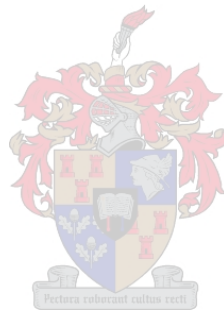


**The evaluation of locally produced canola oilcake  
meal as an alternative protein source in the diets  
of slaughter ostriches  
(*Struthio camelus var. domesticus*)**

by

**Johanet van der Merwe**



Thesis presented in fulfilment of the requirements for the degree of

**MASTER OF SCIENCE IN ANIMAL SCIENCES**

In the Faculty of AgriSciences at Stellenbosch University

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*Co-supervisor:* Prof. Louwrens C. Hoffman

April 2019

## **Declaration**

By submitting this thesis electronically, I declare that the entirety of the work contained therein is my own, original work, that I am the sole author thereof (save to the extent explicitly otherwise stated), that reproduction and publication thereof by Stellenbosch University will not infringe any third party rights and that I have not previously in its entirety or in part submitted it for obtaining any qualification.

March 2019

## Abstract

Feeding costs make out ~75% of all expenses in an intensive ostrich production system. Protein is one of the major components in ostrich diets. Currently, the main source of protein used in animal feed is soybean oilcake meal (SOCM). This protein source is, however, expensive as it is an imported raw material. In order to decrease feeding costs a locally produced alternative source of protein; canola oilcake meal (COCM) was identified. It is important to evaluate the possible influence alternative raw materials might have on the production and slaughter traits of animals as nutrition has a direct influence on production.

The use of COCM is limited in animals' diets due to its glucosinolate content. Glucosinolates are anti-nutritional factors that reduce palatability. Therefore, a study to determine whether or not the glucosinolate content has an influence on ostriches' feed intake was conducted. Grower ostriches in a free-choice system had access to five iso-nutritious diets with different inclusion levels of COCM, replacing SOCM in increments of 0%, 25%, 50%, 75% and 100%. The control diet (the diet with 0% inclusion of COCM) showed to be the preferred diet by having the highest average intake per bird per day over the entire trial period ( $736.1 \pm 74.1$  g/bird/day). The intake of this diet made up to 35% of the total daily intake while the diets containing COCM were consumed at levels lower than 18% of the total DMI per bird.

As the preference trial showed that the inclusion of COCM in ostrich diets might have a negative influence on feed intake in a free-choice system, production and slaughter traits were evaluated in the following trial. Ostriches were reared from 77-337 days of age on five iso-nutritious diets, each with a different inclusion level of COCM, replacing 0%, 25%, 50%, 75% and 100% of SOCM, respectively. Bird weight and feed intake were measured over the entire growth period. Results showed that the replacement of SOCM with COCM had little effect on the performance of ostriches. The ostriches that were reared on the diet replacing 75% of SOCM had the best performance in terms of slaughter and production traits.

Typically, production of the end-products (feather, leather and meat yield and quality) is directly influenced by nutrition. The replacement of SOCM with COCM had no influence on the production and quality characteristics of feathers, skin or meat.

This study concludes that the COCM can be used as a cheaper alternative protein source in the diets of slaughter ostriches without having any detrimental effect on growth, production parameters and slaughter traits. This will not only be beneficial to the ostrich industry but it will also benefit the local grain industry.

## Opsomming

Ongeveer 75% van al die uitgawes wat gepaardgaan met intensiewe volstruisboerdery, word toegeskryf aan voerkoste. Een van die hoofkomponente in volstruisdiëte is proteïen. Sojaboonoliekoek word tans hoofsaaklik gebruik as proteïenbron in dierevoer. Omdat die aanvraag na sojaboonoliekoek hoër is as die produksie daarvan in Suid-Afrika, word groot hoeveelhede sojaboonoliekoek jaarliks ingevoer. Dit laat die koste van voer aansienlik styg. In 'n poging om voerkoste te verlaag, is kanola-oliekoek as alternatiewe, plaaslik beskikbare proteïenbron geïdentifiseer. Omdat voeding 'n direkte invloed op produksie het, is dit belangrik om die moontlike invloed wat alternatiewe rou materiale mag hê, te ondersoek.

Kanola-oliekoek het 'n hoë glikosinolaatinhoud wat die insluiting daarvan in dierevoeding beperk. Glikosinolate is antinutriënte wat die smaaklikheid van voer verlaag. 'n Studie is gedoen om te bepaal tot watter mate die glikosinolaatinhoud van voer 'n invloed het op voerinnamings van volstruise. Volstruise (in die groeifase) is in 'n vrye-keuse sisteem van vyf verskillende diëte voorsien. Elke diëet het 'n verskillende insluitingsvlak van kanola oliekoek bevat. Die kanola-oliekoek het onderskeidelik 0%, 25%, 50%, 75% en 100% van die sojaboonoliekoek wat as proteïenbron in die diëte gedien het, vervang. Die volstruise het 'n duidelike voorkeur vir die kontrole diëet (wat geen kanola-oliekoek bevat het nie) getoon. Oor die hele proeftydperk was die gemiddelde inname van hierdie diëet 35%, terwyl die inname van die ander diëte laer as 18% elk was.

Omdat die voorkeurproef aangedui het dat die insluiting van kanola-oliekoek in volstruisdiëte 'n negatiewe invloed op voerinnamings in 'n vrye-keuse sisteem het, is die produksie- en slageienskappe geëvalueer. In hierdie studie is volstruisruikens vanaf die ouderdom van 77 dae tot 337 dae oud grootgemaak op vyf diëte. Vir elke diëet is die sojaboonoliekoek weereens vervang met kanola-oliekoek (0%, 25%, 50%, 75% en 100%, onderskeidelik). Voerinnamings en die gewigte van die volstruise is gedurende die hele proeftydperk gemonitor. Alhoewel die vervanging van sojaboonoliekoek met kanola-oliekoek in volstruisdiëte min tot geen effek op produksie gehad het, het die volstruise wat grootgemaak is op die diëet waarin 75% van die sojaboonoliekoek vervang is die beste produksie getoon. Die resultate na die evaluering van die eindprodukte (naamlik vere-, leer- en vleiskwaliteit en opbrengs) het getoon dat die vervanging van sojaboonoliekoek met kanola-oliekoek geen invloed op die produksie en kwaliteit van hierdie produkte het nie.

Die algehele gevolgtrekking van hierdie studie is dat kanola-oliekoek geskik om as proteïenbron te dien in diëte van slagvolstruise sonder dat enige nadelige effek op groei, produksie parameters en slageienskappe waargeneem sal word. Die gebruik van kanola-oliekoek sal nie net tot voordeel van die volstruisbedryf wees nie, maar sal ook tot voordeel vir die plaaslike graanindustrie wees.

## Acknowledgements

This study was carried out at the directorate: Animal Sciences, Western Cape Department of Agriculture. Permission to use the results from this project: The evaluation of locally produced canola oilcake meal as an alternative protein source in the diets of slaughter ostriches (*Struthio camelus var. domesticus*) (Project leader: Professor T.S. Brand), for a postgraduate study, is hereby acknowledged and greatly appreciated.

I would also like to thank the following persons and institutions for their contributions in making the completion of this study possible:

**My Heavenly Father** – For bringing this opportunity over my path and blessed me with the wisdom and strength to complete my studies.

**Professor T.S. Brand** – Thank you for providing me with the opportunity to complete my postgrad studies under your supervision. It was a privilege to work under your supervision. The guidance and the knowledge you shared with me is of inestimable value. Thank you for always making time for me when I needed help and for proofreading the final manuscript while you were on holiday.

**Professor L.C. Hoffman** – You taught me to look for an opportunity in each day and to take chances and follow my heart, no matter what others may think. Thank you for guiding me to communicate my thoughts in a scientific manner.

**The Directorate: Animal Sciences at Elsenburg** – Thank you for providing a professional working environment in which I could develop as a young scientist. Thank you to all the staff members for always being willing to help and give guidance.

**Western Cape Agricultural Research Trust** – For the financial support during my postgraduate studies and enabling me to attend conferences and symposiums.

**Protein Research Foundation** – For financial support.

**Mrs Gerty Mostert and Mr Alwyn Benson** – Thank you for always being willing to help me with a smile on administration and finances when I needed accommodation or transport and handling my finances at the trust.

**Marieta van der Rijst, Agricultural Research Council** – Thank you for all the time and effort you spent on the statistical analysis of my data.

**Oudtshoorn Research Farm** – For the use of the facilities over the entire growth period of the ostriches.

**Mr Stefan and Mrs Anel Engelbrecht** – Thank you for making me feel at home on the Oudtshoorn Research Farm. You always had time for me, work related, and non-work related. Your guidance in the time I was on the farm is deeply appreciated and the knowledge you shared with me is of non-estimated value.

**Molatelo Mokoелеle and Bernard Snyman** – It was a privilege to work with you. Thank you for driving me around on the farm and standing in for me when I could not be there. Bernard, thank you for always seeing the bright side and all the joking.

**Luwellin, Justin and the rest of the team at the chick section of the Oudtshoorn Research Farm** – Thank you for having patience with me and teaching me valuable information on the rearing of ostriches – information that cannot be learned in theory.

**Tonny Muvhali** – Thank you for allowing me to share the “verepaleis” with you and all the chats till late in the evenings, the advice and teaching me new skills.

**Ms Marinda Bosch, Mr Eugene de Bod, Mr Richard Pienaar and the staff from the Klein Karoo International Abattoir** – Thank you for the use of your facilities and accommodate and bearing me and my team during the slaughter of the trial ostriches. And the effort in keeping all of the carcasses separately until we were done with sampling.

**Mr Leon Lareman and the staff from the Klein Karoo International Tannery** – Thank you for all the trouble you had in keeping the skins of the trial separate and trusting me with it to use it for evaluation.

**Arthur Muller and the staff from Klein Karoo International** – Thank you for the trouble of keeping all birds’ feathers separate and accommodating us during the data collection.

**Gerhard Niemann** – Thank you for your patience, advice and guidance with the data capturing during the slaughter and providing me with data sheets and tips during the trial.

**Resia Swart** – Your friendship and motivation and willingness to help, no matter what, is deeply appreciated.

**Daniël van der Merwe** – Thank you for editing and proofreading my entire thesis.

**Leanne Jordaan, Nelius Nel, Christof Naudé and Anieka Muller** – Thank you for making the office a happy environment and lifting my mood.

**My family – Dad Johan, Mom Mariet, Maryna, Ouma, Lizbet** – Thank you for all your prayers, motivation and love. The moral support during my undergrad as well as postgrad studies is deeply appreciated. Thank you for always being there for me and for encouraging me to study and all the sacrifices you made to give me this opportunity.

**Hanno Loubser** – Thank you so much for your never-ending support and love. Thank you for always listening when I needed to rant and then encouraging and motivating me to keep going. Your advice and help and all the chocolates, hugs and messages when I needed it most, means the world to me.

## **Notes**

The language and referencing style used in this thesis are in accordance with the requirements of The South African Journal of Animal Sciences. This thesis presents a compilation of manuscripts where each chapter is an individual entity and some repetition between chapters was therefore unavoidable. It should be known that each chapter has its own reference list, instead of one comprehensive list appearing at the end of the thesis.



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## List of Abbreviations

ADF	Acid detergent fibre
ADG	Average daily gain
AFMA	Animal Feed Manufacturers Association
AI	Avian influenza
AgriLASA	Agri Laboratory Association of South Africa
ANOVA	Analysis of Variance
AOAC	Association of Official Analytical Chemists
°C	Degree Celcius
<i>ca.</i>	<i>circa</i> (about/around)
CF	Crude fibre
COCM	Canola oilcake meal
CP	Crude protein
DAFF	Department of Agriculture, Forestry and Fisheries
dm <sup>2</sup>	Cubic decimetre
DM	Dry matter
DMI	Dry matter intake
EU	European Union
FCR	Feed conversion ratio
g	Gram
GLM	General linear model
kg	Kilogram
LC-MS	Liquid chromatography-mass spectrometry
LSD	Least significant difference
LSM	Least square means
m	Meter
MCP	Monocalcium phosphate
ME	Metabolisable energy
MeOH	Methanol (CH <sub>3</sub> OH)
MJ	Megajoules
mL	Milliliter
mm	Millimeter
NaCl	Sodium chloride (common salt)
NAMC	National Agriculture Marketing Council
NDF	Neutral detergent fibre
PUFA	Polyunsaturated fatty acid
SE	Standard error
SOCM	Soybean oilcake meal
µmol	Micromolar

# Chapter 1

## General Introduction

Compared to traditional livestock practices, ostrich farming is a relatively young practice in the agriculture sector. Despite not being as developed, it fills a substantial gap in the local as well as international agricultural markets (Viljoen *et al.*, 2004). World-wide, the highest population of ostriches is found in the Klein Karoo region in South Africa, with 70-75% of all ostrich products being of South African origin (Brand & Jordaan, 2011; DAFF, 2017). About 90% of all ostriches in South Africa are slaughtered in the Oudtshoorn region (NAMC, 2010); which has the ideal hot weather and dry environment conditions for successful ostrich farming (Smit, 1964; DAFF, 2017).

Ostriches are multipurpose monogastric animals; the total income of a slaughter bird can be broken down to 65% of the income derived from the leather (skin), 20% from the meat and 15% from the feathers (Brand, T.S., Pers. Comm., Animal Production, Western Cape Department of Agriculture, Elsenburg, 7607, South Africa, December 2018). Since the domestication of ostriches in the 1800's, ostrich farming has expanded (DAFF, 2017) due to the increasing interest in ostrich products. The ostrich industry is still relatively small and is consistently pressuring producers to supply enough products to fulfil the ever growing demands of consumers. For successful farming of ostriches and to ensure products of high quality, farmers need good intensive production practices in order to ensure decent profit margins (Jordaan *et al.*, 2008).

One of the highest expenses of livestock production is feeding costs, which contributes up to 80% of all expenses (Brand & Jordaan, 2004; Brand & Jordaan, 2011). With properly formulated, well-balanced diets that fulfil the animals' requirements, containing locally produced raw materials, production costs can be reduced without having negative effects on the production and reproduction characteristics of the birds (Brand & Jordaan, 2004; Niknafs & Roura, 2018).

Protein is considered to be the most expensive component of ostrich feeds (Carstens, 2013; Dalle Zotte *et al.*, 2013). It is necessary for the sufficient level of protein to be included in the diet, as this essential nutrient is responsible for the production and maintenance of the muscle, skin and feathers (Smit, 1964). Currently, soybean oilcake meal (SOCM) is used as the predominant protein source in monogastric diets (Snyman, 2016). Despite the yearly increase in the production of local soybean, about two thirds of the soybean that is used in South Africa needs to be imported (Sihlobo & Kapuya, 2016; AFMA, 2017), subsequently leading to high feed prices (Dalle Zotte *et al.*, 2013). Therefore, it is necessary to identify alternative, locally produced protein sources in order to reduce the cost of feed. There is, however, little information available on the nutritive value of alternative protein sources for

ostrich diets (Brand *et al.*, 2000a). Research on the influence of alternative protein sources on the production traits of ostriches is needed.

A potential alternative protein source to SOCM is canola oilcake meal (COCM). Canola oilcake is the by-product of the process of extracting oil from canola seeds (Zheng *et al.*, 2017). With a concentrated protein content of approximately 40%, canola oilcake meal is seen as a good alternative protein source for use in animal feeds (Zeb, 1998; Dingyuan & Jianjun, 2007; De Kock & Agenbag, 2009; DAFF, 2016a; Nega & Woldes, 2018). Currently, South Africa has an oversupply of COCM, while high quantities of protein for animal feeds still have to be imported (DAFF, 2016b). Thus, the use of COCM will benefit the local grain producers as well as ostrich farmers. Furthermore, the highest production of canola in South Africa is in the Western Cape while ostrich farming in South Africa is also mainly based in this province (Brandt, 1998). Thus making this resource easier to obtain at more cost effective rates.

Although high in protein content and locally available, COCM contains high levels of glucosinolates, which are anti-nutrients that give a bitter taste to the feed and might, consequently, have a negative effect on the dry matter intake (DMI). Low DMI will lead to reduced productive performance (Niknafs & Roura, 2018). Therefore it is crucial to determine whether the inclusion of COCM will influence the palatability of the feed and thus the level of feed intake of the ostriches.

The aim of this study was to determine to what extent expensive protein sources (SOCM) in slaughter ostrich diets can be replaced by an alternative, locally produced plant protein source (COCM) without having any detrimental effect on the welfare, production parameters, slaughter traits and end-products. This has the potential to allow producers to formulate least cost diets and thus reduce the input costs in intensive ostrich production systems so as to achieve maximum profitability. It would also allow for an additional marketing channel for the oilseeds industry so as to generate income from the by-products of canola oil extraction.

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## Chapter 2

### Literature Review

#### 2.1 Introduction

Commercial farming of ostriches started in 1863 when ostriches were bred for the production of feathers. It is only recently that people became aware of the value of the other end-products (leather and meat). The ostrich industry is relatively small and young compared to other livestock industries (Brand & Olivier, 2011; Cloete *et al.*, 2012). Despite the size of the industry, it plays a significant role in the agricultural industry, particularly in the Western Cape province. The average gross value over the past decade for ostrich production in South Africa amounted to R391 million (DAFF, 2017). As the ostrich industry is relative small, it is vulnerable to numerous external factors. Therefore, for the successful farming of ostriches, farmers need good intensive production/management practices in order to ensure decent profit margins (Jordaan *et al.*, 2008).

The largest expense of most livestock production systems, including that applicable to ostriches is feeding costs, which contributes up to 80% of all expenses (Brand & Jordaan, 2004). With a properly formulated, well-balanced diet that fulfils the animals' needs, containing locally produced raw materials, production costs can be reduced without having negative effects on the production and reproduction of the animals (Brand & Jordaan, 2004; Niknafs & Roura, 2018). Feeding costs are not only the largest expense, but it is also the most important expense as nutrition has a direct influence on production.

Besides energy, protein makes up the largest component of animal feed and is therefore the second most expensive component of animal feed (Carstens, 2013; Dalle Zotte *et al.*, 2013). With the rapid growth in human population, protein is becoming less available and more expensive due to the competition between humans and animals for protein as food and feed, respectively (Brand *et al.*, 2000; Brand *et al.*, 2004a; Sridhar & Bhat, 2007). In order to maximise profit margins, it might be necessary to consider alternative sources of protein that are available to be used in animal feed.

Currently, soybean is the main source of protein used in animal feeds (Snyman, 2016). The production of soybean in South Africa is, unfortunately, not as high as the demand thereof (Sihlobo & Kapuya, 2016). A large quantity of soybean must therefore be imported. This necessitates the identification of alternative, locally produced raw materials that could be incorporated in stock feeds in order to cut on expenses and amplify profit margins. Possible alternative plant protein sources for animal feed, that are locally grown, include lupins and canola. Furthermore, the price of canola oilcake meal ranges between 60 and 70% of the price of soybean oilcake meal (Hickling, 2007).

Engelbrecht (2016) and Niemann (2018) showed that lupins and full-fat canola could be used as alternative protein sources to soybean meal without having detrimental effects on the production and growth of slaughter ostriches. The current study was conducted in order to determine the effect of locally produced canola oilcake meal (COCM) as protein source on the growth and production of slaughter ostriches and whether ostriches prefer or reject feed containing canola oilcake meal.

## 2.2 South African Canola industry

Canola is an oilseed crop derived from rapeseed. Rapeseed is not considered as a viable food source because of its high levels of undesirable compounds such as glucosinolates, phytase, hulls and phenolics (Naczki *et al.*, 2000). However, rapeseed is an important agriculture crop because of its high levels of oil and protein content (Zeb, 1998). The oil content of rapeseed can be as high as 40%, and after this oil has been extracted, the remaining residue has a high protein content of 38 – 43% (Zeb, 1998).

In 1974, a special biotype of rapeseed was produced through selective breeding in Canada in order to reduce the levels of antinutrients and erucic acid ( $C_{22}H_{42}O_2$ ; cis-13-docosenoic acid) within the rapeseed. This biotype is now commonly known as “canola”, an acronym for the phrase “Canadian oil, low acid” (De Kock & Agenbag, 2009; DAFF, 2016a). In order for rapeseed to be known as “canola”, the erucic acid content in the oil must be less than 2% of all the fatty acids and the oil-free dry matter of the seed must contain no more than 30  $\mu$ moles per gram glucosinolates (Bell, 1993). *Brassica napus* and *Brassica campestris* are currently the two most commonly cultivated types of canola (Thacker, 1990; Unger, 1990).

From 1992, when canola was produced for the first time in South Africa, production increased exponentially with a national production of 1 690 374 tons in 2015 (DAFF, 2016a). The Western Cape province is the main producer of canola in South Africa, producing about 99% of the national crop (DAFF, 2013; 2016a; 2016b). Canola is grown as a winter-crop in crop-rotation with other crops. In such systems where wheat was planted after canola, wheat yields have increased with up to 25% (DAFF, 2013).

