EFFECTS OF MOLASSES-ENHANCED FERMENTATION AND EXOGENOUS ENZYMES ON THE NUTRITIVE VALUE OF CASTOR BEAN (*RICINUS COMMUNIS L*) OIL CAKE FOR GROWING PIGS

By

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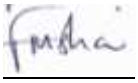
Supervisor: Prof F Fushai **Signature**  **Date** _03/07/2023__

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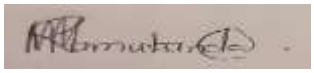
DECLARATION

I, Mulisa Ramukanda, hereby declare that this thesis for Master of Science in Agriculture (MSCANS) sent to the Department of Animal Science, School of Agriculture, at the University of Venda has not previously been given for any degree at this or any other institution of another university. It is original in design and execution, and all reference material has been duly acknowledged.

Signature  …… Date …27/February/2023……

Mulisa Ramukanda

DEDICATION

I dedicate this thesis to my beloved parents, Mr T.L and Mrs K.C Ramukanda, who have always been my guiding light and pillars of strength throughout my academic journey. Their unwavering love, support, and encouragement have been the driving force behind my success.

To my siblings, Mukundi, Ndaedzo, and Luvhani, thank you for being my constant companions, and for always standing by me, no matter what. Your love, laughter, and endless support have been a source of inspiration, and I am grateful to have you in my life.

I also dedicate this thesis to my supervisor, and friends who have played a significant role in shaping my academic and personal growth. Your guidance, wisdom, and encouragement have been invaluable, and I am forever indebted to you.

Lastly, I dedicate this thesis to the future generations, with the hope that the knowledge and insights gained from this research will contribute to the advancement and betterment of society.

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ABSTRACT

Castor bean (*Ricinus communis L*) is highly valued for its oil, which has diverse applications in the pharmaceutical industry. Castor bean oil cake (CBOC) contains high levels of protein, but contains highly toxic compounds that require proper processing before feeding to livestock. The aim of the study was to evaluate optimum solid fermentation for CBOC, and the effects on nutrient digestibility, nitrogen balance, or plasma metabolites of 10% of the optimally fermented CBOC (FCBOC) in a diet fortified with exogenous fibrolytic enzymes. In experiment 1, a micro-fermentation study was conducted to evaluate the optimum level of supplementary Voermol (Product V10257; molasses containing 33 g/kg CP, 300 g/kg moisture, 6-9.2 g/kg Ca, 0.8 g/kg P and, 9.5 MJ ME/kg), 75% BRIX 75) in relation to the period of anaerobic solid fermentation of the CBOC. The experiment followed a completely randomized, 4 (molasses level; 0%, 5%, 10%, and 15%) x 4 (fermentation days; 0, 4, 7, and 10-day) factorial design, replicated three times. The change in proximate and detergent fibre composition was evaluated, and the pH of the fermented CBOC (FCBOC) measured as an indicator of fermentation intensity and extent, which are key determinants of silage quality and anerobic stability. Highest ($P<0.05$) OM was observed in 5% Voermol, 4- day FCBOC, similarly ($P>0.05$) followed by 10% Voermol, 4% fermented FCBOC, and least ($P<0.05$) OM in 15% Voermol, 7- day FCBOC, followed in the increasing ($P<0.05$) order 10% Voermol, 4-day fermented FCBOC>15% Voermol, 4-day fermented FCBOC. Treatment effects on ash were inverse to the OM ($P<0.05$) The fat content decreased ($P<0.05$) with 7, and 10-day fermentation, while 15% Voermol inclusion decreased ($P<0.05$) the fat content. The lowest pH (3.89) was achieved with 5% Voermol inclusion and 7-day fermentation. In experiment 2, the effects of including 10% inclusion of the optimally (5% Voermol, 7-day fermentation) fermented FCBOC in the pig's diet, and the efficacy of fibrolytic enzymes were investigated. Experimental diets were a standard maize-soybean diet (0% FCBOC), and an iso-nutrient, 10% FCBOC diet, each with (+) and without (-) 500g/tonne of Ronozyme® WX CT (EC-3.2.1.8, 1000 FXU/g endo-1,4-β-xylanase). Eight growing (31.88 ± 1.63 kg live weight) male Large White x Landrace pigs in metabolic cages were assigned to diets in a randomized 2 (diet) x 2 (enzyme) factorial arrangement within a two balanced 4 (period) X 4 (diet) Latin squares. Each feeding period consisted of 9 days dietary adaptation, plus five days of feed intake measurement, and the total collection of faeces and urine, from which nutrient digestibility, and parameters of N balance (Nitrogen intake, Urine Nitrogen Output, Feacal Nitrogen Output, Total Nitrogen Excretion, Absorbed Nitrogen, Nitrogen Retention, Nitrogen Utilization, Biological Value Feed Protein , and Apparent N digestibility were calculated. Blood was collected by jugular venepuncture into 10ml serum vacutainers on the last day of each period, from which Glucose, Urea, Creatinine, total protein, albumin, globulin, alanine aminotransferase, aspartate aminotransferase, alkaline phosphatase, bilirubin, cholesterol and triglyceride were analysed. In conclusion, based on the depletion of organic substrates, intensity and extent of CBOC fermentation, optimum fermentation was considered to be through 5% supplementary Voermol and 7-day ensilage. The 10% dietary inclusion of the optimally fermented FCBOC was not detrimental to nutrient digestibility, nitrogen balance, or plasma metabolites, which were not affected by the fibrolytic enzymes.

Key words: Bioprocessing, blood metabolites, castor bean oil cake, digestibility, exogenous enzyme, pigs.

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LIST OF ABBREVIATIONS

AOAC - Association of Official Analytical Chemists

ADF - Acid Detergent Fibre

AID - Apparent Ileal digestibility

ANFs - Antinutritional Factors

CBOC - Castor Bean Oil Cake

CP - Crude Protein

DM - Dry Matter

FAO - Food and Agriculture Organization

FCBOC - Fermented Castor Bean Oil Cake

EE - Ether Extract

GLM - General Linear Model

N - Nitrogen

NRC - National Research Council

NDF - Neutral Detergent Fibre

NI -Nitrogen Intake

UNO - Urine Nitrogen Output

FNO - Feacal Nitrogen Output

TNE - Total Nitrogen Excretion

AN - Absorbed Nitrogen

NR - Nitrogen Retention

NU -Nitrogen Utilisation

BVFP - Biological Value Feed Protein

ND - Apparent N Digestibility

CHAPTER 1 INTRODUCTION

1.1 Background

Feeding accounts for more than 70% of the costs of pig production (Gomes da Silva *et al.*, 2018). Demand from the biofuel and food industries has for long escalated the cost of maize and soybean oil cake (Patience, 2013; Avalos, 2014)). As a result, there is demand for affordable alternative feedstuffs (Woyengo *et al.*, 2014).

Although soybean oil cake meal makes a smaller quantitative contribution to pig diets than maize does, the greater cost per kilogram points to the necessity for full or partial replacements of protein concentrate (Gomes da Silva *et al.*, 2018). By-products from the production of biodiesel, including castor bean (*Ricinus communis L*.) oil cake (CBOC), are an alternative in this situation because of their high protein levels (Carrera *et al.*, 2012). Castor bean oil cake, which has an average oil yield of 50%, is mostly produced by mechanical pressing without the use of solvents (Gomes da Silva *et al.*, 2018). Castor bean oil cake is estimated to have 21-48 % crude protein (CP) (Akande *et al.*, 2012). Due to the presence of ricin, agglutinin-RCA120, ricinine, and allergenic chemicals such chlorogenic acid, Ric c 1, Ric c 2, and Ric c 3, its usage in animal feed is restricted (Akande *et al.*, 2016). Castor bean oil cake must therefore undergo detoxification before being fed to animals (Bueno *et al.*, 2014). Molasses is a fermentation-enhancing additive traditionally included in pig diets as a cost-effective way to increase palatability (Selvam *et al.*, 2016). The feeding value of CBOC in diets for growing pigs can be improved by different methods, including fermentation, auto-calving, heating, and dehulling (Akande and Odunsi, 2012; Akande *et al.*, 2014). Water soaking and fermenting CBOC for five days inactivated the ricin content (Oso *et al.*, 2011). Fermentation of CBOC reduced the fibre content with a potential positive influence on the bioavailability of other nutrients (Akande and Odunsi, 2012). However, the type and extent of fermentation conditions determine the degree of residual anti-nutrient factors (Akande *et al.*, 2016).

Castor bean oil cake has undergone significant research in ruminant animal nutrition, where its high fibre content and toxins are less restrictive (Gomes *et al.*, 2012). Recent research indicated that detoxified CBOC might be safe for pigs (Akande and Odunsi, 2012; Bueno *et al.*, 2014). In piglets, 10% dietary inclusion did not affect growth (Apata *et al.*, (1999).

Enhancing the nutritional value of oilseed byproducts through fermentation is a quick, affordable, and effective method (Duodu *et al.*, 2018). Exogenous enzymes may also enhance nutrient utilization and digestibility (Munir and Maqsood, 2013; Ravindran, 2013). Exogenous enzymes promote cell wall rupture, decrease intestinal viscosity based on non-starch polysaccharides, and mitigate the effects of antinutritional dietary elements (Palhares *et al.*, 2019). Supplemental enzymes reduce nutrient wastage (Bedford *et al.*, 2022), thereby reducing the feeding cost, and the environment footprint.

The objectives of the study were to evaluate the effects of 10% CBOC in a growing pig diet, the effectiveness of fermentation, and efficacy exogenous enzymes in processing on CBOC for feeding growing pigs.

1.2 Problem statement of the research

Protein is the most limiting nutrient in smallholder growing pig diets, mainly due to the cost and scarcity of protein-rich feeds (Wang *et al.*, 2018). Low-cost protein sources should not contain too much fibre or harmful compounds that might impair pig performance and should instead have a suitable amount of highly digestible protein. Previous research points to a 10-15% dietary inclusion limit for CBOC in monogastric diets, due to its fibre and highly toxic components (Akande *et al.*, 2016). Limited research has investigated how monogastric animals like pigs react to CBOC as a protein source. Enzyme supplements and fermentation are efficient, low-cost processing techniques for production systems with limited resources. The effectiveness of these processing techniques in detoxifying CBOC for inclusion in pig diets has not been fully investigated.

