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NUTRITIONAL EVALUATION OF DIFFERENT VARIETIES OF FULL-FAT SOYBEANS IN BROILER CHICKEN STARTER DIETS.

A Thesis

Submitted to the Graduate Faculty

in Partial Fulfillment of the Requirements

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in the Department of Health Management

Faculty of Veterinary Medicine

University of Prince Edward Island

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ABSTRACT

The nutritional quality of new varieties of high protein (HP) (OT 89-16) and low trypsin inhibitor (LTI) (X 2033) full-fat soybean (SB) developed at Agriculture Canada, Ottawa, was evaluated in broiler chicken starter diets. Full-fat SB, because of the high protein and oil contents, can be used as a source of supplementary protein and energy in poultry rations. Use of HP SB has the potential to substitute on an equivalent protein basis in poultry rations and reduce the cost of protein supplementation. Use of LTI SB may eliminate the cost of heat treatment before feeding to poultry. Soybeans used in this study were: conventional raw SB [(cultivar *Maple Isle*, 39% CP DM, 70 Trypsin Inhibitor Units (TIU)/g DM)], LTI SB (42 TIU/g DM), autoclaved HP SB (44%CP DM), autoclaved conventional SB and commercially roasted conventional SB. Commercially produced SBM was included as a control protein source. The amino acid levels (DM basis) of the HP SB were higher than the other SB varieties. Thus, higher crude protein levels observed in HP beans were a result of increased true protein levels. The LTI SB had proximate composition similar to the conventional SB but comparative electrophoresis analysis clearly showed the absence of Kunitz Trypsin Inhibitor.

Nutritional quality of these protein sources was evaluated by incorporating them in broiler chicken starter diets and studying the growth performances of chickens. The corn-wheat based diets were formulated to be isonitrogenous and isoenergetic. Supplementation of these diets with 0 or 0.3 % DL methionine was also studied, as the anti-proteolytic activity of TI increases the requirement for sulphur amino acids. Nutrient availability from these diets was also estimated in chickens and rats. True metabolizable energy (TME) of the beans and the diets was determined with precision-fed cockerel assay using adult roosters. The mean body weight gains, feed efficiencies, body composition and digestibility coefficients in terms of dry matter, crude protein and metabolizable energy of the chickens fed heat treated beans were superior ($P<0.05$) to those fed unprocessed beans. Performance of birds fed HP SB and roasted SB diets was similar. Feed conversion, digestibility coefficients and TME content of these diets were similar to those of the control SBM diet. The digestibility coefficients of raw and LTI SB diets and the feed conversion and performance of chicks fed these diets were similar. However, mean pancreas weight of chickens fed LTI SB was lower ($P<0.01$) than those fed raw SB which could indicate that intestinal proteolysis was impaired less by the lower trypsin inhibitor activity of the LTI beans. Improved utilization of LTI SB diet over the conventional raw SB diet was more evident in the rat digestibility trial. The chickens given methionine supplemented LTI diet made greater weight gains ($P<0.05$) than those on non-supplemented diet but not to the level of the birds fed heat treated beans. It was concluded that TI levels in the LTI beans probably were not low enough to eliminate the heat treatment entirely but perhaps would require lower temperatures and shorter cooking times than for the SB currently grown. HP SB were well utilized by the birds and will result in the reduction of the amount of SB required by subsituting the ordinary SB on an equivalent protein basis.

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1. INTRODUCTION

Soybeans (SB) have been a component of diets, primarily in a fermented form, in the far East since about 1000 B.C. In contrast, this legume has been grown in the West only since the late 19th century. However, during the past five decades it has rapidly gained popularity as a source of oil and protein in human and animal diets. Breeding of improved cultivars have made SB a profitable crop.

Whole SB contain 15-21 % oil. The oil is removed by solvent extraction and consumed in the form of salad oils, cooking oils, shortening, and margarine. It is also used industrially in the production of paints, varnishes, plastics, inks, linoleum and other products. During the process of oil extraction, the meal is "toasted"-- a process which improves the biological value of its protein. The protein content of this meal is standardized to about 44 or 48 % by dilution with SB hulls. This SB meal (SBM) is highly favoured as a protein supplement for animal and poultry feeds because it is palatable, highly digestible and results in excellent growth performance when used as a protein supplement for many animal species (1). It has an excellent balance of amino acids and is commonly used as the standard to which other protein sources are compared (2). In overall nutritional value, SBM has currently become the most important source of plant protein in the diets of monogastric animals (3). It is a standard protein supplement used in many poultry and swine rations.

Considerable interest has developed in recent years in the use of full-fat SB in animal diets to increase the dietary energy content and eliminate the cost of oil extraction (4, 5). These SB are incorporated into rations after appropriate heat treatment and grinding as a supplementary source of protein and energy.

1.1 Full-Fat Soybeans

Full-fat SB contain approximately 37% crude protein and 18 % fat, as compared to 44 or 48 % protein and less than 1 % fat in commercially produced SBM. Unfortunately, a number of thermostable and heat labile substances, capable of eliciting diverse nutritional, biological and physiological responses occur in SB which limits their use in animal diets. The presence of these anti-nutritional factors results in deleterious effects on rats, chicks and other non-ruminant animals (6, 7).

Fortunately, these anti-nutritional factors can be inactivated by heat treatment. The process referred to as "toasting" in the oilseed processing industry, which use live steam, greatly improves the nutritive value of raw soy protein products (7). Other processing techniques such as extrusion, roasting, infrared cooking (micronization), dielectric heating and microwave processing can be equally effective (8).

It is now well established that most raw or unheated vegetable protein foods are of low nutritive value for use as food or feed (8). Thus, heat treatment of SB not only inactivates the anti-nutritional factors but converts raw protein into more digestible forms (6, 9). Overheating, on the other hand, usually results in protein

deterioration which has a negative impact on growth of animals (7, 8). Therefore, precise control of the heating process is critical to the preparation of protein products of maximum nutritive value.

1.2 Animal Feeding Trials

Ham and Standstedt (1944) provided the first evidence that raw SB contained a trypsin inhibiting substance that reduced the growth rate of chicks (2). Trypsin inhibitors (TI) in SB are the major factors responsible for the poor nutritional value of unheated meal (10). Depression of growth and enlargement of the pancreas are two metabolic responses of nutritional importance associated with TI (11). Growth inhibition has been observed in rats, chickens, mice, guinea pigs, goslings, growing swine and calves (12). Animals become less sensitive to the anti-nutritional effects of dietary raw SB with maturity and adults can maintain equally well on diets containing raw or heat treated SB (6, 11, 12). The growth inhibition observed in young animals given raw SB is primarily due to interference with protein metabolism. In contrast, weight gain in mature animals is predominantly associated with lipid deposition. This age-related change in body metabolism may, in part, explain why mature animals are not significantly affected by raw SB in the diet (12).

Pancreatic enlargement due to feeding purified TI or raw SBM has been observed in rats, chickens, mice and the growing guinea pig (7, 12, 13). The presence of TI in the diet leads to hypersecretion of pancreatic enzymes by interfering with the cholecystokinin-mediated feedback control of exocrine pancreatic secretion resulting

in pancreatic hypertrophy (13, 14). The growth inhibition noted under these conditions is attributable to decreased efficiency of digestion of dietary proteins and excessive fecal loss of pancreatic enzymes. The pancreatic enzymes have a high proportion of sulphur-containing amino acids and the loss of these amino acids is further exacerbated by their low levels in SB proteins (14). Supplementation of raw SB meal with certain essential amino acids, especially methionine, improves the growth rate associated with raw SBM (15, 16). Supplementation of raw SB diets with DL-methionine has been shown to improve the growth performance of chickens (17). This improvement is partly due to the fact that methionine is limiting in SB and that the anti-proteolytic activity of the TI in the raw SBM increases the requirements for sulphur amino acids (18, 19, 20).

1.3 Trypsin Inhibitor in Soybeans

Several types of TI are present in SB (21, 22, 23). These TI can be broadly classified into two main categories: Kunitz or Bowman Birk Trypsin Inhibitors (KTI and BBTI, respectively). The KTI have a molecular weight of 20,000 - 25,000 , few disulphide bonds, are heat labile and are specific to trypsin inhibition. On the other hand, the BBTI have a molecular weight of 6000-10,000, a high proportion of disulphide bonds, are heat stable and inhibit both trypsin and chymotrypsin (24). Although the TI make up only about 6 % of the total protein in SB, it has been estimated that they may be responsible for 30 - 50 % of the growth inhibiting effect and much of the pancreatic hypertrophy which occurs when monogastric animals

ingest unheated SB (22, 25, 26). Much of the SB TI activity is thought to be due to the soybean trypsin inhibitor A_2 (SBTI-A_2) which was first crystallized by Kunitz (1945) and is commonly called the Kunitz Trypsin Inhibitor (KTI) (27).

Four forms of KTI have been identified in the U.S. SB germplasm collection (27). Three of the forms designated Ti^a, Ti^b and Ti^c are electrophoretically distinguished from one another by the Rf values of 0.79, 0.75 and 0.83 respectively. (Rf is the mobility relative to a bromophenol blue dye front in a polyacrylamide gel anodic system.) The fourth form found in SB germplasm collection, P.I.157440 and P.I.196168, is the absence of KTI and is designated as "ti" (28, 29).

A standard trypsin assay of crude protein (CP) extracts from soybeans lacking the KTI showed 30-50 % less TI activity per gram of protein than CP extracts from seeds of the cultivar Amsoy 71 which contains KTI (Ti^a) (27). Although other TI may be present, the KTI is lacking in the seeds which do not have the SBTI-A2 protein (27).

1.4 New Varieties of Soybeans

A soybean variant isolated by Singh et al.(30) had markedly less specific KTI activity than the commercially grown cultivars such as Williams. Preliminary studies with rats and young pigs showed that this variant was nutritionally superior to Williams and other regularly produced cultivars (31). In 1986, Hymowitz reported development of a low TI SB cultivar produced by incorporating the germplasm of the variant cultivar into William 82, a commercially available SB cultivar. This resulted

in a cultivar that lacked the KTI allele and contained one half of the TI content of standard SB varieties, but with yield characteristics which were similar to commercial varieties. Feeding trials with chicks and pigs have revealed that the growth performance of animals fed unheated LTI beans was intermediate between those fed raw conventional SB and those fed a diet with SBM as the supplemental protein source (2, 32). The availability of amino acids in the LTI SB are significantly greater than the availability of the amino acid in unheated conventional SB but still considerably less available than they are in SBM (2).

Research has also been directed towards breeding SB of higher protein levels. Protein is one of the critical nutrients for young, rapidly growing animals and high-producing adults. Protein supplements used in animal diets are usually more expensive than energy feeds. The protein content of commercially grown SB is about 37%. Development of new lines of SB with higher protein content will be beneficial. These beans, when subsituted on an equivalent protein basis in animal rations, will reduce the amount of SB needed and, thus, decrease the feed costs.

