Influence of enzyme supplementation on growth performance, serum biochemical parameters and meat quality of broilers fed diets of varying levels of sunflower meal.

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DECLARATION

I, **Gontse Molale**, declare that this dissertation hereby submitted for the degree of Master of Science in Agriculture in Animal Science at the North-West University is my own original and independent work conducted under the supervision of Dr CK Lebopa, and co-supervisors Prof KH Mokoboki and Dr NA Sebola. All assistance towards producing this work and all the references contained herein has been properly acknowledged.

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Contents

LIST OF TABLES AND FIGURES

ABBREVIATIONS

AA’s:	Amino Acids
ADG:	Average Daily Gain
AFI:	Average Feed Intake
ALB:	Albumin
ALKP:	Alkaline Phosphatase
ALT (SGPT):	Alanine Aminotransferase
AMEn:	Apparent Metabolizable Energy for Nitrogen
ANF:	Anti-Nutritional Factor
ANOVA:	Analysis of Variance
AST:	Aspartate Transaminase
AWG:	Average Weight Gain
BUN (UREA):	Blood Urea Nitrogen
Ca:	Calcium
CCW:	Cold Carcass Weight
CF:	Crude Fiber
CHOL:	Cholesterol
CP:	Crude Protein
CPK:	Creatine Phosphokinase
CRD:	Completely Randomized Design
CREA:	Creatinine
DM:	Dry Matter
EE:	Ether Extract
EOM:	Essential Oil Mixture
FA’s:	Fatty Acids

FAOSTAT:	Food and Agriculture Organization Statistical Database
FCR:	Feed Conversion Ratio
FI:	Feed Intake
GGT:	Gamma-glutamyltransferase
GIT:	Gastro Intestinal Tract
GLM:	General Linear Model
GLU:	Glucose
HDL:	High-density Lipoprotein
HCW:	Hot Carcass Weight
KCL:	Potassium Chloride
LBW:	Live Body Weight
LDH:	Lactate Dehydrogenase
LDL:	Low-Density Lipoprotein
LI:	Large Intestine
ME:	Metabolizable Energy
NFE:	Nitrogen Free Extract
NS:	Not Significant
NSP:	Non-Starch Polysaccharides
OM:	Organic Matter
PHOS:	Phosphorus
RH:	Rice Hulls
SA:	South Africa
SAS:	Statistical Analysis System
SBM:	Soybean Meal
SE:	Standard Error

SFM:	Sunflower Meal
SI:	Small Intestine
TBIL:	Total Bilirubin
TP:	Total Protein
TRT:	Treatment
WG:	Weight Gain
WHC:	Water Holding Capacity
WP:	Wheat Pollard

GENERAL ABSTRACT

This study was conducted to determine the effect of enzyme supplementation (500g/ton kenzyme) on growth performance parameters, carcass characteristics, meat quality and serum biochemical parameters of broilers fed diets of varying levels of sunflower meal (SFM). The aim of the study was to investigate the potential use of SFM as a replacement of soybean meal (SBM) for the role of a protein source in broiler diets. A total of two-hundred and eighty-eight (288) day-old Cobb 500 broiler chicks were used for the experiment. A 4 x 2 factorial treatment arrangement in a completely randomized design was conducted with four levels of SFM replacing SBM (Control (0%), 25%, 50%, and 75%) and two enzyme (kemzyme complex, a multi-enzyme (exogenous and endogenous) for improving the digestibility of raw materials and increasing the nutrient levels of the feed) levels (0 and 500 g/ton) with three replications (n= 12 birds/ replicate). The data was analysed using Completely Randomized Design (SAS, 2010). Iso-caloric and iso-nitrogenous grower and finisher treatment diets were prepared with different levels of SFM. A three-phase commercial broiler ration feeding regimen was used, consisting of starter (standard), grower and finisher. All birds received the control starter diet for the first 10 days. From day 11 up to day 42 (32-day period) birds received their respective grower (20CP, 12.62 ME) and finisher (18CP, 12.96 ME) experimental diets. Growth performance, carcass characteristics, meat quality and haematological bio-chemical parameters were determined. Treatment diet did not significantly ($P > 0.05$) influence average weight gain (AWG) and feed conversion ratio (FCR). However, average feed intake (AFI) was significantly ($P < 0.05$) affected by the treatment diets. High AFI was observed in treatments with high SFM inclusion rates, SFM50 and SFM75. No significant differences ($P > 0.05$) were observed on enzyme supplementation and growth performance parameters. Week (time) had a significant effect ($P < 0.05$) on all growth

performance parameters, whereby the AFI, AWG and FCR improved with the age of broilers. There was no 3-way interaction (treatment diet, enzyme supplementation and week) effect reported on growth performance parameters. Hot carcass weight (HCW) was significantly influenced ($P < 0.05$) by treatment diet. The study did not establish any significant variation ($P > 0.05$) in terms of meat yield, meat quality, internal organs and serum biochemical parameters when birds were fed various inclusion rates of SFM, with or without enzyme supplementation. The findings of this current study concluded that SFM is a potential replacement as an alternative for SBM in broiler diets and can be used to improve production performance. From this present study, inclusion of SFM up to 75% with or without enzyme supplementation showed no significant ($P > 0.05$) effect on WG, FCR, carcass yield and meat quality and serum biochemical parameters.

Key words: Growth performance, carcass characteristics, sunflower meal, serum biochemical parameters, soybean meal, water holding capacity, feed conversion ratio.

CHAPTER 1

INTRODUCTION

The rapid growth of the human population coupled with increased urbanization and rising incomes has led to improved livelihoods of the population around the globe thus resulting in escalated growth in the demand for poultry meat. In order to fulfil the increasing demand for poultry meat worldwide, it is essential to improve the feed efficiency of broiler chickens. (Lević *et al.,* 2005). Therefore, this means that approaches in animal nutrition are of fundamental importance in ensuring that this swift growth is achieved whilst maintaining sustainable poultry production (Beski *et al.,* 2015).

Globally, broiler rations are mainly formulated utilizing cereal grains as a source of energy and oil-seed meals to meet the protein requirements in broiler feeds (Solangi *et al.,* 2002; Khan *et al.,* 2006; Beski *et al.,* 2015; Horvatovic *et al.,* 2015). Escalating prices of major feed ingredients used in formulating broiler rations are fundamental in determining the profitability, livelihood and sustainability of the broiler enterprise (Amerah *et al.,* 2015). One of the most effective ways of increasing profit is to decrease the cost of inputs, especially feed which is the main input in broiler production accounting for about 60 – 75% of the total cost of production (Casartelli, *et al.,* 2006; Abbas & Yagoub, 2008; Sayda *et al.,* 2011; Anuradha & Roy, 2015), and this necessitates for developing cost-effective diets for the broilers in order to realize economic gains.

According to Rezaei and Hafezian (2007), Sayda *et al.* (2011), and Shi *et al.* (2012) soybean meal (SBM) is the major source of protein in broiler diets since its superior nutritional value (NV) is recognised throughout the world. Nevertheless, the price variations brought about by global demand and supply, and weather conditions have resulted in researchers looking for cheaper alternative feedstuffs to reduce the production costs and improve the feed efficiency of broilers.

One of the best strategies advocated in decreasing the cost of feeding is to use cheap, alternative and locally available raw materials in ration formulation such as sunflower meal (SFM), which is abundant in South Africa and is usually not utilized to its full potential. SFM is well-known and is a cheap source of protein that can be used for broiler rations (Casartelli *et al.,* 2006) and is the third important source of vegetable oil worldwide (Salari *et al.,* 2009). The SFM belongs to the genus of *Helianthus*. The SFM is a crucial by-product derived after extracting oil from decorticated sunflower seeds (Khan *et al.,* 2006; Raza *et al.,* 2009) and it is economically vital as it is available in large quantities and used as an alternative protein source in broiler nutrition (Araújo *et al.,* 2011; Moghaddam *et al.,* 2012; Sredanovic, *et al.,* 2012). After processing, SFM remains in a surplus amount at a very competitive price as a source of protein for livestock feeding compared with other animal by-products (Salih & Taha, 1989).

The nutritional value (NV) of SFM is influenced by several factors including the weather, soil conditions, seed cultivar and management of the crop during the growing stages, method of oil extraction, oil extraction efficiency, and the degree of dehulling (Tsuzuki *et al.,* 2003; Casartelli, *et al.,* 2006; Rezaei & Hafezian, 2007; Abbas & Yagoub, 2008; Sredanovic *et al.,* 2012). Removing the hull as a source of fibre from the kernel determines the chemical composition of the SFM. However, Casartelli *et al.* (2006)

reported that extreme temperatures during oil extraction cause protein denaturation leading to a reduction on the availability of threonine and lysine.

Sunflower meal is a very good source of vegetable protein and usually contains about 33–43% CP (crude protein). The SFM also contains 1760 kcal/kg metabolizable energy (ME), 2.8% fat, 0.4% calcium, 0.3% phosphorus, 1.65% methionine, 0.4% cystine, 0.5% tryptophan, and 3.5% arginine (Solangi *et al.,* 2002) and contains adequate amounts of B–vitamins. According to Malakian (2010), SFM is high in ether extract (38–40%) and this adds to the high metabolizable energy (ME) per unit of feed. Working on broilers, Araújo *et al.* (2011) reported that SFM is relatively rich in sulphur amino acids. Compared to other vegetable oilseed meals, SFM contains low concentrations of anti-nutritional factors (ANF) (Casartelli *et al.,* 2006; Rezaei & Hafezian, 2007).

Nonetheless, SFM inclusion in broiler diets is restricted by several factors that affect the chemical composition, daily intake, and digestibility of feeds. The major factor to the inclusion of SFM is high fibre content (about 18–23%) caused by residual seed hull. High fibre content results in dietary nutrient dilution, poor digestibility and bulking which may pose difficulties in young broiler chicks since their gastro-intestinal tract (GIT) has limited storage capacity. High fibre content in SFM based diets may result in a diet with low ME (Senkoylu & Dale, 2006).

Using SFM from de-hulled seeds enhances the quality of SFM and results in low fibre content. Nonetheless, it is unknown whether SFM fibre digestibility will be improved when fed de-hulled SFM (Malakian, 2010) and this process would increase the cost of production and therefore diminish the competitiveness of SFM as a possible alternative protein source.

The testa of SFM has a high amount of non-starch polysaccharides (NSP) which reduce the digestibility of SFM (Khan *et al.,* 2006). This NSP has anti-nutritional effect on bird performance as it modifies intestinal viscosity and the time that feed passes through the intestines, thus reducing digestion and absorption rates of certain nutrients (Anuradha & Roy, 2015).

Enzyme supplementation could be the answer to this problem. Senkoylu & Dale (1999) suggested that enzyme supplementation is required in SFM based diets due to the high amounts of NSP present in SFM, especially at high inclusion rates. Birds lack the ability to produce enzymes (beta-glucanase, carbohydrase, cellulase, hemicellulase, lipase, pectinase, phytase) vital for digesting β-linkages in NSP present in cell walls of sunflower (Vooren, 2012). Therefore, broiler diets are supplemented with exogenous enzymes to help breakdown large molecular structures present in feed ingredients into small molecular structures so that they can be readily available to the gastro intestinal tract (GIT) for improved digestion and absorption (Raza *et al.,* 2009).

1.1 Problem statement

Increase in human population coupled with urbanization has led to increased demand for poultry meat per capita. As a result, this necessitates for an increase in the production of meat (broilers) to ensure that production does not lag behind the human population increases. However, the rapid growth in human population and poultry production has led to an increase in the demand for poultry feed which in turn encouraged the increase in the price of raw materials. Consequently, this has resulted in the increase of the cost of feeding broilers for optimum production. The demand for

affordable broiler feed is high, due to high feed costs and a limited supply of broiler rations.

Little research has been conducted on the potential of using SFM as an alternative protein source in poultry production. Sunflower meal contains less protein, lysine and energy than SBM (El Sherif, *et. al.,* 1997). The high fibre content of SFM is also one of the factors that limit its use in poultry diets, owing to low ME and impair in the use of other nutrients. The nutrient composition of SFM is influenced by several factors such as, seed variety, growth conditions, method of processing and degree of dehulling. According to Senkoylo & Dale (1999) SFM can be successfully used to replace SBM up to 100% in broiler rations, provided that diets are supplemented with adequate amount of lysine and energy.

Broiler chickens lack the ability to digest fiber present in high quantities in SFM, and therefore, the use of endogenous and exogenous enzymes are vital in hydrolyzing NSP's that can be used by animals, for example, energy use (Tavernari *et al.,* 2008) and improving bird performance.

1.2 Justification

The ideal way of reducing feed costs is by formulating broiler diets using alternative and local ingredients that are affordable (Casartelli *et al.,* 2006; Abbas & Yagoub, 2008). Due to the high cost of broiler rations, formulating diets using cheap, local ingredients is imperative for the sustainable broiler production (Sebola *et al.,* 2015). The SFM is known as an important vegetable oil and protein source worldwide (Solangi *et al.,* 2002); and it is utilised as an alternative protein source in broiler rations (Moghaddam *et al.,* 2012). However, the use of SFM in broiler diets for efficient and sustainable production is restricted due to its high fibre content (Adebiyi *et al.,* 2010).

Monogastric animals like poultry lack the endogenous capacity to break down NSP, and as a result, enzyme supplementation is vital in breaking down NSP to the form that can be used by broilers, and therefore, improving the availability and use of nutrients such as energy-yielding carbohydrates (Tavernari *et al.,* 2008). Senkoylu and Dale (1999) stated that enzyme supplementation is necessary when SFM is used due to the presence of NSP.

According to Abdelrahman and Saleh (2007), numerous studies have been carried out to evaluate the effect of using SFM in broiler rations with or without the addition or supplementation of some exogenous and endogenous enzymes, and there was a lack of consistency in the findings and recommendations. There is insufficient data on the availability of the anti-nutritional effects of SFM's NSP and the ability of exogenous enzymes to breakdown these large compounds (Kocher *et al.,* 2000).

There has not been a lot of studies conducted on the effects of incorporating SFM in broiler rations at 75% inclusion level supplemented with or without enzyme supplementation on the growth performance, carcass characteristics and meat quality, and biochemical parameters of broiler chickens. The potential of high inclusion levels of dietary SFM with or without supplementation of enzymes on growth performance and carcass characteristics under intensive management system has not been evaluated. Therefore, this current study seeks to address the above shortfalls.

1.3 Research aim and objectives

1.3.1 Research aim

The main aim of this study was to evaluate the effect of dietary inclusion of varying levels of SFM with enzyme supplementation on growth performance, serum biochemical parameters, carcass characteristics, meat quality and internal organs of broilers.

1.3.2 Specific objectives

The specific objectives of the study were to:

1. Determine the influence of varying inclusion levels of SFM with or without enzyme supplementation on feed intake (FI), weight gain (WG) and feed conversion ratio (FCR) of broilers.
2. Determine the influence of varying inclusion levels of SFM with or without enzyme supplementation on carcass characteristics, meat quality and internal organs of broilers.
3. Determine the influence of varying inclusion levels of SFM with or without enzyme supplementation on serum biochemical parameters of broilers.

