Effects of Dietary Fibre on Pig Excreta Characteristics and Odours from Slurry

By

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A Thesis submitted for the requirements for the degree of Masters of Science in Agriculture (Animal Science)

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Scottsville, Pietermaritzburg

SOUTH AFRICA

2012

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Declaration

Regardless of the acknowledgements provided as citations or quotes right across this document, this thesis is the author's own original work under the supervision of Professor Michael Chimonyo at the University of KwaZulu-Natal (Pietermaritzburg campus). This thesis has and will not be submitted to any other institution.

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Abstract

A study was conducted to test the effects that different high fibre sources and their varying inclusion levels had on the characteristics of the excreta (faeces, urine and the slurry), and on odour from the slurry of growing pigs. Faeces and urine characteristics were tested from 52 pigs fed rations containing grass hay (GH), lucerne hay (LH), maize cobs (MC), maize stover (MS) and sunflower husk (SH) diets at inclusion levels up to 400 g/kg as fed basis. Faecal output, faecal consistency and nitrogen were influenced by fibre type ($P < 0.01$) and inclusion level ($P < 0.01$). Nitrogen content in faeces and urine was also affected by dietary fibre inclusion. Increasing fibre inclusion levelled to a reduction in urinary nitrogen content, indicating nitrogen repartitioning from urine to faeces, thereby minimizing nitrogen volatilization.

The slurry from pigs fed on LH, MC and SH at levels up to 160 g/kg was tested for chemical composition and odour offensiveness. The slurry was incubated for 16 days. The pH and nitrogen content varied among fibre types and incubation period ($P < 0.05$). Isobutyrate and butyrate concentrations varied with fibre type and the incubation period tested ($P < 0.01$). Using panellists, the SH containing rations resulted in low odour offensiveness score. Maize cob-containing diets resulted in the largest odour scores, with (mean rank of 2.2 and 4.3 for SH and MC, respectively). To reduce odour offensiveness from piggeries, sunflower husk was recommended as an alternative feed ingredient for growing pigs.

Key words: Dietary fibre, growing pigs, excreta composition, odour offensiveness

Acknowledgements

Firstly, I would like to thank God Almighty for keeping me up day by day and blessing me to be where I am today. Secondly, I would like to thank my supervisor Professor Michael Chimonyo for his guidance throughout the course of this thesis, especially during the times when the going gets tough, he always advised with a positive mode to progress. I would also like to thank Dr James Madzimure and my colleague Saymore Petros Ndou for their support and encouragement.

I also thank the National Research Foundation for the financial assistance towards my welfare during the course of this Master's project.

Also, I would like to thank the staff of the Discipline of Animal and Poultry Science and Microbiology Department, not forgetting my fellow postgraduates, particularly Saymore Petros Ndou for giving me courage, motivation and sharing ideas with me as means of beefing up the standard of this thesis. I would like thank the Feed Evaluation Unit of the Discipline of Animal and Poultry, not forgetting the Department of Microbiology for making the analyses of feeds and the excreta a success. I also thank the staff and the students of the Discipline of Animal and Poultry Science for their participation on the sensory evaluation for odours from slurry, as that activity was not an easy task to do at all.

Deeply, I would like to thank my family, particularly my Mother and my late Father for always believing that I will attain success towards my studies and beyond the academic carrier, not forgetting my close friends for giving me courage and hope that I will complete my thesis with success. Lastly, I would like to thank my daughter for being a very significant inspiration every day, as I will always push harder in life to secure her future.

Dedication

This thesis is a dedication to the whole Mpendulo family, particularly my late father and son who unfortunately could not live long enough to taste the fruits that this thesis will reap in the near future.

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Mpendulo, C. T. and Chimonyo, M., 2012. Effect of fibrous diets and inclusion level on the chemical composition and odours from slurry of growing pigs. Oral Presentation at the 2012 ADSA-AMPA-ASAS-CSAS-WSASAS Joint Annual Meeting, held on the 15-19 July 2012 in Phoenix, Arizona. Abstract: Journal of Animal Science 90, Supplement 3/Journal of Dairy Science, 95, Supplement 2.

CHAPTER 1: General Introduction

1.1 Background

The world pig population stands at over 965 million and keeps on increasing (Bindelle *et al*., 2008). Pig diets are largely composed of maize and soya bean. The demand for these products for human consumption is also high (Ndindana *et al*., 2002; Stein and Lange, 2007), raising concerns about unsustainable commercial pig production. Improved pig genotypes also require diets with high amounts of nutrients, particularly protein (Chimonyo *et al*., 2005). The excreta produced are also likely to have high amounts of nutrients, leading to marked pollution levels of the environment. Excreta production has, therefore, increased as piggeries keep expanding, raising concerns about odours and volatile nutrients emitted from piggeries (Le *et al*., 2009). Aarnink and Verstegen, (2007) defined odours as a combination of various compounds that give an unpleasant smell. Odours are known nuisances rather than the environmental pollutants when pig farms are located nearby residential areas. There is growing interest among researchers to seek for strategies of reducing odours from piggeries. One of the options that have been suggested is to alter pig excreta characteristics through dietary fibre inclusion to pig rations (Canh *et al*., 1998; Kerr *et al*., 2006).

Including dietary fibre in pig rations is one potential strategy to increase anaerobic fibre fermentation and reduce protein content of the excreta (Le *et al*., 2009). Dietary fibre improves the welfare of pigs by reducing persistence hunger, stomach ulcers, and gut health of pigs (Masse *et al*., 2003). In newly weaned pigs, dietary fibre inclusion increases early feed intake due to an increased rate of passage caused by a reduced viscosity of the digesta (Perez-Mendoza, 2010;

Ndou *et al*., 2012). Masse *et al*. (2003) reported an improved faecal production and a reduced urine production as the dietary fibre content of pregnant sows' diet was increased. The reduction in the drinking habits of pigs has been known to be due to adjunctive drinking habits brought about by hunger satiety, because pigs are known to drink more water to allay hunger (Masse et al., 2003; Dryden, 2008). Although various authors have reported conflicting statements concerning water consumption by pigs feeding on high fibre diets, with some authors finding no statistical differences in water consumption when pigs are fed varying levels of dietary fibre (Wilfart *et al*., 2007; Dryden, 2008).

Dietary fibre incorporation also improves microbial fermentation of fibre, thereby increasing the production of short-chain fatty acids (SCFA) mainly acetate, propionate, and butyrate known to be major contributors of energy to the host (Bindelle *et al*., 2008; Le *et al*., 2009; Perez-Mendoza, 2010). Odour forming SCFAs, such as butyrate, are reduced in excreta by a further reduction in the pH of faeces or slurry (Gralapp *et al*., 2002). In addition, the use of fibrous diets also minimises odour emission from slurry due to changes in microbial activities, as microbes access more fibre substrates than the protein substrates in the feed ingested (Gralapp *et al*., 2002; Le *et al*., 2009). When faeces and urine combine, more nitrogen in urine is repartitioned and bonded more with nitrogen in the faeces. This is due to the activity of urease enzyme present in the faeces, as pigs are fed high fibre ingredients; changing the urea nitrogen in urine to bacteria nitrogen in faeces, making it less susceptible to volatilization (Canh *et al*., 1997; Le *et al*., 2008). The influence of different fibre types on excreta characteristics and odour emission is, however, still unclear.

In southern Africa, crop residues and industrial by-products are usually wasted or burnt and can, in most cases, become environmental hazards. Limited utilisation of these by-products in pig feeding is largely caused by their perceived low nutritional quality and negative impacts on growth performance and feed conversion efficiency. Benefits of using fibre sources are usually underestimated. The most common agricultural by-products include grass hay, lucerne hay, maize cob, maize stover, and sunflower husk. Industrial by-products are also produced in large quantities and should be considered when formulating pig diets. The contribution of these fibre-rich sources as ingredients for pig feeds should, thus, consider their influences on the characteristics of excreta. Differences brought about by plant's botanical origin, processing and the age of the pig influence how dietary fibres are utilised differently, yielding varying effects on faecal characteristics (Le Goff *et al*., 2003; Amirkolaie, 2005). For example, notable differences were reported on excreta and odour emission when pigs were fed on maize and dried distiller's grains (Canh *et al*., 1998; Gralapp *et al*., 2002; Le *et al*., 2009). The fact that dietary fibre is a heterogeneous substance, its effect and impact on excreta characteristics and odour emission is likely to vary with source of fibre used in pig ration. The influence of different locally available fibre sources on changes in slurry and odour emission also warrants further investigation.

1.2 Justification

Odour emissions have largely been ignored by nutritionists when formulating livestock feeds. Dietary fibre has been identified as one sustainable approach to manage pollutants from pig excreta. Commercial pig farmers still rely on conventional rations to maximise performance and the economic returns from commercial operations. This is because farmers are still unclear of the impact of odours on surrounding communities and the environment, which can potentially limit expansion of pig production enterprises. Effects of a wide variety of fibres on feed intake, digestibility and growth performance, have been evaluated (e.g. Kanengoni *et al*., 2003; Bindelle *et al*., 2008; Ndou *et al*., 2012), but their effects on excreta composition and odours have largely been ignored. Incubating slurry for 16 days allows for a full understanding of the odours and the changes in chemical characteristics that occur in slurry over standard incubation periods, as animal effluents are stored for a minimum of 15 days (Moller *et al*., 2004). Additionally, Hansen et al. (2007) reported ammonia emissions to have been monitored over a 16 day period of *in vitro* slurry incubation. Therefore, it is crucial to understand how different high fibre diets, especially those that are produced in bulk and are easily accessible to farmers, contribute towards reducing odours emitted from piggeries.

1.3 Objectives

The broad objective of the current study was to determine the effects of different dietary fibre types on the characteristics of pig excreta and odour emissions from slurry of growing pigs. The specific objectives were to:

1. Determine the effects of dietary fibre and its inclusion levels on the physico-chemical composition of the excreta of growing pigs, and
2. Determine the effects of dietary fibre and its inclusion levels on the chemical composition and odour offensiveness from slurry of growing pigs over a 16 d incubation period.

1.4 Hypotheses

The hypotheses to be tested were that:

1. The type of dietary fibre and its inclusion level influences the physico-chemical composition of faeces and urine; and
2. The type of dietary fibre and its inclusion level influences the chemical composition and odours from slurries of growing pigs over a 16 d incubation period.

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CHAPTER 2: Literature Review

2.1 Introduction

There is increasing concern about odour production from commercial pig enterprises. Increasing the protein content of pig diets, leads to elevated levels of protein in faeces. This is one of the major causes of odour emissions, due to the formation of odour intermediates that results from protein fermentation. Dietary fibre inclusion to pig rations is one strategy of minimizing odours from piggeries, as they alter the characteristics of pig excreta (Gralapp *et al*., 2002; Kerr *et al*., 2006). The current review discusses pig production systems practised in South Africa, the environmental impacts of pig excreta, fibre utilisation in pig production and effects of fibre inclusion on excreta output, composition and odour offensiveness of the slurry.

2.2 Pig production systems

In South Africa, pig rearing is categorised into large-scale commercial and communal production systems. For sustainable pig production, there is pressure for large-scale pig producers to use feeding materials produced on the farm so as to promote nutrient cycling (Jongbloed and Lenis, 1998; Moeser and van Kempen, 2002). Barker *et al*. (1996) stated commercial piggeries aim to produce a high number of fast-growing pigs over a small area to maximize production. Commercial piggeries produce large amounts of excreta, which then reduce air quality due to the odours emitted from piggeries (Zahn *et al*., 2000).The intense management and feeding practices for pigs ignores the excreta effect on environmental pollution (Chimonyo *et al*., 2005; Madzimure, 2011).

Commercial pig production systems include the indoor and outdoor production systems (Gentry and McGlone, 2003). In South Africa, the indoor production system is a widely practiced system compared to the outdoor system where pigs are grown on pastures (Thornton, 1990). In the indoor system, pigs are confined in a controlled environment with minimal access to the outside environment, with pigs largely feeding on conventional nutrient dense feeds to maximize performance. Large amounts of excreta are produced, mainly from farms practicing the indoor production system, such that odours from such farms have largely an environmental concern (Honeyman, 2005).

Communal production systems in South Africa involve the use of local and non-descript breeds. Pigs kept under communal production systems survive under low plane nutrition (Petrus *et al*., 2011). The most common communal production system practised in South Africa is the scavenging production system (Madzimure, 2011). Scavenging pigs roam around freely and feed on kitchen wastes, cropping by-products, grasses, rotten maize, green maize, vegetables, pumpkins, fruits and brewers wastes (Chimonyo *et al*., 2005). At night, pigs are usually confined within the back-yard using low-cost materials, and are fed on kitchen wastes and cropping by-products negligible for use by humans. In most communal production systems, pigs are largely kept for household consumption and to ensure the livelihoods of the farmers (Petrus *et al*., 2011). Conditions of the current pig production systems need to be improved to minimize the limitations of pigs on environmental pollution (Honeyman, 1996).

2.3 Environmental impact of pigs

As piggeries intensify, a large amount of excreta is produced from pig houses (Aarnink and Verstegen, 2007). As a result, there is a significant increase in the level of both solid and gaseous environmental loads emitted from piggeries. Environmental loads exist in either liquid or gaseous forms. The solid form affects the quality of soil and water, while the gaseous form reduces the quality of the air. As pig slurries become more difficult to manage, their economic sense is underestimated due to negative impacts that effluents impose on soil, water and air pollution (Jongbloed and Lenis, 1998; Miller and Varel, 2003; Aarnink and Verstegen, 2007). Understanding impacts that the excreta have on the environment will assists policy-makers to develop strategies that can be enforces to commercial farmers as means of minimizing environmental loads from piggeries. Such systems can help minimize negative impacts of pig wastes on water quality as well.

