, on performance response of broilers.

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Effect of Dietary Enzymes Supplementation and Flaxseed Autoclaving on the Performance Response of Broilers Fed Flaxseed

Yingran Shen,* E. R. Chavez, *[1] and P. C. Laguë*

*Department of Animal Science, Macdonald Campus of McGill University, Canada

1 To whom correspondence should be addressed: chavez@macdonald.mcgill.ca

E. R. Chavez

Department of Animal Science, Macdonald Campus of McGill University, 21,111

Lakeshore, Ste Anne de Bellevue, Quebec, Canada, H9X 3V9

Tel: (514) 398 7795; Fax: (514) 398 7964

ABSTRACT Three experiments were carried out to study the effect of enzyme addition to flaxseed (FS) containing diets, or FS autoclaving, on the performance response of young broilers aged day one to 21. The enzymes tested, including single liquid xylanase, arabinofuranosidase and lipase, and powdered feed enzyme as Natugrain, Natugrain Blend, Avizyme 1500, and Allzyme A and Allzyme B, were incorporated into 10% FS containing diets at the level recommended by the manufacturer. Autoclaving of FS was performed with various combinations of pressure, temperature, and duration. There were three or four replicates per treatment with 10 birds per replicate in all the experiments.

The body weight, feed consumption and feed to gain ratio of control birds after three weeks with 10% ground FS, was 493 g, 791 g, and 1.81 in experiment 1, 688 g, 1001 g, and 1.55 in experiment 2, and 810 g, 1140 g, and 1.49 in experiment 3, respectively. No significant performance improvement ($P>0.05$) was observed by the enzyme addition tested. However, when the vitamins and minerals were in marginal as in experiment one, the addition of xylanase and arabinofuranosidase slightly ($P>0.05$) improved the three weeks old broiler body weight by 11%, feed consumption by 8%. The same or higher dosage of enzyme addition failed to improve performance of young broilers when a commercial vitamins and minerals premix was used in experiment two.

Autoclaving of FS at high pressure (16.5 kg/cm^2), high temperature (120 °C), and a longer period (15 min) lead birds to gain more (4.9%) and consume more feed (5.1%), as compared to the control birds. However, autoclaving at lower pressure (13.4 kg/cm^2), and lower temperature (110°C) and shorter time period (7.5 min) reversed this improving effect.

The results seemed to indicate that the antinutritional factors present in FS at 10% inclusion rate might not cause performance reduction when in optimal feeding condition. Autoclaving at higher pressure (16.5 kg/cm^2) and longer time period (15 min) could physically change the structure of FS and/or the antinutritional factors in FS and better benefit broiler in utilizing its nutrient.

(*Key Words*: flaxseed, enzyme, autoclaving, performance, broilers)

INTRODUCTION

The presence of antinutritional factors (ANFs) in flaxseed (FS) reduces its use, especially in monogastric animals. It is well documented that FS contain ANFs, like cyanogenic glycosides (Bhatty 1993, Wanasundara et al. 1993, and Mazza and Oomah 1995), linatine (Kratzer 1954, Klosterman 1967), and soluble non-starch polysaccharides (sNSP) (Oomah et al 1995, Fedeniuk and Biliaderis 1994). The cyanogenic glycosides can release hydrogen cyanide, which is a potent cellular respiratory inhibitor, whereas linatine is an antagonist to vitamin B6, and the sNSP content is closely correlated to viscosity in the intestine (Bhatty et al. 1991). It has been shown that viscous stuffs in animal intestine reduce nutrient utilization (Smits and Annison 1996). ANFs can thus depress body growth due to their toxic effect and their reduction in nutrient utilization.

By removing these ANFs, the feeding value of FS may be improved. This relies on the processing of FS, or counteracting by supplementing additional ingredients to the FS containing diet. One of the processing methods is proper heat treatment. Heating may reduce the content of ANFs in FS, as in most cases the ANFs are heat sensitive. On the other hand, the positive effect in broilers of exogenous enzyme supplementation has also been demonstrated for ingredients with high viscous property. There is very limited information regarding FS in these aspects. It is expected that the effectiveness of these dietary treatments will be obvious for broilers, as fast growing birds tend to be sensitive to ANFs variation. The objectives of the present studies were to screen for effective means to improve feeding value of FS for broilers. Exogenous enzyme supplementation and autoclaving of FS were tested.

MATERIALS AND METHODS

Experiment Design and Diets

Three feeding experiments with young broilers (day 1 to 21) were performed. Experiment 1 tested the effectiveness of exogenous enzymes, xylanase and arabinofuranosidase[2] and various autoclaving and FS processing combinations on the performance response of chicks. A corn-soybean diet and a full-fat soybean diet (FFSB) were used as control groups.

Experiment 2 tested the same dietary treatments but with increased enzyme concentration, or increased autoclaving pressure and temperature, or longer autoclaving times. Liquid lipase[3] was included as another dietary factor in this experiment.

Experiment 3 studied the effect of a variety of commercial feed enzymes on the performance response of chicks. The enzymes were Natugrain and Natugrain Blend[4], Avizyme™[5], and Allzyme A and Allzyme B[6].

All FS-containing diets had 10% FS. The diet with ground FS was regarded as FS control and was used in all 3 experiments. Each treatment had 3 replicates for experiment 1 and 2, and 4 replicates for experiment 3. There were 10 chicks in each replicate (pen). The treatment factors for these experiments are shown in Table 2.1.

The following enzyme mixing procedures were followed to guarantee the even distribution and preservation of activities. All enzymes were mixed with FS first before mixing with other ingredients. For liquid enzymes, they were sprayed onto ground FS, after their dilution with the same amount of water so to make spraying as even as possible. These enzyme-containing FS were kept at 4°C pending their incorporation into other feed mixture, which took place one day prior to the beginning of a feeding trial. The powder feed enzymes were also premixed with FS. These FS-enzyme mixtures were then incorporated into other feed mixes during normal feed mixing. All experimental feed was kept at 4°C until being fed to the birds.

The composition and proximal analysis of basal diets are shown in Table 2. 2.

Birds and Management

One-day-old male broiler chicks (Ross × Ross cross) purchased from a local commercial hatchery were used in the studies. They were raised in thermostatically controlled and electrically heated Petersime batteries for 3 weeks under the 24 hours lighting. They were fed ad libitum and had free access to water. Chicks that were fed whole FS had access to grit ad libitum.

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[2] Institut Armand-Frappier, Université du Québec, Ville de Laval, Qué, Canada H7N 4Z3.
[3] Innu-Science Canada Inc., 1777 Nobel, Ste-Julie de VerchÎres, QC, Canada.
[4] BASF Canada Inc., 80 Todd Road, Georgetown, Ont. Canada.
[5] Finnfeeds International, Missouri 63026, U.S.A.
[6] Alltech Canada, 449 Laid Road, Unit 10-11, Guelph, Canada N1G 4W1.

Table 2.1. Treatment design of experiment 1, 2 and 3.

Experiment 1	Experiment 2	Experiment 3
Controls:	FS control	FS control
Corn-soy diet	FS + Xyl A (8,000 U/kg)	FS + Natugrain, 0.1 kg/MT
FFSB diet	FS + Xyl B (16,000 U/kg)	FS + Natugrain Blend, 0.1 kg/MT
FS control	FS + Ara A (400 U/kg)	FS + Avizyme^{TM} 1500, 1 kg/MT
FS + Enzyme (8000 U Xyl + 400 U Ara./kg)	FS + Ara B (800 U/kg)	FS + Allzyme A: 1 kg/MT
	FS + Xyl B (16,000 U/kg) + Ara B (800 U/kg)	FS + Allzyme B: 1 kg/MT
FS, ground-then-autoclaved A	FS, autoclaved A	FS + Lip: 16,000 U/kg mixed into diet during feed mixing
FS, autoclaved A-then-ground	FS, autoclaved B	
FS, whole seed, autoclaved D	FS, autoclaved C	
	FS, autoclaved D	
	FS + Lip A (8,000 U/kg, mixed into diet during feed mixing)	
	FS + Lip B (16,000 U/kg, mixed into diet during feed mixing)	
	FS + Lip A (8,000 U/kg, mixed into diet right before feeding)	
	FS + Lip B (16,000 U/kg, mixed into diet right before feeding)	

FFSB, full-fat soybean;
FS, flaxseed;
Xyl., xylanase
Ara, arabinofuranosidase
Lip., Lipase

Autoclave A: FS autoclaved at 13.4 kg/cm^2, 110°C for 7.5 min.

Autoclave B: FS autoclaved at 13.4 kg/cm^2, 110°C for 15 min.

Autoclave C: FS autoclaved at 16.5 kg/cm^2, 120°C for 7.5 min.

Autoclave D: FS autoclaved at 16.5 kg/cm^2, 120°C for 15 min.

Claimed enzymes activities: Natugrain, endo-xylanase, minimum 1650 U/g; β-glucanase, 1200 U/g. Natugrain is designed for barley type of feed, while Natugrain Blend for wheat-type feed. Avizyme^{TM}, 300U xylanase, 4000 U protease and U alpha-amylase/g.
Allzyme A and Allzyme B: the specific enzyme activity of these two products is not available

Flaxseed and its Processing Procedures

Brown feed-grade FS purchased from a local feed ingredients supplier was used in the present experiments. The seed was ground prior to feeding with a household coffee grinder,[7] except those fed as whole seed. Autoclaving was performed by using a steam sterilizer[8] at 16.5 kg/cm^2 and 120°C for 15 minutes, except when otherwise specified. During autoclaving, FS were laid 3 cm thick on a tray.

Performance Record

Body weight and feed consumption were recorded weekly for three weeks. Weighing was performed by pen weighing on day one and by individual at the end of first, second and third week under full fed condition. Feed consumption was determined on pen basis. Mortality was recorded. The feed consumed by culled or dead birds was deducted from total feed consumption of the pen. The correction was made by weighing the feed of the pen on the day of culling or death and then adjusting for the share of the culled or dead birds.

Statistics

All data were processed by ANOVA by using SAS (1995) of General Linear model (GLM) procedure (for even number observation), or mixed model (MIXED) (for uneven or missing observation). Pen represented the experimental unit. The observation within the pen were regarded as the sampling unit and nested with the pen. The statistical significance of the difference between least square means was determined by T test.

RESULT

Experiment 1.

The performance of chicks in experiment 1 is shown in Table 2.3. No significant improvement ($P>0.05$) in body weight, feed intake and feed conversion efficiency was detected by xylanase and arabinofuranosidase addition or by the tested FS autoclaving.

When compared to birds fed diets containing 22.4% full-fat soybean, birds fed the 10% FS diet supplemented with 8000 IU xylanase and 400 IU arabinofuranosidase/kg showed a better growth and consumed more feed ($P<0.05$). The chicks fed enzyme added

[7] Black & Decker Canada Inc. Brockville, Ont.
[8] Barnstead Still and Sterilizer Co., Boston, Mass., U.S.A.

FS vs. FFSB diet weighed, at 1 and 3 weeks of age, 145 vs. 128 g, and 548 vs. 426 g respectively; while feed consumption was 855 vs. 653 g for the 3-week-period. This better performance was not obtained when FS-containing diets were not supplemented with enzymes.

Table 2.2. Composition and proximal analysis of diets in experiment 1, 2 and 3

	Control (Experiment 1, 2 and 3)		Flaxseed control	
	Corn-soybean	Full-fat soybean	Experiment 1	Experiment 2, 3
Ingredients: %				
Corn	57.04	53.29	51.69	52.38
Soybean meal (47%)	32.07	18.58	31.24	31.65
Soybean, full-fat	5.00	22.40	0	0
Flaxseed	0	0	10.00	10.00
Wheat bran	0	1.93	3.34	0.77
Animal Fat	2.00	0	0	0
Dical 18.5% Phos	1.73	1.68	1.53	0
Calcium Carbonate	1.38	1.7	1.41	0
Premix A[1]	0.60	0.60	0.60	0
Premix 4050[2]	0	0	0	5.0
D,L. Methionine	0.14	0.14	0.14	0.14
Lysine 98%	0.04	0.01	0.06	0.06
Calculated Composition %				
Crude Protein	22.10	22.10	22.10	22.0
Either Extract	5.50	6.34	6.34	6.26
ME, kcal/kg	3050	3050	3050	3050
Methionine	0.50	0.50	0.50	0.50
L-Lysine	1.27	1.27	1.27	1.27
Ca	1.00	1.00	1.00	1.01
Available P	0.45	0.45	0.45	.39
Actual Analysis, %				
Dry Matter	89.22	89.04	89.64	87.72
Crude Protein	22.96	22.94	22.88	22.19
Ether Extract	5.64	6.60	7.80	6.31
Ash	5.82	5.64	6.45	5.95
Ca	0.95	0.90	1.32	0.85
P	0.73	0.71	0.77	0.63

1. Vitamin and Mineral premix A supplied the following per kilogram diet: vitamin A, 1500 IU; vitamin D_3, 200IU; vitamin K, 0.50 mg; vitamin B_{12}, 0.01mg; Biotin, 0.15mg; Folacin, 0.55mg; Niacin, 35 mg, pantothenic acid, 10mg; Pyridoxine, 3.5gm; Riboflavin, 3.6 mg; Thiamin, 1.8mg; Choline, 1300 mg; Cu, 8 mg; I, 0.35mg; Fe, 80 mg; Mn, 60 mg; Zn, 40 mg; Na, 2 g; Cl, 2 g.
2. Premix 4050 provided by COOPÉRATIVE FÉDÉRÉE DE QUÉBEC, Montr9al, Canada, claimed to supply per kilogram diet: Ca, 9.0g; P, 3.2g; Na, 1.4g; Fe, 250mg; Mn, 90mg; Cu, 10mg; I, 1.25mg; Zn, 90mg; Se, 0.3mg; vitamin A, 10,000 IU; vitamin D_3, 3,000IU; Vitamin E, 25IU.

Table 2.3. Effect of enzyme supplementation and flaxseed autoclaving on performance of chicks fed 10% of flaxseed

Dietary	Body weight, g		Feed intake, g		Feed/gain	
Factor	Wk 1	Wk 3	Wk 1	Wk 1 to 3	Wk 1	Wk 1 to 3
Corn-soy diet	139^{ab}	530^{a}	127	782^{ab}	1.37^{a}	1.71
FFSB[1] diet	128^{b}	426^{b}	119	653^{b}	1.48^{ab}	1.88
10%FS[2]	132^{ab}	493^{ab}	123	791^{ab}	1.45^{ab}	1.81
10%FS + enzyme[3]	145^{a}	548^{a}	136	855^{a}	1.39^{ab}	1.80
10%FS, G then A[4]	137^{ab}	506^{ab}	123	825^{ab}	1.37^{a}	1.88
10%FS, A then G[5]	122^{b}	499^{ab}	117	811^{ab}	1.60^{b}	1.94
10%FS, whole, A[6]	126^{b}	472^{ab}	110	751^{ab}	1.40^{ab}	1.81
SEM	5.8	17.2	7.3	38.5	0.035	0.095
Probability	0.135	0.167	0.339	.048	0.007	0.722

1. FFSB, extruded full-fat soybean.
2. FS, flaxseed.
3. Diet was supplemented with 8000U/kg xylanase and 400U/kg arabinofuranosidase;
4. Flaxseed was ground then autoclaved.
5. Flaxseed was autoclaved then ground.
6. Whole flaxseed, autoclaved.

Means within columns with no common superscripts differ significantly (T test, $P<0.05$).

Autoclave processing at 13.4 kg/cm^2 and 110°C for 7.5 minutes, did not improve performance of birds both in ground and whole seed groups. The birds fed with whole FS had the lowest body weight at 3 weeks of age, and the lowest feed consumption in week 1 and the week 1-to-3 period, even though grit had been provided and the FS had been autoclaved for shorter period.

Experiment 2.

Experiment 2 failed to demonstrate any significant ($P>0.05$) benefit by including xylanase, arabinofuranosidase, and lipase under present supplement level, or by autoclaving the FS under specified protocols as shown in Table 2.4. All birds fed 10% ground FS for 3 weeks had relatively satisfactory growth performance. The birds fed the

FS control diet weighed 143 g at 1 week of age, and 688 g at 3 weeks of age. Their feed intake was 130 g in week 1 and 1001 g for the 3-week period, giving a feed to gain ratio of 1.28 and 1.55, respectively.

Table 2.4. Effect of enzyme supplement and flaxseed autoclaving on performance of chicks fed 10% flaxseed

Dietary Factor	Body weight, g		Feed intake, g		Feed/gain	
	Wk 1	Wk 3	Wk 1	Wk 1 to 3	Wk 1	Wk 1 to 3
FS[1] control	143	688	130^{ab}	1001	1.28^{abc}	1.55^{ab}
Xyl A[2]	143	679	129^{ab}	1021	1.28^{abc}	1.60^{ab}
Xyl B[3]	148	680	134^{bc}	1005	1.26^{ab}	1.58^{ab}
Ara A[4]	146	654	131^{ab}	1046	1.27^{abc}	1.72^{b}
Ara B[5]	148	691	131^{ab}	1002	1.25^{a}	1.55^{ab}
Xyl B + Ara B[6]	149	707	133^{bc}	1008	1.25^{a}	1.52^{a}
Autoclave A[7]	134	660	123^{a}	973	1.35^{c}	1.58^{ab}
Autoclave B[8]	147	711	134^{bc}	1016	1.28^{abc}	1.52^{a}
Autoclave C[9]	138	718	127^{ab}	1001	1.33^{bc}	1.48^{a}
Autoclave D[10]	146	722	135^{bc}	1052	1.31^{abc}	1.55^{ab}
Lipase A[11]	142	678	131^{ab}	1028	1.31^{abc}	1.63^{ab}
Lipase B[12]	138	694	127^{ab}	990	1.32^{abc}	1.52^{ab}
Lipase C[13]	151	710	142^{c}	1027	1.31^{abc}	1.54^{ab}
Lipase D[14]	156	694	135^{abc}	1012	1.24^{a}	1.56^{ab}
SEM	4.5	24.3	3.4	28.0	0.029	0.067
Probability	0.254	0.108	0.068	0.854	0.254	0.691

FS, flaxseed; 2. Xyl A, 8,000 U/kg xylanase; 3. Xyl B, 16,000 U/kg xylanase; 4. Ara A, 400 U/kg arabinofuranosidase; 5. Ara B, 800 U/kg arabinofuranosidase; 6. Xyl B + Ara B, 16,000 U/kg xylanase + 800 U/kg arabinofuranosidase; 7. Autoclave A, FS autoclaved at 13.4 kg/cm^2, 110°C for 7.5 min; 8. Autoclave B: FS autoclaved at 13.4 kg/cm^2, 110°C for 15 min; 9. Autoclave C: FS autoclaved at 16.5 kg/cm^2, 120°C for 7.5 min; 10. Autoclave D: FS autoclaved at 16.5 kg/cm^2, 120°C for 15 min; 11. Lipase A, 8,000 U/kg lipase mixed into diet during feed mixing; 12. Lipase B, 16,000 U/kg lipase mixed into diet during feed mixing; 13. Lipase C, 8,000 U/kg lipase mixed with flaxseed was incorporated into diet prior to feeding; 14. Lipase D, 16,000 U/kg lipase mixed with flaxseed was incorporated into diet prior to feeding.

There was a slight improvement for body weight (4.9%) and feed consumption (5.1%) in week 3 by autoclaving FS for longer periods (15 min) and higher temperature (120°C),

when compared with control FS diet. Both body weight and feed intake were reversed (-4.1 and –2.0% respectively), when FS was autoclaved for a shorter period (7.5 min) and at lower temperature (110°C).

As compared to FS control diet, adding lipase of 8000 U/kg diet during feed mixing slightly improved weight gain by 3.2% and feed intake by 2.6% in the 3-week period, but not in statistically significant level. For all lipase-containing groups, lipase addition did not significantly ($P>0.05$) improve performance.