1.6 Objectives

Due to the lack of an oil extraction plant in Atlantic Canada, SBM must be shipped in from Ontario or imported from the U.S.A. which results in increased feed costs. Thus, use of full-fat soybeans in animal diets is gaining popularity in this region. An increase in soybean production in the Atlantic Provinces can potentially make the region self-sufficient in production of protein supplements (33). As part

of the SB breeding programme at Agriculture Canada (Ottawa) high protein (HP) and low trypsin inhibitor (LTI) lines of SB have been bred, the use of which has potential in animal diets in Atlantic Canada.

The present study was undertaken to evaluate the nutritional quality of these new lines of HP (OT 89-16) and LTI SB (X 2033) as protein supplements in poultry diets. The specific objectives of the study were :

(i) To determine the chemical composition of the new HP and LTI lines of SB in terms of protein, fat, amino acid composition and trypsin inhibitor activity;

(ii) To evaluate the nutritional value of full-fat SB in broiler chicken diets;

(iii) To estimate the bioavailability of nutrients from these SB;

(iv) To determine if the HP SB has the potential to be used in starter diets for broiler chickens.

(v) To evaluate the nutritional value of LTI SB, the use of which may eliminate the cost of heat treatment before feeding to poultry; and

(vi) To determine the effects of supplementation with DL-methionine on the nutritive value of the conventional raw and the new line of LTI SB.

2. CHARACTERISATION OF THE SOYBEANS

2.1 Introduction

Over the past 50 years, SB have gained popularity as a source of oil and protein in human and animal diets. Raw SB, in spite of the high protein and energy content, contain a number of anti-nutritional components which cause several biological and physiological responses in many species of animals (7). It has been established that the nutritional value of SB can be improved by heat treatment (34). The importance of heat treatment in improving the nutritional value of raw SB for non-ruminants was first suggested by Osborne and Mendel in 1917 (35). Maximum protein efficiency of SB has been achieved by treating the raw SB with live steam for about 30 minutes or by autoclaving at 15 lb pressure for 15-20 minutes (36). However, excessive heat treatment markedly impairs the nutritive value of soybeans (7, 34, 36).

Improved cultivars of SB have been developed which contain higher protein levels or lower levels of trypsin inhibitors. High protein SB has the potential to substitute on an equivalent protein basis in animal rations and reduce the cost of protein supplementation. Cultivars with lower levels of TI could eliminate the cost of heat treatment before feeding to poultry. High protein (HP) variety and low trypsin inhibitor (LTI) lines of SB have been bred at Agriculture Canada (Ottawa).

The main objective of this study was to compare the chemical composition of the new lines of HP (OT 89-16) and LTI (X 2033) beans in terms of protein, fat, amino acid composition, in vitro protein solubility and trypsin inhibitor activity to that of conventional SB. Electrophoretic analysis was also conducted to compare the protein profiles of the beans; and to detect the absence or presence of the Kunitz Trypsin Inhibitor in the beans.

2.2 Materials and Methods

2.2.1 Soybeans Selected for the Study

The soybeans selected for the study were : conventional SB (cultivar *Maple Isle*) and the new lines of HP and LTI SB. Commercially produced, solvent extracted SBM was used as the control protein source.

The quantity of HP SB was insufficient for processing in a commercial roaster; therefore, the beans were processed by autoclaving at 89.6 KPa for 15 minutes. Conventional raw SB were also processed the same way to be used as a control. Commercially roasted conventional SB were used as a control for the autoclaved beans in order to determine whether the heat treatment received during autoclaving was sufficient to destroy the anti-nutritional factors or excessive to damage the proteins and decrease the nutritive value of the beans. Roasting was done in a commercial propane flame roaster (GEM Roasting Co., model #7700) having an exit

temperature of 107°C. The LTI SB were not heat treated. Conventional raw SB were used as a control for the LTI beans.

The six protein sources included in the present study were:-

(i) SBM

(ii) autoclaved conventional SB (*Maple Isle*)

(*iii*) autoclaved HP SB

(iv) commercially roasted SB (*Maple Isle*)

(v) LTI SB

(vi) raw SB (*Maple Isle*)

2.2.2 Chemical Analysis

Proximate Analysis

The beans were analysed for dry matter, crude protein and ether extract according to the methods of AOAC (37). An isoperibolic calorimeter (Model #1261, Parr Instruments, Illinois) was used to determine the gross energy content of the beans.

Amino Acid Analysis

Duplicate samples (0.1g) were hydrolysed in Pyrex tubes (18 x 150 mm) under reduced pressure (< 10 μm of Hg) with 5 ml of triple glass-distilled constant-boiling HCl (6.0 M) at 110°C for 24 hours. The small amount of insoluble material formed during acid hydrolysis and the fat plug were removed by filtration (0.22 μm

millipore microfilter). The amino acid analysis of individual filtrates dissolved in 0.2 M sodium citrate solution (pH 2.2) was performed in duplicates by the method of Moore and Stein (38) using a LKB Alpha Plus Amino Acid Analyser equipped with a Hewlett - Packard Model 3396A Integrator. Similarly, methionine and cystine were determined in separate samples after oxidation by the performic acid procedure as described by Moore (39).

Protein Solubility in 0.2% Potassium Hydroxide (KOH)

In vitro protein solubility was determined according to the method of Araba and Dale (40). Solubility of proteins in 0.2% KOH has been used as an indicator of overprocessed soybean meal (40).

Trypsin Inhibitor Content of Soybeans

The anti-tryptic activity of SB was determined by the enzymatic method of Liu and Markakis (41) and expressed as Trypsin Inhibitor Units (TIU)/g sample (dry matter basis) and TIU/g crude protein of the sample. Benzoyl-DL-arginine-*p*-nitroanilide hydrochloride (BAPA; Sigma # B-4875) was used as the protease substrate.

Electrophoresis

Ground soybean (0.5 g) was homogenized in 3 ml buffer (0.06M Tris, 0.015 M calcium chloride, 13% sucrose, pH 8.2) at 4°C with a Brinkman homogenizer

(Model PT10/35) at maximum setting according to the method of Hildebrand and Hymowitz (42). The extract was centrifuged at 3000 rpm (1470 R.C.F.) at 4°C for 15 minutes. The supernatant was analysed for protein according to the method of Lowry et al. (43). Sample was then diluted with distilled water to give protein concentrations of 5 μg/μl. Diluted sample (0.05 ml) was added to an equal volume of sample buffer [62.5 mM Tris (pH 6.8), 2 % sodium dodecyl sulphate, 0.005 % bromophenol blue, 10 % glycerol (v/v) and 5 % 2-mercaptoethanol (v/v)], and 4 ml distilled water) and boiled for seven minutes. Eight microliters (20 μg protein) of this solution was applied to each lane of the gels.

Sodium dodecyl sulphate polyacrylamide gel electrophoresis (SDS-PAGE) using 12.5% polyacrylamide gels was performed on the extracts of the protein sources according to the method of Laemmli (44) and stained with Coomassie Blue solution (0.1% Coomassie Blue, 25% ethanol and 5% acetic acid). Low Molecular Weight (LMW) standard proteins, 14.4 - 94.0 kD, (Pharmacia) were used as reference proteins.

Negative Staining for Determination of TI in Electrophoresis Gels

Negative staining for KTI determination in the gels was done according to the method of Uriel and Berges (45). Conventional PAGE, without SDS, was done using 12.5% polyacrylamide gels to detect the presence of trypsin inhibitors. Gels were immersed in 200 mg/ml trypsin in 100 mM sodium buffer, pH 7.5 for 20 minutes at 37°C, rinsed twice with distilled H_2O and immersed in freshly made protease

substrate for 10 minutes. Protease substrate contained 21 mg N-acetyl-dl-phenylalanine β-naphthyl ester (APNE) in 8.75 ml N,N-dimethylformamide freshly mixed with 42 mg *o*-dianosidine in 77 ml of 100 mM sodium phosphate buffer. Trypsin inhibitor zones appeared clear against a pink background. Kunitz TI (Sigma #T-9003) was used as a standard.

2.3 Results and Discussion

2.3.1 Proximate Analysis

The results of the proximate analysis are shown in Table I. The HP SB had 17% higher protein content compared to the conventional autoclaved beans. Due to the increase in the protein content, the fat content of the HP SB was about 11% less than the similarly treated conventional SB. The heat-treated beans had higher fat values than the unheated conventional and LTI SB indicating that the heat treatment may have increased the extractability of fat from the beans. The LTI SB showed similar proximate composition as the conventional beans. Han et al. (32) also reported similarity in proximate composition of their LTI line of SB and the commercial variety (Williams 82).

2.3.2 Amino Acid Analysis

The amino acid composition of the SB is shown in Table II expressed as a percent of DM and crude protein. The amino acid profiles of the SB, heated or unheated, were similar. Friedman et al.(46) also reported no change in the amino acid composition of heated and unheated beans but this similarity in the amino acid composition does not give an estimate of their availability to the animal.

The amino acid composition of many different varieties and strains of SB have been compared, particularly with regard to methionine, the limiting amino acid of the SB protein. There has been some indication that the level of methionine in the seed increases proportionately with protein content but few significant differences have been found between the amino acid composition of different varieties and strains of SB (36). The HP SB had higher amino acid levels than the other SB varieties when expressed on a dry matter basis but the amino acid levels were similar expressed on a crude protein basis (Table II). Therefore, higher crude protein levels as observed for these beans (Table I) are a result of increased levels of true protein rather than of any other source of nitrogen.

The LTI SB, although lacking in the KTI protein, still showed similar amino acid levels to those found in the other beans. Friedman et al.(46), also found similar amino acid composition between the commercially grown SB, Williams 82, and an isoline lacking KTI (L81-4590). It was suggested that the amino acids otherwise used in the synthesis of KTI are free or are incorporated into other proteins. Moreover, TI make up only about 6% of the total protein in SB (22, 25, 26), therefore, the

absence of KTI in the LTI SB will have little impact on the amino acid content of these beans.

2.3.3 Protein Solubility

The solubility of proteins in certain buffer solutions is used as an indicator of overprocessing in SB. Growth depression in chicks due to increased heat treatment of SBM was found to be correlated to decreased protein solubility in 0.2 % potassium hydroxide (40). Overheating of protein feeds leads to deterioration of proteins by complex reactions between proteins and carbohydrates, by interactions between protein molecules, and by oxidation. The nutritional consequences of these reactions are reduced solubility and digestibility of the proteins (47).

The protein solubility results of the beans are shown in Table III. The per cent protein solubility of the autoclaved SB was higher than that of the commercially produced SBM and similar to the roasted beans indicating that the autoclaved SB did not receive excessive heat treatment. According to Araba and Dale (40), protein solubility values of less than 70 % indicate impaired nutritive value for the chick and values less than 65% almost certainly indicate overprocessing. Parson et al. (48) reported 59 $\pm$ 1.5 % to be the critical level of protein solubility needed for maintaining optimal feed efficiency in chicks. Therefore, the protein solubility results of the protein supplements in this study did not indicate overprocessing of the beans.