Canola is cultivated for purpose of oil production with the remaining oilcake after extraction being widely popular in livestock feeds. Canola oil is popular, specifically for its health benefits as it contains low levels of saturated fats, and has a high omega 3 fatty acid content (DAFF, 2013). As people are becoming more aware of maintaining a healthy lifestyle, the demand for canola oil is expected to increase.

Canola oil is derived from the seeds by mechanical pressing or by solvent extraction. Pressing alone is relatively inefficient, as a large portion of the oil still remains in the oilcake. By solvent extraction, the majority of the oil can be extracted. There are two processes for the

pressing of canola seeds, namely cold and hot pressing, with hot pressing being more effective as it extracts a higher oil yield (Zheng *et al.*, 2017). The process of oil extraction starts with the cleaning of the seeds of dust, weed and foreign particles that might have contaminated between the seeds during harvesting. The cleaning process includes removing foreign particles by aspiration and screen separation to remove over- and undersized particles. The seeds are pre-heated before they are flaked in order to improve flake formation and extraction efficiency. Flaking of the seeds is done to ensure the cell walls surrounding the lipid globules in the seeds can be ruptured and that the lipid can flow out easily with pressing. The seeds are cooked in order to ensure that oil-soluble glucosinolate derivatives are not produced and extracted with the oil. Thereafter, the seeds are pressed to derive the oil which in turn is filtered to remove any solids. The pressed cake that remains after the extraction process undergoes solvent extraction using hexane. The hexane solvent must then be removed after the final extraction via a distillation system (Unger, 1990; Thanaseelaan, 2013). The residue after the extraction of oil, canola oilcake, is a good source of protein (36 – 40%) and is therefore popular for incorporation into animal feeds (Canola Production Guide, 2013; DAFF, 2016a; Nega & Woldes, 2018).

**Table 2.1:** The average nutrient composition of soybean oilcake meal, full fat canola seeds, solvent-extracted canola oilcake meal and mechanical-extracted canola oilcake meal

Nutrient composition	Soybean oilcake meal	Full fat Canola	Solvent extracted canola oilcake meal	Mechanical extracted canola oilcake meal
Dry matter (% as fed)	87.9	92.3	88.8	89.9
Gross energy (MJ/kg feed)	19.7	28.8	19.4	20.8
Crude protein (% DM)	51.8	20.9	38.3	35.6
Crude fibre (% DM)	6.7	10.1	14.1	13.2
NDF <sup>1</sup> (% DM)	13.7	20.4	31.1	29.9
ADF <sup>2</sup> (% DM)	8.3	14.4	20.4	19.7
Lignin (% DM)	0.8	6.3	9.5	9.1
Ether extract (% DM)	2.0	4.6	2.7	9.2
Ash (% DM)	7.1	4.3	7.8	6.9
Total sugars (% DM)	9.4	5.5	10.4	9.8
Amino acid composition (presented as % of total protein)				
Lysine (%)	6.1	6.3	5.5	5.6
Methionine (%)	1.4	2.0	2.0	2.2
Threonine (%)	3.9	4.8	4.3	4.7
Tryptophan (%)	1.3	1.3	1.2	1.3
Arginine (%)	7.4	6.2	6	6.3
Anti-nutritional factors				
Tannins (g/kg DM)	6.9	7.2	5	10.8
Glucosinolates ( $\mu\text{mol/g DM}$ )	0.0	14.5	11.7	15.3

<sup>1</sup> Neutral detergent fibre<sup>2</sup> Acid detergent fibre

### 2.3 Canola oilcake in animal feeds

Canola oilcake (also referred to as canola oilcake meal; COCM) is the residue after the extraction of oil from the seeds via chemical or mechanical pressing processes (Canola Production Guide, 2013; Zheng *et al.*, 2017). To ensure maximum extraction, an organic solvent such as hexane is used in common industrial processes (Thanaseelaan, 2013). The nutritional composition of commercial South African canola oilcake is comparable to the content of soybean oilcake as it has a minimum protein content of 36%, oil content of 1.5% and by-pass protein of 28% (Zeb, 1998; De Kock & Agenbag, 2009; Newkirk, 2009; Canola Production Guide, 2013). The nutritive value of canola oilcake may, however, be influenced by the environmental conditions in which the canola was grown and harvested and also the cultivar and processing of the seeds during oil extraction (Newkirk, 2009). According to Zeb (1998) and Newkirk (2009), canola oilcake has a well-balanced amino acid profile. Although it is, like other vegetable crops, limited in lysine, it still has a higher methionine content than

soya (Yapar & Clandinin, 1972; Newkirk, 2009). In terms of essential amino acids, canola oilcake has a far better profile than most cereals. In comparison to other vegetable oilseed meals, COCM is also a good source of essential minerals (Bell, 1993). Various studies have investigated the use of canola oilcake in the nutrition of livestock species. Depending on the species, age and level of production, canola oilcake can successfully be used as supplement in the feed (Nega, 1998). The fibre content of 11.7% in COCM is higher than that of soybean oilcake meal; this is due to the presence of canola seed hulls which are not removed from the meal, with the hulls making up a relatively high proportion of the seed (Newkirk, 2009). Most of the fibre in COCM is in the form of NDF, with the NDF content being approximately 10% higher than the ADF content (Canola meal feeding guide, 2015). This is important to consider, particularly in ostrich nutrition, as studies by Brand *et al.* (2000b) showed that ostriches fed on high fibre diets had lower daily intakes than those on a diet with lower fibre content. These animals also exhibited a decrease in growth rate, which may lead to a decrease in production of slaughter ostriches. Therefore, it is key to correctly balance the energy and roughage compositions of the diets by not oversupplying COCM.

#### **2.4 Anti-nutritional factors in canola oilcake meal**

Although it is a popular raw material in animal nutrition, the use of canola, especially COCM, in animal feed is limited due to the presence of anti-nutritional factors (ANFs), especially erucic acid and glucosinolates (Dingyuan & Jianjun, 2007).

Glucosinolates are anti-nutritional factors that influence the palatability of feed and causes COCM to have a bitter taste (Dingyuan & Jianjun, 2007). After the extraction of oil from the canola seeds, glucosinolates are concentrated in the oilcake (Zeb, 1998). Consequently, high inclusion levels thereof could have detrimental effects on the animal's production and reproduction due to lower feed intake (Tripathi & Mishra, 2007; Niknafs & Roura, 2018). In previous studies, it was found that high levels of glucosinolates caused impaired thyroid function in growing animals, foetuses and embryos and, liver haemorrhage mortality in laying hens (Campbell & Schöne, 1998). This ANF limits the amount of canola oilcake meal that can be included in animal feed (Dingyuan & Jianjun, 2007). Quinsac *et al.* (1994) found that a glucosinolate content of 15.8  $\mu\text{mol/g}$  was not high enough to have a detrimental effect on the feed intake of broilers. Niemann *et al.* (2018) showed that glucosinolate levels of 2.156  $\mu\text{mol/g}$  were not sufficient to cause a negative effect on the intake and growth of slaughter ostriches when full fat canola was included in their diets. However, the oil extraction process may result in concentrating glucosinolates in the oilcake, which could limit the level at which COCM can be included in the diet (Zeb, 1998). Erucic acid is present in canola oil. This fatty acid is considered to be toxic when excessive amounts are ingested. As COCM has low oil content,

the detrimental effects thereof on the performance and health of livestock is of less concern (Breytenbach, 2005).

Anti-nutritional factors that is of less concern in COCM, but is present in canola includes sinapine, phytic acid, tannins, phenolics and high dietary fibre (Khajali & Slominski, 2012; Naczka *et al.*, 1997). Sinapine makes up 1 – 4% of COCM (Blair & Reichert, 1984). This ANF is a choline ester of sinapic acid and has a bitter taste that may affect feed consumption (Butler *et al.*, 1982). Phosphorus is stored in the form of phytic acid in canola seeds (Khajali & Slominski, 2012). Phytic acid is considered to be an ANF as it forms insoluble complexes with calcium, iron, zinc, manganese and magnesium, making it unavailable to the animal (Cabahuag *et al.*, 1999). Condensed tannins are found in the hulls of canola seeds. About 70 – 96% of the total tannins found in the hulls of canola seeds is insoluble. These tannins form insoluble compounds with the proteolytic enzymes, proteins and fibre in animals' gastrointestinal tracts and cause a decrease in protein ingestion (Naczka *et al.*, 2000; Khajali & Slominski, 2012).

Generally, soybean oilcake is preferred in intensive livestock production systems as it has the highest protein content of up to 48% (BFAP Baseline Agricultural Outlook 2018 - 2027, 2018). Thus, in order to match the same level of protein, a higher inclusion level of canola oilcake will be needed in the diet (Thacker, 1990). However, as mentioned, the use of canola oilcake in animal feed is constrained due to its high fibre content and the presence of ANFs. Therefore, the substitution of soybean with canola oilcake will only be possible when it can be acquired at exceptional low prices (BFAP Baseline Agricultural Outlook 2018 - 2027, 2018).

## **2.5 The ostrich industry in South Africa**

During the 1800's, ostrich feathers became very popular in the fashion industry of Europe. Ostriches were then domesticated and bred exclusively for supplying of feathers to the industry. The increasing demand for ostrich feathers lead to the farming of ostriches world-wide with ostrich farming in South Africa starting around 1865. The oversupply of ostrich feathers resulted in the industry collapse in 1885 with the recovery of the industry being delayed by the Anglo-Boer War (1899-1902). After these setbacks, the ostrich industry became bigger than it was before with its peak being reached in 1913. With the onset of World War I, the feather market collapsed completely and by 1930 and ostrich farming had reached an all-time low. The industry recovered slowly and after World War II ostrich farming in South Africa was revived, not only for the production of feathers but also for the production of skins and meat, especially biltong. The Klein Karoo Landbou Koöperasie was established in 1945 in Oudtshoorn in the Klein Karoo for the buying, exporting and selling of ostrich feathers (Deeming *et al.*, 1999). In the following years (1964), the first ostrich abattoir in South Africa was opened, followed by a tannery in 1970 (Smit, 1964; Jorgensen, 2014). Currently, 90% of



all ostriches slaughtered nationwide are slaughtered in Oudtshoorn (NAMC, 2010). This location has the highest concentration of ostriches. This region has an ideal climate for successful ostrich breeding as these desert animals flourish in hot, arid conditions (DAFF, 2017). South Africa is the largest producer of ostrich products (i.e. feather, leather and meat) and a net exporter of these products. About 75% of all ostrich products world-wide has a South African origin (DAFF, 2017).

Ostriches are multipurpose animals producing feathers, leather and meat that contribute to the income derived from these animals. Ostrich feathers were responsible for the establishment of the ostrich industry, product emphasis has shifted over time resulting in feathers to be of least income nowadays and meat and leather becoming more dominant sources of income (Brand & Cloete, 2015). Feathers are mainly used as feather dusters and in the fashion industry (van Zyl, 2001; DAFF, 2017). The demand for ostrich meat is growing in the western countries as people are more conscious of living a healthy lifestyle. Ostrich meat is regarded as a healthier alternative for red meat as it has low levels of intra-muscular fat, saturated fat and cholesterol and contains high quality protein, iron and vitamin E (Mellett, 1992; Sales & Oliver-Lyons, 1996; Majewska *et al.*, 2009; Poławska *et al.*, 2011). However, the most sought after product derived from the ostrich, is the leather. Ostrich leather is unique and can easily be distinguished from other types of leather due to the raised bumps caused by the feather follicles on the surface of this leather (Meyer *et al.*, 2004; NAMC, 2010; Engelbrecht, 2014). Each and every skin is unique due to the different shapes, sizes and patterns of these nodules. This leather is supple and durable and is regarded as one of the most attractive leather types of all exotic leathers (NAMC, 2010; Engelbrecht, 2014). Currently, the economic value of an ostrich can be broken down into 65% skin, 20% meat and 15% feathers (Brand, T.S., Pers. Comm., Animal Production, Western Cape Department of Agriculture, Elsenburg, 7607, South Africa, December 2018).

Due to the increasing interest in ostrich end-products, ostrich farming expanded, especially in South Africa as this is one of the very few places world-wide where ostriches are commercially farmed (DAFF, 2017). As ostriches are desert animals, they survive best in hot, arid environments. Oudtshoorn in the Klein Karoo region of South Africa adheres to these requirements as this region has the perfect weather conditions for the rearing of these animals (Smit, 1964; DAFF, 2017).

Compared to other livestock practices, the ostrich industry is still relatively small as it is also one of the youngest practices in the agricultural sector. However, this industry has a substantial influence on the national as well as international market (Viljoen *et al.*, 2004). Between 70-75% of all ostrich products world-wide originates from South Africa (Brand & Jordaan, 2011; DAFF, 2017) making South Africa a net exporter of ostrich products. Due to the size of the industry, a relative small setback could have major impacts on the industry and

will influence the economy both on a national and international level. There is thus consistent pressure on producers to supply enough products of a good quality to fulfil the requirements of consumers.

The ostrich industry world-wide experienced setbacks in 2004, 2011 and 2017 due to the outbreak of avian influenza (AI) and the consequent ban of export of fresh meat into the European Union. The value of ostrich meat was affected negatively and the industry as a whole paid the price. Currently, the industry is still recovering from the previous AI outbreak and is under pressure to produce sufficient products. High input costs lead to narrow profit margins in the ostrich industry and the ban on the export of meat due to the occurrence of AI, has left the industry in a vulnerable state (Brand & Jordaan, 2011). Furthermore, the ostrich industry has become relatively small due to these economic realities and through the exasperation of the AI and so any changes in the nutrition or management of the birds will have an impact on the producers' commercial viability. It is therefore crucial for producers to have good management practices in place and to cut down on unnecessary expenses and lower input costs while still sustaining production levels.

Producers are consistently looking for options to amplify their profit margin in order to ensure that ostrich farming remains a sustainable practice. As feed is the largest expense in an intensive production system, approximately 75%, of all expenses in a livestock farming system (Brand *et al.*, 2002; Aganga *et al.*, 2003; Brand & Jordaan, 2011), this is the first expense farmers attempt to reduce. The feeding costs are also the most important expenses as feeding has a direct influence on the growth and maintenance of the animals and, consequently, on the products produced by the animals (Niknafs & Roura, 2018). As the latter, ultimately influences the income that the producer obtains, it is therefore necessary to first consider any influence that feed constituents may have on the products.

## **2.6 Ostrich nutrition**

Studies based on ostrich nutrition revealed that ostriches ingest, like all other animals, according to their nutritional requirements, especially to satisfy their energy requirements with respect to the content of the feed (Bozinovic & Del Rio, 1996; Niknafs & Roura, 2018). Several studies based on ostrich nutrition (Brand *et al.*, 2000a; Brand *et al.*, 2000b, Brand *et al.*, 2006) showed that an ostrich's intake is dependent on the energy content of the feed, with higher intakes being realised when consuming feeds that are low in energy. In a study by Viviers (2015) where ostriches were reared on diets with different levels of protein, it was found that the birds that were reared on diets with higher inclusion levels of protein, had higher DMI. Despite that, the slaughter weights of ostriches reared on diets with low protein levels, did not differ from those of the ostriches that received high protein levels. This was attributed to

compensatory growth. Good nutrition from hatching is essential in ensuring welfare, health, growth, development and production of high quality end-products. An unbalanced diet will result in poor feeding efficiency and thus poor growth (Cooper, 2004; Cooper *et al.*, 2004). It is crucial that optimised consumption of balanced diets can be ensured to meet production requirements (Niknafs & Roura, 2018)

The growth of an ostrich chick can be divided into four nutritionally unique phases: pre-starter (one to eight weeks of age), starter (eight to 16 weeks of age), grower (four to six months of age) and finisher (six to 10 months of age) (Cooper *et al.*, 2004). The nutrient density in the feed of the different phases decreases from pre-starter to finisher. As the young chicks have a smaller capacity, they consume less feed thus the feed needs to be high in nutrients in order to fulfil the chicks' requirements for the high growth rates experienced in this stage (Carstens, 2013). As the nutrient requirements of the ostriches are constantly changing according to their growing phase, their diets need to be adjusted accordingly. Theoretically, the growth rate of an ostrich increases from hatching to six months of age where after the rate of growth decreases till the age of fourteen months. The current practice is to slaughter ostriches at the age of ten to eleven months (Brand & Olivier, 2011) to ensure maximum income and profitability. The minimum protein content in the feed of ostriches for the different growing phases are as follow: 190 g/kg for the pre-starter phase, 170 g/kg for the starter phase, 150 g/kg for the grower phase and 120 g/kg for the finisher phase (Brand, 2016).

Aside from the energy content of the feed, it is argued that palatability of the feed could be a driver in influencing feed intake. Although there is an absence of taste buds in the beaks of ostriches, several studies concluded that taste have an influence on their DMI (Kare *et al.*, 1957; Jackowiak & Ludwig, 2008; Kruger *et al.*, 2008). According to Ganchrow *et al.* (1991), the taste buds may be located on the hard palate of the beak or at the openings of the ducts of the salivary glands. In a study where feed was artificially coloured in order to determine whether or not ostriches can distinguish between feed colour, no differences was found in DMI (Kruger, 2007; Kruger *et al.*, 2008). Janse van Vuuren (2008) determined whether ostriches have preference towards colour and reported no preference towards different coloured feed that were fed to the chicks. Interestingly, feed that were fed to chicks with no previous exposure to feed were artificially flavoured to be sweet, sour, bitter and salty. These chicks showed preference towards the salty feed (Kruger, 2007). In another study, different levels of salt were included in ostrich diets (0.4%, 1.4%, 2.4% and 3.4%). The diet with a salt inclusion level of 1.4% had the highest DMI and the best performance in terms of weight at slaughter and FCR. It was concluded that ostriches will perform better on diets containing higher levels of salt as they have more preference towards higher salt levels (Kruger, 2007). In continuation ostrich chicks were reared in a free-choice system on artificially flavoured feed with the following flavours: meat, seafood, citrus, aniseed, lusern and mint. The intake for the seafood

flavoured feed was the highest and was explained by the higher salt content of this flavour (Janse van Vuuren, 2008). These findings imply that although ostriches cannot distinguish between feed colour, smell and taste of the feed will have an influence on feed intake.

Knowledge on ostrich nutrition is relative scarce as this industry is new in comparison to other livestock practices (Brand & Olivier, 2011). Nutrition makes up the largest component of an intensive ostrich production system (Brand & Jordaan, 2011). It is thus of great importance to investigate the use of various alternative (and cheaper) raw materials that can potentially be incorporated in the diets of ostriches.

## **2.7 Impact of protein nutrition on ostrich products**

Although the production of feathers led to the domestication and farming of ostriches, the emphasis has shifted resulting in feathers being the least important product in terms of economic income, while the income of ostrich meat and leather increased. Despite being the lowest source of income, good quality feathers as a result of good management practices might be the difference between profit or loss (Engelbrecht, 2014). Studies have shown that the level of energy and protein in the diets of slaughter ostriches do not influence the yield or quality of feathers (Carstens, 2013; Viviers, 2015). While the source of protein in the diets also do not carry any affect (Brand *et al.*, 2018; Niemann, 2018).

Ostrich meat is the second largest source of income in the ostrich industry. Ostrich meat is known to be low in cholesterol and has favourable polyunsaturated fatty acid (PUFA) profile with low intramuscularfat concentration and is also rich in iron (Mellett, 1993; Dalle Zotte *et al.*, 2013). This makes ostrich meat a popular red meat alternative for people who are more aware of maintaining a healthy lifestyle. Compared to chicken, beef and turkey, ostrich meat has the most favourable fat:protein ratio with low unsaturated fat and high protein content. The tenderness of ostrich meat is comparable to turkey (Sales & Hayes, 1996; Paleari *et al.*, 1998).

The income from ostrich leather is the most important contributor to the local as well as international economy within the ostrich industry. Grading according to physical appearance is done subjectively by trained graders with only the crust surface area being measured objectively. The minimum requirements for ostrich leather characteristics are yet to be clearly defined (Engelbrecht *et al.*, 2009). Brand *et al.* (2018) and Niemann (2018) concluded that protein source did not influence leather quality. This conclusion is supported further by Brand *et al.* (2004, 2014, 2018), Cloete *et al.* (2006), Engelbrecht *et al.* (2009) and Viviers (2015) who did similar studies to determine whether diet, especially energy level and protein content, has an effect on leather quality.

