DIETARY INCLUSION LEVELS OF ENSILED AVOCADO (*PERSIA AMERICANA*) OIL CAKE INTO PIG DIETS AND THEIR EFFECTS ON THE GROWTH PERFORMANCE, NUTRIENT DIGESTIBILITY, CARCASS CHARACTERISTICS AND MEAT QUALITY

By

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DECLARATION OF THE COPYRIGHT

I, Mmaseala Lynette Seshoka, declare that this thesis "Dietary inclusion levels of ensiled avocado (*Persea americana*) oil cake into pig diets and their effects on the growth performance, nutrient digestibility, carcass characteristics and meat quality", submitted to the Central University of Technology, Bloemfontein for the degree of Doctor of Technology: Agriculture is my own independent work and that all sources used and quoted have been duly acknowledged by means of complete references, and compile codes of academic integrity, as well as other relevant policies, procedures, rules and regulations of the Central University of Technology; and not been submitted before to any institution by myself or any other person in fulfilment (or partial fulfilment) of the requirements for the attainment of any qualification. I also declaim this thesis in favour of the Central University of Technology, Bloemfontein, Free State.

ABSTRACT

The value of ensiled avocado (*Persia Americana*) oil cake (AOC) in pig diets was evaluated through three experiments. The first experiment was conducted to evaluate the effects of an enzyme addition on the fermentation characteristics, nutritive value and aerobic stability of ensiled AOC. An amount of 700 g AOC /kg fresh matter (FM) was mixed with 250 g wheat bran/kg FM, and 50 g sugarcane molasses/kg FM. This mixture was treated without i) additive (control), or with enzyme (Axtra® XB enzyme) at ii) 3 % and iii) 5% levels, making three treatments in total. The enzyme contained endo-1, 4-β-xylanase (12.200 U/g) and endo 1,3β-gluconase, (1.520 U/g). The forage mixtures were thoroughly mixed and compacted into 1.5 L anaerobic jars. Jars were kept at 25-28° C room temperature for 90 days and there were 36 jars with nine jars per treatment. Samples of the pre-ensiled mixtures were collected for the determination of nutritive values, pH and water soluble carbohydrates (WSC). Three jars per treatment were then opened on days 3, 7, 10 and 90 post ensiling to determine pH while those of day 90 were also used to determine nutritive values and fermentation profiles of the treatments. Further, silage samples of day 90 were subjected to an aerobic stability test that lasted for 7 days. It was found that good quality silage can be produced from AOC without (P >0.05) the addition of enzyme. However, the effect of enzyme addition was apparent (P<0.05) on the reduction of NDF and increased WSC of ensiled AOC compared to the untreated silage. Although enzyme addition was applied at two different levels (i.e. 3 and 5 %), increasing enzyme addition to AOC at ensiling was not worthwhile since it did not make any significant contribution.

The second experiment was conducted to evaluate the effect of different dietary inclusion levels of AOC silage on the growth performance, nutrient digestibility and carcass characteristics in pigs. In this experiment, AOC silage was produced without enzyme additive and ensiled in 200

L drums for 90 days. Experimental diets that contained different levels of AOC silage (i.e. 0, 3 and 5%) were formulated and fed to twenty-seven Large White x Landrace (LW x LR) pig crosses, weighing approximately 22 kg live weight. The pigs were randomly allocated to the three experimental diets (9 pigs/ treatment) balanced on their live weights. Pigs were individually weighed at the start and weekly until the end of the trial. Data on daily feed intake, average daily gains and feed conversion rates were recorded. A nutrient digestibility study whereby daily feed intake and the outputs of faeces and urine were measured, was done a week after completion of the growth study. At the end of the nutrient digestibility experiment, pigs were fasted for twelve hours and weighed to determine the final weight. The pigs were then slaughtered at an abattoir and carcass samples were collected and analysed.

The growth performance of pigs was not ($P > 0.05$) affected by dietary inclusion levels of AOC silage, irrespective of increased fibre levels in the AOC silage diets. However, dietary addition of 3 % AOC silage improved ($P<0.05$) the digestibility of fibre fractions. The cold carcass weight and the chop were reduced ($P<0.05$) with AOC silage addition. In addition, AOC silage reduced ($P<0.05$) the back fat thickness of the pig carcasses, which has positive implications for human consumption.

A third experiment was conducted to evaluate the effect of different dietary inclusion levels of enzyme on the growth performance, nutrient digestion and carcass characteristics in pigs. In this experiment, AOC silage was produced without enzyme additive and ensiled in 200 L drums for 90 days. A total mixed ration that contained 5 % AOC silage was formulated. The diet was top dressed with enzyme at 0, 2.5 and 5 % of the daily ration of pigs. Twenty-four Large White x Landrace (LW x LR) crosses, weighing approximately 22 kg live weight were used. The pigs were randomly allocated to the three experimental diets (8 pigs/ treatment) balanced on their live weights. Pigs were individually weighed at the start and weekly until end of the trial. The pigs were fed on experimental diets *ad lib* in the morning, allowing a 10 % of feed refusal, and

free access to water was allowed. Data on daily feed intake, average daily gains and feed conversion rates were recorded. The dietary treatments were fed to growing pigs for 60 days. Growth performance, nutrient digestibility and carcass traits were evaluated. A nutrient digestibility study was done a week after completion of the growth study. Daily feed intake and the outputs of faeces and urine were measured. At the end of the nutrient digestibility experiment, pigs were fasted for twelve hours and weighed to determine the final weight. The pigs were then slaughtered at an abattoir and carcass samples were collected and analysed. This study showed that the addition of enzymes during the feeding of AOC silage diets to growing pigs improved (P <0.05) the dry matter intake (DMI) and nutrient digestibility without (P >0.05) improving average daily gains (ADG) and feed conversion rates (FCR) in pigs. Further, the carcass characteristics of pigs were not (P>0.05) affected by diets, but the small and large intestines were increased (P<0.05) with enzyme addition. The meat colour and the cooking quality were not (P>0.05) affected by dietary treatments. Increasing the enzyme addition to the pig diets was not worthwhile, and enzyme addition only affected the feed intake and nutrient digestion. It was concluded that the AOC can be ensiled and used at less than 5 % in the diets of growing pigs without any detrimental effects on the growth of pigs.

Keywords: additives, digestion, fats, feed, fermentation

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LIST OF ACRONYMS

ADF; acid detergent fibre

ADFI; average feed daily intake

ADG; average daily gain

ADL; acid detergent lignin

ADIN; acid detergent insoluble nitrogen

AIBPs; agro-industrial by-products

AOC; avocado oil cake

ARC; Agricultural Research Council

CCW; cold carcass weight

CF; crude fibre

CLM; cassava leaf meal

CM; canola meal

CP; crude protein

CPC; canola press cake

CSM; cotton seed meal

DDGS; dried distillers grains with solubles

DJM; detoxified jatropha carcus meal

DM; dry matter

DMI; dry matter intake

DL; drip loss

EE; ether extract

FCR; feed conversion ratio

FM; fresh matter

FSM; flax seed meal

FSBM; fermented soybean meal

GE; gross energy

GIT; gastro-intestinal tract

IMF; intramuscular fat

LA; lactic acid

LW; live weight

LW x LR; Large White x Landrace crosses

ME; metabolisable energy

MNC; macadamia nut cake

N; nitrogen

NDF; neutral detergent fibre

NE; net energy

NRC; National Research Council

PKM; palm kernel meal

PM; peanut meal

PUFA; polyunsaturated fatty acid

RSM; rape seed meal

SBM; soybean meal

SFM; sunflower meal

SSF; solid state fermentation

VFA; volatile fatty acid

VFI; voluntary feed intake

WSC; water soluble carbohydrate

WCW; warm carcass weight

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CHAPTER 1

1.1 Background

Pig production in South Africa (SA) is generally intensive, with feed costs accounting for 70 – 80 % of the production costs. Smallholder pig production in SA has the highest pig population in Southern Africa (Phiri et al., 2003), with 25 % of the population free ranging in resource poor areas (Krecek et al., 2004). Although these smallholder farmers contribute towards the national herds, the sector is faced with challenges that include unavailability of less costly nutritive feed (Lekule and Kyvsgaard, 2003, Lemke and Zárate, 2008). The pig production in the country is characterized by the use of conventional feed resources such as plant oilcakes (e.g. soybean meal, sunflower oilcake) and cereal grains (e.g. maize, wheat, barley). Utilization of these conventional feed resources increases the feed costs associated with pig production (Kemm, 1993). Additionally, droughts that took place in this country in recent years have made the prices of maize and soybean to increase further (DAFF, 2016). Moreover, the use of maize in pig production results in competition between animals and human beings. Some commercial pig farmers are however, producing their herds in places that lie in proximity to farms that produce the conventional feed resources. This has been practiced as a strategy to minimize transport costs. For smallholder farmers, the use of non-conventional feed resources such as agro-industrial by-products (AIBPs) is regarded as one of the strategies that can be used to reduce feed costs.

These by-products contain valuable nutrients that can benefit pig production, and can contribute to more sustainable pig production. However, the uses of AIBPs in animal nutrition have their own limitations, which include the presence of anti-nutritional agents (e.g. saponins, tannins, glucosinolates and phytates), high fibre and moisture contents. Fibrous feeds have not been considered suitable for pigs due to their low energy values, and are utilized less efficiently in the gastrointestinal tract (GIT) compared to sugars and starch (Carlson et al., 1999). Feeding high fibre diets to growing pigs is often associated with increased gut fill and gastrointestinal weight, which negatively affect the growth performance and lower the dressing percentage in growing pigs. This is attributed to the limited capacity for feed intake and hindgut fermentation of fibrous feed resources in young pigs. Techniques such as reduction of particle size, pelleting and supplementation of diets with exogenous enzymes have been applied to enhance the nutritional value and utilization of feed resources (Kim et al., 2005, Brufau et al., 2006). These methods are relevant particularly for locally available feed resources that are high in fibre content (de Lange et al., 2010). Reducing particle size has been reported to improve the nutrient digestion and feed efficiency of weaned pigs (Kim et al., 2005; Brufau et al., 2006). Furthermore, addition of multi-enzymes (mixtures of β-glucanase, α-amylase and protease) improves the total tract apparent digestibility of the fibre components in feed resources (Ngoc et al., 2011).

Avocado (*Persea Americana*) oil cake (AOC), a by-product that emanates from the oil extraction from avocado fruits that are unsuitable for the fresh fruit market in South Africa, is one of the AIBPs that causes disposal problems for fruit distributors. This by-product contains < 200 g dry matter (DM)/kg, 20 MJ/kg DM gross energy (GE), 119 g ether extract/kg DM (Nkosi et al., 2018), and *in vitro* organic matter digestibility of 54 % (Skenjana et al., 2006). The high fat and energy contents in AOC show that this by-product can benefit pig production, especially under intensive systems where high energy diets are fed. However, this by-product contains 518 g NDF/kg DM, 393 g ADF/kg DM, 258 g ADL/kg DM and 38 g ADIN/kg DM (Skenjana et al., 2006), making it a high fibre source. Feeding diets that contain > 100 g AOC/kg DM to chickens was reported to reduce the growth performance of the birds, with the high levels of condensed tannins and fibre in AOC diets as a reason for poor performance of birds (Van Ryssen et al., 2013).

1.2 Problem statement

The amount of AOC produced per day may not be fed to pigs in a day and may become rancid and toxic to animals if not properly stored for future use. The production of meal from this by-product is possible but smallholder pig producers in South Africa may not afford the machinery or equipment for drying this resource. Ensiling is one of the cost effective methods that can be easily adopted by smallholder pig farmers. This method involves the use of microbial additives such as enzymes (cellulytic, fibrolyitc, etc) and lactic acid bacterial (LAB) inoculants, which reduce the pH of the ensiled material, by increasing lactic acid production and preserve the forage (McDonald et al., 2010). This fermentation process has been shown to reduce anti-nutritional agents (e.g. phorbol esters in *Jatropha curcas L*. cake, de Oliveir et al., 2012), pathogens and fibre fractions of ensiled materials (Muck, 2010). Although feeding silage to growing pigs may have reduced the growth performance in some studies (e.g. Zanfi and Spanghero, 2012), feeding acid fermented feeds to pigs helps in the reduction of enterobacterial populations in the GIT of pigs (van Winsen et al., 2001, Canibe and Jensen, 2003). Further, supplementation of *Lactobacillus plantarum* to liquid feed has been reported to improve the growth performance of piglets compared to those fed liquid feed without LAB inoculation (Canibe et al., 2008).

In respect of high fibrous feed resources, the use of enzymes has been reported to degrade the fibre fractions of ensiled materials and improved feed intake of silage by animals (Kung et al., 2018). However, high level (70 %) addition of maize silage in the diets of pigs have been reported to increase dietary NDF compared to maize meal, which negatively affected the growth performance of the animals compared to those fed on maize meal (Zanfi and Spanghero, 2012). Feeding a diet containing 40 % potato silage to growing pigs reduced the average daily gains (ADG) of pigs compared to those fed on a control diet (Bohme et al., 2005). This makes it worthwhile to investigate the supplementation of silage based diets with enzymes for feeding to growing pigs.

It is important to note that the supplementation of enzymes to diets is done to target specific non-digestible diet components, especially fibre. Kanengoni et al. (2016) added enzymes when ensiling maize cobs and reported a reduction in silage fibre fractions with the enzymes compared to untreated silage. In contrast, some reports showed that enzyme addition to forage at ensiling did not affect cell wall contents of forages (Faber et al., 1989; Meeske et al., 1993). This shows that the type of enzyme, its dose and the type of forage play significant roles in the effectiveness of an enzyme when used at ensiling.

Research that evaluates the ensilability of AOC, the application of enzymes at ensiling of AOC and dietary supplementation of enzymes in diets that contain AOC silage on the growth performance, nutrient digestion and carcass traits of pigs is scarce.

1.3 Main aim of the study

The main aim of the study was to determine the dietary inclusion levels of avocado oil cake meal into pig diets and their effects on growth performance, nutrient digestibility, carcass characteristics and meat quality.

1.4 Study objectives

i. To determine the nutritive content in AOC
ii. To determine the effects of enzyme addition at ensiling on the fermentation characteristics, nutritive values and aerobic stability of ensiled AOC
iii. To determine the effects of dietary inclusion levels of ensiled AOC on the growth performance, nutrient digestion and carcass characteristics in pigs fed experimental diets
iv. To determine effects of dietary supplementation of enzymes on the growth performance, nutrient digestion and carcass characteristics in pigs fed experimental diets.

1.5 Hypotheses

i. The nutritive value content of AOC is consistent across different batches

ii. Enzyme addition at ensiling will improve the fermentation characteristics, nutritive values and aerobic stability of ensiled AOC

iii. Feeding diets containing dietary inclusion levels of ensiled AOC does not affect the growth performance, nutrient digestion and carcass characteristics of grower pigs fed experimental diets.

iv. Feeding diet containing dietary supplementation of enzymes will not affect the pig growth, nutrient digestion and carcass characteristics of grower pigs fed experimental diets.

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CHAPTER 2

Literature Review

2.1 Introduction

Generally, feed costs constitute approximately 70 % of the total cost of pig production (Gonzalez-Razo et al., 2010). An increase in feed cost translates to decreased profit margins, which negatively impact on the pig production and consequently the production of pork protein for consumers. Monogastric diets are mainly composed of maize and soybean meal as sources of energy and protein, respectively. These two nutrient sources are the most preferred pig feed ingredients owing to their essential feeding value (Stein et al., 2009). While demand for these ingredients is vastly growing in order to cater for the pig production, countries in the Sub-Saharan region rely on maize and soybean (Khojely et al., 2018) as staple food for human population. There is therefore competition for maize and soybean between humans and animals (Szebiotko, 1985). The demand for these resources is further exacerbated by the growing population, which is projected to double from 856 million to 2 billion by 2050 (Bremner, 2012). Seemingly prices for these ingredients is increasing (McGlone, 2013). Subsequently, the availability of these conventional feed ingredients (maize and soybean) will be insufficient and this will place an economic burden to pig producers. Alternatively, agro-industrial by-products (AIBPs) are used to replace conventional feed resources for animal nutrition.

2.2 Use of agro-industrial by-products as alternative feeds

Agro-industrial by-products (AIBPs) are defined as materials that are generated in the processing, production, distribution, transportation or consumption of foodstuff (Westendorf et al., 1996). Research has shown that some of the AIBPs consist of nutrients that could benefit animal nutrition in terms of growth and health (Ristanovic et al., 2009).

Identification of novel and unconventional feedstuffs including agricultural by- and waste products can serve as viable alternatives to traditional ingredients of animal diets (Álvarez-Fuentes et al., 2012). Such feedstuffs must not be directly utilized by humans to avoid competition between humans and livestock for food resources (Bampidis and Robinson, 2006). Another important incentive is to find ways of utilizing agricultural waste products and residues. These products tend to accumulate owing to the concomitant cost of disposing of them, and managing them to limit environmental pollution (Bampidis and Robinson, 2006).

According to Fang et al. (2016), some AIBPs have been suggested as effective alternative sources of nutrients that can meet the nutritional requirements of animals. However, AIBPs that could be of nutritional use and financial value in animal production remain poorly developed or unexploited (Agbo and Prah, 2014). This could be due to scanty information on their nutritive value and or how the nutritive value could be improved. Moreover, the usefulness of these by-products, of which most are of plant origin is restricted by the presence of secondary metabolites (tannins, gossypol, lectins, phytic acid, oxalic acid and glucosinolates) (Francis et al., 2001), as well as the high fibre and fat contents (Bedford and Schulze, 1998).

Several methods that can be used to reduce the concentration of secondary metabolites, include heat treatment, soaking, de-hulling, crushing (Abdelnour et al., 2018) and ensiling (Duodo et al., 2018). While ensiling is another method of preserving feed, it can also improve the digestibility of feedstuff through denaturation of the plant cell wall structure, thus releasing the nutrients for digestion by the host animal (Silva et al., 2016). In scenarios where ensiling is not effective to disrupt the cell wall structures, additives/inoculants are applied to the forage at ensiling (Weinberg et al., 2008). When properly utilized, AIBPs can overcome the high demand of conventional nutrient sources. It is thus important to also investigate the effects of these by-products on growth performance of pigs and quality of pork.

