

**EVALUATION AND STANDARDISATION OF LABORATORY
METHODS USED FOR
DETERMINING THE DEGREE OF SOYA PROCESSING.**

By

Kedibone Yvonne Modika

A thesis submitted to the Department of Animal and Wildlife Sciences, School of
Agricultural and Food Sciences, Faculty of Natural and Agricultural Sciences

In partial fulfillment of the requirement for the
Masters of Science Degree in **Nutrition**

Supervisor
Prof A. Oelofse
Centre for Nutrition
University of Pretoria

Co-supervisor
Dr D. Palic
University of Novi Sad, Serbia



DECLARATION

I declare that this thesis for the degree MSc Nutrition at the University of Pretoria has not been submitted by me for a degree at any other University.



*There is a time for everything,
and everything on earth has its special season.*

*There is a time to cry and a time to laugh. There is a time to be sad
and a time to dance.*

Ecclesiastes 3:1 & 4

ACKNOWLEDGEMENTS

This research project was funded by the Protein Research Foundation (PRF). This thesis is the product of collaboration between the following laboratories and institutions: Agri Enviro Lab (Bethal) Agricultural Research Council (Irene), CAL (Pelindaba), Department of Agriculture, Soil Fertility and Analytical Services (Cedara), Inspectorate McClahen & Lazar (Johannesburg) Quantum Analytical Services (Malmesbury), Meadow Feeds (Pietermaritzburg), Nutri Feeds (Viljoenskroon), SGS (Johannesburg)

The work was supervised by Prof Oelofse from the University of Pretoria, centre for Nutrition. I appreciate his willingness and assistance as a supervisor.

The study was co-supervised by Dr Palic from the University of Novi Sad. I would like to say thanks for all his assistance and for being my mentor for the first 2 years of my MSc study. I have learned a lot from him. Without his assistance and persistence I would not have grown both academically and socially as I did.

It has been such an honor to be part of the ARC- Professional Development Programme (PDP), I would like to thank the ARC for giving young black people an opportunity to study. Through the programme I found an opportunity to further my studies and gain a lot of experience through research.

There are no words to describe how grateful I am to Dr Lorinda Frylinck, my current mentor at the Agricultural Research Council. In her I found a mother and a mentor. I am grateful to her for standing by my side through finishing writing my thesis and all the guidance, motivation and support she gave me.

I also received continuous, immeasurable support, guidance and help from the ARC-animal nutrition staff.

A lot of thanks to my friend Mr Jacob Marola who has offered his support from the beginning of my studies. Thank you very much for all the support and your comments

on some chapters.

Not forgetting the most important person in my life, my spiritual sister and friend, Simphiwe Malaza, thank you for lifting me up when I would fall and for always reminding me that "with God nothing is impossible".

I also received a lot of support and assistance from my friends, Mr Ntuthuko Mkhize, Mr Kgantjie Moloto and Mrs Karabo Kgarimetsa. Thank you guys, you are very good friends and I learnt a lot from you.

And most importantly, I can not thank my family enough for their moral. I thank them for their love, prayers and their understanding. Except for my mom, I will not mention any names for fear of mistakenly missing some. I am profoundly indebted to my dearest and loving mother, Flocia Modika for giving me the gift of life and instilling the positive values in me since my childhood.

It will be a very big mistake to forget thanking the Lord my creator. Without Him none of this would have been possible and thank you for making me strong through the tough challenges I came through during my studies and thank you for giving me the ,perseverance, strength, comfort and support since the beginning of my studies.

LIST OF ABBREIATIONS

AA	-	Amino acid
ABWG	-	Average body weight gain
ANFs	-	Anti-nutrient factors
ANOVA	-	Analysis of variance
AOCS	-	American oil chemist society
Apo	-	apolipoprotein
BAPNA	-	benzoyl-DL-arginine p-nitroanilide
BWG	-	Body weight gain
Ca	-	Calcium
CIAD	-	Apparent amino acid digestibility
CCK	-	Cholecystokinin
CHD	-	Coronary heart disease
CP	-	Crude protein
CV%	-	Coefficient of variation
DM	-	Dry matter
DNFB	-	2,4-dinitrofluorobenzene
FCR	-	Feed Conversion ratio
Fe	-	Iron
FFSB	-	Full fat soybeans
FFSBM	-	Full fat soybean meal
g	-	Gram
H ₂ O ₂	-	Hydrogen peroxide
H ₂ SO ₄	-	Sulphuric acid
HIV	-	Human immuno virus

KOH	-	Potassium hydroxide
Kg	-	Kilo gram
LDL	-	low density lipoprotein
LSD	-	least significant difference
mg/g	-	Milligram per gram
mg/kg	-	Milligram per kilo gram
mm	-	Millimetre
mg	-	Milligram
mmol/L	-	Millimol per litre
NH ₃	-	Ammonium
NSI	-	Nitrogen solubility index
PBWR	-	Pancreas to body weight ratio
PDI	-	Protein Dispersibility index
PSA	-	Prostate specific antigen
PSKOH	-	Protein Solubility in potassium hydroxide
r	-	Repeatability limit
R	-	Reproducibility limit
RSD _r	-	Repeatability relative standard deviation
rpm	-	Revolutions per minute
S	-	Standard deviation
SA	-	South Africa
SBM	-	soya bean meal/soybean meal
SFs	-	soy flakes
SEM	-	Pooled standard error of the means

TIA	-	Trypsin inhibitor activity
t/h	-	tons per hour
TNBS	-	2,4,6-trinitrobenzene sulphonic acid
TVP	-	textured vegetable protein
UAI	-	Urease activity index
WISHH	-	World Initiative for Soy in Human Health
°C	-	Degree Celsius
%	-	percentage
Δ pH	-	Change in pH
>	-	Greater than/above
<	-	Less than or below
μ g	-	Micro gram
\approx	-	Approximately



INDEX

Declaration	i
Acknowledgements	ii
List of abbreviations	v
Index	viii
List of figures	x
List of tables	xi
Abstract	1
Chapter 1: Introduction	3
Chapter 2. Literature review	6
2.1. Anti-nutrient factors	6
2.2. Methods of processing Full Fat Soybeans (FFSB)	9
2.3. Assessing heat treatment	14
2.4. Quality control	15
2.5. Use of soybeans in animal nutrition	21
2.6. Use of soybeans in human nutrition	25
Chapter 3: Materials and methods	29
3.1 Processing of full fat soybeans (FFSB)	29
3.2. <i>In vivo</i> trials with broilers	29
3.3. <i>In vitro</i> analysis of FFSB quality	30
3.3.1. Single laboratory study: Urease Activity Index	30
3.3.2. Protein solubility in Potassium hydroxide (PSKOH)	31
3.3.3 Protein dispersibility index (PDI)	34
Chapter 4: Results	37
4.1. <i>In vivo</i> trials with broilers	37
4.2. <i>In vitro</i> analysis of FFSB	39
4.2.1. UAI method using Δ pH procedure	39
4.2.2. UAI method using released NH_3 procedure	40
4.2.2. Protein solubility in KOH	41



4.2.3. Protein Dispersibility index	47
Chapter 5: Discussion	52
5.1. In <i>vivo</i> trials with broilers	52
5.2. Urease activity index (UAI)	52
5.2.1. UAI using the Δ pH procedure	52
5.2.2. UAI method using released NH_3 procedure	53
5.3. Protein solubility in potassium hydroxide (PSKOH)	54
5. 4. Protein Dispersibility Index (PDI)	56
5.5. Precision parameters of PSKOH and PDI procedures	57
Chapter 6: Conclusions and recommendations	58
Chapter 7. Reference	61

LIST OF FIGURES

FIGURE	PAGE
2.1. Photos showing the difference between adequately extruded (left) and inadequately extruded (right) FFSB using dry extrusion.	12
4.1. Average daily body weight gain in the period from day 0 to day14 and feed conversion on day 14, of broiler chickens fed FFSB processed at different temperatures.	38
4.2. Urease Activity Index (UAI) determined by pH-difference and NH ₃ -released procedures in relation to body weight gain of chickens in the <i>in vivo</i> trials.	41
4.3. The influence of temperature of processing on PSKOH values.	44
4.4. Relationship between PSKOH and the average daily weight gain in trials with broilers.	46
4.5. Relationship between PSKOH values and feed conversion ratio of broilers in the <i>in vivo</i> trials.	46
4.6. The influence of processing temperature on PDI values.	48
4.7. Relationship between PDI values and average daily weight gain (g), in trials with broilers.	50
4.8. Relationship between PDI values and feed conversion ratio (FCR) in trial with broilers.	51

LIST OF TABLES

TABLE	PAGE
2.1. Comparison of nutritional content of different types of beans	6
2.2. Urease activity index values for determining the degree of soy processing	16
2.3. PSKOH values for determining the degree of soy processing at different temperatures	17
2.4. Trypsin inhibitor activity values using the TIA method	19
2.5. Amount of cresol red dye absorbed by heat treated soya beams	20
4.1. Means of average daily weight gain (ADWG) in the period from day 0 to day 14 and feed conversion ratio (FCR) on day 14, of broiler chickens fed FFSB processed at different temperatures.	37
4.2. Relation between the temperature of extruding and the <i>in vivo</i> assessment of the degree of FFSB processing.	38
4.3. Results of the UAI using the Δ pH procedure obtained by Lab A.	39
4.4. Results of the UAI using the Δ pH procedure obtained by Lab B.	39
4.5. UAI of FFSB samples processed at different temperatures, determined by Δ pH and NH_3 -released procedures.	40
4.6. Results of analysis of FFSB sample done by two laboratories, two analysts at each laboratory and in seven replicates, using the	42

original PSKOH method (Araba and Dale, 1990).

4.7.	Results of determination of PSKOH in FFSB samples processed by dry extrusion at different temperatures.	43
4.8.	South African ranges for describing the degree of FFSB processing using PSKOH method.	44
4.9.	Precision parameters of the PSKOH method	45
4.10.	Results of determination of PDI in FFSB samples processed by dry extrusion at different temperatures.	47
4.11.	Precision parameters of PDI method	49
4.12.	South African ranges for describing the degree of FFSB processing using PDI method	50

ABSTRACT

The use of full fat soybeans (FFSB) in animal feeds has, to date, been limited due to the presence of anti-nutritional factors (ANF). It is, therefore, increasingly recognized that, if the full potential of full fat soybeans for the use in diets is to be realized, reliable analytical procedures must be available for the quality control of processed FFSB which would enable feed manufactures to determine the degree of soybean processing. Results of currently available analytical techniques vary widely between laboratories, causing uncertainty and confusion among soybean processors, feed manufacturers and end users.

A collaborative study was conducted to standardize a number of existing analytical procedures used for determining the effects of heat treatment on FFSB and to generate South African ranges for standardized laboratory procedures. Raw soybeans, in a mixture of cultivars, were processed by dry extrusion at eight different temperatures (110°C, 120°C, 127°C, 136°C, 140°C, 145°C, 151°C and 164°C). *In vivo* trials were conducted on broiler chicks which were fed the eight extruded FFBB. Their performance was monitored with regard to average daily gain (ADG) and feed conversion ratio (FCR).

The Protein Solubility in KOH (PSKOH) and the Protein Dispersibility index (PDI) procedures were used for standardization in an inter-laboratory study with the participation of ten South African analytical laboratories.

Statistical analysis of the *in vivo* trials with broilers showed no significant difference ($P>0.05$) between broilers fed FFSB processed at 136 °C, 140 °C and 145 °C. In addition, samples processed at those temperatures (136 °C, 140 °C and 145 °C) showed the best chick performance with regard to average daily gain (ADG) and the feed conversion ratio (FCR). There was a significant difference ($P<0.05$) between broilers fed FFSB processed at 110 °C, 120 °C and 120 °C as well as those fed FFSB processed at 151 °C and 164 °C.

In vitro results showed that the PSKOH and PDI values corresponding to temperatures which showed the best chicken performance were between 67-77% and 10.3-8.5 index units respectively. Therefore, the South African ranges for describing the degree of soybean processing using the PSKOH method are 66-77% with repeatability and reproducibility limits of 3.5 and 10.9 respectively and, when using the PDI method, are 8.5-10.3 index units with repeatability and reproducibility limits of 2.1 and 7.7 respectively.

A very good correlation was established between the animal production parameters and the PSKOH values, while a poor correlation between animal production parameters (ADWG and FCR) and PDI values was established.

The PSKOH method was found to be the most reliable method for FFSSB quality control under standardized South African conditions.

CHAPTER 1

Introduction

The soybean is by far the most important oilseed crop in the world and is grown for a number of industrial and agricultural uses. Soybeans have been used in human and animal nutrition because of their favourable agronomic characteristics, relatively low price, high quality and quantity of protein and oil (Liu, 2000) as well as their important functional properties for the development of different types of foods for humans (Traina and Breene, 1994). Moreover, consumers' awareness of the reported beneficial effects on health has increased their consumption (Machado, 2008). Soybeans contain highly valuable proteins and oils (on average ranging from 39-41% crude protein and 18-21% oil) which make them good feed alternatives to animal proteins and oils. According to their digestibility and amino acid composition, soybean proteins are very similar to proteins of animal origin, except that the sulphur amino acids (cystein and methionine) contents are limited (Anderson and Wolf, 1995).

Nevertheless, raw soybeans contain several factors with anti-nutritional properties (ANFs) and must be processed prior to inclusion in animal and human diets. Anti-nutritional factors contained in soya beans may cause unfavourable physiological effects (Buttle et al. 2001; Vasconcelos et al. 2001) and may decrease weight gain in animals (Palacios et al. 2004). This is an indication of the indigestibility of raw soybeans for both animals and humans. Proper processing is required to inactivate the ANFs to an acceptable level without reducing the availability of nutrients (Van der Poel et al. 1995). Processing of the raw full fat soybeans (FFSB) by means of heat and mechanical treatment destroys the anti-nutrients contained therein, thus making the beans fit for use in monogastric diets for example in chickens, pigs, pets as well as humans. The problem relating to the availability of the amino acids in the heat- treated beans arises due to the fact that only an optimum level of heat treatment will produce the maximum availability of the amino acids to the animal. Under-processing of the FFSB limits amino acid availability due to the partial destruction of the anti-nutrient factors. Over-processing, on

the other hand, decreases the amino acid availability as a result of the Maillard reaction that occurs between aldehyde groups of sugar and free amino groups (Stern, 1989).

The provision of amino acids, either free or as protein, contributes a substantial amount to the cost of animal feedstuffs. The objective of any nutritionist is, therefore, to formulate diets that will provide the correct amounts of nutrients required by the animal at the lowest possible cost. This implies that dietary formulation must be done on an available amino acid basis in order to optimise the dietary amino acid levels, thereby minimizing cost. Because of the effect of processing on the amino acid availability of full fat soybeans, nutritionists using FFSB in diets have been forced to compensate for the possibility of reduced amino acid availability by over-formulating diets on a total amino acid level. It is, therefore, increasingly recognised that, if the full potential of FFSB for use in animal diets is to be realised, reliable analytical procedures must be available for the quality control of processed FFSB, which would enable feed manufacturers to determine the exact degree of the processing of the soybeans. The results would, in turn, provide an estimate of the availability of the amino acids contained in FFSB for use in feed formulations.

It has been clearly illustrated that the results of the analysis of the same sample of soybeans on the content of anti-nutrients obtained by currently available analytical techniques vary widely between laboratories, causing uncertainty and confusion among soya processors, feed manufacturers and end-users (Davies, 1998; Palic and Groove, 2004). It is, thus, of the utmost importance to evaluate and standardize the available laboratory procedures for determining the degree of soybean processing.

In humans, soy foods are known to have a number of health benefits, ranging from the well-documented cholesterol-lowering effects of soy protein, which may lower the risk of cardiovascular diseases, (Lammersfeld et al. 2009) to the potential for decreasing bone loss in healthy, postmenopausal women (Greendale et al, 2002) .

The soybean can also be processed to produce a texture and appearance similar to many

other foods, and they are the primary ingredient in many dairy product substitutes (for example soy milk, margarine, soy ice cream, soy yoghurt, etc.) and meat product substitutes (for example veggie burgers). The oil from processed soybeans may be refined for cooking and other edible uses. Furthermore, research by the World Initiative for Soy in Human Health (WISHH) (<http://www.wishh.org/nutrition/overview.html>), indicated that soy foods may be ideally suited to assist in meeting the nutrient requirements (high-quality protein and calories) required by people infected with the Human Immuno deficiency virus (HIV).

Soybeans are also major ingredients in livestock feed. Almost all soybeans are processed for their oil and protein for the animal feed industry as protein is an essential key ingredient of animal feeds. It is necessary for animal growth, body maintenance, the production of young and the output of such products as milk, meat and eggs. The high-protein fiber (that remains after processing has removed the oil) is toasted and added to animal feed for poultry, pigs, cattle, pets and young ruminants.

The quality of human nutrition is inextricably linked to the quality of the livestock products consumed which, in turn, is significantly influenced by the nature of the raw materials (protein sources) eaten by the animal. This fact can be used as a tool in the development of the following strategy: if non-ruminant species are fed the appropriate nutrients, the desired products (for example eggs and meat) can be obtained for human use (Greendale et al, 2002). The nutritional characteristics of meat and egg products of livestock industries can be enhanced for consumer health by using feed that is high in protein and essential fatty acids especially oilseed crops i.e. soybeans and groundnuts.

The aims of this project are, therefore:

1. To evaluate a number of existing analytical procedures for determining the adequacy of FFSSB processing and for its quality control.
2. To standardize, through an inter-laboratory study, methods which have passed the evaluation stage.
3. To establish South African ranges for standardized methods for describing under-, adequate- and over-processed FFSSB.
4. To establish South African values for the repeatability and reproducibility of standardized methods through inter-laboratory studies and to consequently recommend them to South African feed industries.

CHAPTER 2

Literature review

Legumes play an important role in the diets of many regions throughout the world. In western countries beans play a minor role in the diet, except for the fact that they are excellent sources of protein, dietary fiber and a variety of micronutrients and phytochemicals. Soybeans are unique among the legumes as they are a concentrated source of isoflavones and have been referred to as the miracle bean for their high nutritive value as shown in Table 2.1 where they are compared with other legumes (Messina, 1999). According to Table 2.1, a serving of 90 g of soybeans contains 14.3 g of protein and 7.7g of fat, the same serving contribute about 38% of energy as protein and 47% of energy as fat. Soy protein is a rich source of many amino acids that are deficient in most cereal grains commonly fed as energy sources to poultry and swine. Soybean is often referred to as the gold standard with which all other protein sources are compared (Bruce, 2006).