Experiment 3.

As shown in Table 2.5, no performance improvement was noticed for broilers fed 10% FS in the 3-week period, by the inclusion of the commercial enzymes tested.

Table 2.5. Performance of chick fed 10% of flaxseed supplemented with enzymes (experiment 3)

Dietary Factor	Body weight, g		Feed intake, g		Feed/gain	
	Wk 1	Wk 3	Wk 1	Wk 1 to 3	Wk 1	Wk 1 to 3
10%FS[1]	165	810	138	1140	1.15	1.49
10%FS + Natugrain[2]	161	794	135	1113	1.17	1.49
10%FS + Natugrain Blend[3]	162	801	137	1133	1.16	1.50
10%FS + Avizyme^{TM} 1500[4]	162	787	135	1155	1.15	1.56
10%FS + Allzyme A[5]	159	823	137	1139	1.20	1.46
10%FS + Allzyme B[6]	166	816	145	1168	1.19	1.51
10%FS + Lipase[7]	165	782	142	1112	1.19	1.51
SEM	3.4	14.7	3.5	24.1	0.017	0.026
Probability	0.673	0.411	0.367	0.653	0.337	0.2853

1. FS, flaxseed;
2. Supplemented with 0.1 kg/MT Natugrain.
3. Supplemented with 0.1 kg/MT Natugrain Blend.
4. Supplemented with 1 kg/MT Avizyme^{TM} 1500.
5. Supplemented with 1 kg/MT Allzyme A 1kg/MT.
6. Supplemented with 1 kg/MT Allzyme B 1 kg/MT.
7. Supplemented with 16,000 U/kg lipase mixed into diet during feed mixing.

DISCUSSION

Enzyme Addition

The soluble non-starch polysaccharides (sNSP) in FS are the main target for the dietary enzyme supplement. The water binding capacity exerted by sNSP prevents the action of the digestive enzyme in the intestine. Its influence in reducing nutrient utilization is not limited to starch, but also protein and lipids (Smits and Annison 1996, Smits *et al.* 1997), and particularly in lipids (Choct and Annison 1992). The effectiveness of using exogenous enzyme preparation in broilers has been well documented: xylanase for wheat-containing diet (Veldman and Vahl 1993), xylanase for rye-containing diet (Däniche *et al.* 1997; Boros et al. 1998). Positive response has also been reported for oilseed-diets like Lupins by using mainly the xylanase supplement (Annison *et al.* 1997), and soybean by xylanase and protease (Zanella *et al.* 1999).

FS is known for its mucilaginous properties. Depending on the varieties, FS contains 3.6-8.0% water-soluble polysaccharides without distinction between starch and non-starch proportion (Oomah et al. 1995). Mazza and Oomah (1995) cited from other researchers that 83% of carbohydrate in FS is nonstarch polysaccharides. By this ratio, one can presume that FS contains 2.99-6.63% sNSP, which is substantially more than the sNSP in rye flour (4.61%) and in whole-wheat flour (2.55%) (Englyst et al. 1982). It should therefore be possible to improve the nutrient digestion related to sNSP by using exogenous enzymes. The improvement in weight gain and feed consumption by xylanase and arabinofuranosidase addition, as observed in experiment 1, could partly be the result of the alleviation of the adverse effect caused by sNSP in FS.

Even though there is no evidence that the enzyme chosen for a given sNSP-rich diet should proportionally depend on the constituting sugars, there may be advantages in selecting enzymes capable to digest the relative high amount of sugar in sNSP moiety. In FS, xylose makes up 16-38% of the sNSP (Mazza and Oomah 1995). All the enzyme preparations used in the present studies contained mostly xylanase, however, they failed to improve the productivity of the chicks, except for a slight improvement observed in experiment 1. Hence, one may question whether it is desirable to choose exogenous enzymes based on the constitutive sugar of sNSP.

The effectiveness of enzyme addition is affected by many factors, and may not always be consistent (Boggs et al. 1960). The response of chicks to xylanase and arabinofuranosidase supplement observed in experiment 1 may have been partly related to the low vitamin and mineral addition also. Only minimum vitamins and minerals, as suggested by NRC (1994), were incorporated in experiment 1 diets. The same enzyme inclusion did not reproduce the results when a commercial premix was used in experiment 2. It is claimed that this premix provides ample supply of minerals and vitamins that could satisfy requirement under normal and sub-clinical conditions. That may also explain the satisfactory performance response in FS control groups when no extra treatment of FS was provided besides grinding. The body weight of control birds at 3 weeks of age reached 688 g in experiment 2 and 810 g in experiment 3. Meanwhile, the dilution effect of around 40% oil in FS may reduce the deleterious factors in FS, like sNSP, under the condition of 10% inclusion rate.

There is diversity in the sources and characteristics of xylanase and ∃-glucanase in different enzyme preparations, while the action of exogenous enzyme in the intestinal tract would be type specific and related to fat-type (Däniche et al. 1997). The main enzyme activities for the enzymes used in the present experiments are xylanase and ∃-glucanase. They are designed mainly for wheat and barley type diets. The inclusion of these enzymes preparations strictly followed the manufacturers recommendation. The ineffectiveness of these feed enzymes in FS containing diets could be just another example of specificity of enzyme action.

Autoclaving

Autoclaving works mainly in two areas. The first one could be the removal or destruction of a variety of toxins, or ANFs, like phytate (De Boland *et al.* 1975), anti-thyroid agents (Nakaya 1980), and trypsin and chymotrypsin inhibitors (Rattansi and Dikshit 1997). Another effect is nutrient utilization improvement.

It is well documented that FS may contain high amounts of cyanogenic glycosides, depending on variety and other conditions (Bhatty 1993, Chadha et al. 1995, Mazza and Oomah 1995). As autoclaving is also one kind of heat processing but under high pressure, it is expected that the cellular glycosidase will be inactivated due to excess heat. That leads to believe that the hydrogen cyanide (HCN) content from hydrolysis of cyanogenic

glycosides would be reduced, as shown by Deshmukh *et al.* (1982), Mandolhot and Singh (1983), Kozlowska (1989), and Veldsink *et al.* (1999). Autoclaving reduced effectively HCN content, ranging from a reduction to one eighth of the original amount (85mg/kg), to almost total removal. However, the deactivation of the cellular glycosidase may not be enough. Intestinal microorganisms may produce linamarase activity: Majak and Cheng (1987) demonstrated that the cyanogenic glycoside after heat processing could still by degraded to produce HCN in the intestine. Therefore, *in vitro* reduction of HCN content may not realy represent the in vivo HCN toxic effect. The results in experiments 1 and 2 showed that lower temperature and shorter period of FS autoclaving (13.4 kg/cm^2, 110°C for 7.5 min) did not improve the feeding response. These results support the observation by Lee *et al.* (1991) that dry heating FS for 30 minutes at 85°C did not improve chicks performance, nor the protein retention and ME value of the 10% FS-containing diet.

No improvement or reverse response by heat treatment or autoclaving may be related to the devaluation of protein. It has been reported that improper autoclaving reduces available essential amino acids content (Nath et al. 1981), while excess heating would reduce nitrogen digestibility (Young 1982).

Effective processing such as autoclaving may therefore lie between the devaluation of other nutrients and the effective destruction of toxins. The positive performance response by the autoclaving protocol of 16.5 kg/cm^2 at 120°C for 15 minutes may support this reasoning (experiment 2).

In addition to destroying HCN, heat processing can also reduce other ANFs, like phenolic acids. Heating decreased their concentration from 13.14 g/kg in seed to 8.8 g/kg in meal (Oomah and Mazza 1998).

Autoclaving, however, may have broader effect than a simple cyanogenic glycoside reduction, as excessive heating of FS also reduces nitrogen digestibility. It seems that the autoclaving protocol is critical. While aiming to achieve detoxification, one should also consider the devaluation of other nutrients in FS. Although the present studies indicate the possibility to improve feeding value of FS by autoclaving, further research is required for establishing effective autoclaving protocols.

In conclusion, the feed enzymes tested with 10% FS did not improve the performance of broiler chicks fed these rations between day-old to 3 weeks of age under the present

experimental conditions. Autoclaving of FS under the protocol of 16.5 kg/cm^2 at 120°C for 15 minutes slightly improved the performance of young broilers. Autoclaving at lower temperature and shorter periods showed no performance improvement.

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Section III

Significance of Flaxseed Level, Processing, and Vitamin B_6 Addition on Broiler Performance and/or Nutrients Utilization

Connecting Text for Section III

Previous experiment showed that a level of 10% FS would not cause obvious growth depression for young birds, provided an optimal feeding condition is available. As there could be of nutritional and economical advantage for higher FS inclusion rate, higher FS level in the diet could demonstrate the effectiveness of any processing. This effectiveness may rely on the physical change caused by this given processing, or the combined effect of nutrition availability improvement and antinutritional factors removal. The following experiment therefore tested the effect of processing method as pelleting, autoclaving on performance response, as well as nutrition utilization of broilers, under different levels of FS inclusion rate.

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Significance of Flaxseed Level, Processing, and Vitamin B_6 Addition on Broiler Performance and/or Nutrients Utilization

Yingran Shen,* P. C. Laguë,* and E. R. Chavez, *[1]

**Department of Animal Science, Macdonald Campus of McGill University, Canada*

1 To whom correspondence should be addressed: chavez@macdonald.mcgill.ca

E. R. Chavez

Department of Animal Science, Macdonald Campus of McGill University, 21,111

Lakeshore, Ste Anne de Bellevue, Quebec, Canada, H9X 3V9

Tel: (514) 398 7795; Fax: (514) 398 7964

ABSTRACT The objective of the present study was to study the effect of flaxseed (FS) level and FS processing on broiler performance and nutrient utilization. FS was fed to young broiler (aged day one to 21) as whole seed, whole seed but autoclaved, autoclaved then ground, grinding only, or pelleted together with other ingredients, at the inclusion levels of 0, 10, 12, or 14%. Each dietary treatment had three or four replicates with 10 day-old male birds per replicate. Chicks fed pellet processed FS had significant ($P<0.01$) better body weight, consumed more feed, and had better feed to gain ratio than those fed other FS processing diets, both after week one and after three weeks. FS levels had also very significantly ($P<0.01$) effect on body weight and feed to gain ratio both after first week and after three weeks. Chicks fed 10% FS had significant ($P<0.01$) better body weight and feed to gain ratio over those fed 12 or 14% FS, both after week one and after three week feeding. Birds fed 10% FS that was processed as pelleted together with other ingredients had best performance. They weighed 181 and 829 g after first week and after three weeks, consumed 160 and 1168 g feed, and obtained feed to gain ratios of 1.17 and 1.49, respectively. FS levels higher than 10% in the diets tended to lower the body weight and reduced feed consumption. Birds that consumed whole FS weighed lighter and consumed less feed, with a higher feed to gain ratio, both after first week and after three weeks. Whole FS that had been autoclaved tended to alleviate this performance reduction. The effect of processing and FS level on performance was in agreement with the results of apparent nutrient digestibilities determined. There were very significantly interactions ($P<0.01$) between FS processing and FS level on the apparent digestibilities of dry matter, crude protein, ether extract (EE), ash, and AMEn values. This interaction reached a significant level ($P<0.05$) for ether extract digestion. Among the FS containing diets, the best EE digestion was achieved by pelleting, with values of 77.8 and 77.0% for 10 and 14% FS-containing diets, respectively. The better EE utilization by young broilers may explain the higher AMEn values, 2743 and 2924 kcal/kg for 10 and 14% pelleted FS-containing diets, respectively, as compared to other FS containing diets. Pelleting allowed a higher inclusion rate, as much as 14%, in young broiler diet without adverse effect on performance and AMEn values.

(*Key Words*: flaxseed, flaxseed processing, performance, nutrient utilization, broilers)

INTRODUCTION

The growth depression of flaxseed (FS) caused by the antinutritional factors (ANFs) in flaxseed, like linatine and cyanogenic glycosides (Kratzer 1946, 1947, Madhusudhan et al. 1986, Batterham et al. 1991) and its low nutrient utilization (Barbour and Sim 1991, Lee et al. 1995, Grossu et al. 1998) had long been recognized in chicks. Their influence on chick performance may also be related to FS inclusion level in the diet. Leeson and Summers (1997) suggested that the maximum inclusion rate of FS for broiler to be 8%. Proper processing is an essential step to maximize the potential of FS as an alternative ingredient. This basic requirement is not different than that for most other oilseeds, like soybean. As additional consideration, any processing should bear practicability, and should not alter the unique nutritional feature of FS.

To achieve these objectives, a few processing methods were designed and tested for their influence on young broilers' performance and nutrient utilization. Their interaction with FS inclusion level was also studied.

MATERIALS AND METHODS

Experimental Birds and Management

Six hundred male day-old Ross H Ross chicks were acquired from a commercial hatchery and were raised in thermostatically controlled and electrically heated Petersime batteries for three weeks under 24 hrs lighting. They were fed ad libitum and had free access to water. Chicks fed whole FS had access to grit ad libitum.

Design of the Experiment

A complete random design with factorial arrangement of treatments was used in this experiment, with four levels of FS and six processing methods. The four FS levels were 0, 10, 12 and 14%, while 6 processing or treatment factors included ground seed, autoclaved whole seed, autoclaved whole seed then ground, whole seed without any processing, whole seed pelletted together with other ingredients, ground seed supplemented with extra vitamin B_6 at 40 mg/kg diet. Full-fat soybean at concentration of 14.0 or 23.8% and a commercial pelleted diet were used as controls. There were 3

replicates for the following dietary treatments: commercial ration, full-fat soybean, ground seed with no other processing (10, 12 14% of FS), and whole seed with no other processing (10, 14%). All the other treatments had four replicates. There were 10 chicks per replicate.

Flaxseed, its Processing, and Experimental Diets

The brown feed-grade FS purchased from a local ingredient supplier was used in the present experiment. It was ground with a household coffee grinder, or autoclaved using a steam sterilizer[2] at 16.5 kg/cm2 and 120°C for 15 min. Pelleting was performed with a California Pellet Machine, Master model[3]. The die parameters were: hole diameter, 0.318 cm; die diameter, 30.48 cm; thickness of the die, 3.05 cm. Pelleting was performed twice for the same feed with no steam conditioning. However, prior to the second pelleting, the feed was cooled down to room temperature by placing it on trays under an electric fan for 10 min.

All diets were isocaloric and isonitrogenous except for the commercial diet. Their composition and proximal analysis are shown in Table 3.1.

Apparent Digestibility Determination

Some selected treatments were used for total excreta collection. These treatments included three levels of FS: 0, 10 and 14%; and four FS-processing methods: grinding only, whole seed that had been autoclaved, whole seed without any processing, and pelleting together with other ingredients in the diet. The diets with two level of full-fat soybean, 14% and 23.8%, and the commercial diet were also included in the total excreta collection trial. Three pens from each of these treatments were used for the digestibility trial.

The collection started when the birds were 15 days old and lasted for 4 consecutive days. The feed intake and excreta were recorded daily for each pen. Feathers, scales and spilled feed were carefully excluded from weighing. Fresh feces were freeze-dried with a vacuum freeze dryer[4] at –40°C for 72 hrs.

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[2] Barnstead Still and Sterilizer Co., Boston, Mass., U.S.A.
[3] California Pellet Mill Co. 1114 E. Wabash Avenue, Crawfordsville, IN 47933, U.S.A.
[4] Virtis Freeze Dryer, =278341, Gardiner NY 12525, U.S.A.

Apparent nutrient digestibility of the feeds were calculated by the method described by Farhat et al (1998). The factor of 8.22 kcal/g (Hill and Anderson, 1958) was used for the calculation of apparent metabolizable energy corrected for nitrogen loss (AMEn).

Table 3.1. The composition and proximate analysis of basal diets with different level of flaxseed

	Flaxseed			Full-fat soybean		Commercial starter
	10%	12%	14%	14%	23.8%	
Ingredients %:						
Corn	52.22	49.55	46.83	49.90	53.18	NA
Soybean 47	31.96	31.06	30.14	25.80	17.85	NA
FFSB[1]	0	0	0	14.0	23.81	NA
Flaxseed	10.00	12.00	14.00	0	0	NA
Wheat bran	0.64	2.21	3.84	2.20	0	NA
Animal fat	0	0	0	3.00	0	NA
Premix[2] 4050	5.00	5.00	5.00	5.00	5.00	NA
D,L methionine	0.134	0.134	0.135	0.155	0.156	NA
L-Lysine	0.047	0.046	0.044	0.007	0.010	NA
Calculated composition, % :						
Crude Protein %	22.00	22.00	22.0	22.05	22.00	NA
ME, kcal/kg	3050	3050	3050	3050	3050	3080
Ether Extract	6.26	6.96	7.66	7.80	6.50	NA
Meth. + Cyst.	0.85	0.85	0.85	0.85	0.85	NA
Methionine	0.50	0.50	0.50	0.51	0.51	NA
Lysine	1.27	1.27	1.27	1.27	1.27	NA
Ca	1.01	1.01	1.01	1.01	1.00	NA
Total P	0.87	0.88	0.90	0.85	0.86	NA
Actual analysis, %:						
Dry Matter	87.51	87.60	87.73	87.63	86.95	88.65
Crude Protein	21.94	21.83	23.09	22.60	22.62	21.00
Ether Extract	6.39	7.30	8.62	8.40	6.92	4.79
Ash	6.95	6.62	6.92	6.48	6.58	5.54
GE, Kcal/kg	4047	4122	4107	4114	4044	4022
Ca	1.03	1.00	1.08	0.96	0.93	0.88
P	0.55	0.56	0.60	0.54	0.54	0.53
ADF	4.27	5.92	4.68	4.03	3.91	4.05

1. FFSB, extruded full-fat soybean.
2. Premix 4050 contains: Premix 4050 provided by COOPÉRATIVE FÉDÉRÉE DE QUÉBEC, Montréal, Canada, claimed to supply per kilogram diet: Ca, 9.0g; P, 3.2g; Na, 1.4g; Fe, 250mg; Mn, 90mg; Cu, 10mg; I, 1.25mg; Zn, 90mg; Se, 0.3mg; vitamin A, 10,000 IU; vitamin D_3, 3,000IU; Vitamin E, 25IU.

NA, not available.

Chemical Analysis

Dry matter in feed and freeze-dried fecal samples was determined by weight difference after drying at 100°C for 12 hrs using a vacuum oven[5]; Nitrogen was determined by a Leco Nitrogen Analyser[6]; acid detergent fiber (ADF) was determined with the method developed by Goering and Van Soest (1970); Ash content was determined by using a muffle furnace[7]; gross energy was determined by using an adiabatic oxygen bomb calorimeter[8]; ether extract was determined by dietylether extraction for 20 hrs.

Performance Record

Body weight and feed consumption were recorded weekly for three weeks. Weighing was performed by pen weighing on day one and by individual at the end of first, second and third week. Feed consumption was determined on pen basis. Mortality was recorded. The feed consumed by culled or dead birds was deducted from total feed consumption of the pen. The correction was made by weighing the feed of the pen on the day of culling or death and then adjusting for the share of the culled or dead birds.

Statistics

Statistical analyses were performed with mixed model of SAS (1995). A nested 4 by 6 factorial design was used. Pen represented the experimental unit. The observation within the pen was regarded as the sampling unit and nested with the pen. The statistical significance of the difference of least square means among the treatment groups was determined by multiple comparison adjusted by Scheffe's test and accepted at $P<0.05$.

The statistical model is:

$$Y_{ijkl} = \Phi + Processing_i + Flaxseed\ level_j + Interaction_{ij} + Pen_{ijk} + \varepsilon_{ijkl}$$

[5] National Appliance Co., Portland, OR 97223, U.S.A.
[6] Leco FP-426, Leco Corporation, St-Joseh, MI.
[7] model F-A1730, Sybron Thermolyne, Dubuque, IA 52001, U.S.A.
[8] #1241, Parr Instrument Co., Moline, IL 61625, U.S.A.

