EFFECTS OF DIETARY PRICKLY PEAR (*OPUNTIA FICUS INDICA L.)* SEED CAKE MEAL INCLUSION IN A MAIZE-COWPEA DIET ON ROSS 308 BROILER PERFORMANCE AND CARCASS CHARACTERISTICS

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Declaration

I, Thuso Netili of student number 11630050, hereby declare that this dissertation for a Master of Science in Agriculture in Animal Science (AGMAAS) is submitted to the Department of Animal Science, Faculty of Science, Engineering & Agriculture, at the University of Venda has not been submitted previously for any degree at this or another university. It is original in design and in execution, and all reference material contained therein has been duly acknowledged.

Signed (Student): .... Date: ....27/02/2023...............

Dedication

This dissertation is dedicated to my parents, wife, and children for their LOVE and all the SUPPORT.

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My initial and profound gratitude is to God, who saw me through my graduate studies. To Him alone I give all Glory and Honour, now and forever.

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Abstract

The aim of the study was to investigate the effects of graded inclusion of prickly pear oil seed cake (PPSC) in sprouted cowpea-maize diets for broilers. Diluent (0%PPSC) sprouted cowpea-maize grower and finisher diets, and the respective (10% and 12.5% crude fibre on DM basis) iso-nutrient, PPSC “’summit” grower and finisher diets were formulated and blended to constitute the test diets. Blended grower test diets contained calculated 0, 2.5, 5, 7.5, 8.75 and 10% PPSC, respectively denoted G_0, G_1, G_2, G_3, G_4, and G_5. Blended finisher test diets contained calculated 0, 3.1255, 6.25, 9.375, 10.9375%, 12.5% PPSC, respectively denoted F0, F1, F2, F3, F4, F5. Dietary nutrient profiles were benchmarked to respective commercial grower and finisher maize-soybean positive control (PC) diets. The trial used 504 Ross 308 broiler chicks reared in an open, deep litter house partitioned into 1.5 m long x 1.4 m wide steel framed, mesh wire pens, each holding 18 birds. Chicks fed on the same commercial starter (days 1-24) diet, after which they were assigned to grower (days 25-35), followed by finisher (days 36-42) experimental diets for a completely randomised experiment replicated four times. Birds had free access to feed and water. Feed intake (FI), live weight gain (LWG), and the feed conversion ratio (FCR) were evaluated, along with slaughter weight, carcass characteristics, visceral organ weights, and meat quality. Quadratic regression analysis revealed significant effects of dietary PPSC levels on grower phase intake ($P = 0.044$) and cumulative grower-finisher live weight gain ($P = 0.04$). During the grower phase, feed intake increased ($P < 0.05$) with PPSC inclusion until it matched the control diet at PPSC dietary level G3 and above ($P > 0.05$). The optimum dietary inclusion of PPSC for feed intake during the grower phase was estimated to be 1.74%. For the finisher phase, broilers on the F_0-F_2 PPSC inclusion levels had lower final (42-day) live weights compared to the control ($P < 0.05$). Live weight at and above F_3 PPSC dietary inclusion was intermediate, similar to both the lower level PPSC dietary inclusion and the control ($P > 0.05$). A quadratic estimate of 4.58% dietary optimum PPSC inclusion was predicted for the cumulative live weight gain. Dressed carcass weight increased ($P < 0.05$) with dietary PPSC inclusion at and above the G_3-F_3 PPSC feeding regime, matching the control diet ($P > 0.05$). A quadratic estimate of 3.01% dietary optimum PPSC inclusion was predicted for carcass weight. The dressing percentage increased ($P < 0.05$) with dietary PPSC inclusion above the G_2-F_2 PPSC feeding regime, matching the control diet ($P > 0.05$). A quadratic estimate of 7.58% dietary optimum PPSC inclusion was predicted for dressing percentage. Broilers on the G_5-F_5 feeding regime had higher ($P < 0.05$) abdominal fat compared to those on no or lower PPSC feeding regimes, while broilers on the G_4-F_4 feeding regime had intermediate abdominal fat ($P < 0.05$). A quadratic estimate of 4.73% dietary optimum PPSC inclusion was predicted for abdominal fat. Quadratic regression analysis also showed significant effects of PPSC levels on scaled gizzard weight ($P = 0.007$). The optimal dietary inclusion of PPSC for scaled gizzard weight was estimated to be 4.39%. In conclusion, within the limitations of the recommended dietary fibre content, grower-finisher dietary PPSC inclusion upgraded the sprouted cowpea diets to match the standard diet in terms of grower phase feed intake, finisher phase live weight gain, slaughter weight, abdominal fat, and the dressing percentage, with the predicted optimum dietary inclusion level dependent on the broiler response variable. Based on the carcass yield, approximately 3 % was considered optimum dietary PPSC inclusion in sprouted-cowpea based broiler diets.

Keywords: *broiler chickens, prickly pear, oil seed cakes*

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ABBREVIATIONS AND ACRONYMS

ADG - Average Daily Gain

AOAC - Association of Official Analytical Chemist

BFAP - Bureau for Food and Agricultural Policy

CF - Crude Fiber

CP - Crude Protein

CPI - Centurion Poultry Incorporation

DAFF - Department of Agriculture, Forestry and Fisheries

DM - Dry Matter

FAOSTAT – Food and Agriculture Organization Corporate Statistical Database

FCR - Feed convention ratio

FOA - Food and Agriculture Organization

ha - Hectare.

MDM - Mechanically Deboned Meat

ME - Metabolizable Energy

NRC - Nutrient Requirement Council

PPP - Prickly Pear Powder

SAPA - South African Poultry Association

CHAPTER 1

INTRODUCTION

1.1. BACKGROUND

Broiler feeding accounts for approximately 60%-70% of variable costs (Adino *et al*., 2018). This means profitability is most dependent on efficient feeding management (Al-Sagheer and Naiel, 2019). Legume and cereal grains are the respective main dietary protein and energy sources for broilers (Nalluri *et al*., 2021; Ciurescu *et al*., 2022). These ingredients have for long become increasingly scarce and expensive (Shi, 2012). South Africa is a net importer of animal feeds (Ncube, 2016). The imported feed inflation increases the price of poultry meat products. Therefore, the use of potentially cheaper and more readily available non-conventional substitutes needs concerted investigation.

The cowpea (V*igna unguiculata*) is a potential commercial feed legume which is traditionally widely grown in the small-holder farming sectors in Southern Africa (Hall, 2012). Cowpeas are considered to have relatively good nutritive value for stock feeding (Kirse *et al.,* 2015). Compared to other native grain legumes, cowpeas contain high protein of good quality (Ciurescu *et al*., 2017; Ciurescu and Pana, 2017). Embaye (2018) reported 257.6 g/kg DM crude protein, 62.2 g/kg DM crude fibre, 16.5 g/kg DM fat, 13.8 MJ ME/kg DM, 0.75 g/kg DM tannins and 1.5 g/kg DM. phytates. The cowpea is preferred for its drought tolerance, adaptation to diverse arid agroecological environments, and relatively lower agronomic input costs (Embeye, 2018). Therefore, the cowpea is a climate-smart feed, which can be successfully promoted for large-scale production and inclusion into local feed value chains for sustainable, low-cost feeding. However, compared to soybean, sole cowpea diets may be insufficient (Defang *et al.*, 2008; Chakam *et al.*, 2008; Chakam *et al*., 2010; Embaye *et al*., 2018) for broiler feeding. Though rich in lysine and tryptophan (6.8 and 3.8 g/100g respectively), cowpeas are deficient in methionine and cystine (Chakam *et al.,* 2010; Frota *et al.*, 2017).

Instead of the grain milling by-products ingredients traditionally used to balance least-cost commercial maize-soybean diets, novel, relatively high protein agro-industrial by-product such as prickly pear (*Opuntia ficus-indica* (L.) Mill) oil seed cake (PPSC) might be more economic and better nutritional complements to maize-cowpea diets. Given the abundance of the species in arid and semi-arid regions prickly pear by-products are increasingly considered viable stockfeed alternatives (Cherif *et al*, 2021; Silva *et al.*, 2021). The prickly pear is primarily produced for its fruit, which are sweet and nutritionally rich (Labra, 2003), but is also increasingly utilised as ruminant forage (Orsuna-Martinez, 2014; Cruz-Cansino *et al*., 2015;

Sahoo, 2017; Tadoro and Alabiso, 2020). The fruit can be eaten fresh, or utilized for juice, vinegar, and jam production (Cherif, 2020). Previous findings by Lidetewold *et al*., (2016) and a review by Moula and Humbel (2019) concluded that the entire fruit can be used as broiler feed. The whole seed contains 11.5% ether extract and 12.3% of crude fibre (Kunyanga, 2014). The seed protein is dominated glutelin (60%) and albumins (30%), with more than 24% essential amino acids of the total amino acids (Borchani *et al*., 2021). However, given the high pharmaceutical seed oil value (Karabagias *et al*., 2020), only the oil seed cake can be viably considered for feeding to broilers (Cherif, 2021). Abdel *et al*., (2020) reported 10.7% protein, 46.3% fiber, 4.88% fats and 3.39% ash in prickly pear oil seed cake, while Todaroo *et al.,* (2020) reported 3.36% ash, 8.85% fat, 5.75% crude protein and 57.25% fibre. The variable composition could be due to different cultivars, different harvesting seasons, as well as environmental effects (Matthäus and Özca, 2012). However, the prickly pear seed also contains considerable tannins, oxalates, and phytate (El-Safy, 2012 and Reda, 2019).

In the advent of climate change and its negative implications on future production of stock feeds, diets which are partially or wholly constituted from native legumes and the uniquely chemically complex novel ingredients might become viable options to replace the expensive soybean-maize conventional diet, particularly for low resource small scale poultry producers. Therefore, the objective of the study was to investigate potential complementing effects of graded dietary inclusion of PPSC into cowpea-based grower and finisher diets on broiler performance.

1.2. STATEMENT OF THE RESEARCH PROBLEM

The broiler chicken enterprise is one of the fastest growing livestock enterprises in South Africa, with possibilities of yielding profit in a very short period compared to other livestock sectors. The cost of broiler feeding represents about 65% of the total production cost (Bagopi *et al.,* 2014). The high cost of locally produced chicken meat results in the influx of cheap imported chicken meat. Therefore, there is need to find alternative feed ingredients to reduce the feed cost (Thirumalaisamy *et al.,* 2019. Increasingly, producers may be forced to divert to novel diets which include different, non-conventional diets. Currently, there is growing interest to replace soybean with native legumes such as the cowpea. The prickly pear is climate-smart, potentially low-cost additional substitute ingredient for the increasingly scarce and expensive broiler feed ingredients. Prickly pear seeds contain numerous functional organic compounds, including the antioxidant betaxanthin, phenolic acids and flavonoids, and belatin of proven hypolipidemic and hypoglycemine action (Osorio-Esquivel *et al.,* 2011; Paiz, 2010; Schaffer, 2005), as well as vitamin E, amino acids, and ascorbic acid (Stintzing, 2003). However, prickly pear seed also contain considerable anitinutritional factors such as crude fibre, tannin, oxalate, phytate and protein inhibitors which have detrimental effects if consumed in excessive amounts (Mikiæ, 2009). As a result, there is limited information on the optimum inclusion of the oil seed cake to improve potentially inefficient cowpea-maize broiler diets. Therefore, the aim of the present study was to evaluate the effects of graded inclusion of prickly pear seed cake meal into a maize-cowpea on broiler performance, carcass characteristics and meat quality.