Table 2.1: Comparison of soybeans with other legumes.

TABLE 1
Nutrient content of selected beans (serving size is ≈90 g or ½ c boiled)¹

Bean	Protein	Fat	Dietary fiber	Riboflavin	Folate	Ca	Zn	Fe
	<i>% of energy</i>		<i>g</i>	<i>µg</i>	<i>µg</i>	<i>mg</i>	<i>mg</i>	<i>mg</i>
Black	7.6, 27	0.5, 4	3.6	50	128	24	0.96	1.80
Baby lima	7.3, 25	0.4, 3	3.9	50	137	26	0.94	2.18
Chickpea	7.3, 22	2.2, 15	2.9	50	141	40	1.26	2.37
Kidney	7.7, 27	0.5, 4	3.2	50	115	25	0.95	2.60
Lentil	9.0, 31	0.4, 3	4.0	75	179	19	1.25	3.30
Navy	7.9, 24	0.5, 3	3.3	55	128	64	0.97	2.26
Soybean	14.3, 38	7.7, 47	0.9 ²	25	47	138	0.99	4.42
Pinto	7.0, 24	0.5, 3	3.4	80	147	41	0.93	2.24
Great northern	7.4, 28	0.4, 3	3.0	50	91	61	0.78	1.89
Lima	7.4, 27	0.4, 3	6.8	50	78	16	0.80	2.25

2.1. Anti nutrient factors

The nutritive value and protein digestibility of soybeans is generally poor due to the presence of ANFs (Machlachlan, 1993). ANFs are compounds that interfere with the intake, digestibility, availability or metabolism of nutrients in animals. The effects of these factors on monogastric animals may cause unfavourable physiological effects (Buttle et al, 2001) of the digestive,

absorptive, protective or secretory systems and affect cellular proliferation and turnover (Vasconcelos and Oliviera, 2004) and, in some cases, decreased weight gain (Palacios et al, 2004). The most important factors that are of concern are protease inhibitors and haemagglutinins/ lectins. Protease inhibitors and haemagglutinins/ lectins are heat labile and their presence in raw soybeans may be significantly reduced by processing methods of which thermal treatments such as soaking, boiling, autoclaving, extrusion etc are examples (Vidal-Valverde et al, 2002).

2.1.1. Protease inhibitors

Protease inhibitors are molecules that block the function of enzymes that degrade proteins, the main ones being the trypsin and chymotrypsin inhibitors. Raw soybeans contain larger amounts of these inhibitors and they reduce the activities of both trypsin and chymotrypsin. The inactivation of these enzymes in the gut of monogastric animals induces the endocrine cells in the mucosa to release more cholecystokinin (CCK) which, in turn, stimulates the pancreas to produce more digestive enzymes. Specifically feeding more protease inhibitors to rats and chickens results in hypertrophy of the pancreas (Huisman and Tolman, 1992).

Trypsin and chymotrypsin inhibitors are heat sensitive and their activities in raw soybeans may be reduced to insignificant levels by adequate heat processing making them safe to include in diets for non-ruminants (Pieterse, 2000). Raw soybeans normally have a trypsin inhibitor activity (TIA) of between 20 and 35 mg/g, therefore, it is necessary to process them prior to feeding in order to denature the trypsin inhibitors in order that the residual TIA is below the currently recommended threshold of 4 mg/g. This is the level assumed to have the minimum adverse affects in birds although the basis for such a recommendation is questionable according to Clarke and Wiseman (2007). Although protease inhibitors can reduce the digestive efficiency when the raw beans are fed to animals, it is likely that these anti-nutritive factors have a natural function within the bean by protecting it against bird attacks or microbial invasion.

Haemagglutinins/ lectins

Haemagglutinins are compounds which agglutinate red blood cells and are members of a larger group of compounds named lectins. Lectins are carbohydrate-binding glyco-proteins which are

ubiquitous in nature (Vasconcelos and Oliveira, 2004). In experimental animals fed on diets containing plant lectins, the evident symptoms are loss of appetite, decreased body weight and eventual death (Duranti and Gius, 1997; Lajalo and Genovese, 2002).

Many plant lectins have been found to be resistant to degradation by proteases, both *in vitro* (Carbonaro et al, 2000) and *in vivo* (Vasconcelos and Oliveira, 2004). This property makes lectins bind to the epithelial cells which express carbohydrate moieties which are recognized. Once bound to the digestive tract, the lectin can cause dramatic changes in the cellular morphology and metabolism of the stomach and small intestines and activate a cascade which alters the intermediary metabolism.

An additional effect of lectins is that it stimulates the proliferation of bacteria in the intestinal lumen of monogastric animals (Shulze et al, 1995). The more specific reason for this is not clear but it may be related to an increased nutrient availability and an increase in epithelial cell turnover which, in turn, may increase the number of potential binding sites for bacteria on epithelial cells (Vasconcelos and Oliveira, 2004). Lectins are relatively unstable in heat, therefore heat processing appears to be an effective means to inactivate them (Calvalho and Sgabieri, 1997).

The finding that plant lectins exert significant toxic or growth-inhibitory effects on insects suggests that this plays a role in plant defence against insect attack (Peumans and Van Damme, 1995).

2.1.3. Saponins

Saponins are steroids that are present in many feedstuffs. The feedstuffs containing saponins have a bitter taste, they can form foam in aqueous solutions and hemolyse red blood cells (Machlaclan, 1993). The haemolytic properties of saponins are generally attributed to the interaction between the saponins and sterols of the erythrocyte membrane. As a result the membrane bursts, causing an increase in permeability and a loss of haemoglobin (Sparg, 2004).

Baumann et al. (2000) reported that saponin-lysed erythrocytes do not reseal and that saponin

damage to the lipid-bilayer is irreversible. Although toxic to cold blooded species, if taken orally by warm-blooded species, saponins have only a weak toxicity (Huisman and Tolman, 1992). Due to their toxicity to various organisms, saponins can be utilized for their insecticidal, antibiotic, fungicidal, and pharmacological properties.

2.1.4. Urease

Urease is an enzyme that converts urea to ammonia. Raw soybeans also have variable urease activity, which is not likely to be of great nutritional significance other than as an indirect assessment of the degree of adequacy of processing (Monary, 1996). The urease assay is based on the release of ammonia from urea by the residual urease enzyme in soybeans. The destruction of the urease enzyme through heating is correlated to the destruction of trypsin inhibitors and lectins. This assay is useful for detecting the undercooking of soybean meal but is of limited use for detecting overcooking (Araba and Dale, 1990; Parsons et al, 1991).

2.2. Methods of processing full fat soybeans (FFSB)

Processing of the beans is necessary prior to the inclusion to animal diets because the anti-nutritional factors contained reduce the digestibility and utilization of amino acids (AA) of poultry, swine and immature ruminants (Wiseman, 1986) thus making the nutritive value of this feed low. If fed raw, FFSB may adversely influence animal health. Thermal processing, for example toasting and extrusion cooking, is frequently used to increase the nutritional value of soya bean meal SBM (Liener, 1994). In general, process conditions such as temperature, moisture content, screw-speed, shear forces and duration of heating will determine the effectiveness of inactivation of the heat-labile anti-nutrient factors (ANF) and the degree of denaturation of the storage proteins in SBM (Marsman et al, 1997)

The removal or inactivation of the anti-nutritive factors described above is considered important if they are present at higher levels (Wiseman, 1986). In addition to inactivating the anti-nutritional factors, the processing treatment improves the taste of the end product and increases the use of the energy and proteins contained in the beans. The improved taste results from the heat applied to the beans which trigger the release of additional aromas and flavours which encourage domesticated mammals to eat more feed (Murray, 1987). Part of this improvement could be due to the inactivation of the lipoxygenase in the beans, promoting the quality and

storage life of the end product. However, the exact improvement depends on the processing method used, the processing conditions and the species for which the feedstuff is intended (Leeson and Atteh, 1996).

Different technological processes have been developed for the treatment of FFSB to inactivate the anti-nutritional compounds of this valuable protein source (Senkoylu et al, 2005). They include: cooking, expansion, extrusion, flaking, jet sploding, micronization, microwave and roasting (Barbi, 1996). Factors which vary from one process to another are time, temperature, pressure, humidity, exposed surface, particle size and the type of energy used.

2.2.1. Cooking

This is a relatively simple method to use. The raw beans are immersed in water and cooked for between 30 and 120 minutes. They are then dried mechanically or alternatively spread out on the ground. The beans are then consumed ground, whole or crushed (Barbie, 1996). This processing method is time consuming and expensive. It can only be done in batches and is, therefore, rather inefficient.

2.2.2. Expansion

This process involves preparing and treating the given product using pressure and hot steam (Pipa and Frank, 1989). Temperatures range from 70°C to 170°C and the time of processing from five to 15 seconds. The essential components of an expander are the supply and preconditioning units, the hollow tube complete with screws and vapour injection valves, the hydraulic system cone which regulates the pressure level and the expander shaft which is situated inside the guide tube and is powered by a motor.

2.2.3. Extrusion

An extruder is a high temperature, short processing time bioreactor that can transform a variety of ingredients to intermediate or finished products. The extrusion process involves pushing the material through a series of restriction rings by means of a system of coils, which creates high pressure (30 – 40 bars) and high temperature as the result of the friction and movement of the processed beans (Ristic, 1999). In this process, soybeans are fed into an extruder barrel where a

central revolving shaft forces the beans through the extruder. This technique creates sufficient heat through friction to destroy the anti-nutritional factors.

During extrusion, denaturalization causes the breaking of the hydrogen bridges and the disulphide bonds responsible for the secondary and tertiary structure in proteins resulting in irreversible protein denaturation. This process probably increases the accessibility of proteins to enzymatic breakdown and thus improves *in vitro* digestibility. Extrusion also changes the content, composition and physiological effects of dietary fiber (Marsman, 1997).

The quality of the end product is affected by (Serrano and Valla, 1999):

1. Initial size of the bean particle.
2. Extruder supply speed and duration of treatment.
3. Pre-conditioning moisture and temperature levels.
4. Residence time, percentage of moisture added and temperature reached within the extruder unit.
5. Geometric configuration of the worm gear and bolts.
6. Size and shape of the exit opening of the extruder unit.
7. Dwell time, temperature and air velocity in the dryer.

Two extrusion models are currently available on the market namely dry and moist extrusion.

2.2.3.1. Dry extrusion

The dry extrusion process refers to extrusion without the prior conditioning of raw ingredients and can only be used with ingredients that are rich in fat which will then provide sufficient lubrication for the matrix. This technique developed during the 1960s subjects the ground beans to pressure inside a thick-walled barrel powered by a worm gear. The pressure reaches a level of 35-40 atm and the heat produced by the friction between the beans and the walls of the cylinder heats and sterilizes the product. This treatment takes 120 to 30 seconds and reaches temperatures of some 120°C to 165°C, depending on the machine used (Perilla et al, 1997; Wijeratne, 2000). This treatment is utilized in processing low moisture, highly expanded starch products and in processing whole soybeans for both the food and feed industries.

The main advantage of this treatment being the friction that can cause excessive temperatures which, in turn, influence the level of the available lysine. Dry extrusion is more popular than moist/wet extrusion as it is less expensive and farmers can use it on-site to treat their own soybeans (Wijeratne, 2000). Figure 2.1 shows the difference between properly extruded and improper extruded soya beans using an “Insta-pro” extruder.

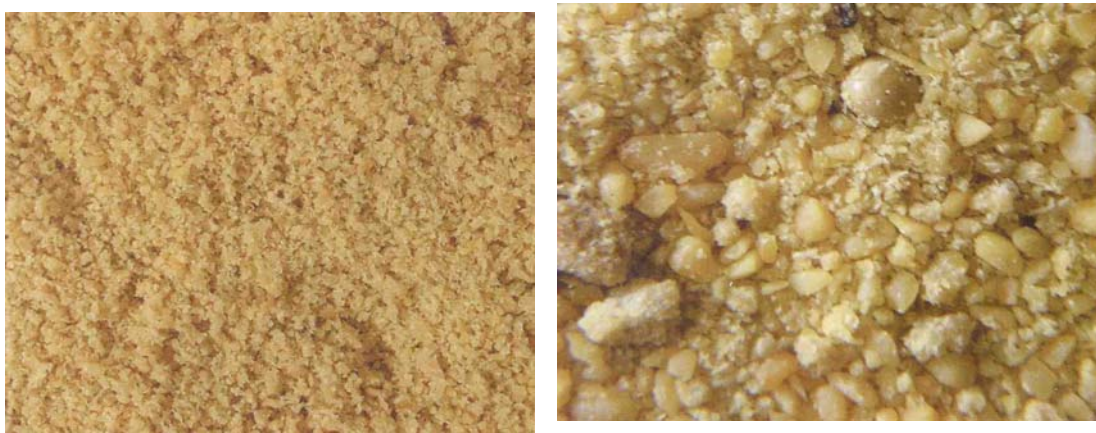


Figure 2.1: Photos showing the difference between adequately extruded (left) and inadequately extruded (right) FFSSB using dry extrusion.

2.2.3.2. Moist/wet extrusion

Moist/wet extrusion involves prior conditioning and the injection of hot steam into the extruder unit. An additional drying stage is required once the treatment is complete. This equipment is more expensive than that used for dry extrusion. On the other hand, it has a superior production capacity of up to 9,000 t/h; (Thomason, 1987) and is more effective in terms of denaturing the anti-nutritional factors (Harper, 1978).

A typical wet extrusion plant comprises a seed filter or cleaner, a preconditioning unit, a supply unit, a hollow tube complete with bolts and steam injection valves and/or several inner shafts powered by a motor, a drier and a cooler. Within the preconditioning unit, water vapor is added to take the mixture to a moisture level of 24 - 28% and a temperature of 80°C - 90°C. The size of the extruder shaft depends on the distance concerned so as to obtain an optimally homogeneous mixture and to ensure that the pressure applied to the mixture is sufficiently high. The pressure level inside the tube is approximately 30 atm, so that the water does not evaporate

in spite of the high temperatures it can reach. Pressure is then swiftly blown to the mixture when it leaves the extruder, this leads to the rapid evaporation of the water and, subsequently, the expansion of the product. As a result the oil cells burst releasing the oil. This oil is then absorbed again as the mixture is cooled and remains locked inside. The bean is then dried to reduce its moisture level.

2.2.4. Flaking

The flaking method is a hydro-thermal treatment based on an injection of low pressure steam into a conditioner. On the exit side the heated soybeans are flaked by forcing them through two rollers (Monary, 1996). In this process, dry heated air (300°C) is blown on to the beans, the moisture within boils and the seeds swell producing a characteristic “popping” noise. The flaking machine consists of three mild steel rollers (knurled and chromium plated surface), mainframe, hopper, stand and collecting tray and drive mechanism. The three rollers have differing diameters and press and elongate the moist grains. This method is suitable for producing flakes from soybean, sorghum, maize and Bengal gram.

2.2.5. Jet-sploding

Jet-sploding is a dry heat technique in which the beans are sent through a stream of air pre-heated to 315°C rather than being directly exposed to the flame. The molecules vibrate in the heat, the intracellular water cooks and the grain heats up from the inside out, reaching inner temperatures of 90°C to 95°C. The seeds swell and pop (Thomason, 1987).

2.2.6. Micronization

Micronization is a dry-heat process using infrared electromagnetic short waves of 1.8-3.4 microns, produced by burning industrial propane over ceramic tile or nichrome wire elements to heat the grains (Lawrence, 1973; McNab and Wilson, 1974). The micronizer consists of a feeder, vibrating unit and infrared radiation source. For the process to take place, infra-red rays must strike an absorbent material. Infrared radiation is not entirely a surface heating method but the energy rather penetrates the kernel in a non-contact mode. Two conventional types of infrared radiators are commercially used, namely, electric radiators and gas-fired radiators, the latter mainly available to the feed meal industries. For conduction heating, the material is

heated at the surface and the heat is then transferred to the interior of the material. Solids are directly contacted with the energy source (Wiriyampaiwong et al, 2004).

2.2.7. Roasting

Roasting is a process that has its origins in prehistoric times, but has been modified somewhat by industry to meet today's needs. Several models exist including conventional "dry" systems, similar to those used to dry out cereals, and moist heat systems. The heat used for the latter can be generated by an oven, a coal burner or directly by a flame. The temperature reached varies between 110 °C and 170 °C depending on the equipment used (Katic et al, 1996). The simplest of these processes involves the direct and intensive application of dry heat (roasting) for around 20 seconds. This process consists of an intense dry heating of the beans, at the end of which they have lost 30% of their initial moisture and their temperature varies from 110 °C-168 °C according to the type of machinery used. The easiest roasting method is carried out in a common drier. Other straight forward but less common methods use heating on solid carriers (sand, salt, ceramic tiles). It is important to divide the different beans into size categories before roasting in order to prevent the overheating of the smallest ones.

2.3. Assessing heat treatment

Heat treatment is an important step in soybean processing. One of the major concerns is: what happens when FFSB are under- or over-processed? Is the one more detrimental than the other? To define under- and over-processing is easy in theoretical terms but not in practical terms.

Under-processing will not sufficiently eliminate trypsin inhibitor activity and will reduce protein digestibility. Over-heating, however, will denature proteins and reduce the availability of amino acids especially the essential ones.

The following mechanisms are involved in under- and over- processing:

Under-processing:

In this process part of the anti-nutritional factors are not destroyed and this leads to a reduction in the use of amino acids. The residual trypsin inhibitor mediates its effects via the digestive

processes, effecting both endogenous and exogenous amino acid losses. It also binds and inactivates the pancreatic enzyme, trypsin. The result is that protein digestibility is reduced and swelling of the pancreas occurs (compensated by the production of additional enzymes - trypsin and chymotrypsin). This lost or bound trypsin is also rich in sulphur amino acids which further reduce the protein status of the animal.

