The evaluation of locally produced full-fat canola seed (*Brassica napus*) as an alternative protein source in the diets of slaughter ostriches (*Struthio camelus* var. *domesticus*)

By

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Declaration

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Abstract

Ostriches are multi-purpose animals, producing feathers, leather and meat that contribute to the income generated from slaughter ostrich production. Compared to other domesticated farm animals, knowledge of the nutritional requirements of ostriches has been very limited, until recently. Together with simulation models this knowledge gained is being used to formulate least cost, nutrient specific rations for ostriches in different production phases. In an effort to reduce feeding costs, which make up the largest expense (*ca.* 75%) in an intensive ostrich production unit, the use of alternative protein sources are being explored to replace the more expensive protein sources that make up a large portion of the diet. Full-fat canola seed (FFCS) is one such locally produced protein source that has the potential to replace current protein sources such as soybean oilcake meal. However, it is unclear whether ostriches will readily consume canola, due to its anti-nutritional factors, and what effect its consumption may have on animal performance.

The feeding preference (Chapter 3) of ostriches towards canola was established by placing 60 South African Black ostriches (82.2 ± 1.06 kg in live weight, 233 days of age) in ten camps of six birds per camp. Each camp had five identical feed troughs, each containing diets, where FFCS incrementally (0, 25, 50%, 75 and 100% of protein source) replaced the soybean oilcake meal (9.8% of the total diet composition in control diet) as protein source. Feed and water were made available *ad libitum*. Dry matter intake (DMI) was measured on a daily basis and feed colour characteristics were measured based on CIE L*, a* and b* colour attributes. Only the 25%FFCS (25% soybean oilcake meal replacement diet) showed a higher DMI (817.4 ± 81.98 g/bird/day) than the other diets (average of 488.8 ± 81.98 g/bird/day). While there were slight differences between some of the colour attributes, it is believed to have had no effect on DMI. Based on the results of this study, FFCS can be used to replace 25% soybean oilcake meal without any negative effect on DMI; resulting in an inclusion level of 6.8% FFCS in ostrich diets.

To evaluate to what extent FFCS can be utilised in the diets of slaughter ostriches, 187 day old South African Black ostrich chicks were randomly divided into 15 groups (9 - 12 animals per group). The growth trial (Chapter 4) commenced at the onset of the starter phase when the chicks were 84 days of age weighing 24.7 ± 0.36 kg. Five iso-nutritional treatment diets with varying levels of FFCS were randomly allocated to the groups with three replications per treatment diet. Birds were reared according to standard practises and slaughtered at 309 days of age (93.2 ± 1.82 kg). Within each feeding phase, FFCS incrementally (0%, 25%, 50%, 75% and 100% of protein source) replaced the soybean oilcake meal as protein source. Feed and water were supplied *ad libitum*. Dry matter intake, average daily gain (ADG), feed conversion ratio (FCR) and end weights were recorded with in each phase and over the entire trial period, as well as slaughter traits. No differences were observed regarding production traits during the starter and finisher phases. Dry matter intake during the grower phase was lowest (P =0.01) for the 100% replacement of soybean oilcake meal (100%FFCS) (1.52 kg/bird/day). The rest of the diets with an average DMI of 1.80 kg /bird/day did not differ. The 100%FFCS also showed the slowest growth (P =0.01) (152.0 g/bird/day) during the grower phase, and did not differ from 25%FFCS (208.9 g/bird/day) and 75%FFCS (209.5 g/bird/day) diets. With the 0%FFCS (236.2 g/bird/day) and 50%FFCS (267.8 g/bird/day) diets resulting in higher ADG. End weights during the grower phase for the 0%FFCS, 25%FFCS, 50%FFCS and 75%FFCS (74.8, 72.2, 76.8 and 72.5 kg respectively) did not differ from each other. The 100%FFCS resulted in lower end weights (67.4 kg), although not differing from the 25%FFCS

and 75%FFCS. For the overall trial period the only differences observed, were within ADG, with the 0%FFCS, 50%FFCS and 75%FFCS replacement diets showing the fastest growth and the 100%FFCS, although not differing from the 25%FFCS and 75%FFCS diets, resulted in the slowest growth. Fat pad weight was the only slaughter trait that revealed differences between diets, with the 50%FFCS resulting in the heaviest fat pad weights. Based on these results, it is recommended that a maximum of 20.6% inclusion (75%FFCS) of FFCS be used in diets during the grower phase as it may lead to reduced performance when exceeded. Although in the other phases, FFCS can be included up to the maximum levels evaluated (100% replacement of soybean oilcake meal) without any detrimental effects.

The aim of the study in Chapter 5 was to evaluate the effect of different dietary FFCS inclusion levels on the feathers, leather and meat composition of slaughter ostriches. The end products of the South African Black ostriches slaughtered in Chapter 4 were used during this trial. The only differences between diets regarding feather yields were within chick body short (P =0.021) and unmarketable feathers (P =0.011). The 50%FFCS resulted in the heavier yields of chick body short feathers (283.2 ± 14.84 g), however, it did not differ from the 0%FFCS, 25%FFCS and 75%FFCS. The 100%FFCS resulted in lighter yields (202.3 ± 14.84 g) of chick body short feathers, although not differing from the 75%FFCS. The 50%FFCS had the lightest yield (97.1 ± 13.18 g) of unmarketable feathers, differing from all the other diets. The rest of the diets did not differ and yielded an average of 161.2 ± 13.18 g unmarketable feathers. Skin thickness was the only leather trait showing differences (P =0.038) between diets. With the 0%FFCS resulting in thicker skins (0.65 ± 0.027 mm), not differing from the 25%FFCS and 50%FFCS diets. The 100%FFCS resulted in thinner skins (0.53 ± 0.027 mm), not differing from the 50%FFCS or 75%FFCS diets. Moisture and protein concentration of the meat were the only chemical components showing differences (P =0.008 and P =0.004, respectively) between diets. Meat from the 100%FFCS was found to have higher moisture concentrations (77.0 ± 0.29%). The 25%FFCS meat resulted in the lowest mean moisture concentration (75.2 ± 0.24%), not differing from the 0% and 50%FFCS meat. The inverse of moisture concentration could be seen with regards to protein concentration where the 25%FFCS diet resulted in the highest meat protein concentration (22.6 ± 0.19%), not differing from the 0%FFCS and 50%FFCS diets' meat. The 100%FFCS diet resulted in the lowest (20.9 ± 0.23%) meat protein concentration.

Dietary FFCS inclusion had beneficial effects on the fatty acid profile of the abdominal fat tissue. Total saturated fatty acids concentrations decreased from 37.8% (0%FFCS) to 20.3% (100%FFCS) of total identified fatty acids. Total MUFA concentrations increased from 39.7% for the 0%FFCS fed birds to 51.0% for the 100%FFCS fed birds. The total PUFA concentrations also increased from 22.1% for the 0%FFCS fed birds to 28.7% for the 100%FFCS fed birds. These changes resulted in the PUFA:SFA ratios to increase from 0.60 (0%FFCS) to 1.43 (100%FFCS). Both n-6 and n-3 fatty acids increased with an increase in FFCS inclusion, however the n-6:n-3 ratio showed a beneficial decrease from 3.20 for the 0%FFCS fed birds to 2.28 for the 100%FFCS birds.

Overall it is recommended that full-fat canola seed can be used to replace up to 75% (not exceeding inclusion levels used within each growth phase in this trial) of the soybean oilcake meal in slaughter ostrich diets. Prescribed replacement of soybean oilcake meal with FFCS can be done without affecting growth of ostriches or the quality of end products, and achieve similar results as current standard commercial diets.

Opsomming

Volstruise is veeldoelige diere, met vere, leer en vleis wat bydra tot die inkomste van slagvolstruisproduksie. In vergelyking met ander kommersiële plaasdiere was kennis ten opsigte van die voedingsbehoeftes van volstruise tot onlangs nog baie beperk. Simulasiemodelle word, saam met die huidige inligting beskikbaar, gebruik om die laagste koste, nutriëntspesifieke rantsoene vir volstruise in verskillende produksiefases te formuleer. Die voerkoste in 'n intensiewe volstruisproduksie-eenheid is verantwoordelik vir ongeveer 75% van die uitgawes, en om sodoende die koste daarvan te verminder, word die benutting van alternatiewe proteïenbronne ondersoek om die duurder proteïenbronne soos sojabone-oliekoekmeel, wat 'n groot deel van die dieet uitmaak, te vervang. Volvet kanolasaad (VVKS) is 'n plaaslike verboude proteïenbron wat die potensiaal het om huidige proteïenbronne soos ingevoerde sojabone-oliekoekmeel te vervang. Dit is egter onduidelik wat die reaksie van volstruise sal wees teenoor VVKS in hul dieet, omdat antinutriënte die vrywillige inname van voer kan beïnvloed en sodoende die diere se produksie vermoë verlaag.

Die voedingsvoorkeure (Hoofstuk 3) van volstruise teenoor kanola in hul dieet was ondersoek deur 60 Suid-Afrikaanse Swart volstruise (82.2 ± 1.06 kg in lewende massa) in tien kampe van ses voëls per kamp te plaas. Elke kamp het vyf identiese voerbakke gehad wat elk 'n dieet bevat het waar VVKS inkrementeel (0, 25, 50, 75 en 100% van die proteïenbron) die sojabone-oliekoekmeel (9.8% van die totale dieetsamestelling in kontrole dieet) as proteïen bron vervang het. Voer en water is *ad libitum* (vrylik en onbeperk) beskikbaar gestel. Droë materiaal inname (DMI) is daagliks gemeet en voerkleur eienskappe is gemeet, wat gebaseer is op L*, a* en b* kleur eienskappe. Slegs die 25% sojabone-oliekoekmeel vervangingsdieet het 'n hoër DMI (817.4 ± 81.98 g/voël/dag) getoon in vergelyking met die ander diëte (gemiddeld 488.8 ± 81.98 g/voël/dag). Alhoewel daar klein verskille was tussen diëte vir sommige kleurkenmerke, word dit nie as die rede vir die verskil in DMI geag nie. Gebaseer op die resultate van hierdie studie, kan VVKS gebruik word om 25% sojabone-oliekoekmeel te vervang sonder enige negatiewe effek op DMI; dit dui op 'n insluiting vlak van 6.8% VVKS in volstruise dieet.