Therefore, to address these questions, this study evaluated the effects of 10% inclusion of fermented CBOC (FCBOC) in a growing pig diet fortified with a cocktail of exogenous enzymes

1.3 Justification of the study

The traditional components of pig diets, soybean oil cake, and cereal grains, are becoming scarce and more expensive. The risk to the viability of pig production is driving the current search for alternative feedstuffs. Although the contribution of soybean in growing pigs is quantitatively smaller than corn, the higher cost per kilogram of this ingredient indicates greater need for total or partial substitutes of protein concentrate. The current pressure on conventional feeds justifies the need to find alternative pig feeds. In this context, high protein, bulk oil extraction by products such as CBOC are considered the suitable alternatives. This study is significant to smallholder farmers who find themselves at the receiving end of high feed costs and scarcity of high protein feedstuffs. Currently, massive quantities of CBOC are produced as a by-product of small-scale pharmaceutical castor oil production, to which small-scale farmers have access at no cost. There is a scarcity of information on the suitability, and on how to use CBOC as a protein source for growing pigs. The study evaluated the effects of 10% dietary inclusion of CBOC in the growing pig diet, and evaluated fermentation, in combination with enzymes as practical CBOC processing methods for adoption by farmers in poorly resourced settings.

1.4 Research objectives

1.4.1 Main objective

The aim of the study was to evaluate the potential of CBOC as feed for growing pigs.

1.4.2 Specific objectives

The objectives of the study were to evaluate the following;

1. Effective level of readily fermentable substrate (molasses) in relation to the period of fermentation of CBOC in terms of the nutrient composition (Dry matter, crude protein, ash, moisture, ADF, and NDF) and the terminal pH.
2. Effects of supplementing exogenous enzymes to growing pigs on fermented CBOC diets on digestibility, and blood metabolite markers for energy and nitrogen metabolism.

1.5 Research hypothesis

i. The level of inclusion of highly fermentable substrates such as molasses, and the period of fermentation do not affect the effectiveness of fermentation (terminal chemical composition and terminal pH) of CBOC.

ii. Supplementing exogenous enzymes to growing pigs fed on diets containing fermented CBOC does not affect *in vivo* dry matter, nutrient and energy digestibility, and N balance and blood metabolite markers for energy and nitrogen metabolism.

CHAPTER 2 LITERATURE REVIEW

2.1 Introduction

Due to constraints in both the quantity and quality of the feed, underfeeding tropical livestock is a major challenge to pig production (Mendieta-Araica *et al.*, 2011). The Castor bean (Ricinus communis L), which originated in tropical Africa, is grown in Asia, Central and North America, Africa, and Europe, r as ornamental, and increasingly, as an oilseed crop (Doan, 2004). The product of its oil extraction is CBOC. Castor remains the most important non-edible oilseed crop in the arid and semi-arid regions (Srinivasa Rao *et al.*, 2012). The high nutritional profile of castor seed meal suggests enormous potential as an important feed resource (Adeniran *et al.*, 2017). However, there is limited information on the value of CBOC as a source of protein for growing pigs, given its content of highly toxic compounds (Mondal *et al.*, 2019).

2.2 Nutrient composition of castor bean oil cake

Castor beans oil cake contains 21-48 percent crude protein (CP), depending on the degree of decortication and oil extraction (Annongu and Joseph, 2008; Ani and Okorie, 2009; Matos Júnior *et al.*, 2011). Whole seed respectively contained 27.2 MJ/kg gross energy (Akande *et al.*, 2012). The mechanically pressed CBOC retains a high fat content and (Matos Júnior *et al.*, 2011),. According to (Matos Júnior *et al.*, 2011), the mineral content, particularly calcium and phosphorus, was similar to other plant protein sources (Akande *et al.*, 2012). However, if the seeds are not dehulled, the defatted meal contains high fibre (Akande *et al.*, 2014).

Table 2.1 Nutrient composition of castor bean oil cake.

Nutrients	Range	Sources
Moisture (%)	5–12	(Annongu and Joseph, 2008; Ani and Okorie, 2009) (Oso and Bamgbose, 2013) (Akande and Odunsi, 2012)
Crude protein (%)	21–48	(Annongu and Joseph, 2008); (Ani and Okorie, 2009), (Matos Júnior *et al.*, 2011), (Akande and Odunsi, 2012)
Crude fat (%)	1.9–50	(Annongu and Joseph, 2008), (Matos Júnior *et al.*, 2011), (SEEDS), (Akande and Odunsi, 2012)
Soluble carbohydrate (%)	9.1–20.5	(Annongu and Joseph, 2008), (Matos Júnior *et al.*, 2011), (SEEDS), (Akande and Odunsi, 2012)
Crude fibre (%)	2.5–24.5	(Annongu and Joseph, 2008), (Matos Júnior *et al.*, 2011), (SEEDS), (Akande and Odunsi, 2012)
Total ash (%)	8.1–19.2	(Annongu and Joseph, 2008), (Matos Júnior *et al.*, 2011), (SEEDS), (Akande and Odunsi, 2012)
GE MJ/kg	13.5–23.8	(Matos Júnior *et al.*, 2011), (Akande and Odunsi, 2012)
Calcium (%)	1.06–5.67	(Matos Júnior *et al.*, 2011), (Akande and Odunsi, 2012)
Phosphorous (%)	0.3–0.73	(Matos Júnior *et al.*, 2011), (Akande and Odunsi, 2012)

2.3 Antinutritional factors in castor bean oil cake

The castor bean contains a variety of anti-nutritional factors, including the poisonous glycoprotein ricin, and the alkaloid ricinoleic acid (12-hydroxylic acid derivative), the levels of which depend on the variety (Mondal *et al.*, 2019). Ricin is a naturally occurring, extremely poisonous lectin (a protein that binds to carbohydrates) found in the seeds of the castor oil plant (Nasr *et al.*, 2014). Even at very small doses, the highly poisonous protein ricin prevents protein synthesis in a cell-free environment (Polito *et al.*, 2019). Ricinine, is an alkaloid that is present in the fruit's pericarps and leaves, it may be isolated in trace levels together with ricin (Mondal *et al.*, 2019). Ricinine is regarded as a stimulant of the central nervous system (Jena and Gupta, 2012). The biopesticide

ricinine (C8H8N2O2) is used in pest control . According to (Bullangpoti *et al.*, 2011), ricinine is used as an alternative to a small number of chemical insecticides. Ricinoleic acid, a fatty acid found in the castor bean is very irritating to the gastrointestinal mucosa and is thought to be the cause of the cathartic effects of ricin oil (Diaz, 2011). Ricinoleic acid changed the intestinal epithelium, which resulted in water and electrolyte loss, increased luminal DNA loss, and decreased enterocyte enzymatic activity (Cires *et al.*, 2017). Though ricinine is commonly analyzed as the surrogate for the presence of castor bean toxins in press cakes in food products (Darby *et al.*, 2001), ricin is the most toxic in mechanically pressed CBOC (Baleta *et al.*, 2015). Ricin is present in every part of the castor plant, though exceptionally abundant in the seeds, and frequently linked to clinical toxicosis (Aslani *et al.*, 2007).

2.4 Effects of type and level additive on fermentation of castor bean oil cake

If effectively processed, CBOC may be utilized as animal feed (Sousa *et al.*, 2017). From an economic standpoint, the available processing techniques are not considered viable and sufficiently effective to be implemented on a broad scale (Severino *et al.*, 2012). Previous studies attempted to detoxify castor cake using physical, chemical, and biological means with varying degrees of success (Mondal *et al.*, 2019). Anandan *et al.* (2005) investigated soaking, steaming, boiling, autoclaving, and heating at various intervals, as well as chemical treatments, such as ammonia, formaldehyde, lime, sodium chloride, tannic acid, and sodium hydroxide at various concentrations. Among other methods, only calcium hydroxide treatment (40 g calcium hydroxide per kilogram castor residue) and autoclaving (15 psi for 60 minutes) totally removed the toxins (Barnes *et al.*, 2009). Autoclaving for different periods (15 or 30 minutes), the use of CaO in place of calcium hydroxide, or combinations of these methods achieved partial or nearly complete detoxification (Borja *et al.*, 2017).

Inexpensive biological detoxification techniques have been used to turn hazardous waste into useful feedstock (Veza *et al.*, 2021). As used as a solid culture medium, fermentation of CBOC eliminated all its toxicity and reduced the allergenicity (Godoy *et al.*, 2011). Sousa *et al.* (2017) investigated genetic manipulation to produce non-toxic castor bean genotypes. They effectively locked ricin coding genes in genetically modified (GM) plants where ricin proteins were not detectable by ELISA.

2.5 The use of exogenous enzymes in novel pig diets

Increased pork consumption demands broader research into cheaper, readily available feed alternatives. The alternative feedstuffs tend to be inferior in quality to conventional feeds, hence the need for processing. On the other hand, selection for larger litter size is linked to low piglet maturation, characterized by low birth weights, compromised immune systems, and immature digestive systems at weaning, with limited endogenous digestive enzyme secretion (Ugwuanyi, 2016) (Torres-Pitarch *et al.*, 2017). This has created opportunity for greater us exogenous enzymes in pig nutrition. Exogenous enzymes are mostly manufactured using fungi of the Aspergillus and Peniophora genera (Rodehutscord and Rosenfelder, 2016). Much of our current knowledge on exogenous digestive enzymes is largely from research in poultry, which is often considered applicable to pigs. However, the application of the technology should be cautious, given the different digestive physiology (Hernández, 2006). Exogenous enzymes improve the digestibility of feed, degrading their complex matrix. In growing pig's carbohydrase supplementation (xylanase, β-glucanase, β-mannanase, α-galactosidase) increased substrate digestibility (Torres-Pitarch *et al*., 2017). Phytase improved the digestibility of minerals, which are otherwise bound to phytate (Dersjant-Li *et al*., 2015; Torres-Pitarch *et al*., 2017). Tiwari *et al (* 2018) reported that xylanase and mannanase can be used together or separately in diets rich in arabinoxylans and mannans, depending on the quantitative and qualitative composition of the feed. Despite the general consensus on exogenous enzyme benefits such as improved nutrient

digestion, intestinal maturity and gut health in weaned piglets, supplementation of the diets of weaned piglets with individual or combined mannanase, phytases, proteases and carbohydrates previously inconsistently affects nutrient digestibility, growth , the histomorphology of the absorptive intestinal epithelium (Notey *et al.,2015 ;*Torres-Pitarch *et al.*, 2017).