RESULT

Effect of Flaxseed Processing and Flaxseed Level on Performance parameters

As shown in Table 3.2, FS processing had very significant effect ($P<0.001$) on chicks' body weight, feed intake and feed conversion ratio in both after first week and after three weeks feeding. Birds fed the pelletted FS containing diet had the heaviest body weight and the highest feed intake over other treatment groups that had the same FS level. The main effect of body weight for pellet processing after first week was 174 g, while after three weeks, it was 784 g. The main effect of feed intake per bird after week-one was 157 g, whereas after three weeks it was 1130 g. The main effect of feed conversion efficiency was also better for birds fed the pelletted FS diets than those fed the non-pelleted FS diets. After week one, feed-to-gain ratio was 1.21, which was statistically significant better than all other flaxseed processing groups. While after three weeks, it was 1.53 which was only statistically significant better than whole flaxseed and ground flaxseed ones, but not than that of autoclaved whole flaxseed.

FS autoclaving improved the performance of for chicks after week one and after three weeks, even though it did not reach significant level. It was of same benefit when the FS level was higher than 10%. This is the case for both ground and whole FS diets. In the 12% whole seed diet, autoclaving increased body weight by 9.6% (137 vs. 125 g) for birds after week one and by 15% (646 vs. 559 g) for birds after three weeks, while for feed intake this improvement was 13.5% (123 vs. 111) for birds after week one and 9.3% (936 vs. 856 g) for birds after three weeks, respectively.

At 14% FS level, FS autoclaving improved performance in a similar manner for birds after three weeks. In the whole seed groups, autoclaving improved body weight by 7.9% (631 vs. 585 g), and feed intake by 2.3%. While in the ground seed group, autoclaving lead to 5.1% improvement in body weight (143 vs. 136 g) for birds after the first week and 7.9% (680 vs. 630 g) for birds after three weeks. Feed to gain ratio was also improved by this processing: 2.3% for birds after the first week (1.29 vs. 1.32) and 9.6% for birds after three weeks (1.50 vs. 1.66).

Table 3 2. Effect of flaxseed level and processing on performance of broiler chicks.

Diet and	FS	Body weight, g		Feed intake, g		Feed/gain	
Processing	%	Wk 1	Wk 3	Wk 1	Wk 1 to 3	Wk 1	Wk 1 to 3
Commercial	0	148^{ab}	676^{bcd}	128^{abcd}	980^{abc}	1.28	1.56^{ab}
FFSB A	0	152^{ab}	766^{ab}	126^{abcd}	994^{abc}	1.28	1.37^{a}
FFSB B	0	151^{ab}	741^{abc}	129^{abcd}	984^{abc}	1.19	1.41^{ab}
FSG	10	145^{b}	650^{bcd}	128^{abcd}	933^{bc}	1.27	1.54^{ab}
Pellet	10	181^{a}	829^{a}	160^{a}	1168^{a}	1.17	1.49^{ab}
FSWA	10	138^{b}	635^{bcd}	122^{cd}	923^{bc}	1.29	1.56^{ab}
WS	10	146^{ab}	654^{bcd}	125^{abcd}	924^{bc}	1.22	1.51^{ab}
FSG	12	134^{b}	630^{bcd}	127^{abcd}	981^{abc}	1.40	1.67^{b}
Pellet	12	167^{a}	766^{ab}	154^{abc}	1119^{ab}	1.24	1.55^{ab}
FSWA	12	137^{b}	646^{bcd}	126^{abcd}	936^{bc}	1.33	1.55^{ab}
FSW	12	125^{b}	559^{d}	111^{d}	856^{c}	1.36	1.66^{b}
FSAG	14	143^{b}	680^{bcd}	130^{abcd}	955^{abc}	1.29	1.50^{ab}
FSG	14	136^{b}	630^{bcd}	123^{bc}	976^{abc}	1.32	1.66^{ab}
$FSGB_6$	14	130^{b}	624^{bcd}	121^{cd}	953^{abc}	1.39	1.64^{ab}
Pellet	14	174^{a}	756^{abc}	158^{ab}	1104^{ab}	1.22	1.55^{ab}
FSWA	14	135^{b}	631^{bcd}	119^{d}	928^{bc}	1.29	1.58^{ab}
FSW	14	135^{b}	585^{cd}	123^{bc}	907^{bc}	1.35	1.67^{b}
	SEM	4.9	20.3	5.1	33.2	0.040	0.038
				Probability			
	Processing	0.000	0.000	0.000	0.000	0.001	0.000
	FS level	0.003	0.004	0.381	0.812	0.003	0.001
Processing * FS level		0.378	0.123	0.403	0.417	0.633	0.305
Main Effect							
FS level	0	150	728	128	986	1.25	1.45
	10	153^{a}	692^{a}	134	987	1.24^{a}	1.53^{a}
	12	141^{b}	650^{b}	130	973	1.33^{b}	1.61^{b}
	14	142^{b}	651^{b}	129	971	1.31^{b}	1.60^{b}
Processing	FSG	138^{b}	634^{b}	125^{b}	961^{b}	1.35^{b}	1.63^{b}
	Pellet	174^{a}	784^{a}	157^{a}	113^{a}	1.21^{a}	1.53^{a}
	FSWA	134^{b}	637^{b}	122^{b}	929^{bc}	1.30^{b}	1.56^{a}
	FSW	135^{b}	599^{c}	120^{b}	896^{c}	1.31^{b}	1.61^{b}

FFSB A: contains 14% full-fat soybean with no flaxseed; FFSB B: contains 23.8% full-fat soybean with no flaxseed; FSAG: flaxseed autoclaved then ground; FSG: ground flaxseed, no autoclaving; FSWA: whole seed but autoclaved; FSW: whole seed without any processing; $FSGB_6$: ground flaxseed, no autoclaving, but supplemented with 40 mg pyridoxine hydrochloride/kg diet.

Means within columns with no common superscripts differ significantly (Scheffe's test, $P<0.05$).

At 10% FS inclusion, the whole seed diet did not cause a significant difference in performance of birds, as compared with the ground seed diet. The body weight, feed consumption and feed-to-gain ratio for the birds fed the whole seed diet was 146 g, 125 g and 1.22 after the first week and 654 g, 923 g and 1.51 after three weeks. While for birds fed the ground seed diet, it was 145 g, 128 g and 1.27 after the first week and 650, 9633 g and 1.54 after three weeks, respectively. When the FS level increased to 12 or 14%, birds fed the whole seed diets had the lowest body weight and feed consumption, and the worst feed efficiency both after week one and after three weeks.

FS level itself had also a very significant effect ($P<0.01$) on body weigh and feed conversion rate, but not on feed intake, as shown in Table 3.2. The main effect of body weight for chicks fed 10% flaxseed was 153 g after week one and 692 g after three weeks statistically significant higher than those fed 12 or 14% flaxseed chicks ($P<0.01$). The main effect of feed to gain ratio was 1.24 after week one, 1.53 after three weeks, which were statistically significant better ($P<0.01$) than those chicks fed 12 or 14% flaxseed.

Effect of Pyridoxine Addition and Full-fat Soybean Inclusion on Performance Response

The addition of extra 40 ppm vitamin B_6 to a diet containing 14% ground FS did not cause any improvement in the birds' performance, as shown in Table 3.2. The body weight for the B_6 group and the non-B_6 group was 130 vs. 136 g for birds after week one, and 624 vs. 630 for birds after three weeks, respectively. The feed intake was 121 vs. 123 g for birds after the first week and 953 vs. 976 g after three weeks, while feed to gain ratio was 1.39 vs. 1.32 after first week and 1.64 vs. 1.66 after three weeks, correspondent to pyridoxine added and non-added diets.

Chicks performed satisfactorily when fed diets containing extruded full-fat soybean (FFSB) at 14 or 23.8%. No significant difference was observed among these groups. These two FFSB inclusion rates are the levels commonly used in ration formulation and supply about the same lipids as 10% FS.

Nutrients Utilization as Affected by Flaxseed Processing and Level

The effect of FS processing and FS level on nutrient utilization and AMEn value of the diets are shown in Table 3.3. There were significant ($P<0.05$) interaction between processing methods and FS level on the utilization of all the nutrients tested.

Table 3 3. Effect of flaxseed level and processing on nutrient utilization of broiler chicks

Diet and Processing	FS %	DM %	CP %	EE %	Ash %	ADF %	GE %	AMEn kcal/g
Commercial	0	74.1^{a}	62.0^{ab}	84.2^{a}	38.7^{ab}	23.0^{ab}	77.3^{a}	2936^{a}
FFSB A	0	71.1^{ab}	66.2^{ab}	79.1^{ab}	35.4^{ab}	21.1^{ab}	74.7^{a}	2878^{a}
FFSB B	0	72.1^{ab}	67.6^{a}	80.3^{ab}	39.3^{ab}	20.3^{ab}	75.8^{a}	2864^{a}
FSG	10	69.7^{ab}	65.5^{ab}	67.0^{b}	40.4^{ab}	18.0^{ab}	71.8^{abc}	2743^{ab}
Pellet	10	68.3^{abc}	58.3^{b}	77.8^{abc}	33.7^{ab}	10.4^{b}	70.7^{abc}	2686^{ab}
FSWA	10	68.2^{abcd}	65.9^{ab}	73.4^{abcd}	12.3^{cd}	17.2^{ab}	71.7^{abc}	2696^{ab}
FSW	10	65.3^{bcd}	61.0^{ab}	60.0^{d}	26.9^{bc}	12.4^{b}	67.5^{bc}	2554^{b}
Pellet	14	71.3^{ab}	64.5^{ab}	77.0^{abc}	48.0^{a}	29.5^{a}	73.8^{ab}	2924^{a}
FSWA	14	61.6^{cd}	60.7^{ab}	59.9^{d}	11.8^{cd}	17.3^{ab}	65.9^{c}	2533^{b}
FSW	14	61.3^{d}	62.7^{ab}	62.0^{cd}	6.5^{d}	16.8^{ab}	65.2^{c}	2489^{b}
SEM		1.07	1.34	2.54	2.70	2.41	1.07	40.5
		Probability						
Processing		0.000	0.018	0.000	0.000	0.186	0.001	0.001
FS level		0.008	0.414	0.063	0.334	0.001	0.072	0.935
Processing * FS level		0.001	0.002	0.014	0.000	0.002	0.002	0.000

FS, flaxseed; DM, dry matter; CP, crude protein; EE, ether extract; ADF, acid detergent fiber; GE, gross energy; FFSB A: contains 14% full-fat soybean with no flaxseed; FFSB B: contains 23.8% full-fat soybean with no flaxseed; FSG: ground flaxseed, no autoclaving; FSWA: whole seed but autoclaved; FSW: whole seed without any processing. Means within columns with no common superscripts differ significantly (Scheffe's test, $P<0.05$).

Pelleting significantly improve the apparent digestibility of dry matter (DM), ether extract (EE), and gross energy (GE), as well as AMEn value over whole seed diet with the same 14% FS. The apparent digestibility for DM, EE, GE and AMEn value for the pelletted diet were 71.3, 77.0, 73.8% and 2924 kcal/kg respectively, and for the whole seed diets, 61.3, 62.0, 65.2% and 2489 kcal/kg, respectively.

DISCUSSION

FS is composed of seed coat (testa, true hull or spermoderm), embryo or germ, a thin endosperm and two cotyledons. Cotyledons make up 55% of the seed and are the storage tissue of flax oil (Dorrell 1970). For whole seed, the seed coat may prevent thorough enzymatic action in the intestine, which may explain the reduced nutrient utilization and decreased performance of birds. Under high pressure and increased temperature during pelleting, the hull structure of FS may undergo changes that permit nutrients to be exposed to extensive intestinal digestion. That may explain the higher EE digestibility of the pelleted FS diet and the commercial diet. As the fatty acids are not excreted in the urine and lipid will contribute to energy the most, the ME value will be related to the absorbability of the lipids (Scott et al. 1982). This explained the higher AMEn value for these two pelleted diets.

Other benefits that animals obtain from pelletted diets are increased feed consumption. It simplifies feed handling and reduces feed waste. Nitrogen efficiencies may also be improved (Calet 1965). Boggs et al. (1960) reported the effect of pelleting on the improvement in weight gain and feed efficiency for chicks, which were observed not only for cottonseed containing diets, but also a soybean diet.

The benefit of pelleting FS containing diets may not be limited simply to improved nutrient utilization. It may also reduce the antinutritional factors (ANFs) in FS. The tissue disruption during pelleting allows the mixing of cyanogenic glycoside with glycosidase, which are kept apart in intact seeds (Bell 1981). The enzymatic reaction releases HCN from cyanogenic glycosides, however, the cooling procedure during pelleting could drive away the HCN formed during pelleting. It may be maximized if steam conditioning is used during pelleting, which was not used in our experiment. We instead pelletted the diets twice in order to maximize this possible effect. Monitoring the HCN level in the diets will be discussed in another paper.

Diets containing whole FS generally caused reduced nutrient utilization, even though grit had been provided, as observed in the present experiment. The reduction effect was magnified with higher FS inclusion rates. When the seed had been autoclaved, this reduction was decreased for the 10% FS level, but not the 14% level. The physical barrier of the seed to enzymatic action in the intestine could be the reason behind the poor

performance and lower fat availability. Leeson and Summers (1997) suggested that the whole FS is not well digested by birds and should be ground prior to inclusion in feed. They suggested that the seed could be mixed with cereal and then ground in a hammer mill.

However, whole seed inclusion in the diet has the advantage that the diet does not need special protection from potential peroxidation of linolenic acid. This benefit seems to be overshadowed by the adverse effect of reduced nutrient utilization and depressed growth.

No benefit of processing FS on apparent crude protein utilization was observed. Barbour and Sim (1991) reported that the availability of leucine, isoleucine, valine, phenylalanine, and methionine in flax products were lower ($P<0.05$) than those of canola and soybean meal. It is generally recognized that the digestibility of FS protein by monogastric animals is lower than that of canola's. Lee et al. (1995) recorded the values of the true amino acid availability (TAAA) for FS and meal between 71-89% for mature roosters. These values are lower than those of canola products, which are between 79 and 94%. Using broiler cockerels, Grossu et al. (1998) reported values of the digestibility of essential amino acid in FS ranging between 74 and 87%; among them, lysine and the methionine had values of 82 and 80%, respectively. Even though the apparent crude protein utilization is not a desirable criterion for judging the quality of FS protein, our results are in agreement with the finding of other researchers that flax protein is less available.

The vitamin B_6 antagonism effect of linatine in linseed meal was demonstrated by Kratzer (1954) and Klosterman et al. (1967). Their experiments showed that chicks reared with linseed meal developed vitamin B_6 deficiency symptoms. These symptoms were overcome by 20 ppm supplement of pyridoxine. The insensitivity to the pyridoxine addition observed in the present study could be explained in two ways. One is the dilution effect of FS oil in FS, which composes about 40% and is known to contain no toxic factors. The other could be that the premix used contained sufficient levels of pyridoxine to overcome the antagonistic action of linatine.

A 23.8% incorporation rate of FFSB in a diet contributes a similar ether extract as 10% FS, while 14% FFSB is a level that normally is used in broiler diet formulation. Leeson and Summers (1997) suggested its maximum use in broiler diets to be 15%. Compared to

other plant oilseed, soybean is highly digestible by chicks. The better nutrient utilization was supported by the results from our present experiment for all nutrients tested, as compared to FS containing diets, although the pelleting of FS containing diets reduced this difference to a minimum margin.

The commercial diet used in the present experiment had better nutrient utilization and a higher AMEn value, as compared to FS-containing diets. The nutrient content in the commercial diet is similar to other diets used in the experiments, except the slightly lower EE content (4.6 vs. 6.4% of 10% FS diet). No ingredients information is available. The diets were pelleted then crumbled. Steam application and preconditioning prior to pelleting is normally used in commercial feed production. Its better nutrient utilization could be related to the ingredients used in feed formulation, and to the commercial pelleting procedure. From the nutrient utilization results of the commercial diet, one may expect a better nutrient utilization for the FS-containing diet, if it were processed under commercial conditions.

CONCLUSION

The processing of FS significantly influenced the performance and nutrient utilization of birds after three weeks. Pelleting seems to be the most efficient and practical method. First, it maximizes lipid utilization, which is a very important nutrient in FS. Secondly, pelleting can be carried out during feed manufacturing, as FS can be pelletted together with other nutrients.

Autoclaving had also some positive response over whole seed or ground seed on performance and nutrient utilization. Its effect is magnified when the FS level is higher than 10% in the diet. However, autoclaving is not as practically applicable commercially as would pelleting.

Whole seed ration did not show a significant reduction in performance for broilers when given at 10%, and with grit provided ad libitum. However, at higher level than 10% in young broiler diets, it depressed growth and lowered nutrients utilization. This depression became statistically significant for lipids and AMEn values, as compared with the diets containing the same level of FS but pelletted.

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Section IV

Influence of Processing on Hydrogen Cyanide Content of Flaxseed

Transition Text for Section IV

Flaxseed processing, like pelleting and autoclaving, had been shown in previous experiments to be positive in improving the nutrient utilization and performance response of broilers fed FS containing diets. Besides the physical change of the seed caused by processing, another benefit could be related to the removal of antinutritional factors (ANFs). One of the main ANFs in flaxseed is the cyanogenic glycoside. It could easily release hydrogen cyanide (HCN) upon hydrolysis. Monitoring the status of HCN could indicate the effectiveness of processing.

Influence of Processing on Hydrogen Cyanide Content of Flaxseed

Dingyuan Feng, † Yingran Shen,* E. R. Chavez, *[1] and P. C. Laguë*

†*Department of Animal Science, South China Agricultural University, China*

**Department of Animal Science, Macdonald Campus of McGill University, Canada*

1 To whom correspondence should be addressed: chavez@macdonald.mcgill.ca

E. R. Chavez

Department of Animal Science, Macdonald Campus of McGill University, 21,111 Lakeshore, Ste Anne de Bellevue, Quebec, Canada, H9X 3V9

Tel: (514) 398 7795; Fax: (514) 398 7964

ABSTRACT The objective of the experiment was to study the effect of flaxseed (FS) processing on its content of hydrogen cyanide (HCN). FS was processed by oven heated, single or repeat pelleted, pelleted together with other ingredient(s), autoclaved, or microwave roasted. The comparative effectiveness in reducing HCN in FS by different processing methods tested was monitored by using alkaline titration. The HCN content was 377 mg/kg in raw feed-grade FS and 139 mg/kg in human grade FS. All the processing methods tested markedly reduced the HCN content in FS. Pelleting one time significantly ($P<0.05$) reduced the HCN content in FS by 13.3%, while repeat pelleting alone (three or six times), or single or repeat pelleting together with other ingredient reached greater HCN reduction with statistically very significant ($P<0.001$) level. Autoclaving and microwave roasting both also very significantly ($P<0.001$) reduced the HCN content in FS. The most efficient FS processing methods tested were microwave roasting and repeated pelleting together with 50% corn. The HCN content in the FS after these two processing methods were 63.5 and 136.5 mg/kg, with 83.2 and 63.8% removal, respectively. The HCN reduction could be the result of deactivation of glycosidase, or the evaporation of HCN from hydrolysis of cyanogenic glycosides. As increasing temperature was detected together with the increase pelleting times, it could be reasonable to think that higher temperature within a certain range, even though not determined, could benefit the efficient reduction of HCN. In the case of HCN reduction by microwave roasting, as there was 5.7% water loss after 4 min processing, it could be result of both deactivation of glycosidase, or evaporation of HCN formed from enzymatic hydrolysis. Proper FS processing, as autoclaving, pelleting, and microwave roasting, can significantly reduce the HCN content in FS.