Over-processing:

In this process, the proteins are denatured and the amino acid availability is reduced. When proteins are exposed to excessive heat treatment or over-processing, the negative effects that cause reduced analytical concentrations and reduced digestibility of amino acids occur for lysine and cystine. Most of the other amino acids are not affected by excessive heat treatment or over-processing. Thus the reduction in protein quality of soybeans as a result of over-processing is primarily due to the combination of the destruction of lysine or cystine and the reduced digestibility of the lysine and cystine that is not destroyed. These effects on lysine and cystine may be largely explained by the Maillard reaction in which proteins are heated in the presence of certain carbohydrates, the sugars (such as xylose and glucose) complex with free amino groups, especially the epsilon amino group of lysine. The sugar and amino acids enter into a series of reactions and, as a consequence, the availability of amino acids is reduced (Anderson-Haferman et al, 1992).

2.4. Quality control

The quality of FFSB is related to heat treatment. Quality control is an extremely important element during soya bean processing. The objective of quality assurance is to prevent and minimize the deterioration of the quality of the products during processing. The main objective of heat processing of the FFSB used for inclusion in diets for poultry and pigs is to achieve an optimum balance between the degradation of the anti-nutritional factors and the maintenance of amino acid availability.

Proper heat treatment results in a product with a low trypsin inhibitor activity and high nutritional value of soybean meal products. The quality of the end product can be affected by three key factors which are the processing conditions, the amount of soy hulls and their origin (Shin, 2002). Fellowship

According to Monary (1989) and Bruce et al, (2006), the following methods are commonly used for determining the degree of FFSB processing: the urease index, the protein solubility in 0.2% KOH, the protein dispersibility index, the nitrogen solubility index, the trypsin inhibitor activity, the cresol red test and the lysine availability. The objectives of these tests are to examine whether:

1. The anti-nutrient factors have been adequately reduced.
2. The protein quality has been maintained.
3. As much oil as possible has been released from the cells (Monary, 1996).

2.4.1. Urease index

The urease index is the test most commonly used to evaluate the quality of the soybean processing treatment. This method determines the residual urease activity of soybean products as an indirect indicator to assess whether the anti-nutritional factors such as trypsin inhibitors that are present in soybeans have been destroyed by heat processing. This test measures the increase in the pH consequence of the release of ammonia into the media arising from the breakdown of urea by the urease present in soybean products (AOAC Official Methods, 1997a). All overheated samples yield urease indices below 0.05 but that does not imply that all samples with urease tests below 0.05 have been overheated (Waldroup et al, 1985). According to Garlich (1988), the following UAI values (Table 2.2) are used to estimate the degree of processing:

Table 2.2: Urease activity index values for determining the degree of soya processing (Garlich 1988)

FFSB	UAI (ΔpH)
Under-processed	> 0.20
Adequately – processed	0.05 - 0.20
Over-processed	< 0.05

Method assessment

When Δ pH is used as an indicator of FFSB processing, the data may be influenced by whether or not the FFSB had been previously treated with organic acids, preservatives or sterilizing

agents. Thus the value of UAI as a reliable indicator of the adequacy of FFSB is questionable. Furthermore, a UAI value of “zero” does not necessarily indicate that the FFSB are over-processed, which has been shown in trials with chickens (Araba and Dale, 1990a). Consequently, the UAI is suitable only for determining under-processed FFSB and its use, therefore, is limited.

2.4.2. Protein solubility in 0.2% potassium hydroxide (PSKOH)

This method consists of determining the percentage of protein that is solubilised in a potassium hydroxide (KOH) solution (Araba and Dale, 1990). The solubility values have been correlated with growth rates in poultry (Araba and Dale, 1990) with a clear decline in growth performance for solubility values below 71% and those with values above 85%. The following solubility values in Table 2.3 are commonly used (Monary, 1989):

It was observed by Shini (2002) that a lower KOH value means lesser digestible amino acids or lesser amino acid availability due to the Maillard reaction and that a higher KOH value means more digestible amino acids but a lesser breakdown of trypsin inhibitors present in soybeans, leading to a lower digestion and absorption of amino acids.

Table 2.3: PSKOH values for determining the degree of soya processing (Monary, 1989)

FFSB	Protein solubility in KOH (%)
Under-processed	> 85
Adequately-processed	71 – 85
Over-processed	< 70

Method assessment

The PSKOH method is relatively inexpensive and simple to perform. Unlike the UAI method, it can be used to determine all degrees of soybean processing from under- to over-processed. The original Araba and Dale (1990) method showed a low reproducibility between South African laboratories. This has been modified by Palic (2005). Parsons et al, (1991) evaluated PSKOH as an indicator of protein quality for chickens and pigs and concluded that protein solubility in KOH is a good indicator of *in vivo* FFSB quality.

2.4.3. Protein dispersibility index

The protein dispersibility index (PDI) is another method for distinguishing the quality of soybean meal (SBM) for feed use. The PDI is simply a measure of protein solubility in water, since the protein solubility of SBM decreases as the heat exposure increases. It was reported by Batal et al, (2000) that the PDI method has proven to be especially useful in the determination of the degree of under-heating of soybean meals to remove anti-nutritional factors. Current recommendations for meals that are considered adequately-heat processed are those with PDI values between 15 and 28%.

The PDI of processed FFSB is a very important quality index, particularly if relative tests are run in relation to the animals to which feeds containing FFSB are destined. Kratzer et al, (1990) demonstrated that flaking FFSB at 121°C for increasing lengths of time brought about a reduction in the contents of protein dispersibility. Studies by Batal et al, (2000) demonstrated the usefulness of the PDI as a quality indicator of SBM. The body weight gain and feed efficiency of broilers exhibited a high positive correlation with the reduction of PDI values.

Method assessment

The PDI method uses a special blender at a speed of 8500 rpm, which makes the method the simplest to perform of all the methods for processed FFSB quality control. Work by Batal et al. (2000) has shown that the PDI procedure demonstrates more consistent results than the urease or PSKOH methods. While the UAI and TIA rapidly decline to “zero” (at which point the FFSB may or may not be over-processed) PDI values do not approach “zero” even with severe over-processing. Batal et al. (2000) reported that the PDI displayed the most constant response to the heating of FFSB and that it may better indicate the processed FFSB quality compared to other methods. For that reason, preference has been given to the PDI over the NSI method.

2.4.4. Nitrogen solubility index (NSI)

The nitrogen solubility index is a measure of the degree of the solubility of the soybean protein in a water solution. This method uses lower quantities of the sample in water and a lower agitation speed of 120 rpm for 120 minutes at 30°C (AOCS Official Methods, 1997b). It was previously reported that the water solubility of raw soybean is approximately 90%, but tends to

decrease in relation to the temperature and duration of heating. Globally accepted NSI values for well-processed soybeans range from 10 to 11%.

2.4.5. Trypsin Inhibitor activity (TIA)

The trypsin inhibitor activity (TIA) method involves the extraction of the trypsin inhibitor from the sample of FFSB at pH 9.5. This test determines the presence of trypsin inhibitors partially bound or in a soluble form (Valdebouze et al, 1980; Smith et al, 1980; Kourtera et al, 1987). This procedure is based on the release of spectrophotometrically determined para-nitroaniline by the tryptic hydrolysis of benzoyl-DL-arginine p-nitroanilide (BAPNA) (Hammerstrand et al, 1981). The test makes use of monoclonal antibodies to locate and bind the trypsin inhibitors which can distinguish between the two types of principal anti-trypsin factors with greater sensitivity than is normally achieved with other methods. The trypsin inhibitor content may be expressed in two ways: mg of trypsin inhibited per gram of a sample and mg per g or kg of nitrogen (Kakade et al, 1974). There is a divided opinion as to what indicates an acceptable trypsin level in processed FFSB. It is thought that a reduction of 90 to 95 % indicates adequate processing. In an attempt at standardization, the European Federation of Feed Manufacturers recommended TIA contents in FFSB as presented in Table 2.4 below to be used in feed preparation (Monary, 1989).

Table 2.4: Trypsin inhibitor activity values using the TIA method (Monary, 1989b).

% protein in FFSB	Trypsin Inhibitor Activity (mg/kg)
50	5
40	4
30	3

Method assessment

As this method specifically determines the level of one of the major anti-nutritive factors present in raw FFSB, it is potentially of considerable value. On the other hand, the method of Kakade (1974) that is mainly used is extremely laborious and, possibly for that reason, low repeatability

has been obtained in some laboratories. In addition, similarly to UIA, a value for TIA of “zero” does not necessarily indicate over-processed FFSB. From a practical point of view, it is suggested that this method should be used only if there is no other choice, such as in case of the development of soybean cultivars with low levels of trypsin inhibitors

2.4.6. Cresol Red Test

This is a fast and reliable indirect test which measures the degree of heating of the soya bean product. This method is based on the capacity of soybean protein to absorb the cresol red dye. The capacity of cresol red to bind proteins increases with more extensive heat processing. As an indirect test, it does not measure the actual content of the anti-nutritional factors as the recommended levels indicating adequate heating are subjective. Table 2.5 shows the amount of dye absorbed (mg/g) by processed FFSB (Monary, 1989):

Table2.5: Amount of cresol red dye absorbed by heat-treated soya beams at different temperatures.

FFSB	Dye absorbed (mg/g)
Under-processed	<3.7
Adequately-processed	3.7 - 4.3
Over-processed	4.3 - 4.5

The Cresol red test is a semi-quantitative method and, therefore, cannot be objective. It can be moderately reliable if used by an experienced person, for example, a soya bean processor but it cannot be recommended for use in analytical laboratories.

2.4.7. Lysine availability

Lysine availability is very important in poultry nutrition. This method attempts to measure chemically the amount of lysine in the feedstuff which is available to the animal. Carpenter (1960) developed a method using 2,4-dinitrofluorobenzene (DNFB) and Kakade and Liener (1969) developed a method using 2,4,6-trinitrobenzene sulphonic acid (TNBS) as the reagents by which to determine the available lysine.

Method assessment

These methods are complicated and do not always correlate well with the lysine availability as determined *in vivo* (Monary, 1989). It has also been reported (Palic, 1998) that female analysts experienced allergic reaction to DNFB.

2.5. Soybeans in animal diets

There is much evidence from studies on experimental animals and human subjects that, by substituting soya protein for animal protein in the diet, the concentration of total and low density lipoprotein (LDL) cholesterol in plasma or serum is reduced. This evidence has been summarized in a number of studies (Carrol, 1991; Sirtori et al, 1993).

Studies show that the poultry and swine industries are the major consumers of soybeans and soybean products, as compared to beef and pets. However, all these studies agree on the necessity of heat treatment before feeding diets including soybeans to animals (Zhang et al, 1991).

Soybean meal can be an important feed ingredient for animals as it has the following attributes (Loon, 1996):

1. High protein content with a well-balanced amino acid profile.
2. High oil content with more than half consisting of linoleic acid, which is an essential PUFA (poly-unsaturated fatty acid).
3. Good mixing property as a feed ingredient.
4. Control of dustiness.
5. Excellent smell and palatability.

Although this study focuses on soybean usage in poultry nutrition, the following section will summarize a number of studies conducted on the use of soybean and soybean products in different monogastric and ruminant animals.

2.5.1. Soybeans in poultry diets

Soybean meal is of superior value because no other common plant protein feedstuff exceeds soybean meal in crude protein content. Soybean meal matches or exceeds all other common plant proteins in both total and digestible amino acid content. It is perhaps the only common protein supplement that is typically included in poultry rations with no limitation as to the quantity used. (Bajalieh, 2002).

Stephenson and Tollett (1959) were the first to evaluate the use of full-fat soybeans in broiler diets. They used beans that had been cooked in a rendering plant cooker and in an autoclave to examine the effect of various cooking times and moisture levels. Their results showed that chicks fed properly cooked soybeans were equal to those of chicks fed the standard diets.

Lessire (1992) confirmed that the inclusion of soybeans increased growth rates and feed conversions and, furthermore, that those improvements were particularly significant with diets containing rather elevated proportions of wheat by-products and saturated fats.

In addition, studies by Senkoylu et al. (2005) examined the effect of various inclusion levels of full fat soybeans (FFSB) on laying hen performance and egg shell quality. They found that egg weight, egg mass and the feed conversion ratio increased at the maximum inclusion levels of FFSB used which were 22%.

Leeson and Atteh (1996) evaluated the response of broiler chicks on dietary FFSB extruded at different temperatures. They found that lower extrusion temperatures do not reduce TIA to a satisfactory level that will enable a high dietary inclusion of such extruded soybeans for broilers. They recommended temperatures around 140°C for adequately-processed full fat soybeans.

Batal et al. (2000) demonstrated that body weight gains and gain-to-feed ratios of chicks fed autoclaved soybean meal (SBM) increased with increasing SBM heating times (0 to 18 min in chick assay 1, 1.0 to 10 min in chick assay 2 and 0 to 9 minutes in chick assay 3) with no additional improvement for longer autoclaving times.

Clarke and Wiseman (2005) evaluated the amino acid digestibility and effect on pancreas size in young broilers using both FFSB and SBM. Their findings demonstrated that the pancreas to body weight ratio (PBWR) increased with the rate of soy inclusion in both the SBM and FFSB, and that pancreas size increased with increasing TIA levels. Recently, Clarke and Wiseman (2008) reported an increase in the concentration of the ileal digestibility of lysine when the extrusion temperature of full-fat soybeans increased from 90 °C to 160 °C. An improvement in the coefficient of the ideal apparent amino acid digestibility (CIAD) of lysine and other amino

acids was also reported.

2.5.2. Soybeans in pig diets

Pigs, being non-ruminants, are similar to poultry in terms of their sensitivity to anti-nutritional factors in raw soybeans. Consequently, adequate processing of this raw material is considered essential before it is fed to all classes of pigs (Wahlstrom et al, 1971).

Since the early reported trials of Jimenez et al. (1960, 1963), a large volume of data has accumulated on the utilization of soybean products and FFSB processed in a variety of ways in diets for pigs. A number of the observations are outlined below:

Zollitsch et al. (1993) reported a lower content of saturated and monosaturated acids and an increased percentage of linoleic and linolenic acids in trials for the evaluation of pig fattening using differently processed full fat soybeans.

Friesen et al, 1993 reported that pigs fed wet-extruded soy products had an improved average daily weight gain and gain-to-feed ratio compared to pigs fed non-extruded soybean products. Wet extrusion was also found to increase the average daily gain (ADG), dry matter (DM) and nitrogen digestibility as compared to dry extrusion.

Furthermore, White et al. (2000), observed that the initial moisture of beans (10% versus 20%) and the processing temperature (110°C for 60 seconds versus 125°C for 90 seconds) influenced the quality of the product obtained using a commercial dry-roasting technique. Piglets fed beans processed at a temperature of 110°C grew at reduced rate when compared with those that consumed beans processed at a temperature of 125°C. A 10% to 20% increase in the moisture of beans prior to processing improved the growth parameters in both groups, particularly when the temperature was not as high

Recently, Lee et al. (2007) evaluated the *in vivo* quality of soy flakes heat treated under different conditions and found that the body weight, average daily gain and feed efficiency of pigs increased with an increasing heat treatment. The best pig performance was achieved at treatment temperatures of 110°C. It was also observed that, at higher temperatures, there was a

reduced growth performance.

2.5.3. Soybeans in ruminant diets

As in monogastric animals, the use of processed soybeans is required in young ruminants. The administration of raw FFSB to cattle below 150-200 kg live weight should be avoided because the ANFs are not degraded in the rumen of young animals and their presence may result in a reduction in the intake and digestibility of diets. Accordingly, for young cattle, the use of heat processed FFSB is recommended. In addition, raw soybeans have a high amount of rumen degradable protein and require processing prior to feeding. Roasting or heating renders the fat more stable, reduces rumen degradability, and increases palatability. Although data on soybeans in cattle feeding is limited, the following section will outline some of the recent studies available.

McNiven et al. (2004) reported that the inclusion of roasted soybeans modified the fatty acid composition of beef as compared to raw and extruded soybeans. The fatty acid composition of the meat from cattle fed roasted soybeans reflected a healthier profile for humans that consume them as the hypocholesterolemic unsaturated fatty acids and lipid peroxidation of the cattle were higher than of those fed raw or extruded soybeans.

Recently, Neves et al. (2007) reported that extruded soybeans decreased milk production, lowered the digestibility of dry matter, neutral detergent fiber and acid detergent fiber as compared to non-extruded soybeans in Holstein cows.

Nasri et al. (2008) reported that the heat processing (roasting at 140°C-145°C and roasting with steeping for 45 min) of whole soybeans reduced the ruminal degradation of nitrogen and the disappearance of individual and total amino acids and increased the small intestinal disappearance of nitrogen and amino acids due to the destruction of the trypsin inhibitor. The authors concluded that the processing methods in this study reduced ruminal degradation of nitrogen and disappearance of individual and total amino acids and increased small intestine disappearance of nitrogen and amino acids which could be due to destruction of trypsin inhibitor. They also found that steeping improved the small intestinal and total tract digestibility of nitrogen, total and individual amino acid beyond the effects of roasting.

2.5.4. Soybeans as a feed ingredient in aquaculture

The high energy value and good protein quality of processed FFSB allow it to be used for formulating fish diets. The replacement of fish meal with soybeans/soybean products partially and sometimes fully indicated no change or improved performance in different fish species. As a consequence of the very high protein requirements necessary to promote the optimum performance coupled with the increasing price of fish meal, an attempt must be made to replace, at least partially, the most expensive ingredients of fish diets, such as fish meals and oils, with other cheaper and locally available products. In the search for alternative feed sources with high contents of digestible protein of good quality, soybean products have been acquiring considerably importance. Saad (1979) evaluated the effect of roasted FFSB in catfish by replacing 50% and 100% of SBM in the diets. No difference in body protein gain on all diets when partially or fully replacing soybeans with fishmeal were found. Tacon et al. (1984) conducted a study with tilapia where a replacement of 70% of brown fish meal with 50% of jet-sploded FFSBM was examined. The results showed that tilapia fed diets containing FFSBM performed as well as those fed on diets based on fish meal. Samocha et al, (2004) found no differences with regard to survival, average body weight and feed efficiency when fish meal was substituted with soya beans in Pacific white shrimp. In addition, recent studies by Sorensen et al. (2009) were conducted where fish meal partially replaced soybeans to evaluate the effect of the processing of soybeans on the physical quality of extruded fish feed. They found that this partial replacement of fish meal with soybeans improved the physical quality of feed in terms of breaking force and durability.