## 2.8 Concluding remarks

Although protein is an expensive commodity which is becoming more scarce, there is little information available on the nutritive value of alternative protein sources for ostrich diets (Brand *et al.*, 2000a). Therefore, it is important to quantify the nutritive value of alternative protein sources by formulating diets that fit the ostriches' specific needs without having a negative influence on production. Locally produced, alternative raw materials to soybean that have been identified include lupins, full-fat canola meal as well as canola oilcake meal (COCM). Brand *et al.* (2018) gradually replaced soybean oilcake meal with sweet lupin (*Lupinus angustifolius*) seed in ostrich diets with successful results. In a similar study by Niemann (2018), soybean oilcake meal was gradually replaced with full-fat canola meal. After evaluating the production characteristics and product qualities, it was noted that up to 75% of soybean oilcake meal in the ostriches' diet could be replaced with full-fat canola meal. The current study aims to evaluate the use of COCM in possibly replacing soybean oilcake meal as the primary protein source in ostrich nutrition. This information will not only be useful to ostrich farmers but also contribute to the canola oilseed industry, as it could provide another marketing opportunity for the by-product of oil extraction.

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## Chapter 3

### The feeding preferences of grower ostriches to feed containing increasing levels of canola oilcake meal

#### Abstract

As canola oilcake meal (COCM), though, contains anti-nutritional factors, specifically glucosinolates, which influence palatability and may negatively impact feed intake and the animal's production, the aim of this trial was to determine to what extent canola oilcake meal can be included in the diets of ostriches without having detrimental effects on dry matter intake (DMI). Sixty ostriches were divided into ten camps where they were exposed to five different diets with increasing levels of canola oilcake meal (0%, 5%, 10%, 15% and 20%) incrementally replacing soybean oilcake meal. The DMI of each of the five diets that were present in each camp was measured on a daily basis for 15 consecutive days. The feed colour characteristics were measured to determine if colour may have an effect on the preference of ostriches to a particular feed. No differences in colour parameters were observed between the different diets ( $P > 0.05$ ). It was found that the ostriches preferred the diet with a 0% inclusion of canola oilcake meal, as the highest intakes were observed with this diet ( $736.1 \pm 74.1$  g/bird/day). Intake of the 0% inclusion diet made up 35.5% of the total daily intake, while the other diets with higher COCM inclusion levels were consumed at levels lower than 18% of the total daily DMI per bird. Based on the results of this study, the inclusion of canola oilcake meal as an alternative to soybean oilcake meal in the diets of ostriches might have a negative influence on feed intake in a free-choice situation and should be used with caution at high levels in the diets of ostriches. Research is necessary to evaluate production of ostriches receiving diets with COCM in a feedlot situation without choice.

#### 3.1 Introduction

For the successful farming of ostriches, farmers need good intensive production practices in order to ensure decent profit margins (Jordaan *et al.*, 2008). One of the largest expenses of livestock production is feeding costs, which contributes up to 80% of all expenses (Brand & Jordaan, 2004). With a properly formulated, well-balanced diet that fulfils the animals' needs, containing locally produced raw materials, production costs can be reduced without having negative effects on the production and reproduction of the animals (Brand & Jordaan, 2004; Niknafs & Roura, 2018).

Protein is a major component of ostrich diets and is also considered the most expensive component of the feed (Carstens, 2014; Dalle Zotte *et al.*, 2013). Protein is essential for the production and maintenance of the muscle, skin and feathers (Smit, 1964).

Currently, soybean oilcake meal is used as the predominant protein source in monogastric diets (Snyman, 2016). According to Sihlobo & Kapuya (2016), locally soybean production only satisfies one third of the country's needs. Therefore, large quantities of soybean are imported, resulting in high costs associated with this raw material (Dalle Zotte *et al.*, 2013).

An alternative to the expensive soybean oilcake meal that is currently used is canola oilcake meal. Canola oilcake is a by-product of the process of extracting oil from canola seeds (Zheng *et al.*, 2017). With a concentrated protein content of approximately 40%, canola oilcake meal (COCM) is seen as a good alternative protein source for use in animal feed (Zeb, 1998; Dingyuan & Jianjun, 2007; De Kock & Agenbag, 2009; DAFF, 2016a; Nega & Woldes, 2018). South Africa, currently has an oversupply of COCM, while high quantities of protein for animal feeds still have to be imported (DAFF, 2016b). Thus, the use of COCM will benefit the local grain producers as well as ostrich farmers. Although high in protein content, COCM contains glucosinolates, which are anti-nutrients that give a bitter taste to the feed. The levels of glucosinolates that cause the undesirable taste in the feed might have a negative effect on the dry matter intake (DMI) that will cause reduced productive performance (Niknafs & Roura, 2018).

Therefore, this study was conducted to determine the effect of COCM inclusion on the feeding preference of ostriches and whether it will have an impact on the DMI of the feed. Slaughter ostriches in the grower phase were exposed to diets with increasing levels of COCM, replacing soybean oilcake meal. The aim of this study was to determine to what extent COCM can be included in the diets of grower ostriches before it results in a detrimental effect on their intake.

### **3.2 Materials and Methods**

This preference trial was conducted during June 2017 on the Oudtshoorn Research Farm (situated at longitude 22°63'E and latitude 33°63'S at an altitude of 307 m above sea level) in the Klein Karoo, Western Cape, South Africa. Ethical clearance was obtained from the Elsenburg departmental ethics committee (DECRA R14/108). The trial ran for a period of 15 consecutive days. Sixty grower ostriches with an age of 188 days and average initial body weight of  $67.4 \pm 8.2$  kg were randomly divided into ten camps (32 m x 30 m) with six birds per camp.

Each of the 10 camps contained five feeding troughs (46 cm x 23 cm x 20 cm) and one water trough (29 cm x 20 cm x 15 cm) in fixed positions around the camp. Each of the feeding troughs was filled with one of the experimental diets. The order of the diets in the troughs were randomised from camp to camp in order to exclude behavioural habits. All five diets as well as water were available *ad libitum* to the ostriches in each camp. Feed and water were

monitored and filled twice daily (morning as well as midday) and the feed in each trough was then mixed by hand in order to stimulate feed intake. The refusals in each of the feeding troughs were weighed back at the same time every day and subtracted from the amount that was fed the previous day in order to calculate the dry matter intake (DMI) of each diet in each camp over 24 hours.

The five experimental diets (Table 3.1) were formulated by Mixit2+ software (Agricultural Software Consultants Inc., San Diego, USA). Diet 1 served as the control and contained no COCM. The consecutive diets were formulated to incrementally replace soybean oilcake meal with increasing levels of COCM, namely at replacement levels of 0%, 25%, 50%, 75% with the final diet containing only COCM as protein source (respectively 0%, 5%, 10%, 15% and 20% COCM in the complete diets).

The feeds were milled, mixed, and pelleted on the Oudtshoorn Research Farm where the trial was conducted. Approximately 1 kg of feed was sampled for each ton of feed that was pelleted. These samples were ground through a 1.5 mm screen by making use of Retsch TM ZM200 sample mill (Haan, Germany). Proximate analyses were performed on these ground samples to determine the dry matter (DM) (method 934.01), crude protein (CP) (method 990.03), crude fibre (CF) (Goering & Van Soest, 1970), acid detergent fibre (ADF) and neutral detergent fibre (NDF) (Van Soest *et al.*, 1991). All the chemical analyses were according to the methods as described by the Association of Official Analytical Chemists (AOAC, 2012). Calcium (Ca) and phosphorous (P) values was obtained according to the methods outlined by AgriLASA (1998). The amino acid composition of each diet was obtained from the formulated diet specifications of the raw materials. The ingredient and nutritional compositions of trial feeds are shown in Table 3.1.

**Table 3.1:** The ingredient and chemical composition of five experimental diets containing increasing levels of canola oilcake meal fed to grower ostriches (as is basis)

Ingredients (kg/ton)	Diets expressed as percentage canola oilcake meal replacing soybean oilcake meal				
	0%	25%	50%	75%	100%
Canola oilcake meal	0.00	50.02	100.05	150.07	200.09
Wheat grain	583.64	571.40	559.17	546.93	534.69
Oats hulls	171.20	155.97	140.74	125.50	110.27
Soybean oilcake meal, 44% CP <sup>1</sup>	134.58	100.94	67.29	33.65	0.00
Lucerne meal, 17% CP <sup>1</sup>	0.00	12.50	25.00	37.50	50.00
Molasses meal	40.00	40.00	40.00	40.00	40.00
Kynofos21/MCP <sup>2</sup>	26.66	26.25	25.84	25.42	25.01
Limestone, ground	17.09	16.22	15.35	14.48	13.61
Sodium Bentonite	10.00	10.00	10.00	10.00	10.00
Common salt/NaCl <sup>3</sup>	10.00	10.00	10.00	10.00	10.00
Vitamin & Mineral Premix <sup>*</sup>	5.00	5.00	5.00	5.00	5.00
Lysine-HCL (L-lysine 95%)	1.83	1.71	1.58	1.46	1.33
Nutrient composition (laboratory analysis)					
Dry matter (g/kg)	903.98	901.58	901.33	883.03	884.00
ME <sup>4</sup> ostrich (MJ/kg feed)	10.42	10.34	10.29	10.01	10.02
Crude protein (g/kg)	162.29	157.60	160.42	146.35	151.67
Crude fibre (g/kg)	82.55	81.55	84.27	87.57	97.07
Acid detergent fibre (g/kg)	105.15	118.23	119.35	118.33	132.45
Neutral detergent fibre (g/kg)	177.87	204.25	205.07	213.57	230.62
Calcium (g/kg)	12.67	12.13	13.17	18.63	16.57
Phosphorous (g/kg)	8.73	9.50	10.30	10.40	10.03
Amino acid composition (formulated)					
Lysine (g/kg)	0.71	0.71	0.71	0.71	0.71
TSAA <sup>5</sup> (g/kg)	0.41	0.45	0.48	0.52	0.55
Threonine (g/kg)	0.44	0.46	0.48	0.50	0.53
Tryptophan (g/kg)	0.18	0.18	0.18	0.17	0.17
Arginine (g/kg)	0.68	0.68	0.68	0.68	0.68

\*Refer to Annexure A for the composition of the vitamin and mineral premix for grower ostriches

<sup>1</sup> Crude protein

<sup>2</sup> Sodium chloride

<sup>3</sup> Monocalcium phosphate

<sup>4</sup> Metabolisable energy, as formulated by the formula ME ostrich = 6.35 + 0.645 × ME poultry (Brand & Gous, 2006)

<sup>5</sup> Total sulphur containing amino acid

The glucosinolate concentration of the dry COCM was determined by an adapted method of liquid chromatography-mass spectrometry (LC-MS) as described by Sasaki *et al.* (2012). The samples were prepared by extracting 1 g of the canola oilcake meal with 25 mL of 50% MeOH/1% formic acid with vortexing and ultrasonification. After extraction, the sample

was centrifuged and the clear supernatant was transferred to glass vials for analysis by LC-MS (Taylor, M.J.C., Pers. Comm., Central Analytic Facilities, Stellenbosch University, Stellenbosch, 7600, South Africa, October 2018). The glucosinolate contents of each of the experimental diets are presented in Table 3.2.

**Table 3.2:** The glucosinolate content of canola oilcake meal (as is basis) and the treatment diets (calculated) in which soybean oilcake meal was gradually replaced by canola oilcake meal

Glucosinolate compound ( $\mu\text{mol/g}$ )	Canola oilcake meal	Diets expressed as percentage canola oilcake meal replacing soybean oilcake meal				
		0%	25%	50%	75%	100%
Progoitrin	1.364	0.000	0.068	0.136	0.205	0.273
Sinigrin	0.035	0.000	0.002	0.004	0.005	0.007
Glucobrassicin	0.372	0.000	0.019	0.037	0.056	0.074
Gluconapin	1.147	0.000	0.057	0.115	0.172	0.229
4-hydroxyglucobrassicin	3.542	0.000	0.177	0.354	0.532	0.709
Epiprogoitrin	2.378	0.000	0.119	0.238	0.357	0.476
Gluconapoleiferin	0.137	0.000	0.007	0.014	0.021	0.027
Glucobrassicinapin	1.308	0.000	0.065	0.131	0.196	0.262
Gluconasturtin	0.134	0.000	0.007	0.013	0.020	0.027
Total ( $\mu\text{mol/g}$ )	10.417	0.000	0.521	1.042	1.563	2.084

The colour of each batch of feed for all five diets was measured in order to establish whether colour differences could have an effect on the diet preference. A colour-guide 45°/0° colorimeter with a 20 mm aperture size and D65/10° illuminant/observer ratio (Catalogue number 6805) (BYK-Gradner GmbH, Geretsried, Germany) was used to take colour measurements according to the CIE Lab-System colour ordinates that include the parameters L\* (white-black), a\* (green-red) and b\* (blue-yellow) (Boccard *et al.*, 1981). The colorimeter was calibrated with standards provided (BYK-Gradner). Readings were taken on the surface colour of the unground pellets (the form fed to the birds). The feed pellets were spread out evenly in a circular container with a diameter of 90 mm before five readings on each sample were taken. This procedure was performed via an established protocol by Brand *et al.* (2018), with the exception that samples were not ground to a fine powder for the current study. In addition, the following formulas were used to calculate the hue angle (colour definition) and chroma (colour intensity) values (Honikel, 1998):

$$\text{Hue } (^{\circ}) = \tan^{-1} \left( \frac{b^*}{a^*} \right)$$

$$\text{Chroma } (C^*) = (a^{*2} + b^{*2})^{-0.5}$$

Statistical analysis was performed using SAS Enterprise Guide (Version 9.4, SAS Institute Inc., Cary, USA) to determine if there were any significant differences, at the  $P \leq 0.05$

level, between the diets. Analysis of variance (ANOVA) was performed on the respective CIE-Lab-System colour ordinates in order to determine whether there were significant colour differences between the five diets. Fisher's least significant difference (LSD) t-test was used to determine whether changes in feed colour could explain the differences in feed intake. The DMI of a specific diet in a certain camp was divided by the total DMI of all diets in that camp multiplied by 100 so as to give the percentage DMI (%DMI) for a specific diet. In order to determine whether there were significant differences for DMI/bird/day and %DMI/bird/day between diets, a randomised block ANOVA was performed using the GLM procedure of SAS Enterprise Guide. Analyses were performed on the average intake per bird per camp for each day for each diet over the 15 trial days. Fisher's LSD was used to determine which diets differed from each other in the case of significant differences.

### 3.3 Results

The total DMI of the birds was  $2129.6 \pm 74.13$  g feed/bird/day over the experimental period. Regarding the feed intake, a difference ( $P \leq 0.05$ ) was observed between the 0% COCM diet and the diets containing COCM. The 0% COCM, which served as the control diet, had the highest DMI/bird/day ( $736.1 \pm 74.1$  g/bird/day) in contrast with the other diets that contained COCM where less than 381.2 g/bird/day were consumed. The diets with higher inclusion levels of COCM made up less than 17.6% of the daily intake per bird while the control diet made up 35.5% of the total daily intake per bird. However, no differences ( $P \geq 0.05$ ) were observed between the DMI/bird/day as well as %DMI/bird/day for diets containing 5% up to 20% COCM (Table 3.3).

**Table 3.3:** The effect of canola oilcake meal inclusion levels on the dry matter intake (DMI) and %DMI of grower ostriches, presented as least square means  $\pm$  standard error of the mean (LSM  $\pm$  SE)

Intake	Diets expressed as the percentage canola oilcake meal replacing soybean oilcake meal				
	0%	25%	50%	75%	100%
DMI/bird/day (g)	736.08 <sup>a</sup> $\pm$ 74.13	366.53 <sup>b</sup> $\pm$ 74.13	381.24 <sup>b</sup> $\pm$ 74.13	321.58 <sup>b</sup> $\pm$ 74.13	324.17 <sup>b</sup> $\pm$ 74.13
Percentage DMI/bird/day (%)	35.46 <sup>a</sup> $\pm$ 3.83	16.92 <sup>b</sup> $\pm$ 3.83	17.56 <sup>b</sup> $\pm$ 3.83	15.18 <sup>b</sup> $\pm$ 3.83	15.03 <sup>b</sup> $\pm$ 3.83

<sup>a,b</sup> Row means with different superscripts differ significantly at  $P \leq 0.05$

As pertaining to the colour of the feed, no differences ( $P > 0.05$ ) were observed amongst the various trial diets for any of the colour ordinates measured (Table 3.4). The positive a\* values is an indication that there is more red than green pigments present in the feed while the positive values of the b\* values indicates that there are more yellow than blue

pigments present in the feed. The hue angle and chroma values also showed no differences ( $P > 0.05$ ) between the different diets.

**Table 3.4:** Colour attribute differences between diets with increasing levels of canola oilcake meal, replacing soybean oilcake meal (least square mean  $\pm$  standard error)

Surface colour attributes	Diets expressed as the percentage canola oilcake meal replacing soybean oilcake meal					P-value
	0%	25%	50%	75%	100%	
L* (Lightness)	47.11 $\pm$ 1.12	46.04 $\pm$ 0.83	45.51 $\pm$ 0.94	45.55 $\pm$ 1.02	44.40 $\pm$ 1.12	0.541
a* (redness)	3.31 $\pm$ 0.37	3.63 $\pm$ 0.28	4.00 $\pm$ 0.31	3.88 $\pm$ 0.34	3.45 $\pm$ 0.37	0.599
b* (blue- yellow)	16.33 $\pm$ 0.64	15.74 $\pm$ 0.48	15.88 $\pm$ 0.55	15.00 $\pm$ 0.59	15.12 $\pm$ 0.64	0.529
Hue ( $^{\circ}$ )	78.24 $\pm$ 1.63	76.77 $\pm$ 1.21	75.74 $\pm$ 1.37	75.54 $\pm$ 1.48	77.08 $\pm$ 1.63	0.730
Chroma (C*)	16.72 $\pm$ 0.59	16.19 $\pm$ 0.44	16.40 $\pm$ 0.50	15.50 $\pm$ 0.54	15.51 $\pm$ 0.59	0.454

### 3.4 Discussion

Due to its high quantity and quality of protein, COCM is a popular source of protein in animal feeds (Zeb, 1998; Dingyuan & Jianjun, 2007; De Kock & Agenbag, 2009; DAFF, 2016b; Nega & Woldes, 2018).

Animals eat according to their nutritional requirements as well as their preference for the available feed ingredients so as to obtain the necessary nutrients to maintain homeostasis and metabolic processes needed for survival, growth, production and reproduction (Bozinovic & Del Rio, 1996; Niknafs & Roura, 2018). Several studies based on ostrich nutrition (Brand *et al.*, 2000a; Brand *et al.*, 2000b, Brand *et al.*, 2006) showed that an ostrich's intake is dependent on the energy content of the feed, with higher intakes being realised when consuming feeds that are low in energy. However, all five of the treatment diets in this study were considered to be iso-energetic (Table 3.1) and available *ad libitum* and therefore it is argued that the differences in feed intake were not directly linked to the the energy levels within the diets.

Regression analysis of the crude fibre (CF) and neutral detergent fibre (NDF) fractions revealed that linear relationships exist between the inclusion level of COCM in the diet and the level of CF ( $R^2 = 77.8\%$ ) and NDF ( $R^2 = 90.41\%$ ) in the diet. COCM does have higher fibre content in relation to other vegetable proteins with most of the fibre in the form of NDF. The NDF content of COCM is approximately 10% higher than the ADF content (Canola meal feeding guide, 2015). In studies by Brand *et al.* (2000b), grower ostriches fed on high fibre diets had a lower daily intake than those on a diet with lower fibre content. These animals showed a decrease in growth rate, which may lead to a decrease in production of slaughter ostriches.



Contradictory results on whether poultry can or cannot distinguish between tastes have been reported. Studies on chicken feed, conducted by Sizemore & Lillie (1956) and Romoser *et al.* (1958), determined that the taste of feed has no effect on feed intake. However, in 1957, Kare *et al.*, found that fowls have the ability to distinguish between tastes. This was also confirmed by Kare & Pick (1960) and Gentle (1971) who stated that poultry indeed have the ability to taste. However, there will only be a significant negative influence on intake when the unpalatable substance is present in high quantities for a prolonged period. Studies showed an absence of beak taste buds in young and mature ostriches (Jackowiak & Ludwig, 2008; Kruger *et al.*, 2008). This implies that the taste buds may be located on the hard palate of the beak or at the openings of the ducts of the salivary glands (Ganchrow *et al.*, 1991). The ostriches in the current study showed preference for the control diet, while there was discrimination towards diets with higher inclusion levels of COCM (Table 3.3). This phenomenon indicates that ostriches have a sense of taste or smell. After the extraction of oil from seeds, the glucosinolates that are found in the canola seeds (Table 3.2), become concentrated within the oilcake (Zeb, 1998). Glucosinolates are ANF's that tend to give a bitter taste to feed containing canola oilseeds and can therefore have a negative effect on feed intake (Brand *et al.*, 2007; Tripathi & Mishra, 2007) and, consequently, reduces growth (Garnsworthy *et al.*, 1992). Furthermore, glucosinolates have been found to suppress the utilization of iodine in monogastric animals which affects the normal function of the thyroid gland as well as the liver (Garnsworthy *et al.*, 1992).