2.2.1 Use of conventional oil cakes or meals in pig nutrition

Conventional oil cakes or meals are AIBPs that are generated from oil extraction from seeds or grains such as soybeans, canola, sunflower, etc. These oilcakes or meals have been commercialized for use as animal feed ingredients owing to their nutritional value (Ramachandran et al., 2007). Their protein content in particular has been reported to range from 15 to 50% (www.seaofindia.com). Their nutritional values however, vary due to their variety, edaphic conditions, extraction methods (Ramachandran et al., 2007, Abdelnour et al., 2018) and treatment methods such as autoclaving, fermentation and soaking (Duodu et al., 2018). Treating of oil cakes might improve the nutrient quality of the resources. For example, Duodu et al. (2018) reported that long-term fermentation and soaking increased the crude protein (CP) content in cottonseed meal and groundnut meal.

2.2.1.1 Effects of dietary inclusion of conventional oil cakes on the growth performance of pigs

Soybean meal (SBM) is the most important protein source in farm animal nutrition, probably due to the high quality of the amino acid profile and the dependable supply (Steinfeld et al., 2006). The SBM represent about two-thirds of the total world output in protein feedstuffs (Oil-World, 2010). It is estimated that SBM accounts for 85 % of the protein supplements fed to pigs (Cortamira et al., 2000). It is usually classified as “high protein” with 490-500 g/kg crude protein and 30 g/kg crude fibre (CF), and “low protein” with 440-460 g/kg crude protein and 60-70 g/kg CF (McDonald et al., 2010). Moreover, SBM has a very good amino acid balance and contains high amounts of lysine, tryptophan, threonine, and isoleucine, which are often lacking in cereal grains. However, the amounts of sulfur amino acids are suboptimal for pigs, so methionine supplementation is necessary (McDonald et al., 2010). Amino acid digestibility in SBM is very high e.g. for lysine it is more than 90 % in pig diets (Anderson and Lardy, 2012). For non-ruminants, SBM is necessary to be heat-treated in order to inactivate several anti-nutritional factors, in particular trypsin inhibitors and lectins (Grala et al., 1998). All the above factors can depress growth rate and decrease efficiency of nutrient utilization when inappropriately heat-treated SBM is fed to pigs (Anderson et al., 1979).

Sunflower oil is a rich source of polyunsaturated fatty acids (PUFA), which have recently gained more interest in human and animal nutrition. According to Vaclavkova et al. (2011), the dietary inclusion of sunflower feed meal to fattening pigs resulted in higher content of PUFA in muscle and fat samples of the animals.

The protein content of rape seed meal (RSM) and canola meal (CM) is relatively high, varying between 310 and 370 g/kg. Noteworthy, CM has much higher lysine content, and comparable to SBM, whereas RSM has less lysine (Khajali and Slominski, 2012). Dehulling of RSM reduces fibre content and increases amino acid and nutrient digestibility (De L'Ange et al., 1998). Other researchers reported that extruded RSM could be an acceptable alternative feed to SBM in growing or finishing pig rations (Xie et al., 2012).

Wang et al. (2017) reported that CM is rated as the second most fed source after SBM in pig production globally. Using CM that contained 37 % crude protein (CP) and 20 % fat, Zhou et al. (2016) did not report any significant difference on the average daily feed intake (ADFI) and average daily gains (ADG) but increased feed efficiency in pigs when canola meal was fed at 20 % dietary inclusion level. This was supported by Wang et al. (2017) who reported that inclusion of canola meal at 20 % in pigs basal diet did not affect the average daily feed intake but improved the feed efficiency of the pigs. Landero et al. (2012) further supplemented diet with expeller-pressed CM in basal diets of pigs and reported reduction in apparent total tract digestibility of energy, dry matter and crude protein. This study shows that expeller-pressed canola meal did not affect body weight gain, feed intake and feed efficiency when added at 20 % in the diet. Zhou et al. (2016) reported that canola press-cake (CPC) contains 370 g/kg CP and 240 g/kg remaining oil. These researchers further indicated that CPC could be a source of amino acid and energy in pig diets. In their study, wheat-based diets were supplemented with 0, 50, 100, 150 and 200 g/kg of CPC replacing SBM in two phases (Phase 1 and 2). Increasing dietary inclusion of CPC linearly reduced the diet apparent total tract digestibility coefficient (CATTD) of gross energy (GE), diet digestible energy (DE) and calculated net energy (NE) values for Phase 1 and 2. Increasing dietary inclusion of CPC did not affect overall average daily feed intake (ADFI) and ADG in pigs. In conclusion, feeding up to 200 g/kg of CPC reduced CATTD of GE and CP, but did not affect overall growth performance of weaned pigs fed phase diets balanced for NE and standardized ileal digestible (SID) lysine/NE ratio.

According to Wang et al. (2017), nutritive quality of CM varies and may affect growth performance of pigs. In comparing the CM with SBM, dietary inclusion of CM decreased diet apparent total tract digestibility coefficient (CATTD) of DM by 0.037, of GE by 0.0036 and of CP by 0.040, whereas DE value of CM diets was maintained. Average daily feed intake (ADFI) and ADG of pigs did not differ between CM diets and the SBM diet for each week and for the entire trial, but gain: feed was greater for the CM diets than the SBM diet for day 1-7 and for the entire trial. In conclusion, inclusion of 200 g CM/kg to replace SBM did not affect ADFI and ADG in weaned pigs.

Landero et al. (2012) evaluated the effects of feeding increasing levels of expeller-pressed (EP) CM in substitution for SBM as an energy and amino acid source in pig diets. Increasing inclusion of EP CM linearly reduced the apparent total tract digestibility of energy, DM and CP and the DE content of diets. The researchers concluded that inclusion of up to 200 g EP CM/kg can replace SBM in diets formulated to equal net energy and standardized ileal digestible amino acid content and fed to nursery pigs starting 1 week after weaning without reducing growth performance.

Chae et al. (1999) conducted a study to compare growth performance of early-weaned pigs fed diet supplemented with SBM, dried skim milk (DSM), isolated soy protein (ISP), spray-dried plasma protein (SDPP) and wheat gluten (WG) as protein sources. The results indicated that at first week post-weaning, the average daily gain (ADG) and average daily feed intake (ADFI) of pigs fed the SDPP-based diet were higher relative to pigs fed diet containing other protein sources. On days 8 to 21 post weaning, the pigs fed SBM-based diet showed the lowest ADG and ADFI compared to others. The researchers concluded that SDPP was an excellent alternative protein source for the early-weaned pigs.

In a study using pea protein concentrate (PPC) as a substitute of SBM, soya protein concentrate (SPC) or full fat soya bean (FFSB) on crude protein basis in diets of piglets to evaluate productive performance and digestive traits of piglets, Valencia et al. (2008) found that piglets fed the SBM and SPC-based diets had higher coefficient of total tract apparent digestibility (CTTAD) of crude protein (CP) and higher coefficient of ileal apparent digestibility (CIAD) of organic matter (OM) and gross energy (GE) than pigs fed diets containing PPC and FFSB. Moreover, the growth performance of piglets fed diets with SBM and SPC was similar. However, the gastrointestinal tract weight of piglets fed diets containing PPC and FFSB was bigger than of those fed SBM and SPC-based diets. It was therefore concluded that substitution of SBM with PPC and FFSB in the diets reduced performance of the piglets from days 26 to 36 of age and impaired the ileal digestibility of OM and GE at 48 days of age.

When assessing the effects of replacing SBM with faba beans in grower and finisher diets for pigs, Smith et al. (2013) reported no effects on average daily gain, average daily feed intake and gain: feed of pigs on grower diet. These researchers concluded that pea and faba bean may be viable alternative to SBM in grower and finisher pig diets.

2.2.1.2 Effects of conventional oil cakes on carcass characteristics of pigs

Many studies have shown that RSM can be incorporated into pig diets with no detrimental effects on their performance or their carcass characteristics (McDonnell et al., 2010). Moreover, Gill et al. (1995) reported that RSM increased back fat thickness, while Warnants et al. (1995) reported that dorsal fat changed to weaker, thinner and pink. According to McDonnell et al. (2010), the substitution of SBM with RSM did not affect the carcass characteristics and the meat quality of the pigs. Moset et al. (2012) reported that the inclusion of RSM in pig diets can affect slurry composition and thus influence gas emission and particularly methane from the animal faeces.

Skoufos et al. (2016) evaluated dietary RSM as an alternative to SBM on growth performance and meat quality of growing-fattening pig. The pigs on the control group were fed 10 and 11 % commercial SBM based growing and fattening rations. The pigs on treatment were fed with isocaloric and isonitrogenous rapeseed meal included at 10.6 and 16.7 % to replace SBM in a ration. Body weight gain and FCR of the pigs did not differ during the growing and the fattening Steak cuts from pigs fed the RSM diet had higher total monounsaturated fatty acids and lower saturated fatty acids and polyunsaturated fatty acids compared to those fed the control diet. No differences were found on the ham and steak meat lipid oxidative stability after 4 or 7 days of refrigerated storage.

According to Yun et al. (2017), replacing SBM with 4 % RSM or 4 % CM has no effects on ADG, ADFI, gain: feed ratio, concentration of blood urea nitrogen and creatinine and apparent total tract digestibility of DM, N and GE. They concluded that inclusion of 4 % of RSM or CM in finishing pig diets had not negative effects on growth performance, nutrient digestibility, faecal noxious gas emission, blood characteristics and meat quality.

A study by Little et al. (2015) showed that the replacement of SBM in growing finishing pigs diets with high-protein canola meal (CM-HP) or conventional canola meal (CM-CV) at 33 %, 66 % and 100 % did not affect the warm carcass weight, loin eye area, carcass yield, and 10th rib back-fat thickness or carcass lean of pigs. Carellos et al. (2005) fed growing pigs diets that contained 16 % SM and reported no detrimental effects on carcass traits. However, the replacement of SBM with either sweet lupins, CM or full-fat canola in pig diets resulted in heavier cold and warm carcass weight in pigs fed diets containing 17 % of full-fat canola and 8 % of CM than those that were fed the control diet. Pigs that were fed a diet containing 8 % of sweet lupin as a dietary protein source had leaner carcasses compared to those that were fed 17 % of CM containing diet (Smith, 2005).

According to Shelton et al. (2001), comparison of the effect of SBM with other protein sources [crystalline AA (corn-AA), extruded soybeans (ESB), CM, peanut meal (PNT), SFM, ground peas, meat and bone meal (MBM), and poultry by-product meal (PLTY)] on growth performance and carcass traits of pigs showed that pigs fed diets with SBM had higher ADG than those fed diets with corn-AA, SFLR, MBM, or PLTY. Additionally, this study showed that pigs fed SBM-based diet had higher ADFI than those fed diet with corn-AA, ESB, MBM or PLTY as protein sources. Pigs fed diets with corn-AA, peas and MBM had greater tenth-rib back fat thickness compared to those that were fed the SBM-based diet. These results suggest that pigs fed SBM supplemented diet have equal or better growth performance and carcass traits relative to pigs fed diet with other protein sources (Shelton et al., 2001).

2.2.2 Use of non-conventional oil seed by-products in pig nutrition

Non-conventional oilseed by-products are used as alternative feed resources or replacement for conventional oil cakes/meals in animal nutrition. These include amongst others, palm kernel meal (PKM), macadamia nut cake (MNC), copra kernel meal and RSM etc. (Fatufe et al., 2007). Some of these by-product have been shown to contain valuable nutrients that can benefit animal nutrition (Table 2.1). The data shows that these by-products had a DM, CP and ether extract (EE) that range from 155 to 940, 90 to 179 and 13 to 182 g/kg DM, respectively.

Table 2.1 Chemical composition (g/kg DM, unless stated otherwise) of non-conventional by-products for animal nutrition

By-products	DM	CP	GE (MJ/kg)	EE	NDF	ADF	ADL	Ash	References
Olive oil cake	470	-	-	13	691	551	278	13	Hadjipanayiotou (1994)
Palm kernel meal	883	179.9	19.9	126.3	682.0	460.5	-	39.4	Agunbiade et al. (1999)
Avocado pulp	186	147	-	182	652	-	269	45.0	Eliyahu et al. (2015)
Citrus pulp	155	90	-	-	301	198	-	74	Moset et al. (2015)
Macadamia oilcake meal	940	132	-	-	-	-	-	28	van Ryssen et al. (2014)
Rapeseed oil cake	929	169	20.23	192	-	-	-	-	Jorgensen et al. (1999)

DM = dry matter; CP = crude protein; GE = gross energy; EE = ether extract; NDF = neutral detergent fibre; ADF = acid detergent fibre; ADL = acid detergent lignin

2.2.2.1 Effects of dietary addition of non-conventional by-products on the growth performance and nutrient digestibility in pigs

Palm kernel meal (PKM), a by-product that contains 23 % CP, 5 % crude fat (CF) and 3 % of ME was added in a grower feed at 20 %, and was reported to reduce the gain-to-feed ratio, but increased ADG and ADFI in pigs compared to those fed the control diet (Seo et al., 2015). However, Rhule (1996) added PKM at 0, 20, 30 and 40 % in pigs' grower and finisher diets, and reported to have lack of effect on ADG, but increased FCR at grower phase. Replacing maize with PKM in diets of pigs (Adesehinwa, 2007) and the FCR of the pigs decreased with increasing levels of PKM. The PKM can effectively replace maize at 30 % without depressing growth performance of pigs and efficient utilization of diets.

Avocado (*Persea americana*) oil cake (AOC), is another non-conventional feed resource that contains 949 g DM/kg, 156 g CP/kg, 63 g CF/kg and 349 g CF/kg, and has an *in vitro* organic matter digestibility (IVOMD) of 54 % (Skenjana et al. 2006). Van Ryssen et al. (2013) fed chickens diets containing 7.3, 14.7, 22 and 29.3% of AOC and reported increased feed intake in chickens, but the ADG and FCR in chickens fed the diets were poor compared to those on the control diet. The reason for the poor performance was attributed to the high fibre and presence of tannins in diets containing AOC. The researchers concluded that AOC was not a suitable feed ingredient for chickens, especially at more than 15 % dietary inclusion level. This problem of tannin content can be sorted by treating the resource as indicated by Duodo et al. (2018).

Berrocoso et al. (2017) carried out a study wherein an optimum level of macadamia nut cake (MNC) inclusion in pastured broiler diet on growth performance was determined. The results showed that the MNC can be incorporated as high as 15 % in the broiler diets without any adverse effect on their growth performance. Van Ryssen et al. (2014) conducted a study to determine the potential of MNC and wood ash as feed ingredients for poultry. Three basic diets were formulated: one without MNC and the other two containing 10 and 50 % MNC. Each of these diets was split into two: one receiving feed lime ($CACO_3$) as the main source of calcium (Ca), and the other wood ash, which contained 257 g Ca/kg. In the two Ca diets containing 50 % MNC, the Ca and phosphorus (P) concentrations of the tibiae were significantly lower than in the diets containing lower levels of MNC.

Replacing 0, 25 and 50 % of SBM with 0, 54, 102 g/kg of detoxified *Jatropha curcas* kernel meal (DJM) in diets of growing pigs on their growth, visceral organ weight and serum biochemical parameters, revealed that DJM had no effect on weight, weight gain and feed to gain ration of the pigs (Wang et al., 2011). Additionally, the replacement of SBM with DJM in diets had no significant effects on visceral organ weights, serum biochemical parameters and histomorphology of kidneys and livers of the pigs. These results indicate that the nutritive value of the DJM supplemented with additional lysine is equivalent to that of SBM for pigs (Wang et al., 2011).

Babatunde et al. (1990) studied the effects of replacing graded levels of SBM with rubber seed meal containing 16 % CP at 10, 20 and 30 % in pig diets on the growth performance, plasma metabolites hematocrit and nitrogen utilization of pigs. The results revealed that there were no differences in ADG, ADFI and feed: gain ratios. However, the ADG and feed: gain ratio decreased with increasing levels of rubber seed meal. While hematocrit was similar across the treatments, plasma protein and albumin were lowered by the inclusion of rubber seed meal above 10 %. Apparent digestibilities of GE, DM and nitrogen (N) were lower in pigs fed diets containing 20 % of rubber seed meal, but the apparent N retained and percent of digested N retained were not reduced. The researchers concluded that although rubber seed meal is of poor quality for growing pigs compared to SBM, at least 10 % of the meal can be included in pigs' diets without adversely affecting growth and N utilization.

Cotton seed meal (CSM), a by-product of the oil extraction from the cotton seeds, is limited in pig nutrition due to its high fibre content (110-130 g/kg), variable protein amount (average 410 g/kg), lower amino acid content and availability and the presence of gossypol (Adeniji and Azeez, 2008).

Traditionally, flax seed is crushed to produce linseed oil and the resultant flax seed meal (FSM) can be used as a protein source in animal feeding (McDonald et al., 2011). Flax contains approximately 200 g/kg α-linolenic acid, essential n-3 fatty acid, which is associated with several possible health benefits (Simopoulos, 2008). Some researchers (Kouba et al., 2003, Juarez et al., 2010) reported that pigs fed dietary flax showed no differences in performance or carcass traits, whereas they had increased n-3 fatty acid concentrations in muscle and fat tissues.

Dietary peas and faba beans may be viable alternative protein source to SBM, especially during the growing and finishing periods of pigs (Smith et al., 2013). Peas have high levels of lysine and reasonable levels of other essential amino acids, with protein digestibility being lower than that of SBM (Gatel, 1994).

2.2.2.2 Effects of dietary inclusion of non-conventional by-products on carcass characteristics of pigs

Inclusion of 15 % high fat rape seed press cake (34 % CP, 18 % EE and 13 % CF) in pig diet did not affect muscle leanness, but increased drip loss and back fat thickness and reduced sensory evaluation score (Schönet et al., 2002). However, supplementing diets with 3 % of kapok (*ceiba pentandra*) seed meal improved meat quality and enhanced the fatty acid content in carcass fat and muscles without causing any adverse effect on growth performance of pigs (Li et al. 2018). Dietary inclusion levels of PKM up to 40 % in pigs' grower diets decreased carcass dressing percentage but did not affect the back fat content of the pig carcasses (Rhule, 1996). Feeding pigs on avocado wastes also had a significant effect on the intramuscular fat content and composition by reducing the lipid content in the muscles and increasing the unsaturation degree, but did not increase the oxidative instability of the muscles (Hernández-López et al., 2016).