1.3.3 Hypothesis tested

1. Dietary inclusion of varying levels of SFM with or without enzyme supplementation will have some influence on FI, WG and FCR of broilers.
2. Dietary inclusion of varying levels of SFM with or without enzyme supplementation will have an influence on carcass characteristics, meat quality and internal organs of broilers.
3. Dietary inclusion of varying levels of SFM with or without enzyme supplementation will have some influence on serum biochemical parameters of broilers.

1.3.4 The research questions:

1. Does supplementing broiler rations with varying SFM levels supplemented with or without enzymes influence FI, WG and FCR of broilers?
2. Does supplementing broiler rations with varying SFM levels supplemented with or without enzymes influence carcass characteristics, meat quality and or internal organs of broilers?
3. Does supplementing broiler rations with varying SFM levels supplemented with or without enzymes influence serum biochemical parameters of broilers?

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CHAPTER 2
LITERATURE REVIEW

2.1 Introduction

The South African poultry meat industry dominates the agricultural sector in South Africa and it is the main supplier of animal protein in terms of food exceeding all other animal proteins combined. Over the past decades, the poultry industry has undergone enormous expansion in order to meet the world's ever-increasing demand for animal protein. One of the major challenges in poultry production is the availability of good quality feed on a sustainable basis at unwavering costs. Feed constitutes the major input production cost in poultry production and accounts for 60 – 75% of the total cost of broiler production (Anuradha & Roy, 2015). Increasing prices of feed ingredients remain the greatest determinants of economic gains in poultry production. The best strategy in reducing feed costs is the development of diet formulation using cheap, alternative and locally available feed ingredients, thereby reducing feed costs (Abbas & Yagoub, 2008). Therefore, sunflower has been suggested as an alternative vegetable protein source for poultry (Ravindran & Blair, 1992). SFM is a well-established and relatively inexpensive protein source for poultry diets (Casartelli *et al.,* 2006) and is cultivated in different parts of South Africa in abundance, and available at lower prices than other oilseed meals. SFM has been used extensively in poultry diets and has been described as a good protein source for poultry, provided that some of its nutritional characteristics are taken into considerations (Senkoylu & Dale, 1999).

2.2 Taxonomy and origin of the domesticated sunflower

Sunflower (*Helianthus annuus*) belongs to a genus *Helianthus* of the Compositae family and is one of 67 species of genus *Helianthus* in the world. It is native to Middle American region later being commercially available at a global level (Anjum *et al.,* 2012). It is one of the most widely cultivated oilseeds in the world and ranks third in importance as a source of vegetable oil and although referred to as sunflower seed, it is more correctly described as a type of indehiscent fruit (Salari *et al.,* 2009).

Native Americans cultivated sunflower and found it useful for several things: the nutritious seeds were eaten raw, made into a meal, or used as a source of hair oil; and fibre from the stems, and the roots of other species were eaten. Up to this day, the common sunflower is widely established and valued around the globe. The seeds are almost universally used as poultry feed and principally as the source of oil utilized for such purposes as cooking, soap making, and the oil cake is fed to stock (Encyclopaedia, 2016: 6).

It is estimated that annual worldwide production of sunflower seeds is 24.9 million tons (FAOSTAT, 1999) and 10.8 million tons are available for use in poultry production (Kocher *et al.,* 2000). South Africa (SA) is not a significant role player in the production and trade of oilseeds in the international market since it contributes only 3% to the sunflower seed produced in the world. Sunflower is cultivated worldwide for oil extraction because of its great capability for adaptation to different climate and soil conditions (Ravindran & Blair, 1992). Sunflower is adapted to vast environmental conditions in SA. In addition, it can be harvested up to three times a year in tropical areas and is a good alternative for oil producers and for the feed mill industry (Vieira *et al.,* 1992).

2.3 Description of the sunflower plant

The sunflower is a broad-leafed plant that emerges from the soil with two large cotyledons (Boshoff, 2008). Sunflower is an annual, tall and erect plant with a strong taproot and prolific lateral spread of surface roots. Stems are usually round early in the season, angular and woody later in the season, and normally un-branched. The length of the stem is mostly determined by the number of internodes. However, they vary between 1.5 and 2.1 metres depending on the planting date and prevailing soil and environmental conditions (Boshoff, 2008). Sunflower leaves are phototropic and will follow the sun's rays, this is so as to increase photosynthesis and the colour intensity could vary from light to dark green.

The growth period of sunflower takes about 120 days (PANNAR, 2007). The sunflower head is not a single flower. The head is radiate and the ray flowers are neutral or pistillate. They are usually large and yellow but the colour can range from lemon-yellow, orange to reddish. The head shape varies, being concave, convex or flat. Once mature, the head will turn downwards. This is to prevent birds from eating the seeds (Meyer, 2005).

The inflorescence is a capitulum or head, characteristic of the Compositae family. Each inflorescence is composed of two types, (a) yellow petals around the edge of the head and (b) face of the head is comprised of hundred disk-like flowers each forming a seed (Boshoff, 2008). Pollen is shed at the periphery and proceeds to the centre of the head. A lot of sunflower cultivars have a degree of self-incompatibility and pollen movement among plants by either wind or insects is important, and bee colonies have generally increased production.

2.4 Chemical composition of SFM

Sunflower has been recognized as a viable feed ingredient in poultry diets (Viveros *et al.,* 2009). The nutritional value of SFM for poultry has not been extensively studied (Elzubeir & Ibrahim, 1991). SFM is a by-product of sunflower seed oil extraction, and it is economically important due to its abundance and use as an alternative protein source in broiler feeds (Araújo *et al.,* 2011). Literature studies present variations in the chemical composition of SFM, and this may be ascribed to growing conditions, seed cultivar, different processing methods, the extent of dehulling of decortication, and the efficiency of oil extraction (Tsuzuki *et al.,* 2003; Rezaei & Hafezian, 2007; Sredanović *et al.,* 2012).

The seed of high-oil sunflower varieties, if properly processed, has valuable protein and is rich in essential fatty acids and amino acid (AA). Sunflower contains 45% oil and is a good source of protein and B-group vitamins (Rezaei & Hafezian, 2007; Ahmed *et al.,* 2013). According to Alagawany *et al.* (2015), SFM is commonly produced with 60 – 65% protein core (kernel) and 35 – 40% hull (shell). Its crude protein (CP) content ranges between 29 – 45%, depending on the dehulling and oil extraction process. Corresponding crude fibre (CF) contents may range between 14 – 32% (Moghaddam *et al.,* 2012). Thus, an inverse relationship is observed between the CP and CF content of SFM (Lević *et al.,* 2005; Sredanović *et al.,* 2012). As a result of high CF content, the nutritive value of SFM is reduced drastically in poultry nutrition. Dry matter (DM) of SFM ranges from 88 – 93% with an average of 90% (Alagawany *et al.,* 2015).

Hybrid varieties contain 380 – 540g of oil/kg (Crum *et al*., 1993), which is very rich in linoleic acid (Salari *et al.,* 2009) and oleic acid. A potential commercial source of oleic

acid (18:1) is whole high oleic acid sunflower seed, which contains about one-half oil, of which just over 80% is oleic acid. According to Alagawany *et al.* (2015), SFM showed limiting amounts of lysine, but good concentrations of arginine, glutamic acid, aspartic acid, and sulphur containing amino acids (methionine and cystine).

Solangi *et al.* (2002) reported SFM to have a high energy content of about 1760 kcal/kg metabolizable energy (ME). They further explained that it usually contains 2.8% fat, 0.4% calcium, 0.3% available phosphorus, 1.65% methionine and 3.5% arginine. As compared to other oilseeds available, SFM contains more ether extract (EE) (38 – 40%). This high EE content contributes to a high ME per unit or high energy density of feed (Selvaraj & Purushothaman, 2004; Salari *et al.,* 2009; Malakian, 2010). Unlike most other oilseed meals, SFM does not contain high concentrations of anti-nutritive factors (Casartelli *et al.,* 2006).

Table 2.1. Chemical composition and metabolizable energy content of high fibre SFM

Authors	*DM %*	*CP %*	*CF %*	*EE %*	*Ash %*	*NFE %*	*ME (kcal/kg)*	*Fat*	*Ca*	*Phos*	*Moisture%*
Tsuzuki *et al.* (2003)	93	22	16	40	-	-	4,9	-	0.3	0.7	-
Senkoylu & Dale (2006)	90	32	12	-	6.3	-	5,2	19	-	-	-
Rezaei & Hafezian (2007)	92	36	26	1.2	6.8	30	1221	-	0.4	0.7	-
Abbas & Yagoub (2008)	93	41.	10.	-	7.6	-	-	17	-	-	-
Salari *et al.* (2009)	-	18	14	38	-	-	16.2	-	0.3	0.2	8.5
Malakian (2010)	-	17	15	38	3.4	26	-	-	0.3	0.2	-
Araújo *et al.* (2011)	-	31	23	-	-	-	1.8	-	0.3	1.0	
Ahmed *et al.* (2013)	-	42	8.9	-	7.1	-	-	15	-	-	

(-) not indicated

Table 2.2. Amino acids profile of SFM

Amino acids	**Authors**		
	Senkoylu & Dale (2006)	**Malakian (2010)**	**Araújo *et al*. (2011)**
Argininie	2.48	-	-
Histidine	0.77	-	-
Lysine	1.14	0.55	1.22
Leucine	2.02	-	-
Methionine	0.68	0.45	0.72
Cystine	0.66	-	1.25
Phenylalanine	1.44	-	-
Threonine	1.15	-	1.19
Tryptophan	0.41	-	-
Tyrosine	0.80	-	-
Glycine	1.77	-	-
Valine	1.58	-	-

2.5 Anti-nutritional factors of SFM

Plant proteins contain some anti-nutritional factors (ANF) that naturally exist within their structures, which can adversely affect the quality of the protein and limit its value in animal nutrition (Beski *et al.,* 2015). They further explained that ANFs are by-products of different metabolic processes of plants that detract from the nutritive value of the feed. ANFs limit feed intake, inhibit digestion and utilization of dietary nutrients, and therefore, negatively affect the intestinal health and animal performance. According to Akande *et al.* (2010) the most commonly found ANFs in plant protein sources includes toxic amino acids, saponins, cyanogenic glycosides, tannins, phytic acid, gossypol, oxalates, goitrogens, lectins, protease inhibitors, chlorogenic acid, and amylase inhibitors.

SFM is almost free of toxic compounds that may impede their use in human and/or animal nutrition. However, the presence of high fibre (non-starch polysaccharides), lysine and threonine deficiency, and associated polyphenolic compounds such as chlorogenic acid in hulls may restrict its use in poultry feed. It is well known that non-starch polysaccharides (NSP) are anti-nutrients that inhibit the digestion and utilization of dietary nutrients by the animal and therefore reduce animal performance (Choct, 2006). NSP of SFM has been extensively studied. Senkoylu & Dale (1999) stated that SFM cell wall contains NSP such as β-glucans, xylans, pectins and oligosaccharides which tend to increase the viscosity of the digesta, lower nutrient utilization, and lead to depressed growth in chickens. NSP of SFM consists of 42% cellulose, 24% pectic polysaccharides, 24% 4-0-methyl-glucuronoxylans, 5% glucomannans, and 4.5% fucoxyloglucans (Senkoylu & Dale, 2006; Viveros *et al.,* 2009). Poultry being

monogastric, lack enzymes (cellulose, hemicellulase) to digest fibre that make up the cell wall of sunflower.

SFM, being a good source of CP is restricted by the presence of polyphenolic compounds (Chlorogenic acid) in poultry feed. In their study, Milic *et al.* (1968) detected 1.56% tannin-like Chlorogenic acid compound. Chlorogenic acid is bound to low molecular weight proteins by a hydrogen bond between the hydroxyl groups of phenolic compounds and peptide bond in proteins. Chlorogenic acid causes a reduction in the digestibility and bioavailability of the protein content of SFM. The content of Chlorogenic acid in sunflower seeds may vary from 1.4 to 4.0% (Dorrell, 1976). The content seems to be affected by early seeding and warm temperatures during seed maturation.

Reduction of Chlorogenic acid content in the seed through genetical selection is difficult because oil and Chlorogenic acid contents are positively correlated. However, according to Dorrell (1976), there exists sufficient genetic variation to justify future selection for lower levels of Chlorogenic acid. Furthermore, the difficulty would be compounded by a strong environmental effect on Chlorogenic acid concentration. Irrespectively of chlorogenic acid, SFM contains other phenolic compounds such as caffeic, rosmarinic, and ferulic acids, as well as myricetin and rutin, all these compounds present in quantities of less than 0.15ppm (Alagawany *et al.,* 2015).

Presence of ANF's in SFM limits its use in poultry nutrition. However, removing the hull after oil extraction reduces CF and consequently improves nutrient availability and digestibility of SFM, which then results in improved animal performance. Another method of tackling the ANFs would be heating the seeds. The heat-liable ANF's in plant proteins can be decreased by heat treatment and this process increases the quality and protein level of plant proteins (Adeyemo & Longe, 2007). Rezaie & Hafezian (2007)

observed that heating sunflower seeds at 100ºC for 5 hours destroyed at least 43% of chlorogenic acid.

2.6 Reduction or removal of anti-nutritional factors in SFM

Fafiolu *et al.* (2015) indicated that SFM is a good source of CP with a relatively high CF and EE content. However, this relatively high fibre content is a major concern in poultry production as it limits SFM use in poultry nutrition. An advance in poultry nutrition has made it possible to improve the utilization of SFM. One of these advances includes; (1) physically removing the hull during the process of oil extraction, and (2) the use of commercially available exogenous enzymes.

2.6.1 Physical and chemical processing

Very little information exists on the processing or heat treatment of sunflower. Processing conditions of a sunflower during oil extraction are critical for maximizing oil yield and producing a good quality SFM that can be successfully used in animal and/or human nutrition. As a result of processing, SFM nutritional composition may vary greatly. The use of SFM in animal feed has been limited due to high fibre content caused by residual seed hulls (Abbas & Yagoub, 2008) because the nutritive value of SFM is largely influenced by the fibre content (14 – 33%) of the meal. However, this can be solved by the removal of the hull through the decortication process. Separating the hull, as the main source of fibre, from the kernel is the processing solution for improving the nutritional and commercial values of SFM (Sredanović *et al.,* 2012). A strong negative correlation exists between CF content and the whole sunflower seed true metabolizable energy.

Decortication or dehulling reduces the fibre content of SFM and consequently increases the protein content and digestibility of the diet. It is very important that the hulls are not crushed into too small particles so that it could later be separated by mechanical fractionation. This process, however, may result in increased cost of feeds and therefore, hinder SFM as a possible alternative in poultry nutrition. Another method used for plant and animal processing is thermal treatment. The possible benefits of heat-treating sunflower may be the release of nutrients due to the disruption of cell wall structures during processing. This process increases the protein content and quality of plant proteins (Adeyemo & Longe, 2007). The temperature (° C) at cooking has an influence on the efficiency of oil extraction and the nutritive value of sunflower meal. Carsatelli *et al.* (2006) reported that heating the seed at 100 ºC, or at 135 ºC for 5 hours, destroyed about 43% of chlorogenic acid. However, excessive heating of SFM may denature proteins and result in decreased amino acid levels, especially lysine and methionine.