The preference of grower ostriches towards the replacement of soybean meal with full fat canola meal were also evaluated by Brand *et al.* (2018). It was found that up to 25% of soybean can be replaced by full fat canola without having a negative impact on the DMI. The total glucosinolate content of the COCM used in the current study (Table 3.2) were compared to the total glucosinolate content of full fat canola seed that was used by Brand *et al.* (2018). It was found that the total glucosinolate content of the COCM used in the current study was 33.9% higher than the total glucosinolate content of the full fat canola seeds used in studies performed by Brand *et al.* (2018); this would indicate that there might be a level above which ostriches can detect glucosinolates. However, more research is required to see what this level is and which of the numerous glucosinolates, or combinations thereof, influence the aversion response in ostriches.

It has also been speculated that ostriches are able to distinguish between colour and have a preference for certain colour wave lengths. Cooper & Palmer (1994) studied the dietary choices of juvenile ostriches in the wild and found that the ostriches were attracted to dark green foliage and plants. However, unpalatable plants were rejected regardless of whether they were dark green. This indicates that ostriches will primarily base their selection of feed on visual stimuli and then secondarily, on the taste of the feed. The finding that ostriches

showed preference to the colour green was confirmed in the colour preference study of Bubier *et al.* (1996) which could possibly be explained by their herbivorous nature. The second preferred colour was white (Bubier *et al.*, 1996). This phenomenon might be related to juvenile ostriches' habit of eating adult dung that consists of white urate deposits (Holtzhausen & Kotze, 1990 as cited by Bubier *et al.*, 1996). According to Jarvis (1994, as cited by Bubier *et al.*, 1996), this coprophagy of eating mature ostriches' dung will benefit the intestinal microflora of the juveniles. However, when feed was artificially coloured and intake of the different coloured feed was measured, there was no colour preference shown by the juvenile ostriches (Kruger, 2007). Hill (1979) also noted that the feed's colour holds no importance to poultry, as poultry have poorly developed smell and visual senses. The treatment diets in this trial did not show any significant difference in colour (Table 3.4). Therefore, it can be concluded that the colour of feed cannot be used to explain the differences observed in DMI in this trial, regardless whether ostriches have the ability to react on visual stimuli or not.

### 3.5 Conclusion

The control diet, which did not contain any COCM, was the only favoured diet. This diet had the highest DMI (35.5% of the daily intake), while the DMI of the diets that contained COCM did not differ from each other (*ca.* 16% each). No obvious differences in the chemical composition of the complete feeds were observed, except for crude fibre and NDF content which gradually increased with an increased amount of COCM in the diets. The treatment diets in the current study were iso-energetic and showed no differences in colour, these two attributes therefore cannot explain the higher DMI of the control diet. Therefore, it is safe to conclude that the anti-nutritional factors, specifically the glucosinolate content (0.52 up to 2.1  $\mu\text{mol/g}$ ), of the COCM had an influence on the feed intake of the growing ostriches. This may either be due to taste or smell. The higher CF and NDF content of the feed that contained COCM also explains the decreasing DMI, as ostriches will have a lower intake on feed with higher fibre content in the diet. Further studies are required, specifically on the effect of different inclusion levels of COCM on the growth (Chapter 4) and production (Chapter 5) of slaughter ostriches when the birds have no choice of diets that contain different levels of COCM.

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## Chapter 4

### **The effect of varying canola oilcake meal dietary inclusion levels on the production and slaughter traits of slaughter ostriches (*Struthio camelus var. domesticus*)**

#### **Abstract**

Feeding costs make up the largest expense on a livestock farm as nutrition has a direct influence on the performance of animals. In order to decrease feeding costs, canola oilcake meal (COCM) has been identified as a possible replacement to imported soybean oilcake meal (SOCM) as a protein source in the diet of slaughter ostriches and as such, needs to be evaluated for its suitability for inclusion. Ostriches were reared from 77-337 days of age on diets with different inclusion levels of COCM, replacing SOCM (replacement of 0%, 25%, 50%, 75% and 100% of SOCM with COCM). The ostriches were weighed and monitored during the entire growth period up until slaughter, where after slaughter traits were examined. It was concluded that the replacement of SOCM with COCM had little effect on the performance of these ostriches.

#### **4.1 Introduction**

The Klein Karoo in South Africa, specifically Oudtshoorn, is one of a few places world-wide that have the perfect weather conditions and environment for ostrich farming (Smit, 1964; DAFF, 2017). Currently, Oudtshoorn has the highest population of ostriches world-wide with about 90% of all ostriches in South Africa produced and slaughtered here (NAMC, 2010). Despite the ostrich industry being so small, it has a substantial influence on the national as well as international economy, as approximately 70-75% of all ostrich products used world-wide originates from South Africa (Brand & Jordaan, 2011; DAFF, 2017). A relative small setback will have a detrimental impact on the industry and will, consequently, influence the local as well as the international economy. Therefore it is crucial for producers to have good management practices in place and to cut down on unnecessary expenses.

The largest expense (about 75% of all expenses) of an intensive ostrich farm is the feeding cost (Brand & Jordaan, 2011). Feeding costs are the most important expense in any livestock industry as nutrition has a direct influence on the growth and production of the animals and can therefore not be neglected. The only feasible approach to reduce feeding costs is to identify alternative, locally available raw materials that can be incorporated in the diets of ostriches. Research of the influence on these alternative raw materials on the production characteristics is essential to ensure profitability.

Protein makes up a large component of ostrich feed and represents thus a large portion of the feeding cost (Carstens, 2013; Dalle Zotte *et al.*, 2013). The price of protein sources are on the increase as protein becomes more and more scarce due to a rapid increase in the human population (Brand *et al.*, 2000a, Brand *et al.*, 2004a). Currently, soybean meal is used as the main protein source in animal feeds in South Africa (Dalle Zotte *et al.*, 2013; Snyman, 2016). The current demand for soybean in the country is higher than the production thereof, therefore, large quantities of soybean is imported to fulfil the needs for protein, which leads to increased feed prices (Sihlobo & Kapuya, 2016; AFMA, 2017). Although protein is expensive and scarce, there is little information available on the nutritive value of alternative protein sources for ostrich diets (Brand *et al.*, 2000a). Therefore, it is important to quantify the nutritive value of alternative protein sources by formulating diets that fit the ostriches' specific needs without having any negative influence on production.

South Africa, especially the Western Cape, has high production of canola. Canola is used predominantly for the production of vegetable oil. The by-product after the extraction of oil from the seeds is canola oilcake meal (COCM) (Zheng *et al.*, 2017). Canola oilcake meal has a protein content of approximately 36% and is ideal to use as protein source in animal feed (Newkirk, 2009). However, it has been shown that the anti-nutritional factors in COCM influence DMI (Chapter 3) when birds are provided with a choice feeding system over a limited period and it was thus argued that the effect of COCM when no choice is available on the production parameters be evaluated.

The current study was therefore conducted in order to determine whether, and to what extent, COCM can be included in the diets of slaughter ostriches without having any negative influence on the growth, production and slaughter traits of ostriches.

## 4.2 Materials and Methods

Ethical clearance for the current study was obtained from the Elsenburg departmental ethics committee (DECRA R14/108). The 230 South African Black ostriches that were used for this trial were hatched during November 2016 on the Oudtshoorn Research Farm. The trial ran until November 2017 when the remaining 197 ostriches in the study were slaughtered. *Post mortem* evaluations were conducted by an experienced veterinarian on the ostrich chicks that had died in order to determine the cause of death and whether mortalities were related to the nutrition of the ostriches. If mortalities indicated nutrition as the cause of death, then the trial would have been terminated immediately.

After hatching, the chicks were divided into five groups, representing the treatment groups, of the same size with an average chick weight of  $0.894 \pm 0.007$  kg. These five groups were further sub-divided into three sub-groups, resulting in fifteen small groups ( $\pm 15$  chicks

per group) to have three replicates per diet treatment. Each of the fifteen groups were allocated to similar camps of 10 x 5 m with adequate shelter and indoor housing (5 x 3 m). All the groups received the same pre-starter diet (Table 4.1) up to an age of 76 days. Water and feed were made available *ad libitum* at all times throughout the trial.

**Table 4.1:** The ingredient and chemical composition of the pre-starter diet fed to the ostrich chicks in this trial for the age of 0 – 76 days (as is basis)

Ingredients	Amount (kg/ton)
Maize (yellow grain)	504.36
Lucerne meal, 17% CP <sup>1</sup>	100.87
Soybean oilcake meal, 44% CP <sup>1</sup>	172.82
Fish Meal	75.65
Canola oilcake meal	50.44
Canola oil	50.44
Limestone, ground	24.31
Kynofos 21/MCP <sup>2</sup>	4.01
Common salt/NaCl <sup>3</sup>	10.09
Vitamin & Mineral Premix <sup>*</sup>	5.04
Lysine-HCl	1.97
<b>Nutrients (as formulated)</b>	
Dry matter (g/kg)	907.40
ME <sup>4</sup> ostrich (MJ/kg feed)	14.36
Crude protein (g/kg)	205.68
Crude fibre (g/kg)	54.31
Crude fat (g/kg)	78.46
Calcium (g/kg)	15.18
Phosphorous (g/kg)	6.03

\* Refer to Annexure A for the full vitamin and mineral premix

<sup>1</sup> Crude protein

<sup>2</sup> Monocalcium phosphate

<sup>3</sup> Sodium chloride

<sup>4</sup> Metabolisable energy, as formulated by the formula ME ostrich = 6.35 + 0.645 × ME poultry (Gous & Brand, 2008)

Five experimental diets (Tables 4.2 – 4.4) were formulated for each of the growing phases (i.e. starter, grower and finisher) according to a formulation model developed by Brand & Gous (2008) using Mixit 2+ software. All of these diets were formulated to be iso-nutritious in terms of energy and certain essential amino acids (i.e. lysine, total sulphur containing amino acids, threonine, tryptophan and arginine). Each diet had different inclusion levels of COCM, replacing soybean meal in the following increments: 0:100, 25:75, 50:50, 75:25, 100:0.

The trial feeds were milled and pelleted on the Oudtshoorn Research Farm. From each batch of feed that was made, approximately one kilogram was sampled and sent for laboratory analysis to ensure uniform nutrient composition between the batches. The methods of analysis



as described by the Association of Official Analytical Chemists (AOAC, 2012) were followed to determine the dry matter (method 934.01), crude protein (method 990.03), crude fibre (Goering & Van Soest, 1970), acid detergent fibre (ADF) and neutral detergent fibre (NDF)(Van Soest *et al.*, 1991). The Agri Laboratory Association of South Africa guidelines (AgriLASA, 1998) were followed to determine the calcium and phosphorous values (method 6.1.1).

**Table 4.2:** The ingredient and chemical composition of five starter diets containing increasing levels of canola oilcake meal fed to ostrich chicks from 76 – 146 days of age (as is basis)

Ingredients (kg/ton)	Diets expressed as percentage canola oilcake meal replacing soybean oilcake meal				
	0%	25%	50%	75%	100%
Yellow maize	572.16	529.12	486.08	443.04	400.00
Soybean oilcake meal 44% CP <sup>1</sup>	179.51	134.63	89.76	44.88	0.00
Canola oilcake meal	0.00	78.20	156.40	234.60	312.80
Lucerne meal, 17% CP <sup>1</sup>	123.97	125.48	126.99	128.50	130.00
Molasses meal	38.14	38.61	39.07	39.54	40.00
Fat, animal	28.61	35.31	42.01	48.70	55.40
Limestone, ground	11.47	11.43	11.39	11.34	11.30
Kynofos 21/MCP <sup>2</sup>	21.47	21.40	21.34	21.27	21.20
Bentonite clay	9.54	9.66	9.77	9.89	10.00
Common salt/NaCl <sup>3</sup>	9.54	9.66	9.77	9.89	10.00
Vitamin & mineral premix <sup>*</sup>	3.34	3.38	3.42	3.46	3.50
Lysine-HCl	2.26	2.10	1.93	1.77	1.60
Nutrient composition (laboratory analysis)					
Dry matter (g/kg)	900.70	895.45	891.58	899.43	899.20
ME <sup>4</sup> ostrich (MJ/kg feed)	10.89	10.72	10.50	10.60	10.32
Crude protein (g/kg)	155.31	158.59	168.91	170.47	175.47
Crude fibre (g/kg)	78.13	80.05	90.73	93.53	116.78
Acid detergent fibre (g/kg)	110.05	113.30	122.45	134.05	170.53
Neutral detergent fibre (g/kg)	149.28	151.45	160.15	165.98	209.70
Calcium (g/kg)	12.15	12.20	14.55	15.45	17.35
Phosphorous (g/kg)	8.80	9.20	10.55	10.45	10.25
Amino acid composition (formulated)					
Lysine (g/kg)	0.93	0.95	0.97	0.99	1.01
TSAA <sup>5</sup> (g/kg)	0.70	0.71	0.73	0.74	0.76
Threonine (g/kg)	0.69	0.70	0.72	0.73	0.75
Tryptophan (g/kg)	0.19	0.20	0.20	0.20	0.21
Arginine (g/kg)	0.89	0.91	0.93	0.95	0.97

\* Refer to Annexure A for the full vitamin and mineral premix

<sup>1</sup> Crude protein

<sup>2</sup> Monocalcium phosphate

<sup>3</sup> Sodium chloride

<sup>4</sup> Metabolisable energy, as formulated by the formula ME ostrich = 6.35 + 0.645 × ME poultry (Gous & Brand, 2008)

<sup>5</sup> Total sulphur containing amino acids

**Table 4.3:** The ingredient and chemical composition of five grower diets containing increasing levels of canola oilcake meal fed to ostrich chicks from 147 – 230 days of age (as is basis)

Ingredients (kg/ton)	Diets expressed as percentage canola oilcake meal replacing soybean oilcake meal				
	0%	25%	50%	75%	100%
Wheat grain	583.64	571.40	559.17	546.93	534.69
Oats hulls	171.20	155.97	140.74	125.50	110.27
Soybean oilcake meal, 44% CP <sup>1</sup>	134.58	100.94	67.29	33.65	0.00
Canola oilcake meal	0.00	50.02	100.05	150.07	200.09
Molasses meal	40.00	40.00	40.00	40.00	40.00
Lucerne meal, 17% CP <sup>1</sup>	0.00	12.50	25.00	37.50	50.00
Kynofos 21/MCP <sup>2</sup>	26.66	26.25	25.84	25.42	25.01
Limestone, ground	17.09	16.22	15.35	14.48	13.61
Bentonite clay	10.00	10.00	10.00	10.00	10.00
Common salt/NaCl <sup>3</sup>	10.00	10.00	10.00	10.00	10.00
Vitamin & Mineral Premix <sup>*</sup>	5.00	5.00	5.00	5.00	5.00
Lysine-HCl	1.83	1.71	1.58	1.46	1.33
Nutrient composition (laboratory analysis)					
Dry matter (g/kg)	903.98	901.58	901.33	883.03	884.00
ME <sup>4</sup> ostrich (MJ/kg feed)	10.42	10.34	10.29	10.01	10.02
Crude protein (g/kg)	162.29	157.60	160.42	146.35	151.67
Crude fibre (g/kg)	82.55	81.55	84.27	87.57	97.07
Acid detergent fibre (g/kg)	105.15	118.23	119.35	118.33	132.45
Neutral detergent fibre (g/kg)	177.87	204.25	205.07	213.57	230.62
Calcium (g/kg)	12.67	12.13	13.17	18.63	16.57
Phosphorous (g/kg)	8.73	9.50	10.30	10.40	10.03
Amino acid composition (formulated)					
Lysine (g/kg)	0.71	0.71	0.71	0.71	0.71
TSAA <sup>5</sup> (g/kg)	0.41	0.45	0.48	0.52	0.55
Threonine (g/kg)	0.44	0.46	0.48	0.50	0.53
Tryptophan (g/kg)	0.18	0.18	0.18	0.17	0.17
Arginine (g/kg)	0.68	0.68	0.68	0.68	0.68

\* Refer to Annexure A for the full vitamin and mineral premix

<sup>1</sup> Crude protein

<sup>2</sup> Monocalcium phosphate

<sup>3</sup> Sodium chloride

<sup>4</sup> Metabolisable energy, as formulated by the formula ME ostrich = 6.35 + 0.645 × ME poultry (Gous & Brand, 2008)

<sup>5</sup> Total sulphur containing amino acids

**Table 4.4:** The ingredient and chemical composition of five finisher diets containing increasing levels of canola oilcake meal fed to ostrich chicks from 231 – 337 days of age (as is basis)

Ingredients (kg/ton)	Diets expressed as percentage canola oilcake meal replacing soybean oilcake meal				
	0%	25%	50%	75%	100%
Wheat grain	463.82	447.44	431.07	414.69	398.31
Oats hulls	322.98	314.51	306.04	297.56	289.09
Soybean oilcake meal, 44% CP <sup>1</sup>	104.86	78.65	52.43	26.22	0.00
Canola oilcake meal	0.00	49.84	99.69	149.53	199.37
Molasses meal	40.00	39.97	39.94	39.90	39.87
Lucerne meal, 17% CP <sup>1</sup>	0.00	2.49	4.99	7.48	9.97
Kynofos 21/MCP <sup>2</sup>	27.04	26.63	26.21	25.80	25.38
Limestone, ground	16.80	16.25	15.69	15.14	14.58
Bentonite clay	10.00	9.99	9.99	9.98	9.97
Common salt/NaCl <sup>3</sup>	10.00	9.99	9.99	9.98	9.97
Vitamin & Mineral Premix <sup>*</sup>	3.50	3.50	3.50	3.49	3.49
Lysine-HCl	1.01	0.76	0.51	0.25	0.00
Nutrient composition (laboratory analysis)					
Dry matter (g/kg)	892.92	901.97	916.90	916.50	915.57
ME <sup>4</sup> ostrich (MJ/kg feed)	10.08	10.35	10.40	10.31	10.35
Crude protein (g/kg)	137.92	134.38	138.13	138.23	138.65
Crude fibre (g/kg)	98.47	104.52	112.78	119.52	125.50
Acid detergent fibre (g/kg)	129.27	136.88	153.92	162.87	178.30
Neutral detergent fibre (g/kg)	240.82	251.82	262.67	281.15	288.32
Calcium (g/kg)	14.57	13.07	13.30	13.47	13.50
Phosphorous (g/kg)	9.13	9.63	10.10	10.13	9.93
Amino acid composition (formulated)					
Lysine (g/kg)	0.55	0.55	0.55	0.55	0.55
TSAA <sup>5</sup> (g/kg)	0.35	0.39	0.43	0.47	0.50
Threonine (g/kg)	0.37	0.40	0.42	0.45	0.48
Tryptophan (g/kg)	0.16	0.16	0.16	0.16	0.16
Arginine (g/kg)	0.56	0.58	0.59	0.61	0.62

\* Refer to Annexure A for the full vitamin and mineral premix

<sup>1</sup> Crude protein

<sup>2</sup> Monocalcium phosphate

<sup>3</sup> Sodium chloride

<sup>4</sup> Metabolisable energy, as formulated by the formula ME ostrich = 6.35 + 0.645 × ME poultry (Gous & Brand, 2008)

<sup>5</sup> Total sulphur containing amino acids

The glucosinolate concentration of the dry COCM was determined by an adapted method of liquid chromatography-mass spectrometry (LC-MS) as described by Sasaki *et al.* (2012). The samples were prepared by extracting 1 g of the canola oilcake meal with 25 mL of 50% MeOH/1% formic acid with vortexing and ultrasonification. After extraction, the sample was centrifuged and the clear supernatant was transferred to glass vials for analysis by LC-MS (Taylor, M.J.C., Pers. Comm., Central Analytic Facilities, Stellenbosch University,

Stellenbosch, 7600, South Africa, October 2018). The glucosinolate contents of each of the trial diets are presented in Table 4.5.