Vir die evaluasie om vas te stel tot watter mate VVKS in die dieet van volstruise ingesluit kan word, was 187, dagoud Suid-Afrikaanse Swart volstruiskuikens ewekansig verdeel in 15 groepe (9-12 voëls per groep). Die groeiproef (Hoofstuk 4) het begin met die aanvangsfase toe die kuikens 84 dae oud was en 24.7 ± 0.36 kg geweeg het. Vyf diëte met dieselfde nutriëntwaardes maar met verskillende vlakke van VVKS was ewekansig toegeken aan elk van die groepe, wat dan drie herhalings per behandelingsdieet tot gevolg gehad het. Voëls is volgens standaardpraktyke gevoer waarna hul geslag was op die ouderdom van 309 dae (93.2 ± 1.82 kg). Binne elke voedingsfase het VVKS inkrementeel (0%, 25%, 50%, 75% en 100% van die proteïenbron) die sojabone-oliekoekmeel as proteïenbron vervang, die voer en water was *ad libitum* beskikbaar gestel. Droë materiaal inname, gemiddelde daaglikse toename (GDT), voeromsetverhouding (VOV) en eindmassa is aangeteken binne elke fase asook oor die hele proefperiode, waarna slagopbrengste ook aangeteken was. Geen verskille is waargeneem ten opsigte van produksie eienskappe gedurende die aanvangs- en afrondingsfase nie. Droë materiaal inname tydens die groeifase was die laagste vir die 100% vervanging (100%VVKS) (1.52 kg/voël/dag) van sojabone-oliekoekmeel (27.5% totale VVKS-insluiting), verder het die res van die diëte met 'n gemiddelde DMI van 1.80 kg/voël/dag geen verskille tussen mekaar getoon nie. Die 100%VVKS het ook tydens die groeifase die stadigste groei getoon (152.0 g/voël/dag), maar het egter nie

verskil van die 25%VVKS (6.9% totale insluiting) (208.9 g/voël/dag) en 75%VVKS (20.6% totale VVKS insluiting) (209.5 g/voël/dag) nie. Die 0%VVKS (0% totale FFCS insluiting) (236.2 g/voël/dag) en 50%VVKS (13.8% totale FFCS insluiting) (267.8 g/voël/dag) diëte het gelei tot ’n hoër GDT in die groeifase. Eindmassas gedurende die groeifase vir onderskeidelik die 25%VVKS, 75%VVKS en 100%VVKS (onderskeidelik 72.2, 72.5 en 67.4 kg) het nie verskil van mekaar nie, die 0%VVKS (74.8 kg), 25%VVKS, 50%VVKS (76.8 kg) en 75%VVKS het ook nie van mekaar verskil nie. Gemiddelde daaglikse toename was die enigste eienskap wat verskille getoon het tussen diëte, met die 0%VVKS, 50%VVKS en 75%VVKS wat die vinnigste groei getoon het en die 100%VVKS die stadigste groei, alhoewel die 100%VVKS nie verskil het van die 25%VVKS en 75%VVKS nie. Die abdominale vetmassas was die enigste slagopbrengs wat verskille tussen diëte getoon het, die 50%VVKS het die swaarste vetgewigte tot gevolg gehad. Op grond van die resultate wat verkry is in die huidige studie, word aanbeveel om nie meer as 20.6% VVKS (75%VVKS) in diëte in te sluit tydens die groeifase nie, aangesien dit tot swakker produksie kan lei. Verder, in die aanvangs- en afrondfase kan VVKS tot die maksimum insluitings vlakke (100%VVKS diëte) wat in die studie geëvalueer was in volstruis diëte ingesluit word, sonder enige nadelige effekte.

Die doel van die studie in Hoofstuk 5 was om te evalueer wat die effek van verskillende VVKS insluiting vlakke op die vere, leer en vleissamestelling van slagvolstruise is. Eindprodukte van die voëls wat in Hoofstuk 4 gebruik was, is tydens hierdie proef gebruik. Die enigste verskille tussen diëte ten opsigte van veeropbrengste was binne kuikenliggaam-kort (Chick body short) en onbemarkbare veerklasse. Die 50%VVKS wat die hoër opbrengste (283.2 ± 14.84 g) tot gevolg gehad het, het nie verskil van die 0%VVKS, 25%VVKS en 75%VVKS nie. Verder was bevind, dat die 100%VVKS gelei het tot laer opbrengste (202.3 ± 14.84 g) van kuikenliggaam-kort vere, alhoewel dit nie van die 75%VVKS verskil het nie. Die 50%VVKS het die laagste opbrengs (97.1 ± 13.18 g) onbemarkbare vere getoon. Die res van die diëte wat nie verskil het van mekaar nie en ’n gemiddeld van 161.2 ± 13.18 g onbemarkbare vere gelewer. Vel dikte was die enigste leer eienskap wat verskille tussen diëte getoon het. Die 0%VVKS het dikker velle (0.65 ± 0.027 mm) opgelewer, maar het nie verskil van die 25%VVKS en 50%VVKS diëte nie. Die 100%VVKS wat dunner velle tot gevolg gehad het (0.53 ± 0.027 mm) het nie verskil van die 50%VVKS of 75%VVKS diëte nie. Vog en proteïen konsentrasie van die vleis was die enigste chemiese komponente wat verskille tussen diëte getoon het. Die 100%VVKS het die hoogste gemiddelde vog konsentrasie getoon (77.0 ± 0.29%) en 25%VVKS die laagste (75.2 ± 0.24%), met geen verskille tussen 0%VVKS 25%VVKS en 50%VVKS vleis nie. Die invers van die vog konsentrasie kan gesien word met betrekking tot proteïen konsentrasie. Die 25%VVKS het die hoogste proteïen konsentrasie (22.6 ± 0.19%) tot gevolg gehad, maar nie verskil van die 0%VVKS en 50%VVKS vleis nie. Die 100%VVKS dieet het die laagste proteïen konsentrasie (20.9 ± 0.23%) opgelewer.

Insluiting van VVKS het voordelige veranderinge op die vetsuurprofiel van die abdominale vetweefsel gehad. Totale versadigde vetsure konsentrasies het van 37.8% (0%VVKS) tot 20.3% (100%VVKS) van totale geïdentifiseerde vetsure afgeneem. Totale mono-onversadigde vetsure (MUFA) konsentrasies het toegeneem van 39.7% vir die 0%VVKS gevoerde voëls tot 51.0% vir die 100%VVKS gevoerde voëls. Die totale poli-onversadigde vetsure (PUFA) konsentrasies het ook toegeneem van 22.1% vir die 0%VVKS gevoerde voëls tot 28.7% vir die 100%VVKS gevoerde voëls. Hierdie veranderinge het gelei tot die PUFA:SFA verhoudings se verhoging van 0.60 (0%VVKS) tot 1.43 (100%VVKS). Beide n-6 en n-3-vetsure het toegeneem met 'n

toename in VVKS-insluiting in die dieet, maar die n-6:n-3 verhouding het 'n voordelige afname van 3.20 vir die 0%VVKS gevoerde voëls tot 2.28 vir die 100%VVKS gevoerde voëls getoon.

Die bevindinge afgelei uit die huidige studie is dus dat, volvet kanolasaad gebruik kan word om 75% (nie die insluiting vlakke wat in elke fase in hierdie proef gebruik word nie) van die sojabone-oliekoekmeel in die diëte van slagvolstruis te vervang. Dieselfde resultate as wat verkry word van die huidige standaard kommersiële diëte kan dus opgelewer word met insluiting van VVKS, sonder om die eindprodukte wat deur volstruise geproduseer word negatief te beïnvloed.

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Notes

The language and referencing style used in this thesis are in accordance with the requirements of The South African Journal of Animal Science. 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.

The following parts arising from this thesis have been presented at the following conference and submitted to the following journal for publication:

1. **The 50th South African Society for Animal Science Congress (SASAS), 18-21 September, Port Elizabeth, Eastern Cape, South Africa**

   Niemann, G.J., Brand, T.S. & Hoffman L.C., (2017). Feeding preferences of grower ostriches towards diets containing increasing levels of full-fat canola seed. Proc. 50th South African Society for Animal Science Cong. Port Elizabeth, South Africa, pp. 453-454 (Presentation).