2.3.1 Water quality

The production of slurries without proper re-cycling systems has transformed slurries into burdens to the environment. This is due to the ever-increasing nutrient and energy flow from the ever-expanding piggeries, (Prapaspongsa, 2010). Usually, slurry contains 65 to 85% water content (Dhawan and Kaur, 2002). Farmers apply slurry on land to aid crop growth or fed to fish kept in ponds as a mean of practising nutrient re-cycling. The over-use of slurry results in the seepage of nutrients though the soil to reach the ground water, destroying the soil structure as well (Dhawan and Kaur, 2002; Vu *et al*., 2007). This has been fuelled by farmers using largely conventional diets to feed pigs to maximize performance, ignoring the negative impacts that the over-flow of pig slurries might pose on the environment (Zahn *et al*., 2000). Two possible means of limiting the over-flow of energy and nutrients from piggeries exist, and can be undertaken to creatively make waste a useful by-product. One of such is to feed slurry to fish to reduce the feeding costs, also ensuring nutrient re-cycling (Vu *et al*., 2007). Another option is by dietary fibre inclusion to pig rations, as one significant means of minimizing the water content of the slurry. This is because pigs fed on high fibre diets reduce their drinking habits, producing more faeces and less urine (Masse *et al*., 2003). This has been attributed to gut fill brought about by the bulky feedstuffs, because pigs are known to drink water as a mean of overcoming hunger (Dryden, 2008). All together, these management systems need monitoring to reduce negative air systems that affect residences nearby commercial pig farms.

2.3.2 Air quality

Air pollution results from the slow anaerobic digestion of organic substances in faeces and a fast hydrolysis of urinary compounds, forming odour intermediates (Miller and Varel, 2003; Aarnink and Verstegen, 2007). Odours form from a combination of various compounds that give an unpleasant scent, fuelled by microbial activities (Bottcher, 2001). The production of odour intermediates starts in the digestive system and continues during the storage of slurry (Applegate *et al*., 2008). Odours from piggeries reduce air quality, cause nuisances, trigger symptoms such as irritation of the nose, and throat, vomiting, stress, negative mood, and many more, which mainly affect farm workers and residents nearby farms (Schiffman, 1998; Schiffman *et al*., 2000). Most commercial piggeries are located near the low-income class communities. These nearby farm residents fear to report odours to the health and environmental authorities as most farm owners are socially powerful (Wing *et al*., 2008).

It is necessary to develop strategies to reduce odours from pig farms. One feasible way of minimizing such burden is by including dietary fibre to pig rations to establish a system of minimizing odour intermediates (Gralapp *et al*., 2002; Kerr *et al*., 2006). This means that an increasing amount of research on this particular aspect is necessary as piggeries keep expanding, as means of yielding sound agricultural practices. Making good use of panels for odour assessment should be seriously considered as one way of addressing issues relating to malodours from pig farms, which can be used to manipulate odour thresholds. It is important to conduct experiments that the determine odour intensities from pig slurries from a variety of fibre types.

2.4 Fibre utilization in pig production

Dietary fibre is the plant portion that is resistant to digestion, with the large molecular size of the cellulose giving the fibrous appearance for many fibres. The increase in prices of cereals has led to the identification of alternative feedstuffs to include in pig rations (Noblet *et al*., 2001; Bindelle *et al*., 2008). Use of dietary fibres is a potential alternative that can be exploited to minimize pollution when fed to pigs (Kerr *et al*., 2006; Ziemer *et al*., 2009).

Dietary fibre is the non-digestible component of the diet. It is found in abundance in by-products of agricultural and bio-fuel industries. Dietary fibre inclusion increase the bulkiness of the ration, resulting into pigs eating more to compensate for nutrient requirements (Noblet and Goff, 2001; Reese *et al*., 2008; Ndou *et al*., 2012). Increasing dietary fibre is associated with reduced pig performance when incorporated in rations, due to a reduced nutrient density (Noblet *et al*., 2001). Inclusion of dietary fibre into pig ration is one of the known effective means of reducing odours emitted from piggeries (Gralapp *et al*., 2002; Miller and Varel, 2003; Ziemer *et al*., 2009). Pigs fed on high fibre diets exhibit increased hindgut fermentation and reduced odour emissions (Le *et al*., 2008).

Pigs possess a high potential to ferment fibre in the gut (Johnston *et al*., 2003; Bindelle *et al*., 2008; Ziemer *et al*., 2009). Microbial fermentation in the large intestines promotes bacterial growth depending on the source of fibre fed to pigs (Metzler and Mosenthin, 2008). Non-starch polysaccharides, oligosaccharides and the resistant starch are categorized as dietary fibre fractions. Fermentation of non-starch polysaccharides is poorly predictable and is closely related to the physico-chemical properties of the feed (Ndou *et al*., 2012). It is, therefore, necessary to understand the physico-chemical properties of different fibre sources so as to determine optimal inclusion level in the diet.

2.4.1 Types of dietary fibres

Agricultural and bio-fuel industrial by-products have a great potential for use as pig feed ingredients, and are largely available (Chimonyo *et al*., 2005; Mashatise *et al*., 2005). Use of these fibrous ingredients increases sustainability of agricultural enterprises. By-products that are commonly available include maize cobs, maize stover, lucerne hay, grass hay and sunflower husk. Characterizing such fibre sources not only provides cost effective means of maximizing pig performance, but also to endorse changes in the excreta of pigs to help come up with fibres that impose minimum impacts on the environment. Moreover, their inclusion in pig ration reduces feed costs for pigs, as their presence reduces the proportion of the fairly expensive cereals and grain ingredients present in complete of pig rations (Nagadi *et al*., 2000; Mashatise *et al*., 2005). Looking at these benefits coupled with dietary inclusion may be used to expand the

Table 2.1: Some physico-chemical characteristics of maize cobs, maize stover, lucerne hay, grass hay and sunflower husk

Parameter	Fibre type				
	MC	GH	LH	MS	SH
Crude fibre (g/kg DM)	236	287	168	320.0	408
Neutral detergent fibre (g/kg DM)	775	398	873	724	558
Acid detergent fibre	654	386	501	482	279
Bulk density (g DM/ml)	0.9	1.4	1.2	1.0	0.7
Water holding capacity (g water/g DM)	6.8	12.1	7.8	18.3	6.8

(Adopted from Ndou *et al*., 2012)

Abbreviations: MC = maize cobs; GH = grass hay; LH = lucerne hay; MS = maize stover; SH = sunflower husk.

scope of animal feed formulation programmes.

2.4.2 Benefits of dietary fibre inclusion to pig rations

Incorporating dietary fibre to pig rations yields a number of benefits to pigs (Jongbloed and Lenis, 1998; Metzler and Mosenthin, 2008). The potential of fibres to bind water increases the bulk of the digesta and the rate of passage, which improves intestinal development. In newly weaned pigs, dietary fibre inclusion increases feed intake due to a reduced viscosity of the pig rations (Perez-Mendoza, 2010). As the fibre content of the feed increases, nutrient exposure of diet to the pig's enzymes reduces. This is due to a reduced retention time of the diet in both the large and the small intestines (Bindelle *et al*., 2008). Dietary fibre inclusion also improves litter sizes of sows (Johnston *et al*., 2003; Renteria-Flores *et al*., 2008). Dietary fibre also improves the welfare of pregnant sows by reducing persistent hunger and energy losses due to stereotypic behaviours (Johnston *et al*., 2003; Masse *et al*., 2003).

Dietary fibre inclusion improves the fibre fermentative capacity of their large intestines and during storage of the excreta (Metzler and Mosenthin, 2008; Ziemer *et al*., 2009). In the hind gut of pigs, fibre fermentation yields SCFAs and lactic acid, which can supply up to 30% of energy needs for growing pigs (Johnston *et al*., 2003). This is due to the changes in the population and the composition of the microbial community, yielding more of cellulolytic and hemicellulolytic bacteria that actively act on fibrous materials. The proliferation of these bacteria minimizes the fermentation of protein substrates (Metzler and Mosenthin, 2008). The reduction in protein fermentation thereby reduces pollutants from piggeries. It is therefore necessary to consider the benefits that dietary fibres have on pig production to exploit strategies that minimize environmental impacts of odours from piggeries. Understanding the role of different fibre sources on pollutant emission thereby becomes crucial.

2.5 Effects of fibre inclusion on excreta output and composition

Minimising pollutants from piggeries is influenced by possible changes in characteristics of pig excreta. Various factors that influence changes in the physico-chemical composition of the pig excreta exist (Canh *et al*., 1997; 1998; Mroz *et al*., 2000), particularly the diet, influencing the microbial community and the enzyme activities. They all impact on the removal pattern of nutrients in the excreta of pigs. Their understanding may result in successful pig feed formulation programmes that aim at attaining minimal effects on water and air pollution.

2.5.1 Effects of fibre on physico-chemical properties of excreta

Diet composition influences changes in the physico-chemical composition of faeces, urine, and slurry. It also impacts on the microbial activities that take place in the large intestines and the excreta (Ziemer *et al*., 2009; Jarret *et al*., 2011). Under standard conditions, faecal output is 2.3 times lesser than the amount of urine produced by growing pigs (Canh *et al*., 1998). However, faecal production becomes much more than the production of urine when pigs are fed dietary fibre. This could be attributed to the increased amount of fibre (ADF) levels contained in faeces and the reduced drinking habits, as pigs are fed on more fibrous feeds. The NDF and ADF are generally the indigestible portions of plants resistant to digestion (Jarret *et al*, 2011). Therefore, their availability in large amounts reduces the potential of feed that an animal can consume. Varying the processing of fibre also affects the fibre content in pig faeces (Table 2.2). Table 2.2 is based on the characteristics that pig excreta fed a fibre source such as dried distiller's grains at inclusion levels up to 25 g/kg, with varying fibre levels that give their characteristic fibre content in rations, as categorised (low fibre, medium fibre and high fibre rations). The characterisation of these faecal parameters have not been dealt with on a wide spectrum of dietary fibres, such that studies have been conducted and decisions made based on a few fibres, ignoring that dietary fibre is not a homogeneous substance.

Dietary fibre also affects the faecal consistency, which is a measure of the fluid or coarseness of the faeces as affected by the type of diet ingested by an animal. Ingestion of high fibre diets improves faecal consistency due to a reduced liquid content and a large accumulation of fibres that become present in faeces (Strickling *et al*., 2000; Nijboer *et al*., 2006). Dietary fibre inclusion to pig rations also reduces the amount of nitrogen excreted with urine, also increasing the content of SCFAs in faeces and slurry. High levels of SCFAs causes a drop in the pH content of the slurry (Canh *et al*., 1998). While, the reduction of the pH in pig excreta is influenced by the dietary electrolyte balance (Canh *et al*., 1997). Dietary inclusion shifts nitrogen from its urea origin in urine to bacterial nitrogen in faeces by the action of urease enzyme during excretion (Canh *et al*., 1997; Otto *et al*., 2003; Bindelle *et al*., 2008). Bacterial nitrogen contained in faeces, during nitrogen re-partitioning is less susceptible to volatilization, reducing the amount of odour-forming compounds.

Dietary fibre inclusion alters the composition of slurry (Canh *et al*., 1998). Changes that occur in the excreta are dependent on the type dietary fibre included in pig rations, due to the varying physico-chemical properties that different fibre source. Chemical changes in pig excreta that affect odour emissions from slurry, are shown in Table 2.2. The varying levels of dietary fibre

Table 2.2: Excreta physico-chemical composition as affected by dietary fibre inclusion to pig rations

Parameters	High fibre	Medium fibre	Low fibre	Source
Dry matter (g/kg)	272.2	273.3	269.0	Vu *et al*., 2010
Faecal output (kg DM/d)	0.38	0.3	0.3	Vu *et al*., 2010
Urine output (L/d)	1.75	3.5	5.8	Leek *et al*., 2005
Faecal consistency (scale of 1-5)	3-5	2-3	1-2	Strickling *et al*., 2000
Crude protein (g/kg)	34.7	29.7	27.6	Le *et al*., 2008
Crude fat (g/kg)	20.7	21.7	27.5	Canh *et al*., 1998
Total short chain fatty acids (g/kg)	2.2	1.7	1.1	Jarret *et al*., 2011
Faecal nitrogen (g/d)	5.6	4.7	4.4	Le *et al*., 2008
Urine nitrogen (g/d)	12.5	15.3	17.5	Vu *et al*., 2010

Table 2.3: Dietary fibre effects on fresh slurry composition from pigs fed low fibre, high fibre and very-high fibre diets

Parameter	Very-high fibre	High fibre	Low fibre	Source of data
pH	8.24	8.4	8.6	Masse *et al*., 2003
Chemical oxygen demand (g O_2/L)	147.8	110.9	38.0	Masse *et al*., 2003
Nitrogen (%)	0.5	0.5	0.5	Vu *et al*., 2010
Acetate (g/kg)	7.8	6.1	6.3	Le *et al*., 2008
Propionate (g/kg)	1.6	1.2	1.2	Le *et al*., 2008
Isobutyrate (g/kg)	0.5	0.4	0.4	Le *et al*., 2008
Butyrate (g/kg)	0.7	0.4	0.5	Le *et al*., 2008
Valerate (g/kg)	0.1	0.1	0.1	Masse *et al*., 2003

shown on Table 2.3 are based on dietary treatments that varied in their crude protein (CP) and crude fibre (CF) levels. Pigs were fed reduced CP levels, included at (low 120; high 180 g/kg feed, as fed basis) and incorporated with increasing CF levels that were included at (low 95; medium 145; high 195 g/kg feed, as fed basis). During excretion, nitrogen is largely contained in the urine, and is vulnerable to emission before and after mixing with faeces, also during land application of manure as fertilizer for cropping (Aarnink and Verstegen, 2007). Possible partitioning pathways that nitrogen undergoes exist from ingestion of nitrogen with the feed, to the point where the nitrogen is contained and volatilizes at storage and during land application as fertilizer, as shown in Figure 2.1. Nitrogen partitioning is influenced by the microbe and enzyme activities altogether need understanding.