2.6 Optimum dietary inclusion of CBOC oil cake for pigs

Given residual toxins, the optimum dietary inclusion of CBOC for growing pigs depends on the detoxification process. Previous studies evaluated the maximum tolerable levels of detoxified CBOC in pig diets, with the objective optimize the nutritional benefits, while minimizing the negative effects. Ferreira *et al*. (2013) evaluated the inclusion of detoxified castor bean meal in growing pig diets at levels of 0%, 5%, 10%, and 15%. The 5-10% dietary inclusion did not negatively impact the performance of the animals, nutrient digestibility, or carcass traits. Higher inclusion (15%) resulted in reduced performance. Oliveira et al. (2016) investigated the effects of including detoxified CBOC in the diets of growing pigs at levels of 0%, 4%, 8%, and 12%. The results demonstrated that pigs fed diets containing up to 8% detoxified CBOC showed did not affect the average daily gain, feed intake, and feed conversion ratio compared to the control group. The inclusion of 12% detoxified CBOC negatively impacted these parameters. Based on these studies, it appears that, subject to the processing method, the optimum dietary inclusion of detoxified CBOC for growing pigs lies between 8% and 12 %. Further research is therefore required to optimize the detoxification process, and the dietary inclusion of CBOC inclusion in pig diets.

2.7 Effects of fermentation period on the fermentation of castor bean oil cake

Fermentation improves the quality of edible grains (Ishiwu *et al.*, 2015), and has been traditionally used for food processing (Onofiok *et al.*, 1996).. The digestibility, nutritional value, and flavors of the raw seeds were significantly enhanced by fermentation, in addition to the reduction of antinutritional factors (Odunfa, 1985). An initial (three-day) decline in protein typically occurs due

fermentation (Chikwendu *et al.*, 2014; Ishiwu *et al.*, 2015), which is compensated with prolonged fermentation (Ishiwu *et al.*, 2015). Total ash was also observed to increase with fermentation period (Alemawor *et al.*, 2009). The microbial enzyme activities determine the rate of chemical change with progressive fermentation (ODUNFA, 1985).

2.8 Summary of Literature Review

Protein is usually the most limiting in small holder growing pig diets, largely due to both cost and scarcity of protein feeds. There is limited information on the use of CBOC in pig diets in terms of chemical composition, efficacy of fermentation in mitigating the antinutritional factors, and the benefit of exogenous enzymes when supplemented to complex growing pig diets. Therefore, to facilitate the effective utilization of CBOC in growing pigs diets, there is a need to evaluate the optimum fermentation, effects of dietary inclusion, , and the benefit of exogenous enzymes.

CHAPTER 3

EFFECTS OF SUPPLEMENTARY MOLASSES AND FERMENTATION PERIOD ON THE QAULITY OF FERMENTED CASTOR BEAN (*RICINUS COMMUNIS L*) OIL CAKE.

Abstract

The Castor bean (*Ricinus communis l*) is valued for its oil, which has diverse applications in the pharmaceutical industry. The cold oil expeller castor bean oil cake (CBOC) retains toxic compounds which require effective processing prior to feeding to livestock. The objective of the study was to evaluate the effects of fermenting CBOC for different fermentation periods and different levels of supplementary molasses on the terminal pH and nutrient composition of the fermented product (FCBOC). In a micro fermentation study, 250g CBOC samples treated with 0, 5, 10 and 15% Voermol (Molasses Product V10257; containing 33 g/kg CP, 300 g/kg moisture, 6-9.2 g/kg Ca, 0.8 g/kg P and, 9.5 MJ ME/kg), 75% BRIX 75) were subjected to 0, 4, 7, and 10 days anerobic fermentation in tight sealed, 250ml glass bottles in a completely randomized 4 (molasses level) x 4 (fermentation days) factorial experiment replicated three times. The terminal pH of FCBOC, and proximate components, and detergent fibre in washed FCBOC were measured. Significant fermentation period * molasses inclusion level interaction occurred for washed FCBOC DM (P=0.05), OM (P< 0.001), ash (P< 0.001) and the terminal FCBOC pH (P< 0.001). Highest (P<0.05) OM was observed in 5% Voermol, 4- day FCBOC, similarly (P>0.05) followed by 10% Voermol, 4% fermented FCBOC, and least (P<0.05) OM in 15% Voermol, 7- day FCBOC, followed in the increasing (P<0.05) order 10% Voermol, 4-day fermented FCBOC>15% Voermol, 4-day fermented FCBOC. Significant (P<0.05) levels of the OM content were observed in all other treatments. Treatment effects on ash were inverse to the OM, whereby highest (P<0.05) ash content was in 10% Voermol, 4- day FCBOC, similarly P>0.05) followed by 15% Voermol, 7% fermented FCBOC, with least (P<0.05) ash in 15% Voermol, 7- day FCBOC,

increasing in the order 0% Voermol, 10-day fermented FCBOC<10% Voermol, 0-day fermented FCBOC. Significant ash (P<0.05) content was observed for in the other treatments. The fat content decreased (P<0.05) to similar (P>0.05) extent with 7, and 10-day fermentation, while 15% Voermol inclusion decreased (P<0.05) the fat content. Compared to an initial, initial 0% Voermol, 0-days fermentation high (5.60) pH, the lowest pH achieved was 3.89, which was recorded with 5% Voermol inclusion, 7-day fermentation, which increased to a stable, intermediate pH range at high treatment levels thereafter. Considering pH as the surrogate indicator for the intensity and extent of fermentation necessary for effective fermentation and aerobic stability, it was concluded that the best fermentation was achieved with 5% Voermol inclusion, 7-day fermentation.

Keywords: Bioprocessing, Castor by-products, Pigs, Ricin.

3.1 Introduction

Feeding typically contributes approximately 70% of the variable pig production cost (Zijlstra and Beltranena, 2013). Demand for animal protein to meet the needs of a rapidly increasing human population imposes immense pressure on producers to find suitable alternatives to augment the constrained supply of conventional feeds (Woyengo et al., 2014). The castor bean (*Ricinus communis L*.) is among oilseeds which are highly sought after for either pharmaceutical (Wang et al., 2021) or biodiesel (Ávila Vázquez et al., 2020) manufacturing. Its advantages include minimal management, wide ecological adaptability (Salihu et al., 2012) and high yields of expensive oil (Silva et al., 2014). In livestock production, CBOC is considered a potential high protein source (Abdalla et al., 2012), typically containing 33.7% CP (Abdalla et al., 2012). However, whether solvent or expeller oil extracted, CBOC retains allergens such chlorogenic acid, Ric c 1, Ric c 2, and Ric 3, as well as poisonous residual ricin, agglutinin-

RCA120, and ricinine (Akande and Odunsi, 2012; Akande et al., 2014). Fermentation is among different methods considered which can be used to detoxify and improve the nutrient profile of CBOC (Abdalla et al., 2012; Akande and Odunsi, 2012; Akande et al., 2014). Enhancing the nutritional value of oilseed by-products through fermentation is quick, affordable, and effective (Duodu et al., 2018). Fermentation of CBOC reduces the fibre and improves the bioavailability of nutrients (Akande and Odunsi, 2012). Molasses is an abundant, low fermentation-enhancing additive that is routinely included in stock feeds to provide readily available energy, and to increase palatability (Selvam et al., 2016).

The objective of the study is to determine the effective molasses inclusion level in relation to the fermentation period required for optimum fermentation and improvement in nutrient composition.

3.2 Methods and materials

3.3 Ethical consideration

The protocols used in this study were approved by the University of Venda Ethics Committee (SARDF/19/ANS/14/2001). The fermentation study was conducted in the Month of May, at the University of Venda Animal Science Nutrition laboratory. The location experiences summer temperatures ranging from 11^{o}C to 38^{o}C and winter with average minimum and maximum temperatures of 11^{o}C and 27^{o}C respectively (South African Weather Bureau (SAWB), 2014).

3.4 Source and preparation of Castor bean oil cake

Castor bean oil cake (CBOC) was supplied for scientific testing on behalf of small-scale oil producers in the Mbangwane, Nkomazi region, Mpumalanga province. It was a by-product from

cold expeller oil extraction from castor bean, with nutrient composition as presented in Table 3.1.

Table 3.1 Analyzed chemical composition of Castor bean oil cake.

Nutrient	g kg^{-1} DM
Dry matter	.
Organic matter	955.9
Ash	44.1
Crude protein	222.1
Fat (ether extract)	270.4
Neutral detergent fibre	295.0
Acid detergent fibre	172.2
Ca	4.10
P	6.67

3.5 Fermentation additive

Voermol (Product V10257; containing 33 g/kg CP, 300 g/kg moisture, 6-9.2 g/kg Ca, 0.8 g/kg P and, 9.5 MJ ME/kg), 75% BRIX 75) manufactured by Voermol feeds (Pty) Ltd, Maid-stone, KwaZulu-Natal, South Africa was used as the fermentation additive. The product was molasses-based, recommended for use as an energy supplement, and to increase feed palatability in place of normal molasses.

3.6 Castor bean oil cake processing, treatments, and experimental design

Samples (250g) of 90.4% dry matter CBOC were treated (v/m) with 0% (0 ml), 5% (12.5 ml), 10% (25 ml), 15 (37.5 ml) Voermol for fermentation over 0, 4, 7, or 10-day periods in a 4 x 4 factorial arrangement of the treatments in a completely randomized experiment replicated three times. Measured additive was placed in a beaker which was made up to 37.5ml with boiling distilled water and stirred to homogeneity, after which the solutions were mixed into the CBOC. The treated CBOC was compacted into tight-sealing glass bottles using a wooden stick. The bottles were sealed, placed in a dark cupboard for anaerobic fermentation, after which samples of

fermented CBOC (FCBOC) were retrieved for pH measurement, and the bulk retained for chemical analyses.

3.6 Measurement of pH

The terminal pH of FCBOC was measured as the sole surrogate indicator of effective fermentation. To determine pH, 20g of the FCBOC were mixed with 50ml of distilled water. The mixture was stirred for 10 minutes, left to stand for 30 minutes, and remixed for 2 minutes, after which the pH was measured.