2. **The South African Journal of Animal Science**

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List of abbreviations

ADF	Acid detergent fibre
ADG	Average daily gain
AFMA	Animal Feed Manufacturers Association
AgriLASA	Agri Laboratoy Association of South Africa
AI	Avian influenza
ANOVA	Analysis of variance
AOAC	Association of Official Analytical Chemists
AQC	6-aminoquinolyl-N-hydroxysuccinimidyl carbamate
avg	Average
BSE	*Bovine spongiform encephalopathy*
BW	Bird weight
°C	Degree Celsius
ca	*circa* (around/about)
CAF	Central Analytical Facilities
CF	Crude fibre
CLA	Conjugated linoleic acid
cm	Centimetre
CP	Crude protein
DAFF	Department of Agriculture, Forestry and Fisheries
dm^2	Cubic decimetre
DM	Dry matter
DMI	Dry matter intake
etc	*et cetera* (so on/forth)
EE	Ether extract (crude fat)
EU	European Union
FAME	Fatty acid methyl esters
FCR	Feed conversion ratio
FFCS	Full-fat canola seed
g	Gram
GC	Gas chromatograph
GDT	Gemiddelde daaglikse toename (Average daily gain)
GLM	General linear model
GLS	Glucosinolates
ICP	Inductively Coupled Plasma
kg	Kilogram
km	Kilometre
LC-MS	Liquid chromatography-mass spectrometry
LSD	Least significant difference

LSM	Least square means
m	Metre
MCP	Monocalcium phosphate
ME	Metabolisable energy
Min	Minute
MJ	Megajoules
mL	millilitre
mm	Millimetre
MUFA	Monounsaturated fatty acids
NaCl	Sodium chloride
NAMC	National Agricultural Marketing Council
NDF	Neutral detergent fibre
NFE	Nitrogen free extract
ppm	Parts per million
PUFA	Polyunsaturated fatty acid
SE	Standard error
SFA	Saturated fatty acids
SOM	Soybean oilcake meal
TAAA	True amino acid availability
TME	True metabolisable energy
TMEn	Nitrogen-corrected true metabolisable energy
TMR	Total mixed ration
T4	Tetraiodothyronine
V	Volt
viz	*videlicet* (namely)
VFA	Volatile fatty acids
VOV	Voeromsetverhouding (Feed conversion rate)
VVKS	Volvet kanolasaad (Full-fat canola seed)
UPLC	Ultra performance liquid chromatography
UV	Ultra violet
µL	Microlitre
µm	Micrometre
µmol	Micromolar

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Chapter 1

General Introduction

Ostrich farming is a relatively new practice compared to cattle, sheep and other livestock. Smit (1964) noted that ostrich farming in South Africa only started between 1857 and 1864. This shows the relative youth of the industry, considering that in England and Wales in the year 1086, farmers were already rearing livestock with numbers of over 100 cattle and 9000 sheep on a single farm (Thirsk & Hallam, 1989). Owing to the relative young age of the ostrich industry there is still room for improvement as pertaining to production practices.

Ostriches are multi-purpose animals producing feathers, skins (leather) and meat. Initially ostriches were mainly reared for their feathers, which were incorporated in the fashion industry (Osterhoff, 1979). Intensive ostrich farming only started in the 1960's, after which the industry began to shift focus, with leather becoming the dominate commodity in 1975 and later on in the 1980's the increase in popularity of ostrich meat were observed (van Zyl, 2001).

Food products consumption of animal origin has increased at a very high rate, it is predicted that *per capita* demand for these products will increase even more in developing countries (Bradford, 1999). With population growth, the increase of higher living standards and urbanization, the demand for higher quality protein grows. China and other Asian countries already show an increase in demand for animal derived food products (Bradford, 1999; Cao & Li 2013). Between 1997 and 1999 worldwide, total meat production was 218 million tons with predictions that it will rise to 376 million tons in 2030 (Gardner, 2013). In order to meet the fast growing demand for animal food products, production systems on the farms need to be optimized and input costs reduced. Ostrich meat is considered to be healthier than other meats due to the low fat content and composition of fatty acids (Harris *et al*., 1994; Sales & Hayes, 1996; Girolami *et al*., 2003) and can make a contribution to supplying animal protein to the local populations and international markets.

Ostrich diet formulation was initially based on the knowledge and standards of poultry in terms of energy and amino acid requirements, as there was limited available information regarding ostrich requirements. This lead to inaccurate formulation, causing an oversupply of metabolisable energy (ME) to ostriches, which increased cost or reduced animal performance (Angel, 1996; Brand *et al*., 2014). Up until early to mid-1990's this was found to be the case. Mellett (1993) reported that poor feed conversion ratios (FCR) were observed amongst ostriches in South Africa, which may have been the result of the type of feed provided to the birds. However, pioneering work by Swart (1988) was done to better understand the metabolism of ostriches, with Cilliers *et al*. (1994) and Cilliers (1995) establishing the true ME values for ostriches. Since then much research on ostrich nutrition has been conducted (Ullrey & Allen, 1996; Cilliers *et al*., 1998; Brand *et al*., 2000c; Glatz *et al*., 2003; Gous & Brand, 2008; Carstens, 2013; Brand *et al*., 2014; Viviers, 2015; Engelbrecht, 2016). With the ostrich industry experiencing a decline during 1997 and 1998, the focus of nutritional studies shifted to cost efficient diet formulation and feeding in order to lower the input cost of feeding. Currently the ostrich industry is under pressure due to the outbreak of avian influenza (AI) where birds that are found to be positive are maintained in feedlots under quarantine, which further emphasizes the importance of finding solutions to lower production/feed costs.

The largest expense of an intensive ostrich production system is feed cost (Aganga *et al*., 2003; Brand & Jordaan, 2004), this can account for ca. 75% of total input costs (Brand *et al*., 2002). The profitability of an intensive ostrich production unit can thus be improved by attempting to lower the feeding costs (Brand & Jordaan, 2004; Jordaan *et al*., 2008). There are several factors other than feed cots influencing profitability that the farmer cannot control, thus it is of key importance to optimize factors such as nutrition which can be to some degree controlled by the producer (Smit, 1964; Cooper, 2001; Carstens, 2013). Brand & Jordaan (2004) noted that feed costs can be reduced by making use of well formulated least cost diets and incorporating the use of locally produced feedstuffs. The use of mathematical simulation models, developed by Gous & Brand (2008), are very effective tools in determining the exact nutritional requirement of ostriches and formulating least cost diets.

Protein (amino acids) is essential for growth and optimal production of animals. According to Brand & Gous (2006), depending on the feeding phase the ostriches are in, protein can constitute up to 22.8% of a diet. With global population growth, protein is becoming scarcer, more expensive and particularly less available to be utilised in animal feeds; this necessitates the research in order to find alternative protein sources (Brand *et al*., 2000a; Brand *et al*., 2004; Sridhar & Bhat, 2007).

Soybean oilcake meal is currently used as the main protein source in ostrich diets, however full-fat canola seed has the potential to replace soybean oilcake meal and is locally produced in the Western Cape region of South Africa (Brand *et al*., 2007). Brand *et al*. (2000) evaluated FFCS as a potential protein and energy source for ostriches and found that FFCS contained 192 g crude protein (CP)/kg feed and 11.2 g lysine/kg feed. Canola has long been used in other animal feeds such as poultry (Ajuyah *et al*., 1991; Lee *et al*., 1991), pigs (Busboom *et al*., 1991) and ruminants (Mir *et al*., 1984). However, no information is available about the suitability of canola in ostrich diets. The use of rapeseed in animal feed has also been limited, due to high levels of glucosinolates and erucic acid (Bell, 1993). However, the development of double zero canola cultivars (a rapeseed cultivar containing lower levels of these anti-nutrients) made it possible to include higher levels into diets (Dale, 1996).

Therefore the aim of this study is to evaluate to what extent full-fat canola seed can be included in slaughter ostrich diets as a locally produced protein source, replacing soybean oilcake meal, without having any detrimental effect on the health, production parameters, slaughter traits and end products of growing ostriches.

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Chapter 2

Literature Review

2.1 Introduction

Ostriches (*Struthio camelus*) belong to the ratite group. Ratites are flightless birds that have underdeveloped or non-existing breast muscles with a raft-like breastbone and no keel (Angel, 1996). Ostriches are well adapted to dry arid regions (Ullrey & Allen, 1996) and can be reared to produce feathers, leather and meat (Engelbrecht, 2016a).

Compared to other livestock systems, ostrich farming is a relatively small and new enterprise (Brand & Olivier, 2011; Cloete *et al*., 2012). The first extensive ostrich farming systems only starting in the mid 1800's, when ostriches were farmed primarily for their feathers (Smit, 1964). Intensive ostrich production began in the 1960's, after which the industry started transforming, with leather becoming the dominate commodity in 1975. Later on in the 1980's ostrich meat saw an increased in popularity (van Zyl, 2001). Currently, South Africa is the largest producer of ostrich products and holds ca. 75% of the global market share, with ostriches mainly being reared in semi-intensive or intensive systems until slaughter at 10 to 14 months of age (Brand, 2016; DAFF, 2016a), depending on market trends.

Compared to that of other monogastric animals, the knowledge of ostrich nutrition is still somewhat limited (Gous & Brand, 2008). Numerus studies have been conducted on ostrich nutrition with the pioneering work on determining true metabolisable energy (TME) for ostriches being done by Swart (1988), Swart *et al*. (1993), Cilliers *et al*. (1994) and Cilliers (1995). Since this pioneering work, more research has been conducted and is still being conducted in order to expand the knowledge of ostrich nutrition (Ullrey & Allen, 1996; Cilliers *et al*., 1998; Brand *et al*., 2000c; Glatz *et al*., 2003; Gous & Brand, 2008; Carstens *et al*., 2014; Brand *et al*., 2014; Viviers, 2015; Engelbrecht, 2016b). Initially, due to the limited knowledge regarding ostrich nutrition, poultry energy and amino acid requirement standards were used to formulate ostrich rations. This lead to lower profitability as inaccurate formulation would cause an over- or undersupply of metabolisable energy (ME), resulting in increased feeding costs or a reduction in animal performance (Angel, 1996; Brand *et al*., 2014). Carstens *et al*. (2014) stated that optimal growth can be achieved through the optimum supply of nutrients, which will have an economic advantage for ostrich producers.