2.6. Use of soybeans in human diets

Soybeans can be classified into food beans and oil beans, based on their usage in different regions despite small fundamental differences (Lui, 2000). There are a number of different methods whereby the beans may be prepared (processed) for human consumption. They may be eaten sprinkled with salt and boiled, in a form usually referred to by the Japanese name *edamame*. They may be turned into soy milk, a process involving soaking in water, then grinding and boiling them into a milky substance. The curds from this soy milk may then be pressed into blocks of tofu in a process similar to that of making cheese from dairy milk. Whole soybeans may also be slightly cooked and then fermented using vinegar and the fungus *Rhizopus*

oligosporus to make tempeh. The leftover soy flour from extracting the oil from soybeans is also used to create textured vegetable protein (TVP) which is often used in meat.

The consumption of soybeans and soy products has many health benefits including cholesterol reduction, protection against certain types of cancers, menopausal symptoms, and osteoporosis (Greendale et al, 2002). Many of the health benefits of soy are derived from its isoflavones. Isoflavones remain the subject of many scientific studies. Most of these show that isoflavones may have some health benefit. The following section will focus on the health benefits of soybeans in humans.

2.6.1. Cholesterol reduction

Vegetable proteins, mostly soy proteins, reduce plasma cholesterol particularly when cholesterol is elevated by dietary means (high cholesterol intake, semi-synthetic diets, etc.) (Kim et al, 1980; Terpstra et al. 1982).

There is much evidence from studies on experimental animals and human subjects that substituting soy protein for animal protein in the diet reduces the concentration of total and lower density lipoprotein (LDL) cholesterol in plasma or serum. (Carroll, 1991; Sirtori et al, 1993). The earliest study demonstrating the significant cholesterol-lowering properties of a soy-protein-based diet was carried out in a prison setting by Hodges et al, (1967). They showed a serum cholesterol decrease, from a mean of 7.66 mmol/L to approximately 5.0 mmol/L, in six subjects with hypercholesterolemia after a four week regimen containing protein, fat, and carbohydrate, formulated with a textured soy product. Soy protein directly lowers blood cholesterol levels, a major risk factor for coronary heart disease (CHD). Greaves et al. (1999) reported that isolated soy protein containing naturally occurring isoflavones was associated with lowering the LDL cholesterol in cynomolgus monkeys. When compared to a casein diet, soy protein has been shown to reduce apolipoprotein (Apo) B synthesis and to stimulate LDL receptors.

The other area of clinical use of soy proteins is in hypercholesterolemia, secondary to the nephritic syndrome. In this condition, a change from an animal to a vegetarian soy diet

markedly reduces serum cholesterol and urinary protein excretion (D'Amico et al, 1992). From ongoing long-term studies, it appears that this type of dietary treatment may favourably influence the progression of renal disease (Sirtori et al, 1995). Carrol et al. (1991) reviewed studies on the cholesterol-lowering properties of soy proteins which involved normolipidemic volunteers. The studies confirmed the hypothesis that only subjects with elevated cholesterol levels show a clear cut response to the dietary protein change.

2.6.2. Cancer

Soybeans are one of most frequently studied legumes for their anti-cancer activity. Components found in the beans that exert biological activities are isoflavones, saponins, phytic acid, fiber, phytosterols, protease inhibitors, inositol and hexaphosphate (Messina and Barnes, 1991; Messina, 1999). These components may contribute individually or synergistically to the aforementioned health benefits. Messina et al. (1994) reported that the majority of the animal studies on experimental carcinogenesis report an inhibitory effect on tumor growth when soya was used in the diet. In contrast, Hawrylewicz et al. (1995) suggested that several biological mechanisms were involved. However, the isoflavones were considered to be the most active anti-tumor constituent in the soy diet. A study conducted in the United States to examine the relation between soy intake and breast cancer risk found that tofu consumption reduced the risk of breast cancer in Asian women of all ages (Wu et al, 1998).

Rodent studies done by Messina (2003) indicated that soy protein and isoflavones suppress the development of *in vivo*-induced prostate cancer. Other studies have shown that soy protein and isoflavones may inhibit the growth of existing tumors. In addition, epidemiologic research showed that men who eat soy foods daily are less likely to develop prostate cancer than those who do not and that soy protein and isoflavones may slow the rise of prostate specific antigen (PSA) levels in men diagnosed with prostate cancer (Messina., 2006). Recent studies by Hwang et al, (2009) evaluated a comprehensive meta-analysis on the extent of the possible association between soy-based food consumption and the risk of prostate cancer. Their studies suggested that soy food consumption, in fact, did lower the risk of prostate cancer in subjects who consumed soy-based diets. They also reported that genistein and daidzein were the main

isoflavones found in soybeans which play major roles in prostate cancer prevention.

2.6.13. Osteoporosis

As a rich source of high-quality protein, soy foods can aid in the promotion of bone health. In addition, the calcium in fortified soymilk is well absorbed and the skeletal effects of soy isoflavones are of interest (Dawson-Hughes, 2003). A study by Messina et al. (2004) suggested that exposure to isoflavones results in a reduction in bone loss. In addition, studies by Chiechi et al, (2002) confirmed that soy products could be effective in reducing the risk of osteoporosis in asymptomatic postmenopausal women.

Therefore, vegetable proteins are recommended over animal proteins as they are cost effective and contain approximately the same amount of protein. Animal proteins have high levels of fat and cholesterol which can lead to serious medical conditions. In addition, increases in dietary animal protein are associated with increases in urinary calcium excretion and osteoporotic fracture, while increases in vegetable protein have shown no side effects (Massey, 2003).

CHAPTER 3

Materials and Methods

3.1 Processing of full fat soybeans (FFSB)

In this study, dry extrusion was used as a method of processing FFSB. The reason for selecting this processing method was because it was the most commonly used method for FFSB processing in South Africa (representing about 90%) at the commencement of this study.

Full fat soybeans, as a mixture of cultivars were processed by dry extrusion at eight different temperatures of 110°C, 120°C, 127°C, 136°C, 140°C, 145°C, 151°C, and 165 C using an “Insta-Pro” extruder. A range of temperatures was used in order to obtain a range of samples that would represent under-, adequate- and over-processed FFSB.

3.2. *In vivo* trials with broilers

The aims of the *in vivo* trials with broilers were:

- 1 To establish the optimum temperature for FFSB processing
- 2 To establish the temperature range corresponding to adequately-processed soybeans.

Procedure

A total of 384 male Ross broilers was randomly allocated to 48 pens, each containing eight birds. On arrival, all broilers were sorted into equal weight groups, and assigned at random to the different treatment pens, such that the initial average weight and weight distribution were similar for all pens. The birds were allocated to one of eight dietary treatments containing the processed FFSB. The average body weight gains (ADWG) in the period from day 0 to day 14 and feed conversion ratio (FCR) on day 14 were monitored as the production parameters.

Data were analysed using the statistical programme, SAS/STAT (1981). The experiment was designed as a randomized complete block with five replicates per treatment. Analysis of variance (ANOVA) was used to test the difference between treatments. Treatment means were separated using Fisher’s protected t-test least difference (LSD) at the 5% level of significance.

3.3. *In vitro* analysis of FFSB quality

Quality control plays an important part in ensuring that raw soybeans have been adequately processed. This is to ensure that anti-nutritional factors are sufficiently reduced, protein quality is not lowered and as much oil as possible is released from the cells (Loon, 1996).

Based on the assessment of commonly used methods for FFSB quality control (stated in Chapter 2) and the overall objectives of this study, the following plan was drawn up for the current study:

- 1 A separate, single laboratory study on the Urease Activity index (UIA) was to be conducted taking in to account the fact that it is the most commonly used procedure for FFSB quality assessment in the laboratories of South Africa.
- 2 The Protein Solubility in KOH (PSKOH) and the Protein Dispersibility Index (PDI) methods must be standardized through inter-laboratory study due to the fact that protein solubility is the best indicator of FFSB quality control in the assessment of methods (Chapter 2).
- 3 To establish a South African analytical range which corresponds to under-, adequately- and over-processed FFSB for both PSKOH and PDI methods.
- 4 To establish the precision (repeatability and reproducibility) of PSKOH and PDI methods.
- 5 To regress the PSKOH and PDI results against values obtained in the *in vivo* trials and to establish which of the two methods has better a correlation with animal production parameters.

3.3.1. Single laboratory study: Urease Activity Index

The AOCS method (AOCS official methods, 1997a) for the determination of the Urease activity index (UAI) is based on the measurement of the pH change/differences (ΔpH) and its simplicity could be the reason that this method is probably the most generally used method for FFSB quality control in laboratories in South Africa. Nevertheless, wide differences in the results obtained between laboratories using this method were reported by Davies (1998) and Palic (2004) and were confirmed by the following preliminary investigation.

Procedure

Raw soybeans were dry extruded at 115°C, 125°C, and 135°C in order to obtain a range of under-processed samples to which Urease activity can apply. Each of the extruded samples was analyzed by two laboratories and by two analysts at each laboratory in seven replicates.

The Urease activity was determined according to the AOCS official procedure (1997 a) as follows:

Approximately 200mg of the FFSB sample was incubated in 10 ml of phosphate buffered urea solution at 30°C for 30 minutes after which the difference in pH units from pH 7.0 was recorded.

In order to reach a deeper understanding of the differences in results obtained by the laboratories, a single-laboratory follow-up study was conducted by the Urease Activity Index using both the pH-difference (ΔpH) method and the NH_3 -released method.

Full fat soybean was processed by dry extrusion on eight different temperatures: 110°C, 120°C, 127°C, 136°C, 140°C, 145°C, 151°C and 164°C. Apart from determining the UAI by measuring the resulting pH difference, as in the investigation shown above, it can also be quantified by determining the amount of released NH_3 . The UAI was, therefore, determined according to the following procedures:

1. The pH-difference (ΔpH) method (AOCS Official Methods, 1997a) as described above
2. The NH_3 -released method (Laboratory methods, 1989):

200 mg of FFSB sample was incubated in 10 ml of phosphate buffered urea solution at 30°C for 30 minutes, followed by the addition of 10 ml of concentrated H_2SO_4 , bringing the solution volume to a total of 20 ml. The sample was filtered and the concentration of NH_3 (mmol/L) in the supernatant was determined using a Technicon Auto Analyzer II.

3.3.2. Protein solubility in Potassium hydroxide (PSKOH)

3.3.2.1. Preliminary study

A full fat soybean (FFSB) sample was processed at 14°C. Two analysts at two different laboratories analyzed the sample in seven replicates using the original PSKOH method described by Araba and Dale (1990).

Procedure

Approximately 1.5 g of a FFSB sample was ground in an Udy mill (Udy Corporation, Boulder, Co), in order to pass it through a 0.5 mm screen. The sample was mixed with 75 ml of 0.2% (0.036 N, pH 12) potassium hydroxide, stirred for 20 minutes on a magnetic stir plate and centrifuged at 2700 rpm for 15 minutes. The supernatant was decanted through glass wool and approximately 40 ml was recovered in a 50ml beaker. Approximately 15 ml in duplicates were transferred to Kjeldahl tubes giving a 0.3 g aliquot of the original sample (1.5 g x 15 ml per 75 ml) and 12.5 ml of concentrated H₂SO₄. Two Kjeltab and 2 ml of 30% H₂O₂ were added to each tube. The total nitrogen was determined by the Kjeldahl method and the total protein content was determined. The protein solubility was expressed as a percentage of the total protein soluble in a 0.2% solution of potassium hydroxide.

3.3.3.2. Inter-laboratory study

Inter-laboratory studies were conducted according to the AOCS Collaborative study procedure (1997d) in order to standardize (harmonize) the Protein solubility in potassium hydroxide (PSKOH) and Protein Dispersibility Index (PDI) methods, i.e. to establish South African analytical unit ranges for describing under-, well- and over-processed FFSB and to establish the precision parameters of these two methods.

Eight samples of FFSB which were used in the *in vivo* trials with chickens (FFSB samples dry extruded at 110°C, 120°C, 127°C, 136°C, 140°C, 145°C, 151°C, and 165°C) were sent to nine participating laboratories together with the modified PSKOH method. Each laboratory was assigned a number known only to the study leader.

Sample preparation

The entire sample of FFSB was taken from a bag and homogenized by mixing thoroughly prior to grinding. Each of the extruded samples was milled using a cyclone mill equipped with a 1.0 mm sieve making sure that:

- 1 During the milling process, the mill was allowed to cool in between milling samples.
- 2 The mill was cleaned properly before grinding the next sample
- 3 Small aliquots of the sample were ground at a time.

The ground sample was homogenized by mixing thoroughly on a flat, hard surface and sieved through 1.00 mm and 0.600 mm sieves. The fraction that passed through the 0.600 mm sieve was homogenized by mixing thoroughly on a flat hard surface. The fraction that passed through the 0.600 mm sieve was mixed thoroughly and stored in a refrigerator prior to protein analysis.

Procedure

The determination was performed in triplicate for each of the eight extruded FFSB samples. Laboratories conducted the PSKOH determination according to the modified method of Araba and Dale (1990) as follows:

Approximately 1.5 g of FFSB sample was placed in a 250 ml Erlenmeyer flask with an 85 mm bottom diameter and a 145 mm height. A solution of 0.2% KOH was added and the mixture was sealed with a stopper, ensuring that no sample particles adhered to the sides of the beaker. Immediately after addition of the KOH solution, the flask was placed in a lateral (horizontal) shaker and shaken at 100 cycles per minute for exactly 20 minutes. The mixture was left for exactly two minutes to settle. Approximately 50 ml of the liquid was collected and filtered through 150 mm wide-pore filter paper with a 150 mm diameter (e.g. Schleicher & Schuell 520B / Whatman 113 / G.I.C. Scientific EconoFilt IHD or equivalent). A 15-ml aliquot of the supernatant was collected and the nitrogen content of the supernatant and the original FFSB was determined. The nitrogen values were multiplied by 6.25 to yield crude protein (CP) and the protein solubility was then calculated as a percentage of the total CP in the original FFSB sample. Digestion of the supernatant and original soybean sample was conducted

simultaneously.

Calculation

% Soluble protein in original soybean sample =
= % Protein in supernatant (using 15 ml) x {Volume (75ml) / Sample mass (1.5g)}

% Protein solubility in KOH =
= (% Soluble protein in original soybean sample / % Total protein in original soybean sample) x
100

3.3.3 Protein dispersibility index (PDI)

The PDI method (AOCS Official Methods, 1997c) is used for FFSB protein dispersion using a special blender, at the speed of 8500 rpm, which makes this method potentially the quickest and simplest to perform of all the methods for processed FFSB quality control.

Furthermore, Batal et al, (2000) reported that the PDI displayed the most constant response to the heating of FFSB and that it “may better indicate processed FFSB quality compared to other methods”.

For adequately processed soybeans, globally accepted values for PDI are between 15 and 28% (Monary, 1989). However, these values are not specified in the description of the PDI method (AOCS Official Methods, 1997c) and, in fact, no values were specified. Attempts to source original publication(s) which cited the PDI values of 15 – 28 for adequately processed FFSB have failed. This fact provided an additional justification for the need to determine these PDI values for South African conditions.

3.3.3.1. Preliminary study

As stated, a special blender (designed according to AOCS Official Methods, 1997c) is required to perform the PDI procedure. This blender (“Hamilton Beach” Commercial Blender, model G936) is manufactured by “Vos Instrumenten”, The Netherlands, and costs approximately 2500 euro.

A survey was conducted between laboratories and only one was prepared to purchase a

Hamilton Beach blender. In order to be able to conduct an inter-laboratory study of the PDI method, an alternative solution was identified for purchasing a much less expensive variable-speed “Waring” Commercial blender, model LB20E (available in South Africa) and for calibrating it against the Hamilton Beach blender.

In a preliminary study, the official PDI method was followed, while the samples were blended by both the “Hamilton” and “Waring” blenders. Blending with the Hamilton blender was conducted using the official method at a speed of 8500 rpm. Blending with the “Waring” blender was done at different speeds, until the speed at which the PDI result of the sample matched the PDI values obtained by the “Hamilton” blender was determined. That speed for the “Waring” blender was 12000 rpm.

3.3.2. Inter-laboratory study

Sample preparation

The entire sample of full fat soybean was taken from a bag and was homogenized by mixing thoroughly prior to grinding. Each of the extruded samples (processed at different temperatures of 110°C, 120°C, 127°C, 136°C, 140°C, 145°C, 151°C and 164°C) was milled through a cyclone mill equipped with a 1.0 mm sieve making sure that:

- 4 during the milling process, the mill was allowed to cool between milling samples
- 5 the mill was cleaned properly before grinding the next sample
- 6 small aliquots of the sample were ground at a time
- 7 the sample did not heat up during the milling process

Standardization of the blenders

The blender cup was filled with 300ml of distilled water and placed in position on the mixer. A small self-adhesive strip of reflective tape was placed in the upper part of the blender shaft, with the switch in the medium position. The transformer setting was gradually increased until the shaft showed 8500 rpm and 12,000 rpm for the official and the “Waring” blenders respectively

on the strobe tachometer (MT 952). The blender was standardized before each series of tests to eliminate errors due to fluctuation in the line voltage. A minimum time of 20 minutes was allowed between samples to allow the blender to cool.

Procedure

Approximately 20g of FFSB sample was placed in a laboratory blender cup and mixed with 50 ml distilled water to form a paste. Approximately 250 ml of water was added to the mixture to make a total of 300 ml distilled water. The blender cup was placed in position for blending and the sample was blended for exactly ten minutes. The slurry was poured into a 600 ml beaker to settle for 15 minutes. A portion of the upper layer was poured on to 50 ml centrifuge tubes and centrifuged for ten minutes at 2700 rpm. A 15ml aliquot of the supernatant was collected and the nitrogen content of the supernatant and the original FFSB sample was determined. The nitrogen values were multiplied by 6.25 to yield crude protein (CP) and protein solubility was calculated as a percentage of the total CP in the original sample.