3.7 Chemical analyses

The FCBOC was thoroughly rinsed in 500 ml tap water three times to reduce its sour taste and foul odor. The washed FCBOC was sun-dried for storage prior to chemical analyses. Oven DM of CBOC and FCBOC was determined at 60º C (AOAC, 1990; method 930.15). Ash was determined by combustion at 500 °C overnight (AOAC., 1990; method 942.05). Neutral detergent fibre and acid detergent fibre were determined according to Van Soest *et al*. (1991). Crude protein was determined by the Kjeldahl procedure (AOAC, 1990; method 984.13).

3.8 Statistical analysis

Data were subjected to analysis of variance (ANOVA) for a 2-factor experiment using the general linear models (GLM) of Minitab version 21.1.0 (Minitab, I., 2020). Different ($P<0.05$), means were separated using Tukey's test.

Data were analyzed based on the model:

- $Y_{ij=}\mu + \alpha_i + \beta_j + \alpha\beta_{ij} + \varepsilon_{ij}$

Where, Y_{ij} = the observation – Ash, DM, CP, EE, NDF, ADF and PH.

- μ = overall mean common to all observations:

- α_i = effect of the i^{th} molasses inclusion
- β_j = effect of the j^{th} fermentation period
- $\alpha\beta_{ij}$ = interaction of molasses and fermentation processing
- ε_{ij} = random residual error.

3.9 Results

Table 3.2 presents the effects of Voermol (Molasses) inclusion, and of the fermentation period on the terminal pH, and the washed chemical composition of FCBOC. Significant fermentation period * molasses inclusion level interaction occurred for washed FCBOC DM (P=0.05), OM (P< 0.001), ash (P< 0.001) and the terminal FCBOC pH (P< 0.001). Highest (P<0.05) OM was observed in 5% Voermol, 4- day FCBOC, similarly (P>0.05) followed by 10% Voermol, 4% fermented FCBOC, and least (P<0.05) OM in 15% Voermol, 7- day FCBOC, followed in the increasing (P<0.05) order 10% Voermol, 4-day fermented FCBOC>15% Voermol, 4-day fermented FCBOC. Intermediate (P<0.05) levels of the OM content were observed in all other treatments. Treatment effects on ash were inverse to the OM, whereby highest (P<0.05) ash content was in 10% Voermol, 4- day FCBOC, similarly P>0.05) followed by 15% Voermol, 7% fermented FCBOC, with least (P<0.05) ash in 15% Voermol, 7- day FCBOC, increasing in the order 0% Voermol, 10-day fermented FCBOC<10% Voermol, 0-day fermented FCBOC. Intermediate ash (P<0.05) content was observed in the other treatments. The fat content decreased (P<0.05) to similar (P>0.05) extent with 7, and 10-day fermentation, while 15% Voermol inclusion decreased (P<0.05) the fat content. Compared to the control (0% Voermol, 0-days fermentation) high (5.60) pH, the lowest pH achieved was 3.89, which was recorded with 5% Voermol inclusion, 7-day fermentation.

Table 3.2 Effects of molasses inclusion, and the fermentation period on the chemical composition the terminal pH and fermented castor bean oil cake

Treatment		Chemical Composition							pH
		DM%	OM%	Ash%	CP%	EE%	ADF%	NDF%	
Fermentation period (FP)									
0		93.53^{ab}	94.03	5.96	26.48	27.96^{a}	30.61	39.77	5.35^{a}
4		93.66^{ab}	94.30	5.70	25.49	25.04^{ab}	30.69	38.98	5.42^{a}
7		93.84^{a}	94.23	5.76	24.52	23.35^{b}	32.64	40.27	4.90^{c}
10		93.07^{b}	94.27	5.73	24.20	23.03^{b}	29.27	35.91	5.12^{b}
SEM		0.148	0.085	0.085	1.087	0.973	1.370	1.378	0.033
Molasses inclusion (MI)									
0%		93.77^{a}	94.44^{a}	5.56^{b}	26.78	27.71^{a}	32.52	38.59	5.60^{a}
5%		93.87^{a}	94.40^{a}	5.57^{b}	26.10	24.53^{ab}	31.59	40.35	4.95^{c}
10%		93.59^{a}	94.23^{a}	5.77^{b}	24.40	25.26^{ab}	30.17	35.55	5.16^{b}
15%		92.87^{b}	93.68^{b}	6.32^{a}	23.41	21.94^{b}	28.92	40.45	5.09^{bc}
SEM		0.148	0.085	0.085	1.087	0.973	1.370	1.378	0.033
Molasses inclusion	Fermentation Period								
0%	0	93.46^{ab}	93.65^{cde}	6.35^{abc}	29.60	27.41	31.74	34.71	5.64^{abc}
	4	93.69^{ab}	94.68^{abc}	5.32^{cde}	30.61	27.17	31.81	38.50	5.77^{a}
	7	94.48^{a}	94.53^{abc}	5.47^{cde}	24.33	27.72	35.69	43.95	5.67^{ab}
	10	93.44^{ab}	94.89^{ab}	5.11^{de}	22.59	28.52	30.85	37.20	5.33^{abcd}
5%	0	93.51^{ab}	93.83^{cde}	6.17^{abc}	28.35	25.67	30.62	42.92	5.40^{abcd}
	4	94.50^{a}	95.32^{a}	4.68^{e}	24.57	27.58	32.43	39.74	5.41^{abcd}
	7	93.84^{ab}	94.50^{abcd}	5.50^{bcde}	25.38	23.28	33.57	39.54	3.89^{e}
	10	93.64^{ab}	94.32^{abcde}	5.68^{abcde}	26.10	21.57	29.77	39.18	5.09^{d}
10%	0	93.25^{abc}	94.66^{abc}	5.34^{cde}	23.98	32.94	26.40	34.74	5.24^{bcd}
	4	93.76^{ab}	93.31^{de}	6.54^{ab}	23.41	24.28	30.54	35.42	5.30^{bcd}
	7	93.70^{ab}	94.60^{abc}	5.39^{cde}	25.48	22.42	32.48	40.25	5.06^{d}
	10	93.65^{ab}	94.19^{bcde}	5.81^{abcd}	24.73	21.41	31.24	31.78	5.03^{d}
15%	0	93.89^{ab}	94.01^{bcde}	5.99^{abcd}	23.98	25.82	33.69	46.69	5.11^{d}
	4	92.68^{bc}	93.74^{cde}	6.26^{abc}	23.33	21.13	27.95	42.24	5.20^{cd}
	7	93.33^{abc}	93.31^{e}	6.69^{a}	22.90	19.98	28.81	37.35	5.00^{d}
	10	91.56^{c}	93.68^{cde}	6.32^{abc}	23.36	20.85	25.24	35.51	5.05^{d}
SEM		0.256	0.148	0.148	1.890	1.690	2.370	2.380	0.066
P values									
Fermentation Period		0.023	0.251	0.251	0.577	0.020	0.520	0.231	0.000
Molasses inclusion		0.001	0.000	0.000	0.232	0.010	0.403	0.123	0.000
Interaction		0.010	0.000	0.000	0.638	0.215	0.751	0.254	0.000

^{abcd}= Means in the same rows with different superscripts are significantly different at P<0.05; CBOC= Castor bean oil cake; DM= Dry matter; CP= Crude protein; ADF=Acid detergent fibre; NDF= Neutral detergent fibre; EE= Ether extract; SEM= Standard error of the mean.

3.10 Discussion

The chemical CBOC as determined in this study was considered as typical (Ishiwu *et al.*, 2015). Previously (Akande *et al.*, 2014; Akande *et al.*, 2012) suggested the composition of CBOC is variable due to genetic and agro-ecological factors, and the methods used in oil extraction. The composition of FCBOC is further subject to fermentation conditions (Silva *et al.*, 2014; Silva *et al.*, 2016; Silva *et al.*, 2018). In the present study, the CP content of the unfermented CBOC was 29.60%, while that of the FCBOC ranged from 22.59% to 30.61%. Fermentation did not affect the CP content, which was inconsistent with expected progressive increase as reported in previous studies (Madeira Jr *et al.*, 2011).

Residual fat content in CBOC depends on the oil extraction process (Freitas *et al.*, 2016), and may further depend on the analytical methods(Ishiwu *et al.*, 2015). In contrast to previous studies (Ojimelukwe *et al.*, 2011), in this study, the fat in FCBOC decreased as fermentation progressed. Fat content decreased to similar extents with 7, and 10-day fermentation. A 15% Voermol inclusion level also decreased the fat content. The disparity with other studies could relate to both the level and composition of the substrate fat, or the quantum and composition of fatty acids produced during fermentation.

The ash content of CBOC of the current study ranged from (4.68% - 6.69%), lower than previous study reported by (Akande and Odunsi, 2012). The pH is expected to drop with progression and intensity of fermentation due to accumulation of fermentation acids, and then gradually rise if nitrogenous become fermentation substrates (Wasnin *et al.*, 2014). High terminal pH of protein-rich oil seeds is linked to microbial protease activity to yield amino acids, peptides, whose metabolism yields alkaline ammonia (Sharma *et al.*, 2020). Low pH confers a pleasant flavor and aerobic stability (Ojimelukwe *et al.*, 2011). As expected, the pH of FCBOC decreased with the

fermentation period. Overall, the pH ranged from 3.89 to 5.77 %, with the lowest pH value obtained with seven-day fermentation period and 5% molasses inclusion.

3.11 Conclusion

The level of supplementary molasses and the period of fermentation both progressively reduced the fat content, and interacted to affect the pH, and the dry matter, organic matter and ash content of FCBOC. Fermentation did not affect the CP, and the fibre components. The lowest pH of the FCBOC was observed with 5% molasses, and 7 days fermentation, indicative of the most extensive fermentation, and potentially, best quality in terms of detoxification, and aerobic stability.

CHAPTER 4

EFFECTS OF DIETARY INCLUSION OF FERMENTED CASTOR BEAN (*RICINUS COMMUNIS I*) OIL CAKE WITH SUPPLEMENTARY EXOGENOUS ENZYMES ON DIGESTIBILITY, NITROGEN BALANCE, AND BLOOD METABOLIC MARKER PROFILES OF GROWING PIGS.