According to Kim et al. (2001), the effects of partially replacing soybean meal (SBM) with 2 to 4 % PKM and copra meal (CPM) reduced the nutrient digestibility. The carcass length of pigs fed diets with 2 % CPM was longer than those fed 4 % PKM-based diets. The inclusion of PKM or CM in the diet did not affect the total saturated fatty acids and unsaturated fatty acids in the back fat of finishing pigs. It was then concluded that copra meal can be a valuable source of protein in the diet for finishing pigs and may replace other protein sources in pig diets to a considerable extent.

In a study by Rhule (1995), Large White pigs were fed from 25 to 90 kg live weight on diets containing PKM at 0, 200, 300 and 400 g/kg inclusion levels. Dressing percentage and eye muscle reduced with increasing levels of PKM in the diet, while the back fat thickness was not affected. Yaophakdee et al. (2018) conducted a study that determined the effects of feeding different levels of PKM on live performance and gut morphology of broilers (1 to 35 days of age). The birds were fed a maize-SBM basal diet with low (5 and 7.5 %) and high (10 and 15 %) PKM levels in starter and grower diets, respectively. The results showed that the birds fed high PKM diets had higher FCR than those fed the low PKM diet. It was concluded that feeding high levels of PKM had a negative effect on the feed efficiency but not on the gut health and litter quality of broilers.

In a study investigating the performance and carcass traits of pigs on diets containing varying amounts of peanut meal (PM) by Hale and McCormick (1979), it was found that pigs on diets 1 (consisting of 100 % SBM) and 2 (consisting of 75 % SBM and 25 % PM) had lower FCR than the other pigs. Carcass length and back fat depth were similar for pigs on all diets. Area of the *longissimus dorsi* muscle at the 10^{th} rib (loin-eye area) was also similar for pigs on diets 1, 2 and 3 (consisting of 50 % SBM and 50 % PM), but smaller in pigs on diet 4 (consisting of 25 % SBM and 75 % PM) and a further decrease occurred in pigs on diet 5 (comprising of 100 % PM).

Grageola et al. (2010) replaced maize/SBM with AOC at 200 g/kg and compared the growth performance in two pig breeds. They reported poor N retention in Pelon Mexicano pigs compared to Yorkshire x Landarace breeds. Dietary avocado wastes had significant impact on the content and composition of intramuscular fat (IMF), reducing the lipid content in *longissimus thoracis et lumborum* (LTL) muscles and increasing the degree of unsaturation. The muscles from pigs fed on diets containing avocado wastes had significantly lower lipid and protein oxidation rates during chilled storage (Hernández-López et al., 2016). In particular, pigs treated with the avocado wastes had significantly smaller amount of IMF and as a result, significantly higher moisture content. The lower tendency of animals fed on the avocado waste to deposit fat was unexpected given the higher fat, energy and daily intake (1.49 ± 0.23 kg/day vs 2.04 ± 0.24 kg/day for control and treated, respectively) in the treated group compared to the control counterparts. On the other hand, increasing the gross level, has a negligible effect on IMF as reported by D'Souza et al. (2007). According to D'Souza et al. (2007) including avocado wastes in an isoprotein diet leads to a significant reduction in IMF content of LTL muscles. In fact, abudant literature indicates that IMF is less affected by differences in the diet than back-fat thickness (Hocquett et al., 2010). The IMF content has relevant consequences for meat quality in terms of eating quality traits, nutritional value and caloric delivery to humans. It is generally known that IMF reduction compromises consumer acceptability of pig meat (Lawrie, 1998). A significant reduction of the lipid content in loins from pigs fed on avocado wastes may be seen as an advantage as current consumers' trends indicate preferences to leaner pork (Ngapo et al., 2007).

Pigs fed diets containing avocado wastes had significantly higher levels of α-tocopherol than those from control pigs. This result could be explained because avocado tissues including the peel, the seed and the pulp are rich in diverse natural antioxidants such as α-tocopherol (Rodríguez-Carpena et al., 2011). AOC and its oil have high content of unsaturated fatty acids (Rodríguez-Carpena et al., 2011) and hence the differences found in muscles from both treatments were expected and consistent with the fatty acid composition of the diets. Pigs fed the treated diet had higher levels of linoleic acid in IMF because linoleic acid is one of the most abundant in the avocado oil (Rodríguez-Carpena et al., 2011).

Two experiments were conducted Diarra et al. (2017) to investigate the effects of replacing feed protein (fish and SBM) with cassava leaf meal (CLM: a non-conventional protein source) protein in weaner and growing pigs' diets. In experiment 1, CLM protein replaced the feed protein at 0, 15 and 30 % in weaner diets. The results in experiment 1 showed that feed intake (FI), body weight gain (BWG) and FCR were improved and feed cost of gain was reduced on the 30 % protein replacement, while dressing percentage was maximized on the 15 % protein replacement diets. In experiment 2, feed protein was replaced with CLM protein at 0, 30 and 45 % in pigs' grower diets. The FI and BGW were reduced while FCR and feed cost of gain were increased above 30 % protein replacement. The researchers concluded that replacing 30 % of feed protein with sun-dried CLM protein will maintain growth and reduce cost of pork production.

In determining the digestibility of DM and GE of macadamia nut cake (MNC) using an *in vitro* model, Tiwari and Jha (2017) found that the concentration of lysine was 0.7 %. The DM and GE digestibility were 76 and 71 %, respectively. The GE content of MNC is comparable with that of maize and higher than that of SBM (Tiwari and Jha, 2017). A study was conducted to evaluate the potential of MNC as a replacement of SBM in Ross broiler diets (Acheampong-Boateng et al., 2016). The broilers fed diet with 100 % MNC had the least feed intake, final body weight and weight gain compared to those fed other diets. The increased abdominal fat of broilers fed more 50 % level of MNC was attributed to high levels of lipids in MNC compared to SBM. It was concluded that the threshold of 25 % MNC can replace SBM in the diets of broiler provided that tis alternative feed ingredient is readily available at an affordable cost (Acheampong-Boateng et al., 2016).

2.3 Effect of feeding high fibre diets in pig nutrition

Dietary fibre is usually defined as the indigestible portion of food derived from plants and it forms a key component of many animal diets. This includes a wide range of carbohydrates (non-starch polysaccharides) that are resistant to hydrolysis in the small intestines of animals (Low, 1993). The effect of dietary fibre concentration on feed intake is variable and related to factors such as age of a pig, botanical origin of the fibre, processing method and the chemical composition of the diet (Low, 1993). However, if growing pigs are fed *ad lib*, dietary energy content is the factor in controlling feed intake, and differences in fibre content should not affect DM intake provided that the feed bulk and palatability are acceptable (Coffey et al, 1982).

According to Machin (1990), feeding pigs fibrous feeds is economical for matured pigs with a body weight of above 50 kg due to their ability to efficiently digest fibre compared to young pigs. Young pigs from 21 to 28 day-old, require time for the development of the gastro-intestinal tract and size enlargement of the secretory capacity to digest fibrous feed post weaning (Cranwell and Moughan, 1989). In young pigs, the digestibility of fibre reduces as the quantity of dietary fibre increases and consequently there is limited provision of nutrients particularly energy to the pig (Noblet and Le Goff, 2001). High fibre diet can reduce the feed intake, thus growth performance and increase incidents of intestinal diseases especially in piglets whose gastro-intestinal tract is not well developed with optimum amount enzymes and microorganisms to efficiently utilize the fibre (Bartoloni and Bortolozzo, 2004).

While Manchin (1990) regards mature pigs as economical in terms of fibre digestibility, several authors (Noblet and Goff, 2001; Knudsen et al., 2012, Rojas and Stein, 2017) indicated that high fibre feeds are not appropriate for pigs of any age due to low energy content associated with high fibre diets. It is therefore imperative to know the energy value of a roughage prior to feeding pigs. In contrast, feeds high in fibre content have beneficial effects on the health status of pigs (Elbers et al., 1995). Pigs need a minimum amount of fibre in the gastro-intestinal tract to maintain normal physiological status of pigs (Wenk, 2001). Fibrous diets might have prebiotic effects on pigs through the interaction between the gut and immune system (Lindberg, 2014).

2.3.1. Effects of feeding high fibre diets on growth performance of pigs

Several research has been conducted in monogastrics to attest that dietary fibre is considered a diluent in the diet (Rougière and Carrè, 2010), with negative implications associated to voluntary feed intake (Mateos et al., 2002). Consequently, commercial diets particularly those for young broilers or pigs are compounded to contain less than 3 % CF. However, it has been scientifically proven that inclusion of moderate quantities of different fibre sources in the diets improves digestive organ development (Hetland and Svihus, 2007) and increases hydrochloric acid, enzyme secretion and bile acids (Svihus, 2011). These variations can result in improvements in growth performance (González-Alvarado et al., 2010). Moreover, depending on the amount of dietary fibre, as well as the basal diet composition, the existing microbiota profile in the distal part of the gastrointestinal tract might be affected (Shakouri et al., 2006). Sklan et al. (2003) reported that an increase in dietary fibre content (from 3 to 9 %) in the diet increased ADFI in pigs of 1 to 4 weeks old but reduced body weight and FCR at 11 to 14 weeks old. It was evident from the study that the level of fibre content and the age of the animal affected the animal performance in terms of feed intake, body weight and FCR.

Inclusion of dietary fibre to pig diets increases fibre fermentation and the availability of desirable microbiota in the gut (Nahm, 2003). There is evidence that inclusion of dietary fibre to pig diets results in dietary N shifting from the urea form in urine to bacterial form in faeces, which is known to be less volatile (Otto et al., 2003). In addition, fibre inclusion to pig diets reduces pollutants which contribute to global warming (Le, 2009). Common fibre sources (maize cob, grass hay, lucerne hay, maize stover and sunflower husk on VFI have been examined (Ndou et al., 2013). These authors reported that feed intake decreased uniformly across fibre sources tested up to 160 g/kg inclusion level. At high fibre inclusion levels, pigs are expected to consume more feed to compensate for the reduction in nutrient density and for them to meet nutrient satiety. The gut capacity of the pigs, however prevents them from continuing to increase VFI. Consequently, growth performance starts to drop (Le Goff et al., 2003).

2.3.2. Effects of feeding high fibre diets on nutrient digestibility in pigs

Several researchers investigated the digestibility of high fibre feeds such as lucerne (Anderson and Lindberg, 1997), clover, grass silage and sugar beet pulp (Just et al., 1983) in pigs. Despite the differences in the DM content of the feeds, their dry matter daily intake and digestibility was similar (Anderson and Lindberg, 1997; Just et al., 1983). The effect of fibre on protein digestion in the small intestines partially depends on the solubility of fibre in water (Li et al., 1994). High fibre diets decrease energy and nutrient utilization in pigs due to lack of the enzymes necessary to break down dietary fibre, and are known to increase intestinal weight. Glucose is a major energy source in pig diets, and feeding high fibre diets reduces active glucose transport. This results in reduced nutrient absorption (Agyekum et al., 2015). Diets supplemented with high amounts of insoluble dietary fibre (e.g. soybean hulls or lucerne meal) contain significantly low ME (13 MJ/kg DM) in comparison with a diet supplemented with sugar beet pulp (14 MJ/kg ME), which is characterized by high nutrient digestibility (Freire et al., 2000). This shows that nutrient digestibility decreased due to lower dietary energy concentrations.

2.3.3. Effects of feeding high fibre diet on carcass characteristics of pigs

Partanen et al. (2001) reported that carcass characteristics are not affected by the fibre content in basal diets. However, high concentration of insoluble dietary fibre in diets can increase the weights of the stomach and large intestines. When supplementing substrates rich in insoluble dietary fibre (e.g. wheat bran, barley hulls, oats hulls) in pig diets, the activity of enzymes in the small intestines is enhanced, while diet supplementation with soluble dietary fibre (e.g. potato starch and wheat middling) tends to decrease intestinal maltase activity. Jorgensen et al (1996) found that animals fed diets high in fibre had higher ADG compared to those on a low fibre diet. Their reasoning was based on that the weight and size of the visceral organs and gastrointestinal tract increase mainly due to the higher water holding capacity of fiber. However, high fibre diets negatively affects the hot carcass and the dressing percentage in pigs (Len et al., 2008). This is probably due to the increased weight of visceral organs and the gastrointestinal tract.

2.4 Strategies used to reduce high fibre in forage

2.4.1 Silage making

Ensiling forage is a conservation method consisting of storing feed under anaerobic conditions resulting in a fermentation process. During the fermentation process, several energy substrates, including organic acids, soluble sugars and soluble nitrogen compounds are utilized by fermentative bacteria to produce volatile fatty acids such as butyrate, propionate, lactate and acetate (Santos et al., 2010). The ensiled materials have abundant quantity of nutrients than the dry collected material stored as hay (McDonald et al., 2010). Hence, silage is considered the better way to conserve forage crops (Chedly and Lee, 2000), and has been a preferential method to maintain the energy nutrient content of crops, ensuring a good nutritional value when used as feed (Vervaeren et al., 2010).

An advantage of ensiling AIBPs is that since by-products have the potential of being utilized as feed ingredients owing to their nutritional value, ensiling by-products possess an environmental significance by way of recycling material and thus reducing pollution (Huber, 1980). However, the disadvantages are that the use of by-products as ingredients in animal feed is discouraged by the presence of secondary metabolites such as saponins, phytic acid, flavonoids and mycotoxins that can interfere with the absorption of nutrients in the intestines. Reduced nutrient absorption compromises the growth performance of pigs (Pluske et al., 2018). In addition, although ensiling is viewed as a vital method for long term preservation of feedstuffs including the by-products, ensiling by-products that are high in moisture content (> 600g/kg) is difficult (Kim et al., 2014). The neutral detergent fibre content of the by-product can also decrease due to cell wall degradation by acid hydrolysis or cellulolytic clostridia during the ensiling process (McDonald et al., 1991).

2.4.1.1 Effects of feeding silage on growth performance of pigs

According to Presto et al (2013), feeding pigs silage has the potential to improve pig welfare. Jones et al. (1980) fed growing pigs (average 25.5 kg LW) maize silage treated without or with propionic acid. Maize silage was added at 70 % fresh form of total diet. Pigs fed on diets containing treated maize silage had faster gains and improved feed efficiency. Zanfi and Spanghero (2012) substituted maize meal with maize silage at 15 and 30 % in diets of pigs and reported high palatability and no differences in DMI of pigs fed the maize silage. Previous studies (e.g. Zanfi et al., 2014) demonstrated that maize silage can be included at moderate levels (20 – 40 % DM) in diets for finishing heavy pigs without detrimental effects on growth and slaughtering traits. However, high (50 % DM) dietary inclusion of maize silage has been reported to reduce the growth performance in fattening pigs (Scipioni and Martelli, 2001).

Bocian et al (2017) fed fattening pigs on diets that contained 1.4 % of steamed potato silage and reported reduced feed intake and growth rate compared to pigs fed on the control diet. Feeding *ad lib* a diet that contained 20 % silage (either chicory or red clover) resulted in reduced growth rate compared to pigs on a commercial liquid feed (Akerfeldt et al., 2018). However, the researchers reported an improved feed efficiency and growth performance in pigs fed diets containing the 20 % silage. Under adequate feeding and management systems, growing pigs are expected to have an ADG of 640 g/d (Payne, 1990). Dom and Ayalew (2009) fed pigs on diets that contained 50 % sweet potato silage and obtained ADG of 850 g/d. According to Sarria and Martens (2013), growing pigs (> 45 kg live weight) can ingest bulk feed when the DM content is > 440 g DM/kg FM without presenting physiological constraints to the pig. The researchers further reported that the DM content of silages can affect feed intake in pigs. At 12 kg live weight, pigs fed silage based diet achieved similar ADG to that in pigs fed the commercial diet (Carter et al., 2017). In the growing and finishing phase, Carvhalo et al (2011) reported that the inclusion of levels of sticky coffee hull silage did not affect feed intake and weight gain in pigs. However, in the finishing phase, FCR improved as the levels of sticky coffee hull silage increased.

Lee et al (2009) added 40 and 60 g/kg fresh matter (FM) of fermented apple pomace in diet of pigs and reported improved feed intake while FCR was decreased when the by-product was added at 20 g/kg FM. Chamorro-Ramirez et al. (2017) fed diets containing 50 and 100 g/kg FM to growing pigs and reported no reduction in feed intake, which indicated that pigs were able to consume fermented apple pomace at these levels, probably because the by-product contained quality protein.

In a study evaluating the nutritional quality and bioactivity of SBM by solid-state fermentation (SSF) with *Bacillus subtilis*, Dai et al. (2017) indicated that the contents of crude and soluble proteins, total phenol and flavonoid all increased distinctly, while trypsin inhibitor content decreased after fermentation. The fermented FSBM was found having a strong inhibition on angiotensin I-converting enzyme, revealing that FSBM has antihypertensive activity. These results suggest that SSF with *Bacillus subtilis* can substantially improve both the nutritional quality and bioactivity of SBM.

Piglets were used to study the effects of *Aspergillus oryzae* on FSBM and unfermented SBM on growth performance, digestibility and activities of intestinal enzymes (Feng et al., 2007). The results showed that the piglets fed FSBM diet had an increase in ADG and a reduction in feed: gain ratio compared to the control. There were significant effects of FSBM fed piglets on coefficients of total tract apparent digestibility of DM, CP and energy that were observed in piglets than those fed the SBM diet. In conclusion, Feng et al. (2007) reported that the FSBM is beneficial to growth performance, digestibility of dietary components and activities of intestinal enzymes in piglets.

