UNIVERSITEIT VAN PRETORIA
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The potential nutritive value of waste products from the sub-tropical fruit processing industry as livestock feed

by

AKHO SKENJANA

Submitted in partial fulfillment of the requirements for the degree

M.Sc. (Agriculture)
Animal Nutrition

In the Faculty of Natural & Agricultural Sciences
Department of Animal & Wildlife Sciences
University of Pretoria
Pretoria

November 2011

DECLARATION

I Akho Skenjana declare that the dissertation, which I hereby submit for the degree, Magister Scientiae (Agriculturae) 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.

Date: ……

Signature: ……

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LIST OF ABBREVIATIONS

AD	- acid detergent
ADF	- acid detergent fibre
ADG	- average daily gain
ADIN	- acid detergent insoluble nitrogen
ADL	- acid detergent lignin
ADS	- acid detergent solution
AM	- Avocado meal
ANF	- antinutritional factor
BWC	- weekly body weight per chick
Ca	- calcium
CF	- crude fibre
CNCPS	- *cornell net carbohydrate and protein system*
CP	- *crude protein*
CT	- condensed tannin
Cu	- copper
DM	- dry matter
EE	- ether extract
FCR	- feed conversion ratio
Fe	- iron
FID	- flame ionization detector
g	- gram
GC	- gas chromatograph
H_2BO_3	- boric acid
H_2SO_4	- sulphuric acid
HNO_3	- nitric acid
IP	- insoluble protein
IVOMD	- *in vitro* organic matter digestibility
K	- potassium
KCl	- potassium chloride
kg	- kilogram
kJ	- kilojoules
KOH	- potassium hydroxide

L - litre
LCFA - long chain fatty acid
LI - large intestine
MCH - Macadamia chips
ME - metabolisable energy
Mg - magnesium
mg - milligram
mL - millilitre
Mn - manganese
MOC - Macadamia oil cake
MUFA - mono-unsaturated fatty acid
N - nitrogen
Na - sodium
Na_2SO_4 - sodium sulphate
$NaBH_4$ - Sodium borohydroxide
NaCl - sodium chloride
NaOH - sodium hydroxide
ND - neutral detergent
NDF - neutral detergent fibre
NDIP - neutral detergent insoluble protein
NDS - neutral detergent solution
ng - nanogram
nm - nanometer
NPN - non-protein nitrogen
OM - organic matter
P - phosphorus
POCM - Peanut oil cake meal
PUFA - polyunsaturated fatty acid
S.D - standard deviation
SBM - Soya bean meal
Se - selenium
SFA - saturated fatty acid
SI - small intestine

SRM - Standard reference material

TFA - total fatty acid

TP - true protein

UDP-D - rumen undegradable protein digestibility

Zn - zinc

LIST OF TABLES AND FIGURES

ACKNOWLEDGEMENTS

First and foremost, I would like to give thanks and praise to my Lord Jesus Christ who gave me the strength and courage to complete this research project.

I want to thank my promoters, Prof J.B.J Van Ryssen and Prof W.A Van Niekerk for their support, guidance and patience. It is highly appreciated.

I want to thank the personnel of the Nutrition lab, Farm Services and especially Mr. Roelf Coertzee for his support and assistance with the statistical analysis

A special thanks to my aunt (Thami Skenjana), my brothers and sisters for their support and prayers during the course of this project.

Last but not least, I want to thank my wife, Nolitha together with my boys, for her constant support, prayers, love and encouragement. Thanks my love, you are such a blessing.

ABSTRACT

The potential nutritive value of waste products from the subtropical fruit processing industry as livestock feed

by

Akho Skenjana

Supervisor	**:**	**Prof. W.A. van Niekerk**
Co-supervisor	**:**	**Prof. J.B.J. van Ryssen**
Department	**:**	**Animal and Wildlife Sciences**
Degree	**:**	**M.Sc. (Agric) Animal Science**

The scarcity of feed resources often imposes a major challenge to the development of animal production in the tropics and subtropics. However, by-products have long been recognised in providing cheaper alternative feed ingredients relative to conventional feed ingredients, thus alleviating the challenge. The aim of the present study was to investigate the potential of three waste products from the subtropical fruit processing industry in animal feeding. The products include avocado meal (AM), macadamia oil cake (MOC) and macadamia chips (MCH). The samples were obtained from the processing plants in Nelspruit of Mpumalanga Province, Makhado (Louis Trichardt) and Tzaneen of Limpompo Province. Eight samples of each waste product were collected, prepared and their chemical composition, *in vitro* dry matter digestibility and, *in situ* ruminal dry matter and crude protein (CP) degradability were determined. A broiler growth trial was conducted as well to determine the effect of replacing maize with AM on performance of broilers under commercial production thus establishing the replacement value of avocado meal in broiler feeds.

As the products under study were from the oil extraction process of the two fruits, there were high remnants of oil in all three waste products. As a result the ether extract (EE) content of the three waste products was higher compared to any of the commonly used oilseed meals. The MCH had the highest EE concentration, followed by the MOC and the AM had the lowest. However, after defatting the AM had the highest ($P<0.05$) EE concentration compared to either the MOC or MCH. The CP concentration of the MOC was significantly higher than that of the AM and

MCH. There was no significant difference observed between the AM and MCH in CP concentration. A better amino acid profile was observed with the MOC compared to the AM and MCH. None of the products can be regarded as a protein source.

The fibre fractions of the MCH were significantly higher than that of AM and MOC, with the exception of the acid detergent lignin (ADL) concentration. The ADL concentration of the AM and MCH did not differ significantly although the MCH concentration was higher. The MOC had the lowest ($P<0.05$) ADL concentration compared among these waste products. Generally, the fibre concentration in the MOC was lower compared to other waste products but higher compared to the SBM and POCM.

The acid detergent insoluble nitrogen (ADIN) concentration of the AM was significantly higher than that of MOC and MCH. There was no significant difference observed between MOC and MCH. The condensed tannin (CT) concentration of the waste products differed significantly with the AM being the highest and the MOC the lowest. The ADIN concentration of the MOC could be compared to that of the SBM and lower than that of the POCM. There were some significant differences observed in the mineral composition between the waste products under study. The mineral concentrations were below the maximum tolerable levels of animals except for iron (Fe) in AM which can be toxic to sheep as it was above the maximum tolerable level of 500mg/kg.

The *in vitro* dry matter digestibility of the three waste products differed significantly, with the MOC being the highest and the MCH the lowest. Huge variation within waste products was observed and it could be due to the contamination levels of the products with the indigestible portions of the parent fruits. The *in situ* degradability of dry matter and CP of the AM and MOC differed significantly, with the MOC surpassing the AM in most of the degradability characteristics. The potential degradable fraction (“b”) and the degradation rate of the b fraction (“c”) fractions of the AM and MOC did not differ significantly. The MCH had more indigestible particles of the kernel and as a result it could not be analysed statistically and was therefore omitted.

The effect of replacing maize with AM at different inclusion rates led to decreased feed intake and the final mass of the broilers during the trial period. The feed intake of the broilers on commercial diet was significantly higher than that of the broilers on commercial diets with avocado meal, except for the inclusion rate of 10% AM. The final mass, the ADG and the FCE of the broilers on commercial diet were significantly higher compared to the broilers on diet with AM irrespective of the inclusion rate. No mortalities observed during the experimental period.

It is concluded that the waste products under study have the potential as animal feed ingredients, except for the MCH that showed a high fibre concentration, very low *in vitro* organic matter digestibility and very poor dry matter degradability *in situ*. The fibre, in both AM and MOC, was digestible although different in extent. The MOC fibre was highly digestible. The fibre and the EE concentration of these products provide them with the potential of being good energy sources. These products can only be considered as intermediate products as they cannot be classified as protein sources.

The AM was found to be of limited nutritive value in broiler rations as it led to depressed intake and growth of broilers, even at 10% inclusion rate. This can be linked to the high fibre content in the product. However, further feed intake studies would be necessary to evaluate the AM and MOC in ruminant animal nutrition, *in vivo* in order to ascertain their nutritive for inclusion as animal feed.

Introduction

The international livestock industry is changing rapidly. Animal production units are increasing in size and becoming more specialised. Furthermore, due to genetic selection and better environmental management, animal performance potentials continue to increase. In addition, consumers are becoming increasingly demanding with regards to the quality of animal products, the use of feed additives and the welfare of animals. As a result, and also because of economic and environmental pressures (Linton, 1973; Ørskov, 1980; Schingoethe, 1991), the need to develop effective and situation specific feeding programmes for individual livestock units will continue to increase. Concurrently, the range in the quality and type of feed ingredients that can be included in livestock feeds is increasing. This can be attributed to genetic modification of plants, greater variation in growing conditions, the availability of increasing amounts of by-products from the food industry and advances in feed and food processing. For an effective use of feed ingredients in diets for the various classes of livestock, it is important that the feeding value of feed ingredients be properly estimated (Madsen *et al.*, 1997; Weiss & St-Pierre, 2005). Insufficient knowledge of the feeding value of feed ingredients will result in variable and lower than anticipated animal performance levels. Otherwise, it will increase the cost of animal feeds, as a result of the need to raise safety margins for target nutrient levels, or as a result of limiting the inclusion level of ingredients for which the feeding value is not well defined (Schingoethe, 1991).

Feed evaluation is basically a description of feeds in terms that allow for a prediction of the performance of the animals offered the feeds (Madsen *et al.*, 1997). Feed evaluation in its simpler – and also very useful form; is just an evaluation of the feeds with the aim of giving guidance about the best feeding methods and, if possible, how the feeds should be stored best, fed and combined or supplemented with purchased feeds. Feed evaluation is needed by: (1) farmers trying to optimise feed rations for animals; (2) feed manufacturers trying to produce the best and cheapest feeds; (3) agronomists trying to choose the best plants for fodder production; (4) plant breeders trying to select the most nutritious plants; and (5) researchers trying to evaluate results from experiments and, for instance, to evaluate different methods of treatment to improve the nutritive value of straw. The kind of information and accuracy needed is different in different situations (Madsen *et al.*, 1997).

The term nutritive value, together with others, like feeding value or feed quality is used to describe the usefulness of a feed to animals. The ultimate criterion of feed quality must be the performance of the animal consuming the feed. Such values are based on the utilisation of nutrients at any of the various stages of processing and metabolism of nutrients in the body. The nutritive

value of feeds is conventionally classified under three general components; digestibility, feed consumption (voluntary intake) and the efficiency with which feed energy is utilised (Raymond, 1969). Feed efficiency can, therefore, be deduced from the chemical composition of the feed, the level of voluntary feed intake, rate and extent of feed and nutrient digestion and absorption, or from the efficiency and utilisation of specific nutrients in the body. A given nutritive value is in fact at best a prediction of potential of the feed (Van Ryssen, Unpublished).

Chapter One

Literature Review

1.1 By-products as animal feeds

Shortages of feed resources often impose major constraints on the development of animal production in the tropics and sub-tropics. However, from the production and processing of animals and plants for food production for humans and feed for animals many by-products and crop residues tend to accumulate and can be utilised as livestock feeds (Ensminger *et al*., 1990). Considerable quantities of crop residues and agro-industrial by-products (by-product feedstuffs, BPF) are generated every year in most developing countries in the tropics and sub-tropics. These are potentially suitable for the feeding of livestock. However, because of the lack of technical know-how they are lost or under-utilised (Aregheore & Chimwano, 1991).

By definition, a by-product feedstuff is a product that has value as an animal feed and is obtained during the harvesting or processing of a commodity in which human food or fibre is derived. By-product feedstuffs can be either of plant or animal origin (Fadel, 1999). Many by-products have a substantial potential value as animal feedstuff, though mostly in ruminant nutrition. The main reason is that ruminants have the unique ability to utilise fibre because of their rumen microbes. This means that cereals can largely be replaced by these by-products (Boucqué & Fiems, 1988) thus reducing the competition between humans and animals for cereal products. .

Furthermore, recovering by-products for use as animal feed can benefit food processors by saving money while preventing pollution at the same time. Waste management and water quality have become key environmental and economical issues in agriculture and industry. Therefore offering by-products for use as animal feeds is an economically and environmentally sound way for food processors to reduce waste discharges and cut waste management costs, and also provide additional revenue to processors. Livestock farmers, as well, can save money if by-products provide a less expensive source of nutrients than traditional feeds provided they support acceptable animal performance.

The use of by-products and wastes as sources of nutrients for domesticated animals has always reflected the ability of these animals to scavenge. From the early days of domestication those parts of plants that were unsuitable for human consumption because they were too fibrous or had an undesirable taste, or carried a high risk of infection, were given to animals which lived nearby. With the development of organised crop and animal production, the process of keeping animals on crop by-products developed to a considerable extent (Wilkinson, 1988). In recent years, by-products are

receiving increasing attention from livestock producers and nutritionists. The growth of the animal feed industry has allowed considerable use to be made of by-products and wastes, some of which, although containing potentially toxic components, can be safely included in compounded feeds in relatively low proportions (Wilkinson, 1988).

1. 2 Classification of by-products in animal feeding

Various classifications of by-products are possible. By-products can be classified based on moisture content and fermentable organic matter content (Preston, 1981), their origin (Boucqué & Fiems, 1988) or their nutritional value (∅rskov, 1980).

The latter one is of relevance to animal nutrition. This form of classification is particularly important in relation to least-cost ration formulation where different ingredients are evaluated as sources of energy, protein, minerals and vitamins in relation to their cost, and then included subject to constraints in relation to specific nutrients or anti-nutrient properties. ∅rskov (1980) proposed that by-products and wastes should be considered in four main categories in relation to their contents of metabolisable energy and protein (nitrogen, N). This is particularly relevant in relation to ruminants, where there is a degree of inter-dependence between these two major nutrients. The microbial population of the rumen thus has a requirement for nitrogen that varies according to the proportion of the organic matter which is digested in the rumen (Fig. 1.1).

A. By-products of low digestibility and low nitrogen content

Products in this category are mainly fed to ruminants and include straws, husks and pods. While it may be observed from Figure 1.1 that these products require low concentrations of nitrogen for optimal digestion to be achieved, their N content is often so low that supplementary rumen degradable N is required to speed up both the rate and extent of digestion in the rumen. When these by-products are given as a high proportion of the diet, they have slow digestion rates and long retention times in the rumen, and as a result the voluntary intake levels of ruminants are low. These by-products are therefore regarded as of little feed value unless they are supplemented with N.

B. By-products of low digestibility but relatively high in nitrogen

Waste products from excreta (e.g., poultry litter) of different kinds fall into this category, as they tend to contain excess nitrogen in relation to the available energy. Also, some by-products of the food industry such as coffee residues, grape pulp and cocoa meal are of low available energy value but are relatively high in N. These waste products are mainly fed to ruminants (∅rskov, 1980). These by-

products are not recommended for use as sole diets, but as ingredients of diets containing other products. They are well suited to be included in mixtures or combinations with feeds high in energy but with a low N content.

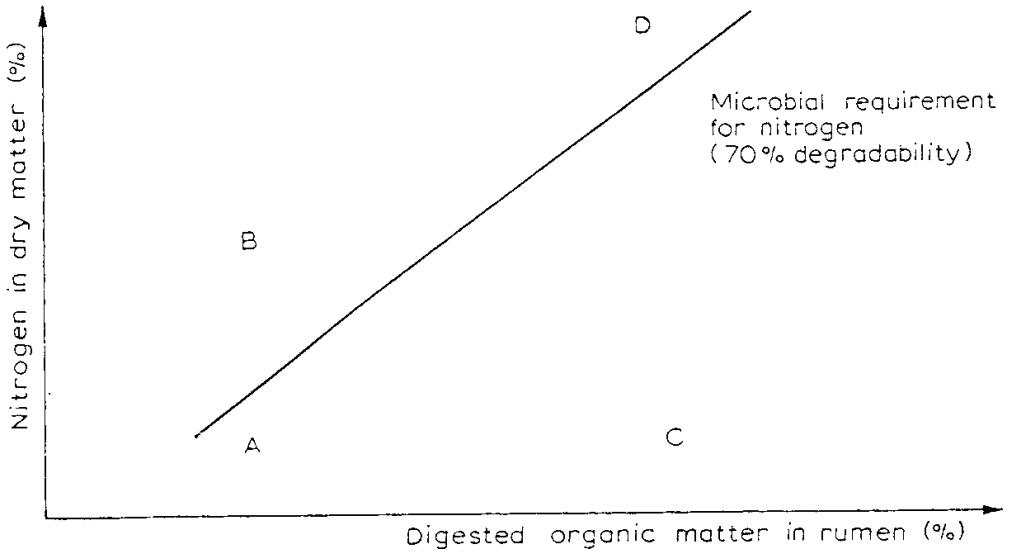

Fig. 1.1 The effect of the proportion of organic matter digested in the rumen on the minimal requirement for nitrogen. The letters refer to type of by-product – see text (adapted from Ørskov, 1980)
A – By-products of low digestibility and low content of nitrogen
B – By-products of low digestibility but relatively high in nitrogen
C – By-products of high energy value but low in nitrogen
D – By-products high in energy and nitrogen

C. By-products of high energy value, but low in nitrogen

By-products from sugar processing (molasses and sugar beet pulp), and also most of the by-products from the fruit and cereal processing industry fall in this category. As they are characterised by rapid rate of digestion there is usually a substantial requirement for supplementary sources of readily available nitrogen to be given to balance the excess of energy. With monogastric animals this may take the form of skim milk, blood meal, fish meal, soyabean meal or dried yeast (Wilkinson, 1988). When they are fed to ruminants the N required to support these diets must be highly degradable in the rumen. For most of the products concerned, urea is the most useful and cheapest N source to use (Ørskov, 1980)

D. By-products high in energy and nitrogen

By-products in this category include products such as waste material from the processing of vegetables, extracted oilseed meals, and also yeasts, whey and meat, and fish by-products. These by-products often present few problems nutritionally. Problems related to them if any, are mainly in

handling, seasonality of production, moisture content, storage problems and some toxic substances. Proper care is needed to ensure that potentially toxic compounds are recognised and that the inclusion of the by-product in the diet will not adversely affect flavour and acceptability. Thus, it is not recommended to use blood meal for chickens (McDonald *et al*., 1995) and calves (Cooke & Pugh, 1980). Those based on leaves (e.g. cauliflowers, cabbages) may have short shelf lives and in consequence are better suited for use near the place of their production (Wilkinson, 1988).

1.3 Nutritional limitations of by-product feeds

The efficient utilisation of cereal crop residues and industrial by-products is important in both developing and developed countries. Crop residues and by-products have been used and will continue to be used as alternative feeds. Some by-products, such as crop residues are potentially rich sources of energy because up to 80% of the dry matter consists of polysaccharides; and some, such as industrial by-products contain high levels of protein. However, plants and even by-products that are used in animal feeding often contain substances which affect or reduce the nutritive value of the ration, and even limit their utilisation in animal feeding.

1.3.1 Antinutritional Factors

Antinutritional factors (ANFs) can be defined as substances which either by themselves, or through their metabolic products, interfere with the utilisation of dietary nutrients, and cause depressions in growth and feed efficiency and/or affect animal health (Huisman & Tolman, 1992). In plants and seeds these ANFs primarily act as biopesticides, protecting them against moulds, bacteria and birds, and they are, therefore, of interest to plant breeders. Some examples of these so-called ANFs in the more commonly employed ingredients in animal feedstuffs derived from plant materials, are shown in Table 1.1. Each of these will be briefly discussed with respect to their nutritional significance.

1.3.1.1 Protease Inhibitors

Plants contain a number of proteinacious factors which are potent inhibitors of digestive enzymes such as trypsin and chymotrypsin (Liener, 1990). These inhibitors are usually in high concentrations in legumes. Historically, and because of their economic importance, the protease inhibitors in soya beans have been the object of considerable study, and have served as models for all other plant protease inhibitors.