2.5.2 Microbial activities

Inclusion of dietary fibre to pig rations changes the activities of the microbial community in the gut and in the excreta. These changes include the increase in the population of the microbes, their diversity as substrates such as dietary fibre become available in the diets of pigs (Ziemer *et al*., 2009). Cellulose degrading bacteria such as *Fibrobacter intestinalis (succinogenes)*, *Ruminococcus flavefaciens* and *Butyrivibrio* species, dominate when dietary fibre is included in pig rations (Metzler and Mosenthin, 2008). Growth and basal activities of microbes result from the hydrolysis and the metabolism of the carbohydrate constituents of dietary fibre, leading to a production of ATP from a series of energy-yielding processes (Bindelle *et al*., 2008). The anaerobic fermentation of the undigested feedstuffs occurs in faeces and slurry of pigs during storage.

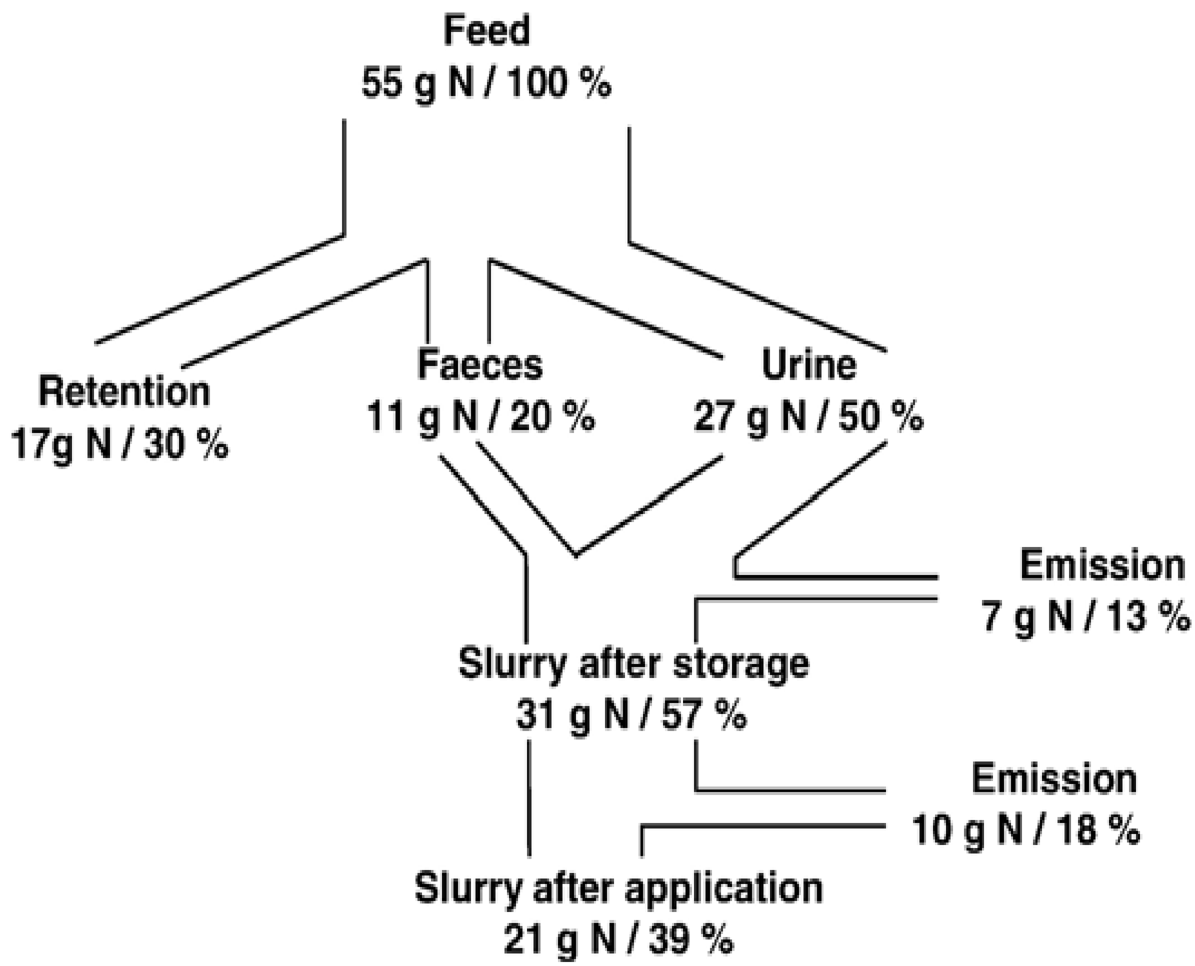

Figure 2.1: Nitrogen flow chain and emission from the feed to the excreta of growing-finishing pigs fed a standard ration, adapted from Aarnink and Verstegen, (2007).

The increase in nitrogen content of faeces when dietary fibre is included in pigs' rations is due to an increase in the population of the fibre degrading bacteria. This is done to yield more nitrogen to stimulate bacterial growth, resulting in the suppression of growth of other kinds of bacteria (Nahm, 2003). Activities of the microbial communities increase the SCFAs content in the pig excreta and that of stored slurry (Otto *et al*., 2003; Metzler and Mosenthin, 2008). Microbes also affect the pH content of excreta due to an increased production of lactic acid from lactate bacterial activities, creating a comfortable environment for microbes (Le *et al*., 2008). Microbes ferment protein substrates to produce odour mediators when the pigs are fed on diets containing a large amount of protein, as compared to the fibre content in the rations. As the protein content of the feed increase, microbes utilize the protein substrates as energy sources and produce odour precursors. In other words, an increase in the fibre content of pig diets minimizes protein fermentation, and therefore, odour emissions. Dietary fibre inclusion may, therefore, be used to manipulate microbial populations in the gut and, consequently, in slurry during storage to yield fermentation products that result in minimal odour intermediates. These changes in enzyme activity, nutrient losses and odour emissions, thus need to considered when incorporating dietary fibres in pig diets.

2.5.3 Effect of fibre on enzyme activity in slurry

As the nutrients such as nitrogen are excreted, enzyme activities help to minimize nitrogen volatilization during excretion (Canh *et al*., 1997; Singh *et al*., 2009). Nitrogen excreted with urine (also known as urea nitrogen) is vulnerable to volatilization than the bacterial nitrogen contained in the faeces (Le *et al*., 2008). The volatilization of nitrogen occurs by the hydrolysis of nitrogen from its ammonium nature to ammonia within hours and days after excretion. Urease enzyme activities in the faeces of pigs increase more when pigs are fed on high fibre rations to limit the amount of nitrogen contained in urine. The urea form of nitrogen contributes to pollutants emitted to the environment (Miller and Varel, 2003; Le *et al*., 2008). Minimizing the intake of dietary nitrogen is, therefore, a convenient way of reducing nitrogen losses to the environment (Canh *et al*., 1997; Singh *et al*., 2009).

2.6 Effects of dietary fibre on odour emissions

Odour production from pig houses has long been a dreaded constraint to the pig industry, due to increased environmental concerns about odours from piggeries (Kerr *et al*., 2006; Le *et al*., 2008). Today, possible and significant means of minimizing odour impacts from piggeries have been by diet modification, through the inclusion of dietary fibres to pig rations to inhibit odour intermediates from their sources (Miller and Varel, 2003; Kerr *et al*., 2006; Le *et al*., 2008). Dietary fibre hinders microbial protein fermentation which reduces the production of odour forming compounds in the intestine and excreta of pigs.

Reducing the protein content in the pig diet minimizes odour mediators by changes in the population and the activities of the microbial community (Otto *et al*., 2003; Le *et al*., 2008; Ziemer *et al*., 2009). An increased content of dietary fibre in pig rations favours a less microbial fermentation of protein substrates under anaerobic conditions, resulting in low concentrations of odours emitted from the excreta of pigs (Le *et al*., 2008). It is, therefore, highly possible to minimize pollution burdens from piggeries by increasing fibre levels in the diet (Leek *et al*., 2007).

2.7 Summary

Inclusion of dietary fibre changes the characteristics of pig faeces and urine, and minimizes odours from pig effluents. This is due to a reduction in the fermentation of protein substrates. Increasing the fibre content of pig feeds tends to decrease the proportion of nitrogen lost via urine. This results in minimum nitrogen volatilization, because faecal nitrogen is less susceptible to volatilization, minimizing odour precursors. Therefore, pig farmers need to understand the importance of including dietary fibre to pig rations as pollution can hinder production levels in the future. This could be motivated by the ever-increasing concerns about the environmental pollution, particularly odours emitted from large commercial piggeries. Understanding odours can be emphasised more by including a wide variety of largely available fibre sources to pig ration, to try and source fibres that have minimum impact on pig performance. By so doing, we can try to understand the changes that occur in the excreta so as to minimize odours from their source. The aim of the current study was, therefore, to determine the effects of dietary fibre on the excreta composition and odours from pig slurry.

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CHAPTER 3: Effects of dietary fibre type and level on the physico-chemical composition of the excreta of growing pigs

Abstract

Dietary fibre to pig rations has been known to alter the physico-chemical components of pig excreta. A study was carried out to test whether including varying fibrous ingredients at varying inclusion levels would change the excreta profiles of growing pigs. A total of 52 pigs were kept in individual cages, averaging 20 ± 1.37 kg, until they reached a maximum average of 40 ± 1.37 kg body weight. Dietary fibres used include grass hay GH), lucerne hay (LH), maize cob (MC), maize stover (MS) and sunflower husk. They were included at 0, 80, 160, 240, 320 and at 400 g/kg. There were a total of 26 treatments, with two pigs receiving each treatment. Pigs were fed ad libitum, and were let to adapt for a period of 10 days to allow the pigs to get accustomed to treatments before any samples could be collected. Dietary fibre inclusion influenced nutrient removal patterns of both faeces and urine. The large neutral detergent fibre and the acid detergent fibre content of rations containing GH and MS resulted in the largest faeces removal, due to the high content indigestible fibre substrates in the ration of pigs with (1805 ± 25.8) and (1335 ± 25.8), respectively. Pigs fed increasing fibre content excreted more faeces and less urine removal. As dietary fibre content increased, faecal nitrogen increased, resulting into a shift of nitrogen from urine to faeces. The optimum fibre inclusion level was 160 g/kg, as further inclusion resulted in excess of some odour intermediates, such as butyrate.

Key words: Dietary fibre, excreta, growing pigs

3.1 Introduction

Commercial pig operations depend on conventional diets to feed pigs (Small Livestock Component, 2003; Vu *et al*., 2010). Farmers focus on increasing pig growth performance, ignoring the sustainability and environmental challenges that pork production may pose to the environment. Recent concerns about a clean environment have fuelled needs for better excreta nutrient management to reduce the loss of nutrients to the environment (Moeser and Kempen, 2002; Jarret *et al*., 2012). Inclusion of dietary fibre to pig rations at optimal levels is one potential strategies of reducing pollutants from piggeries. Increasing fibre content reduces protein content of complete rations and increase microbial fermentation (Strickling *et al*., 2000).Dietary fibre alters the physico-chemical properties of pig excreta by inhibiting protein fermentation in the gut (Mroz *et al*., 2000; Zervas and Zijlstra, 2002; Nahm, 2003).

Dietary fibre inclusion affects faecal output (Kerr *et al*., 2006; Ly, 2008). As more indigestible feedstuffs are included to pig rations, a more profound faecal production results than the production of urine (Dryden, 2008). When pigs are fed on conventional rations, half of the nitrogen ingested by pigs is contained in urine during excretion, and is susceptible to volatilization (Aarnink and Verstegen, 2007). Inclusion of dietary fibre shifts a portion of the urea form of nitrogen excreted with the urine and binds it to the bacterial form of nitrogen contained in the faeces, known to be of low volatility (Canh *et al*., 1997; Kerr *et al*., 2006). Fibre inclusion, therefore, minimizes the loss of nitrogen from excretion to the point where the excreta is land applied. Apart from only maximising pig performance when formulating pig rations, the impact of the fibre on excreta characteristics, should, therefore, be considered to increase the sustainability of pig enterprises. The effects of locally available fibre sources on the physico-chemical changes in excreta of pigs are poorly understood. Dietary fibre sources differ and are likely to be utilized differently by pigs, yielding varying excreta profiles (Strickling *et al*., 2000; Amaefule *et al*., 2006).

Commonly used agricultural by-products in Southern Africa include grass hay, lucerne hay, maize cob, maize stover and sunflower husk. The effects of these locally available fibre sources on scaled feed intake and gut capacity of weaner pigs have been reported (Ndou *et al*., 2012). Maize cob inclusion, for example, did not reduce pig performance at inclusion levels up to 300 g/kg (Chimonyo *et al*., 2001; Kanengoni *et al*., 2004). The role of these fibres on excreta production and characteristics is crucial to design sustainable pig production enterprises. The objective of the current study was to determine whether incorporating dietary fibre, with their varying inclusion levels would affect the physico-chemical characteristics of excreta of growing pigs. It was hypothesized that including varying sources of dietary fibre at varying levels would alter the physico-chemical characteristics of faeces.