The largest expense of an intensive ostrich production system is associated with the cost of feed (Aganga *et al*., 2003; Brand & Jordaan, 2004), this can account for ca. 75% of total input costs (Brand & Jordaan, 2004). The profitability can thus be improved by lowering the high cost of feeding (Brand & Jordaan, 2004; Jordaan *et al*., 2008). With global population growth, protein is becoming scarcer, more expensive and especially less available to be utilised in animal feed (Brand *et al*., 2000a; Brand *et al*., 2004a; Sridhar & Bhat, 2007). According to Brand & Gous (2006), depending on the feeding phase the ostriches are in, protein can constitute up to 22.8% of a diet. This means that protein sources are major contributors to the high cost of feed which necessitates research in finding alternative protein sources.

Canola is locally produced in the Western Cape province of South Africa and has the promising potential to be utilised as a protein source in animal rations, replacing soybean oilcake meal (Brand *et al*., 2007). Canola has long been used in other animal feeds such as poultry (Ajuyah *et al*., 1991; Lee *et al*., 1991), pigs (Castell

& Falk, 1980; Busboom *et al*., 1991) and ruminants (Mir *et al*., 1984; Rule *et al*., 1994). However, no information is available on the suitability of canola in ostrich diets. In the past, the use of rapeseed in animal feed was very limited due to high levels of glucosinolates and erucic acid (Bell, 1993), however, the development of double zero canola cultivars (rapeseed cultivars containing lower levels of these anti-nutrients) made it possible to include higher levels into diets (Dale, 1996).

The aim of this research is therefore; to evaluate the use of locally produced full-fat canola seed as an alternative protein source in slaughter ostrich diets, to replace imported soybean oilcake meal. The study will also determine dietary inclusion levels of full-fat canola seed, to prevent potential production or product quality inhibition.

2.2 Ostrich production in South Africa

The South African ostrich industry has experienced huge fluctuations throughout the years. This has mainly been due to the birds being very susceptible to Avian Influenza (AI) outbreaks and cyclic market trends of ostrich products, as they are considered as luxury goods (Brand & Jordaan, 2011; DAFF, 2016a). This causes limitations on the export of ostrich meat and other products. Nonetheless, South Africa remains the largest producer of ostrich products and holds ca. 75% of the global market share. Ostriches are mainly reared in semi-intensive (lucerne grazing with concentrate supplementation) or intensive systems (fed a total mixed ration) and slaughtered between 10 to 14 months of age (Brand, 2016; DAFF, 2016a). The highest profit margins are obtained when slaughtering ostriches at 10.5 month of age (Jordaan *et al*., 2008). In 2016 it was estimated that there were 588 registered ostrich farms in South Africa, with 453 of those farms being found in the Western Cape, 102 in the Eastern Cape and 33 farms in other provinces (DAFF, 2016a). The Klein Karoo region in the Western Cape has the ideal arid climate that ostriches are adapted to (Ullrey & Allen, 1996) and so has the highest contribution to ostrich production in the Western Cape, where 80% of the country's ostrich production occurs. Over the past 10 years, the industry had an average gross value of R370 million, and in 2014 the industry created 20,000 direct jobs, mainly in the rural areas as most production areas are within rural communities (DAFF, 2016a).

South Africa is a net exporter of ostrich products, and so the ostrich industry plays a major part in earning foreign exchange. In 2011, a ban was placed on the export of raw ostrich meat to Europe. Before this ban 98% of ostrich meat was exported which along with other ostrich products contributed to ca. R1.2 billion annually (DAFF, 2016a). The ban on ostrich meat export had a devastating effect on the gross value of the industry, however, the industry recovered by increasing the leather value, developing pre-cooked (*sous vide*) meat products for exported and promoting local ostrich meat consumption (DAFF, 2014; Booysen, 2015). In 2015 the ban on raw ostrich meat export was lifted, resulting in the increased growth of the industry which by that stage created 50,000 jobs (Booysen, 2015). Under the circumstances, the ostrich industry is ever growing with many opportunities. In the period of 2014 to 2015, the South African ostrich meat exports increased by 175% (DAFF, 2016a), but is currently again limited by a recent outbreak of AI (DAFF, 2017).

2.2.1 Brief history of the ostrich industry

Commercial ostrich farming is a relatively new and small enterprise when compared to other livestock species (Brand & Olivier, 2011; Cloete *et al*., 2012). Smit (1964) reports that ostrich farming only began

between 1857 and 1864 with a few farmers succeeding in breeding with tamed captive ostriches. The young age of the ostrich farming industry is emphasised when compared to other livestock animal farming practices. Reports of 9,000 sheep and 100 head of cattle on a single farm in Europe in the year 1086 show how developed production of these species was at this period in time, long before ostriches were being reared (Thirsk, 1989).

Ostrich farming became popular due to the bird's feathers being highly sought-after and becoming very valuable in the 19th century. Ostrich farming especially took place, and grew, in the Karoo and Eastern Cape areas of the country, with the Oudtshoorn region that showed the fastest expansion. After the development of the first ostrich egg incubator by Arthur Douglas in 1881 the industry rapidly grew as little capital was needed and high numbers of chicks could be produced (Smit, 1964).

During the late 1800's and early 1900's efforts were made to improve feather quality as well as animal temperament. This led to the importation of North African ostriches (*Struthio camelus camelus*) with superior feather quality from the Barbary Coast and cross-breeding them with South African ostriches (*Struthio camelus australis*) (Osterhoff, 1979; Strydom, 2007). The cross-breeding of these two strains lead to the development of the South African Black ostrich (*Struthio camelus* var*. domesticus*) (Swart *et al*., 1987) which is currently still regarded as a breed with superior feather quality.

In the year 1913, ostrich feather demands reached an all-time high due to the superior feathers being produced by the newly bred South African Black ostriches. Feathers became one of South Africa's major export products, ranking fourth after gold, diamonds and wool (Smit, 1964; ANON, 2004).The feather frenzy however did not last long due to the outbreak of World War One in 1914, as well as a shift in the fashion industry, which lead to the collapse of feather exports and ultimately the South African ostrich industry as a whole. After the Great War ended in 1918, ostrich numbers in South Africa stood at 314,000, this number rapidly decreased to just 32,000 in 1930 and by 1940 only 2,000 ostriches were left in the Oudtshoorn district, which was considered as the ostrich capital of the country. It is only after the Second World War in 1945 that the ostrich industry slowly regained momentum, but by this stage ostrich leather had become a popular product and together with tourism, aided in the recovery of the industry (ANON, 2004). The first ostrich leather tannery was built in 1970 which contributed greatly to leather being the leading source of income for the industry (NAMC, 2010).

Ostrich meat at first did not contribute much to the industry and was mainly used for making biltong (Osterhoff, 1979). The popularity of ostrich meat slowly grew with the first abattoir being built in 1964 (Jorgensen, 2016), this lead to larger ostrich sub-species such as the Kenyan Red Necks (*Struthio camelus massaicus*) and Zimbabwean Blue Necks (*Struthio camelus australis*) being domesticated for meat production (Horbañczuk *et al*., 1998). In the early 1990's ostrich meat became internationally popular especially in the First World countries as a niche product (NAMC, 2010) due to the low fat content, causing meat prices to increase (ANON, 2004). This gave rise to the first abattoir being built for meat exports to Europe in 1993. With the outbreak of *Bovine spongiform encephalopathy* (BSE), commonly known as mad cow disease, in Britain in the early 2000's, ostrich meat prices increased rapidly by 40%. This along with the weak value of the Rand, resulted in high incomes per ostrich, allowing the industry to thrive. However after the BSE incident was under control, ostrich meat prices dropped by 30%, resulting in new farms closing down (NAMC, 2010).

Throughout the development of the ostrich industry, the ostrich became a multi-purpose animal which produces feathers, leather and meat (DAFF, 2016a). As seen through the history of this industry, ostrich feathers and meat income have fluctuated immensely, however, that of leather has remained relatively stable

during the years, which lead to Engelbrecht *et al*. (2005) stating that the ostrich leather sector is the strongest pillar supporting the ostrich industry.

2.3 Ostrich end products and their utilisation

Considering that the ostrich is a multi-purpose animal it is important to devote time and effort into optimizing the production of all three major products produced by the ostrich industry, especially when taking into account how profit margins have decreased substantially in the past decade (Engelbrecht, 2016a). Ideal slaughter age and weight of ostriches varies considerably due to factors such as feed prices and the market trends towards certain end product features, which can have a major impact on the profitability of a production system. Therefore, when deciding on a slaughter age these factors need to be kept in mind (Brand, 2016). Table 2.1 gives a perspective of the influence of slaughter mass on the three end products produced by ostriches. End product contribution to the world market share has shifted significantly since the South African ostrich industry started. In 2016, feathers, which was once the major contributor to the income, only held 10% of the market share for ostrich products being produced in South Africa, with leather and meat respectively contributing 45% each (DAFF, 2016a).