2.4.1.2 Effects of feeding silage on carcass characteristics of pigs

Some forages (e.g. chicory) have the ability to stimulate hindgut development, which resulted in heavier colons in pigs (Ivarsson, 2012). This could lead to lower dressing percentage in pigs fed the chicory diet. Compared to pigs fed diet containing 1.4% of steamed potato silage, those that were fed untreated silage (control diet) had thinner back-fat, higher loin muscle and higher meatiness (Ivarsson, 2012). Thacker (2006) reported a linear decrease in dressing percentage and loin as dietary inclusion level of dried distillers grains with solubles (DDGS) from wheat was increased without any adverse effect on growth performance. When restricting concentrate diet with a combination of *ad lib* supply of silage to pigs, carcasses of higher lean meat were reported in comparison with those on concentrate feed (Hansen et al., 2006). Moset et al. (2015) fed pigs (77 kg live weight) on diets containing citrus pulp silage and observed a decreased carcass yield while increasing the back fat of the carcasses. The polyunsaturated fatty acid content of the subcutaneous fat layer decreased with citrus pulp silage inclusion. The nutritive value of citrus pulp silage for finishing pigs is low and levels of 150 g/kg FM can negatively affect carcass yield. The inclusion of 100 g citrus pulp silage/kg did not affect carcass yield and subcutaneous fat in pigs. In comparison to a concentrate diet, feeding pigs with either barley and or pea silage resulted in lean meat of high quality (Hansen et al., 2006).

Capraro et al. (2017) fed heavy pigs (90 – 160 kg live weight) on diets supplemented with 30 % maize silage and found that the hams from pigs fed maize silage diets were lighter compared to those fed on concentrate diet. Further, the pigs on the maize silage diets had a significant increase of saturated fraction of the back fat, mainly due to the increase of the C18:0 fatty acid. These researchers concluded that supplementing heavy pigs with maize silage improved the back fat quality in terms of fatty acid composition. In terms of consumer health, it would be preferable to have a decrease of saturated fatty acids to reduce several risks, primarily represented by coronary disease. Sticky coffee hull silage has good nutritional value and if used in levels up to 16 % of the diet, it does not impair performance of pigs in the growing and finishing phases and it results in leaner carcasses (Carvhalo et al., 2011).

2.4.2 Dietary inclusion of enzymes in silage

Dietary fibre components are not digested by endogenous digestive enzymes, and consequently are the main substrates for bacterial fermentation in the distal part of the gut (Lindberg, 2014). Treatment of ensiled material with enzymes increases the availability of nutrients. Furthermore, enzymes are used as silage additives in order to increase the energy source available for lactic acid bacteria by promoting the breakdown of cellulose and thus availing cell wall contents. The cell wall contents can be moderately degraded by cellulolytic enzymes during fermentation, which will result in increased forage DMI (McDonald et al, 1991). Failure of enzymes to degrade the forage cell wall could be as a result of enzyme denaturation in the stomach or high population of lactobacilli with β-glucanase activity in the small intestines of pigs (Kung et al., 2003). The presence of lignin, poor solubility and crystalline structure of cellulose also contribute to poor enzymatic forage degradation (Jayasekara and Ratnayake, 2019).

2.4.2.1 Effects of feeding diets treated with enzymes on growth performance of pigs

Several studies have been carried out to evaluate the effect of dietary enzymes on growth performance (Table 2.2) and carcass characteristics of pigs (Table 2.3). Morgan (1995) for instance, found that supplementation of dietary xylanase in pig diets resulted in improvement of ADG, DFI and feed: gain (F: G). Supplementation of dietary xylanase in by-products based diets of pigs was reported to improve ADG, ADFI and FCR in pigs (Fang et al., 2007, Borges et al., 2014).

In a study by Tangendjaja et al. (1988), both cellulase and amylase inclusion in diet increased the NDF digestibility but reduced the FI. Mellange et al. (1992) however, reported that dietary enzymes in wheat-based diet had no beneficial effects on the growth performance of pigs. This could be attributed to the type and or concentration of the enzyme included in the diet. A study using broilers has shown that not only is the type of enzyme significant but also the concentration of enzymes, to the size and direction (negative or positive) of the animal's response to the included enzyme (Bedford and Inborr, 1993).

Shim et al. (2003) conducted a study to investigate the effects of microbial phytase (Natuphos®) supplementing in combination with carbohydrates [composed of enzymes targeted to SBM dietary components such as α-galactosides and galactomannans Endo-Power®] to maize-SBM based diet and complex diet (CD) with partial replacement of SBM with RSM and cotton seed meal (CSM) on growth performance and nutrient digestibilities of growing pigs. Results showed that dietary phytase and carbohydrates supplementation significantly improved gain: feed ratio, nutrient digestibility, and overall ileal amino acid digestibilities of SBM, RSM and CSM based diets by growing pigs. The results suggest that 2.5 to 3 % of RSM and CSM, respectively, might be used as a protein source in growing pig diets without having an adverse effect on the growth performance and nutrient digestibility.

The effects of diets containing 20 % sweet lupin (*L. luteus* or *L. augustifolius*) with and without enzyme supplementation (Rovabio ™ Excel AP) on growth performance and carcass characteristics were evaluated (Roth-Maier et al. 2004). Lupin-based diets showed higher daily gains and better FCR in the growing period. A significant enzyme effect was observed in feed efficiency in the supplemented groups. The carcass characteristics were not affected by either lupin variety or by enzyme supplementation. Pettey et al. (2002) carried out a study wherein effects of the inclusion of 0.05 % *β*-mannanase in maize-SBM diet (control) on growth performance and nutrient digestibility of weaning and growing-finishing pigs was determined. Addition of soybean oil to increase ME in the diet was also evaluated. The results showed that soybean meal oil addition in corn-soybean meal diet improved gain: feed compared to those that were fed the control diet. The gain: feed was similar for pigs fed diets with soybean oil or *β*-mannanase. Addition of *β*-mannanase in corn-soybean meal diet increased average daily gain and lean gain of the pigs than those on control or soybean oil-based diets. However, the *longissimus* muscle area or back fat of pigs among the treatments were similar. The results suggest that while *β*-mannanase may improve growth performance in weanling and growing-finishing pigs, it has minimal effects on the nutrient digestibility.

2.4.2.2 Effects of feeding diets treated with enzymes on carcass characteristics of pigs

In a study by Morgan (1995), the use of xylanase in wheat and rice by-products resulted in heavy back fat but more lean percentage in pigs. Enzyme complex in citrus pulp based diet improved carcass traits of pigs (Borges et al., 2014). The use of enzyme complex in diets comprised of sunflower meal improved back fat, carcass muscularity and percentage of carcass lean meat in pigs (De Araujo et al., 2014).

2.5 Effect of high dietary fat in pig nutrition

Energy is the most expensive nutrient for formulating diets in livestock feed industry (Kerr et al., 2015). Lipids, fats and oils are highly digestible energy sources that when included in livestock diets can affect feed efficiency and growth rate as well as diet palatability, provision of fat soluble vitamins, essential fatty acids, reduce diet dustiness, and affect pellets quality of the diets. Additionally, their inclusion in diets can affect amino acid absorption and nitrogen digestibility and retention (Jorgensen and Fernandez, 2000). However, lipids that are utilized in pig diets are variable in terms of their composition (Kerr et al., 2015). Factors related to the processing and origins of the lipid products can affect lipid digestibility and utilization. While new types of lipids are becoming available to be used in pig feed industry, there are by-products from the biodiesel industry and vegetable oil processing plants that can be blended with the conventional fats and oils resulting in a surplus of animal-vegetable blends available for formulating diets (Kerr et al., 2015).

2.5.1 Effect of feeding high fat diet on growth performance and nutrient digestion in pigs

In an experiment where 2, 4 and 6% of white grease was used in pig diet to evaluate the effects of fat growth performance (De la Llata et al., 2001), it was reported that FCR increased linearly due to fat inclusion over the total experiment. The effect of the fat on ADG was not consistent among the weight phases where a linear improvement was found only from 36 to 59 kg, and not on weight heavier than 59 kg. However, the ADG of the pigs over the total experiment was improved. This result suggests that addition of up to 6 % of fat to diets for growing-finishing pigs can improve ADG and FCR, but reduce ADFI of the pigs (De la Llata et al., 2001).

The use of RSM compromising of 18 % of CF reduced the digestibility of DM, CP, CF, organic matter (OM) and nitrogen-free extract except that of fat, which was higher than the digestibility of other nutrients (Schöen et al., 1996).

Table 2.2 Effect of dietary enzyme on the growth performance of pigs.

By-products	Enzyme used	ADG	ADFI (g/d)	Daily Feed intake (g/d)	Feed: gain (g)	FCR(g/g)	Weight Gain (g/day)	Reference
Wheat and Rice by-products	Xylanase 4000 U/g (Porzyme 9300)	Positive	-	Positive	Positive	-	-	Morgan (1995)
Citrus pulp	Enzyme complex	Positive	Positive	-	Positive	-	-	Borges et al. (2014)
Sunflower meal	Enzyme complex	Positive	Positive	-	Positive	Positive	Positive	De Araujo et al. (2014)
Rapeseed meal	Xylanase	Positive	Positive	-	Positive	-	-	Fang et al. (2007)
Canola oil cake	Roxazyme® enzyme	Positive	Positive	-	-	Positive	-	Smith (2005)
Wheat by-products	Xylanase	Positive	-	Positive	-	Positive	-	I'Anson et al. (2014)

ADG = average daily gain; ADFI = average daily feed intake; FCR = feed conversion ratio

Table 2.3 Effect of dietary enzyme on the carcass characteristics of pigs.

By-products	Parameters	Response	Enzyme used	References
Wheat and rice by-products	Back fat	Negative	Xylanase 4000 (Porzyme 9300)	Morgan (1995)
	Lean %	Positive		
Citrus pulp	Carcass traits	Positive	Enzyme complex	Borges et al. (2014)
Sunflower meal	Back fat	Positive	Enzyme complex	De Araujo et al. (2014)
	Carcass muscularity	Positive		
	% of carcass lean meat	Positive		

2.5.2 Effect of feeding high dietary fat on meat quality from pigs

The pork industry is continually seeking feasible methods that will increase production effectiveness and carcass quality of pigs (Weber et al., 2005). Addition of 5 % animal fat to pig diets increased carcass weight and the 10th rib back fat depth, but did not impact the percent lean (Weber et al., 2005). The use of three dietary oils namely palm kernel, palm and soybean oil comprising of 4.6, 5 and 6 % did not affect the carcass quality of the pigs. The palm kernel oil significantly reduced the PUFA: SFA ratio in the *longissimus* muscle of the pigs, however, the lauric, myristic, palmitic and stearic fatty acids concentration was increased, while linoleic acid decreased (Teye et al., 2006). The results of the study suggest that palm kernel and palm oil could be utilized as alternatives to SBM for the production of good quality and healthy pork, however their limit of inclusion needs to be determined (Teye et al., 2006).

2.6 Summary

Various oil cakes/meals were discussed in substitution of SBM. Different methods (e.g. ensiling, use of enzymes, solid state fermentation etc). were discussed as possible solutions to improve the use of non-conventional AIBPs in the diets of pigs. This is due to the high fibre, fat and moisture contents, and the presence of anti-nutritional factors in AIBPs. The focus of the present study is therefore to develop a strategy that can be applied by smallholder pig farmers on the utilization of AOC.

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CHAPTER 3

Effects of fibrolytic enzyme addition on the nutritive value, fermentation characteristics and aerobic stability of avocado oil cake silage

Abstract

The present experiment was conducted to evaluate the effects of an enzyme addition on the fermentation characteristics, nutritive value and aerobic stability of ensiled avocado (*Persia Americana*) oil cake (AOC). An amount of 700 g AOC /kg fresh matter (FM) was mixed with 250 g wheat bran/kg FM wheat bran, and 50 g sugarcane molasses/kg FM. This mixture was treated without i) additive (control), or with enzyme (Axtra® XB enzyme) at ii) 3 % and iii) 5% levels, making three treatments in total. The enzyme contains endo-1, 4-β-xylanase (12.200 U/g) and endo 1,3β-gluconase, (1.520 U/g). The forage mixtures were thoroughly mixed and ensiled into 1.5 L anaerobic jars that were kept at 25-28° C room temperature for 90 days. Samples of the pre-ensiled mixtures were collected for the determination of nutritive values, pH and water soluble carbohydrates (WSC). Three jars per treatment were then opened on days 3, 7, 10 and 90 post ensiling to determine pH while those of day 90 were also used to determine nutritive values and fermentation profiles of the treatments. Further, silage samples of day 90 were subjected to an aerobic stability test that lasted for 7 days. It was found that good quality silage can be produced from AOC without ($P > 0.05$) the addition of enzyme. However, the effect of enzyme addition was apparent ($P < 0.05$) on the reduction of NDF and increased WSC of ensiled AOC compared to the untreated silage. Although enzyme addition was applied at two different levels (i.e. 3 and 5 %), increasing enzyme addition to AOC at ensiling was not worthwhile since it did not make any significant contribution.

Keywords: acids, additives, by-products, ensiling, feed, nutrients,

3.1. Introduction

Avocado (*Persia Americana*) oil cake (AOC), is a by-product that emanates from the production of oil from discarded avocados. This by-product contains 260 g dry matter (DM)/kg, 81 g crude protein (CP)/kg DM, 632 g crude fat/kg DM, 612 g monounsaturated fatty acids/kg DM and 146 g polyunsaturated fatty acids/kg DM (Grageola et al., 2010, Nkosi et al., 2018); *in vitro* organic matter digestibility (IVOMD) of 540 g /kg DM, 518 g neutral detergent fibre (NDF)/kg DM, 393 g acid detergent fibre (ADF)/kg DM, 258 g acid detergent lignin (ADL)/kg DM and 38 g acid detergent insoluble nitrogen (ADIN)/kg DM (Skenjana et al., 2006), making it a high fibre feed source. These constituents provide some valuable nutrients that can benefit animal nutrition, especially those under smallholder production. However, storing AOC for future use is a challenge because of its high moisture content but ensiling may be advocated for because it has been seen to be the easiest and most efficient preservation method for preserving high moisture by-products. Ensiling has also been reported to reduce anti-nutritional agents such as glucosinolates in carinata meal (Rodriguez-Hernandez et al., 2015) and polyphenol content in olive cake (Weinberg et al., 2008).

However, the ensiling of AOC cannot be done successfully without the addition of dry resources or absorbents (e.g. ground hay, wheat bran, etc.) that will improve its DM content for efficient fermentation. The addition of these resources to AOC at ensiling conversely increase the fibre content of AOC silage, making it less suitable for feeding to growing pigs. Although bacterial inoculants have improved potato hash silage fermentation quality (Nkosi et al., 2010), their effects on fibre degradation is not consistent because lactic acid bacteria (LAB) cannot effectively use fibre as an energy source to produce lactic acid (LA). Consequently, it has been proposed that fibrolytic enzymes be added during the ensiling of high fibre forages to degrade cell walls and increase the availability of WSC to be consumed by LAB (McDonald et al., 1991, Selmer-Olsen et al., 1993). Avocado oil cake also contains low levels of water-soluble carbohydrates **(**WSC) (Eliyahu et al., 2015) which concentrations may be further reduced during oil extraction. Because WSC are critical for successful ensiling, the boosting of the sugar content by addition of such products as sugarcane molasses has been advocated (Weinberg et al., 2008, Abarghoei et al., 2011). The objective of the study was therefore to evaluate the effects of fibrolytic enzyme addition to AOC on the fermentation characteristics and aerobic stability of silage.

3.2 Materials and Methods

3.2.1 Collection of avocado oil cake

Batches of avocado oil cake (AOC) were collected from Westfalia in Pietermaritzburg, South Africa and brought to ARC-Irene for proximate analysis and silage production. The silage was produced by mixing 700 g AOC /kg fresh matter (FM) of AOC, 250 g wheat bran/kg FM and 50 g sugarcane molasses/kg FM. This mixture was treated without (control), and with either 3% enzyme or 5% enzyme, making three treatments in total. The enzyme (Axtra® XB enzyme) was purchased from Pennyville LTD (corner Kirkney Ext 6 and Richards Bay road), Pretoria west. The enzyme contains endo-1, 4-β-xylanase (12.200 U/g) and endo 1,3β-gluconase, (1.520 U/g). The forage materials were thoroughly mixed before being compacted into 1.5 L anaerobic jars (J. Weck, GmBHu. Co., Wehr-Oflingen, Germany). Each treatment had 12 jars, resulting in 36 jars. Each jar was filled with approximately 850 g (wet weight) forage mixture without headspace, and a packing density of 567 kg DM/m^3 was achieved. Jars were kept at 25-28° C room temperature for 90 days. Samples of the pre-ensiled mixtures were collected for the determination of nutritive values, pH and water soluble carbohydrates (WSC). Three jars per treatment were then opened on days 3, 7, 10 and 90 post ensiling to determine pH while those of day 90 were also used to determine nutritive values and fermentation profiles of the treatments. Further, silage samples of day 90 were subjected to an aerobic stability test that lasted for 7 days, following the procedures of Ashbell *et al.* (1991).

3.2.2. Chemical analysis procedures

A 40 g representative sample of pre-ensiled mixture and that of silages were taken from each treatment to determine the fermentation characteristics. The 40 g silage sample (three samples/treatment) was mixed with 360ml of distilled water in a stomacher bag, homogenized for 4 min and pH was determined immediately with a pH metre (Thermo Orion Model 525, Thermo Fisher Scientific, Waltham, MA, USA). The sample was then filtered through a Whatman No. 54 filter paper (G.I.C. Scientific, Midrand, Gauteng, South Africa). The extract was used for determination of WSC and lactic acid (LA). The WSC were determined by the phenol-sulphuric acid method of Dubois et al. (1956) and LA was determined by the modified colorimetric method of Pryce (1969). Lactic acid bacteria were measured by planting serial dilutions on de Man, Rogosa, sharpe agar (HongzhouBaisi Biotechnology Co., Ltd., Hangzhou, China). Incubated at 30˚C for 72 hours under anaerobic conditions (Amaerobic box; Yiheng Technical co., Ltd, Shanghai, China).

The dry matter (DM) of pre-ensiled mixtures and that of silages was determined by drying the samples at 60˚C until a constant mass was achieved, and was corrected for loss of volatiles using the equation of Weissbach and Strubelt (2008). After drying, the samples were ground through a 1-mm screen (Wiley mill, Standard Model 3, Arthur H. Thomas Co., Philadelphia, PA) for crude protein (CP), gross energy (GE), fibre (aNDF, ADF and ADL) and ether extract (EE) analyses. The OM and EE (ID 963.15) were determined according to the procedure of AOAC (1990). The aNDF, ADF and the ADL were determined according to the procedures of Van Soest *et al.* (1991). The aNDF was determined using heat stable α-amylase (Sigma-Aldrich Co. LTD., Gillingham, UK, no. A-1278) with sodium sulfite, and the ADF was determined using the Fibertec TM System equipment (Tecator LTD., Thornbury, Bristol, UK).