3.2 Materials and Methods

3.2.1 Study site

The study was conducted at Ukulinga Research Farm of the University of KwaZulu-Natal, Pietermaritzburg, KwaZulu-Natal, South Africa. The farm lies at 29°40′ S, 30°24′ E with an elevation of about 775 m above sea level. The daily temperatures average 29°C, with variation ranging from 28.2 to 43°C. The farm consists of a Dohne sourveld, characterized by savannah which is dominated by *Acacia karroo*, *A. nilotica* and *A. sieberiana*, with the grasslands dominated by *Themeda triandra*. The mean annual rainfall is 735 mm, falling mostly in summer with light to moderate frost occurring occasionally in winter (Devereux *et al*., 2000; Bengaly *et al*., 2007).

3.2.2 Pigs, diets and experimental design

A total of 52 weaner pigs weighing between 20±1.37 kg were kept in individual pens until they attained a body weight of 40±1.37 kg. Pigs were kept in individual cages and randomly allocated to diets, with two pigs receiving each diet. Five fibre types namely; lucerne hay (LH), maize cobs (MC), maize stover (MS), grass hay (GH) and sunflower husk (SH). Lucerne hay was purchased from Agricultural Products Supply at Mkondeni, Pietermaritzburg. Maize cobs were sourced from Pannar Seed Company (Pty) Ltd., in Grey town, KwaZulu-Natal. The maize cobs were sun-dried together with grass hay and maize stover from Ukulinga Research Farm, University of KwaZulu-Natal in Pietermaritzburg. Sunflower husk was sourced from a food processing industry at Willowton, Pietermaritzburg. The conventional diet, used as the control, was sourced from Meadow Feeds Company (Pty) Ltd., in Pietermaritzburg. Table 3.1 shows the chemical composition of the ingredients used.

Conventional feed contained yellow maize, soybean, soybean oil cake, cotton seed cake, sunflower oil cake, fishmeal, whole wheat; molasses syrup and starch. Yellow maize, soybean and whole wheat were harvested and sun-dried at Ukulinga Research farm, Pietermaritzburg. Soybean oil cake, cotton seed cake and sunflower oil cake were obtained from oil making industry at Willowton, Pietermaritzburg. Fishmeal, molasses syrup, and starch were purchased from Agricultural Products Supply, Mkondeni, Pietermaritzburg. Fibre types used were diluted to the conventional diet at 80, 160, 240, 320 and 400 g/kg, including a control diet, making a

Table 3.1: Chemical composition of dietary ingredients

Fibre source	Moisture (g/kg)	CP (g/kg)	EE (g/kg)	Ash (g/kg)	NDF (g/kg)	ADF (g/kg)
Lucerne hay	110.1	18.9	1.2	8.7	398.3	372.4
Sunflower husk	93.1	6.0	6.4	3.3	558.1	279.3
Maize cob	83.6	5.2	0.8	4.2	272.6	501.1
Maize stover	149.4	5.3	0.5	6.2	723.9	481.6
Grass hay	71.1	6.0	1.6	7.1	749.1	347.9

CP: crude protein, EE: ether extracts, NDF: neutral detergent fibre, ADF: acid detergent fibre total of 26 complete rations as shown in Table 3.2. The 25 compete experimental rations were fed to pigs *ad libitum* in their individual pens. An adaptation period of 10 d was allowed. Pigs were randomly allocated to treatments.

3.2.3 Excreta collection and storage

A total of 250 g of faeces and 250 ml of urine were collected from each pig within an hour of excretion. Faeces were collected over a 24 h period from the edges of the pen such that they did not mix with urine. Urine was collected from every two pigs receiving 80 and 160 g/kg MC, MS, SH, GH and LH and a control diet making a total of 22 pigs. There were inadequate containers to collect urine from all pigs; hence urine was collected from a limited number of pigs. Additionally, the inclusion threshold for the same fibres was found to have no impact on pig performance by Ndou *et al*. (2012), such that the collection of urine beyond the 160 g/kg threshold would not be sensible. Plastic trays were positioned below pens to allow for total collection of urine that would have oozed through a 1 mm sieve suspended under the pen of each pig. To reduce the volatilization of nitrogenous compounds in the urine collected, 2 ml of 25% sulphuric acid was added to each tray within 5 minutes of collection. Collected faeces and urine samples were stored at 4°C, pending analyses.

3.2.4 Measurements

3.2.4.1 Amount of faeces and urine

Faeces and urine were collected from each pig on different days. To minimised contamination of faeces, faeces for chemical analyses were collected immediately after defecation. Sacks were suspended underneath the pens, covering the whole surface area to ensure a total recovery of

Table 3.2: Chemical composition of the conventional ration and diluted rations as fed basis at six inclusion levels

Fibre type	Inclusion level (g/kg)	Dry matter (g/kg)	Gross energy (kJ)	Crude protein (g/kg)	Ether extracts (g/kg)	Ash (g/kg)	Crude fibre (g/kg)	Neutral detergent fibre (g/kg)	Acid detergent fibre (g/kg)	Nitrogen (g/kg)
Maize cob	80	989.3	18.0	181.7	51.2	59.0	36.4	210.6	101.5	29.7
	160	990.4	17.9	168.1	45.9	54.9	47.9	234.4	127.4	26.9
	240	990.5	17.9	152.8	45.2	53.2	59.3	355.3	181.2	24.7
	320	990.2	17.9	139.4	41.3	52.1	70.8	401.3	218.4	22.3
	400	991.0	17.8	116.4	39.9	46.7	82.2	457.0	250.5	18.6
Maize stover	80	990.1	18.0	178.6	50.1	62.3	48.6	192.3	110.7	28.6
	160	990.0	17.9	169.8	44.9	64.2	72.2	228.1	142.9	27.2
	240	989.9	17.8	147.8	39.3	65.3	95.8	279.8	175.2	23.7
	320	989.8	17.7	143.0	37.1	66.0	119.4	332.3	207.4	22.9
	400	989.8	17.6	141.3	31.9	66.5	143.0	383.2	239.7	22.6
Sunflower husk	80	989.3	18.2	184.9	54.1	56.6	55.6	243.1	94.5	26.7
	160	989.4	18.3	166.7	55.4	53.8	86.3	269.8	110.7	23.3
	240	989.6	18.5	134.6	55.7	51.6	116.9	316.9	126.2	22.9
	320	989.7	18.7	132.1	56.6	48.5	147.6	363.3	142.1	21.1
	400	989.8	19.0	123.5	57.3	44.9	178.2	409.6	157.8	19.8
Grass hay	80	989.5	18.0	186.4	51.7	60.4	40.1	230.3	121.8	29.8
	160	990.0	18.0	145.3	49.3	61.9	55.1	284.2	166.7	23.2
	240	990.5	17.9	135.5	40.7	63.9	70.2	337.6	214.3	21.7
	320	990.5	17.9	120.8	36.6	64.9	85.2	391.2	259.4	19.3
	400	990.8	17.8	112.9	32.4	65.9	100.3	444.0	304.0	18.1
Lucerne hay	80	989.3	18.1	192.6	49.2	63.1	46.0	228.1	101.2	30.8
	160	990.0	18.0	187.0	45.2	65.1	67.0	261.1	126.0	29.9
	240	990.0	18.0	174.0	41.5	67.2	87.9	301.9	150.7	27.8
	320	989.9	17.9	168.5	39.2	69.2	108.9	343.9	175.5	27.0
	400	989.9	17.9	165.9	33.3	71.3	129.9	385.0	200.2	26.5
Control diet	0	989.3	18.1	195.7	52.9	61.1	26.1	192.3	88.4	31.3

faeces (Ouellet *et al*., 2004). The collection of wet faeces from each pig was done once a week, over 24 hours. Faeces were sampled for each pig and dried using 25 g plastic containers to get the dry weight of the faeces. The DM of faeces was determined by oven drying. A 2 g sample of faeces was dried in an oven for 24 h at 103°C, according to Kerr *et al*. (2006). After 24 h of oven drying, each faecal sample was weighed again. The difference in the two weights resulted in the DM of faeces, expressed as a percentage of the initial weight.

3.2.4.2 Faecal consistency

Faecal consistency for all the faecal samples was determined on fresh faeces by hand scoring within an hour of excretion according to Strickling *et al*. (2000). The description of faecal consistency scores are described in Table 3.3.

3.2.4.3 Fibre levels in faeces

Neutral detergent fibre (NDF) and acid-detergent fibre (ADF) were determined for each faecal sample. Samples from each pig were analysed in duplicate. The same variables were determined from complete rations on as fed basis. The NDF and ADF contents of the faeces were determined using a Fibretec extraction unit according to the method of Van Soest*et al*. (1991).

3.2.4.4 Crude fat in the faeces

A 5g sample of faeces was weighed in a 150 ml Erlenmeyer flask. After adding 10 ml of 33% alkali solution and 40 ml of ethanol containing 0.4% amyl alcohol, the mixture was then boiled for 20 minutes under a reflux condenser, and then thoroughly cooled. A 17 ml of 25% hydrochloric acid was also added using a graduated cylinder, after which the mixture was then

Table 3.3: Score for faecal consistency (1 to 5)

Faecal consistency scoring scale	Description
Scoring scale 1	Watery faeces
Scoring scale 2	Smooth faeces, with a fairly high moisture content
Scoring scale 3	Faeces is a bit granular in texture, with a fair moisture content
Scoring scale 4	Firm faeces with traces of fibre consumed
Scoring scale 5	Completely firm and coarse faeces

cooled. Exactly 50 ml of petroleum ether was then added, and the flask was closed with a rubber stopper and shaken vigorously for 1 min. After a complete separation, 25 ml of the petroleum ether layer were transferred into a small Erlenmeyer flask by using the pressure pipette. Thereafter, a piece of filter paper was added and the petroleum ether evaporated and 10 ml of neutral ethanol was also added. The fatty acids were then titrated with 0.1 N NaOH from a micro burette, with thymol blue as an indicator until the yellow colour began to change. The calculations followed methods by Beath *et al*. (1993).

3.2.4.5 Short chain fatty acids in faeces

Faeces from each pig were analyzed for SCFAs according to modifications of protocols followed by Otto *et al*. (2003). A 2g faecal sample for each pig was diluted with 8 mL of distilled water and two drops of concentrated hydrochloric acid. The solution was mixed and let to stand for 30 min and centrifuged at 17000 x g min at 4°C for 10 min. The supernatant was filtered using a 0.45μm filter and pipetted into 2.0-mL gas chromatography vials. Concentrations of the SCFAs were determined using a gas chromatograph system equipped with a 2 m x 3.2 mm i.d. glass column. Nitrogen was used as a carrier gas with a flow rate of 25 mL/min. Air and hydrogen gas was used for combustion with a flow rate of 30 mL/min. Samples were automatically injected (2 μL. The temperature programming initiated at 110°Cfor 5 min and to 127°C at a rate of 3°C/min to halt at 127°C for three minutes to ensure complete volatilization of short chain fatty acids. A standard solution containing 10 mmol/mL of each of acetate, propionate, isobutyrate, butyrate, isovalerate and valerate were injected (2μ) to determine a standard curve.

3.2.4.6 Nitrogen levels in faeces and urine

The concentration of N of the faeces and urine for the total Kjedhahl nitrogen was determined according to the macro-Kjeldahl technique widely known as the Buchii distillation apparatus (Leek *et al*., 2005). The CP content was determined by weighing 0.05 g faecal or urine sample, transferred on a tissue paper to the Kjeldahl digestion flask. A catalyst powder and 2 mL of concentrated sulphuric acid were added to the flask. Then the flask was connected to fume trap and attached to the pump. Samples were then allowed to digest for four hours, until a clear solution was obtained. Then samples were cooled for an hour. Blank digestion was also carried out. Samples were then dissolved in a minimum amount of ammonia free distilled water and transferred to a semi micro Kjeldahl distillation apparatus which was previously conditioned by passing steam for several minutes. Eight millilitres of sodium hydroxide was added to the solution Kjeldahl apparatus. After that, 5 mL of 4% boric acid solution and 3 drops of indicator were added into a titration flask and kept at the end of the apparatus to trap the ammonia liberated. Steam was then passed through the flask until 15 mL of distillate was received. This solution was collected at the titration flask and titrated with standard hydrochloric acid solution. Endpoint for samples and blank tested was a pink colour (AOAC, 1990). The CP in the faeces was estimated by calculation ($N \times 6.25$) from the concentration of CP that was analysed.

3.2.5 Statistical analyses

A general linear model (GLM) procedure of SAS (2008) was run to analyse the data. Pair-wise comparisons of means were performed using the PDIFF procedure. The effects of type of dietary fibre and its inclusion level on the physico-chemical composition of the excreta were analyzed using PROC GLM of SAS (2006). The model used was:

$Y_{ijk} = \mu + F_i + L_j + (F \times L)_{ij} + E_{ijk}$, Where:

Y_{ijk}– is the response variable

μ - is the overall mean common to all observations

F_i – is the effect of dietary fibre type

L_i – is the effect of fibre inclusion level

$(F \times L)_{ij}$– is the interaction between the dietary fibre type and its inclusion level

E_{ijk}– is the residual error

Relationships between fibre inclusion level with DM, FO, FC, nitrogen, CP, crude fat, NDF and ADF were determined using regression analyses in SAS (2006).