1.3. JUSTIFICATION OF THE STUDY

Escalating broiler feeding costs remain one of the biggest challenges for both commercial and emerging farmers in countries which are net importers of animal feeds. This is because of high cost of conventional feed ingredients such as soybean. It is anticipated that the price of soybean will increase by 2.2% yearly until 2030 (Agralytica, 2012). This challenge leads to a low profit margin, which impedes the small holder farmers to grow up to a level of commercialization. Therefore, using agro-ecologically adaptable, climate resilient unconventional alternative feeds such as cowpea and prickly pear by-products can help to minimize the feed cost for broilers. The study will expand the existing knowledge on the feeding value and complementarity of cowpea and prickly pear seed cake meal as potential low-cost substitute feed ingredients in commercial broiler soybean-maize diets. Cheaper feeding is key to increased productivity by local small-scale farmers, creating more jobs and other downstream business opportunities.

1.4. RESEARCH OBJECTIVE

1.4.1. MAIN OBJECTIVE

The aim of the study was to investigate the responses to graded inclusion of prickly pear (PPSC) oil seed cake into sprouted cowpea-maize grower (G) and finisher (F) diets.

1.4.2. SPECIFIC OBJECTIVES

The specific objective of the study was to evaluate the following broiler responses to graded inclusion of PPSC in maize – cowpea grower and finisher diets;

i. Growth performance (feed intake, feed convention ratio and weight gain).

ii. Carcass (hot dressed) and carcass components (breast, thigh, and wing) characteristics

iii. Abdominal visceral organs/tissues (liver, and gizzard, heart, and fat) weights.

iv. Meat quality (pH, colour, drip loss and water holding capacity).

1.5. RESEARCH HYPOTHESIS

Inclusion of PPSC at incremental levels of up to 10% in grower and 12.5% in finisher diets fed to Ross 308 broiler chickens will not affect the following.

i. growth performance of Ross 308 broiler chickens.

ii. carcass characteristics

iii. abdominal visceral organs/tissues

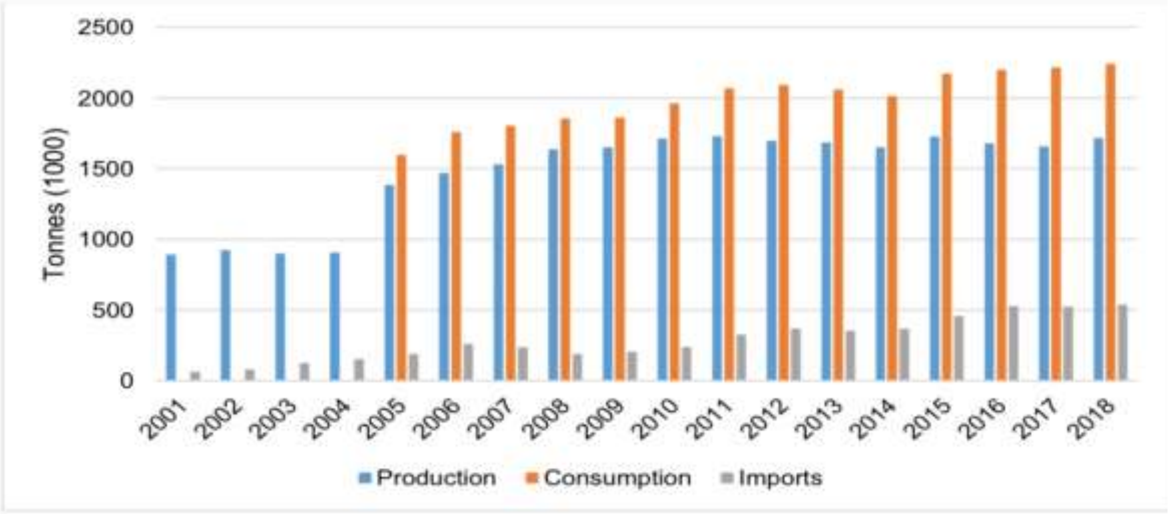
iv. meat quality

CHAPTER 2

LITERATURE REVIEW

2.1. Poultry production in South Africa

Poultry meat is one of the main animal protein sources in many developing (USDA, 2008). Its production is therefore key to the advancement of rural, semi-urban and urban nutrition and economic activity. The poultry industry in South Africa is relatively small with 1.7 million tons of poultry produced in 2018, compared to 2.3 million tons of poultry meat consumption. Poultry meat consumption has been on the rise since 2005, but the growth trend is below the poultry meat needs of the nation (SAPA, 2019). From 2011 to 2018, the deficit between production and demand (Figure 1) has been met by increasing imports from major producing countries like Brazil.



Source: SAPA, 2019

Figure 1: South African chicken production, imports, and consumption.

2.2. Nutrient requirements of broilers

Feed is the main cost in broiler production, representing about 65% of the total cost of producing poultry meat. The broiler diet should be formulated to supply adequate nutrients to allow optimal growth (Aviagen, 2009). The nutrient requirements of poultry depend on the genotype, age, weight, and sex, as well as the purpose of production (eggs or meat), while the nutrient composition and availability is highly variable among feed ingredients, such that correct feed formulation is crucial in obtaining optimal production, to maximise profitability (NRC, 1994).

Chickens need sufficient supply of energy, protein, essential amino acids, essential fatty acids, minerals, vitamins, and most importantly water (Holden, 2018). They get protein, other organic nutrients, and energy from the main organic feed ingredients, with supplementary manufactured minerals, vitamins, and some key essential amino acids such as tryptophan, methionine, threonine, and lysine (Aviagen, 2009)**Error! Bookmark not defined.**. Birds can get energy from simple carbohydrates, protein, and fats (NRC, 1994), with minimal ability to digest the complex carbohydrates which constitute the dietary fibre (McDonald, 2010).

The purpose of dietary protein is to provide amino acids for maintenance, muscle growth and the synthesis of egg protein (Sleman *et al.,* 2015). The synthesis of muscle requires 20 primary amino acids (Guoyao *et al.,* 2014). Ten of these (lysine, methionine, tryptophan, threonine, arginine, isoleucine, leucine, histidine, phenylalanine, and valine) are either not synthesized or are synthesized too slowly to meet the metabolic requirements and are designated as essential (amino acids) elements of the diet (Table 1) (David, 2020). These should be supplied in the diet. Water is the most critical nutrient in poultry production, and the daily supply clean water is essential. Table 1 shows the recommended nutrient requirements for broilers (NRC, 1994),

Table 1: The nutrient requirements for broilers (DM basis).

Nutrients	Unit	Starter 0 -14 days	Grower 15 – 28 days	Finisher >29 days
Metabolizable energy	MJ/kg	13.38	13.38	13.38
Crude Protein	%	23	20	18
Fibre	%	5	5	5
Amino acids				
Arginine	%	1.25	1.10	1.00
Glycine + Serine	%	1.25	1.14	0.97
Histidine	%	0.35	0.32	0.27
Isoleucine	%	0.80	0.73	0.62
Leucine	%	1.20	1.09	0.93
Lysine	%	1.10	1.00	0.85
Methionine	%	0.50	0.38	0.32
Methionine + Cysteine	%	0.90	0.72	0.60
Phenylalanine	%	0.72	0.65	0.56
Phenylalanine + Tyrosine	%	1.34	1.22	1.04
Threonine	%	0.80	0.74	0.68
Tryptophan	%	0.20	0.18	0.16
Valine	%	0.90	0.82	0.70
Fatty Acids				
Linoleic acid	%	1.00	1.00	1.00
Major minerals				
Calcium	%	1.00	0.90	0.80
Chlorine	%	0.20	0.15	0.12
Non-phytate phosphate	%	0.45	0.35	0.30
Potassium	%	0.30	0.30	0.30
Sodium	%	0.20	0.15	0.12
Trace minerals				
Copper	mg	8.0	8.0	8.0
Iodine	mg	0.35	0.35	0.35
Iron	mg	80.0	80.0	80.0
Manganese	mg	60.0	60.0	60.0
Selenium	mg	0.15	0.15	0.15
Zink	mg	40.0	40.0	40.0

Source: (NRC, 1994)

Broilers require nutrients to maintain body tissues and processes in addition to supporting productive purposes, which may include growth, and thus productivity level of broilers certainly affects nutrient requirements (Ferket, 2006). Protein and energy need and, consequently, feed intake varies considerably with environmental temperature and amount of physical activity. A bird's daily need for amino acids, vitamins, and minerals are mostly independent of these factors (NRC, 1994). If a bird consumes a diet that has a higher energy content, it will decrease its feed intake; consequently, that diet must contain a proportionally higher number of amino acids, vitamins, and minerals (Applegate and Angel, 2014). Thus, nutrient density in the ration should be adjusted to provide appropriate nutrient intake based on requirements and the actual feed intake. It is impossible to set the energy requirements in terms of unit/kg diet because birds adjust their feed intake to achieve their daily energy intake (NRC, 2000).

2.3. Broiler feeds

Broiler diets should supply all the required nutrients for growth and maintenance. Nesheim *et al., (1979)* indicated that broiler diets require the select ingredients whose components are most digestible, efficiently absorbed, and utilized. In the broiler diets, cereal grains such as corn, sorghum, barley, and wheat are the primary energy sources, while the protein sources include fishmeal, soybean, meat, and bone meal, with complimentary energy and nutrients from by-product feeds (Emmanuel, 2018). Trace minerals, vitamins and amino acids are also available from synthetic sources on the local market to supplement the nutrients from the natural ingredients.

2.4. Nutritive value of cowpeas

The nutrient profile of cowpeas is presented in Table 2. Henshaw (2008) and Tshovhote *et al.,* (2003) reported crude protein ranging from 25.35 to 26.43%. The different cowpea chemical composition may be due to different varieties, treatment as well as environment factors (Giami, 2005).

Table 2: Chemical and energy composition of cowpeas

Component	Composition
Dry matter (%)	89.9
Crude protein (% DM)	25.75
Crude fibre (% DM)	6.22
Ether extract (% DM)	1.65
Ash (% DM)	4.6
Metabolizable energy (MJ ME/kg)	13.8
Ca (% DM)	0.15
P (%)	0.5
Tannin (g/kg)	0.75
Phytate (g/kg)	1.5

Embaye *et al.* (2018)

2.5. Anti-nutritional factors of cowpea seeds

According to Tshovhote (2003), nutrient composition of cowpea is like that of other plant protein sources such as peas and lentin seeds. Despite being potential source of protein in broiler diets, cowpea has considerable amount of anti-nutritional factors (such as trypsin inhibitors, phenols, and tannins) and mineral chelanting agents (like phytic acid and oxalic acid) and these factors limit its nutritive value and consumption by animals (Shaheen, 2019). The content of tannins and trypsin inhibitors in cowpeas may result in retarded chicken growth as they limit the acceptability and utilization by chickens (Peiretti, 2018). Similarly, Ramakrishna (2008), Ghumman (2016) and Parmar (2017) indicated that the presence of these anti-nutritional factors (ANF) has negative effects on the digestibility and absorption of nutrients by animals. However, several methods such as sprouting, dehulling, cooking or roasting can be used to minimize the effects of the ANF thereby increasing nutrients digestibility (Medugu *et al.,* 2012 and Linh *et al.,* 2020). Successful minimization of ANF cowpeas can help in realizing their full potential (Gonçalves *et al.* 2016). Chinma (2017) in his study, reported that sprouting of Moring oliefera seed has increased protein and mineral content and reduced crude fiber content. Similarly, Martinez (2013) reported higher nutritive value of sprouted soybean that unsprouted one. Fouad and rehab (2015) also reported the increased protein content, amino acids, and protein solubility of legume seeds.