Separate samples were used for ADF and aNDF analysis and both included residual ash. The gross energy (GE) in diets and faeces was determined with a bomb calorimeter (MC-1000 modular calorimeter, Energy Instrumentation, 135 Knoppieslaagte, Centurion, South Africa).

3.2.3 Aerobic stability determination

Samples of d 90 were subjected to an aerobic stability test in which 500 g sample from each jar was packed loosely in an open plastic jar, which was covered with two layers of cheesecloth and kept at 28 ^{o}C. Thermocouples (T-type copper constantan, 20-gauge wire) were placed in the geometric centre of the silage mass in each jar and in the room where the jars were stored to record temperature. Room temperature and the temperature in each jar were simultaneously recorded at one-hour intervals using a CR7X data logger (Campbell Scientific, Logan, Utah) for 7 d. Carbon dioxide (CO_2) production (Ashbell, et al., 1991), pH and yeasts and moulds were determined after the seven-day exposure following the IDF procedure (1990). The aerobic stability was denoted as the time (h) that the silage remained stable before rising at least 2^{0}C above the ambient temperature.

3.3 Statistical analysis

Data on effects of treatments on fermentation, chemical composition and aerobic stability of AOC silage were analysed in a completely randomized design by ANOVA using Genstat (2011). Differences among treatment means were compared with least significant difference (LSD) and significance was declared at the 0.05 % probability level. Data was fitted to the model: $Yij = \mu + ti + \varepsilon ij$ where: Yij is the individual observations of the i-th treatment and the j-th replicate, μ is the general effect, ti is the effect of the i-th treatment and εij is the random variation or residual error.

3.4 Results and Discussion

3.4.1. Chemical composition of forages at pre-ensiling

Data on the chemical composition of the by-products (AOC and wheat bran) and their mixture is shown in Table 3.1. The DM content of feedstuffs before ensiling has a strong influence on the rate and extent of the resulting fermentation. When silage DM content is less than 30 %, conditions for clostridial activity are favourable, resulting in high losses and silage of low nutritional value (McDonald et al., 2002). In this study, the DM content of pre-ensiled AOC was 24 %, making it difficult to ensile. However, the DM content of AOC was higher than 18.6 % in AOC reported by Eliyahu et al. (2015), but was consistent to 26 % DM reported by Grageola et al. (2010). In order to ensile AOC well, the by-product was mixed with wheat bran and sugarcane molasses to improve both the DM and the fermentable substrate. This resulted in a DM of 44.9 %, sufficient for ensiling. The AOC is rich in fibre, but the fibre fractions are lower than 930 g NDF/kg DM and 573 g ADF/kg DM reported in maize cobs by Kanengoni et al. (2002).

Water-soluble carbohydrates are regarded as essential substrates for growth of LAB for proper fermentation, and low concentration may restrict LAB growth (McDonald et al., 1991). Wilkinson (1990) indicated that the WSC content in a forage at pre-ensiling should range between 8 to 12 % of DM for good fermentation. The WSC content of AOC in this present study was 6 % of DM (Table 3.1), insufficient for good fermentation. However, the WSC in AOC in the present study is higher than 1.5 % WSC of DM reported by Eliyahu et al. (2015) in AOC. The differences in the WSC from the AOC in these studies might be attributed to different cultivars and oil extraction methods. Mixing AOC with wheat bran plus the addition of sugarcane molasses increased the WSC of AOC to 10 % (Table 3.1) sufficient for efficient fermentation.

Table 3.1 Chemical composition of forages and their mixture at pre-ensiling (n=3)

Parameter (%)	AOC	Wheat bran	AOC + Wheat bran mixture#
DM	24.0	88.8	44.9
CP	10.6	15.0	14.7
EE	12.3	4.0	5.1
GE (MJ/kg DM)	20.4	-	23.4
Ash	3.98	6.57	0.41
aNDF	45.9	57.0	47.6
ADF	26.98	33.00	25.27
ADL	9.81	3.00	13.32
WSC	6.2	-	9.8
pH	5.43	5.45	5.91

DM = dry matter, CP = crude protein, EE = ether extract, GE = gross energy, aNDF = neutral detergent fibre (ash residual inclusive), ADF = acid detergent fibre, ADL = acid detergent lignin, WSC = water-soluble carbohydrates, LAB = lactic acid LAB= DM=dry matter, CP=crude protein, EE=ether extracts, GE=gross energy, NDF=neutral detergent fibre, ADF=acid detergent fibre, ADL=acid detergent lignin, WSC=water soluble carbohydrates

#mixture = 700 kg AOC + 250 kg wheat bran (WB)

Dietary protein is measured as crude protein, which is composed of amino acids in numerous proportions (van Lunen and Cole, 2001). The crude protein content of a forage or feed is one of the important components to enhance animal welfare. Adequate amount of crude protein should be provided to pigs in an optimal ratio to dietary energy to enhance growth, health and lean muscle deposition (van Lunen and Cole, 2001). Additionally, in pig nutrition, a good quality protein provides the 10 essential amino acids that are required for normal body function in the quantity and proportions critical for the particular need of the pig (Adesehinwa and Ogunmodede, 1995). Failure to supplement low protein pig diet with adequate amount of high quality protein source results in inefficient feed utilization, poor growth, high carcass fat and reduced reproductive performance (Adesehinwa and Ogunmodede (1995).

The CP content of AOC in the present study was 10.6 % of DM (Table 3.1), higher than 7 % (Van Soest, 1994) but lower than 14. 7 % of DM and 15. 6 % of DM reported by Eliyahu et al. (2015) and Skenjana et al. (2006) in AOC, respectively. This difference might be related to the different processing methods used during oil production from avocados and differences in avocado cultivars used. Mixing AOC with wheat bran and sugarcane molasses increased the CP content to 14.7 %.

It is well known that lipids are energy dense compared to proteins and carbohydrates (McDonald et al., 2010). The EE content of AOC in the present study was 12.3 %, which is lower than 49.8 % recorded in AOC by Nkosi et al. (2019). However, mixing AOC with wheat bran reduced the EE content of the mixture to 5 %, which was favourable for ensiling. The high fat content in AOC makes it a good source of energy especially for animals that are in intensive fattening systems, since these systems depend on energy dense diets for animals to deposit intramuscular fat that would enhance product quality and taste. Nevertheless, the high residual fat in AOC means that it has high energy content, which makes it a supplement for both protein and energy.

3.4.2. Silage fermentation and aerobic stability

The content of ash, CP, energy, ADF and ADL were not affected (P>0.05) by treatments after 90 days of ensiling (Table 3.2). However, the fat content of the silage was higher (P<0.05) in the treated silage compared to the control. This shows that the enzyme addition have no effect on fat reduction in silage. This concurs with Alves et al. (2011) who reported lack of response on the fat composition when silage inoculants were added to ryegrass at ensiling.

After 90 d of fermentation, higher (P<0.05) residual WSC were obtained in the enzyme treated silage compared to the control (Table 3.2). This might be attributed to the higher (P<0.05) degradation of NDF in the silage treated with enzymes in relation to the control treatment, making more sugars available to stimulate fermentation in the AOC silage. Higher residual WSC in silage may be beneficial to animals since it supplies energy to animals (Tava et al., 1995, McDonald et al., 2010), but may serve as substrate for aerobic microbes during feeding of silage to livestock (Weinberg et al., 1993).

Lactic acid (LA) is the strongest of all silage acids and its presence will reduce the pH more effectively than VFAs. Achieving an increase in the content of LA is the most reliable indicator for the success of a microbial additive in improving silage quality. The addition of enzyme to AOC at ensiling resulted in higher ($P<0.05$) concentration of LA compared to the control. This is related to the increase LAB population (Table 3.2) in the enzyme treated silage in comparison with the control treatment. This concurs with the reports of Mari et al. (2009) and Nkosi et al. (2009). In contrast, other studies reported a lack of response on the influence of microbial inoculation to LA content of silage in relation to untreated silage (McAllister et al., 1995). According to McDonald et al. (2010), LA concentrations in silages should range between 8 and 12 % of DM, and only that of enzyme treated AOC was within this threshold.

Silage pH is one of the main criteria reflecting the extent of fermentation and quality of ensiled forages. A rapid decline of pH during the early stages of ensiling is required to ensure good silage quality (McDonald et al., 1991). The pH of AOC at pre-ensiling was 5.5 but gradually dropped to less than 4 as the fermentation process progressed (Figure 3.1), indicative of a good silage (Kung and Shaver, 2001). The addition of enzyme to AOC at

ensiling did not affect the terminal pH of the silage. Working on capim-marandu pastures (*Brachiara brizanthia cv Marandu*), Bergamsachine et al. (2006) reported no difference in the pH in the control treatment compared with microbial treated silages, which is consistent with the results of the present study.

Table 3.2 Effect of enzyme inclusion on the nutritive value, fermentation characteristics and aerobic stability of avocado oil cake after 90 days of ensiling (n= 3)

Parameter (%)	**Treatment**			**SEM**	***P* value**
	Control	**3%**	**5%**		
		Nutritive value (% of DM)			
DM	37.9^{a}	35.7^{b}	38.1^{a}	0.15	0.033
Ash	2.47	2.41	2.68	0.13	0.360
CP	13.6	13.6	13.5	0.03	0.140
EE	5.10^{b}	5.22^{a}	5.23^{a}	0.01	0.0001
GE (MJ/kg DM)	20.6	20.6	20.7	0.01	0.115
aNDF	23.8^{a}	21.1^{b}	19.5^{c}	0.15	0.0001
ADF	14.8	14.7	13.9	0.39	0.308
ADL	7.55	8.41	7.45	0.64	0.539
		Fermentation characteristics (% of DM)			
WSC	0.84^{b}	0.92^{a}	0.91^{a}	0.01	0.002
pH	3.9	3.9	3.9	0.04	0.794
LAB cfu/g/kg	2.1^{3b}	4.1^{3a}	4.5^{3a}	122	0.038
LA	5.6^{b}	8.9^{a}	8.7^{a}	0.25	0.001
		Aerobic stability			
pH	5.4	5.5	5.4	0.21	0.796
CO_2, g/kg DM	2.04	2.64	2.11	1.18	0.282
Yeast & moulds log^{10} CFU/kg	2.4×10^{3}	2.4×10^{3}	2.1×10^{3}	441	0.190

$^{a\text{-}b}$ Means of the same row with different superscripts differ significantly ($P<0.05$) while those without superscripts did not differ ($P>0.05$)

Treatments: control = no enzyme, 3 % = 3 % enzyme addition, 5 % = 5 % enzyme addition

DM = dry matter, CP = crude protein, EE = ether extract, GE = gross energy, aNDF = neutral detergent fibre (ash residual inclusive), ADF = acid detergent fibre, ADL = acid detergent lignin, WSC = water-soluble carbohydrates, LAB = lactic acid LAB, LA=lactic acid, CFU = colony forming unit, CO_2 = carbon dioxide

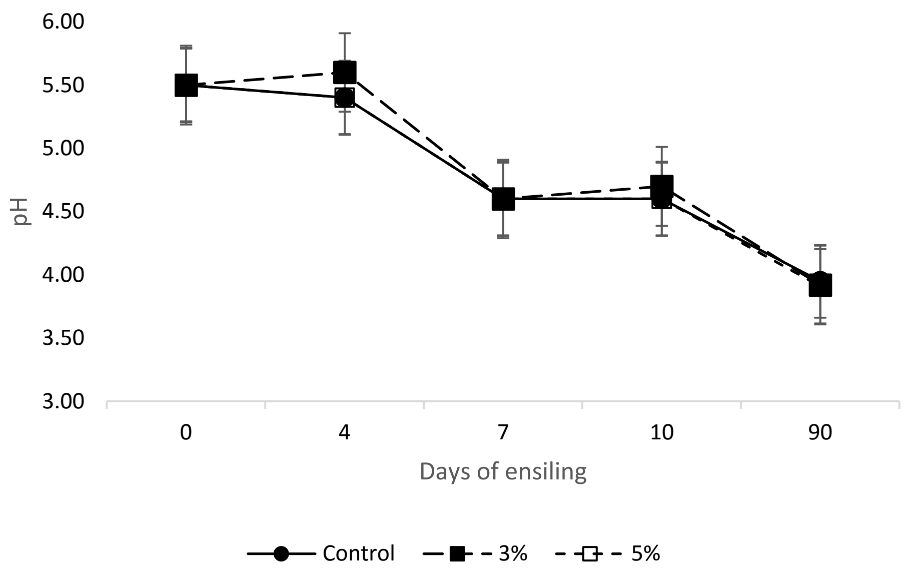

Figure 3.1 Effects of treatments on the pH reduction in ensiled AOC

The aerobic deterioration of silage may increase the risk of proliferation of potential pathogenic or undesirable micro-organisms thus affecting the performance of animals fed the silage. A higher CO_2 production in silage indicates the activity of yeasts and moulds, which cause a rise in temperature and deteriorates the quality of silage (Woolford, 1990, Ashbell et al., 1991). The aerobic stability of the AOC silage was not affected by enzyme addition. Although the enzyme treated AOC had higher residual sugar and LA compared to the control, the differences in these fermentation indices were too small to affect the aerobic stability of the silage.

3.5. Conclusion

Good quality silage can be produced from AOC without the addition of enzyme. However, the effect of enzyme addition was apparent on the reduction of NDF and increase in the WSC of ensiled AOC. Although enzyme addition was applied at two different levels (i.e. 3 and 5 %), increasing enzyme addition to AOC at ensiling was not worthwhile since it did not make any significant contribution.

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CHAPTER 4

Effects of different dietary inclusion levels of ensiled avocado oil cake on the growth performance, nutrient digestion and carcass characteristics of weaner pigs

Abstract

This experiment was conducted to evaluate the effect of different dietary inclusion levels of avocado oil cake (AOC) silage on the growth performance, nutrient digestibility and carcass characteristics in pigs. An amount of 700 g AOC /kg fresh matter (FM) was mixed with 250 g wheat bran/kg FM wheat bran, and 50 g sugarcane molasses/kg FM. The mixture was ensiled in 200 L drums for 90 days, and was incorporated at different levels (i.e. 0, 3 and 5%) in diets. The diets were fed to twenty seven Large White x Landrace (LW x LR) pig crosses, weighing approximately 22 kg live weight, which were randomly allocated to the three experimental diets (9 pigs/ treatment) on their live weights. Pigs were individually weighed at the start and weekly until the end of the trial. Data on daily feed intake, average daily gains (ADG) and feed conversion rates (FCR) were recorded. A nutrient digestibility study was done a week after completion of the growth study. At the end of the nutrient digestibility experiment, pigs were fasted for twelve hours and weighed to determine the final weight. The pigs were then slaughtered and carcass samples were collected and analysed. The growth performance of pigs was not affected by dietary inclusion levels of AOC silage, irrespective of increased fibre levels in the AOC silage diets. However, dietary addition of 3 % AOC silage improved the digestibility of fibre fractions. The cold carcass weight and the chop were reduced with AOC silage addition. In addition, AOC silage reduced the back fat thickness of carcasses. It was concluded that silage from AOC can be incorporated in the diets of pigs at not more than 5 %.

Keywords: energy, forages, inoculants, nutrients, silage, wastes

4.1. Introduction

The intensive pig production systems in South Africa are costly due to the increased prices for feed ingredients. Smallholder pig farmers therefore rely on non-conventional feed resources to sustain their farm production. These resources are fibrous, which negatively affect pig performance, especially in growing pigs. Feeding high fiber diets to growing pigs is often associated with increased gut fill and gastrointestinal weight, which negatively affect the growth performance and lowers the dressing percentage in growing pigs. This is attributed to the limited capacity for feed intake and hindgut fermentation of fibrous feed resources in young pigs.

Avocado (*Persea Americana*) oil cake (AOC), a by-product that derives from the oil extraction from avocado fruits in South Africa, contains 518 g neutral detergent fibre (NDF), 393 g acid detergent fibre (ADF), 258 g acid detergent lignin (ADL) and 38 g acid detergent insoluble nitrogen (ADIN) (Skenjana et al., 2006). Brai et al. (2017) reported that AOC contains lysine of 59 mg/100 g, threonine of 40 mg/100 g and methionine of 29 mg/100 g, making it a poor source of amino acids. According to van Ryssen et al. (2013) dietary inclusion of > 100 g AOC/kg DM reduced the growth performance of the birds due to the high levels of condensed tannins and fibre in AOC diets.

The making of silage has been reported to reduce fibre fractions in forages due to the activity of enzymes during fermentation (McDonald et al., 2010). This method can be used to preserve AOC to be fed to animals. Duodo et al. (2018) reported that the fermentation of oil cakes during silage production is a simple, cost effective and efficient way of improving the nutritional value of oilseed by-products. However, the feeding of silage to pigs has been practised for many years in other countries but is less common to emerging pig farmers in South Africa. This practice is limited because of the high content of fibre in the feed, which negatively affects pig growth performance. For example, dietary inclusion of 500 g maize silage/kg DM reduced the growth performance and feed conversion in fattening pigs (Scipioni and Martelli, 2001). However, some advantages of feeding silage to pigs include better feed efficiency and more active pigs on diets containing silage (Akerfeldt et al., 2018). In addition, Wustholz et al. (2017) fed pigs on diets that contained 20 to 50 % inclusion of lucerne silage and the pigs on lucerne silage diets consumed more fibre than those on the control diet but the daily weight gains were not affected by the diets.

The objectives of the present study were to evaluate the effects of feeding AOC silage at different dietary inclusion levels on the growth performance, nutrient digestion and carcass characteristics of growing pigs

4.2 Materials and methods

4.2.1 Silage production

The study was conducted at the Agricultural Research Council Institute in Irene, South Africa (Longitude 28°C12ˈ40 ˈE: Latitude 25° 54ˈ36 S; Altitude 1526 m) about 15 km South of Pretoria. Batches of avocado oil cake (AOC) were collected from Westfalia in Pietermaritzburg, South Africa and brought to ARC-Irene for proximate analysis and silage production. The silage was produced by mixing 700 g AOC/kg fresh matter (FM), 250 g wheat bran/kg FM and 50 g sugarcane molasses/kg FM. This mixture was ensiled in 210 L drums that were lined with plastic bags which were enclosed with a rubber lid to prevent damages to the bag by rodent. A packing density of 820 + 29.8 kg/m^3 was obtained and the drums were stored at 25-28°C. After 90 days of ensiling, drums were opened and silage samples were collected for the determination of nutrient composition and fermentation characteristics. Experimental diets that contained different levels of AOC silage (i.e. 0, 3 and 5%) were formulated as shown in Table 4.1.