**Table 4.5:** The glucosinolate content (as is basis) of the treatment diets in which soybean oilcake meal was gradually replaced by canola oilcake meal

Glucosinolate compound ( $\mu\text{mol/g}$ feed)	Diets expressed as percentage canola oilcake meal replacing soybean oilcake meal				
	0%	25%	50%	75%	100%
Starter diet (COCM inclusion levels)	0%	7.8%	15.6%	23.5%	31.2%
Progoitrin	0.00	0.11	0.21	0.32	0.43
Sinigrin	0.00	0.00	0.01	0.01	0.01
Glucobrassicin	0.00	0.03	0.06	0.09	0.12
Gluconapin	0.00	0.09	0.18	0.27	0.36
4-hydroxyglucobrassicin	0.00	0.28	0.55	0.83	1.11
Epiprogoitrin	0.00	0.19	0.37	0.56	0.74
Gluconapoleiferin	0.00	0.01	0.02	0.03	0.04
Glucobrassicinapin	0.00	0.10	0.20	0.31	0.41
Gluconasturtin	0.00	0.01	0.02	0.03	0.04
<b>Total (<math>\mu\text{mol/g}</math>)</b>	<b>0.00</b>	<b>0.81</b>	<b>1.63</b>	<b>2.44</b>	<b>3.26</b>
Grower diet (COCM inclusion levels)	0%	5%	10%	15%	20%
Progoitrin	0.00	0.07	0.14	0.20	0.27
Sinigrin	0.00	0.00	0.00	0.01	0.01
Glucobrassicin	0.00	0.02	0.04	0.06	0.07
Gluconapin	0.00	0.06	0.11	0.17	0.23
4-hydroxyglucobrassicin	0.00	0.18	0.35	0.53	0.71
Epiprogoitrin	0.00	0.12	0.24	0.36	0.48
Gluconapoleiferin	0.00	0.01	0.01	0.02	0.03
Glucobrassicinapin	0.00	0.07	0.13	0.20	0.26
Gluconasturtin	0.00	0.01	0.01	0.02	0.03
<b>Total (<math>\mu\text{mol/g}</math>)</b>	<b>0.00</b>	<b>0.52</b>	<b>1.04</b>	<b>1.56</b>	<b>2.08</b>
Finisher diet (COCM inclusion levels)	0%	5%	10%	15%	20%
Progoitrin	0.00	0.07	0.14	0.20	0.27
Sinigrin	0.00	0.00	0.00	0.01	0.01
Glucobrassicin	0.00	0.02	0.04	0.06	0.07
Gluconapin	0.00	0.06	0.11	0.17	0.23
4-hydroxyglucobrassicin	0.00	0.18	0.35	0.53	0.71
Epiprogoitrin	0.00	0.12	0.24	0.36	0.47
Gluconapoleiferin	0.00	0.01	0.01	0.02	0.03
Glucobrassicinapin	0.00	0.07	0.13	0.20	0.26
Gluconasturtin	0.00	0.01	0.01	0.02	0.03
<b>Total (<math>\mu\text{mol/g}</math>)</b>	<b>0.00</b>	<b>0.52</b>	<b>1.04</b>	<b>1.56</b>	<b>2.08</b>

At the age of 77 days, with the onset of the starter phase, the chicks were moved to fifteen larger pens (25 x 6 m). At this point, the trial started and the birds received the respective starter diets (Table 4.2). During the starter phase, all of the birds as well as the feed refusals were weighed on a weekly base in order to determine growth and feed intake. The chicks entered the grower phase at the age of 147 days and were supplied the respective grower trial diets (Table 4.3). During this phase the groups were moved to larger camps (40 x 30 m) in order to allow for growth and reduce the risk of skin damage. Due to the risk of injuries to the birds as well as the handlers, each bird and the feed refusals were weighed once every three weeks. The finisher diets (Table 4.4) were fed from the age of 231 days until slaughter. Before the ostriches were slaughtered at 337 days of age, the fifteen experimental groups were sorted into the five respective treatment groups and relocated to five large quarantine camps as obligated by the European Union (EU) meat quality standards (DAFF, 2017). During the quarantine time, the birds received treatment for possible external parasites and blood samples were tested for avian influenza (AI). The birds tested negative for AI and could thus be taken for slaughter at the abattoir. One day prior to slaughter, the birds were moved according to protocol to the Klein Karoo International abattoir where they were slaughtered. During this lairage period the birds received no feed but had *ad lib* fresh water available.

Similar slaughtering procedures as those described in Hoffman (2012) were followed. The birds were electrically stunned and exsanguinated and the weight of the dead bird was recorded. This bled weight was taken as the slaughter weight. After the birds were exsanguinated, the feathers were plucked by hand and placed in clearly marked bags. Following the plucking of the feathers, the carcasses were skinned. Each skin was marked clearly and sent for tanning at the Klein Karoo International tannery. Following evisceration, the abdominal fat (fat pad) was removed and weighed. The liver and thyroid glands' weights were recorded in order to determine whether the diets (specifically the glucosinolate content of the diets) had an influence on the development and function of these organs. The carcasses were then chilled overnight in a cold room at 0 – 2°C. The cold carcass weight was obtained the next morning ( $\pm$  24 hours *post mortem*), prior to deboning. These weights were used to calculate the dressing percentage. Dressing percentage was calculated by dividing the cold carcass weights by the bled out weight, after exsanguination, multiplied by 100. The pH of the big drum muscles (*Muscularis gastrocnemius*) were measured prior to deboning. The right thigh of each bird was weighed to obtain the contribution of the thighs to the whole carcass. It was assumed that the weight of the right thigh would have the same weight as that of the left thigh. Samples of the big drum muscle (*M. gastrocnemius*) were excised from the right thigh and weighed.

SAS Enterprise Guide (Version 9.4, SAS Institute Inc., Cary, NC, USA) was used to statistically analyse the production and slaughter data. The general linear model procedure

was used to test for significant differences between the various COCM inclusion levels at the  $P \leq 0.05$  level. Detailed treatment differences were investigated by Fisher's least significant difference (LSD) t-test. In the analyses, camps were used as experimental units and thus the random replicates of treatment diets. The production traits of the birds were analysed within each of the phases as well as over the entire production period. The end weight of each feeding phase was taken as the covariate, starting weight, for the traits analysed in the following phase. Regression models were fitted to the data in order to describe the trends shown for each production trait in response to the level of COCM included in the diet. The growth curves for ostriches on each of the trial diets were developed by applying the nonlinear Gompertz function in the form given below:

$$W = a \times \exp(-\exp(-b(t - c)))$$

Where,  $W$  represents the weight of the ostrich at time  $t$ , and  $a$  represents the asymptotic mature weight. The  $b$  parameter represents the growth coefficient while  $c$  denotes the age of the birds at the point of maximum growth. A one way analysis of variance (ANOVA) was used to test for differences of the parameter means between the dietary treatments.

During the course of this trial, all of the birds were monitored every day to ensure their well-being. The Code of Conduct for the Commercial Production of Ostriches (2011) of the South African Ostrich Business Chamber with regards to the rearing, handling, vaccination and transport was followed throughout the entire trial.

### 4.3 Results

The mortality rate of ostriches in this trial was 14.8%. Most of these mortalities (11.3%) occurred during the starter phase. *Post mortem* analyses indicated that the main cause of deaths during this phase was the occurrence of prolapses due to the ingestion of sticks and gravel. The mortalities in the grower phase (2.5%) were due to gravel in the stomachs that caused prolapses, as well as due to leg injuries. In the finisher phase, a mortality rate of 1% was observed as a result of ostriches injuring themselves while getting stuck in the fencing. The mortalities occurred across the different treatments and so it was determined that mortalities were not as a result of ingesting the inclusion levels of COCM. Mortality rates were well below the industry norm of 40% (Brand, 2016).

The production traits of ostriches reared on diets with COCM incrementally replacing SOCM as the protein source are given in Table 4.6. The starting weights at the beginning of each phase were taken as the average end weights of the birds in the previous feeding phase. The average starting weights of the birds on the starter, grower and finisher diets were 4.53 kg, 42.06 kg and 77.65 kg respectively. Dry matter intake (DMI) did not vary between the diets within each of the feeding phases nor over the entire rearing period ( $P > 0.05$ ). The average

DMI during the starter, grower and finisher phases were 1.34, 1.89 and 2.65 kg/bird/day, respectively, with the overall average DMI being 2.02 kg/bird/day. Differences were observed for the average daily gain (ADG) of ostriches in the starter phase, with birds on the 75% replacement diet (0.43 kg/bird/day) exhibiting higher growth rates ( $P=0.031$ ) than birds on the 0% (0.38 kg/bird/day), 25% (0.36 kg/bird/day) and 100% replacement (0.35 kg/bird/day) diets, which in turn did not differ from each other ( $P > 0.05$ ). The ADG in the other phases did not differ between the diets with average ADG for the grower phase  $0.41 \pm 0.02$  kg/bird/day and for the finisher phase  $0.29 \pm 0.02$  kg/bird/day.

The feed conversion ratio of grower ostriches ( $5.38 \pm 0.30$ ) was highest for the diet with 15% COCM and 33.7% SOCM (75% replacement diet). It did not differ from the diet with 10% COCM inclusion (50% replacement of SOCM) ( $4.78 \pm 0.30$ ) but differed from the diets that had COCM inclusion levels of 0% ( $4.28 \pm 0.30$ ), 25% ( $4.51 \pm 0.30$ ) and 100% replacement ( $4.05 \pm 0.30$ ).

Differences in end weights were observed during the starter and finisher phases with the starter phase having the highest end weight in birds reared on the 75% replacement diet ( $45.53 \pm 1.36$  kg for the starter phase and  $104.41 \pm 1.35$  kg for the finisher phase) and the lowest end weight in birds that received the diet with 0% inclusion of SOCM and 100% COCM inclusion ( $39.12 \pm 1.42$  kg for the starter phase and  $100.76 \pm 1.31$  kg for the finisher phase). The average end weight for the grower phase was  $77.65 \pm 2.66$  kg.



**Table 4.6:** The effect of replacing soybean oilcake meal with increasing levels of canola oilcake meal in the diets of slaughter ostriches on the production traits in different production phases, presented as least square means  $\pm$  standard error (LSM  $\pm$  SE)

Production traits	Phase	Diets expressed as percentage canola oilcake meal replacing soybean oilcake meal					P-value
		0%	25%	50%	75%	100%	
Start weight <sup>1</sup> (kg)	Starter	4.53	4.53	4.53	4.53	4.53	-
	Grower	42.06	42.06	42.06	42.06	42.06	-
	Finisher	77.65	77.65	77.65	77.65	77.65	-
Dry Matter Intake (kg/bird/day)	Starter	1.25 $\pm$ 0.08	1.26 $\pm$ 0.08	1.37 $\pm$ 0.08	1.55 $\pm$ 0.08	1.25 $\pm$ 0.08	0.155
	Grower	1.89 $\pm$ 0.15	1.86 $\pm$ 0.16	1.84 $\pm$ 0.16	2.02 $\pm$ 1.20	1.86 $\pm$ 0.19	0.364
	Finisher	2.62 $\pm$ 0.13	2.62 $\pm$ 0.14	2.59 $\pm$ 0.13	2.75 $\pm$ 0.14	2.66 $\pm$ 0.13	0.834
	<b>Overall</b>	<b>1.98 <math>\pm</math> 0.09</b>	<b>1.96 <math>\pm</math> 0.09</b>	<b>1.98 <math>\pm</math> 0.10</b>	<b>2.19 <math>\pm</math> 0.90</b>	<b>1.97 <math>\pm</math> 0.10</b>	<b>0.491</b>
Average Daily Gain (kg/bird/dag)	Starter	0.38 <sup>a</sup> $\pm$ 0.01	0.36 <sup>a</sup> $\pm$ 0.01	0.39 <sup>ab</sup> $\pm$ 0.01	0.43 <sup>b</sup> $\pm$ 0.01	0.35 <sup>a</sup> $\pm$ 0.01	0.031
	Grower	0.43 $\pm$ 0.02	0.41 $\pm$ 0.02	0.39 $\pm$ 0.02	0.40 $\pm$ 0.02	0.44 $\pm$ 0.02	0.397
	Finisher	0.28 $\pm$ 0.02	0.31 $\pm$ 0.02	0.30 $\pm$ 0.02	0.30 $\pm$ 0.02	0.27 $\pm$ 0.02	0.450
	<b>Overall</b>	<b>0.39 <math>\pm</math> 0.01</b>	<b>0.38 <math>\pm</math> 0.01</b>	<b>0.39 <math>\pm</math> 0.01</b>	<b>0.40 <math>\pm</math> 0.01</b>	<b>0.38 <math>\pm</math> 0.01</b>	<b>0.722</b>
Feed Conversion Ratio (feed in kg/weight gain in kg)	Starter	3.31 $\pm$ 0.21	3.47 $\pm$ 0.21	3.53 $\pm$ 0.21	3.64 $\pm$ 0.21	3.49 $\pm$ 0.21	0.857
	Grower	4.28 <sup>a</sup> $\pm$ 0.30	4.51 <sup>a</sup> $\pm$ 0.30	4.78 <sup>ab</sup> $\pm$ 0.30	5.38 <sup>b</sup> $\pm$ 0.30	4.05 <sup>a</sup> $\pm$ 0.30	0.078
	Finisher	9.21 $\pm$ 0.49	8.43 $\pm$ 0.49	8.73 $\pm$ 0.49	9.10 $\pm$ 0.49	9.91 $\pm$ 0.49	0.327
	<b>Overall</b>	<b>5.08 <math>\pm</math> 0.21</b>	<b>5.10 <math>\pm</math> 0.21</b>	<b>5.14 <math>\pm</math> 0.21</b>	<b>5.50 <math>\pm</math> 0.21</b>	<b>5.18 <math>\pm</math> 0.21</b>	<b>0.646</b>
End weight (kg)	Starter	41.76 <sup>ab</sup> $\pm$ 1.36	40.72 <sup>b</sup> $\pm$ 1.36	43.17 <sup>ab</sup> $\pm$ 1.40	45.53 <sup>a</sup> $\pm$ 1.36	39.12 <sup>b</sup> $\pm$ 1.42	0.042
	Grower	78.37 $\pm$ 2.36	77.10 $\pm$ 2.47	76.88 $\pm$ 2.46	76.27 $\pm$ 3.07	79.61 $\pm$ 2.95	0.126
	Finisher	101.75 <sup>ab</sup> $\pm$ 1.30	103.91 <sup>ab</sup> $\pm$ 1.33	103.80 <sup>ab</sup> $\pm$ 1.30	104.41 <sup>c</sup> $\pm$ 1.35	100.76 <sup>a</sup> $\pm$ 1.31	0.005
	<b>Overall</b>	<b>102.13 <math>\pm</math> 2.33</b>	<b>102.49 <math>\pm</math> 2.33</b>	<b>104.74 <math>\pm</math> 2.40</b>	<b>106.18 <math>\pm</math> 2.33</b>	<b>99.11 <math>\pm</math> 2.43</b>	<b>0.458</b>

<sup>1</sup> End weight of previous phase used as covariate for start weightsa,b,c Row means with different superscripts differed significantly ( $P \leq 0.05$ )

In order to describe possible trends with increasing levels of COCM replacing SOCM in the diets of ostriches, the production traits were analysed by fitting the most appropriate regression models to the data during the different growth phases (i.e. starter, grower and finisher) (Table 4.7).

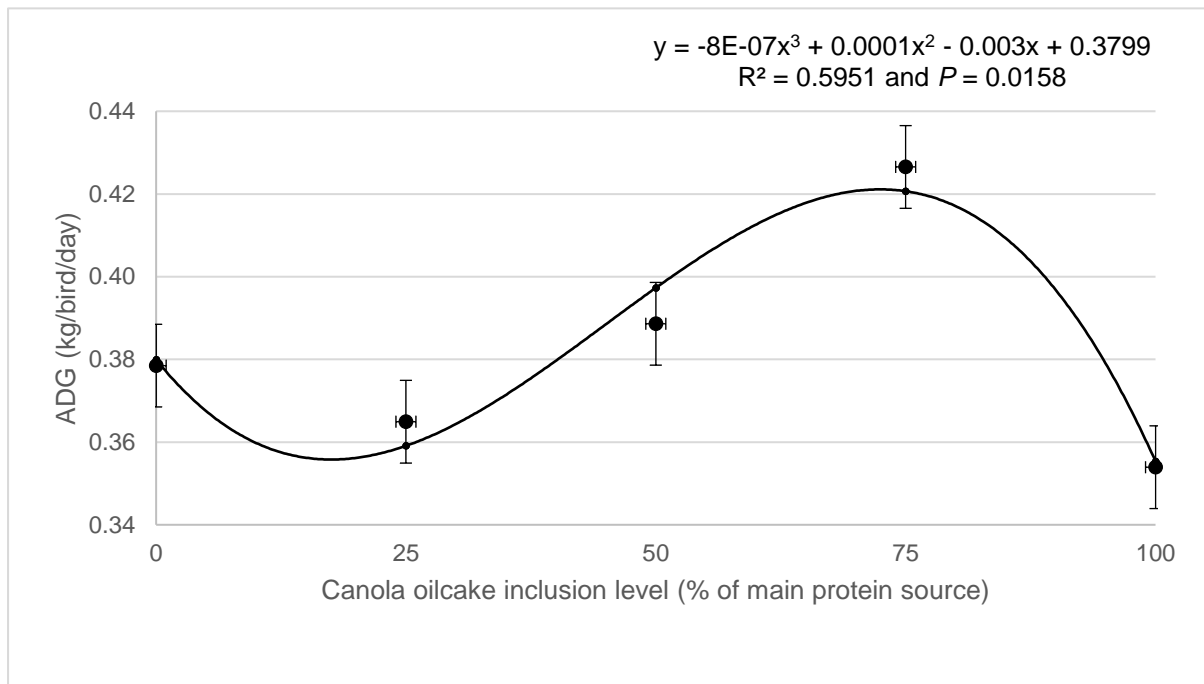
**Table 4.7:** Regression models fitted to the data of production traits of slaughter ostriches describing the trends due to the change in canola oilcake meal inclusion in the diets within each production phase and the overall trial period ( $x$  = canola oilcake meal as percentage of total protein source in the diet)

Production traits	Phase	Function	Equation	R <sup>2</sup>	P value
Dry matter intake (g/bird/day)	Starter	Cubic	$y = -3E-06x^3 + 0.0004x^2 - 0.0096x + 1.2604$	0.4997	NS <sup>1</sup>
	Grower	Linear	$y = 0.0004x + 1.8756$	0.2400	NS <sup>1</sup>
	Finisher	Linear	$y = 0.0008x + 2.6068$	0.0299	NS <sup>1</sup>
Average daily gain (g/bird/day)	Starter	Cubic	$y = -8E-07x^3 + 0.0001x^2 - 0.003x + 0.3799$	0.5951	0.016
	Grower	Linear	$y = -4E-05x + 0.4182$	0.0020	NS <sup>1</sup>
	Finisher	Linear	$y = -0.0001x + 0.3006$	0.0385	NS <sup>1</sup>
Feed conversion ratio (feed in g/weight gain in g)	Starter	Linear	$y = 0.0022x + 3.3788$	0.0572	NS <sup>1</sup>
	Grower	Cubic	$y = -1E-05x^3 + 0.0014x^2 - 0.0297x + 4.313$	0.5053	0.045
	Finisher	Linear	$y = 0.0083x + 8.6642$	0.1182	NS <sup>1</sup>
End weight (kg)	Starter	Cubic	$y = -7E-05x^3 + 0.0086x^2 - 0.2295x + 41.833$	0.5984	0.042
	Grower	Linear	$y = 0.0056x + 77.365$	0.3559	NS <sup>1</sup>
	Finisher	Quadratic	$y = -0.0012x^2 + 0.1176x + 101.68$	0.7866	NS <sup>1</sup>

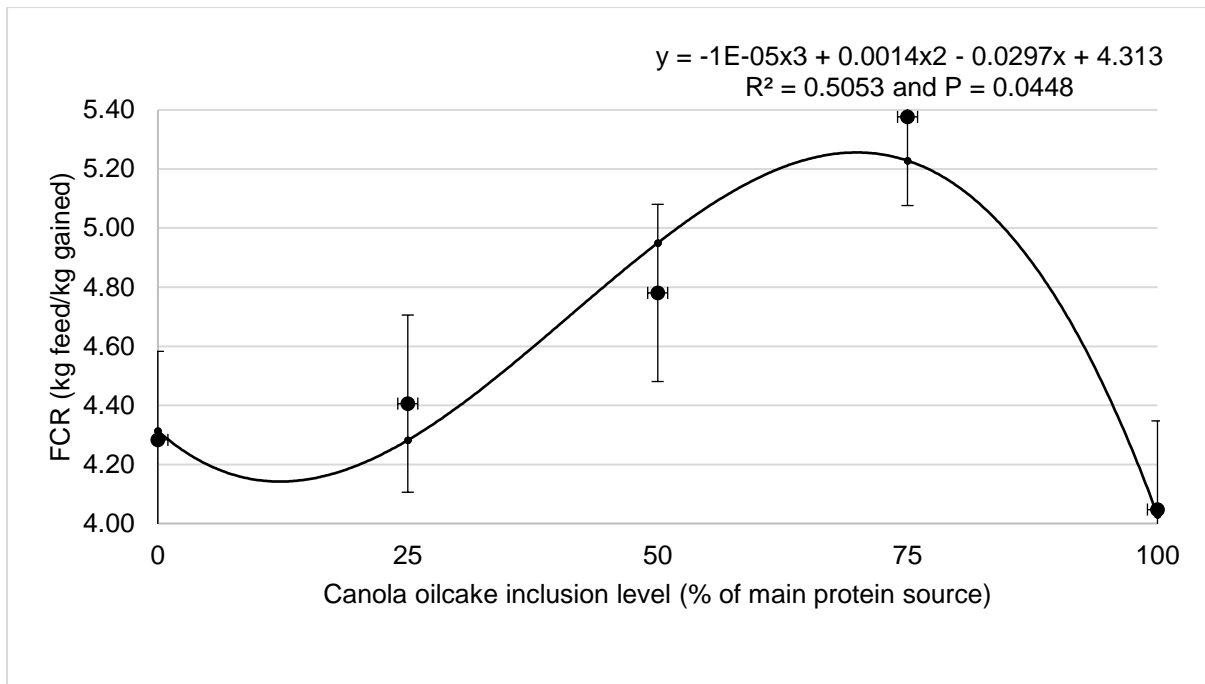
<sup>1</sup> Non-significant ( $P > 0.05$ )