2.6.2 Use of exogenous enzymes

The presence of ANF's and NSP in SFM, which are responsible for the reduction of digestibility and nutrient availability in broilers have compelled nutritionists to explore a viable method to incorporate SFM in poultry diets at higher levels. Dietary supplementation with enzymes is not a new concept, but it becomes more fine-tuned with the production of specific enzyme preparations (Sredanović *et al.,* 2012). A number of studies have been carried out to evaluate the influence of SFM with enzyme supplementation in broiler diets.

Enzymes are included in diets to facilitate the breakdown of larger molecular structures of the feed ingredients into smaller ones by their specific actions, making these

nutrients more readily available in the gastro-intestinal tract (GIT) for improved absorption (Raza *et al.,* 2009). SFM contain considerable amounts of cell-wall material and a high concentration of fat that could affect the nutritive value of the meal, therefore, the use of crude enzyme preparation might be justified to improve the accessibility of cell contents to digestive enzymes (Brenes *et al.,* 2008). Enzyme supplementation has increased considerably in poultry nutrition over the past years, however, only a few reports are available on the effect of exogenous enzyme supplementation of SFM utilization in broiler production. Birds being monogastrics, lack the ability to produce enzymes that breakdown NSP present in SFM. The difficulty in fibre digestion, besides reducing feed energy, may also impair the use of all other nutrients (Neto *et al.,* 2012), especially when the feed is soluble. Use of exogenous enzymes is important as they hydrolyse NSP that can potentially be used by birds, improving, for instance, energy use (Tavernari *et al.,* 2008) and feed conversion ratio (Amerah *et al.,* 2015). Other benefits of enzymes supplementation are the reduction of viscosity of gut contents, (Khan *et al.,* 2006; Neto *et al.,* 2012; Anuradha & Roy, 2015), which changes the intestinal micro-flora and reduces the excreta moisture and improves the conditions of the litter. Enzyme supplementation has also shown to improve weight gain with several studies having observed improved performance and weight gain on birds fed diets supplemented with enzymes than birds fed control diets (Khan *et al.,* 2006; Raza *et al.,* 2009; Anuradha & Roy, 2015; Horvatovic *et al.,* 2015).

2.7 SFM in poultry Production

2.7.1 Growth performance

Few studies have been reported to evaluate the use of SFM at different inclusion levels in broiler production; however, there is scarcity in the research studying the effects of SFM at high inclusion rate (up to 75%) with enzyme supplementation. Attia *et al.* (2003) reported that feed intake and weight gain were not adversely affected by including SFM at 5%; however, growth and feed intake was decreased when SFM was included in the diet at 10 or 15% compared to 0 or 5%. This may be explained by the high CF content of the diet and high oil level of the experimental feeds (Tavernari *et al.,* 2008).

The use of exogenous enzymes in poultry has been shown to have a positive effect on feed conversion ratio (FCR), feed intake (FI), and growth rate. In their study, Horvatovic *et al.* (2015) observed improved weight gain on birds fed diets supplemented with dietary enzyme during the entire experimental period. This suggests the role of enzymes in degrading fibre present in structures of SFM. Increased nutrient availability and ME may also be reasons for these improvements (Raza *et al.,* 2009) However, neither SFM nor enzyme supplementation had any effect on feed intake (g/day) (Horvatovic *et al.,* 2015). Without the enzyme, indigestible fibre promotes the growth of 'harmful' bacteria but with the enzyme, the fibre is broken down and promotes the growth of 'useful' bacteria (Khan *et al*., 2006).

2.7.2 Carcass characteristics and meat quality

Horvatovic *et al*. (2015) studied the effects of SFM inclusion and enzyme supplementation on carcass traits (dressing percentage, and breast, thigh, drumstick, and abdominal fat yields). They did not find any significant effect of SFM or enzyme

supplementation on the evaluated parameters. Similarly, Malakian (2010) reported that liver, giblet percentage, and dressing percentage did not differ among treatments.

On the contrary, Salari *et al.* (2009) reported that the relative weight of liver decreased significantly in birds fed full-fat sunflower seed compared to those fed on a control diet. This might be due to the nature of the fat in SFM, which is composed mainly of unsaturated fatty acids mainly linoleic acid). Oleic acid prevented fat accumulation in the liver (Salari *et al.,* 2009).

Studies suggest that enzyme supplementation may improve carcass yield. Khan *et al.* (2006) observed that the use of NSPase in diets with high SFM inclusion levels resulted in better carcass yield. However, in a broader context, carcass quality is not affected by enzyme supplementation.

2.7.3 Blood constituents

Few reports are available on the effect of inclusion of SFM with or without enzyme supplementation on blood parameters (cholesterol, triglycerides, and lipoprotein profiles). Selvaraj and Purushothaman (2004) reported that SFM inclusion in the diet did not significantly affect muscle lipid, serum lipid, and total and high-density lipoprotein (HDL) cholesterol. In their study, liver lipid content was on average 9.5%, muscle lipid 2.8%, plasma total cholesterol 200 mg/dL, muscle total cholesterol 102 mg%, and plasma HDL 46 mg/dL. Similar results were produced by Malakian (2010).

Conflicting results were produced by Brenes *et al.* (2008) who reported that the inclusion of unsupplemented sunflower seed in the diet increased plasma uric acid, cholesterol and glucose concentrations by 5, 29 and 15% respectively and reduced serum lactate dehydrogenase (LDH) and creatine phosphokinase (CPK) by 6 and 16%,

respectively. They further reported that inclusion of enzyme in SFM diets increased plasma uric acid, calcium, serum LDH, serum CPK, and total protein concentrations by 6, 12, 7, 52 and 15%, respectively; and reduced plasma cholesterol and glucose by 8 and 5%, respectively.

The uric acid increment in diets supplemented with exogenous enzymes may suggest amino acid imbalance that could justify the decrease in amino acid digestibility (Brenes *et al.,* 2008). Moghaddam *et al.* (2012) also reported that glucose, phosphorus, triglyceride and HDL concentrations increased with an increase in SFM inclusion.

2.7.4 Nutrients digestibility

Apparent digestibility of DM, organic matter (OM), CP, EE, starch, and energy were increased with enzyme supplementation (Khan *et al.,* 2006); however, enzyme supplementation did not improve EE digestibility (Fafiolu *et al.,* 2015). CF digestibility was low in starter diets (Selvaraj & Purushothaman, 2004; Malakian, 2010). Solubilisation and disruption of sunflower endosperm cell walls by enzyme addition are primarily responsible for the improvement in digestibility.

According to Tavernari *et al.* (2008) and Salari *et al.* (2009) dietary inclusion of SFM increased apparent metabolisable energy corrected for nitrogen (AMEn), which explains the improvement in the feed: gain ratio of broilers. The inclusion of unsupplemented SFM reduced fat digestibility by 7% and amylase and lipase activities by 22 and 19%, respectively (Brenes *et al.,* 2008). Reduced digestibility is a pointer to the fact that diets may not have met the nutritional requirements of the birds for these essential nutrients.

2.7.5 Intestinal enzyme activities and gut viscosity

Amerah *et al.* (2015) observed that SFM inclusion and enzyme supplementation did not significantly affect jejunal digesta viscosity. On the contrary, high level of SFM inclusion rate increased gut viscosity, particularly ileum (Horvatovic *et al.,* 2015) and villus heights of the duodenum and jejunum (Moghaddam *et al.,* 2012). An increase in villus length increases surface area, therefore, this might improve absorption of nutrients and enhance nutrient digestibility. According to Horvatovic *et al.* (2015), the effect of the diet and of the interaction between diet and enzyme supplementation on gut viscosity is significant. This indicates that enzymes reduced digesta viscosity only when the diet high SFM inclusion was fed, and therefore, the diet contained high NSP levels. Enzymes break down some NSP factors in SFM and help promote the growth of useful bacteria.

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CHAPTER 3

THE EFFECT OF DIETARY INCLUSION OF VARYING LEVELS OF SFM IN BROILER DIETS WITH OR WITHOUT ENZYME SUPPLEMENTATION ON GROWTH PERFORMANCE OF BROILERS

Abstract

A 42-day feeding trial was conducted to determine the effect of varying levels of sunflower meal (SFM) (0, 25, 50, 75% inclusion rate) with enzyme supplementation on growth performance parameters (average feed intake (AFI), average weight gain (AWG), and feed conversion ratio (FCR) of broilers. Two hundred and eighty eight (288) broiler chickens were reared on a starter ration from day 1 up to 10 days of age. On the 11^{th} day, birds were randomly allocated to four (4) treatment diets (n = 36 birds per treatment replicated three times): control, SFM25, SFM50, and SFM75. Growth performance data was collected over a 32-day period (day 11 up to day 42) in grower and finisher treatment diets. AFI was measured daily, AWG was measured weekly, whilst FCR was measured at the end of the study period and calculated on a weekly basis. Diet had a significant effect ($P < 0.05$) on FI. However, WG and FCR were not significantly influenced by diet ($P > 0.05$). Enzyme supplementation did not significantly influence ($P > 0.05$) growth performance. The effect of time significantly affected ($P < 0.05$) all growth performance parameters, weight (kg) of birds drastically improved with age (time). There was no two-way interaction observed ($P > 0.05$) on AWG and FCR, however, treatment diet and enzyme effect affected AFI. Three-way interaction (treatment diet x enzyme x weeks) did not significantly influence ($P > 0.05$) for AFI, AWG and FCR. Birds fed diets with 50% and 75% SFM inclusion rate with enzyme supplementation had a higher FI, and therefore, resulted in a direct relationship (the

higher the intake, the high the AWG was) with AWG although treatment diet did not have any significant effect on AWG. Therefore, the results of this study suggest that replacing SBM with SFM in broiler diets up to 75% inclusion rate supplemented with Kemzyme can be effectively used without any adverse effects on bird performance (AFI, WG, & FCR).

Key words: Sunflower meal, growth performance, feed intake, feed conversion ratio, weight gain, enzyme supplementation, serum biochemical parameters, broilers

3.1. Introduction

Globally, there is an increasing demand for poultry products (Chisoro *et al*., 2018). This may be attributed to an increased world population that is increasing at an alarming rate and is projected to continue growing in the years to come. As a result, this leaves producers with a responsibility to ensure that production does not lag behind population growth. However, high input costs such as feed, which contributes to about 60 – 75% of the input costs are a limiting factor in poultry production as they have a negative impact on the profitability and sustainability of the poultry enterprise. As a result, this has encouraged scientists to search for alternative, cheap and locally produced feed ingredients in order to reduce feed costs and ultimately increase the profitability of the poultry enterprise.

Currently, soybean is used as a main protein source in poultry rations. Nevertheless, its inadequate supply has led to a price increase in poultry rations (Chisoro *et al*., 2018). According to Solangi *et al*. (2002) nutritional science has taken so much uncertainty out of this, resulting in feeds now formulated in such a manner that they produce economical gains. Hence, the need to find alternative protein source in poultry rations. Several alternatives have been identified and only sunflower meal (SFM) has a close chemical composition to soybean meal (SBM) as a more conventional protein source in poultry nutrition (Fafiolu *et al*., 2015). Numerous tests have been conducted on the value of SFM for poultry feed and were found entirely satisfactory and an excellent substitute for cereal grains (Solangi *et al*., 2002). The chemical composition of SFM depends on a number of factors, including but not limited to cultivar choice, growth conditions, oil extraction process and degree of dehulling.

Crude protein (CP) of conventional SFM usually varies between 33 – 37%, and fibre content my range between 18 – 23%, thus, an inverse relation is seen between the protein and fibre contents of SFM (Lević *et al*., 2005). If properly processed, SFM has a valuable protein with amino acid (AA) availability similar to that of SBM (Green & Kiener, 1989) and high metabolizable energy (Solangi *et al*., 2002).

However, the use of SFM in monogastrics has been restricted due to high fibre content caused by residual seed hulls. On the other hand, advances in animal nutrition have made it possible for improvement in the utilization of lesser known feedstuffs (Fafiolu *et al*., 2015). These includes removing excess hulls from un-decorticated SFM seeds and inclusion of exogenous enzymes in order to break down non-starch polysaccrides (NSP) into small molecular structures, making them readily available for better absorption in the gastro-intestinal tract of poultry.

Monogastrics lack the ability to produce some ruminant enzymes (cellulase, hemi-cellulase, beta-glucanase, etc) which are vital in digesting beta type linkages in NSP which is rich in cell walls of plant materials (Vooren, 2012). According to Neto *et al* (2012) as well as Anuradha and Roy (2015), soluble NSP has an anti-nutritional effect in poultry by modifying intestinal viscosity and intestinal transit time, resulting in reduced diffusion and assimilation rates of various nutrients.

Recently, there has been increased investigation into enzyme supplementation in poultry feeds in order to improve production efficiency and effective nutrient utilization. Enzyme supplementation reduces the viscosity of the intestinal digesta that changes intestinal microflora and reduces the excreta moisture, improving the conditions of the litter (Oliveira & Moraes, 2007) and improving availability of energy and other nutrients for poultry to digest and absorb, therefore, improving feed conversion ratio (FCR) and

consequently weight gain (Shirmohammad & Mehr, 2011; Harvatovic *et al*., 2015). This study was therefore designed to investigate the effects of dietary inclusion of varying levels of SFM in poultry diets with or without enzyme supplementation on growth performance of broiler chickens.

3.2 Materials and Methods

3.2.1 Description of study site

The study was carried out at the North West University (Mafikeng Campus) experiential farm (Molelwane), Mahikeng (25.8^0S, 25.5^0E), North West Province, South Africa, with an altitude of about 1 290 m above sea level. The prevailing temperatures were from 17° to 39° C during summer and between 4° and 19° C during winter. The area receives an annual rainfall of about 550 mm on average and most of the rainfall is received during the summer.

3.2.2 Experimental Animals and dietary treatments

A total of two hundred and eighty-eight (288) day-old Cobb 500 chicks were purchased from Mimosa chicks in Mahikeng and fully vaccinated against Newcastle disease and infectious bronchitis from the hatchery. Eight (8) iso-nitrogenous and iso-caloric grower and finisher diets were formulated to contain specific levels (0%, 25%, 50% & 75%) of SFM as a protein source replacing it with SBM with or without enzyme supplementation (Table 3.1a and Table 3.1b).

The treatment diets with SFM inclusion were supplemented with 0g/ton (0%) and 500g/ton of an exogenous and endogenous enzyme (Kemzyme) complex (carbohydrase, protease, cellulase, xylanase, glucanase, NSPases). From day 1 up to day 10, all birds were fed a similar balanced ration (starter). Then from day 11 up to day

24 birds were fed a grower treatment diet and finisher treatment diet from day 25 up to day 42.

