A comparison of *in vitro* quality parameters and digestibility between locally produced and imported soya oilcake meal destined for the South African pig industry

By

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I, Chantelle Cronje, declare that this dissertation, which I hereby submit for the degree of MSc Agric Animal Science: Animal Nutrition at the University of Pretoria, is my own work and has not previously been submitted by me for a degree at this or any other tertiary institution.

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Abstract

Soya oilcakes are the residues from oilseeds from which most of the oil has been extracted. Undesirably, these meals contain antinutritive factors such as allergenic, goitrogenic and anticoagulant substances as well as protease inhibitors. The most relevant antinutritive factor to consider in monogastric animal feeding is trypsin inhibitor. Trypsin inhibitor is a crystalline globular protein, which restrains the trypsin protein activities, forming an irreversible stoichiometric compound. This results in a high concentration of undigested protein compounds in the lower digestive tract of the pig, and inflammation and other digestive upsets may occur. To reduce the impact of anti-nutritive factors in soya and increase its digestibility, soya needs to be processed by heat. When soya is overheated, the Maillard reaction occurs. This causes the availability of lysine and other essential amino acids to be reduced. The processing of soya is thus of utmost importance to ensure protein availability and high digestibility of the soya proteins. Pig producers in South Africa are concerned about the quality of locally processed soya oilcakes and the effect that it may have on the intestinal health and lifetime production of the pig.

The primary aim of this study was to compare the quality of locally processed soya oilcake to soya oilcake imported from Argentina. The secondary aim of this study was to analyse the locally processed soya oilcake, to ensure that correct nutrient values are used during feed formulation. Several nutrient analyses were done on the soybean oilcakes to compare the quality of the locally processed versus imported soya oilcakes. A digestibility trial was also conduced, to compare the total tract and ileal digestibility of protein in diets formulated with locally produced and imported soya oilcakes.

The nutrient analyses suggested that the imported soya oilcake is of better quality than the locally produced product. All the results for the imported soya oilcakes were very consistent, with minor variations. The imported soya oilcake had lower trypsin inhibitor activity, but caution should be taken since the urease value for the imported soya oilcake was below the recommended values and this could be an indicator of over-processed soya oilcake. Crude protein (CP) values were higher and amino acid profiles had minor variations among the imported soya oilcake samples. A high variation was found for antinutritive factors measured in the soya oilcakes sourced from local processing plants. This may be attributed to different processing methods used between plants and perhaps poor quality-control within plants. The results obtained from this study showed that some of the local soya oilcake processing plants in South Africa produced products of higher quality than others. One of the locally processed soya oilcakes, named soya oilcake 3 in this study, was identified as a good replacement for the imported soya oilcake. Soya oilcake 3

had a CP value of 53.11% which compared well with the imported soya oilcake with a similar CP value of 53.51% (all values based on an as is basis). The protein dispersibility index (PDI) values of the imported soya oilcake and locally processed soya oilcake 3 did not differ from each other ($P<0.05$). The potassium hydroxide percentage (KOH%) value for the imported soya oilcake was 83.94% and 84.20% for soya oilcake 3. Their urease values also did not differ from each other ($P<0.05$). The trypsin inhibitor concentration in the imported soya oilcake was the lowest and the second lowest in soya oilcake 3. Soya oilcake 3 had the second highest methionine value of 0.725 g/kg and the imported soya oilcake had a methionine value of 0.730 g/kg. The two soya oilcakes also did not differ significantly in their lysine values. Due to the high variance found in the results obtained from the *in vivo* digestibility study, it was not possible to make a conclusion on the effect of feeding lower quality soya oilcakes to weaner pigs. Further research is needed on the effect of trypsin inhibitor on gut health and digestibility of crude protein in weaner pigs.

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List of abbreviations

ADF	Acid detergent fibre
AA	Amino acids
ANF	Anti-nutritive factor
ADI	Apparent ileal digestibility
CLA	Conjugated linoleic acids
CF	Crude fibre
CP	Crude protein
DE	Digestible energy
EE	Ether extract
GLM	General linear model
GM	Genetically modified
IgA+	Immunoglobulin A+
IgG	Immunoglobulin G
iDM	Initial dry matter
IL	Interleukin
MCFA's	Medium chain fatty acids
ME	Metabolisable Energy
NIR	Near infrared spectroscopy
NE	Net energy
NDF	Neutral detergent fibre
PUFA	Polyunsaturated fatty acid
KOH test	Potassium hydroxide solubility test
PDI test	Protein dispersibility index test
SCFA	Short chain fatty acids
SA	South Africa
SPC	Soya protein concentrate
SPF	Specific pathogen free
SID	Standardised ileal digestibility
SAS	Statistical Analysis System
TN60	Topigs Norsvin 60 breed
TNC	Total non- structural carbohydrates
TID	True ileal digestibility
TNF	Tumour necrosis factor
USA	United States of Amerika
UI	Urease index

CHAPTER 1

Introduction

Soya oilcake is the protein source mostly used in pig diets. In most pig diets, maize is the main energy source. Maize meals are deficient in amino acids such as lysine and tryptophan. These amino acids are readily available in soya oilcakes and are therefore an important protein source to include in a pig diet. Weaner diets are mostly milk protein-based, hereafter they are gradually introduced to soybean proteins (Shelton, *et al*., 2001).

Many pig producers are concerned about the quality of soya oilcake processed in South Africa and the effect thereof on the gut-health and growth performance of weaner and grower pigs. During the last couple of years there were speculations of an increased risk for infectious enteropathogens, causing scouring in weaner and grower pigs as well as loss in carcass weight at slaughter (Jacobsen *et al*., 2010). Enteritis is caused by high concentrations of undigested proteins in the hindgut due to the trypsin inhibitor binding its free carboxyl groups to the free amino groups on the trypsin molecule (protein molecule). This forms an irreversible stoichiometric compound (Kunitz, 1947). Enteropathogens damages the villi in the small intestine and decrease gut health and growth performance throughout the lifetime of the pig (Zijlstra *et al*.,1997; Moeser *et al*., 2007). Trypsin inhibitors decrease protein digestion and causes hypertrophy of the pancreas, which impairs its secretive function, thus decreasing the utilisation of nitrogen and increases endogenous losses (Friedman & Brandon, 2001; Pacheco *et al*., 2014).

During the last few years, there was an increase in local soya production and soya processing. South Africa (SA) was producing between 450 000 and 500 000 tons per annum in 2010, yielding 2.5 to 3 tons per hectare under dry-land conditions (Department of Agriculture Research Centre, 2010). In 2018, SA produced over 1 200 000 tons per year. The soybean industry in South Africa is currently experiencing a bottle neck effect due to the low yields per hectare, and high demands for processed soya oilcakes. Improved technology and techniques, already implemented in America, must be incorporated in SA. A new Roundup Ready 2 strain soybean of Monsanto is being developed and should be available in SA by 2020. This could possibly increase South African soybean yield to 2 500 000 tons per year. South Africa increased their soybean crushing capacity from 600 000 tons per year in 2012 to more than 2 200 000 tons per annum in 2017 (Protein Research Foundation report, 2016/2017).

The substantial increase in soybean production and processing in South Africa encourages producers to make use of these soybeans in pig diets, due to the higher availability and lower prices of the local processed soya oilcakes. It is therefore important to know the composition and quality of the soya oilcakes in terms of amino acid profiles, presence of anti-nutritive factors and availability of protein, to formulate diets according to the correct nutrient specifications. By comparing the quality of soya oilcakes processed in SA with soya oilcakes imported from Argentina, one can determine whether it is more profitable to use the cheaper South African product. Argentine soya oilcake was used since this is the soya oilcake mostly imported into South Africa. Local soya oilcake prices for September 2019 were R5590/ton and imported soya oilcakes were R5720/ton (Commodity Derivatives – JSE, 2019).

The quality of the soya oilcakes can also affect the digestibility of the proteins present in the feed, which can be measured *in vivo*, using methods such as the indigestible marker technique. Soya oilcakes quality can also be measured directly via *in vitro* analysis of amino acid concentrations and anti-nutritive factors. The primary aim of this study was to compare the quality of South African produced soya oilcake to soya oilcake imported from Argentina, to enable pig producers to make an inform decision whether to use imported soya oilcake or cheaper locally produced soya oilcake. The secondary aim of this study was to analyse the nutrient content of processed soya oilcake from SA, to ensure the use of accurate matrix values during feed formulation to avoid over or under supplying of nutrients to pigs.

The null hypothesis of this trial was that soya oilcake processed in South Africa is of lower quality than imported soybean oilcake. The alternative hypothesis for this study states that locally produced soybean oilcake is of equivalent quality compared to imported soybean oilcake. It is also possible that the lower quality soybean oilcake can successfully be incorporated into weaner diets, without compromising growth performance or intestinal health, by adjusting feed formulations.

CHAPTER 2

Literature review

2.1 Introduction

Soybeans are mainly grown for the use of human consumption, but the by-products are used for animal nutrition (Rodica & Adrian, 2010). Soybeans contains all essential amino acids and is therefore an important source of protein. Soya oilcake, full fat soya, soybean concentrates, and soybean hulls are mostly used in monogastric animal feed (Cromwell, 1999). Imported soya oilcakes make up a large portion of soya oilcakes used in the South African pig feed industry, due to the high demand for soya oilcakes and the concern about lower quality locally processed soya oilcakes. It is important to know the exact chemical composition and nutritive values of soya oilcakes, to be able to incorporate it into the diets of the pigs without compromising health or production. Antinutritive factors present in soya, affects intestinal health and overall production in a pig herd, but the correct processing procedures will reduce the negative effect of antinutritive factors. Soya oilcake quality affects the physiological and immunological processes of the weaner pig and may affect lifetime production of the pig.

2.2 Soybean cultivation and production

Soya is a legume with the scientific name, *Glycine max(L.) Merril* (Morse, 1947). Soybeans, also known as the greater bean, originated in Manchuria, China (Gai, 1997). Soybeans are one of five oldest cultivated crops in the world and were planted by the Chinese 2500 BC. The western parts of the world only started growing soybeans commercially in the late 1800s (Fletcher, 1950). The top producers of soya worldwide, are the United States of America, Brazil, Argentina, China and India (Morse, 1947). The largest crop of soybeans is grown in Brazil, on more than twenty-nine million hectares. There are many different varieties of soybeans in existence. Genetically modified (GM) soybeans were cultivated since 1996 and became the major option for soybean production in most countries. Traits of these GM soybeans include herbicide resistance and good oil extraction properties, which are particularly important in the processing of soya oilcakes. Genetically modified soybeans are low in saturated and trans-fatty acids and may also be beneficial to human health by lowering blood cholesterol levels and reducing the risk for different cancers (Stacey, 2008). Genetically modified soybeans are selected for higher amino acid concentrations and this induces better balanced diets for poultry and pigs (Baker & Stein, 2009; Baker *et al*., 2011).

The first recorded soybean production in South Africa was in 1903, but germination of the seeds was very poor, and the growth of the plants were too fast, the pods shattered before harvesting took place. Later, soybeans were grown on a research farm in Potchefstroom where a better breeding line was developed (Department of Agriculture Research Centre, 2010). Currently, SA are producing over 2.5 tons of soya per hectare on dryland (Protein Research Foundation report, 2017/2018). Mpumalanga produces around 42% percent, Free State 22%, KwaZulu-Natal 15%, Limpopo 8%, North West Province 5% and Gauteng 2%. The different cultivars in South Africa are adapted to specific areas. Cultivars differ in resistance to diseases, climate adaptation, weed resistance and other production properties. Conventional cultivars include among others, Sonop (150), Stork (254), Dumela (305), Kiaat (489), Mopanie (489), Knap (150) and Tambotie (489). Genetically modified cultivars include, PAN 538 RR (1412), LS 6164 R (484), AS 4801 R (1076), AG 5601 (80), Phb 95Y20 R (411) and Phb 95B53 R (411) (Department of Agriculture Research Centre, 2010).

Considering South Africa's current economic status, importing products are becoming too expensive for local feed producers and consumers are increasingly considering the use of cheaper locally processed soya oilcakes. It is important, however, for the consumer to have confidence in the quality of the local product. Even when paying a lower price per ton for locally processed soya oilcakes, inferior quality may cause more digestive upsets in weaner pigs, lower growth in baconers and porkers and thus resulting in a lower net income. The reason behind this is the lower quality of processing due to incorrect or partially incorrect procedures being followed at the processing plants. It is therefore important to weigh these factors up against each other, to ensure optimum profit. The processing capacity in South Africa has grown up to 2.2 million tons per year in 2018 (Department of Agriculture research centre, 2018). This increased the locally produced soya oilcakes to more than 1 million tons. The imports of soya oilcakes have decreased by 33% since 2012. This is due to the increased number of soya processing plants, but even though processing is at an all-time high, there is still a lot of processed soya being imported from Argentina, to meet local demand. Due to low rainfall and other socio-economic factors, a decrease of 27% soya production in 2016/2017 were seen, this increased the need for importing soya oilcakes. Increased processing potential in South Africa will urge feed processors and farmers to make use of these locally processed soya oilcakes. Quality assurance is therefore of high importance when processing these soya oilcakes (Sihlobo & Kapuya, 2016).

2.3 Soybean processing and resultant products

The main producers of soybean oilcakes are China, USA, Argentina and Brazil (ASA, 2002). Soya oilcakes are mainly residues from oilseeds, which most of the oil has already been extracted. There are mainly two

methods used for processing soybeans. The first is the solvent extraction method which is very effective in separating the beans from the oil and is also easier to use when excessive amounts of soybeans need to be processed. However, solvent extracted soybean products are not suitable for human consumption; firstly because it is difficult to maintain proper hygienic conditions during processing and secondly because of the high concentration of chemical residues in the product. It is therefore mainly used in animal feed (Bargale *et al.*, 1999). The solvent extraction method is most commonly used, as it has a better extraction percentage of about 99%. The mechanical oil extraction method is the oldest method used for soya processing. This method is not as efficient in removing the oil from the bean as the solvent extraction method (<70% vs. 99%). The mechanical oil extraction methods have been modified to increase efficiencies by using extrusion methods as a pre-treatment (Isobe *et al.*, 1992). The extrusion methods involve the heating of the soybeans over a short period to disrupt the tissues of the bean. High temperature (125-135˚C) and pressure are used to push soybeans through little openings to cook them and convert them to particles called collets. These collets are easier to process in the mechanical screw presses. The high temperatures used for short periods, help to retain the nutritional value of the soya oilcakes and decrease the activity of specific anti-nutritive factors and enzymes. A study was done by Nelson *et al.* (1987) on the coupling of the extrusion method with the mechanical screw pressing method. The soybeans were cooked via extrusion and then pushed through the continuous screw press. This technique significantly increased the oil recovery and throughput rates. During this trail, soya oilcake products had crude protein values of 50% (on an as is basis), oil content of 6% and 90% inactivation of the anti-nutritive factor, trypsin inhibitor (Nelson *et al.*, 1987). Some soybean processing plants have already implemented these modifications with extruders but most of the machinery is restricted to the solvent extractions. The soya oilcakes are cooked via extruders before the oil is extracted via solvents. This increase throughput rates in the plants, as well as the quality of the oil and the soya oilcakes (Bargale *et al.*,1999). The soya oilcakes can either be dehulled before processing or the hulls can be included in the soya oilcakes.

The different nutritive values for the different soybean oilcakes can be seen in Table 2.1 Soya oilcake has the highest protein value of all plant protein sources and is considered the “gold standard” among the plant protein sources (Cromwell, 1999). Soya oilcakes are commonly used in cereal based monogastric animal diets, since it has a very good amino acid profile, with only methionine limiting for poultry (NRC,1998). Undesirably, the soya oilcakes contain antinutritive factors such as allergenic, goitrogenic and anticoagulant substances as well as protease inhibitors (Parsons *et al.*, 2000).

Table 2.1 The chemical composition (%) of different soybean products (Agunbiade *et al.*, 2004)

	Raw soybean	Full fat soybean	Expeller soybean oilcake	Solvent extracted soybean oilcake	Dehulled solvent extracted meal	Soybean hulls
Dry matter	87.0-88.0	91.0	89.0	90.0	88.0	90.9
Crude protein	40.0-45.0	43.0	42.0	44.0	47.8	13.9
ME, Swine (MJ/kg)	N/A	14.811	15.690	12.929	13.138	7.728
DE, Swine (MJ/kg)	N/A	17.321	15.765	14.602	15.418	8.393
Acid detergent fibre	14.8	14.7	10.4	10.0	6.2	44.6
Acid ether extract	18.0-20.0	19.0	8.1	0.5	1.0	1.4
Cysteine	1.33	0.55	0.62	0.67	0.71	0.19
Lysine	6.38	2.40	2.70	2.70	3.02	0.71
Tryptophan	1.28	0.52	0.58	0.60	0.70	0.14
Threonine	3.86	1.69	1.70	1.70	2.00	0.43
Isoleucine	4.54	2.18	2.80	2.50	2.60	0.44
Histidine	2.53	1.01	1.10	1.10	1.30	0.28
Valine	4.80	2.02	2.20	2.40	2.70	0.51
Leucine	7.78	2.80	3.80	3.40	3.80	0.74
Arginine	7.23	2.80	3.20	3.40	3.60	0.59
Phenylalanine	4.94	2.10	2.10	2.20	2.70	0.45

Values on a DM basis

The following is a description of soya processing procedures followed at Russel Stone, a large soybean processing plant near Bronkhorstspruit, Gauteng, South Africa. The soybeans are transferred from the outside storage bin to the bin situated inside the plant. Beans are then softened by means of heat exposure through the vertical seed conditioner. Moisture from the beans are released. The soybeans are then moved via a conveyer belt to the Jet Dryer. The dryer uses warm air, to heat the beans evenly and ensure the shrinking of the hull, which loosens the hull from the bean. The beans are then transferred to the hull screener. The screener removes solid particles by cyclones and divides them into “overs”, “mids”, and “fines”. Thereafter, beans are rolled into smaller pieces and at the same time any remaining hulls from the beans are released. The secondary dehulling aspirator divides the “mids” particles into hulls and core pieces. This is done based on the density differences. The core particles fall through the secondary dehulling aspirator, to the flaking rolls. Here the beans are rolled to a desired thickness. The flakes are then

transferred to the expander bin. This process involves compression of the flakes by the addition of steam. The oil from the beans moves to the surface with the steam, expanding the collets. The collets are then mixed with a hexane solvent. The solvent moves into the collet and the combination of the solvent and the oil forms a miscella. The miscella complex, containing a high amount of oil, moves out of the collet and into the solvent bath. The desolvenitiser-toaster is then used to remove the solvent from the soybean meal. This is done by means of evaporation from steam-heated trays. The meal is then cooled and dried by the dryer cooler. A vapour forms as the solvent is separated from the meal and the oil. The vapour then needs to be condensed. This process is done in the first stage evaporator, the vapour contractor and the tube condensers, where water is the cooling agent. The exit vapours in the first evaporator are used as the heating medium. The second stage of the evaporation process uses the rising and falling film evaporator, where steam is the heating agent. The last stage is where a falling film disk and donut stripping column are used to separate the last solvent from the oil. This is done by a vacuum under very high temperatures. The lighter solvent is then removed from the heavier water in the tank, where after the content is further heated to remove the last solvents still present in the water. Lastly the soya oilcake is grounded using a hammermill and the final product is transported to storage bins (Russell Stone Protein, 2015).