Table 1.1 Examples of ANFs that occur in plant materials commonly used as ingredients in animal feedstuffs

Antinutritional factor	Physiological effect
Protease inhibitors	Impaired growth, panreatic hypertrophy, pancreas carcinogen.
Lectins	Impaired growth/death
Goitrogens	Hyperthyroidism
Vicine/Convicine	Adverse effect on egg production
Phytate	Forms complexes with minerals and proteins Depress absorption of minerals
Tannins	Reduce voluntary feed intake Forms complexes with enzymes or feed proteins and carbohydrates Reduces feed digestibility
Alkaloids	Neural disturbances Reduced feed palatability
Gossypol	Anaemia due to iron complexing Reduced egg weight

The soya bean protease inhibitors occur in two forms or classes; that is, Kunitz inhibitors and Bowman-Birk inhibitors. They differ in molecular weight, the number of disulphide bonds and their specificity. Kunitz inhibitors are of high molecular weight (20 000) with only two disulphide bonds and specificity directed mainly towards trypsin, while the Bowman-Birk inhibitors have a low molecular weight (8 000) with seven disulphide bonds. They possess the capability of inhibiting both trypsin and chymotrypsin at independent binding sites (Liener & Kakade, 1980).

Variants of these two types of inhibitors are found in most legumes (Liener, 1990) and their by-products used in animal feeding, such as soyabean meal (McDonald *et al*., 1995). Upon their ingestion, these enzyme inhibitors have the potential of reducing or preventing digestion of nutrients and possibly impair body metabolism, growth and health. The explanation of their growth inhibiting action is derived from the fact that they interfere with the digestibility of dietary protein (Liener, 1990). There does not appear to be major species differences in the effects of protease inhibitors on nutrient utilisation. In general, feed conversion efficiency and weight gain are reduced when young

animals are fed diets containing protease inhibitors whereas body weights of older animals appear to be unaffected by these dietary compounds (Grant, 1999).

Heat treatment is still regarded as the most successful method of reducing the activity of trypsin inhibitors (Huisman & Tolman, 1992). With heat the protein, including the proteinaceous ANFs (trypsin inhibitors, lectins and amylase inhibitors), will be degraded. The efficiency of heat treatment depends on various factors such as the temperature applied, duration of heating, and the use of pressure during heating, particle size and moisture content (Leiner & Kakade, 1980; Rackis *et al*., 1986). However, it must always be borne in mind that overheating of feeds may reduce the nutritional value due to the Maillard reaction (Voragen *et al*., 1995).

1.3.1.2 Lectins

Lectins are defined as carbohydrate-binding proteins/glycoproteins other than enzymes or antibodies (Baroneds, 1988). The primary effect of lectins is related to the fact that they bind to the mucosa of the intestinal wall. This binding can result in damage to the intestinal epithelial cells, which can result in a decreased absorption of nutrients, a change in the activity of brush border enzymes and hypersecretion of endogenous protein due to shedding of damaged cells, increased production of mucins and a loss of plasma proteins to the intestinal lumen (Jaffé, 1980). In all, these effects may cause decreased nutrient digestibility, decreased nitrogen retention and sometimes scours (Huisman & Tolman, 1992), thus leading to reduced weight gain and a less efficient feed conversion.

As is the case with trypsin inhibitors, heat treatment is the method most frequently used to reduce lectin activities. From the review of Van der Poel (1990) on various possibilities of reducing lectin activity, the results have shown that lectins are more sensitive to heat than trypsin inhibitors. It was also concluded that inactivation of trypsin inhibitors and lectins depends on type of treatment. With dry roasting the inactivation was less effective than with steam heating, autoclaving and extrusion (Huisman & Tolman, 1992).

1.3.1.3 Vicine and Convicine

Vicine and convincine are the causative agents of a haemolytic disease in humans known as favism (haemolytic anaemia) (Mager *et al*., 1980; Huisman & Tolman, 1992; De Lange *et al*., 2000). The toxins that are directly responsible for the disease include aglycones, divicine and isoumaril; which are products formed when vicine and convicine, respectively, undergo hydrolysis by intestinal anaerobic microflora (Mager *et al*., 1980).

The only animal which appears to be sensitive to these two compounds is the laying hen. These compounds can cause decreased egg size with an increased incidence of blood spots, the latter presumably being due to erythrocyte haemolysis (Muduuli *et al*., 1982). Unlike many other antinutritional factors which are proteinaceous in nature, vicine and convicine are thermostable and unaffected by heat treatment, but may be reduced by 56% and 34% respectively if cooking is preceded by soaking (Hussein *et al*., 1986). Breeding of faba bean varieties which are low in vicine and convicine, is the most promising approach to overcome this problem (Huisman & Tolman, 1992; De Lange *et al*., 2000).

1.3.1.4 Goitrogens

Goitre producing agents in the form of thioglucosides (referred to as glucosinolates) are present in most cruciferous plants including rapeseed, which is a commonly used feed ingredient in many parts of the world. Rapeseed meal has been found to be high in glucosinolates, thus limiting its use in animal feeding, especially with the monogastric animals (McDonald *et al*., 1995). Its use as a feed ingredient has been limited, however, by the fact that the glucosinolates although innocuous in them, are enzymatically hydrolysed to yield goitrogenic products and act as growth depressants. Other undesirable consequences include cytotoxicity and the tainting of poultry eggs and dairy milk. The goitrogens in rapeseed meal inhibit the uptake of iodine by the thyroid gland so that iodine supplementation is relatively ineffective.

The toxic effects of these goitrogens can be eliminated partially by heat treatment and also by the use of low glucosinolate strains of rapeseed. Again, it can be reduced by one or a combination of several different processing techniques including the removal of glucosinolates or their end-products by extraction with hot water, dilute alkali or acetone or by decomposition with iron salts (Liener, 1983).

1.3.1.5 Phytate

Phytate, a cyclic compound (inositol) containing six phosphate groups, occurs in most legumes and oilseeds to the extent of 1% to 5% of the dry weight. It is generally regarded as an antinutritional factor because it interferes with the bioavailability of minerals (Reddy *et al*., 1982). It is known to reduce the availability of phosphorus. However, phytate can also form complexes with a variety of minerals, including calcium, copper, cobalt, iron, magnesium, manganese, selenium and zinc, thus reducing the availability of these nutrients (Pallauf & Rimbach, 1997). Phytic acid, therefore, results in increased requirements of the animal for minerals, which are essential for optimum growth

(Liener, 1990). Phytate has also been shown to interact and form complexes with the basic residues of proteins (Gifford & Clydesdale, 1990; Liener, 1990; Honning & Wolf, 1991). Therefore, it should be expected that phytate would inhibit a number of digestive enzymes such as pepsin, pancreatin and amylase, leading to a negative effect on the digestibility of protein and carbohydrates. Inhibition of digestion may also result from the chelation of calcium ions that are essential for the activity of trypsin and amylase. The complexing of phytate with proteins could also render these substances more resistant to enzymatic attack (Liener, 1990).

The phytate content of legumes is minimally affected by heat treatment but can be reduced by taking advantage of the endogenous enzyme, phytase, which is located in a separate compartment of the plant tissue, or by providing an exogenous source of the enzyme from microbial sources (Liener, 1987).

1.3.1.6 Tannins

Tannins belong to a diverse group of polyphenolic substances that are not derived from any single biogenic origin. Due to their diversity, some tannins react specifically with certain proteins and not with others. Tannins are among the plant protective factors elicited and elaborated to avoid predation, generally by herbivores (Salam Abdullah & Rajon, 1997). Tannins are classified into two major groups, the hydrolysable and condensed tannins. The hydrolysable tannins split under mild or alkaline conditions into sugars and phenolic carboxylic acids, most of which are either gallic or derivatives of gallic acid, whereas condensed tannins do not hydrolyse (Salam Abdullah & Rajon, 1997).

Tannins present in many different feedstuffs do not only affect the feed quality adversely but can also be toxic to animals. However the scarcity of livestock feeds in developing countries has made it obligatory to incorporate tannin-rich feeds in livestock rations (McLoed, 1974). Tannins are often perceived as anti-nutritional compounds. This view stems from research with tannins or tanniferous feeds given to monogastric animals (Lowry *et al*., 1996). However, the nutritional effects on ruminants are far more complicated and interesting as many wildlife and domestic animals have adapted to tannins in feeds (Muller-Harvey, 1999). In monogastrics, feeding tannin-containing diets is generally associated with adverse effects on animal performance. This arises from reduced nutrient availability either directly, as a result of tannin binding, or through alterations to the physiology of the animal, causing inappetence, intestinal mucosal breakdown and at toxic concentrations, degeneration of different organs in the body (Makkar *et al*., 1987).

It has been shown by many researchers (Chang & Fuller, 1964; Ford & Hewitt, 1979) that tannins have adverse effects on the growth of chicks and rats. Tannins tend to depress the nutritive value of fodder for ruminants by reducing the voluntary feed intake and digestibility (McLoed, 1974). Tannins also diminish the permeability of the gut wall by reacting with the outer cellular layer of the gut (Mitjavila *et al*., 1977). Consequently the passage of the nutrients through the gut wall is reduced, thus leading to the reduced voluntary feed intake by ruminants (Kumar & Singh, 1984).

Tannins are also the limiting factors in the digestibility of many plant forages and agricultural and industrial waste products of low biodegradability. Tannins in feed reduce the digestibility of dry matter (Burns & Cope, 1974) and of N (Van Soest, 1982). This is the most likely consequence of the interaction of tannins with either starch or protein to the formation of enzyme-resistant substrates as they are capable of binding enzyme proteins as well as with the substrate (Kumar & Singh, 1984). The inhibition of trypsin by tannins of *Vicia faba* (Griffith, 1979) and field beans (Griffith & Mosley, 1980) has been reported. In contrast to monogastric animals, feeding low concentrations of condensed tannins in the diet of ruminants (~10 – 40g/kg) has been reported to improve animal productivity (Butter *et al*., 1999). The mechanism that has been postulated for this is the complexation of soluble protein and tannin in the near neutral pH of the rumen, preventing microbial degradation (Jones & Mangan, 1977; Barry & Manley, 1986). Thus, low tannin levels may increase the absorption of amino acids as they protect protein from bacterial degradation and make them available for absorption in the duodenum (Reid *et al*., 1974). The optimum tannin concentration in feed at which this protection occurs has been presumed to vary with the level of crude protein in the feed, the energy available for the synthesis of microbial crude protein and the extent in which the tannin depresses voluntary feed intake (Minson, 1990).

Animals and humans that consume high tannin diets develop physiological means to counteract the adverse effects of tannins. This occurs through the production of a family of proline-rich protein in the salivary gland (Jansman *et al*., 1994; Nyachoti *et al.*, 1997). These proteins bind tannins in a highly specific manner, thereby reducing their toxicity. However, pigs and birds are unable to completely eliminate the toxic effects of dietary tannins. Alternative means to detoxify tannins have been described in detail by Kumar & Singh (1984), Jansman *et al*. (1994) and Nyachoti *et al*. (1997). These include the soaking of feedstuffs in water or alkaline solution; addition of chemicals in the diet (such as polyethylene glycol) that have a high affinity for tannins (Kumar & Singh, 1984; Nyachoti *et al*., 1998); anaerobic fermentation, formalin and urea supplementation (Kumar & Singh, 1984). However, none of these methods has been proven to be cost effective (De Lange *et al*., 2000)

1.3.1.7 Alkaloids

Alkaloids are compounds that contain N in a heterocyclic ring, are generally basic and often have a bitter taste. The word alkaloid simply means "alkali-like" (Cheeke, 1988). Alkaloids are present in many plants where they are thought to serve as a chemical defence against herbivory (Cheeke & Shull, 1985). There is limited information about the toxicity of alkaloids in animal nutrition. However, they are reported to stimulate copper uptake by liver cells thus leading to copper toxicity (Swick *et al*., 1982 a, b). The growth performance of pigs and broiler hens and the egg production of laying hens are adversely affected beyond a certain threshold level of lupins in the diet (Waldroup & Smith, 1989). Pigs appear to be more sensitive to alkaloids than poultry (De Lange *et al*., 2000). The literature also suggests that sheep and rabbits are quite resistant to alkaloids from *Datura*, due to the presence of the enzyme atropine esterase. Boiling and steeping lupin seeds effectively reduce the alkaloid content, but it has been the genetic development of low-alkaloid or "sweet" lupins that has increased the acceptability of lupins as a feed ingredient (Liener, 1990).

1.3.1.8 Gossypol

Gossypol is a yellow, phenolic pigment normally found in the glands of cottonseed from the genus *Gossypium* (Beradi & Goldblatt, 1980; Price *et al*., 1993). The main physiological effects in poultry are loss of bodyweight, decreased feed intake, decreased haemoglobin content in the blood, decreased egg size and decreased egg hatchability. Free gossypol can form complexes with proteins, which may result in a decreased protein digestibility.

Cardiac, reproductive, pulmonary, and hepatic lesions have been observed in animals poisoned by gossypol (Price *et al*., 1993). Non-ruminants and immature ruminants (functionally underdeveloped rumen) are particularly susceptible to gossypol toxicity (Morgan, 1989). Mortality rates due to gossypol poisoning can be as high as 80% in piglets. In pigs, gossypol can cause death if dietary levels exceed 0.015%, which is the highest level of free gossypol considered safe for them (Nesser *et al*., 1988). Other effects of high dietary levels of free gossypol include respiratory distress, abdominal distension and infertility in male pigs (Randel *et al*., 1992).

However, there are cases of gossypol toxicity in mature dairy cows and the death of cows has been reported (Randel *et al*., 1992). Adverse physiological effects of gossypol may be counteracted by binding of free gossypol during processing with cottonseed, and by the use of certain minerals, especially iron salts (Beradi & Goldblatt, 1980; De Lange *et al*., 2000). Other possibilities of elimination are heat treatment and extraction (Huisman & Tolman, 1992).

1.3.2 Moisture

Moisture content of by-products may limit dry matter intake, how far the product can be economically transported, and how long the by-product can be stored without spoilage. Total dry matter intake of ruminants is often reduced as the dry matter content of the total ration falls below 50%. This is especially true when the moist feeds include fermented feeds such as wet distiller's grains, wet brewer's grain or wet corn gluten feed along with ensiled forages (Schingoethe, 1991). Moisture content of by-products can also limit the potential market area of the material. One cannot afford to haul the large amounts of water in wet products great distances. Thus, feeding products such as liquid whey, wet brewer's grain or cannery wastes may be viable alternatives only to livestock feeders near a source of such products. The marketability of dried by-products is greater than for the same by-product in wet form because of an expanded market area and longer storage times. Dried by-products are however usually more expensive (Schingoethe, 1991).

1.3.3 Variation in composition

Variability in composition of a feed source, by-product or otherwise, can present problems for livestock producers attempting to accurately formulate nutritionally balanced diets. Therefore, the product must be analysed to ascertain its chemical composition, especially if protein, fibre or other critical nutrients vary considerably (Schingoethe, 1991). Variation in the nutrient content of feedstuffs has cost implications (Weiss & St-Pierre, 2005)

1.3.4 Minerals

The mineral content can be a limitation to the use of some by-products and on the other hand it can serve as an inexpensive source of nutrients to balance a deficiency for the rest of the diet. Ruminants, in particular, are sensitive to an excess of heavy metals such as copper, lead and even zinc (Ørskov, 1977). An excess of these could render the products unsuitable. For instance, copper is known to affect entero-hepatic function in small ruminants, especially sheep and goats. Copper toxicity occurs in sheep when the copper content of feed, pasture or soil is high, when the molybdenum content of the plant is low, or when there is liver damage from consumption of certain poisonous plants (Church & Pond, 1988). Liver damage predisposes sheep to copper poisoning by decreasing the ability of liver to metabolise ingested copper (Church & Pond, 1988).

1.4 Evaluation of by-product feeds

Nutritional evaluation of by-products is essentially not different from evaluation of other feeds (Ørskov, 1977). By-products, however, differ from other feeds in so far as they are not, as mentioned in their definition earlier, the primary product. The starting point in evaluating the usefulness of a feed in animal nutrition is the determination of different chemical components (called nutrients) it contains, which are assumed to be essential to the animal. The chemical composition of a feed is usually relatively easy to measure and consequently widely used to predict nutritive value. However, some analyses have been adopted because they are relatively simple to carry out in a laboratory, instead of expensive and complicated but nutritionally more accurate analysis.

Feed analysis has a long and interesting history. The best-known scheme of analysis that developed along this line is the Weende method, generally called Proximate Feed Analysis, and developed in the 19th century. While this method is simple, repeatable and relatively inexpensive, there are several problems associated with the method that caution against its use. For these reasons, crude fibre and the nitrogen-free extract are not and should not be routinely used (Cherney, 2000). The problem components and ash included are the ones that have been mostly criticised (McDonald *et al.*, 1995). Van Soest (1967) developed alternative procedure for fibre and the classification of forage fractions is presented in Table 1.2.

Feed evaluation describing the potential of a feed should include figures that describe the potential feed intake, energy value, protein value, fat value, carbohydrate composition, physical structure, mineral and vitamin value and the content of specific anti-nutritional components. It is said that the production potential of feedstuffs have to be expressed above all by the energy content of the feed, representing the whole of the organic matter. It is well known that, besides this, potential has to be defined simultaneously by the protein content as a specific component of the organic matter, and more precisely by the contents of amino acids, especially in feeds for non-ruminants (Bickel, 1988). Thus feed evaluation was, and is, always orientated on the energy and the protein value of the feed, although other components are to be considered for their specific effects in nutrition, e.g. lipids, dietary fibre, vitamins, minerals, trace elements, etc.

Table 1.2 Classification of forage using the detergent methods of Van Soest (Van Soest 1967; Van Soest, 1982).

Fraction	Component
Cell contents (soluble in neutral detergent)	Lipids
	Sugars, organic acids and
	Water-soluble matter
	Pectin, starch
	Non-protein nitrogen
	Soluble protein
Cell wall constituents (fibre insoluble in neutral detergent)	
1. Soluble in acid detergent	
	Hemicellulose
2. Acid detergent fibre	Fibre-bound protein
	Cellulose
	Lignin
	Lignified N
	Silica

1.5 PREDICTING ENERGY VALUE OF FEEDS

The energy value of a feed is expressed either as digestible energy, metabolisable energy or net energy depending on the evaluation system used. Irrespective of the system, the digestibility of the organic components in the feed is the first step in estimating the energy value and by far the most important factor for determination of energy value of feedstuffs (Madsen *et al.*, 1997). However, digestibility trials with sheep and cattle are very costly and time consuming and many attempts have been made to describe relationships between different chemical analyses and the digestibility measured in animals. None of these relationships can be used universally as the standard deviations of regressions of sheep digestibilities on a wide range of feeds on the basis of crude fibre, NDF, ADF or lignin content is usually 9 – 11% units (Van Soest, 1982). This is not acceptable in feed evaluation (Madsen *et al.*, 1997).

For non-ruminants, the energy is measured directly from the energy determinations (kJ), in the case of digestible energy or metabolisable energy, or more generally based on regression equations derived from digestible nutrients in the Weende analytical system. In this respect, distinction is to be made between single feedstuffs and compound feeds, for which ingredient

composition may be unknown, so that the prediction of energy value is restricted to data on the chemical composition (Henry *et.al.*, 1988).

1.5.1 MEASURING DIGESTIBILITY OF FEEDS

Different techniques have been developed and used in estimating the digestibility of feeds, simulating the digestion process. Biological techniques currently available are: (1) digestion with rumen micro-organisms as in the work of Tilley & Terry (1963) or gas production method (Menke *et al.*, 1979), (2) cell-free fungal cellulase, (Jones & Hayward, 1975; Dowman & Collins, 1982; De Boever *et al.*, 1986) and *in situ* degradation technique (Mehrez & Ørskov, 1977).

***In vitro* technique:** The technique of Tilley & Terry (1963) became an important tool for the evaluation of ruminant feeds and is widely used because of its convenience, particularly when large scale testing of feeds is required. Although the method of Tilley & Terry (1963) has been extensively validated with *in vivo* values (Van Soest, 1994), it appears to have some disadvantages (Getachew *et al.*, 1998).