Abstract

The objective of the study was to evaluate the effects of 10% dietary inclusion of fermented castor bean (*Ricinus Communis L)* oil cake (FCBOC) with supplementary exogenous enzymes on digestibility, nitrogen balance and blood metabolic markers of energy and nitrogen metabolism in growing pigs. Experimental diets were a standard maize-soybean diet (0% FCBOC), and an iso-nutrient, 10% FCBOC diet, each with (+) and without (-) 500g/tonne of Ronozyme® WX CT (EC-3.2.1.8, 1,000 FXU/g endo-1,4-β-xylanase). Eight growing (31.88 ± 1.63 kg live weight) male Large White x Landrace pigs were placed in metabolic cages and assigned to diets in a randomized 2 (diet) x 2 (enzyme) factorial arrangement within a duplicate, balanced 4 (period) X 4 (diet) Latin square design. Each feeding period consisted of 9 days dietary adaptation, plus five days of feed intake measurement, and the total collection of faeces and urine, from which nutrient digestibility, and parameters of N balance (Nitrogen intake (NI) Urine Nitrogen Output (UNO), Feacal Nitrogen Output (FNO), Total Nitrogen Excretion (TNE); Absorbed Nitrogen (AN), Nitrogen Retention (NR), Nitrogen Utilization (NU) BVFP: Biological Value Feed Protein (BVFP), and Apparent N digestibility (ND)) were calculated. Blood was collected by jugular venepuncture into 10ml serum vacutainers on the last day of each period, from which Glucose, Urea, Creatinine (Creat), total protein (TP), albumin, globulin, alanine aminotransferase (ALT), aspartate aminotransferase (AST), alkaline phosphatase (ALP), bilirubin, cholesterol (Chol) and Triglyceride (TG) were analysed. The 10% dietary inclusion of FCBOC increased ($P<0.05$) the EE and NDF digestibility. Highest NDF digestibility was observed in period 4, significantly ($P<0.05$) more than for period 3. When scaled to the live weight, nitrogen retention was low ($P<0.05$) in period 4, compared to periods 1 and 2. When scaled to the metabolic weight, NI was

higher on the 10% FCBOC diet. Higher (P<0.05) UNO was observed in period 4, compared to period 2. The AN was higher (P<0.05) on the 10% FCBOC diet. The NR was low (P<0.05) in period 4. Serum albumin dropped (P<0.05) in period 4. Serum creatinine was higher for period 1, compared to period 4. The 10% FCBOC diet reduced (P<0.05) serum ALP. In conclusion. a 10% dietary inclusion of FBCOC had minimal effects on nutrient digestion, N utilization and plasma metabolites to suggest deleterious effects on overall nitrogen metabolism and pig health. The fibrolytic enzyme was not beneficial to the efficient utilization of a 10 FCBOC diet by growing pigs.

Key Words: Blood metabolites, Castor bean oil cake, digestibility, exogenous enzyme.

4.1 Introduction

Feeding constitutes 70% of the overall pig production costs (Zijlstra and Beltranena, 2013). Over the past decade, increasing demand for animal protein from a rapidly increasing human population has reduced the feed available for animal production (Woyengo *et al.*, 2014). Native to tropical east Africa, Castor bean (*Ricinus communis L.)* has spread and naturalized to become a weed in most tropical and subtropical regions (Poland *et al.*, 2021), where it is also cultivated for industrial, and ornamental applications (Kreissig, 2019). Castor bean oil cake (CBOC) is a by-product from oil extraction in the biofuel and cosmetic (Osorio-González *et al.*, 2020) industries. Though primarily valued as a high-quality organic fertilizer, given 33.7% crude protein (CP) (Abdalla *et al.*, 2012), CBOC has drawn attention as potential stock feed (Makkar, 2013). However, its dietary inclusion is limited by poisonous ricin, agglutinin-RCA120, ricinine, and allergens (Akande *et al.*, 2015), and trypsin inhibitors (Ramos *et al.*, 2013). In addition, given the high fibre, high levels of dietary inclusion may affect both dietary intake and nutrient extraction (Agyekum and Nyachoti, 2017). These deleterious physio-chemical properties are influenced by both the variety and agro-ecosystem in which the crop is grown (Megueni et al. 2016).

The CBOC can be detoxified, and the fibre reduced by different methods. An ecofriendly, thermally and chemically mild bioprocessing option is fermentation (Akande and Odunsi, 2012; Akande *et al.*, 2014). Fermentation is a convenient, affordable, and effective processing method commonly used to enhance the overall nutritive value of oilcakes (Duodu *et al.*, 2018). In addition to detoxification, fermentation reduced the fibre content of CBOC, implying a positive influence on nutrient availability (Akande and Odunsi, 2012; Akande *et al.*, 2012). Fermentation of the castor seed increased amino acids, coupled to significant production of organic acids, indicative of extensive detoxification (Ojinnaka et al., 2013). Fermentation can be enhanced by additives which speedup the fermentative microbial metabolism through desirable biochemical pathways. An additive commonly used to provide readily fermentable energy to high protein fermenting substrates is molasses. Molasses enhances the fermentation process, and increases product palatability (Selvam *et al.*, 2016). Given the high fibre content in CBOC, for feeding to growing pigs, correctly matched fibrolytic enzymes should enhance dietary utilization (Ravindran, 2013). Exogenous fibrolytic enzymes promote cell wall rupture, decrease the intestinal viscosity imposed by soluble non-starch polysaccharides, and might degrade some antinutritional factors (Palhares *et al.*, 2019; Bedford *et al.*, 2022).

Despite fermentation, dietary FCBOC may still present some metabolic or clinical risks to growing pigs, effects which can be tracked by monitoring the profile of some key blood metabolite and enzyme markers. Blood markers of disturbed pig protein metabolism include the total protein, albumin, urea nitrogen and creatinine, while indicators for altered energy metabolism or status include glucose, triglycerides, and cholesterol (Montoro *et al.,* 2022). Profiles of alanine transaminase (ALT), aspartate transaminase (AST), alkaline phosphatase (ALP) activities collectively track the clinical status, including vital organ functions, in combination with changes bilirubin, total protein and albumin (Ozer *et al.,* 2008).

The objective of the present study was to evaluate the effects of 10% dietary inclusion of fermented castor been (*Ricinus Communis L)* oil cake (FCBOC) along with a supplementary

fibrolytic exogenous enzyme on nutrient digestibility, N balance and serum metabolic and health markers in growing pigs.

4.2 Method and materials

4.2.1 Ethical consideration

The experimental procedures were approved by the University of Venda Ethics Committee (SARDF/19/ANS/14/2001).

4.3 Description of the study

The study was conducted in winter, within a metabolic house at the University of Venda experimental farm in South Africa, Limpopo province, Vhembe District, Thulamela Municipality, Thohoyandou. The study area experiences winter average minimum and maximum temperatures of 11^{o}C to 27^{o}C respectively (South African Weather Bureau (SAWB), 2014).

4.4 Fermentation additive

Voermol (Product V10257; containing 33 g/kg CP, 300 g/kg moisture, 6-9.2 g/kg Ca, 0.8 g/kg P and, 9.5 MJ ME/kg), 75% BRIX 75) manufactured by Voermol feeds (Pty) Ltd, Maid-stone, KwaZulu-Natal, South Africa was used as the fermentation additive. The product is molasses-based, recommended for use as an energy supplement, and to increase feed palatability in place of normal molasses.

4.5 Source and processing of castor bean oil cake.

The CBOC utilized in the study was obtained from oil manufacturers in the South African province of Mpumalanga's Mbangwane, Nkomazi region, as a by-product of expeller-pressed oil for pharmaceutical applications. Local farmers traditionally use the cake as a garden fertilizer and plan to expand their castor oil production value chain though manufacturing stock feeds. The

CBOC was weighed in 50kg batches, each of which was treated with 2.5 l (i.e., 5% additive v/m) of Voermol diluted to 7.5.l in boiling water. The homogenous mixtures were compacted into 100-liter, top-screw, airtight plastic drums The drums were tightly sealed for anaerobic fermentation over seven days. The fermented product (FCBOC) was thoroughly rinsed in running tap water to reduce the sour taste and foul odor. The composition of raw CBOC and the washed fermented (FCBOC) products are presented in Table 4.1.

4.6 Preparations of diets

Experimental diets were constituted from a standard maize-soybean basal mix, a 0% FCBOC (control) and an iso nutrient, 10% FCBOC diet, each with (+) and without (-) 500g/tonne Ronozyme® WX CT (EC-3.2.1.8 -1000 FXU/g endo-1,4-β-xylanase). The four dietary treatments were manufactured at Brennco feed company (Pty) Ltd, Louis Trichardt, Limpopo, South Africa. All diets were formulated to be iso-nutritive, according to the NRC (1998) feeding standards for growing pigs.

Table 4.1 Ingredients and nutrient composition of the experimental diets

Components	Castor bean products		Experimental diets	
	[1]CBOC	[2]FCBOC	[2]FCBOC inclusion	
			0 %	10%
Ingredients (% as is)				
Yellow maize- Fine Ground			58.4	53.8
Yellow maize-Gluten Feed			20.0	13.5
Yellow maize- Hominy Feed			7.5	10.0
[2]FCBOC			0.0	10.0
Soybean oil cake-Protein>46.5%			9.5	8.0
Corn Gluten Meal 60% Protein			3.0	3.0
Limestone powder			0.4	0.4
Pig Rearing Base mix			1.2	1.3
Total			100.0	100.0
Nutrient composition (g kg^{-1}DM)				
Dry matter	940.40	933.8	870.2	873.2
Ash	44.1	44.1	43.50	79.0
Crude protein	222.1	218.4	179.7	167.1
Crude fat (ether extract)	270.40	258.8	299.0	348.0
Crude fibre	281.40	277.7	128.0	221.0
NDF	295.0	388.8	274.96	270.23
ADF	172.2	366.1	172.20	203.46
Calcium	4.10	6.1	6.0	7.0
Phosphorus	6.67	5.4	5.0	5.0
Essential amino acids (g/100g DM)				
Arginine	2.69	2.89	0.50	0.56
Alanine	0.85	1.05	0.75	0.81
Asparagine	1.66	2.21	0.77	0.79
Glutamine	3.66	3.97	2.08	2.26
Glycine	1.17	1.21	0.57	0.58
Histidine	0.64	0.74	0.40	0.40
Isoleucine	0.93	1.16	0.41	0.48
Leucine	1.50	1.78	1.30	1.53
Lysine	0.37	0.58	0.34	0.29
Methionine	0.36	0.34	0.28	0.41
Phenylalanine	1.15	1.25	0.61	0.74
Proline	0.78	0.93	0.93	1.06
Serine	1.24	1.35	0.59	0.65
Threonine	0.74	0.93	0.47	0.49
Tyrosine	1.02	0.96	0.51	0.58
Valine	1.11	1.38	0.53	0.60

[1]Castor bean (*Ricinus Communis L)* oil cake, a by-product from expeller oil extraction. [2]Fermented castor bean oil cake – 5% additive (v/m)– prepared in 50 kg batches of oil cake, 2.5 l of Voermol diluted to 7.5.l in boiling water, homogenous

mixtures compacted into 100-liter, top-screw, airtight plastic drums, drums tightly sealed, 7-day anaerobic fermentation. Voermol is a molasses based liquid feed from Voermol feeds (Pty) Ltd, Maidstone, KwaZulu-Natal, South Africa (Product V10257- 33 g/kg CP, 300 g/kg moisture, 6-9.2 g/kg Ca, 0.8 g/kg P and, 9.5 MJ ME/kg), 75% BRIX 75).