2.3.4 Trypsin Inhibitor Content of the Beans

The levels of TIU, as expected, were higher in the raw than the heat treated beans (Table IV). The raw HP SB had about 28% higher TIU than the conventional raw SB. The higher levels of crude protein and amino acids in the HP SB versus the conventional SB as seen in Tables I and II, respectively, could be the result of higher levels of TI measured in the raw HP SB. However, the LTI beans, had 40 % less TIU than the conventional beans which could be attributed to the lack of the KTI in the LTI beans. Friedman et al. (46) found that trypsin inhibitory activity in their variety of LTI SB was 54% of the commercial variety (Williams 82). Animals fed SB with low levels of trypsin inhibitor activity had better growth performances than those fed conventional SB (17, 32, 49, 50).

2.2.5 Electrophoresis

Figure 1 shows the protein profiles of the SB used for the study. The quantitative differences in TI activity of the SB have been associated with qualitative variation, that is, the presence or absence of TI bands, in the electrophoretic pattern of the SB extracts (51). Most of the electrophoretic profiles seen in Figure 1 are similar. Proteins extracted for the gels from heat-treated beans were less than those extracted from the unheated raw or LTI SB which is attributable to the less solubility of heat-treated SB. A band at ~20 kD is missing in the LTI beans when compared to the protein profile of the raw SB (Figure 2). To be certain whether the KTI was absent in the LTI SB, negative staining of the gels was done (Figure 3). The results

showed the absence of the molecular entity known as KTI in the LTI SB as compared with the KTI standard, thus, explaining the low trypsin inhibitor activity of these beans (Table IV). Unlike the SDS-PAGE gels (Figures 1 and 2) where separation was based on molecular weight, the protein molecules in the negatively stained gels (Figure 3) were separated according to their isoelectric charges. Therefore, other bands of activity seen in the negatively stained gels (Figure 3) were due to the presence of other protease inhibitors, such as the isotypes of Bowman Birk TI (52).

2.4 Conclusions

The results showed that the HP beans have 17% more protein than the autoclaved conventional variety. Analysis of the TI content of raw HP SB showed higher levels than conventional beans indicating that heat treating the HP beans was necessary before being fed to the animals. The amino acid profiles of the SB were similar among the sources examined but they could differ in terms of bioavailability. The protein solubility results of the autoclaved SB were similar to those of roasted SB and did not indicate excessive protein damage during autoclaving. The conventional autoclaved SB showed higher protein solubility and TI activity than the HP SB which were similarly heat treated indicating that the HP SB received more heat treatment than the conventional beans. Overall, TI activity was negligible in the heat treated beans compared to the unheated beans. The LTI beans had 60 % of

the TI activity of the conventional raw beans and the electrophoretic analysis of the LTI SB clearly showed the absence of the KTI band. These results suggest that the use of LTI beans may result in better growth performance of the birds when compared to the conventional beans and may eliminate the cost of heat treatment.

TABLE I. Proximate composition of the beans (DM)

	SBM	Autoclaved SB	Autoclaved HP SB	Roasted SB	Raw SB	LTI SB
Dry matter, %	91.7	96.1	95.6	96.3	95.8	95.4
Crude Protein, % (N x 6.25)	54.2	37.2	43.8	37.7	38.6	37.6
Ether Extract, %	0.73	19.47	17.60	20.30	16.32	16.46
Gross Energy, MJ/kg	19.46	23.33	23.13	23.23	23.18	23.13

TABLE II. Amino acid composition of SB used in the study (mean value of four readings)

	SBM		Autoclaved SB		Autoclaved HP SB		Roasted SB		Raw SB		LTI SB		SEM	
Amino Acid	**% DM**	**% CP**	**% DM**	**% CP**	**% DM**	**% CP**	**% DM**	**% CP**	**% DM**	**% CP**	**% DM**	**% CP**	**% DM**	**% CP**
Aspartic acid	5.36	9.89	4.02	10.81	4.64	10.59	3.95	10.48	3.92	10.16	3.94	10.48	0.24	0.13
Threonine	1.58	2.92	1.27	3.41	1.42	3.24	1.28	3.40	1.17	3.03	1.30	3.46	0.06	0.09
Serine	2.21	4.08	1.60	4.30	1.82	4.16	1.64	4.35	1.48	3.83	1.61	4.28	0.11	0.08
Glutamic acid	8.29	15.30	5.93	15.94	6.80	15.53	6.18	16.39	5.75	14.90	5.99	15.93	0.39	0.22
Proline	2.79	5.15	2.04	5.48	2.28	5.21	2.07	5.49	1.91	4.95	2.07	5.51	0.13	0.09
Glycine	1.90	3.51	1.42	3.82	1.61	3.68	1.43	3.79	1.32	3.42	1.42	3.78	0.09	0.07
Alanine	2.02	3.73	1.47	3.95	1.67	3.81	1.48	3.93	1.36	3.52	1.50	3.99	0.10	0.07
Cystine	0.33	0.61	0.25	0.67	0.31	0.71	0.25	0.66	0.25	0.65	0.26	0.69	0.01	0.01
Valine	2.25	4.15	1.70	4.57	1.88	4.29	1.69	4.48	1.57	4.07	1.66	4.41	0.10	0.08
Methionine	1.07	1.97	0.84	2.26	0.97	2.21	0.97	2.57	0.83	2.15	0.92	2.45	0.04	0.09
Isoleucine	2.06	3.80	1.54	4.14	1.76	4.02	1.55	4.11	1.45	3.76	1.51	4.02	0.09	0.06
Leucine	3.46	6.38	2.60	6.99	2.94	6.71	2.61	6.92	2.40	6.22	2.59	6.89	0.16	0.13
Tyrosine	1.32	2.44	0.99	2.66	1.15	2.63	1.00	2.65	0.88	2.28	1.02	2.71	0.06	0.07
Phenylalanine	2.32	4.28	1.72	4.62	1.97	4.50	1.72	4.56	1.59	4.12	1.70	4.52	0.11	0.08
Histidine	1.01	1.86	0.89	2.39	0.93	2.12	0.90	2.39	0.82	2.12	0.89	2.37	0.03	0.09
Lysine	3.22	5.94	3.83	10.39	3.65	8.33	3.30	8.75	3.01	7.80	4.20	11.17	0.18	0.76
Arginine	3.08	5.68	2.31	6.21	2.72	6.21	2.31	6.13	2.13	5.52	2.30	6.12	0.14	0.12

TABLE III. Protein solubility in 0.2% KOH (mean value for triplicate analyses)

SB	Solubility (% of total CP)	SEM
SBM	64.1	0.6
Autoclaved	71.1	0.5
Autoclaved HP	65.3	0.4
Roasted	71.3	0.7
LTI	72.3	0.5
Raw	70.9	0.1

TABLE IV. Trypsin inhibitor content of the beans (mean of triplicate determinations)

SB	TIU*/g DM ± SEM	TIU/g Protein ± SEM
SBM	0	0
Autoclaved	11.8 ± 1.2	31.7 ± 3.3
Autoclaved HP	4.9 ± 0.8	11.2 ± 1.7
Raw HP	98.3 ± 3.0	203.5 ± 6.8
Roasted	0	0
LTI	41.9 ± 0.6	111.4 ± 1.6
Raw	70.5 ± 1.7	182.6 ± 4.4

*TIU are the micromoles of the substrate (BAPA) hydrolyzed per minute at 37°C.

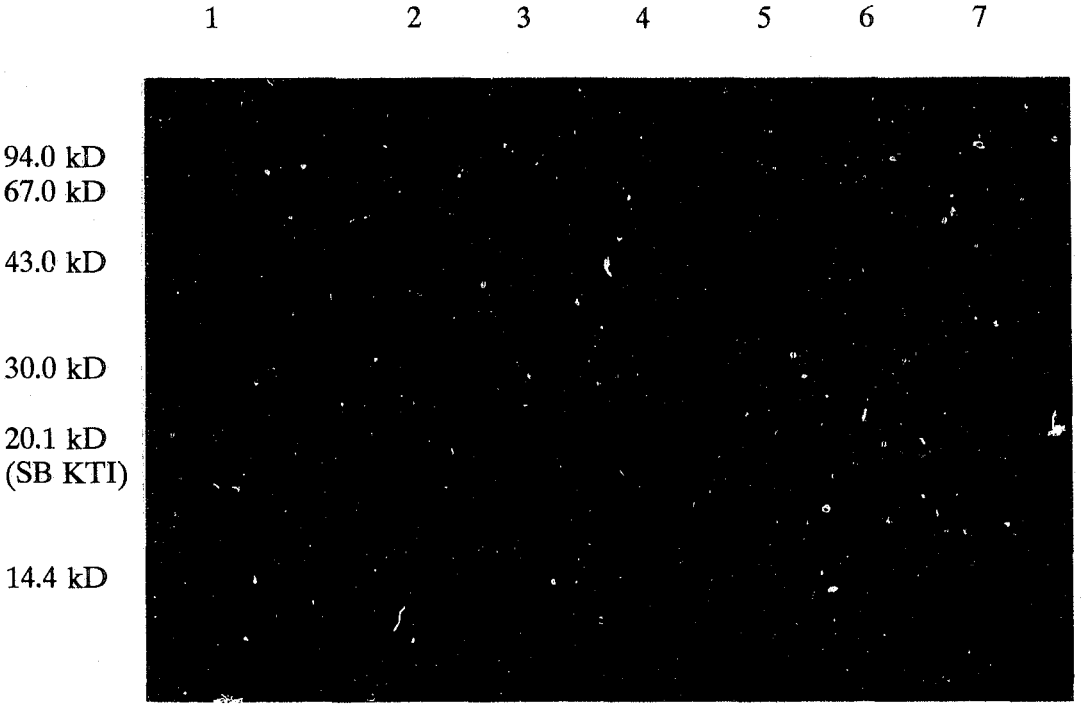

Figure 1. SDS-PAGE pattern of the soybeans (SB) used in the study.

Lane 1: Standard low molecular weight proteins; Lane2: SBM; Lane 3: Raw (*MapleIsle*) SB; Lane 4: Autoclaved (*MapleIsle*) SB; Lane 5: Autoclaved HP SB; Lane 6: LTI SB; Lane 7: Roasted (*MapleIsle*) SB

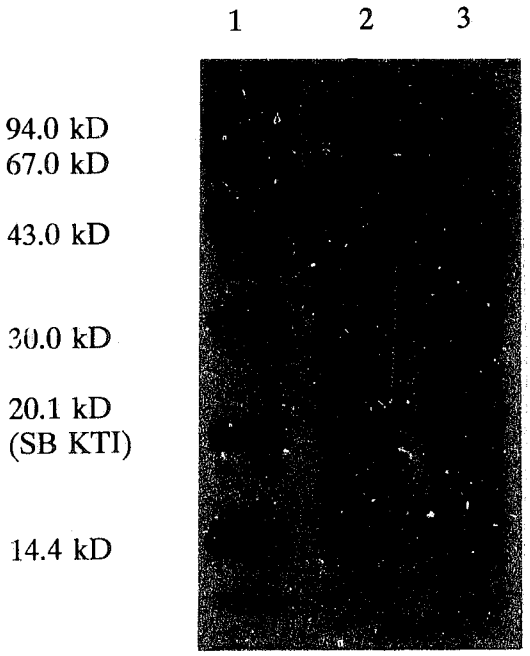

Figure 2. SDS-PAGE pattern of the raw (*Maple Isle*) SB and LTI SB.