Enzymatic methods: Enzymatic digestibility assays (Jones & Hayward, 1975; Dowman & Collins, 1982; De Boever *et al.*, 1986) which use enzymes instead of microorganisms have appeared largely as a result of the increased availability of commercially produced enzymes. Enzymatic methods of evaluation are routinely used as end-point digestibility procedures and suffer from similar disadvantages as the Tilley & Terry (1963) technique. The main advantage of the enzymatic method over rumen fluid is that it does not require a fistulated animal as inoculum donor. However, recent studies have indicated that faecal inoculum has a potential to replace the rumen fluid and therefore reduces the dependence of *in vitro* technique on fistulated animals as inoculum donors (Jones & Barnes, 1996; Macheboeuf & Jestin, 1997). However, results from the enzymatic method have not been extensively validated with *in vivo* values (Getachew *et al.*, 1998).

***In situ* technique:** The nylon bag technique has been used widely for many years to provide estimates of both the rate and extent of disappearance of feed constituents (Mehrez & Ørskov, 1977). This technique provides a useful means to estimate rates of disappearance and potential degradability of feedstuff and feed constituents. The disadvantage of the method is that only a small number of feed samples can be assessed at any one time, and it also requires at least three fistulated animals to account for variations due to animals. It is, therefore, of limited value in laboratories undertaking routine screening of a large number of samples. Substantial error could result in values obtained at early stages of digestion due to low weight loss and for poor quality roughage feeds adherence at early stages can lead to higher weights and thus distortion of results. Dewhurst *et al*. (1995), from

their comparison of the nylon bag technique with the Tilley & Terry (1963), have found that the nylon bag method overestimated the fermentation. On the other hand, Ørskov & Ryle (1990) indicated the possible underestimation of dry matter loss from the nylon bag at early periods of incubation due to adherence of microbes. Both *in vitro* method (Tilley & Terry, 1963) and nylon bag technique (Mehrez & Ørskov, 1977) which are based on residue determinations may result in overestimation of dry matter digestibilities for tannin-rich feeds. In such systems, tannins are solubilised but might be indigestible (Makkar *et al.*, 1993).

***In vitro* gas production technique:** The gas measuring technique was considered to be a routine method of feed evaluation after the work of Menke *et al.* (1979), where a high correlation between gas production *in vitro* and *in vivo* apparent digestibility was reported. The advantages of the gas measuring technique over other *in vitro* techniques (Tilley & Terry, 1963) for feed evaluation have been outlined by Blummel & Ørskov (1993) and Makkar *et al.* (1995). In the gas method, kinetics of fermentation can be studied on a single sample and therefore a relatively small amount of sample is required or a large number of samples can be evaluated at a time.

Using the *in vitro* gas measurement and chemical composition in multiple regression Menke *et al.* (1979) (data from 89 experiments), found a high precision (R = 0.98; S.D. = 0.25) in prediction of *in vivo* organic matter digestibility. This group further extended its work and eventually concluded that the prediction of metabolisable energy is more accurate when based on gas and chemical constituent measurements as compared to calculations based on chemical constituents only.

1.5.2 FRACTIONATION OF CARBOHYDRATES

Carbohydrates are extremely important from a nutritional perspective, providing the primary source of energy in ruminant diets (Moore & Hatfield, 1994). Sniffen *et al*. (1992) developed a method of determining carbohydrate fractions. Carbohydrates in feeds are differentiated into two groups, the non-fibrous carbohydrates (NFC) or non-structural carbohydrates (NSC) and the soluble fibre, according to their degradation rates in the rumen (Cruywagen, 1999). The NFC part of the feed is complex, because there are many fractions that digest at different rates, producing different digestion products and are difficult to isolate analytically (Sniffen *et al*., 1992).

Numerous modifications in methods to determine feed fractions have been documented in the last two decades. Much attention has recently been given to the importance and methods of analysis of neutral detergent soluble carbohydrates (Hall *et al.*, 1997). The NFC or NSC and the NDF provide the majority of energy that ruminants derive from their diets. NDF is commonly measured by chemical analysis. In contrast, NFC/NSC is a calculated value, estimated by difference (Cruywagen,

1999). A single value does not adequately describe the compositionally and nutritionally diverse carbohydrates found in NSC. Determination of the energy values of feeds analytically and the prediction of how they will be digested and fermented by ruminants depends upon our ability to accurately separate carbohydrate fractions based upon their digestion characteristics. A proposed composition and digestion characteristic of carbohydrate fractions is in Table 1.3.

Table 1.3 Proposed composition, ruminal degradation and intestinal digestion of carbohydrate fractions (Sniffen *et. al*., 1992)

Fraction	Composition	Rumen degradability (%h)	Intestinal digestibility (%)
A_1	Sugars	200 - 300	100
A_2	Organic acids	1 - 2	100
B_1	Starch	20 - 40	SI 50-85 LI 50-100
B_2	Soluble fibre	40 - 60	SI 0 LI 100[1]
B_3	Insoluble avail. fibre	2 - 20	SI 0 LI 100
C	Lignin + fibre assoc. With lignin	0	0

[1]SI = small intestine, LI = large intestine and caecal/colon area

The NFC values currently used were derived from the Weende system of proximate analysis. Cruywagen (1999) gives the current method for calculation of the NFC.

1.6 PREDICTING PROTEIN VALUE OF FEEDS

Protein is one of the major determinants of the nutritive value of feeds. The nutritional value of individual dietary protein sources for ruminants and non-ruminants differs markedly because of differences in anatomy and digestive processes. This, therefore, necessitates that the protein value be evaluated for both non-ruminant animals and ruminants. Common to both animal types, however, is a fundamental demand that feed proteins must be digestible (Boisen *et. al*., 2000).

1.6.1 Protein value for non-ruminants

Of most significance to non-ruminants regarding the protein value is the amino acid composition of feed protein and the digestibility and more generally the availability of protein and amino acids (Henry *et al.*, 1988).

1.6.1.1 Amino acid composition of feeds

Prediction equations are available for estimating amino acid content in feed from protein content. The ultimate point in protein evaluation of individual feedstuffs or of complete diets is to assess the hierarchy of the successive limiting amino acids by reference to the corresponding requirements of the animals, expressed as a percentage of the diet or in relation to energy supply (Henry *et al*., 1988).

1.6.1.2 Availability of protein and amino acids

The animal growth and digestibility assays are the two major evaluation systems for assessing the bioavailability of amino acids in feeds for pigs (Sauer *et al*., 2000). The animal growth assay (the slope-ratio assay) is the most direct approach for the estimation of amino acid availability in protein in feedstuffs since it provides a combined estimation of digestibility and post-absorptive utilisation of amino acids at tissue level (Batterham *et al*., 1979). However, this assay is expensive, time-consuming and provides an estimate of the availability of only one amino acid per assay (Henry, 1985; Sibbald, 1987).

Digestibility is said to be the most important single determinant of amino acid availability. Amino acid digestibility values can be determined according to the ileal or faecal analysis method. The faecal analysis method, developed by Kuiken & Lyman (1948), has been used extensively in studies with pigs. Following pioneering work with poultry (Payne *et al.*, 1968), it was suggested that the appropriate method would be the ileal analysis method (Henry *et al.*, 1988). Evidence that the ileal rather than the faecal analysis method should be used for determining amino acid digestibility values was also provided by Dierick *et al.* (1988) in studies in which the performance of pigs was related to digestibility measurements (Sauer *et al.*, 2000).

1.6.2 Protein value for ruminants

The realisation that ruminants have a need for absorbed amino acids like monogastrics and, moreover, need protein for their rumen microbes, led to the systems to consider the same factors to predict the amount of amino acids absorbed and the amount of nitrogen available for the rumen microbes (Cruywagen, 1999). As a result of the changes in protein rationing systems that have taken

place during the past decade, the evaluation of dietary protein sources in terms of crude protein content and digestibility of crude protein has now been superseded by methods which provide an assessment of rumen-degradability of the dietary protein source (Thomas, 1990). Thus the pepsin/HCl test (AOAC, 1980), traditionally used for digestibility evaluation, has progressively been supplemented by other tests designed to determine rumen degradability.

1.6.2.1 Estimation of protein degradation

The extent of degradation in the rumen is an important value to be used for the prediction of protein passing not degraded to the small intestine for the calculation of protein utilisation and protein requirements of ruminants (Raab *et al.*, 1983). Various techniques have been developed for the estimation of protein degradation in the rumen and include *in vivo*, *in situ* and *in vitro* methods. *In situ* procedures by Mehrez & Ørskov (1977) and Ørskov & MacDonald (1979) are well accepted in many countries for estimating the degree of ruminal CP degradation of feedstuffs (Cottrill, 1993; Broderick, 1994; Michalet-Doreau & Nozière, 1998)

***In situ* procedures:** The technique gives characteristic disappearance curves for different feedstuffs combining the rate of degradation with an appropriate outflow rate from the rumen provides estimates of effective degradability (Ørskov & McDonald, 1979). *In situ* measures can be used to obtain estimates of rumen undegradable protein (UDP) values of feedstuffs within a relatively short period of time, but still, this method requires cannulated animals. A variation of the *in situ* technique, the mobile bag procedure, has been used for the estimation of intestinal digestibility of UDP (De Boer *et al*., 1987). There are also other limitations with this method. Firstly, the material remaining in the nylon bag after passing through the alimentary canal is, as with *in situ* technique, subject to microbial contamination, leading to potential under-estimation of digestibility (Jarosz *et al.*, 1991). A second and more basic drawback is that it again relies on surgically modified animals, making it inappropriate as routine tool for feed analyses.

1.6.2.2 Fractionation of Protein

Protein fractionation is derived from the fact that total protein (CP) may be only 60 – 80% true protein in fresh and ensiled forages, with the rest being non-protein N (NPN) and unavailable N (Van Soest, 1994). Ideally, any method of assessing protein quality should be able to describe the degree to which a protein contributes to bacterial crude protein and undegraded intake protein (Broderick, 1994). Protein solubility has long been used as a technique for determining degradation

characteristics of protein (Broderick, 1994). Solubility and extraction methods for protein fractions are subject to many of the limitations of carbohydrate solubility methods (Van Soest, 1994).

Sniffen *et al.* (1992) proposed the use of a series of chemical fractionation to identify five components in crude protein. It provides a rational basis for the fractionation of protein, based on nutritional availability, much like carbohydrate fractionation system by Van Soest (1994). A suggested methodology for these components is reported by Licitra *et al.* (1996). The methodology is outlined in Table 1.4. Shannak *et al*. (2000) concluded that there is a correlation between in situ UDP values and chemical fractionation of feed protein according to the Cornell Net Carbohydrate and Protein System (CNCPS), but there is a need for further studies with the objective of increasing precision and accuracy of estimates.

Table 1.4 A suggested partitioning of N and protein fractions in forages (adapted from Licitra *et al*., 1996)

Fraction	Estimation or definition	Enzymatic degradation	Classification*
Non-protein N	Not precipitable	Not applicable	A
True protein	Precipitate with tungistic acid		
True soluble protein	Buffer soluble but precipitable (TP-IP)	Fast	B_1
Insoluble protein	Buffer insoluble	Variable	B_2
Neutral-detergent Soluble protein	IP – NDIP		
ND-insoluble protein but, soluble in AD	Protein insoluble in ND, but soluble in AD	Variable to slow	B_3
Insoluble in AD	Includes heat-damaged protein and N associated with lignin	Indigestible	C

*From Van Soest, 1994. TP, true protein; IP, insoluble protein; ND, Neutral detergent; AD, acid detergent; NIDP, neutral detergent-insoluble protein.

1.7 Justification and objective of the study

Conventional feedstuffs are expensive and livestock farmers are always looking for cheaper, alternative sources of quality feed for their livestock. The considerable costs of feed ingredients have necessitated the use of agro-industrial by-products in animal feeds; ingredients such as wheat offal, dried brewer's grain, maize bran among others hitherto considered as wastes have been evaluated and found suitable for incorporation into poultry and livestock feeds. By-product feeds have been used extensively in animal rations in many countries when economical as substitutes for maize and oilcakes, especially soyabean oilcake.

Tropical and subtropical fruit waste materials from commercial and small-scale fruit industries have been identified as possible alternative feed resources. The materials are from avocado and macadamia nut processing. Currently, there is limited information on their nutritive value and even their potential as animal feeds, and therefore there is a need to determine their potential nutritive value. However, to optimise the nutritive value of by-product feedstuffs, knowledge of their chemical composition and ruminal degradation characteristics is desired for proper inclusion in the ration formulations. In highlighting the significance of ruminal degradation characteristics of by-products, Nocek & Russell (1988) have indicated that by-product feedstuffs with very rapid ruminal rates of starch degradation may result in low ruminal pH and lactic acidosis, if this degradation property is not accounted for in ration formulation. Also, by-product feedstuffs that are low in rumen-available carbohydrate may reduce microbial protein output (Stokes, *et al.*, 1991a, b). Currently, there are models of carbohydrate and protein digestion which rely on estimates of kinetics of ruminal degradation of feeds (Russell *et al.*, 1992). The objective of the current study was to evaluate the nutritive value of avocado oilcake, macadamia oilcake and macadamia chips from chemical composition and determine the effect of replacing maize with avocado meal on the performance of broilers; and promote the use of by-products in animal feeding since they are assumed to provide a relatively cheaper source of feed and are environmentally friendly. To achieve the objective, chemical composition, *in vitro* digestibility, and *in situ* dry matter and protein degradability of these by-products were determined. A broiler growth trial was conducted as well to determine the effect of replacing maize with avocado meal on performance of broilers under commercial production thus establishing the replacement value of avocado meal in broiler feeds.

Chapter Two

Materials and Methods

2.1 Introduction

The objective of this study was to determine the chemical composition and the rumen degradation characteristics of the three waste products from the sub-tropical fruit processing industry, namely, avocado meal, macadamia oilcake and macadamia chips. The waste products were collected from factories in Mpumalaga and Limpompo Provinces of South Africa. Eight samples of each waste product were collected for this study. The products were obtained and prepared for the chemical analyses in the laboratory, the determination of digestibility *in vitro* of dry matter and *in situ* dry matter and CP degradability. A broiler growth trial aimed at testing the performance of broilers on diets with avocado meal in different inclusion rates was conducted.

2.2 Materials

2.2.1 Waste products

Waste products from the subtropical fruit processing industry were collected. The products were from the processing of macadamia nuts and avocado. Two products were obtained from the macadamia processing, namely, macadamia oilcake and macadamia chips.

2.2.2 Description of the waste products

2.2.2.1 Avocado meal

The waste products from Nelspruit (4 samples) were derived from the oil extraction from the lower grade avocados not suitable for commercial avocado fruit market. The fruit is ripened, crushed as is (seed, peel and the flesh included) and mixed with hot water. It is then pumped into a mixing tank where retention time allows the oil cells to break. The product left over is put through a Kiln dryer (70 ^{o}C) to remove the moisture. Waste products from Tzaneen (4 samples) were derived using the same method except for the drying stage where some were dried in the sun (sun dried) (2 samples) and some were supplied in the wet form (2 samples).

2.2.2.2 Macadamia oilcake

The product was derived from the oil extraction of the lower grade macadamia nuts. The lower grade nuts include the insect damaged nuts, immature nuts, internal discoloured nuts, malformed nuts and mechanically damaged nuts. Mouldy nuts are discarded completely and therefore not used for this

product. The nuts are pressed mechanically with some soya bean hulls as a base and then centrifuged. The oil is removed and the left-over product is regarded as the oilcake.

2.2.2.3 Macadamia chips

The product is mainly the pieces of both the kernel and nuts that come from the nut cracking process. The pieces were obtained as a mixture of pieces of the nutshell and the kernel in a variety of proportions.

2.2.3 Sample collection

The avocado meal samples were obtained from the Da Gama Avocado oil factory in Hazyview in Nelspruit of Mpumalanga Province and from the Levubu district in Tzaneen of the Limpopo Province.

Macadamia oilcake and macadamia chips samples were collected from the Royal Macadamia processing plant and originated from the Green Farm and Royal Macadamia farms at Makhado (Louis Trichardt) in the Limpopo Province.

2.2.4 Sample preparation

The total fat content of the samples was determined on arrival at the laboratory. All samples had high fat content. They were defatted using chloroform, until the fat content was below 10%. Soaking the samples in chloroform for overnight did the defatting process. The samples were then sieved through cheesecloth so as to avoid the loss of particles of the products. The samples were then dried for 48 hours at 30 ^{0}C to remove all the chloroform. Eight samples from each product were obtained. Each sample was divided into two portions, one for chemical analyses in the laboratory and the other for digestibility studies. The samples for chemical analyses were milled through a 1 mm sieve and kept in capped sample bottles. The sample bottles were stored in a cool place. The samples for the digestibility trial were kept in big capped sample bottles in a cool room.

2.2.5 Treatments

Treatments were the three by-products:

Treatment 1 = Avocado meal (AM) – 8 samples

Treatment 2 = Macadamia oilcake (MOC) – 8 samples

Treatment 3 = Macadamia chips (MCH) – 8 samples

2.2.6 Parameters

Parameters determined include chemical composition of collected by-product samples:

Dry matter, ash content, crude protein (CP), crude fibre (CF), acid detergent fibre (ADF), neutral detergent fibre (NDF), acid detergent lignin (ADL), hemicellulose content, cellulose content, acid detergent insoluble nitrogen (ADIN), mineral content (calcium, magnesium, phosphorus, potassium, sodium, cobalt, copper, manganese, zinc, iron and selenium), amino acids, condensed tannins and *in vitro* organic matter digestibility (IVOMD), *in situ* dry matter and CP degradability in the rumen of a sheep, intake of avocado meal by broilers, growth performance of broilers on feeds with avocado meal, feed conversion ratio and optimal replacement value of avocado meal.

2.3 Methods

2.3.1 Chemical Analyses

2.3.1.1 Dry Matter content

The DM content analysis was done according to the AOAC (1995) method. A porcelain crucible was put into an oven for about one-hour in order to dry it completely after which it was put into a desiccator to cool. One gram of each sample of the products was weighed into a porcelain crucible, and then placed in an oven at 100 °C for 24 hours. The crucibles were then put in a desiccator that contained silica gel for 30 minutes to cool before weighing. The dry matter content (%) was calculated as recommended by AOAC (1995).

$$\%DM = A/B \times 100$$

Where: A = dry sample mass, B = wet sample mass

2.3.1.2 The ash content

The ash content analysis was done according to the AOAC (1995) method. Oven-dried samples of the by-products in porcelain crucibles from the DM procedure were incinerated in a muffle furnace for four hours at 600 °C. The furnace was then allowed to cool down to approximately 250 ^{0}C. The crucibles were removed and placed in a desiccator for 30 minutes and weighed. The ash content of the samples was calculated as follows:

$$\% \text{ Ash} = (A/B) \times 100$$

Where: A = ash mass, B = wet sample mass

The ash content was corrected for dry matter as follows:

$$\% \text{ Ash (dry basis)} = \frac{\% \text{ Ash} \times 100}{\% \text{ DM}}$$

2.3.1.3 Organic Matter content

The organic matter (OM) content of the samples for the *in vitro* digestibility of OM determinations was calculated as follows:

$$\% \text{ OM} = \frac{\text{Dry matter (g)} - \text{Ash mass (g)}}{\text{Sample mass (g)}} \times 100$$

2.3.1.4 Crude Protein concentration

The nitrogen (N) concentration of the by-product samples was determined by the Macro Kjeldahl method (AOAC, 1995). One gram of each sample was weighed into a digestion flask. A digestion mixture made of 10 g sodium sulphate (Na_2SO_4) and 0.4 g elemental selenium was added together with 25 mL of concentrated (98%) sulphuric acid (H_2SO_4) into the same digestion flask with the sample. The flasks were then put on a block digester until the solution was clear (±45 minutes) for the digestion of the sample. After cooling of the solution, 35 mL of boric acid solution (40 g solution of H_2BO_3 in 10 mL methyl red and 25 mL methyl blue made up to a 1000 mL) was added. Three hundred and fifty millilitres of distilled water, zinc granules and 100 mL NaOH (45%) were added as well. Then the solution was allowed to boil for about 10 minutes until about 200 mL of the distillate remained and a Tecator kjeltec system model 1002 for the distillation. The distillate was titrated with 0.1 N H_2SO_4. The values were corrected by the titration of a blank sample.