4.7 Pigs, housing, experimental design, and management

Eight male Large White x landrace growing (31.88 ± 1.63 kg) pigs were used in the study. The trial facility was an open house equipped with elevated 117.5 cm X 57.8 cm X 83.9cm width and length adjustable steel metabolic cages. The cages were fitted with frontal individual feeders, a nipple drinker, and were adequately structured and equipped for separate, total recovery of urine and faeces. Prior to the experiment, all pigs were dewormed using 1*ml* injectable Virbamec ® LA. The four experimental diets were assigned to the eight experimental pigs in a randomized 2 (diet) x 2 (enzyme) factorial arrangement within two balanced Latin squares. Each feeding period extended over 14 days, consisting of nine days of dietary adaptation and five days of feed intake, faeces and urine measurements, and blood sampling.

4.8 Measurements

Pigs were weighed at the start, and at each period changeover. Feed intake was estimated over the 5-day excreta collection period. The total faeces and urine were collected daily between 08:00 – 09:00. Urine was collected in floor plastic buckets into 10%, 1M HCI solution to reduce nitrogen volatilisation. After weighing the daily collection, 10% of faeces and urine were retained for cold storage at -18°C pending chemical analyses. At the end of the sampling periods, the faeces and urine of each pig were thawed, and the faeces mixed thoroughly by gloved hand. The pooled faecal samples were oven-dried to constant weight at 65 ^{0}C and milled for homogeneity prior to sampling for chemical analyses.

Blood was collected from each pig into 10l, serum vacutainers by jugular venipuncture on the last day of each period, between 08:00 – 09:00. The samples were stored in ice during transport to

the IDEXX laboratory. Samples were centrifuged at 3,000 x g for 15 min at -20 °C for serum isolation.

4.9 Chemical analyses

Faecal samples, CBOC, FCBOC and the experimental diet DM were determined according to the AOAC (1990, method 930.15). Ash determined according to AOAC (1990, method 942.05). Neutral detergent fibre and acid detergent fibre were determined according to Van Soest *et al*., (1991). Fats and oils were determined by Soxhlet ether extraction (AOAC, 1990) 920, 39). Faecal and urine nnitrogen were determined using the Kjeldahl procedure (AOAC., 1990, method 984.13). The FCBOC pH was measured according to the AOAC (2002, method 981.12).

An IDEXX VetTest® Chemistry Analyzer was used to determine Glucose, Urea, Creatinine (Creat), total protein (TP), albumin, globulin, alanine aminotransferase (ALT), aspartate aminotransferase (AST), alkaline phosphatase (ALP), bilirubin-total (BT), cholesterol (Chol) and Triglyceride (TG).

4.10 Mathematical and Statistical analysis

Nitrogen balance parameters were computed using the equations listed in Table 3. The weight (W)-dependent parameters (NI, FNO, UNO, TNE, NR, AN) were further scaled to the pig live (g kg^{-1} of W) or metabolic (g kg^{-1} of $W^{0.75}$) weight.

Table 4. 2 Equations for estimating nitrogen balance.

Component	Formula
Nitrogen intake (NI)	(N feed/100) ×daily feed intake
Faecal nitrogen output (FNO)	(N faeces/100) ×daily faecal output
Urinary nitrogen output (UNO)	(N urine/100) ×daily urine output
Total nitrogen excretion (TNE)	FNO+UNO
Nitrogen retention (NR)	NI – TNE
Absorbed nitrogen (AN)	NI – FNO
Apparent nitrogen digestibility (ND)	(AN/ NI)
Nitrogen utilization (NU)	NR/ NI×100
The biological value of feed protein (BVFP)	NR/ND×100

4.11 Statistical analysis

All data were subjected to ANOVA for a 2(diet) X 2 (enzyme) X 4 (feeding period) factorial experiment, in a replicated 4 X 4 Latin square design using the General Linear Models (GLM) procedure of Minitab software vision 19 using the following statistical model.

$$Y_{ijklmn} = \mu + D_i + E_j + S_k + P_i + A_m + (\alpha\beta)_{ij} + \varepsilon_{ijklmn}$$

Where, Y_{ijklmn} is the observed parameter value,

μ the overall mean,

D_i the fixed effect of the i^{th} diet,

E_j the fixed effect of the j^{th}enzyme dosage,

S_k the random effect of the k^{th} Latin Square,

P_l The Radom effect of the l^{th} period within a Latin square,

A_m the random effect of the m^{th} animal within a Latin Square,

$(\alpha\beta)_{ij}$ The Diet x enzyme interaction, and

ε_{ijklmn} the residual error.

Means were separated using Tukey's procedure, at the $p<0.05$ level of significance.

4.12 Results

Effects of 10% dietary inclusion of FCBOC with supplementary exogenous enzymes on feed intake and digestibility were presented in Table 4.3. The 10% dietary inclusion of FCBOC increased (P<0.05) the EE and NDF digestibility. Highest NDF digestibility was observed in period 4, significantly (P<0.05) more than for period 3.

Table 4. 3 The effects of 10% dietary inclusion of fermented castor bean oil cake with supplementary exogenous enzymes on digestibility coefficients

Treatments		Digestibility coefficients					
		DM	OM	CP	EE	NDF	ADF
[1]Diet	[2]Enzyme						
0% FCBOC	+	0.48	0.49	0.50	0.63	0.51	0.34
	-	0.56	0.57	0.59	0.56	0.64	0.45
10% FCBOC	+	0.51	0.52	0.47	0.72	0.79	0.42
	-	0.54	0.56	0.46	0.74	0.78	0.55
SEM		0.019	0.018	0.018	0.026	0.026	0.039
[1]Diet							
0% FCBOC		0.52	0.54	0.55	0.60[b]	0.57[b]	0.39
10% FCBOC		0.53	0.54	0.47	0.73[a]	0.79[a]	0.49
SEM		0.019	0.018	0.018	0.026	0.026	0.039
Enzymes							
-		0.55	0.57	0.53	0.65	0.71	0.50
+		0.49	0.51	0.49	0.68	0.65	0.38
SEM		0.019	0.018	0.018	0.026	0.026	0.039
Period							
1		0.51	0.52	0.49	0.68	0.70[ab]	0.47
2		0.53	0.55	0.50	0.60	0.65[ab]	0.48
3		0.53	0.55	0.55	0.71	0.56[b]	0.43
4		0.51	0.52	0.49	0.66	0.82[a]	0.39
SEM		0.033	0.031	0.031	0.045	0.044	0.067
P-Values							
Diet		0.839	0.933	0.055	0.032	0.002	0.270
Enzyme		0.150	0.134	0.280	0.579	0.285	0.163
Period		0.933	0.871	0.662	0.515	0.034	0.868
Diet*Enzyme		0.586	0.685	0.223	0.400	0.241	0.917

[ab] Within each factor or factor interaction, for each parameter, means with different superscripts differ significantly at (P <0.05).DM-Dry matter, OM- Organic matter,,CP-Crude protein, EE- Ether extract, NDF- Neutral detergent fibre, ADF-Acid detergent fibre. [1]Standard maize-soybean basal mix, a 0% fermented castor bean oil cake (FCBOC) and an iso-nutrient, 10% FCBOC diet – 5 % additive (v/m) - 50 kg batches of oil cake, 2.5 l of Voermol diluted to 7.5.l in boiling water, homogenous mixtures compacted into 100-liter, top-screw, airtight plastic drums, drums tightly sealed, 7-day

anaerobic fermentation. Voermol is a molasses based liquid feed from Voermol feeds (Pty) Ltd, Maidstone, KwaZulu-Natal, South Africa (Product V10257- 33 g/kg CP, 300 g/kg moisture, 6-9.2 g/kg Ca, 0.8 g/kg P and, 9.5 MJ ME/kg), 75% BRIX 75). [2]Enzyme: with (+) or without (-) 500g/tonne Ronozyme® WX CT (EC-3.2. 1.8 - 1,000 FXU/g endo-1,4-β-xylanase. SEM: Standard error of the mean.

The effects of 10% dietary inclusion of FCBOC with supplementary exogenous enzymes on unscaled, live weight and metabolic weight scaled N utilization are outlined in Table 4.4, Table 4.5, and Table 4.6, respectively. When scaled to the live weight, nitrogen retention was low ($P<0.05$) in period 4, compared to periods 1 and 2. When scaled to the metabolic weight, N intake was higher on the 10% FCBOC diet. Higher ($P<0.05$) urinary N output was observed in period 4, compared to period 2. The absorbed N was higher ($P<0.05$) on the 10% FCBOC diet. Nitrogen retention was low ($P<0.05$) in period 4.