2.5. Production performance of broilers fed cowpea diets.

Baliel (2014) in his study reported low feed intake and body weight gain in broilers fed raw cowpeas at 0%, 5%, 10% and 15% inclusion level which they attributed to anti-nutritional factors. Similarly, Teguia *et al.,* (2005) observed poor feed intake and depressed growth, and this is linked to tannins and protease inhibitors. Embeye *et al.,* (2018) reported an increased final body weight gain and average daily weight gain in starter phase in Cobb 500 broilers fed. raw cowpea at 0%, 5%, 10%, 15% and 20% inclusion level. Diets containing 0, 10, 20 and

30% inclusion level of cooked cowpea had no significant effect on feed intake during the finishing phase (Chakam *et al.,* 2010).

2.6. Prickly pear as poultry feed

Figure 2 shows the prickly pear plant and its fruits. The prickly pear (also known as cactus pear) is a fruit of the genus Opuntia, belonging to the Cactaceae family (Lidetewold, 2016) (Figure 3). The fruit presents a thick pericarp with small prickles, covering a pulp, which has a predictable number of seeds (Lidetewold, 2016). The prickly pear plant originated in the tropical and subtropical of America and can be found in a wide assortment of agro-climatic conditions across the whole of the American mainland (FAO, 2013). It is believed to be native to central Mexico, but its cultivation is expanding worldwide throughout semi-arid and warm arid areas**Error! Bookmark not defined.**. Todaro *et al*., (2020) indicated that there are about 1500 species of prickly pear plants and are dispersed mainly in the United States, Africa, Northern Mexico, Mediterranean nations, and other areas. Figure 3 shows the world distribution of Prickly pear plant.

Figure 2: The Prickly Pear plant

Source: FAO, 2013

Figure 3: The world distribution of Prickly pear plant

2.7. Morphology and chemical composition of prickly pear fruit

The prickly pear fruit (Figure 4) possesses an egg-shaped, expanded shape, like an oval pear, and is in fact a meaty berry (Forster, 2001). Moβhammer *et al.,* (2006) reported weight range between 67 and 216g. The fruit products are obtainable (removed or even unpeeled) in fresh, yellow, purple, reddish, which comparison like the amount of betalains colour content (Nesheim, 1979; Stintzing *et al.,* 2005). Under optimum conditions, the upper parts of the plant can reach 50 tons of DM/ha/year. Fresh fruit yield from Cacti is nearly 40 t/ha/year (Nobel, 1992).

Source: theothersideofthetortilla.com/2019/cactas-fruit/

Figure 4: The prickly pear fruits

Subject to the origin of the fruit and factors of the site of cultivation, the nutrient composition of the prickly pear can be highly variable.

Table 3: Chemical composition of Prickly Pear fruit (%DM)

Parameters	Pulp	Skin	Seed
Water	95.4	90.33	18.05
Protein	1.45	1.45	4.48
Lipids	0.7	1.06	3.66
Ash	1	3.05	12.66

Source: (Nebbache, 2010)

Abdel (2020) reported seed protein content of 10.70%, with fat content of 4.88%, fibre content of 46.31% and ash content of 3.39% ash. These results contrast with findings of Tadoro *et al.,* (2020), who reported seed crude protein content of 5.75% with ash and fats of 3.36% and 8.85% respectively, similarly, (Nebbache, 2010) reported seed protein content of 4.48%. Kunyanga (2014) reported 4.13% protein, 11.5% fats and 12.3% fibre. El-safy and Salem (2012) reported seed 16.6% CP, 49.6% CF, 17.21% crude fat and 3.14% ash. Moβhammer (2006) reported 85% water, 0.3% ash, 1% protein and 15% sugar in prickly pear whole fruit, similar to the same findings by Saenz-Hernandez *et al.,* (1995), who reported that water and protein content of the fruit ranges from 84% to 90% and 0.21% to 1.6% respectively with 3% ash and 14% carbohydrates.

2.7. Antinutritional factors of prickly pear seed cake

Generally, antinutritional factors are toxic and have negative effects on nutritive value of prickly pear seed cake by limiting protein digestibility and mineral availability (Reda, 2019). They can also be fatal if consumed in high amount (Mikiæ *et al*., 2009). Like other seed cakes, the prickly pear seed cake contains several antinutritional factors (such as crude fibre, tannins, oxalate, phytate, and trypsin inhibitors). Reda (2019) in his study reported higher phytate content (259.20 mg/100g, DM basis) than tannin and oxalate with 0.13 mg/100g and 0.11 gm/100 g respectively. These results agree with the findings by El-Safy *et al*., (2012).

2.8. Production performance of broilers fed prickly pear by-products

Imene *et al.,* (2020) investigated the effects of prickly pear by-products (husk and cake) in broiler feed. A 10% dietary inclusion of prickly pear by-products during the early growth phase (10 and 20 days) increased weight, confirming a similar response observed by Belghiti *et al.,* (2021). In the finishing phase (day 34 to day 48), 30% prickly pear by-products dietary inclusion reduced weight at day 48, while 20% inclusion level increased the weight at day 48, and 10% inclusion level had higher weight than all groups (Imene, 2020). Badr and Fattah (2019) reported highest weight at 15% dietary inclusion of prickly pear peel meal during all growth stages while the 0% inclusion level was the least.

In the study by Imene (2020), was reduced feed intake at 20% prickly pear by-products dietary inclusion at day 20, with no differences during day 34 to 48 compared to 10% inclusion, with significantly low intake at 30% inclusion. Badr *et al.,* (2019) reported reduction of feed intake in broilers fed starter diets with 5%, 10% and 15% prickly pear peel inclusion.

Inclusion of prickly pear by-products (husk and cake) in broiler's diet increased cold and hot carcass, liver, and gizzards (Imene, 2020). In rabbits, similar results were reported by Zedan *et al.,* (2019). Moula and Humbel *et al.,* (2019) reported increased cold carcass weight and carcass parameters (breast, thigh, heart, and gizzard), reduced abdominal fat weight.

2.9. Summary of literature review

South Africa has a relatively well-developed broiler industry, which contributes significantly to the per capita meat consumption, though remains chicken is disproportionately consumed by the low-income population, with a deficit covered by cheap imports, at the disadvantage of local producers. Findings from literature suggest that, considering the comparative protein content of cowpea to that of soybean, cowpea can also be used as a source of protein in broilers diet. Different researchers reported that the use of cowpea diet produced positive results in performance and carcass characteristics of broilers and did not have any negative impact on the overall performance. To the best of my knowledge there is no information about incorporation of cowpea and prickly pear seed cake in broilers diets. Previous studies reported variable quality in the nutrient composition of prickly pear seed as poultry feed, with overall beneficial effects on broiler growth performance and carcass quality at 10% dietary inclusion levels. There is limited information on the efficiency of seed cake utilization in broiler diets in the South African context.

CHAPTER 3

RESEARCH METHODOLOGY

3.1. Description of the study area

The study was conducted during summer of 2022 at the University of Venda Poultry Experimental Unit. The site location is 22^{0}58.6’S and 30^{0}26.4’E, at elevation of 592 m above sea level. The climate is semi-arid tropical, with a mean annual precipitation of 800 mm. The rain predominantly (83%) falls from November to March. Temperatures drop to an average minimum average of 12 ^{0}C in the winter months and rise to a maximum average of 40 ^{0}C in summer (Mzezewa and Gwata, 2012).

3.2. Processing of cowpea

Cowpeas were processed by sprouting. Bulk grain was manually cleaned of debris and damaged grain. The cleaned grain was sterilized by soaking in 0.5% Sodium Hypochlorite (NaClO) solution for 30 minutes, after which the grain was rinsed in tap water. Thereafter, the sterilized grain was soaked in water for 12 hours, prior to open air irrigated sprouting on porous plastic sheets for 96 hours. The sprouted cowpeas were sundried to approximately 90% DM.

3.2.1. Production and processing of prickly pear oil seed cake

Prickly pear (*Opuntia ficus indica L. cv Morado*) oil seed cake (PPSC) was sourced from Sweat-Water farm, in Bloemfontein, Free State Province, South Africa. Seed from the Morado variety of the prickly pear plant is mechanically extracted from hand-harvested fruit, specifically the damaged or over-ripe, fermenting fruit. Extracted seed is rinsed in clean water, and sundried over 3 days. The cake is an oil by-product from cold pressing of the seed though dies. The by-product is currently discarded or used to produce fire logs, or manure.

The trial used Prime Broiler Starter (Product V17767) manufactured by Meadow Feeds (Pty) Ltd, Delmas, South Africa. The product contains 200.0 g/Kg/Min protein, 25.0 g/Kg/Min fat, 50.0 g/Kg/Max fiber and 12.0 g/Kg/Min total Lysine). Test grower (days 25-35) and finisher (days 36-41) diets were custom made, which were designed to contain similar nutrient (crude protein, fat (ether extract), Ca, P, lysine, methionine+Cystine), energy (ME) and fibre (crude fibre, acid detergent fibre, neutral detergent fibre) profiles despite graded levels of PPSC. For compositional uniformity, test diets were blended using a “summit dilution” technique (Gous and Morris, 1985). The chemical composition of the three organic ingredients used in constituting the test diets are presented in Table 4. Diet formulation was in two steps. A set of maize-based grower and finisher diets were first constituted using sprouted cowpeas as the protein source in place of soybean cake. A second set of iso-nutrient grower and finisher diets was similarly formulated to respectively include upper limits of 10% and 12.5% PPSC, limitations imposed by the recommended, and respective control diet crude fibre content, consistent with previous studies (Cherif *et al.*, 2021). The resultant grower and finisher diet pairs (Table 2) were then blended (Table 3) to respectively contain 0%, 25%, 50%, 75%, 75%, 87.5% and 100% of the PPSC mixes. These blending ratios generated dietary treatments which respectively contained 0%, 2.5%, 5%, 7.5%, 8.75% and 10% PPSC, and 0%, 3.1255, 6.25, 9.375%, 10.9375%, 12.5% PPSC on a dry matter basis, which were conveniently denoted G_0, G_1, G_2, G_3, G_4, G_5, and F_0, F_1, F_2, F_3, F_4 and F_5. To prepare the test diets, yellow maize, sprouted cowpeas and the PPSC were hammer-milled (Jacobson hammer mill, model P160 Teordrop 10HP, USA) through a 3mm screen, and mixed (approximately 618 kg lots) along with the amino acid, mineral, and vitamin supplements for 20 minutes in a vertical mixer (MORHLANG VERTA MIX, 1200VM, USA). Tests diets were formulated to match the composition of Meadow Feeds' Prime Broiler Grower (Product V17768, 180.0 g/Kg/Min protein, 25.0 g/Kg/Min fat, 50.0 g/Kg/Max fibre and 10.0 g/Kg/Min total Lysine) and Prime Broiler Finisher (Product: V17810, 160.0 g/Kg/Min protein, 25.0 g/Kg/Min fat, 70.0 g/Kg/Max, 60.0 g/Kg/Max fibre and 9.0 g/Kg/Min total Lysine) commercial diets, the respective maize-soybean positive controls. All diets complied to the nutrient standards for grower and finisher broiler diets (National Research Council (NRC), 1994).