Calculations

$$\begin{aligned} \% \text{ Soluble protein in original soybean sample} &= \\ &= \% \text{ Protein in supernatant (using 15.0 mL)} \times \left\{ \text{Volume (300 mL)} / \text{Sample mass (20 g)} \right\} \end{aligned}$$

Precision of the KOH and the PDI methods

The precision of the KOH and PDI methods was determined using the AOCS official methods (1997e). This is defined by the repeatability and reproducibility limits, which give the maximum allowed difference between the results of analysis in a single laboratory and between laboratories.

Repeatability limit (r) is the absolute difference between two independent single test results, obtained with the same method on the identical test material in the same laboratory by the same operator using the same equipment within short intervals of time.

Reproducibility limit (R) is the absolute difference between two single test results, obtained with

the same method on the identical test material in different laboratories with different operators using different equipment.

CHAPTER 4

Results

The means of average daily weight gain and feed conversion ratio of broiler chickens fed full fat soya beans (FFSB) processed at different temperatures are reported in Table 4.1.

Treatment	ADWG (g)	FCR
110°C	87.8 ^{bc}	2.081 ^d
120°C	96.0 ^{bc}	1.893 ^{cd}
127°C	108.0 ^{bc}	1.768 ^c
136°C	138.3 ^a	1.382 ^a
140°C	132.0 ^a	1.466 ^a
145°C	123.0 ^a	1.529 ^a
151°C	97.2 ^b	1.679 ^c
164°C	79.8 ^c	1.891 ^{cd}
SEM¹	7.94	0.081
LSD²	22.81	0.232
CV%³	19.1	11.5

^{a,b,c,d}Values in the same column with different superscript differ significantly (P<0.05)

¹SEM = Pooled standard error of the means

²LSD = Least significant difference

³CV% = Coefficient of variation

Statistical analysis of the results showed that the best performance was achieved by chickens that were fed the FFSB processed at 136°C, 140°C and 145°C and that there was no significant difference between them (P>0.05). However, the difference between the groups that were fed the FFSB processed at 127°C and 136°C as well as well as 145°C and 151°C was significant (P<0.05). Based on these parameters, a relation between the temperature of extruding and the *in vivo* assessment of the degree of FFSB processing was derived and is shown in Table 4.2.

Table 4.2: Relation between the temperature of extruding and the *in vivo* assessment of the degree of FFSB processing.

Degree of FFSB processing	Temperature of extrusion (°C)
Under-processed	<136°C
Adequately-processed	136°C-145°C
Over-processed	>145°C

The relation between the temperature of extruding and the average daily body weight gain in the period from day 0 to day 14 and the feed conversion ratio on day 14, of broiler chickens is illustrated in figure 4.1.

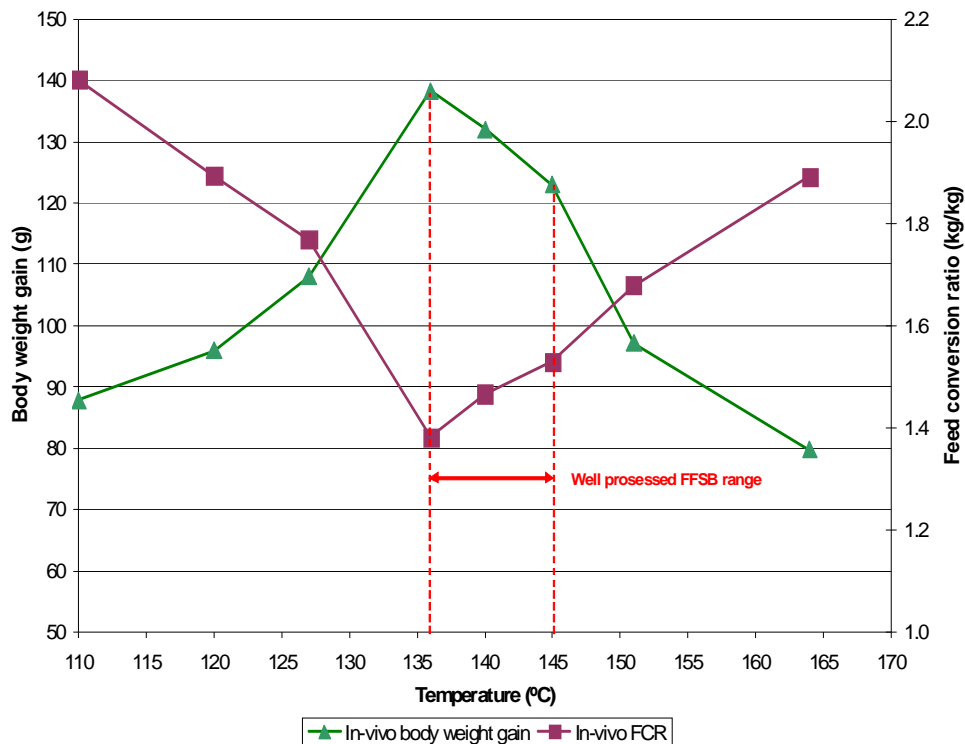


Figure 4.1. Average daily body weight gain in the period from day 0 to day 14 and feed conversion ratio on day 14, of broiler chickens fed FFSB processed at different temperatures.

Based on the results in Table 4.1 and Figure 4.1, broilers fed FFSB processed at temperatures between 136°C to 145°C resulted in the best broiler performance with regard to ADWG and FCR. A decrease in broiler performance was observed in temperatures below 136°C and above 145°C.

4.2. Urease activity index (UAI)

4.2.1. Urease Activity Index using the pH difference (Δ pH) method

The results of UAI using the Δ pH method obtained by Lab A and Lab B are shown in Table 4.3 and 4.4 with two analysts per laboratory.

Table 4.3. Results of the UAI using the Δ pH procedure obtained by Lab A

115 ⁰ C		125 ⁰ C		135 ⁰ C	
Analyst 1	Analyst 2	Analyst 1	Analyst 2	Analyst 1	Analyst 2
2.16	2.22	0.42	0.45	0.07	0.08
2.10	2.27	0.40	0.44	0.07	0.09
2.13	2.26	0.40	0.41	0.06	0.09
2.19	2.28	0.45	0.42	0.08	0.09
2.18	2.19	0.47	0.43	0.08	0.08
2.19	2.13	0.43	0.43	0.08	0.09
2.19	2.15	0.48	0.43	0.07	0.09

Table 4.4. Results of the UAI using the Δ pH procedure obtained by Lab B

115 ⁰ C		125 ⁰ C		135 ⁰ C	
Analyst 1	Analyst 2	Analyst 1	Analyst 2	Analyst 1	Analyst 2
2.17	2.18	0.28	0.25	0.05	0.06
1.99	2.16	0.22	0.27	0.06	0.07
2.12	1.12	0.24	0.25	0.06	0.06
2.18	2.12	0.27	0.27	0.08	0.05
2.10	2.16	0.20	0.24	0.04	0.06
2.00	2.11	0.21	0.23	0.07	0.05
2.04	2.01	0.22	0.22	0.07	0.06

By using the Urease Activity method based on Δ pH, the two analysts at both laboratories obtained comparable results ($P > 0.05$). However, the results between two laboratories were significantly different ($P < 0.05$) for the sample processed at 125⁰C.

4.2.2. UAI using both the pH difference and the NH₃ released methods

A comparison of the UAI results obtained by the Δ pH and NH₃ released methods is shown in Table 4.5.

Table 4.5. UAI in full fat soya bean (FFSB) samples processed at different temperatures, determined by Δ pH and NH_3 -released procedures.

Temperature ($^{\circ}\text{C}$)	*Urease Activity Index (UAI)	
	Δ pH	NH_3 (mmol /L)
110	2.12	521.00
120	1.94	338.37
127	0.16	187.03
136	0.15	25.13
140	0.13	21.60
145	0.13	17.97
151	0.13	17.97
164	0.13	17.97
¹ SEM	0.01	2.23
² S	0.61	184.76

*Means of values obtained on 3 different days;

¹SEM = Standard error of the means;

²S = Standard deviation.

Based on the average daily gain (ADG) of broilers in the *in vivo* trials and the UIA using both the Δ pH and NH_3 -released procedures, a relationship was drawn and is shown in Figure 4.2.

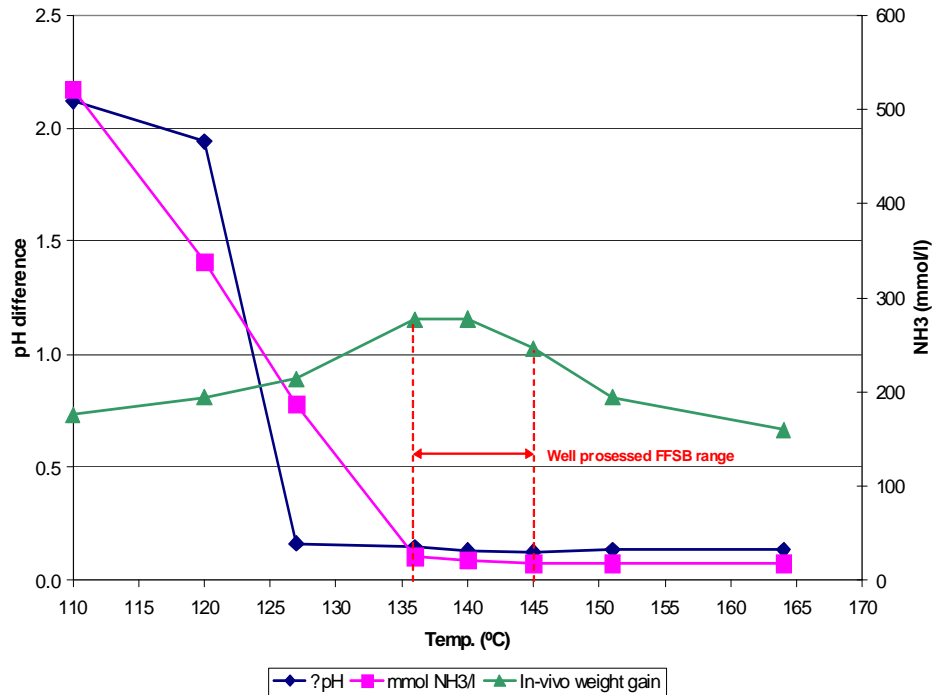


Figure 4.2: Urease Activity Index (UAI) determined by pH-difference and NH₃-released procedures in relation to body weight gain of chickens in the *in vivo* trials.

As extrusion temperatures increased, a decrease in the pH and released NH₃ was observed. A value close to zero was reached from a temperature of 127°C when the Δ pH procedure was used and the pH decreased from 1.92 at 120°C to a value close to zero (0.16) from a temperature of 127°C. Using the NH₃-released procedure, a linear response in released NH₃ with regard to increased temperatures was observed and from 136°C a value close to zero was reached.

4.3. Protein solubility in potassium hydroxide (PSKOH)

4.3.1. Preliminary studies

The results of the full fat soybeans (FFSB) analysis for the FFSB processed at 145°C using the original PSKOH method (Araba and Dale, 1990) are shown in Table 4.6.

Table 4.6. Results of analysis of FFSB sample conducted by two laboratories, two analysts at each laboratory and in seven replicates, using the original PSKOH method (Araba and Dale, 1990a).

FFSB processed at 145⁰C			
PSKOH (%)			
LAB 1		LAB 2	
Analyst 1	Analyst 2	Analyst 1	Analyst 2
71.50	72.00	54.06	48.75
72.44	72.47	53.45	49.16
73.40	73.40	50.18	51.34
72.94	73.78	51.88	51.61
71.75	73.09	51.27	50.59
73.34	73.18	53.11	50.11
71.22	72.06	53.11	48.55
\bar{x} 72.37	72.85	52.44	50.02
SD 0.894	0.686	1.374	1.233

\bar{x} = Means; SD = Standard deviation

The application of the Student's t-test on PSKOH results obtained by the two analysts at Lab 1 and Lab 2 confirmed that the two sets of results at each laboratory were not significantly different ($P > 0.05$). However, the application of the Student's t-test on the PSKOH results of the two laboratories showed that there was a significant difference ($P < 0.01$) in results. The relative average difference in results obtained by the two laboratories was in excess of 41%.

4.3.2. Inter-laboratory study

The results of the inter-laboratory study in which nine South African laboratories participated using the PSKOH method are shown in Table 4.7.

Table 4.7. Results of the determination of PSKOH in FFSB samples processed by dry extrusion at different temperatures

Lab No	PSKOH (%)							
	110°C	120°C	127°C	136°C	140°C	145°C	151°C	164°C
1	89.74	85.74	84.70	77.50	74.68	67.04	68.27	57.84
	89.85	85.79	85.35	76.77	74.77	67.41	68.01	59.48
2	87.10	87.64	88.05	76.91	68.94	65.84	66.18	56.70
	87.42	89.09	88.20	76.19	68.65	66.49	66.53	57.34
3	93.57	88.06	86.57	77.70	72.40	69.63	72.47	61.62
	95.45	88.79	85.15	78.23	71.71	71.25	73.90	60.57
4	95.70	89.82	88.63	75.12	80.05	68.39	69.66	62.69
	95.88	93.34	87.73	78.40	78.02	67.44	71.99	63.97
5	89.72	90.00	87.01	71.45	67.37	58.14	60.83	51.94
	87.37	90.10	87.35	74.13	68.30	57.22	61.14	50.18
6	85.76	91.91	87.12	76.13	76.64	72.41	71.21	65.08
	83.54	91.79	88.96	75.90	76.64	70.77	71.80	65.55
7	88.42	82.67	82.31	74.64	73.35	65.36	73.60	61.23
	88.71	83.33	82.35	73.73	73.54	65.36	73.12	60.01
8	94.18	92.75	92.48	77.70	74.27	70.63	58.88	68.61
	96.23	97.57	88.31	78.40	77.00	66.14	57.26	70.00
9	90.45	91.85	88.38	79.47	77.88	70.78	69.33	64.54
	88.93	85.62	84.99	78.74	75.48	68.23	69.56	61.46
Average	99.45	89.21	86.87	76.51	73.87	67.14	67.99	61.01

The influence of the processing temperatures for the FFSB processed at eight different temperatures on PSKOH values is shown in Figure 4.3.

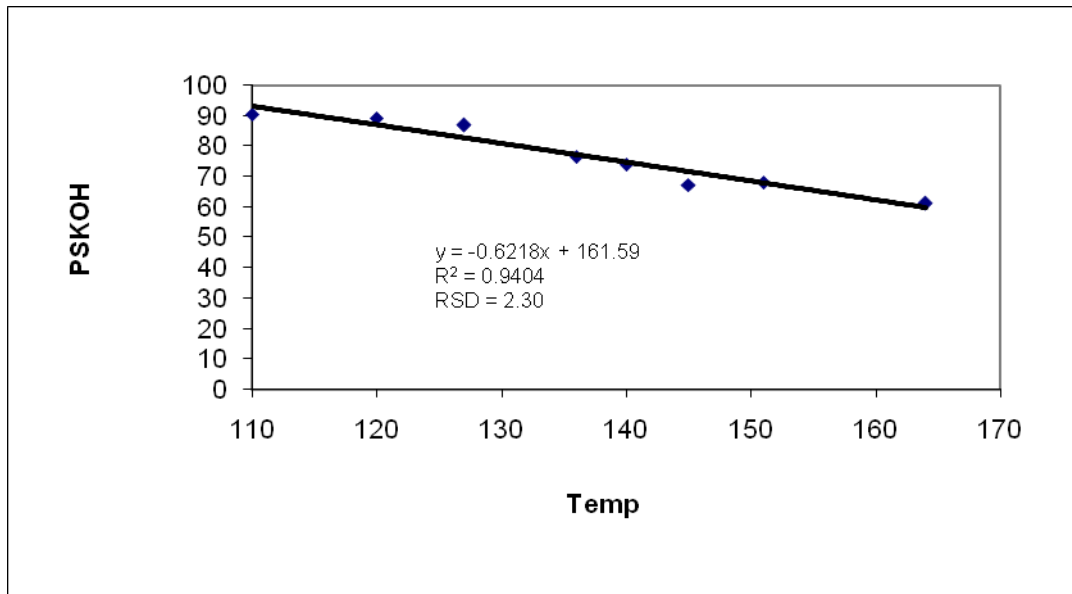


Figure 4.3: The influence of the temperature of processing on PSKOH values.

A very high negative correlation ($R^2 = 0.94$) was established between PSKOH values and treatment temperatures. A summary of the relationship between the processing conditions based on the *in vivo* trials and the PSKOH values is tabulated in Table 4.8.

Table 4.8: South African ranges for describing the degree of FFSB processing using the PSKOH project method.

Degree of FFSB processing	PSKOH (%)
Under-processed	>77%
Adequately-processed	67%-77%
Over-processed	<67%

According to the results indicated in Table 4.7, adequately processed FFSB have PSKOH values between 67% and 77%. PSKOH values below 67% and above 77% index units are associated with under- and over-processed soybeans respectively (Table 4.8).

4.3.3. Precision of the PSKOH method

The precision parameters of the PSKOH method were determined based on results from nine (Table 4.7) national laboratories and are tabulated in table 4.9.

Table 4.9: Precision parameters of the PSKOH method

Parameter	Sample temperature (°C)								
	110	120	127	136	140	145	151	164	
Number of laboratories	9	9	9	9	9	9	9	9	
Number of laboratories retained after eliminating outliers	9	9	9	9	9	9	9	9	
PSKOH values (%), average of 9 laboratories	90.45	89.21	86.87	76.51	73.87	67.14	67.99	61.05	
Repeatability standard deviation (s_r), %	1,07	2,08	1,41	1,09	1,02	1,38	0,78	1,11	1,24
Repeatability relative standard deviation (RSD_r), %	1,19	2,33	1,62	1,42	1,38	2,06	1,15	1,81	1,63
Repeatability limit (r) [$r = 2,8 \times s_r$], %	3,01	5,81	3,94	3,04	2,86	3,86	2,19	3,10	3,48
Reproducibility standard deviation (s_R), %	3,94	3,84	2,50	2,12	3,81	4,18	5,37	5,27	3,88
Reproducibility relative standard deviation (RSD_R), %	4,36	4,30	2,88	2,77	5,15	6,22	7,903	8,63	5,06
Reproducibility limit (R) [$R = 2,8 \times s_R$], %	11,03	10,75	7,01	5,93	10,66	11,70	15,04	14,76	10,86

When the PSKOH method, as described in this study, is used by South African role players, the repeatability (r) defined according to AOCS Official Methods, (1997e) which is the absolute difference between two single results of analysis of the same sample obtained in one laboratory, should not exceed 3.48%. The reproducibility (R), which is the absolute difference between the single results of analysis of the same sample obtained in different laboratories, should not exceed 10.86% as calculated using the values in Table 4.9.