Table 4. 4 Effects of 10% dietary inclusion of fermented castor bean oil cake with supplementary enzymes on unscaled intake, balance, and efficiency of nitrogen utilization

Treatments		NI	N Utilization							
			UNO	FNO	TNE	AN	NR	NU	ND	BVFP
Diet	Enzyme	g/d	g/d	g/d	g/d	g/d	g/d	%		%
0%	+	655.27	18.69	303.99	322.69	332.58	56.39	9.26	0.53	0.17
	-	730.18	13.59	296.26	309.83	420.35	58.46	8.85	0.60	0.14
10%	+	675.92	24.28	319.65	343.93	331.99	40.31	16.54	0.48	0.24
	_	467.05	17.46	187.57	205.03	262.01	67.14	25.49	0.71	0.31
	SEM	56.100	1.180	31.900	32.700	28.800	4.350	3.280	0.037	0.044
Diet	0%	692.73	16.14	300.12	316.26	376.46	54..43	9.06	0.57	0.16
	10%	571.49	20.87	253.61	274.48	297.00	53.72	18.01	0.59	0.28
	SEM	56.100	1.180	31.900	32.700	28.800	4.350	3.280	0.037	0.044
Enzymes	-	598.61	15.53	241.90	257.43	341.18	62.80	17.17	0.65	0.23
	+	665.60	21.48	311.82	333.31	332.28	45.35	9.90	0.51	0.21
	SEM	56.100	1.180	31.900	32.700	28.800	4.350	3.280	0.037	0.044
Period	1	604.56	22.38	246.33	268.72	355.85	47.88	17.82	0.57	0.30
	2	928.15	18.69	405.63	424.32	503.82	50.61	7.52	0.54	0.15
	3	526.49	12.51	217.19	229.70	296.74	60.14	13.01	0.62	0.18
	4	469.25	20.45	238.29	258.74	210.51	57.68	15.79	0.59	0.20
	SEM	214.000	4.490	121.000	124.000	110.000	16.600	12.500	0.140	0.166
P Values										
Diet		0.393	0.182	0.542	0.588	0.302	0.942	0.306	0.762	0.303
Enzymes		0.611	0.127	0.387	0.366	0.891	0.183	0.384	0.187	0.842
Period		0.351	0.217	0.217	0.427	0.326	0.937	0.685	0.961	0.524
Diet*Enzyme		0.334	0.753	0.753	0.437	0.304	0.393	0.363	0.400	0.644

[ab] Within each factor or factor interaction, for each parameter, means with different superscripts differ significantly at ($P < 0.05$). NI: Nitrogen intake; UNO: Urine Nitrogen Output; FNO: Feacal Nitrogen Output; TNE: Total Nitrogen Excretion; AN: Absorbed Nitrogen; NR: nitrogen retention; NU: Nitrogen utilization; BVFP: Biological Value Feed Protein; ND: Apparent N digestibility; [1]FCBOC - fermented castor bean oil – 5 % additive (v/m) - 50 kg batches of oil cake, 2.5 l of Voermol diluted to 7.5.l in boiling water, homogenous mixtures compacted into 100-liter, top-screw, airtight plastic drums, drums tightly sealed, 7-day anaerobic fermentation. Voermol is a molasses based liquid feed from Voermol feeds (Pty) Ltd, Maidstone, KwaZulu-Natal, South Africa (Product V10257- 33 g/kg CP, 300 g/kg moisture, 6-9.2 g/kg Ca, 0.8 g/kg P and , 9.5 MJ ME/kg), 75% BRIX 75). [2]Enzyme: with (+) or without (-) 500g/tonne Ronozyme® WX CT (EC-3.2. 1.8 - 1,000 FXU/g endo-1,4-β-xylanase. SEM: Standard error of the mean.

Table 4. 5 Effects of 10% dietary inclusion of fermented castor bean oil cake with supplementary enzymes on N balance when scaled on live weight (LW) basis.

Treatments		NI	N Utilization							
			UNO	FNO	TNE	AN	NR	NU	ND	BVFP
Diet	Enzyme	g/kg LW	g/kg LW	g/kg LW	g/kg LW	g/kg LW	g/kg LW	%		%
0%	+	7.46	0.22	3.47	3.68	7.24	0.78	9.26	0.54	0.17
	-	9.17	0.16	3.59	3.75	9.00	0.86	8.85	0.60	0.15
10%	+	7.66	0.31	3.57	3.88	7.34	0.83	16.54	0.52	0.29
	_	4.97	0.73	1.70	1.88	4.61	0.82	25.49	0.71	0.31
SEM		0.503	0.015	0.278	0.285	0.500	0.061	2.840	0.031	0.035
Diet	0%	8.31	0.17	3.53	3.72	8.12	0.82	9.06	0.57	0.16
	10%	6.22	0.26	2.64	2.88	5.98	0.83	18.01	0.61	0.30
SEM		0.503	0.015	0.278	0.285	0.500	0.061	2.840	0.031	0.035
Enzymes	-	6.97	0.19	2.65	2.82	6.81	0.84	17.17	0.65	0.23
	+	7.56	0.24	3.52	3.79	7.29	0.80	9.90	0.53	0.23
SEM		0.503	0.015	0.278	0.285	0.500	0.061	2.840	0.031	0.035
Period	1	6.91	0.33	2.84	3.17	6.58	1.08[a]	17.82	0.64	0.42
	2	9.75	0.23	4.35	4.58	9.52	0.97[a]	7.52	0.62	0.24
	3	6.89	0.18	2.57	2.70	6.76	0.74[ab]	13.01	0.58	0.14
	4	5.52	0.13	2.57	2.75	5.34	0.49[b]	15.79	0.52	0.11
SEM		0.872	0.026	0.482	0.493	0.867	0.094	4.920	0.055	0.061
Significance										
Diet		0.399	0.140	0.412	0.556	0.394	0.982	0.306	0.451	0.116
Enzymes		0.766	0.075	0.418	0.380	0.804	0.749	0.384	0.058	0.982
Period		0.613	0.121	0.545	0.426	0.602	0.010	0.685	0.445	0.098
Diet x Enzymes		0.372	0.175	0.378	0.361	0.379	0.705	0.363	0.313	0.752

[ab] Within each factor or factor interaction, for each parameter, means with different superscripts differ significantly at (P <0.05). NI: Nitrogen intake; UNO: Urine Nitrogen Output; FNO: Feacal Nitrogen Output; TNE: Total Nitrogen Excretion; AN: Absorbed Nitrogen; NR: nitrogen retention; NU: Nitrogen utilization; BVFP: Biological Value Feed Protein; ND: Apparent N digestibility; [1]FCBOC - fermented castor bean oil – 5 % additive (v/m) - 50 kg batches of oil cake, 2.5 l of Voermol diluted to 7.5.l in boiling water, homogenous mixtures compacted into 100-liter, top-screw, airtight plastic drums, drums tightly sealed, 7-day anaerobic fermentation. Voermol is a molasses based liquid feed from Voermol feeds (Pty) Ltd, Maidstone, KwaZulu-Natal, South Africa (Product V10257- 33 g/kg CP, 300 g/kg moisture, 6-9.2 g/kg Ca, 0.8 g/kg P and , 9.5 MJ ME/kg), 75% BRIX 75). [2]Enzyme: with (+) or without (-) 500g/tonne Ronozyme® WX CT (EC-3.2. 1.8 - 1,000 FXU/g endo-1,4-β-xylanase. SEM: Standard error of the mean

Table 4 6 Effects of 10% dietary inclusion of fermented castor bean oil cake with supplementary enzymes on the N balance when scaled on metabolic weight ($W^{0.75}$) basis.

Treatments		NI	N Utilization							
			UNO	FNO	TNE	AN	NR	NU	ND	BVFP
Diet	Enzyme	g/kg $W^{0.75}$	g/kg $W^{0.75}$	g/kg $W^{0.75}$	g/kg $W^{0.75}$	g/kg $W^{0.75}$	g/kg $W^{0.75}$	% $W^{0.75}$		%
0%	+	22.69	0.66	10.54	11.20	11.49	2.24	9.26	0.54	0.17
	-	27.16	0.49	10.73	11.22	15.94	2.43	8.86	0.60	0.15
10%	+	19.24	0.75	9.23	9.98	9.25	2.31	15.44	0.52	0.29
	_	14.94	0.55	5.51	6.05	8.88	2.34	25.48	0.71	0.31
	SEM	1.570	0.051	0.800	0.835	0.942	0.142	2.970	0.027	0.039
Diet	0%	24.92[a]	0.57	10.64	11.21	13.72[a]	2.33	9.06	0.56	0.16
	10%	17.09[b]	0.65	7.37	8.02	9.07[b]	2.33	20.46	0.61	0.31
	SEM	1.570	0.051	0.800	0.835	0.942	0.142	2.970	0.027	0.039
Enzymes	+	20.67	0.71	9.89	10.59	10.37	2.27	12.36	0.52	0.23
	-	21.05	0.57	8.12	8.64	12.42	2.39	17.13	0.65	0.23
	SEM	1.570	0.051	0.800	0.835	0.942	0.142	2.970	0.027	0.039
Period	1	12.64	0.60[ab]	4.67	5.27	7.37	2.86[a]	27.63	0.65	0.42
	2	22.12	0.35[b]	9.58	9.93	12.19	2.72[a]	17.34	0.62	0.24
	3	24.39	0.56[ab]	9.95	10.52	13.86	2.19[ab]	8.10	0.58	0.14
	4	24.89	0.93[a]	11.81	12.74	12.15	1.55[b]	5.98	0.52	0.12
	SEM	2.720	0.088	1.390	1.450	1.630	0.242	5.140	0.047	0.067
Significance										
Diet		0.047	0.449	0.088	0.105	0.049	0.977	0.103	0.451	0.116
Enzymes		0.979	0.114	0.311	0.285	0.319	0.695	0.448	0.058	0.982
Period		0.099	0.036	0.084	0.088	0.188	0.017	0.133	0.445	0.098
Diet*Enzyme		0.212	0.892	0.267	0.282	0.248	0.772	0.413	0.313	0.752

[ab] Within each factor or factor interaction, for each parameter, means with different superscripts differ significantly at ($P < 0.05$). NI: Nitrogen intake; UNO: Urine Nitrogen Output; FNO: Feacal Nitrogen Output; TNE: Total Nitrogen Excretion; AN: Absorbed Nitrogen; NR: nitrogen retention; NU: Nitrogen utilization; BVFP: Biological Value Feed Protein; ND: Apparent N digestibility; [1]FCBOC - fermented castor bean oil – 5 % additive (v/m) - 50 kg batches of oil cake, 2.5 l of Voermol diluted to 7.5.l in boiling water, homogenous mixtures compacted into 100-liter, top-screw, airtight plastic drums, drums tightly sealed, 7-day anaerobic fermentation. Voermol is a molasses based liquid feed from Voermol feeds (Pty) Ltd, Maidstone, KwaZulu-Natal, South Africa (Product V10257- 33 g/kg CP, 300 g/kg moisture, 6-9.2 g/kg Ca, 0.8 g/kg P and , 9.5 MJ ME/kg), 75% BRIX 75). [2]Enzyme: with (+) or without (-) 500g/tonne Ronozyme® WX CT (EC-3.2. 1.8 - 1,000 FXU/g endo-1,4-β-xylanase. SEM: Standard error of the mean

Blood metabolic marker profiles of growing pigs fed the 0% and 10% FCBOC diets with supplementary exogenous enzymes are presented in Table 4.7. Serum albumin dropped ($P<0.05$) in period 4. Serum creatinine was higher for period 1, compared to period 4. The 10% FCBOC diet reduced ($P<0.05$) serum ALP.