Table 4.1 Formulation of experimental diets

Ingredients (%)	Treatments		
	0% AOC	3% AOC	5% AOC
Maize meal	73.97	73.02	71.02
Wheat bran	2.7	0	0
Feed lime	0.6	0.5	0.5
Monocalcium phosphate	1.85	2.00	2.00
Soyabean meal	19.6	20.2	20.2
Vitamin premix[1]	0.20	0.20	0.20
Salt	1.00	1.00	1.00
Lysine	0.08	0.08	0.08
AOC silage	0	3	5
Total	100	100	100

[1]Provided the following per kilogram of diet: 6,500 IU vitamin A, 1,200 IU vitamin D_3, 40 IU vitamin E, 2 mg vitamin K_3, 1-5 mg vitamin B_1, 4.5 mg vitamin B_2, 0.03 mg vitamin B_{12}, 2.5 mg vitamin B6, 25 mg niacin, 12 mg calcium pantothenate, 190.5 mg choline, 0.6 mg folic acid, 0.05 mg biotin, 40 mg manganese, 100 mg zinc, 125mg copper,1 mg iodine,100 mg ferrous, and 0.3 mg selenium.

AOC= avocado oil cake silage

4.2.2 Animals, housing and feeding

This experiment was approved by the Animal Ethics Committee of the ARC-Irene with the approval no: APIECIS/027. Twenty seven Large White x Landrace (LW x LR) crosses, weighing approximately 22 kg live weight were used. The pigs were randomly allocated to the three experimental diets (9 pigs/ treatment) balanced on their live weights. Pigs were housed individually in pens (1.96 x 1m) and the feeders checked and adjusted twice each day to ensure constant access to fresh feed, and minimize any possible wastage. Water was freely available through nipple drinkers. The pigs had an adaptation period to the experimental condition of 14 days and the experiment took 60 days. Pigs were individually weighed at the start and weekly until the end of the trial. The pigs were fed on experimental diets *ad-lib* in the morning, allowing a 10 % of feed refusal. Data on daily feed intake, average daily gains and feed conversion rates were recorded. The following formulae were used to calculate the growth performance parameters.

$$\text{Dry matter intake (DMI, kg)} = \frac{\text{(Total feed intake x Dry matter)}}{100}$$

$$\text{Average daily gain (ADG, kg/d)} = \frac{\text{(Final weight – Initial weight)}}{\text{Experimental period}}$$

$$\text{Feed conversion ratio (FCR)} = \frac{\text{Average daily feed intake (ADFI)}}{\text{ADG}}$$

4.2.3 Nutrient digestibility study

The digestibility study was done a week after completion of the growth study. Chromic oxide was mixed with the feed at a concentration of 3 g chromic oxide/kg feed as an indigestible marker (Brandy et al., 2017). The pigs were allowed an adaptation period of 3 days to the chromic oxide mixed diet before faecal collection for 5 days using the grab sampling methods. Faecal samples were collected once per day, weighed and the DM determined. The samples were then kept in 250 ml plastic container and frozen at -20°C for further analysis. The samples of the 5 day period were pooled per pig and stored. Digestibility was calculated according to McDonald et al. (2010) using the formula: Digestibility (%) = 100 – (100 x % Indicator feed / % Indicator faeces x % Nutrient faeces / % Nutrient feed).

4.3 Post slaughter measurements

At the end of the digestibility study, pigs were fasted for twelve hours and weighed to determine the final weight. The pigs were then transported to the ARC Institute’s abattoir for slaughter. Pig carcasses were processed according to the routine abattoir procedures, which included an ante-mortem inspection and rest for the pigs before slaughter. The pigs were then stunned with an electrical stunner set at 220 V and 1.8 A with a current flow for 6 s and exsanguinated within 10 s of stunning. Dehairing and evisceration were done according to the abattoir`s standard operating procedures.

4.4 Carcass measurements

Warm carcass weight (WCW) was measured after dressing using an overhead scale. Dressing percentage (DP) was calculated by taking the WCW as a percentage of final weight. The carcasses were then placed in a cold room and kept at an approximate temperature of 0 ^{0}C for 24 hours, after which cold carcass weights (CCW) were measured. After this, ultimate pH (pH at 24 h (pH_{24})) and temperature readings were taken from the *Longissimus thoracis* muscle (eye muscle) with a portable pH meter (EUTECH Instruments, Thermo Fisher Scientific Inc. Singapore) between the third and the fourth rib, 60 mm from the midline. The pH meter had an automatic temperature compensator to adjust the pH for temperature. Before use, the pH meter and electrode were calibrated at pH 4 and pH 7 and was recalibrated in pH buffers after every fourth reading.

The head from each carcass was then removed at the *atlanto-occipital* joint and the tail at the junction of the third and fourth sacral vertebrae, and the flare fat, kidneys, fat, glands and remaining parts of the diaphragm were also removed. Carcasses were then split into 2 parts along the median plane from the remaining sacral vertebra to the cervical vertebra with a carcass splitting band saw. Back fat measurements were taken at the first rib (dorsal fat thickness at first rib), last rib (dorsal fat thickness at last rib). All other carcass measurements were taken from the left side.

Drip losses were measured from chops that were cut from the *Longissimus dorsi* muscle between the 4th and 8th ribs. Samples of 80 g were trimmed and weighed using a Mettle scale (range: 0.01 kg-15 kg). Each sample was placed in a nylon mesh and sealed in a plastic bag for a period of 24 hrs at 2 $^{\circ}$C. After 24 hrs, the chop was re-weighed separately without the plastic bag. The drip loss was calculated by dividing the water loss on the meat by the original weight x 100. Back fat were measured between the 2nd and 3rd ribs about 60 mm from the midline on the left carcass using a pair of Vernier Calipers.

4.5 Chemical analysis

Three 40 g representative samples of silage were taken from each drum to determine fermentation characteristics. The 40 g silage samples were mixed with 360 ml of distilled water in a stomacher bag, homogenized for 4 min and pH was determined immediately with a pH meter (Thermo Orion Model 525, Thermo Fisher Scientific, Waltham, MA, USA). The samples were then filtered through Whatman No. 54 filter paper (G.I.C. Scientific, Midrand, Gauteng, South Africa). The extract was used for determination of water soluble carbohydrates (WSC) and lactic acid (LA). The WSC were determined by the phenol-sulphuric acid method of Dubois et al. (1956) and LA was determined by the modified colorimetric method of Pryce (1969). Lactic acid bacteria were measured by planting serial dilutions on de Man, Rogosa, sharpe agar (HongzhouBaisi Biotechnology Co., Ltd., Hangzhou, China). Incubated at 30 ˚C for 72 hours under anaerobic conditions (Amaerobic box; Yiheng Technical co., Ltd, Shangai, China).

The dry matter (DM) of silage was determined by drying the samples at 60 ˚C until a constant mass was achieved, and was corrected for loss of volatiles using the equation of Weissbach and Strubelt (2008). After drying, the silage and the experimental feeds samples were ground through a 1-mm screen (Wiley mill, Standard Model 3, Arthur H. Thomas Co., Philadelphia, PA) for crude protein (CP), gross energy (GE), fibre (aNDF, ADF and ADL) and ether extract (EE) analyses. The OM and EE (ID 963.15) were determined according to the procedure of AOAC (1990). The aNDF, ADF and the ADL were determined according to the procedures of Van Soest et al. (1991). The aNDF was determined using heat stable α-amylase

(Sigma-Aldrich Co. LTD., Gillingham, UK, no. A-1278) with sodium sulfite, and the ADF was determined using the Fibertec TM System equipment (Tecator LTD., Thornbury, Bristol, UK). Separate samples were used for ADF and aNDF analysis and both included residual ash. The gross energy (GE) in diets and faeces was determined with a bomb calorimeter (MC-1000 modular calorimeter, Energy Instrumentation, 135 Knoppieslaagte, Centurion, South Africa).

4.6 Statistical analysis

Data on effects of treatments on the growth performance, nutrient digestion and carcass characteristics of pigs fed the AOC silage were analysed in a completely randomized design by ANOVA using Genstat (2011). Differences among treatment means were compared with least significant difference (LSD) and significance was declared at the 0.05 % probability level. The data were analysed using the model:

$Y_{ij} = \mu + t_i + ß_j + \varepsilon_{ij}$ where: Yij is the individual observations of the i-th treatment (i=1, 3) and the j-th replicate (j=1, 3), μ is the general effect, ti is the effect of the i-th treatment, βj is the effect of the j-th replicate, ε ij is the random variation or experimental error.

4.7 Results and Discussions

Data on the nutritive values and silage fermentation characteristics of ensiled AOC is shown in Table 4.2. The silage was well fermented as indicated by low pH (< 4.0) (McDonald et al., 2010). The formulated diets had similar contents of energy and CP (Table 4.3). However, the addition of AOC silage reduced (P< 0.05) the DM content while increasing the NDF content of the diets. This was expected since the AOC silage had high moisture and fiber contents.

Table 4.2 AOC silage fermentation and chemical composition (n=3)

Parameter	%
DM	37.9 ±5.986
Ash	2.47 ± 0.452
CP	13.6 ± 3.411
EE	5.10 ± 1.331
GE (MJ/kg DM)	20.6 ± 2.451
αNDF	23.8 ± 3.847
ADL	14.8 ± 2.668
WSC	0.84 ± 0.046
pH	3.9 ± 1.341
LA	5.7 ± 1.894

DM=dry matter, CP=crude protein, EE=ether extracts, GE=gross energy, αNDF= alpha-amylase neutral detergent fibre, ADF=acid detergent fibre, ADL=acid detergent lignin, WSC=water soluble carbohydrates, LA= lactic acid

Table 4.3 Chemical composition of the experimental diets (n=3)

Parameter	**Treatment**			**SEM**	***P***
(%)	**Control**	**3% AOC**	**5% AOC**		
DM	88.4^{c}	78.6^{b}	74.7^{a}	0.12	0.001
Ash	7.9^{a}	7.1^{c}	7.2^{b}	0.02	0.001
CP	12.6	12.5	12.5	0.03	0.252
EE	4.7	4.9	4.8	0.06	0.184
GE (MJ/kg DM)	21.8	21.9	21.8	0.03	0.243
aNDF	45.5^{b}	48.6^{a}	51.4^{a}	0.23	0.004
ADF	25.7	26.3	28.8	0.51	0.232
ADL	14.4	13.3	13.1	0.29	0.061

^{a,b,c} means with different superscripts differ significantly, DM = dry matter, CP = crude protein, EE = ether extract, GE = gross energy, αNDF = alpha-amylase neutral detergent fiber, (ash residual inclusive), ADF = acid detergent fiber, ADL = acid detergent lignin

Data on the growth performance of pigs fed the experimental diets is shown in Table 4.4. The allocation of pigs in the experimental diets was balanced since the live weights of pigs in the diets were similar (P > 0.05) at the start of the experiment. There were no differences (P > 0.05) in the final body weights, dry matter intake (DMI), ADG and FCR amongst the diets.

Just (1984) reported high DMI in pigs fed a diet that contained high DM and low fibre compared to those fed on a low DM and high fibre diets. Shimazawa et al. (2007) did not report differences in feed intake between pigs that were fed on a concentrate and those on total mixed potato silage diet. Ndindana et al. (2002) reported a decrease in ADG and feed intake in pigs with increasing dietary inclusion levels of maize cobs, but Kanengoni et al. (2014) did not observe this when maize cobs were ensiled. This means that ensiling improved the nutritive values of forages. This is consistent with the present study since DMI and ADG in pigs were not affected by the inclusion levels of ensiled AOC. Feeding Large White x Landrace pig crosses on diets containing ensiled maize cobs (200 g maize cob silage/kg FM), Kanengoni et al. (2014) reported a DMI of 1.4 kg/d, which is comparable to that of pigs in the present study.

Under adequate feeding and management systems, growing pigs are expected to have average daily gains of 640 g/d (Payne, 1990). Pigs in the present study had ADG of 750 g/d, which is higher than this threshold. This might be attributed to the type of breed used since voluntary feed intake varies with pig breed and ages. Dom and Ayalew (2009) fed pigs on diets that contained 50 % sweet potato silage and obtained ADG of 850 g/d, higher than that of pigs in the present study. According to the National Research Council (1998), an intake of 2320 g/d is recommended for finishing pigs. Pigs in the present study had a daily intake that ranged between 1600 to 1900 g/d, less than this threshold.

Table 4.4 Growth performance of pigs fed diets that contained different levels of avocado silage (n=9).

Parameter	**Treatment**			**SEM**	***P***
	Control	**3% AOC**	**5% AOC**		
Initial body weight, kg	22.5	23.3	23.9	1.47	0.793
Final body weight, kg	67.6	67.7	69.4	0.89	0.287
Body weight gain, kg	45.1	44.4	45.6	0.471	0.250
Dry matter intake, kg/day	1.9	1.7	1.6	0.02	0.268
Average daily gain, kg/day	0.75	0.74	0.76	0.04	0.589
Feed conversion ratio, kg/kg	2.5	2.3	2.1	0.05	0.572

The addition of maize silage in the diets of pigs has been reported to increase dietary NDF compared to maize meal, which negatively affected the growth performance of the animals (Zanfi and Spanghero, 2012). In this study the NDF of the diets was increased when AOC silage was added in the diets (see Table 4.3). However, when growing pigs are fed *ad lib*, dietary energy content is the factor in controlling feed intake, and differences in fibre content should not affect DM intake provided that the feed bulk and palatability are acceptable (Coffey et al, 1982). The experimental diets of the present study had differences in NDF contents but had similar energy concentrations (Table 4.3), hence the daily intake was not affected, which corroborates findings by Len et al. (2008) who observed no differences in daily intakes when diets were having similar energy content. In contrast, Campbell and Taverner (1986) reported a low feed intake in diets that contained high level of fibre (120 g ADF/kg DM) compared to those with low fibre (62 g ADF/kg DM), but the energy content of the diets was not similar. The reduced growth rate and poor feed efficiency in pigs fed high fibre diets might be related to an increased energy requirements for maintenance purposes at the expense of body growth (Agyekum et al., 2015).

Table 4.5 Digestibility coefficients (%) of nutrients in pigs fed diets containing different levels of avocado silage (n = 9)

Parameter	Treatment			SEM	*P*
	Control	3% AOC	5% AOC		
DM	83.3^{a}	77.7^{b}	76.9^{b}	0.93	<0.001
CP	82.4^{a}	71.1^{b}	74.9^{b}	0.360	<0.001
EE	76.4	76.4	75.7	0.77	0.206
Andf	70.2^{b}	73.6^{a}	70.9^{b}	0.443	<0.001
ADF	70.9^{c}	74.6^{a}	72.7^{b}	0.161	<0.001
ADL	68.1^{c}	73.5^{a}	60.8^{b}	0.401	<0.001

^{a,b,c} Means of the same row with different superscripts differ significantly (P<0.05) while those without superscripts did not differ (P>0.05)
DM = dry matter, CP = crude protein, EE = ether extract, aNDF = alpha-amylase neutral detergent fiber (ash residual inclusive), ADF = acid detergent fiber, ADL = acid detergent lignin

Data on the nutrient digestibility of the experimental diets is shown in Table 4.5. The digestibility of DM and CP was higher ($P<0.05$) in pigs fed the control diets compared to those fed on the AOC silage diets. Several studies reported a trend of a reduction of digestibility with increasing fibre content (Galassi et al., 2010), consistent with the present study. However, this higher digestibility of DM and CP in the control diet did not translate into better growth performance in pigs fed the control diet (see Table 4.4). Barrera et al. (2004) observed that an improved nutrient digestibility does not always translate to an improvement in pig performance, consistent with the present study. According to Agyekum et al. (2015) high fibre diets decrease energy and nutrient utilization in pigs due to lack of the enzymes necessary to break down dietary fibre, and are known to increase intestinal weight. In contrast, Souffrant (2001) concluded that the effect of dietary fibre on digestibility is variable due to the high variability in the physio/chemical characteristics of the diet. The digestibility of the fibre fractions in pigs was higher ($P<0.05$) with the diet that contained the 3 % AOC silage compared to other diets.

Data on the effects of dietary treatments on the carcass characteristics of pigs fed experimental diets is shown in Table 4.6. The warm carcass weight (WCW) was not affected ($P>0.05$) by the dietary treatments, but pigs in the control diet had heavier ($P<0.05$) WCW compared to those from AOC silage diets. It is well documented that pigs fed on high fibre diets are heavier in relation to those fed on low fibre diets (Thacker, 2006). This is probably due to the increased weight of visceral organs and the gastro-intestinal tract of pigs fed on high fibre diets (Len et al., 2008). Since the AOC silage diets contained higher fibre compared to the control, it was expected that pigs fed the AOC silage diets would have carcasses of a low dressing percentage (DP). However, the DP of pig carcasses in the present study were not affected ($P>0.05$) by dietary treatments (Table 4.5). This is inconsistent with Thacker (2006) who reported a decrease in DP and loin lean as dietary inclusion level of wheat DDGS was increased without any adverse effect on growth performance.