For DMI, the  $P$  values of the models used in each feeding phase were found to be non-significant, with low  $R^2$  values being obtained ( $< 0.50$ ) (Table 4.7). Figure 4.1 depicts a cubic regression fitted between the ADG and the COCM inclusion level during the starter phase, describing 59.5% of the variation amongst the data ( $P = 0.016$ ). The trend shows a slight decrease in ADG as the COCM inclusion level increase from 0% to 7.8%. With the increase in COCM levels, the ADG increases to reach the highest ADG for the diet with the 23.5% COCM inclusion. This diet differs from all the other diets, except the diet with 15.6% COCM inclusion level. There is a sharp decrease in ADG from the 23.5% COCM diet to the 31.3% COCM diet, where the ADG is the lowest at  $0.35 \pm 0.01$  kg/bird/day. When examining Figure 4.2, a similar cubic regression trend ( $R^2 = 50.5\%$ ) can be drawn between FCR and the inclusion level of COCM during the grower phase ( $P = 0.045$ ). As the COCM level increases from 0% to 5%, there is a slight decrease in FCR where after the FCR increases until it reaches its peak at 15% COCM inclusion level. Again, the FCR of this diet differs from all the other diets, except from the diets that have 50% SOCM replaced with COCM. A sharp decrease in FCR is observed when the COCM level increases from 75% to its maximum of 100%. The

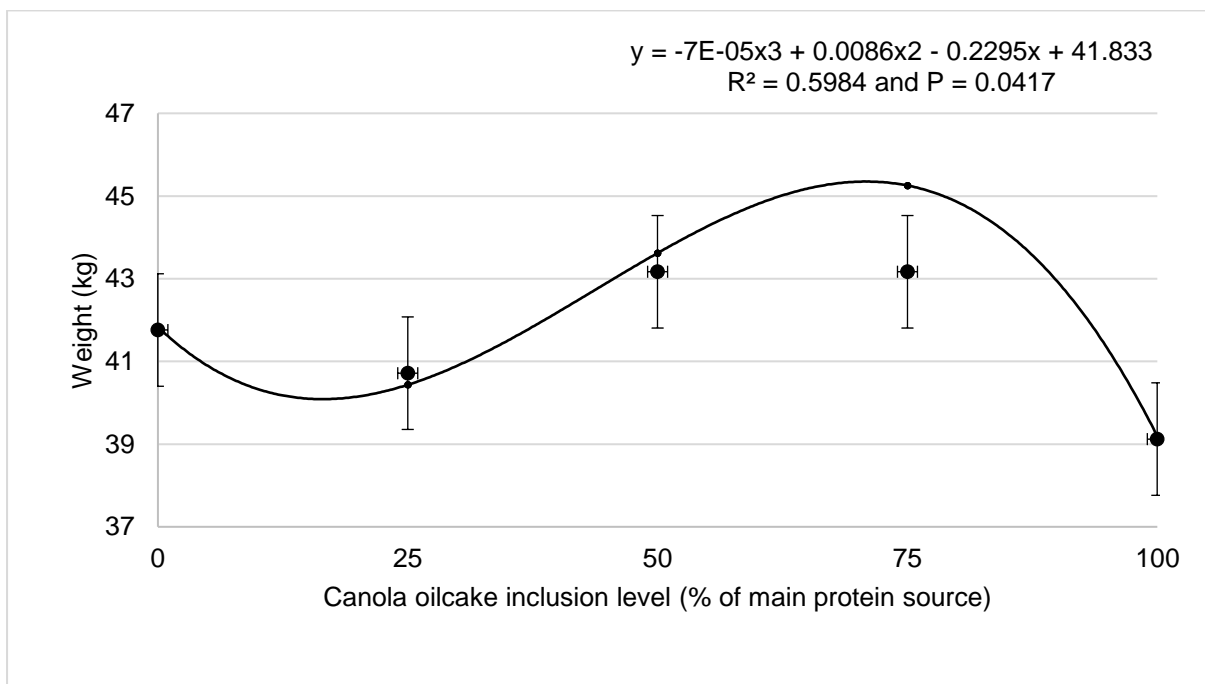
weights of each diet at the end of the starter phase also fits a similar cubic regression (Figure 4.3) describing 59.8% of the variation in the data ( $P=0.042$ ). Similar to the regression of above mentioned figures, the trend slightly decreases with the increase of COCM from 0% to 7.8%, where after the weights increased to reach its maximum at the 23.5% COCM inclusion level. There is a sharp decrease in end weight when the COCM inclusion level increase from 23.5% to 31.3%.



**Figure 4.1:** Cubic function fitted to the least square mean average daily gain of slaughter ostriches in the starter phase with varying levels of canola oilcake meal in the diets



**Figure 4.2:** Cubic function fitted to the least square mean feed conversion ratios of slaughter ostriches in the grower phase with varying levels of canola oilcake meal in the diets



**Figure 4.3:** Cubic function fitted to the least square mean of the end weights of slaughter ostriches in the starter phase with varying levels of canola oilcake meal in the diets

The sigmoidal Gompertz growth curves from hatching to slaughter, as presented in Figure 4.4, explains 94% of the variation in the growth data (Table 4.8). No differences ( $P \geq 0.05$ ) were observed between diets among any of the parameters.

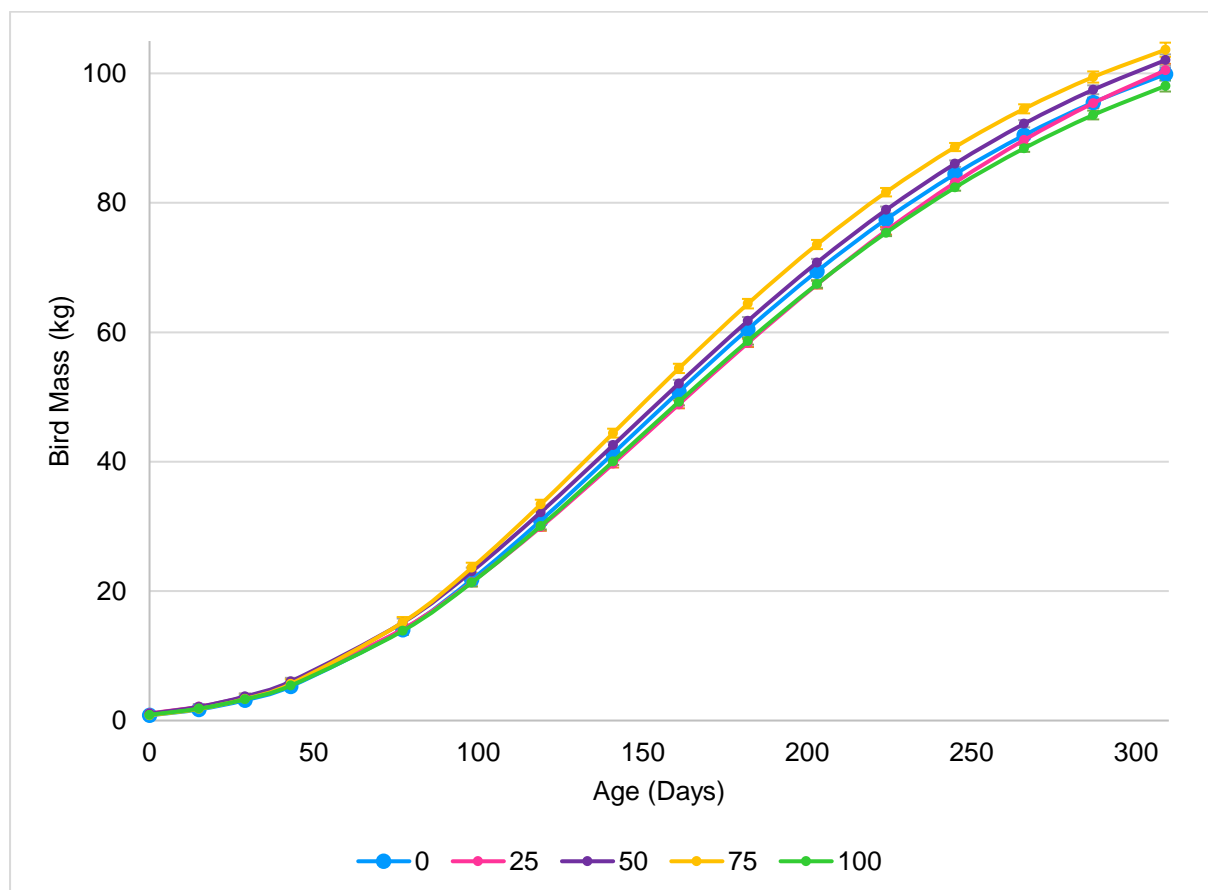
**Table 4.8:** Predicted growth parameters ( $\pm$  standard error) of slaughter ostriches fed diets with varying levels of canola oilcake meal incrementally replacing soybean oilcake meal as protein source based on the Gompertz growth curve

Gompertz growth parameters	Diets expressed as percentage canola oilcake meal replacing soybean oilcake meal					<i>P</i> value
	0%	25%	50%	75%	100%	
<i>a</i>	119.32 $\pm$ 3.60	125.21 $\pm$ 3.60	121.89 $\pm$ 3.60	119.97 $\pm$ 3.60	116.30 $\pm$ 3.60	0.531
<i>b</i>	0.1 $\pm$ 0.0007	0.01 $\pm$ 0.0007	0.01 $\pm$ 0.0007	0.01 $\pm$ 0.0007	0.01 $\pm$ 0.0007	0.605
<i>c</i>	145.79 $\pm$ 4.39	155.23 $\pm$ 4.39	146.60 $\pm$ 4.39	141.01 $\pm$ 4.39	147.23 $\pm$ 4.39	0.313
R <sup>2</sup>	0.936	0.940	0.951	0.938	0.944	-

*a* = mature weight (kg)

*b* = rate of maturing parameter (growth coefficient)

*c* = age at maximum growth (days)



**Figure 4.4:** Gompertz growth curves fitted to the mean body weights (kg) of slaughter ostriches that consumed diets with varying levels of canola oilcake meal over the entire growth period (from the age of 0 days to the age of 337 days)

With regards to the slaughter traits, differences ( $P < 0.05$ ) were observed between diets for the slaughter weights with the heaviest birds reared on the 50% COCM ( $93.60 \pm 2.00$ ) diet and the lightest birds on the diet with 100% ( $83.38 \pm 2.00$ ) inclusion of COCM. No differences

( $P > 0.05$ ) between diets were observed for the weight of the abdominal fat pad, warm and cold carcasses' weights, dressing percentage, weights of the right thighs and the weights of the big drum muscles.

The lowest liver weight ( $1.26 \pm 0.04$  kg) was observed for the 100% COCM diet while the heaviest liver weight was for the 25% diet ( $1.61 \pm 0.04$  kg). The liver weights for the rest of the diets did not differ. The lowest thyroid gland weight was measured in the diet with 75% COCM replacement ( $36.48 \pm 2.92$  kg), not differing from the diet with 100% COCM inclusion level ( $39.05 \pm 2.92$  kg), but differing from the rest of the diets (0%:  $44.86 \pm 2.92$  kg, 25%:  $58.57 \pm 2.92$  kg and 50%:  $49.50 \pm 2.92$  kg) (Table 4.9).

**Table 4.9:** The effect of the replacement of soybean oilcake meal with increasing amounts of canola oilcake meal in the diets of slaughter ostriches that were slaughtered on an age of 337 days, expressed as least square means  $\pm$  standard error (LSM  $\pm$  SE)

Slaughter trait	Diets expressed as percentage canola oilcake meal replacing soybean oilcake meal					P-value
	0%	25%	50%	75%	100%	
Slaughter weight <sup>1</sup> (kg)	91.30 <sup>a</sup> $\pm$ 2.00	90.36 <sup>a</sup> $\pm$ 2.00	93.60 <sup>a</sup> $\pm$ 2.00	89.01 <sup>ab</sup> $\pm$ 2.00	83.38 <sup>b</sup> $\pm$ 2.00	0.045
Fat pad <sup>2</sup> weight (kg)	5.36 $\pm$ 0.37	5.66 $\pm$ 0.37	5.88 $\pm$ 0.37	4.88 $\pm$ 0.37	4.40 $\pm$ 0.37	0.101
Liver weight (kg)	1.40 <sup>b</sup> $\pm$ 0.04	1.61 <sup>a</sup> $\pm$ 0.04	1.42 <sup>b</sup> $\pm$ 0.04	1.34 <sup>bc</sup> $\pm$ 0.04	1.26 <sup>c</sup> $\pm$ 0.04	0.0007
Thyroid gland weight (g)	44.86 <sup>bc</sup> $\pm$ 2.92	58.57 <sup>a</sup> $\pm$ 2.92	49.20 <sup>b</sup> $\pm$ 2.92	36.48 <sup>c</sup> $\pm$ 2.92	39.05 <sup>c</sup> $\pm$ 2.92	0.0024
Warm carcass weight <sup>3</sup> (kg)	45.51 $\pm$ 1.14	45.97 $\pm$ 1.14	46.43 $\pm$ 1.14	45.27 $\pm$ 1.14	42.27 $\pm$ 1.14	0.161
Cold carcass weight (kg)	44.60 $\pm$ 1.11	44.82 $\pm$ 1.11	45.39 $\pm$ 1.11	44.30 $\pm$ 1.11	41.31 $\pm$ 1.11	0.156
Dressing percentage (%)	51.80 $\pm$ 0.39	51.99 $\pm$ 0.39	51.43 $\pm$ 0.39	52.47 $\pm$ 0.39	52.53 $\pm$ 0.39	0.309
Right thigh weight (kg)	16.51 $\pm$ 0.41	16.59 $\pm$ 0.41	16.76 $\pm$ 0.41	16.39 $\pm$ 0.41	15.24 $\pm$ 0.41	0.137
Big drum muscle <sup>4</sup> weight (kg)	1.14 $\pm$ 0.03	1.16 $\pm$ 0.03	1.14 $\pm$ 0.03	1.12 $\pm$ 0.03	1.03 $\pm$ 0.03	0.069
Big drum muscle <sup>4</sup> contribution to right thigh weight (%)	6.88 $\pm$ 0.08	6.96 $\pm$ 0.08	6.75 $\pm$ 0.08	6.82 $\pm$ 0.08	6.76 $\pm$ 0.08	0.342
Cold carcass pH	5.72 <sup>a</sup> $\pm$ 0.03	5.57 <sup>b</sup> $\pm$ 0.03	5.62 <sup>b</sup> $\pm$ 0.03	5.61 <sup>b</sup> $\pm$ 0.03	5.65 <sup>ab</sup> $\pm$ 0.03	0.028

<sup>a,b,c</sup> Row means with different superscripts differed significantly ( $P \leq 0.05$ )

<sup>1</sup> Bled out weight; feathers and skin still attached

<sup>2</sup> Abdominal fat pad

<sup>3</sup> Bled out weight; feathers removed but skin still attached, just before evisceration

<sup>4</sup> *Muscularis gastrocnemius*

## 4.4 Discussion

The ostrich industry is one of the smallest and youngest commercial agricultural practices including domestic animals today; therefore, knowledge on ostrich nutrition is limited. Any alternative raw materials need to be investigated to determine what their influence on growth and production will be. The biggest challenge that producers face is to constantly try to reduce expenses in order to maximise profit margins. This is also the reason for identifying and investigating cheaper raw materials that can be used in ostrich diets. Canola oilcake meal is a good source of protein and is currently cheaper than the SOCM that is mainly used as a protein source in animal feed; as COCM is locally available while soybean needs to be imported (De Kock & Agenbag, 2009; DAFF, 2016b; Nega & Woldes, 2018).

The concern of using COCM as raw material in animal feeds is its glucosinolate content. Glucosinolates are anti-nutrients (ANF) which causes feed to be unpalatable. These ANF's are concentrated in COCM after the extraction of oil from seeds (Zeb, 1998). As pertaining to the treatment diets in the current study, the highest glucosinolate concentration was to be found in the starter diet with 31.3% COCM inclusion (3.26  $\mu\text{mol/g}$ ) in the diet where the SOCM was completely replaced with COCM (Table 4.5). However, this had no clear effect as no differences in DMI were detected ( $P > 0.05$ , Table 4.6) with no significant trends in DMI being observed (Table 4.7). The growth rate and end weights of birds in the starter phase receiving the 100% replacement diet had the lowest values, however, these values did not differ from that of the 0% replacement diet and therefore the differences observed cannot be due to the levels of glucosinolates in the feeds. This suggests that the levels of ANFs in the 100% replacement diet are below a critical level that would negatively impact the production of the ostriches. Results obtained from a previous study where SOCM was replaced with full-fat canola concluded that the diet with the highest glucosinolate content (2.156  $\mu\text{mol/g}$ ) was too low to have an influence on the DMI, ADG, FCR of end weight of the birds fed on the specific diet (Niemann *et al.*, 2018) which is confirmed by findings of the current study. Quinsac *et al.* (1994) found that a higher glucosinolate content of 15.8  $\mu\text{mol/g}$  was not high enough to have a detrimental effect on the feed intake of broilers.

Studies based on ostrich nutrition revealed that ostriches eat, like all other animals, according to their nutritional requirements, especially the energy content of the feed (Bozinovic & Del Rio, 1996; Niknafs & Roura, 2018). All treatment diets in this study were formulated to have similar energy levels in order to not influence dietary intake of the birds.

Most of the differences in production traits occurred in the starter phase. Birds receiving the diet with 75% replacement of SOCM (respectively 23.5% COCM and 44.9% SOCM) had the highest ADG and end weights while the ostriches receiving the diet where 100% of the SOCM was replaced had the lowest ADG as well as end weights. Although no



significant differences were observed in the DMI between the diets, the higher ADG of the 75% COCM replacement diet is likely due to the higher DMI of this diet. It might be concluded that the starter phase was the adaptation phase. The chicks were used to the pre-starter diet that contained only a small amount (5%) of COCM. This amount of COCM was too minute to have any effect on the taste of the feed. Thus, during the starter phase the birds adapted to diets with less (or no) SOCM containing COCM that tasted different to the pre-starter diet.

In the grower phase, differences between diets for FCR were observed. There were, however, no differences in DMI or ADG for this phase. Seeing that there were no differences in production traits between the diets for the finisher phase, it can be concluded that the different treatment groups adapted to the diets they were fed on with its different inclusion levels of COCM.

When considering the overall performance for each production trait, no differences between traits were observed. The diet in which 75% of SOCM was replaced with COCM showed the highest DMI, ADG, FCR and end weights.

The Gompertz growth curve in Figure 4.4 shows that the birds of all the treatment groups grew according to similar sigmoidal growth patterns with no differences for the model parameters being observed between the diets. Therefore it can be concluded that the COCM inclusion level in the diets had no influence on the growth of slaughter ostriches. A study by Niemann *et al.* (2018) where SOCM was replaced with incrementally increasing levels of full-fat canola, showed that the diet where 50% SOCM was replaced with full fat canola had the heaviest mature weight ( $P < 0.0001$ ).

The inclusion level of COCM to the diets had little effect on the slaughter traits of ostriches (Table 4.7). The slaughter traits are directly influenced by the production traits, thus it is not unexpected that there are slight differences between diets with regards to the slaughter traits. The birds on the control diet had the highest live weight at slaughter (Table 4.7) whilst the birds that received the diet with 100% replacement of SOCM with COCM had the lowest live weight at slaughter (Table 4.9) and also the lowest ADG and end weights (Table 4.7) and growth rate (Table 4.6). The various carcass yields (dressing percentage, thigh and big drum muscle weights) did not differ between the various diets and are similar to that noted for birds of this live weight.

With regards to the weights of the liver and thyroid glands, significant differences were observed resulting in the 25% COCM diet to have the highest weights of both these organs (Table 4.9). The findings of the current study is contradicting the conclusions of Ibrahim & Hill (1980), Butler *et al.* (1982), Opalka *et al.* (2001) and Maroufyan & Kermanshahi (2006). In the abovementioned studies, chickens and pigs were reared on canola meal and rapeseed meal where it was found that high levels of glucosinolates caused enlargement of the animals' livers and thyroid glands. However, these conclusions were refuted by a study by Roth-Maier *et al.*

(2004) who noted that different levels of canola meal in the diets of pigs had no influence on the weights of thyroid glands. It was reported that feed with a glucosinolate concentration above 8.0  $\mu\text{mol/g}$  of feed, will result in growth depression in broilers (Tripathi & Misha, 2007). In the current study, the 100% COCM diet of the starter phase had the highest glucosinolate concentration of 3.26  $\mu\text{mol/g}$  of feed. This is 41% of the total that is needed to have an influence on the thyroid glands and livers of chickens; glucosinolate concentration values that could have similar effects are not known for ostriches.