The relationship between PSKOH and the average daily weight gain (ADWG) and feed conversion ratio (FCR) of broilers is shown in Figures 4.4 and 4.5.

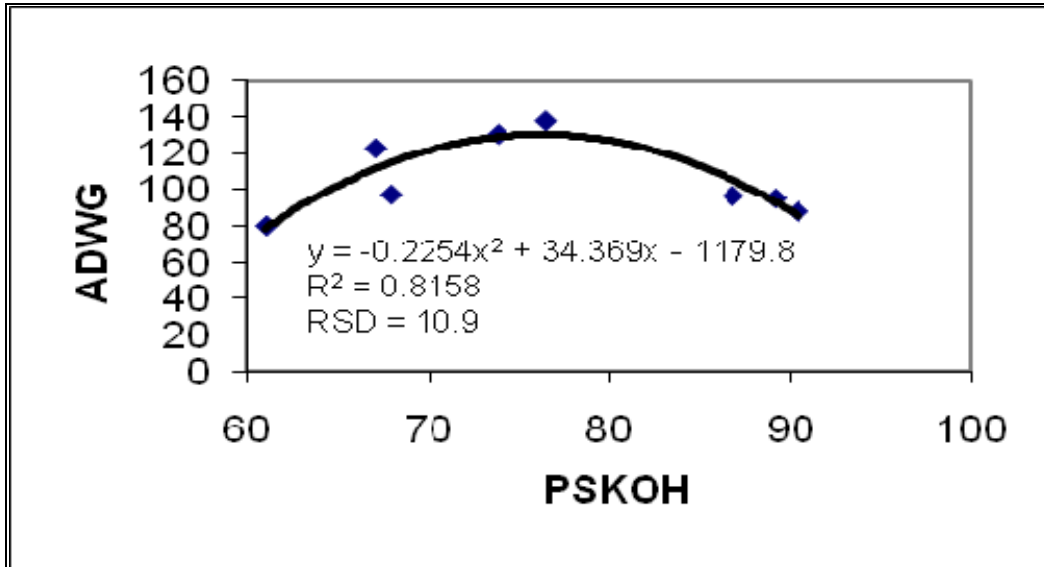


Figure 4.4: Relationship between PSKOH and the average daily weight gain in trials with broilers.

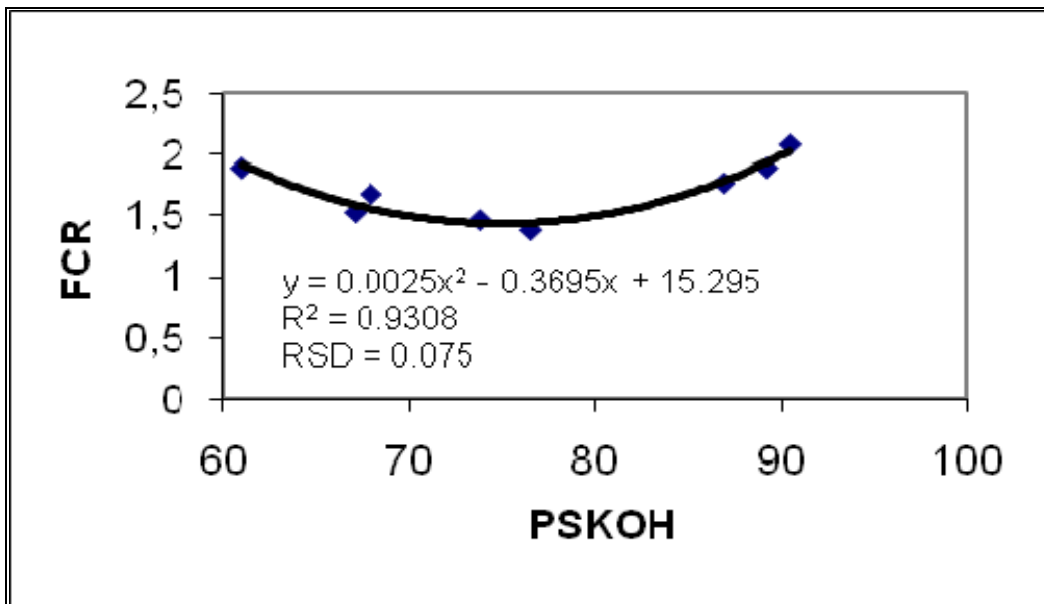


Figure 4.5: Relationship between PSKOH values and feed conversion ratio of broilers in the *in vivo* trials.

The results showed a very good correlation, i.e. $R^2 = 0.82$ and $R^2 = 0.93$ between the PSKOH values and the animal production parameters ADWG and FCR respectively.

4.4. Protein Dispersibility Index (PDI)

4.4.1. Inter-laboratory study

The results of the inter-laboratory study on the PDI determination of eight FFSSB samples processed at different temperatures by eight national laboratories are shown in Table 4.10. Based on these results a relationship was derived between the PDI values and the processing temperatures (Figure 4.6)

Table 4.10: Results of determination of Protein Dispersibility Index (PDI) in FFSSB samples processed by dry extrusion at different temperatures.

Lab No	PDI						
	110°C	127°C	136°C	140°C	145°C	151°C	164°C
1	38.21	25.77	11.62	9.67	8.42	7.25	6.21
	36.66	22.10	9.22	10.55	8.89	7.82	6.75
2	37.26	21.32	10.34	9.07	8.74	7.77	6.74
	36.15	21.46	10.01	9.27	8.81	7.30	6.09
3	47.63	26.39	9.64	8.91	8.79	7.27	4.09
	47.18	28.20	10.01	8.36	9.39	6.80	4.23
4	44.72	31.10	14.69	10.87	9.10	3.96	1.62
	48.42	30.10	10.64	8.51	8.86	4.57	2.43
5	45.50	19.83	11.88	9.55	10.08	8.45	8.66
	47.62	18.17	12.54	9.50	9.90	8.26	8.38
6	46.24	27.79	10.17	9.12	9.02	8.89	7.78
	45.61	28.58	10.18	8.92	8.70	8.23	8.10
7	41.80	25.40	10.10	8.70	8.60	7.90	7.20
	41.90	25.25	10.70	8.80	8.10	8.20	7.10
8	35.86	11.84	6.15	6.90	5.01	4.73	4.66
	36.80	12.25	6.81	6.10	5.42	5.48	5.39
Average	42.35	23.47	10.30	8.92	8.49	7.06	5.96

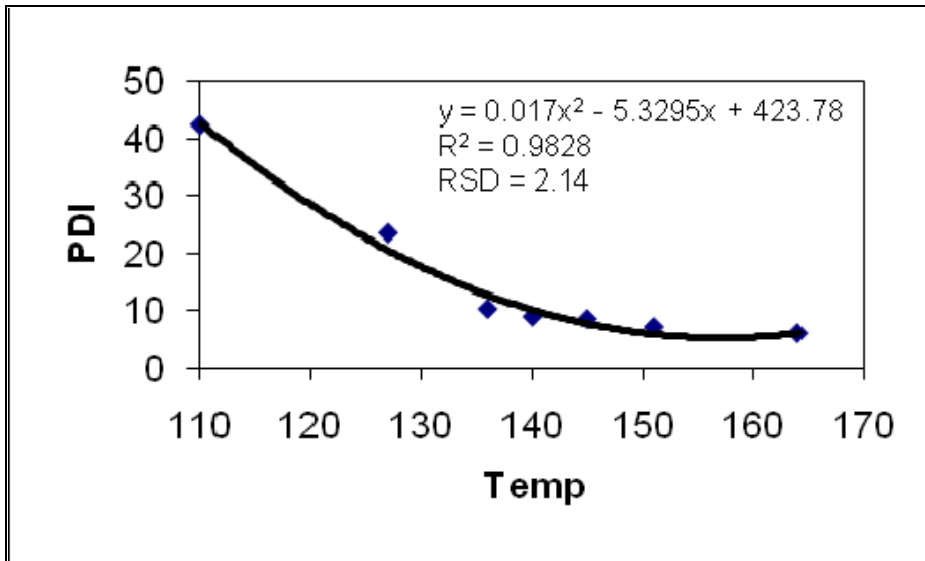


Figure 4.6: The influence of processing temperature on PDI values.

A high negative correlation ($R^2=0.98$) was established between the FF SB processing temperature and the PDI (Figure 4.6).

The precision parameters of the PDI method were determined based on results from the eight national laboratories that participated in the inter-laboratory study (Table 4.10) and are tabulated in Table 4.11.

Table 4.11. Precision parameters of project PDI method

Parameter	Sample							
	110°C	127°C	136°C	140°C	145°C	151°C	164°C	
Number of Laboratories	8	8	8	8	8	8	8	
Number of laboratories retained after eliminating outliers	8	8	8	8	8	8	8	
PDI values (index units), average for 8 laboratories	42.35	23.47	10.30	8.92	8.49	7.06	5.96	
Repeatability standard deviation (s_r), index units	1.21	1.16	1.22	0.68	0.27	0.38	0.36	0.75
Repeatability relative standard deviation (RSD_r), index units	2.85	4.92	11.81	7.61	3.22	5.33	6.10	4.94
Repeatability limit (r) [$r = 2,8 \times s_r$], index units	3.38	3.23	3.40	1.90	0.77	1.05	1.02	2.11
Reproducibility standard deviation (s_R), index units	4.95	5.99	2.05	1.20	1.42	1.58	2.15	2.76
Reproducibility relative standard deviation (RSD_R), index units	11.69	25.51	19.87	13.41	16.72	22.40	36.09	18.14
Reproducibility limit (R) [$R = 2,8 \times s_R$], index units	13.86	16.76	5.73	3.35	3.97	4.43	6.03	7.73

When using the PDI method as described in this study, the **repeatability** (r) i.e. absolute difference between two single results of analysis of the same sample obtained in **one laboratory**, should not exceed **2.11** index units.

The **reproducibility** of the PDI method (R), i.e. the absolute difference between single results of analysis of the same sample obtained in **different laboratories**, should not exceed **7.73** index units, as calculated in Table 4.11.

Table 4.12: South African ranges for describing the degree of FFSB processing using the project PDI method (Summarized from Table 4.11)

Degree of FFSB processing	PDI (index units)
Under-processed	>10.30
Adequately-processed	8.50– 10.30
Over-processed	<8.50

According to the results, adequately-processed soybeans have PDI values between 8.50 and 10.30 index units. PDI values below 8.50 and above 10.30 index units are associated with under- and over-processed soybeans respectively (Table 4.12).

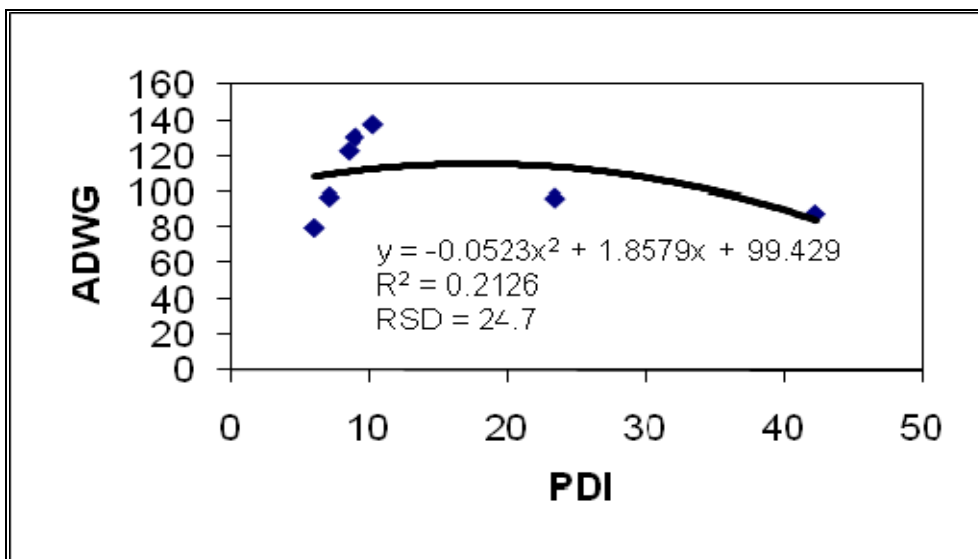


Figure 4.7: Relationship between PDI values and average daily weight gain (g), in the trial with broilers

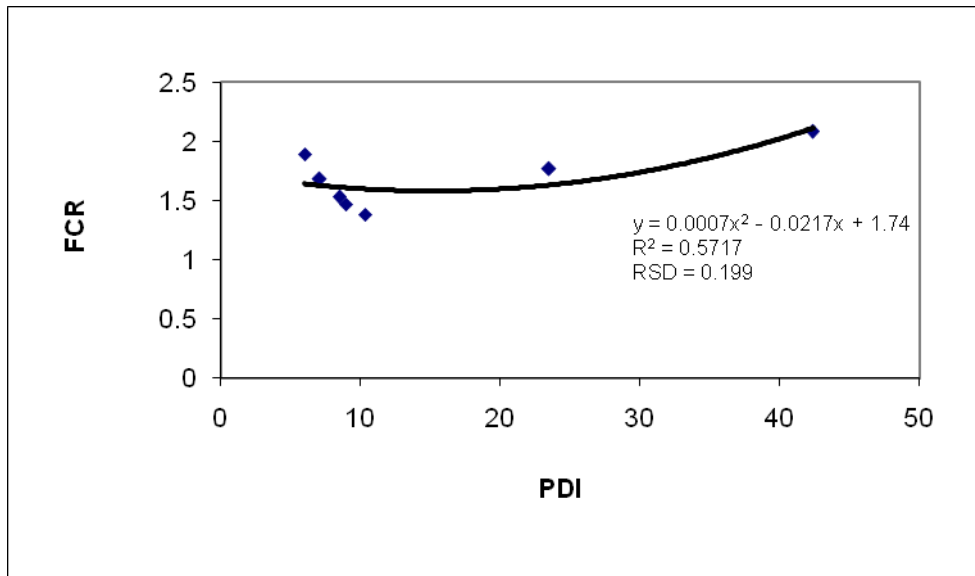


Figure 4.8: Relationship between PDI values and feed conversion ratio (FCR) in the trial with broilers

The results (Figure 4.7 and 4.8) showed a very low correlation ($R^2 = 0.21$ for ADWG and $R^2 = 0.57$ for FCR) between the PDI values and the animal production parameters when using the PDI method as described in this study.

CHAPTER 5

Discussion

5.1. *In vivo* trials with broilers to determine the adequacy of the processed full fat soybeans (FFSB)

The *in vivo* trials with broilers conducted in the present study showed that the best performance with regard to the average daily gain (ADG) and feed conversion ratio (FCR) was achieved at the FFSB which were processed between 136⁰C -145⁰C as summarized in Table 4.2. Heat treatment has been shown to remove the detrimental effects of anti-nutrient factors, especially trypsin inhibitors in FFSB (Leesson and Atteh, 1996) and to improve the protein quality by the denaturation of the anti-nutrient factors. From the present study it was observed that increasing heat treatment improved the growth performance of broilers, but FFSB processed at higher temperatures of above 145⁰C resulted in a decreased growth performance.

In correlation with the above mentioned results, studies by Leeson and Atteh (1996) observed that extrusion temperatures of 140 °C are regarded as adequate to process FFSB and are recommended for inclusion in broiler diets as they result in the highest weight gain in broilers. In addition, Meyer and Froseth (1988) reported an improved body weight gain and FCR in broiler chicks fed a diet containing soybeans extruded at 138 °C-154 °C.

5.2. Urease activity index (UAI) as an indication of the degree of adequacy of full fat soybean (FFSB) processing.

5.2.1. Urease activity index (UAI) using the change in pH (Δ pH) method

According to the results indicated in Table 4.3 and 4.4 there was a significant difference ($P < 0.05$) for the sample processed at 125 °C between test laboratories A and B. These observations raised many questions regarding the reliability of the UAI method using the (Δ pH) method especially as the same protocol was followed in both laboratories. Therefore, in order to have a deeper understanding of the differences in the results obtained by the two laboratories, a single-laboratory follow-up study was conducted whereby the Δ pH method and the NH_3

released method were compared.

5.2.2. Urease activity index (UAI) using the Δ pH and NH_3 released methods

The Urease activity index (UAI) determined by the NH_3 released method had a linear response to an increase in temperature as compared to the UAI using the Δ pH method. The released NH_3 decreased linearly up to the entry point (136 °C) of adequately-processed FFSB and remained constant throughout.

When using the Δ pH method, there was a steep decrease in urease activity and a value close to zero was reached from the FFSB processed at 127 °C, which is assessed as under-processed according to the *in vivo* trials with broilers (Table 2). A significant number of studies reported that the UAI method is only effective in assessing under-processed FFSB (Waldroup, 1982; Araba and Dale, 1990; Batal et al, 2000; Lee et al, 2007). Waldroup (1982); Araba and Dale (1990), Batal et al. (2000) and Lee et al. (2007) indicated that the Δ pH reaches a value close to zero at the entry point of adequately-processed FFSB. A value close to zero was expected to be reached at 136 °C which is regarded as the entry point of adequately-processed FFSB in this study. Similar observations were made when the NH_3 released method was used at temperatures below 136 °C where the urease activity was low (Table 4.5). McNaughton et al. (1981), Araba and Dale (1990), Anderson-Haferman et al. (1992) and Batal et al. (2000) also reported little or no change in the urease activity for the high extrusion temperatures that produced the largest changes in chick growth performance.

The fact that the UAI is not linear (using the Δ pH method) and that it rapidly falls from approximately 1.92 units of pH to a value near zero (Figure 4.2) as the extrusion temperatures increase, contributes to the difficulty in determining a precise maximum acceptable level of urease activity using the Δ pH method.

The Urease activity index (UAI) as a quality control indicator using the released NH_3 method can be used to identify under-processed FFSB only. It was also observed that the NH_3 -released procedure showed more reliability in assessing under-processed FFSB than the Δ pH procedure.