Table 4: Chemical composition of organic ingredients in test grower and finisher broiler diets.

Components	Maize	[1]Sprouted cowpeas	Prickly pear oil seed cake
Dry matter (g/kg)	898.1	890.4	932.5
Crude protein(g/kg)	70.2	280.9	100.0
Fat (ether extract) (g/kg)	34.8	9.2	42.8
Gross energy (MJ/kg)	16.2	16.8	19.3
Crude fibre F(g/kg)	34.5	64.0	607.5
Neutral detergent fibre (g/kg)	107.0	112.0	730.0
Acid detergent fibre (g/kg)	60.0	28.5	542.0
Ca (g/kg)	2.0	1.0	0.5
P (g/kg)	2.8	3.0	1.6
Amino acids (g/100g)			
His	0.40	1.07	0.39
Arg	0.46	1.90	1.36
Ser	0.46	1.34	0.36
Gly	0.40	1.21	0.71
Asp	0.54	3.40	0.68
Glu	1.57	4.18	1.86
Thr	0.35	1.03	0.31
Ala	0.64	1.17	0.35
Pro	0.76	1.30	0.42
Lys	0.21	1.20	0.18
Tyr	0.41	1.13	0.38
Met	0.13	0.52	0.14
Val	0.45	1.33	0.39
ILe	0.30	1.13	0.29
Leu	1.14	2.25	0.66
Phe	0.52	1.98	0.48

[1]Soaked for 12 hours, 96- hour open air irrigated sprouting, sundried to approximately 90% DM.

Table 5: Ingredient and chemical composition of blending grower and finisher broiler diets.

Composition	Blending diets			
	Grower phase		Finisher Phase	
	Diluent	[2]Summit	Diluent	[2]Summit
Ingredients (% as is)				
[1]Sprouted cowpeas	50.8	52.6	42.7	42.6
Maize	39.2	30.9	47.3	38.4
[3]Broiler micro-pack	2.4	2.6	2.4	2.6
Sand	4.0	0.9	4.9	0.9
Prickly pear oils seed cake meal	0.0	9.4	0.0	11.3
Methionine	0.3	0.3	0.2	0.2
Mono Dicalcium Phosphate	1.1	1.3	1.2	1.3
Limestone	1.3	1.6	0.9	1.0
Salt	0.8	0.4	0.4	1.7
Total	100.0	100.0	100.0	100.0
Calculated nutrients ($g\ kg^{-1}$ DM)				
Crude protein(g/kg)	180.5	180.2	160.6	160.3
Gross energy (MJ/Kg	14.5	15.5	14.5	15.4
Fat (ether extract) (g/kg)	16.0	19.1	18.5	21.4
Crude fibre (g/kg)	96.9	114.8	104.2	124.6
Neutral detergent fibre(g/kg)	146.5	173.3	156.2	185.9
Acid detergent fibre (g/kg)	84.7	96.5	97.8	109.8
Ca(g/kg)	8.9	9.6	7.4	7.7
P(g/kg)	5.6	5.8	5.8	5.7
Lysine (g/kg)	9.2	9.0	8.3	8.0
Methionine (g/kg)	11.4	10.3	8.7	9.1

[1] Soaked for 12 hours, 96- hour open air irrigated sprouting, sundried to approximately 90% DM.

[2] Grower and finisher diets formulated to include prickly pear oils seed cake meal at limits imposed by the recommended crude fibre content, and to match the positive control dietary fibre.

Table 6: Diet dilutions and analysed chemical composition of maize-sprouted cowpeas grower and finisher diets blended to contain graded levels of prickly pear oil seed cake meal.

Components	Diets											
	Grower						Finisher					
	G_0	G_1	G_2	G_3	G_4	G_5	F_0	F_1	$F_{2,}$	$F_{3,}$	F_4	$F_{5.}$
Diet dilution (% as is)												
Diluent	100	75	50	25	12.5	0	100	75	50	25	12.5	0
Summit	0	25	50	75	87.5	100	0	25	50	75	87.5	100
Total	100	100	100	100	100	100	100	100	100	100	100	100
Calculated chemical composition (DM basis)												
Crude protein(g/kg)	180.5	180.2	180.4	180.3	180.3	180.2	160.6	160.3	160.5	160.4	160.3	160.3
Gross energy (MJ/kg)	16.0	19.1	16.8	17.5	18.3	18.7	18.5	21.4	19.2	19.9	20.7	21.0
Fat (ether extract) (g/kg)	14.5	15.5	14.8	15.0	15.3	15.4	14.5	15.4	14.7	14.9	15.2	15.3
Crude fibre (g/kg)	96.9	114.8	101.4	105.9	110.3	112.6	104.2	124.6	109.3	114.4	119.5	122.1
Neutral detergent fibre(g/kg)	146.5	173.3	153.2	159.9	166.6	170.0	156.2	185.9	163.6	171.1	178.5	182.2
Acid detergent fibre (g/kg)	84.7	96.5	87.6	90.6	93.5	95.0	97.8	109.8	100.8	103.8	106.8	108.3
Ca(g/kg)	8.9	9.6	9.1	9.2	9.4	9.5	7.4	7.7	7.5	7.6	7.6	7.6
P(g/kg)	5.6	5.8	5.6	5.7	5.7	5.8	5.8	5.7	5.8	5.8	5.7	5.7
Lysine (g/kg)	9.2	9.0	9.1	9.1	9.0	9.0	8.3	8.0	8.2	8.1	8.1	8.0
Methionine (g/kg)	11.4	10.3	11.1	10.8	10.6	10.4	8.7	9.1	8.8	8.9	9.0	9.0

[1] Soaked for 12 hours, 96- hour open air irrigated sprouting, sundried to approximately 90% DM.

[2] Grower and finisher diets formulated to include prickly pear oils seed cake meal at 10% and 12.5% respectively, limits imposed by the recommended crude fibre content, and to match the positive control dietary fibre.

3.3. Experimental Design and Broiler management

A total of 504-day-old Ross 308 mixed sex chicks were used in the trial. The rearing house was a deep litter, naturally ventilated, 17.0 m x 9.0 m broiler facility with a section divided into twenty-eight 1.5 m x 1.4 m steel framed mesh wire pens, each stocked with 18 birds. During brooding (1 – 15 days), a 175-watt infrared Brooding lamp was suspended at a height of 1 m over each cage. All birds were on the same standard starter (days 1-24) diet. Chicks received a Phenix Stress pack in drinking water on days 1 to 6. Chicks were on the same standard commercial starter (days 1-24) prior to experimental grower (day 25-35) and finisher (day 36 -41) diets. Pens were randomly assigned to experimental diets with four (4) replicates per treatment combination. One tube feeder (15 kg) and one manual drinker (10 L) were provided in each pen, and heights were adjusted for unhindered access. Birds were fed and supplied the feed and water *ad libitum*. Feeders and drinker's height were adjusted every day in relation to the bird's height.

3.4. Growth and feed intake

Initial (day 25), and endpoint grower (day 35) and finisher (day 42) phase broiler live weight were estimated from six (6) random birds per pen. Feed intake was determined for each feeding phases, and the feed conversion ratios calculated.

Feed intake was calculated as follows:

$$Total\ feed\ intake\ (kg) = Total\ feed\ given\ (kg) - Total\ feed\ leftover\ (kg)$$

$$Average\ daily\ feed\ intake\ (g) = \frac{Total\ feed\ intake}{Total\ number\ of\ days\ of\ the\ experiment}$$

The weight gain was calculated as follows:

$$Body\ weight\ gain\ (g) = Final\ body\ weight\ (g) - initial\ body\ weight\ (g)$$

$$Daily\ body\ weight\ gain\ (g) = \frac{Final\ body\ weight\ gain\ (g) - Initial\ body\ weight\ gain\ (g)}{Total\ number\ of\ days\ of\ experiment}$$

Feed conversion ratio was calculated as follows:

$$Feed\ convention\ rstio\ (FCR) = \frac{Total\ feed\ consumed\ (kg)}{Total\ body\ weight\ gain\ (kg)}$$

3.5. Carcass characteristics

Feed was withdrawn for 12 hours prior to slaughter on day 42. At slaughter, six random birds were selected, which were weighed individually before slaughtering. These sampled birds were tagged by plastic leg cable ties for identification during carcass evaluation. Birds were slaughtered by standard, humane procedures (Osei, 2010). The gizzard, liver, heart, and abdominal fat were removed and weighed. The carcass was weighed upon slaughter to estimate the warm dressing percentage. The breast, thighs, and wings were removed from the warm carcass and weighed. Meat cuts and organ weights were scaled to percentages of the carcass, and the live weight, respectively.

3.6. Meat quality

3.6.1. Colour analysis

Meat colour was evaluated following methods described by Benli (2015). Approximately 30 mm thick x 20 mm wide x 10 mm of breast meat samples were dissected, and the meat colour attributes (*L*, a** and *b*)* evaluated using the Lab Scan-XE (Hunter Associates Laboratory Inc. USA) spectrophotometer.

3.6.2. Drip loss

Breast meat samples (20 mm thick x 30 mm wide x 50 mm long) were dissected immediately after slaughter and placed in a zip sealable plastic bag and refrigerated at 4 ^{0}C for reweighing after 72 hours (Bowker and Zhuang, 2015).

3.6.3. Water holding capacity

Water holding capacity was determined according to Wardlaw *et al*., (1973). About 8g of breast meat was centrifuged for 15 minutes in 12ml of 0.6 M aqueous NaCl at 4^0 C and 10 000 rmp, after which supernatant was measured and the retained fluid (ml of 0.6 M NaCl) expressed as the water holding capacity (%).

3.6.4. Determination of pH

Breast meat pH was measured according to Zhang *et al*., (2012) using a pH meter (BASIC 2.0 pH meter, Crison instrument, S.A. EU). The pH meter was calibrated using 4.0 and 7.0 buffers. Thereafter, about 10 g of sample was weighed and placed in a beaker containing 100 mL of 5 mM NaIAc and 150 mM KCl. The sample was mixed for 30 seconds, and the pH measured. The measurement was conducted in triplicate. After every trial, the pH meter electrode was cleaned with diluted soap water and pat dried with a paper tissue.

3.7. Chemical analysis

Prickly Pear Seed Cake meal, maize, sprouted cowpeas were subjected to proximate analysis using the Association of Official Analytical Chemist (AOAC, 2016) methods for dry matter (DM) (method 945.32), crude protein (method 978.02), crude fiber (method 978.10), Crude fat (method 920.39) and ash (method 923.03). Acid detergent fibre (ADF) and Neutral detergent fibre (NDF) were determined using the method of Goering and van Soest (1970).