Soybean hulls makes up 8% of the total soybean seed and consists mostly of the soybean coat. The hulls are one of the by-products produced during the extraction of soybean oil. Soybean hulls are high in dietary fibre and pectin and low in crude protein; it contains 59.9 - 72.2% insoluble fibre, 3.9 - 12.7% soluble fibre and 12% crude protein (CP) (Cole *et al*., 1999; Monsoor, 2005). The soybean hulls are used as a source of fibre in monogastric animal and ruminant diets (Banaszkiewicz, 2000). Full fat soya is a heat-treated soybean product that did not undergo the oil extraction process and has a very high energy value, because of its high oil content. Only the outer layer of the soya seed is removed. The two cotyledons of the soybean are then crushed to fine particles (Pringle, 1974). Soya protein concentrates (SPC) are another popular soya product, produced from defatted soybean flakes, with the carbohydrates removed. These concentrates contain little to zero trypsin inhibitor anti-nutritive factors (Lenehan, 2007).

2.4 The components of soybeans

2.4.1 Proteins and amino acids

The proteins found in the soybean are globular, subcellular packed structures in the cotyledons of the soybean (Bair & Snyder, 1980). The soybean has two storage proteins, namely glycinin and β-conglycinin. These are tightly placed to ensure that the optimal packing of proteins can take place (Shewry *et al.*, 1995). In raw soybeans, the proteins in the cotyledons are soluble in water at a pH of 7. As the solution increases

in acidity, the solubility of the soya proteins decreases. A pH of between 4.2 and 4.6 is known as the isoelectric section of the soya proteins. This characteristic of the soya is used to isolate the soybean protein. A defatted, unheated meal is removed from the soybean with water at neutral pH, and the protein is then precipitated from a filtered extract by acidification to the isoelectric section (Saio & Watanabe, 1969; Wolf, 1970). Research have found that even with an increase yield of soybeans over the past decade, the protein percentage decreased *(*Mahmoud *et al.*, 2006*)*. They also found that soybean cultivars with higher percentage proteins, contain higher amounts of the specific storage proteins (Yaklich, 2001). Therefore, the increased protein concentration in the specific soya cultivars is due to a higher expression of the storage protein genes rather than any other proteins found in the soybean (Krishnan *et al.*, 2007).

There are many proteins present in the soybean, each with special properties. The storage proteins glycinin and P- conglycinin, form the largest part of the soybean proteins and are important for the storage of amino-nitrogen, necessary for the germination process. These proteins accounts for 65-85% of the proteins in the soybeans, where the seed proteins consist of 30-50% of the proteins. Seed proteins include, lipo-oxygenase, trypsin inhibitors (Knutz and Bowman-Brink) and soya lectins. The storage proteins can be divided into two families, namely the vivilins and the legumins. Glycinin storage protein is part of the legumins protein family and have a larger molecular weight than the β-conglycinin storage protein, which forms part of the vivilins family (Johnson *et al,* 2008). Wolf and Cowen (1971) used ultracentrifugation sedimentation to describe the molecular weights of these storage protein segments. Svedberg units were allocated to these proteins. Proteins include 11S, 7S, 2S and 15S, with the 7S and 11S fractions accounting for more than seventy percent of the soybean protein. The 15S protein is the dimer of glycinin. The 2S fraction consists mainly of trypsin inhibitors and other enzymes (Wolf & Nelsen, 1996). The glycinin storage protein consists of twelve different polypeptides (Badley *et al.*, 1975) with special acid-basic peptide pairs, covalently bound by a disulphide bond (Scott *et al.,* 1992). The glycinin protein is fixed in the seed around sixty days after flowering of the plant has taken place (Plietz *et al.*, 1987). β-conglycinin consists of three peptides, α, α' and β. The α and α' peptides are produced before the β peptide, after the flowering process occurred (Galyer & Sykes, 1981). According to Thanh and Shibasaki (1977), these three β-conglycinin peptides are non-randomly associated with each other. There are no disulphide bonds or interpeptide links in the β peptide but there is one cysteine bond in each of the other two peptides. Strong hydrophobic and hydrogen bonds are present to fix these trimers together. There is one methionine in the α peptide and four in the α' peptide (Utsumi *et al.*, 1997). All the units of the storage proteins have special heating properties and abilities to interact with each other. Ionic strength, pH, and the reducing agent affect the way these proteins behave in response to the addition of heat. Legumins denature at higher temperatures than vicilins. Glycinin denature at 90˚C and β-conglycinin denatures at 75˚C (Pernollet & Mosse 1983). Trypsin

inhibitors are found in the 2S fraction of the soybean seed, together with smaller enzymes (Friedman & Brandon, 2001).

Functional characteristics of soybean proteins include increased viscosity, emulsification, gel and foam formations and the absorption of water or fat. These properties are possible due to the amino acid profiles of the soybeans, the three-dimensional shape of the protein molecules and their secondary and tertiary structures (Maruyama *et al.*, 2003). The most limiting amino acids in soybeans are methionine and cystine, which are sulphur containing amino acids. Soybeans contain high concentrations of lysine and complement cereal feeds, where lysine is limited. The digestibility coefficients of lysine, threonine and methionine are 88%, 81% and 90%, respectively. The availability of these amino acids depends on the processing procedures of the soybeans since over-processing binds amino acids and makes them unavailable to the animal, and under-processing ensures trypsin inhibitors to bind the trypsin amino acids (Grieshop *et al*., 2003). The origin, the bean variety and processing procedures influence the protein and amino acid concentrations of the soybean oilcakes (Parsons *et al.*, 1991, 2000; de Coca-Sinova, 2008, 2010; Baker *et al.*, 2011).

2.4.2 Antinutritive factors

2.4.2.1 Trypsin Inhibitor

The most relevant antinutritive factor (ANF) to consider in monogastric animal feeding, is trypsin inhibitor (Kunitz, 1947). The first isolation of the soybean trypsin inhibitor protein was done in the early forties (Koide & Ikenaka, 1973). The trypsin inhibitor is a crystalline globular protein, which restrains trypsin protein activities. This protein inhibitor binds its free carboxyl groups to the free amino groups on the trypsin molecule (Kunitz, 1947). This forms an irreversible stoichiometric compound. It causes a high concentration of undigested protein compounds to be found in the lower digestive tract of the pig and inflammation and other digestive upsets may occur. The inhibitor protein can only constrain the soya protein in its native state. During processing of the soya, these proteins are destroyed. Experiments with rodents have shown an improved protein utilisation in the application of moisture and heat but not in the event of dry treatments. Ten to twenty percent of the trypsin inhibitor will always remain in the treated soybeans (Koepke *et al*., 2000).

In soybeans, there are two types of trypsin inhibitors, the Kunitz inhibitor that has a molecular weight of around 20000 da with two disulphide bonds and the Bowman-Birk inhibitor with a molecular weight of around 8000 da (Koide & Ikenaka, 1973). The Kunitz inhibitor is the most abundant inhibitor of the two

inhibitors. The Kunitz trypsin inhibitor has less disulphide bonds than the Bowman Brik inhibitor and is therefore more heat liable. Very little inhibitor activity is seen towards chymotrypsin from the Kunitz trypsin inhibitor (Friedman & Brandon, 2001). The Kunitz inhibitors are found in low concentrations in heat treated soya oilcakes (Dipietro & Liener, 1989). The Bowman Brink inhibitor can bind both trypsin and chymotrypsin (Birk, 1985). The trypsin inhibitor impairs protein digestibility and can therefore restrict growth in young pigs. Trypsin inhibitors also increase the secretion of pancreatic substances and this leads to hypertrophy of the pancreas. Higher amounts of enzymes released from the pancreas increase the losses of endogenous amino acids in the digestive tract. These endogenous amino acids are mostly sulphur containing which are very important in diets low in methionine or cystine (Chernick *et al.*, 1948).

2.4.2.2 Lectins

Lectins, also known as hemagglutinins, are an ANF in soybeans that bind glycoprotein receptors in the gastrointestinal tract and restricts nutrient absorption. This causes damage to the intestinal lining and may result in poor animal performance (de Mejia & Prisecaru, 2005). Lectins are composed of a tetrameric glycoprotein and contains one oligo-mannose chain for every monomer. These haemagglutinins may also bind to blood cells, causing agglutination. This is due to their strong attraction to cellular carbohydrates. Lectins are destroyed during processing, which reduces their effect on animal performance. If soybean meals are not heat treated, lectins can survive the digestive tract of the animal, and then bind to the gastrointestinal cells and enter the blood circulation, fully functional (Abdullaev & de Mejia, 1997). Research, however, has shown that lectins are less susceptible to heat denaturation than trypsin inhibitors. The residue lectins left after the processing of raw soybeans, may contribute to lower than optimal animal performances (de Mejia & Prisecaru, 2005). Lectins can be divided into three groups; mitogenic, non-mitogenic and anti-mitogenic. Mitogenic lectins stimulate cell division in a normal cell that would normally not divide and this mitogenic activity may cause cancers. Low concentration of lectins will promote production of cells and high concentration of lectins promote inhibition of cell division (Abdullaev & de Mejia, 1997). A study done on chickens showed that diets containing lectins up to 0.048%, increased intestinal development by increasing the villus crypt, but the integrity of the lymphoid were compromised. The urease concentration is also linked to the lectin content in soybean. If urease activity is less than 0.2 pH units, lectin concentration is low enough not to have a negative effect on animal welfare (Fasina *et al.*, 2006).

2.4.2.3 Other antinutrient factors

Other antinutritive substances found in soybeans are tannins. These however have little to no effect on monogastric animal feeding (de Mejia & Prisecaru, 2005). Phytoestrogens are compounds that have equivalent properties to the hormone oestrogen and may influence reproduction. The concentrations however are very low and have no effect on the reproduction functions of a pig. Saponins are also found in the raw soybean. This ANF may decrease intake due to bitter taste thus influencing nutrient absorption. The concentrations again are too low to have any significant negative effect on the animal. Allergenic protein compounds in soybeans may decrease animal performance due to allergic reactions in the intestines. These proteins seem to have very little influence on swine performance. Phytic acid is another ANF to consider in soybeans. This phosphorus compound is indigestible and may cause interference of absorption of minerals like calcium an iron. Phytase enzyme can be used in feed to increase the utilisation of phytic acid (Ishaaya *et al.*, 1969). Non-digestible short chain carbohydrates or oligosaccharides such as raffinose and stachyose cause excessive gasses and bloat in the hind gut of monogastric animals. This is due to undesirable micro-organisms fermenting undigested compounds in the lower gut (Parsons *et al.*, 2000).

2.4.2.4 Measuring antinutritive factors in soya oilcake

Soya based diets are often associated with ANF and may increase chances of intestinal upsets (Richter *et al.*, 2014). The quality of soybeans is measured via the quality of the protein content of the soya. This is associated with the availability of the amino acids of the protein and also the presence of ANF (Rodica & Adrian, 2010). The most relevant ANF here is the trypsin inhibitor (protease inhibitor). To reduce the amount of ANF in soya and increase digestibility, soya needs to be processed by heat. Over processing of soya, causes the Millard reaction to occur. This causes the availability of lysine and some other essential amino acids to be reduced. Thus, the processing of soya is of utmost importance to ensure protein availability and high degradability of the soya proteins (Richter *et al.*, 2014). Trypsin inhibitors can be measured directly via chemical analysis by measuring the loss of activity when the soya sample is added to either a tris buffer (6.05 g Tris-(hydroxymethyl)-methylamine and 2.94 g calcium chloride dehydrate dissolved in distilled water, BAPA solution (0.04 g of N-α-benzoyl-DL-arginine-p-nitroanilide hydrochloride dissolved in dimethyl sulphoxide)) or The Standard Trypsin solution (0.04 g of crystalline bovine trypsin dissolved in 0.001 M HCl). The activity of trypsin inhibitor can also be measured indirectly via other methods, such as the urease index, protein dispersibility index, nitrogen solubility index or the KOH protein solubility index (Zarkadas & Wiseman, 2005).

The urease index method (UI) measures the pH increase due to the release of ammonia from the urease enzyme in soybean oilcake. This method is used to determine if the soybean has been processed to the full extent to reduce ANF such as trypsin inhibitors. This method is not a good indication of over-processed soya oilcakes (Rodica & Adrian, 2010). Urease activity is measured by changes in pH, which is an easier method than analysis of trypsin inhibitor concentrations. There is a direct correlation between the urease value and trypsin inhibitor concentrations. It is, however, important to be cautious since the kinetics of trypsin inhibitor destruction and urease may vary. The urease test does not indicate the level of heat treatment on the soybean quality after the urease enzyme has been deactivated (Albrecht *et al.*, 1966). In the case of high initial moisture content, both trypsin inhibitor and urease concentrations decrease quickly. The particle size affects the urease concentration but has no effect on the trypsin inhibitor activity (Wright, 1981). Moreover, research in chickens showed that the total deactivation of the urease enzyme did not result in decreased growth performance (McNaughton & Reece, 1980; Dale *et al*., 1986). Another study showed no decreased growth in chicks fed diets containing soya oilcakes with an urease activity value of less than 0.01, compared to diets high in urease values. Low urease values may also be due to long duration storage of soya oilcakes that were under-processed (de Schrijver, 1977). The urease index test is a fast and effective indicator for trypsin inhibitor activity, but by measuring the trypsin inhibitor activity directly, one will decrease variability. This method is more complicated with higher expenses involved (Rodica & Adrian, 2010). Figure 2.1 shows the linear relationship between trypsin inhibitor concentrations (mg/g) and urease activity (pH units) of 104 soybean oilcake samples (Belalcázar & Otálora, 2012).

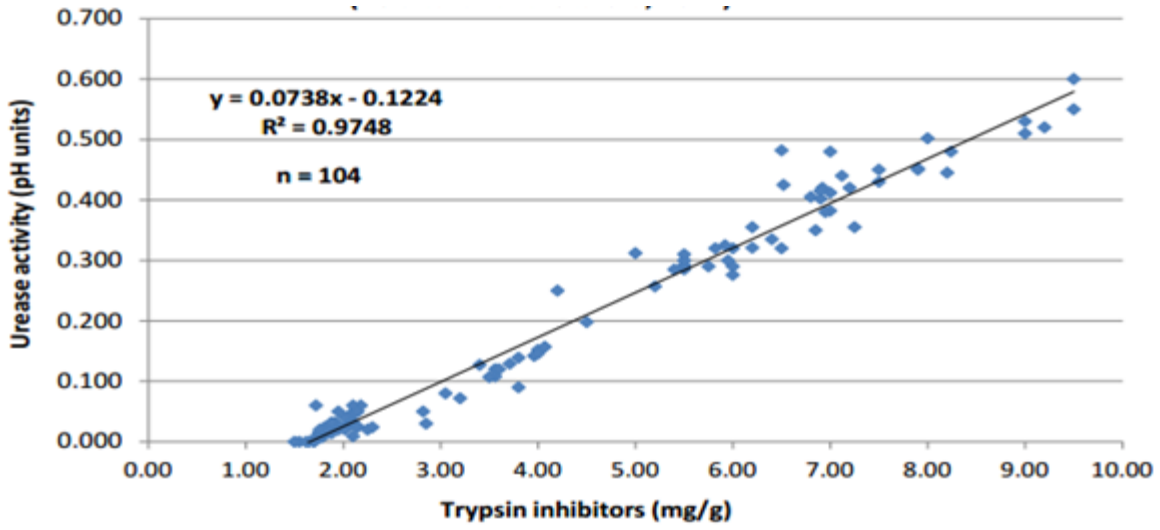

Figure 2.1 The linear correlation between the urease activity (pH units) of soya oilcakes and trypsin inhibitor concentrations (mg/g) (Belalcázar & Otálora, 2012)

There is no biological relationship between urease and trypsin inhibitors but both trypsin inhibitors and urease in the presence of enough moisture, are inactivated when heat is applied. Thus, there is an informal relationship between them, and it is easier to measure urease concentrations than trypsin inhibitor concentrations (Kakade *et al.*, 1969). The indirect urease method was invented in 1944 by Caskey and Knapp to detect the under-processing of soybean oilcakes (Caskey & Knapp, 1944). The change of pH units between 0.05 to 0.20 is an indicator for adequately processed soybean oilcake. When the change of the pH is below 0.05 units, the soya oilcake is overheated and above 0.20 pH units, the soybean oilcake is considered undertreated, thus high anti-nutritive factors will be active in the soybean oilcake (Caskey & Knapp, 1944). Dale *et al*. (1987) proved that the urease test is, however, not the right method to use to test soybean oilcake for over-processing. They proved the correlation between solubility of soybean oilcake in potassium hydroxide (KOH) and broiler chicken performances and this became the preferred method for analysing over-processed soybean oilcake (Dale, *et al*., 1987).

The KOH test measures the solubility of the protein in the soya oilcake in a 2% KOH solution. The lower the solubility value, the higher the chance of over-processed oilcake (Rodica & Adrian, 2010). A KOH protein solubility of 75-85% is acceptable for optimal animal performance (Araba & Dale, 1990). This method is good for determining over-processed soya, but it is not a good indicator of under-processing. Guzman *et al.* (2016), tested the effect of soya concentrates (SPC) and soya oilcakes (SBM) from different origins on the growth performance and nutrient digestibility of weaning pigs. Pigs from treatment 1 and 2 were fed SPC containing diets, originated from the USA and Brazil, respectively. Treatment groups 3, 4 and 5 were fed diets containing SBM originating from the USA, Brazil and Argentina respectively. The KOH % values were 66.3, 64.0, 78.6, 67.9 and 77.3%, respectively, for the 5 treatment groups. The urease values for treatment group 1 and 2 were not detected and was 0.02, 0.06, 0.00, 0.01 and 0.06 for the remaining treatments respectively. The trypsin inhibitor activity for the five treatments were 1.07, 1.50, 3.15, 2.39, 2.67, 3.62, 2.76 and 2.71 mg/g DM respectively. Growth performance was similar for the pigs fed the diets containing SPC than those fed diets containing SBM. Post-weaning diarrhoea was higher in the SBM treatments groups than the SPC treatment groups. The total tract digestibility was also higher for the SPC treatment groups. Thus, specific performance parameters such as post weaning diarrhoea and total tract digestibility were influenced by trypsin inhibitors present in the SMB treatments but overall did not influence growth performance (Guzman, *et al.*, 2016).