3.3 Results

3.3.1 Summary statistics and levels of significance

Table 3.4 shows the summary statistics for the physico-chemical parameters tested on the faeces of pigs. Both fibre type and inclusion level influenced DM, faecal output, nitrogen, crude protein, NDF and ADF (P<0.01). The inclusion level affected (P<0.05) crude fat levels, but fibre type did not. The total short chain fatty acids (TSCFAs) and acetate were only affected by fibre type (P<0.05). There was a significant fibre type × inclusion level interaction on iso-butyrate and butyrate concentrations in faeces at (P<0.05) and (P <0.01), respectively.

3.3.2 Faecal composition

The DM varied greatly amongst the type of fibres tested, with SH resulting in the largest DM, as compared to the least from the faeces of pigs fed MS containing rations (Table 3.5). Pigs fed on diets rations containing GH, MS and SH excreted much more faeces than pigs fed on either MC or LH containing diets (Table 3.5). Faecal consistency and nitrogen were similar across all types of fibres tested, except that for pigs fed on SH (Table 3.5). Nitrogen and CP were highest in faeces produced from pigs fed on SH (Table 3.5). The NDF and ADF contents were highest in the faeces from pigs fed on both GH and MS. Pigs fed on MS and MC produced the highest SCFA concentrations. Acetate concentration was higher in pigs fed on MS than GH and LH (Table 3.5).

Dry matter content of faeces decreased linearly as fibre inclusion level increased ($P<0.05$; Figure 3.1). Faecal output, however, increased exponentially as the level of fibre inclusion increased. As the level of fibre inclusion increased, faecal consistency and nitrogen content increased ($P<0.05$) in an exponential and linear manner, respectively. The CP content of the faeces increased linearly, while the fat content was highest at 0 and 400 g/kg inclusion level. Both the NDF and the ADF increased linearly as the level of fibre inclusion increased (Figure 3.1).

Generally, isobutyrate concentration from pigs fed on MC and GH increased with the level of fibre and reached a peak at 240 and 320 g/kg inclusion level (Table 3.6). Isobutyrate for MS reached a maximum and peaked at 160 g/kg inclusion level. For SH containing rations, isobutyrate was across all inclusion levels (Table 3.6). N-butyrate for LH increased was low at high fibre inclusion levels. For both MS and SH, butyrate was highest at 240 g/kg inclusion level (Table 3.6).

Table 3.7 shows the nitrogen content in the urine, faecal to urine nitrogen ratio and the amount of urine produced from pigs per day. Fibre type had no effect on the urine nitrogen content and output. As fibre levels increased, both the urine content and output were reduced. The faecal to urine nitrogen ratio was affected by both fibre type and the inclusion level ($P < 0.05$). The fibre type × inclusion level interaction was not significant for all the parameters tested on urine.

3.4 Discussion

The linear decrease in DM content as the level of fibre inclusion increased agrees with literature (Hansen *et al*., 2007; Vu *et al*., 2010). As fibre content in faeces increases, moisture content is expected to increase, since fibre has a high water holding capacity (Vu *et al*., 2010). As the pig diets are diluted with high fibre ingredients, pigs eat more to compensate for nutrient satiety, because bulky feedstuffs decrease nutrient density (Ndou *et al*., 2012). This results in more faecal excretion, as pigs tend to excrete more of the indigestible components of the ration or any other non-digestible fibre substrates other than fibre ingested (Vu *et al*., 2010; Jarret *et al*., 2011). Faecal consistency has been found to improve with the addition of fibre in animal rations (Nijboer *et al*., 2006). This is due to large amounts of the indigestible portions that fibrous feeds contain, or the ability of the ingredients to hold water, or the availability of the undigested feed particles either than fibre available contained in faeces of pigs (Jarret *et al*., 2011). Therefore, the texture of faeces can be affected by one or many of these factors, as solely or together, they give the faeces that characteristic fibrous texture with their varying moisture contents. Improved faecal consistency is due to constipation, possibly by large distribution of short fibre available for fermentation in the gastrointestinal tract (Nijboer *et al*., 2006; Masse *et al*., 2003). This is because pigs fed high protein rations result to rumen gut acidosis results, yielding smeary faeces. This is indicated by faecal consistency, as faecal consistency can be a measure to predict constipation of pigs.

Table 3.4: Significance levels for fibre type and inclusion level on faecal parameters such as the dry matter, faecal output, faecal consistency, nitrogen, crude protein, crude fat, neutral detergent fibre, acid detergent fibre, total short chain fatty acids, acetate, propionate, iso-butyrate, butyrate and valerate

Parameter	Fibre type	Inclusion level	Fibre type × inclusion level interaction
Dry matter (g/kg)	**	**	NS
FO (g/kgDM/d)	**	**	NS
FC (1-5 score)	NS	**	NS
Nitrogen (g/kg)	**	**	NS
Crude protein(g/kg)	**	**	NS
Crude fat (g/kg)	NS	*	NS
NDF (g/kg)	*	**	NS
ADF (g/kg)	**	**	NS
TSCFAs (g/kg)	*	NS	NS
Acetate (g/kg)	*	NS	NS
Propionate (g/kg)	NS	NS	NS
Isobutyrate (g/kg)	**	**	**
N-butyrate (g/kg)	*	NS	*
Valerate (g/kg)	NS	NS	NS

****** indicates significance (*P <0.01*)

***** indicates significance (*P <0.05*)

NS indicates not significant (*P >0.05*)

Abbreviations: FO = faecal output; FC = faecal consistency; NDF = neutral detergent fibre; ADF = acid detergent fibre; TSCFAs = total short chain fatty acids.

Table 3.5: Least square means (± SE) for the effect of fibre type on faecal parameters such as the dry matter, faecal output, faecal consistency, nitrogen, crude protein, crude fat, neutral detergent fibre, acid detergent fibre, total short chain fatty acids, acetate, propionate, and valerate

Parameter	Fibre type					SEM
	MC	GH	LH	MS	SH	
DM (g/kg)	196.3^{b}	219.2^{bc}	201.8^{b}	163.7^{a}	234.1^{c}	10.85
FO (g/kgDM/d)	586.4^{a}	1804.9^{c}	790.8^{a}	1335.4^{b}	1196.8^{b}	125.79
FC (1-5 score)	3.1^{a}	3.3^{ab}	2.8^{a}	3.1^{a}	3.6^{b}	0.22
Nitrogen (g/kg)	2.2^{ab}	2.4^{bc}	2.1^{a}	2.3^{ab}	2.6^{c}	0.08
CP (g/kg)	13.8^{ab}	14.9^{bc}	13.2^{a}	14.7^{ab}	16.2^{c}	0.51
Fat (g/kg)	5.5^{a}	5.3^{a}	5.3^{a}	5.7^{a}	5.1^{a}	0.70
NDF (g/kg)	816.1^{ab}	829.0^{ab}	798.0^{a}	896.0^{b}	751.5^{a}	30.34
ADF (g/kg)	182.2^{a}	274.4^{bc}	238.6^{ab}	289.9^{c}	231.9^{ab}	19.78
TSCFAs (g/kg)	2.6^{b}	2.3^{ab}	1.9^{a}	2. 6^{b}	2.2^{ab}	0.18
Ac (g/kg)	1.1^{ab}	0.9^{a}	0.8^{a}	1.4^{b}	1.2^{ab}	0.15
Pr (g/kg)	0.5^{a}	0.5^{a}	0.5^{a}	0.5^{a}	0.5^{a}	0.05
Val (g/kg)	0.1^{a}	0.2^{a}	0.1^{a}	0.1^{a}	0.1^{a}	0.04

^{a,b,c,d}Within a row, means with the different superscripts differ (*P* <0.05)

Abbreviations: DM = dry matter; FO = faecal output; FC = faecal consistency; CP = crude protein; Fat = crude fat; NDF = neutral detergent fibre; ADF = acid detergent fibre; TSCFAs = total short chain fatty acids; Ac = acetate; Pr = propionate; Val = valerate; SEM = standard error of mean.

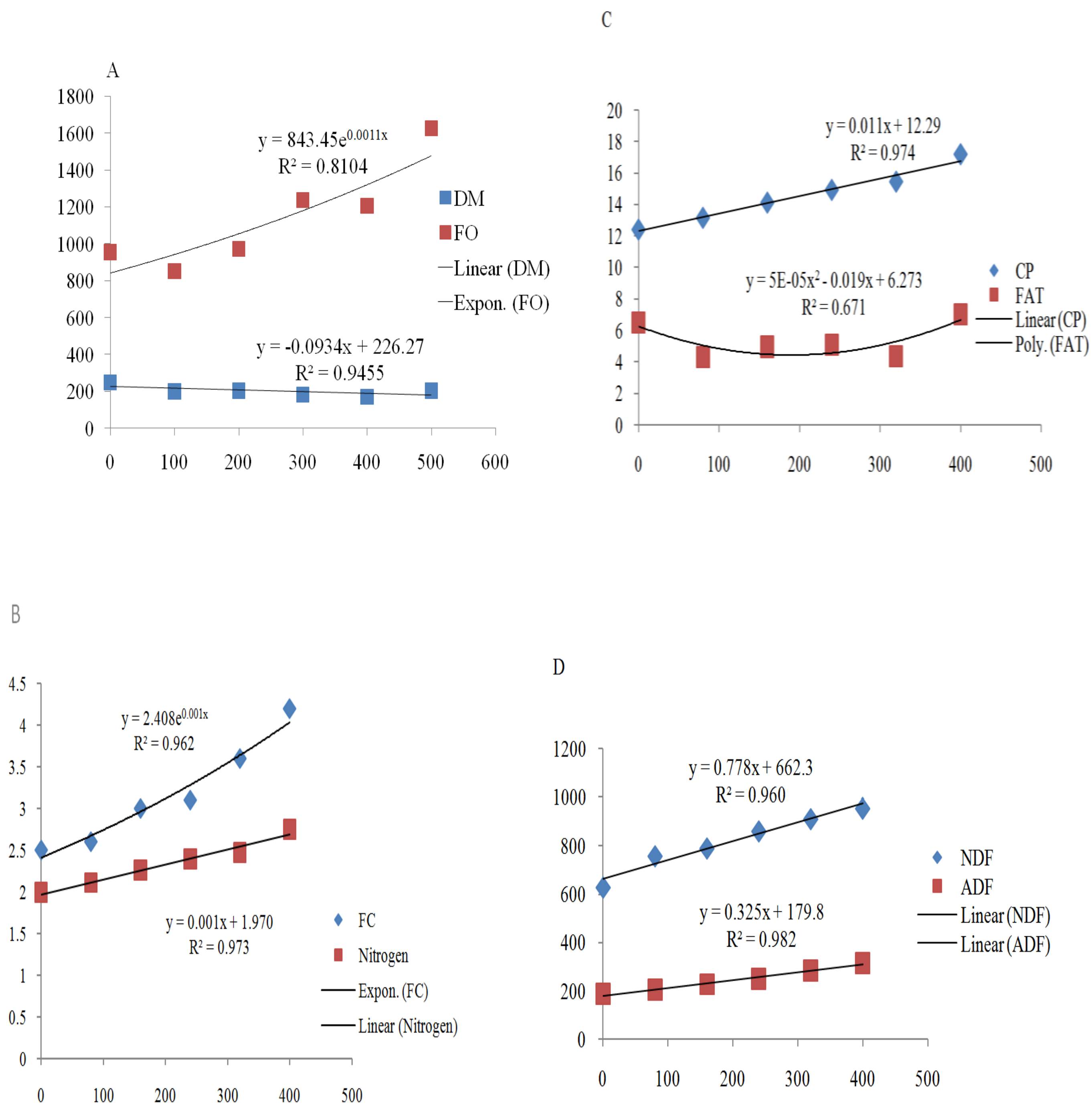

Figure 3.1: Changes in dry matter (DM), faecal output (FO), faecal consistency (FC), nitrogen, crude protein (CP), crude fat (FAT), neutral detergent fibre (NDF) and acid detergent fibre (ADF) as the dietary fibre inclusion level increased.

Table 3.6: Least square means (±SEM) for the effect of fibre type × inclusion level on isobutyrate and butyrate

Parameter	Fibre type	Inclusion level (g/kg) 0	80	160	240	320	400	SEM
I-but	MC	0.1^{a}	0.2^{b}	0.2^{b}	0.4^{bc}	0.4^{c}	0.4^{c}	0.03
	GH	0.1^{a}	0.2^{b}	0.3^{bc}	0.3^{bc}	0.1^{ab}	0.2^{b}	0.03
	LH	0.1^{a}	0.2^{abc}	0.2^{bcd}	0.2^{cd}	0.3^{cd}	0.3^{d}	0.03
	MS	0.1^{a}	0.1^{ab}	0.3^{c}	`0.1^{ab}	0.1^{a}	0.1^{a}	0.03
	SH	0.1^{a}	0.1^{a}	0.1^{ab}	0.1^{ab}	0.1^{a}	0.1^{a}	0.03
N-but	MC	0.2^{a}	0.6^{b}	0.6^{b}	0.2^{a}	0.8^{c}	0.6^{b}	0.10
	GH	0.2^{a}	0.4^{ab}	0.3^{a}	0.7^{a}	0.3^{a}	0.5^{b}	0.10
	LH	0.2^{a}	0.4^{a}	0.5^{ab}	0.5^{b}	0.4^{a}	0.3^{a}	0.10
	MS	0.2^{a}	0.2^{a}	0.2^{a}	0.3^{b}	0.3^{b}	0.3^{b}	0.10
	SH	0.2^{a}	0.2^{a}	0.3^{ab}	0.4^{b}	0.2^{a}	0.2^{a}	0.10

[a,b,c,d,e,f,g,h,i]Within a row, means with the different superscripts differ (P <0.05)

Abbreviations: I-but = isobutyrate; N-but = butyrate; MC = maize cob; GH = grass hay; LH = lucerne hay; MS = maize stover; SH = sunflower husk; SEM = standard error of mean.