Lane 1: Standard low molecular proteins; Lane 2: Raw SB; Lane 3: LTI SB.
Note the absence of ~ 20 KD band in lane 3.

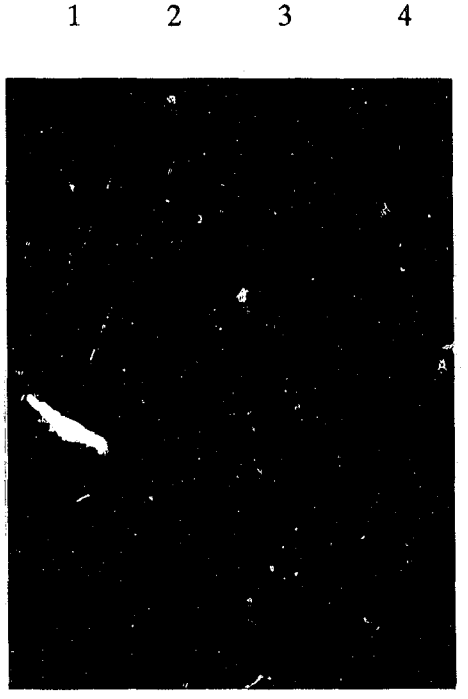

Figure 3. Negative staining of 12.5% polyacrylamide gels to detect the presence or absence of Kunitz Trypsin Inhibitor (KTI) in raw (*Maple Isle*) SB and LTI SB.

Lane 1: Raw SB; Lane 2: LTI SB; Lane 3: KTI Standard; Lane 4: Standard low molecular weight proteins.
Note the absence of the KTI band in the LTI SB (lane 2).

3. EVALUATION OF THE NUTRITIONAL QUALITY OF SOYBEANS

3.1 Introduction

The chemical composition of the SB, as described in chapter 2, gives the potential feeding value but does not give an estimate of nutrient bioavailability. A number of factors such as the digestibility of the nutrient, its rate of absorption from the gastro-intestinal tract, and complex interactions with other nutrients can alter the physiological availability of a nutrient. For example, a knowledge of the amino acid composition of a protein can provide a valuable index as to its potential nutritive value, but it is the actual performance of an animal fed that protein which needs to be assessed. The digestibility of the protein relates to the extent to which amino acids are released from the protein in the digestive system of the animal. The rate at which these amino acids are absorbed from the gastro-intestinal tract and the complex interactions with other nutrients may then affect the digestibility of the protein and the availability of the amino acids to the animal (36).

The use of full-fat SB, because of the high protein and energy content, is generating considerable interest as a replacement for SBM in chicken diets (53, 54). The presence of heat-labile TI in raw SB flour, however, causes growth depression and pancreatic enlargement due to increased secretory activity in rats, chicks and mice (24). This pancreatic hypersecretion leads to increased losses of essential amino

acids, in particular the sulphur amino acids, which are in high concentration in pancreatic secretions in these species (24). Thus, the growth depression observed in growing animals fed raw SB is a combined effect of impaired intestinal proteolysis due to the inhibition of trypsin and the drain of essential amino acids in pancreatic secretions (14). Amino acid supplementation, particularly cystine or methionine, of raw SB diets for rats and chickens alleviates some of the growth depression (7, 55). The effective use of SB depends on the heat treatment received to reduce the TI activity in the beans before being fed to the animal (54).

In diets containing adequate amounts of all required nutrients, the efficiency of feed utilization depends upon the metabolizable energy content of the diet since birds eat to satisfy their energy requirements (56). Knowledge of the bioavailable energy from the feed to the bird is of value to the nutritionist in formulating poultry rations (57). The use of accurate bioavailable energy values to formulate rations will result in more effective use of resources, reduce bioavailable energy input costs and increase the efficiency with which other nutrients are utilized, thereby leading to increased animal productivity at reduced costs (57).

The present study was undertaken to evaluate the utilization of full-fat SB, in particular the new HP and LTI varieties, by broiler chickens. The specific objectives were the following:

(i) To evaluate the nutritional quality of the full-fat SB described in Chapter 2, when incorporated in chicken broiler starter diets, by studying the growth performance of the chickens;

(ii) To estimate the nutrient bioavailability of the diets containing these beans to the birds; and

(iii) To determine if supplementation of conventional raw and LTI SB diets with 0.3% DL-methionine improves the nutritional quality of the beans.

3.2 Materials and Methods

3.2.1 Diets / Treatments

The experimental diets were formulated to contain 240 g protein and 12.96 MJ metabolizable energy (ME) per kg of dry matter. The protein sources were included in the diets at the level of 25 % by weight. The diets, as listed below, were corn and wheat based and were formulated to be isoenergetic and isonitrogenous.

SBM diet (control)

Autoclaved SB diet

Autoclaved HP SB diet

Roasted SB diet

Raw SB diet

LTI SB diet

The composition of the diets is shown in Table V. Crude SB oil was added to the HP SB diet to compensate for the lower fat content of these beans. Effect of

methionine supplementation was also studied by supplementing the diets with 0 or 0.3% DL-methionine.

3.2.2 Chicken Growth and Digestibility Trial

Two trials of randomized block design were conducted to study the growth performance of chickens fed the experimental diets. Day old male broiler chickens were obtained from ABS Hatchery, New Minas, Nova Scotia, and were fed the Kentville broiler starter diet (24% CP and 12.5 MJ/kg DM ME) for seven days posthatching. The birds were housed in thermostatically controlled starter batteries in a room where the temperature was controlled. The temperature in the battery cages was 32°C until the chickens were three days of age, 29°C until seven days afterwhich the temperature was reduced by 3°C weekly. Feed and water were available ad libitum and the light program was 23L:1D throughout the experiment.

On Day 7 posthatching, after a 2 hour fast, the birds were weighed and assigned to the treatments so that the weight distribution for each replicate was similar. Each pen served as a replicate with 5 chickens per pen in Trial I and 4 chickens per pen in Trial II. There were 5 replicates of each diet in trial I and 2 replicates for each diet in trial II, arranged in a random block design. The test diets were fed for 14 days.

Estimation of Digestibility of the Diets

Excreta were collected from each pen during the last three days of the experiment. Care was taken not to include feed and feathers during the excreta collection. The excreta were freeze-dried and ground before analysis for dry matter and Kjeldahl nitrogen according to the methods of AOAC (37). Gross energy was determined by an isoperibolic calorimeter (Model #1261, Parr Instruments, Illinois). Dry matter digestibility (DMD), crude protein digestibility (CPD) and the metabolizable energy (ME) of the diets were determined using acid insoluble ash as an internal indicator.

$$\textit{Nutrient Digestibility (\%)} = 100\ X\ \left(1-\frac{\textit{\% indicator in feed}}{\textit{\% indicator in excreta}}\ X\ \frac{\textit{\% nutrient in excreta}}{\textit{\% nutrient in feed}}\right)$$

Acid insoluble ash was measured in the diets and the excreta according to the method of Vogtmann et al.(58).

Feed Intake and Weight Gain

At the termination of the experiment, the birds were individually weighed after a two hour fast. Feed intake per pen, over the experimental period of 14 days, was also recorded.

Weight of Pancreas

The birds were killed by cervical dislocation. The pancreas was removed from 3 birds from each pen in trial I and 2 birds from each pen in trial II , weighed (wet weight), dried at 100°C for 24 hours and reweighed (dry weight).

Body Composition

To determine the body composition, whole birds (including blood, feathers, legs and head) were autoclaved for 200 minutes, cooled and homogenized in a Waring blender with 100 ml of distilled water. The homogenate was analysed for dry matter, protein, fat and ash according to the standard methods of AOAC (37). Fat was determined on freeze-dried samples. The carcass energy was calculated using the values of 23.4 and 38.9 kJ/g for protein and fat, respectively (59).

Statistical Analysis

Values for individual birds were used for the statistical analysis of body weight gain, pancreas weight and body composition. Pen means were used to statistically evaluate feed intake and feed efficiency data.

Statistical analysis of the data was performed as a 6x2 factorial design, consisting of diet and the methionine level as the factors, using the GLM procedure of the Statistical Analysis System (60). The results obtained from the two trials were combined for the analysis as the trial x diet interaction was not significant.

3.2.3 Rat Digestibility Trial

Twelve adult, male Sprague Dawley rats, weighing about 250g, were housed individually in Nalgene metabolic cages that allowed separate collection of feces and urine. The diets used in this study were the same as those used in the chicken digestibility trial. Two 6X6 Latin Square designs were run simultaneously. One group was fed the non-supplemented diets and the other group was fed the methionine supplemented diets. Feed and water were provided ad libitum throughout the experiment. The experimental period for each diet lasted for 8 days which included a three-day adjustment period followed by a five-day collection period. During the collection period, daily records were kept for the amount of feed consumed, weight of the feces and the volume of the urine excreted. A protein-free diet was fed to the rats for 5 days immediately following the completion of the experiment to determine metabolic nitrogen losses to calculate the metabolizable protein (MP).

The fecal and urine samples collected each day during the experimental periods were pooled and frozen until analyzed. Diets and ground feces were analysed for dry matter, nitrogen and energy according to the methods previously cited. Urine was diluted to a known volume with distilled water and analysed for nitrogen. Dry matter digestibility (DMD), apparent protein digestibility (APD), true protein digestibility (TPD) and apparent digestible energy (ADE) of the diets were calculated by the following equations:

$$\textit{Apparent Nutrient Digestibility (\%)} = 100 \times \frac{I-F}{I}$$

$$\textit{True Protein Digestibility (\%)} = 100 \times \frac{I-(F-Fo)}{I}$$

$$\textit{Metabolizable Protein (\%)} = 100 \times \frac{I-(F-Fo)-(U-Uo)}{I}$$

where, 'I' is the amount of nutrient ingested; 'F' is the amount of nutrient excreted in the feces; 'U' is the urinary nitrogen; 'Fo' and 'Uo' represent the amount of endogenous nitrogen losses in the feces and urine, respectively.

Statistical analysis of the data was performed as a 6x2 factorial design, consisting of diet and the methionine levels as the factors, using the GLM procedure of the Statistical Analysis System (60).

3.2.4 Determination of True Metabolizable Energy

The true metabolizable energy (TME) content of the diets and the protein sources was determined using adult Single Comb White Leghorn roosters according to the 'precision feeding' method described by Sibbald (61) modified to a 48 hour collection period (62). Five replicate measurements were done on each diet and protein source. Four samples were tested using twenty four roosters chosen at random. In addition, four birds during each assay period were fasted for 48 hours and used to measure metabolic and endogenous losses. The birds were allowed to

recover for 2 weeks between each assay period. All birds were fed a maintenance diet between assays.

Trays were placed under each cage to collect excreta voided for 48 hours exactly. The excreta were collected quantitatively and frozen until analysed. Care was taken not to include feathers and scales during the collection. Any sample showing regurgitation of feed was discarded. After being oven dried for 24 hours at 55°C the excreta were ground and analysed for dry matter, nitrogen and gross energy. TME and TME corrected to zero nitrogen balance (TME_n) were calculated as described by Sibbald (62).

Statistical analysis of the data was performed with the GLM procedure of the Statistical Analysis Systems (60).