Protein dispersibility index (PDI) also measures the solubility of protein, but in this case, water is used as the solvent. Palic *et al* (2011) recommend the following parameters for PDI values: >10.3 indicates under-processed soya oilcake, 8.5-10.3 indicates adequately processed soya oilcake, while soya oilcake with a

PDI value of <8.5 is likely to be over-processed. Nitrogen solubility index is obtained when nitrogen is extracted from the grinded soya in distilled water, using a slow stirring technique. It has been suggested that using the UI together with the PDI is a good indicator for soya quality (Rodica & Adrian, 2010). Qin *et al.* (1997), tested for trypsin inhibitor activity in soya oilcakes originating from China and Argentina. The study showed differences in trypsin inhibitor activity (TIA) and PDI values between the two sources. The raw soya from Argentina had an average TIA value of 15.2 mg/g and a PDI value of 85.6%. The raw beans originating from China had a TIA value of 20.6 mg/g and a PDI value of 87.6%, indicating that genetic components also play a key role in the concentration of trypsin inhibitor present in the different origin soybeans. The TIA and PDI value of the soya from Argentina declined as the temperature and duration of processing increased. At 100˚C for 5 minutes, the TIA was 7.3 mg/g and the PDI value was 55.4%. At 100˚C for 40 minutes, the TIA was 1.6mg/g and the PDI value was 25%. When the temperature was 118 ˚C for 5 minutes, the TIA was 2.0 mg/g and the PDI value was 15.8%. At 118 ˚C for 20 minutes the TIA was 0.4 mg/g and the PDI was 9.8%. At 136 ˚C for 10 minutes the TIA value became 0 mg/g and the PDI value was 10.4%. The beans originating from China had the following results after processing. The TIA value at 100˚C for 5 minutes was 13.1mg/g and the PDI value was 64.8%. At 100˚C for 40 minutes, the TIA was 2.4 mg/g and a PDI value of 29.9%. When the temperature was 118 ˚C for 5 minutes, the TIA was 4.4 mg/g and the PDI was 29.5%. At 118 ˚C for 20 minutes the TIA was 0.7mg/g and the PDI value was 9.9%. At 136 ˚C for 10 minutes the TIA reached 0mg/g and the PDI value was 9.7%. When the soya oilcake from the different origins are heated at different temperatures, both origins of soybeans' TIA and PDI values decreased. During the heating process, different patterns where observed between the two sources. The TIA values for the soya from China seemed to be higher when samples are heated for a brief period, at the different temperatures, compared to the soya from Argentina (Qin *et all.*, 1997).

Lee *et al.* 2007 did an experiment where soybeans were heat treated under four different conditions and the performance data of 60 pigs were recorded, when fed these different soya oilcake treatments. Treatment 1 had no heat applied to the soybean flakes. For treatment 2, the soya flakes were heated for 5 minutes at 95 ˚C. Treatment 3 the soya flakes were heated for 5 minutes at 110 ˚C. Soya flakes were heated for 15 minutes at 110 ˚C for treatment 4 and treatment 5 the soya flakes were heated for 60 minutes at 110 ˚C. The PDI values decreased drastically from treatment 1 to 3 and gradually decreased with the rest of the treatments. As the PDI values decreased the ADG (average daily gain) increased up to treatment 4 and decreased again in treatment 5. The ADG for the five treatments were as follow: 323 g/day, 554 g/day, 583 g/day, 625 g/day and 550 g/day. The PDI value for treatment 5 was 8% and thus an indication of over-processed soya oilcake. This negatively influenced ADG and feed efficiency (FE). The FE values for treatment 1-5 were as follow: 380 g/kg, 541g/kg, 564 g/kg, 562 g/kg and 520 g/kg, respectively. In this

study it was concluded that over-processed and under-processed soya negatively influenced growth performance of grower pigs (Lee *et al.*, 2007).

2.4.3 Lipids and fatty acids

The lipids in soybeans are very important since it provides over twenty-nine percent of the world's oils and fats (Golbitz, 2007). Most of the lipids are found in the cotyledon of the soybean, and this contributes to twenty percent of the soybean's weight. Lipids play an important an important role in membrane function, and serves as energy reserves and solvent for lipid soluble materials (Johnson *et al*, 2008). Sphingolipids are a polar lipid found in the soybean plasma membranes (Merril *et al.*, 1997). Soybeans contains two types of sphingolipids, namely ceramide and cerebroside. The cerebroside class have the highest concentration in the soybean and is classified a simple glycosphingolipid due to a single sugar residue (Vesper *et al.*, 1999). Sphingolipids are mainly involved in cell structure, but their metabolites are important for signalling, cell development, differentiation and apoptosis (Schmelz, 2000). Phospholipids are polar lipids located on the external parts of the membrane and is present in higher concentrations in the cell membrane compared to sphingolipids. Soybean oil contain high quantities of phospholipids in relation to other vegetable oils, because of the small size of the soya oil body that ensures a larger surface area per unit weight. Phospholipids in the soybean can be divided into three groups, namely phosphatidylcholine (55.3%), phosphatidylethanolamine (26.3%) and phosphatidylinositol (18.4%) (Wang *et al.*, 1997). Lecithin is the collective term used for the different substances of the phospholipids. Lecithin is used in many industries as an emulsifier and antioxidant. Phospholipids are removed from crude oil by degumming before refining the oil (Nasner, 1985).

Fatty acids mostly found in the soybean are palmitic, stearic, oleic, linoleic and linolenic fatty acids. Smaller amounts of arachidic and behenic acids are found in the soybean. Palmitic fatty acids and stearic fatty acids are saturated with no double bonds. Oleic acids have one double bond, linoleic acids two double bonds and linolenic acids have three double bonds. All these configurations are in the *cis* form, and methylene interrupts the bonds where there is more than one double bond (Brown *et al.*, 1962). The fatty acids in the soya is produced in the stroma of the plastids (Lynen, 1961; Voelker & Kinney, 2001). The polyunsaturated fatty acids (PUFA) are responsible for the soya oils' oxidative instability and degradation during heating. When considering PUFA, essential fatty acids are important. These are fatty acids which cannot be synthesised by the animals' body and must be added to the diet (Beare-Rogers *et al.*, 2001). Essential fatty acids can be divided into two groups, n-6 (linoleic acid) and n-3 (linolenic acid). Raw materials such as maize, sunflower oils and soybean oils contain n-6 fatty acids, mainly linoleic acids, where linseed contains

linolenic acids. Both linolenic and linoleic acids are metabolised to long chain polyunsaturated fatty acids. This conversion is very inefficient since there is a low activity of desaturase in the digestive tract of pigs. Long chain PUFA are very important for efficient growth in pigs. These fatty acids may also have a positive effect on gut health of the pigs, improving the integrity of the intestine membranes (Xie & Innis, 2008; Boudry *et al.,* 2009; Jacobi *et al.,* 2011). Even though n-6 PUFA are not normally considered as the group of fatty acids providing health benefits, studies have proven the opposite, for example arachidonic acid may help with the repairing of damaged gut intestinal linings (Ruthig & Meckling-Gill, 1999). Lipids like sterols, tocopherols and chlorophyll are also found in the soybean in low quantities. These, together with the free fatty acids, are removed during the removal of the refined oil from the soybean (Lynch & Dunn, 2004).

2.4.3 Carbohydrates

The carbohydrate content of soybeans is between 30 and 35% and that of soya oilcake can be up to 40% (NRC, 1998). In soybeans, the main fibre sources are cellulose and hemicelluloses, pectin and glycoproteins, found in the parenchymal cells of the cotyledons of the bean (Selvendran *et al.,* 1987). The fibre components of the soybean hulls are galactomannans, xylan hemicelluloses, uronic acids and cellulose (Aspinall & White, 1964). Soybeans contain 27% nitrogen free extract (NFE) and 6% crude fibre (CF), where soya oilcakes contain 36% NFE and 8% CF with the hulls intact and 34% NFE and 4% CF without the soya hulls (NRC, 1998). Non-structural carbohydrates can be grouped as mono-and disaccharides, oligosaccharides and polysaccharides. These three groups together are called total non-structural carbohydrates (TNC). The TNC are more than half of the total carbohydrates found in soybeans and soya oilcakes. In soybeans the TNC contributes 12.3-16% of dry matter and 18.3-21.2% in soybean oilcakes. Low molecular weight sugars make up 40-45% of the total carbohydrates and in soya oilcakes the percentage goes up to 50%. Monosaccharides in soybeans, like glucose (0.12-0.47%), galactose (0.07-0.4%) and fructose (0.11-0.47%) are not present in soya oilcakes due to processing procedures. Sucrose is the most abundant sugar in soya oilcakes and can be up to 9.5% of the dry matter (Grieshop *et al.,* 2003). Oligosaccharides in soybeans contribute up to 5% of the dry matter. In soya oilcakes the contribution is between 7% and 8% (Grieshop *et al.,* 2003; Van Kempen *et al.,* 2006). Oligosaccharide concentrations differ between cultivars and is of importance since they have anti-nutritional characteristics of oligosaccharides like stachyose and raffinose (Parsons *et al.,* 2000). Non-structural polysaccharides are mostly storage saccharides. Starch is the main storage saccharide in most legumes and can be as high as 60% of dry matter, but soybeans contain a very low concentration of starch (only 5% of dry matter) (Wilson *et al.,* 1978; Thomas *et al.,* 2003). The starch in soybeans contains more amylopectin than amylose and the

ratio is normally 7.5:1 depending on the cultivar (Stevenson *et al.*, 2006). Structural polysaccharides consist of cellulosic and non-cellulosic polysaccharides and have complex structures in soybeans. Crude fibre content of soybeans is between 40.9 - 41.6%, while dehulled soya oilcakes contain 2.6-6.2% CF and soya oilcakes with the hulls contain 32.1-37.3% CF (Brillouet & Carre, 1983).

2.4.5 Other

The mineral content of soybeans is around five percent and is found in higher concentrations in the soya oilcake after the removal of the soya oil. The main minerals present in the soya oilcakes are potassium, calcium and magnesium. Lower concentrations of iron, zinc and copper are present in the oilcakes (Stevenson *et al.*, 2006).

Carotenoids are pigments found in soybeans in low concentrations and can be divided into two classes namely lutein and p-carotene. During processing of soybeans, the carotenoids are often destroyed by the oil refining procedures. Lutein is found in soybeans with a yellow seed coat and soybeans with a green seed coating contains lutein and xanthophylls (Simonne *et al.*, 2000).

The most important enzyme found in soybeans is lipoxygenase, which is responsible for the oxidation of poly-unsaturated fatty acids by molecular oxygen, which cause rancidity and bean flavour. Products from oxidation of fatty acids include pentanol, hexanol, ethyl-vinyl-ketone and trans-2-octenal (Hill & Hammond, 1965; Arai *et al.*, 1967 & Boatright & Crum, 1997). Phytases are enzymes that removes phosphate groups from myo-inositol-hexakis-phosphate, catalysing the hydrolysis of phytic acid (Lei & Porres, 2007). The purple-acid-phosphatases class is found in the cotyledons of germinating soybeans (Hegeman & Grabau, 2001).

2.5 The nutritive value of soybeans from different origins

When comparing soybeans from different countries in the world, studies have shown chemical differences in both soybeans and soybean oilcakes (Grieshop & Fahey, 2001). Karr-Lilienthal *et al.* (2004) compared the chemical composition and ileum digestibility in pigs of soybean oilcakes from five the USA, Brazil, Argentina, China and India. The soybeans were all processed in the USA using the same procedures. The study revealed differences in the chemical composition and digestibility of the soybeans from the different countries. The soybean oilcakes from Argentina and Brazil had a lower digestibility than that from the USA, where the soya oilcakes from China, India and the USA all had similar digestibility. These results

suggest that the quality and digestibility of the soya oilcakes do not only depend on the processing procedures but also on the chemical composition of the soybean itself, which is influenced by its origin and genetics (Karr- Lilienthal *et al*, 2004). In a different study, amino acid concentrations were measured from soya oilcake originating from Argentina, Brazil, Spain and the United States. The crude protein measurements ranged from 45.2% to 50.6% and the lysine concentrations expressed as a percentage of the crude protein, was between 5.51% and 6.26%. The soya oilcake samples from Spain showed the highest crude protein values and those from the United State (US) had the highest lysine values (de Coca-Sinova, 2008). Some soybean cultivars are genetically modified and selected to have higher amino acid concentrations to add more nutritional value when included in animal diets (Baker & Stein, 2009; Baker *et al.*, 2011).

Li *et al*. (2015) observed crude protein values on a dry matter basis, for soya oilcakes of 50.2% for China, 49.4% for the USA, 51.1% for Brazil and 48.8% for Argentina. The soya oilcake from Argentina had the lowest dry matter and NDF values of all the sources. Soybean oilcake from China had the highest phosphorous values and the lowest raffinose concentrations. Brazil soya oilcakes had the lowest sucrose values and the USA had the highest concentration of stachyose. The soybean oilcake originating from China had the highest arginine, cysteine and lysine concentrations. In this study, the digestible energy (DE) and metabolisable energy (ME) of the soya oilcakes in pigs, from the different origins, were also measured. The average DE of soya oilcake from Brazil, China, Argentina and the US were 15.64, 15.73, 15.90 and 15.93 MJ/kg, respectively, on a DM basis. Average values for ME were 14.97, 15.10, 15.42 and 15.31 MJ/kg, respectively. There were no significant differences in the DE and ME of the raw soybeans from the different origins (Li *et al.*, 2015). In another study, Mateos *et al.* (2011), collected 385 soya oilcake samples from different origins over a period of four years. These countries included the US, Brazil and Argentina. In this study the soya oilcake from the USA had higher crude protein concentrations (53.9%) than the soya oilcake from Argentina (51.6%) and Brazil (52.7%). The NDF values for the soya from the USA (8%) were lower compared to thos from Brazil (12%) and Argentina (10.7%). The USA soya oilcake also had higher sucrose and stachyose concentrations than those from the other two countries. The soybean oilcakes from the USA had higher KOH solubility values (87.3%) compared to Brazil (83.6%) and Argentina (82.5%). Protein dispersibility index was 19.9% for the USA, 17.1% for Brazil and 15.3% for Argentina. The trypsin inhibitor activity for the USA, Brazil and Argentina was 3.9, 3.0, 3.0 mg/g, respectively. It was concluded that the soybean oilcake from the USA had higher feeding value than the soya oilcakes originating from Brazil and Argentina (Mateos *et al.*,2011). In a study done by Frikha *et al.* (2012), soya oilcakes from Brazil had higher CP content that the soya oilcakes from the USA, but both had higher CP values than Argentina. The differences in CP values were partly due to the processing procedures where the hulls were

removed, the genetic profile of the soybean and the environmental conditions during cultivation of the soybeans (Wilcox & Shibles, 2001). Soybean oilcake from the USA had higher lysine, methionine and cystine per unit CP than the oilcakes from Brazil and Argentina. Stachyose and sucrose levels wer lower for the soya from Brazil compared to the USA and Argentina. The KOH solubility percentage was higher for the USA oilcakes while Argentina soya oilcakes showed the lowest solubility, which could point to more intense processing procedures occurring in Argentina in comparison to the USA. The trypsin inhibitor values were very variable, but lowest values were seen for the soya oilcakes from Argentina. (Fikha *et al.,* 2012).

2.6 Feeding soybean products to pigs

In most pig diets, maize is the main energy source. Maize meals are deficient in amino acids such as lysine and tryptophan (Mahan, 1991; Owen *et al.,* 1996). These amino acids are readily available in soybean products (Owen *et al.*, 1996) and are therefore an important protein to include in a pig diet.

2.6.1 Grower pigs and sows

Soybean oilcakes are used in the diets of pregnant and lactating sows as well as grower pigs, to provide all the essential amino acids for optimal production (Li *et al.,* 1990; 1991 & Sohn *et al.,* 1994). Sovent-extracted soya oilcake with a crude protein content of 44% is commonly used in South African pig diets. However, the dehulled solvent-extracted meal has higher energy and lower lysine concentration levels due to the higher oil content. Most processing plants combine the extrusion and expelling methods for increased oil extraction efficiency and better utilisation properties of the meal by the animals. The oil content of such meals is less and can therefore be used at higher inclusion rates, without problems of oxidation and lower carcass quality (Shelton *et al.,* 2001). Full fat soybeans have a high energy value and can be used in the diets of lactating sows. Although no maximum inclusion level is necessary for soybean oilcakes, it is important to limit the inclusion of full fat soya in grower pigs' diets to avoid a negative effect on the carcass quality. Full fat soybean meals cause deposition of soft fat due to its high polyunsaturated fat content, which is unappealing to consumers (Van Lunen *et al.,* 2003). Higher concentrations of polyunsaturated fat, linoleic (C18:2) and linolenic (C18:3) acids in the diet, cause the meat to be more prone to oxidation after slaughtering. This is due to the presence of double bonds in the polyunsaturated fatty acids. The fats have lower stability and may decrease shelf life of meat (Morel *et al.,* 2006).

Soybean oil can be added to the diets as an energy source (Mahan, 1991; Owen *et al.*, 1996). Soybean hulls should not be included at levels higher than 15% in the diets of sows and growers, to avoid a decrease in digestibility and production due to higher fibre content (Kornegay, 1981 & Dilger *et al.*, 2004).

2.6.2 Weaner pigs

During the weaning process, the piglet experiences elevated levels of stress. Factors contributing to these stress levels include diet changes, gut development processes, environmental adjustments, removal from their mother, fighting and ranking in weaner houses and immune challenges (Rodica & Adrian, 2010). The most critical period for the development of a healthy gut system is just after weaning, where the piglets' diets are converted from milk proteins, in a liquid phase, to soya proteins in a dry form (Rodica & Adrian, 2010; Richter *et al*., 2014). The gastrointestinal tract of the pig is very important for nutrient digestion, absorption and metabolism, but it is also an essential organ for immune function (Liu, 2015). The gastrointestinal tract contains more than 70% of the body's immune cells. Stressors such as the weaning process and pathogenic invasions cause damage to the intestinal linings and this may result in poor growth and lower slaughter weights (Liu, 2015). The diet of the newly weaned piglet consists of carbohydrates and plant proteins. During the fermentation process of these undigested carbohydrates in the digestive tract of the piglet, short chain fatty acids are produced, and they prevent enterogens from multiplying, helps with the formation of tight junctions and prevents inflammation in the digestive tract of the pig. Branched chain fatty acids, sulphides, amines and phenols are produced during the fermentation of proteins in the gut and these products restrict the function of the tight junctions and causes enteritis and other digestive upsets in weaner pigs (Richer *et al*., 2014). The tight junctions or gut barriers are defined as the ability of the gastrointestinal tract's epithelial lining to prevent pathogenic bacteria and other allergens entering the gut mucosa (McOrist. & Mellits, 2010). Imbalances between digestible proteins and fermentable carbohydrates can cause digestive problems, thus feeding the correct ratio of proteins to carbohydrates will ensure a healthy digestive system in newly weaned piglets (Yegani & Korver, 2008).

In addition, newly weaned piglets do not tolerate soya proteins well. Just after weaning, the inclusion rates of soybean oilcakes must be restricted, due to hypersensitivity of the piglets towards larger amounts of the soya oilcake in the diets. Alternative protein sources should be used to gradually introduce the soya proteins in the piglets' diet (Li *et al*., 1990; 1991; Sohn et al., 1994). Antinutritive factors present in these soybean proteins, creates a cell-driven immune response and causes the villus height in the small intestine to decreases, and crypt depth to increase, damaging the intestinal lining of the piglet (Newby, et al., 1984).

During inflammatory responses, different specialised immune molecules such as tumour necrosis factor (TNF) – α, interleukin (IL) - 1β and IL-6 are released. An overproduction of these molecules may cause damage to the intestinal mucosal lining and this may result in lower feed intakes, poor absorption of nutrients and ultimately the reduction of growth performance of pigs (Liu, 2015). During the weaning period of the piglet, it is important that the gastrointestinal tract's immune function is activated, to ensure the protection of the piglet against newly encountered pathogens. Overproduction of these immune molecules can be limited by balanced nutrition and lower inclusions of soya proteins. Bacterial infections cause damages to the top half of the villi in the small intestine, and this reduces the piglets' ability to absorb nutrients and in effect, reduce growth rates (Sanford & Josephson, 1981). Most nutrient absorption is restricted to the top part of the villi in the small intestine. The state of the villi, microvilli and the enzymes involved has a direct correlation with gut health, which also correlates to growth performance. Damage to these parts in the gut will cause lower growth performance throughout the lifetime of the pig (Zijlstra *et al.,* 1997; Moeser *et al.,* 2007).