Table 3.7: Least square means (± SE) for the effect of fibre type on urine nitrogen content, urine to nitrogen ratio and urine output

Fibre type	Inclusion level (g/kg)	Urine nitrogen (g/kg)	Faecal: urine nitrogen	Urine output (kg/day)
Control	0	4.3^{d}	0.5^{a}	2.5^{ab}
MC	80	3.5^{cd}	0.6^{a}	1.9^{a}
	160	3.0^{bcd}	0.8^{ab}	0.9^{a}
GH	80	0.8^{a}	2.9^{d}	1.5a
	160	0.8^{a}	2.9^{d}	1.2a
LH	80	2.6^{abcd}	0.7^{ab}	4.9^{b}
	160	1.0^{a}	2.0^{bcd}	1.1a
MS	80	1.8^{abc}	1.3^{abc}	1.9^{a}
	160	1.6^{abc}	2.3^{cd}	0.8^{a}
SH	80	1.1^{ab}	2.2^{cd}	1.0^{a}
	160	1.4^{ab}	2.1^{bcd}	0.7^{a}
SEM		0.68	0.5	0.87

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

Abbreviations: MC = maize cob; GH = grass hay; LH = lucerne hay; MS = maize stover; SH = sunflower husk; SEM = standard error of mean.

As pigs are fed rations high in protein, more nitrogen is contained in faeces due to nitrogen repartitioning that results when pigs are fed high fibre rations. This minimises nitrogen volatilization, as faecal nitrogen is known as less susceptible to volatilization (Aarnink and Verstegen, 2007). A reduction in the nitrogen content of the urine explains the excretory patterns of nitrogen known to be affected by dietary fibre inclusion, as increasing fibre content in pig rations is known to reduce urinary nitrogen. Increments in faecal nitrogen with increasing dietary fibre explain nitrogen repartitioning during excretion. This results in the minimization of losses of nutrients through volatilization, as faecal nitrogen is less volatile because it is of bacterial origin when contained in faeces (Hansen *et al*., 2007). The production of less urine with increasing fibre contents explain the behavioural aspects of pigs that tend to reduce drinking ability as dietary fibre is increased. This can be attributed to reduced persistence hunger brought about by fibrous feeds, as pigs normally increase their water intake to overcome hunger (Dryden, 2008).

Fibre inclusion affects the amount of nitrogen excreted in urine. The nitrogen content in the faeces is of bacterial origin and is less volatile. An increase in the amount of faecal nitrogen is due to the increased dietary fibre content of rations that later results in nitrogen shift, resulting into more nitrogen contained in the faeces during excretion (Canh *et al*., 1997; Nahm, 2003; Jarret *et al*., 2011). In the current study, the observed linear relationship was expected. Increasing fibre level is expected to have a negative effect on nitrogen digestibility. Urine nitrogen increases odour precursors more than faecal nitrogen, because urine nitrogen is highly volatile. This is because urea nitrogen conversion is a much faster process than the breakdown of faecal nitrogen which is of bacterial origin (Canh *et al*., 1998). Fibre levels contained in grass hay and maize stover rations suggests digestibility and microbial activities when fed to pigs compare to maize cob, lucerne hay and the sunflower. This indicates a lesser ability of microbes that could have utilized the diet, such that the presence of large amounts of fibres in faeces may be indicative of the indigestibility states that feeds might have during digestion. This is because the fermentation in the gut is largely dependent on the dietary fibre content of the ration, as influenced by varying fibre ingredients (Nagadi *et al*., 2000; Hansen *et al*., 2007).

Acetate varied with varying fibre sources, rather than the amount of fibre included in diet; indicating the varying extent of lignifications of fibres (lignin was not measured in this study). This can be attributed to the microbes producing fermentation products (SCFAs) in varying proportions, as different fibre sources are fermented (Nagadi *et al*., 2000; Kerr *et al*., 2006). The largest proportion of the total short chain fatty acids that resulted with might be attributed to the smallest ADF and other non-digestible dietary substrates that improve fermentation. The production of large total short chain fatty acids, isobutyrate and butyrate, as in the case of MC, GH and LH, may contribute significantly to the amount of pollutants that result from piggeries, as SCFAs represent the large portion of fermentation products responsible for odour (Otto *et al*., 2003). Ideally, including dietary fibre to pig faeces is one means of reducing the protein fermentation of pig rations so as to minimize the production of odour intermediates such as isobutyrate and butyrate. In the current study, the increase in SCFAs content with an increasing fibre content of the pig rations depicts findings by Otto *et al*. (2003), who found the SCFAs to have increased as the protein content of the pig diet was reduced. Furthermore, fibre inclusion limited to thresholds, as further inclusion for fibres such as MC resulted in high content of butyrate's known to be odour precursors.

3.5 Conclusions

Excreta properties were influenced by dietary inclusion of varying fibre sources. Maize stover and grass hay resulted in high fibre content than the other fibres tested, such that, their use in pig rations minimizes gut microbial utility efficiency. To improve the efficiency of these fibres for use in agriculture, technologies and the use of enzyme inclusion to pig rations that can improve their degradability need to be developed as well, as these are sources largely available in agriculture. It is not advisable to include dietary fibre beyond inclusion levels such as 160 g/kg for maize cobs, lucerne hay and sunflower husk, as they are known not to have any impact on pig performance. For example, maize cob yielded much more isobutyrate and butyrate in the current study, which is known as one of the malodour fermentation by-products. Therefore, the use of maize cobs, lucerne hay and sunflower husk should be limited to inclusion level thresholds such as 160 g/ kg, as to ensure maximum performance of the animal, as the goal of minimizing pollutants is being attained in the pig commercial sector.

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CHAPTER 4: Effects of fibre type and its inclusion level on chemical composition and odours from pig slurry

Abstract

Incorporation of high fibre ingredients into pig rations has the potential to reduce odours emitted from pig slurry tanks. The current study was conducted to determine whether diets containing 0, 80 and 160 g/kg of each of lucerne hay (LH), sunflower husks (SH) and maize cobs (MC) influenced the chemical characteristics and odours from pig slurries. Fourteen pigs weighted 20±1.37 kg were kept in individual cages until they reached an average weight of 36±1.37 kg to allow the total collection of faeces and urine. All pigs were fed *ad libitum*. Faeces and urine were mixed in a 1: 2.3 ratio (w/w), stored and fermented for 16 days in a temperature controlled room at 22 to 24 ºC. Slurry was sampled (on day 1 and on day 16) and analyzed for pH, chemical oxygen demand (COD), nitrogen and the short chain fatty acids (SCFAs) on a wet basis. All samples were tested for odour offensiveness using 18 panellists. Slurry pH and COD varied with fibre source. After day 16, the COD for LH, SH and MC were 369, 512 and 425±34.2 mgO/L. Total SCFA concentration was higher on day 16 than day 1. Odour offensiveness varied with fibre source. SH and LH-based diets that were rated as less offensive (mean rank = 2.2) than MC diets (mean rank of 4.3). No differences were found between inclusion levels. It was concluded that characteristics of pig slurries were only influenced by fibre source. More work still needs to be done to characterize compounds from a large variety of locally available fibre sources that reduce water and air pollution from pig enterprises.

Keywords: Dietary fibre, Slurry characteristics, Odour offensiveness

4.1 Introduction

Large amounts of nutrients and odours are emitted from piggeries (Bundy *et al*., 2007). Odour emission results from the anaerobic breakdown of nutrients, especially proteins in the gut and in the slurry (Bindelle *et al*., 2008; Le *et al*., 2008). This has prompted nutritionists to formulate pig diets that are high in fibre content and have reduced crude protein (CP) content. The use of fast-growing pigs and the increase in levels of intensification forces farmers to increase the protein contents in pig diets. The impact of such practices on the environment is often ignored. Odours are regarded as one of the main problems that emerge from pig slurry. As such, the production of odours from slurry is associated with microbes activities, and the chemical changes that occur over time when the slurry is stored (Snell-Castro *et al*., 2005).

Intensification of pig units has resulted in large quantities of manure being produced from pig operations when faeces and urine of pigs come together to form slurry. Slurry characteristics depend on the quality of the diet fed to pigs, pig characteristics, and the extent of storage of the slurry in the ponds. Various chemical components such as pH, chemical oxygen demand (COD), nitrogen and SCFA influence the chemical composition and odour emissions from slurry. The increase in content of the SCFAs, particularly butyrate, increases the offensiveness of odours (Otto *et al*., 2003; Ryan, 2005). Large amounts of nutrients in the slurry increases microbial activities, consequently increasing SCFA production, causing a reduction in slurry pH to a comfortable threshold for microbes (Salminen and Rintala, 2002; Bundy *et al*., 2007; El-Jalil *et al*., 2008). Low slurry pH results in a shift of nitrogen, from its urea form contained in urine to its bacterial form contained in faeces, known to be less volatile. This mechanism results more when pigs are fed rations with a high content of dietary fibre and occurs by the presence of urease enzyme in faeces, minimizing environmental pollution (Canh *et al*., 1997). The COD is the most widely used parameter to estimate organic content in waste water and it determines the effects of organic pollutants in aqueous systems (Zupancic and Ros, 2011).

Incorporating high fibre diets to pig rations is one alternative to reducing odour emissions from pig slurries. Apart from altering the characteristics of the excreta by permitting less anaerobic fermentation of proteins, high fibre inclusion inhibits the incomplete anaerobic fermentation of proteins (Shriver *et al*., 2003). Examples of widely available fibre sources with a huge potential to be used as ingredients in pig diets include, lucerne hay, maize cob and sunflower husk. Nagadi *et al*. (2000) reported marked variations amongst different high fibre sources due to varying extent of lignifications and chemistry, in addition to geographic origins. Different fibres are known to be utilised differently in the gut of pigs due to varying properties (Ndou *et al*., 2012). The influence of dietary fibre sources on slurry chemical changes and odours over time is not well understood. Odour precursors may be reduced by including locally available dietary fibres in pig rations at levels at which optimum pig performance is achieved. In the previous study (Chapter 3), grass hay and maize stover rations contained quite a large amount of faecal fibre content which suggests that, a lot of fibre substrates escaped digestion. This was due to a content of dietary fibre which can hinder the microbes to aid their maximum activities required for efficient fermentation.

Effects of various fibrous diets on scaled feed intake and gut capacity have recently been studied and found not to affect performance on weaner pig when included at 80 and 160 g/kg inclusion levels for fibre such as lucerne hay, maize cob and sunflower husk (Ndou *et al*., 2012). As odours are necessary to understand, the fibre inclusion threshold that a pig can withstand needs consideration as well. This could be adopted to avoid tampering with animal performance and welfare. Studying odour offensiveness from effluents increases our understanding of the effect of these fibre sources on the environmental impacts. The objective of the current study was to determine whether type of dietary fibre and its inclusion level would influence the chemical composition and odours from slurries of growing pigs over a 16 d incubation period. It was hypothesized that varying dietary fibre and their inclusion levels would affect the chemical composition and odours from slurry of growing pigs over a 16 d incubation period.

4.2 Materials and Methods

4.2.1 Study site

The study was conducted at the UKZN. Details on the study site are described in Section 3.2.

4.2.2 Pig management, diets and design

Fourteen weaner pigs weighing an average of 20 ± 1.37 kg were kept in an environmentally controlled room until they reached an average of 36 ± 1.37 kg. Lucerne hay (LH), maize cob (MC) and sunflower husk (SH) were each included in pig rations at 0, 80 and 160 g/kg. A period of 10 days was allowed for pigs to get accustomed to the treatments that were offered. There were two pigs per treatment, and they were assigned randomly to treatments. For the chemical composition of LH, MC and SH, refer Table 3.1.

4.2.3 Excreta collection, storage and analyses

Faeces and urine samples were collected according to the protocols described in section 3.2.3.

The faeces and urine were sampled on day 1 (d 1) and on day 16 (d 16). Faeces and urine were collected from 14 pigs, 2 from each treatment. Excreta from each pig receiving the same treatment was mixed in 3 l buckets to make slurry for all the faecal and urine samples collected. Amounts of 200 and 460 g of faeces and urine respectively were sampled. Slurry was made from a mixture of faeces and urine in a ratio of 1: 2.3 (w/w basis of faeces and urine) as was done by Canh *et al*. (1998). This is because pigs feeding on standard rations produce excreta with similar ratios. The slurry was left to stand and ferment in a temperature-controlled room at 20 to 23 °C conditions for a 16 d period. Sampling for chemical analyses was done on day 1 and on d 16 of the incubation period.

4.2.4 Measurements

4.2.4.1 Nitrogen content

Two gram sample of slurry was added to a 125 ml Erlenmeyer flask, to which 20 ml of 6M HCl was added. The Erlenmeyer flask was sealed with a cap and heated to a temperature of 48 to 50°C on an electric hot steam plate for 16 h. It was then allowed to cool down and get filtered into a 250 ml sample bottle using hardened Whatman paper (No. 50) and refrigerated at 5°C overnight. After overnight refrigeration, each sample was removed from the refrigerator and placed in a freezer at 4°C for 1 h and placed in an oversized beaker which, was surrounded by ice water. The pH was raised to 5.5 using sodium hydroxide without raising the temperature above 12°C. After titration, samples were transferred to 250 ml volumetric flasks, brought to volume using distilled water and then transferred to a 250 ml sample bottle. The determination of the nitrogen content of the slurry was based on the Mulvaney *et al*. (2001) diffusion technique for soil nitrogen fractionation.