Table 3.1a: Gross (%) composition of nine experimental diets offered to broilers

Ingredients	Starter	Grower				Finisher			
		0%	25%	50%	75%	0%	25%	50%	75%
Extruded FF Soya	80,00	40,00	80,00	100,00	120,00	34,28	60,00	80,00	100,00
Prime Gluten	16,89	24,66	15,00	16,05		10,00			
Yellow Maize 8.0%	598,41	627,76	611,51	602,72	566,45	698,62	660,72	627,99	590,38
Bran 15%		39,04	20,00						
Soya O/C 47%	268,83	230,00	177,52	127,42	93,18	220,00	180,47	133,00	90,00
Sunflower O/C 36%			57,50	115,00	172,50	0,00	55,00	110,00	165,00
Limestone	12,63	12,17	11,95	11,76	11,53	11,21	11,01	10,86	10,70
Monocalcium Phos	9,07	7,74	7,63	7,63	7,42	6,02	5,79	5,63	5,42
Salt	1,92	1,85	1,75	1,58	1,52	1,68	1,66	1,63	1,60
Sodium Bicarbonate	2,00	2,07	2,19	2,40	2,47	2,00	2,00	2,00	2,00
DL Methionine	2,80	2,34	2,26	2,08	2,06	2,30	2,22	2,06	1,88
L Threonine	0,53	0,52	0,54	0,53	0,61	0,55	0,56	0,54	0,46
L Tryptophan		0,16	0,10	0,08	0,02	0,17	0,10	0,06	
Lysine HCL	2,33	2,59	2,73	3,15	3,18	2,01	2,06	2,38	2,57
Crude Soya Oil Mixer		5,00	5,22	5,50	14,96	7,56	14,83	20,25	26,39
BSGF	3,00	2,50	2,50	2,50	2,50	2,00	2,00	2,00	2,00
AxtraPhy10000 Broiler (100g87)	0,10	0,10	0,10	0,10	0,10	0,10	0,10	0,10	0,10
Choline CL	0,50	0,50	0,50	0,50	0,50	0,50	0,50	0,50	0,50
Salinomycin 12%	0,50	0,50	0,50	0,50	0,50	0,50	0,50	0,50	0,50
Zinc Bacitracin	0,50	0,50	0,50	0,50	0,50	0,50	0,50	0,50	0,50
TOTAL	1000, 00	1000,00	1000,00	1000,00	1000,00	1000,00	1000,00	1000,00	1000,00

Table 3.1b: Chemical composition of nine experimental diets offered to chickens

Nutrients	Units	Starter	Grower				Finisher			
			0%	25%	50%	75%	0%	25%	50%	75%
VOLUME	-	100,00	100,00	100,00	100,00	100,00	100,00	100,00	100,00	100,00
Dry Matter	g/kg	884,96	883,64	884,50	885,20	887,19	882,24	884,05	885,55	887,24
ME Poultry	MJ/kg	12,59	12,62	12,62	12,62	12,62	12,96	12,96	12,96	12,96
Crude Protein	g/kg	220,00	200,00	200,00	201,09	200,00	183,56	184,53	186,33	189,67
Soluble Protein	g/kg	35,57	33,15	30,75	27,66	26,31	28,72	27,59	25,95	24,65
NSC	g/kg	464,39	477,75	460,81	446,73	421,72	507,11	481,52	457,71	431,85
AP Arginine	g/kg	12,93	11,32	11,90	12,30	12,93	10,52	11,16	11,63	12,22
AP Isoleucine	g/kg	8,69	7,70	7,68	7,67	7,59	7,10	7,10	7,10	7,18
AP Lysine	g/kg	11,80	10,50	10,50	10,50	10,50	9,50	9,50	9,50	9,50
AP Methionine	g/kg	5,94	5,31	5,31	5,31	5,31	5,00	5,00	5,00	5,00
AP Threonine	g/kg	7,70	6,96	6,96	6,96	6,96	6,50	6,50	6,50	6,50
AP Tryptophan	g/kg	2,18	2,05	2,05	2,05	2,05	1,92	1,92	1,92	1,92
AP TSAA	g/kg	8,80	8,00	8,00	8,06	8,03	7,47	7,47	7,53	7,61
Arginine	g/kg	14,01	12,29	12,87	13,25	13,92	11,39	12,07	12,54	13,14
Histidine	g/kg	5,63	5,10	5,12	5,13	5,13	4,74	4,78	4,81	4,89
Isoleucine	g/kg	9,53	8,45	8,47	8,47	8,42	7,78	7,82	7,85	7,96
Lysine	g/kg	13,08	11,62	11,64	11,63	11,66	10,53	10,57	10,57	10,59
Methionine	g/kg	6,24	5,58	5,60	5,61	5,63	5,24	5,26	5,28	5,30
T.S.A.A.	g/kg	9,75	8,88	8,92	9,01	9,01	8,26	8,32	8,42	8,55
Threonine	g/kg	8,84	7,98	8,01	8,02	8,05	7,44	7,48	7,50	7,53
Tryptophan	g/kg	2,55	2,39	2,37	2,34	2,34	2,21	2,21	2,20	2,19
Valine	g/kg	10,44	9,46	9,62	9,81	9,92	8,71	8,90	9,13	9,44
18:2 n-6 EFA	g/kg	9,28	8,61	11,78	13,20	19,54	8,44	14,25	18,66	23,45
18:3 n-3 EFA	g/kg		0,34	0,35	0,37	1,02	0,51	1,01	1,38	1,79
Fat	g/kg	41,06	41,29	48,08	51,81	64,07	43,68	54,76	63,31	72,41
Total Omega 6 (n-6) EFA	g/kg		3,28	3,43	3,61	9,83	4,96	9,74	13,30	17,34
ADF	g/kg	47,24	47,67	59,26	70,06	82,22	43,64	55,62	67,39	79,26
Fibre	g/kg	30,57	31,80	40,73	49,01	58,95	28,83	38,40	47,65	56,95
NDF	g/kg	103,87	115,39	125,18	134,24	148,28	104,50	118,21	131,98	145,63
Ash	g/kg	26,83	24,86	26,09	26,79	28,67	22,71	24,49	25,80	27,32
Available P (New)	g/kg	34,88	27,00	25,75	22,74	21,32	25,43	24,05	21,30	19,00
Avl Phosphorus	g/kg	4,50	4,20	4,20	4,20	4,20	3,80	3,80	3,80	3,80
Calcium	g/kg	9,00	8,40	8,40	8,40	8,40	7,60	7,60	7,60	7,60
Chloride	g/kg	1,94	1,95	1,95	1,95	1,95	1,76	1,79	1,86	1,90
Magnesium	g/kg	1,72	1,75	1,96	2,14	2,42	1,60	1,86	2,10	2,34
Potassium	g/kg	8,87	8,03	7,89	7,50	7,59	7,42	7,47	7,29	7,19
Sodium	g/kg	1,70	1,70	1,70	1,70	1,70	1,60	1,60	1,60	1,60
Sulphur	g/kg	2,10	2,00	2,03	2,08	2,11	1,85	1,89	1,96	2,04
Total Phosphorus	g/kg	5,82	5,52	5,65	5,76	5,97	4,83	5,03	5,21	5,40
oP Poultry	g/kg	167,71	154,05	151,95	151,58	147,82	142,47	140,79	140,51	141,43
oP Poultry/Pigs	g/kg	4,75	4,27	4,25	4,23	4,19	3,99	3,99	3,97	3,99
Xanthophyll	mg/kg	27,68	23,98	27,53	30,58	29,33	21,31	22,21	24,56	26,81
Choline	mg/kg	1241,83	1101,46	1157,81	1181,25	1251,37	1047,66	1111,17	1145,39	1189,69
Density	kg/m	693,19	683,99	683,43	681,42	673,21	708,45	697,60	684,35	669,91

3.2.3 Experimental design

A completely randomized design (CRD) with 4 (treatment diets) x 2 (enzyme levels) factorial treatment arrangements was used for this study. The experimental unit was a pen containing 12 birds, which was replicated 3 times, resulting in a total of 24-pens measuring 1.5m x 1.5m x 1.5m (L x B x H).

3.2.4 Management of birds

On arrival, birds were randomly allocated to clean and disinfected experimental pens. A stress pack was administered after the first two (2) days of the birds' arrival at the poultry house and one day after changing to the experimental diets through drinking water. Sunflower hulls were used as a form of bedding material and litter stirred every week to keep it dry. Each pen had a water trough which was exchanged twice daily and a feed trough with feed provided daily. Light was provided by a combination of natural daylight and artificial light (lamp).

Ambient temperature was maintained at 32° C using infra-red lamps at the start of the study and gradually decreased to 27° C at 14 days of age and 24 °C at day 21 and then kept constant until the end of the experiment. Daily, temperature was recorded at 0800h, 1200h, and 1700h. A minimum–maximum thermometer was used to record the temperature in the poultry house. Proper management practices in terms of ventilation and sanitation were adhered to throughout the study period.

Birds were vaccinated against Newcastle and Gumboro disease at day 9 and day 14 respectively. Mortalities, behaviour and the health status were visually observed and recorded daily throughout the entire experimental period and mortality was used to

adjust the total number of birds to determine total feed intake and feed conversion ratio (FCR).

3.2.5 Data collection

Experimental diets were weighed daily to determine the daily average feed intake (AFI) and birds were weighed weekly to determine average weight gain (AWG) from day 11 until the end of the study. Average daily feed intake per bird was measured by subtracting the weight of the feed refusals from that of the feed offered per day, and dividing the difference by the total number of birds in the pen. The total feed intake per week and/or over the experimental period was calculated by adding the daily feeding intake. ADG was determined weekly by subtracting the weight of birds at the end of the week by the initial weight at the beginning of the week and dividing it by number of days of the week (I.e., Finish weight – start weight / days (age). Data on ADG and FI was used to calculate FCR (FCR = FI (g)/ ADG (g) of birds.

3.3 Statistical analysis

Weekly feed intake, weight gain, and FCR data were analysed using the repeated measures procedure of (SAS, 2010). Overall feed intake, weight gain, and FCR data were analysed using the general linear model (GLM) procedure for a 4 SFM levels (0%, 25%, 50%, & 75%) x 2 enzyme levels (0g, & 500g/ton) in a completely randomized design (CRD). For traits measured once during the experimental period, the data was subjected to analysis of variance (ANOVA) using the following model;

$$Y_{ijkl} = \mu + T_i + E_j + W_k + (T_j \times E_{ij}) + (T_j \times E_{ij} \times W_k) + E_{ijkl}$$

Where Y_{ijk} = dependent variable $_{ijk}$ (parameters analysed), μ = the overall mean, T_i = treatment diet effects, E_j = enzyme level effect, W_k = week effect, (T_j x E_{ij}) = interaction effect between treatment diet and enzyme level, (T_j x E_{ij} x W_k) = interaction effect between treatment diet, enzyme level and week effect, E_{ijk} = random experimental errors associated with observation.

The significance was tested at P <0.05. The Turkey procedure was used to separate means among levels within a significant factor. Traits measured repeatedly during the experimental period, such as body weight and feed intake, were subjected to a repeated measured analysis of variance for CRD in factorial arrangement in SAS.

3.4 Results

3.4.1 Feed intake and growth performance

Statistical differences (P values) of the effect of treatment diets, enzyme supplementation, week effect, and their interactions on AFI, AWG and FCR are presented in Table 3.2. Treatment diet significantly influenced AFI ($P < 0.05$) but did not have any significant effect ($P > 0.05$) on AWG and FCR. Enzyme supplementation did not significantly influence ($P > 0.05$) AFI and growth performance on any of the SFM inclusion levels. Significant differences ($P < 0.05$) were observed on the effect of time on FI, growth performance parameters (AWG and FCR). The two-way interaction (diet x weeks) did not significantly affect ($P > 0.05$) AWG and FCR, however, significant differences ($P < 0.05$) were observed on AFI. AFI, AWG and FCR were not significantly influenced by the three-way interaction (TRT x enzyme x weeks).

Table 3.2: Statistical significance (P values) of the effects of treatment diets, enzyme level and time (weekly performance) on feed intake and growth performance.

Parameter	*TRT*	*Enzyme*	*Week*	*TRT x Enzyme*	*TRT x Enzyme x Week*
FI	*	NS	*	*	NS
WG	NS	NS	*	NS	NS
FCR	NS	NS	*	NS	NS

* = Significant, NS = non-significant, TRT = treatment diet, FI = feed intake, WG = weight gain, FCR = feed conversion ratio

Data showing the response of birds to treatment diet on AFI is presented in table 3.3. Significant differences (P < 0.05) were observed on AFI as affected by treatment diets. High AFI was observed in diets with high SFM inclusion rates (50% & 75% SFM), whilst the control diet and SFM25 had significantly lower AFI.

Table 3.3 shows data on the effect of treatment diet on AWG and FCR. Inclusion level of SFM did not significantly affect (P > 0.05) growth performance parameters. Treatment diets with 50% and 75% SFM inclusion rate had a high AWG; however, treatment diet had no significant effect on AWG. Low FCR was observed on birds fed SFM50 treatment diet. AWG ranges were between 437.40 g and 486.37 g.

Table 3.3: Effect of inclusion levels of Sunflower meal on AWG and FCR

	TRT					
Parameter	*Control*	*SFM25*	*SFM50*	*SFM75*	***SEM***	***Pr > F***
AFI (g)	116.36^{b}	113.23^{b}	120.69^{a}	124.18^{a}	0.937	<.0001
AWG (g)	453.51	437.40	478.73	486.37	15.534	0.1041
FCR	3.1	3.2	2.8	3.2	0.030	0.8397

TRT = treatment diet, SFM = sunflower meal, AWG = average weight gain, FCR = feed conversion ratio

The effects of enzyme supplementation on AFI and growth performance are presented in table 3.4. Although no significant differences (P > 0.05) were observed, AFI and AWG were improved in birds fed diets supplemented with enzymes, however, FCR was higher for birds fed diets without enzyme supplementation.

Table 3.4 Effect of enzyme level on AFI, AWG and FCR

	Enzyme level			
Parameter	*0*	*500*	*SEM*	*Pr > F*
AFI (g)	117.70	119.53	1.0781	0.2313
AWG (g)	455.38	472.62	10.984	0.2705
FCR	0.33	0.29	0.212	0.1790

AFI = average feed intake, AWG = average weight gain, FCR = feed conversion ratio

The results indicate that there was a significant difference (P < 0.05) on growth performance as affected by week effect (Table 3.5). AFI of birds was significantly higher on week IV (28 – 34 days) and low AFI were observed in week I. AFI ranges were between 66.90 g and 149.41 g. Week III (22 – 27 days) had significantly high AWG, whilst low AWG was in week V. Significantly higher values of FCR were observed in week 5.

Table 3.5: Effect of week on AFI, AWG and FCR

	Week						
Parameter	*1*	*2*	*3*	*4*	*5*	***SEM***	***Pr > F***
AFI (g)	66.90^{d}	111.42^{c}	134.89^{b}	149.41^{a}	130.47^{b}	1.704	<.0001
AWG (g)	363.82^{c}	544.30^{b}	608.38^{a}	556.30^{b}	247.20^{d}	17.367	<.0001
FCR	1.8^{c}	2.1^{c}	2.3^{bc}	3.2^{b}	6.1^{a}	0.034	<.0001

^{abc} = significant values, AFI = average feed intake, AWG = average weight gain, FCR = feed conversion ratio

Table 3.6 shows the interaction effect of treatment diet and enzyme supplementation on AFI, AWG and FCR. No interactions (P > 0.05) were observed on AWG and FCR, however, significant differences (P < 0.05) were observed on the interaction of treatment diet and enzyme supplementation on AFI. Although no significant differences were observed treatment diets without enzyme supplementation had higher values of FCR, whereas AWG was high in birds fed SFM50 and SFM75 treatment diets with enzyme supplementation. Birds fed SFM50 and SFM75 supplemented with enzyme had significantly higher (P < 0.05) AFI values, whilst low values were observed in SFM25 and SFM50 without enzyme supplementation and control diet as well as SFM25 supplemented with enzymes.