2.6.3 Amino acid digestibility and bioavailability of soybean oilcake in pigs

Ileal digestibility is the most commonly used method to determine the amino acid digestibility and absorption rates in monogastric animals (Sibbald, 1987*)*. Ileal amino acid digestibility can be described as apparent (AID), true (TID) or standardised (SID) ileal digestibility. Apparent amino acid digestibility refers to the net dietary amino acids that is absorbed from the lower part of the ileum and is measured as follows: AID (%) = $[(AA_{intake} - \text{ileal } AA_{outflow})/ AA_{intake}] \times 100$. The apparent digestibility considers the dietary amino acids that was not absorbed, and the endogenous amino acids released in the lower part of the ileum. These endogenous amino acids contribute to the total outflow of ileal amino acids (Stein *et al.,* 2007). Methods to measure AID include surgical methods by inserting a T-cannula in the distal ileum of the small intestine. The T-cannula method only allows collects of a portion of the ileum outflow and standard errors are high. By combining the T-cannula method with indigestible markers in the feed will reduce standard errors (Jagger *et al.,* 1992; Yin & McCracken, 1996). Markers mostly used are chromium oxide and titanium dioxide. Research has shown a recovery rate of 71-85%, obtained with the chromium oxide marker but recovery can be optimised by increasing the inclusion rate of the chromium marker to 5 g/kg diet (Mroz *et al.,* 1996).

Marker recovery rates are influenced by the type of diet fed to the pigs and diets high in fibre seem to have lower recovery rates (Yin & McCracken, 1996). Apparent amino acid digestibility is calculated based on the assumption of full marker recovery and this is not always the case. The equation used to calculate

apparent digestibility when using a marker (M) is as follow: AID (%) = [1 – ($AA_{digesta}$/ AA_{diet}) x (M_{diet}/ $M_{digesta}$)] x 100. The main problem with using AID in formulating diets, is the difficulty of adding the AID values of the different feed ingredients together. This is due to the different ingredients having different effects on endogenous amino acid losses and the total amino acid outflow. Increasing dietary amino acid concentration will increase endogenous amino acid losses non-linearly (Stein *et al.,* 2005).

Ileal endogenous amino acid losses include shedded cells, serum, enzymes and amides not digested and absorbed in the distal ileum (Nyachoti *et al.,* 1997). Endogenous ileal amino acid losses can be as much as 50% of the total ileum outflow. Ileal endogenous amino acid losses can be divided into two groups, basal losses and specific losses. The basal losses occur due to the movement of the digesta through the gut and is not predisposed by die dietary components (Jansman *et al.,* 2002). Basal endogenous losses increase with increase in feed intake and decrease with the body weight of the animal (Furuya & Kaji, 1992; Butts *et al.,* 1993). Specific endogenous losses are losses that occur due to different feed ingredient characteristics, which includes anti-nutritive factors and fibre compositions. Feed containing high levels of fibre and anti-nutritive factors like trypsin inhibitors, causes the specific endogenous losses to increase to more than 50% of the total endogenous amino acid losses (Schulze *et al.,* 1995).

True ileal amino acid digestibility (TID) measures the amino acids originating from the diet, that are removed from the gut before the distal ileum. The TID are calculated the same way as AID, only the ileal endogenous losses are subtracted from the total amino acid outflow: TID % = [AA_{diet} – (ileal amino outflow – total ileal endogenous AA losses) / AA_{intake} x 100 (Stein *et al.,* 2005). When using TID to formulate diets, the amino acid requirements will differ with different feed ingredients used, due to the diet effect on endogenous losses in the ileum. The main reason why TID is not often used in pig formulations, is the lack of values for the different feed ingredients. Standardised ileal amino acid digestibility is used instead of TID. Using SID, only the basal endogenous amino acid losses are subtracted and the formula is as follow: SID % = [(AA_{diet} – (ileal AA outflow – basal ileal endogenous losses) / AA_{diet}] x 100 (Stein *et al.,* 2005). It is important to indicate the basal endogenous losses used in the equation, since values differ from one group of pigs to the next. By using SID instead of AID the variation caused by the effect of proteins on ileal digestibly is lowered. The advantage of using SID instead of AID is that the values for the different feedstuffs can be added to give a representative SID value for the whole diet (Stein *et al.,* 2005).

The main objective for nutritionists is to ensure that a formula promotes optimal production at the least costs. It is therefore important to know the bioavailability of amino acids in feedstuffs, to ensure that the animals' requirements are met. The concentrations of amino acids in raw materials differ significantly.

Reasons for this include, species differences, genotypes, different plant fractions, stage of maturity, soil fertility, the season of growth, the year of growth and where the plant is grown (Sibbald, 1987). The bioavailability of amino acids is a part of the dietary amino acids used by cells for metabolism and protein synthesis (Batterham, 1992). Bioavailability of amino acids is of great concern in animal nutrition. The form in which the amino acids are present in the feed will ensure the uptake thereof. Bioavailability, however, does not only depend on the absorption of the nutrient in the lumen of the small intestine, but also by the uptake of the amino acids in the cells. Proteins damaged by heat may be absorbed in the gut but secreted in the urine due to the inability of the cell to absorb and utilise them (Bjarnason & Carpenter 1969; Ford & Shorrock, 1971). Table 2.2 shows the relative amino acid bioavailability of different soya products (Shelton *et al.*, 2001).

Table 2.2 The relative availabilities (%) of amino acids in different soybean meals (Shelton *et al.*, 2001)

Amino acids	Solvent extracted soybean meal (44%CP)	Dehulled solvent extracted (48% CP)	Extruded/ Expeller meal (43% CP)	Extruded full fat	Roasted full fat
Methionine	88	90	73	83	76
Cysteine	81	84	77	77	73
Met + Cys	85	87	75	80	75
Lysine	87	89	84	86	77
Threonine	82	84	74	81	73
Isoleucine	87	89	83	83	73
Tryptophan	84	87	n/a	74	81
Histidine	88	90	84	87	80
Valine	85	86	80	82	72
Leucine	86	88	83	83	74
Arginine	92	94	88	90	82
Phenylalanine	88	90	84	85	76

The bioavailability of amino acids was originally measured using a slope-ratio assay. Grouped amino acid levels are created by including various levels of a specific feed ingredient. The response of the animals fed the specific test feed ingredient are then measured as protein deposited in the body (Batterham, 1992) or

amino acid oxidation (Moehn *et al.*, 2005). These responses are related to the amino acid intake and the slope of the regression line when compared to the control group, fed a reference protein source. The bioavailability is determined by comparing the ratio of the slope for the test feed, to the slope of the reference protein feed. All the feeds used in this assay, must have the specific amino acid as first limiting and the inclusion levels of the amino acid must be below required levels for the animals. In this assay it is assumed that the response by the animal is linear and not influenced by other nutrients in the diet. The advantage of this assay is that the metabolic losses due to digestion and absorption are considered when measuring the amino acid bioavailability, but this gives an underestimation of amino acid availability. The disadvantages of this assay are that it is expensive and labourious. It only represents relative values with high standard errors (Moehn *et al.*, 2005). Bioavailability of amino acids can also be measured via amino acid digestibility. The amino acid digestibility reflects the digestion of dietary proteins and the absorption of the amino acids and peptides from the gut (Fuller, 2003). Ileal digestibility of amino acid is a more accurate estimate of amino acid availability than total tract digestibility because amino acids are absorbed from the small intestine (Sauer & Ozimek, 1986). Amino acid digestibility is measured by the disappearance of the specific amino acid in the gut, but these values do not consider all the amino acids synthesised and broken down, or the form in which it is absorbed in the small intestine. Over-processed soya products contain amino acids like lysine, that is in a chemical form unavailable to the animal but are still absorbed in the intestines. Here the bioavailability of the amino acids is overestimated (Carpenter, 1960; Moughan & Rutherfurd, 1996). The synthesis and break down of amino acids by microorganisms in the small intestine may also cause differences in the bioavailability and ileal digestibility values (Fuller, 2003). It is important to also consider the effect of dietary ingredients on the efficiency of utilising the amino acid for growth and milk production. High concentrations of dietary amino acids broken down in the gut causes substantial amounts of endogenous amino acid losses (Tammininga *et al.*, 1995). Amino acids released into the hindgut from endogenous losses are not taken into account when ileal digestibility is calculated. When an essential amino acid is limited in a pigs' diet, it causes higher endogenous amino acid losses and reduces overall protein deposition in the body (Lahaye *et al.*, 2004).

2.7 Conclusion

Soybeans (*Glycine max (L.) Merril*), are grown for human consumption and the by-products are used in animal nutrition. One of these by-products includes soya oilcakes, residues from the soybean where most of the oil has been extracted. China, USA, Argentina and Brazil are the main producers of soya oilcakes worldwide. Soya oilcakes have a very good amino acid profile with only the amino acid methionine limiting for poultry and pigs. Soya oilcakes contains anti-nutritive factors that include, among others, the trypsin

inhibitor (protease inhibitor). There are two types of trypsin inhibitors namely the Kunitz inhibitor and the trypsin-chymotrypsin Bowman Brink inhibitor. This protein inhibitor causes a high concentration of undigested protein compounds to be found in the lower digestive tract of the pig and inflammation and other digestive upsets may occur. During inflammatory responses, different specialised immune factors are released. An overproduction of these molecules may cause damage to the intestinal mucosal lining and ultimately reduce performance of the pigs.

The soya oilcakes from different origins vary in CP values and this is part due to processing procedures, the genetic profile of the soybeans and the environmental conditions during the growth process of the soybeans. There are mainly two methods used in the processing of soybeans. The first is the solvent extraction method which is very effective in separating the beans from the oil and is also easy to use with excessive amounts of soybeans. The mechanical oil extraction method is the oldest method used for soya processing. This method is not as efficient as the solvent extraction method in removing the oil from the bean (<70% vs. 99%). The mechanical oil extraction methods have been modified to increase efficiencies by using extrusion methods as a pre-treatment. There is an increasing potential for soya oilcake processing in South Africa, but caution should be taken not to compromise production and overall health of the pig herd by using lower quality products.

In the following chapter the Materials and Methods of the trail will be presented, followed by a chapter on the results and discussion an ended off with conclusions and a critical review.

CHAPTER 3
Materials and methods

Due to the increased processing of soya oilcakes in South Africa and high import costs, pig producers are forced to make use of locally processed soya oilcakes. The amino acid profiles and quality of these oilcakes needs to be considered to safely incorporate into pig diets. This trail was conducted to measure the quality of locally produced soya oilcakes against the oilcakes imported from Argentina. The trail consisted of two parts. In part one of the trail, eighty-eight samples of soya oilcakes from three different processing plants in South Africa and one from Argentina were analysed for their nutritive value and antinutritive factors. The results from the *in vitro* analyses were used to conduct the second part of this trail. Thirty-two piglets were divided into four treatment groups, the control group (diet containing no trypsin inhibitors), a low trypsin inhibitor group, a medium trypsin inhibitor group and a high trypsin inhibitor group. A digestibility trail was conducted to determine the effect of antinutritive factors on the digestibility of CP in pigs.

All procedures and animal husbandry applied in this trial were approved by the University of Pretoria Animal Ethics Committee (EC025-17).

3.1 Trial 1: Nutrient content and anti-nutritive factor concentrations in soya oilcakes from different origins

3.1.1 Processing and sampling

Soya oilcakes from four sources were collected during this trail. Soya oilcake 1 was imported soya oilcake from Argentina and soya oilcake 2, soya oilcake 3 and soya oilcake 4 were all from different processing plants in South Africa.

The following processing procedures were used in a soya oilcake processing plant in Argentina: The soybeans were crushed to reduce the size of the particles and were then heated to give it plasticity. Thereafter, the soybeans were rolled, to ensure the breaking of the fat globules and allow the solvent to directly pass through the particles and extract the oil. After the oil has been removed, the soya oilcake was separated from the solvent at the meal desolventiser-toaster. The soya oilcake was then dried, cooled and grinded (Bunge Argentina, 2012).

In South Africa, a few soybean processing plants uses the mechanical extraction method to remove the oil from the soybean, which allows the products to be used for both animal nutrition and human consumption. The soybeans are cracked, dried, steam heated and the passed through a mechanical screw press. The flakes are then dried and grinded. The heat caused by the friction of the press reduces the anti-nutritive factors, but the soya oilcake has higher residual oil, lower protein and higher by-pass values. The high residual oil can cause problems with rancidity during storage (Irwing Soya, 2018).

Most of the soybean processing plants in South Africa use the solvent extraction method to remove the oil from the soybeans. Soybeans are cleaned and then transferred into hoppers through a chain conveyor. The beans are then cracked, heated, flaked and thereafter passed through an expander to remove excess oil. This is done in a temperature-controlled environment to maintain the pungency of the soya oil. The impurities of the expeller solvent are removed through pre-cleaner and destoner processes and then placed through the cracker breaker to further decrease the particle size. These flakes are then sprayed with a hexane solvent extractor. The soya flakes are then transferred to the desolventiser-toaster to evaporate the hexane from the flakes. The soya oilcake is then released at the end of the toaster section where it is collected and conveyed to storage bins. Before the extraction process, the soybeans are dehulled and those hulls can be added back at the end of the process, this reduces the protein concentration and increases the fibre content of the soya oilcakes (Soya foods SA, 2009).

A total of twenty-two (20 kg each) soya oilcake samples from each of the four sources were collected during this trail. These samples were all collected from different batches that arrived on participating farms over a period of eight months. Each sample were taken at random from different batches of soya oilcakes that arrived on the farm. This ensured collection of representative samples typically produced by each of the sources. Samples were mixed by means of quartering. Each representative sample were mixed thoroughly on a hard, clean surface and then formed into a cone in the centre of the surface. The cone was then flattened and divided into four quarters. The quarters opposite from each other were removed and the other two quarters mixed together again. This process was repeated until the desired sample size was acquired. After thorough mixing, representative samples of 250 g each, were collected from each of the 88 samples in the containers. These samples were sent to five different laboratories for analyses. Table 3.1 summarises the specific nutritive analyses and methods used by each laboratory. The names of the five laboratories are not revealed in this dissertation and are only referred to as laboratory A-E.

3.1.2 Nutrient analyses

3.1.2.1 Nutrient values of soya oilcakes samples

Laboratory A used the official AOAC methods (AOAC, 2005) to measure dry matter (DM), crude protein (CP), crude fat (EE), crude fibre (CF), neutral detergent fibre (NDF), acid detergent fibre (ADF) and starch on all 88 soya oilcake samples.

DM was determined according to AOAC official method 934.01 (AOAC, 2005). Porcelain crucibles were dried in an oven after which the weights were recorded. Two grams of each sample were placed in the crucible and dried in an oven at 95-100 ˚C for 5 hours. The samples were then measured, and the moisture content and DM were calculated as follow: Moisture % = {(Initial weight – Final weight)/ Final weight} x 100 and DM % = (100 –moisture, %).

The CP was determined using the Dumas official method of analyses 992.23 (AOAC, 2005,). Hundred and fifty grams of each soya oilcake sample were ground to pass through a number 20 sieve. Protein was determined using the TruSpec CHNS Macro (Leco, St.Joseph, MI, USA) instrument. The TruSpec determined the carbon, nitrogen, sulphur and hydrogen in the soya oilcakes. Zero point two grams of the ground soya oilcake samples were weighed into a tin foil and placed on an automated loader where after the samples were placed into an oven at 950 ˚C. The samples were then flushed with oxygen, for rapid combustion. The products of the combustion process were then placed in a second oven at 850 ˚C for additional oxidation to take place. Here the fine soya oilcake particles were also removed. The remaining sample were collected at the collection container. A representative sample was then taken and placed into the helium carrier flow, where it moved through hot copper to remove the carbon dioxide and water and convert nitrogen oxide to nitrogen. The nitrogen was then measured by passing the gas through a thermal conductivity cell. The results were calculated using a calibration curve and using EDTA as the nitrogen calibration standard. Calibration of the system was done on a daily basis before any analyses was done by determining blanks and performing a drift correction.

The EE was determined by using the methods described by the AOAC official method of analyses 920.39 (AOAC, 2005). Two grams of each ground sample were weighed into an Erlenmeyer flask. 100 mL HCL (3 N) were added to the sample and then boiled for one hour. The mixture was left to cool at room temperature, where after the mixture was filtered through filter paper and rinsed with distilled water in order to remove all the HCl. The filtered mixture was then dried in an oven at 105 ˚C for twenty-four hours. The sample was then placed in an anhydrous diethyl ether solution, in the Soxhlet extractor. The coil of the heater was adjusted to ensure the mixture evaporated 2-3 drops per second in the condenser. This extraction

process takes 24 hours to be completed. After the 24 hours, all the ether was removed and replaced with more clean ether for extraction of an additional 8 hours. The mixture was then removed from the Soxhlet system and air dried for two hours thereafter it was placed in a 105 ˚C oven for twelve hours. The crude fat was then calculated as follow: Crude fat % = {(Final weight after extraction (g) / Original weight (g))} x 100.

The official method of analyses number 978.10 for CF analyses was used (AOAC, 2005). Two grams of each sample (A) were weighed out and an ether solution was used to remove moisture and fat. The samples were then placed into a 600 mL beaker and 1 g of a prepared asbestos solution was added. Thereafter, 200 mL of boiling 1.25% H_2SO_4 was added together with a drop of diluted antifoamer. The beaker was then placed on a hot digestion instrument plate for 30 minutes. The beaker was moved regularly to prevent solids adhering to the sides of the beaker. The beaker was then removed from the heat and the contend was filtered through a Buchner filter. The beaker was then rinsed with 50 mL of boiling water. The rinsing process was repeated three times with 50 mL of boiling water. A vacuum was then applied to dry the sample. The residue and mat were removed and placed into the beaker again. 200 mL of boiling 1.25% H_2SO_4 was added and left to boil for 30 minutes. The beaker was again removed from the heat and filtered as described above. The beaker was then washed with 25 ml of boiling 1.25% H_2SO_4 and then 50 ml of water (repeat three times). Lastly the beaker was washed out with 25 ml of alcohol. The dry mat and residue were then dried at 130 ˚C for 2 hours. Afterwards the dry mat was placed in a desiccator, cooled and the weights were recorded (B). The samples were transferred to an ashing dish and ignited at 600 ˚C for 30 minutes. The samples were then cooled down in a desiccator and weighed again (C). The CF was calculated as follow: CF % = {(weight after acid and base extraction (B) – weight after ashing (C)) / (original weight (A) x % dry matter)} x 100.

The NDF was determined by laboratory A by making use of the official method of analyses number 2002.04 (AOAC, 2005). One gran of each sample was weighed out in a 600 mL Berzelius beaker (A) and 100 mL of the neutral detergent fibre solution (30 g sodium lauryl sulphate, 18.61 g di-sodium dihydrogen ethylene di-amine tetra-acetic di-hydrate, 6.81 g sodium borate deca-hydrate, 4.56 g disodium hydrogen phosphate and 10 mL tri-ethylene glycol 65, dissolved in 1 litre of de-ionized water) was added to the sample. The solution was then heated for 5 to 10 minutes. The heat was decreased as the solution started to boil and left to boil for 60 minutes. After the 60 minutes the content were filtered under a vacuum, onto a pre-weighed filter paper (B). The content was then washed with distilled hot water and filtered again. This process was repeated twice, where after the content was rinsed twice with acetone. The filtered contend was then dried in an oven at 100 ˚C for 24 hours. The filter paper and the residue were cooled in a desiccator and the weights were recorded. The filter paper was folded and placed in an aluminium pan of which the weight

was already recorded. This content was then ashed in a muffle for 4 hours in a 500 ˚C oven. After the ashing processes the weights were recorded (D). NDF was then calculated as follow: NDF % = {[(weight of NDF residue (C) – weight of filter paper (B)) – weight after ashing (D)] / original weight of sample (A) x drymatter %} x 100.