Table 4.1: Chemical composition of the conventional ration and diluted rations (lucerne hay, sunflower husk and maize cob) as fed basis at different inclusion levels

Composition	Inclusion level (g/kg)	Lucerne hay	Sunflower husk	Maize cob	Control diet
Dry matter (g/kg)	80	989.3	989.3	989.3	989.4
	160	990.1	989.4	990.4	
Gross energy (kJ)	80	18.1	18.2	18.0	18.1
	160	18.0	18.3	17.9	
Crude protein (g/kg)	80	192.7	184.9	181.8	195.7
	160	187.1	166.7	168.1	
Ether extracts (g/kg)	80	49.2	54.1	51.3	52.9
	160	45.3	55.5	45.9	
Ash (g/kg)	80	63.2	56.6	59.1	61.2
	160	65.1	53.9	54.9	
Crude fibre (g/kg)	80	46.1	55.6	36.5	26.2
	160	67.1	86.3	47.9	
Neutral detergent fibre (g/kg)	80	228.1	243.1	210.7	192.4
	160	261.2	269.8	234.4	
Acid detergent fibre (g/kg)	80	101.2	94.6	101.6	88.5
	160	126.0	110.7	127.5	
Nitrogen (g/kg)	80	30.9	26.8	29.8	31.4
	160	29.9	23.3	26.9	

4.2.4.2 pH

Samples of slurry were taken and placed in universal containers to test for pH. The pH of the slurry was recorded twice, that is: on Day 1 of slurry storage and after 16 days of storage. The pH measurements were taken as duplicates for all the samples on a pH meter, calibrated with certified pH 4 and 7 buffer solutions (Lynch *et al*., 2007).

4.2.4.3 Short chain fatty acids

The slurry samples collected from each pig were analyzed for the concentration of the total short chain fatty acids (TSCFAs) according to Otto *et al*. (2003), see 3.2.4.5.

4.2.4.4 Chemical oxygen demand

Slurry samples collected from each pig and analysed for the chemical oxygen demand (COD). One ml of sample was diluted with 99 ml of distilled water, centrifuged at 17 400 x g for 10 min at 4°C and supernatant was extracted. The cap from a MERC COD vials provided in the kit was removed and 3 ml of each of the extracted supernatant were pipetted into MERC COD vials (range 0-15000 mg O_2/l). The cap was secured tightly and vials were inverted several times to mix the contents and homogenized for 2 min in a vortex mixer. The vial were placed in the preheated digester block, heated to 150ºC to heat for 2 h and allowed to digest for 2 h. The block was turned off and allowed to cool for 15-20 min. The digested samples and reagent blanks were measured in a pre-programmed CHEMmetrics photometer, where the absorbencies were recorded (Quayle *et al*., 2009).

4.2.4.5 Odour offensiveness

Odour offensiveness was tested using 18 panellists from the Discipline of Animal and Poultry Science at the University of Kwa-Zulu Natal. The same panellists tested for odour offensiveness for both sessions; one being at the beginning of the incubation period and after the 16 d incubation period. Odour offensiveness represented one of the four factors describing odour nuisance, namely FIDO (frequency, intensity, duration and offensiveness), as described by Otto *et al*. (2003). Ten millilitres of slurry was sampled and put on plastic vials, capped with cotton wool and allowed to rest. Odour offensiveness was ranked on a scale of 1 to 5, where 1 was described as not offensive, 2 being mildly offensive, 3 being moderately offensive, 4 being strongly offensive and 5 described as extremely offensive. The panellists tested for odour nuisance in temperature controlled room at 20 to 23 ^{0}C where there would be no detectable odours that would somehow affect the sensory evaluation (Otto *et al*., 2003).

4.2.5 Statistical analyses

The effect of dietary fibre type and the inclusion level of fibre on the chemical composition of the slurry for the two incubation periods was analysed using PROC GLM of SAS (2008). The PDIFF procedure of SAS (2008) was used to compare treatment means. Effects could be considered significant when probabilities were below 0.05. A Pearson's correlation test was run to cater for the relationship that could exist between the chemical composition of the slurry and the odour offensiveness numerical scales obtained by olfactory sensory evaluation. The model used was:

$Y_{ijkl} = \mu + F_i + L_j + P_k + (F \times L)_{ij} + (F{\times}P)_{ik} + (L \times P)_{jk} + (F{\times}L{\times}P)_{ijk} + E_{ijkl}$, where:

Y_{ijk} = dependent variable (pH, chemical oxygen demand, nitrogen, total short chain fatty acids, acetate, propionate, iso-butyrate, butyrate, valerate, and odour),

μ = the overall mean,

F_i = the effect of the type of dietary fibre, (i = lucerne hay, maize cob and sunflower husk),

L_j = inclusion level (j = 0, 80, and 160 g/kg),

P_k = incubation period, (k = Day 1 and Day 16),

$(F\times L)_{ij}$= interaction of fibre type and the inclusion level,

$(F\times P)_{ik}$= interaction of fibre type and the incubation period

$(L \times P)_{jk}$ = interaction of inclusion level and the incubation period

$(F \times L\times P)_{ijk}$= interaction of fibre type, inclusion level and the two incubation periods tested, and

E_{ijkl} = residual error.

4.3 Results

4.3.1 Summary statistics and levels of significance

The summary statistics for the variables tested in the slurry are presented in Table 4.2. The parameters tested on the slurry were the pH, COD, nitrogen, TSCFAs and individual SCFAs namely, acetate, propionate, isobutyrate, butyrate, and valerate as shown in Table 4.2. The pH was significant for the fibre type × incubation period interaction ($P < 0.05$). There was an incubation period effect on the COD at ($P < 0.01$). Nitrogen content in the slurry was significant for fibre type × incubation period interactions at ($P < 0.05$). The TSCFAs were significant for fibre type × incubation period at ($P < 0.05$). The individual SCFAs such as acetate and propionate were significant for the incubation period at ($P < 0.01$). Iso-butyrate and butyrate were significant for fibre type × incubation period at (P < 0.01). And valerate was significant for the fibre type × inclusion level × incubation period interaction at (P < 0.05).

4.3.2 Slurry composition

The pH of the slurry was different for all fibres during incubation period d 1, but the pH was similar for all the fibres on Day 16 (Table 4.3). The COD was the same for all types of fibres on Day 1. On Day 16, the COD was largest for the slurry containing SH as compared to the smallest for the slurry containing LH (Table 4.3). Nitrogen contained in the slurry was largest for LH (Table 4.3). The total short chain fatty acids were largest for SH on Day 1. On Day 16, the total short chain fatty acids the same for all fibres tested. Acetate, propionate and valerate were similar across all the fibres (Table 4.3). Isobutyrate and butyrate were the same on Day 1, but was largest for MC on Day 16 (Table 4.3).

4.3.3 Odour offensiveness

Odour offensiveness of the slurry varied amongst all types of fibres (P< 0.01). The slurry from pigs fed on rations containing MC resulted in higher odour offensiveness, compared to that from pigs fed SH (Figure 4.1). On Day 1, the slurry from pigs fed MC was less offensive than the slurry on Day 16. The offensiveness of the slurries from pigs fed rations containing LH and SH was similar for both days (Figure 4.1).

4.3.4 Correlations

The results presented on correlations are based on Table 4.4. There was a positive correlation amongst all of the slurry chemical parameters tested, except for pH which was negatively

Table 4.2: Significance levels for fibre type, level of inclusion, and the incubation period on the pH, COD, nitrogen, acetate, propionate, isobutyrate, butyrate, valerate and the total short chain fatty acids

	Parameters								
	pH	COD (mgO_2/L)	Nitrogen (%)	TSCFAs (g/kg)	Acetate (g/kg)	Propionate (g/kg)	Iso-butyrate (g/kg)	Butyrate (g/kg)	Valerate (g/kg)
Fibre type	*	**	**	NS	NS	*	*	*	NS
Inclusion Level	NS	NS	NS	*	NS	NS	NS	*	**
Incubation period	**	**	NS	**	**	**	**	**	**
Fibre type × inclusion level	**	NS	*	NS	NS	NS	NS	NS	NS
Fibre type× incubation period	*	NS	*	*	NS	NS	**	**	NS
Fibre type× inclusion level× incubation period	NS	NS	NS	NS	NS	NS	NS	**	*

****** indicates significance (*P <0.01*)

***** indicates significance (*P <0.05*)

NS indicates not significant (*P >0.05*)

Abbreviations: pH = hydrogen potential; COD = chemical oxygen demand; TSCFAs = total short chain fatty acids.

correlated to all the chemical parameters tested.

4.4 Discussion

The reduced pH content of the slurry of pigs fed on SH compared to those fed on MC, reflected the fermentable fibre content fed to the pigs. Dietary fibre inclusion increases the content of the fermentable carbohydrate in pig rations, by so doing, the pH of the slurry from pigs fed high fibre rations is reduced compared to the slurry of pigs fed low fibre rations (Shriver *et al*., 2003; Bundy *et al*., 2007). These changes are attributed to the lactic acid bacteria that consume a low amount of glycosides from fibre substrates to produce as much lactic acid as possible, to attain a comfortable threshold for the microbes' environment, also preserving nutrients such as nitrogen from volatilization (Salminen and Rintala, 2002; El-Jalil *et al*., 2008). The decline in slurry pH over time was due to an increase in acidic parameters such as the SCFAs contained in slurry from the current study.

These findings compare greatly with those by El-Jalil *et al*. (2008), who found a decline in slurry pH from poultry fed rations containing molasses when the slurry was incubated for 14 days. This means the longer the incubation period of slurry, the low the pH gets. Low pH that resulted in slurry containing SH and LH as compared to that from pigs fed rations containing MC might have influenced nitrogen shift and ionization balance, resulting in a more efficient retention of nitrogen in the slurry for SH and LH in the current study. Hankins *et al*., 2008 found diets with a high content of fermentable carbohydrates to result into a higher content of nitrogen in the slurry. This is because that, diets with a larger content of fermentable carbohydrate are known to influence a great shift in nitrogen, from urine nitrogen of urea origin to faecal nitrogen of

Table 4.3: Least square means (± SE) for the effects of period of incubation and fibre type on the pH, COD, Nitrogen, acetate, propionate, isobutyrate, butyrate, valerate and the total short chain fatty acids

	Day 1			Day 16			
Parameter	Fibre type			Fibre type			
	LH	MC	SH	LH	MC	SH	SEM
pH (pH unit)	8.0^{ab}	8.8^{c}	8.2^{b}	7.7^{a}	8.0^{ab}	8.0^{ab}	0.12
Chemical oxygen demand (mg O_2/l)	366^{ab}	348^{a}	362^{ab}	369^{ab}	425^{b}	512^{c}	21.4
Nitrogen (g/kg)	5.0^{bcd}	4.7^{ab}	4.9^{abc}	5.6^{d}	4.4^{a}	5.4^{cd}	0.20
Total short chain fatty acids (g/kg)	10.3^{ab}	9.6^{a}	11.3^{b}	33.1^{c}	33.1^{c}	32.1^{c}	0.42
Acetate (g/kg)	6.8^{a}	6.6^{a}	7.9^{a}	24.9^{b}	23.7^{b}	23.9^{b}	0.59
Propionate (g/kg)	1.7^{ab}	1.4^{a}	2.4^{bc}	3.0^{cd}	3.4^{d}	3.7^{d}	0.27
Isobutyrate (g/kg)	0.2^{a}	0.2^{a}	0.2^{a}	0.9^{b}	1.2^{c}	1.0^{b}	0.06
Butyrate (g/kg)	1.4^{a}	1.0^{a}	1.3^{a}	2.5^{b}	3.5^{c}	2.3^{b}	0.20
Valerate (g/kg)	0.2^{a}	0.2^{a}	0.2^{a}	0.8^{b}	1.0^{bc}	1.1^{c}	0.09

^{a,b,c,d}Within a row, means with the different superscripts differ (P<0.05)