The percentage of N in a sample was calculated as follows:

$$\%N = \frac{F \times (\text{Titration} - \text{Blank})}{\text{Sample mass}} \times 100$$

Where F - factor associated with the strength of the H_2SO_4.

Percentage CP was calculated as follows:

$$\% \text{ CP} = \% \text{ N} \times 6.25$$

% CP was corrected for dry matter as follows:

$$\% \text{ CP (dry basis)} = \frac{\% \text{ CP} \times 100}{\% \text{ DM}}$$

2.3.1.5 Crude Fibre

The CF concentration of the by-product samples was determined according to Goering & Van Soest (1970) using the Tecator fibretec system. One gram of each sample was weighed into a filter crucible and placed on the hot extraction units of the system. The extraction was carried with a 30 mL of 98% sulphuric acid H_2SO_4 (following Weende method) for 14 minutes, by boiling. The H_2SO_4 was

removed by switching on the vacuum pump and washed out (three times) with warm distilled water. Sequential to the H_2SO_4 removal, 100 mL of sodium hydroxide (NaOH) was added and boiled for 14 minutes and removed as performed with the H_2SO_4. The residues in the crucibles were dried at 100 °C overnight, then cooled in a desiccator for 30 minutes and weighed. After weighing they were ashed in a muffle furnace at 600 °C for 3 hours. The furnace was allowed to cool to at least 250 °C, and then the crucibles were cooled in a desiccator for 30 minutes and weighed.

Percentage CF was calculated as follows:

$$\% \text{ CF} = \{[W_{1\,(g)} - W_{2\,(g)}]/ W_{3\,(g)}\} \times 100$$

Where: W_1 = dry mass of sample after NDS extraction

W_2 = mass of ash

W_3 = sample mass

% CF was corrected for dry matter as follows:

$$\% \text{ CF (dry basis)} = \frac{\%\text{CF} \times 100}{\%\text{DM}}$$

2.3.1.6 Neutral Detergent Fibre

The NDF concentration of the by-product samples was determined according to Goering & Van Soest (1970) using the Tecator fibretec system. One gram of each sample was weighed into a filter crucible and placed on the hot extraction units of the system. The extraction was carried with a 100 mL of neutral detergent solution (NDS) (following alpha amylase method) for one hour, after boiling had commenced. The NDS was removed by washing out with hot distilled water. The residues in the crucibles were dried at 100 °C overnight, then cooled in a desiccator for 30 minutes and weighed. After weighing they were ashed in a muffle furnace at 600 °C for 3 hours. The furnace was allowed to cool to at least 250 °C, and then the crucibles were cooled in a desiccator for 30 minutes and weighed.

Percentage NDF was calculated as follows:

$$\% \text{ NDF} = \{[W_{1\,(g)} - W_{2\,(g)}]/ W_{3\,(g)}\} \times 100$$

Where: W_1 = dry mass of sample after NDS extraction

W_2 = mass of ash

W_3 = sample mass

% NDF was corrected for dry matter as follows:

$$\% \text{ NDF (dry basis)} = \frac{\%\text{NDF} \times 100}{\%\text{DM}}$$

2.3.1.7 Acid Detergent Fibre

The acid detergent fibre (ADF) content of the by-products was determined according to Goering & Van Soest (1970) using a Tecator Fibretec system as outlined in the application in the Application Note AN 03/78; exactly like NDF except that the acid detergent solution (ADS) (Van Soest, 1963) was used. Percentage ADF was calculated as follows:

$$\% \text{ ADF} = \{[W_{1\,(g)} - W_{2\,(g)}]/ W_{3\,(g)}\} \times 100$$

Where: W_1 = dry mass of sample after ADF extraction

W_2 = mass of ash

W_3 = sample mass

% ADF was corrected for dry matter as follows:

$$\% \text{ ADF (dry basis)} = \frac{\%\text{ADF} \times 100}{\%\text{DM}}$$

2.3.1.8 Acid Detergent Insoluble Nitrogen (ADIN)

Acid detergent fibre in a sample was extracted using the same procedure as in 2.2.6, except that the sample mass was 2 g and 150 mL of ADS were used (to yield a sample large enough for N determination after extraction with ADS). Nitrogen content of the residue was determined according to the Dumas method (AOAC, 2000) using a Leco machine (FP 428 model), where a 0.20 g of a sample was weighed into an aluminium foil and then combusted to get the reading of the nitrogen content of the sample. The ADIN was calculated as follows:

$$\%\text{ADIN} = \frac{\%\text{ N in residue} \times 100}{\%\text{ N in sample}}$$

Expressed as % of N

% ADIN was then corrected for dry matter as follows:

$$\%\text{ADIN (dry basis)} = \frac{\%\text{ADIN} \times 100}{\%\text{ N in sample}}$$

2.3.1.9 Acid Detergent Lignin (ADL)

The acid detergent lignin was determined as outlined in Application Note of the Tecator Fibretec System. The samples were prepared with the ADF procedure as in 2.3.1.6 but not ashed. A sequential extraction with 72% sulphuric acid was done for 3 hours. The sample remaining after filtration and

washing with hot water was dried overnight, weighed and ashed in a muffle furnace at 550 °C for three hours. The residue was then cooled in a desiccator for 30 minutes and weighed.

% ADL of total sample was calculated as follows:

$$\% \text{ ADL} = \{[W_{1\,(g)} - W_{2\,(g)}]/ W_{3\,(g)}\} \times 100$$

Where: W_1 = dry mass of the residue after filtration and washing

W_2 = mass of ash

W_3 = sample mass (before extraction)

2.3.1.10 Hemicellulose

Hemicellulose was calculated as:

% Hemicellulose = NDF % - ADF %

2.3.1.11 Cellulose

Cellulose was calculated as:

% Cellulose = ADF % - ADL %

2.3.1.12 Ether Extract

Total fat content was determined according to the ADSIR ether extraction method (AOAC, 1995) using the Soxtec HT6 Model. A 3 g sample was weighed onto a filter paper and then put into a thimble. The thimble was then placed on an extraction unit. Petroleum ether of 60 – 80% was used for extraction. The extracts were collected into extraction cups for 2 hours of boiling. The cups with EE were then dried in a 70 ^{0}C oven overnight. They were then removed and placed in a dessicator for 30 minutes to cool and then weighed. The total fat was calculated as follows:

$$\%EE = (A/B) \times 100$$

Where: A = fat mass (g)

B = mass of the original sample (g)

The percentage EE was corrected for DM as follows:

$$\%EE \text{ (dry basis)} = \frac{\%EE \times 100}{\%DM}$$

2.3.1.13 Long Chain Fatty Acids

Reagents:

Internal Standard: 1 mg/mL pentadecanoic acid in methanol, Supelco 37 Component FAME mis Std, Cat no 4-7885, 0.1N HCl, chloroform, 30% (w/v) methanolic KOH, 14% BF3 in methanol standard

reagent (Aldrich cat no: 26,412-1), borontrifluoride complex in methanol, saturated sodium chloride in water, hexane, anhydrous Na_2SO_4, phosphate buffered saline.

Sample preparation

A 0.01 g of the original sample of each by-product (before defatting) was weighed into a 30 mL test tube. Ten millilitres of phosphate buffered saline was added into the test tube. Hundred millilitres of the internal standard was added into the solution as well. The solution was then homogenised in a mechanical homogeniser at low speed for 30 seconds and then at maximum speed for 30 seconds until the sample was fully homogenised. The solution was then centrifuged in a bench top centrifuge at maximum speed for 15 minutes. Into a clean 10 mL test tube, 5 mL of the supernatant was aspirated. Then 2 mL of chloroform was added with 1 mL of 0.1 N HCl. This was then mixed well and phases were allowed to separate. The chloroform (lower layer) was transferred into a clean 10 mL test tube with a Teflon lined screw cap. The extraction was repeated with 2 mL aliquot chloroform and the extract was combined with the previous chloroform extract. The chloroform was then evaporated under a gentle stream of nitrogen until there was no liquid visible.

Derivatisation

1 mL of methanolic KOH was added into a test tube with the extract, and closed. The test tube was then heated at 60 ^{0}C for 20 minutes. The extract was then allowed to cool and 1 mL of saturated NaCl in water was added. 1 mL of hexane was also added into the test tube with the cool extract. The tube was then closed and vortexed. The phases were allowed to separate and the hexane (upper layer) was aspirated into a clean 1 mL autosampler vial containing ±100 mg (1 mm layer) of anhydrous Na_2SO_4. This was then allowed to stand for 15 minutes, after which it was injected into a Gas Chromatograph (GC) Varian 3300 model from Varian Associates, Inc. 1985, United States of America.

GC conditions were as follows:

Initial column temp 140 ^{0}C

Initial column-holding time - 5.00 minutes

Temp program column - Yes

Program 1 final column temp 240 ^{0}C

Program 1 column rate in ^{0}/min 2 min

Program 1 column hold time 15 min

Add next column program - No

Injector 250 ^{0}C

Detector 250 ^{0}C

Flame Ionization Detector (FID) A initial attenuation 2

FID A initial range 11

FID autozero on - Yes

Time program FID A - No

Initial relays - No

Program 1 relay time in min 0.60

Program 1 relays - 1

Add next relay program - No

Method complete end time 70 min

Helium pressure 4000 kPa

Psi column A 50

Varian column Wcot Fused silica coating CP- Sil 88, 100 mx 0.25 mm DF 0.2 μm

Computer software - Empower build 1154 program 2002

Standard Fames obtained from Sigma

2.3.1.14 Minerals

The concentration of minerals, calcium (Ca), phosphorus (P), potassium (K), sodium (Na), magnesium (Mg), selenium (Se), manganese (Mn), cobalt (Co), copper (Cu), zinc (Zn) and iron (Fe) was determined in all the samples. A sample of 1 gram was digested in a block digester at 230 ^{0}C using the wet digestion technique. A sample of 1 g was weighed and put into a test tube together with 25 mL nitric acid (HNO_3) (65%) and heated afterwards. Five mL HOCl was added into the tubes after about 10 minutes when almost half of the HNO_3 had evaporated. The solution was further allowed to boil for another 40 minutes until the solution was clear. The solution was then allowed to cool down and made up to 50 mL with distilled water. Each sample was prepared in duplicates and analysed for the different minerals. Ca, Mg, K, Na, Mn, Cu, Zn and Fe concentrations were then determined using the atomic absorption spectrophotometer (Perkin Elmer 2380 model). P concentration was determined using a Technicon auto analyser and the concentration obtained from a calibration curve. Peach leaves were used as a standard reference material (SRM 1547) to verify the accuracy of the mineral assays.

The Se analysis was done as follows: 0.3 g of each by-product sample was weighed in duplicates into test tubes, as well as 0.1 g of bovine liver sample (SRM 1577b) into separate test

tubes to be used as a reference standard. Five mL of a digestion mixture (1:4 v/v of 55% nitric acid - HNO_3 and 72% perchloric acid - HOCl) was added to samples in the test tubes. The tubes were then put on a programmable digestion block for 16 hours. The tubes were allowed to cool down to room temperature. A 2.5 mL of 20% hydrochloric acid (HCl) were added in each tube. The tubes were put on the block for another 40 minutes at 130 °C. The solutions in test tubes were made up to 20 mL with 20% HCl for the standards and 10% HCl for the samples. The solutions were put through a hydride generator (VGA -77), with 20% HCl as an oxidising agent and sodium borohydride ($NaBH_4$) in 0.5% sodium hydroxide (NaOH) solution (1.2 g $NaBH_4$/200 mL 0.5% NaOH) as a reducing agent. The readings of the Se concentration were obtained through the atomic absorption spectrophotometer (Perkin-Elmer 2380 model).

The macro minerals are reported in g/kg DM and micro minerals in mg/kg DM. Selenium concentrations are presented in ng/g DM.

Macro minerals: % = (ng/kg x D / M) / 100

Micro minerals:

Where: D = Dilution factor, M = sample mass

Se: ng/g (1000/% DM)

2.3.1.15 Amino acids

The amino acids composition of the samples was determined using the Pico.tag method (Bidlingmeyer *et al.*, 1984), with courtesy of Ms Exley from the Biochemistry Department of the University of Pretoria. A 10 mg of defatted sample was weighed into a hydrolysis flask and 1 mL of 6 N HCl plus 1-% phenol was added. The flask was evacuated and blown with N_2 to remove O_2 and then sealed off under vacuum (0.01 mm Hg). They were then placed in an oven for 24 hours at 110 ^{0}C. After cooling, deionized water was added up to 5 mL. The samples were then derivatized. After that, they were filtered and placed in WSIP (automatic loader). The amino acids were separated by pumping the solutions through a reverse phase column. Two pumps were used to form a gradient for optimum separation. SYSTEM GOLD was used for calculations.

Amino acids were reported in g/kg and corrected for DM.

2.3.1.16 Condensed tannins

The concentration of condensed tannins was determined according to Makkar (1995), with the courtesy of Ms H.K. Mokoboki of the University of the Limpopo, Polokwane. A 200 g of feed

sample was weighed into a 25 mL glass beaker. A 10 mL of aqueous acetone (70%) was added and the beaker was then suspended in an ultrasonic water bath for 20 minutes. The contents of the beaker were transferred to centrifuge tubes. They were centrifuged for 10 minutes at approximately 3000 rpm at 4 ^{0}C. The supernatant was then collected into a glass test tube. A 0.2 mL of the tannin extract was diluted with 0.3 mL of 70% acetone, and then pipetted to a test tube. A 0.3 m of butanol-HCl reagent and 0.1 mL of the ferric reagent were added. The tubes were then vortexed. The mouth of each tube was covered with a glass marble and the tubes were then placed on a heating block at 100 ^{0}C for 1 hour. The tubes were then cooled and the absorbance of condensed tannins (extracted) was recorded at 550 nm. The extracted tannin absorbance was recorded as leucocynadin equivalent and calculated as follows:

(A550nm x 78.26 x Dilution Factor)/ (%DM)

Where: Dilution factor: - = 2.5 (0.5 mL/0.2 mL)

The condensed tannins were reported in g/kg and corrected for DM.

2.3.2 The digestibility and degradability techniques

2.3.2.1 *In vitro* organic matter digestibility (IVOMD)

The two- staged *in vitro* technique of Tilley & Terry (1963) was used to determine the IVOMD. A 0.2 g milled dry sample was incubated in a test tube with rumen fluid urea solution, artificial saliva and carbon dioxide for 48 hours at 39 ^{0}C in a water bath. The rumen fluid was collected from mature sheep wether fitted with rumen cannulae fed on a diet of 100% lucerne (*ad libitum*). The samples were shaken continuously. After this period the tubes were centrifuged at 1000 rpm for 15 minutes.

The initial solution was decanted and then a 20 mL solution of HCl and pepsin was added. This solution was made of a 4 g of pepsin and a litre of 0.1 M HCl. The tubes were further incubated for another 48 hours. After incubation the samples were centrifuged and decanted as in the first stage, then dried overnight at 100 ^{0}C in an oven. They were then placed in a desiccator to cool for 30 minutes, and weighed. The tubes with the residues were ashed in a muffle furnace at 550 ^{0}C for 3 hours, then cooled in a desiccator for 30 minutes and weighed. A lucerne sample with an IVOMD of 75% was used as a control.

The % IVOMD was calculated as follows:

D = 100 – [Undigested residue (OM)/ sample mass (g)] x 100

Where: D = digestibility of OM (% IVOMD)

The masses of the incubated sample and the undigested residue are expressed in terms of OM content

2.3.2.2 Rumen degradability of the waste products

Samples and nylon bags preparation

Wet by-products from the industry were freeze dried for 48 hours. Dry samples were used as they were after defatting. All the samples for this study were milled through a 2.5 mm screen. Synthetic polyester bags of 53 μm pore-size and approximately 26% open area and 10 x 21cm dimension were used (AFRC, 1992; Michalet-Dorau & Nozière, 1998). Bags were dried for 2 hours at 60 ^{0}C and their masses were recorded for calculations (AFRC, 1992).

Animal preparation

Three mature wether sheep fitted with rumen cannulae were used. They were fed a diet of 100% lucerne (*ad lib.*). They were adapted to the lucerne for a period of two weeks before the trial commenced.

Parameters

Variables under investigation were as follows:

Dry matter degradation rate

Percentage disappearance of N at each incubation period

The constants a, b, and c in the equation suggested by Ørskov & MacDonald (1979).

$$P = a + b\ (1\text{-}e^{-ct})$$

Where p = the amount of protein/dry matter degraded at time t, a = the rapidly soluble fraction, b = insoluble but fermentable fraction in time, c = degradation rate constant of the b fraction and the predicted degradation (PD) = the extent of degradation (a+b). The effective degradability p to be estimated from a, b and c fractions by introducing the fractional outflow rate k into the equation:

$$p = a + (bc/c + k)$$

***In situ* procedure**

Approximately 5 g of a sample, in triplicates were weighed out and put into bags (one bag for each sheep). DM was determined on a separate sample. The bags with samples were securely tied with cotton twines onto metal rings. The rings with bags were securely tied by fish lines, which were held outside the cannulae. Samples were incubated for 0, 2, 5, 8, 12, 24 and 48 hours in the rumen of sheep. The 0-hour incubation was achieved by soaking the bags with samples in tap water for 10

minutes and then hand washed until the water was clear. Lucerne was used as a control for all the incubations by including a bag with 5 g of lucerne sample with the samples under study.

Incubations were done at different times for each incubation period in the rumen of sheep. On removal the bags were immediately hand washed under running tap water until the rinsing water was clean. After washing, the bags with residues were dried in an oven at 60 ^{0}C for 48 hours. They were then put into a desiccator for 30 minutes, and then weighed. The residues from the bags were emptied into capped sample bottles and then further analysed for N according to the Dumas method (2000) using a Leco machine (1996) (FP 428 model), where 0.20 g of a sample was weighed into an aluminium foil and then combusted to get the reading of the nitrogen content of the sample.

2.3.3 BROILER GROWTH TRIAL

As per the definition of a nutritive value of feed by Raymond (1969), the three components used for classification of feeds are digestibility, feed consumption (voluntary intake) and the utilisation efficiency. Feed efficiency can, therefore, be deduced from the chemical composition of the feed, the level of voluntary feed intake, rate and extent of feed and nutrient digestion and absorption, or from the efficiency and utilisation of specific nutrients in the body. This implies that a feeding trial is necessary to ascertain the nutritive of the AM for broilers so as to determine the intake and its utilisation efficiency in monogastric animals, especially poultry. However, the chemical composition for AM has shown that the product is high in fibre, lignin, ADIN and condensed tannin. The hypothesis for the feeding trial is that the AM can be used in monogastric animals as an energy source at low inclusion rates in least cost ration formulated diets.

Animals used

A total of 400 day-old Ross 308 broiler chickens were obtained from National Chicks, Pretoria, South Africa. Unsexed chicks were used and randomly assigned to pens.

Treatments

The treatment design was a completely randomised design with total of 100 chickens allocated per treatment, with four replications, and randomly distributed. Five diets were be formulated, containing five levels of AM replacing maize, viz. 0, 10, 20, 30 and 40%AM. The experimental design is presented in Table 2.1.

Experimental terrain

The study was conducted in an environmentally controlled broiler house at the experimental farm of the University of Pretoria, Hatfield, Pretoria, South Africa. In the broiler house the replicate groups of chickens were placed randomly in pens (3 x 1.5 m) enclosed with chicken mesh. Sawdust was be used as litter material. Light was provided for 24 hours per day. Infrared bulbs were used as heating equipment and with the temperature ranging from 32 °C during the early stages to 22 °C as they reach 1.5 kg weight. The trial ran for 6 weeks.