ADF was determined by using the official method of analyses number 973.18 (AOAC, 2005). One gram of each sample was measured and placed into a Berzelius beaker (A), after the sample was air dried. 100 mL of the acid detergent solution (27.84 mL of H2SO4 was added to a volumetric flask and brought to 1 litre volume with de-ionized water, and the 20 g of a Quaternary ammonium salt solution, CH3(CH2)15N(CH3)3Br, was added to this solution) was added. The solution was heated to the boil and boiled for 60 minutes. The solution was then filtered under a light suction into crucibles (pre-weighed). The beaker was then washed two to three times, with de-ionized hot water, and washed with acetone until all the colour was removed, thereafter it was suction dried. The samples were then placed in an oven at 100 ˚C for 24 hours. The samples were weighed and recorded (B). Thereafter the samples were ashed in a muffle at 500 ˚C for 4 hours and cooled in a desiccator. The samples were weighed again (C). The ADF was then calculated as follow: ADF % = {(weight of ADF residue and crucible (B) – weight after ashing (C)) / (original weight (A) x dry matter %)} x 100.

Starch was measured using the official method of analyses 996.11 (AOAC, 2005). Samples were milled to pass through a 0.5 mm screen. 0.1 g of each sample was weighed (in duplicated, one was used for the blank) into Corning culture tubes, and the weights were recorded. 10 mL of a 100 mM sodium acetate buffer solution (pH 5.0) was added to each tube by using a brand bottle-top dispensette. Each of the tubes were stirred on a vortex mixer for 5 seconds. 0.1 mL of the undiluted thermostable α-amylase solution was added to the sample tube using a handy step dispenser with a 5 mL tip. 0.1 mL of the 100 mM sodium acetate buffer (pH 5.0) was added to the blank tube. All the tubes were then stirred again on the vortex mixer for 3 seconds. Caps were placed on all the tubes and transferred to a boiling water bath for two minutes. After the two minutes the caps were tightened, and the tubes were placed on the vortex to stir for another 5 minutes. Hereafter the tubes were placed back into the water bath for another 15 minutes. After 15 minutes the tubes were stirred on the vortex mixer for another 5 seconds. The tubes were then placed in a water bath at 50 ˚C and allowed to equilibrate to temperature over a period of 5 minutes. 0.1 mL Megayme (cat. No. E-AMGDF; 3,300 U/mL), was then added to the sample tubes using a handystep dispenser with a 5 mL tip. The sample tubes were then mixed on the vortex for 3 seconds. 0.1 mL of the 100 mM sodium acetate (pH 5.0) buffer was added to the blank tubes. All the tubes were incubated in the water bath for 30 minutes at 50 ˚C. The tubes were then removed from the water bath and allowed to cool over a period of 10 minutes. The tubes were then flipped to ensure condensed water on the inside of the lid, mixed with the

content in the tube. 2.0 mL of each tube was then transferred to a microfuge tube and centrifuged at 13 000 rpm for 5 minutes. A Gilson pipetman dispenser was used to transfer 1.0 mL of the sample to twelve 120mm tubes that contains 4 mL of the 100 mM sodium acetate buffer (pH 5.0) and each tube was mixed thoroughly. A duplicate sample of 0.1 mL was transferred to sixteen empty 120 mm glass tubes. 0.1 mL of the blank samples were also transferred to sixteen empty 120 mm glass tubes. 3.0 mL of the GOPOD reagent (p-hydroxybenzoic acid and sodium azide) was added to each tube and incubated for 20 minutes at 50 ˚C. The absorbance of each tube was measured at 510 nm. Glucose controls and blank tubes were also incubated to calculate the starch content.

Laboratory C determined gross energy by using a bomb calorimeter, using the official method of analyses 945.46 (AOAC, 2005). The bomb calorimetry measures the heat released during the burning of organic materials. 2g of soya oilcake were placed in the sealed container of the bomb calorimeter and filled with oxygen. Hereafter the soya oilcake is set alight using a hot wire. During the combustion a chain of reactions takes place. The carbon molecules convert to carbon dioxide, the hydrogen molecules are converted to water and nitrogen molecules converts to a gaseous solution. The bomb calorimeter records the change in enthalpy, which is the sum of the bonds broken and made by the conversion of the soya particles to gaseous molecules.

Near infrared spectrometry (NIR) analysis was used by laboratory B, laboratory C and laboratory D for analyses of CP, CF, EE and DM. The NIR is a spectroscopic analytic technique that uses the electromagnetic spectrum for quantitative analysis of different feed nutrients. The NIR spectrum have wavelengths between 700 nm and 2500 nm (Rodica & Adrian, 2010). All the samples were divided into three parts. One-hundred-and-fifty grams of each unground sample was placed in a bottle and kept as a backup. Another 100 g of each sample was ground and placed into a bottle as a retention ground sample. 100 g of the unground sample was then milled through a sieve size of 0.5 mm and a 6-tooth blade. After milling, the sample was placed into a container. The grinder was cleaned thoroughly between samples by using a vacuum cleaner. The sample was then placed inside the round quartz glass cup and it was sealed with the lid to compress the sample. The glass was cleaned with a microfibre cloth to ensure that there were no fingerprints on the glass cup, since this would interfere with the light of the NIR. The sample was then scanned by the NIR spectrophotometer and the computer programme ISIScan analysed the data obtained from the NIR. After the NIR scan, the spectra were exported via Mosaic into a NIR file. Results were then exported to an excel spreadsheet for data analysis (NIRMaster, Pro IP65).

Table 3.1 The nutrient analyses and methods used by the different laboratories

Laboratory	Nutrient analyses	Method of analyses	AOAC
A	DM	Wet chemistry	934.01
	CP	Wet chemistry (Dumas)	992.23
	EE	Wet chemistry	920.39
	CF	Wet chemistry	978.10
	NDF	Wet chemistry	2002.04
	ADF	Wet chemistry	973.18
	Starch	Wet chemistry	996.11
B, C, D	DM	NIR	N/A
	CP	NIR	N/A
	EE	NIR	N/A
	CF	NIR	N/A
C	GE	Bomb calorimeter	945.46

3.1.2.2 Antinutritive factors

Antinutritive factors were measured by laboratory A, D and E. In Table 3.2 is summarized the methods used by each laboratory to measure the antinutritive factors. Laboratory A and D measured the urease values by using the method described by Caskey & Knapp (1944). The urease index method measures the pH increase, due to the release of ammonia from the urease enzyme in the soya oilcake. This method is used to determine if the soya oilcake was processed to the full extent to reduce ANF, such as trypsin inhibitors. Laboratory A evaluated the efficiency of the processing techniques of each soya oilcake source by evaluating the KOH values using the method by Araba & Dale (1990). This method measures the solubility of protein in a potassium hydroxide solution. The solubility of protein was determined by laboratory D using the protein dispersibility index (PDI) as described by AOAC (2005). Protein dispersibility index, also measures the solubility of protein, but water is used as the solvent. Laboratory E measured trypsin inhibitor concentrations in the soya oilcake. The method used was based on Senanayake *et al.* (2013). An extract was taken from the soya samples via a buffered aqueous solution. The extract was then allowed to react with a trypsin solution at 37 ˚C. BAPNA (trypsin substrate) was added to solution. The reaction was stopped when acetic acid was added. Using high performance liquid chromatography (HPLC), the 4-nitroaniline (4-NA) released, was measured at 410 nm. HPLC was used instead of direct spectrophotometry because it allows large numbers of samples to be measured without having to observe every sample

analysed. Reagent blank and standard samples were run with each batch of samples. Each sample had its own blank and all samples were analysed in duplicate. When elevated levels of trypsin inhibitor concentrations were present in the soya sample, it showed a small response and low levels of trypsin inhibitor led to high responses. When adding trypsin to a soya oilcake extract with high concentration of trypsin inhibitor, it resulted in low concentration of free trypsin inhibitor remaining in the solution, adding BAPNA caused a low response of the HPLC. When adding trypsin to a soya oilcake with low levels of trypsin inhibitor, it resulted in high levels of free trypsin inhibitors; adding BABNA caused a high peak on the HPLC. The trypsin inhibitor peak values of the different soya oilcakes can only be interpreted in relation to one another.

Laboratory A, B and C analysed amino acids by using HPLC, where the amino acids were all separated from each other by means of column chromatography. This technique is based on an ion exchange resins (Davidson & Hepburn, 1970).

Feed samples were analysed by Nutrilab at the University of Pretoria. Nutrilab used Dumas method to determine the CP content of the different feed samples, as described above (AOAC, 2005). The official method of analyses 992.23 were used to determine the CP values. The chromium oxide concentrations of the different feedstuffs were also measured by means of inductively coupled plasma-mass spectrometry, official method of analyse 2015.06 (AOAC, 2005).

Table 3.2 The methods used to measure antinutritive factors by the different laboratories

Laboratory	Antinutritive factor determining method	Method of analyses
A	Urease index	Described by Caskey & Knapp (1944)
	KOH solubility	Described by Araba & Dale (1990)
D	Urease index	Described by Caskey & Knapp (1944)
	PDI solubility	AOAC (2005)
E	Trypsin inhibitor	Described by Senanayake (2013)

3.2 Trail 2: *In vivo* dry matter and crude protein digestibility of soya oilcakes from different origins

3.2.1 Animal husbandry and housing

The trail was conducted in the pig grower house on the Hillcrest Experimental Farm, University of Pretoria, in early autumn months. The pig house was disinfected a month before the arrival of the animals and again a week before they arrived. The pens were 2.9 meters in length, 1.2 meters in width and fences were 1.2 meters high. The floor area was 3.48 square meters. Each pen was fitted with a single infrared light that was switched on during night times to ensure that the piglets would not experience cold stress. The house contained two extractor fans. The floors were concrete, one side of the floor having concrete slats for the manure to fall into the slurry system. Each pen was equipped with a feeding trough and one nipple drinker. Since pigs are social animals, they were placed together in pairs, to limit social stress. Pens were cleaned daily by removing the excess manure with shovels and rubber scrapers. The pig house was also swept clean daily. Minimal water was used to avoid the spreading of infectious bacteria.

Thirty-two TN60 male piglets, four weeks-of-age, between the weights of 10 and 15 kg, were used for this trial. TN60 male piglets were used at this age, since gut development and digestive upsets are most prone at this age. Only male piglets were used, to limit variation. The piglets used in this experiment came from a specific pathogen free (SPF) Topigs farm on the outskirts of Pretoria, Gauteng. Upon arrival of the piglets on the experimental farm, they were individually weighed and ear-tagged. Each piglet was assigned a unique number, clearly visible on the tag. After weighing, the piglets were divided according to their weights (light, medium and heavy). The piglets were placed into their allocated pens using a randomised block design. This was done to ensure that each treatment group had pen replicates of light, medium and heavy weight pigs, spread evenly throughout the house. There were eight piglets assigned to each of the four treatment groups and two piglets per pen, thus four pens were assigned to each treatment group. Piglets were allowed an adaptation period of one week during which they received the same feed fed in the nursing pens on the farm where they were reared. This ensured fewer digestive upsets and helped them adapt to their unfamiliar environment. After a week of adaptation, the pigs were fed the experimental diet, *ad libitum*, for two weeks. The control diet contained no soya oilcake. The soya oilcake was replaced by HP300, a soya protein concentrate, which is a very specialised processed soya that contains little to no trypsin inhibitors. Treatment A contained soya oilcake with low trypsin inhibitor activity. Treatment B contained soya oilcake with medium trypsin inhibitor activity while treatment C, contained soya oilcake with high trypsin inhibitor activity. The pigs were fed twice a day, 7 o'clock in the morning and 5 o'clock in the afternoon. The feed was fed in a pelleted form and samples of the feed was taken to test the nutrient

values of the actual feed mixed. After the two-week treatment period, a veterinarian euthanatised all the pigs.

3.2.2 Experimental design

The results obtained from the *in vitro* trail (Part 1 of the study) were used to create four treatment groups for Part 2 of the study. The soya oilcake samples collected during Part 1 were classed according to its level of trypsin inhibitor activity, as measured by laboratory E. The urease values and the KOH% were also considered during the assignment of the treatment groups (See appendix 1), although the trypsin peak values carried the most weight. After considering all the quality measurements of the eighty-eighty soya oilcake samples, two samples were selected and mixed in equal parts to form a specific treatment group. Thus, two soya oilcake samples of the lowest trypsin inhibitor activity (or highest trypsin peak value) were mixed in equal parts to be used in treatment group A. Two replicant soya oilcake samples of medium concentration trypsin inhibitor activity was mixed in equal parts to be used in the diet for treatment group B. And two replicants of the soya oilcake samples of the highest trypsin inhibitor activity were mixed in equal parts to be used in treatment group C. The control group contained no soya oilcake but instead include HP300. A summary of the treatment groups can be seen in Table 3.3.

Table 3.3 Quality measurements of the soya oilcakes used in the treatment groups

Soya source	Replicate	Urease (pH value)	KOH %	Trypsin inhibitor average peak*
Treatment A				
1	5	0.0176	66.04	206.4
1	6	0.0263	65.32	204.9
Treatment B				
4	13	0.0357	77.88	160.9
4	16	0.1174	69.87	160.8
Treatment C				
2	21	0.1520	74.70	75.6
2	22	0.0980	73.83	72.6

*A higher trypsin inhibitor peak value indicates lower trypsin inhibitor activity

The feed formulations for the different diets and their calculated nutrient values are shown in Table 3.4. The diets were formulated according to nutrient values of typical commercial weaner feed used in South Africa. To determine the protein digestibility of the feed, the indigestible marker technique was used. The pigs were fed a diet containing a chromium oxide concentration of 0.3%. Faecal samples were taken and frozen at -20°C until analysed. The digestibility of protein was then calculated by using the concentration

of the chromium oxide detected in the faeces and the concentration of the chromium oxide in the feed (McCarthy *et al.*,1974).

Table 3.4 The feed ingredient composition and calculated nutrient values of the different treatment diets (%)

Ingredients	Control diet	Dietary treatments
Maize	38.83	36.35
Soya protein concentrates[1]	13.31	-
Soya oilcake	-	17.46
Cooked maize	20	20
Whey powder	13.81	13.81
Fish meal (66% CP)	5	5
Dextrose	2	2
Wheat bran	2	0
Bergafat	1.5	1.5
Soya oil	0.67	1.04
Monocalcium phosphate	0.92	0.93
Limestone	0.59	0.55
Premix	0.3	0.3
L-Lys HCL 78%	0.28	0.27
Salt	0.23	0.24
ZnO	0.2	0.2
DL- Methionine	0.15	0.16
L-Threonine 98.5%	0.11	0.11
L Tryptophan	0.7	0.7
Pigortek Raspberry[2]	0.02	0.02
Sucram[3]	0.01	0.01
Chromium Oxcide (CrO_3)	0.3	0.3
Calculate nutrient values (g/kg)		
Dry matter	908.04	901.12
NE pig (MJ/kg)	10.5 MJ/kg	10.5 MJ/kg
Crude protein	180.0	181.31
Crude ash	53.81	54.15
Crude fat	50.11	52.39
Crude fibre	19.98	19.13
Calcium	6.5	6.5
Total phosphorus	7.09	6.9
Lysine (total)	12.01	12.21
Methionine (total)	4.76	4.81
Methionine + Cystine (total)	7.61	7.65
Threonine (total)	8.1	8.17
Tryptophan (total)	2.68	2.7

[1]HP 300, Hamlet Protein, Hamlet Protein Denmark

[2]Pigortec Raspberry 656, flavouring agent used in weaner pig diets to increase palatability, Allied Nutrition, 89 Jean Ave, Doringkloof, Centurion, 0157

[3]Sucram C150, sweeting agent used in weaner diets to increase palatability, Allied Nutrition

3.2.3 Sampling and measurements

After euthanasia of the piglets, they were transferred to the abattoir unit at the University of Pretoria's Hillcrest experimental farm. Each pig was dissected to remove the ileal content from the small intestine, as well as the manure from the rectum. Each of these samples were collected in containers and marked. After all the samples were collected, the samples were placed in the freezer for chemical analysis later. A feed sample from each treatment group was also collected for chemical analysis. Each rectal and ileum sample were thawed before the start of the analysis. Initial dry matter (iDM) of each sample was determined. Crucibles were dried in a 105˚C oven for one hour. The crucibles were removed from the oven and cooled down in a desiccator for one hour. Each crucible's weight was recorded, and one gram of each sample was weighed into the crucible. Each sample was duplicated. All the crucibles were then dried at 105˚C for 24 hours. After twenty-four hours the crucibles were removed, and the dry weight was measured. The remainder of the samples were placed in a 55˚C oven for 48 hours to completely dry out and to ensure no breakdown of important protein structures occurred. After the 48 hours, the samples were all ground, bottled and labelled. These bottled samples were then used for further analysis. The chromium oxide of the ileum and rectum samples where measured using an atomic absorption spectrophotometer according to the official method of analyses 990.03 (AOAC, 2005). AOAC Dumas official method of analyses 992.23 (2005) was used to measure the crude protein in the different digesta samples. With dissection of pigs, only small amount of digesta were present in the ileum and rectum for most of the piglets. Therefore, two samples from the ileum and two from the rectum for each treatment were pooled to get sufficient a volume of sample for analyses of CP and Cr_2O_3. Samples were pooled by mixing together 2 samples, from the same treatment, in equal parts (see appendix 2 and 3).

The pooled ileal samples were used to calculate the protein digestibly of each treatment. The following formula was used to calculate the digestibility of each sample:

$$\% \text{ Protein digestibility} = 100 - \left(100 \times \frac{\% \text{ indicator}_{feed}}{\% \text{ indicator}_{ileum}} \times \frac{\% \text{ nutrient}_{ileum}}{\% \text{ nutrient}_{feed}}\right)$$

The following formula was used to calculate the total tract digestibility from each pooled rectum sample:

$$\% \text{ Protein digestibility} = 100 - \left(100 \times \frac{\% \text{ indicator}_{feed}}{\% \text{ indicator}_{manure}} \times \frac{\% \text{ nutrient}_{manure}}{\% \text{ nutrient}_{feed}}\right)$$

3.2.4 Statistical analyses

All the statistical analyses were conducted using SAS software (Statistical Analysis System, 2004). The significant differences between the treatments and laboratories were determined by the GLM (general linear model), using an analysis of variance. The Fishers test was used to calculate means, standard errors and significance of the differences between the means. The confidence level was at 95% and level of statistical significance was $P<0.05$. The GLM model was also used for repeated measures of analyses using the SAS model, where the nutrient measurements were repeated. The means and standard errors for the different treatment groups were calculated and the significant differences were analysed using the Fishers test ($P<0.05$) (Fisher *et al*. 1973).

CHAPTER 4

Results

4.1 Trial 1: Nutrient content and antinutritive factor concentrations in soya oilcakes from different origins

4.1.1 Nutrient content of the soya oilcake

4.1.1.1 Crude protein concentrations of the different soya oilcakes

Table 4.1 shows the results from laboratory A, B, C and D for crude protein values of the different soya sources.