3.8. Statistical Analysis

Broiler responses to incremental PPSC inclusion were analysed using Minitab (2020, version 21.1.0) to fit the data into linear or quadratic regression models of the following respective forms;

$$\mathrm{Y} = \mathrm{a} + \beta_1 \mathrm{x}$$

$$\mathrm{Y} = \mathrm{a} + \beta_1 \mathrm{x} + \beta_2 \mathrm{x}^2$$

Where Y is the response available, a is intercept, β_1 and β_2 are the regression coefficients, x the dietary PPSC inclusion (%) level. The optimal PPSC dietary inclusion within the dietary fibre limitations were estimated by quadratic prediction as $-\beta_1 \div 2\beta_2$.

The data was further subjected to one-way analyses of variance using the model:

$$\mathbf{Y}_{\boldsymbol{ijk}} = \mu + \boldsymbol{\alpha_i} + \boldsymbol{e_{ij}}$$

Where: Y_{ijk} is the response variable, μ is the overall mean, α_i the effect of diet and e_{ij} the random error. The Tukey test was used for post hoc pairwise mean comparisons, with mean separation for significant effects at $P<0.05$.

3.9. Ethical considerations

Chickens were raised and managed under the standard conditions that comply with the animal welfare requirements of the University of Venda Animal Research Ethics committee (Ethical clearance no: FSEA/22/ANS/07/1611).

CHAPTER 4

RESULTS

4.1. The effects of prickly pear seed cake meal inclusion levels on the performance of broiler chickens.

Means of the growth parameters, and all quadratic and the significant linear regression equations, and the statistical test parameters are presented in Table 7. Quadratic regression on the dietary PPSC level was significant for grower phase intake ($P=0.044$) and for the cumulative grower-finisher live weight gain ($P=0.04$). A significant linear regression was observed for daily intake during the grower phase ($P= 0.02$). The relationships are depicted in Figures 5, 6, and 7, respectively. Considering the dietary fibre limitations, the predicted minimum PPSC dietary inclusion for improved grower phase feed intake was 1.74%, with a predicted 4.54% optimum dietary inclusion for the cumulative live weight gain. During the grower phase, feed intake increased ($P<0.05$) with PPSC inclusion, to match the PC on the G_3 diet, with the peak intake maintained ($P>0.05$) up to the G_5 diet. During the finisher phase, broilers on $F_{0,}$ $F_{2,}$ $F_{3,}$ diets had low final (42-day) live weight compared to the PC. Live weight at $F_{4,}$ $F_{4,}$ F_5, was similar to both the lower level PPSC, and the to the PC ($P>0.05$). Feed intake was high ($P<0.05$) on the F_0 and F_4 diets compared to the F_3 diet, with similar ($P>0.05$) intake to the PC for all the diets. Over the 42-day grower-finisher production cycle, broilers had low weight gains ($P<0.05$) on G_0-F_0, G_1-F_1 and G_2-F_2) diets compared to broilers on the PC diets, though despite the difference, all these diets had similar ($P>0.05$) weight gains to the higher PPSC feeding regimes. Feed intake increased with PPSC inclusion to match the PC on the G_3-F_3 and G_4-F4 feeding regimes ($P>0.05$) and was maintained ($P>0.05$) up to the G5-F_5 feeding regime.

Table 7: Ross 308 broiler growth responses to graded dietary prickly pear oil cake inclusion in a maize-cowpea diet during grower (days 25 - 35) and finisher (days 36 -42).

Variables	[1]PC	Diets [2]Sprouted cowpeas + maize -+ PPSC							SEM	P Values	Regression analyses [3]Equation	R^2	*P*
		G_0	G_1	G_2	G_3	G_4	G_5						
Grower phase													
25-day weight (g)	947.3	840.3	883.5	871.8	951.8	876.8	0.013	0.828	55.00	0.590	$Y = 885.7 + 111.10x – 143.6x^2$	0.025	0.727
35-day weight (g)	1578.7	1357.3	1365	1398	1650.5	1425.5	0.243	0.321	54.90	0.050	$Y = 1510.0 – 398.80x + 467.2x^2$	0.084	0.334
Weight gain (g/day/bird)	63.2	51.7	48.2	52.6	59.0	54.9	0.637	0.057	3.45	0.060	$Y = 55.78 – 20.78x + 24.19x^2$	0.111	0.231
Feed Intake (g/bird/day)	99.3	87.1	88.0	90.6	103.3	97.8	0.709	0.035	2.24	0.000	$Y = 92.03 – 4.62x + 13.25x^2$	0.221	0.044
											$Y = 90.92 + 0.08046x$	0.192	0.020
Feed conversion ratio	1.58	1.71	1.84	1.74	1.75	1.8	0.089	0.566	0.10	0.580	$Y = 1.676 + 0.564x - 527x^2$	0.084	0.334
Finisher phase	PC	F_0	F_1	$F_{2,}$	F_3	F_4	F_5						
42-day weight	2010.8	1659.7	1692.6	1739.0	1905.3	1823.0	0.774	0.021	55.60	0.000	$Y = 1815 – 288.4x + 354 x^2$	0.066	0.426
Weight gain (g/bird/day)	72.0	50.4	54.6	56.8	59.1	66.3	0.919	0.003	4.93	0.050	$Y = 60.92 – 28.89x + 37.33x^2$	0.149	0.132
Feed intake (g/bird/day)	159.4	163.2	156.4	159.8	149.6	169.1	0.010	0.962	2.96	0.010	$Y = 161.1 – 18.83x + 19.72x^2$	0.063	0.441
Feed conversion ratio	2.8	3.3	2.9	2.8	2.6	2.6	0.928	0.002	0.21	0.060	$Y = 2.789 + 0.3799x – 0.7368x^2$	0.089	0.322
Grower + finisher													
Weight gain (g/bird/day)	66.8	51.3	51.1	52.8	58.5	57.8	0.857	0.008	2.91	0.010	$Y = 58.35 – 24.82x + 30.76 x^2$	0.114	0.040
Feed intake (g/bird/day)	125.8	116.9	117.5	119.1	127.1	128.8	0.704	0.037	1.51	0.000	$Y = 126.6 – 11.77x + 16.48 x^2$	0.04	0.894
Feed conversion ratio	1.9	2.3	2.3	2.3	2.2	2.3	0.509	0.111	0.11	0.070	$Y = 2.232 + 0.4717x – 0.6317 x^2$	0.017	0.637

SEM= Standard error of the mean.

[1] Positive controls - Meadow Feeds' Prime Broiler Grower (Product V17768), Prime Broiler Finisher (Product: V17810

[2] Grower (G_n) and finisher (F_n) maize-sprouted cowpea diets constituted using cowpeas processed by soaking for 12 hours, subjected to 96- hour open air irrigated sprouting and sundried, blended with 0%, 25%, 55%, 75%, 87.5% and 100% of iso-nutrient, diets reformulated using same basal ingredients plus 10% and 12.5% prickly pear seed oil cake (PPSC) meal, respectively.

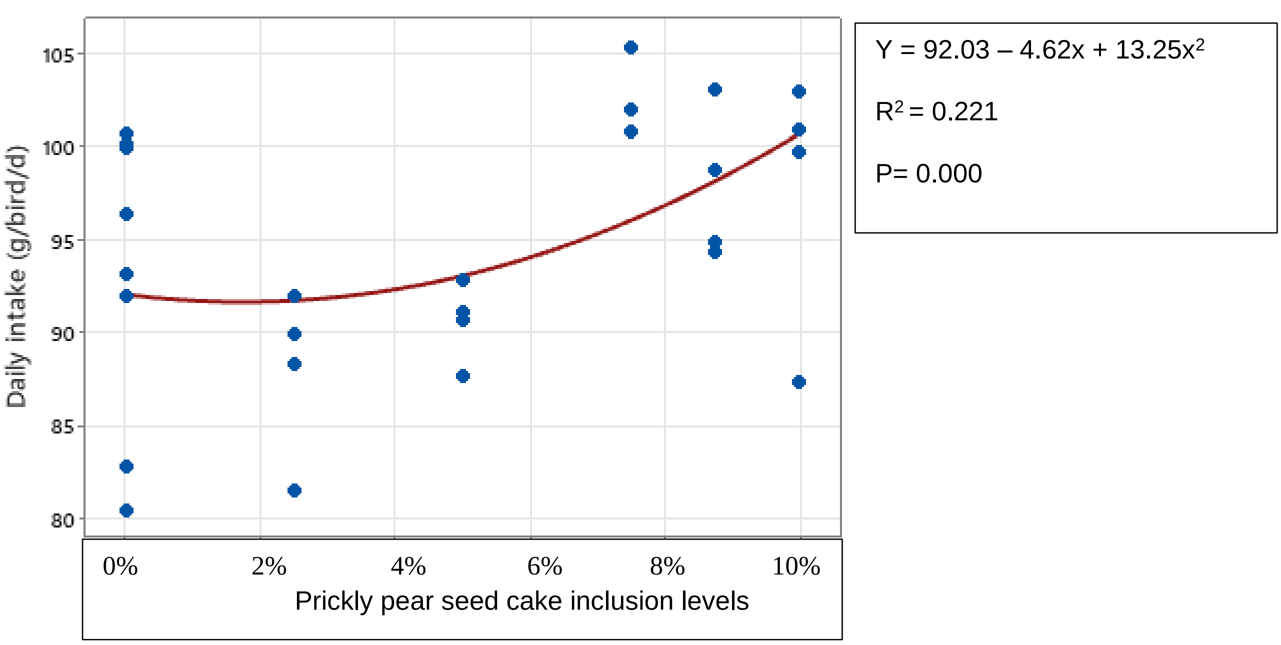


Figure 5: Effect of prickly pear seed cake dietary inclusion levels on the daily feed intake (g/bird/day) of Ross 308 broiler chickens at grower phase (day 25 - 35).