Table 3.6 Effect of inclusion level of SFM x enzyme level on AFI, AWG & FCR

		Enzyme level			
Parameter	***TRT***	*0*	*500*	*SEM*	*Pr > F*
AFI (g)	Control	118.33^{bc}	114.40^{cd}	2.156	0.0035
	SFM25	114.78^{cd}	111.67^{cd}		
	SFM50	115.45^{cd}	125.93^{a}		
	SFM75	122.22^{ab}	126.14^{a}		
AWG (g)	Control	465.73	441.27	21.968	0.2383
	SFM25	437.55	437.25		
	SFM50	448.58	508.88		
	SFM75	469.68	503.06		
FCR	Control	3.3	3.0	0.042	0.6851
	SFM25	3.2	3.2		
	SFM50	3.0	2.7		
	SFM75	3.7	2.7		

^{abc} = significant values, TRT = treatment diet, AFI = average feed intake, AWG = average weight gain, FCR = feed conversion ratio

Data on the interaction effect of treatment diet, enzyme supplementation and week on growth performance is presented in table 3.7. No significant differences (P > 0.05) were observed on AFI and growth performance.

Table 3.7: Growth performance of birds fed inclusion levels of SFM with or without enzyme levels at 1, 2, 3, 4 and 5 weeks of age.

			Week						
Parameter	***TRT***	***Enzyme level***	***1***	***2***	***3***	***4***	***5***	***SEM***	***Pr > F***
AFI (g)	Control	0	60.16	107.05	127.56	150.40	146.46	4.820	0.0537
		500	65.93	111.70	134.80	138.27	121.28		
	SFM25	0	68.54	106.37	130.96	144.01	124.03		
		500	58.08	114.80	133.88	141.00	110.60		
	SFM50	0	69.17	105.52	131.50	147.91	123.15		
		500	73.12	117.39	139.24	156.38	143.52		
	SFM75	0	70.50	111.27	140.24	156.28	132.79		
		500	69.66	117.22	140.90	160.98	141.94		
AWG (g)	Control	0	362.50	486.11	622.78	614.17	243.08	49.122	0.2604
		500	370.84	574.44	505.94	507.29	247.93		
	SFM25	0	358.69	516.54	579.62	547.10	185.79		
		500	335.83	568.61	560.83	514.50	206.46		
	SFM50	0	357.22	508.06	575.56	590.00	212.04		
		500	408.91	596.19	627.58	592.07	319.66		
	SFM75	0	353.22	537.78	778.89	430.00	248.51		
		500	363.33	566.67	615.83	655.28	314.17		
FCR	Control	0	1.7	2.2	2.0	2.5	8.1	0.095	0.5900
		500	1.8	2.0	2.7	2.7	5.8		
	SFM25	0	1.9	2.1	2.3	2.8	7.0		
		500	1.7	2.0	2.4	2.8	6.8		
	SFM50	0	1.9	2.1	2.3	2.5	6.1		
		500	1.8	2.0	2.2	2.7	4.9		
	SFM75	0	2.0	2.1	1.9	6.9	5.4		
		500	1.9	21	2.3	2.5	4.5		

TRT = treatment diet, SFM = sunflower meal, AFI = average feed intake, average weight gain, FCR = feed conversion ratio

3.5 Discussion

Although several studies (Solangi *et al.,* 2002, Salari *et al.,* 2009, Moghaddam *et al.,* 2012, and Horvatovic *et al.,* 2015) have been conducted to determine the effect of dietary inclusion of varying levels of SFM on growth performance parameters of broilers, there has been conflicting results throughout. Furthermore, it appears that only limited studies have been conducted to determine the effect of SFM at high inclusion rates with or without enzyme supplementation in diets of broiler chickens.

In this present study, diets with 50% and 75% SFM had a higher AFI while diets containing <50% SFM (SFM25 and control) had the least AFI. The observation that the inclusion of SFM at higher levels increased FI compares well with those reported by several authors (Solangi *et al.,* 2002, Salari *et al.,* 2009, Moghaddam *et al.,* 2012, and Horvatovic *et al.,* 2015). A trend towards increasing cumulative feed consumption with increasing SFM in the diet was also observed by Abdelrahman and Saleh (2007) so also did Fafiolo *et al.* (2015).

According to Tsuzuki *et al.* (2003) along with Fafiolo *et al.* (2015), high FI in SFM diets with high inclusion rates can be attributed to palatability of SFM (Sayda *et al.,* 2011) and high fibre content in sunflower seeds. As a result, there is reduced ME in sunflower based diets (Rezaei & Hafezian, 2007) which ultimately causes a deficiency of energy in SFM diets (Rehman *et al.,* 2002), thus increasing intake to counter the deficiency.

However, the results from this present study contradict those of Selvaraj and Purushothaman (2004), Tavernari *et al.* (2008) along with those of Malakian (2010)

who observed that FI was not affected by SFM inclusion rate. Similar findings were observed by Casartelli *et al.* (2006) in layers. Senkoylu and Dale (2006) also observed low FI in diets with SFM compared to control diets.

The possible explanation for these contrary findings may be due to the quality (metabolizable energy, protein content, fibre content) of SFM used in diets which is influenced by cultivar choice, degree of dehulling, method of oil extraction and micro and macro environmental factors (Abbas & Yagoub, 2008, Salari *et al.,* 2009, Sayda *et al.,* 2011, together with Raza *et al.,* 2009).

Different inclusion levels of SFM showed no significant differences ($P > 0.05$) on AWG and FCR. Results from this present study indicate that diets with 50% and 75% SFM resulted in the numerically higher ($P > 0.05$) AWG values (478.73 and 486.37 g/bird respectively) while diet with 25% SFM resulted in the lowest AWG (437.38 g/bird). These results are in keeping with those reported by El-Sheriff *et al.* (1997) as well as Abbas and Yagoub, (2008) who observed that treatment diet had no significant differences ($P > 0.05$) on AWG and FCR.

However, contrarily, several authors (Solangi *et al.,* (2002), Raza *et al.,* (2009), Ahmed *et al.,* (2013)) found that different inclusion levels of SFM had a significant effect on WG and FCR. The conflicting results in the present study on WG and FCR could be due to the high fibre content in SFM which is responsible for poor FCR (Rehman *et al.,* 2002).

It can be argued that differences in results of the current study compared to those of other authors may be due to the fact that they used lower inclusion levels of SFM (0-30%) while the current study used higher levels (25-75%). Higher inclusion levels

resulted in high fibre content which reduced the FCR and WG (Rehman *et al.,* 2002). However as the FCR and WG between the control and treatments were not significantly different, it can be further argued that SFM can be included in broiler rations at higher levels (50-75%). This will reduce the higher inclusion level of SBM and thus reduce the feed cost. Data for AFI, AWG & FCR showed no significant differences ($P > 0.05$) as influenced by enzyme supplementation. These results are in agreement with those obtained in a study by Brenes *et al.* (2008) who observed that birds fed diets supplemented with enzymes improved feed consumption by 25%. Findings of the current study highlight the role played by the enzyme in degrading crude fibre present in SFM-based diets thus resulting in an increase in nutrient availability and ME (Raza *et al.,* 2009).

The results are consistent with findings by Abbas and Yagoub (2008), Abdelrahman and Saleh (2007), El-Sheriff *et al.* (1997) and Tavernari *et al.* (2008) who observed that inclusion of SFM with or without enzyme supplementation had no significant effect ($P > 0.05$) on WG & FCR. Furthermore, Tsuzuki *et al.* (2003) and Casartelli *et al.,* (2006) also observed similar results in layers where different SFM inclusion levels showed no significant difference ($P > 0.05$) on FCR. However, El-katcha *et al.* (2017) observed that 0%, 15% or 30% replacement of soybean protein by SFM with essential oil mixture (EOM) (enzyme) supplementation significantly ($P < 0.05$) increased body weight throughout the study by about 13.9%, 5,4% and 7.1% respectively when compared with the group fed the same diets without EOM enzyme supplementation.

The age of the birds (week) had a significant effect ($P < 0.05$) on all growth performance parameters. AFI, AWG and FCR were directly proportional to the age of the birds. In this present study, AFI and AWG increased with age up to week 4 and then decreased in the last week. AFI values were significantly higher in week 5 whilst AWG values were higher during week 3. Feed consumption increased as birds attempted to obtain more nutrients to satisfy their growth requirements, and as a result this increased AWG. FCR numerically increased from week 1 up to week 5 and was significantly higher in week 5.

These results are in agreement with data reported by Moghaddam *et al.,* 2012 who observed a quadratic increase ($P < 0.01$) in FI, AWG and FCR in grower and finisher phases. Abdelrahman and Saleh (2007) also observed an increase in feed consumption and AWG from week 1 up to week 6. Salari *et al.* (2009) in their investigation also observed that WG increased in the different stages (1 – 49 days). A significant improvement in FCR was observed by Tavernari *et al.* (2008) during the starter and grower phases. According to these authors, this could be due to the immature digestive system when birds were still young as their functional digestion and absorption capabilities were still immature. However, controversial results have been reported by Furlan *et al.* (2001) in their study who found no significant differences ($P > 0.05$) in AFI for both starter and growth phases.

The results of the present study show no interaction effect ($P > 0.05$) between SFM inclusion and enzyme supplementation on AWG and FCR. However the interaction between treatment diet and enzyme supplementation significantly affected ($P < 0.05$) AFI. The range of AFI was between 111.67 g and 126.14 g as affected by the two-way interaction effect. SFM75 and SFM50 both supplemented with enzymes

had significantly higher ($P < 0.05$) AFI values. The same treatment diets also resulted in high AWG, although these findings were not significant. These results show that combination of SF and enzyme supplementation increase AFI and consequently AWG.

The results of this present study are in agreement with those of Attia *et al.* (2003) and Abdelrahman and Saleh (2007) who observed no enzyme by SFM interaction on growth and FCR of broilers. Furthermore, Raza *et al.* (2009), Neto *et al.* (2012) together with Amerah *et al.* (2015) also observed diet and enzyme supplementation to have no significant effect ($P > 0.05$) on FCR. These results are also in line with Abdelrahman and Saleh (2007) who reported significant differences ($P < 0.05$) on FI among treatment diet and enzyme supplementation.

Conflicting results were presented by several authors (Khan *et al.,* (2006), Raza *et al.,* 2009, Horvatovic *et al.,* (2015)) who observed dietary treatment and enzyme supplementation did not influence ($P > 0.05$) cumulative FI. Data presented on AWG by several authors (Khan *et al.,* (2006), Brenes *et al.,* (2008), Raza *et al.,* (2009)) also showed conflicting results. In their studies, these authors observed that AWG was significantly influenced ($P < 0.05$) by diet and enzyme interaction effect. Kocher *et al.* (2000), Khan *et al.* (2006) as well as Brenes *et al.* (2008) observed the interaction effect (diet x enzyme) ($P < 005$) on FCR. These findings contradict results from this present study.

Findings of this current study showed no three-way interaction effect (treatment diet x enzyme supplementation x week (age)) on growth performance parameters. These are in agreement with Tavernari *et al.* (2008) who observed no significant

differences (P > 0.05) between SFM inclusion rate and enzyme complex on FI, WG and FCR for the starter (1 – 21 days) and grower (22 – 42 days) phases. Neto *et al.* (2012) observed no interaction (P > 0.05) on production performance between diet and enzyme inclusion for the period of 1 to 21 days, however, a significant interaction (P < 0.05) was observed for the period between 21 to 42 days on production performance.

3.6 Conclusion

This current study did not establish any significant effects of different inclusion levels of SFM supplemented with or without enzyme on AWG and FCR. However, different inclusion levels significantly influenced the AFI. Birds fed diets with high SFM inclusion rates with enzymes showed high AFI while diets containing lower SFM supplemented with or without enzyme supplementation had the least AFI. These findings indicates that SFM can be used up to 75% inclusion rate as an alternative dietary protein source in commercial broiler diets with no adverse effects on growth performance parameters.

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CHAPTER 4

THE INCLUSION EFFECTS OF VARYING LEVELS OF SFM IN BROILER DIETS WITH OR WITHOUT ENZYME SUPPLEMENTATION ON CARCASS CHARACTERISTICS, MEAT QUALITY PARAMETERS AND INTERNAL ORGANS OF BROILERS

Abstract

The aim of this study was to examine the effect of varying levels of sunflower meal (SFM) with or without enzyme supplementation on carcass characteristics, meat quality parameters and internal organs of broilers. At the end of the 42-day growth trial, four (4) birds were randomly selected from each treatment, totalling 96 birds. A 4 x 2 factorial treatment arrangement in a completely randomized design was conducted with four levels of SFM replacing SBM (Control, 25%, 50%, and 75%) and two enzyme levels (0 and 500 g/ton) were used and data was analysed using SAS (SAS, 2010). Commercial cuts (drumstick, thigh, wings, and breast), internal organs (liver, spleen, heart, and gizzard, small and large intestines) and meat quality parameters (pH and water holding capacity) were measured. Treatment diet had a significant effect ($P < 0.05$) on hot carcass weight (HCW) of slaughtered birds. The HCW ranged from 1 944.79 g – 2 118.29 g, whilst cold carcass weight (CCW) ranged from 1 908.96 g – 2 068.00 g. Meat portions, internal organs and meat quality parameters were not significantly ($P > 0.05$) influenced by treatment diets. Although there were significant differences, better weights were observed for carcass characteristics and meat quality parameters for birds fed SFM with 50% inclusion rate. Also, birds fed diets supplemented with enzyme supplementation showed higher weights for carcass characteristics and high values for

meat quality parameters, however enzyme supplementation had no significant effect on carcass and meat quality characteristics. No interaction effect was observed ($P > 0.05$) between treatment diet and enzyme effect, however, treatment diets with high SFM inclusion level (SFM50 and SFM75) supplemented with exogenous enzymes showed higher weights and values for carcass and meat quality respectively. Although heavier weights and long intestinal length of internal organs were observed in birds which had diets supplemented with exogenous enzymes across all levels; treatment diet, enzyme supplementation and the interaction of treatment diet and enzyme had no significant effect on weights of internal organs and length of intestines of broilers. From the results, it can be concluded that inclusion of SFM up to 75% with or without Kemzyme supplementation can be incorporated in broiler diets without negatively affecting carcass characteristics, meat quality parameters and internal organs traits.

Key words: Sunflower meal, enzyme supplementation, carcass characteristics, organ characteristics, meat quality, commercial cuts

4.1 Introduction

Poultry plays a key role in promoting food security and eliminating food scarcity. Amongst other domestic livestock species, poultry provides high quality protein and micronutrients through meat at a very affordable price. In poultry production, feed constitutes 70 – 75% of the total costs of production and is primarily based on cereal grains, mainly maize, wheat, sorghum and vegetable protein meals in order to meet the energy and protein requirements of birds (Raza *et al*., 2019).