Table 4.1 Crude protein (%) concentrations for the soya oilcakes tested at laboratory A, B, C and D (on as is basis)

Soya source*	Laboratory A	Laboratory B	Laboratory C	Laboratory D	Mean (X)
1	53.108^{Ac}	52.817^{Ac}	54.539^{Aa}	53.556^{Ab}	53.505^{A}
2	52.468^{Bc}	52.823^{Ab}	54.289^{Aba}	52.658^{Cb}	53.060^{B}
3	52.731^{Bc}	52.456^{Bc}	54.161^{Ba}	53.091^{Bb}	53.110^{B}
4	51.320^{Cc}	51.990^{Cb}	53.090^{Ca}	51.909^{Db}	52.078^{C}
Mean (X)	52.407^{c}	52.522^{c}	54.02^{a}	52.804^{b}	

$^{A-D}$ Column means with same superscript do not differ significantly (P>0.05)

$^{a-c}$ Row means with the same superscript do not differ significantly (P>0.05)

* Source 1 was imported from Argentina; sources 2-4 were from South African soya processors (values are the means of 22 samples for each source)

The results obtained from laboratory A and B showed similar CP values for the soya oilcakes. Laboratory C reported the highest average CP value for the different soya oilcake sources and laboratory A and B showed the lowest values. On average, soya oilcake 1 had the highest CP value and soya oilcake 4 had the lowest average CP value of the various soya oilcakes.

4.1.1.2 Crude fat concentrations of the different soya oilcakes

Table 4.2 shows the different crude fat values for the four soya sources.

Table 4.2 Crude fat (%) concentrations for the soya oilcakes tested at laboratory A, B, C and D (on as is basis)

Soya source*	Laboratory A	Laboratory B	Laboratory C	Laboratory D	Mean (X)
1	1.286^{Cd}	1.661^{Bc}	2.478^{Ba}	1.949^{Bb}	1.843^{C}
2	1.797^{Bc}	2.137^{Ab}	2.957^{Aa}	2.344^{Ab}	2.309^{B}
3	1.965^{Abc}	2.227^{Ac}	3.157^{Aa}	2.513^{Ab}	2.466^{A}
4	2.142^{Ac}	2.222^{Ac}	2.996^{Aa}	2.533^{Ab}	2.473^{A}
Mean (X)	1.780^{d}	2.062^{c}	2.897^{a}	2.335^{b}	

$^{A-C}$ Column means with same superscript do not differ significantly (P>0.05)

$^{a-d}$ Row means with the same superscript do not differ significantly (P>0.05)

* Source 1 was imported from Argentina; sources 2-4 were from South African soya processors (values are the means of 22 samples for each source)

Laboratory C reported the highest crude fat values for the soya oilcake samples and laboratory A reported the lowest crude fat values for the various soya oilcake samples. On average, the crude fat value was the highest for soya oilcake 3 and soya oilcake 4 and lowest for soya oilcake 1.

4.1.1.3 Starch concentrations of the different soya oilcakes

Table 4.3 indicates the starch values for the four soya oilcake sources measured by laboratory C.

Table 4.3 Starch (%) concentrations for the soya oilcakes tested at laboratory C (on as is basis)

Soya source*	Starch
1	0.883^{A}
2	0.782^{B}
3	0.898^{A}
4	0.863^{A}

A-B Column means with same superscript do not differ significantly (P>0.05)

* Source 1 was imported from Argentina; sources 2-4 were from South African soya processors (values are the means of 22 samples for each source)

The starch values for soya oilcake 1, 3 and 4 did not differ significantly from each other. The starch level of soya oilcake 2 was significantly lower than the starch level in soya oilcake 1, 3 and 4.

4.1.1.4 Crude fibre concentrations of the different soya oilcakes

Crude fibre concentrations measured by laboratory A, B, C and D are shown in Table 4.4.

Table 4.4 Crude fibre (%) concentrations for the soya oilcakes tested at laboratory A, B, C and D (on as is basis)

Soya source*	Laboratory A	Laboratory B	Laboratory C	Laboratory D	Mean (X)
1	4.402^{Abc}	4.905^{Ab}	3.349^{Bd}	5.231^{Aa}	4.472^{B}
2	3.660^{Cc}	3.999^{Cb}	3.591^{Bc}	4.322^{Ca}	3.893^{D}
3	4.120^{Bc}	4.505^{Bb}	3.321^{Bd}	4.909^{Ba}	4.233^{C}
4	4.609^{Ab}	5.087^{Aa}	4.141^{Ac}	5.145^{ABa}	4.745^{A}
Mean (X)	4.217^{c}	4.624^{b}	3.600^{d}	4.902^{a}	

A-D Column means with same superscript do not differ significantly (P>0.05)

a-d Row means with the same superscript do not differ significantly (P>0.05)

* Source 1 was imported from Argentina; sources 2-4 were from South African soya processors (values are the means of 22 samples for each source)

Laboratory D reported the highest CF values for the soya oilcake samples and laboratory C reported the lowest values. All four soya oilcake samples differed significantly in their CF values. Soya oilcake 4 had the highest CF value followed by soya oilcake 1 and soya oilcake 3, while soya oilcake 2 had the lowest CF value.

4.1.1.5 Acid detergent fibre (%) concentrations of the different soya oilcakes

Table 4.5 shows the ADF values for the four soya oilcakes tested at laboratory C.

Table 4.5 Acid detergent fibre concentrations for the soya oilcakes tested at laboratory C (on as is basis)

Soya source*	ADF
1	6.716^{B}
2	6.389^{C}
3	6.649^{B}
4	7.372^{A}

A-C Column means with same superscript do not differ significantly (P>0.05)

* Source 1 was imported from Argentina; sources 2-4 were from South African soya processors (values are the means of 22 samples for each source)

Laboratory C reported ADF values for soya oilcake 4 to be significantly higher than the other soya oilcake samples. Soya oilcake 1 and 3 had the second highest ADF value and did not differ significantly from each other. Soya oilcake 2 had an ADF value of 6.389% that was the lowest value compared to the other three samples.

4.1.1.6 Neutral detergent fibre concentrations of the different soya oilcakes

Table 4.6 indicates the NDF values for the four soya oilcake groups, analysed at laboratory C.

Table 4.6 Neutral detergent fibre (%) concentrations for the soya oilcakes tested at laboratory C (on as is basis)

Soya source*	NDF
1	11.453^{B}
2	10.561^{C}
3	10.564^{C}
4	13.322^{A}

A-C Column means with same superscript do not differ significantly (P>0.05)

* Source 1 was imported from Argentina; sources 2-4 were from South African soya processors (values are the means of 22 samples for each source)

The highest NDF value was reported by laboratory C for soya oilcake 4. Soya oilcake 1 had the second highest NDF value. Soya oilcake 2 and 3 did not differ significantly from each other and showed the lowest NDF value of all four soya oilcake sources.

4.1.1.7 Gross energy values of the different soya oilcakes

All the results for the GE values from laboratory C are shown in table 4.7.

Table 4.7 Gross energy values (MJ/kg) for the soya oilcakes tested at laboratory C (on an as is basis)

Soya source*	GE
1	19.935^{C}
2	20.164^{A}
3	20.067^{B}
4	20.072^{B}

$^{A-C}$ Column means with same superscript do not differ significantly (P>0.05)

* Source 1 was imported from Argentina; sources 2-4 were from South African soya processors (values are the means of 22 samples for each source)

The GE reported by laboratory C showed that soya oilcake 2 had the highest GE value. Soya oilcake 3 and 4 reportedly had the second highest GE values and did not differ significantly from each other. Soya oilcake 1 had the lowest GE value of all four the soya oilcake sources.

4.1.2 Antinutritive factor concentrations

4.1.2.1 Protein dispersibility index test values for the different soya sources

Table 4.8 gives the results for the protein dispersibility values tested for by laboratory D.

Table 4.8 Protein dispersibility index values for the soya oilcakes tested at laboratory D (on an as is basis)

Soya source*	PDI values
1	10.027^{B}
2	12.430^{A}
3	9.616^{B}
4	12.820^{A}

$^{A-B}$ Column means with same superscript do not differ significantly (P>0.05)

* Source 1 was imported from Argentina; sources 2-4 were from South African soya processors (values are the means of 22 samples for each source)

Soya oilcake 1 and 3 did not differ significantly from each other, and soya oilcake 2 and 4 also did not differ from each other. Soya oilcake 2 and 4 had the highest PDI values and soya oilcake 1 and 3 had the lowest PDI values.

4.1.2.2 Potassium hydroxide solubility percentages for the different soya sources

The results for the KOH solubility % obtained from laboratory A, are shown in Table 4.9.

Table 4.9 Potassium hydroxide solubility (%) for the soya oilcakes tested at laboratory A (on an as is basis)

Soya source*	KOH
1	83.943^{C}
2	94.279^{A}
3	84.203^{C}
4	89.915^{B}

$^{A-C}$ Column means with same superscript do not differ significantly (P>0.05)

* Source 1 was imported from Argentina; sources 2-4 were from South African soya processors (values are the means of 22 samples for each source)

Soya oilcake 1 and 3 did not differ significantly from each other, and they had lower KOH solubility values than the other soya oilcake sources. Soya oilcake 2 showed the highest KOH solubility percentage and soya oilcake 4 showed the second highest KOH solubility percentage.

4.1.2.3 Urease pH values for the different soya sources

The different urease pH values analysed at laboratory A and D are shown in Table 4.10.

Table 4.10 Urease pH values for the soya oilcakes tested at laboratory A and D (on an as is basis)

Soya source*	Laboratory A	Laboratory D	Mean (X)
1	0.046^{bB}	0.167^{a}	0.107^{B}
2	0.161^{A}	0.168	0.164^{A}
3	0.050^{bB}	0.134^{a}	0.092^{B}
4	0.075^{B}	0. 117	0.096^{B}
Mean (x)	0.083^{b}	0.147^{a}	

$^{A-B}$ Column means with same superscript do not differ significantly (P>0.05)

$^{a-b}$ Row means with the same superscript do not differ significantly (P>0.05)

* Source 1 was imported from Argentina; sources 2-4 were from South African soya processors (values are the means of 22 samples for each source)

The urease values for soya oilcake 1 and 3 differed significantly between laboratory A and laboratory D. Laboratory A had lower values for all four the soya oilcake sources. According to laboratory A, soya oilcake 1, soya oilcake 3 and soya oilcake 4 did not differ significantly in its urease pH values. Soya oilcake 1 had a value below 0.05. Laboratory D showed that soya oilcake 1, soya oilcake 2, soya oilcake 3 and soya oilcake 4 did not differ significantly from each other. On average soya oilcake 2 had the highest urease pH value and soya oilcake 1, 3 and 4 had the lowest urease pH values and did not differ significantly from each other.

4.1.2.4 Trypsin inhibitor activity for the different soya oilcakes

The trypsin inhibitor peak values reported by laboratory E, are shown in Table 4.11.

Table 4.11 Trypsin inhibitor peak values for the soya oilcakes tested at laboratory E (on an as is basis)

Soya source*	Trypsin peak values*
1	178.68^{A}
2	124.59^{C}
3	155.95^{B}
4	164.42^{AB}

$^{A-B}$ Column means with same superscript do not differ significantly (P>0.05)

* Source 1 was imported from Argentina; sources 2-4 were from South African soya processors (values are the means of 22 samples for each source)

*The higher the trypsin inhibitor peak value, the lower the trypsin inhibitor activity level

Soya oilcake 1 and 4 did not differ significantly from each other. Soya oilcake 3 and soya oilcake 4 also did not differ from each other. Soya oilcake 1 and 4 had the highest trypsin inhibitor peak values, thus the lowest trypsin inhibitor activity. Soya oilcake 2 had the lowest trypsin inhibitor peak value, thus the highest trypsin inhibitor activity.

Figure 4.1 shows the relationship between the urease pH values and trypsin inhibitor concentrations. Urease pH values are directly correlated to the 1/trypsin inhibitor peak value, which means the urease concentration is directly related to the trypsin inhibitor concentration.

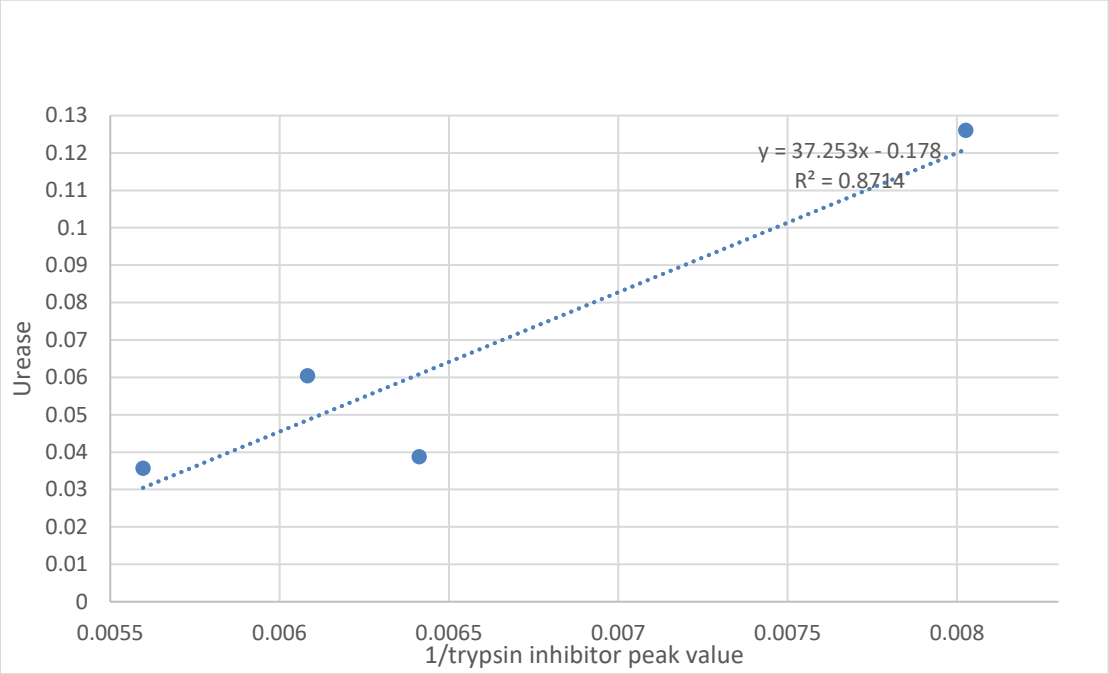

Figure 4.1 The relationship between Urease pH value and 1/trypsin peak values

4.1.3 Amino acid concentrations of the different soya oilcake sources

Laboratory B and C tested for the different total amino acid concentrations of the four soya oilcake sources and the results can be seen in Table 4.12- 4.22.

Table 4.12 Lysine concentrations (g/kg) for soya oilcakes tested at laboratory B and C

Soya source	Laboratory B	Laboratory C	Mean (X)
1	3.404^{Aa}	3.304^{Ab}	3.354^{A}
2	3.389^{Aa}	3.259^{Bb}	3.324^{B}
3	3.399^{Aa}	3.296^{Ab}	3.347^{A}
4	3.366^{Ba}	3.185^{Cb}	3.275^{C}
Mean (x)	3.390^{a}	3.261^{b}	

$^{A-C}$ Column means with same superscript do not differ significantly (P>0.05)

$^{a-b}$ Row means with the same superscript do not differ significantly (P>0.05)

* Source 1 was imported from Argentina; sources 2-4 were from South African soya processors (values are the means of 22 samples for each source)

Laboratory B showed on average the highest lysine concentrations for the four soya sources. Soya oilcake 1 and 3 had on average the highest lysine concentrations and did not differ significantly from each other. Soya oilcake 4 had on average the lowest lysine concentration.

Table 4.13 Methionine concentrations (g/kg) for soya oilcakes tested at laboratory B and C (on an as is basis)

Soya source*	Laboratory B	Laboratory C	Mean (X)
1	0.732^{A}	0.728^{B}	0.730^{A}
2	0.725^{Bb}	0.735^{Aa}	0.730^{A}
3	0.727^{AB}	0.723^{B}	0.725^{B}
4	0.715^{Cb}	0.729^{Ba}	0.722^{B}
Mean (X)	0.725^{b}	0.729^{a}	

$^{A-C}$ Column means with same superscript do not differ significantly (P>0.05)

$^{a-b}$ Row means with the same superscript do not differ significantly (P>0.05)

* Source 1 was imported from Argentina; sources 2-4 were from South African soya processors (values are the means of 22 samples for each source)

Laboratory C showed on average the highest concentrations for the methionine amino acid. Soya oilcake 1 and 2 did not differ significantly from each other and had the highest methionine concentration. Soya oilcake 3 and 4 did not differ significantly from each other and showed on average the lowest methionine concentration.

Table 4.14 Cystine concentrations (g/kg) for soya oilcakes tested at laboratory B and C (on an as is basis)

Soya source*	Laboratory B	Laboratory C	Mean (X)
1	0.798^{Ba}	0.783^{Bb}	0.791^{B}
2	0.811^{Aa}	0.796^{Ab}	0.803^{A}
3	0.794^{BC}	0.789^{B}	0.791^{B}
4	0.792^{Ca}	0.773^{Cb}	0.782^{C}
Mean (X)	0.799^{a}	0.785^{b}	

$^{A-C}$ Column means with same superscript do not differ significantly (P>0.05)

$^{a-b}$ Row means with the same superscript do not differ significantly (P>0.05)

* Source 1 was imported from Argentina; sources 2-4 were from South African soya processors (values are the means of 22 samples for each source)

Laboratory B reported the highest average value for cystine amino acid. On average soya oilcake 2 had the highest concentration cystine amino acid. Soya oilcake 1 and 2 did not differ significantly from each other and reported the second highest cystine concentration. Soya oilcake 4 had on average the lowest cystine concentration.

Table 4.15 Threonine concentrations (g/kg) for soya oilcakes tested at laboratory B and C (on as is basis)

Soya source*	Laboratory B	Laboratory C	Mean (X)
1	2.102^{Aa}	2.081^{Ab}	2.091^{A}
2	2.070^{Ca}	2.054^{Cb}	2.062^{C}
3	2.082^{Ba}	2.068^{Bb}	2.075^{B}
4	2.049^{Da}	2.026^{Db}	2.037^{D}
Mean(X)	2.076^{a}	2.057^{b}	

$^{A-D}$ Column means with same superscript do not differ significantly (P>0.05)

$^{a-b}$ Row means with the same superscript do not differ significantly (P>0.05)

* Source 1 was imported from Argentina; sources 2-4 were from South African soya processors (values are the means of 22 samples for each source)

Laboratory B reported the highest threonine value on average. Soya oilcake 1 had on average the highest threonine concentration followed by soya oilcake 3 and then soya oilcake 2. Soya oilcake 4 reported the lowest threonine concentration on average.

Table 4.16 Tryptophan concentrations (g/kg) for soya oilcakes tested at laboratory B and (on an as is basis)

Soya source*	Laboratory B	Laboratory C	Mean (X)
1	0.779^{a}	0.741^{Ab}	0.760^{A}
2	0.778^{a}	0.743^{Ab}	0.761^{A}
3	0.776^{a}	0.736^{Bb}	0.756^{B}
4	0.776^{a}	0.728^{Cb}	0.752^{C}
Mean (X)	0.777^{a}	0.734^{b}	

$^{A-C}$ Column means with same superscript do not differ significantly (P>0.05)

$^{a-b}$ Row means with the same superscript do not differ significantly (P>0.05)

* Source 1 was imported from Argentina; sources 2-4 were from South African soya processors (values are the means of 22 samples for each source)

Laboratory B showed the highest average tryptophan concentration. Soya oilcake 1 and 2 did not differ significantly from each other and showed on average the highest tryptophan concentration. Soya oilcake 3 had the second highest concentration followed by soya oilcake 4.