(*Key Words*: flaxseed, processing, hydrogen cyanide)

INTRODUCTION

Cyanogenic glycosides are glycoside of aldehyde or ketone cyanohydrin. Their presence serves for the plant self-protection, as plants are static and animals are mobile (Bell 1981). When the plant tissue is damaged, cyanogenic glycosides are hydrolyzed and HCN, as the hydrolyzed product, is released. The liberation of HCN from cyanogenic glycosides is an inhibitor of cytochrome oxidase and consequently a cell respiration inhibitor. The minimum lethal dose to man is quoted at between 0.5 to 3.5mg/kg body weight (Bell 1981). Over 1000 species of plant are known to produce hydrogen cyanide (Bell 1981), FS obviously one of them.

There are four forms of cyanogenic glycosides in FS: linamarin, linustatin, lotaustralin and neolinustatin. Linustatin and neolinstatin are diglycosides, while linamarin and lotaustralin are monoglycosides (Seigler 1981). FS contains very low level of linamarin, but considerable amount of the diglycosides linustatin and neolinustatin (Mazza and Oomah 1995). Young flax contains mainly the monoglycosides, linamarin and lotaustralin, at levels as high as 90% of total cyanogenic glycosides. Older flax contains about 30% of the diglycosides linustatin and neolinustatin (Frehner et al 1971).

The detection of cyanogenic glycosides in FS can be performed by two methods. One is the quantitative determination of individual cyanogenic glycosides by HPLC. The other is the colorimetric titration of HCN, which is used normally for the comparative study between samples or cutivars.

It is well established scientifically that proper processing is required before linseed meal can be satisfactorily utilized in diets for monogastric animals. Kratzer reported (1946, 1947) that water soaking of linseed meal overcame its growth depression effect, when used in chick diets at level over 5%. MacGregor and McGwnis (1948) demonstrated a similar result. They reported that water soaked linseed meal did not cause depressed growth when used at a level up to 30% in chicks diet, whereas 4.5% untreated linseed meal did. Other processing of linseed meal, like wet autoclaving, lead to a similar performance response (Mandokhot and Singh 1983). One of the benefits of processing is the reduced HCN content in linseed meal (Deshmukh et al. 1982). As it is well recognized that flaxseed (FS) or linseed meal contain relative high amounts of hydrogen cyanide (HCN), a potent respiratory inhibitor, it will be of interest to know the HCN status in processed flax

products. Likewise, by monitoring the HCN level, one can evaluate the comparative effectiveness of a FS processing.

Based on the necessity to process linseed meal, the same reasoning can be applied to FS, as there is limited information on the subject. The present study intended to establish the efficiency of various processing methods in removing HCN from FS.

MATERIALS AND METHODS

Flaxseed and Its Processing

Two brown FSs lots purchased from a local feed market, one feed grade and one human grade, were used in this study. They were subjected to different processing methods, which included autoclaving, microwave oven roasting, pelleting, and grinding. Water soaking of FS was also evaluated. The processing procedures were as follow:

Autoclaving was performed at 16.5 kg/cm^2 and 120°C for 15 min., by using a steam sterilizer[2]. Microwave processing was performed with a household microwave oven[3] with 750Watt output, under the operating frequency of 2450MHz. Two hundred grams of FS were laid on a 20 by 20 c cm^2 plastic tray and cooked under maximum output for 4 min. Water soaking consisted of adding 100 g of FS to 200ml of water in a 400-ml beaker for 2 h.

Pellet processing was performed with a California pellet machine of laboratory model or Master Model[4]. The hole diameter of the die in CPM laboratory model is 0.31 cm. The parameters of the Master Model were: hole diameter, 0.318 cm; die diameter, 30.48 cm; thickness of the die, 3.05 cm. When using the CPM Master model, FS was pelleted twice. While the laboratory model was used, FSs were pelletted either once, 3 times, or 6 times, alone or with 50% corn. In between the pelleting, the pelleted feed was laid on the ground for about 15 min. for cooling.

The temperature reached during pelleting was recorded by measuring the pellet temperature at the pelleter's outlet, which was obtained with a Raytek noncontact infrared thermometer (Raytek, TS-2, USA).

FS, including the pelleted seed, was ground by using a coffee grinder prior to HCN analysis.

Chemical Analysis

The HCN content in FS was determined by alkaline titration (AOAC 1990). Twenty grams of air-dried FS was ground with a coffee grinder (specified in 2.2 of section 2) and transferred to a Kjeldahl flask. Two hundred ml H_2O were added and mixed with the

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[2] Barnstead Still and Sterilizer Co., Boston, Mass., U.S.A.
[3] Kenmore microwave oven manufactured by Goldstar Co. Ltd, Korea.
[4] California Pellet Mill Co. 1114 E. Wabash Avenue, Crawfordsville, IN 47933, U.S.A.

sample. After 2 h, the solution was then distilled. Distillate was collected in a flask containing 20ml 2.5% NaOH solution (0.5 g in 20 mL H_2O), until distilled to a definite volume. The distillate was added with 8 mL 6*N* NH_4OH and 2 mL 5% KI solution and titrated with 0.02*N* $AgNO_3$, using a microburet. HCN was calculated as:

HCN (mg) = mL of 0.02*N* $AgNO_3$ / 1.08.

Statistics

The General linear Model of SAS (1995) was used for the statistical analysis. The statistical significance of the difference between least square means of HCN in raw FS and that of processed ones was determined by T test. The statistical significance was accepted when $P<0.05$.

RESULT

Processing of FS by autoclaving, microwave roasting, pelleting, and pelleting together with other ingredients, significantly reduced HCN in FS. The ground FS had 377.0 mg/kg HCN. When FS was one-time pelletted with a CPM laboratory pellet mill, HCN was reduced ($P<0.05$) by 13.3%. Repeated pelleting, both by three or six times, very significantly ($P<0.001$) reduced HCN content to a greater extent both for FS alone and for the combined mixture with corn. Three times and six times pelleting caused around 30 and 60% HCN reduction, respectively. When FS was pelletted with other ingredients, as would be the case in commercial pelleting operations, the HCN in the FS reduced to 98.9 mg/kg, which was 73.8% less than that in the ground FS. Oven-heated processing also reduced the HCN content, but to a lesser extent (16.2% reduction), while autoclaving lowered HCN value by 29.7%. Among all the processing methods tested, FS processing by microwave oven seemed to be the most effective in this regard, which lowered HCN to 63.5 mg/kg. The results are shown in Table 4.1.

The temperature of the feed when leaving the pellet machine outlet was between 30 to 56°C. These values were increased with the increase of re-pelleting frequency and were highest when FS was pelletted with other ingredients.

Water soaking of whole FS was not achievable due to the slurry effect of the outer layer mucilage, which prevented complete drying. Hence no HCN measurement was performed for the soaked FS.

Table 4.1. Effect of flaxseed (FS) autoclaving, microwave roasting, pelleting and oven roasting on its hydrogen cyanide content

Ingredient and processing	DM %	HCN[1] mg/kg	Compared with raw FS		Pellet Temperature[2] °C					
			reduction	Pr > t	1st	2nd	3rd	4th	5th	6th
Flaxseed (FS)	93.3	377.0								
FS, autoclaved	89.4	265.0	-29.7	0.0001						
FS, microwave	87.6	63.5	-83.2	0.0001						
FS, pelleted 1 time		327.0	-13.3	0.0494	31.5					
FS, pelleted 3 times	91.7	267.5	-29.0	0.0001	29.0	31.7	33.9			
FS, pelleted 6 times	91.9	170.0	-54.9	0.0001						
FS, pelleted 1 time with 50% corn		292.3	-22.5	0.0001	34.0					
FS, pelleted 3 times with 50%corn		264.5	-29.8	0.0001	34.0	36.0	37.1			
FS, pelleted 6 times with 50% corn	90.0	136.5	-63.8	0.0001	33.7	36.0	37.0	38.1	39.5	40.3
FS, pelleted together with other ingredients		98.8	-73.8	0.0001	48.5	56.0				
FS, oven heated[3] A		316.0	-16.2	NT[4]						
FS, oven heated[3] B		291.0	-22.8	NT						
FS, human grade		138.8		0.0001						
Canola		68.0		0.0001						
SEM		7.41								

1. Values are least square means of 5 measurement for each sample; HCN content of flaxseed in compound feed had been converted back to 100% flaxseed basis.

2. Pellet temperature were the feed temperature when leaving the outlet of pellet machine. They were the average of three measurements of each pellet, in the order from first to last pellet.

3. Flaxseed was oven-heated for 10, 20 min. at 130°C for A and B, respectively. The values are the average of two measurements.

4. NT: not tested.

DISCUSSION

The HCN Content in Flaxseed

The reported values of hydrogen cyanide in FS vary greatly, due to cultivar variation, difference in detection methods, or as their expression in different compounds. Mandokhot and Singh (1983) reported values of 470-490 mg/kg. Rosling (1993) reported that the cyanide (CN) content varied from 4 to 12 mmol/kg (104 to 312 mg/kg). Chadha et al. (1995) detected 124-196 mg/kg of cyanide by using HPLC. These values are much less than HCN found in cassava, which is 2450 mg/kg (Montgomery 1969). The present study determined the HCN content at 377 mg/kg, which is generally in agreement with reported values.

Flaxseed Processing and HCN Removal

Pelleting The removal of HCN from FS processing is based on the enzymatic hydrolysis of cyanogenic glycosides, or the deactivation of glycosidase. The hydrolysis reactants, cyanogenic glycosides and glycosidase, are kept apart when the seed is intact (Bell 1981). The disruption of tissue during ingestion by animals or processing makes this enzymatic reaction possible, and HCN could be formed.

Once formed, HCN must be released from the seed or flax products. HCN is volatile (boiling point 26°C) and can readily diffuse through plant tissue (Davis 1991). It is known that to guarantee the successful removal of HCN from cassava during processing, running water, or boiling vapor, must be provided. Obviously this contaminated water and vapor could be toxic (Montgomery 1969). This knowledge on cassava detoxification can be applied to FS processing by pelleting.

Pellets are formed with high pressure and increased temperature. Tissue disruption when ingredients are passed through the pellet die may encourage the enzymatic formation of HCN, while cooling the pelleted feed could help the removal of HCN. By controlling these two procedures, one may achieve the efficient removal of HCN from FS.

The present study tested repeated pelleting and pelleting together with other ingredients. It was expected that by altering the condition of pelleting, the pellet temperature could be raised to maximize the formation of HCN. This assumption was formulated since steam was not available on the experimental pellet machine and, therefore, the real pellet temperature would be lower than that attained under commercial

production. When steam is provided, as in commercial feed pelleting, repeated pelleting may not be necessary. As shown in Table 4.1, our results demonstrated that increased temperature was achieved by repeated pelleting and caused a further reduction of HCN.

Some researchers reported that heat processing did not change the HCN content (Mazza and Oomah 1995), because the HCN formed from hydrolysis could remain in flax products, or the deactivation of glycosidase in plant tissue could be replaced by the β-glycosidase formed by gut micro flora (Poultin 1989, Majak et al 1990). It is reasonable to state that effective FS processing requires more than just cyanogenic glycosides hydrolysis or deactivation of glycosidase in tissue alone. That may also partly explain the ineffectiveness of dry heating in improving the feeding value of FS for chicks (Kratzer et al. 1954, Lee et al. 1991).

High oil content in FS and the low storing temperature (4°C) of the FS used in this experiment prior to pelleting may contribute to the lower temperature measured during pelleting, which was between 30 to 56°C. High oil content reduces the friction between feed and die. Such a condition would not have happened under commercial conditions, as the lipid content in animal feed would normally not exceed 6%, and the temperature would mostly be between 65-80°C or higher (Decksheimer 2000). If steam is provided for preconditioning, beneficial effects of both increased temperature and consequent glycosidase enzymatic reaction might lead to a more complete removal of the formed HCN, even though the optimum temperatures have not been determined.

Autoclaving is one kind of heat processing under high pressure. The toxic potential of FS cyanogenic glycoside may be reduced, as the result of inactivation of glycosidase by high temperature. This is supported by reported results of HCN reduction after autoclaving (Deshmukh et al. 1982, Mandolhot and Singh 1983), as well as by the results of the present study.

Besides high temperature, autoclaving differs from dry heating due to the presence of vapor. Even though autoclaving for 30 min. reduced the HCN level in linseed meal from 470-490 mg/kg to a negligible amount, water played a role in improving feeding value of linseed meal, as revealed by Mandolhot and Singh (1983). They observed that when linseed meal was soaked first then autoclaved, which was defined as wet autoclaving, chicks performed better, as compared to dry autoclaving. The benefits of vapor may be

tissue moisturing, which may encourage enzymatic reaction or its HCN carrying capacity as seen in pelleting. The present study showed that both autoclaving and dry oven heating reduced the HCN content of FS, while autoclaving showed greater efficiency. Our findings are in agreement with the results reported by Deshmukh et al. (1982) and Mandolhot and Singh (1983).

High pressure may be an additional benefit for autoclaving, namely the possible destruction of toxic chemicals such as not only the cyanogenic glycosides but also other ANFs present in FS. Besides, the hydrolyzing activity catalyzed by glycosidase may be replaced by other enzymes produced by gut micro flora, as demonstrated by Majak and Cheng (1987), Poultin (1989), Majak et al (1990). The studies discussed in previous experiments conducted in our laboratory in which birds fed FS autoclaved for a short period and low temperature (13.4 kg/cm^2, 110°C for 7.5 min) showed no positive but even a slightly adverse response, while autoclaving for a longer period and at a higher temperature (16.5 kg/cm^2, 120°C for 15 min) had a positive performance response, tend to support the toxic chemical destruction, rather than the deactivation of glycosidase alone.

Microwave processing is an interaction process between microwave and processed material. Microwaves lie between radiowave frequencies and infrared frequencies. They are reflectable, absorbable and transmittable. The interaction between microwave and material generates heat that is used for materials processing (Clark 1996). This heat generation is from within the processed material itself, induced by the "cold" microwave, in contrast with the heat obtained from the outer layer as in conventional oven heating. It is fast and efficient, because of the high penetrating power (Decareau 1985), and has been extensively used for material and food industry.

It is worthy to be used in food processing for the inactivation of some enzymes and antinutritional factors. There had been examples of polyphenoloxidase inactivation in mushroom Devece et al. (1999), inactivation of the enzymes lipase and lipoxygenase in cereal bran, germ and soybean (Vetrimani et al. 1992), inactivation of trypsin-chymotrypsin inhibitor of Blackgram (*Phaseolus mungo L.*) (Hajela et al. 1998), reduction of the thermolabile antinutritive constitutes in soybean, such as trypsin inhibitor and urease (Szabo et al. 1998). Similar effects could be reached by FS microwave roasting.

As observed in the present study, microwave roasting achieved the highest level of HCN reduction in FS among all the processing methods tested. The HCN was decreased from 377 to 63.5 mg/kg, a reduction of 83.2%. This reduction could either be the benefit of the heat deactivation of glycosidase, or evaporation of HCN after formation from hydrolysis, or the combination of both.

The evaporation of HCN was supported by the 5.7% water loss in FS after 4 min microwave roasting, which was higher than the 3.9% by autoclaving, and the 1.4% by pelleting 6 times. One explanation for this water loss is the strong heat induction power of microwave. Together with the rearrangement of charge groups induced by microwave-material interaction, heat induces great formation of HCN. The vaporization of this volatile HCN is then possible, which resulted in the greatest reduction of HCN among all processings methods.

The application of microwave roasting in soybean revealed that this processing method causes no major changes in main nutrients and fatty acids profile. Yoshida et al. (1999) studied the tocopherol distribution and oxidative stability of oils prepared from the hypocotyl of soybean roasted in a microwave oven. The oil characters in hypotyls changed slightly in carbonyl value, and anisidine value, with increased roasting time. As compared to original level, more than 80% tocopherols remain after 20 min roasting. The same researchers reported (Takagi, et al 1999) that microwave heating (2450 MHz for 6, 12, or 20 min) significantly increased free fatty acids FFA and 1,3- and 1,2- diacylglycerols (DAG). There were significant differences ($P<0.05$) in fatty acids distributions when soybeans were microwave roasted for 12 min or more. The influence of microwave processing on nutrients composition other than HCN removal merits more research.

CONCLUSION

The present study demonstrates that a series of FS processing methods reduce the HCN content in FS. The extent of reduction varies among methods. Microwave processing and pelleting with other ingredients produced the greatest reduction, while dry oven heating is less effective. The result of reduced HCN content could either be the result of glycosidase inactivation, or the vaporization of HCN after it is formed from cyanogenic glycosides hydrolysis, or the destruction of the cyanogenic glycoside compounds. The latter two would be of greatest potential in commercial production application.

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Section V

The Effect of Flaxseed Processing on its ME and Nutrient Digestibility for Adult Chicken

Connecting Text for Section V

Previous experiments demonstrated that proper flaxseed processing allowed high flaxseed inclusion rate without obvious performance depression. One benefit was the reduction of hydrogen cyanide content in flaxseed. Young broiler experiments also showed that the nutrient utilization such as ether extract, which contributes most to the energy value, was affected by processing. The influences of flaxseed processing on specific fatty acids, as well as other nutrients including the TME values are worthy for further study.

To be submitted to Poultry Science

Running Title:

FLAXSEED PROCESSING AND NUTRIENT UTILIZATION FOR CHICKEN

Title:

The Effect of Flaxseed Processing on Its True Metabolizable Energy Values and Nutrients Digestibility for Adult Chicken

Yingran Shen,* E. R. Chavez, *1 P. C. Laguë, * and Dingyuan Feng†

**Department of Animal Science, Macdonald Campus of McGill University, Canada*

†*Department of Animal Science, South China Agricultural University, China*

1 To whom correspondence should be addressed: chavez@macdonald.mcgill.ca

E. R. Chavez

Department of Animal Science, Macdonald Campus of McGill University

21,111 Lakeshore, Ste Anne de Bellevue, Quebec, Canada, H9X 3V9

Tel: (514) 398 7795; Fax: (514) 398 7964

Section: Metabolism and Nutrition

Abbreviation Key: AD = apparent digestibility; ADF = acid detergent fiber; ANFs = anti-nutritional factors; EE = ether extract; FA = fatty acid; FAs = fatty acids; FFSB = extruded full-fat soybean; FS = flaxseed; FSA = flaxseed batch A; FSB = flaxseed batch B; LM = linseed meal; TFA = total fatty acids.

ABSTRACT The objective of the present study was to study the effect of flaxseed (FS) processing on TMEn values, and nutrient digestibility for adult chicken. FS was pelleted alone or together with 50 or 70% corn, autoclaved, or microwave roasted. Leghorn roosters were used for the TME determination procedure. The TMEn value of FS batch A, 3462 kcal/kg, was significantly (P<0.05) increased by three-time repeat pelleting (P3T) or autoclaving, 4277 and 4318 kg/kg, respectively. The improvement effect of processing was highly significant (P<0.01) with a 40 and 34% increase by six-times repeat pelleting (P6T) FS alone (4840 kcal/kg) and with 70% corn (4640 kcal/kg), respectively. Microwave roasting also significantly (P<0.05) increased the TMEn value for FS batch B from 3146 to 3833 kcal/kg. The TMEn improvement observed due to processing was accompanied with increased ether extract (EE) utilization. P3T, P6T, and autoclaving very significantly (P<0.01) improved the apparent digestibility (AD) of EE for FS batch A from 61.2% to 83.2, 82.2, and 81.5%, respectively. Microwave roasting also significantly (P<0.05) increased the AD of EE for FS batch B from 49.1% to 64.4%. The AD of total and individual fatty acid (FA) was influenced by processing in a similar pattern as EE. The experiment showed that roosters can utilize linolenic acid better than other monounsaturated FA or saturated FA. In the case of raw FS batch A, the AD of total saturated, total monounsaturated, total polyunsaturated FA, and linolenic acid were 55.7, 61.2, 68.9, and 69.5%, respectively. Proper FS processing will benefit the utilization of linolenic acid most, as it makes up 53.6% of the total FA in FS.