3.3 Results

3.3.1 Nutrient Bioavailability

Chicken Digestibility Trial

The average dry matter digestibility (DMD), crude protein digestibility (CPD) and energy metabolizability values of the diets containing heat-treated beans were higher than those containing raw beans (Table VI). Among the diets containing heat-treated beans, the HP SB diet had highest digestibilility values which were similar to the control SBM diet. The digestibility values of the autoclaved SB diet and the

roasted SB diets were similar. The ME values were lowest for the raw SB diet. There was no significant difference observed between the digestibility values of the diets containing raw versus LTI beans. Methionine supplementation of the diets did not show any significant differences between the non-supplemented and the respective supplemented diet (Appendix A) but overall there was an improvement in the digestibility values with methionine supplementation.

Rat Digestibility Trial

Rat digestibility trial results are shown in Table VII. The dry matter digestibility (DMD), apparent protein digestibility (APD), true protein digestibility (TPD) and the apparent digestible energy (ADE) of the HP and roasted SB diets were higher than the values for the autoclaved conventional SB diet and similar to those of the control SBM diet. The raw SB diet consistently showed lowest digestibility values ($P<0.05$). Overall, the digestibility values for the LTI SB diet were higher than for the raw SB diet and similar to the autoclaved conventional SB diet. Significant differences ($P<0.05$) were observed for the APD and TPD values between the raw and the LTI beans which were 80.82% and 85.63% for the raw SB and 83.07% and 88.08% for the LTI SB, respectively.

Supplemented LTI SB diet showed higher values, though not significant, for DMD, APD, ADE and MP than the raw SB diet. The TPD of the supplemented LTI SB diet, however, was significantly ($P<0.05$) improved compared to the supplemented raw SB diet.

True Metaboilizable Energy

The TME and TME_n values of the full-fat SB, as expected, were higher ($P<0.05$) than the defatted SBM (Table VIII). The TME_n value of the autoclaved SB among the full-fat SB was the highest ($P<0.05$). The LTI SB had a higher TME value than the raw SB ($P< 0.05$), although the difference was not observed in the TME_n values of these beans. The TME for the HP SB was the least which could be attributed to the lower fat content of these beans when compared to the other beans in the study (Table I).

Although the diets were formulated to be isoenergetic, they varied in the TME values. The TME values of the diets (Table IX) agree well with the TME values of the SB as seen in Table VIII. The diet containing the autoclaved SB had the highest bioavailable energy. The TME and TME_n values of the diets containing unheated SB were less ($P<0.05$) than those of the diets containing heated SB. The HP and the roasted SB diets showed similar TME_n values as the control SBM diet. The TME values of raw and the LTI SB diets were not significantly different. Overall, methionine supplementation did not improve the TME values of the diets (Appendix B).

3.3.2 Growth Performance of Broiler Chickens

The productive performance and the pancreas weights of the birds fed the diets containing different sources of SB as protein supplements are shown in Table X. Highest body weights and gains were obtained with the birds fed the diet that

contained SBM. Among the birds fed the full-fat SB containing diets, those fed the heat treated SB performed better than those fed the diets containing the unheated raw or LTI SB ($P<0.05$). The weights and gains of chickens fed autoclaved HP and roasted SB diets were similar, but greater than the values for those fed the conventional autoclaved beans; the difference was significant ($P<0.05$) between those fed autoclaved and roasted SB diets.

The feed intake of the roasted SB diet was highest among the full-fat SB diets ($P<0.05$) and similar to that of SBM diet. The consumptions of the raw and LTI SB diets were the least ($P<0.05$). The feed efficiency of the HP SB diet was higher ($P<0.05$) than that of conventional autoclaved SB diet. The feed efficiency values for the autoclaved HP and roasted SB diets were similar to that of the control SBM diet. The lowest feed efficiency values occurred with the raw and LTI SB diets and were not different from one another .

Birds fed methionine-supplemented LTI SB diet showed significantly ($P<0.05$) higher weight gains and improved feed efficiency than those fed the non-supplemented LTI SB diet (Appendix C). This improved effect was not observed in methionine-supplemented raw conventional SB diet. Overall, the effect of methionine supplementation was not significant (Appendix C).

The pancreas weights of the birds fed unheated beans were greater ($P<0.01$) than those fed heated SB containing diets (Table X). Significant difference was observed ($P<0.01$) between the pancreas weight of the birds fed the raw and LTI SB diets, even though the performance of these birds was similar. The pancreas

weight of the birds fed LTI SB diet was less than that of the birds fed the raw conventional SB diet ($P<0.01$). Pancreas weight when expressed as percentage of body weight also showed similar differences ($P<0.05$). Methionine supplementation had no effect on the pancreas weight (Appendix C).

Body composition of the birds is presented in Table XI. The protein, fat, ash and carcass energy contents of the birds fed SBM diet were higher ($P<0.05$) than those fed full-fat SB diets. Among the full-fat SB diets, the body composition of the chickens fed autoclaved, HP and roasted SB diet were similar and had higher mean values with regards to protein, fat, ash and carcass energy contents than those for the birds fed raw or LTI SB diets. The body compositions of the chicks fed raw and LTI SB diets were similar. Overall, diet and methionine interaction was not significant (Appendix D).

3.4 Discussion

3.4.1 Nutrient Bioavailability

The improved nutritive value of heat-treated beans is partly a result of inactivation of anti-nutritional factors such as trypsin inhibitors and lectins (63). The presence of TI in the small intestine induces the formation of a trypsin- trypsin inhibitor complex which passes through the digestive tract and is excreted in the feces. The decrease in the free trypsin concentration and presence of the undigested

proteins in the small intestine results in increased secretory activity of the pancreas. This compensatory reaction leads to a loss of essential sulphur-amino acids to secrete additional pancreatic enzymes for the digestion of proteins. These phenomena contribute to an increase of the endogenous fecal nitrogen excretion and would account for low digestibility of proteins (64). This is evident in the low values for the raw SB diets in the present study (Tables VI and VII). The presence of chymotrypsin inhibitors and the nature of the raw soy protein further enhances the low digestibility of the raw SB (26, 65). It has also been reported that the release of amino acids from raw SB is slow compared to heat treated beans (24). In chickens, a 20 % difference in the overall protein absorption between heat-treated and raw SBM has been reported (66). It was suggested that in chicks fed heat treated SBM, the proteolytic enzymes remain active beyond the duodenum, resulting in additional digestion. On the other hand, in the raw SBM fed group the increased enzymes secreted into the duodenum are inactivated by the inhibitors resulting in impaired digestion.

In addition, heating is necessary for partial denaturation of the soybean proteins prior to digestion by pancreatic enzymes so as to render the availability of amino acids more readily for the animal (11, 26, 67). Therefore, the higher digestibility values obtained for the diets containing heat treated beans than the raw SB diets (Tables VI and VII) are attributed to the beneficial effects of heat treatment as discussed.

The chicken and rat digestibility trials showed that the digestibility coefficients of the HP and roasted SB diet were similar to those of the control SBM diet. The protein solubility results presented in Table III support the digestibility data (Tables VI and VII) which indicate that the beans were not overheated during processing. The lower digestibility values of the conventional autoclaved SB diet than those for HP SB diet could be due to some residual TI activity (Table IV).

Carroll et al.(68) measured net nitrogen absorption from a section of the small intestine and found that rats fed raw SBM absorbed less than half the nitrogen of those fed heated meal. Nitsan and Liener (67) reported that the main site of absorption of amino acids of heated soy flour was the jejunum and ileum in rats, whereas the absorption from raw SB occurred mainly in the ileum. This delay in absorption of nitrogen and amino acids from raw soy flour might affect the quantity of amino acids absorbed by the animal (11, 67). Higher digestibility values of the LTI SB than the raw conventional beans (Table VII) is then attributed to the lower TI activity of the former since they otherwise showed similar proximate and amino acid composition (Tables I and II). The difference between the raw and the LTI SB was more evident in the methionine supplemented diet (Table VII), as the supplementation could have partially corrected the sulphur amino acid imbalance leading to better utilization of the proteins of the LTI SB. The proteins of the supplemented LTI SB seemed to be utilized better than the supplemented conventional raw SB diet indicating detrimental effects of high levels of TI in the latter diet.

The inferiority of the LTI beans to heat treated beans was due to the fact that in spite of the absence of KTI in these beans (Figures 2 and 3, Chapter 2), the trypsin inhibitor activity was still significant (Table IV). The presence of other protease inhibitors, as seen in the negatively stained gels in Figure 3 likely contributed to decreased protein digestion. Furthermore, as stated previously, some heat treatment is necessary for denaturation of the SB protein for subsequent digestion by pancreatic enzymes (26).

The MP values did not show any significant differences among the diets although the supplemented LTI SB diet values were higher than those of the raw SB diet (Table VII). Aguilera et al. (69) reported MP values of 51.1 % for rats fed a 12% CP (DM basis) diet containing SBM. The lower values found in the present study could be due to the high protein content of the diets (Table V), relative to the rat's requirement, which resulted in increased metabolizable losses of proteins.

The bioavailable energy results showed higher values for the heat treated beans compared with those for the raw beans (Table VIII). The low TME values of the raw and the LTI SB are attributable to the presence of anti-nutritional factors, such as TI, which interfere with the utilization of fat from the beans and, thus, decrease the ME (12, 54, 70, 71). Further, heat treatment makes the fat more accessible for digestion and absorption (54, 71, 72). This is in agreement with the fat analysis results of the beans in the present study (Table I) which showed higher fat values for the heat-treated beans. The TME values for the full-fat SB were higher than that for the SBM due to their higher fat contents. Han et al.(32) found higher

TME_n value for their LTI variety of SB compared with the conventional beans. This difference was attributed to the higher availability of the amino acids in the LTI SB. However, no difference in the TME values was observed between the two varieties in this study even though the LTI SB diet did show better crude protein digestibility than the raw conventional SB diet.

3.4.2 Growth Performance of the Birds

The chickens fed the heat-treated beans showed higher weight gains than those fed unheated beans (Table X). The superior performance of birds fed heat-treated beans is likely due to the destruction of heat-labile trypsin inhibitor factors (6, 24) and SB hemagglutinin, or lectin, which possibly reduces the feed intake (73). Lectins also combine with the cells of the intestinal wall and cause a non-specific interference with the absorption of nutrients (74). The superior growth performance of the chicks fed the heat-treated beans can also be attributed to the higher digestibility values of dry matter and protein, and metabolizable energy than the values for unheated beans as seen in the previous section (Tables VI, VII and IX).

The protein solubility (Table III) and digestibility (Tables VI and VII) values of the roasted SB and the autoclaved SB were comparable, indicating that the heat treatment during autoclaving was not excessive to cause deterioration of the proteins. The performance of the birds fed HP SB diet was similar to the performance of birds fed commercially roasted SB diet. The amino acid composition of the HP and the roasted SB were similar on a per cent protein basis (Table II) but when expressed

on dry matter basis, the HP SB contained higher levels than the conventional roasted SB. Therefore, the extra 17% protein in the HP SB (Table I) is composed of amino acids and has the potential to be well utilized by the birds.