Abbreviations: LH = lucerne hay; MC = maize cob; SH = sunflower husk

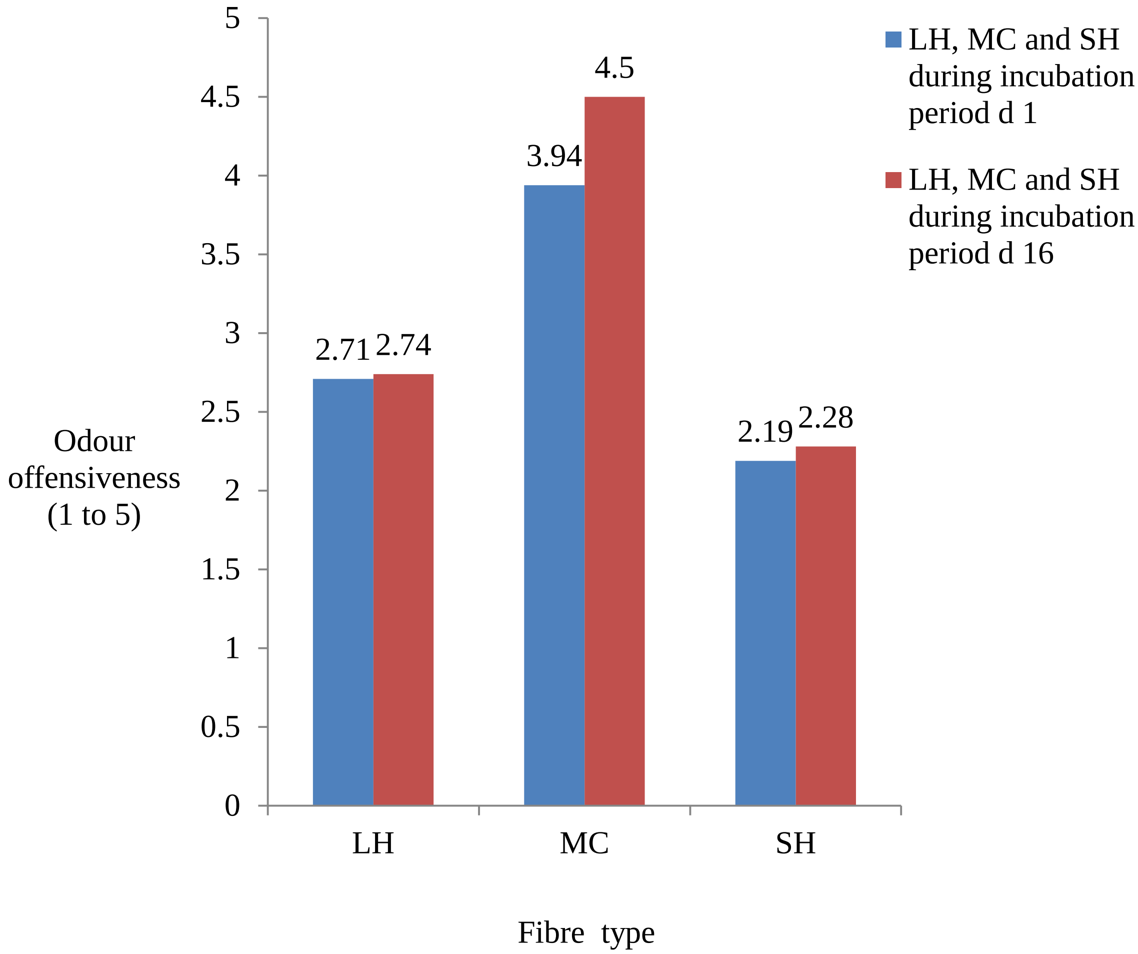

Figure 4.1: Odour offensiveness from slurry treated with lucerne hay (LH), maize cob (MC) and sunflower husk (SH) over an incubation period of 16 days

bacterial origin in the faeces. This is by the action of the urease enzyme contained in the faeces (Cahn *et al*., 1997; Bundy *et al*., 2007). This mechanism occurs when the faeces and the urine combine to form slurry. This bacterial nitrogen in faeces is known for being less volatile when urine and the faeces combine (Shriver *et al*., 2003). The increased content of the nitrogen as the slurry with time was due to a further decline in the pH numerical values of the slurry from the pigs fed on rations containing both LH and SH that contained a larger content of the fermentable carbohydrate compared to MC. Low pH numerical values result in an efficient retention of the nitrogen in the faeces when urine and faeces combine, minimizing volatilization (Cahn *et al*., 1997; Hankins *et al*., 2008).

The observed increase in the content of the COD with time reflects an expected lesser potential of the slurry contamination from pig fed high fibre containing diets such as the SH. This is because an increase in the COD content of the slurry with time is known to accelerate the de-nitrification process. Similar to the current study, the piggery slurries are said to fuel the nutrient removal process, increasing odours with a decrease in the COD content of the slurry (Knowlton *et al*., 2005). Small odour emissions relate well with large COD contents in slurry with increased fibre content in pig rations (Gralapp *et al*., 2002). This is due to an increased rate of change of ammonia to nitrate by chemical oxidation, minimizing nutrient volatilization more when pigs are fed diets with a large content of dietary fibre. Hence; small odours from SH could also be explained by the high content of fibre contained in the SH containing pig rations compared to MC which yielded the largest odours from panellists.

The large SCFAs that resulted from slurries of pigs fed rations containing LH and SH due to a

Table 4.4: Pearson's correlation coefficients among slurry characteristics

Measure	pH	COD	N	Ac	Pr	iBut	nBut	Val	TSCFA
pH		-0.2**	-0.4**	-0.5**	-0.4**	-0.4**	-0.4**	-0.4**	-0.5**
COD			0.1**	0.5**	0.6**	0.4**	0.2**	0.4**	0.5**
N				0.2**	0.2**	NS	NS	0.2**	0.2**
Ac					0.8**	0.9**	0.7**	0.8**	1.0**
Pr						0.7**	0.5**	0. 7**	0.7**
iBut							0.8**	0.8**	0.9**
But								0.6**	0.7**
Val									0.8**

Significance level: ** *P*< 0.01; * *P*< 0.05; ns = not significant (P > 0.05)

Abbreviations: pH = hydrogen potential, N = Nitrogen, Ac = acetate, Pr = propionate, iBut = isobutyrate, nBut = butyrate, Val = valerate and TSCFA = total short chain fatty acids.

resultant reduced pH numerical values that are said to accelerate the production of the SCFAs, as there was a strong negative relationship in pH and TSCFAs was observed. This is due to microbial synthesis in the gut and in the slurry during incubation. The small concentration of the SCFAs observed with maize cob containing diets might have been due possible fewer substrates in the slurry required by the microbial to increase their activities. As such, the microbes tend to utilize the available SCFAs to stimulate their activities, as the SCFAs can serve as potential energy sources to the host. This is due to a relatively smaller content of the fermentable carbohydrate in the pig rations known for inhibiting the microbial activities in the hindgut of the pigs, where the production of the SCFAs occurs (Canh*et al*., 1998; Hernandez *et al*., 2011). The increase in the TSCFAs over time could have been indicative of the increased microbial activities that result due to a relatively larger content on nutrients available in the slurry. Hankins *et al*., (2008) stated the reduction in the microbial activities over time is due to fewer nutrients available in the slurry and a continuous drop of the pH of the slurry. The increased production of isobutyrate and butyrate may suggest the increased odour strength that the slurry from pigs fed MC contained, as they are one of the leading SCFAs that largely contribute to odour formation from slurry.

The offensiveness of the slurry is attributed by various parameters such as the COD, Nitrogen and the SCFAs contained in slurry. The COD of the slurry is known to be an important factor in determining odour assessment from the stored slurry (Gralapp *et al*., 2002). Small COD numerical values are known to reflect increased odour strength, therefore, MC might have yielded more odour forming intermediates from the decomposition the slurry as compared to that from pigs fed SH. The largest odour numerical scales that resulted from MC on Day 16 reflect a relatively large proportion of the iso-butyric and butyric acids that resulted with the slurry from pigs fed rations containing MC. This is because that, the production of much more iso-butyric and butyric acids is known to highlight an improvement in the level of malodours produced from the slurry (Masse *et al*., 2003). Smaller slurry pH numerical values might have also influenced the balance strike between the volatile and the non-volatile compounds such as nitrogen from the slurry of pigs fed rations containing SH, minimising odour forming compounds (Cahn *et al*. 1998; Hernandez *et al*., 2011).

The smaller odour offensiveness numerical scales from the slurry from pigs fed rations containing SH were due to a possible CP to fibre balanced in the diet compared rations containing MC. Pig rations with a high CP and fibre content are known to result in small odours, as compared to rations with an unbalanced protein to fibre ration (Le *et al*., 2008; Le *et al*., 2009). As a result; the slurry from pigs fed rations containing SH resulted in the smallest odour numerical scale than that from the slurries from pigs fed rations containing MC. This could be attributed by a possible equivalent utilization of both protein and fibre substrates, during fermentation in the gut. Although the rations containing MC and SH contained a similar content of CP content, the variation in the odour offensiveness numerical scales might have been due to their varying contents of fermentable carbohydrates contained in both ingredients.

4.5 Conclusions

The fibres used (maize cobs, lucerne hay and sunflower husk) resulted in varying chemical components of the slurry, and were attributed to varying physico-chemical properties of the diets ingested by the pigs. Sunflower husk was less offensive. The sunflower husk containing diet was effective at minimizing the odours from the slurry, the levels at which it was included were not important to explain the odour strength. The intensity of the odour prolonged with time for the slurry from pigs fed on rations containing maize cobs than any other diet fed. The large carbohydrate content of the SH diet created an enough environment at which the microbial population and activities would need to produce and reduce the utilization of protein substrates, yielding small odour emissions. This resulted in an effective change in the parameters such as the chemical oxygen demand, nitrogen and the short chain fatty acids that greatly interact to influence the odours from the slurry.

4.6 References

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Chapter 5: General discussion, conclusions and recommendations

5.1 General Discussion

The use of conventional high protein feeds in feeding growing pigs has been used for a long time to maximise growth performance and feed conversion efficiencies. Development of these feeds has, however, ignored the impact of the excreta and slurry on environmental pollution from piggeries. Feeding high protein diets generally make pigs produce excreta rich in nutrients. These nutrients consequently produce odours and cause eutrophication in surface water bodies. High fibre diets, therefore, are likely to reduce pig performance, largely because most fibre sources have high water holding capacities, thereby limiting the amount of feed and nutrients that pigs can consume (Jarret *et al*., 2011; Ndou *et al*., 2012). High fibre levels also limit the digestibility and absorption of nutrients. Variations exist on the effect of dietary fibre on pig performance. For example, maize cob meal has been shown to have little impact on feed intake.

On the other hand, including dietary fibre to pig rations is one sustainable way of reducing the crude protein content of excreta. When making decisions on appropriate fibre levels to incorporate in pig rations, the excreta characteristics should not be ignored. On the whole, the changes that occur in the excreta and slurry characteristics have not been explored. It should also be borne in mind that dietary fibre is not a homogeneous substance. The main hypothesis tested in the study was that fibre type and inclusion levels have huge influences on pig excreta and slurry characteristics.

In Chapter 3, the study was conducted to test whether including grass hay, lucerne hay, maize cob, maize stover and sunflower husk, included at levels up to 400 g/kg would alter the physico-chemical composition of the faeces and urine of growing pigs. The excreta profiles varied with fibre type and inclusion level. For all fibre types urinary nitrogen levels decreased as dietary fibre increased, up to 160 g/kg. Generally, increasing the level of fibre beyond 160 g/kg had a great impact on the excreta profiles, such as isobutyrate and butyrate concentrations. The ratio of faecal to urinary nitrogen increased as fibre level increased, suggesting that the increasing fibre contents of pig rations promote nitrogen partitioning from urine to faeces (Hansen et al., 2007).

Slurries in South Africa are normally kept for about two weeks. In Chapter 4, lucerne hay, maize cob and sunflower husk were included in pig rations up to 160 g/kg to test whether they influences the characteristics and odour emissions of the slurry incubated for 16 days. The offensiveness of odours from the pigs fed on rations containing maize cob was attributed to the butyrate and the nitrogen content of the slurry. Butyrate and nitrogen form large portions of odour forming compounds. Using untrained panellists, slurry incubation period had no significant influence on odour scores. Slurry from diets containing SH had the least odour scores, making SH the best fibre to use in pig rations to reduce odour emissions from piggeries.

5.2 Conclusions

Dietary fibre inclusion to pig rations influence on the excreta, slurry characteristics and odours from growing pigs. The desirable levels of fibre inclusion have been found to a limit of 160 g/kg, as some excreta profiles, known to impact negatively to the environment increase unreasonably beyond the fibre inclusion limits. Sunflower-husk containing diets proved to impact less on the environment, as the pigs fed on such rations resulted in less odours, compared to those fed rations containing maize cob or lucerne hay. These are considerations that need to be made during feed formulation to minimize odour impacts from slurry.

5.3 Recommendations

The use of other fibre sources, other than the ones used in the current experiment need attention, as this widens the scope of dietary fibre effects on pig excreta and odours. Quantifying the odour forming intermediates as the hydrogen sulphide and phenols could be another way of expanding the scope of understanding fibre effects on odours. It is also important to investigate the changes in the microbial activities in the faeces and the slurry, as odours result from microbial fermentation.

5.4 References

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Appendix

Consent form

I am Conference Thando Mpendulo, a student at the University of KwaZulu-Natal doing MSc in Animal Science. I am conducting a study (as the requirement of experiment 2 of my Masters thesis), to evaluate the effects dietary fibre and their varying inclusion levels on the odour offensiveness of growing pig slurries stored for 16 days. All data collected from this study will be confidential and will only be used as part of this research project. Each individual is urged to rate each of the 14 slurry samples provided using the 5 odour offensiveness scales and indicate these scores on the evaluation sheet provided.

I, Mpendulo, C. T........ (Name), hereby confirm that the questionnaire has been clearly explained to me and I understand the purpose of this study and how this information is going to be tested.

I therefore agree to voluntarily participate in this research study.

……………………. …………………………

Signature Date

Odour offensiveness questionnaire

Sensory evaluation of Pig slurry____
Sex: ___________________________ Age (OPTIONAL): _______________ Panellist Number: ________________

Samples code	Sensory Characteristics					Comments
	Not offensive	Mildly offensive	Moderately offensive	Strongly offensive	Extremely offensive	
	NB: Mark with **X** where relevant					
1						
2						
3						
4						
5						
6						
7						
8						
9						
10						
11						
12						
13						
14						

Sensory evaluation of slurry from pigs.

Instructions:

1. Please do not eat an hour before starting.

2. Please wait for 2 minutes before going to the next sample.