(Key words: flaxseed, processing, true nitrogen-corrected metabolizable energy, apparent fatty acid digestibility, chicken)

INTRODUCTION

Flaxseed (FS) is generally regarded as an inferior feed ingredient for monogastric animals, as compared to canola that has similar nutrient content. It is lower in nutrient availability, and contains antinutritional factors (ANFs) that limit its inclusion rate in animal diets, despite the fact that it in general contains some 37% oil and 24% protein (Bhatty1997). For chicken, the reported TME values, 4156 (Barbour and Sim 1991), 3960 (Lee et al. 1995), and 3600 kcal/kg (Leeson and Summers 1997), are generally lower than those for canola seed, 4623 (Barbour and Sim 1991), and 4730 kcal/kg (Lee et al. 1995). Lee et al. (1995) reported that true amino acid availability for FS and Canola was between 71-89%, and 79-94%, respectively. These values are obtained from ground but raw FS. The physical structure of FS, as well as the presence of ANFs, could be the reasons that contribute to these inferior properties.

Besides the energy and protein potential, FS draws attention by nutritionists in recent year for its high content of linolenic acid, which makes up 46-71% of its oil (Bhatty 1997). Linolenic acid, an T-3 fatty acid (FA), is known to bear health functional properties in regulation of blood platelets, blood vessels and leukocytes in preventing cardiovascular disease (Galli and Simopoulos 1988). While low nutrient utilization and low inclusion rate deter the use of FS in monogastric animal, the functional health property of linolenic acid will likewise be restricted. In other words, if nutrient utilization could be improved, it could benefit linolenic acid the most.

One possibility to improve FS nutrient utilization is through its proper processing. Processing normally involves pressure or heat that may modify the physical structure of the seed, as well as the possible removal or destruction of ANFs. The present experiment intended to study the effect of various processing methods on energy and nutrients utilization for chicken.

MATERIALS AND METHODS

Experimental Ingredients and Processing

Two batches of feed grade brown FS (FS A and FS B) purchased from the local feed ingredient market were used in this experiment. Canola seed, corn, extruded full-fat soybean, linseed meal were also tested as controls. The processing of FS A included

pelleting alone, pelleting with 50% or 70% corn, autoclaving, while FS B was microwave roasted. Pelleting was done with repeat process (repeat pelleting) of 3 or 6 times for FS, and 6 times for flax-corn mixtures. The FS processing procedure were as follow:

Autoclaving: using a steam sterilizer[2] at 16.5 kg/cm^2 and 120°C for 15 minutes with a layer of 3 cm thick on a tray.

Pelleting: using a CPM Laboratory Pellet Mill[3] with no steam or binding agent. The hole diameter for the CPM Lab model was 0.31cm.

Microwave roasting: using a household microwave oven[4] with 750Watt output, under the operating frequency of 2450 MHz. Two hundred grams of FS were laid on a 20 by 20 cm^2 plastic tray and roasted for 4 min. under the maximum output.

Whole seed, either canola or FS was ground before being fed to birds using a household coffee grinder[5] .The nutrient content of the FS before and after processing, and that of the control ingredients are shown in Table 5.1.

Experimental Birds and Management

Thirty healthy mature Leghorn roosters were housed in individual metabolic cages measuring 38, 50, and 50 cm (width, length, and height). They were fed ad libitum in the metabolic cages for 48 hours for adaptation. Following this adaptation period, they were deprived of feed for 24 hours to empty their alimentary tract of feed residue. Excreta collection was then commenced. Birds had free access to water during all periods. They were kept under a photoperiod of 15 h light and 9 h darkness.

Digestibility Assay

Precise feeding and TME collection procedures were used. Each bird served as an experimental unit and had its own control and testing periods. There were 6 birds (units) for each tested ingredient. For estimating metabolic and endogenous excretion, control period started upon completion of the 24 hours fast and lasted for 36 hours. Precise feeding was then performed with a stainless steel funnel, as described by Sibbald (1986). Thirty grams of each tested ingredient were used. The testing period lasted also 36 hours. The total excreta collection for the control or testing periods was performed twice a day,

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[2] Barnstead Still and Sterilizer Co., Boston, Mass.
[3] California Pellet Mill Co. 1114 E. Wabash Avenue, Crawfordsville, IN 47933.
[4] Kenmore, manufactured by Goldstar Co. Ltd, Korea.
[5] Black & Decker Canada Inc. Brockville, Ontario, Canada.

Table 5.1. The nutrient composition and fatty acids profile of flaxseed before and after its pelleting, autoclaving, and microwave roasting[1]

Ingredients & processing	DM %	N %	EE[2] %	ADF[3] %	TFA[4] mg/g	FA (% in weight)							
						C10:0	C12:0	C16:0	C16:1	C18:0	C18:1	C18:2	C18:3
FSA[5]	93.2	3.47	37.6	8.73	376.1	0.08	0.06	6.49	0.13	4.21	17.67	17.77	53.61
FSA P3T[6]	91.6	3.38	36.3	7.97	352.5	0.10	0.09	6.56	0.18	4.19	17.46	17.83	53.59
FSA P6T[7]	91.9	3.39	35.5	9.39	347.4	0.10	0.08	6.55	0.13	4.19	17.49	17.80	53.66
FSA ATC[8]	89.4	3.36	36.4	9.16	351.5	0.10	0.03	6.55	0.13	4.20	17.55	17.81	53.62
FSB[5]	92.0	3.32	34.2	11.50	360.3	0.08	0.06	6.00	0.00	2.75	16.22	15.70	59.22
FSB M[9]	97.2	3.59	38.1	10.37	393.0	0.06	0.06	5.85	0.00	2.69	15.73	15.29	60.32
FFSB[10]	83.1	5.76	18.1	7.94	173.3	2.06	1.40	10.43	0.09	3.29	19.76	53.50	8.85
Canola seed	94.0	3.79	37.4	13.97	353.1	0.63	0.14	4.84	0.30	2.24	40.10	18.65	9.22

1. Results are the average of two analyses per sample.

2. EE, ether extract.

3. ADF, acid detergent fiber.

4. TFA, total fatty acids.

5. FSA, raw flaxseed batch A; FSB, raw flaxseed batch B.

6. FSA P3T, flaxseed batch A pelletted 3 times.

7. FSA P6T, flaxseed batch A pelletted 6 times.

8. FSA ATC, flaxseed batch A autoclaved at 16 kg/cm^2, 120°C for 15 min.

9. FSB M, Flaxseed batch B processed by microwave roasting for 4 min.

10. FFSB, extruded full-fat soybean.

one in the morning, and one in the evening. A plastic tray, measuring 45 H 55 H 5 cm (width H length H height), was used for this collection by placing it under the metabolic cage. At the end of each testing program, the birds were returned to their original cages and new birds were used for the next test program.

To prevent the contamination of excreta, feather and scales were removed from excreta prior to the collection. The samples were then placed in a vacuum freeze dryer at -50°C. After 3 days of freeze drying, the samples were placed at room temperature for 30 minutes and then weighed. They were then ground through 0.5 mm sieve for chemical analysis.

Chemical Analysis

For feed and freeze-dried fecal samples, DM was determined by using a vacuum oven[6]; Nitrogen by Leco Nitrogen Analyser[7]; Acid detergent fiber (ADF) by the method developed by Goering and Van Soest (1970); GE by adiabatic oxygen bomb calorimeter[8]; Ether extract (EE) by diethylether extraction.

Total and individual FA contents of feed and fecal samples were determined by the method of one-step extraction and esterification described by Sukjija and Palmquist (1987) with some modifications. Freeze dried samples of approximately 50 mg lipids were accurately weighed and transferred into culture tubes. One ml of benzene, 1 ml of benzene containing internal standard (C17:0, 1 mg/ml) and 3 ml of freshly prepared methanolic HCL were added to each sample. The tubes were then capped and vortexed at medium speed for 30 seconds. The tubes were placed in a water bath at 70°C for 2 hours, followed by cooling to room temperature. Five ml of 6% K_2CO_3 and 1 ml of benzene were added and each sample was again vortexed for 20 seconds. The sample tubes were then centrifuged for 15 min. at 2000 gravity. The supernatant of the organic upper layer (benzene containing methyl esters) was transferred with a Pasteur pipet to a 1 ml auto sample vial. The capped sampled vial was used for FA qualified analysis by automated

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[6] National Appliance Co., Portland, OR 97223.
[7] Leco FP-428, Leco Corporation, St-Joseh, MI.
[8] No. 1241, Parr Instrument Co., Moline, IL 61625.

gas liquid chromatography[9]. The individual FA was then identified by comparing their retention time with those of methylated FAs of standard mixtures[10].

Calculation

For the calculation of the apparent digestibility of DM, ADF, the N retention, the values of

AME, TME, AMEn, and TMEn of FS, the following formulas were applied:

Apparent digestibility = (nutrient intake – nutrient in feces)/nutrient intake.

N retention = N intake – N in excreta of testing period + N in excreta of control period

AME = Energy intake – Energy in feces

TME = AME + Energy in control excreta

AMEn = AME – 8.22 apparent N retention/feed intake

TMEn = TME –8.22 (N in control excreta + apparent N retention)/feed intake

A nitrogen correction value of 8.22 kcal/g (Hill and Anderson 1958) was used for AMEn and TMEn calculation, as described by Farhat et al. (1998). The values of nutrient digestibilities or ME of FS in FS-corn mixture were converted back to 100% FS basis, presuming that the relevant values of corn, which was determined from control testing of corn, did not change.

The weight percentage of individual FA acid was obtained after adjusting by the total areas of FAs in the samples. The total FAs were calculated from the total peak areas corrected by the internal standard area, as described by Sukjija and Palmquist (1987). The following formula was used to calculate total FAs:

Total FAs = (total areas – area int. std.)/area int. std. * conc. int. std./dry wt of sample (g)

Where: total areas = total areas under peaks;

area int. std.= area of internal standard;

conc. Int. std.= concentration of internal standard, mg.

The apparent digestibility (AD) of total or individual FA was calculated as:

AD of FA=(FA intake-FA in fecal sample of testing period)/FA intake

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[9] Series 2, # 5890, Hewlett Packard, Palo Alto, CA 94304 – 1181.

[10] GLC-461, Nu-Check_Prep, Inc., Elsyian, MN 56028.

Statistical Analysis

Data were subject to ANOVA by using general linear model of SAS (1995). T test was used to compare the nutrients (AME, AMEn, TME, TMEn, DM, EE, ADF, and N retention) utilization difference between least square means of ground raw FS and that of other processed FS. Multiple comparisons adjusted by Scheffe's test were used to compare the difference among the treatment groups for FA utilization. Significance was accepted at $P<0.05$.

RESULTS AND DISCUSSION

Flaxseed TME and TMEn Values as Affected by Processing

FS pelleting, autoclaving, and microwave roasting all remarkably improved FS ME values. As shown in Table 5.2, the TME and TMEn values of FS were 3343 and 3225 kcal/kg for batch A (37.6% oil), and 3022 and 2893 kcal/kg for batch B (34.20% oil), respectively. The improvement ($P<0.01$) was greatest by 6 times repeated pelleting. The energy values of FS after pelleting were 4599 and 4446 kcal/kg for TME and TMEn, with improvement of 39.5 and 39.8%, respectively, when compared to the values of raw FS on a DM basis. Conversion to dry matter basis was made for an equal comparison, due to water loss during processing. Three times repeated pelleting only improved by 23 to 24% ($P<0.05$).

FS processing as repeat pelleting with 70% corn, autoclaving, and microwave roasting also significantly ($P<0.05$) improved the ME values. The improvement was 34% by repeat pelleting with 70% corn, 25% by autoclaving, and 22% by microwave roasting.

The control ingredients, canola and full-fat soybean, had higher ME values than those of raw FS, as measured in the present experiment. These differences were reversed when FS was processed by all the methods used. In the case of linseed meal, 3 times repeat pelleting slightly improved its ME values with 5% for TME and 8% for TMEn, but not at a significant level ($P>0.05$).

The difference of TME and TMEn between FS batch A (FSA) and FS batch B (FSB), as shown in Table 5.2, could be the quality difference, mainly the variation in EE and ADF content. FSA contains 37.6% EE and 8.7% ADF, while FSB has 34.2% EE and

Table 5.2. The effect of flaxseed pelleting, autoclaving, and microwave roasting on its AME, AMEn, TME, and TMEn values for Leghorn roosters, kcal/kg

Ingredients & processing	AME as fed	AMEn as fed	TME as fed	TMEn as fed	TME In DM	Compared to raw flaxseed		TMEn in DM	Compared to raw flaxseed	
						%	Pr>|t|		%	Pr>|t|
FSA[1]	2848	2991	3343	3225	3589	100		3462	100	
FSA P3T[2]	3440	3658	4060	3918	4432	123	0.016	4277	124	0.011
FSA P6T[2]	4061	4197	4599	4446	5006	139	0.000	4840	140	0.000
FSA ATC[3]	3477	3621	4007	3858	4484	125	0.011	4318	125	0.008
FSA + C50 P6T[4]	3010	3185	3686	3491	3831	107	0.481	3623	105	0.607
FSA + C70 P6T[4]	3963	4212	4635	4465	4818	134	0.001	4640	134	0.000
FSB[1]	2393	2629	3022	2893	3286	100		3146	100	
FSB M[5]	3346	3493	3905	3726	4016	122	0.036	3833	122	0.031
Corn	3142	3285	3582	3463	4248			4106		
FFSB[6]	3110	3190	3846	3523	4626			4238		
Canola seed	3175	3383	3840	3623	4087			3856		
LM[7]	1541	1640	2108	1911	2400	100		2176	100	
LM P3T[8]	1858	1957	2296	2144	2525	105[9]	0.715	2358	108[9]	0.561
SEM	207	200			242			220		

1. FSA, raw flaxseed batch A (37.6% oil); FSB, raw flaxseed batch B (34.2% oil). 2. FSA P3T, flaxseed batch A pelletted 3 times; P6T, 6 times, by CPM laboratory pellet mill. 3. FSA ATC, flaxseed batch A autoclaved at 16.5 kg/cm^2, 120°C for 15 min.

4. FSA C50 P6T, flaxseed batch A pelleted 6 times with 50% corn; FSA C70 P6T, flaxseed batch A pelleted 6 times with 70% corn. The energy values of flaxseed were converted back to 100% flaxseed. 5. FSB M, flaxseed batch B processed by microwave roasting for 4 min. 6. FFSB, extruded full-fat soybean.

7. LM, linseed meal. 8. LM P3T, linseed meal pelletted with CPM laboratory pellet mill for 3 times.

9. The value of pelleted linseed meal was compared with that of raw linseed meal.

11.5% ADF. It has also been noticed that there was lower DM and EE apparent digestibility for FSB than FSA.

The TME and TMEn values obtained in the present study were lower than those reported by others. FS TME and TMEn values have been calculated to be 4156 and 3957 kcal/kg by Barbour and Sim (1991), 3960 and 3750 kcal/kg by Lee et al. (1995). When expressed as ME, the value is 3600 kcal/kg as reported by Leeson and Summers (1997), and 4779 kcal/kg by Grossu et al (1998). Difference among FS cultivars, like nutrient content, ANFs presence, or unit expression (whether expressed on DM basis), could be some of the reasons for the values difference, while variations in analytical methodology could be another source of differences.

The TME and TMEn of ground raw canola seed measured in the present experiment were 3840 and 3623 kcal/kg, respectively. These values were obtained without processing by pelleting or any other methods used in the present study. They were higher than that of ground raw FS in the present study, but lower than 4623 and 4487 kcal/kg reported by Barbour and Sim (1991), 4730 and 4560 kcal/kg by Lee et al. (1995) respectively. The lower canola seed ME value obtained in the present study compared to published data could be due to the same reasons as for raw FS, namely analytical methodology differences among laboratories.

As compared to canola seed, which has a similar nutrient content as FS, Barbour and Sim (1991) attributed the low ME of FS to the difference in hull coat thickness, indigestible crude fiber content, shape, structure and presence of mucilage and other deleterious factors. Proper processing could modify these characteristics and therefore improve its ME values.

Flaxseed Apparent Ether Extract Digestibility and Its Relationship to ME Values

Since there was an insignificant amount (0.89 to 1.39%) of EE detected in the excreta of the control period, with a total EE excretion of 0.053 to 0.098 g per bird, the AD of EE and FA for the test ingredients could represent their true availability.

As shown in Table 5.3, the AD of EE for FSA, 61.2%, was significantly ($P<0.01$) increased by both FS repeat pelleting, either 3 or 6 times, and autoclaving. Its improvement was between 33 to 36%. A significant effect ($P<0.05$) was also observed for FSB by microwave roasting, with 31% improvement from its original AD of 49.1%.

Table 5.3. The effect of flaxseed pelleting, autoclaving, and microwave roasting on its apparent digestibility of nutrients and nitrogen retention for leghorn roosters

Ingredient & processing	DM			EE[1]			ADF[2]			N		
	AD[3] %	Compared to raw form		AD %	Compared to Raw form		AD %	Compared to raw form		True N retention	Compared to raw form	
		%	Pr >t		%	Pr > t		%	Pr > t	g	%	Pr > t
FSA[4]	30.8	100		61.2	100		13.17	100		0.431	100	
FSA P3T[5]	33.6	109	0.643	83.2	136	0.001	2.67	20	0.018	0.519	120	0.618
FSA P6T[5]	45.7	148	0.018	82.2	134	0.002	14.83	113	0.699	0.559	130	0.467
FSA ATC[6]	41.4	134	0.087	81.5	133	0.002	7.00	53	0.156	0.542	126	0.527
FSB[4]	19.7	100		49.1	100		23.67	100		0.473	100	
FSB M[7]	32.9	167	0.034	64.4	131	0.019	23.83	101	0.969	0.652	138	0.311
FFSB[8]	43.1			90.1			MVD[11]			1.179		
Canola seed	28.6			60.3			18.3			0.790		
LM[9]	24.2	100		76.6	100		5.2	100		0.716	100	
LM P3T[10]	27.3	113	0.606	79.0	103	0.708	7.7	148	0.561	0.555	78	0.363
SEM	4.3			4.5			3.0			.154		

1. EE, ether extract. 2. ADF, acid detergent fiber. 3. AD, apparent digestibility. 4. FSA, raw flaxseed batch A (37.6% oil); FSB, flaxseed batch B (34.2% oil). 5. FSA P3T, flaxseed batch A pelletted 3 times or FSA P6T, 6 times, by CPM laboratory pellet mill.

6. FSA ACT, flaxseed batch A autoclaved at 16 kg/cm^2, 120°C for 15 min. 7. FSB M, flaxseed batch B processed by microwave roasting for 4 min. 8. FFSB, extruded full-fat soybean. 9. LM, Linseed meal.

10. LM P3T, linseed meal pelletted with CPM laboratory pellet mill for 3 times.

11. MVD, minus value detected.

Pelleting imposes high pressure on FS when passing through the pellet die. Carew et al. (1959) suggested that pressing altered the cell wall and whole cellular structure. This may make oil in FS more exposed to digestive enzyme in the intestine of the bird and explain the improved EE utilization. Its effect may be related to the extent of friction obtained during pelleting. Friction may be reduced by high oil content in FS, and increased by repeated pelleting, or by inclusion of other ingredient. Since EE contributes most to the energy content, its increased absorption augments the ME content of FS. Repeated pelleting, 3 and 6 times, of FS increased EE utilization the most (from 61.2% to 83.2 and 82.2%), so its TMEn value increased from 3589 to 4432 and 5006 kcal/kg DM, respectively. When 70% corn was mixed with FS, a similar increase was achieved for TMEn value of FS (4818 kcal/kg DM).