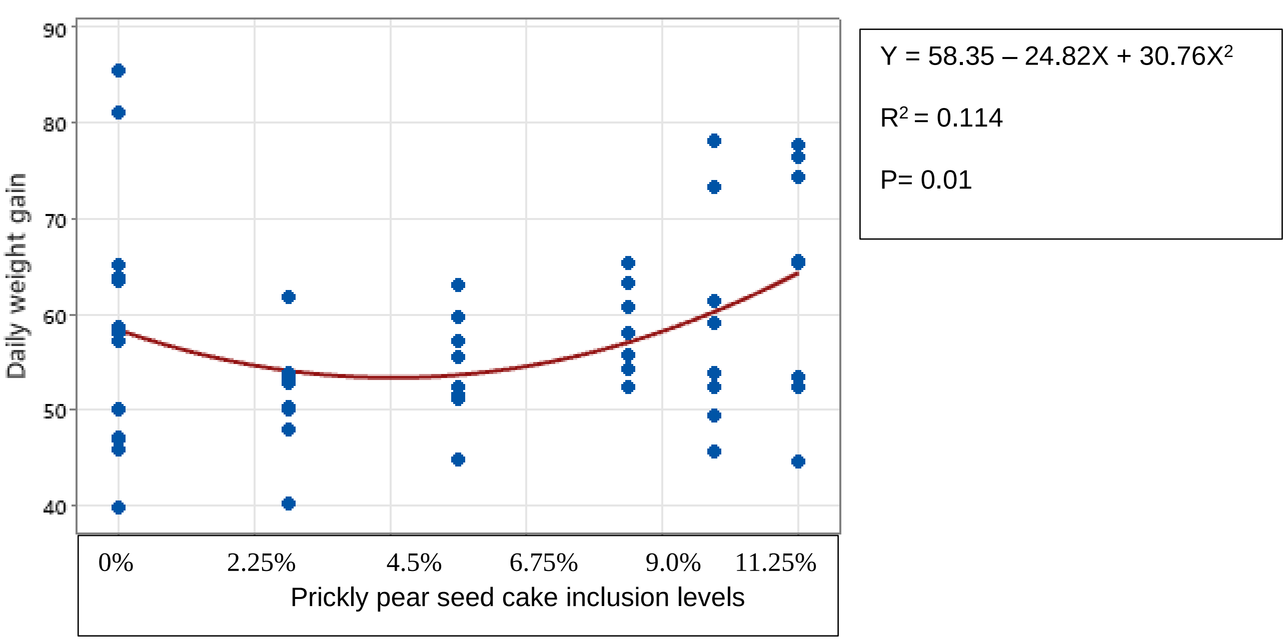


Figure 6: Effect of prickly pear seed cake dietary inclusion levels on daily weight gain (g/day) of Ross 308 broilers for full production cycle (day 25 – 41).

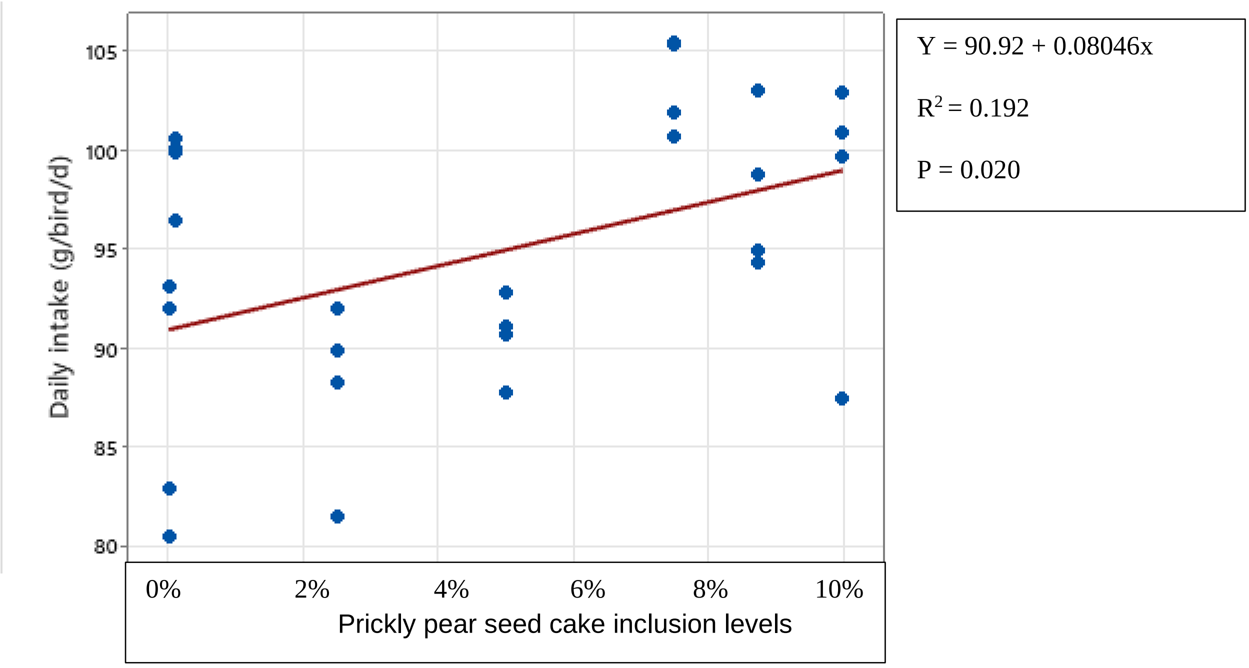


Figure 7: Effect of dietary prickly pear seed cake inclusion on daily feed intake (g/bird/day) of Ross 308 broilers during the grower phase (day 25 – 35).

4.2. The effects of prickly pear seed cake meal on the carcass characteristics of broiler chickens.

Means of the carcass parameters, and regression equations and statistical test parameters are presented in Table 8. Quadratic regression on the dietary PPSC level was significant for the scaled gizzard weight (P=0.05), dressed carcass (P=0.018) and abdominal fat (P=0.001), which are depicted in figures 7.8-9, respectively. Considering the dietary fibre inclusion limitations, the predicted optimal PPSC dietary inclusions were 4.39% for the scaled gizzard size, 7.58% for the dressed carcass, and 4.78% for abdominal fat. The carcass weight increased to match the PC on the G_3-F_3 feed regime (P>0.05) and was maintained (P>0.05) up to the G_5-F_5 feeding regime. Similarly, the dressing percentage increased (P<0.05) with dietary PPSC inclusion, to match the PC on G_2-F_2 feed regime and was maintained up to the G5-F_{10} feeding regime. Similar (P>0.05) to the PC, broilers on the G_5-F_5 feeding regime had high (P<0.05) abdominal fat compared to broilers on G_0-F_0, G_1-F_1, G_2-F_2, G_3-F_3 feeding regimes, all of which had similar (P>0.05) abdominal fat to broilers on the G_4-F_4 feeding regime. Broilers on the G_0-F_0 feeding regime had relatively small livers (P<0.05).

Table 8: Effects of prickly pear seed cake meal on the carcass characteristics of broilers.

Variables	Diets							SEM	P Value	Regression		
	[1]PC	[2]Sprouted cowpeas + maize -+ PPSC										
		G_0-F_0	G_1-F_1	G_2-F_2	G_3-F_3	G_4-F_4	G_5-F_5			Equations	R^2	*P*
Dressed carcass (g)	1427.38	1130.25	1162.50	1350.00	1380.50	1364.63	1341.44	44.10	0.00	$Y = 1130 – 154.3 x + 288.67 x^2$	0.151	0.341
Dressed carcass (%)	71.03	68.09	68.78	77.89	72.43	74.86	72.95	1.58	0.01	$Y = 68.99 + 16.34 x – 12.12 x^2$	0.274	0.018
Carcass components (% carcass weight)												
Breast	26.07	25.95	27.06	27.24	31.26	25.61	30.00	1.97	0.33	$Y = 25.99 + 4.727 x – 1.777 x^2$	0.087	0.319
Thigh	13.01	11.50	10.87	10.96	12.04	11.04	12.53	0.66	0.25	$Y = 12.21 – 5.292x + 5.601 x^2$	0.155	0.122
Wing	3.74	4.86	5.17	4.76	4.44	3.95	4.51	0.33	0.06	$Y = 4.410 + 1.818x – 2.115 x^2$	0.085	0.328
Viscera (% Live weight)												
Abdominal fat	2.26	1.56	1.44	1.54	1.48	1.82	2.41	0.14	0.00	$Y = 1.923 – 2.590x + 2.952 x^2$	0.451	0.001
Liver	1.95	2.56	2.43	2.48	2.26	2.24	2.34	0.12	0.04	$Y = 2.277 + 0.5347x - 0,5607x^2$	0.0367	0.627
Gizzard	2.91	2.48	2.53	2.52	2.72	2.80	2.90	0.12	0.07	$Y = 2.693 – 0.8577x + 1.098x^2$	0.2137	0.050
Heart	0.44	0.50	0.52	0.50	0.51	0.45	0.42	0.04	0.47	$Y = 0.468 + 0.255x – 0.301x^2$	0.155	0.121

PC= Positive control, NS= No significant difference (P>0.05)

SEM= Standard error of the mean.

[1]PC= Positive controls - Meadow Feeds' Prime Broiler Grower (Product V17768), Prime Broiler Finisher (Product: V17810

[2]Grower (G_n) and finisher (F_n) maize-sprouted cowpea diets constituted using cowpeas processed by soaking for 12 hours, subjected to 96- hour open air irrigated sprouting and sundried, blended with 0%, 25%, 50%, 75%, 87.5% and 100% of iso-nutrient, diets reformulated using same basal ingredients plus 10% and 12.5% prickly pear seed oil cake (PPSC) meal, respectively.

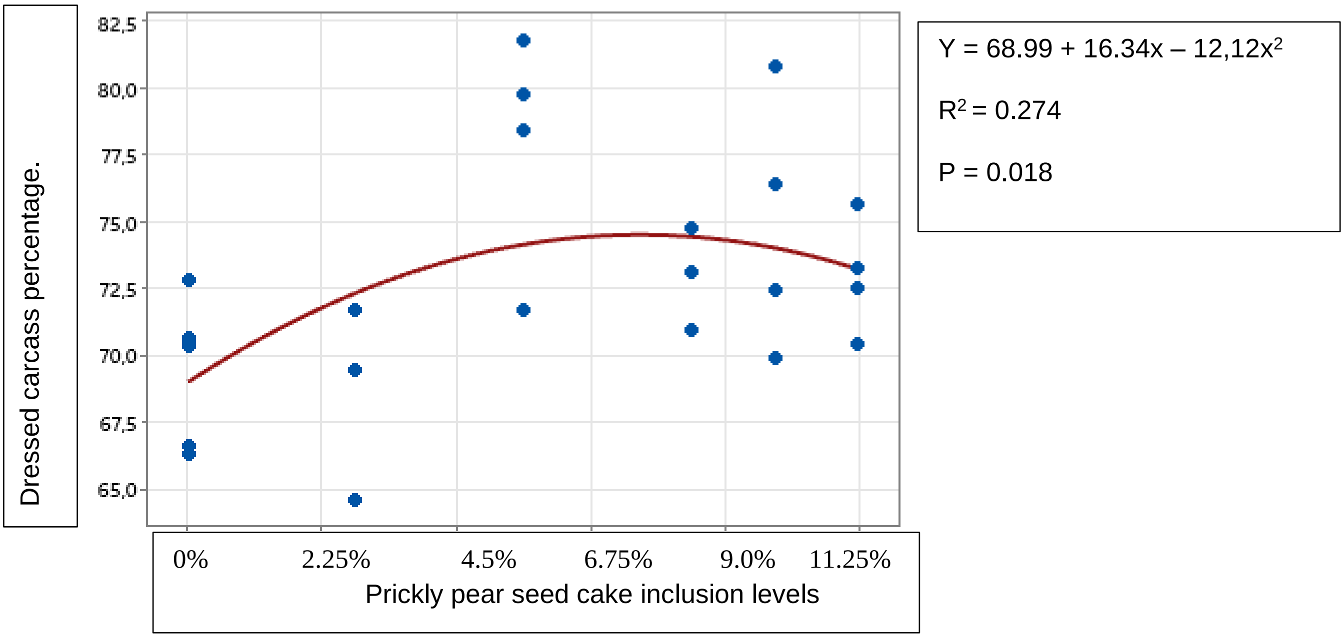


Figure 8: Effect of dietary prickly pear seed cake inclusion levels (treatment) on dressed carcass weight percentage of Ross 308 broilers.