Table 4. 7 The effects of 10% dietary inclusion of fermented castor bean oil cake with supplementary exogenous enzymes on blood metabolic marker profile of growing pigs.

Treatments		Parameters											
		Globulin	Albumin	Total Protein	Urea	Triglycerides	Glucose	Creatinine	Cholesterol	Bilirubin	AST	ALT	ALP
		g/L	g/L		mmol/L	mmol/L	mmol/L	µmol/L	µmol/L	µmol/L	U/L	U/L	U/L
Diet	Enzyme												
0% FCBOC	-	28.87	31.25	60.12	8.22	0.39	5.02	75.12	2.51	1.25	57.25	57.37	215.25
	+	25.75	31.63	57.37	3.86	0.35	3.95	74.37	2.36	1.50	89.75	49.25	215.50
10% FCBOC	-	28.00	30.87	59.00	3.85	0.96	6.90	77.73	2.26	2.12	114.00	47.12	147.12
	+	27.62	30.25	57.87	3.78	0.33	5.12	82.25	2.23	2.12	69.62	43.25	140.62
SEM		0.909	0.568	0.818	0.214	0.157	0.669	1.920	0.083	0.221	23.800	2.930	14.400
Diet													
0% FCBOC		27.31	31.44	58.75	4.04	0.37	4.48	74.75	2.44	1.38	73.50	53.31	215.38^{a}
10% FCBOC		27.81	30.56	58.44	3.82	0.65	6.01	79.81	2.25	2.13	91.81	45.19	143.88^{b}
SEM		0.91	0.57	0.82	0.21	0.16	0.67	1.92	0.08	0.22	23.8	2.93	14.4
Enzyme													
-		28.44	31.06	59.56	4.04	0.68	5.96	76.25	2.39	1.69	85.63	52.25	181.19
+		26.69	30.94	57.63	3.82	0.34	4.54	78.31	2.30	1.81	79.69	46.25	178.06
SEM		0.909	0.568	0.818	0.214	0.157	0.669	1.920	0.083	0.221	23.800	2.930	14.400
Period													
1		28.63	28.63^{b}	57.25	4.44	0.19	5.10	87.00^{a}	2.09	2.63	96.75	62.25	173.75
2		28.25	29.00^{b}	57.25	4.34	0.54	5.01	72.13^{ab}	2.56	1.75	44.50	46.63	200.50
3		28.86	30.63^{b}	59.50	3.20	1.01	7.09	70.50^{b}	2.27	1.38	73.86	41.63	188.25
4		24.50	35.75^{a}	60.38	3.74	0.29	3.80	79.50^{ab}	2.45	1.25	115.50	46.50	156.00
SEM		1.570	0.983	1.420	0.371	0.272	1.160	3.320	0.143	0.383	41.200	5.070	25.000
P-value													
Diet		0.787	0.452	0.851	0.609	0.390	0.270	0.205	0.273	0.109	0.705	0.184	0.025
Period		0.315	0.001	0.441	0.192	0.295	0.401	0.029	0.232	0.159	0.746	0.111	0.726
Enzyme		0.350	0.914	0.254	0.619	0.304	0.301	0.598	0.603	0.781	0.902	0.321	0.915
Diet*Enzyme		0.460	0.666	0.626	0.742	0.356	0.796	0.474	0.710	0.781	0.431	0.721	0.908

[ab] Within each factor or factor interaction, for each parameter, means with different superscripts differ significantly at (P <0.05). [1]FBCBOC - fermented castor bean oil – 5 % additive (v/m) - 50 kg batches of oil cake, 2.5 l of Voermol diluted to 7.5.l in boiling water, homogenous mixtures compacted into 100-liter, top-screw, airtight plastic drums, drums tightly sealed, 7-day anaerobic fermentation. Voermol is a molasses based liquid feed from Voermol feeds (Pty) Ltd, Maidstone, KwaZulu-Natal, South Africa (Product V10257- 33 g/kg CP, 300 g/kg moisture, 6-9.2 g/kg Ca, 0.8 g/kg P and , 9.5 MJ ME/kg), 75% BRIX 75). [2]Enzyme: with (+) or without (-) 500g/tonne Ronozyme® WX CT (EC-3.2. 1.8 - 1,000 FXU/g endo-1,4-β-xylanase. SEM: Standard error of the mean. AST- Aspartate transferase; ALT- Alanine Transferase; ALKP-Alkaline phosphate SEM: Standard error of the mean.

4.13 Discussion

The chemical composition of FCBOC depends on the source ,and the processing (da Silva César and Batalha, 2010). Castor bean allergens, the poisonous glycoprotein ricin, the alkaloid ricinine, and ricinoleic acid are well documented ((Woyengo *et al.*, 2014). (Worbs *et al.*, 2011) previously indicated the risk of ricin induced diarrhea, vomiting, tachycardia, and a decrease in feed intake in pigs. These factors impair nutrient digestion, absorption, and utilization (Akande *et al.*, 2012). However, in this study, two pigs vomited after feeding the CBOC diet, suggesting some clinical effects.

The pig's ability to digest and absorb nutrients decreases with increased dietary fibre level (Galassi *et al.*, 2010). (Landero *et al.*, 2011) reported a depressed nutrient digestibility with increasing inclusion of canola meal, which they attributed the higher fibre content. To avoid confounding deleterious fibre effects, in this study, diets were formulated to contain similar fibre content, despite the inclusion of high fibre FCBOC in the test diets. The 10% dietary inclusion of FCBOC had minimal effects on nutrient digestibility, which was limited to increased EE and NDF digestibility, which suggested better digestibility of these fractions compared to the basal dietary matrix. Pigs fed diets with higher levels of FCBOC had higher fibre digestibility, which was attributed to fibre pre-fermentation (Mwesigwa *et al.*, 2013). Highest NDF digestibility was observed in period 4, consistent with a more developed digestive system. Contrary to this study, when fed fermented, autoclaved CBOC, at 5% and 10% dietary inclusion, pigs on the higher level had low fibre digestibility (Jha,R *et al.,2016*).

In this study, when scaled to the live weight, nitrogen retention was low in period 4, compared to periods 1 and 2. Nitrogen retention in period 4 was confirmed low on metabolic basis. The low nitrogen retention was consistent with higher urinary N output. Without change in the faecal output, this suggested more wastage of metabolic N, likely due to lower N requirements over time.

When scaled to the metabolic weight, N intake was higher on the 10% FCBOC diet. The absorbed N was also higher on the 10% FCBOC, which implied better protein extraction compared to the basal dietary matrix.

Numerous factors, including nutrition, can affect an animal's physiology and hematological characteristics (Etim *et al.*, 2014). Dietary effects on plasma metabolites were minimal. Except for 10% FCBOC diet reduction of serum ALP. Since alkaline phosphatase is produced by osteoblasts and some of it is expelled in bile, its activity could be utilized to evaluate the condition of the liver (Thabethe *et al.*, 2018). (Ncobela *et al.*, 2018) suggested ALP activity is an indicator of liver health. In this study, serum albumin dropped by period 4. These period effects on N utilisation were attributed to growth related changes in N metabolism and tissue accretion. However, the increase in serum albumin with the period of feeding was contrary to the findings by (Chedea *et al.*, 2019). in this study, serum creatinine was higher for period 1, compared to period 4. Creatinine, and urea are two non-protein nitrogen compounds associated with protein degradation (Hăbeanu *et al.*, 2019). The main source of creatinine in serum is the breakdown of creatine in animal muscle (López-Carlos *et al.*, 2010). Therefore, there may be a connection between the drop in serum creatinine and the drop in nutrient availability (Caprarulo *et al.*, 2020). Diarrhea may also increase blood creatinine due to increased mobilization of muscle protein to make up for the decreased absorption of nutrients (Caprarulo *et al.*, 2020).

In this study, the exogenous enzyme supplementation did not affect any of the measured parameters.

4.13 Conclusion

A 10% dietary inclusion of FBCOC had minimal effects on nutrient digestion, N utilization and plasma metabolites. This suggested such levels of dietary inclusion of FCBOC is possible without deleterious effects on overall energy, protein, and nitrogen metabolism and pig health. The fibrolytic enzyme had no effect on the utilization of both the standard control, and the 10 FCBOC diet by growing pigs.

CHAPTER 5 GENERAL DISCUSSION, CONCLUSION and RECOMMENDATIONS

The overall study aimed to evaluate the potential of Castor bean (*Ricinus Communis L)* oil cake as feed for growing pigs. The fermentation test indicated that the level of supplementary molasses and the period of fermentation both had variable effects on the chemical composition of fermented CBOC. Surprisingly, fermentation did not affect the CP content, which was inconsistent with progressive increase in previous studies (Madeira *et al.*, 2011).

As expected, in this study, the pH of FCBOC decreased with the fermentation period. The pH is expected to drop with progression and intensity of fermentation due to accumulation of fermentation acids, and then gradually rise if nitrogenous compounds become the fermentation substrates (Wasnin *et al.*, 2014). High terminal pH of protein-rich oil seeds is linked to microbial protease activity to yield amino acids, peptides, whose metabolism yields alkaline ammonia (Sharma *et al.*, 2020). Low pH confers a pleasant flavor and aerobic stability (Ojimelukwe *et al.*, 2011).

The results of this study indicate that the level of supplementary molasses and the period of fermentation both had variable effects on the chemical composition of FCBOC. Lowest pH of the FCBOC was observed with 5% molasses, and 7 days fermentation, indicative of potentially best quality in terms of detoxification, and aerobic stability. A subsequent 10% dietary inclusion of FBCOC had minimal effects on nutrient digestion, N utilization and plasma metabolites to suggest deleterious effects on overall nitrogen metabolism and pig health. The fibrolytic enzymes were not beneficial to the efficient utilization of a 10% FCBOC diet by growing pigs.

Based on the results of the study, it can be concluded that FCBOC can replace the conventional growing pig's dietary protein at 10% dietary inclusion, provided the cake is effectively processed to reduce toxicity. Further research is required to test higher levels of dietary inclusion.

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