The back fat thickness was higher ($P<0.05$) in carcasses of pigs from the control diets compared to those on AOC silage diets (Table 4.6). According to Hernandez-Lopez et al. (2016), back fat thickness is usually affected by dietary composition and Galassi et al. (2017) reported a reduced back fat deposition in the carcass of pigs with dietary addition of maize silage, consistent with the present study. In contrast, Moset et al. (2015) fed pigs on diets containing citrus pulp silage and observed a decreased carcass yield while increasing the back fat of the carcasses. In addition, Bocian et al. (2017) fed fattening pigs with a diet that contained 14 kg steamed potato silage and reported that pigs fed the control diet had thinner back fat, higher loin muscle and higher meatiness than the carcass of pigs fed diets that contained silage. The different effects of silage diets on the back fat thickness in pig carcasses might be attributed to the differences in the dietary compositions between the present study and that of literature. The reduced back fat thickness with the AOC silage diets could probably be related to a balanced dietary amino acids which are responsible for protein synthesis, and the shortage of these nutrients could result in a greater fat and a lower lean meat deposition in pigs fed silage (Zanfi et al., 2014). Feeding Large White x Landrace crossbred pigs in diets containing 200 g maize cob silage/kg resulted in carcass back fat thickness of 10 mm (Kanengoni et al., 2014), which is comparable to those of the present study. According to Kanengoni et al. (2014) drip loss in pork is affected by various and complex factors which include, among other factors, the rate of pH decline and the ultimate pH. The ultimate pH of the pig carcasses in the present study was similar ($P>0.05$) across treatments, hence the drip loss percentage was not affected. Other carcass traits were not affected by dietary treatments.

Table 4.6 Effects of different dietary inclusion levels of avocado silage on carcass characteristics and physical attributes of pigs (n = 9)

	Treatments				
Parameter	**Control**	**3% AOC**	**5% AOC**	**SEM**	***P***
Warm carcass weight, kg	51.4	50.9	50.6	2.72	0.130
Cold carcass weight, kg	50.4^{a}	48.8^{b}	48.6^{b}	9.33	0.003
Dressing percentage %	74.6	75.2	72.9	4.07	0.174
Warm muscle pH_i	6.6	6.7	6.5	0.10	0.543
Cold muscle pH_u	6.4	6.4	6.4	0.28	0.970
Back fat thickness, mm	12.7^{a}	9.2^{b}	9.8^{b}	1.43	0.005
Chop, kg	54.0^{a}	47.8^{b}	49.2^{b}	1.90	0.011
Loin, kg	6.1	5.9	5.9	0.18	0.914
Rib, kg	6.0	6.3	6.3	0.17	0.456
Thick rib, kg	6.6	6.5	6.4	0.16	0.659
Leg, kg	6.4	6.3	6.4	0.12	0.627
Breast, kg	1.8	1.8	1.8	0.01	0.814
Belly, kg	1.3	1.3	1.3	0.01	0.548
Drip loss %	2.0	2.2	2.2	0.02	0.712

[a-b] Means of the same row with different superscripts differ significantly ($P<0.05$) while those without superscripts did not differ ($P>0.05$)

4.8 Conclusion

The growth performance in pigs was not affected by dietary inclusion levels of AOC silage, irrespective of increased fibre levels in the AOC silage diets. However, dietary addition of 3 % AOC silage improved the digestibility of fibre fractions. Among the carcass characteristics and physical attributes of the pigs, only cold carcass weight, back fat thickness and chop weight were reduced by the inclusion on AOC silage in pig diets. While it is imperative that the back fat thickness is reduced for consumer health purposes, the reduction of cold carcass and chop weights could be attributed to the high fibre level in the AOC silage, which reduced the muscle fibres after being chilled for 24 hrs. The use of AOC in the form of silage is a strategy that can be adopted by smallholder pig farmers in this country to resolve feed shortages and reduce the disposal of agro-industrial wastes into the environment and water. Further research to determine the amino acid profile and minerals in AOC is needed.

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CHAPTER 5

Effects of dietary exogenous enzyme addition on the growth performance, nutrient digestion and carcass characteristics in Large White x Landrace crossbred pigs fed diets containing Avocado (*Persia Americana*) oil cake (AOC) silage

Abstract

This experiment was conducted to evaluate the effect of different dietary inclusion levels of enzyme on the growth performance, nutrient digestion and carcass characteristics in pigs. Avocado oil cake (AOC) silage was produced and ensiled in 200 L drums for 90 days. A total mixed ration that contained 5 % AOC silage was formulated. The diet was top dressed with enzyme (Axtra® XB enzyme) at 0, 2.5 and 5 % of the daily ration of pigs. Twenty four Large White x Landrace (LW x LR) crosses, weighing approximately 22 kg live weight were used. The pigs were randomly allocated to the three experimental diets (8 pigs/ treatment) balanced on their live weights. Pigs were individually weighed at the start and weekly until end of the trial. The pigs were fed on experimental diets *ad lib* in the morning, allowing a 10 % of feed refusal, and free access to water was allowed. Data on daily feed intake, average daily gains and feed conversion rates were recorded. The dietary treatments were fed to growing pigs for 60 days. Growth performance, nutrient digestibility and carcass traits were evaluated. A nutrient digestibility study was done a week after completion of the growth study. At the end of the nutrient digestibility experiment, pigs were fasted for twelve hours and weighed to determine the final weight. The pigs were then slaughtered and carcass samples were collected and analysed. The addition of enzymes during the feeding of AOC silage diets to growing pigs improved ($P < 0.05$) the dry matter intake (DMI) and nutrient digestibility without ($P > 0.05$) improving average daily gains (ADG) and feed conversion rates (FCR) in pigs. Further, the carcass characteristics of pigs were not ($P > 0.05$) affected by diets, but the small and large

intestines were increased (P<0.05) with enzyme addition. The meat colour and the cooking quality were not (P>0.05) affected by dietary treatments. Increasing the enzyme addition to the pig diets was not worth, and enzyme addition only affected the feed intake and nutrient digestion. It was concluded that the AOC can be ensiled and used at less than 5 % in the diets of growing pigs without any detrimental effects on the growth of pigs.

Keywords: additives, backfat, daily gains, digestion, drip loss, feed intake

5.1 Introduction

The lack of animal feed resources is a challenge to smallholder pig producers in South Africa. Feeding pigs on commercialized diets is a very expensive exercise and these farmers resort to feeding their pigs with agro-industrial by-products, which are fibrous in nature. This leads to poor growth rates and carcass composition of the pigs, which do not meet market specifications. According to Mukumbo et al. (2014), the composition of feed consumed by pigs has a major influence on the physio-chemical and nutritional quality of pork. Avocado (*Persia Americana*) oil cake (AOC), is one of the agro-industrial by-products that contains valuable nutrients and is available for use in animal nutrition. However, this by-product contains high fibre (518 g neutral detergent fibre (NDF)/kg DM, 393 g acid detergent fibre (ADF)/kg DM, 258 g acid detergent lignin (ADL)/kg DM and 38 g acid detergent insoluble nitrogen (ADIN)/kg DM) (Skenjana et al., 2006). Feeding this by-product to growing pigs may have a negative impact on the growth performance of these pigs.

Research has shown that dietary addition of starch and fibre degrading multi-enzymes improved the growth performance of piglets (Officer, 1995, Emiola et al., 2009, Gandra et al., 2017). The positive effects of exogenous enzyme addition are due to disruption of intact cell walls and release of entrapped nutrients or due to the changes in the physical properties on non-starch polysaccharides (Officer, 1995). It has been reported that silage making from high fibre forages tends to reduce forage fibre fractions due to the activity of enzymes during fermentation (McDonald et al., 2010). This approach can be implemented to preserve AOC, which will be fed to animals. Research on the use of exogenous enzymes in pigs fed diets that contain ensiled avocado oil cake is limited. The present study was therefore conducted to evaluate the effects of exogenous enzyme addition on the growth performance, nutrient digestion and carcass characteristics of growing pigs.

5.2 Materials and methods

5.2.1 Silage production

The study was conducted at the Agricultural Research Council Institute in Irene, South Africa (Longitude 28°C12ˈ40 ˈE: Latitude 25° 54ˈ36 S; Altitude 1526 m) about 15 km South of Pretoria. Batches of avocado oil cake (AOC) were collected from Westfalia in Pietermaritzburg, South Africa and brought to ARC-Irene for proximate analysis and silage production. The silage was produced by mixing 700 g AOC/kg fresh matter (FM), 250 g wheat bran/kg FM and 50 g sugarcane molasses/kg FM. This mixture was ensiled in 210 L drums that were lined with plastic bags which were enclosed with rubber lids to prevent damages to the bags by rodents. A packing density of 820 ± 29.8 kg/m^3 was obtained and the drums were stored at 25-28°C. After 90 days of ensiling, drums were opened and silage samples were collected for the determination of nutrient composition and fermentation characteristics. An experimental diet that contained 5 % of AOC silage was formulated as shown in Table 5.1.

Table 5.1 Chemical composition of the experimental diet and its nutrients

Ingredients	kg
Maize	709.5
Feed lime	4.8
Monocalcium	20.1
Soybean meal	202.8
Premix[1]	2
Salt	10
AOC Silage	50
Lysine	0.8
Nutrient Composition (n=3)	**%**
DM	74.7
Ash	7.2
CP	12.5
EE	4.8
GE MJ/kg DM	21.8
aNDF	51.4
ADF	28.8
ADL	13.1

[1]Provided the following per kilogram of diet: 6,500 IU vitamin A, 1,200 IU vitamin D_3, 40 IU vitamin E, 2 mg vitamin K_3, 1-5 mg vitamin B_1, 4.5 mg vitamin B_2, 0.03 mg vitamin B_{12}, 2.5 mg vitamin B6, 25 mg niacin, 12 mg calcium pantothenate, 190.5 mg choline, 0.6 mg folic acid, 0.05 mg biotin, 40 mg manganese, 100 mg zinc, 125mg copper,1 mg iodine,100 mg ferrous, and 0.3 mg selenium.
AOC= avocado oil cake silage

5.2.2 Animals, housing and feeding

This experiment was approved by the Animal Ethics Committee of the ARC-Irene with the approval no: APIECIS/027. Three diets containing 5 % AOC silage were treated as follows: i) control diet (no enzyme addition), ii) 2.5 % enzyme addition, and iii) 5 % enzyme addition. The enzyme (Axtra® XB enzyme) was purchased from Pennyville LTD (corner Kirkney Ext 6 and Richards Bay road), Pretoria West. The enzyme contains endo-1, 4-β-

xylanase (12 200 U/g) and endo 1,3β-gluconase (1520 U/g), and was applied by top dressing on the daily ration in accordance with the daily intake of the pigs.

Twenty four Large White x Landrace (LW x LR) crosses, weighing approximately 22 kg live weight were used. The pigs were randomly allocated to the three experimental diets (8 pigs/ treatment) using their live weights. Pigs were housed individually in pens (1.96 x 1m) and the feeders checked and adjusted twice each day to ensure constant access to fresh feed, and minimize any possible wastage. Water was freely available through nipple drinkers. An adaptation to the experimental condition of 14 days was given and the experiment took 60 days. Pigs were individually weighed at the start and weekly until the end of the trial. The pigs were fed on experimental diets *ad lib* in the morning, allowing a 10 % of feed refusal, and free access to water was allowed. Data on daily feed intake (DFI), average daily gains (ADG) and feed conversion rates (FCR) were recorded. The following formulae were used to calculate the growth performance parameters of the pigs.

$$\text{Dry matter intake (DMI, kg)} = \frac{\text{(Total feed intake x Dry matter)}}{100}$$

$$\text{Average daily gain (ADG, kg/d)} = \frac{\text{(Final weight – Initial weight)}}{\text{Experimental period}}$$

$$\text{Feed conversion ratio (FCR)} = \frac{\text{Average daily feed intake (ADFI)}}{\text{ADG}}$$

5.2.3 Digestibility study

A digestibility study was done a week after completion of the growth study. Chromic oxide was mixed with the feed at a concentration of 3 g chromic oxide/kg feed as an indigestible marker (Brandy et al., 2017). The pigs were allowed an adaptation period of 3 days to the chromic oxide mixed diet before faecal collection for 5 days using the grab sampling method. Faecal samples were collected once per day, weighed and the DM determined. The samples were then kept in 250 ml plastic containers and frozen at -20°C until further analysis. The samples of the 5 day period were pooled per pig and stored. The samples of the diets and faeces were analysed for DM, crude protein (CP), energy, ash, fat and fibre fractions (NDF, ADF and ADL) by standard procedures (AOAC, 1990). Nutrient digestibility was calculated as described by McDonald et al. (2011) as follows:

Digestibility (%) = 100 – (100 x % Indicator feed / % Indicator faeces x % Nutrient faeces / Nutrient feed)

5.3 Post slaughter measurements

At the end of the nutrient digestibility study, pigs were fasted for 12 hours and weighed to determine the final weight. The pigs were then transported to the ARC Institute's abattoir for slaughter. Pig carcasses were processed according to the routine abattoir procedures, which included an ante-mortem inspection and rest for the pigs before slaughter. The pigs were then stunned with an electrical stunner set at 220 V and 1.8 A with a current flow for 6 s and exsanguinated within 10 s of stunning. Dehairing and evisceration were done according to the abattoir`s standard operating procedures.

5.4 Carcass measurements

Following carcass dressing and evisceration, warm carcass weight (WCW) was measured using an overhead scale while warm carcass pH (pH_i) was measured on each of the carcasses' *Longissimus thoracis* muscle (eye muscle) with a portable pH meter (EUTECH Instruments, Thermo Fisher Scientific Inc. Singapore) between the third and the fourth rib, 60 mm from the midline. Immediately after determination of warm carcass weight and pH, dressing percentage (DP) was calculated as described by Bonvillani et al. (2010) and expressed as percentage of live weight using the equation: Dressing (%) = weight of warm carcass / final weight of pig x 100

The carcasses were then placed in a cold room and kept at an approximate temperature of $0^{\circ}C$ for 24 hours, after which cold carcass weight (CCW) of each carcass was measured. After determination of cold carcass weight, cold carcass pH (pH_u) as measured on the *Longissimus thoracis* muscle using a pH meter. Back fat thickness was measured between the 2^{nd} and 3^{rd} ribs about 60 mm from the midline on the left carcass using pair of Vernier Calipers (Bruwer et al., 1991).

The head from each carcass was then removed at the *atlanto-occipital* joint and the tail at the junction of the third and fourth sacral vertebrae. The flare fat, kidneys, fat, glands and remaining parts of the diaphragm were also removed. Carcasses were then split into 2 parts along the median plane from the remaining sacral vertebra to the cervical vertebra with a carcass splitting band saw. Backfat thickness was measured at the first rib (dorsal fat thickness at first rib) and last rib (dorsal fat thickness at last rib) of each carcass. Other carcass samples (loin, thick rib, leg, breast and belly) were cut from the left side of the carcass and weighed using digital scale (DIGI DS160 industrial scale, Toronto, Canada).

5.5 Sample collection and measurements.

5.5.1 Drip loss determination.

An 80 g chop was cut from the *Longissimus dorsi* muscle between the 4^{th} and 8^{th} ribs of each carcass and used to determine drip loss (DL) of the muscles. Each chop sample was placed in a nylon mesh and sealed in a plastic bag for a period of 24 hrs at $2^{\circ}C$. After 24 hours the sample was taken out of the plastic and re-weighed separately, while the plastic bag was also re-weighed to determine the weight of the empty bag. Drip loss was calculated according to the method described by Choi and Oh, (2016) using the equation: Drip loss = Final weight of sample / Initial weight of sample x 100.

5.5.2 Meat colour determination.

Meat colour was measured using a Minolta colour meter (Model CR200, Osaka, Japan) as described by Krzywicki, (1979). Six chop samples from the *Longissimus dorsi* muscle at the last lumber vertebra were used to measure the meat colour following the CIE colour convention (CIE, 1978). The three fundamental colour components (L*, a* and b*) were determined. The L* demonstrates lightness on a scale of 0 (all light absorbed) to 100 (all light reflected), a* indicates red to green colour and b*indicates yellow to blue colour. After putting the meat samples on a tray to bloom for 1 hour at 4˚C to allow for oxygenation, three colour measurements were carried out on each of the six samples at three different locations. Values for the colours measurements were used to calculate Hue (H* spectral colour) defined as tan -1 (b/a) and chroma (C* colour saturation) defined as the square root of a*2 + b*2 of the meat according to Young et al. (1991).

5.5.3 Thaw and cooking losses determination.

Thaw and cooking losses were done as a precondition for the determination of Warner Bratzler shear force. In summary, 60 mm of *Longissimus loin* from the left side of each carcass was sub-sampled. The sub-samples were vacuum packed and chilled for 1 day at 5˚C. Another 60 mm sub-samples of the left *Longissimus loin* was chilled for 7 days at 4˚C. Both sub-samples were removed from the chiller after 1 and 7 days, respectively and frozen at -20˚C until the evaluation of Warner Bratzler shear force.

5.5.3.1 Thaw loss determination

Following freezing the two 60 mm sub-samples, each was weighed using a digital balance scale (Model GM-501, Lutron Electronic, Coopersburg, USA) to measure the initial weights. The sub-samples were then thawed at 4˚C for 24 hrs following which the final weights were similarly measured using the digital balance scale. Thaw loss was then calculated as described by Jama et al. (2008) using the equation: Thaw loss (%) = weight of frozen sub-sample – weight of thawed sub-sample / weight of frozen sub-sample x 100.

5.5.3.2 Cooking losses

Immediately after evaluation of thaw loss, each thawed sub-sample was broiled in an oven using direct radiant heat according to AMSA (1978). The electric oven was set on "fan heat'' at 160˚C for 10 minutes prior to broiling of the sub-samples. The steaks were placed on an oven pan on a rack to allow meat juiciness to drain during cooking and placed in the preheated oven in the middle of the oven. The steaks were broiled until an internal temperature of ±50˚C was reached. After broiling, the final weight of each sub-sample was measured. Cooking loss was then calculated according to Hwang et al. (2004) using the equation: Cooking loss (%) = weight of thawed raw sub-sample – weight of broiled sub-sample / weight of thawed raw sub-sample x 100.

5.5.4 Warner Bratzler shear force

The Warner Bratzler shear force (tenderness measurement) of the meat samples was determined as described by Raharjo et al. (1992). In brief, after boiling each sub-sample was cooled to room temperature for at least 2-3 hrs after which six cylindrical round cores (12.5 mm diameter) were cored parallel to the grain of the each sub-sample using a coring device.

The device was mounted on an Instron Universal Testing Machine (Model 4301). Each of the six cores from the sub-samples was sheared once through the centre at the crosshead speed of 200 mm/min with a 1 kN load cell perpendicular to the muscle fiber direction. From the six cores per sub-sample, an average shear force was recorded.