Table 2.1 Experimental design for broiler growth trail

	Number of birds per treatment					
Reps	0% AM	10% AM	20% AM	30 % AM	40% AM	Total
1	20	20	20	20	20	100
2	20	20	20	20	20	100
3	20	20	20	20	20	100
4	20	20	20	20	20	100
Totals	80	80	80	80	80	400

AM = avocado meal

Diet formulation and feeding facilities

Five treatment diets containing the different levels avocado with a commercial concentrate for broilers and maize used as the main ingredients were compiled. The commercial starter and finisher were obtained from Epol and are known as Farmix Broiler Starter and Finisher. The Farmix Broiler Starter and Finisher are classified as Protein Concentrate for broiler starter and finisher feed, respectively. Treatment rations are presented in Table 2.2 and 2.3. The calculated chemical composition of the treatment rations are as presented in Table 2.4 and 2.5. Water was provided *ad libitum* with the bell drinkers adjusted to the height of the chicks. The chickens were fed a commercial starter (68% maize and 32% Epol farmix broiler starter concentrate) for the first two weeks. This was to provide a good start in life for the chickens.

Table 2.2 Ingredient composition of the starter rations for the broiler growth trial

	Treatments				
Ingredients	**0%AM**	**10%AM**	**20%AM**	**30%AM**	**40%AM**
Yellow maize (%)	68.00	61.2	54.41	47.59	40.80
Epol Farmix Broiler Starter (%)	32.00	32.00	32.00	32.00	32.00
Avocado meal% (% of maize)	-	6.8(10%)	13.59(20%)	20.41(30%)	27.20(40%)
TOTAL	100	100	100	100	100

Table 2.3 Ingredient composition of the finisher rations for the broiler growth trial (%)

	Treatments				
Ingredients	**0%AM**	**10%AM**	**20%AM**	**30%AM**	**40%AM**
Yellow maize	75.00	67.50	60.00	52.50	45.00
Epol Farmix Broiler Finisher	25.00	25.00	25.00	25.00	25.00
Avocado meal%(% of maize)	-	7.50(10%)	15.00(20%)	22.50(30%)	30.00(40%)
TOTAL		100	100	100	100

Table: 2.4 The chemical composition of the avocado meal used in the study (g/kg)

Nutrient	Content(g/kg "DM basis")
Dry matter	949.42
Crude protein	156.00
Crude fat	63.00
Crude fibre	349.00
Calcium	2..00
Total Phophorus	2.10
[1]Lysine	2.40

[1]As in Table 3.3, an average value for AM

Table: 2.5 Calculated chemical composition of broiler starter experimental rations (g/kg)

Nutrient	% inclusion of avocado meal				
	0.00	10.00	20.00	30.00	40.00
DM	866.40	872.45	878.50	884.56	890.61
CP	229.20	232.60	236.00	239.40	242.80
EE	31.76	32.92	34.07	35.23	36.38
CF	41.92	62.86	83.81	104.75	125.70
Ca	12.11	12.25	12.38	12.52	12.66
Total P	7.40	7.39	7.34	7.29	7.25
Lysine	12.00	11.99	11.97	11.96	11.95

Table: 2.6 Calculated chemical composition of broiler finisher experimental rations (g/kg)

Nutrient	% inclusion of avocado meal				
	0.00	10.00	20.00	30.00	40.00
DM	865.00	871.68	878.35	885.03	891.70
CP	191.00	194.75	198.50	202.25	206.00
EE	34.00	35.28	36.55	37.83	39.10
CF	38.00	61.10	84.20	107.30	130.40
Ca	12.20	12.35	12.50	12.65	12.81
Total P	6.43	6.37	6.32	6.27	6.22
Lysine	9.00	8.99	8.97	8.96	8.94

Data collection

Feed Intake

Feed intake was recorded every Monday morning on a weekly basis. Diets were offered per replicates during the week and leftovers at the end of the week were weighed. Feed intake, on a dry matter basis, was calculated by subtracting feed leftovers from total feed offered.

Body Weight

Body weights of the birds per pen were recorded on a weekly basis every Monday morning, and an average was taken as weight per pen.

Feed Conversion efficiency (FCE)

Feed conversion efficiency was calculated as gain per chick over feed intake. The FCE was calculated as g gain/kg feed consumed.

Final Body Weight

The final body weight was obtained by weighing of the individual broilers at the end of the trial.

Mortalities

Mortalities were recorded daily over the experimental period to allow for in calculations.

2.3.4 Statistical analysis

An analysis of variance with the ANOVA model (Statistical Analysis Systems, 1994) was used to determine the significance between different treatments (by-products) for the balanced chemical composition and *in vitro* digestibility data. Means and standard deviations were calculated. Significance of difference (5%) between means was determined by multiple comparisons using Tukey's Studentized Range test (Samuels, 1989). The level of significance used for the correlations was 1%. An analysis of variance with the ANOVA model was used to determine the significant between different treatment means on a broiler growth trial for the balanced data, with the mortalities taken into consideration. Significance difference of (5%) between means was determined using Bonferroni t-test. Repeated measures ANOVA was conducted on weekly body weights as contrast variables with significance difference (1%) between treatments was determined using the Bonferonni t-test. Regression analysis on the feed intake and final mass of broilers was done using SAS (1994).

CHAPTER THREE

RESULTS

3.1 Introduction

As some of the by-product samples were received with remnants of oil after the processing of the fruit and some of the avocado meal samples were also wet, the original dry matter, moisture content and the ether extract are presented in Table 3.1. The moisture content of the by-products ranged from 56.0 g/kg "as is", in macadamia chips (MCH) to 277.0 g/kg in avocado meal (AM). The AM had a significantly higher moisture content compared to the macadamia oil cake (MOC) and MCH but there was no significant difference between the MOC and MCH. The moisture content within AM samples varied significantly compared to other products. The original ether extracts ranged from 154.0 g/kg in AM to 409.0 g/kg in MCH. The original EE of the MCH was significantly higher than the other by-products and there was no significant difference between the MOC and AM. As the samples were still high in oil content they were then further subjected to oil extraction. This means that all the other results, with the exception of Table 3.1, are on a fat free basis.

Table 3.1: The dry matter, moisture content and the fat content (mean g/kg) of the subtropical fruit processing industry waste products before oil extraction on "as is" basis

By-product	n	DM	Moisture	Ether extract
Avocado meal	8	723.0^{b}±26.6	277.0^{a}±26.6	154.0^{b}±10.4
Macadamia oil cake	8	941.0^{a}±1.1	59.0^{b}±1.1	265.0^{b}±10.0
Macadamia chips	8	943.0^{a}±1.2	56.0^{b}±1.2	409.0^{a}±8.7

$^{a, b, c}$ Means with different superscripts, within rows, differed significantly ($P<0.05$)

3.2 CHEMICAL COMPOSITION

The chemical composition of all three by-products under study is presented in Table 3.2. The ash content ranged from 14.5 g/kg DM in macadamia chips (MCH) to 77.5 g/kg in avocado meal (AM). The differences between all three by-products were significant, with the AM having the highest ash content. The CP content ranged from 97.0 g/kg in AM to 209.4 g/kg in MOC, with no significant difference between the AM and MCH. The MOC had a significantly high CP compared to the MCH and AM.

The CF concentration ranged from 265.6 g/kg in AM to 606.6 g/kg in MCH. The MCH differed significantly with the AM and MOC, and there was no significant difference between the AM and MOC.

3.2 Chemical composition (mean g/kg ± SD) of waste products from the subtropical fruit processing industry (DM basis, Fat-free basis)

	By-products		
	Avocado meal	Macadamia oil cake	Macadamia chips
N	8	8	8
Crude ash	77.5[a] ±34.9	42.9[b] ±10.1	14.5[c] ±4.0
Crude protein	99.2[b] ±16.1	209.3[a] ±53.3	97.0[b] ±26.1
Crude fibre	265.6[b] ±35.0	286.9[b] ±71.1	606.6[a] ±79.4
Neutral detergent fibre	602.8[b] ±56.1	498.4[b] ±84.2	796.2[a] ±83.0
Acid detergent fibre	458.4[b] ±41.2	400.4[b] ±91.3	751.7[a] ±77.9
Acid detergent lignin (% of DM)	301.2[a] ±38.3	150.6[b] ±75.2	363.2[a] ±55.1
Acid detergent insoluble nitrogen (% of total N)	25.7[a] ±5.1	4.1[b] ±0.3	5.1[b] ±0.2
Hemicellulose	144.4[a] ±55.1	98.9[a] ±39.2	44.5[b] ±13.4
Cellulose	157.2[c] ±41.4	267.1[b] ±74.9	388.6[a] ±27.2
Condensed tannins	258.4[a] ±86.4	46.1[c]±21.2	93.3 [b] ±27.6

[a, b, c] Means with different superscripts, within rows, differed significantly (P<0.05)

The NDF concentration ranged from 498.4 g/kg DM in MOC to 796.2 g/kg DM in MCH, with the MCH being significantly higher than the MOC and AM. There was no significant difference between the AM and MOC. The ADF concentration ranged from 400.4 g/kg in MOC to 751.7 g/kg in MCH, with the MCH being significantly higher than the MOC and AM. There was no significant difference (P<0.05) between the MOC and AM in the ADF concentration. The ADL ranged from 150.6 g/kg in MOC to 363.2 g/kg in MCH, with both the MCH and AM being significantly higher than the MOC and there was no significant difference between MCH and AM in ADL concentration.

The ADIN concentration ranged from 4.1 % of N DM in MOC to 25.7 % of N in AM. All three by-products differed significantly in ADIN, with AM being the highest and MOC the lowest. Among the macadamia processing waste products MCH had a significantly higher ADIN concentration (1 %N) than MOC. The hemicellulose concentration ranged from 44.5 g/kg in MCH to 144.4 g/kg in AM, with no significant difference between the AM and MOC. The AM and the MOC were both significantly higher than the MCH in hemicellulose concentration. The cellulose concentration ranged from 157.2 g/kg in AM to 388.6 g/kg in MCH. All three by-products (AM, MOC and MCH) differed significantly in cellulose concentration with the MCH being the highest in cellulose and AM the lowest. The condensed tannin (CT) concentration ranged from 46.1 g/kg DM in MOC to 258.4 g/kg in AM, all three by-products differed significantly. The AM was the highest in CT concentration and the MOC the lowest.

3.3 AMINO ACIDS

The amino acid profile and concentrations of the three by-products are presented in Table 3.3.

Table 3.3 Amino acid profile (mean g/kg DM ± SD) of the waste products from the subtropical fruit processing industry (fat-free, DM basis)

	By-products		
	Avocado meal	Macadamia oil cake	Macadamia chips
N	8	8	8
Aspartic acid	5.9 [b] ±1.5	16.1 [a] ±4.6	6.0 [b] ±1.4
Glutamic acid	7.4 [b] ±2.6	33.1 [a] ±9.2	7.3 [b] ±2.4
Serine	4.3 [b] ±1.4	9.5 [a] ±2.2	4.4 [b] ±1.2
Glycine	3.6 [b] ±1	9.4 [a] ±2.2	3.5 [b] ±0.9
Histidine	1.4 [b] ±0.3	3.7 [a] ±0.8	1.4 [b] ±0.3
Arginine	3.2 [b] ±0.6	15.7 [a] ±4.7	3.2 [b] ±0.5
Threonine	3.3 [b] ±1	6.5 [a] ±1.5	3.3 [b] ±0.9
Alanine	3.8 [b] ±1.2	8.6 [a] ±2.2	3.8 [b] ±1.1
Proline	3.9 [b] ±1	9.3 [a] ±2.2	3.9 [b] ±0.9
Tyrosine	2.0 [b] ±0.5	6.6 [a] ±1.2	2.0 [b] ±0.4
Valine	3.6 [b] ±0.9	7.0 [a] ±1.3	3.6 [b] ±0.8
Methionine	1.1 [b] ±0.3	2.4 [a] ±0.5	1.1 [b] ±0.3
Isoleucine	2.9 [b] ±0.7	5.4 [a] ±1.2	2.9 [b] ±0.7
Leucine	5.0 [b] ±1.5	10.3 [a] ±2.6	5.0 [b] ±1.4
Phenylalanine	3.1 [b] ±0.9	5.9 [a] ±1.6	3.1 [b] ±0.8
Lysine	2.4 [b] ±0.5	8.5 [a] ±2.5	2.4 [b] ±0.4

[a, b, c] Means with different superscripts, within rows, differed significantly ($P<0.05$)

The amino acid profile and concentration of the MOC was significantly higher compared to AM and MCH. There was no significant difference between the AM and MCH in amino acid concentration. The total essential amino acid concentration of the MOC was higher than that of the AM and MCH.

3.4 LONG CHAIN FATTY ACIDS

The results of the long chain fatty acids of the three by-products are presented in Table 3.4. The total fatty acid concentration of the subtropical fruit processing industry by-products differed significantly. The concentration ranged from 43.87 mg/g with AM to 308.19 mg/g with MCH. There was also a significant difference between the MOC and MCH. However, with individual fatty acids, there was no significant difference between the MOC and MCH except with the linolaidic acid (C18:2n6t), gondoic acid (C20:1) and α-linolenic acid (C18:3n3). The AM fatty acid concentration differed significantly with the MOC and MCH but there was no significant difference with the MCH with respect to the linolaidic acid, gondoic acid and α-linolenic acid.

Table 3.4: The long chain fatty acid concentration (as percentage of total fatty acids identified, % TFA) of the subtropical fruit processing industry waste products

LCFA	AM (n=8)	MOC (n=8)	MCH (n=8)
C14:0	2.21^{a} ±1.0	1.08^{b} ±0.4	1.12^{b} ±0.3
C16:0	20.6^{a} ±3.3	10.1^{b} ±0.8	10.7^{b} ±1.3
C16:1	8.30^{b} ±3.3	21.7^{a} 2±1.0	23.36^{a} ±2.6
C17:0	0.76^{a} ±1.8	0.14^{a} ±0.2	0.06^{a} ±0.0
C18:0	12.56^{a} ±5.7^{a}	5.73^{b} ±1.4	4.89^{b} ±0.6
C20:0	0.58^{b} ±0.4	2.24^{a} ±0.3	2.33^{a} ±0.3
C22:0	0.71^{a} ±0.9	0.60^{a} ±0.2	0.58^{a} ±0.1
C24:0	0.01^{b} ±0.0	0.24^{a} ±0.1	0.19^{a} ±0.1
C20:1	0.25^{c} ±0.2	2.08^{a} ±0.2	1.79^{b} ±0.2
C18:1n9t	1.00^{a} ±2.3	0.09^{a} ±0.1	0.09^{b} ±0.1
C18:1n9c	36.75^{b} ±15.1	51.91^{a} ±2.5	51.37^{a} ±1.9
C22:1n9	0.00^{b} ±0	0.16^{a} ±0.1	0.12^{a} ±0.1
C18:2n6t	1.67^{a} ±1.4	0.14^{b} ±0.3	0.06^{b} ±0.1
C18:2n6c	7.0^{a} 5±3.3	2.69^{b} ±0.6	2.34^{b} ±0.6
C20:2	1.06^{a} ±1.0	0.05^{b} ±0.0	0.17^{b} ±0.2
C18:3n3	0.54^{a} ±0.2	0.49^{a} ±0.2	0.29^{b} ±0.1
C20:3n6	0.35^{a} ±0.6	0.11^{a} ±0.3	0.24^{a} ±0.4
C20:5n3	1.33^{a} ±0.9	0.20^{b} ±0.3	0.11^{b} ±0.1
C22:6n3	0.63^{a} ±0.3	0.13^{b} ±0.1	0.12^{b} ±0.1
TFA	43.87^{c} ±27.0	228.40^{b} ±104.9	380.19^{a} ±136.8
SFA	37.38^{a} ±1.3	20.16^{b} ±2.4	19.81^{b} ±2.0
MUFA	46.30^{b} ±13.4	75.96^{a} ±3.3	76.73^{a} ±2.9
PUFA	12.62^{a} ±1.3	3.80^{b} ±1.2	3.32^{b} ±1.1
n-6	9.07^{a} ±2.8	2.94^{b} ±0.8	2.6^{b} 4±0.9
n-3	2.49^{a} ±1.1	0.82^{b} ±0.5	0.51^{b} ±0.2
PUFA:SFA	0.39^{a} ±0.2	0.19^{b} ±0.1	0.17^{b} ±0.04
n-6:n-3	5.04^{a} ±4.2	3.99^{a} ±1.1	5.55^{a} ±2.6

$^{a, b, c}$ Means with different superscripts, within rows, differed significantly (P<0.05)
LCFA – Long Chain Fatty Acids; SFA – Saturated fatty acids; MUFA – Mono-unsaturated fatty acids; PUFA – Polyunsaturated fatty acids; n-6 – omega-6 fatty acids; n-3 – omega-3 fatty acids; PUFA:SFA – Ratio of polyunsaturated fatty acids and saturated fatty acid;, n-6:n-3 – Ratio of omega-6 and omega-3 fatty acids.

The oleic acid (C18:1n9c) was the fatty acid that occurred at the highest concentrations in all the three by-products at 36.75 % TFA for AM, 51.91 %TFA for MOC and 51.37%TFA for MCH. The macadamia by-products (MOC and MCH) differed significantly with the AM. The MOC and MCH did not differ significantly in oleic acid concentration. The palmitic acid (C16:0) was the second highest with the AM at 20.6% while it was the third highest with the MOC and MCH. The palmitoleic acid (C16:1) was the second highest with the MOC and MCH at 21.72% and 23.36%, respectively. The linoleic acid (C18:2n6c) concentration of the AM differed significantly with the

MOC and MCH. The AM had the highest concentration of this fatty acid at 7.05% while the MOC and MCH had 2.69% and 2.34%, respectively.

The saturated fatty acid (SFA) concentration of the AM differed significantly with that of MOC and MCH. There was no significant difference between MOC and MCH. The SFA ranged from 19.81% to 37.38% with MCH being the lowest and AM the highest. The monounsaturated fatty acids ranged from 46.30% with AM to 76.73% with the AM being the lowest and MCH the highest. Both of the macadamia waste products (MOC and MCH) differed significantly with AM. There was no significant difference between MCH and MOC. The polyunsaturated fatty acids (PUFA) ranged from 3.32% with MCH to 12.62% with AM. The AM differed significantly with both MOC and MCH but there was no significant difference between MOC and MCH. The AM had the highest concentration of the PUFA and the MCH had the lowest. The omega-6 fatty acid (n-6) concentration ranged from 2.64% in MCH to 9.07% in AM. There was no significant difference between the MOC and MCH. The AM was significantly higher than the MOC and MCH. Omega-3 fatty acid (n-3) concentration of the waste products ranged from 0.51% in MCH to 2.49% in AM. The AM was significantly higher than MOC and MCH in n-3 fatty acid concentration. There was no significant difference between MCH and MOC.

The PUFA/SFA ratio of the waste products ranged from 0.17 in MCH to 0.39 in AM. The PUFA/SFA ratio of the AM was significantly higher than that of the MOC and MCH. There was no significant difference between the MOC and MCH. However, the MCH had the lowest ratio. The n-6/n-3 ratio of the waste products ranged from 3.99 with MOC to 5.55 with MCH. There was no significant difference among the waste products. The MOC had the lowest and MCH had the highest ratio.

3.5 MINERALS

The results of both the macro and micro minerals of all the three by-products are presented in Table 3.5.

3.5.1 Macro minerals

The Ca concentration ranged from 1.1 g/kg DM in AM to 3.3 g/kg DM in MOC. The MOC Ca concentration was significantly higher than that of the other by-products. There was no significant difference in Ca concentration between the AM and MCH. The P concentration ranged from 1.4 g/kg DM in MCH to 3.0 g/kg DM in MOC. The MOC had a significantly higher P concentration compared to the AM and MCH. There was no significant difference in P between the AM and the MCH. The P concentration of the macadamia by-products (MOC and MCH) differed significantly.