Table 4.17 Valine concentrations (g/kg) for soya oilcakes tested at laboratory B and C (on an as is basis)

Soya source*	Laboratory B	Laboratory C	Mean (X)
1	2.681^{Aa}	2.562^{Ab}	2.622^{A}
2	2.684^{Aa}	2.514^{Cb}	2.599^{B}
3	2.661^{Ba}	2.546^{Bb}	2.603^{B}
4	2.606^{Ca}	2.470^{Db}	2.538^{C}
Mean (X)	2.658^{a}	2.523^{b}	

$^{A-D}$ Column means with same superscript do not differ significantly (P>0.05)

$^{a-b}$ Row means with the same superscript do not differ significantly (P>0.05)

* Source 1 was imported from Argentina; sources 2-4 were from South African soya processors (values are the means of 22 samples for each source)

Laboratory B reported the highest average valine concentration. Soya oilcake 1 had the highest average valine concentration. Soya oilcake 2 and 3 showed no significant differences and had the second highest valine concentration. Soya oilcake 4 showed the lowest valine amino acid concentration.

Table 4.18 Isoleucine concentrations (g/kg) for soya oilcakes tested at laboratory B and C (on an as is basis)

Soya source*	Laboratory B	Laboratory C	Mean (X)
1	2.603^{Aa}	2.475^{Ab}	2.539^{A}
2	2.555^{Ca}	2.432^{Bb}	2.493^{C}
3	2.579^{Ba}	2.450^{Bb}	2.514^{B}
4	2.539^{Ca}	2.393^{Cb}	2.466^{D}
Mean (x)	2.569^{a}	2.437^{b}	

$^{A-D}$ Column means with same superscript do not differ significantly (P>0.05)

$^{a-b}$ Row means with the same superscript do not differ significantly (P>0.05)

* Source 1 was imported from Argentina; sources 2-4 were from South African soya processors (values are the means of 22 samples for each source)

Laboratory B showed on average the highest isoleucine concentrations. All the soya oilcake isoleucine concentrations differed significantly from each other. Soya oilcake 1 had the highest isoleucine concentration followed by soya oilcake 3 and then soya oilcake 2. Soya oilcake 4 had on average the lowest isoleucine concentration.

Table 4.19 Leucine concentrations (g/kg) for soya oilcakes tested at laboratory B and C (on an as is basis)

Soya source*	Laboratory B	Laboratory C	Mean (X)
1	4.127^{A}	4.121^{A}	4.124^{A}
2	4.067^{B}	4.054^{A}	4.060^{C}
3	4.098^{A}	4.090^{B}	4.094^{B}
4	4.063^{Ba}	3.981^{Cb}	4.022^{D}
Mean (X)	4.089^{a}	4.061^{b}	

$^{A-D}$ Column means with same superscript do not differ significantly (P>0.05)

$^{a-b}$ Row means with the same superscript do not differ significantly (P>0.05)

* Source 1 was imported from Argentina; sources 2-4 were from South African soya processors (values are the means of 22 samples for each source)

Laboratory B had on average the highest leucine concentration. All four the soya oilcake sources differed, on average, significantly from each other. Soya oilcake 1 had on average the highest leucine value followed by soya oilcake 2 and 3. Soya oilcake 4 had on average the lowest concentration leucine.

Table 4.20 Phenylalanine concentrations (g/kg) for soya oilcakes tested at laboratory B and C on an as is basis)

Soya source*	Laboratory B	Laboratory C	Mean (X)
1	2.792^{Aa}	2.739^{Ab}	2.766^{A}
2	2.719^{C}	2.703^{B}	2.711^{C}
3	2.767^{Ba}	2.710^{Bb}	2.739^{B}
4	2.725^{Ca}	2.647^{Cb}	2.686^{D}
Mean (X)	2.751^{a}	2.700^{b}	

$^{A-D}$ Column means with same superscript do not differ significantly (P>0.05)

$^{a-b}$ Row means with the same superscript do not differ significantly (P>0.05)

* Source 1 was imported from Argentina; sources 2-4 were from South African soya processors (values are the means of 22 samples for each source)

Laboratory B had the highest phenylalanine concentrations. The four soya oilcake sources differed significantly from each other. Soya oilcake 1 had on average the highest phenylalanine concentration followed by soya oilcake 3 and then soya oilcake 2. Soya oilcake 4 had on average the lowest phenylalanine concentration.

Table 4.21 Histidine concentrations (g/kg) for soya oilcakes tested at laboratory B and C (on an as is basis)

Soya source*	Laboratory B	Laboratory C	Mean (X)
1	1.360^{Ab}	1.386^{Aa}	1.373^{A}
2	1.353^{AB}	1.359^{B}	1.356^{C}
3	1.347^{Bb}	1.378^{Aa}	1.363^{B}
4	1.335^{C}	1.341^{C}	1.338^{D}
Mean (X)	1.349^{b}	1.366^{a}	

$^{A-D}$ Column means with same superscript do not differ significantly (P>0.05)

$^{a-b}$ Row means with the same superscript do not differ significantly (P>0.05)

* Source 1 was imported from Argentina; sources 2-4 were from South African soya processors (values are the means of 22 samples for each source)

On average laboratory C reported the highest histidine concentration. Soya oilcake 1 had the highest histidine concentration followed by soya oilcake 3 and then soya oilcake 2. Soya oilcake 4 had on average the lowest histidine concentration. All the soya oilcake sources differed significantly from each other.

Table 4.22 Arginine concentrations (g/kg) for soya oilcakes tested at laboratory B and C (on an as is basis)

Soya source*	Laboratory B	Laboratory C	Mean (X)
1	3.956^{Aa}	3.923^{Ab}	3.940^{A}
2	3.918^{Ba}	3.885^{Bb}	3.902^{B}
3	3.936^{Aba}	3.903^{ABb}	3.920^{AB}
4	3.918^{Ba}	3.820^{Cb}	3.869^{C}
Mean (X)	3.932^{a}	3.883^{b}	

$^{A-B}$ Column means with same superscript do not differ significantly (P>0.05)

$^{a-b}$ Row means with the same superscript do not differ significantly (P>0.05)

* Source 1 was imported from Argentina; sources 2-4 were from South African soya processors (values are the means of 22 samples for each source)

The highest arginine concentration was reported by laboratory B. The arginine concentration for soya oilcake 1 and 3 did not differ significantly from each other. Soya oilcake 2 and 3 also did not differ significantly. Soya oilcake 4 had on average the lowest arginine concentration.

Laboratory C also measured the methionine: cystine ratio, glycine, serine, proline, alanine, glutamate and aspartate concentrations for the different soya oilcake sources. These results are shown in Table 4.23 – 4.29.

Table 4.23 Methionine: Cystine ratio for soya oilcakes tested at laboratory C (on an as is basis)

Soya sources*	Laboratory C
1	1.512^{AB}
2	1.516^{A}
3	1.509^{AB}
4	1.506^{B}

$^{A-B}$ Column means with same superscript do not differ significantly (P>0.05)

* Source 1 was imported from Argentina; sources 2-4 were from South African soya processors (values are the means of 22 samples for each source)

Soya oilcake 1 and 3 did not differ significantly from soya oilcake 2. Soya oilcake 1 and 3 also did not differ significantly from soya oilcake 4. Soya oilcake 4 had on average the lowest methionine to cystine ratio.

Table 4.24 Glycine concentrations (g/kg) for soya oilcakes tested at laboratory C (on an as is basis)

Soya source*	Laboratory C
1	2.259^{A}
2	2.224^{C}
3	2.241^{B}
4	2.193^{D}

A-D Column means with same superscript do not differ significantly (P>0.05)

* Source 1 was imported from Argentina; sources 2-4 were from South African soya processors (values are the means of 22 samples for each source)

Laboratory C reported that soya oilcake 1 had the highest glycine concentration, followed by soya oilcake 3 and then soya oilcake 2. Soya oilcake 4 had the lowest glycine concentration of all four the soya oilcake sources.

Table 4.25 Serine concentrations (g/kg) for soya oilcakes tested at laboratory C (on an as is basis)

Soya source*	Laboratory C
1	2.690^{A}
2	2.615^{B}
3	2.671^{A}
4	2.598^{B}

A-B Column means with same superscript do not differ significantly (P>0.05)

* Source 1 was imported from Argentina; sources 2-4 were from South African soya processors (values are the means of 22 samples for each source)

According to results obtained from laboratory C, soya oilcake 1 and 3 did not differ significantly from each other and had the highest serine concentrations. Soya oilcake 2 and 4 did not differ significantly and had the lowest serine concentration.

Table 4.26 Proline concentrations (g/kg) for soya oilcakes tested at laboratory C (on an as is basis)

Soya source*	Laboratory C
1	2.724^{A}
2	2.735^{A}
3	2.704^{B}
4	2.688^{B}

A-B Column means with same superscript do not differ significantly (P>0.05)

* Source 1 was imported from Argentina; sources 2-4 were from South African soya processors (values are the means of 22 samples for each source)

Soya oilcake 1 and 2 did not differ significantly and had the highest proline concentration. Soya oilcake 3 and 4 also did not differ significantly from each other, showing the lowest proline concentration.

Table 4.27 Alanine concentrations (g/kg) for soya oilcakes tested at laboratory C (on an as is basis)

Soya source*	Laboratory C
1	2.355^{A}
2	2.312^{B}
3	2.339^{A}
4	2.269^{C}

A-C Column means with same superscript do not differ significantly (P>0.05)

* Source 1 was imported from Argentina; sources 2-4 were from South African soya processors (values are the means of 22 samples for each source)

Laboratory C reported that soya oilcake 1 and 3 showed no significant differences and had the highest alanine concentration. Soya oilcake 2 had the second highest alanine concentration and soya oilcake 4 had the lowest alanine concentration of all the soya oilcakes analysed.

Table 4.28 Aspartate concentrations (g/kg) for soya oilcakes tested at laboratory C (on an as is basis)

Soya source*	Laboratory C
1	6.100^{A}
2	6.076^{AB}
3	6.049^{B}
4	5.954^{C}

A-C Column means with same superscript do not differ significantly (P>0.05)

* Source 1 was imported from Argentina; sources 2-4 were from South African soya processors (values are the means of 22 samples for each source)

The aspartate concentrations analysed by laboratory C showed that soya oilcake 2 did not differ significantly from soya oilcake 1. It also showed that soya oilcake 2 did not differ significantly from soya oilcake 3. Soya oilcake 1 had the highest aspartate concentration and soya oilcake 4 had the lowest aspartate concentration.

Table 4.29 Glutamate concentrations (g/kg) for soya oilcakes tested at laboratory C (on an as is basis)

Soya source*	Laboratory C
1	9.628^{A}
2	9.582^{AB}
3	9.547^{B}
4	9.388^{C}

$^{A-B}$ Column means with same superscript do not differ significantly (P>0.05)

* Source 1 was imported from Argentina; sources 2-4 were from South African soya processors (values are the means of 22 samples for each source)

Laboratory C reported that soya oilcake 2 did not differ significantly from soya oilcake 1 and it also did not differ significantly from soya oilcake 3. Soya oilcake 1 had reportedly the highest concentration glutamate and soya oilcake 4 had the lowest concentration.

Table 4.30 summarises all the average values for the different amino acids tested at the different laboratories.

Table 4.30 Average amino acid concentrations of the different soya sources (on an as is basis)

Amino acid	Soya source*			
	1	**2**	**3**	**4**
Lys	3.354^{A}	3.324^{B}	3.347^{A}	3.275^{C}
Met	0.730^{A}	0.730^{A}	0.725^{B}	0.722^{B}
Tryp	0.760^{A}	0.761^{A}	0.756^{B}	0.752^{C}
Thr	2.091^{A}	2.062^{C}	2.075^{B}	2.037^{D}
Cys	0.791^{B}	0.803^{A}	0.791^{B}	0.782^{C}
Val	2.622^{A}	2.599^{B}	2.603^{B}	2.538^{C}
Ile	2.539^{A}	2.539^{A}	2.514^{B}	2.466^{D}
Leu	4.124^{A}	4.060^{C}	4.094^{B}	4.022^{D}
Phe	2.766^{A}	2.711^{C}	2.739^{B}	2.686^{D}
His	1.373^{A}	1.356^{C}	1.363^{B}	1.363^{B}
Arg	3.940^{A}	3.902^{B}	3.920^{AB}	3.869^{C}
Ser	2.690^{A}	2.615^{B}	2.671^{A}	2.598^{B}
Pro	2.724^{A}	2.735^{A}	2.704^{B}	2.688^{B}
Ala	2.355^{A}	2.312^{B}	2.339^{A}	2.269^{C}
Asp	6.100^{A}	6.076^{AB}	6.049^{B}	5.954^{C}
Glu	9.628^{A}	9.582^{AB}	9.547^{B}	9.388^{C}
Met:Cys	1.512^{AB}	1.516^{A}	1.509^{AB}	1.506^{B}
Gly	2.259^{A}	2.224^{C}	2.241^{B}	2.193^{D}

$^{A-D}$ Column means with same superscript do not differ significantly (P>0.05)

* Source 1 was imported from Argentina; sources 2-4 were from South African soya processors (values are the means of 22 samples for each source)

Soya oilcake 1 showed on average the highest amino acid concentrations for all the different amino acids. Soya oilcake 4 had on average the lowest amino acid concentrations for all the amino acids analysed for.

4.2 Trial 2: *In vivo* dry matter and crude protein digestibility of soya oilcakes from different origins

4.2.1 Feed analysis

Table 4.31 shows formulated and analysed concentrations for CP and chromium oxide in the different feeds. The control diet had no soya oilcake incorporated into the diet and only contained the HP300 soya concentrates, with little to no trypsin inhibitor present in the control diet. Treatment A was the diet fed to the weaner pigs which contained low trypsin inhibitor activity. Treatment B contained medium trypsin inhibitor activity soya oilcake and treatment C had high trypsin inhibitor activity soya oilcake incorporated into the diet. Nutrilab at the University of Pretoria detected a CP value of 20% in all four diets, and a 3% inclusion of chromium oxide. The CP values and the chromium oxide marker values correlated with the initial feed formulations for the different treatment groups.

Table 4.31 Chrome marker and crude protein concentrations of the formulated diets

Treatment	Formulated CP (%)	Analysed CP (%)	Formulated Chromium oxide (mg/kg)	Analysed Chromium oxide (mg/kg)
Control	18.000	20.055	3000	3209.6
A	18.131	19.747	3000	2955.0
B	18.131	20.692	3000	3230.1
C	18.131	20.677	3000	3073.0

4.2.2 Ileal protein digestibility of the different treatment groups

Table 4.32 indicates the average digestibility of crude protein in the ileum of the pigs. The control group had an average digestibility of 61.46%. The *in vivo* average ileal digestibility for treatment A, indicated that the diet containing the soya oilcake with low trypsin inhibitor concentration, was 59.06%. Treatment B containing medium trypsin inhibitor soya oilcake, had a digestibility of 47.61%. High trypsin inhibitor treatment D had a digestibility of 64.93%.

Table 4.32 Ileal protein digestibility of the four treatment groups

Treatment group	Average digestibility (%)
Control	61.468
A	59.064
B	47.608
C	64.929

[AB] Column means with same superscript do not differ significantly (P>0.05)

The results showed that there were no significant differences between the treatment groups. Due to the high variations between replicates within treatments and between treatments. These results were rejected for the purpose of this study.

4.2.3 Total tract protein digestibility of the different treatment groups

Table 4.33 indicates the total tract digestibility of the different treatment groups. The control group for the *in vivo* analysis, containing no trypsin inhibitors, had an average digestibility of 79.45%. For treatment A, the diet containing soya oilcake with low trypsin inhibitor activity, the digestibility was 82.48%. Treatment B, with medium trypsin inhibitor activity soya oilcake, had a digestibility of 80.76%. High trypsin inhibitor activity treatment C had a digestibility value of 82.03%.

Table 4.33 Total tract digestibility of the four treatment groups

Treatment group	Average digestibility (%)
Control	79.449
A	82.479
B	80.761
C	82.029

[AB] Column means with same superscript do not differ significantly (P>0.05)

There were no significant differences between the different treatment groups for total tract digestibility for crude protein.

CHAPTER 5
Discussion

5.1 Laboratory comparisons

Laboratories A to D, analysed the soya oilcakes for nutrient content, using different analytical methods. Laboratory A used wet chemistry to analyse nutrient content of the soya oilcakes. On average, laboratory A showed the lowest crude protein values (52.41%), the lowest crude fat values (1.78%) and the second lowest CF values (4.22%). Laboratory B and C used NIR to quantify the nutrient values for the different soya oilcakes. Wet chemistry uses official laboratory methods to quantify CP, crude fat and crude fibres, where NIR uses reflectance spectrometry to measure these nutrient values. The NIR instrument however needs to be calibrated on recent wet chemistry results. Thus, wet chemistry is the more accurate method to use when the correct official methods are followed. Laboratory B and C used similar methods to evaluate the amino acid profiles of the different soya oilcakes. Laboratory B showed on average the higher amino acid values except for methionine, and histidine. The differences could be due to the use of different equipment and solutions in the different laboratories or the human error factor. Urease values were measured by laboratory A and D. The results obtained from laboratory A were in line with the other antinutritive factor evaluations, but the urease values from laboratory D did not correlate with the other quality tests. Laboratory D's urease results could not be used for this trial. The reason for the invalid results could be due to laboratory personal not did not apply the correct official method of analyse.

5.2 Nutrient content of the soya oilcakes

The CP values for soya oilcake 1 (imported from Argentina) was on average the highest (53.51%) and soya oilcake 4 (52.08%) had the lowest CP value. Soya oilcake 2 and 3 showed similar CP concentrations (53.06% and 53.11%). A study done by Li *et al.* (2015) showed CP values for soya oilcakes from China, the USA, Brazil and Argentina to be 50.22%, 49.39%, 51.12% and 48.79% respectively. Mateos *et al.* (2011) did a study on 385 soya oilcake samples originating from the USA, Brazil and Argentina. The samples were collected over a period of four years. The soya oilcake from the USA had higher CP values than the oilcakes from Brazil and Argentina (53.9% vs. 51.6% vs. 52.7%; $P \leq 0.001$). The differences in CP values of the soya oilcakes was due to differences in CP content of the beans and the amount of soya hulls that was removed or added during processing. The more hulls added to the soya oilcakes, the lower

the CP value and the higher the NDF, ADF and CF values. The CP values are also influenced by the genetic profiles of the beans and their growing conditions (Grieshop *et al.*, 2003), and how effective oil extraction was (Li *et al*., 2015).

On average soya oilcake 3 and 4 had the highest crude fat (2.47%) values and soya oilcake 1 had the lowest crude fat value (1.84%). Different processing procedures used for soya oilcakes caused the oil content of the oilcakes to vary among the different processing plants. The processing plants for soya oilcakes 3 and 4 had low extraction efficiencies and a higher percentages oil remained in the soya oilcakes. One of these processing plants used only the mechanical extraction method to remove the oil from the bean. The other plant used the solvent extraction method but the efficiency of removing the oil was lower than for soya oilcake 1 and 2. The study done by Li *et al.*(2015) showed that CP was negatively correlated with crude fat, which was confirmed by the results of this current study.