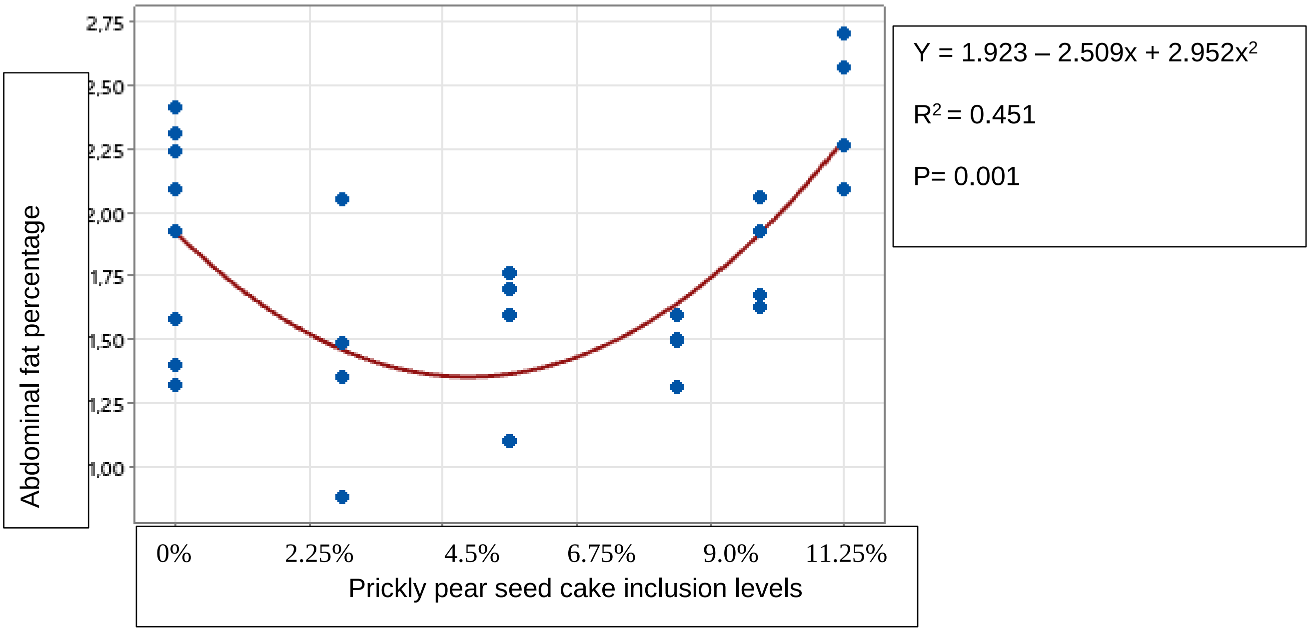


Figure 9: Effect of dietary prickly pear seed cake inclusion levels on abdominal fat percentage of Ross 308 broilers.

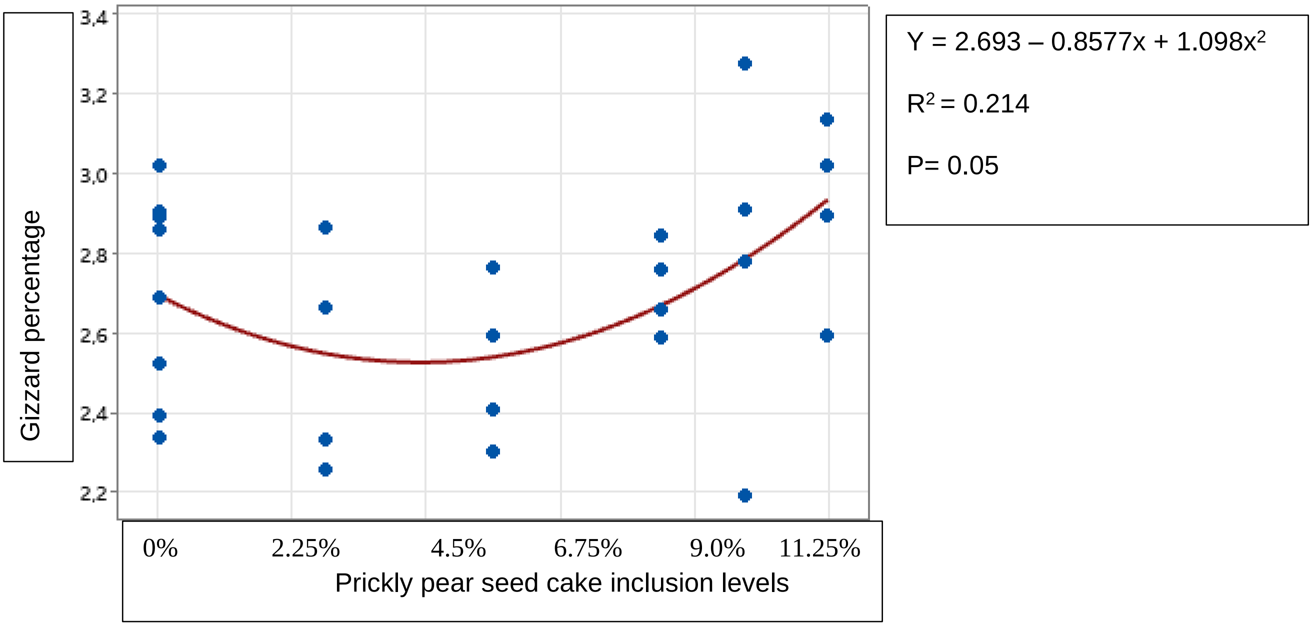


Figure 10: Effect of dietary prickly pear seed cake inclusion levels on gizzard percentage of Ross 308 broilers.

4.3. The effects of prickly pear seed cake meal on the meat quality of broiler chickens.

Mean meat quality parameters, the significant linear regression, and all quadratic regression equations and statistical test parameters are presented in Table 9. Low drip loss ($P>0.05$) was recorded in broilers on the G_0-F_0 and G_1-F_{2S} dietary regimes compared to those on the G_3-F3 dietary regime, which had similar ($P>0.05$) high drip loss compared to broilers on the PC, and on high dietary PPSC inclusion levels. A linear positive relationship ($r^2 = 0.176$, P= 0.026) was observed between the PPSC inclusion level and the drip loss.

Table 9: Effects of prickly pear seed cake meal on eat quality of chickens fed experimental diet.

[1]Meat quality attributes	Diets							SEM	P Values	Regression		
	[2]PC	[2]Sprouted cowpeas + maize -+ PPSC										
		G_0-F_0	G_1-F_1	G_2-F_2	G_3-F_3	G_4-F_4	G_5-F_5			Equation	R^2	*P*
L*	30.94	35.53	29.16	31.38	29.42	30.98	32.45	2.57	0.66	$Y = 33.03 – 12.68x + 11.94x^2$	0.067	0.419
a*	11.19	11.20	10.65	14.06	11.37	10.61	11.58	1.04	0.32	$Y = 11.02 + 5.052x – 5.137x^2$	0.056	0.487
b*	19.21	21.90	19.91	21.83	19.12	18.56	21.25	0.99	0.12	$Y = 20.60 – 1.137x + 0.551x^2$	0.013	0.853
pH	5.64	5.67	5.65	5.66	5.67	5.69	5.64	0.05	0.90	$Y = 5.652 + 0.0429x – 0.0289\ x^2$	0.047	0.942
Drip loss (%)	5.64	4.95	4.44	5.56	7.41	6.07	5.92	0.53	0.22	$Y = 5.038 + 1.484x – 0.106x^2$	0.174	0.091
										$Y = 5.039 + 0.01395x$	0.176	0.026
Water holding capacity (%)	49.19	50.14	50.78	53.65	50.00	51.56	49.91	1.87	0.71	$Y = 49.6 + 10.09x - 9.846x^2$	0.080	0.354

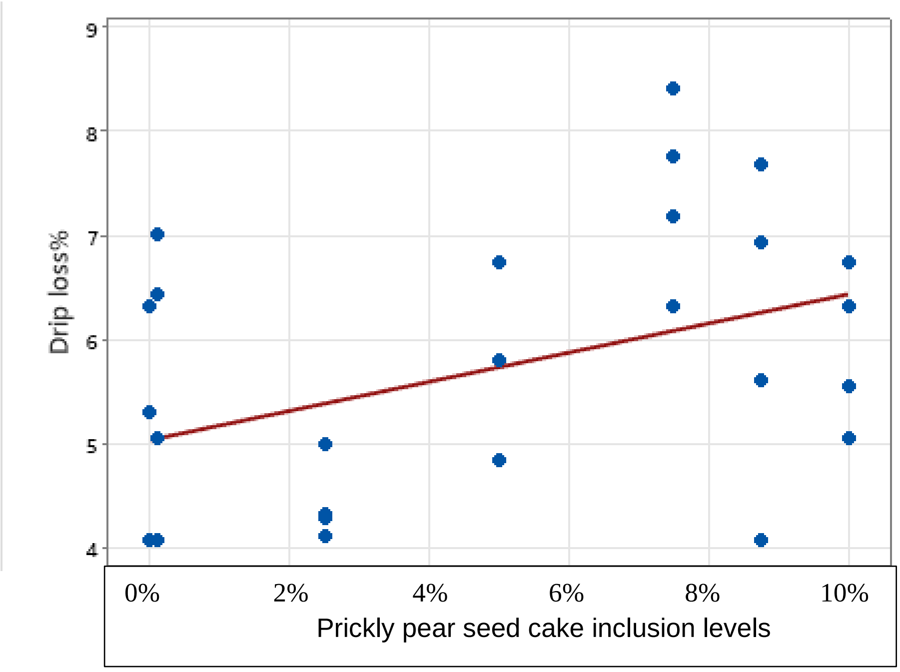
SEM= Standard error of the mean.

[1] Breast meat colour coordinates, - L*= Lightness, a*= Redness, b*= Darkness

[2] Positive controls - Meadow Feeds' Prime Broiler Grower (Product V17768), Prime Broiler Finisher (Product: V17810

[2] Grower (G_n) and finisher (F_n) maize-sprouted cowpea diets constituted using cowpeas processed by soaking for 12 hours, subjected to 96- hour open air irrigated sprouting and sundried, blended with 0%, 25%, 55%, 75%, 87.5% and 100% of iso-nutrient, diets reformulated using same basal ingredients plus 10% and 12.5% prickly pear seed oil cake (PPSC) meal, respectively.

SEM= Standard error of the mean



$Y = 5.039 + 0.01395x$

$R^2 = 0.176$

$P = 0.026$

Figure 11: The relationship between dietary prickly pear seed cake inclusion levels and drip loss percentage (%)/day) of Ross 308 broilers.