It has been reported that heated ground unextracted SB are less efficacious in terms of growth rate and feed efficiency compared to SBM (54, 75). Similar results were observed in this study although not with respect to feed efficiency (Table X). The HP SB and roasted SB diets had feed efficiencies (Table X) and digestibility values (Tables VI and VII) comparable to SBM. The poor performance observed in the birds fed heated, ground SB by Carew et al. (75) has been attributed to the inability of the birds to extract oil from the cellular structure, resulting in poor fat absorption and low ME. They suggested that beans must be flaked, to cause a greater disruption of cellular structure for effective utilization by chicks as birds fed heated, SB flakes showed growth performance comparable to those fed SBM. White et al. (76) reported similar findings but observed no significant difference in the growth performance of birds fed heated ground versus flaked soybeans.

The increased pancreas weights of birds fed unprocessed SB (Table X) are the result of the presence of TI in these beans (77). Yen et al. (49) also observed lower dry pancreas weights, as percent of body weight, in rats fed SB of lower TI content compared to those fed the conventional variety. The results of the pancreas weights, as percent of body weight, in the present study (Table X) are similar to those quoted by Bajjalieh et al.(77). Higher pancreas weight observed for the birds fed autoclaved conventional SB diet (Table X) versus those fed HP or roasted SB diets

could be due to higher residual TI activity (Table IV) obtained in the autoclaved SB as compared to the autoclaved HP or roasted SB.

By chemical analysis, the TI levels of the LTI beans used was 40% less than the TI level of conventional SB (Table IV). In addition, the proximate analysis and the amino acid composition of these beans were similar (Tables I and II). The lower mean pancreas weight of the birds fed the LTI SB could then be a result of lower TI levels and, therefore, less pancreatic hypersecretion compared to the effect of higher levels of TI in the conventional raw SB. This would also suggest less impairment of intestinal proteolysis in the birds fed LTI SB.

Overall, the DMD, APD, TPD, MP and ADE values of the LTI SB diets, from the rat digestibility trial (Table VII) were higher than those of the conventional raw SB diet with that of TPD being significantly ($P<0.05$) higher for LTI SB diet. The lower pancreatic stimulating effect of the LTI beans, as mentioned above, would also be associated with lower endogenous loss of fecal nitrogen and essential amino acids which would explain the higher digestibility values and better utilization of LTI SB protein than that of the raw SB (Table VII).

The presence of TI in raw SB depresses proteolytic activity in the small intestine and decreases the release of free amino acids from the soy protein (32). Trypsin inhibitors also stimulate hypersecretion of the pancreas in response to the inhibited trypsin activity due to their binding effect with the enzyme, trypsin. This hyperactivity of the pancreas results in hypertrophy of the pancreatic cells and excessive use of certain essential amino acids, in particular the sulphur amino acids,

in the formation of pancreatic enzymes. Thus, the growth depression is a combined effect of excessive loss of essential amino acids and decreased intestinal proteolysis (11, 14, 49). This growth depression can partially be corrected with methionine supplementation through stimulating the pancreatic biosynthesis of cystine from the added dietary methionine and its subsequent incorporation into pancreatic enzymes (16, 17, 49, 50, 77). Therefore, increased enzyme synthesis would improve intestinal proteolysis and overall performance. This positive effect of addition of methionine on growth (Table XI) was observed for the LTI SB diet but not with the raw SB diet indicating a more detrimental effect of the high levels of TI in the conventional raw SB. Methionine supplementation improves growth response of the birds but does not correct pancreatic enlargement (17). The results of the present study agree with these reported findings.

It has been reported that the growth performance of chicks fed heated full-fat SB diets is comparable to the performance of those fed SBM diets (53, 76, 78). Superior performance of the birds fed SBM diet compared to those fed full-fat SB diets was observed in the present study (Tables X and XI). The analysis of the diets showed that the gross energy and ME values of the full-fat SB diets were slightly higher than the SBM diet (Table V). Since birds eat to meet their energy requirements (56), then it is possible that due to the higher energy levels of the SB diets the birds satisfied their energy requirements but did not consume enough feed to meet their protein requirements for body tissue deposition. Waldroup and Cotton (78) recommended that the inclusion of cooked full-fat SB in all-mash broiler chicken

diets should be limited to 25%. Inclusion of higher levels of SB resulted in decreased feed intake due to the higher density of these feeds leading to impaired growth of the birds. Leeson et al. (54) reported impaired growth in broiler chickens fed diets containing more than 20% of cooked full-fat SB. In the present study, although inclusion of full-fat SB was at 25% level in the diets, inclusion of lower levels of other energy sources in the diets would probably have resulted in similar performance of the birds fed full-fat SB diets to those fed SBM diet as the digestibility values and the feed efficiency values of the heated full-fat SB diets were comparable to those of the SBM diet.

The higher body protein, fat, ash and carcass energy contents of birds fed heat treated SB as compared to those fed unheated SB (Table XI) could be due to the destruction of the anti-nutritional factors in the former and, hence, better utilization of the nutrients as was seen in the digestibility results of the previous section for these beans. The higher ash content of the birds fed heat treated SB than of those fed unheated SB, could be due to the fact that raw SB contain some factors, such as phytates, which interfere with the absorption of certain minerals (67). No difference was observed between the body composition of the birds fed raw and LTI beans. Yen et al.(17) also found no difference in the body composition of chickens fed raw SB with different TI activities.

3.5 Conclusions

The bioavailability of nutrients from heat treated beans was higher than that from raw and LTI SB. The HP SB showed greater bioavailability of nutrients compared to conventional beans and similar to that of the SBM and roasted SB diets. The growth performance of the birds agrees well with the results of nutrient bioavailability from the diets. The birds fed HP and roasted SB diets showed similar growth performance and their feed efficiencies were the same as the SBM diet. The results of the present study also showed that the extra 17% protein in the HP SB was well utilized by the birds and that these beans will be of economic value when substituted on an equivalent protein basis for conventional SB.

The LTI SB diet showed a tendency towards improved utilization compared to conventional raw SB. This difference was more evident when the diets were supplemented with 0.3% DL-methionine. However, the growth performances of the birds fed LTI and conventional raw SB were similar indicating that the levels of TI in the LTI are still high enough to cause impaired growth performance of the birds. Methionine supplementation resulted in an improved weight gain in birds fed LTI SB but not to the level of heat treated conventional SB. Therefore, the TI levels in LTI SB were not low enough to eliminate heat treatment entirely, but perhaps would require lower temperatures and shorter cooking times than for the SB currently grown.

TABLE V. Composition of the diets (g/100 g)

Ingredients	SBM	Autoclaved SB	Autoclaved HP SB	Roasted SB	LTI SB	Raw SB
Soybean source	25.00	25.00	25.00	25.00	25.00	25.00
Corn	49.75	20.07	36.08	20.07	20.09	22.09
Wheat	10.00	38.28	23.59	38.28	38.25	36.72
Poultry fat	2.44	1.00	1.00	1.00	1.00	1.00
SB Crude Oil	-	-	0.75	-	-	-
Fish Meal	5.00	5.00	5.00	5.00	5.00	5.00
Corn Gluten	4.56	7.41	6.04	7.41	7.42	6.06
Vitamin-mineral premix[a]	3.11	3.23	3.15	3.13	3.14	3.41
Methionine	0.14	0.11	0.14	0.11	0.11	0.17
TOTAL	100.00	100.00	100.00	100.00	100.00	100.00
Dry matter, %	90.45	91.59	91.86	92.23	91.45	91.42
Crude Protein, % DM	28.74	28.04	28.39	27.74	27.10	27.48
Ether Extract, % DM	5.46	8.36	8.16	8.39	7.40	7.77
Gross Energy, MJ/kg DM	19.55	20.17	19.87	20.19	20.08	19.91
Metabolizable Energy, MJ/kg DM[b]	18.53	18.97	18.99	18.94	18.98	18.62

[a]Amount per kilogram of diet: 12,000 IU vitamin A; 2,000 ICU vitamin D_3; 8 mg riboflavin; 12 mg calcium pantothenate; 12 mg vitamin B_{12}; 30 mg niacin; 3 mg vitamin K; 1 mg folic acid; 400 mg choline chloride; 200 μg biotin; 5 mg pyridoxine; 3 mg thiamine; 15 IU vitamin E; 750 mg Amprol High E (25%); 100 mg ethoxyquin; 120 mg manganese oxide (60% Mn); 90 mg zinc oxide (80% Zn); 25 mg cooper sulphate (25% Cu); 500 μg calcium iodate (65% I); 200 mg ferrous sulphate (36% Fe); and 200 μg sodium selenite (45% Se).
[b]Calculated by Gross Energy x Energy Metabolizability

TABLE VI. Mean digestibility coefficients (%) and energy metabolizability (%) of the diets fed to broiler chickens

	SBM	Autoclaved SB	Autoclaved HP SB	Roasted SB	Raw SB	LTI SB	SEM
Dry Matter Digestibility	93.98[ab]	93.43[b]	94.40[a]	93.75[ab]	93.39[b]	93.59[b]	0.24
Crude Protein Digestibility	90.98[ab]	90.27[b]	91.51[a]	90.34[ab]	90.45[b]	89.82[b]	0.44
Energy Metabolizability	95.37[a]	94.23[c]	95.22[ab]	94.63[bc]	93.99[c]	94.44[c]	0.25

[abc]Means within a row with same superscripts are not significantly different ($P<0.05$)

TABLE VII. Digestibility coefficients (%) of the methionine supplemented and non-supplemented diets as obtained from the rat trial

	% Meth	SBM	Autoclaved SB	Autoclaved HP SB	Roasted SB	Raw SB	LTI SB	SEM
Dry matter Digestibility	0	84.2	82.5	83.6	83.3	81.2	82.0	0.28
	0.3	83.9	81.6	83.2	83.5	81.0	82.1	0.28
	Mean	84.0^{a}	82.1^{ab}	83.4^{ab}	83.4^{ab}	81.1^{c}	82.0^{c}	0.48
Apparent Protein Digestibility	0	85.8	84.2	85.6	86.4	80.9	83.6	0.30
	0.3	85.8	83.3	84.4	86.0	80.8	82.6	0.30
	Mean	85.8^{a}	83.7^{b}	85.0^{ab}	86.2^{a}	80.8^{c}	83.0^{b}	0.52
True Protein Digestibility	0	90.1	88.6	90.1	90.8	85.4	88.1	0.32
	0.3	90.7	88.0	89.5	90.9	85.9	88.1	0.32
	Mean	90.4^{a}	88.3^{bc}	89.8^{ab}	90.9^{a}	85.6^{d}	88.1^{c}	0.56
Metabolizable Protein	0	40.8	40.8	42.9	39.3	38.7	41.0	2.01
	0.3	45.3	43.3	42.1	41.9	43.6	44.3	2.01
	Mean	43.0	42.0	42.5	40.6	41.2	42.6	3.48
Apparent Digestible Energy	0	72.0	71.7	74.3	73.4	70.0	70.0	0.68
	0.3	70.7	67.4	72.0	71.7	65.9	69.8	0.68
	Mean	71.4^{ab}	69.5^{bc}	73.2^{a}	72.5^{ab}	68.0^{c}	70.0^{bc}	1.18

^{abcd}Means within a row with same superscripts are not significantly different ($P<0.05$).