5.3. Protein solubility of FFSB in KOH (PSKOH) for determining the degree of heat treated full fat soya bean (FFSB)

Globally accepted ranges for describing the degree of FFSB processing using the Protein solubility in KOH method (Monary, 1989) are as follows:

In the preliminary study on these values, according to the results of laboratories A and B (Table 4.6), the FFSB sample processed at 145 °C was assessed as over-processed. The same sample was assessed as adequately-processed by means of the *in vivo* trials with broilers.

Following the obtained results, a critical review of the original PSKOH method (Araba and Dale, 1990) used by test laboratories A and B was undertaken and four major possible sources of errors in analysis were revealed:

1. Milling through a 0.5 mm screen.

It was very difficult to obtain a representative sample, uniform in the size of the particles of FFSB by milling through a 0.5 mm screen. This has been shown to be especially true for samples processed above 130 °C.

2. Mixing of FFSB sample with 75 ml of 0.2% KOH.

The volume and shape of the beaker, which both affect the protein solubility, were not specified in the method.

3. Stirring of the sample on a magnetic stirring palate.

The speed of stirring and the size of the magnetic stir bar, both of which have a great effect on protein solubility, were not specified the method.

4. Centrifugation of mixture at 2700 rpm (revolutions per minute).

Specification of “rpm” value means little in practice where different types of centrifuges are used. The g-value (centrifugal force) which is the acceleration constant between centrifuges should be specified instead of the rotational speed (rpm). Specifying the

acceleration is important as two rotors with different diameters running at the same rotational speed will subject samples to different accelerations.

Based on a review of the method, modifications to the original KOHPS method were made.

5.3.1 Inter-laboratory studies to standardize the degree of adequately-heat treated FFSB using the PSKOH method for use in South African laboratories

A reduction in the protein solubility in 0.2% KOH was found to be associated with an increased heat treatment in soybeans and a decreased growth performance in chickens (Wiriyaumpaiwong et al, 2004). As indicated in Table 2, FFSB processed at temperatures between 136 °C and 145⁰C represented adequately-processed FFSB. PSKOH values for these samples obtained in the inter-laboratory study were 77 % and 67% respectively as shown in Table 4.7. Therefore, these are the South African ranges for describing the degree of FFSB processing using the PSKOH method.

The PSKOH did not change consistently as soybeans were heated at higher temperatures. The inconsistent response for FFSB protein solubility in KOH was observed at higher temperatures of above 145 °C at which the growth performance of broilers decreased. According to the observed results, the FFSB processed at 151⁰C had a PSKOH value of 68% which falls within the range of adequately-processed FFSB. The same FFSB is assessed as over-processed in the *in vivo* trials. Previous studies by Parsons et al. (1991), Anderson-Haferman et al. (1992), and Batal et al. (2002) reported that PSKOH is a good indicator of the over-processing rather than the under-processing of soybeans. On the other hand, results of the inter-laboratory study showed that PSKOH is a reliable indicator of the under-processing rather than the over-processing of soybeans.

Recently, Lee et al. (2007) reported a very good negative correlation ($R^2=0.97$) between PSKOH and treatment temperatures. In agreement with the present study, a very good negative correlation ($R^2=0.94$) was achieved between PSKOH and treatment temperatures.

There was a good correlation between PSKOH values and the animal production parameters (Figures 4.4. and 4.5). This is in agreement with Parsons et al. (1991) who also reported a good

correlation between the KOH and the growth performance of chicks and pigs especially in the case of over-heat-treated soybeans.

5.3.2 Precision of the PSKOH method in terms of repeatability and reproducibility of the method

When the PSKOH project method, as described in this study, is used by South Africa laboratories, the repeatability (absolute difference between two single results of analysis of the same sample obtained in one laboratory) should not exceed 3.48% and the reproducibility (absolute difference between single results of analysis of the same sample obtained in different laboratories) should not exceed 10.86% as shown in Table 4.9.

5.4. Inter-laboratory studies using the protein dispersibility index (PDI) as an indicator of the degree of heat treated full fat soya beans (FFSB).

The PDI measures the protein solubility in water with high speed mixing (Lee et al. 2007). According to the *in vivo* trials with broilers (Table 4.2), the samples which represent adequately-processed FFSB were between 136 °C and 145 °C. The PDI values for those temperatures were between 10.3 and 8.5 index units. Therefore, South African ranges for describing the degree of FFSB processing using the PDI method applied in this study were found to be between 8.5 and 10.3 index units (Table 4.10). Nevertheless, these results are much lower than the globally accepted PDI values which are between 15 and 28 index units (Monary, 1989). At this stage, there is no firm explanation for the difference between the globally accepted and the values established in the present study.

There was a good correlation between the PDI values and extrusion temperatures. Studies by Lee et al. (2007) also reported a good correlation between treatment temperatures and the PDI values. The present study showed that there was a steep decrease in the PDI from a temperature of 110 °C to 127 °C and, thereafter, a slight decrease was observed throughout. In addition, Lee et al. (2007) reported a decrease when the PDI was used to determine the quality of soya flakes (SFs). They reported that the PDI decreased steeply when the SFs were heat treated for five minutes at 95 °C and 110 °C, but decreased only slightly thereafter. Nevertheless, Batal et al. (2000) reported a consistent pattern of decrease of the PDI in response to the temperature.

Previous studies have shown that the PDI is a better indicator of the adequate-heating of soybean meal than either the UIA or the PSKOH (Batal et al, 2000). However it was observed that the PDI has no meaning when compared with the *in vivo* quality of heat treated soya flakes (Lee et al, 2007), which is in line with the results of this study as presented in Figures 4.7 and 4.8, as a low correlation was observed between the PDI and animal production parameters (ADWG and FCR).

5.5. Precision of the PDI method in terms of the repeatability and reproducibility of the method

When the PDI method as described in this study is used the repeatability (r), i.e. absolute difference between two single results of analysis of the same sample obtained in one laboratory, should not exceed 2.11 index units.

The reproducibility of the PDI method (R), i.e. the absolute difference between the single results of analysis of the same sample obtained in different laboratories, should not exceed 7.73 index units. The repeatability limit of the PDI method is very good. Nevertheless the reproducibility value of 7.73 is too broad, considering the narrow (8.50-10.30) range for adequately processed FFSSB.

CHAPTER 6

Conclusions and recommendations

Although the urease activity index (UAI) using the change in pH (Δ pH) procedure is used as an indicator of under-processed full fat soybeans (FFSB), the test has met with some criticism in a number of studies. Araba and Dale (1990) found that the UAI of zero does not always indicate heat damaged FFSB and that the assay is of no value in determining over-processed FFSB. Therefore, the assay was declared not suitable for determining over-processed FFSB and only suitable for determining the degree of under-processed soybeans. An alternative UAI method that can be used is the released NH_3 procedure which measures the amount of NH_3 released in a soybean sample to determine the amount of toxic factors.

Results of the present study did not agree with the conclusion made by Araba and Dale (1990) regarding the suitability of UAI using the Δ pH method for determining under-processed FFSB. The results showed that the UAI determined by the Δ pH procedure cannot be considered as a reliable indicator for the FFSB quality control. The NH_3 -released method showed better results when compared to the Δ pH procedure in determining the degree of under-processed FFSB. The UAI determined by the NH_3 -released procedure has potential as a quality control indicator, but for under-processed FFSB only. Therefore, the UAI using NH_3 -released procedure is recommended for use in South African laboratories over the Δ pH procedure in the case where only under-processed soybeans are to be determined.

Scientific studies indicated that the FFSB processors are using the protein dispersibility index (PDI) test to aid in obtaining a high quality soybean product. This is because the PDI procedure is relatively easy and fairly correlated to *in vitro* protein degradability although it tends to lose sensitivity as the optimum heat treatment is approached. Satter et al. (1994) suggested that a PDI value of 9-11 index units be considered as optimally heated, those with a PDI value of 11-14 as marginally under-heated, and others with a PDI value of greater than 14 index units as under-heated. On the other hand, globally accepted PDI ranges are between 15 and 28 index units (Monary, 1989) and a relatively large number of studies indicate different PDI values for

adequately-processed soybeans which caused confusion among soy processors, feed manufacturers, laboratories and end users.

It has been established in the present study that the protein dispersibility index (PDI) range for adequately-processed FFSB is between 8.5 and 10.3 index units, with repeatability and reproducibility limits of 2.1 and 7.7 index units respectively. The reproducibility value of 7.7 index units creates a concern with regard to the use of the PDI method. The PDI method produced a good repeatability limit of 2.1 index units, but the reproducibility limit was too wide (7.7 index units), taking into account the range of 8.5 – 10.3 index units for adequately-processed FFSB. A low correlation between animal production parameters (average daily weight gain and feed conversion ration) and PDI values was established. Therefore, the PDI method in this study did not prove to be the most reliable indicator of processed FFSB despite its simplicity and initial indications that it might be the best indicator.

Protein solubility in potassium hydroxide (PSKOH) is also one of the methods used for FFSB quality control. This method has also indicated variations in the values obtained for adequately processed FFSB. Whittle and Araba (1992) attempted to evaluate a number of the factors that could lead to differences in the PSKOH values obtained in different laboratories. The authors attempted to bring a solution to the confusion experienced by assessing certain variables such as the particle size of SBM, the fat content, the processing method and the duration of extraction in a 0.2% KOH solution. Despite their efforts to standardize the assay, different values were nevertheless obtained for the PSKOH method. Other alternative efforts were made in the present study to attempt to solve the problem further.

When using the protein solubility in potassium hydroxide (PSKOH), the values obtained for adequately-processed FFSB were found to be between 67% and 77% with the absolute difference between two single results of analysis of the same sample obtained in one laboratory not exceeding 3.5% and in different laboratories not exceeding 10.9%.

A very good correlation between the PSKOH and the animal production parameters PSKOH values (average daily weight gain and feed conversion ratio) was established. These factors

have proven that PSKOH is a reliable indicator of the degree of processed FFSB.

Other methods, for example trypsin inhibitor, available lysine and cresol red test were less suitable as they are complicated, require a great amount of effort and, therefore, cannot be recommended for a routine, everyday quality control of FFSB.

The results of the present study will be able to address an effective resolution to the confusion between the following parties: Soya processors- feed manufacturers –laboratories- and end users.

Protein solubility in KOH is recommended as the best method for the analysis of extruded FFSB in South African laboratories because of the fact that it showed good repeatability and reproducibility values when evaluated in the inter-laboratory study and also a very good correlation with the *in vivo* trials using broilers.

It is also recommended that an initiative for establishing the PSKOH method used in this study as an official method for determining the degree of FFSB processing in South Africa is put forward.

REFERENCES

- Anderson, RL & Wolf, WJ 1995, 'Compositional changes in trypsin inhibitors phytic acid, saponins and isoflavones related to soybean processing', *Journal of Nutrition*, vol. 125, pp. 581-8.
- Anderson-Haferman, JC, Ahang, Y, Parsons, CM & Hymowitz, T 1992, 'Effect of heating on the nutritional quality of Kunitz-trypsin-inhibitor-free and conventional soybeans for chicks', *Poultry Science*, vol.711, pp.700–1709.
- A.O.C.S Official Methods 1997a, 'Sampling and analysis of Oilseed by-products', Method BA 9-65 - Urease Activity, Champaign, IL.
- A.O.C.S Official Methods 1997b, 'Sampling and analysis of Oilseed by-products', Method BA 10-65 - Nitrogen Solubility Index (NSI), Champaign, IL.
- A.O.C.S Official Methods, 1997c, 'Sampling and analysis of Oilseed by-products', Method BA 11-65 - Protein Dispersibility Index (PDI), Champaign, IL.
- A.O.C.S Official Methods, 1997d, 'Collaborative Study Procedures', AOCS Procedure M48-6, Champaign, IL.
- A.O.C.S. Official Methods, 1997e. Determination of precision of analytical methods, AOCS procedure m4-86, Champaign, IL.
- Araba, M & Dale, NM 1990, Evaluation of protein solubility as an indicator of over-processing of soybean meal', *Poultry Science*, vol. 69, pp. 76-83.
- Barbi, JW 1996, 'Technology comparisons and results on processing and nutrition of fullfat soya, Second International Fullfat Soya Conference, Budapest, Hungary.

Batal, AB, Douglas, MW, Engram, AE & Parsons, CM 2000, 'Protein dispersibility index as an indicator of adequate processed soybean meal', *Poultry Science*, vol.79, pp. 1592-1596.

Baumann, E, Stoya, G, Völkner, A, Richter, W, Lemke, C & Linss, W 2000, 'Hemolysis of human erythrocytes with saponin affects the membrane structure', *Journal of Acta Histochemica*, vol. 102, pp. 21–35.

Bruce, KJ, Karr-Lilienthal, LK, Zinn, KE, Pope, LL, Mahan, DC, Fastinger, ND, Watts, M, Utterback, PL, Parsons, CM, Castaneda, EO, Ellis M & Fahey, GC jr 2006, 'Evaluation of the inclusion of soybean oil and soybean processing by-products to soybean meal on nutrient composition and digestibility in swine and poultry', *Journal of Animal Science*, vol. 84, pp. 1403-1414.

Buttle, LG, Burrells, AC, Good, JE, Williams, PD, Southgate, PJ & Burrells, C 2001, 'The binding of soybean agglutinin (SBA) to the intestinal epithelium of Atlantic salmon, *Salmo salar* and Rainbow trout, *Oncorhynchus mykiss*, fed high levels of soybean meal', *Journal of Veterinary Immunology and Immunopathology*, vol. 80, no. 3, pp. 237–244.

Calvalho, MRB & Sgabieri, VC 1997, 'Heat treatment and inactivation of trypsin-chymotrypsin inhibitors and lectins from faba beans (*Phaseolus vulgaris* L)', *Journal of Food Biochemistry*, vol. 21, pp. 219-233.

Carbonaro, M, Grant, G, Cappeloni, M, Pusztai, A 2000, 'Perspectives into factors limiting in vivo digestion of legume proteins: antinutritional compounds or storage proteins?' *Journal of Agricultural Food Chemistry*, vol. 48, pp. 742–749.

Carpenter, KJ, McDonald, I & Miller, WS 1972, 'Protein quality of feedstuffs: Collaborative studies on the biological assays of available methionine using chicks', *British Journal of Nutrition*, vol. 27, pp. 7-17.

Carroll, KA 1991, 'Review of clinical studies on cholesterol lowering response to soy protein', *Journal of American Diet Association*, vol. 91, pp. 820- 827.

Chiechi, LM, Secreto G, D'Amore, M, Fanelli, M, Venturelli, E, Cantatore, F, Valerio, T, Laselva, G, Loizzi, P 2002, 'Efficacy of a soy rich diet in preventing postmenopausal osteoporosis: the Menfis randomized trial', *The European Menopause Journal*, vol. 42, pp. 295-300.

Clarke, E & Wiseman, J 2005, 'Effects of variability in trypsin inhibitor content of soya bean meals on true and apparent ileal digestibility of amino acids and pancreas size in broiler chicks', *Journal of Animal Feed Science and Technology*, vol. 121, pp. 125–138.

Clarke, E & Wiseman, J 2007, 'Effects of extrusion conditions on trypsin inhibitor activity of full fat soybeans and subsequent effects on their nutritional value for young broilers', *Journal of British Poultry Science* vol.48, no.6, pp.703-712

Clarke, E & Wiseman, J 2008, 'Protein dispersibility index as a predictor of the nutritional value of soybeans fed to broiler chicks', *Journal of British Poultry Science*, Vol. 4, Suppl. 1, pp. 6–7.

D'Amico, G, Gentile, MG, Manna, G, Fellin, G, Ciceri, R, Cafano, F, Petrini, C, Lavarda, F, Perolini, S & Porrini, M 1992, 'Effect of vegetarian soy diet on hyperlipidaemia in nephrotic syndrome', *Lancet*, vol. 339, no. 8802, pp. 1131-1134.

Dawson-Hughes, B 2003, 'Interaction of dietary calcium and protein in bone health in humans', *Journal of Nutrition*, vol. 133. suppl. 3, pp. 852-854.

Davies, H 1998, Processing of full-fat soybeans, Proceedings of a workshop on the processing of full-fat soya, Irene, South Africa.

Duranti, M, Gius, C 1997, 'Legume seeds: protein content and nutritional value'. *Journal of Field Crop Research*, vol. 53, pp. 31–45.

Friesen, KG, Nelsson, JL, Goodband, RD, Behnke, KC & Kats, LJ 1993, 'The effect of moist extrusion of soy products on growth performance and nutrient utilization in the early-weaned pig', *Journal of Animal Science*, vol. 71, pp. 2099–2109.

Garlich, G 1988, Soyabean meal and measures of its quality, The Pacific Northwest Animal Nutrition Conference, Spokana, W.A, USA.

Greaves, KA, Parks, JS, Williams, KJ & Wagner, JD 1999, 'Intact Dietary Soy Protein, but Not Adding an Isoflavone Rich Soy Extract to Casein, Improves Plasma Lipids in Ovariectomized Cynomolgus Monkeys', *Journal of Nutrition*, vol. 129, pp. 1585–1592.

Greendale, GA, Fitzgerald, G, Huang, MH, Sternfeld, B, Gold, E, Seeman, T, Sherman, S & Sowers, M 2002, 'Dietary soy isoflavones and bone mineral density: Results from the study of women's health across the nation', *American Journal of Epidemiology*, vol. 155, pp. 746-754.

Hamerstrand, GE, Black, LT & Glover, JD 1981, 'Trypsin inhibitor in soy products: Modification of the standard analytical procedure', *Journal of Cereal Chemistry*, vol. 58, pp.42-45.

Harper, JM 1978, 'Food extrusion'. *Critical Review Food Science Nutrition*, vol. 11, pp. 115-123.

Hodges, RE, Krehl, WA, Stone, DB & Lopez, A 1967, 'Dietary carbohydrates and low cholesterol diets: effects on serum lipids of man', *American Journal of Clinical Nutrition*, vol. 20, pp. 198-208.

Huisman, J & Tolman, GH 1992, Anti-nutritional factors in plant proteins of diets for non-ruminants. In: Garnsworthy, PC, Haresign, W & Cole, DJA (Eds), *Recent Advances in Animal Nutrition*, Butterworth – Heinemann Ltd., Oxford.