CHAPTER 5

5.1. DISCUSSION

The current study hypothesised that dietary inclusion of the highly fibrous PPSC within the limits of recommend dietary fibre levels will improve the quality of maize-sprouted cowpea grower and finisher diets to improve broiler growth, slaughter performance, and meat quality, given its nutrient profile and potential functional properties. Despite the iso-nutrient dietary composition, quadratic regression on the dietary PPSC level suggested different threshold benefits for different response parameters. Where significant, linear models suggested the inclusion of higher PPSC levels could have dictated non-liner responses without the limits imposed by the dietary fibre. Currently, there is limited information on these assumptions. An important consideration of feeding novel feed products to livestock is the clinical risk. In this study, no adverse clinical effects (i.e., no ill health or mortality) were recorded, consistent to reports by Badr *et al.,* (2019) on broilers fed diets containing prickly pear fruit peel. This was similar to findings in rabbit diets similar diets (Bakr *et al*., 2017). In contrast, Moula *et al*., (2019) reported 10% mortality in broilers fed prickly pear cladodes. Ragab (2007) reported 3.3% mortality in quails fed diets containing prickly pear shells. This could be due to high fibre content in the cladodes of the prickly pear plant and envelopes.

In this study, despite the iso-nutrient dietary composition, quadratic regression on the dietary PPSC level suggested benefit in grower phase intake and the cumulative grower-finisher live weight gains. Within the tested range of dietary inclusion, the findings suggested that dietary PPSC is incrementally beneficial to different optimums for these parameters. Further research is required to explain these beneficial effects. Due to the relative protein and fibre content in the ingredients, the diet formulation effectively resulted in PPSC replacement of maize, and not the sprouted cowpeas. To support local communities, the maize was sourced from local producers, who produce it under suboptimal, dryland farming, which explained the relatively low protein. Previous studies (Fernandez *et al*, 1994) indicated a limiting order of amino acids in maize of Lys, Thr, Trp, Arg, He, and Val, Met + cystine, Phe + Tyr, and His, and in soybean meal, in the order Met + Cystine, Thr, Lys and Val, and His, and in the complete maize-soybean diet, the limiting order Met, Thr, Lys, Val, Arg, and Trp. There are no similar studies on limiting amino acids in sprouted cowpea-based diets. The PPSC protein is typically dominated by glutamic acid, arginine and aspartic acid (Borchani *et al*., 2021), in a profile confirmed in this study. In this study, the dietary substitution of PPSC for maize had minimal effects on the amino acid profile, save for an increase in the non-essential glutamate, arginine, and glycine, and reduction of non-essential alanine and proline. Therefore, given relatively low dietary inclusion, and given similar and total dietary amino acid profiles, assuming similar amino acid bioavailability, the positive influence of

PPSC likely did not relate to limiting essential amino acids. Therefore, other PPSC functional components which could hypothetically explain the beneficial effect need investigation, such as essential fatty acids, immunogenic compounds, and, given potential thermal stress, antioxidant activity.

Broiler performance is dependent on many factors, which include the diet, health, stocking density, ambient temperature, water and feed supply and lighting, among other factors (Aviagen, 2009). These factors complicate comparative analyses among studies. Performance is however invariably maximised in controlled environments, and on high precision feeding. This experiment was controlled by including a standard commercial grower and finisher diets. The study also used a popular hybrid broiler strain for the suboptimal production conditions. Pairwise mean comparison suggested an approximate minimum of G_3 dietary PPSC to match the intake on the PC diet. The quadratic estimate of the dietary PPSC inclusion for optimum growth during this phase was 4.30%. During the finisher phase, the data suggested an approximate minimum F4 dietary PPSC to match the 42-day slaughter weight on the PC diet. The quadratic estimate of the dietary PPSC inclusion for optimum slaughter weight was 5.08%. Feed intake was reduced by an approximate 6.5% dietary PPSC (F_3 diet). The quadratic estimate of the dietary PPSC inclusion for optimum feed intake was 5.97%. A positive linear relationship (r^2 =0.192; P=0.020) was observed between the PPSC dietary inclusion and the daily feed intake. Over the 42-day grower-finisher production cycle, the data suggested approximate minimums of 5% (G_3 diet) and 6.25 (F_3 diets) PPSC grower-finisher dietary inclusion to match the live weight gain on the PC control. The quadratic estimates of the dietary PPSC inclusion for optimum 42-day growth was 4.54%. Feed intake required minimum 7.5% (G_4 diet) 9% (F_4 diet) PPSC grower-finisher diets to match the PC diet. The quadratic estimates of the dietary PPSC inclusion for optimum 42-day feed intake was 4.02%. Previously, increased dietary intake was similarly reported by Badr (2019) in broilers fed 5%, 10% and 15% dietary prickly pear envelopes and by El-Neney (2019) in broilers fed diets containing 20% and 30% prickly pear envelopes. Moula (2019) reported that low inclusion levels of prickly pear cladodes did not affect broiler performance. In broilers on prickly pear fruit meal, Lidetewold *et al*., (2016) reported an increase in daily weight gain with an increase in prickly pear fruit meal inclusion level (0, 2.25, 4.5 and 6.75%). Hassan (2019) reported improved weight gain and FCR at 50% prickly pear fruit meal in rabbit's diet. High (15%) prickly pear cladode dietary inclusion improved FCR compared to 5% inclusion (Moula, 2019). Similarly, 20% and 30% dietary inclusion prickly pear fruit meal improved broiler FCR (Lidetewold, 2020). Cherif (2021) reported 2.3 compared to 2.6) day-42) FCR at 10% inclusion level in broilers fed prickly pear by-products.

Broiler performance is dependent on many factors, which include the diet, health, stocking density, ambient temperature, water and feed supply and lighting, among other factors (Aviagen, 2009). These factors complicate comparative analyses among studies. Performance is however invariably maximised in controlled environments, and on high precision feeding. This experiment was controlled by including a standard commercial grower and finisher diets. The study also used a popular hybrid broiler strain for the suboptimal production conditions. Pairwise mean comparison suggested an approximate minimum of G_3 dietary PPSC to match the intake on the PC diet. During the finisher phase, the data suggested an approximate minimum F_4 dietary PPSC to match the 42-day slaughter weight on the PC diet. Feed intake was reduced by an approximate 6.5% dietary PPSC (F_3 diet). Over the 42-day grower-finisher production cycle, the data suggested approximate minimums of 5% (G_3 diet) and 6.25 (F_3 diets) PPSC grower-finisher dietary inclusion to match the live weight gain on the PC control. Feed intake required minimum 7.5% (G_4 diet) 9% (F_4 diet) PPSC grower-finisher diets to match the PC diet. Previously, increased dietary intake was similarly reported by Badr (2019) in broilers fed 5%, 10% and 15% dietary prickly pear envelopes and by El-Neney (2019) in broilers fed diets containing 20% and 30% prickly pear envelopes. Moula (2019) reported that low inclusion levels of prickly pear cladodes did not affect broiler performance. In broilers on prickly pear fruit meal, Lidetewold *et al*., (2016) reported an increase in daily weight gain with an increase in prickly pear fruit meal inclusion level (0, 2.25, 4.5 and 6.75%). Hassan (2019) reported improved weight gain and FCR at 50% prickly pear fruit meal in rabbit's diet. High (15%) prickly pear cladode dietary inclusion improved FCR compared to 5% inclusion (Moula, 2019). Similarly, 20% and 30% dietary inclusion prickly pear fruit meal improved broiler FCR (Lidetewold, 2020). Cherif (2021) reported 2.3 compared to 2.6 day-42 FCR at 10% inclusion level in broilers fed prickly pear by-products.

Cherif (2021) reported increased live weight with 10% and 20% dietary inclusion of prickly pear husks and seed cake. Similarly, in Cobb broilers Badr *et al* (2019) reported increase in live weight at 5% to 15% inclusion of prickly pear envelopes. In contrast, Moula (2019) indicated that 5% and 10% prickly pear cladode inclusion level in broilers diet did not affect the daily weight gain and body weight, however, Ragab (2012) reported that 10% and 20% prickly pear fruit peels inclusion improve daily weight gain but did not affect the body weight of Hy-Line W-36 cocks at 70 days. In quails, 15% and 30% prickly pear envelopes had no effect on average daily gain weight and final body weight (day 42) (Ragab, 2007). El-Neney *et al*., (2019) on the other hand reported increased live weight with 20% and 30% dietary prickly pear envelopes inclusion in rabbits.

In the present study, quadratic regression on the dietary PPSC level was significant for scaled gizzard weight. The quadratic estimate optimum for the dietary PPSC for the gizzard size was 4.39%. Enlargement of the gizzard could be due to both quantitative and qualitative (NDF, ADF) ingredient (Table 4), and consequently, dietary (Table 5-6) fibre effects. Broilers fed sprouted cowpea-maize diets without the PPSC (G_0-F_0 feeding regime) also had relatively small livers, which suggested less metabolic load.

The data suggested approximate minimum 6.5-7.5% (G_3 diet) and 6.5-9 (F_3 diet) PPSC grower and finisher dietary inclusion to match the carcass yield on the PC. The quadratic estimate of the optimum dietary PPSC inclusion for carcass yield was 7.58%. Previously, (Badr, 2019) 5%, 10% and 15% dietary prickly pear peels differentiated carcass parameters. Dietary inclusion of prickly pear cladodes also affected broiler dressing percentage (El-Neney *et al*., 2019), contrary to findings by Moula (2019). Similarly, dietary inclusion of prickly pear peels did not affect carcass characteristics of Japanese quails (Ragab, 2007), and of broilers (Ragab, 2012)

High, approximately 8.75% (diet G_5) and 10.9375 (diet F_5) PPSC in grower and finisher diets increased abdominal fat, comparable to the PC. The quadratic estimate optimum dietary PPSC inclusion for abdominal fat was 4.66%. In contrast, Moula (2019) observed a decrease in abdominal fat with 5% and 10% dietary prickly pear cladodes. Isikwenu *et al*., (2015) also reported the decrease in abdominal fat of broilers fed prickly pear fruit meal. This difference could be due to high methionine concentration in the experimental diet. Xie *et al*., (2006) and Wang *et al*., (2010) suggested high dietary methionine decreased the abdominal fat of broilers.

Low drip loss was recorded in broilers fed diets without or on low PPSC (G_0-F_0 and G_1-F_1 dietary regimes), like the PC, and to the highest dietary PPSC inclusion levels. This could have been caused by the time it took the meat samples to freeze after slaughter. Overall., PPSC had minimal effects on meat quality.

5.2. Conclusion

Within dietary fibre limitations, regression analyses indicated beneficial effects of graded inclusion of PPSC into sprouted cowpea-maize diets in terms of feed intake, live weight gain, gizzard size, meat colour and meat pH. The predicted optimum dietary inclusion level dependent on the broiler response variable. Pairwise comparisons for these parameters indicated the efficacy of the sprouted cowpea-based broiler diets can be upgraded to match the control, soybean-maize broiler diets. Similar to the regression predictions, parity was achieved at different PPSC inclusion level for different parameters. However, based on the carcass yield, approximately 3 % was considered optimum dietary PPSC inclusion in sprouted-cowpea based broiler diets. Hypothetically, given dietary formulation on a total amino acid basis, such benefit might result from improved availability of the limiting amino acids, or the effect of functional compounds, none of which were evaluated in this study. Therefore, further, dose-response studies are recommended to investigate the specific dietary components which account for the complementing effect of PPSC.

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