However, the high demand of soybean meal (SBM) for human consumption and animal feed has resulted in increased price of high quality protein source (SBM) on the world market. As a result, nutritionists have opted for affordable and readily available alternatives such as canola, barley, oats and sunflower meal (SFM). SFM is a by-product that is obtained after oil extraction from sunflower seeds and provides energy and protein that plays an important role in replacing SBM in animal feed (Santos *et al*., 2012). SFM is a good source of oil, crude fibre, carbohydrate and essential amino acids (AA's) (Satish & Shrivastava, 2011) and unlike most other oilseed meals, SFM does not contain high concentrations of anti-nutritional factors (ANF's) (Razaei & Hafezian, 2007) which may affect growth performance and carcass yield.

According to Senkoylu and Dale (1999) the use of SFM in poultry diets is limited by variations in its chemical composition and the two main components, namely, high fibre and low lysine content. The lysine deficiency leads to AA imbalance, that is, insufficient utilization of rich AA potential contained in sunflower protein (Sredanovic *et al*., 2006).

High fibre components such as arabio-xylans, cellulose, β-Glucans, pectins and other ANF's are responsible for poor digestibility and bio-availability of protein and other

nutrients to broilers (Abdelrahman & Saleh, 2007) because birds lack the exogenous enzymes necessary to digest the beta type of linkages present in SFM (Raza *et al*., 2019). As a result, this leads to poor absorption of nutrients, poor growth and health and consequently increases the cost of production.

The addition of exogenous feed enzymes (phytase, hemi-cellulase, cellulase, pectinase, carbohydrase, protease, lipase, β-glucanase, etc) offers numerous possibilities for breakdown and “liberation” of these nutrients, their easier digestion and absorption (Acamovic., 2001) and results in improved performance (Kocher *et al*., 2003). Mandal *et al*. (2006) reported that enzyme inclusion of SFM based diets improved eviscerated yield of broiler and the yield of giblets also increased due to increased yield of gizzard and liver.

There are conflicting results on the effect of SFM inclusion in poultry diets supplemented with or without exogenous enzymes on the carcass and organ characteristics of broilers. There is also a lack of information on the effect of inclusion of SFM at high levels (50 and 75%) on carcass and organ characteristics of broilers. Therefore the aim of this study was to evaluate the inclusion of different levels of SFM on poultry diets with or without enzyme supplementation on carcass characteristics, meat quality parameters (pH and water holding capacity) and internal organs.

4.2 Materials and methods

4.2.1 Data collection

4.2.1.1 Carcass characteristics and meat yield

The carcass characteristics, such as carcass yield; breast, thigh, drumstick and wings were assessed. At six weeks of age, four birds from each replicate, totalling 96 birds with body weight similar to the mean body weight of each replicate were selected and delivered to the slaughter house. At the slaughter house, the birds were electrically stunned, exsanguinated, de-feathered and eviscerated. Live body weight (LBW) (before slaughter), weight of fresh eviscerated carcass (without the feet, head and abdominal fat and internal organs) and the yield of prime cuts, from the whole breast (with skin and bones) and legs (thighs and drumsticks) were weighed immediately after slaughter to express hot carcass weight (HCW) and 24-hours post slaughter to express cold carcass weight (CCW). Carcass percentage was expressed as a percentage of live body.

4.2.4.2 Meat pH measurement

The pH of the birds was taken from the breast muscle of the chickens using a pH meter with 0.01 precision (Sentron, model 1001) coupled to a probe (Sentron, type Lance FET, model 1074001) with a thin penetrating needle inserted in the centre of the pectoralis major muscle, 0.5 to 1.0 cm below the muscle surface (Garcia, 2010). The pH meter probe was placed directly into the left breast muscle and the instrument was given time to stabilise before the pH reading was taken. Between the measurements, the instrument probe was rinsed with distilled water and stored in a 3M potassium chloride (KCL) electrolytic solution. The measurements were taken 24 hours after slaughter.

4.2.4.3 Water holding capacity

Water holding capacity (WHC) was determined using a press (compression) method. Four pieces of meat (approximately 30 g) from each replicate were placed on filter paper, between two plastic sheets, and pressure was applied to the meat for 20 minutes. Weights of meat samples were measured before and after applying pressure and the difference (amount of water lost) used to determine the water holding capacity of the meat samples. The water squeezed out was absorbed by the filter paper and was related to the amount of free water in the sample.

4.3 Statistical analysis

Carcass characteristics, meat quality parameters and internal organs data were analysed using the repeated measures procedure of SAS (2010). The experiment takes the form of a 4 (SFM levels) x 2 (enzymes) factorial treatment arrangement in a completely randomized design. Variation in carcass characteristics, meat quality parameters and internal organs were analysed using SAS (2010) software according to the following general linear model:

$$Y_{ijk} = \mu + T_i + E_j + (T \times E)_{ij} + E_{ijk}$$

Where Y_{ijk} = dependent variable (parameters analysed), μ = the overall mean, T_i = treatment diet effect, E_j = enzyme level effect, $(T \times E)_{ij}$ = treatment diet x enzyme level interaction effect and E_{ij} = random experimental errors associated with observation.

The level of significance was set at $P < 0.05$. For parameters where significant variation was detected, multiple comparisons of treatment means were carried out using the

probability of difference (pdiff) option of the General Linear Models (GLM) procedures of SAS.

4.4 Results

4.4.1 Carcass characteristics and meat quality parameters

Table 4.1 shows the effects of treatment diets on carcass characteristics (LBW, carcass weights and meat portions) and meat quality parameters (ultimate pH and WHC). A significant difference ($P < 0.05$) was observed on the effect of treatment diet and WHC. Birds fed diets with high SFM inclusion rate (50% and 75%) had the highest ($P<0.05$) HCW. HCW ranged between 1 944.79 g – 2 118.33g. Diet did not have any significant effect ($P > 0.05$) on carcass characteristics (LBW, CCW, drumstick, thigh, wings, and breast) and meat quality parameters. The pH values ranged between 5.54 and 5.63, whilst WHC was 2.13 g in SFM75 to 2.63 g in SFM50 and control diets. Although there was no significant difference ($P > 0.05$) observed, birds fed diets supplemented with 50% SFM showed better results for both carcass characteristics and meat quality parameters.

Table 4.1 Effect of inclusion level of Sunflower meal on carcass characteristics and meat quality parameters

	TRT					
Parameter	***Control***	***SFM25***	***SFM50***	***SFM75***	***SEM***	***Pr > F***
LBW	2835.58	2724.79	2881.08	2872.67	53.454	0.1858
HCW	1987.75^{bc}	1944.79^{c}	2118.33^{a}	2089.29^{ab}	43.287	0.0367
CCW	1951.88	1908.96	2068.00	2038.79	42.625	0.0604
PH	5.54	5.58	5.63	5.55	0.072	0.8235
Drumstick	256.79	255.21	270.21	272.29	6.358	0.1629
Thigh	293.67	279.92	307.83	294.83	6.579	0.0612
Wing	194.58	206.33	203.54	199.13	4.397	0.2872
Breast	816.25	820.13	884.29	819.63	21.367	0.1094
WHC	2.63	2.42	2.63	2.13	0.284	0.5693

^{abc} = means within rows with different superscripts differ significantly (P < 0.05).,,, TRT = treatment diet, SFM = sunflower meal, LBW = live body weight, HCW = hot carcass weight, CCW = cold carcass weight, WHC = water holding capacity

Data on the effect of enzyme supplementation on carcass characteristics and meat quality parameters is presented on table 4.2. High carcass weight characteristics and values of meat quality parameters were obtained in treatment diets supplemented with 500g/ton enzyme level, however, these differences were not statistically significant (P > 0.05).

Table 4.2 Effect of enzyme level on carcass characteristics and meat quality parameters

	Enzyme level			
Parameter	***0***	***500***	***SEM***	***Pr > F***
LBW (g)	2789.90	2867.17	37.797	0.1676
HCW (g)	2009.42	2060.67	30.61	0.2537
CCW (g)	1962.83	2020.98	30.141	0.1914
PH	5.58	5.57	0.051	0.8207
Drumstick (g)	262.81	264.44	4.496	0.8015
Thigh (g)	292.90	295.23	4.652	0.7275
Wing (g)	199.96	201.83	3.109	0.6754
Breast (g)	816.02	854.13	15.109	0.0935
WHC (g)	2.29	2.60	0.201	0.2877

LBW = live body weight, HCW = hot carcass weight, CCW = cold carcass weight, WHC = water holding capacity

The interaction effect of “treatment diets x enzyme levels” on carcass characteristics and meat quality parameters is presented in table 4.3. The results showed no significant differences (P > 0.05) for carcass characteristics and meat quality parameters.

Table 4.3 Effect of inclusion level of Sunflower meal x enzyme level on carcass characteristics and meat quality parameters

		Enzyme level			
Parameter	***TRT***	***0***	***500***	***SEM***	***Pr > F***
LBW (g)	Control	2863.08	2808.08	75.595	0.2039
	SFM25	2705.92	2743.67		
	SFM50	2855.42	2906.75		
	SFM75	2735.17	3010.17		
HCW (g)	Control	2015.92	1959.58	61.217	0.1013
	SFM25	1969.58	1920.00		
	SFM50	2078.67	2158.00		
	SFM75	1973.50	2205.08		
CCW (g)	Control	1980.25	1923.50	60.281	0.1228
	SFM25	1920.92	1897.00		
	SFM50	2024.25	2111.75		
	SFM75	1925.92	2151.67		
PH	Control	5.57	5.50	0.102	0.5815
	SFM25	5.66	5.50		
	SFM50	5.58	5.68		
	SFM75	5.52	5.58		
Drumstick (g)	Control	260.83	252.75	8.992	0.2859
	SFM25	256.58	253.83		
	SFM50	273.42	267.00		
	SFM75	260.42	284.17		
Thigh (g)	Control	301.00	286.33	9.304	0.3745
	SFM25	281.25	278.58		
	SFM50	302.75	312.92		
	SFM75	286.58	303.08		
Wing (g)	Control	198.83	190.33	6.218	0.5676
	SFM25	203.58	209.08		
	SFM50	202.42	204.67		
	SFM75	195.00	203.25		
Breast (g)	Control	831.50	801.00	30.217	0.2828
	SFM25	801.00	839.25		
	SFM50	839.83	928.75		
	SFM75	791.75	847.50		
WHC (g)	Control	2.50	2.75	0.402	0.6111
	SFM25	2.50	2.33		
	SFM50	2.17	3.08		
	SFM75	2.00	2.25		

TRT = treatment diet, SFM = sunflower meal, LBW = live body weight, HCW = hot carcass weight, CCW = cold carcass weight, WHC = water holding capacity

4.4.2 Internal organs

Results of SFM inclusion rate in broiler diets on relative organ weights (liver, spleen, heart, gizzard, small intestine, and large intestine) and intestinal length (small intestine and large intestine) are shown in Table 4.4. Results showed no significant (P > 0.05) effect on relative organ weights and intestinal length for birds fed varying levels of SFM.

Table 4.4: Effect of inclusion level of Sunflower meal on internal organs

	TRT					
Parameter	***Control***	***SFM25***	***SFM50***	***SFM75***	***SEM***	***Pr > F***
Liver (g)	54.54	59.54	56.58	54.08	1.919	0.2168
Spleen (g)	1.94	2.63	2.70	2.74	6.024	0.1675
Heart (g)	13.71	13.75	13.54	13.46	0.771	0.9921
Full gizzard (g)	66.67	63.71	66.92	75.21	3.247	0.1144
Empty gizzard (g)	48.17	46.79	48.75	49.83	2.097	0.7791
SI length (m)	1.87	1.92	1.96	1.95	0.034	0.2153
SI weight (g)	121.75	123.63	117.46	119.83	5.305	0.8618
LI length (cm)	8.26	8.90	9.45	8.33	0.392	0.1530
LI weight (g)	3.26	3.40	3.96	3.81	0.281	0.2880

TRT = treatment diet, SFM = sunflower meal, SI = small intestine, LI = large intestine

Data on enzyme inclusion rate on internal organs is presented on table 4.5. Enzyme supplementation did not significantly affect internal organ weights and length of the intestines. Although birds fed varying levels of SFM supplemented with kemzyme ® showed numerically heavy weights for all internal organs and longer intestinal lengths than those not supplemented with an enzyme, the differences were however not significantly different.

Table 4.5 Effect of enzyme level on internal organs

	Enzyme level			
Parameter	***0***	***500***	***SEM***	***Pr > F***
Liver (g)	55.42	56.94	1.357	0.4396
Spleen (g)	6.55	7.17	4.260	0.9200
Heart (g)	13.13	14.10	0.545	0.2225
Full gizzard (g)	65.60	70.65	2.296	0.1400
Empty gizzard (g)	46.35	50.42	1.483	0.0706
SI length (m)	1.93	1.92	0.024	0.7386
SI weight (g)	116.75	124.58	3.751	0.1592
LI length (cm)	8.66	8.81	0.277	0.7086
LI weight (g)	3.43	3.79	0.199	0.2169

SI = small intestine, LI = large intestine

The interaction effect of treatment diets x enzyme levels on weights of internal organs and length of intestines is presented in table 4.6. The results showed no significant differences ($P > 0.05$) for internal organs. Although no significant differences were observed ($P > 0.05$), birds fed diets supplemented with 500g/ton enzyme level generally yielded better results. This means that enzyme supplementation generally has a positive impact in internal organs weights.

Table 4.6 Effect of inclusion level of Sunflower meal x enzyme level on internal organs

		Enzyme level			
Parameter	***TRT***	***0***	***500***	***SEM***	***Pr > F***
Liver (g)	Control	50.42	58.67	2.714	0.0964
	SFM25	58.50	60.50		
	SFM50	59.83	53.33		
	SFM75	52.92	55.25		
Spleen (g)	Control	1.85	2.21	8.519	0.9997
	SFM25	2.49	2.77		
	SFM50	2.70	2.69		
	SFM75	2.49	3.00		
Heart (g)	Control	13.17	14.25	1.091	0.9941
	SFM25	13.42	14.08		
	SFM50	12.92	14.17		
	SFM75	13.00	13.91		
Full gizzard (g)	Control	65.83	67.50	4.591	0.0893
	SFM25	59.67	67.75		
	SFM50	70.50	63.33		
	SFM75	66.42	84.00		
Empty gizzard (g)	Control	44.67	51.67	2.966	0.1026
	SFM25	44.00	49.58		
	SFM50	51.50	46.00		
	SFM75	45.25	54.42		
SI length (m)	Control	1.84	1.89	0.048	0.3243
	SFM25	1.90	1.94		
	SFM50	2.01	1.91		
	SFM75	1.97	1.93		
SI weight (g)	Control	109.58	133.92	7.503	0.3184
	SFM25	120.50	126.75		
	SFM50	119.58	115.33		
	SFM75	117.33	122.33		
LI length (cm)	Control	8.02	8.50	0.555	0.0888
	SFM25	8.14	9.67		
	SFM50	9.39	9.50		
	SFM75	9.08	7.57		
LI weight (g)	Control	3.04	3.48	0.397	0.8456
	SFM25	3.08	3.73		
	SFM50	3.75	4.17		
	SFM75	3.83	3.78		

TRT = treatment diet, SFM = sunflower meal, SI = small intestine, LI = large intestine

4.5. Discussion

4.5.1 Carcass characteristics and meat quality parameters

The results indicates that HCW was significantly influenced ($P < 0.05$) by SFM inclusion in treatment diets. HCW of birds fed treatment diet containing SFM50 and SFM75 were significantly higher. The results of this study are in agreement with Rehman *et al*. (2002) who observed a significant ($P < 0.05$) difference in carcass weight of birds fed different levels of SFM. Similar findings were observed by Khan *et al*. (2006) who reported that dressing percentage of birds was improved ($P < 0.05$) in sunflower-corn based diets. Significant differences ($P < 0.05$) in HCW and a lack thereof in CCW could be attributed to differences in drip loss.