#### 4.5 Conclusion

When considering the results obtained from this trial, it can be concluded that COCM can be used as a suitable replacement for SOCM in the diets of ostriches without negative effects on performance. In fact, the ostriches in this trial had a very low mortality rate when compared to industrial norms. Overall (production and slaughter traits) birds exhibited similar growth, with the diet that had 100% replacement of SOCM resulting in slightly lower end weights at slaughter, although there were no differences in meat yields. The diet with 75% COCM replacement level would be more beneficial, especially in the starter phase. Further research is, however, warranted to evaluate the influence of COCM inclusion in the diets on the quality of end products (i.e. meat, skin, feathers) of the slaughter ostriches.

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## Chapter 5

### The effect of varying canola oilcake meal dietary inclusion levels on the feather yield, leather traits and meat characteristics of slaughter ostriches (*Struthio camelus var. domesticus*)

#### Abstract

Canola oilcake meal (COCM) has been identified as alternative locally produced protein source and has been shown to have minimal influence in production performance when fed to ostriches although the effect thereof on product quality is unknown. The current study was conducted to determine the influence of COCM inclusion in diets on the end-products (meat, skin and feathers) of ostriches. Ostrich chicks were divided in fifteen groups ( $\pm 15$  chicks per group) that received one of five experimental diets with different inclusion levels of COCM, replacing soybean oilcake meal (SOCM) gradually (respectively 0%, 25%, 50%, 75% and 100% COCM in total diet) during their production span. It was found that COCM had no ( $P > 0.05$ ) influence on the production and quality characteristics of feathers, skin or meat. Thus, COCM can be used as replacement for SOCM in ostrich diets without having negative effect on the quantity and quality of the end-products.

#### 5.1 Introduction

During the 1800's, ostrich feathers became very popular in the European fashion industry. Ostriches were then domesticated and bred exclusively to supply the industry with feathers. However, today, ostrich farming is not primarily driven by producing feathers for the fashion industry, but also by leather and meat production. Nowadays, feathers have the lowest income and are used mostly as industrial and household feather dusters (van Zyl, 2001; DAFF, 2017). Ostrich leather is currently the most sought after product of the ostrich, and has the highest economic value. Ostrich leather is unique due to the quill follicles that cause raised bumps in unique patterns that makes this distinctive from all types of leather (Meyer *et al.*, 2004; NAMC, 2010; Engelbrecht, 2014). The different shapes, sizes and patterns of the nodules contribute to its quality and ensure each skin to be unique. Furthermore, ostrich leather is regarded as one of the most supple, durable and attractive of all exotic leather (NAMC, 2010; Engelbrecht, 2014). Together with snake and crocodile, ostrich leather is classified as 'exotic leather' and is marked as a luxury product (Cooper, 2001; Adams & Revell, 2003).

As people became more aware of a healthy lifestyle, there was an exponential increase in research on food that will benefit these healthy lifestyles. Ostrich meat is seen as an ideal meat to maintain a healthy lifestyle as it has a low intra-muscular fat content, favourable fatty

acid profile, is low in sodium and cholesterol and high in iron and vitamin E (Mellett, 1992; Sales & Oliver-Lyons, 1996; Majewska *et al.*, 2009; Poławska *et al.*, 2011; Dalle Zotte *et al.*, 2013). Currently, the economic income ratio of skins, meat (local consumption) and feathers is approximately 65:20:15.

As the ostrich industry is relative small, there is consistent pressure on producers to supply enough products to fulfil the increasing demands of the consumers that is ever growing. Producers are consistently looking for options to enlarge their profit margin. As feed is the largest expense, approximately 75%, of all expenses in a livestock farming system (Brand *et al.*, 2002; Aganga *et al.*, 2003; Brand & Jordaan, 2011), this is the first expense farmers want to cut. But this is also the most important expense as feeding has a direct influence on the growth and maintenance of the animals and, consequently, on the products produced by the animals (Niknafs & Roura, 2018). As the latter, ultimately influences the price that the producer receives, it is therefore necessary to first consider any influence alternative feed ingredients might have on quantity and quality of the products.

Protein is one of the most important ingredients in animal feed. After energy, protein is the largest and the second most expensive component of the diet (Kleyn, 2013; Brand *et al.*, 2014). This nutrient is essential for the growth and maintenance of the muscles and the production and maintenance of feathers (Smit, 1964; Kleyn, 2013). Currently, soybean meal is used as main protein source in the diets of ostriches world-wide (Dalle Zotte *et al.*, 2013; Snyman, 2016). But, the demand in South Africa for soybean is much more than the supply thereof (DAFF, 2016a). Therefore, soybean is imported, resulting in larger expenses for feed (Sihlobo & Kapuya, 2016; AFMA, 2017).

Alternative raw materials to soybean were identified to be lupins, full-fat canola meal as well as canola oilcake meal (COCM). Brand *et al.* (2018) reared ostriches on five diets with different inclusion levels of sweet lupin (*Lupinus angustifolius*) seed, gradually replacing soybean oilcake meal (SOCM). The conclusion were made that the different diets had little influence on the feather classes, leather traits and the meat composition of the ostriches in the trial. In a similar study by Niemann (2018), SOCM were gradually replaced with full-fat canola meal. After evaluating the leather quality, feather yield and meat characteristics of the ostriches in this trial, it was noted that up to 75% of SOCM in the ostriches' diet could be replaced with full-fat canola meal without affecting the leather, feather and meat characteristics. The current study evaluates the influence of COCM (the residue of extracting oil from canola seeds) on the end-products (i.e. feathers, leather and meat) of slaughter ostriches. Ostriches were reared on five different diets containing increasing levels of COCM, gradually replacing SOCM.

## 5.2 Materials and Methods

South African Black ostriches were used in this trial to determine the effect of including COCM in the diets of slaughter ostriches on feather, leather and meat production aspects. These birds were bred and hatched on the Oudtshoorn Research Farm (situated at latitude 33°63'S and longitude 22°26'E in the Klein Karoo region of the Western Cape, South Africa) in November 2016. After hatching, the chicks were individually marked and randomly divided into five dietary treatments (0%, 25%, 50%, 75% and 100% replacement of SOCM, respectively) with three replicates each, resulting in 15 groups with approximately 15 chicks per group.

Initially, all the groups were reared on the same pre-starter diet (Table 5.1) from hatching to 76 days of age. During this time, the groups were reared in fifteen identical camps (10 x 5 m) with sufficient shade as well as shelter (5 x 3 m), where they were kept at night. During daytime they had free access to the outdoor camp. During the entire growth period, the chicks had *ad libitum* access to feed and water.

**Table 5.1:** The ingredient and chemical composition of the pre-starter diet fed to the ostrich chicks in this trial for the age of 0 – 76 days (as is basis)

Ingredients	Amount (kg/ton)
Maize (yellow grain)	504.36
Lucerne meal, 17% CP <sup>1</sup>	100.87
Soybean oilcake meal, 44% CP <sup>1</sup>	172.82
Fish Meal	75.65
Canola oilcake meal	50.44
Canola oil	50.44
Limestone, ground	24.31
Kynofos 21/MCP <sup>2</sup>	4.01
Common salt/NaCl <sup>3</sup>	10.09
Vitamin & Mineral Premix <sup>*</sup>	5.04
Lysine-HCl	1.97
<b>Nutrients (as formulated)</b>	
Dry matter (g/kg)	907.40
ME <sup>4</sup> ostrich (MJ/kg feed)	14.36
Crude protein (g/kg)	205.68
Crude fibre (g/kg)	54.31
Crude fat (g/kg)	78.46
Calcium (g/kg)	15.18
Phosphorous (g/kg)	6.03

\* Refer to Annexure A for the full vitamin and mineral premix

<sup>1</sup> Crude protein

<sup>2</sup> Monocalcium phosphate

<sup>3</sup> Sodium chloride

<sup>4</sup> Metabolisable energy, as formulated by the formula ME ostrich = 6.35 + 0.645 × ME poultry (Gous & Brand, 2008)

The trial started with the onset of the starter phase, at the age of 77 days. On this day all the groups were moved to fifteen larger camps (25 x 6 m) to accommodate for growth.

Five diets (Tables 5.2 – 5.4), each with a different inclusion level of COCM replacing SOCM, were formulated for each of the growth phases (i.e. starter, grower and finisher) by Mixit2+ software (Agricultural Software Consultants Inc., San Diego, USA). These formulations were based on the optimization model predictions, as developed by Brand & Gous (2008). All the diets met the recommended specifications for that phase.



**Table 5.2:** The ingredient and chemical composition of five starter diets containing increasing levels of canola oilcake meal fed to ostrich chicks from 76 – 146 days of age (as is basis)

Ingredients (kg/ton)	Diets expressed as percentage canola oilcake meal replacing soybean oilcake meal				
	0%	25%	50%	75%	100%
Yellow maize	572.16	529.12	486.08	443.04	400.00
Soybean oilcake meal 44% CP <sup>1</sup>	179.51	134.63	89.76	44.88	0.00
Canola oilcake meal	0.00	78.20	156.40	234.60	312.80
Lucerne meal, 17% CP <sup>1</sup>	123.97	125.48	126.99	128.50	130.00
Molasses meal	38.14	38.61	39.07	39.54	40.00
Fat, animal	28.61	35.31	42.01	48.70	55.40
Limestone, ground	11.47	11.43	11.39	11.34	11.30
Kynofos 21/MCP <sup>2</sup>	21.47	21.40	21.34	21.27	21.20
Bentonite Clay	9.54	9.66	9.77	9.89	10.00
Common salt/NaCl <sup>3</sup>	9.54	9.66	9.77	9.89	10.00
Vitamin & mineral premix <sup>*</sup>	3.34	3.38	3.42	3.46	3.50
Lysine-HCl	2.26	2.10	1.93	1.77	1.60
Nutrient composition (laboratory analysis)					
Dry matter (g/kg)	900.70	895.45	891.58	899.43	899.20
ME <sup>4</sup> ostrich (MJ/kg feed)	10.89	10.72	10.50	10.60	10.32
Crude protein (g/kg)	155.31	158.59	168.91	170.47	175.47
Crude fibre (g/kg)	78.13	80.05	90.73	93.53	116.78
Acid detergent fibre (g/kg)	110.05	113.30	122.45	134.05	170.53
Neutral detergent fibre (g/kg)	149.28	151.45	160.15	165.98	209.70
Calcium (g/kg)	12.15	12.20	14.55	15.45	17.35
Phosphorous (g/kg)	8.80	9.20	10.55	10.45	10.25
Amino acid composition (formulated)					
Lysine (g/kg)	0.93	0.95	0.97	0.99	1.01
TSAA <sup>5</sup> (g/kg)	0.70	0.71	0.73	0.74	0.76
Threonine (g/kg)	0.69	0.70	0.72	0.73	0.75
Tryptophan (g/kg)	0.19	0.20	0.20	0.20	0.21
Arginine (g/kg)	0.89	0.91	0.93	0.95	0.97

\* Refer to Annexure A for the full vitamin and mineral premix

<sup>1</sup> Crude protein

<sup>2</sup> Monocalcium phosphate

<sup>3</sup> Sodium chloride

<sup>4</sup> Metabolisable energy, as formulated by the formula ME ostrich = 6.35 + 0.645 × ME poultry (Gous & Brand, 2008)

<sup>5</sup> Total sulphur containing amino acids

**Table 5.3:** The ingredient and chemical composition of five iso-nutritious grower diets containing increasing levels of canola oilcake meal fed to ostrich chicks from 147 – 230 days of age (as is basis)

Ingredients (kg/ton)	Diets expressed as percentage canola oilcake meal replacing soybean oilcake meal				
	0%	25%	50%	75%	100%
Wheat grain	463.82	447.44	431.07	414.69	398.31
Oats hulls	322.98	314.51	306.04	297.56	289.09
Soybean oilcake meal, 44% CP <sup>1</sup>	104.86	78.65	52.43	26.22	0.00
Canola oilcake meal	0.00	49.84	99.69	149.53	199.37
Molasses meal	40.00	39.97	39.94	39.90	39.87
Lucerne meal, 17% CP <sup>1</sup>	0.00	2.49	4.99	7.48	9.97
Kynofos 21/MCP <sup>2</sup>	27.04	26.63	26.21	25.80	25.38
Limestone, ground	16.80	16.25	15.69	15.14	14.58
Bentonite clay	10.00	9.99	9.99	9.98	9.97
Common salt/NaCl <sup>3</sup>	10.00	9.99	9.99	9.98	9.97
Vitamin & Mineral Premix <sup>*</sup>	3.50	3.50	3.50	3.49	3.49
Lysine-HCl	1.01	0.76	0.51	0.25	0.00
Nutrient composition (laboratory analysis)					
Dry matter (g/kg)	892.92	901.97	916.90	916.50	915.57
ME <sup>4</sup> ostrich (MJ/kg feed)	10.08	10.35	10.40	10.31	10.35
Crude protein (g/kg)	137.92	134.38	138.13	138.23	138.65
Crude fibre (g/kg)	98.47	104.52	112.78	119.52	125.50
Acid detergent fibre (g/kg)	129.27	136.88	153.92	162.87	178.30
Neutral detergent fibre (g/kg)	240.82	251.82	262.67	281.15	288.32
Calcium (g/kg)	14.57	13.07	13.30	13.47	13.50
Phosphorous (g/kg)	9.13	9.63	10.10	10.13	9.93
Amino acid composition (formulated)					
Lysine (g/kg)	0.55	0.55	0.55	0.55	0.55
TSAA <sup>5</sup> (g/kg)	0.35	0.39	0.43	0.47	0.50
Threonine (g/kg)	0.37	0.40	0.42	0.45	0.48
Tryptophan (g/kg)	0.16	0.16	0.16	0.16	0.16
Arginine (g/kg)	0.56	0.58	0.59	0.61	0.62

\* Refer to Annexure A for the full vitamin and mineral premix

<sup>1</sup> Crude protein

<sup>2</sup> Monocalcium phosphate

<sup>3</sup> Sodium chloride

<sup>4</sup> Metabolisable energy, as formulated by the formula ME ostrich = 6.35 + 0.645 × ME poultry (Gous & Brand, 2008)

<sup>5</sup> Total sulphur containing amino acids

**Table 5.4:** The ingredient and chemical composition of five iso-nutritious finisher diets containing increasing levels of canola oilcake meal fed to ostrich chicks from 231 – 337 days of age (as is basis)

Ingredients (kg/ton)	Diets expressed as percentage canola oilcake meal replacing soybean oilcake meal				
	0%	25%	50%	75%	100%
Wheat grain	463.82	447.44	431.07	414.69	398.31
Oats hulls	322.98	314.51	306.04	297.56	289.09
Soybean oilcake meal, 44% CP <sup>1</sup>	104.86	78.65	52.43	26.22	0.00
Canola oilcake meal	0.00	49.84	99.69	149.53	199.37
Molasses meal	40.00	39.97	39.94	39.90	39.87
Lucerne meal, 17% CP <sup>1</sup>	0.00	2.49	4.99	7.48	9.97
Kynofos 21/MCP <sup>2</sup>	27.04	26.63	26.21	25.80	25.38
Limestone, ground	16.80	16.25	15.69	15.14	14.58
Bentonite clay	10.00	9.99	9.99	9.98	9.97
Common salt/NaCl <sup>3</sup>	10.00	9.99	9.99	9.98	9.97
Vitamin & Mineral Premix <sup>*</sup>	3.50	3.50	3.50	3.49	3.49
Lysine-HCl	1.01	0.76	0.51	0.25	0.00
Nutrient composition (laboratory analysis)					
Dry matter (g/kg)	892.92	901.97	916.90	916.50	915.57
ME <sup>4</sup> ostrich (MJ/kg feed)	10.08	10.35	10.40	10.31	10.35
Crude protein (g/kg)	137.92	134.38	138.13	138.23	138.65
Crude fibre (g/kg)	98.47	104.52	112.78	119.52	125.50
Acid detergent fibre (g/kg)	129.27	136.88	153.92	162.87	178.30
Neutral detergent fibre (g/kg)	240.82	251.82	262.67	281.15	288.32
Calcium (g/kg)	14.57	13.07	13.30	13.47	13.50
Phosphorous (g/kg)	9.13	9.63	10.10	10.13	9.93
Amino acid composition (formulated)					
Lysine (g/kg)	0.55	0.55	0.55	0.55	0.55
TSAA <sup>5</sup> (g/kg)	0.35	0.39	0.43	0.47	0.50
Threonine (g/kg)	0.37	0.40	0.42	0.45	0.48
Tryptophan (g/kg)	0.16	0.16	0.16	0.16	0.16
Arginine (g/kg)	0.56	0.58	0.59	0.61	0.62

\* Refer to Annexure A for the full vitamin and mineral premix

<sup>1</sup> Crude protein

<sup>2</sup> Monocalcium phosphate

<sup>3</sup> Sodium chloride

<sup>4</sup> Metabolisable energy, as formulated by the formula ME ostrich = 6.35 + 0.645 × ME poultry (Gous & Brand, 2008)

<sup>5</sup> Total sulphur containing amino acids

During the starter phase, each chick was weighed every week. Feed intake was measured each week by weighing the feed refusals. The starter phase was terminated at the age of 146 days, followed by the grower phase. Thereafter the ostriches were moved to larger camps (40 x 30 m) at the age of 156 days, to reduce the risk of injuries and skin damage and to create more space for the growing birds, the birds as well as the feed refusals were weighed

once every three weeks as too much handling of the birds increases stress and can lead to injuries of the birds as well as the handlers. The finisher phase started at day 231 and ended with the slaughtering of the birds on day 338.

Four weeks before slaughter, all the birds were vaccinated for external parasites. Blood was drawn to test for avian influenza and they were moved to five different quarantine camps, according to their treatment groups. These specifications were followed according to the prescriptions of the European Union meat quality standards (DAFF, 2014). During this time they continued receiving the five finisher experimental diets.

On the day before slaughter, the remaining 197 ostriches (see Chapter 4 for detail on causes of death) were transported by vehicle to the registered Klein Karoo International abattoir (registration number: 7/159) in Oudtshoorn where they were slaughtered at the age of 338 days. The standard slaughtering procedures that were followed to slaughter these ostriches are described in detail by Hoffman (2012).

After exsanguination, the feathers of each bird were plucked by hand. Each bird's feathers were kept in separate, individually marked bags. The feathers were cleaned and dried at the feather department of Klein Karoo International Limited, situated in Oudtshoorn. The processing of the feathers is described in Viviers (2015) as well as Brand *et al.* (2018). All birds' feathers were kept separate throughout the whole process. Feathers were graded and classed into the respective economical classes by qualified graders according to the appearance and quality thereof.

The skins were individually marked and sent off for tanning as described in Brand *et al.* (2018). After the skins were tanned, the crust size (dm<sup>2</sup>) was measured and each skin was graded by the subjective evaluation through qualified and experienced leather graders from the tannery. A digital calliper (Mitutoyo 0-200 mm) was used to measure the thickness (mm) of each skin. Five different localities on each skin, as indicated on Figure 5.1, were allocated to evaluate the leather. The nodules in a 10 x 10 cm square at each of these localities were counted. The number of pinholes in a smaller (5 x 5 cm) square within the bigger square were counted at each of these localities. A digital calliper was used to measure the diameter of five randomly selected nodules at each of these localities.

The birds in each treatment group were sorted in ascending order according to their last live weight that was measured on the farm. Ten birds around the median of each group were then selected (resulting in a group of 50 selected ostriches) and the meat and fat sampled for further chemical analysis.

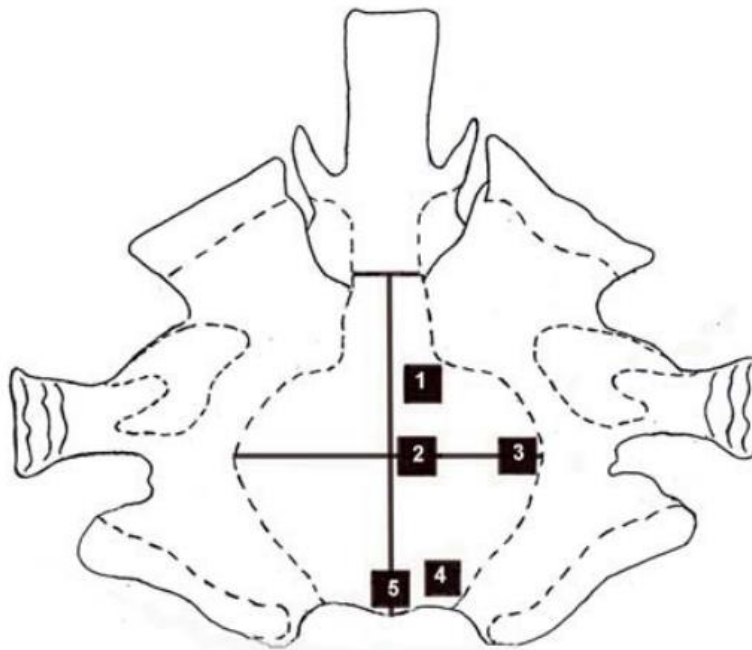
Directly after dressing, the warm carcasses were weighed and then chilled overnight in a cold room at 2°C. After 24 hours, the muscle pH as well as carcass temperatures were measured in the big drum muscle (*Muscularis gastrocnemius*). The cold carcass weight was

measured before deboning ( $\pm$  24 hours post mortem) of each carcass to calculate the dressing percentage.