Table 2.1 Experimental information on the end products produced by slaughter ostriches at different slaughter weights (Brand, 2016)

Slaughter weight (kg)	Meat			Leather			Feathers
	Carcass mass (kg)	Dressing percentage (%)	Total high-price meat cut yield per carcass (kg)	Skin surface (dm^2)	Follicle size (mm)	Average grade (1 - 4)	Feather yield (kg)
65	31	48	14.2	127	3.03	1.68	1.12
72	34	47	15.2	130	3.10	1.81	1.20
79	37	47	16.2	134	3.17	1.94	1.28
86	39	45	17.2	138	3.24	2.07	1.36
94	42	45	18.2	141	3.31	2.20	1.44
101	45	45	19.2	146	3.38	2.33	1.52
107	47	44	20.1	149	3.45	2.46	1.60
115	50	44	21.1	153	3.52	2.59	1.68
122	52	43	22.1	157	3.59	2.72	1.76

2.3.1 Feathers

Feathers have the lowest contribution to the market value of ostriches (DAFF, 2016a), however, it still remains a valuable end product in aiding the industry through tough times such as the current situation with the outbreak of AI, which is threatening the exportation of raw meat (Viviers, 2015). Not all feathers have the same commercial value; plumes (white feathers) which originate from the wings hold the highest value. The

value of feathers is also dependent on the quality, which is determined by various factors such as management, as described in detail by Smit (1964). Although fashion industries were the major users of ostrich feathers in the early developing stages of the ostrich industry, other applications for feathers such as feather dusters, artificial flowers and fans were also developed (Smit, 1964). This aids as a buffer to ensure a steady income with the fluctuating nature of the fashion industry. Sales (1999) reported on feather clipping practices being implemented in South Africa, which are currently still the production norm. At six months of age the feathers are clipped above the blood line of the shaft, the rest of the shaft is left in the wing to dry and removed two month later (Smit, 1964).This practice is done to enhance feather production and optimise the amount of feathers produced by ostriches.

Although limited, some studies have investigated the influence of dietary energy and protein concentrations on the yield and quality of ostrich feathers (Brand *et al*., 2004b; Brand *et al*., 2014; Viviers, 2015). Brand *et al*. (2004b) found that varying protein concentrations had no effect of saleable feather yields, however higher energy concentrations in the diets lead to higher saleable feather yields. The findings of Brand *et al*. (2014) are in accordance to the above mentioned study in regards with dietary energy concentrations affecting saleable feather yields, however the low energy and high energy diets resulted in the same yields with the medium energy diets resulting in the higher saleable feather yields. Viviers (2015) however, did find in a trial that birds receiving lower protein concentrations in their diet had lower tail feather yields. To date, limited literature is available on the influence of specific raw materials on ostrich feather production. Although the study of Brand *et al*. (2018) reported no differences between the varying dietary lupin inclusions within feather traits, which may be attributed to the diets being iso-nutritional.

2.3.2 Leather

Ostrich leather is a very sought after product in the fashion industry due to the unique quill patterns, suppleness and durability. Attention needs to be given to numerous factors when aiming to optimise yield and quality of ostrich leather, which includes nutrition and management (Cooper, 2001). The diamond shaped, nodulated area found on the back of an ostrich skin is called the crown area (Fig. 2.1), which has the highest commercial value (Engelbrecht *et al*., 2009) as the nodules gives the skin it uniqueness and is the reason for the high consumer demand (Meyer *et al*., 2004). When grading ostrich skins three parameters are evaluated, one of these is skin size which is objective and the other two being subjective are visible damage and the appearance of the feathers nodules (Engelbrecht *et al*., 2005). The guidelines for ostrich skin and leather grading is set out by the World Ostrich Association (WOA) (ANON, 2006).

Care should be taken when handling or transporting ostriches, for if it is done inadequately it may result in skin damage (Angel, 1996). This damage inflicted on the skins will lead to permanent scaring and ultimately the downgrading of the leather (Meyer *et al*., 2003). Hair follicles in excessive amounts on the skin also contribute to the downgrading of skins. These follicles form small pinholes in the leather as a result of bristle hairs being removed during the tanning process (Engelbrecht *et al*., 2005). All of the above-mentioned factors contribute to a skin's grade. Grade 1 skins are regarded as the best quality and grade 4 as having the lowest quality.

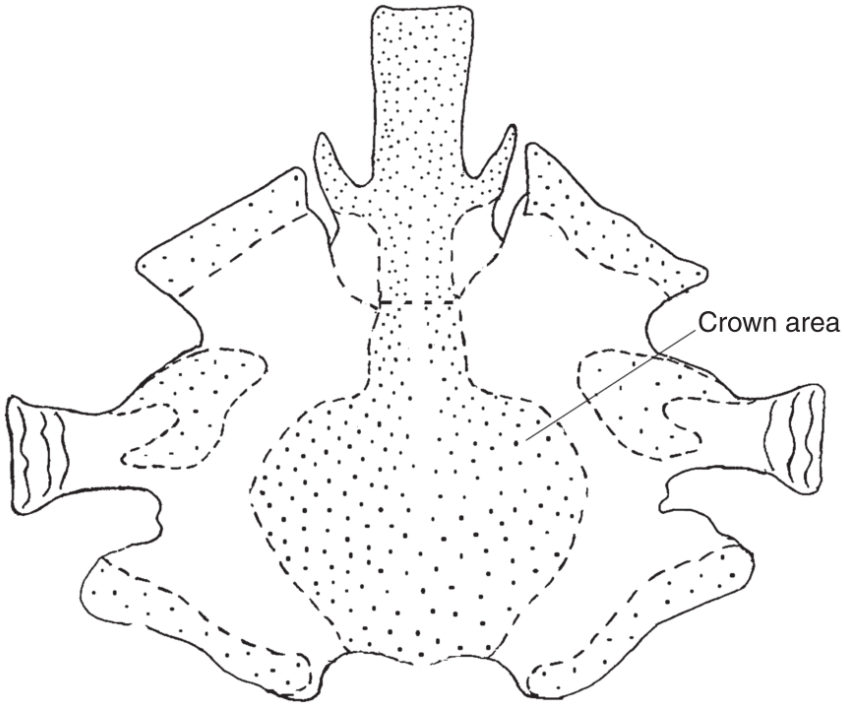

Figure 2.1 Sketch of an ostrich skin indicating the crown area which is the most valuable area on ostrich skins (Engelbrecht *et al*., 2009)

Studies on the effect of nutrition on leather yields and quality have been conducted in recent years. Brand *et al*. (2000c), found that an increase in dietary protein from 13 to 17% resulted in a decrease in the number of grade 1 skins. In their study, it was also found that an increase in dietary energy from 9 to 12 MJ/kg ME resulted in larger skin surface areas. Brand *et al*. (2004b) had similar findings as higher protein concentrations resulted in lower skin grading and higher energy concentrations resulted in lager skin sizes. Cloete *et al*. (2006) however, found that dietary protein concentrations from 13 to 17% had no effect on any leather traits, although they did find that the high energy diets (12 MJ/kg ME) resulted in thicker skins and larger skin sizes than the low energy diets (9 MJ/kg ME). Brand *et al*. (2014) on the other hand found in one

part of their study that the high energy diet resulted in smaller skin sizes than the low energy diet, it was also found that the high energy diet increased the number of nodules on the skins. However, in the second part of their study it was found that the high energy diet resulted in larger skin sizes and that the lower energy diets resulted in higher pinhole counts. Although studies have been done on the effect of nutrition on the leather quality of ostriches, more research needs to be done to better understand these effects.

2.3.1 Meat

Consumers are more aware of living healthier lifestyles and want to be better informed on the nutritional value of the products that they consume (Sales & Hayes, 1996; Horbańczuk & Sales, 1998), especially with regards to lipid consumption of animal origin (Hoffman *et al*., 2005). Ostrich meat is considered as a healthier red meat (Sales & Hayes, 1996), with a lower intramuscular fat concentration (Mellett, 1992), favourable fatty acids profile containing 16.5% polyunsaturated omega-3 fatty acids (Sales, 1998), high protein concentration and is rich in iron and vitamin E (Hoffman *et al*., 2005; Majewska *et al*., 2009; Dalle Zotte *et al*., 2013). Paleari *et al*. (1998) found that ostrich meat, compared to beef and turkey, has the lowest fat concentration, highest protein and lowest cholesterol concentration as well as the highest concentration of polyunsaturated fatty acids (PUFA). Table 2.2 shows the chemical composition and PUFA content of ostrich meat that has been reported by previous studies (Sales & Hayes, 1996; Paleari *et al*., 1998; Sales, 1998; Hoffman *et al*., 2005; Hoffman *et al*., 2012; Poławska *et al*., 2012; Horbańczuk *et al*., 2015) as well as a comparison of ostrich meat composition with that of beef, chicken and turkey. Studies have found that PUFAs in ostrich meat mainly consist of omega-3 fatty acids, which could aid in prevention of cardiovascular diseases and depression in humans and is essential for normal growth and reproduction (Connor, 2000; Simopoulos, 2002).

Table 2.2 Chemical composition (as is basis) and PUFA content (%) of ostrich meat obtained in different studies and in certain cases compared to chicken, turkey and beef (Mean ± SD)

Chemical composition (%)	Studies Conducted on different species									
	Sales & Hayes (1996)		Sales (1998)	Hoffman *et al.* (2005)	Paleari *et al.* (1998)			Poławska *et al.* (2012)	Horbańczuk *et al.* (2015)	Hoffman *et al.* (2012)
	Ostrich	Chicken	Ostrich	Ostrich	Ostrich	Turkey	Beef	Ostrich	Ostrich	Ostrich
Moisture	76.27	71.6	-	76.96	75.10	74.80	74.20	76.15	-	-
Protein[1]	21.12	20.94	-	21.65	22.20	20.40	20.10	21.56	-	-
Fat	0.65	6.33	-	1.95	1.60	3.80	4.50	1.18	-	-
Ash	1.07	1.03	-	1.20	1.10	1.00	1.20	1.11	-	-
PUFA[2]	-	-	31.71	32.99	50.80*	47.20*	48.70*	-	26.63	26.50

*Total unsaturated fatty acid concentration (%)
[1]Defated
[2]Polyunsaturated fatty acids

When examining Table 2.2 it is clear from the previous studies that ostrich meat has a higher protein and lower fat concentration than that of chicken, beef or turkey. Evaluating the findings of Paleari *et al*. (1998), it is noticeable that ostrich meat a have higher total unsaturated fatty acid content than beef or turkey.