TABLE VIII. True metabolizable energy (TME, MJ/kg DM) of the protein sources

Protein Source	TME ± SEM	TME_n ± SEM	N*
SBM	11.35 ± 0.30[d]	11.99 ± 0.24[c]	5
Autoclaved SB	17.07 ± 0.33[a]	17.32 ± 0.27[a]	4
Autoclaved HP SB	15.42 ± 0.33[bc]	15.91 ± 0.27[b]	4
Roasted SB	16.55 ± 0.39[a]	15.95 ± 0.31[b]	3
Raw SB	14.82 ± 0.33[c]	15.61 ± 0.27[b]	4
LTI SB	16.14 ± 0.39[ab]	16.14 ± 0.31[b]	3

*Number of replicates.
[abc]Means within a column with same superscripts are not significantly different ($P<0.05$).

TABLE IX. Mean values of TME (MJ/kg DM) of the diets

Diets	TME ± SEM	TME_n ± SEM	N*
SBM	14.79 ± 0.17[b]	15.66 ± 0.13[b]	8
Autoclaved SB	15.58 ± 0.17[a]	16.82 ± 0.12[a]	8
Autoclaved HP SB	15.42 ± 0.15[a]	15.94 ± 0.11[b]	10
Roasted SB	15.46 ± 0.18[a]	16.12 ± 0.31[b]	7
Raw SB	14.88 ± 0.16[b]	15.35 ± 0.12[c]	9
LTI SB	14.72 ± 0.15[b]	15.52 ± 0.11[c]	10

*Number of replicates.
[abc]Means within a column with same superscripts are not significantly different ($P<0.05$)

TABLE X. **Effect of diets containing the protein sources on growth, feed intake, feed efficiency, and pancreas weights of the broiler chickens**

	SBM	Autoclaved SB	Autoclaved HP SB	Roasted SB	Raw SB	LTI SB	SEM
Final weight, g	629.4[a]	550.7[c]	567.1[bc]	581.6[b]	463.4[d]	453.7[d]	20.6
Gain, g	499.6[a]	422.1[c]	439.5[bc]	452.9[b]	333.1[d]	325.9[d]	18.72
Feed intake, g	729.4[a]	660.3[bc]	643.2[c]	688.4[ab]	579.8[d]	565.7[d]	14.9
Feed efficiency (Intake/gain)	1.46[a]	1.56[b]	1.47[a]	1.51[ab]	1.75[c]	1.76[c]	0.03
Pancreas Weight, g DM	0.67[c]	0.69[c]	0.65[c]	0.63[c]	0.96[a]	0.78[b]	0.01
Pancreas Weight, % of body weight	0.12[d]	0.14[c]	0.12[d]	0.12[d]	0.21[a]	0.18[b]	0.01

[abcd]Means within a row with same superscripts are not significantly different ($P<0.05$)

TABLE XI. Body composition of the broiler chickens fed diets containing the protein sources

Diet	Total carcass dry matter, g	Protein		Fat		Ash		Carcass Energy*	
		g	% DM	g	% DM	g	% DM	kJ	kJ/g DM
SBM	173.7[a]	135.5[a]	78.0	18.2[a]	10.5	20.0[a]	11.5	3880[a]	22.3
Autoclaved SB	145.8[b]	113.4[b]	77.8	15.2[b]	10.4	17.2[b]	11.8	3244[b]	22.2
Autoclaved HP SB	148.9[b]	115.7[b]	77.7	16.0[ab]	10.7	18.0[b]	12.1	3329[b]	22.4
Roasted SB	155.5[b]	120.4[b]	77.5	17.2[ab]	11.1	18.6[ab]	11.9	3487[b]	22.4
Raw SB	116.2[c]	90.9[c]	78.3	11.7[c]	10.1	13.5[c]	11.6	2583[c]	22.2
LTI SB	116.3[c]	90.5[c]	77.8	12.4[c]	10.6	13.4[c]	11.6	2599[c]	22.4

[abc]Means within a column with same superscripts are not significantly different ($P<0.05$).
*Calculated using the values of 23.4 and 38.9 kJ/g for protein and fat, respectively (Farrell, 1974).

4. SUMMARY AND CONCLUSIONS

In Atlantic Canada, increased soybean production has the potential to make the region self-sufficient in production of protein supplements. Therefore, there is the possibility of SB being produced locally and incorporated in an "on farm" mixing program.

The presence of heat labile trypsin inhibitors (TI) and other anti-nutritional factors limits the use of raw SB in animal diets (6, 7). Two metabolic responses of nutritional importance associated with TI are growth depression and pancreatic enlargement due to hypersecretion of the pancreas which leads to excess loss of essential amino acids, especially the sulphur amino acids (13). These responses are more pronounced in growing animals due to their higher requirements for protein for tissue deposition. Adult animals appear to be less sensitive to the anti-nutritional effects of raw SB and can maintain well on diets containing raw or heat treated SB (6, 11, 12).

The growth depression observed in growing animals is a combined effect of loss of essential amino acids and decreased intestinal proteolysis (14). The growth depression can be partially corrected with methionine supplementation (15, 16). This improvement is partly due to the fact that methionine is limiting in SB and the anti-

proteolytic activity of the TI in the raw SB increases the requirement for sulphur amino acids (18, 19, 20).

The effective use of SB in animal diets then depends on the heat treatment received before being fed to the animals (54). Heating is also necessary for denaturation of SB proteins and subsequent digestion by pancreatic enzymes (8, 26). Thus, heat treatment is required not only to inactivate the anti-nutritional factors but also to transform the raw proteins to more digestible forms. Overheating, on the other hand, usually results in protein deterioration which decreases the nutritive value and has a negative impact on the growth of the animals (7, 8). Therefore, precise control of heating process is critical to the preparation of protein products of maximum nutritive value.

The present study was conducted to evaluate the nutritional quality of the new varieties of high protein (HP) and low trypsin inhibitor (LTI) SB, in broiler chicken starter diets. High Protein SB has the potential to be substituted on an equivalent protein basis in animal rations, thereby, decreasing the amount of SB needed. The use of LTI Sb may eliminate the cost of heat treatment before being fed to poultry.

The HP SB were processed by autoclaving at 89.6 KPa for 15 minutes, to destroy TI and other anti-nutritional factors, since the quantity was insufficient to process in a commercial roaster. Conventional SB were similarly processed to be used as a control. Commercially roasted conventional SB were included as a control for the autoclaved beans to determine whether the heat treatment during autoclaving was sufficient to destroy the anti-nutritional factors or excessive to damage the

proteins. The LTI SB were not processed and conventional raw SB were used as the control. Commercially produced, solvent extracted SBM was used as a control protein source.

Study of the chemical composition of the beans showed that the HP SB had 17% higher crude protein levels compared to the conventional beans (Table I). The proximate composition of the LTI SB was similar to that of the conventional beans (Table I). Han et al. (32) also reported similar proximate composition of their variety of LTI SB and the conventional raw SB (Williams 82). The amino acid levels in the HP SB expressed on dry matter basis were higher than in the conventional beans (Table II), indicating that the higher crude protein level is a result of good quality protein. When expressed on protein basis, the amino acid profiles of the conventional, HP and LTI SB were similar. No difference between the amino acid profiles of the heated and the unheated SB was observed. Freidman et al.(46) also reported similar observations. The protein solubility results (Table III) of the beans indicated that none of the heat treated beans were overprocessed when compared to the values reported by Araba and Dale (40). The TI activity was negligible in the the heat treated beans (Table IV). The LTI beans showed 40% less trypsin inhibitor activity compared to the conventional beans and the electrophoretic analysis clearly showed the absence of the KTI band in these beans (Figures 2 and 3). Apart from this, electrophoretic profiles of the beans used in the present study were similar (Figure 1).

The beans selected for the study were incorporated in broiler chicken starter diets. Digestibility and growth trials with rats and chickens were conducted to evaluate the nutritional quality of these beans. True metabolizable energy (TME) of the beans and the experimental diets was determined by the 'precision feeding' method using adult White Leghorn roosters (62).

The bioavailability of nutrients from heat-treated beans was higher than the unheated beans (Tables VI, VII, VIII and IX) which is attributable to the inactivation of anti-nutritional factors such as TI and lectins and denaturation of the SB proteins for subsequent digestion by pancreatic enzymes (11, 26, 63). The growth performance of the birds fed heat treated beans in terms of weight gains, feed efficiencies and body composition was also superior to those fed unheated LTI or raw SB (Table X). The estimation of TME of the protein sources showed higher values for the full-fat SB than the SBM due to the higher fat content of the beans (Tables VIII and IX). Among the full-fat SB, higher TME values were obtained for the heat treated beans compared to those for unheated LTI and conventional SB. Heat treatment makes the lipid fraction from SB more accessible for digestion and absorption (54, 71). Although the gross energy values of the SB were similar, higher fat values for the heat treated beans than the unheated beans (Table I) were observed in the present study. Therefore, heating SB seems to bring about some structural changes in the SB fat which makes them more accessible for absorption and could account for the higher TME values obtained for the heat-treated beans.

The TME values of the experimental diets agreed with those obtained for the protein sources.

The digestibility coefficients with regards to dry matter, crude protein and metabolizable energy of the HP SB diet were greater than those of the autoclaved conventional SB diet and similar to those of the control SBM diet (Table VI). Results of the growth performance of broiler chickens (Tables X) showed higher weight gain for the birds fed SBM diet than those fed full-fat SB diets but the feed efficiency values of the HP and roasted SB diets were similar to that of the control SBM diet. The performance of the birds fed roasted and HP SB diets were similar and their weight gains were higher than for those fed conventional autoclaved SB diet (Tables X and XI). These results agree well with the digestibility results obtained in this study (Tables VI and VII). It has been recommended that SB with more than 3.9 mg/g Trypsin Inhibitor Activity, which is equivalent to 9.5 TIU/g, should not be used in chicken starter diets (54, 79). The high residual trypsin inhibitor activity of 11.8 TIU/g observed in the autoclaved beans in the present study would ascribe to the lower digestibility values and the inferior performance of the birds fed these beans. The detrimental effects of this residual TI activity in these beans was also observed in the higher pancreas weights of the birds fed diets containing these beans as compared to those fed HP or roasted SB containing diets (Table X).

It has been reported that the growth performance of chickens fed heated full-fat SB diets is comparable to those fed SBM diets (53, 76, 78). The performance of the birds fed SBM containing diet, in the present study, was superior in terms of

weight gain (Table X) and body composition content (Table XI) versus performance of those fed heat-treated full-fat SB containing diets. The analysis of the experimental diets showed that the gross energy and the ME values of the full-fat SB diets were slightly higher than those of the SBM diet. The mean energy-protein ratio of full-fat SB diets in the present study was 0.68 (MJ ME per kg DM / CP %) compared to the recommended ratio of 0.58 (80). Since birds eat to meet their energy requirements (Scott et al.,1982), then it is possible that due to the higher energy levels of the SB diets the birds satisfied their energy needs and did not consume enough feed to meet their protein requirements for tissue deposition. It has been recommended that inclusion of cooked full-fat SB in all-mash broiler chicken diets should be limited to the level of 25% (54, 78). Inclusion of higher levels of SB resulted in decreased feed intake due to the higher density of these feeds leading to impaired growth of the birds. In the present study, although the inclusion of full-fat SB was at 25% level in the diets, inclusion of lower levels of other energy sources in the diets would probably have resulted in similar growth performance of the birds fed full-fat SB diets to that of birds fed SBM diet as the digestibility values and the feed efficiency values of the heated full-fat SB diets were comparable to those of the SBM diet.