The effect of pelleting on EE improvement observed from the present experiment was in agreement with the pelleting of soybeans as reported by Carew and Nesheim (1962). As compared to ground soybean, pelleting soybean alone increased the oil availability from 73% to 78%, while pelleting together with whole diet further increased the oil absorbability to 91%. The ME value of soybean for chicks was 3320, 3470, and 3800 kcal/kg, respectively, when used in ground soybean, soybean pellet alone, soybean pellet together with whole diet. Maximization of oil utilization was achieved only through proper processing for soybean.

The consideration for repeated pelleting and FS-corn mixture in the present study was to maximize the possible pressure and heat effect caused by pelleting, based on the fact that no steam and preconditioning treatment were used in the present pelleting conditions. In commercial operations, repeated pelleting will be impractical, and probably unnecessary. Through the adjustment in pellet parameters, inclusion of steam preconditioning, and inclusion of other ingredients, the same effects could be achieved.

Physical changes caused by autoclaving and microwave roasting may not be as great as by pelleting, but still could be expected due to heat, pressure, and microwave induced interaction. Microwave is known to cause polarization of treated materials, which further leads to displacement of charge groups and produces heat within materials (Clark 1996).

The low EE utilization for FSB of 49.1%, as compared to that of FSA of 61.2%, could be related to its lower quality. It contains lower EE (34.2%) and higher ADF (11.5%).

High ADF content could be the main factor affecting the EE utilization. That could also explain the lower ME values of FSB as well.

Fatty Acids Digestibility in Flaxseed

Table 5.4 contains the results of FA AD. The AD of TFA was 65.5 and 48.5%, respectively, for raw FSA and FSB. They were influenced by processing in a similar patter as for EE. The AD of linolenic acid, 69.5% for FSA and 50.7% for FSB, was higher than their AD of TFA. The improvement for FS A was 11.6, 24.9 and 20.3% for 3-times pelleting, 6 times pelleting and autoclaving respectively. Microwave roasting increased the AD of linolenic acid for FSB by 32.7%.

The AD of total polyunsaturated FAs, which includes linoleic acid and linolenic acid, is 68.9% for FS A and 50.1% for FSB. They were identical to that of linolenic acid. The AD of this total polyunsaturated FA was affected by the processing used in the same manner as for linolenic acid.

For total monounsaturated FAs, which is mostly oleic acid, the AD was 55.7% and 44.2%, respectively, for FSA and FSB. Pelleting 3 times, 6 times, and autoclaving increased the AD of monounsaturated FAs for FSA from its 61.2% to 81.7, 83.6, and 80.3%, with 33.5%, 36.6% and 31.2% improvement, respectively. For FSB, microwave roasting significantly ($P<0.05$) improved the digestibility from its 46.9 to 68.0%, with a 45% increase.

Processing also increased the utilization of total saturated FAs, which are composed of mainly palmitic and stearic acids. From a value of 55.7% of FSA, the AD was increased with 37.7, 42.0, and 43.1% improvement, by pelleted 3 times, pelleted 6 times, and autoclaving, respectively. In the case of FSB, microwave roasting significantly ($P<0.05$) increased the utilization of saturated FAs from 44.2 to 66.6% with 50.7% improvement.

Linolenic acid (53.6%) is the major FA, followed by linoleic acid (17.8%) and oleic acid (17.7%), as shown in Table 5.1. Higher utilization rate for total monounsaturated FAs and total polyunsaturated FAs than that of total saturated FAs were noticed in the present study. Ketels et al. (1989), Leeson and Attech (1995) and Vila and Esteve-Garcia (1996) concluded to a similar finding that young poultry can use unsaturated FAs better than saturated ones.

Table 5.4. The effect of processing on the apparent fatty acid digestibility (%) of flaxseed for Leghorn roosters

Ingredient & Processing	TFA[1]	C10:0	C12:0	C16:0	C16:1	C18:0	C18:1	C18:2	C18:3	Σ SAT[2]	Σ mono[3]	Σ poly[4]
FSA[5]	65.5^{bcd}	04.4^{e}	100.0	56.9^{cd}	94.0^{a}	58.5^{bcd}	61.1^{bcd}	66.9^{bcd}	69.5^{abc}	55.7^{cd}	61.2^{bcd}	68.9^{abc}
FSA P3T[6]	84.7^{ab}	23.2^{bc}	98.4	77.9^{abc}	85.1^{a}	77.7^{abc}	81.8^{ab}	85.7^{ab}	87.8^{a}	76.7^{abc}	81.7^{ab}	87.3^{a}
FSA P6T[6]	84.8^{ab}	30.6^{bc}	97.1	80.8^{ab}	83.6^{a}	79.7^{abc}	83.6^{ab}	85.4^{ab}	86.8^{ab}	79.1^{abc}	83.6^{ab}	86.5^{a}
FSA ATC[7]	82.3^{abc}	50.8^{b}	87.6	79.1^{abc}	78.6^{a}	82.2^{ab}	80.3^{ab}	83.8^{abc}	83.6^{ab}	79.7^{ab}	80.3^{ab}	83.7^{ab}
FSB[5]	48.5^{d}	10.1^{bc}	100.0	46.1^{d}	NG[10]	46.5^{d}	46.9^{cd}	48.2^{d}	50.7^{c}	44.2^{d}	46.9^{cd}	50.1^{c}
FSB M[8]	67.3^{abcd}	41.6^{bc}	100.0	67.7^{abcd}	NG[10]	66.9^{abc}	68.0^{abc}	69.3^{abcd}	67.3^{abc}	66.6^{abc}	68.0^{abc}	67.7^{abc}
FFSB[9]	89.5^{a}	98.4^{a}	100.0	87.0^{a}	56.9^{a}	83.4^{a}	87.6^{a}	90.6^{a}	91.5^{a}	88.7^{a}	87.4^{a}	90.8^{a}
Canola seed	61.2^{cd}	99.4^{a}	100.0	60.8^{bcd}	61.1^{a}	57.3^{c}	39.6^{d}	61.6^{cd}	62.5^{cd}	63.2^{bcd}	40.3^{d}	61.9^{bc}
SEM	4.16	7.58	2.9	4.2	7.0	4.3	4.5	4.2	4.5	4.1	4.5	4.4
Pr> F	0.0001	0.0001	0.0574	0.0001	0.001	0.0001	0.0001	0.0001	0.0001	0.0001	0.0001	0.0001

1. TFA, total fatty acids. 2. Σ SAT[2], total of saturated fatty acids. 3. Σ mono, total of monounsaturated fatty acids.

4. Σ poly, total of polyunsaturated fatty acids. 5. FSA, raw flaxseed batch A (37.6% oil); FSB, raw flaxseed batch B (34.2% oil).

6. FSA P3T, flaxseed batch A pelletted 3 times; or FSA P6T, flaxseed batch A pelleted 6 times, by CPM laboratory pellet mill.

7. FSA ATC, flaxseed batch A autoclaved at 16.5 kg/cm^2, 120°C for 15 min.

8. FSB M, flaxseed batch B microwave roasted for 4 min. 9. FFSB, extruded full-fat soybean.

10. NG, negligible value detected.

Means within the same column with different superscripts differ significantly by using Scheffe's test, $P<0.05$.

As reviewed by Scott et al. (1982), the unsaturated FAs can readily form mixed micelles with bile salts and become highly dispersed in the aqueous medium of the intestinal lumen. The mixed micelles then can pass across the membrane of mucosal cells, completing rapidly the process of absorption. These stated reasons may also explain the result observed in the present experiment on the difference between saturated and unsaturated FAs.

Based on the fact that a greater proportion of FS oil is polyunsaturated FA, we can also suggest that FAs themselves in FS would not reduce FAs utilization. In other words, the reduced nutrient utilization, including EE, as compared to soybean or canola seed, could be related to other reasons than the FA profile of FS. While linolenic acid in FS oil makes up more than 50% of the FAs, the improvement in FA utilization by proper processing would benefit linolenic acid most. The exploitation of this T-3 FA reserve could be maximized either by pelleting or by microwave roasting the FS, as observed in the present study.

Pelleting and autoclaving slightly decreased the TFA of FS, as shown in Table 5.1. This was not observed when processed by microwave. The processing listed did not alter the FA profiles of FS. This effect may relate to various duration of the processing tested. Hiromi et al. (1999) reported that soybean lost 2, 7 and 15% of FA during 6, 12 and 20 minutes microwave roasting, respectively. The present microwave roasting time was only 4 minutes and this period may not cause obvious differences in total FA content.

The FA profile of FS was not affected by the processing methods tested in the present study. One of the reasons could be that the FS still had the protection of its seed hull during processing, hence preventing peroxidation. While the physical structure of FS is changed during pelleting, the temperature may not reach a degree that would favor peroxidation. The temperature recorded from repeated pelleting was around 43°C.

Effect of Flaxseed Processing on DM and ADF Utilization, and N Retention

Processing in general increased the utilization of dry matter, and ether extract, and improved nitrogen retention for FS, as shown in Table 5.3. The apparent dry matter digestibility was 30.8% for ground FSA, 19.7% for ground FSB. Pelleting FSA 6 times significantly improved its digestibility to 45.7% ($P<0.05$). When FSB was microwave roasted, its apparent dry matter digestibility increased to 32.9% ($P<0.05$).

The nitrogen retention for FSA and FSB was 0.431 g and 0.473 g, respectively. Even though all the processing methods slightly improved the N retention, this improvement was not statistically significant ($P>0.05$).

The effect of FS processing on the utilization of DM and ADF or N retention was only marginal. The slight improvement in apparent DM digestibility and N retention could be the effect of heat, together with the reduction in ANFs after processing. These results were not in agreement with those reported by Lee et al. (1991), who demonstrated the ineffectiveness of heat processing (85°C for 30 min.) of FS on protein retention, DM retention, and ME and MEn value of 10% FS containing diets for broiler.

The utilization of ADF was not affected by processing. The ADF content in FSA was 8.7%, while 11.5%, in FSB. The ADF apparent digestibility of FSA was 13.2%, and 23.7% for FSB. We adopted in the TME determination a fasting period of 24 hours prior to the control period collection of 36 hours. We observed that some feces from the excreta of control period could contain feed residue after the adaptation periods. There were also great variations for ADF content when these samples were analyzed. Longer than 24 hours of fasting may be required for more accurate ADF digestibility determination.

Another benefit of processing FS, besides nutrient utilization improvement, is the possible reduction of ANFs. FS is known to contain high amount of cyanogenic glycosides, linatine, and some other ANFs. Proper processing would be essential, if one expects to include it in greater amount in young animal diets.

In conclusion, the TME and TMEn values determined in the present experiment were 3343 and 3225 kcal/kg, respectively, for FS containing 37.6% EE. They were 3840 and 3623 kcal/kg for canola seed, 3582 and 3463 kcal/kg for extruded FFSB, and 2108 and 1911 kcal/kg for linseed meal, respectively. FS processing, such as repeat pelleting, autoclaving, or microwave roasting, effectively increased ME value, DM and EE utilization, and N retention. Regarding TME values of FS, the greatest improvement was achieved by repeat pelleting ($P<0.01$), followed by pelleting together with 70% corn. The least effective, but nonetheless significant ($P<0.05$), was autoclaving or microwave roasting. This ME improvement was supported by improved EE utilization for their relevant FS processing.

Adult roosters can use polyunsaturated FA better, followed by monounsaturated FA, and use saturated FA with less efficiency. The processing slightly reduced the total FA content, but increased total and individual FA utilization of FS in a way similar to the EE improvement. The AD of linolenic acid was improved by 26.3% with FS repeated pelleting alone, and 32.7% with microwave roasting. This improvement will benefit the linolenic acid most, due to the fact that this T-3 FA makes up more than 50% of total FAs in FS.

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Section VI

Performance, Carcass Cut-Up Percentage and Fatty Acids Deposition of Broilers Fed Different Levels of Pellet-Processed Flaxseed

Connecting Text for Section VI

Proper flaxseed processing had been shown to be effective in reducing the content of hydrogen cyanide, one of the main antinutritional factors in flaxseed. It could also significantly increase the TMEn value of flaxseed. Therefore, the nutritive value of flaxseed could be greatly improved by proper processing. However, how broilers respond to a diet that contains higher amounts of processed flaxseed had not yet been tested. The following experiments intended to evaluate the performance and fatty acid deposition response of broiler up to market age when fed a pellet-processed flaxseed containing diet.

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FLAXSEED, PERFORMANCE, AND ω-3 FATTY ACID IN BROILERS

Title:

Performance, Carcass Cut-Up Percentage and Fatty Acids Deposition of Broilers Fed Different Levels of Pellet-Processed Flaxseed

Yingran Shen,* P. C. Laguë,* Dingyuan Feng,† and E. R. Chavez *[1]

**Department of Animal Science, Macdonald Campus of McGill University, Canada*

†*Department of Animal Science, South China Agricultural University, China*

1 To whom correspondence should be addressed: chavez@macdonald.mcgill.ca

E. R. Chavez

Department of Animal Science, Macdonald Campus of McGill University, 21,111 Lakeshore, Ste Anne de Bellevue, Quebec, Canada, H9X 3V9

Tel: (514) 398 7795; Fax: (514) 398 7964

Scientific Section: Metabolism and Nutrition

Abbreviation Key: ANFs = anti-nutritional factors; FA = fatty acid; FAs = fatty acids; FS = flaxseed; FFSB = extruded full-fat soybean; HCN = hydrogen cyanide; PM = pellet-then-reground; RTC = ready to cook carcass.

ABSTRACT A feeding trial was performed to study the effect of pellet-processed flaxseed (FS) on performance and fatty acid (FA) deposition in broilers. Two replicates with 50 day-old male broilers per replicate were used for each treatment. Birds were fed with either pellet-then-reground FS containing diets, or diets with ground or whole FS. Regrinding after pelleting was performed so as to make diets the same form among the treatments. The FS inclusion rate was 0, 12, and 14% in the starter-grower period (day one-to-21), and 0, 15, or 17% in the finisher period (day 22-to-40). The performance data showed that there were significant (P<0.05) interaction between diet processing and FS level on birds' body weight by day 21 and day 40. Birds fed diets of less FS but processed by pellet-then-reground gained more, consumed more feed in the starter-grower period. They weighed 839 g, consumed 1022 g feed with 1.37 feed-to-gain ratio for birds fed 12% FS processed by pellet-then-reground after 21 days feeding. The effect of performance improvement caused by pellet then-reground diet processing diminished in the finisher period, when FS level was increased. The highest total ω-3 FA deposition was achieved with higher FS inclusion rate but pellet-then-reground processed. The ω-3 FA composition was 23.04 and 26.46% of total lipid in breast and thigh meat, respectively, after 40 day of feeding. These values were significantly (P<0.05) higher than those found in birds fed whole FS diets, or in the control birds fed no FS, and were comparable to what is reported in fish products. In conclusion, moderate amount of processed FS, as 12% in the present experiment, can be incorporated into broiler diets with satisfactory growth, while achieving a desired ω-3 FA deposition very close to the values in fish products.

(*Key words*: flaxseed level, flaxseed processing, performance, fatty acid deposition, broiler)

INTRODUCTION

The main expectation for using flaxseed (FS) in broiler diets is to obtain satisfactory growth while achieving desired fatty acids (FAs) deposition in meat tissue. It is well known that more than 50% of FS oil is linolenic acid. The inclusion of FS or FS oil in rations of laying hens and broilers has been proven effective to proportionally improve ω-3 FAs in the products. In addition, FS could be regarded as an alternative feedstuff, due to its less expensive and high oil and protein contents. Both the FAs enriching and economic benefit could be maximized, if FS inclusion rate is at a relatively high level. Data show that more than 5 to 10% FS may depress broiler growth (Mandokhot and Singh 1983; Ajuyah et al. 1990; Ajuyah et al. 1993; Bond et al. 1997; Ochrimenko et al. 1997; Roth-Maier et al. 1998). Mostly, the reported results for broilers were based on raw ground FS. If FS is processed further than just grinding, the nutritional response could be different. The present experiment was designed to study the effect of processing and FS level on the performance response, as well as on the FAs deposition in breast and thigh tissues of broiler chickens.

MATERIALS AND METHODS

Experimental Birds and Management

Six hundred day-old male Cobbs by Cobbs broiler chicks were purchased form a local commercial hatchery. They were randomly assigned to 12 floor pens (1.8 m × 3.0 m) on wood shaving litter, with 50 birds per pen in an environmentally control building. They were fed experimental diets from day one until 40 days of age. All birds had access to water and experimental feed ad libitum. They were provided with two cylindrical type feeders and one bell-shaped waterer for each pen. Birds fed whole FS diets had access to grit ad libitum.

The birds were raised at initial temperature of 32°C, which was progressively reduced to 20 – 22°C by day 21. The lighting program was as follows for light (L) and dark (D) in hours: the first four days, 20L: 4D; from 5 to 14 days of age: 6L: 18D; from 15 to 21 days of age, 10L: 14D.

Experimental Design, Diets, and Feed Processing Procedures

As shown in Table 6.1, a completely randomized design with a factorial arrangement of some treatments was followed in this two-period feeding experiment. For FS level, diets

contained 0, or 12%, or 14% FS in starting-growing period, and 0, or 15%, or 17% in finishing period. Canola seed and extruded full-fat soybean were used in 0% FS diets as control. For diet processing, it was mashed (extruded full-fat soybean and ground FS diets), or pellet-then-reground (FS and canola seed diets), or whole FS diets. Whole FS diets were regarded as negative control. Each dietary treatment (group) had two replicates (pens). These diets were fed to birds in two feeding periods: the starter-grower period (day one to 21) and the finishing period (day 22 to 40).

Table 6.1. The factorial design of the experiment: the effect of flaxseed level and pellet processing on performance and fatty acids deposition for broilers

	FS[1] Level					
	Starting and growing (D 1 –21)			Finishing (D 22 – 40)		
FS Processing	0	12%	14%	0	15%	17%
Mash	✔ (14% FFSB[2])			✔ (28%FFSB)	✔	
Pellet-then-reground	✔ (14% canola seed)	✔	✔	✔ (17% canola seed)	✔	✔
Whole seed		✔	✔		✔	

1. FS = flaxseed.

2. FFSB = extruded full-fat soybean.

Adjustment was made for whole FS-fed groups after the starter-grower period, due to the obvious depressing growth caused by the two whole FS diets. The regrouping of these birds was made as follow: at the end of the starting-growing period and after weighing, the birds originally fed whole seed diets (12 and 14% FS) were mixed and randomly assigned to 4 new pens. The average pen weight was adjusted so to have equal pen weight. Then two of these four pens were given 15% whole FS diets, while the other, 15% ground FS diets. As shown in Table 6.2, the diets and processing were variables included in the design of the different feeding programs included in this study.

The feed processing procedure was as follow: Pelleting was performed with California Pellet Machine, Master model[2], by pelleting twice for the same feed with no steam conditioning. The die parameters used for starter-grower diet are: hole diameter, 0.318 cm; die diameter, 30.48 cm; thickness of the die, 3.05 cm. While for finisher diet, all die

parameters are the same except hole diameter of 0.476 cm. This feed was then mashed after cooling by using a comminuting machine[3], and was fed as such to birds. For mash FS diet, the FS was ground together with coarse corn first and then mixed into the other ingredients.

Table 6.2 The feeding program of male broiler chicks fed with different levels of flaxseed (FS) and diet processing[1]

Diet and processing	Level of inclusion	
	Period 0-21 days	Period 22-40 days
FS PM[2]	12%	15%
FS PM	14%	17%
FS Whole	12% Whole	15% whole
FS Whole	14% Whole	15% mashed
FFSB[3]	14%	28%
Canola, PM[6]	14%	17%

1. All diets during the first period contained 23% crude protein and 19% during the second period. The two groups containing 12 and 14% of whole FS performed similarly poor during the first 21 days. Then, after these two groups were mixed together, they were randomly distributed into the next two groups containing 15% whole FS, one maintaining the whole FS and the other with the FS being mashed in the diet.
2. FS PM: flaxseed was pelleted together with other ingredients in the diet and then reground.
3. FFSB: extruded full-fat soybean.
4. Canola seed was processed in the same manner it was done for the FS, pellet and then reground.