Table 3.5 Mineral concentration (mean ±SD) of waste products from the subtropical fruit processing industry (DM basis, fat-free basis)

By-products			
	Avocado meal	Macadamia oil cake	Macadamia chips
n	8	8	8
Macro minerals			
Ca (g/kg)	1.1^{b} ±0.3	3.3^{a} ±1.0	1.5^{b} ±0.5
P (g/kg)	2.1^{ab} ±0.9	3.0^{a} ±0.9	1.4^{b} ±0.4
Mg (g/kg)	9.2^{a} ±21.9	2.4^{a} ±0.6	2.0^{a} ±0.5
K (g/kg)	21.3^{a} ±11.5	13.9^{ab} ±4.3	6.9^{b} ±2.4
Na (g/kg)	0.7^{a} ±1.3	1.8^{a} ±1.6	0.8^{a} ±0.3
Micro minerals			
Cu (mg/kg)	21.7^{a} ±4.4	13.7^{b} ±3.5	17.1^{b} ±1.2
Zn (mg/kg)	28.2^{b} ±9.8	55.6^{a} ±23.6	21.1^{b} ±4.7
Mn (mg/kg)	44.5^{b} ±48.6	267.2^{a} ±175.7	151.2^{ab} ±55.9
Fe (mg/kg)	732.4^{a} ±579.2	248.9^{b} ±71.4	187.3^{b} ±44.3
Se (ng/g)	173.0^{b} ±186.0	600.2^{a} ±248.8	444.1^{a} ±144.6

$^{a, b, c}$ Means with different superscripts, within rows, differed significantly ($P<0.05$)

The Mg concentration ranged from 2.0 g/kg DM in MCH to 9.2 g/kg DM in AM, with the AM Mg concentration being the highest compared to the MOC and MCH. The Mg concentration of MOC and MCH did not differ significantly. The K concentration ranged from 6.9 g/kg DM MCH to 21.3 g/kg DM in AM. There was no significant difference between the AM and the MOC and even between the MOC and MCH, but the K concentration of the AM was significantly higher than that of the MCH. The Na concentration ranged from 0.7 g/kg DM in AM to 1.8 g/kg DM in MOC, with no significant difference between the three by-products.

3.5.2 Micro minerals

The Cu concentration ranged from 13.7 mg/kg DM in MOC to 21.7 mg/kg DM in AM. The Cu concentration of the AM was significantly higher than that of the MOC and even MCH, but there was no significant difference between the MOC and MCH. The Zn concentrations ranged from 21.1 mg/kg DM to 55.6 mg/kg DM in MOC. There was no significant difference between the AM and the MCH. The MOC had a significantly higher Zn concentration compared to the other by-products. The Mn concentrations ranged from 44.5 mg/kg DM in AM to 267.2 mg/kg DM in MOC, with the MOC being significantly higher compared to that of AM. There was no significant difference between MOC and MCH in the concentration of Mn. Also, the Mn concentration in MCH and AM did not differ significantly.

The Fe concentration ranged from 187.3 mg/kg DM in MCH to 732.4 mg/kg DM in AM. The Fe concentration in AM was significantly higher compared to MOC and MCH. There was no significant difference between MOC and MCH in the concentration of Fe. The Se concentration ranged from 173.0 ng/g DM in AM to 600.2 ng/g DM in MOC, with no significant difference between the macadamia by-products (MOC and MCH) in the concentration of this mineral. The Se concentration in the macadamia by-products was significantly higher than that of the AM.

3.6 DIGESTIBILITY

3.6.1 THE *IN VITRO* ORGANIC MATTER DIGESTIBILITY (IVOMD)

The IVOMD results of the subtropical fruit by-products are presented in Table 3.6.

Table 3.6 *In vitro* organic matter digestibility (%±SD) of the waste products from the subtropical fruit processing industry (DM basis)

By-product	Mean (IVOMD)
Avocado meal (n=8)	54.3^{b} ±81.5
Macadamia oil cake (n = 8)	79.2^{a} ±19.8
Macadamia chips (n = 8)	29.2^{c} ± 72.4

$^{a, b, c}$ Means with different superscripts differed significantly (P<0.05)

The IVOMD values ranged from 29.2 % for MCH to 79.2 % for MOC. There was a highly significant difference (P<0.05) between the three by-products in the IVOMD averages observed. There was high variation observed within AM and MCH samples, with the AM being the highest.

3.7 RUMEN DEGRADABILITY

3.7.1 THE *IN SITU* DRY MATTER DEGRADABILITY

The results of the *in situ* rumen dry matter degradability trial of the AM and MOC are presented in Table 3.7 and Figure 3.1. The results were obtained using the Ørskov & McDonald (1979) formula.

Table 3.7 Mean *in situ* ruminal dry matter degradability values (mean ±SD) of waste products from the subtropical fruit processing industry (avocado meal and macadamia oil cake)

Parameters	By-products	
	Avocado meal	Macadamia oil cake
A	32.3^{b} ±0.2	47.6^{a} ±1.2
B	34.9^{a} ±2.1	36.7^{a} ±7.3
C	0.07^{a} ±0.01	0.04^{a} ±0.01
PD	67.1^{b} ±2.0	84.3^{a} ±6.3
ED_k		
$ED_{0.03}$	56.9^{b} ±1.3	69.2^{a} ±1.9
$ED_{0.05}$	52.9^{b} ±1.2	64.6^{a} ±1.1

a - soluble fraction; b - potentially degradable fraction; c - degradation rate constant of the b fraction; PD - extent of degradation (a + b); ED - effective degradability, k - outflow rate

$^{a, b}$ Means with different superscripts, within rows, differed significantly (P<0.05)

The MCH was omitted in Table 3.7 because the by-product seemed to be highly indigestible and resulted in problems during the statistical analysis stage as it could not give a characteristic curve. The MOC was significantly higher in the soluble fraction (a) compared to the AM. There was no significant difference with the other non-linear parameters, the potentially degradable fraction (b) and the rate of degradation (c) between the AM and MOC. The potential degradability (PD) of the MOC was significantly higher than that of the AM. The effective degradability of MOC was also significantly higher compared to that of the AM at all outflow rates

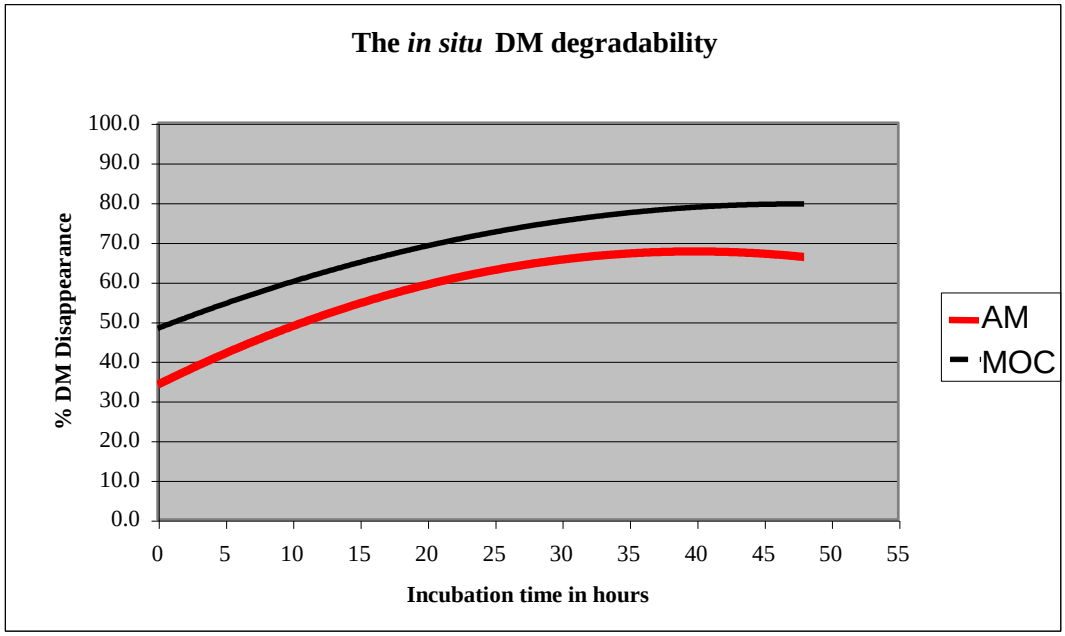

Figure 3.1 *In situ* ruminal dry matter degradability of the subtropical fruit processing industry waste products in the rumen of a sheep. AM – Avocado meal, MOC - Macadamia oil-cake

3.7.2 *IN SITU* CRUDE PROTEIN DEGRADABILITY

The ruminal CP degradability results of the subtropical fruit industry by-products are presented in Table 3.8 and Figure 3.2. The Ørskov & McDonald (1979) formula was used to obtain the results.

All the non-linear parameters differed significantly between by-products. The MOC had the highest significant soluble fraction of 73.5% compared to the AM which had 28.2%. The potential degradable fraction (b) of the AM was significantly higher than that of the MOC. The extent of degradation CP fraction of the MOC was significantly higher than that of the AM. The effective degradability of CP differed significantly between the by-products at all outflow rates, with the MOC being higher than AM.

Table 3.8 Mean ruminal *in situ* CP degradability values (Mean ±SD) of waste products from the subtropical fruit processing industry

Parameters	By-products	
	Avocado meal	Macadamia oil cake
A	28.2[a] ±2.4	73.5[b] ±1.1
B	33.5[a] ±1.9	18.7[b] ±2.5
C	0.09[a] ±0.03	0.19[b] ±0.04
PD	61.7[a] ±0.6	92.2[b] ±1.5
ED_k		
$ED_{0.03}$	53.2[a] ±1.6	89.6[b] ±0.9
$ED_{0.05}$	49.7[a] ±1.8	88.2[b] ±0.7

a - soluble fraction; b - potentially degradable fraction; c - degradation rate constant of the b fraction; PD - extent of degradation (a + b); ED - effective degradability, k = outflow rate. [a, b c] Means with different superscripts, within rows, differed significantly (P<0.05).

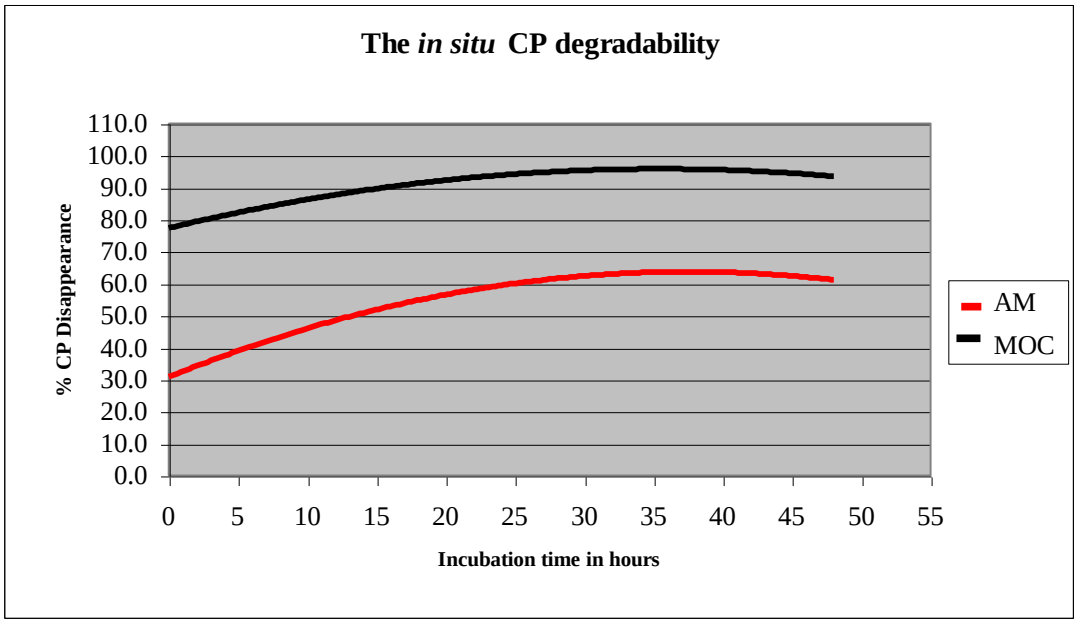

Figure 3.2 *In situ* ruminal CP degradability of the subtropical fruit processing industry waste products in the rumen of a sheep. AM – Avocado meal, MOC - Macadamia oil cake

3.8 GROWTH PERFORMANCE OF BROILERS ON DIETS WITH AVOCADO MEAL

The growth performance of broilers on commercial diets with avocado replacing maize at different levels are presented in Table 3.9 and 3.10

Table 3.9 Effects of including avocado meal in commercial broiler diets on broiler performance

	0%AM	10%AM	20%AM	30%AM	40%AM	SEM
Initial mass (g)	308.24	326.74	316.84	342.83	316.02	±21.085
Feed intake	116.28[a]	110.24[ab]	100.49[b]	93.39[bc]	87.70[cd]	±3.287
Final mass (g)	1986.73[a]	1742.16[b]	1510.34[c]	1198.55[d]	1000.67[e]	±39.476
Average daily gain	59.45[a]	50.72[b]	42.44[c]	31.30[d]	24.23[e]	± 1.409
FCE (g gain/kg feed)	510[a]	460[b]	420[b]	330[c]	280[d]	±0.008

[a,b,c,d,e] Row means with the same superscript do not differ significantly (P>0.05, Bonferroni test)

The feed intake of the broilers ranged from 87.70 g at 40% inclusion of AM to 116.28 g at 0% inclusion. The final mass of broilers as well ranged from 1000.67g at 40% inclusion of AM to 1986.73 at 0% inclusion of AM. The feed intake of the broilers on commercial diet was significantly higher (P>0.05) than that of the broilers on commercial diets with avocado meal, except for the inclusion rate of 10% AM. However, the final mass differed significantly (P>0.05) with the broilers on 100% commercial diet outperforming the ones on diets with avocado meal. Also the average daily gain (ADG) differed significantly (P>0.05) between all the treatments with the commercial diet supporting high ADG compared to the diets with avocado meal. The feed conversion efficieny (FCE) of broilers on commercial ration was significantly higher (P>0.05) to all treatments. There was no significant difference between treatment 2 and treatment 3 where the inclusion rate was 10 and 20%, respectively.

The results presented in Table 3.10 indicate that there were differences between treatments weekly. Significant differences (P>0.05) between weekly body weights (BWC) of chicks per pen were observed. In week three, there were no significant difference (P>0.05) between chicks on commercial ration and chicks on ration with 10% avocado meal. Treatment three differed significantly (P>0.05) to treatment four and five. There were no significant differences (P>0.05) observed between treatment four and five. All treatment differed significantly (P>0.05) in week four, with treatment one being the one with the highest body weights. In week five there were highly significant difference (P>0.05) observed with the exception of treatment four and five. In week six, there were no significant differences (P>0.05) between broilers in treatment one and two. Treatment two did not differ significantly (P>0.05) with treatment three, although treatment one differed significantly (P>0.05) to treatment three. Treatment four differed significantly (P>0.05) with treatment five.

Table 3.10 Body weight changes (BWC) between weeks of broilers on commercial diets with avocado meal

	0%AM	10%AM	20%AM	30%AM	40%AM	SEM
BWC3	1053.29^{a}	945.39^{ab}	871.25^{b}	735.81^{c}	672.12^{c}	±24.37
BWC4	1372.97^{a}	1232.48^{b}	1107.89^{c}	935.23^{d}	822.69^{e}	±22.17
BWC5	1947.73^{a}	1750.18^{b}	1533.57^{c}	1181.81^{d}	1025.16^{d}	±37.71
BWC6	2518.74^{a}	2300.45^{ab}	2071.31^{b}	1531.32^{c}	1166.39^{d}	±52.42

^{a,b,c,d,e} Row means with the same superscript do not differ significantly (P>0.005, Bonferroni test)
BWC3, 4, 5, 6 – Body weight change in week 3, 4, 5 and 6

The effect of replacing maize with AM in broiler rations on the performance of broiler chicken is also presented in Figure 3.3. There was a decline in feed intake with every increase in AM in the

ration. For every unit increase of AM, there was a decline of 0.74 units with the intake of the broilers. The effect replacing maize with AM was more prominent on the final mass of the broilers, as presented in Figure 3.4. For every unit increase of AM in the ration, there was a 25.9 unit decrease on final mass of the broilers.

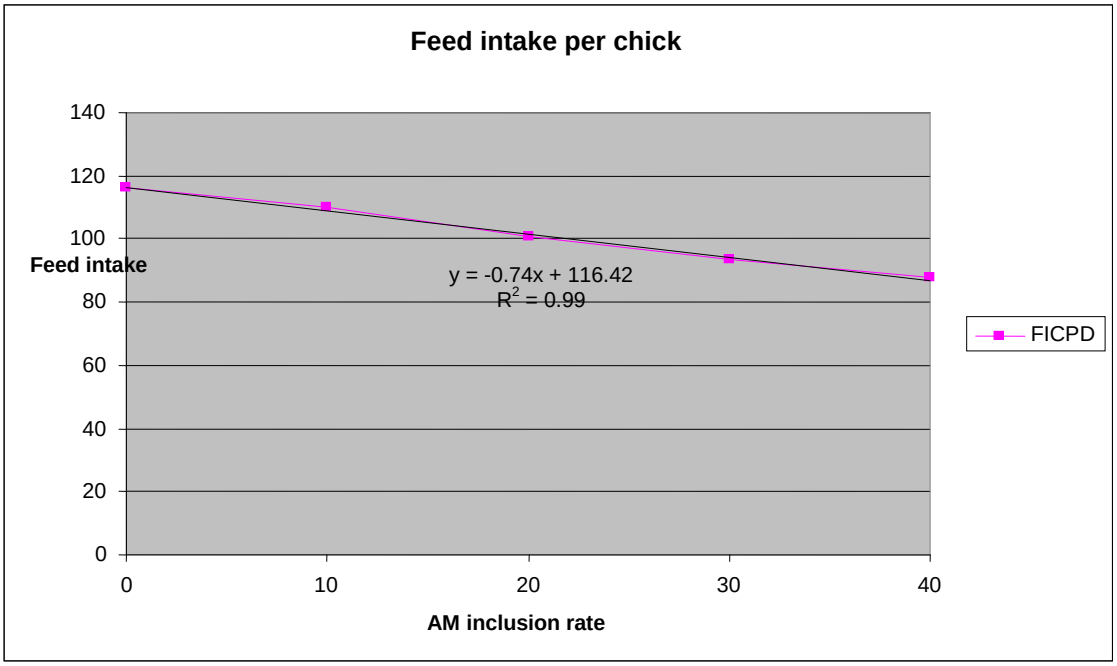

Figure 3.3: Cumulative feed intake of chicken at different inclusion rates of AM

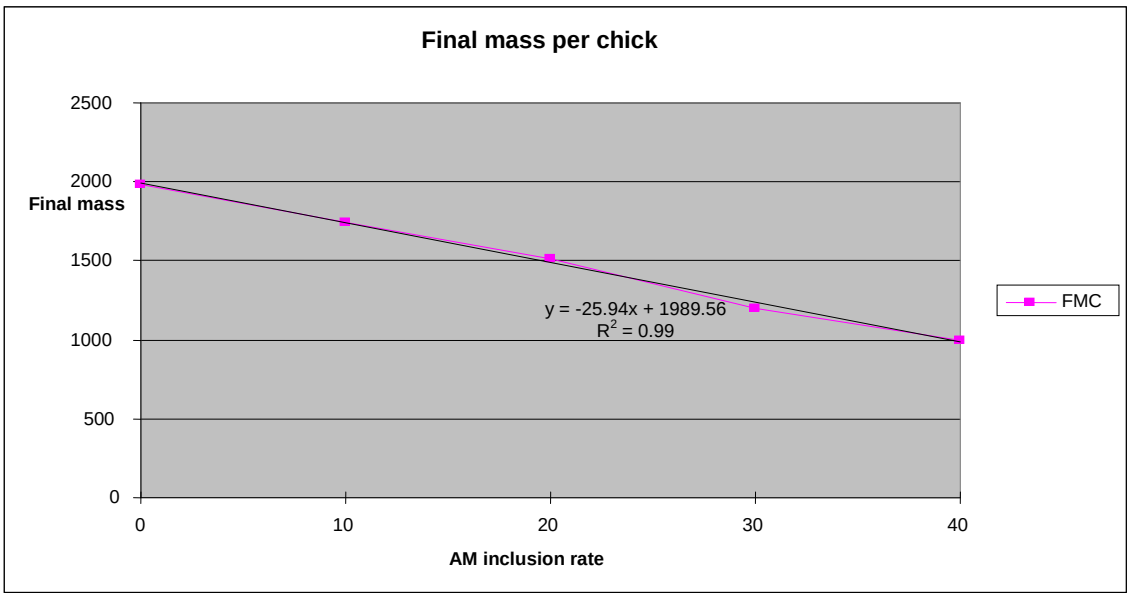

Figure 3.4: Final mass of chicken at different inclusion rates of AM

With respect to the survival rate of broilers on rations with AM, there were no mortalities during the experiment phases. Mortalities were mainly experienced during the first week of the feeding trial and the first two weeks were mainly for back grounding of broilers on a commercial ration.