5.6 Statistical analysis

The experiment was carried out as a randomized blocked design (RBD) with three treatments randomly allocated in 8 blocks. ANOVA (Analysis of Variance) was performed to test the differences between the three treatments effects (Control, 2.5% and 5% enzyme addition) in order to determine the growth performance, nutrient digestibility and carcass characteristics in pigs (Snedecor and Cochran, 1980). Shapiro-Wilk's test was performed on the standardized residuals to test for deviations from normality (Shapiro and Wilk, 1965). In cases where significant deviation from normality was evident and the deviation was due to skewness, the outliers were removed until the data was normally or symmetrically distributed (Glass et.al. 1972). Student's t-LSD (Least significant difference) were calculated at a 5% confidence level to compare treatment means (Snedecor and Cochran, 1967). All the above data analysis was performed with SAS version 9.3 statistical software (SAS, 1999).

The data was fitted with the following model: $Y_{ij} = \mu + t_i + ß_j + \varepsilon_{ij}$

where: Y_{ij} is the individual observations of the i-th treatment and the j-th replicate, μ is the overall mean, t_i is the effect of the i-th treatment, , β_j is the effect of the j-th block replicate, and ε_{ij} is the residual error.

5.7 Results and Discussions

The potential of the nutritive value of ensiled AOC in diet for pigs is not fully realized because of certain physical and chemical characteristics of the resource. In order to improve the performance of pigs, the present study evaluated the use of an enzyme, Axtra® XB enzyme in 2 different dietary doses during the growth of pigs. High fibre diets decrease energy and nutrient utilization in pigs due to lack of enzymes necessary to break down dietary fibre (Agyekum et al., 2015).The use of fibre degrading enzymes have however, had inconsistent effects on growth performance in pigs. For example, Gandra et al. (2017) reported positive effects of xylanase and cellulase addition on fibre digestibility when animals were fed with sugarcane silage, whereas no improvement in pig growth performance when DDGS diet was supplemented with xylanase due to the complex structure of DDGS from wheat, which resists a single substrate enzyme (Widyaratne and Zijlstra, 2007).

Data on the growth performance of pigs fed experimental diets is shown in Table 5.2. Increasing enzyme addition improved ($P<0.05$) the dry matter intake (DMI) in pigs but did not improve the ADG and FCR in the pigs. Consistent to the present study, Widyaratne and Zijlstra (2007) reported lack of significant improvement in pig growth performance when DDGS diet was supplemented with xylanase. The lack of response from enzyme supplementation in the diet of pigs in the present study suggests that endogenous enzyme activity was not limiting piglet performance (Officer, 1995). In contrast, Rho et al. (2018) treated maize DDGS with Axtra XB enzyme (i.e. same product used in the present study), and reported improved feed efficiency, suggesting degradation of dietary fibrous components that may have limited nutrient utilization in younger pigs. The improved DMI with enzyme supplementation might be related to the fermentation of the fibre in the hind gut of pigs (Mwesigwa et al., 2013). Supplementation of Axtra XB enzyme to high fibre (22 % NDF) diets that were fed on growing pigs did not affect feed intake compared to the control diet (Agyekum et al., 2015), contradicting the improved DMI in the present study.

Table 5.2 Effect of different dietary inclusion levels of enzyme on growth performance of pigs (n = 8)

Parameter	Treatment			SEM	*P* value
	Control	2.5%	5%		
Initial body weight, kg	24.6	25.4	24.8	1.31	0.908
Final body weight, kg	67.4	68.0	69.1	0.82	0.354
Body weight gain, kg	42.9	42.6	44.3	0.219	0.707
Average daily gain, g/day	0.714	0.714	0.742	0.02	0.340
Dry matter intake, kg/day	1.64^{b}	1.63^{b}	1.72^{a}	0.02	0.018
Feed conversion ratio, kg/g	2.29	2.29	2.32	0.05	0.571

Treatments: control = no enzyme, 2.5 % enzyme, 5 % enzyme

Enzyme addition improved the digestibility of nutrients, more noticeably on the fibre fractions (Table 5.3). The higher fibre digestibility observed for pigs fed the enzyme treated diets could have resulted from fermentation of the fibre in the hind gut with the volatile fatty acids (VFAs) produced, contributing to the net energy requirements of the pigs (Mwesigwa et al., 2013). The enhanced digestibility of CP in the enzyme treated diet is likely to be associated with the activity of the components of the enzyme product used. This product could disrupt the cell wall matrix of the fibrous components in the feed, thereby causing easy access of the endogenous proteolytic and cellulolytic enzymes to digest the entrapped protein and carbohydrate (Agyekum et al., 2015). In contrast, Owusu-Asiedu et al. (2010) added a combination of β-glucanase and xylanase enzymes in the diet of pigs and reported no improvement in the digestibility of DM, CP and energy in pigs. The extent to which enzyme supplementation improves nutrient digestibility tends to be low when using highly digestible ingredients (Rho et al., 2018). This might be the reason for inconsistent effects of enzyme supplementation in the diet of pigs in the literature. Although enzyme addition improved nutrient digestion in the present study, it did not improve the growth performance of pigs (Table 5.2). According to Barrera et al. (2004), an improved nutrient digestibility due to enzyme supplementation does not always translate to an improvement in pig performance, consistent with the present study.

Table 5.3 Effect of different dietary inclusion levels of enzyme on nutrient digestion by pigs (n = 8)

Parameter (%)	Treatment			SEM	*P* value
	Control	2.5%	5%		
DM	87.9	87.3	88.2	0.37	0.236
CP	72.9^{b}	70.8^{b}	75.0^{a}	0.15	0.001
EE	71.5^{b}	74.2^{a}	73.8^{a}	0.35	0.001
aNDF	58.7^{b}	67.9^{a}	67.9^{a}	0.12	0.001
ADF	56.1^{b}	58.8^{a}	59.7^{a}	0.09	0.001
ADL	49.7^{c}	54.1^{b}	58.3^{a}	0.14	0.001

[a,b,c] Means of the same row with different superscripts differ significantly (P<0.05) while those without superscripts did not differ (P>0.05)
Treatments: control = no enzyme, 2.5 % enzyme, 5 % enzyme
DM=dry matter, CP=crude protein, EE= ether extract, αNDF= alpha-amylase neutral detergent fibre, ADF= acid detergent fibre, ADL=acid detergent lignin

Addition of enzyme to the AOC silage diet did not (P >0.05) affect the carcasses of the pigs (Table 5.4). The back fat thickness in pig`s carcasses is estimated to range between 1.5 – 2.5 mm for each 10 kg live weight, and is related to the genetic influence of pigs since rustic unimproved genotypes have thicker back fat compared with improved pig breeds (Temperan et al., 2014). The back fat thickness of the pig carcasses in the present study falls within this range, but were not affected (P> 0.05) by dietary treatment. According to Hernandez-Lopez et al. (2016), back fat thickness is usually affected by dietary composition and the experimental diets were of similar nutrient compositions (see Table 5.1). Consistent to the present study, Mukumbo et al. (2014) reported no effect on the back fat thickness in the carcasses of pigs fed diets containing moringa leaf meal. However, the back fat thickness in their pigs ranged between 27-29 mm, which is higher than that of pigs in the present study. Responses by back fat thickness and marbling were significant, whereas empty stomach weight increased when sticky coffee hull silage was included in the diet of growing pigs (Carvhalo et al., 2011). Adding enzyme protease to diets of growing pigs did not affect carcass characteristics (carcass weight, back fat, lean meat and dressing %) (O`Shea et al., 2014), consistent with the present study.

The warm carcass weights and the dressing % were not affected (P>0.05) by dietary treatments. The present study is inconsistent with Chamorro-Ramirez et al. (2017) who fed growing pigs diets containing 50 and 100 g/kg fresh mass fermented apple pomace and reported that the addition of enzyme negatively affected the carcass dressing percentage of pigs.

Table 5.4 Effects of different dietary enzyme inclusion on carcass characteristics and physical attributes of pigs (*n* = 8)

Parameter	Treatment			SEM	*P* value
	Control	2.5%	5%		
Warm carcass weight, kg	48.9	49.9	47.7	1.73	0.670
Cold carcass weight, kg	47.5	48.5	46.4	1.69	0.670
Dressing percentage	72.4	73.5	68.9	2.56	0.440
Warm muscle pH_i	5.43	5.46	5.45	0.02	0.522
Cold muscle pH_u	6.13	6.22	6.22	0.17	0.532
Back fat thickness, mm	11.3	11.6	11.0	0.84	0.405
Loin, kg	2.00	2.40	2.50	2.01	0.373
Rib, kg	2.28	2.14	2.18	0.07	0.408
Thick rib, kg	2.85	2.55	2.72	0.13	0.316
Leg, kg	6.58	6.29	6.31	0.28	0.727
Breast, kg	2.5^{a}	2.3^{ab}	2.1^{b}	0.12	0.046
Belly, kg	3.2	3.3	3.2	0.11	0.824

$^{a-b}$ Means of the same row with different superscripts differ significantly (P<0.05) while those without superscripts did not differ (P>0.05)

Treatments: control = no enzyme, 2.5 % enzyme, 5 % enzyme

pHi = Initial pH at 45 minutes, pHu=Ultimate pH at 24 hours

The observation that feeding the enzyme treated diets increased the visceral organs weights in pigs was not expected since pigs fed the enzyme treated diets have improved fibre digestibility. According to Augustine et al. (2011), internal organs in broiler chickens decreased with increasing levels of enzyme supplemented cassava peel meal (Augustine et al., 2011), inconsistent with the present study. The increased organ weights (i.e. caecum, large intestines and small intestines) shown by these pigs (Table 5.5) may be attributed to increased intake of fibre compared to pigs that were fed the control diet (Table 5.2), which is consistent to Galassi et al. (2010) findings. Bharathidhassan et al. (2009) reported an increase in the dressing % and carcass yields in chickens fed enzyme supplemented diets, contrasting the present study. Although pigs fed the enzyme treated diet had larger small and large intestines compared to those fed the control diet, the difference was very small and did not affect the carcass dressing % (Table 5.4). There was a progressive increase in length of the small and large intestines of rabbits with increasing levels of enzyme supplemented yam peel meal (Yakubu et al., 2017), consistent with the present study.

Table 5.5 Effect of different dietary inclusion levels of enzyme on visceral organs of pigs fed diets containing different levels of AOC silage (n = 8)

Parameter	Treatment			SEM	*P* value
	Control	3%	5%		
Liver, kg	0.99	1.14	1.00	0.05	0.076
Heart, kg	0.27	0.31	0.26	0.02	0.371
Large intestine with waste, kg	1.11^{b}	1.25^{a}	1.24^{a}	0.01	0.0001
Large intestine without waste, kg	0.76^{b}	0.85^{a}	0.86^{a}	0.02	0.002
Stomach with waste, kg	0.62^{ab}	0.72^{a}	0.56^{b}	0.03	0.016
Stomach without waste, kg	0.47	0.52	0.44	0.02	0.126
Caecum with waste, kg	0.33	0.29	0.40	0.04	0.138
Caecum without waste, kg	0.14	0.13	0.14	0.01	0.835
Small intestine with waste, kg	1.15^{b}	1.23^{ab}	1.33^{a}	0.14	0.0001
Small intestine without waste, kg	0.69^{b}	0.82^{a}	0.78^{a}	0.07	0.007
Large intestine, cm	3.57	3.55	5.00	2.47	0.360
Small intestine, cm	19.10^{b}	21.48^{a}	21.95^{a}	0.26	0.0001

$^{a-b}$ Means of the same row with different superscripts differ significantly (P<0.05) while those without superscripts did not differ (P>0.05)

Treatments: control = no enzyme, 2.5 % enzyme, 5 % enzyme

Data on the effects of enzyme supplementation on carcass colour from pigs is shown in Table 5.6. The colour of meat from the pork carcasses did not differ (P>0.05) amongst the diets, consistent with Fang et al. (2016) who reported lack of differences in the colour of meat from pork carcasses fed apple pomace mixed silage compared to those from the control diet. In contrast, Martelli et al. (2002) reported a lower "L" and "a" values in carcasses of pigs fed sugar beet pulp silage compared to those on control diet. The "a' value is related to the concentration of pigments and to the pH value, while the lightness (L) value is related to the moisture and fat contents of the carcass, and is also affected by the pH of the meat. The average values of the colour for the pork carcasses are consistent with the report of Temperan et al. (2014) who reported colour "L", "a" and "b" in meat from pigs of different breeds to be in the ranges of 44-58, 5-10 and 4-9, respectively. Some researchers found that low protein diets increased the L, a, and b values in pork carcasses (e.g. Goerl et al., 1995), which did not happen in the present study since the CP content of the diets was sufficient for the pigs (Table 5.1) (NRC, 1998).

Table 5.6 Effect of different inclusion levels of enzyme on meat colour of pigs fed diets containing different levels of avocado oil cake (n = 8)

Parameter	Treatment			SEM	*P* value
	Control	2.5%	5%		
L* (D_{65})	49.85	49.74	49.95	0.70	0.979
a* (D_{65})	3.13	2.69	2.37	0.32	0.272
b* (D_{65})	12.44	12.11	11.83	0.26	0.267

L, lightness; a, redness; b, yellowness

Treatments: control = no enzyme, 2.5 % enzyme, 5 % enzyme

Data on the cooking and thaw losses of pork carcasses are shown in Table 5.7. The cooking and the thaw losses did not differ (P > 0.05) amongst the dietary treatments. Lee et al. (2016) cooked pork samples from pigs fed diets supplemented with persimmon peel, and recorded cooking losses that ranged between 19 to 23 %, higher than that of the present study. According to Koohmaraie et al. (2002) fluid loss during thawing and cooking are critical processing traits used to evaluate meat eating quality. High cooking losses produce tough meat (Gregory, 2007), while low cooking losses yield juicy meat (Ameha, 2006). This means that the pork carcasses produced in the present study are of good quality compared to some reported in the literature

(Lee et al., 2016). According to Kanengoni et al. (2014) drip loss in pork is affected by various and complex factors which include, among other factors, the rate of pH decline and the ultimate pH. The ultimate pH of the pig carcasses in the present study was similar (P>0.05) across treatments, hence the drip loss percentage was not affected.

Table 5. 7 Cooking and thaw losses from pig carcasses fed diets containing different levels of avocado oil cake (n=3

Parameters %	Days	Treatments			SEM	*P* value
		Control	2.5%	5%		
Drip loss		4.89	5.08	5.59	0.70	0.768
Cooking loss	D1	4.98	4.58	4.85	1.064	0.740
Cooking loss	D7	3.93	3.95	3.69	0.431	0.436
Thawing loss	D1	2.98	2.62	3.19	1.251	0.107
Thawing loss	D7	3.86	3.81	3.27	1.365	0.639

Treatments: control = no enzyme, 2.5 % enzyme, 5 % enzyme

The similarity in the thawing and cooking loss percentages, and the drip loss % of pork loin chop samples among the dietary treatments in the present study means that consumers will not get a different pork product if ensiled AOC is used with enzyme.

5.8 Conclusion

This study showed that the addition of enzymes during the feeding of AOC silage diets to growing pigs improved the DMI and nutrient digestibility without improving the ADG and FCR of pigs. Further, the carcass characteristics of pigs were not affected by diets, but the small and large intestines were increased with enzyme addition. The meat colour and the cooking quality were not affected by dietary treatments. It can be concluded that increasing the enzyme addition to the pig diets was not worthwhile with regards to improving growth performance of pigs. However, since enzyme addition only affected the feed intake and nutrient digestion, further research on its effect on nutrient excretion and by extension environmental pollution may be warranted. Further research to study the amino acid and fatty acid profiles on both the diets and pork carcasses is needed.

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CHAPTER 6

Conclusions and Recommendations

6.1. Conclusions

Ensiling of AOC was first tested in 1.5 L anaerobic jars, whereby the fermentation dynamics, nutritive values and the aerobic stability of the silage were evaluated. The AOC silage was produced by mixing 700 g AOC /kg fresh matter (FM) of AOC, 250 g wheat bran/kg FM wheat bran and 50 g sugarcane molasses/kg FM, and treated with either enzyme (3 and 5%) or without enzyme. It was found that good quality silage can be produced from AOC without the addition of enzyme. However, the effect of enzyme addition was apparent on the reduction of NDF and increase in the WSC of ensiled AOC. Although enzyme addition was applied at two different levels (i.e. 3 and 5 %), increasing enzyme addition to AOC at ensiling was not worth it since it did not make any significant contribution.

In the second study, AOC silage was produced without enzyme additive and ensiled in 200 L drums for 90 days. Totally mixed rations that contained 0, 3 and 5 % AOC silage were formulated and fed to growing pigs in a 60 days feeding study. The growth performance of pigs was not affected by dietary inclusion levels of AOC silage, irrespective of increased fiber levels in the AOC silage diets. However, dietary addition of 3 % AOC silage improved the digestibility of fiber fractions. The cold carcass weight and the chop were reduced with AOC silage addition. However, AOC silage reduced the back fat thickness of the pig carcasses, which has positive implications for human health.

In the third study, silage from AOC was added at 5 % in a concentrate diet, and a fibrolytic exogenous enzyme was top-dressed at 0, 2.5 and 5 % of the daily ration of pigs. The dietary treatments were fed to growing pigs for 60 days. Growth performance, nutrient digestibility and carcass traits were evaluated. This study showed that the addition of enzymes during the feeding of AOC silage diets to growing pigs improved the DMI and nutrient digestibility without improving the ADG and FCR of pigs. Further, the carcass characteristics of pigs were not affected by diets, but the small and large intestines were increased with enzyme addition. The meat colour and the cooking quality were not affected by dietary treatments. Increasing the enzyme addition to the pig diets was not worth it, and enzyme addition only affected the feed intake and nutrient digestion.

6.2. Recommendations

The following recommendations can be drawn from the present study:

i) The use of AOC in the form of silage is a strategy that can be adopted by smallholder pig farmers in this country to resolve feed shortages and reduce environmental pollution.

ii) Additional studies that evaluate the use of AOC in diets of growing pigs in a farm setting should be done.

iii) The minerals, fatty acid and amino acids contents in the AOC should be determined

iv) The energy and N balance study in pigs fed diets containing AOC should be conducted

v) The fatty acid and amino acid profiles in the pork carcasses of pigs fed diets containing AOC should be evaluated