The composition and actual analysis of the experimental diets are shown in Table 6.3. They were formulated to have similar ME values and protein content within the feeding periods and to satisfy the nutrient requirements set by NRC (1994).

Performance Record

Weighing of body weight was performed by pen on day one, followed by individual on day 7, 21 and 40, on full-fed basis. Feed consumption was recorded weekly by pen. The weight gain and feed conversion were calculated on pen basis at the end of each feeding period.

[2] California Pellet Mill Co. 1114 E. Wabash Avenue, Crawfordsville, IN 47933.

Table 6.3. The composition and proximal nutrient content of experimental diets[1]

	Starting and growing period (1 to 21 D)				Finishing period (22 - 40 D)			
	12% FS[2]	14% FS	14%CS[2]	FFSB[2]	15% FS	17% FS	17%CS	FFSB
Ingredients,% :								
Corn	52.5	51.3	47.8	55.7	57.5	55.4	50.9	60.8
Soybean meal 48	30.4	29.6	30.2	25.2	21.3	20.3	21.4	15.2
Flaxseed	12.0	14.0	0	0	15.0	17.0	0	0
Canola seed	0	0	14.0	0	0	0	17.0	0
FFSB	0	0	0	14.0	0	0	0	28.0
Wheat bran	0	0	2.9	0	1.1	2.2	5.7	1.9
Premix[3] 4050	5.0	5.0	5.0	5.0	5.0	5.0	5.0	5.0
D,L methionine	0.180	0.180	0.152	0	0.170	0.110	0.080	0.100
L-Lysine	0.040	0.040	0.020	0	0.030	0.030	0	0
Calculated composition, %:								
Crude protein	22.11	22.10	22.11	22.12	19.08	19.08	19.02	19.03
ME, kcal/kg	3023	3045	3047	3040	3097	3098	3100	3097
Ether Extract	6.59	7.28	7.70	4.90	7.89	8.60	9.11	5.88
Ca	1.03	1.03	1.05	1.03	1.05	1.01	1.04	1.01
Total P	0.73	0.73	0.72	0.73	0.71	0.72	0.71	0.71
Meth & cyst.	0.854	0.855	0.851	0.861	0.775	0.716	0.719	0.718
Methionine	0.501	0.502	0.501	0.496	0.408	0.408	0.401	0.410
Lysine	1.150	1.151	1.156	1.265	0.954	0.954	0.963	0.986
Actual analysis, %:								
Crude protein	23.76	23.59	23.59	23.62	19.68	19.01	20.32	19.4
GE, kcal/kg	4314	4288	4273	3968	4190	4204	4222	3915
ADF[4]	3.6	3.78	3.78	4.04	5.53	NA[5]	NA	NA
Ether extract	7.24	8.26	8.26	4.74	9.31	9.53	9.49	5.49
Ash	5.79	6.57	6.57	6.64	5.53	5.40	5.95	5.68
Ca	1.12	1.05	1.23	1.07	1.12	1.11	1.12	1.16
Total P	0.60	0.60	0.64	0.59	0.55	0.57	0.67	0.59

1. Diets were supplemented with 1.25% premix P25 containing Clinacox (COOPÉRATIVE FÉDÉRÉE DE QUÉBEC, Montr9al), by replacing corn in formula.
2. FS: flaxseed; CS: canola seed; FFSB: extruded full-fat soybean.
3. Premix 4050: vitamins and minerals premix (COOPÉRATIVE FÉDÉRÉE DE QUÉBEC, Montr9al) claimed to have: calcium, 18.00%; phosphorus, 6.30%; sodium, 2.80%; iron, 5,000mg/kg; copper, 200 mg/kg; iodine, 25 mg/kg, zinc, 1,800 mg/kg; vitamin A, (min) 200,000 iu/kg; vitamin D-3, (min) 60,000 iu/kg; vitamin E, (min) 500 iu/kg.
4. ADF, acid detergent fiber.
5. NA, not available.

[3] Model D, the W.J. Fitzpatrick Company, Chicago.

Carcass Cut-up Measurement and Breast and Thigh Meat Sampling

Three birds of about average pen weight were chosen from each pen for carcass cut-up measurement and tissue sampling on day 40. After a 10-hours fast, their body weight was recorded. They were then slaughtered by electrical stunning and sectioning of the neck blood vessel. Once bled, the birds were scalded at 59°C for 75 seconds, and plucked with a semi-automatic plucker. The birds were then chilled at 4°C for ten hours, after the removal of their head, neck and shanks. After chilling, the birds were eviscerated and carcass weight recorded. The drumsticks, wings, back, breast with keel bone, fat-pad, and liver as of each bird were cut and weighed. Their percentage was calculated against the carcass weight.

Breast and thigh meat sample were collected from each bird by dissecting meat from the same position after removal of the skin, and was freeze-dried for 72 hrs.

Total Lipids and Fatty Acid Composition of Breast and Thigh Tissue

The freeze-dried breast and thigh meat samples were ground with coffee grinder and extracted with ether to determine total lipids. FA analysis was performed following the procedure described by Sukhija and Palmquist (1987), by using automated gas liquid chromatography.[4]

Statistics

Mixed factorial model of SAS (1995) were used for variance analysis. The fixed effect of the statistical model included diet processing, FS level, and the interaction between diet processing and FS level, while the random effect was variance of pen within a treatment and the random error associated with each observation. The least square mean differences were adjusted by Scheffe's test. The statistical significance was accepted with $P<0.05$.

RESULTS AND DISCUSSION

Performance

The results recorded during the first 21 days are shown in Table 6.4. The best performance in body weight was observed in the control diet containing Canola seed. It was significantly ($P<0.025$) superior to the control diet containing full-fat soybean; the

[4] Series 2, No. 5890, Hewlett Packard, Palo Alto, CA 94304-1181.

12% FS pellet-meal diet gave the best body weight after 3 weeks, similar to the Canola control diet. However the 14% FS pellet-then-reground diet was significantly ($P<0.025$) inferior to the Canola control, although no significantly ($P>0.05$) difference was detected from the 12% FS pellet-then-reground diet. Diets containing whole FS either 12 or 14% were significantly improved in their response on body weight after being pelleted and then mashed; the improvement for the 12% diet was much greater than the 14% diet (+31% vs. + 21%). Overall, the process of pelleting FS resulted in a 26% improvement in body weight.

Table 6.4 Performance of 21 days old male broiler chicks fed diets of different flaxseed levels and diet processing starting from day one

Diet (FS) processing	FS %	Body weight	Feed intake	Feed/gain
FS PM[1]	12	839^{ab}	1022	1.37^{bc}
FS PM	14	783^{b}	1052	1.43^{c}
Whole FS[2]	12	640^{c}	904	1.53^{d}
Whole FS	14	646^{c}	933	1.55^{d}
FFSB[3] Mashed	0	781^{ab}	1007	1.36^{b}
Canola, PM[4]	0	872^{a}	1064	1.28^{a}
	SEM	9.1	29.2	.009
		Probability		
Processing		.0001	.0149	.0001
Flaxseed level		.0025	.4939	.0001
Interaction		.0144	.9935	.1374

1. FS PM: flaxseed was pelletted together with other ingredients in the diet and then reground.

2. Whole FS: flaxseed was provided as whole seed in the diet.

3. FFSB: diet contained 14% extruded full-fat soybean;

4. Canola PM: diet contained 14% canola seed, which was treated the same as FS PM.

Means within columns with no common superscripts differ significantly (Scheffe's test, $P<0.05$).

The results presented in table 6.5 summarized the performance data obtained with the broiler chickens after 40 days. The feeding program with Canola diets gave the best performance of the broiler chickens after 40 days feeding period. However, the best gain during the second period (22-40 days) was observed with the feeding program with the FFSB. At the end of the 40 days feeding program the two control groups were similar,

although the group receiving the FFSB required more feed. The best feeding program with FS was the combination 12% to 15% of pelleted FS. Nonetheless, this feeding program was significantly ($P<0.001$) inferior to either control. The 14% FS pelleted showed the lowest performance during the second period, given a similar final body weight to the two whole FS groups. The significant interaction between FS level and processing seems to indicate that the response in performance after pelleting FS is much greater at a lower inclusion of FS in the diet. It appears also evident that 14% FS during the first period and 17% FS during the second period induced a detrimental effect on broiler chickens performance recorded at 40 days of age.

Body weight was significantly ($P<0.05$) affected by an interaction between FS processing and FS level. The body weight of birds fed diets with low FS (12% in starting-growing period and 15% in finishing period) but pellet-then-reground was 839 and 2198 g after 21 and 40 days, respectively. These birds were slightly lighter than the birds fed canola seed, similar to the birds fed FFSB, but significantly heavier than the birds fed whole FS after 21 days. Whereas after 40 days, they were significantly lighter than those fed canola seed and FFSB diets, and significantly heavier than those fed either pelleted-then-reground diet but with higher FS content, or whole or ground FS diets with the same FS level.

Feed intake was significantly affected by FS processing ($P<0.05$), but not by FS level Birds fed low FS (12% in starting-growing period and 15% in finishing period) but pellet-then-reground diets consumed 1022 and 2942 g feed for 21 and 40 day-periods, respectively. Whole FS diets caused birds to consume less feed in the first 3 weeks, while both whole and ground FS diets lead to higher feed consumption than other FS containing diets in the period of 22 to 40 days.

Feed conversion efficiency was very significantly affected by both FS processing and FS level ($P<0.01$) in the first 21 days. While in the period of 22 to 40 days, it was only affected by FS level ($P<0.01$). The feed-to-gain ratios for birds fed low FS but pellet-then-reground diet were 1.37 and 2.27 for 21 and 40 days of age, respectively. They were higher than those fed canola seed and FFSB diets, but lower than those fed whole seed diets in the first 21-day period. While in the 22-to-40 day period, they were higher than those fed canola seed and FFSB, also higher than birds fed both whole and mash FS. Within the

same diet processing, higher (14% in starting-growing period and 17% in finishing period) FS level slightly increased the feed-to-gain ratio.

Table 6.5 Performance of 40 days old male broiler chicks fed diets of different flaxseed levels and diet processing starting from day 21

Diet (FS) processing	Feeding program FS (FFSB, Canola)		Body weight	Feed intake	Feed/gain
	1-21 D	22-40 D			
FS PM[1]	12%	15%	2198[b]	2942[b]	2.27[c]
FS PM[2]	14%	17%	2014[c]	2896[b]	2.39[c]
FS[3]	12/14% whole	15% whole	2056[c]	3024[ab]	2.14[bc]
FS[4]	12/14% whole	15% mashed	2023[c]	2972[ab]	2.15[bc]
FFSB[5]	0(14%)	0(28%)	2413[a]	3129[a]	1.91[ab]
Canola, PM[6]	0(14%)	0(17%)	2463[a]	2898[b]	1.83[a]
	SEM		25.4	26.2	.040
		Probability			
		Processing	.000	.0050	.4100
		Flaxseed level	.000	.1554	.0001
		Interaction	.011	.0087	.0478

1. FS PM: flaxseed was pelletted together with other ingredients in the diet and then reground. Chicks were fed 12% FS PM diet during day one-21 period.

2. Chicks were fed 14% FS PM diet during day one-21 period.

3. Whole FS: flaxseed was provided as whole seed in the diet. Chicks were fed 12 or 14% whole FS during day one-21 period.

4. Mash FS: flaxseed was provided as mashed form in the diet. Chicks were fed 12 or 14% whole FS during day one-21 period.

5. FFSB: diet contained 28% extruded full-fat soybean. Chicks were fed 14% FFSB during day one-21 period.

6. Canola PM: diet contained 17% canola seed, which was treated the same as FS PM. Chicks were fed 14% Canola seed that had been treated the same as FS PM during day one-21 period.

Means within columns with no common superscripts differ significantly (Scheffe's test, $P<0.05$).

The performance results from the present study revealed that broilers fed moderate amount of FS, like 12%, could have satisfactory growth, if FS had been properly processed. However, higher than 12% FS depressed growth, even if the diet had been processed by pellet-then-reground. The tolerance level obtained in the present study with

12% FS contrasts with the results reported by other researchers. Broilers fed diets containing 10% FS led to 4.9% to 6.7% lighter body weight, as compared to birds fed 10% canola seed (Ajuyah et al. 1991 and Lee et al. 1991). When compared to control corn-soybean diet, 15% FS caused a 17% reduction in body weight (Ajuyah et al. 1993). Bond et al. (1997) concluded that FS was not a practical oil source, as over 10% FS caused significant performance reduction. Roth-Maier et al. (1998) showed that as low as 5% of FS both in ground or whole form slightly reduced the body weight and feed conversion rate, and this reduction was further enhanced at 7.5%, even though grit had been provided *ad libitum*. Whole seed diet also slightly increased the feed to gain ratio, but the feed intake was not affected. These results seemed to indicate that broilers could only tolerate at levels between 5 to 10% in the diet, which is not in agreement with our findings from the present experiment.

In the present study, 12% FS caused a slightly (3.9%) but not statistically significant body weight reduction at 21 days of age, as compared to those fed 14% canola seed. The difference between the results of the present study and those reported by other researchers could be that the FS used in those studies was not processed properly, with mostly just grinding. Proper FS processing may diminish the presence of some ANFs, change the physical structure of the seed, and improve nutrient utilization, hence allowing its usage in broiler diets for up to 12% with satisfactory performance.

In the finishing period, however, 15% FS caused a 12% reduction in body weight, as compared to canola seed diet that had the same processing. As age advances, birds are supposed to be more tolerant to ANFs, and their digestive ability should improve. The observed results seemed to be contradicting these expectations. These adverse results could be explained by the poor pellet quality due to the high oil content (7.9 to 9.1%) in the finishing diets, and/or the bigger pellet size (0.476 vs 0.318 cm). When we re-mashed these pelleted feeds, so to make the feed form equal among all the dietary groups, we noticed that the pellets were more fragile. The feed after this mashing contained very small granules, as compared to the re-mashed diets for the in starter-grower period. As a result, birds under these pellet-then-reground finishing diets consumed less. Also, poorer pellet quality may have caused less reduction in ANFs content, which was discussed in section IV, hence affecting negatively the performance of these birds. A better pellet quality could

be obtained by using different pelleting procedures such as binding agents and steam preconditioning. It may further affect performance of birds.

All the birds fed with FS diets used feed less efficiently than those fed the control diets containing either extruded FFSB or canola seed. It is recognized that processed soybean has higher available lysine and other essential amino acids content (Leeson and Summers 1997). The true available amino acids of canola seed are higher than those in FS and canola protein was regarded as of better quality than that of FS (Barbour and Sim 1991, Lee et al. 1995). That may explain the better feed conversion rate for these control diets.

Diets with 12 or 14% whole FS depressed growth and feed consumption in the early period (before 3 weeks of age). The whole-seed depression was reduced in the finishing period (day 22 to 40). Birds fed whole or mashed FS diets (15%) consumed more feed and had better feed conversion efficiency than birds fed FS diet with similar FS level but processed (pellet-then-reground) in the finishing period. All birds fed whole seed diets were provided grit ad libitum so to improve the digestion of seed. Even so, the young broilers in day one-to-21 had problems in digesting these whole seed diets. But the whole seed diet consumption was greatly increased in the finishing period, as compared to birds fed diets with similar FS level but processed with pellet-then-reground method. Broilers can therefore adapt to use whole FS in finishing period at moderate levels, when grit is available continuously.

Carcass Cut-up Percentage and Lipid Content in Meat Tissue

Except for carcass weight, the cut-up parts for these birds, expressed as percentage of carcass, are not affected by processing or FS level. The results are shown in Table 6.6. The breast-plus-keel yield, around 29% of carcass, was about the same for all treatment groups. The fat-pad seemed to be reduced by the whole seed treatment, as well as the total lipids content in breast tissue. This reduction reached statistical significance for breast lipids content. There was no significant difference in lipid content between birds fed FS pellet-then-reground diets and no FS control diets.

There were inconsistent results regarding the effect of FS on lipids in thigh and breast meat tissue. Ajuyah et al. (1990) reported that addition of 10 or 20% of either FS or FS meal decreased significantly the tissue lipid content both in white and dark meats. But Olomu and Baracos (1991) demonstrated that up to 4.5% FS oil did not alter the lipid

Table 6.6. The effect of flaxseed level and processing on carcass cut-up percentage and fat content in breast and thigh meat of broiler.

	Flaxseed level, %			Cut-up or organ percentage as to carcass, %						Total lipids in meat[2], %	
Processing	1-21 D	22-40 D	RTC[1] g	Breast & keel	Drumstick	Wing	Fat-pad	Back	Liver	Breast	Thigh
Flaxseed PM[3]	12	15	1494^{bc}	29.1	14.9	11.4	2.18	41.4	3.10	2.64^{a}	6.99
Flaxseed PM[3]	14	17	1392^{c}	28.1	15.0	11.7	2.02	42.2	3.42	2.55^{a}	6.64
Whole seed[4]	12 or 14	15	1372^{c}	29.3	15.1	12.2	1.07	41.5	3.36	1.17^{b}	5.53
Flaxseed-Whole 1-21 D[4] -Mashed, 22-40 D	12 or 14	15	1354^{c}	29.2	15.5	12.0	1.13	41.2	3.33	1.07^{b}	5.93
FFSB[5] Mashed	0	0	1597^{ab}	29.5	13.8	11.3	2.50	42.7	2.96	2.75^{a}	6.53
Canola, PM	0	0	1640^{a}	28.5	14.5	11.8	2.42	42.4	2.78	2.89^{a}	8.43
		SEM	29.8	.58	.52	.26	.36	0.61	.17	.23	.96
						Probability					
		Processing	.0061	.5605	.9901	.3863	.1657	.9627	.3548	.0008	.2158
		Flaxseed level	.0001	.6325	.1191	.6649	.0701	.1360	.0120	.0009	.3482
		Interaction	.1020	.4161	.2216	.0452	.1145	.6207	.8619	.0044	.6491

1. RTC, ready to cook carcass, included the weight of eviscerated carcass without head and shank, but with fat-pad.
2. Total lipids content in meat tissue are in absolute dry matter basis; the average dry matter in fresh breast and thigh meat was: 24.9% and 22.7%, respectively.
3. PM: pelletted then mashed
4. Birds were regrouped after 3 weeks of feeding whole flaxseed diets of 12 or 14%.
5. FFSB; extruded full-fat soybean;

Means within columns with no common superscripts differ significantly (Scheffe's test, $P<.05$).

content in both white and dark muscles. Roth-Maier et al. (1998) also reported that 5 or 7.5% FS, either ground of whole, did not affect the total lipids in thigh tissue.

Due to the presence of ANFs and low FS oil utilization by birds, high FS inclusion will easily lead to reduced body weight. As 10 or 20% FS obviously depressed growth, and 5 to 7.5% FS only slightly reduced body weight, it could therefore be reasonable to think that body weight is related to lipid content in meat. If this depressed body weight could be diminished through proper FS processing or moderate FS inclusion, proper body size could be maintained. In other words, the lipid content in meat tissue could be an indicator of performance response to FS containing diets for broilers, other than caused by FS itself.