CHAPTER FOUR

DISCUSSION

4.1 Introduction

Considerable quantities of crop residues and by-product feedstuffs which are suitable for feeding livestock are generated every year in most developing countries in the tropics and sub-tropics. Due to the lack of the technical know-how, they are often lost or under-utilised. However, livestock farmers are always in search of cheaper alternative sources of quality feed for their livestock, as the conventional feedstuffs are usually expensive. It is therefore desirable, if not essential, that the potential of any or all products that can be used as livestock feed be fully explored, and hence used as a by-product feedstuff. For many years, by-products from the vegetable, food and fruit processing industries have been evaluated for their potential in livestock feeding. The group of by-products that have made a huge impact in the feeding of livestock is oil-seed meals. However, there seem to be only a limited number of the by-products from the fruit processing industry that have thus far proved to be outstanding as nutrient suppliers in the feeding of livestock. It was the intention of this study to evaluate the potential feeding value of some waste products from the subtropical fruit industry of which limited information regarding their nutritive value is available.

4.2 Chemical composition

The determination of the chemical composition of a feed is routinely used as a rapid and economical method of predicting the nutritive value and digestibility of feeds. This is based on a statistical association between the content of analysed constituents and feed quality (Van Soest, 1982). This means that there is no compositional parameter that can adequately predict nutritive value for a range of feeds although combining results from several analyses may improve its prediction. It is known that the chemical composition of feedstuffs depends on many factors, such as climate, soil type, fertiliser application, seed variety and storage of feed and processing methods. However, the chemical composition of fibres particularly, affects and alters the protein and energy digestibility. Therefore, it is important to determine the chemical composition and digestibility of nutrients in feedstuffs so as to identify the best way to use the feeds.

As soya bean meal (SBM) is regarded as a universally accepted reference for the composition of the other oilseeds, the chemical composition of the oilcake meals under study was evaluated against that of SBM. Peanut oilcake meal (POCM), however, which can be regarded as the most prominent meal from the subtropical fruit industry, has been used in some comparisons as well. For

comparative purposes a table of the chemical composition of the by-products under study is presented as Table 4.1.

Table 4.1 Comparative chemical composition of the subtropical fruit processing industry waste products with the commonly used by-product feedstuffs (SBM and POCM) (g/kg dry, fat free basis)

	SBM[a]	POCM[a]	AM	MOC	MCH
Ash	62.0	58.0	77.5	42.9	14.5
CP	503.0	518.0	99.2	209.3	97.0
CF	58.0	133.0	265.6	286.9	606.6
NDF	217.0	214.0	602.8	498.4	796.2
ADF	104.0	135.0	458.4	400.4	751.7
ADL	15.0	46.0	301.2	150.6	363.2
ADIN(%N)	0.4	1.1	25.7	4.1	5.1

SBM – Soya Bean Meal, POCM – Peanut oilcake meal, AM – Avocado meal, MOC – Macadamia oilcake, MCH – Macadamia chips. [a]NRC (2005)

The CP concentration of all three by-products is low compared to that of SBM which is published to be 503 g/kg DM, and that of POCM, as 518 g/kg CP (NRC, 2005). However, MOC compared favourably with coconut meal which contained about 213 g CP/kg DM (NRC, 2005). However, coconut meal is regarded as a less commonly used feedstuff. Of the by-products from the subtropical fruit industry, POCM contained the highest level of protein. The CP concentrations of the by-products under study were far below that of POCM. The MOC had the highest CP concentration compared to other products under investigation, but it was still 38.9% below that of POCM. However, in using the feed classification of Hutjens (2008) none of the by-product feed stuffs can be regarded as a protein source as all have protein content lower than 25%. There was a significant difference in CP concentration between MOC, AM and MCH, with the latter by-products being low. The CP concentration of the MOC, however, varied significantly compared to the other products. This variation could be linked to the contamination of the product with the kernel pieces which then dilute the most important nutrient concentrations. The CP concentration of AM and MCH could be compared to that of maize grain which is primarily an energy source (Church & Pond, 1988; McDonald *et al.*, 1995). The CP concentration of AM did not vary much and this can be considered as the sign of consistency in the process of oil extract and product generation. The CP concentration of MCH varied as well but varied less compared to the MOC. The source of variation could be the

proportion of nut pieces and kernel pieces in the product. The more the pieces of the kernel, the lesser the pieces of the nut and this then can result in lower the CP concentration.

The CP concentration of MOC, however, can meet the protein requirements of most of the animal classes, including monogastric animals. The quality of the protein is crucial in the nutrition of monogastric animal and in high producing dairy cows. The quality of protein in feedstuffs is affected by the processing conditions to which they have been subjected. Three major factors affecting the nutritional protein quality are (a) amino acid composition (b) amino acid availability or digestibility, and (c) the presence or absence of biological active components, sometimes referred to as anti-nutritional factors (Aherne & Kenelley, 1982). Heat treatment during processing may adversely affect the final protein quality for monogastric animals. However, it may be beneficial in ruminant nutrition because of the reduction in the rumen degradability of the protein (Aherne & Kenelly, 1982, Schroeder, 1997).

Due to the lower protein content (<25%) and high fibre content, the MOC can be classified as "intermediate" products and be grouped with products such as brewer's and gluten 20. The AM and MCH were significantly high in fibre compared to the MOC. The low protein content and high fibre content with AM and MCH qualify these products to be compared to wheaten bran which is also an intermediate product. The fibre content of the MOC, however, is mostly in the form of NDF and less of ADL compared to the other products (AM and MCH). The AM and MCH had high NDF as well high ADL and this then result in a high proportion of less digestible component of the products and the availability of energy thereof.

The energy content of by-product feedstuffs is, however, usually determined by the level of residual oil and percentage of fibre in the meal (Aherne & Kennelly, 1982; Schingoethe, 1991). The digestibility of the fibre in the by-product is another important determinant of available energy, and is best indicated by NDF and ADF concentrations (Schingoethe, 1991).

Various forms of fats or oils are found in plants. The long chain fatty acids serve a function of being part of the triacylglycerols in plants. Triacylglycerol contains about twice as much energy concentration per gram as protein or carbohydrate and is the form in which energy is stored (Goodridge, 1985). Therefore, fats constitute a concentrated form of energy in animal diets, yielding approximately 2.25 times as much energy concentration as an equal weight of carbohydrate (Schingoethe, 1991; McDonald *et al.*, 1995). Apart from the general importance of dietary fat as a concentrated energy source, nutritionally, fatty acids are important as a source of essential fatty acids for the animal (Rooke *et al.*, 2003). Prominent among the essential fatty acids are the unsaturated fatty acids containing more than one double bond, the linoleic (C18:2n-6), linolenic (C18:3n-3) and

arachidonic (C20:2) acids (Baum, 1982). Significant differences between the total fatty acid concentrations of AM, MOC and MCH were observed. The MCH had the highest concentration of fatty acids and AM the lowest. The variation within the products in different fatty acids was fairly low; although the variation in total fatty acids of was high in MCH compared to the MOC and AM with the AM varied less. The linoleic concentration in AM was higher ($P<0.05$) than either those of MOC and MCH. There was no significant difference between the fatty acid concentrations of MOC and MCH. Linoleic acid is regarded as the only essential fatty acid required by poultry (Watkins, 1991 & NRC, 1994). The linoleic concentrations of the three by-products can meet the requirements for poultry (NRC, 1994). In poultry, long chain (n-3) polyunsaturated fatty acids are necessary for normal development of the brain and the retina (Watkins, 1991).

The C18:2 concentrations of all three the by-products were far below the concentration of this fatty acid in SBM or POCM (NRC, 2005). The C18:1n9t was the most prominent fatty acids in all three by-products, with the concentrations in MOC and MCH significantly different from that in AM. The C18:1 concentration of the MOC and MCH were higher than those of the SBM and PM, as reported by the NRC (2005). The 18:2n-6 and 18:3n-3 fatty acids are regarded as the parent compounds from which all other metabolically important fatty acids are synthesised (Rooke *et al*., 2003).

The monounsaturated fatty acids constitute the greater portion of the long chain fatty acids in these by-products, with the macadamia by-products being the highest. The saturated fatty acids of the AM were the highest amongst the waste products under study. The palmitic acid (C16:0) occurred at the second highest concentration in AM. However, it was the third highest with the MOC and MCH. The palmitoleic acid (C16:1) concentration was the second highest with the MOC and MCH. Stearic acid (C18:0) concentration was the third highest of the fatty acids in AM. The concentrations of the C14:0, C16:0, C16:1 and C18:0 fatty acids in the three by-products were higher than the respective concentrations in the SBM and POCM, as reported by the NRC (2005). The AM had the highest PUFA concentration compared to the MOC and MCH. Even the PUFA/SFA ratio of the AM was higher compared to that of the MOC and MCH. The n-6 and n-3 concentrations of the AM were higher than that of the MOC and MCH, however, the n-6/n-3 ratio of the MCH was the highest. The n-6 concentration of the AM is higher than that of the soya bean oil of 4 as reported by Barroeta & Xalabarder (1994).

The biological significance of the essential fatty acids, however, resides in the longer chain polyunsaturated fatty acids (PUFA) that can be synthesised from the 18 carbon fatty acids (Rooke *et al*., 2003). This could imply that the high proportion of 18 carbon fatty acids in these by-products

could be of high significance in the synthesis of PUFA. The polyunsaturated fatty acids, however, are more susceptible to oxidation than monosaturated fatty acids. Oxidative rancidity leads to the formation of both unpalatable and toxic components and destroys nutrients (Rooke *et al*., 2003). Therefore, rancidity decreases the nutritional quality of feed. However, the concentration of PUFA is lower than the SFA and MUFA concentration, with the MUFA being the highest in concentration. The lower concentration of PUFA does not mean that the waste products are not susceptible to rancidity. The MUFA are also susceptible to oxidation due to the presence of a double bond but they are fairly resistant compared to the PUFA (Christie, 1982). Fats containing high levels of unsaturated fatty acids are sensitive to oxidation which normally results in a less pleasant taste, as well as reduced shelf-life of meat (Marchello *et al.*, 1967). The diet affects the fat quality in pig meat more than any other known factors (breed, sex, or age) (Scherf *et. al*., 1990). Therefore, feeding of feedstuffs with high concentrations of unsaturated fatty acids, especially monogastric animals can cause softening of fat deposits and off-flavours development in the edible tissues of the meat (Chow, 1980), thus reducing the quality and acceptability of the animal products.

The fibre fractions of the by-products under study were all higher than published values for SBM and POCM (Table 4.1). The fibre, as CF, NDF and ADF, levels were higher in MCH compared to the other by-products. The MCH differed significantly in CF levels from the other by-products. There was no significant difference between the AM and MOC. There were no huge variations in fibre fractions within the products. However, the variation in MOC and MCH were higher compared to AM. This could be linked to the presence of the indigestible components of the shell. The more the contamination of the product by the shell pieces the higher the fibre content in the product.

The MOC had the lowest ADF of all the three by-products under study. The ADF fraction contains cellulose whose digestibility in feeds varies, and lignin, which is almost indigestible. The ADL concentrations of these by-products were high, with the MCH and AM being significantly higher than the MOC. The MCH had the highest of all the fibre fractions compared to the other by-products. A possible reason for this is that during the processing, the MCH is derived from pieces of the shell and nut lost during the cracking process. The pieces of the shell, therefore, might be the main source of the fibre. In supporting this assumption, there was a huge variation within all the fibre fractions of the MOC which is presumed to have been derived from the oil extraction process performed on the low grade macadamia kernels. The indigestibility of the macadamia shell pieces is also confirmed by the low IVOMD of the MCH.

The higher ADL concentration could result in a reduced availability of some fractions during digestion of the feed. The by-products would therefore need longer periods to be digested in the

animal. This implies more retention time and lower intakes in the case of ruminant animals. The high cellulose concentration of the MCH could also be contributed to the lower availability of nutrients from this by-product. The by-products differed significantly in cellulose concentration. Fibrous by-products with a high lignin concentration are poorly utilised, even by the ruminants (Boucqué & Fiems, 1988). There was no significant difference across by-products in hemicellulose concentrations. The AM had the highest hemicullulose and the lowest cellulose compared to the other by-products. A similar situation with the MCH was observed, where the hemicellulose was the lowest compared to other by-products, and the cellulose was the highest. The high lignin, hemicellulose and cellulose in the AM could be linked to the presence of the skin and the pit in the remaining material from the extraction process.

The fibre bound N (ADIN) of the AM was higher compared to that of the SBM and POCM. The concentration in AM differed significantly from that in the MOC and MCH. There was also a significant difference between MOC and MCH in ADIN concentration. ADIN concentration of the MOC can be compared to that of the SBM reported by NRC (2001).The MOC had the lowest ADIN concentration. The higher ADIN levels could suggest lower solubility or availability of N of the by-product in rumen, and therefore the availability of N in the rumen of the AM could be lower compared to other by-products. The high ADIN content of the AM could be linked to the presence of the skin (peel) and the seed in remaining solid material from the extraction process. The ADIN content of AM did not vary much (±5.1). This could be a confirmation on the possible source of ADIN in this product as the products were subjected to the same treatment of extraction.

4.3 Condensed tannins

The condensed tannin (CT) concentrations of the three by-products under study differed significantly. The MOC had the lowest concentration of 46.1 g/kg DM and the AM had the highest at 258.4 g/kg DM. The CT concentration in AM varied widely and the variation was the highest compared to other products. The high CT concentration and variation could be from the fruit skin and the seed as the fruit was pressed as is, after ripening for extraction. The maturity of the fruit, the skin and the seed could be the possible sources of variation within the AM samples. This high concentration of CT could have a negative effect in the availability of protein from this product and also lead to reduced intake and digestibility especially with monogastric animal.

Among the macadamia by-products, MOC and MCH there was a significant difference in CT concentration. The difference between the macadamia by-products could be due to the high proportion of the shell in the MCH. This could be seen from variations within the MOC samples. The

more pieces of the shell, the higher were the CT concentration in the by-product. The CTs are mainly concentrated on the shell and therefore high proportion of the shell in a by-product can render the by-product unsuitable for livestock feeding.

The effects of CT on animal performance are vast and vary within species. They mainly affect animal performance by reducing the voluntary feed intake and digestibility of the feed (McLoed, 1974). Although CTs are non-toxic secondary phenolic compounds, they lower the digestibility and palatability of feeds at high concentrations (> 20g/kg DM) (McDowell & Valle, 2000). However, Butter *et al*. (1999) reported improved animal productivity in ruminant animals by feeding low concentrations of CT (~10 – 40 g/kg DM). When CP intake is high, lower levels of CT have a positive effect on N retention and increase the flow of essential amino acids to the duodenum (Thomson *et al*., 1971; Harrison et al., 1973; Egan & Ulyatt, 1980; John & Lancashire, 1981; Barry & Manley, 1984; Beever & Siddons, 1985). Low CT concentrations are associated with better CP digestion and metabolism in ruminants (Reed, 1995). The postulated mechanism is the complexation of soluble protein and tannins in the near neutral pH of the rumen, preventing microbial degradation (Jones & Mangan, 1977; Barry & Manley, 1986). Thus, low CT levels may increase the absorption of amino acids as they protect protein from the bacterial degradation and make them available for absorption in the duodenum (Ried *et.al.*, 1974). The optimum tannin concentration in feed at which this protection occurs has been presumed to vary with the level of CP in the feed, the energy available for the synthesis of microbial protein and the extent in which the tannin depresses voluntary feed intake (Minson, 1990).

The ash concentration of the subtropical fruit industry by-products was lower than that of the SBM, reported in NRC (2001), with the exception of AM which contained 77.5 g ash/kg DM. The ash concentration of the three by-products differed significantly, with the MCH containing the lowest and the AM the highest. This difference could possibly be due to the storage sites of the fruits. The ash content of the AM varied significantly within samples compared to other products. The ash content indicates the total mineral content of feed and not individual minerals. However, ash concentration values may indicate the quality of the products, such as soil contamination of the products in some situations.

4.4 Minerals

The macro mineral concentrations of the three subtropical fruit processing industry by-products are relatively lower than that of the SBM (see Table 4.2). The Mg and Na concentrations of the AM are higher compared to the SBM and even to POCM.

Table 4.2 Mineral element concentration of the commonly used oilseed meals and subtropical fruit processing industry waste products

	Feedstuff				
Mineral	SBM [a]	POCM [a]	AM	MOC	MCH
Macro minerals (g/kg)					
Ca	3.6	2.0	1.1	3.3	1.5
P	6.6	6.4	2.1	3.0	1.4
Mg	3.0	3.2	21.9	2.4	2.0
K	21.2	13.2	21.3	13.9	6.9
Na	0.4	0.3	0.8	1.8	0.8
Ca:P ratio	0.35:0.65	0.24:0.76	0.34:0.66	0.52:0.48	0.52:0.48
Trace minerals (mg/kg)					
Cu	17.0	13.0	21.7	13.7	17.0
Zn	72.0	5.0	28.2	55.6	21.1
Mn	39.0	33.0	44.5	267.2	151.2
Fe	169.0	302.0	732.4	248.9	187.3
Se	0.21	0.21	0.17	0.60	0.44

SBM – Soya Bean Meal, POCM – Peanut oilcake meal, AM – Avocado meal, MOC – Macadamia oilcake, MCH – Macadamia chips
[a]NRC (2005)

The Ca and P concentration of the MOC was higher than that of the AM and MCH. The concentrations in MOC and MCH compare favourably with POCM in macro mineral concentration. The Ca concentration of the MOC is, however, higher than that in POCM. The K concentration of the AM compares favourably with that of the SBM. The Na concentrations of the three by-products are higher than published values in SBM and POCM. In most circumstances, farm animals obtain a high proportion of their mineral nutrients from the feeds they consume. Therefore, the factors that affect the mineral content of the vegetative plant parts and seeds will affect the mineral content available to the animals (Underwood & Suttle, 1999). The concentration of minerals in plants depends on five interdependent factors, a) the genetics of the plant (strain or variety), b) the type of the soil on which the plant was grown, c) climate and weather during growth, d) the stage of maturity of the plant (Miller, 1979, Underwood & Suttle, 1999) and (e) the part of the plant (Miller, 1979).

The macro mineral concentrations of all three by-products cannot meet the requirements of the monogastric animals, except those of Mg and K. The MOC can meet the Na requirements of all the monogastric animal classes. However, the macro mineral concentration of all these by-products

can meet the requirements of certain ruminant livestock classes, although these waste products cannot be fed alone to animals. The macro mineral requirements of beef cattle can be met with the exception of the Ca requirements (NRC, 1994). The mineral requirements of different animal types are presented in Table 4.3.