The Crude fibre values for soybean oilcake 4 was the highest (4.75%) and the lowest for soybean oilcake 2 (3.89%). These results were also in line with the CP values. The lower crude protein and higher crude fibre, ADF and NDF values for soya oilcake 4 were due to addition of more soya hulls during processing procedures. Growing conditions also plays a key role in crude fibre concentrations of the soybean (Grieshop *et al.*, 2003).

The starch content between the soya oilcakes did not differ significantly from each other (0.88%,0.90% & 0.86%) except for soya oilcake 2, having a lower starch value (0.78%). A study by Kumar *et al.* (2010) compared different strains of soybeans to each other, analysing their starch and raffinose values. The different genotypes were planted in different environmental temperatures and conditions. The results concluded that the influence of environmental conditions on starch and raffinose was genotype dependent. Frikha *et al.* (2012) showed higher concentrations starch and sucrose values for the USA and Argentina soya oilcakes than for soya oilcakes from Brazil. Higher starch and sucrose values were seen here, due to the lower temperatures in the USA and Argentina (Frikha *et al.*, 2012). Thus, soya oilcake 2 could have been exposed to lower temperatures during the developmental stage of the soybean plant.

The gross energy of soya oilcake 1 was the lowest (19.94 MJ/kg) and the highest for soya oilcake 2 (20.16 MJ/kg). This was due to the lower residual oil from processing of the imported soya oilcake 1 (Li *et al*., 2015).

Frikha *et al.* (20120 also tested for different amino acid concentrations in the soya oilcakes from different origins. Soya oilcake from Argentina showed the lowest arginine, isoleucine, methionine, phenylalanine, cystine and glutamine values compared to the USA and Brazil. The soya beans originating from the USA had the highest concentrations for lysine, methionine and tryptophan. Beans from Brazil had the highest concentrations for isoleucine, phenylalanine, asparagine and tyrosine. When considering all the different amino acid profiles, imported soya oilcake 1 and locally produced soya oilcake 3 had very similar amino acid profiles. Further research is needed to evaluate the cause of these results. The amino acid profiles of the locally processed soya oilcakes are important to know, to ensure that diets are formulated accordingly. This will ensure optimal performance where local soya oilcake is used in the pig diets.

5.3 Antinutritive factors present in the soya oilcake treatments

Soya oilcake 1 and 3, had lower PDI values (10.03% and 9.62% respectively) than soya oilcake 2 and 4 (PDI values of 12.43% and 12.82%, respectively). Both soya oilcake 1 and 3 were therefore adequately processed. Soya oilcake 2 and 4 had PDI values above 10.3 and could be under-processed, since the PDI test is a good indicator of under-processed soya oilcakes, (Rodica & Adrian, 2010), one can assume that higher trypsin inhibitors are present in these soya oilcakes. It was therefore likely that soya oilcake 2 and 4 contained higher levels of trypsin inhibitor activity.

The KOH method is a good indicator for over-processed soya oilcakes. According to Dale *et al.* (1987) KOH protein solubility values of 75-85% is acceptable for use in monogastic animal nutrition. KOH % values for soya source 1 and 3 were adequate for animal use (83.94% & 84.20%). However, soya oilcake 2 and 4 had KOH% values of above 85% and this could be an indication for under-processed soya oilcake (94.30% & 89.92%). The results indicated a low chance for over-processing of these soya oilcakes.

Optimum urease values for soya oilcakes are between 0.2 and 0.05. The change of pH units from 0.05 to 0.20 is an indicator for the adequate processing of soybean oilcakes. When the change of the pH is below 0.05 units, the soya oilcake was overheated and above 0.20 pH units the soybean oilcake can be considered to be underheated, thus antinutritive factors are likely to be active in the soybean oilcake (Rodica & Adrian, 2010). According to Laboratory D all the soya samples fell into the desired urease range, which did not correlate with the values of the other quality measurements conducted. The method used to test for the urease values by this laboratory should be questioned. According to results from laboratory A, soya oilcake 1 had a very low urease value (0.046), which could have indicated overprocessing. Over-processed soya oilcake may cause amino acids to be unavailable to the animal and lowering performance and growth. Soya

oilcakes 2, 3 and 4 had urease values of 0.161, 0.050 and 0.075 respectfully, which may be considered adequately processed soya oilcake.

The trypsin inhibitor activity of soya oilcakes from the USA, Brazil and Argentina was tested in a study done by Frikha *et al.* (2012). The soya oilcakes from the USA and Brazil both had an average TIA (mg/g DM) of 3.3 and Argentinian soya oilcake had a TIA value of 2.5. These results correlate with studies done by Mateos *et al.* (2011) and correlated with the PDI and KOH values tested for in this study. Laboratory E used trypsin peaks to indicate trypsin inhibitor activities. The higher the peak value, the lower the trypsin inhibitor activity. Soya oilcake 1 and soya oilcake 4 had the highest values (178.68 & 164.45), hence the lowest trypsin inhibitor activities and soya oilcake 2 and 3 (124.59 & 155.95) showed the lowest peak values, with the highest trypsin inhibitor concentrations. Thus, the imported soya oilcake 1 had the lowest trypsin inhibitor values and would be the best soya oilcake to incorporate into pig diets. Locally processed soya oilcake 4 had the second lowest trypsin inhibitor concentration followed by locally processed soya oilcake 3.

Considering all the nutrient analyses, soya oilcake 1 (imported from Argentina) had the highest CP values and the lowest crude fat values, which indicates good processing procedures and oil removal efficiencies. Soya oilcake 1 also had the highest total amino acid concentration for each amino acid. When evaluating the quality measurements, soya oilcake 1 had a PDI value that indicated adequate processing. The KOH value for soya oilcake 1 was in the range which is acceptable for animal nutrition. The urease value for the imported soya oilcake was 0.046, which is below 0.05 the recommend urease value for monogastic nutrition, and this could indicate over-processing. The imported soya oilcake had the most consistent results with minor variations. These results could indicate that the imported soya oilcakes are superior to the locally produced product, due to lower trypsin inhibitor activity.

Locally produced soya oilcake 3 showed similar crude protein values than the imported soya oilcake. The crude fat value for soya oilcake 3 were higher which could indicate poorer oil extraction efficiencies. The amino acid profile for soya oilcake 3 were very similar to that of soya oilcake 1. All the quality measurements indicated that soya oilcake 3 were adequately processed. The trypsin inhibitor activity was higher than that of soya oilcake 1. Taking all these results into consideration, soya oilcake 3 can be considered the best replacement for the imported soya oilcake. Soya oilcake 2 could be considered the lowest quality soya oilcake, due to under-processing and low amino acid concentrations.

5.4 In *vivo* dry matter and crude protein digestibility of soya oilcakes from different origins

The ileal protein digestibility was the highest for treatment group C (containing soya oilcake with high trypsin inhibitor activity) (64.93%) and was the lowest for treatment group B (containing soya oilcake with medium trypsin inhibitor activity) (47.61%). The results obtained from the ileal protein digestibility did not correlate with previous studies. Kaewtapee and co-workers did a study on the digestibility of crude protein of different soya products in grower pigs. The standardized ileal digestibility for full fat soya bean (not roasted) was 53%, full fat soya roasted was 72%, soya oilcake was 85%. The results showed that the roasting of the soya increased the digestibility of the crude protein in the pigs' digestive tract. (Kaewtapee, *et al.*, 2017). The results obtained from trial 2, suggested that the trypsin inhibitor activity had no effect on the digestibility of the protein in the ileum. It is important to not that the results from these analyses were highly variable. Due to the low volumes of illeal digesta found in the digestive tract of the piglets after they were euthanized, standard error and variation were high. The sample volumes were too small to repeat the analyses and the data obtained were not reliable. Samples were pooled which further decreased the number of replicates.

The results obtained from the total tract digestibility analyse, showed no significant differences in the digestibly values for the 4 different treatment groups. The control group had an average digestibility of 79.45%. Treatment group A had an average digestibility was 82.48%. The average digestibility for treatment group B was 80.76%. The high trypsin inhibitor concentration, treatment group C, had an average crude protein digestibility of 82.03%. The results suggested that there is little to no correlation between the trypsin inhibitor concentration in the soya oilcake and total tract crude protein digestibility. There was no correlation between the *in vitro* analysis results and *in vivo* digestibly percentages.

CHAPTER 6

Conclusions and recommendations

Pig producers are concerned about the quality of soya oilcake processed in South Africa and how this influences the overall health and performance of their pig herd. The presence of antinutritive factors such as trypsin inhibitors, increases risk for infectious enteropathogens, causing scouring in weaner and grower pigs.

The feed analysis suggests that the imported soya oilcake is of better quality than the locally produced product. The crude protein and amino acid profiles for the imported soya oilcake showed better values than the locally produced soya oilcakes. All the results of the imported soya oilcakes had very little variations. The imported soya oilcakes had lower trypsin inhibitor activity, but caution should be taken, since the urease value for the imported soya oilcake were below the recommended values and this could be an indicator of over-processing. Even with lower trypsin inhibitor activity, over-processing causes the Maillard reaction and the availability of specific amino acids, decreases. Most of the locally produced soya oilcake falls into the adequately processed margins, however, the results of both quality measurements and proximate analysis were variable. The variable quality measurements are due to the non-uniform processing procedures followed by the local processing plants. Locally processed soya oilcake 3 had similar crude protein, crude fat and amino acid profiles to the imported soya oilcakes. This soya oilcake can be considered the best replacement for the imported soya oilcake, if the amino acid profiles of this soya oilcake are balanced to ensure optimal performance and health for weaner and grower pigs. Taking all the feed analyses into account, the more expensive imported soya oilcake 1 is the best quality soya oilcake to use in the weaner and grower diets. Locally produced soya oilcake 3 can be considered the best replacement for the imported soya oilcake, but regular nutrient analyses should be done to ensure the quality for each batch of soya oilcakes processed.

The *in vivo* analysis was variable, and this could indicate that there is no correlation between trypsin inhibitor and protein digestibility. Ileal digestibility values could not be used due to the high variance and standard error. Other studies suggested that the higher amount of fermentable carbohydrates available in the diet decreased the risk of digestive infections and lower performances This study suggests that trypsin inhibitor concentration alone does not influence crude protein digestion and overall performance, but further research is needed to find the correlation between trypsin inhibitor and pig performance. Factors

that may influence pig production and performance, based on the results from this study and similar studies done on weaner and grower pigs, include the initial trypsin inhibitor concentration in the soybeans from different origins, the amount of fermentable carbohydrates in die diet, the uniformity of heat processing procedures and the duration of heat treatment on the soya oilcakes.

The null hypothesis of this study was approved since the imported soya oilcake are of better quality than the locally produced product. It is however still possible to incorporate the lower quality soya oilcakes into the weaner and grower diets, without compromising health and growth, by using the correct amino acid profiles as analysed in this study.

CHAPTER 7
Critical review

It is important to note that the *in vivo* pig trail included a limited number of replicates (8). The pigs were only fed for a short period of time. Slaughter characteristics, weight gain, immunological parameters or intestinal health were not measured during this trial. Pigs were also placed in a house with no controlled temperatures and only infrared lights were used for heating. Other factors that may have affected the outcome of the pig trial include the time of feeding the morning before slaughtering. Pigs were fed too late the morning before slaughtering and this resulted in too little digesta found in the ileum and rectum. The samples needed to be pooled to increase the volumes for proper analyses and this might have caused higher variations and standard errors.

To improve this trial, one could use more pigs and feed them over a longer period. Growth rates, intestinal health and carcass quality can also be measured. The availability of fermentable carbohydrates can also be measured to indicate the effect it would have on the overall gut health of the pigs. The trypsin inhibitor activity of the soybeans from different origins before processing, should be considered. The trypsin inhibitor activity were only measured in relation to one another. The laboratory only tested for the trypsin inhibitor activity in relation to the other samples and no specific concentration of the trypsin inhibitor was given. The concentrations of trypsin inhibitor could not be compared to standards or previous studies. The control group diet was not analysed, and low trypsin inhibitor concentrations were only assumed, the control diet should also have been tested for anti-nutritive factors. The results from the *in vitro* trail can be used to conduct a full grower pig trial to test the long-term effect of trypsin inhibitor on animal performance, intestinal health and carcass quality.

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APPENDIX 1

Appendix 1 Quality measurements of the eighty-eight soya oilcake samples sorted by their trypsin inhibitor peak values concentrations (high to low)

Soya source	Replicate	Urease (pH value)	KOH (%)	Trypsin inhibitor peak value	Treatment allocated
1	5	0.0176	66.04	206.4	Treatment
1	6	0.0263	65.32	204.9	group A
1	4	0.0351	63.56	204.3	
1	1	0.0352	63.66	200.5	
4	1	0.0621	68.66	199.7	
1	2	0.0438	62.71	199.2	
4	2	0.0178	69.22	197.5	
1	3	0.0352	62.11	197.5	
1	7	0.0176	68.35	197.0	
2	1	0.2001	73.99	191.0	
1	9	0.0264	68.72	189.4	
2	3	0.0263	73.30	188.1	
4	3	0.0178	69.93	187.3	
3	13	0.0262	63.98	186.8	
1	10	0.0176	66.60	186.0	
4	5	0.0267	72.99	185.7	
4	4	0.0717	72.55	185.2	
1	11	0.0264	62.74	184.7	
2	2	0.2988	78.74	183.9	
1	8	0.0264	64.92	181.5	
4	6	0.0984	74.06	177.5	
4	9	0.0268	70.50	173.4	
3	11	0.0526	57.48	172.7	
1	13	0.0264	60.57	172.5	
3	9	0.0354	75.51	171.8	
1	14	0.0439	59.07	171.5	
3	1	0.0351	62.32	169.6	
3	3	0.0438	65.74	168.9	
1	21	0.0351	60.43	167.4	
1	12	0.1431	75.04	167.2	
3	14	0.0262	64.60	166.7	
1	22	0.0440	57.91	166.4	
4	7	0.0536	72.26	165.7	
4	8	0.0445	68.28	165.3	
2	7	0.1159	72.88	164.8	
3	2	0.0440	59.77	164.4	
3	8	0.0263	76.25	164.0	
3	7	0.0263	75.58	163.8	
3	12	0.0262	62.67	163.6	
3	10	0.0175	65.68	162.5	

2	4	0.1490	76.56	161.7	
4	14	0.0359	76.97	161.0	
4	13	0.0357	77.88	160.9	Treatment
4	16	0.1174	69.87	160.8	group B
2	5	0.1432	70.45	160.2	
4	17	0.1355	74.08	159.9	
1	19	0.0176	58.52	158.9	
1	20	0.0351	57.61	158.5	
3	16	0.0437	64.94	158.1	
1	16	0.0176	76.99	157.3	
4	15	0.0536	76.54	156.8	
2	8	0.1430	69.59	156.4	
1	17	0.0351	71.25	156.2	
4	11	0.0538	70.20	154.1	
3	6	0.0438	65.07	153.5	
1	15	0.0438	67.04	153.0	
4	12	0.0807	69.94	152.7	
3	5	0.0527	55.14	152.0	
1	18	0.0351	69.72	151.1	
4	18	0.0726	71.37	150.5	
2	9	0.1246	78.01	146.4	
3	17	0.0354	65.27	146.3	
3	18	0.0437	64.32	145.4	
3	4	0.0702	56.88	144.9	
4	22	0.0814	72.52	143.9	
3	15	0.0526	62.37	142.9	
4	19	0.0627	75.12	142.6	
3	20	0.0267	68.56	142.0	
3	19	0.0437	64.37	141.5	
4	20	0.1263	69.73	138.1	
4	21	0.0178	72.34	134.7	
3	21	0.0537	72.13	134.6	
2	10	0.0542	74.18	124.4	
2	11	0.1847	72.07	124.4	
2	13	0.2055	80.67	123.1	
2	12	0.1509	77.15	120.8	
3	22	0.0269	62.80	115.4	
2	14	0.1243	73.67	109.5	
2	15	0.0359	74.92	102.4	
2	16	0.2096	70.00	94.6	
2	17	0.0721	75.39	81.9	
2	19	0.0531	73.38	80.5	
2	18	0.0798	76.54	78.0	
2	20	0.0979	71.69	76.4	
2	21	0.1520	74.70	75.6	Treatment
2	22	0.0980	73.83	72.6	group C
2	6	0.0534	73.25	73.2	
4	10	0.0357	69.77	69.8	

APPENDIX 2

Appendix 2 The sample numbers of the ileum samples that were pooled together for chemical analyses

First ileum sample number	Second ileum sample number	Pooled sample name	Treatment group
1	4	A	D
2	9	B	C
3	23	C	B
5	26	D	B
6	7	E	B
8	31	F	A
10	14	G	D
11	13	H	C
12	17	I	C
15	18	J	A
16	27	K	D
19	22	L	A
20	28	M	A
21	32	N	D
24	30	O	O
25	29	P	B

APPENDIX 3

Appendix 3 The sample numbers of the colon samples that were pooled together for chemical analyses

First colon sample number	Second colon sample number	Pooled sample name	Treatment group
1	4	A	D
2	9	B	C
3	23	C	B
5	26	D	B
6	7	E	B
8	31	F	A
10	14	G	D
11	13	H	C
12	17	I	C
15	18	J	A
16	27	K	D
19	22	L	A
20	28	M	A
21	32	N	D
24	30	O	O
25	29	P	B

APPENDIX 4

Ileal protein digestibility for the different treatment groups

Ileum samples	Treatment group	%Cr in feed	% Cr in illeum	% CP in feed	% CP in illeum	% Protein digested
F	A	0.3	1.2	20.055	27.912	63.264
J	A	0.3	1.1	20.055	33.759	49.077
L	A	0.3	1.4	20.055	23.294	72.884
M	A	0.3	1.0	20.055	24.538	60.608
C	B	0.3	1.3	19.747	29.750	64.886
D	B	0.3	0.9	19.747	38.614	38.500
E	B	0.3	1.5	19.747	32.692	66.925
P	B	0.3	2.2	19.747	50.456	65.949
B	C	0.3	1.4	20.692	31.256	65.314
H	C	0.3	1.1	20.692	26.765	61.087
I	C	0.3	0.7	20.692	33.622	27.961
O	C	0.3	1.1	20.692	45.586	36.067
A	D	0.3	1.2	20.677	33.980	58.307
G	D	0.3	1.1	20.677	26.597	62.609
K	D	0.3	1.3	20.677	25.550	70.118
N	D	0.3	1.4	20.677	29.278	68.683

Total track digestibility for the different treatment groups

Colon samples	Feed	%Cr in feed	% Cr in Colon	% CP in feed	% CP in colon	% Protein digest
F	A	0.3	2.3	20.055	30.057	79.297
J	A	0.3	2.1	20.055	29.711	77.570
L	A	0.3	2.4	20.055	28.635	80.716
M	A	0.3	2.3	20.055	28.000	80.212
C	B	0.3	2.5	19.747	28.573	83.214
D	B	0.3	2.7	19.747	28.382	84.326
E	B	0.3	2.5	19.747	29.540	82.429
P	B	0.3	2.2	19.747	29.712	79.948
B	C	0.3	2.7	20.692	27.527	84.042
H	C	0.3	2.3	20.692	31.211	79.038
I	C	0.3	2.2	20.692	32.088	77.145
O	C	0.3	2.7	20.692	29.989	82.817
A	D	0.3	2.4	20.677	28.189	82.236
G	D	0.3	2.3	20.677	29.163	81.521
K	D	0.3	2.9	20.677	30.699	84.030
N	D	0.3	2.3	20.677	30.984	80.326