The big drum muscle (*M. gastrocnemius*) of the right thighs of each of the selected ostriches were sampled and homogenized and used for chemical analysis. For this chemical analysis of the meat, the methods of the Association of Official Analytical Chemists (AOAC) were followed to determine the moisture (method 934.01), crude protein (method 992.15) and ash (method 942.05) content of the meat (AOAC, 2002). The crude fat content of the meat was determined by a method developed by Lee *et al.* (1996) with the use of chloroform/methanol extraction. The ratio of chloroform to methanol that were used in this case, was 1:2.

SAS Enterprise Guide (Version 9.2; SAS Institute Inc., Cary, USA) was used to perform statistical analysis on dry feather weight, leather traits and the chemical composition of the meat to determine treatment differences with one-way analysis of variances (ANOVAs) using the general linear model (GLM). In the case where significant differences occurred at the  $P \leq 0.05$  level, Fisher's least significant difference (LSD) t-test was used to determine which diets differed significantly. In cases where data showed possible trends, regression analysis were performed on the means of the data by the use of Microsoft Office Excel 2010 (Version 14.0.7163.5000, Microsoft Corporation by Imprensa Systems, Santa Rosa, California).

During the course of the entire trial, all the ostriches were handled, vaccinated and treated by well-experienced personnel according to the Code of Conduct for the Commercial Production of Ostriches (2011) of the Ostrich Business Chamber. Ethical clearance (DECRA R14/108) was granted by the ethical committee of the Western Cape Department of Agriculture.



**Figure 5.1:** The five locations on the ostrich skin where measurements and counts were taken for the evaluation of the skin (Cloete *et al.*, 2004)

### 5.3 Results

As pertaining to the feather traits (Table 5.5), diet had no effect on either the total dry feather weights ( $P=0.799$ ), nor the marketable feather weights ( $P=0.286$ ).

**Table 5.5:** Least square means  $\pm$  standard error (LSM  $\pm$  SE) for the effect of the replacement of soybean oilcake meal with increasing levels of canola oilcake meal (respectively 0%, 25%, 50%, 75% and 100%) on the feather weight of slaughter ostriches

Dry feather weight	Diets (expressed as the percentage canola oilcake meal replacing the soybean oilcake meal)					<i>P</i> -values
	0%	25%	50%	75%	100%	
Total (g)	936.1 $\pm$ 32.4	974.2 $\pm$ 32.4	921.8 $\pm$ 32.4	945.9 $\pm$ 32.4	961.7 $\pm$ 32.4	0.799
Marketable (g)	794.4 $\pm$ 42.2	852.6 $\pm$ 42.2	763.8 $\pm$ 42.2	715.1 $\pm$ 42.2	759.2 $\pm$ 42.2	0.286
Non-marketable (g)	135.7 <sup>c</sup> $\pm$ 14.2	147.3 <sup>c</sup> $\pm$ 14.2	180.4 <sup>bc</sup> $\pm$ 14.2	230.7 <sup>a</sup> $\pm$ 14.2	205.8 <sup>ab</sup> $\pm$ 14.2	0.004

<sup>a,b,c</sup> Row means with different superscripts differ significantly at  $P < 0.05$

The average total for dry feather weight was  $947.9 \pm 32.4$  g and the average for marketable feather weight was  $777.0 \pm 42.2$  g. However, diet had an effect on the amount of non-marketable feathers ( $P = 0.004$ ). The highest non-marketable feather yields were observed for birds on the 75% and 100% diets (230.7 g and 205.8 g, respectively) followed by birds on the 50% COCM diet (180.4 g), with the lowest yields being observed with the 0% and 25% diets (135.7 g and 147.3 g, respectively).

No differences ( $P > 0.05$ ) were observed between the different diets for the crust size ( $142.5 \pm 1.1 \text{ dm}^2$ ), skin thickness ( $1.4 \pm 0.1 \text{ mm}$ ), pinhole count ( $16.7 \pm 2.4$ ), nodule count ( $41.8 \pm 0.6$ ) and nodule diameter ( $3.4 \pm 0.1 \text{ mm}$ ) between the birds that were fed on different diets (Table 5.6).

**Table 5.6:** Least square means  $\pm$  standard error (LSM  $\pm$  SE) for the effect of the replacement of soybean oilcake meal with increasing levels of canola oilcake meal (respectively 0%, 25%, 50%, 75% and 100%) on the leather traits (crust traits) of slaughter ostriches

Leather traits	Diets (expressed as the percentage canola oilcake meal replacing the soybean oilcake meal)					P-values
	0%	25%	50%	75%	100%	
Crust size ( $\text{dm}^2$ )	$143.5 \pm 1.1$	$142.2 \pm 1.1$	$142.6 \pm 1.1$	$142.7 \pm 1.1$	$141.2 \pm 1.1$	0.641
Leather thickness (mm)	$1.4 \pm 0.1$	$1.3 \pm 0.1$	$1.4 \pm 0.1$	$1.4 \pm 0.1$	$1.4 \pm 0.1$	0.743
Pinhole count	$13.6 \pm 2.4$	$18.7 \pm 2.4$	$20.5 \pm 2.4$	$12.4 \pm 2.4$	$18.1 \pm 2.4$	0.168
Nodule count	$41.5 \pm 0.6$	$41.7 \pm 0.6$	$42.1 \pm 0.6$	$41.4 \pm 0.6$	$42.3 \pm 0.6$	0.824
Nodule diameter (mm)	$3.4 \pm 0.1$	$3.4 \pm 0.1$	$3.5 \pm 0.1$	$3.4 \pm 0.1$	$3.4 \pm 0.1$	0.621
<b>Grades</b>						
Average grade	$2.8 \pm 0.2$	$2.9 \pm 0.2$	$3.2 \pm 0.2$	$3.1 \pm 0.2$	$3.2 \pm 0.2$	0.364
Grade 1 (%)	$5.3 \pm 4.2$	$13.9 \pm 4.2$	$6.3 \pm 4.2$	$0.0 \pm 4.2$	$2.8 \pm 4.2$	0.270
Grade 2 (%)	$34.6^a \pm 3.4$	$20.3^{ab} \pm 3.4$	$16.5^b \pm 3.4$	$17.7^b \pm 3.4$	$20.4^{ab} \pm 3.4$	0.024
Grade 3 (%)	$32.1 \pm 8.1$	$25.1 \pm 8.1$	$26.9 \pm 8.1$	$53.0 \pm 8.1$	$36.1 \pm 8.1$	0.190
Grade 4 (%)	$28.0 \pm 8.2$	$40.7 \pm 8.2$	$50.4 \pm 8.2$	$29.3 \pm 8.2$	$40.7 \pm 8.2$	0.336

<sup>a,b</sup> Row means with different superscripts differ significantly at  $P \leq 0.05$

With regards to the average skin grading, no differences were observed ( $P > 0.05$ ) resulting in the skins having an average grade of  $3.1 \pm 0.2$ . When considering the frequencies of the skin grades within the dietary treatments, it was seen that the majority of the skins were given a grade of 3 or 4. The frequencies of the grades generally did not vary between the diets, except that the 50% and 75% diets had lower frequencies of grade 2 skins than the 0% diet ( $P = 0.024$ ).

With respect to the chemical composition of the meat (Table 5.7), again, no differences were observed for any of the components between the diets ( $P > 0.05$ ). Therefore, the pooled means of the chemical components of ostrich meat from this study can be given as:  $76.5 \pm 0.3 \text{ g/kg}$  for moisture,  $21.1 \pm 0.3 \text{ g/kg}$  for crude protein,  $2.1 \pm 0.2 \text{ g/kg}$  for crude fat and  $1.1 \pm 0.04 \text{ g/kg}$  for ash.

**Table 5.7:** Least square means  $\pm$  standard error (LSM  $\pm$  SE) for the proximate analysis of the big drum muscle of ostriches affected by the replacement of soybean oilcake meal (respectively 0%, 25%, 50%, 75% and 100%) with increasing levels of canola oilcake meal in the diets of slaughter ostriches on an as is basis

Chemical component (%)	Diets (expressed as the percentage canola oilcake meal replacing the soybean oilcake meal)					P-values
	0%	25%	50%	75%	100%	
Moisture	76.8 $\pm$ 0.30	76.4 $\pm$ 0.30	76.8 $\pm$ 0.30	76.2 $\pm$ 0.3	76.2 $\pm$ 0.3	0.250
Crude protein	20.7 $\pm$ 0.30	21.1 $\pm$ 0.30	20.9 $\pm$ 0.30	21.4 $\pm$ 0.3	21.5 $\pm$ 0.3	0.313
Crude fat	2.2 $\pm$ 0.20	2.3 $\pm$ 0.20	2.2 $\pm$ 0.20	2.1 $\pm$ 0.2	2.0 $\pm$ 0.2	0.701
Ash	1.1 $\pm$ 0.04	1.1 $\pm$ 0.04	1.15 $\pm$ 0.04	1.17 $\pm$ 0.04	1.16 $\pm$ 0.04	0.848

## 5.4 Discussion

About 75% of all ostrich products world-wide originates from South Africa (Brand & Jordaan, 2011; DAFF, 2017). South Africa is thus a net exporter of ostrich products even though high input costs lead to narrow profit margins in the ostrich industry and the ban on the export of meat due to the occurrence of avian influenza has left it in a vulnerable state (Brand & Jordaan, 2011). Furthermore, the ostrich industry has become relatively small due to these economic realities and through the exasperation of the avian influenza and so any changes in the nutrition or management of the birds will have an impact on the producers' profitability.

Although the production of feathers led to the domestication and farming of ostriches, the emphasis had shifted post the Great War (WWI) resulting in feathers being the product with the lowest income. Despite being the lowest source of income, good quality feathers as a result of good management practices might be the difference between profit or loss (Engelbrecht, 2014). As the income from feathers is relatively small, research is no longer focussed on the improvement of feather quality and yield (Brand & Cloete, 2015) but may still warrant further research in the future. In this study, no statistically significant differences were observed for the total feather yield as well as the marketable feather yield between the diets fed with varying inclusion levels of COCM. In the past, several studies have shown that nutrition does not influence feather production or quality, which corresponds with the findings of the current study (Carstens, 2013; Viviers, 2015; Brand *et al.*, 2018a; Niemann, 2018). The mean marketable feather yield of the current study was 777.0 g which was higher than the marketable feather yield of other studies investigating the potential of alternative protein sources in ostrich diets, such as sweet lupins (646.6 g) (Brand *et al.*, 2018) and full fat canola meal (716.8 g) (Niemann, 2018).

The income from ostrich leather is the most important contributor to the local as well as international economy within the ostrich industry. Grading according to physical



appearance is done subjectively by trained graders with only the crust surface area being measured objectively. The minimum requirements for ostrich leather characteristics are yet to be clearly defined (Engelbrecht *et al.*, 2009). In this study it was seen that the level of COCM inclusion did not affect the visual or physical traits of the leather, with the exception of the number of pinholes (Table 5.6). In previous studies, the inclusion of lupins (Brand *et al.*, 2018) and full fat canola seed (Niemann, 2018) affected the thickness of the crust, however, this was not seen in this study.

The only difference with regard to the leather traits was found amongst the number of pinholes. Pinholes (also called hair follicles) are tiny holes on the surface of leather that are the result of the removal of bristle hairs during processing (Engelbrecht *et al.*, 2009). When pinholes are present in two or more quarters over the crown area of the skin, the skin will be downgraded (ANON, 2006). In this trial, the number of pinholes on the leather of the ostriches receiving the 75% diet was 39.2% lower than the number of pinholes found on the skins of ostriches fed on the 50% diet (Table 5.6). However, it cannot be concluded that diet have an influence on pinhole density due to a lack of research on the factors that influence on the presence of these bristle hairs. Brand *et al.* (2018) and Niemann (2018) showed that protein source did not influence leather quality. This conclusion is supported further by Brand *et al.* (2004, 2014, 2018), Cloete *et al.* (2006), Engelbrecht *et al.* (2009) and Viviers (2015) who did similar studies to determine whether diet, especially energy level and protein content, has an effect on leather quality.

Ostrich meat is the second largest source of income in the ostrich industry. Ostrich meat is known to be low in cholesterol and has favourable polyunsaturated fatty acid profile and is also rich in iron (Dalle Zotte *et al.*, 2013). This makes ostrich meat a popular red meat alternative for people who are more aware of maintaining a healthy lifestyle. Results regarding the meat traits (Table 5.7) indicate that diet had no effect on the chemical composition of the big drum muscles and that the proximate chemical composition is similar to that found in other studies (Dalle Zotte *et al.*, 2013; Brand *et al.*, 2018a; Niemann, 2018).

## 5.5 Conclusion

Canola oilcake meal (COCM) can be included in the diets of slaughter ostriches (up to 20% in finisher diets) to replace SOCM, without negatively impacting the quality of meat, leather and feathers derived from ostrich production. Thus, locally produced COCM can be used as alternative protein source to reduce the feeding costs of slaughter ostriches while maintaining product quality. Profit margins will, however, be determined by production levels.

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## Chapter 6

### General conclusion and future prospects

#### 6.1 General conclusion

Over the past decade, the world's human population showed an increase of 75 – 77 million. Currently, the human population in the world is 7 billion and is growing at a rate of 1.09% yearly, with about 1.2 billion people on the African continent. With the rapid increase in human population world-wide, the competition for protein between animals and the human population is increasing (Brand *et al.*, 2000; Brand *et al.*, 2004; Sridhar & Bhat, 2007). It is estimated that the soybean consumption in South Africa for the 2018/2019 season will rise to 1.2 million tons which is 11% higher than the volume processed in the 2017/2018 season; 83% of this will be allocated for processing to oilcake for the use in animal feed. A slight decrease (2.58%) for the need of soybean oilcake meal (SOCM) between the 2016/2017 season (1 467 093 tons) and the 2017/2018 (1 429 250 tons) were observed (AFMA, 2018). Although the need for the use of SOCM decreased, the commodity remains expensive. This necessitates the need for cheaper, alternative protein sources in animal feed that will maintain production to make the industry more competitive. Apart from energy sources, protein makes out a large part of feed. Soybean oilcake meal, which is currently the main source of protein in animal feed, does not meet the demand thereof in South Africa and needs to be imported resulting in extra costs for feed. Alternative protein sources that are produced in the Western Cape, the same region where ostriches are primarily farmed with, were identified as lupins, full-fat canola as well as canola oilcake meal (COCM). These sources of protein are not suitable to supplement protein in human diets but can be used to produce high quality protein from livestock that can be utilised by humans more efficiently.

The aim of this study was to determine to what extent SOCM can be replaced with COCM, a locally produced alternative protein source, in the diets of slaughter ostriches without negative effects on production. The use of COCM will benefit the ostrich industry as it will decrease feeding costs and it will be of benefit to the local grain producers.

At first, a preference trial (Chapter 3) was conducted to determine whether the inclusion of COCM in the ostriches' diets will have an influence on dry matter intake (DMI). COCM contains high levels of glucosinolates that may present a bitter taste to the feed. The ostriches in this trial showed preference towards the control diet (with an inclusion of 0% COCM and 100% SOCM). The ostriches consumed 53% more of the control diet than the other diets. In addition, feed colour was measured for all five diets but showed no significant differences. As the diets were iso-energetic and showed no differences in colour, it is safe to conclude that

the taste and/or smell of the glucosinolates in the COCM had an influence on the preference and the intake exhibited by the grower ostriches.

The focus of Chapter 4 was on the effect of different COCM inclusion levels on the production and slaughter traits over the entire growth period. In comparison to the industry norm, the mortality rate for this trial was very low (11.3%). The birds in this trial showed similar growth and the differences in the production and slaughter traits were non-significant. However, the diet with a COCM inclusion level of 20%, where 100% of the SOCM was replaced, resulted in ostriches with slightly lower end weights at slaughter, however when the carcass yields were analysed, diet had no effect. Diets with different COCM will therefore have no meaningful impact on production performance indicators.

The end-products of the birds that were reared and slaughtered in the growth and production trial of Chapter 4 were evaluated to determine the effect of dietary inclusion of COCM (Chapter 5). Dietary inclusion of COCM had no effect on the feather, leather and meat production characteristics. This shows that COCM can be included up to a level of 20% (where COCM represents 100% of the total protein in the diet) in the diet of ostriches without having a negative impact on any of the three products' quality.

Although the designs of these trials adhere to the minimum of 10 degrees of freedom for the computing of the experimental error variance, it would have been beneficial to increase the levels of the existing treatments while the number of replications could also be increased in order to increase the sensitivity of the experiment. With more inclusion levels, trends would also be determined with greater precision.

To conclude, the results obtained by this study showed that COCM is a suitable replacement for SOCM in the diets of slaughter ostriches. The replacement of SOCM with COCM will not negatively impact production or the quality of the products. The use of COCM in the diets of ostriches will benefit the local as well as international ostrich industry as it will decrease feeding costs without having negative impact on the welfare, production and the quality of end-products. The local grain industry will also benefit from this.

## **6.2 Future prospects**

As mentioned above, protein sources are becoming less available and more expensive due to the competition that exists between humans and monogastric animals with regards to feed ingredients as their need for balanced amino acids is similar. As people are more and more aware of healthy lifestyles and show increasing interest in ostrich meat due to its health benefits, the demand for ostrich products is increasing. Although the ostrich industry is currently stagnant, the increasing demand for ostrich products may lead to expansion of the industry. With lower feed prices that will lead to larger profit margins for producers, the

expansion of the industry will be more rapid. With a healthy lifestyle in mind, people show more interest in the use of canola oil in everyday activities. This will result in more oilcake becoming available for the feeding industry at competitive prices.

With regards to the current study, it is advised that the preference study should be repeated within each of the growth phases (i.e. starter, grower and finisher) in order to establish whether the ostriches are more sensitive to specific tastes at certain stages/ages. Such an investigation can also determine whether ostriches become habituated to the tastes, as it is possible that the ostriches preferred the control diet because they were used to a diet that contained no COCM.

Continuing research can be conducted by investigating other alternative protein sources in the diets of slaughter ostriches. Other possible sources of protein includes legumes such as field peas, narbon beans, chickpeas and other oil seed crops such as sunflower, palm kernel cake, sesame, coconut or more novel insect meal.

Another possibility for decreasing feeding costs is intensively rearing of ostrich chicks on cultivated pastures that have high levels of protein such as lucerne (*Medicago sativa*) or other legume pastures.

The local availability of abovementioned raw materials and pastures will be beneficial for feed production and offer future potential. Research of the use of these resources is warranted for, especially, their use in the rearing of ostriches.

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## Annexure A

The composition of the vitamin and mineral premix used in the four feeding phases (i.e. pre-starter, starter, grower and finisher). Formulated per ton of feed.

Ingredients (composition per unit of premix)	Units	Growth phase		
		Pre-starter & Starter	Grower	Finisher
Vitamin A	IU	15 000 000	12 000 000	8 000 000
Vitamin D3	IU	4 000 000	3 000 000	2 000 000
Vitamin E	mg	60 000	45 000	40 000
Vitamin K3 stab	mg	3 000	3 000	2 000
Vitamin B1	mg	5 000	3 000	2 000
Vitamin B2	mg	10 000	8 000	5 000
Vitamin B6	mg	8 000	6 000	4 000
Vitamin B12	mg	100	100	50
Niacin	mg	100 000	80 000	60 000
Pantothenic Acid	mg	15 000	12 000	12 000
Folic Acid	mg	3 000	2 000	1 500
Biotin	mg	300	200	100
Choline	mg	800 000	600 000	300 000
Magnesium	mg	50 000	50 000	50 000
Manganese	mg	120 000	120 000	100 000
Iron	mg	30 000	25 000	40 000
Zinc	mg	120 000	80 000	100 000
Copper	mg	8 000	8 000	10 000
Cobalt	mg	300	300	500
Iodine	mg	2 000	1 000	2000
Selenium	mg	300	300	300
Antioxidant	mg	125 000	125 000	125 000

RECOMMENDATION: Mix 1 unit of 2.5 kg into 1 ton of feed, on an as fed basis