LBW, CCW and meat portions were not significantly ($P > 0.05$) affected by treatment diets. These results are in agreement with those of several studies: Horvatovic *et al.* (2015), Moghaddam *et al*. (2012), Aroújo *et al*. (2011), Mikulec *et al.* (2004), and El-Sheriff *et al*. (1997) who observed that SFM inclusion rate had no significant effect on carcass characteristics and cut yields. Although there was significant differences ($P > 0.05$) observed, birds fed diets supplemented with 50% SFM showed heavier weights for LBW, CCW, drumstick, thigh, and breast while pH values and WHC were high in the SFM50 treatment diet. These results demonstrate that SFM can be included in broiler rations.

Conflicting results were reported by Rehman *et al.* (2002) who observed carcass weights to be significantly ($P < 0.05$) higher in birds fed control diets than diets with SFM inclusion, they observed the lowest carcass weights in diets with 15% SFM

inclusion rate. Anuradha and Roy (2015) also observed thigh muscle to be significantly higher ($P < 0.05$) in birds fed SFM based diets compared to the control group.

Furthermore, Oliveira *et al.* (2016) observed wing yields to have linearly increased ($P<0.05$) with increasing levels of SFM. Differences in carcass weights can be attributed to differences in amino acid profile or biological value of the different oilseed meals used (SFM and SBM) (Rehman *et al.,* 2002).

The current study also showed that SFM inclusion rate had no significant effect ($P > 0.05$) on ultimate pH of the cold carcass and WHC. The values for ultimate pH ranged from 5.54 to 5.63. This is well within the ultimate range of 5.3 to 6.5 post-slaughter. The results are in agreement with Kalmendal *et al.* (2011) who observed the pH of meat samples to be 6.54 and was not affected by treatment diets. The values for WHC ranged from 2.13 g to 2.63 g among the treatment diets. Tested on Turkey, Laudadio *et al.* (2013) also observed no significant differences on meat pH and WHC among the dietary treatment groups.

The pH reached after the muscle is in rigor influences the WHC and/or drip loss of meat. Poultry meat containing high ultimate pH (≥ 6.3) negatively affects meat colour and texture, influencing meat colour to be dark and the surface to be relatively firm and dry, indicating a very high WHC (Mir *et al.,* 2017). On the other hand, relatively low ultimate pH (5.4 - 5.3) can result in meat that has relatively greater drip loss than product with a normal ultimate pH (5.6-5.8) (Lonergan *et al.,* 2001), this may be due to the presence of a high level of glycogen in the muscle.

Water holding capacity refers to the ability of the meat to retain its inherent moisture even though external pressures (gravity, heating, centrifuging, fabrication, processing and storage) are applied to it (Honikel & Hamm, 1994; Honikel, 1998; Bowker & Zhuang, 2016). WHC has a direct bearing on meat tenderness, colour, yield and quality and is one of the most important functional properties of raw meat (Mir et al., 2017). Poor WHC in poultry meat results in poor visual appeal and meat that is not palatable (acceptable) to consumers. Poor WHC also reduces ingredient retention, protein functionality, and product yields for processors. Broiler breast muscles (pectoralis major) are comprised of nearly 100% fast-twitch glycolytic muscle fibers making them particularly susceptible to undergoing a rapid postmortem pH decline and exhibiting inferior WHC characteristics (Bowker & Zhuang, 2016).

Data on enzyme effect on carcass traits and meat quality was not significant ($P > 0.05$). These results demonstrate that enzyme supplementation did not have any adverse effect on the yield and quality of meat. The results of the present study are in agreement with Horvatic *et al.* (2015) who observed that enzyme supplementation had no significant effect ($P > 0.05$) on carcass traits. Kocher *et al.,* (2000) Raza *et al.,* (2009) along with Mursi and Mukhtar (2015) further reported that inclusion of varying levels of SFM in broiler diets supplemented with commercially available enzymes has no negative effects on carcass yield of broilers.

Results from this present study are not in agreement with findings of Khan *et al.* (2006) who observed that birds fed diets without enzyme supplementation have lower carcass weights compared to those supplemented with enzymes. Working with rice hulls (RH) and wheat pollard (WP), Hartini and Purwaningsih (2017) also observed that addition of a single enzyme or combination of enzymes resulted in significantly ($P > 0.05$) higher

carcass weights than the control group. These results show that enzyme supplementation improved carcass characteristics. However, Oliveira *et al.* (2016) observed that thigh and leg yields were higher in birds fed the non-supplemented diet than those fed the diets containing exogenous enzymes at different SFM inclusion rates.

The current study did not establish any significant interaction ($P > 0.05$) between SFM inclusion rate and enzyme level, however, high values and higher weights of carcass and commercial cuts were observed in birds fed treatment diets with high SFM inclusion rate (50% and 75%) supplemented with enzyme. These results are in agreement with Tavernari *et al.* (2008), Sredanovic *et al.* (2012), as well as Horvatovic *et al.* (2015) who observed no significant differences ($P > 0.05$) of SFM inclusion rate and enzyme supplementation on carcass traits. Contradicting results, however, were observed by Oliveira *et al.* (2016) who observed thighs and legs to be significantly influenced by some interaction ($P<0.05$) between SFM levels and enzyme supplementation.

4.5.2 Internal organs

No statistical difference ($P > 0.05$) was observed on relative organ weight and intestinal length. Although there was no statistical significance in the differences, birds fed diets supplemented with exogenous enzymes had heavier weights for internal organs and long large intestines compared to diets without enzyme supplementation. These results are in line with Ozturk (2017) who observed that treatment diets did not affect ($P > 0.05$) internal organs. The results also agree with published reports on quails (Moghaddam *et al*., 2012) and on broilers (Raza *et al*., 2009; Salari *et al.,* 2009).

Conflicting results were observed by Solangi *et al.* (2002) also observed by Amerah *et al.* (2015) who perceived significant differences ($P < 0.05$) of gizzard weight among treatment diets. Salari *et al*. (2009) observed that liver weight was decreased significantly ($P < 0.05$) in birds fed SFM based diets compared to those fed control diets, however, gizzard and gastrointestinal tract (GIT) were not affected by inclusion of SFM.

These can be attributed to the nature of fat in SFM, which comprises of unsaturated FA's (particularly linoleic acid) since FA's prevents fat accumulation in the liver (Salari *et al.,* 2009). Senkoylu & Dale (2006) also observed that treatment diets demonstrated significant ($P < 0.05$) effect on digestive organ measurements (an increase in gizzard weight and large intestine length). According to the authors, this is because insoluble fibre (NSP) accumulates in the gizzard and is retained longer than other nutrients because it has to be ground to a critical particle size (stimulating gizzard function) prior to entering the small intestine.

Data on enzyme effect showed no significant differences ($P > 0.05$) on internal organs. Neither diets with or without enzyme supplementation influenced the weights of edible internal parts and the length of the GIT. Although enzyme supplementation showed no significant differences on internal organs, enzyme supplementation increased the weights of internal organs and length of intestines. As in the present study, Alagawany *et al.* (2017), Mohammed & Mukhtar (2017) and Ozturk (2017) observed no significant differences in internal organ weights and length of intestines to be affected by enzyme supplementation. On the contrary, Amerah *et al.* (2015) and Khan *et al.* (2006) observed treatment diets without enzyme supplementation to have significantly higher weights of the proventriculus and gizzard.

The current study observed no significant differences (P > 0.05) on interaction effect of SFM inclusion and enzyme supplementation. SFM inclusion rate nor enzyme supplementation did not have any impact of internal organ weights and intestinal length, however, control diets and treatment diets with 25% and 75% SFM supplemented with enzymes increased weights of liver, spleen, heart, gizzard and small intestines. This is in line with Amerah *et al.* (2015) who observed no interaction (P > 0.05) between SFM and enzyme supplementation on gizzard weights and Mohammed and Mukhtar (2017) on the weights of liver and heart. Ozturk (2017) also observed no significant differences (P > 0.05) on edible inner organs. This data is in contrast with that obtained by El-katcha *et al.* (2017) who observed that inclusion of SFM with or without enzyme supplementation improved liver, heart, gizzard, proventiculus weights when compared with the control group.

4.6 Conclusion

The results from the current study showed that carcass yield and cuts, internal organs and meat quality parameters of broilers fed varying levels of SFM with enzyme supplementation compared well with those from birds fed the control diet as there were no significant differences. It can therefore be concluded that inclusion of SFM at higher levels (up to 75%) supplemented with enzyme to replace SBM can be used in broiler rations as it will give the same results as when SBM is used as a sole protein source. The higher inclusion levels of SFM replacing SBM will benefit the farmers as sunflower is much cheaper than SBM. Results from this study suggest that SFM can be included in broiler rations up to 75% SFM with or without enzyme supplementation from day 10 up to 42 days of age.

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CHAPTER 5

THE EFFECT OF DIETARY INCLUSION OF VARYING LEVELS OF SFM IN BROILER DIETS WITH OR WITHOUT ENZYME SUPPLEMENTATION ON SERUM BIOCHEMICAL PARAMETERS OF BROILERS

Abstract

The present study aimed to determine the effect of varying inclusion levels of SFM with or without enzyme supplementation on serum biochemical parameters (albumin, alkaline phosphate, alanine aminotransferase, calcium, cholesterol, gamma-glutamyltransferase, glucose, phosphorus, and total protein). A 4 x 2 factorial treatment arrangement in a completely randomized design was conducted with four levels of SFM replacing SBM (Control, 25%, 50%, and 75%) and two enzyme levels (0 and 500 g/ton). At the end of the 42-day experimental period of the birds used in chapters 3 and 4, two birds were randomly selected from each replicate, totalling 48 birds. Birds were fasted overnight and the blood collected the next morning for serum biochemical parameters analysis. Treatment diet, enzyme level and the treatment diet x enzyme level interaction effect did not significantly influence ($P > 0.05$) any of the serum biochemical parameters. Overall, the results from the current study suggest that replacement of SBM with SFM with enzyme supplementation up to 75% inclusion rate have no adverse effect on the health of broilers. Enzyme did not have effect on serum biochemical parameters.

Keywords: serum biochemical parameters, sunflower meal, factorial design

5.1 INTRODUCTION

Sunflower meal (SFM) has been recognised as a viable feed ingredient in poultry diets for many years (Viveros *et al*., 2009) and can be used to replace soybean meal (SBM). However, its use in poultry diets is limited due to the presence of soluble and un-soluble NSP (lysine and fibre). The increased production and availability at low cost, coupled with high oil content, ether extract (EE) content contributing to high metabolisable energy (ME) per unit or high energy density of feed has made SFM a potentially desirable protein and energy ingredient in poultry diets (Salari *et al*., 2009).

SFM is also reported to be rich in unsaturated fatty acids (FA's) (Malakian, 2010), and trends towards formulating high-energy poultry diets make it necessary to include fats and oils up to 10% in poultry diets (Selvaraj & Purushothaman, 2004) and this has prompted SFM to be evaluated as a protein and energy source (Brenes *et al*., 2008). Newman *et al*. (2002) have reported that high unsaturated FA's reduce fat and cholesterol contents in broilers. Inclusion in poultry diets can be valuable to increase the degree of un-saturation of intramuscular fat without any negative impact on lipid oxidation associated with dietary poly unsaturated FA's (Salari *et al*., 2009).

The reduction of fat and cholesterol content in poultry meat has a much higher consumer acceptance (Malakian, 2010). According to Roche (2001), dietary unsaturated FA's have a positive impact on cardiovascular health, decreasing low-density lipoprotein cholesterol but not high-density lipoprotein cholesterol in blood plasma, and decreasing the susceptibility of low-density lipoprotein to oxidation. In general, dietary saturated FA's increase serum low-density lipoprotein content, whereas dietary polyunsaturated FA's decrease serum very low-density lipoprotein, and

cholesterol concentrations (Viveros *et al.,* 2009). In their study, however, Selvaraj and Purushothaman (2004) observed that the muscle lipid, serum lipid and total and high-density lipoprotein cholesterol contents were not significantly affected by varying SFM inclusion rates.

Although a lot of studies have been conducted on the inclusion of SFM with or without enzyme supplementation on broiler diets, very little studies have been conducted to study the serum biochemical parameters, more so included at very high levels. Therefore, this present study aims to evaluate the effects of various inclusion levels of SFM with or without enzyme supplementation on serum biochemical parameters of broiler chickens.

5.2. MATERIALS AND METHODS

5.2.1 Blood sample collection and analysis

At 6 weeks of age, all birds were fasted for 12 hours prior to being weighed. Two birds per replicate (48 birds) were randomly selected and blood samples collected from the cutaneous ulna using sterile needles and syringes into (5ml) non-heparinized tubes. The blood was then stored for 1 hour at room temperature. Blood samples were then centrifuged at 1 500 rpm for 20 minutes and then stored in 1.5 ml Eppendorf tubes at -20° C pending determination of serum metabolites. The serum metabolites (albumin (ALB), alkaline phosphate (ALKP), alanine aminotransferase (ALT), calcium (Ca), cholesterol (CHOL), gamma-glutamyltransferase (GGT), glucose (GLU), phosphorus (PHOS), total protein (TP) and total bilirubin (TBIL) were analysed using the automated Idexx Vet Test Chemistry Analyser (Gilford Impact 4041E, Ciba Coming Diagnostic Corp., Gilford Systems, Oberlin, OH 44774).

5.3 Statistical analysis

The study took the form of a 4 (SBM levels) x 2 (enzymes) factorial treatment arrangement in a completely randomized design. Variation in serum biochemical parameters were analysed using SAS (2010) software according to the following general linear model:

$$Y_{ijk} = \mu + T_i + E_j + (T \times E)_{ij} + E_{ijk}$$

Where Y_{ijk} = dependent variable (parameters analysed), μ = the overall mean, T_i = treatment diet effect, E_j = enzyme level effect, $(T \times E)_{ij}$ = treatment diet x enzyme level interaction effect and E_{ij} = random experimental errors associated with observation.

The significance was tested at $P < 0.05$. The Turkey procedure was used to separate means among levels within a significant factor.

5.4 RESULTS

Data on treatment diet effect on serum biochemical parameters is presented on table 5.1. The results showed that inclusion of SFM in treatment diets had no significant ($P > 0.05$) effect on serum biochemical parameters (ALB, ALKP, ALT, Ca, CHOL, GGT, GLU, PHOS, TP and TBIL). The results on albumin (ALB), alkaline phosphatise (ALKP), alanine aminotransferase (ALT), calcium (CA), cholesterol (CHOL), creatinine (CREA), gamma-glutamyltransferase (GGT) glucose (GLU), phosphorus (PHOS), total protein (TP) and total bilirubin (TBIL) showed no significant difference ($P > 0.05$) as affected by enzyme inclusion level as shown in table 5.2.