Hwang, YW, Kim, SY, Jee, SH, Youn Nam Kim, YN, Chung Mo Nam, CM 2009, 'Soy Food Consumption and Risk of Prostate Cancer: A Meta-Analysis of Observational Studies', *Journal of Nutrition and Cancer*, Vol. 61, no. 5, pp. 598–606.

Jimenez, AA, Perry, TW & Beeson, WM 1960, 'Nutritive value of raw or heated unextracted soybeans as supplemental protein for swine'. *Journal of Animal Science*, vol. 20, pp. 936 -937.

Jimenez, AA, Perry, TW, Picket, RA & Beeson, WM 1963, 'Raw and heat treated soybeans for growing-finishing swine, and their effect on fat firmness'. *Journal of Animal Science*, vol. 22, pp. 471-475.

Kakade, ML & Liener, IE 1969, 'Determination of available lysine in proteins', *Journal of Analytical Biochemistry*, vol. 27, pp. 273 – 280.

Kakade, MI, Rakis, JJ, McGee, JE & Puskai, G 1974, 'Determination of trypsin inhibitor activity of soy products: a collaborative analysis of an important procedure', *Journal of Cereal Chemistry*, vol. 51, pp. 376-382.

Katic, Z, Kritizic, N, Pliestic, S & Tajana, K 1996, 'Optimizing roasting process of full fat soya, 2nd International Full-fat Soya Conference, Hungary. pp 90-98.

Kim, DN, Lee, KT, Reiner, JM & Thomas, WA 1980, 'Increased steroid excretion in swine fed high-fat high cholesterol diet with soy protein', *Journal of Experimental Molecular Pathology*, vol. 33, pp. 25-35.

Kourteva, I, Sleigh, RW, Hjerten, S 1987, 'Assay for enzyme inhibition: detection of natural inhibitor trypsin and chymotrypsin', *Journal of Analytical Biochemistry*, vol. 162, pp. 345-349.

Kratzer, FM, Bersh, S, Vohra, P & Ernst, RA 1990, 'Chemical and biological evaluation of soyabean flakes autoclaved for different durations', *Journal of Feed Science and Technology*, vol. 31, pp. 247-259.

Kung, L jr, & Huber, JT, 1983, 'Performance of high producing cows in early lactation feed protein of varying amounts, sources and degradability', *Journal of Dairy Science*, vol. 66, pp. 227-234.

Lajolo, FM & Genovese, MI 2002, 'Nutritional significance of lectins and enzyme inhibitors from legumes', *Journal of Agricultural Food Chemistry*, vol. 50, pp. 6592–6598.

Lammersfeld, CA, King, J, Walker, S, Vashi, P G, Grutsch, James F, Lis, CG and Gupta, D 2009, 'Prevalence, sources, and predictors of soy consumption in breast cancer', *Journal of nutrition*, vol. 8, no. 2, pp. 1-7.

Lawrence, TLJ, 1973, 'An evaluation of the micronization process for preparing cereals for growing pigs. 1. Effects on digestibility and nitrogen retention', *Journal of Animal Production*, vol. 16, pp. 99–107.

Lee, H.S, Kim, JG, Shin, YW, Park YH., You, SK, Kim, SH & Whang KY 2007, 'Comparison of laboratory analytical values and *in vivo* soybean meal quality on pigs by employing soyflakes heat-treated under different conditions', *Short Communication*, vol. 134, pp. 337-346.

Leeson, S & Atteh, JO 1996, 'Response of broiler chicks to dietary full-fat soybeans extruded at different temperatures prior to or after grinding', *Journal of Animal feed science & technology*, vol. 57, pp. 239-245.

Liener, IE 1994, 'Implications of anti-nutritional components in soybean foods', *Critical Reviews in Food Science & Nutrition*, vol. 34, pp. 31–67.

Liu, K 2000, 'Expanding soybean food utilization', *Journal of Food Technology*, vol. 54, no. 7, pp. 46–58.

Loon, CY 1996, 'Full fat soybean meal production and utilization', American soybean association, Singapore.

Machado, FPP, Queirz, JH, Oliveira, MGA, Piovesan ND, Peluzio MCG, Costa NMB & Moreira, MA 2008, 'Effects of heating on protein quality of soybean flour devoid of Kunitz inhibitor and lectin', *Journal of Food Chemistry*, vol. 107, pp. 649–655.

Machlachlan, R. 1993, 'The use of full fat soybeans in poultry feeds', AFMA Symposium, Alternative raw materials in Animal feeds, South Africa, pp. 31-47.

MarsmanA, GJP, Gruppen, H, Van Der Poel, AFB, Kwakkel, R P, Verstegen, MWA & Voragen, AGJ, 1997, 'The Effect of Thermal Processing and Enzyme Treatments of Soybean Meal on Growth Performance, Ileal Nutrient Digestibilities, and Chyme Characteristics in Broiler Chicks', *Journal of Poultry Science*, vol. 76, pp. 864–872.

Massey, LK 2003, 'Dietary animal and plant protein and human bone health: A whole foods approach', *Journal of Nutrition*, vol. 23, pp. 862-865.

McNab, JM. & Wilson, BJ 1974, 'The nutritive value of triticale and rye in broiler diets containing field beans', *British Poultry Science*, vol. 16, pp. 17-22.

McNaughton, JM, Reece, FN & Deaton, JW 1980, 'Effect of moisture content and cooking time on soybean meal urease index, trypsin inhibitor content, and broiler performance' *Journal of Poultry Science*, vol. 59, pp. 2300-2306.

McNivena, MA, Duynisveld, J, Charmley, E & Mitchella, A 2004, 'Processing of soybean affects meat fatty acid composition and lipid peroxidation in beef cattle', *Journal of Animal feed science*, vol. 116, pp. 175–184.

Messina. MJ 1999, 'Legumes and soybeans: overview of their nutritional profiles and health effects'. *America. Journal of Clinical Nutrition*, vol. 70, Suppl, pp. 439-450.

Messina, MJ 2003, 'Emerging evidence on the role of soy in reducing prostate cancer risk', *Journal of Nutrition Reviews*, vol. 61, pp. 117-31.

Messina, M & Barnes, S 1991, 'The role of soy products in reducing risk of cancer', *Journal of National Cancer Institute*, vol. 83, no. 8, pp. 541-6.

Messina, M, Ho, S & Alekel DL 2004, 'Skeletal benefits of soy isoflavones: a review of the clinical trial and epidemiologic data', *Journal of Current Opinion in Clinical Nutrition & Metabolic Care*, vol. 7, no. 6, pp. 649-58.

Messina, M, Kucuk & O, Lampe, JW 2006, 'An overview of the health effects of isoflavones with an emphasis on prostate cancer risk and prostate-specific antigen levels. *Journal of AOAC International*, vol. 89, no. 4, pp. 1121-34.

Messina MJ, Persky V & Setchell KD, Barnes S 1994 'Soy intake and cancer risk: a review of the in vitro and in vivo data', *Journal of Nutritional Cancer*, vol. 21, pp. 113-131.

Meyer, RO, & Froseth, JA 1988, 'Extruded mixtures of beans (*Phaseolus vulgaris*) and soybeans as protein sources in swine and chick diets', pp. 869, In: MarsmanA, GJP, Gruppen, H, Van Der Poel, AFB, Kwakkel, R P, Verstegen, MWA & Voragen, AGJ, 1997, 'The Effect of Thermal Processing and Enzyme Treatments of Soybean Meal on Growth Performance', Ileal Nutrient Digestibilities, and Chyme Characteristics in Broiler Chicks', *Journal of Poultry Science*, vol. 76, pp. 864–872.

Monary, S 1989 'Fullfat Soya Handbook, American Soybean Association, Brussels. pp. 6.

Monary, S (2nd ed.), 1996, Fullfat Soya Handbook, American Soybean Assosiation, Brussels. pp 22-28.

Murray, JH, 1987, Unlocking new products opportunities with infrared processing, 2nd International Conference on designing and engineering new food plants, United State of America, pp 8.

Nasri, MHF, France, J, Mesgaran, MD, Kebreab, E 2008, 'Effect of heat processing on ruminal degradability and intestinal disappearance of nitrogen and amino acids in Iranian whole soybean', *Journal of Live stock Science*, vol. 113, pp. 43-51.

Neves, CA, Santos, GT, Matsushita, M, Alves, EM, Oliveira, RL & Branco, AF, Silva, DC Furlan, AC, Petit, HV 2007, 'Intake, whole tract digestibility, milk production, and milk composition of Holstein cows fed extruded soybeans treated with or without lignosulfonate', *Journal of Animal feed science and technology*, vol. 134, pp. 32–44.

Palacios, MF, Easter, RA, Soltwedel, KT, Parsons, CM, Douglas, T, Hymowitz, MW & Pettigrew, JE 2004, 'Effect of soybean variety and processing on growth performance of young chicks and pigs'. *Journal of Animal Science*, vol. 82, pp.1108-1114.

Palic, 1998, Optimal processing of full fat soya: Chemical techniques for quality control, Proceedings of Workshop of Processing of Full Fat Soya, Irene, South Africa, pp 17-19.

Palic, D 2005, Quality control of processed full-fat soybeans using protein solubility in KOH: Critical review and modification of method, Proc, XI International Feed Technology Symposium, Vrnjacka Banja, pp. 106-110.

Palic, D & Grove, AA 2004, Validation of Methods for Determining the Degree of Soya Processing, Joint GSSA – SASAS Congress, Gaudini Spa, South Africa.

Parsons, CM, Hashimoto, K, Wedekind, KJ & Baker, DH 1991, 'Soybean protein solubility in potassium hydroxide: An *in-vitro* test of *in-vivo* protein quality' *Journal of Animal Science*, vol.69, pp.2918–2924.

Perilla, NS, Cruz, MP, De Belalcazar, F, & Diaz, GJ 1997, 'Effect of temperature of wet extrusion on the nutritional value of full-fat soybeans for broiler diets', *Journal of British Poultry Science*, vol. 38, pp. 412-416.

Peumans, WJ & van Damme, EJM 1995, 'Lectins as plant defense proteins', *Journal of Plant Physiology*, vol. 109, pp. 347-352.

Pieterse, H, 2000, 'Soy processing technology', *Proceedings of American Soybean Association*. 2nd International Soy Conference, University of Pretoria, South Africa.

Pipa, F & Frank, G 1989, High-Pressure Conditioning with Annular Gap Expander, *Journal of Advances in Feed Technoly*, vol. 2, pp. 22-30.

Ristic, M, Brana, R, Filipovic, S, Marijana, S, Veselina, RP, & Dordevic, M1999, 'Protein meal obtained from small fish by extrusion', *Journal of Acta Veterinaria – Beograd*, vol. 49, no. 1, pp. 57-64.

Saad, CRB 1979, Use of fullfat roasted soybeans in practical catfish diets, Master Thesis, Auburn University, AL, USA, pp. 30, In: Monary, S (2nd ed.), 1996, Full fat Soya Handbook, American Soybean Assosiation, Brussels. pp 22-28.

Samocha, TM, Davisb, DA, Saoudc, IP, DeBault, K 2004, 'Substitution of fish meal by co-extruded soybean poultry by-product meal in practical diets for the Pacific white shrimp, *Litopenaeus vannamei*', *Journal of Aquaculture*, vol. 231, pp. 197–203.

Satter, LD, Dhiman, TR. & Hsu, JT 1994, Use of heat processed soybeans in dairy rations, Proceedings of the Cornell Nutrition Conference for Feed Manufactures, Rochester, USA.

Schulze, H, Saini, HS, Huisman, J, Hessing, M, Van Den Berg, W & Verstegen, MWA 1995, 'Increased nitrogen secretion by inclusion of soy lectin in the diets of pigs', *Journal of Science and Food in Agriculture*, vol. 69, pp. 501-510

Senkoylu, N, Samli, HE, Akyurek, H, Agma, A & Yasar, S 2005, 'Use of High Levels of Full-Fat Soybeans in Laying Hen Diets' *Journal of Applied Poultry Research*, vol. 14, pp. 32–37.

Serrano, X & Villa, E 1999 The extrusion cooking process in piglet feeding: Nutritional

implications, Homatge al Dr. F. Puchal, Univesidad Aut a de Barcelona. Bellaterra, Barcelona.

Shu XO, Jin F & Dai Q. Soyfood Intake during Adolescence and Subsequent Risk of Breast Cancer among Chinese Women, *Cancer Epidemiology Biomarkers Preview*, vol. 10, no. 5, pp. 483-488.

Sirtori, CR, Lovati, MR, Manzoni, AC, Mohetti, M & Pazzucogh, F 1995, 'Soy and Cholesterol Reduction: Clinical Experience1', *Journal of Nutrition*, vol. 125, suppl. Pp. 598-605.

Smith, C, Van Megen, W, Twaalfhoven, L & Hitchcock, C 1980, 'The determination of trypsin inhibitor levels in foodstuffs', *Journal of Scientific Food Agriculture*, vol. 31, pp. 341-350.

Sørensen, M, Stjepanovic, N, Romarheim, OH, Krekling, T & Storebakken, T 2009, 'Soybean meal improves the physical quality of extruded fish feed'. *Journal of Animal Feed Science and Technology*, vol. 149, pp. 149–161.

Sparg, SG Light, ME & van Staden, J 2004, 'Biological activities and distribution of plant Saponins', *Journal of Ethnopharmacology*, vol. 94, pp. 219–243.

Stephenson, EL & Tollett, L 1959, 'Processing of soybeans for broiler feeding', *Journal of Feedstuffs*, p 8.

Stern, MC 1989, 'Protein metabolism in the lactating dairy cow', Proc. Dairy Research Conference, Minnesota Department of Animal Science, University of Minnesota, U.S.A.

Tacon, AGJ, Webster, JL, Martinez, CA 1984, 'Use of solvent extracted sunflower seed meal in complete diets for fingerling rainbow trout *ŽSalmo gairdneri* Richardson', *Journal of Aquaculture*, vol. 43, pp. 381–389

Terpstra, AHM, Woodward, CJH, West, CE & Van Boven, JG 1982, 'A longitudinal cross-over study of serum cholesterol and lipoproteins in rabbits fed on semipurified diets containing either

casein or soya-bean protein', *British Journal of Nutrition*, vol. 47, pp. 213-219.

Thomason, DM 1987, Review of processing systems for full-fat soy, Full fat soya:A regional Conference, ASA Milan, Italy, Pp.114-122.

Traina, MS & Breene, WM 1994, 'Composition, functionality and some chemical and physical properties of eight commercial full-fat soy flours', *Journal of Food Processing and Preservation*, vol. 18, pp. 229–252.

Valdebouze, P, Bergeron, E, Gaborit, T, Delort -Laval, J 1980, 'Content and distribution of trypsin inhibitor and haemagglutinins in some legume seeds'. *Canadian Journal of Plant Science*, vol. 60, pp. 695-701.

Van der Poel, AFB, Verstegen, MWA & Tamminga, S 1995, Chemical physical and nutritional effects of feed processing technology, Proceedings of the 16th Western Nutrition Conference, Saskatoon, Canada.

Vasconcelos, IM, Maia, AAB, Siebra, EA, Oliveira, JTA, Carvalho, AFFU, Melo, VMM, Carlini, CR & Castelar, LIM 2001, 'Nutritional study of two Brazilian soybean (*Glycine max*) cultivars differing in the contents of anti-nutritional and toxic proteins', *Journal of Nutritional Biochemistry*, vol. 12, pp. 1–8.

Vasconcelos, IM & Oliveira, JTA 2004, 'Anti-nutritional properties of plant lectins', *Journal of Toxicology*, vol. 44, pp. 385–403.

Vidal-Valverde, C, Frias, J, Sierra, I, Blazquez, I, Lambein, F & Kuo, YH 2002, 'New functional legume foods by germination: effect on the nutritive value of beans, lentils and peas', *Journal of European Food Research & Technology*, vol. 215, pp. 472-477.

Wahlstrom, RC, Libal, GW & Berns, RL 1971, 'Effect of cooked soybeans on performance, fatty acid composition and pork carcass characteristics', *Journal of Animal Science*. Vol. 32,

pp. 891-894.

Waldroup. PW 1982, 'Whole soybeans for poultry feeds', *Journal of World's poultry science association*, vol. 38, pp. 28-35

Waldroup, PW, Ransey, BE, Hellwig, HM & Smith, NK1985, 'Optimum Processing for soybean meal used in broiler diets', *Journal of Poultry Science*, vol. 64, pp. 2314-2320.

Wijeratne, J 2000, 'The process of dry extrusion', *Journal of Feed Technology*, vol. 4, no. 2, Pp.10-12.

Wiriyampaiwong, S, Soponronnarit, S, Prachayawarakorn, S 2004, 'Comparative study of heating processes for full-fat soybeans', *Journal of Food Engineering*, vol. 65, pp. 371–382.

Wiseman, J, 1986, *Recent Advances in Animal Nutrition*, Butterworths, London, Pp2.

White, C.E, Campbell, DR. & Mcdowell, LR 2000, 'Effects of dry matter content on trypsin inhibitors and urease activity in heat treated soya beans fed to weaned piglets', *Journal of Animal Feed Science and Technology*, vol. 87, no. 1–2, pp. 105–115.

Whittle, E, & Araba, M, 1992, 'Sources of variability in the protein Solubility assay for soybean meal', *Journal of Applied Poultry Research*, vol. 1, pp. 221-225.

World Initiative for Soy in Human Health, 2004, American Soybean Association, St. Louis, Missouri USA, Viewed 13 October 2009, <http://www.wishh.org/nutrition/overview.html>

Wu, AH., Ziegler, RG., Nomura, AMY, West, DW, Kolonel, LN, Horn-Ross, PL, Hoover, RN, & Pike, MC 1998, 'Soy intake and risk of breast cancer in Asians and Asian Americans', *American Journal of Clinical Nutrition*, vol. 68, pp. 1437S–1443S.

Zhang, Y, Parsons, CM. & Hymowitz, T 1991, 'Research Note: Effect of soybeans varying in

trypsin inhibitor content on performance of laying hens', *Journal of Poultry Science*, vol. 70, pp. 2210–2213.

Zollitsch, W, Wetscherek, W & Lettner, F 1993, 'Use of differently processed full-fat soya beans in a diet for pig fattening', *Journal of Animal Feed Science and Technology*, vol. 41, pp. 237-246