Hoffman *et al*. (2005) found that dietary supplementation of fish oil in the diet of ostriches had no significant effect on the proximate chemical composition of the meat. Although, the dietary fish oil inclusions did have an effect on the fatty acid profile of the meat, resulting in a decrease of saturated fatty acids (SFA) and an increase in PUFA as the inclusion of fish oil increased. Raes *et al*. (2004) reported that the fatty acid profile of the diet fed to ruminants does not necessarily resemble the fatty acid profile that is reflected in the meat or fat tissues. In monogastric animals, this however is the case, as the feed they consume and the animal's tissue will have a similar fatty acid profile. This is because in ruminants the fatty acid profile of meat and fat are influenced by the microorganisms in the rumen and their ability to hydrolyse and hydrogenate unsaturated fatty acids. It was however found, that a more favourable n-6/n-3 fatty acid ratio can be achieved by supplying less n-6 fatty acids in ruminant diets, which would result in higher n-3 concentrations (Raes *et al*., 2004). Swart *et al*. (1993) found that the ostrich's unique ability to digest fibre in its hindgut is similar to the forestomach fermentation of ruminants, with fatty acids being utilised by the ostrich in a similar manner. Thus, it is reasonable to assume that the fatty acids within the meat and fat of the ostriches can be manipulated to some extent through nutrition.

2.4 Ostrich nutrition

With the ostrich industry experiencing a decline during 1997 and 1998, the focus of nutritional studies then shifted to cost efficient diet formulation and feeding, in order to lower the input cost of feed which is the largest expense (ca. 75%) of an intensive ostrich production unit (Brand *et al*., 2002; Jordaan *et al*., 2008). There are several factors other than feed cost that influence the profitability of an intensive ostrich production system. Most of these factors are uncontrollable by the producer, thus it is of key importance to optimize factors such as nutrition which can be controlled, to some degree (Smit, 1964; Cooper, 2001; Carstens, 2013).

The knowledge with regards to ostrich nutrition is still somewhat limited and in the infant stages when compared to that of other monogastric animals such as pigs and poultry (Gous & Brand, 2008). Numerus studies have been conducted on ostrich nutrition, with the pioneering work on determining true metabolisable energy (TME) for ostriches being done by Swart (1988), Swart *et al*. (1993), Cilliers *et al*. (1994) and Cilliers (1995). Since this pioneering work, much research has been conducted and is still being conducted on this topic (Ullrey & Allen, 1996; Cilliers *et al*., 1998; Brand *et al*., 2000c; Glatz *et al*., 2003; Gous & Brand, 2008; Carstens *et al*., 2014; Brand *et al*., 2014; Viviers, 2015; Engelbrecht, 2016b).

The unique ability of ostriches to digest fibrous plant material in their enlarged hindguts, results in better utilisation of lower quality feeds or feedstuffs with higher fibre concentrations (Swart *et al*., 1993b; Hoffman *et al*., 2005; Brand & Gous, 2006). This enables ostriches to sustain higher ME values from feed with high fibre concentrations compared to pigs, poultry and ruminants (Brand *et al*., 2000b).

2.4.1 Anatomy of the ostrich digestive system and feed digestion

Ostriches are monogastric herbivores with a large digestive tract, which enables them to digest fibrous plant material (Swart *et al*., 1993b; Brand & Gous, 2006). This ability distinguishes ostriches from other

monogastric animals such as poultry and pigs, with ostriches obtaining 20% higher metabolisable energy values for the same raw materials than that of pigs and up to 30% higher than that of poultry (Brand *et al*., 2000a; Brand *et al*., 2002). Another distinction from other avian species, is that the ostrich does not have a crop. This means that feed accumulation then happens in an enlarged upper oesophagus and the proventriculus. Other than this, the ostrich still being a bird, has a similar digestive tract anatomy to that of poultry, consisting of a beak, mouth, oesophagus, proventriculus (glandular stomach), gizzard (smooth muscle stomach) also known as ventriculus, small intestine, large intestine and cloaca (Swart *et al*., 1993a; Brand & Gous, 2006) as seen in Figure 2.2.

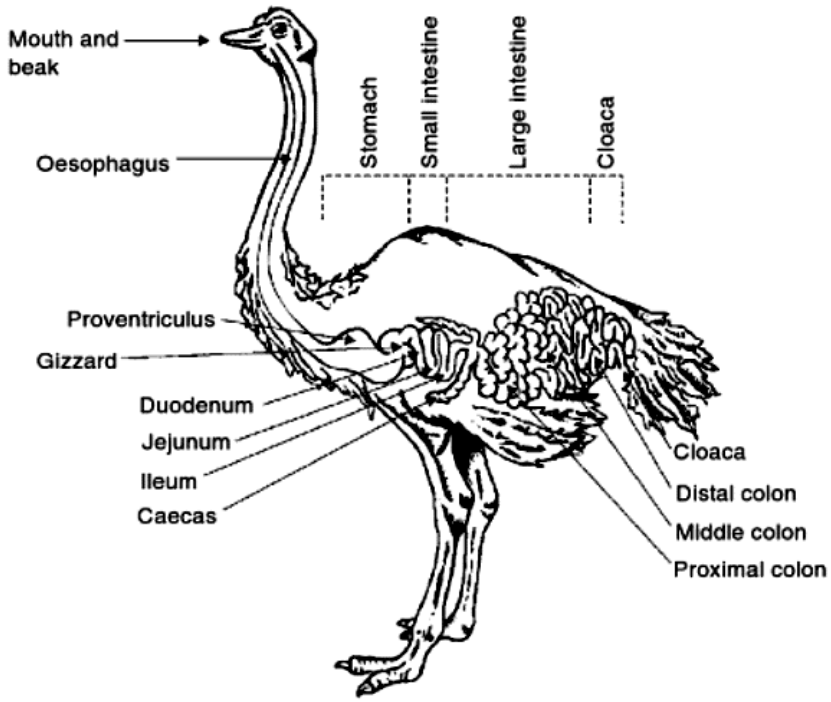

Figure 2.2 Digestive system of the Ostrich (Brand & Gous, 2006)

The digestion process is initiated as swallowed feed or plant material moves into the proventriculus where hydrochloric acid and enzymes are secreted, creating a strong acidic (pH 1.2 -2.1) environment that starts breaking down the digesta and exposes fibre for microbial fermentation, which occurs later on in the digestive system (Swart *et al*., 1993b; Brand & Gous, 2006). Ostriches have a habit of ingesting stones and pebbles (50 – 70% the size of their toenails) which aids with digestion in the gizzard. The gizzard has thick muscular walls and a horny like interior epithelium. As the digesta moves into the gizzard it contracts, and with the aid of the stones and pebbles, mechanically grinds the digesta to a finer state. Digestion in the gizzard is further enhanced by the low pH (ca. 2.2) environment (Brand & Gous, 2006; Brand & Olivier, 2011). Fermentation of digesta in the proventriculus and gizzard may occur to some degree, as Swart *et al*. (1993a) found volatile fatty acid (VFA) concentrations of 139.3 mM and 158.8 mM in these organs, respectively.

Although, acetate concentrations were high, it is unclear whether these VFAs are of microbial origin, as the pH in these organs is relatively low.

The small intestine can be divided into three parts, as is the case in most animals. Digesta moves from the gizzard into the duodenum and through the jejunum before reaching the ileum. While in the small intestine, the partially digested matter is further digested by digestive enzymes in an alkaline environment (pH 6.9 - 7.5) and nutrient absorption starts (Swart *et al*., 1993a; Brand & Gous, 2006). Digestive enzymes present in the small intestine include amylase, trypsin, arginase, maltase, sucrase, lipase, chymotrypsin and alkaline phosphates (Iji *et al*., 2003), fibre digesting enzymes such as cellulase is however lacking in the ostrich's digestive system and fibre needs to be broken down by fermentation (Cilliers & Angel, 1999).

The large and well-developed hindgut of the ostrich (Bezuidenhout, 1986; Swart *et al*., 1993a), together with a long retention time of digesta and a favourable pH of 7.3 – 8.2 in the colon, creates an ideal environment for microbial fermentation of fibrous material, making the ostrich very effective in digesting fibre (Swart *et al*., 1993a; b). The ostrich is definitely the most efficient hindgut fermenter when compared with other avian species (Cilliers & Angel, 1999), giving it the advantage to digest 66% of hemi-cellulose and 38% of cellulose in their diet, producing VFA which can contribute up to 76% of the ME requirements of the bird (Swart *et al*., 1993a; b). This fibre digesting ability of the ostrich however only develops after about 10 weeks of age (Angel, 1996), as the large intestine's size increases with age and the ratio of colon to small intestine increases from 1:1 at hatching to 1.5:1 at 3 months of age and 2:1 at 6 months of age (Bezuidenhout, 1986). What then started out as a typical bird neonate, develops into a hindgut fermenter at 70 - 80 days of age. These changes in the gastrointestinal tract indicate and emphasise the importance of feeding ostriches according to different feeding phases (pre-starter, starter, grower, finisher and maintenance) (Brand & Gous, 2006).

2.4.2 Nutritional requirements

As the ostrich grows, its body composition of protein:fat ratio changes (Brand & Olivier, 2011), together with physiological changes of the gastrointestinal tract (Bezuidenhout, 1986). These changes result in the ostrich having different nutritional requirements, depending on the growth phase that the birds are in. Therefore ostriches are fed in a certain growth phase based on the age of the birds. As the birds age, their live weight also increases, which will affect the level of dry matter intake (DMI) of the birds. These factors will also affect the growth rate of the birds as well as the energy requirements, as seen in Table 2.3 (Brand & Olivier, 2011). Cilliers & Angel (1999) stated that better knowledge regarding the nutritional requirement of ostriches could be gained when understanding the species' natural diet as well as the physical and functional properties of its gastrointestinal tract.