Table 5.1 Effect of inclusion level of Sunflower meal on serum biochemical parameters

	TRT					
Parameter	***Control***	***SFM25***	***SFM50***	***SFM75***	***SEM***	***Pr > F***
ALB (g/L)	12.25	13.25	12.00	13.50	0.433	0.1023
ALKP (U/L)	190.25	150.00	221.00	218.50	27.795	0.3084
ALT (U/L)	17.50	17.50	15.00	15.50	2.391	0.8230
CA (mmol/L)	1.94	1.81	1.75	1.85	0.107	0.6517
CHOL (mmol/L)	3.02	3.16	2.82	2.81	0.311	0.8285
GGT (U/L)	19.75	18.50	20.25	23.25	3.314	0.7774
GLU (mmol/L)	10.05	9.15	9.64	9.04	0.424	0.3695
PHOS (mmol/L)	1.78	.1.74	1.59	1.69	0.069	0.3014
TP (g/L)	31.75	34.50	22.00	29.25	4.153	0.2501
TBIL (mmol/L)	3.50	8.75	4.50	4.75	1.436	0.0772

TRT = treatment, SFM = sunflower meal, ALB = albumin, ALKP = alkaline phosphatase, ALT = alanine aminotransferase, Ca = calcium, CHOL = cholesterol, GGT = gamma-glutamyltransferase, GLU = glucose, PHOS = phosphorus, TP = total protein, TBIL= bilirubin, g/L = gram/litre, U/L = international unit/litre, mmol/L = micromole/litre

Table 5.2 Effect of enzyme level on serum biochemical parameters

	Enzyme level			
Parameter	***0***	***500***	***SEM***	***Pr > F***
ALB (g/L)	12.50	13.00	0.306	0.2815
ALKP (U/L)	217.63	172.25	19.654	0.1412
ALT (U/L)	14.13	18.63	1.691	0.0966
CA (mmol/L)	1.93	1.74	0.075	0.1235
CHOL (mmol/L)	2.84	3.06	0.220	0.5066
GGT (U/L)	22.75	18.13	2.344	0.2004
GLU (mmol/L)	9.39	9.55	0.300	0.7179
PHOS (mmol/L)	1.74	1.66	0.049	0.3001
TP (g/L)	30.88	27.88	2.937	0.4907
TBIL (mmol/L)	4.75	6.00	1.020	0.3772

ALB = albumin, ALKP = alkaline phosphatase, ALT = alanine aminotransferase, Ca = calcium, CHOL = cholesterol, GGT = gamma-glutamyltransferase, GLU = glucose, PHOS = phosphorus, TP = total protein, TBIL= bilirubin, g/L = gram/litre, U/L = international unit/litre, mmol/L = micromole/litre

Data on the interaction between treatment diets x enzyme inclusion level did not significantly affect (P > 0.05) any of the serum biochemical parameters (Table 5.3).

Table 5.3 Effect of inclusion level of Sunflower meal x enzyme level on serum biochemical parameters

		Enzyme level			
Parameter	***TRT***	***0***	***500***	***SEM***	***Pr > F***
ALB (g/L)	Control	12.00	12.50	0.612	0.5957
	SFM25	12.50	14.00		
	SFM50	12.00	12.00		
	SFM75	13.50	13.50		
ALKP (U/L)	Control	212.00	168.50	39.308	0.3194
	SFM25	151.50	148.50		
	SFM50	219.50	222.50		
	SFM75	287.50	149.50		
ALT (U/L)	Control	12.50	22.50	3.382	0.4624
	SFM25	14.50	20.50		
	SFM50	13.50	16.50		
	SFM75	16.00	15.00		
CA (mmol/L)	Control	2.05	1.84	0.151	0.6760
	SFM25	1.87	1.75		
	SFM50	1.94	1.56		
	SFM75	1.86	1.84		
CHOL (mmol/L)	Control	2.81	3.23	0.440	0.7253
	SFM25	2.92	3.40		
	SFM50	3.02	2.62		
	SFM75	2.63	3.00		
GGT (U/L)	Control	19.50	20.00	4.687	0.7799
	SFM25	22.00	15.00		
	SFM50	22.00	18.50		
	SFM75	27.50	19.00		
GLU (mmol/L)	Control	10.92	9.18	0.600	0.1479
	SFM25	8.86	9.45		
	SFM50	9.02	10.26		
	SFM75	8.77	9.31		
PHOS (mmol/L)	Control	1.95	1.62	0.097	0.2210
	SFM25	1.68	1.80		
	SFM50	1.62	1.56		
	SFM75	1.71	1.67		
TP (g/L)	Control	36.50	27.00	5.874	0.3403
	SFM25	31.00	38.00		
	SFM50	20.50	23.50		
	SFM75	35.50	23.00		
TBIL	Control	4.00	3.00	1.658	0.1222
	SFM25	5.00	12.5		
	SFM50	5.00	4.00		
	SFM75	5.00	4.00		

TRT = treatment, SFM = sunflower meal, ALB = albumin, ALKP = alkaline phosphatase, ALT = alanine aminotransferase, Ca = calcium, CHOL = cholesterol, GGT = gamma-glutamyltransferase, GLU = glucose, PHOS = phosphorus, TP = total protein, TBIL= bilirubin, g/L = gram/litre, U/L = international unit/litre, mmol/L = micromole/litre

5.5 DISCUSSIONS

In the present study, the effects of various levels of SFM had no significant effect (P > 0.05) on serum biochemical parameters. ALT tended to be lower and phosphorus and total protein were higher in treatment diets with low SFM inclusion rate (control and SFM25), however this effect was not significant. Although there was no significant difference, ALKP and GGT numerically showed high content whilst cholesterol was low in diets with high SFM inclusion rate. Low cholesterol is appealing to consumers because high cholesterol in food is a major health risk causing heart diseases (Laudadio *et al.,* 2015), therefore SFM inclusion in broiler diets can be beneficial as the effect of fibre present in SFM caused a reduction in cholesterol (Baghban-kanani *et al.,* 2018) and this may be due to the ability to enhance faecal excretion of cholesterol and bile acids (Sarikhan *et al.,* 2009). The results showed no significant difference (P > 0.05) observed in ALT, and therefore this means that treatment diet has no detrimental impacts on the liver of birds (Abdallah *et al.,* 2016).

The present results are in line with the findings of Selvaraj and Purushothaman (2004) in addition to those of Malakian (2010) who reported that serum lipid, total and high-density lipoprotein (HDL) cholesterol contents were not significantly influenced by the level of SFM inclusion rate. Salari *et al*. (2009) as well as Alagawany *et al.* (2017a) also observed that serum biochemical parameters such as glucose, total cholesterol, low-density lipoprotein (LDL), HDL, ALKP, protein, calcium and phosphorus were not significantly affected by the level of SFM inclusion rate.

A similar trend was reported by Moghaddam *et al.* (2012) along with Rabie *et al*., (2013) who observed that treatment diet had no significant effect on blood parameters (total protein, albumin, globulin, cholesterol, triglycerides and activity of AST and ALT in blood plasma), however, they reported that SFM inclusion in broiler diets significantly ($P < 0.05$) increased phosphorus and glucose levels.

Conflicting results were observed by Brenes *et al*. (2008) who observed that the effects of SFM inclusion decreased cholesterol and glucose ($P < 0.01$) concentrations by 20% and 15% respectively; and enzyme supplementation increased calcium and total protein levels by 12% and 15% respectively compared to those fed control diets. The authors also observed SFM based diets increased plasma uric acid, calcium, lactate dehydrogenase (LDH), creatine phosphokinase (CPK) and total protein concentrations in serum. In their study, Viveros *et al.* (2009) also observed SFM inclusion to have some effect on cholesterol, triglycerides and lipoprotein. Conflicting results may be due to various inclusion levels of SFM and the fibre content of the diet (Alagawany *et al.,* 2017a).

The effect of enzyme inclusion rate on serum biochemical parameters showed no significant differences ($P > 0.05$). Total protein, phosphorus and calcium were numerically higher in treatment diets without enzyme supplementation, however, cholesterol and glucose showed high values in birds fed diets with enzyme inclusion, even though there was no significant difference. Similar results were observed by Ciurescu *et al.* (2019) who observed that enzyme (phytase) addition had no significant differences ($P > 0.05$) on ALT, GGT and liver health. However, on the contrary Viveros *et al.* (2002) observed that enzyme addition increased ($P < 0.05$) protein level whilst reducing ($P < 0.05$) ALKP, ALT and calcium. Alagawany *et al.* (2017a) also observed

enzyme addition to have significantly ($P < 0.01$) decreased in all serum biochemical parameters except for serum globulin. Working on layers, conflicting results were observed by Baghban-kanani *et al.* (2018) who observed that total cholesterol increased significantly ($P < 0.05$) in laying hens fed diets supplemented with exogenous enzymes at the rate of 250g/ton.

There was no significant difference ($P > 0.05$) among the treatments because of the interaction effects between treatment diet and enzyme interaction on serum biochemical parameters (table 5.3). Generally, high concentrations of ALT, cholesterol and glucose were observed for all treatment diets supplemented with enzyme, however, there was no significant interaction effect observed. The results of the present study are in agreement with Ciurescu *et al.* (2019) who showed that neither SFM nor enzyme inclusion in broiler diets had any significant effect ($P > 0.05$) on liver health.

Contradicting results, however, were observed by Alagawany *et al.* (2017b) who showed that the interaction between SFM inclusion rate and enzyme supplementation significantly ($P < 0.01$) influenced serum biochemical parameters. In their study, birds fed diets with 50% SFM inclusion rate without enzyme supplementation had the highest concentration of ALT, TP, albumin, cholesterol and triglyceride, whilst high concentration of globulin and aspartate transaminase (AST) were observed in birds fed control diets without enzyme and 75% SFM inclusion rate with enzyme supplementation respectively.

5.6 CONCLUSION

Inclusion of SFM in broiler diets with or without enzyme supplementation had no significant effect on serum biochemical parameters. These findings indicate that SFM can be used as an alternative dietary protein source up to 75% inclusion rate in commercial broiler diets without any negative effects on the health of broiler chickens.

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CHAPTER 6

GENERAL DISCUSSION, CONCLUSION AND RECOMMENDATIONS

6.1 General conclusion

The study was carried out to evaluate the influence of enzyme supplementation on growth performance, carcass characteristics, meat quality and serum biochemical parameters of broiler chickens fed diets of varying levels of SFM as a replacement of SBM as a protein source. A 4 (SFM (0%, 25%, 50%, and 75%) x 2 (0% and 500g/ton) factorial arrangement in CRD was used resulting in eight iso-nitrogenous and iso-caloric grower and finisher treatment diets.

In chapter 3, the study hypothesized that treatment diets would have an impact on AFI, AWG and FCR. The study failed to reject the hypothesis and can confirm that treatment diet significantly influenced AFI ($P < 0.05$), birds fed treatment diets with high SFM inclusion rate significantly ($P < 0.05$) had high AFI compared to the control group. However, AWG and FCR were not significantly influenced ($P > 0.05$) by treatment diets. Although there were no significant differences ($P > 0.05$), it was however observed that the higher the SFM inclusion level, the higher the weight gained. Enzyme supplementation did not have any significant effect ($P > 0.05$) on growth performance parameters. Two-way interaction (SFM inclusion rate and enzyme supplementation) was observed on AFI, whereby birds fed treatment diets with high SFM inclusion rate (50% and 75%) supplemented with enzymes had the highest AFI and the least AFI was observed in birds fed SFM50 without enzymes, those fed SFM25 with and without enzyme supplementation as well as those fed on the control diet supplemented with enzymes. These findings conclude that SFM can be utilized as an alternative protein

source and incorporated in commercial grower and finisher broiler diets with no adverse effects on growth performance parameters.

The hypothesis tested that treatment diets would significantly influence carcass characteristics, internal organs and meat quality parameters of broiler chickens. It was evident in chapter 4 that the study could not accept the hypothesis and demonstrated that treatment diets without or with enzyme supplementation and their interactions had no significant influence ($P > 0.05$) on meat quality parameters (WHC and pH), internal organs (liver, heart, spleen, gizzard and intestines), meat yield (drumstick, wings, breast, and thighs), LBW and CCW. Birds fed treatment diet with 50% SFM inclusion rate numerically had heavier weights of carcass and meat quality values, although treatment diet had no significant effect on these parameters.

No significant difference ($P > 0.05$) was observed for internal organs; however, birds fed diets with enzyme supplementation showed higher weights for internal organs whilst intestinal length was longer in birds fed treatment diets without enzyme supplementation. This proves the ability of enzymes to hydrolyze large molecular compounds to a form that can be utilized by animals, improving the performance and health of broiler chickens.

HCW was significantly influenced ($P < 0.05$) by treatment diet, and this could be attributed to differences in drip loss. This signifies that inclusion of SFM affected the weight of the carcasses post-slaughter. At high SFM inclusion rates (50% and 75%), birds obtained ($P < 0.05$) higher weights compared to birds fed diets with low SFM inclusion rate. These results conclude that SFM can be used up to 75% inclusion rate

with or without enzyme supplementation without hampering carcass traits, internal organs and meat quality parameters.

The results for serum biochemical parameters (chapter 5) showed that treatment diets, enzyme supplementation and the two-way interaction (diet and enzyme supplementation) had no significant influence ($P > 0.05$) on serum biochemical parameters ((albumin (ALB), alkaline phosphate (ALKP), alanine aminotransferase (ALT), calcium (Ca), cholesterol (CHOL), gamma-glutamyltransferase (GGT), glucose (GLU), phosphorus (PHOS), and total protein (TP)). The study rejects the hypothesis and confirms that the treatment diets did not affect serum biochemical parameters of broiler chickens. This signifies that the health of broiler chickens was not negatively influenced by inclusion of SFM with or without enzyme supplementation.

Growth performance, carcass characteristics, meat quality, internal organs and serum biochemical parameters of broiler chickens fed varying levels of SFM with or without enzyme inclusion compares well with those of birds fed the control diet. It can therefore be concluded that inclusion of SFM at higher levels to replace SBM can be used in broiler rations as it will give the same results as when SBM is used as a sole protein source.

6.2 Recommendations

SFM is a good source with a very high nutritive value and it is a by-product of sunflower oil extraction. It is widely available and often underutilized in South Africa and is a cheap source of protein compared to SBM. Results from this study suggest that SFM can be included in broiler rations up to 75% with or without inclusion of Kemzyme ® from day 10 up to 42 days of age and this will not affect growth performance, carcass characteristics, internal organs, meat quality and serum biochemical parameters of broilers. The higher inclusion levels of SFM replacing SBM will benefit the farmers and ultimately improve the profitability of the poultry enterprise. It is suggested that SFM be incorporated into the broiler production systems and therefore, producers be capacitated on the proper inclusion rates of this alternative protein source.

6.3 Further research recommendations

- It is imperative to determine the nutrient utilization (digestibility) and hematological parameters of SFM in broilers.
- Effect of SFM inclusion rate with or without enzyme supplementation on pellets strength and durability.