The results of this study have shown that the commercial heat treatment given to the beans is sufficient to destroy the anti-nutritional factors and to improve the nutritional value of the beans in terms of metabolizable energy and protein quality. The protein solubility results of the roasted and the autoclaved SB were similar

(Table III) but the feed efficiency (Table X) and the digestibility (Tables VI and VII) of the roasted SB containing diet were similar to that of the SBM diet. The birds fed roasted and HP SB diets showed similar growth performances that were comparable to those fed SBM diet. The HP SB were autoclaved for the present study. The results of the present study have also shown that the repeatability of the process of autoclaving is questionable. It is possible that the HP beans when commercially heat treated and incorporated in starter diets may result in even better performance of the birds than observed in the present study since the feed efficiency of the diet containing these beans was similar to that of the SBM diet (Table X). Further, the growth performance (Table X) and the digestibility results (Table VI) showed that the higher protein levels in the HP SB were well utilized by the birds. Therefore, commercial production of HP SB can be of economic value as these beans, when subsituted on an equivalent protein basis in animal diets, will decrease the amount of SB currently needed to formulate a ration.

Between the unheated LTI and raw SB diets, the LTI SB showed a tendency to better utilization than the conventional raw SB (Table VII). However, no difference was observed between the TME values of these beans or the diets containing these beans (Tables VII and IX). Supplementation of these diets with 0.3% DL-methionine was also studied since the anti-proteolytic activity of TI increases the requirements for sulphur amino acids. Improved utilization of nutrients from the LTI SB diet in the rat digestibility trial was more evident when the diets were supplemented with methionine.

Although the LTI SB diet showed a tendency towards better utilization of nutrients than the conventional raw SB, no significant difference was observed between the growth performance and the body composition of the birds fed these diets (Tables X and XI). The mean dry pancreas weight of the birds fed LTI SB was significantly less ($P<0.05$) than that of the birds fed raw SB diet (Table X). Lower mean pancreas weights of the birds fed LTI SB was due to lower levels of TI as compared to those in the conventional raw SB. This would also suggest less impairment of intestinal proteolysis in the birds fed LTI SB and could account for the higher digestibility values obtained for the LTI SB diet when compared to the conventional raw SB diet. Similar results regarding the mean pancreas weights of the birds fed SB varying in TI contents have been reported (49, 77). These authors and Han et al.(32) also reported higher weight gains in birds fed their variety of LTI SB compared to those fed the commercial variety, which was not observed in the present study.

Supplementation of raw SB diets with methionine has been found to partially correct growth depression (16, 17, 49, 50, 77). The positive effect of added methionine in the present study was observed in the birds fed the LTI SB diet but not in those fed the conventional raw SB diet (Appendix C) indicating the detrimental effects of higher levels of TI in the conventional beans.

The performance of the birds fed LTI SB indicate that the levels of TI are still high, resulting in impaired growth performance of the birds. Supplementation of the diet with 0.3% DL-methionine improved the feed efficiency and weight gains of the

birds but not to the level of those fed heated full-fat SB diets. Therefore, the TI levels in the LTI SB are not low enough to eliminate heat treatment entirely, but perhaps would require lower temperatures and shorter cooking times than for the SB currently grown.

Overall, the results of the present study have shown that the higher crude protein levels observed in the HP SB than the conventional SB are a result of increased levels of true protein and that the nutrients from these beans were well utilized by the birds. Commercial production of HP SB can be of economic importance as, these beans, when substituted on equivalent protein basis in animal diets will decrease the amount of SB currently needed to formulate a ration, thus, reducing the cost of protein supplementation. On the other hand, further research is needed on the amount of heat treatment required for the LTI SB so as to improve their economic viability compared to the conventional soybeans.

APPENDIX A. Effect of methionine supplementation on the digestibility coefficients (%) and energy metabolizability (%) of the diets fed to broiler chickens

	% Meth	SBM	Autoclaved SB	Autoclaved HP SB	Roasted SB	Raw SB	LTI SB	SEM
Dry matter Digestibility	0	93.45	93.29	93.83	93.37	93.28	93.99	0.14
	0.3	94.52	93.56	94.97	94.14	93.49	93.09	0.14
	Mean	93.98^{ab}	93.43^{b}	94.40^{a}	93.75^{ab}	93.39^{b}	93.59^{b}	0.24
Crude Protein Digestibility	0	89.95	90.01	91.04	89.59	90.26	90.25	0.26
	0.3	92.01	90.53	91.97	91.09	90.65	89.39	0.26
	Mean	90.98^{ab}	90.27^{b}	91.51^{a}	90.34^{ab}	90.45^{ab}	89.82^{b}	0.44
Energy Metabolizability	0	94.70	94.17	94.73	94.38	93.81	94.76	0.15
	0.3	96.50	94.30	95.71	94.89	94.18	94.13	0.15
	Mean	95.37^{a}	94.23^{c}	95.22^{ab}	94.63^{bc}	93.99^{c}	94.44^{c}	0.25

^{abc}Means within a row with same superscripts are not significantly different ($P<0.05$).

APPENDIX B. Effect of methionine supplementation on the TME (MJ/kg, DM) values of the diets

Diet	Meth	TME ± SEM	TME_n ± SEM	N*
SBM	0	14.54 ± 0.21	16.07 ± 0.16	5
	0.3	15.04 ± 0.27	15.26 ± 0.20	3
	Mean	14.79 ± 0.17[b]	15.66 ± 0.13[b]	
Autoclaved SB	0	15.73 ± 0.23	16.87 ± 0.17	4
	0.3	15.43 ± 0.23	16.78 ± 0.17	4
	Mean	15.58 ± 0.17[a]	16.82 ± 0.12[a]	
Autoclaved HP SB	0	15.56 ± 0.12	15.86 ± 0.16	5
	0.3	15.29 ± 0.21	16.02 ± 0.16	5
	Mean	15.42 ± 0.15[a]	15.94 ± 0.11[b]	
Roasted SB	0	15.48 ± 0.23	16.12 ± 0.17	4
	0.3	15.44 ± 0.27	16.13 ± 0.20	3
	Mean	15.46 ± 0.18[a]	16.12 ± 0.13[b]	
Raw SB	0	15.28 ± 0.23	15.63 ± 0.17	4
	0.3	14.49 ± 0.21	15.08 ± 0.16	5
	Mean	14.88 ±0.16[b]	15.35 ± 0.12[c]	
LTI SB	0	14.82 ±0.21	15.50 ±0.16	5
	0.3	14.63 ± 0.21	15.55 ± 0.16	5
	Mean	14.72 ± 0.15[b]	15.52 ± 0.11[b]	

*Number of replicates

[abc]Means within a column with same superscripts are not significantly different ($P<0.05$)

APPENDIX C. Effect of methionine supplementation on the production parameters and the pancreas weights of the broiler chickens

	% Meth	SBM	Autoclaved SB	Autoclaved HP SB	Roasted SB	Raw SB	LTI SB	SEM
Final weight, g	0	631.5	539.2	566.6	572.6	478.7	434.6	5.8
	0.3	627.3	562.1	567.6	590.5	448.1	472.8	5.8
	Mean	629.4[a]	550.7[c]	567.1[bc]	581.6[b]	463.4[d]	453.7[d]	20.67
Weight gain, g	0	501.7	410.5	440.5	445.0	347.3	308.2	5.3
	0.3	497.6	433.6	438.4	460.9	319.0	343.7	5.3
	Mean	499.6[a]	422.1[c]	439.5[b]	452.9[b]	333.1[d]	325.9[d]	18.72
Intake, g	0	734.2	653.2	657.7	689.3	597.8	557.3	21.0
	0.3	724.6	667.5	628.6	687.6	561.7	574.2	21.0
	Mean	729.4[a]	660.3[bc]	643.2[c]	688.4[ab]	579.8[d]	565.7[d]	14.9
Feed Efficiency (Intake/gain)	0	1.46	1.59	1.49	1.54	1.74	1.83	0.04
	0.3	1.46	1.53	1.44	1.49	1.77	1.68	0.04
	Mean	1.46[a]	1.56[b]	1.47[a]	1.51[ab]	1.75[c]	1.76[c]	0.03
Pancreas weight, g DM	0	0.69	0.67	0.63	0.60	0.99	0.73	0.02
	0.3	0.63	0.71	0.67	0.67	0.93	0.84	0.02
	Mean	0.67[c]	0.69[c]	0.65[c]	0.64[c]	0.96[a]	0.78[b]	0.01
Pancreas weight, % body weight	0	0.12	0.16	0.12	0.11	0.22	0.18	0.01
	0.3	0.11	0.13	0.12	0.13	0.21	0.18	0.01
	Mean	0.12[d]	0.14[c]	0.12[d]	0.12[d]	0.21[a]	0.18[b]	0.01

[abcd]Means within a row with same superscripts are not significantly different ($P<0.05$).

APPENDIX D. Effect of methionine supplementation on the body composition of the birds

	% Meth	SBM	Autoclaved SB	Autoclaved HP SB	Roasted SB	Raw SB	LTI SB	SEM
Total Carcass dry matter, g	0	174.8	147.8	150.2	153.7	125.1	113.2	2.72
	0.3	172.6	143.7	147.7	157.2	107.3	119.4	2.76
	Mean	173.8[a]	145.8[b]	148.9[b]	155.5[b]	116.2[c]	116.3[c]	6.86
Protein, g	0	136.1	114.9	117.0	118.8	97.7	88.4	2.02
	0.3	135.0	111.9	114.4	122.0	84.2	92.6	2.07
	Mean	135.5[a]	113.4[b]	115.7[b]	120.4[b]	90.9[c]	90.5[c]	5.10
Fat, g	0	19.4	15.5	16.6	16.7	13.0	11.9	0.51
	0.3	17.1	14.9	15.3	17.8	10.5	12.9	0.53
	Mean	18.2[a]	15.2[b]	16.0[ab]	17.2[ab]	11.7[c]	12.4[c]	1.30
Ash, g	0	19.4	17.4	18.0	18.2	14.5	12.9	0.33
	0.3	20.6	17.0	18.0	18.9	12.6	14.0	0.33
	Mean	20.09[a]	17.2[b]	18.0[b]	18.6[ab]	13.5[c]	13.4[c]	0.82
Carcass Energy, kJ*	0	3939	3292	3384	3429	2790	2531	64.0
	0.3	3821	3196	3276	3547	2377	2667	66.0
	Mean	3880[a]	3244[b]	3329[b]	3487[b]	2583[c]	2599[c]	162.0

[abc]Means within a row with same superscripts are not significantly different ($P<0.05$).
*Calculated using the values of 23.4 and 38.9 kJ/g for protein and fat; respectively (Farrell 1974).

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