Table 4.3 Mineral requirements for different domestic animal types

	Animal type					
Mineral	Beef cattle[a]	Dairy cattle[b]	Sheep[c]	Pigs[d]	Poultry[e]	
					Layers	Broilers
Macro minerals (g/kg)						
Ca	2.0 – 4.0	3.0 – 7.7	2.0 – 8.2	7.5	34.0	10.0
P	2.0	1.9 – 4.8	1.6 – 3.8	3.5	3.2	3.5 - 4.5
Mg	1.0	1.6 – 2.0	1.2 – 1.8	0.4	500 mg/kg	600 mg/kg
K	6.5	6.5 – 10.0	5.0 – 8.0	2.0	1.5	3.0 – 4.0
Na	0.8	1.0 – 1.8	0.9 – 1.8	1.5-2.0	1.5	1.5
Trace minerals (mg/kg)						
Cu	8.0	10.0	7.0 – 11.0	5.0	6.0	8.0
Zn	30.0	40.0	20.0 – 33.0	50.0	50.0	40.0
Mn	40.0	40.0	20.0 – 40.0	10.0	30.0	60.0
Fe	50.0	50.0	30.0 – 50.0	80.0	50.0	80.0
Se	0.2	0.3	0.1 – 0.2	0.15	0.10	0.15

a - NRC (1994), b – NRC (2005), c - McDowell (1992), d – NRC (1998), e – NRC (1994)

The macro mineral concentration of these by-products falls within tolerable levels by animals, with the exception of the Mg concentration of the AM for all animal types (see Table 4.4). The lower macro mineral concentrations of the by-products could mean that the by-products cannot provide the minerals required by the animals and their use as sole feeds could be limited. Therefore mineral supplementation, especially for Ca and P, should be provided if better animal production is desired.

Table 4.4 Maximum tolerable levels of dietary minerals for domestic animals (adapted from McDowell, 1992)

	Animal Species			
Mineral	Cattle	Sheep	Pigs	Poultry
Macro minerals (g/kg)				
Ca	20.0	20.0	10.0	40.0
P	10.0	6.0	15.0	10.0
Mg	5.0	5.0	3.0	3.0
K	30.0	30.0	20.0	20.0
Na in NaCl	40.0 – lactating 90.0 – non-lactating	90.0	80.0	20.0
Trace minerals (mg/kg)				
Cu	100.0	25.0	250.0	300.0
Zn	500.0	300.0	1000.0	1000.0
Mn	1000.0	1000.0	400.0	2000.0
Fe	1000.0	500.0	3000.0	1000.0
Se	20.0	20.0	20.0	20.0

The trace mineral concentrations of all the subtropical fruit processing industry by-products can meet the requirements of all the domestic animal types (monogastric and ruminants) (McDowell, 1992; NRC, 1994; NRC, 1998; NRC, 2005). However, the Zn concentrations of the MCH and AM were below the requirements of cattle (beef & dairy) and all the monogastric animals, and can be regarded as marginal for sheep as it cannot cover all classes of this species. The MOC differed significantly with the MCH and AM in Zn concentration. The Se concentration of the AM cannot meet the requirements of dairy cattle.

The trace mineral concentration of the subtropical fruit processing industry by-products compares favourably with those of the SBM and POCM (Table 4.2). The only exception is with the Zn concentration, where the concentration in SBM surpasses those of all the by-products under study (Table 4.2). However, the Zn concentration of the POCM is far below those of the three by-products. The AM has the highest Fe concentration of the subtropical fruit processing industry by-products and is also higher than that of the SBM and POCM (NRC, 2005). The Fe concentrations of the MOC and MCH are higher than that of the SBM but lower than that of the POCM, reported by NRC (2005).

However, the Fe concentration of the AM can be toxic to sheep as the maximum tolerable level for sheep is 500 mg/kg (see Table 4.4).

The Se concentrations of the macadamia by-products are higher compared to the Se concentration of SBM and POCM (NRC, 2005), with the MOC being the highest. The MOC differed significantly with the MCH and AM in Se concentration. The Se concentration of the AM is low. However, it compared favourably with the Se concentration of SBM and PM even though they are slightly higher.

The Mn concentration of the macadamia by-products is higher than that of SBM and POCM, with the MOC being the highest. The concentration of Mn is far above the requirements of all domestic animal types and classes. The Mn concentration is, however, below the maximum tolerable level of dietary mineral for domestic animals. The lowest of the maximum tolerable levels is found for pigs at 400 mg/kg (Mc Dowell, 1992).

4.5 IN VITRO DIGESTIBILITY

The IVOMD of the MOC was far superior to those of the other by-products, that is, AM and MCH. The difference was highly significant even between the MOC and MCH, even though they are both derived from macadamia nuts. The difference could be linked to the derivation process of the by-products where the MOC is mainly derived from the low-grade macadamia nut kernels and the MCH from the pieces of the shell and the nut. The pieces of the shell can be regarded as indigestible because they reduced the IVODM and resulted in a huge variation with the MOC that can be linked to the contamination of the by-products with the pieces of the shell. Therefore due to large proportions of the pieces of the shell present in the MCH led to the low in vitro digestibility. The IVOMD of MOC was the highest with an average of 79.2% and was 31.4% and 63.1% higher than those of AM and the MCH, respectively. The AM had IVOMD of 54%, which could be compared with the IVOMD of some forages and is lower than that of lucerne which was used as a control. However, there was a huge variation (±81.5) within the AM products. This variation could be linked to the oil extraction method, where the fruit is pressed as a whole, that is, the skin and the seed. The skin and the seed are expected to be high in fibre and contain high levels of condensed tannin. This then affect the digestibility of the product. At high concentrations fibre reduces the organic matter digestibility of feeds (Khaazal *et al*., 1994). However, `acording to Meissner & Paulsmeier (1995) the intake decreases with a decrease in IVOMD caused by an increase in fibre fractions. This could imply low intake can be expected for the high fibrous products, especially the MCH.

The low IVOMD of the MCH, therefore, renders this by-product useless. The reason for the low digestibility could be linked to the presence of the nut shell which proved to be indigestible, thus rendering the product less digestible as well. However, the high fat content of these products may affect the digestion of samples by the microbes.

4.6 DRY MATTER AND CRUDE PROTEIN DEGRADABILITY

A significantly high soluble fraction, a, of the dry matter was observed with the MOC. The rate was significantly higher than that of the AM of 32.3%. This fraction of AM is higher than that of SBM, reported by Ha & Kennelly (1984), Susmel *et al*. (1993) and Batajoo & Shaver (1998). The soluble fraction of the AM is lower than that of SBM reported. The potentially degradable fraction (b) of the MOC was also significantly higher than that of the AM but the difference was not significant. The high a fraction of MOC implies that this by-product, although high in fibre, is highly digestible. The b fractions of the MOC and AM were lower compared to that of SBM. The rate of degradation of the b fraction of the MOC was lower than that of the AM, but the difference was not significant. The b fraction degradation rate for AM, however, is similar to that of SBM, as reported by Batajoo & Shaver (1998) but higher than the reported rate by Susmel *et al*. (1993). Almost 35% of the DM of the MOC and AM could be regarded as insoluble but degradable in the rumen. The rate of feed degradation often increases with feed particle fineness, as more surface area tends to be exposed (Michalet-Doreau & Ould-Bah, 1992). The AM was very fine and dusty after milling, hence a higher rate of degradation of the b fraction.

As a result of the relatively high soluble and insoluble but rumen degradable fraction of MOC and AM, the extent of degradation (PD) is high for these by-products. However, the extent of degradation for MOC was significantly higher compared to the AM. The effective degradability, representing the fraction that has actually degraded in the rumen, is fairly high. The MOC is significantly higher than the AM in this fraction. The effective degradability decreased with an increase in outflow rate

A similar situation to the DM degradation was observed with the *in situ* CP degradation in the rumen of sheep. The CP soluble fraction of MOC was significantly higher than that of the AM. The soluble fraction, a, of the MOC (73.5%) is very high compared to the, a, fraction of the SBM (27%) reported by Batajoo & Shaver (1998) and NRC (2005) (22.5%). The AM, a, fraction, however, was also higher than that of the SBM reported by Batajoo & Shaver (1998) and the NRC (2005). Compared to the POCM reported by NRC (2005) the, a, fraction of the MOC is higher (73.5% vs. 61.7%). The “a” fraction of the AM was lower than that of the PM (28.2% vs. 61.7%).

The potentially degradable fraction, b, of the MOC was significantly lower than that of the AM (18.7% vs. 33.5%). The b fraction of the CP for MOC was very low compared to the same fraction of SBM reported by Batajoo & Shaver (1998) (18.7% vs. 72.4%) and NRC (2005) (18.7% vs. 76.8%). Even with POCM, the b fraction of the MOC was lower (18.7% vs. 36.6%). For the AM, the b fraction was low compared to that of the SBM reported by Batajoo & Shaver (1998) (33.5% vs. 72.4%) and NRC (2005) (33.5% vs. 76.8%). The rate of degradation for the b fraction of the MOC was higher ($P<0.05$) than that of the AM. It was also higher than that of the SBM, reported by Batajoo & Shaver (1998) (0.19 vs. 0.069). The, c, fraction for AM was higher than that of the SBM, reported by Batajoo & Shaver (1998). The CP fraction that was actually rumen degradable for MOC was higher ($P<0.05$) than that of the AM.

The CP of the MOC was highly degradable compared to that of the AM. This implies that, most of the CP of the MOC was mainly available for digestion in the rumen. The protein will be absorbed as microbial protein. However, with the AM, almost 50% of the CP was not digested in the rumen. The possible explanation for the moderate to low CP degradation in the rumen for the AM might be the heating during the drying process of the waste product at 70 °C during the preparation stage and a relatively high ADIN concentration. Heat and ADIN reduces the protein degradability in the reticulo-rumen while not affecting digestibility in the small intestine (Schroeder *et al.*, 1996). Protein sources can be moderately heated to increase their nutritional value by decreasing ruminal DM and CP degradability (Schroeder, 1997). This change in degradability occurs as a result of the formation of certain compounds (Maillard reaction) between the reducing sugars and the amino acids present in the protein source (Ljϕkjel *et al*., 2000). On the other hand, overheating can be detrimental as it will not only decrease the degradability of protein source in the reticulo-rumen, but in the rest of the digestive tract as well (Schroeder *et al*., 1996). According to Reddy & Morris (1993) the ADIN concentration is a good indicator of the rumen undegradable protein digestibility (UDP-D) fraction of plant proteins and the amount of heat damage in the plant protein. The ADIN concentration of these waste products cannot affect the digestibility of the protein in the small intestines as their concentrations are below the levels that reduce the availability of protein as reported by Schroeder *et al.* (1996)

4.7 GROWTH PERFORMANCE OF BROILERS

Broilers on commercial diet outperformed the ones on diets with AM. The feed intake of broilers on commercial diet was significantly higher than that of those on other treatment diets with AM except at 10% inclusion. The results shown in Table 3.9 demonstrate that inclusion of AM in diets for

broiler chicks depressed final body weight and feed conversion efficiency. The AM substitution reduced feed intake and weight gain. As there is no information available on the performance of animals on AM, the reason for the depressed body weight and feed conversion ratio could be linked to high fibre, high ADIN and high CT concentration as presented in Table 3. 2. With the inclusion of AM in any diet, an increase in the fibre content of the diet should be expected (see Table 2.3 and 2.4) and this explains the poor weight gain and feed utilization, which seemed to be due to an interaction between the feeding levels and the digestibility of the nutrients. This can be further supported by the *in vitro* digestibility and rumen degradation of AM in the rumen of sheep which were fairly high and expected to be of low digestibility with monogastric animals. This could be so because ruminants are animals that are known of possessing rumen microbes which help in digesting fibre.

However, at low inclusion rates (10%) the feed intake of broilers did not differ significantly although the intake of broilers on a control diet was higher compared to the intake of broilers on a diet with 10% AM. On the contrary, the final mass, ADG and FCE of broilers on 100% commercial diet were significantly higher compared to the same variables with broilers on a diet with 10% AM. This proves that the AM is palatable and acceptable to broilers but not good enough as a feed as it led to depressed growth. This is further demonstrated on the regression analysis of the data as presented in Fig 3.3 for intake and 3.4 for final mass. The depression on the intake was not as high as on the final mass of the broilers. On feed intake, there was slight decline (-0.74) for every unit increase of AM in the ration. However, with the final mass of broilers, a sharp decline (-25.9) was observed with every unit increase of the AM in the ration.

CHAPTER FIVE

CONCLUSION AND RECOMMENDATIONS

According to the objectives of the study, the potential nutritive value of the subtropical fruit processing industry by-products (AM, MOC and MCH) was evaluated and the focus was on the determination of the chemical composition, *in vitro* digestibility, rumen degradability of these by-products and the growth performance of broilers in rations with AM as a maize replacement. Based on these analyses it can be concluded that these by-products have the potential as animal feeds with the exception of the MCH. As feeds are usually classified based on the major nutrients (protein, energy or minerals) found in them, the same could be done with these by-products.

The potential of MOC as animal feed lies with its fairly high CP concentration which is 209 g/kg DM on average. However, this product cannot be regarded as a protein source as it is below 250 g/kg CP (Hutjens, 2008). The high fibre concentration and the residual oil-content of MOC provide an opportunity for this by-product to be included in animal feed and be viewed as a valuable energy feed source. It is well known that the energy density of the feed increases with an increase in fat content of the product. This is based on the fact that the energy concentration stored in fat is 2.25 times higher than that provided by carbohydrate of the same unit. This then puts this by-product in the same category as brewer's grain and therefore can be regarded as an intermediate feedstuff. From the *in vitro* digestibility, the by-product showed a high level of digestibility. The high *in situ* degradability of DM and CP of this waste product shows that the fibre in this product is highly digestible, implying high availability of nutrients upon its ingestion. The by-product is suitable for both monogastric and high producing ruminants. According to Phosa *et al*. (2004a), the MOC can be included in poultry diets at 10% inclusion level without any negative effects on the performance. This means that the MOC replaced 10% of maize in the diet providing a cheaper but high quality alternative ration. The MOC can be included in the rations for both layers and broilers at the same rate (Phosa *et al.,* 2004b).

The potential of AM as animal feed lies in its residual oil content and fibre concentration. The AM can be regarded as a valuable energy source. This stems from the fairly high DM degradation in the rumen of sheep. The *in vitro* digestibility, as well, is fairly high. The high fibre content of this waste product can therefore limit its usage in monogastric animals to ruminant animals mainly. This was confirmed in this study as the performance of broilers (ADG, final body mass and FCE) on a control commercial ration was significantly higher compared to treatment

rations. The fibre content of the rations increased with the increase of AM in the ration. This then led to a depression of the feed intake (-0.74) and final mass of broilers (-25.9) as presented in Fig 3.3 and Fig 3.4. Upon establishing this, the apparent metabolisable energy (AME) of AM was analysed using the McNab & Blair (1988) method with the courtesy of the University of KwaZulu-Natal laboratory and was found to be 2.802MJ/kg and this far lower compared to 16 MJ/kg of maize (NRC, 2005; McDonald, *et al*., 2005). However, in total mixed rations for dairy cows the AM could be used to replace forage fibre in the diet. The above assumption is based on the findings of Zhu *et al*. (1997) that the forage NDF can be replaced successfully with the NDF from the by-product feedstuffs. The relatively slow ruminal CP degradation, meaning a low CP digestion in the rumen and more availability in the small intestine, increases the potential of the AM as a source of rumen undegradable protein.

The MCH potential in animal feeding is reduced by the high proportion of the ADF and ADL which then result in low *in vitro* digestibility and *in situ* DM degradability of the by-product, even though it had a high residual oil content. From the *in situ* degradability trial, the pieces of the macadamia shell could not be digested at all and hence a low degradability. The by-product is regarded as not promising because the pieces will always be part of the by-product and they seemed to be completely indigestible as their presence in the MOC affected its composition negatively which led to increased variation in fibre fractions (CF, NDF, ADF, & ADL) and IVOMD.

In terms of cost per unit of CP or energy, the products cannot be compared properly with the available oil cake meals due to the fact that currently the products are just regarded as waste and therefore have no price attached to them. However, in using the equation of Graham (1983) to estimate the ME from chemical composition, the ME value of MOC (13 MJ/kg) is higher compared to that of SBM and sunflower meal but lower than that of maize (14 MJ/kg for ruminants and 16 MJ/kg for poultry) (McDonald *et al.*, 1995). This could imply that the cost per unit of energy for MOC is lower than any of the oil cake meals and can be compared to that of maize. The AM (2.8 MJ/kg) and MCH (5 MJ/kg) are lower in ME than maize. With respect to cost per unit of CP, none of the waste of products could be compared with either SBM or sunflower meal because of the higher CP concentration of the meals in relation to MOC, MCH and AM. However, the MOC has a higher CP concentration than maize and the ME is comparable to that of maize. This then presents this product with an advantage over maize, that is, a higher CP content. This supports, in theory, the findings of Phosa *et. al.* (2004a, b).

The nutritive value of feeds is conventionally classified under the nutrient content, digestibility and intake (Raymond, 1969). Therefore, an intake trial with ruminants to determine the

voluntary intake by the animals and *in vivo* digestibility of the AM should be done in order to predict their value as ruminant animal feeds. From this study it was observed that AM is palatable and acceptable to poultry as the intake did not differ significantly with the commercial diet at 10% replacement of maize with AM. There were no mortalities observed, as well. This could imply that AM does not have harmful factors for poultry and can be used as one of the ingredients in poultry rations. A comprehensive study, however, on the anti-nutritional factors in these by-products (AM and MOC) should be done, together with the extent to which their concentration can affect digestibility, starting with CT as they have been found to be in high concentrations in these waste products.

Variation in chemical composition and the fibrousness of these waste products, especially the AM, could be considered as potential limitation in the use of these by-products as animal feedstuffs. This could be expected as it has been cited to be the limitation of by-product feedstuffs in many instances (Boucque & Fiems, 1991; Schingoethe, 1991, Weiss & St-Pierre, 2005). Weiss & St-Pierre (2005) suggested that use of a wide variety of feedstuffs in formulation of total mixed rations; reduces the impact of variation in nutrient composition and costs associated with variation. The routine analysis of each batch of by-product from the processing industry is recommended as another way of reducing variation. In the case of MOC, I would recommend that the buyer should be careful with each and every batch, as the by-product can be contaminated with high concentrations of shell. The shell is indigestible and can therefore lead to a poor quality MOC and hence poor performance of animals when included in a ration. The pieces of shell increase the indigestible fibre fraction in the MOC.

The fibre in AM has been proved to be limitation with poultry as lower growth rates and poor feed conversion efficiency was observed as the broilers on commercial diet outperformed the ones on diets with AM replacing maize. This was observed, even, at 10% inclusion rate. The AM fibre content might not be a limitation in ruminant animals as they have the ability in the form of rumen microbes to digest fibre, thus releasing the fibre-bound nutrients. This was observed with the *in vitro* digestibility which was fairly high, that is, above 50%.

If this product is to be used with monogastric animals, the adoption of biochemical technology is a promising option. The use of certain microbial enzymes to increase the nutrient availability of cell-wall carbohydrates has been found to hold promise in improving efficient utilisation of available feedstuffs. Wenk *et al*. (1993) reported a significant increase in digestible energy, nitrogen, NDF and ADF through enzyme supplementation. Enzyme supplementation reduced the content of fibre components in the diet by 7% (Wenk *et al*., 1993). The successful use

of enzyme supplementation in poultry has been reported by several authors (Campbell & Bedford, 1992; Bedford, 1995; Dierick & Decuypere, 1996; Pluske, 1999; Partridge, 2001). The use of exogenous enzyme specific to the fibres or less digestible components of the by-products can improve their nutritive value. However, the costs of including enzymes should be weighed against the costs of using an alternative feed instead of AM which is fibrous.

Rancidity of these products is another problem that needs to be monitored closely as rancid feedstuffs affect the quality of produce from the animals that ingested the feed. Rancidity is mainly caused by environmental factors (ambient temperature and humidity) (Chow, 1980), therefore exposure to the elements should be prevented by providing cool storage areas. However, the inclusion of anti-oxidants like ethoxyquin and butylated hydroxytoluene can inhibit lipid oxidation (Chow, 1980).

It will always be the challenge of the animal nutritionists, together with biochemists to increase the use of less conventional feeding stuffs in the light of long term decline in availability of more conventional feed ingredients (Pulske, 1999). This is of most relevance to intensive livestock production systems, although the by-product feedstuffs are required as well under extensive livestock production systems especially where they offer cheaper supplements to poor grazing compared to the conventional feed ingredients.

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