There was a significant effect of FS level on liver percentage over carcass weight ($P<0.05$). The highest liver percentage (3.42%) was obtained with birds fed the highest FS (14% in starter-grower period, and 17% in finishing period). As hydrogen cyanide (HCN) is one of the most significant toxicants in FS, its detoxification is critical for normal metabolism of cyanogenic glycosides. Even though pelleting may reduce the HCN content, there may still be considerable level of HCN remaining when the FS is included in the diet at high percentage. The catabolism of HCN in the animal body is through the formation of a less toxic compound, thiocyanate, as explained by Mazza and Oomah (1995). The process is catabolized by the enzyme rhodonase (thiosulfate sulfurtransferase), which is present in liver, kidney and thyroid tissue. The compensatory enlargement of liver could be the result of active detoxification process.

Fatty Acids Profile in Breast and Thigh Meat as Affected by Flaxseed Processing

The tissue FA profiles were the typical result of "they are what they eat." Inclusion of FS in the diets significantly increased the amount of linolenic acid, the total amount of ω-3 FAs, and reduced the ω-6/ω-3 ratio. The changes caused by FS inclusion were more obvious in thigh meat than in breast. There was also a significant interaction between the FS level and processing on their effect of ω-3 FAs deposition and ω-6/ω-3 ratio. Pellet-then-reground processing with higher FS level had the greatest influence on these FAs deposition, while this effect was minimum for the whole FS diet among the FS-containing diets. The results are shown in Tables 6.7 and 6.8.

Studies on both hens and broilers demonstrated a similar response to dietary inclusion of FS or flax oil. Eder *et al.* (1998) reported that as low as 5% FS in the diet caused hens to

Table 6.7. Fatty acid composition of breast muscle lipids (% weight) of 40 days old male broilers fed different level of flaxseed with pellet processing.

	Flaxseed %		Fatty acid (% weight)															
Processing	1-21 Day	22-40 Day	14:0	16:0	16:1	18:0	18:1	18:2 ω-6	18:3 ω-3	Homo 20:3ω-6	20:4 ω-3	20:5 ω-3	22:5 ω-3	22:6 ω-3	Σ SAT	Σ Mono	Σ ω-6	Σ ω-3	ω-6 /ω-3
FS PM	12	15	.12	18.9^{bc}	2.59	8.06^{bc}	25.95^{bc}	18.90^{bc}	14.39^{a}	1.73^{c}	0.42	2.53	1.93^{ab}	1.29	27.98^{cd}	28.66^{d}	18.88^{bc}	22.08^{a}	0.86^{c}
FS PM	14	17	.22	19.8^{ab}	3.98	8.11^{abc}	24.05^{c}	18.38^{bc}	15.22^{a}	1.51^{c}	0.52	2.54	3.48^{a}	1.01	28.90^{bc}	28.04^{d}	18.36^{bc}	23.04^{a}	0.81^{c}
WS	12 or 14	15	.26	21.7^{a}	3.87	9.80^{a}	30.22^{b}	17.23^{c}	4.43^{c}	3.37^{b}	0.00	2.13	2.45^{ab}	0.00	33.02^{a}	34.18^{bc}	17.25^{c}	11.03^{c}	1.58^{bc}
WS 1-21 D M 22-40 D	12 or 14	15	.26	21.0^{ab}	3.63	9.41^{ab}	25.59^{c}	18.46^{bc}	8.32^{b}	2.89^{c}	0.63	2.39	1.72^{ab}	2.78	31.83^{ab}	29.30^{d}	18.45^{bc}	16.68^{b}	1.11^{bc}
FFSB M	0	0	.40	21.5^{a}	4.84	7.90^{bc}	30.33^{b}	22.96^{a}	1.95^{c}	5.60^{a}	0.00	0.00	0.48^{b}	0.00	30.78^{abc}	35.23^{b}	24.24^{a}	2.44^{e}	10.27^{a}
CS, PM	0	0	.25	16.6^{c}	2.82	6.93^{c}	40.89^{a}	19.41^{b}	3.43^{c}	4.33^{ab}	0.00	1.73	1.35^{ab}	1.51	24.48^{d}	43.85^{a}	19.42^{b}	7.82^{cd}	2.77^{b}
		SEM	.075	.52	.51	.36	.91	.42	.83	.30	.16	.62	.58		.78	1.00			1.04
			probability																
	Processing		.115	.000	.010	.001	.000	.000	.000	000	.024	.342	.394	.232	.000	.000	.000	.000	000
	Flaxseed level		.182	.003	.063	.002	.000	.000	.000	000	.003	.056	.015	.414	.001	.000	.000	.000	.000
	Interaction		.958	.010	.330	.605	.000	.000	.008	.852	.523	.231	.557	.893	.112	.000	.000	.992	.000

FFSB: extruded full-fat soybean;
FS: flaxseed;
CS: canola seed;
M: mashed
PM: pelletted then mashed;
WS: whole seed

Σ SAT: total of saturated fatty acids;
Σ Mono: total of monounsaturated fatty acids;
Σ ω-3: total of ω-3 fatty acids;
Σ ω-6: total of ω-6 fatty acids.

Means within columns with no common superscripts differ significantly (Scheffe's test, $P<.05$).

Table 6.8. Fatty acid composition of thigh muscle lipids (% weight) of 40 days old male broilers fed different level of flaxseed with pellet processing.

	Flaxseed %		Fatty acid (% weight)															
Processing	1-21 Day	22-40 Day	14:0	16:0	16:1	18:0	18:1	18:2 ω-6	18:3 ω-3	Homo 20:3 ω-6	20:5 ω-3	22:5 ω-3	22:6 ω-3	Σ SAT	Σ Mono	Σ ω-6	Σ ω-3	ω-6 /ω-3
FS PM	12	15	0.30	16.54^{bc}	3.60^{bc}	6.78^{bc}	26.36^{c}	20.22^{bc}	20.28^{a}	1.28^{c}	1.68^{a}	2.14^{a}	0.22	23.98^{c}	30.20^{d}	21.48^{bc}	24.36^{a}	0.88^{d}
FS PM	14	17	0.24	15.90^{c}	3.36^{c}	7.26^{abc}	25.14^{d}	19.90^{bc}	22.06^{a}	1.18^{c}	1.72^{a}	2.30^{a}	0.28	23.74^{c}	28.68^{d}	21.08^{c}	26.46^{a}	0.80^{d}
WS	12 or 14	15	0.27	20.36^{a}	5.22^{ab}	9.02^{a}	32.35^{b}	18.70^{c}	6.97^{c}	2.85^{ab}	1.25^{b}	1.77^{ab}	0.32	30.12^{a}	37.93^{bc}	21.57^{bc}	10.30^{c}	2.17^{c}
WS 1-21 D M 22-40 D	12 or 14	15	0.38	18.21^{b}	4.07^{bc}	8.52^{ab}	28.15^{cd}	20.12^{bc}	13.20^{b}	2.08^{bc}	1.50^{ab}	2.07^{a}	0.86	27.50^{b}	32.50^{cd}	22.18^{bc}	17.65^{b}	1.26^{d}
FFSB M	0	0	0.42	21.24^{a}	6.14^{a}	7.38^{abc}	32.08^{bc}	25.14^{a}	2.72^{d}	4.14^{a}	0.00^{c}	0.00^{c}	.000	29.50^{a}	38.40^{b}	29.26^{a}	2.72^{d}	10.86^{a}
CS, PM	0	0	0.27	15.36^{c}	3.13^{c}	6.38^{c}	43.83^{a}	21.60^{b}	4.37^{cd}	3.28^{ab}	0.00^{c}	0.97^{bc}	0.00	22.37^{c}	47.35^{a}	24.88^{b}	5.37^{d}	4.65^{b}
		SEM	.05	.36	.31	.40	.91	.52	.60	.30	.83	.21	0.20	.52	1.21			.67
			Probability															
	Processing		.046	.000	.000	.000	.000	.001	.000	.001	.004	.026	.153	.000	.000	.004	.000	.000
	Flaxseed level		.803	.042	.068	.062	.000	.000	.000	.000	.000	.000	.022	.628	.000	.000	.000	.000
	Interaction		.505	.000	.001	.343	.000	.001	.000	.927	.268	.036	.101	.001	.000	.013	.004	.000

FFSB: extruded full-fat soybean;
FS: flaxseed;
CS: canola seed;
M: mashed
PM: pelletted then mashed;
WS: whole seed

Σ SAT: total of saturated fatty acids;
Σ Mono: total of monounsaturated fatty acids;
Σ ω-3: total of ω-3 fatty acids;
Σ ω-6: total of ω-6 fatty acids.

Means within columns with no common superscripts differ significantly (Scheffe's test, $P<.05$).

produce eggs with increased linolenic acid, at the expense of saturated and monounsaturated FAs.

The response of tissue FA profiles to dietary FS follows a proportional pattern that reflects dosage and period of dietary FS treatment, as well as the processing of FS. Roth-Maier et al. (1998) reported that by using 5 or 7.5% FS in broiler diets, the tissue polyunsaturated FA content was proportional to the dietary FS level, whereas the whole FS only resulted in a moderate effect in this regard. The insensitive response to whole seed could be related to its low FA availability.

Greater FA deposition response was noticed from the present experiment, as compared to those reported by other researchers. Ajuyah et al. (1991) demonstrated a dose response of tissue FA profile by FS inclusion in broiler diet. They studied the effect of 10 or 20% ground FS on both white and dark meat FA content, and found that after 6 weeks feeding, the total ω-3 FAs in white (breast) meat was increased from 6.4% in control chicks to 14.1 and 19.2% when FS was fed at 10 or 20%, respectively. In the present study, total ω-3 FAs reached 17.7% for birds fed 15% ground FS in the finishing period, (the FS in the diets of the starter-grower period was 12 or 14%). When the diet was processed as pellet-then-reground, the ω-3 FAs increased to 24.4% for chicks fed the same level of FS. The highest value was 26.5% attained with birds that were fed 14% FS in the starter-grower period and 17% in the finisher period. This value was higher than that in cultured trout meat (20%), but lower than that (30%) in wild trout meat (van Vliet and Katan 1990). It is also higher than what was found in the oil of Herring, Menhaden and salmon, which have total ω-3 FAs content of 12.0, 21.7, and 20.9% (USDA 1986), respectively.

In the case of thigh meat tissue, Ajuyah et al. (1991) reported values of 5.4% total ω-3 FAs for control birds and 15.6 and 18.2% for birds fed 10 or 20% full-fat FS, respectively. In the present study, total ω-3 FAs were 2.4% for birds fed FFSB control diet, and 16.7% for 15% ground FS in the finisher period (12 or 14% in the starter-grower period). When FS was processed by pellet-then-reground, total ω-3 FAs were 22.1% for birds fed a 15% FS diet (12% FS in the starter-grower period), and 23.0% from 17% pellet-then-reground FS diet (14% FS in the starter-grower period). Again, the increase of ω-3 FAs in thigh meat tissue caused by the inclusion of similar FS level was greater in the present experiment than reported values, when FS was processed by pellet-then-reground method.

The difference in ω-3 FAs deposition in meat between the present experiment and those reported by other researchers indirectly reflects the FS processing effect. This difference could be attributed to variations in FA digestibility caused by processing.

One of the goals of feeding FS to broilers is to incorporate ω-3 FAs into meat tissues. ω-3 FAs draw intensive interest beyond the understanding of their essentiality for cell membrane development and maintenance. Leaf and Weber (1988) stated that T3-FAs, including α-linolenic acid, eicosapentaenoic acid and docosahexaenoic acid, reduce the risk of coronary heart disease. There is a competitive nature between ω-3 FAs and ω-6 FAs. Galli and Simopoulos (1988) suggested that eicosanoids, transformed from eicosapentaenoic acid ($C_{20:5\omega3}$) and arachidonic acid ($C_{20:4\omega6}$), are involved in the regulation of blood platelets, blood vessels and leukocytes in preventing cardiovascular disease. On the contrary, ω-6 FAs, especially arachidonic acid, have been linked to the production of metabolites that causes vasoconstriction and platelet aggregation, which are involved in the formation of arterial thrombosis (Holub 1995).

A higher ω-6 to ω-3 FAs ratio leads to a high level of arachidonic acid production, inhibits the synthesis of the ω-3 FAs family and restricts the conversion of $C_{18:3\omega3}$ to longer carbon chain ω-3 FAs. For the prevention of cardiovascular disease, it is generally recommended we consume diets that have an ω-6 to ω-3 ratio of no more than 4 to1, while diets for North American adults range from 10 even 50 to1. It is obvious that animal products that have lower ω-6 to ω-3 FAs could have large health benefits (Galli and Simopoulos 1988). This could be achieved by feeding ingredients with high ω-3 FAs, as we observed from the present experiment, and those reported by others who also included FS in the diets.

Further more, as demonstrated by the present experiment, this ω-6 to ω-3 ratio can be further reduced by FS processing as pellet-then-reground, using the same quantity in the ration. The ratio of 1.11 by feeding 12 to 15% whole FS in the starter-grower period and 15% ground FS in the finisher period was further lowered to were 0.86 or 0.81 when similar FS containing diets were processed as pellet-then-reground. These ratios are lower than the ratio reported by Ajuyah et al. (1991) of 1.9 or 1.5 in breast meat tissue, when birds were fed 10 or 20% FS, respectively.

From the differences in FAs concentration in tissue of birds fed FS containing diets, we could suggest that maximization of ω-3 deposition could be possible, if the FAs utilization is improved and FS inclusion rate increased. This could be achieved only through proper FS processing. The present study indicated that pelleting is effective in this regard. While diet grinding after pelleting was intended to make all feed form equal, it may not be necessary in practical application.

Conclusion

Broilers could have satisfactory growth when fed diets containing FS for up to 12%, provided the FS is properly processed. Twelve or 14% whole FS significantly depressed growth for birds under 3 weeks of age. Birds from 4 to 6 week of age could better tolerate 15% whole seed in the diet, when grit was available ad libitum. Compared to the same amount of FS, canola seed lead to better performance for broilers in both the starter-grower and finisher periods.

Carcass cut-ups, expressed by percentage, was not significantly affected by FS inclusion in the diets. But fat-pads and lipid content in breast and thigh meat were slightly reduced by FS in the diet. There was a significant effect of FS level on liver percentage of carcass, suggesting the compensatory enlargement of liver for ANFs detoxification.

By the inclusion of FS in the diets at the levels studied, both linolenate and total ω-3 FAs were significantly increased in breast and thigh meat, while ω-6/ω-3 ratios were significantly reduced. These changes were greatly affected by the processing of FS. As compared to whole seed diet in regard to desired FAs deposition in meat, pellet-then-reground FS processing lead to the best results. To satisfy the expectation of high ω-3 FAs deposition in meat tissue while achieving acceptable performance, broilers could use pellet-processed FS in their diets for up to 12%.

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Section VII
General Conclusion

Under the present experimental conditions, the feed enzymes tested (Natugrain, Natugrain Blend, Avizyme^{TM} 1500, Allzyme A and Allzyme B), and the enzymes xylanase, arabinofuranosidase, or lipase, failed to improve the performance of broilers fed rations containing 10% flaxseed between day-old to 3 weeks of age.

Flaxseed processing as autoclaving under the protocol of 16.5 kg/cm^2 at 120°C for 15 minutes slightly improved the performance of young broilers fed either whole or ground flaxseed. This effect was magnified when flaxseed level in the diet was higher than 10%. Autoclaving at lower temperature and shorter period showed no performance improvement.

The best effect of flaxseed processing on broiler performance was achieved with pelleting. A three-week feeding trial revealed that broilers consumed more feed and gained more weight when the flaxseed-containing diet was pelleted together with other ingredients.

Whole flaxseed in the diet did not cause significant reduction in performance over ground seed at the level of 10%, when grit was continually available. However, higher than 10% in young broiler diet caused depressed growth and lowered apparent nutrient digestibilities. This depressing effect reached statistical significance for lipids digestibility and AMEn values ($P<0.05$). Older broilers (22 to 40 days) seemed to be able to tolerate whole seed diets better, judged from feed consumption and feed conversion efficiency.

The deposition of ω-3 fatty acids in breast or thigh meat was significantly influenced by flaxseed inclusion ($P<0.001$) and processing ($P<0.001$). Whole flaxseed (12 to 14% for the starter-grower and 15% in the finishing period) significantly ($P<0.05$) increased the deposition of ω-3 fatty acids in breast and thigh meat of the birds after 40 days feeding, as compared to non-flaxseed diets. However, this deposition of ω-3 fatty acids caused by whole seed diets was significantly ($P<0.05$) lower than those diets containing the same level of flaxseed but processed as pellet-then-mash.

The HCN content in flaxseed (377mg/kg) was significantly ($P<0.05$) reduced by all the processings tested in the present study (autoclaving, pelleting, oven heating and microwave roasting), with the best effect achieved by pelleting together with other ingredients (reduced by 73.8%) and microwave roasting (reduced by 83.2%).

The apparent digestibilities of lipids, total and main individual fatty acids, and the ME values were also effectively increased by these flaxseed processing methods as tested with Leghorn roosters. The AMEn and TMEn values of 2991 and 3225 kcal/kg of raw flaxseed A (contains 37.6% EE) was increased to 4197 and 4446 kcal/kg by repeated (3 and 6 times)

pelleting, respectively. Microwave roasting increased the AMEn and TMEn values of 2629 and 2893 kcal/kg of raw flaxseed B (contains 34.2% EE) to 3493 and 3726 kcal/kg, respectively. The higher ME values of processed flaxseed were the result of increased digestibilities of EE ($P<0.05$).

Proper flaxseed processing such as pelleting reduced HCN content, increased nutrient utilization and TME and TMEn values. It allowed higher flaxseed inclusion rate for up to 12% for broiler without obvious growth depression, while achieving higher desired ω-3 fatty acids deposition.

Section VIII

Summary

SUMMARY

Flaxseed (FS) can be used in broiler diets for up to 12% with satisfactory growth, if it has been properly pellet-processed. Pelleting of FS reduced its hydrogen cyanide (HCN) content by 13.3% to 63.8%, depending on the pelleting condition. Another benefit of FS pelleting was the nutrient digestibility improvement. The TMEn of raw FS (37.6% ether extract), 3225 kcal/kg, was increased to 3918, 4446, 3858, and 4465 kcal/kg by three times repeated-pelleting, six times repeated-pelleting, autoclaving, and six times repeated-pelleting together with 50% corn, respectively. Microwave roasting of FS also significantly ($P<0.01$) reduced its HCN content by 83.2% and increased the TMEn value of another batch of FS (34.2% ether extract) from 2893 to 3726 kcal/kg.

After pelleting, a diet that contains 12% FS for day 1 to 21 and 15% for day 22 to 40 lead to a total T-3 fatty acids deposition in breast and thigh meat of 22.08 and 24.36%, respectively, while attaining satisfactory growth performance. After three weeks of feeding, broilers attained the body weight of 839 g and the feed consumption of 1022 g with no significant ($P>0.05$) differences as compared to birds fed similar amounts of canola seed. However, the feed to gain ratio was significantly higher than that of the canola seed diet. In the period from day 22 to 40, 15% or higher FS in the diet significantly ($P<0.05$) reduced body weight, and increased feed to gain ratio.

FS autoclaving at 16.5 kg/cm^2 and 120°C for 15 min slightly improved the performance of young birds, as compared to those fed same amounts of ground or whole FS. FS autoclaving lead to more obvious performance improvement when diets contained more than 10% FS, but reversed when autoclaved at lower pressure and temperature for a shorter period of time.

When pelleted FS-containing diets were not mashed, young broilers fed on these pelleted diets attained better performance than the birds fed on diets containing the same amount of FS which was processed by grinding, or autoclaving, or just whole seed. Better performance results were also obtained as compared to birds fed a pelleted commercial diet. The better performance of the pelleted FS-fed birds was supported with the higher apparent ether extract digestibility and higher AMEn values, as compared to other FS-containing diets.

Enzyme preparations, including feed enzymes of Natugrain, Natugrain Blend, Avizyme 1500, Allzyme A and Allzyme B, or liquid enzyme of xylanase and lipase, failed to improved the performance of broiler fed 10% FS in the period of day one to 21.