Ostrich diet formulation was initially based on the knowledge and standards of poultry energy and amino acid requirements, as there was limited available information regarding ostrich requirements at the time. This led to inaccurate formulation causing an over- or undersupply of metabolisable energy (ME) to ostriches, increasing costs or reducing animal performance, leading to lower profitability (Angel, 1996; Brand *et al*., 2014). This was seen to be the norm up until early to the mid-1990's. Mellett (1993) reported that poor feed conversion ratios (FCR) were observed amongst ostriches in South Africa, which may have been as a result of the type of feed or poor management that ostriches were subjected to.

Profitable ostrich production depends on scientific feeding of the birds. This can only be achieved with sound knowledge regarding the nutritional requirements of the birds during each feeding phase and precise evaluation and knowledge regarding the nutrient content of raw materials used in feed formulation (Cilliers & Angel, 1999). The information gained from studies by Swart (1988), Swart *et al*. (1993), Cilliers *et al*. (1994) and Cilliers (1995) on the nutrients that ostriches retain from raw materials, as well as the mathematical simulation models (effective tools in determining the exact nutritional requirement of ostriches in specific feeding phases) developed by Gous & Brand (2008), can be combined to formulate well balanced least cost diets.

Energy

As mentioned earlier, ostriches have the advantageous ability over most other monogastric animals to retain ME from fibre through fermentation in their hindguts (Swart *et al*., 1993a; b; Brand *et al*., 2000a). The use of poultry specifications for formulating ostrich diets lead to the under prediction of energy values in raw materials. This phenomenon caused obesity problems in intensive ostrich rearing systems, due to an oversupply of energy (Angel, 1996). A study by Swart *et al*. (1993c) determined that the energy requirement of ostriches was 0.44 MJ/kg W0.75 per day for maintenance, with a ME utilisation efficiency for growth of 0.32. Cilliers *et al*. (1994) and Cilliers (1995) determined the true metabolisable energy, corrected for nitrogen retention (TME_n) values of feedstuffs for ostriches, which are more accurate estimations of the energy values of feeds.

Dietary feed intake of ostriches is inversely related to the energy concentration of the feed, as the energy concentration in the feed increases the feed intake will decrease (Brand & Olivier, 2011). In Table 2.3 the prescribed TME values for ostrich feeds in each growth phase are depicted. As shown, as an ostrich ages, the TME concentrations of the diets are formulated at lower levels. Younger birds have higher energy requirements than that of older birds; as the younger birds need this energy to supplement high levels of protein that is required for rapid growth of bone and muscle. Younger birds also have a lower feed intake and thus require a more energy rich diet. Whereas older birds mainly require energy for maintenance.

Table 2.3 Predicted mean dry matter intake and energy requirement of ostriches in different growth phases (Brand & Gous, 2006; Brand & Olivier, 2011; Brand, 2016)

Growth phase	Age (months)	Live mass (kg)	TME[1] (MJ/kg feed)	Feed intake* (g/bird/day)	Predicted growth rate (g/bird/day)	FCR[2*] (kg feed/kg gain)
Pre-starter	0 - 2	0.85 - 10.0	14.5	275	150	1.80
Starter	2 - 4.5	10 - 40	13.5	1100	400	2.75
Grower	4.5 - 6.5	40 - 60	11.5	1650	330	5.00
Finisher	6.5 - 10.5	60 - 90	9.5	2500	250	10.00
Maintenance	10.5 - 12.0	90 - 120	8.5	3000	200	15.00

[1] True metabolisable energy
[2] Feed conversion ratio
* Based on feeding a pelleted ration

Protein and amino acids

It is important to feed ostriches a well-balanced diet, with special attention given to the energy:protein ratio and the amino acid profile of the feed (Brand *et al*., 2004c). Not only will animals have optimal performance on these well balanced diets, but the producer will also achieve higher profit margins. Brand *et al*. (2002) observed a tendency that diets were more optimally utilized by ostriches when it contains the correct amino acid profile. In this case a diet with lower crude protein content can be fed. Negative financial consequences as well as health problems in animals could occur due to oversupply of protein (>28% of total diet) to ostriches. Thus when protein and amino acid requirements of ostriches are determined, the following factors need to be considered; age, growth phase, growth rate, feed intake and live mass (Brand & Olivier, 2011; Carstens *et al*., 2014) to prevent over or under-supply of protein and amino acids.

Proteins and amino acids are essential components for normal bodily functions such as tissue synthesis and hormone and enzyme production. Table 2.4 summarises the protein and amino acid requirements of ostriches in different growth phases, found in several studies.

Vitamins and minerals

Currently vitamin and mineral requirements of ostriches are based on values for other species due to a lack of information on the specific requirements of ostriches, however commercial vitamin and mineral premixes are available (Brand & Olivier, 2011). Table 2.5 provides commercial guidelines for the nutritional composition of ostrich feeds during each growth phase, which includes the prescribed calcium and phosphate inclusions of the diets.

Production norms

The feed and nutrients that are provided to ostriches will influence the production rate of the birds (Brand & Gous, 2006). When using high quality feedstuffs, providing the feed in the correct form (Table 2.6) and implementing the guidelines for ostrich feed composition (Table 2.5), it is reasonable to assume that the birds will perform in accordance with the production norms provided in Table 2.3.

Table 2.4 Protein and amino acid requirements of ostriches at specific live mass and age intervals and mean dry matter intake in each growth phase. Adapted from Du Preeze (1991), Du Preeze *et al*. (1992), Cilliers *et al*. (1998) and Brand & Olivier (2011)

Predicted parameter	Growth phases				
	Pre-starter	Starter	Grower	Finisher	Maintenance
Live mass (kg)	0.85 - 10	10 - 40	40 - 60	60 - 90	90 - 120
Age (months)	0 - 2	2 - 5	5 - 7	7 - 10	10 - 20
Feed intake (g/day)	275	875	1603	1915	2440
Crude protein (g/100 g feed)	22.89	19.72	14.71	12.15	6.92
Lysine (g/100 g feed)	1.10	1.02	0.84	0.79	0.58
Methionine (g/100 g feed)	0.33	0.33	0.29	0.28	0.24
Cystine (g/100 g feed)	0.23	0.22	0.18	0.17	0.14
Total SAA (g/100 g feed)	0.56	0.55	0.47	0.45	0.38
Threonine (g/100 g feed)	0.63	0.59	0.49	0.47	0.36
Arginine (g/100 g feed)	0.97	0.93	0.80	0.78	0.63
Leucine (g/100 g feed)	1.38	1.24	0.99	0.88	0.59
Isoleucine (g/100 g feed)	0.70	0.65	0.54	0.51	0.38
Valine (g/100 g feed)	0.74	0.69	0.57	0.53	0.36
Histidine (g/100 g feed)	0.40	0.43	0.40	0.40	0.37
Phenylalanine (g/100 g feed)	0.85	0.79	0.65	0.61	0.45
Tyrosine (g/100 g feed)	0.45	0.44	0.38	0.38	0.31
Phenylalanine and tyrosine (g/100 g feed)	1.30	1.23	1.03	0.99	0.76

Table 2.5 Commercial guidelines for the nutritional composition of ostrich feeds in each growth phase, on an air dry basis (Brand & Olivier, 2011; Brand, 2016)

Growth phase	Nutrients								
	Prescribed energy (MJ TME/kg feed)	Min. Crude protein (g/kg)	Min. Lysine (g/kg)	Max. Moisture (g/kg)	Min. Crude fat (g/kg)	Max. Roughage (g/kg)	Calcium		Min. Phosphate (g/kg)
							Min. (g/kg)	Max. (g/kg)	
Pre-starter	14.5	190	10	120	25	100	12	15	6
Starter	13.5	170	9	120	25	100	12	15	6
Grower	11.5	150	7.5	120	25	175	10	16	5
Finisher	9.5	120	5.5	120	25	225	9	18	5
Maintenance	8.5	100	3	120	20	300	8	18	5

Table 2.6 Recommended form in which feed should be provided to ostriches in different growth phases (Brand, 2016)

Growth phase	Feed processing (sieve gauge)
Pre-starter	Meal
Starter	Crumbs
Grower	Pellets (6 - 8 mm)
Finisher	Pellets (6 - 8 mm)
Maintenance	Pellets (6 - 8 mm)

2.4.3 Feedstuffs used in ostrich ration formulations

Slaughter ostriches are predominantly reared in intensive systems, receiving a fully balanced total mixed ration (TMR). In some cases ostriches are reared semi-intensively, mostly on cultivated lucerne pastures, receiving feed concentrate as a supplement (Brand & Gous, 2006). There are some important feedstuffs that are used when ostrich feeds are formulated and mixed, providing the birds with the correct nutrients to meet there demands and optimise their production. Table 2.7 lists some of the most important feedstuffs that are typically used in ostrich feeds.

Table 2.7 The most important feedstuffs used in ostrich ration formulation (Brand, 2016)

Energy sources	Roughage	Protein sources	Mineral sources	Other
Maize	Lucerne hay	Soybean oilcake	Feed lime	Synthetic lysine
Barley	Wheat bran	Canola oilcake	Di-calcium phosphate	Synthetic methionine
Wheat	Oat bran	Sunflower oilcake	Mono-calcium phosphate	Plant oil
Triticale	Barley hay	Fish meal	Salt	Molasses products
Oats	Oat hay	Full-fat soy (roasted)	Mineral and vitamin premix	Binding agents
Brewer's grain	Oat straw	Full-fat canola		Medicines (e.g. antibiotics)
	Wheat straw	Sunflower seeds		Additives (e.g. growth promoters, pre- and probiotics etc.)
	Silage	Sweet lupins		
		Peas		
		Beans		
		Gluten		

2.5 Protein source production and utilisation is South Africa

With global population growth, protein is becoming scarcer, more expensive and in particular, less available to be used in animal feeds; which necessitates the research in finding alternative protein sources (Brand *et al*., 2000a; Brand *et al*., 2004a; Sridhar & Bhat, 2007).

Brand & Jordaan (2004) noted that by incorporating locally produced feedstuffs in well formulated least cost diets, the feeding cost of intensive ostrich production units could be reduced. Typically, protein sources make up to 22.8% of the composition of an ostrich diet (Brand & Gous, 2006). Bearing this in mind, along with the fact that available protein is becoming scarcer and more expensive (Brand *et al*., 2000a; Brand *et al*.,2004a); it is thus important to explore alternative locally produced protein sources to incorporate in ostrich diets in order to reduce feed costs.

When providing feed to animals, the aim is to provide a well-balanced diet containing high quality energy and protein sources that are essential for growth and production. Oilcake meals are the main sources of protein being used in producing animal feeds, with soybean oilcake meal being the predominant source of protein in diets. Other oilcakes that are used in animal feeds, include sunflower, peanut, cottonseed and canola oilcake. Though canola and peanut oilcake are used to a lesser extent and cottonseed oilcake used in very limited amounts (DAFF, 2016a).

In 2011/2012 a national strategy aiding in the development of the local soybean industry in South Africa was implemented by the Department of Trade and Industry (DTI) and the International Trade Administration Commission of South Africa (ITAC) (DAFF, 2016a). This strategy aims to increase local soybean production and to increase the crushing capacity of the industry, enabling a higher production of soybean oilcake meal that can be used in the animal feed industry. Since the implementation of the local soybean strategy, the local soybean oilcake meal availability has increased from 444,720 tons in 2012/2013 (AFMA, 2014) to 671,201 tons in 2016/2017 (AFMA, 2017). The importation of soybean oilcake meal was also positively influenced, showing a decrease from 610,022 tons in 2012/2013 (AFMA, 2014) to 487,919 tons in 2014/2015 (AFMA, 2015). However, due to the drought experienced in South Africa, the import of soybean oilcake meal has increased to 595,197 during 2016/2017 (AFMA, 2017). Although soybean oilcake meal availability in South Africa (imported and locally produced) for 2016/2017 is estimated at 1,467,093 tons, and far exceeds the usage of soybean oilcake meal by the animal feed manufactures association (AFMA) members, which is 953,750 tons (AFMA, 2017), the commodity remains expensive.

Most of the locally produced protein sources are cultivated in the northern parts of South Africa and need to be transported to the Western Cape Province for inclusion in ostrich diets. This combined with the high costs of importation associated with procurement of the remaining protein sources, results in protein sources being more expensive (Brandt, 1998). Therefore, researchers in the Western Cape are driven to lower feed costs by evaluating alternative locally produced protein sources that can be used in animal diets (Brandt, 1998; Brand *et al*., 2002). Canola is a crop that is locally produced in the Western Cape province of South Africa and has promising potential to be utilised as a protein source in animal rations, replacing soybean oilcake meal (Smith, 2005; Brand *et al*., 2007).

2.6 Full-fat canola seed as a locally produced alternative plant protein sources in slaughter ostrich diets

In the 1990's canola (*Brassica napus*) was introduced into South Africa as an alternative oilseed crop. Although canola is mainly cultivated for the extraction of edible oil, the crop has the potential to be used in animal feeds (Brand *et al*., 1999) as it has long been used in other animal feeds such as poultry (Ajuyah *et al*., 1991; Lee *et al*., 1991), pigs (Busboom *et al*., 1991) and ruminants (Mir *et al*., 1984). However, no information is available regarding the suitability of canola in ostrich diets. Full-fat canola is one of the important alternative protein sources that can successfully be cultivated in the Western Cape Province of South Africa (Brandt, 1998), with this province producing 99.8% of the country's canola (DAFF, 2016b).

In the year 1992, only 400 hectares of canola was grown in the country, producing ca. 500 tons of seed. Over the years canola production has fluctuated, as a result of low seasonal yields as well as canola also being cultivated in rotational crop systems. However, the overall production of canola is steadily increasing (De Kock & Agenbag, 2009). The year 2015 saw canola being cultivated on 78,000 hectares, producing 98,000 tons of seed (DAFF, 2016b). Since 2011, canola production in South Africa has met the local canola demand, with an average of 62,000 tons of the 65,000 tons produced being processed to produce oil (DAFF, 2016b). With the demand for processing being met through local production, the potential for using canola in animal feeds has increased.

In the years 2016/2017, 126,386 tons of canola (locally produced and imported) was available for marketing, with 120,000 tons being crushed for oil extraction. Of the available 6,386 tons of full-fat canola seed not being crushed, only 713 tons was used as feedstuff in animal feeds by AFMA members (AFMA, 2017). This indicates that there is still a lot of room for improvement when it comes to the utilisation of full-fat canola in animal feeds. Brandt (1998) emphasised the importance of exploiting the nutritional potential of all protein sources that can be produced in South Africa and used in animal feeds. Canola however, has not reached its full capacity for use in animal feeds as of yet.

The use of rapeseed in animal feed has been limited, due to high levels of glucosinolates and erucic acid contained within the plant and seed (Bell, 1993). However, the development of double zero canola cultivars (a rapeseed cultivar containing lower levels of these anti-nutrients) made it possible to include higher levels into animal rations (Dale, 1996).

2.6.1 Double zero canola cultivar development

In the 1950's the issues regarding erucic acid and glucosinolates contained within rapeseed were fully realised and breeding laboratories developed more sophisticated analytical capabilities in order to work towards zero erucic acid varieties. In 1970, myocardial lesions were observed in laboratory animals that consumed erucic acid, which renewed the concern over erucic acid levels contained within rapeseed. From the late 1960's to the mid-1970's, breakthroughs in developing rapeseed varieties with very low glucosinolate concentrations were made, which was the start of the "double low" (double zero) rapeseed varieties era, now commonly known as canola (Bell, 1982). Rapeseed varieties can now only be called canola, if the erucic acid concentration is below 2% of the total fatty acid content of the seed, and if glucosinolate concentrations are lower than 30 µmol/g of defatted meal (oilcake meal) (DeBonte *et al*., 2001).

2.6.2 Chemical and nutritional composition of full-fat canola

Nwokolo & Sim (1988) investigated the use of full-fat canola seed (FFCS) as an alternative energy and protein source in laying hen diets, as FFCS contains 42% fat and 21% protein. They concluded that the FFCS and barley mixtures evaluated in their study were excellent sources of nutrients to laying hens.

Full-fat canola seed can not only replace soybean oilcake meal as a protein source, but can contribute as an energy source as well, considering that it has a much higher energy value than soybean oilcake meal (Table 2.8) which is even higher than that of maize (15.22 MJ/kg) (Cilliers *et al*., 1997). This is due to the high fat content as well as the high fibre content of FFCS (Brand *et al*., 2000a). The high fibre concentration is an advantage with regards to ostrich nutrition, as it was previously mentioned that ostriches have the ability to digest fibre, to some degree (Swart *et al*., 1993a; b).

The crude protein concentration of FFCS is considerably lower than that of soybean oilcake meal, and when comparing the amino acid concentrations of FFCS and soybean oilcake meal (Table 2.8), it is clear that FFCS is somewhat lacking with regards to essential amino acid concentrations. This shortcoming can however be overcome by including higher levels of FFCS in the diet, compared to that of soybean oilcake meal, or by supplementing the diet with synthetic amino acids. Although FFCS has these shortfalls regarding protein and amino acids, the availability of these amino acids are high. Lee *et al*. (1995) reported that the true amino acid availability (TAAA) of FFCS for poultry is between 81.6 and 90.5%. These values are somewhat lower but still in line with the study of Muztar *et al*. (1979), where it was found that the TAAA of Tower and Candle rapeseed was between 86 and 96%, which is close to that of soybean oilcake meal (90 to 97%). When considering the individual essential amino acid availability, it is clear that the two rapeseed cultivars showed similar availabilities to that of soybean oilcake meal (Muztar *et al*., 1979).

Calcium and phosphorous concentrations of FFCS are very close to that of soybean oilcake meal (Table 2.8). Other minerals that may be lacking can also be supplemented by the inclusion of well-balanced mineral and vitamin premix packs.

Nwokolo & Sim (1988) postulated that canola is an important source of omega-3 fatty acids, as higher concentrations of linolenic and docosahexaenoic acid were found in in the yolk of eggs produced by hens that received diets containing FFCS. It was also found that linoleic acid (omega-6 fatty acid) in the egg yolk increased as FFCS inclusion increased in the diet. In accordance with the findings above, Lee *et al*. (1995) noted that oil seeds such as canola are rich in α-linolenic acid and that these seeds can therefore be used as sources of omega-3 fatty acids in animal diets.

Overall it seems that FFCS is a suitable alterative protein source that can be incorporated in well-balanced ostrich diets. Although, strategic supplementation of essential amino acids as well as trace minerals may be necessary to facilitate any shortcomings.

Table 2.8 Chemical and nutritional composition of full-fat canola seed and soybean oilcake meal on an as fed basis adapted from Muztar *et al*. (1979), Brand *et al*. (2000a) and Centraal Veevoerderbureau (2004)

Chemical and Nutrient components	Full-fat canola seed	Soybean oilcake meal
True metabolisable energy (MJ/kg)	22.5	15.13
Dry matter (g/kg)	923.0	878.0
Crude protein (g/kg)	198.0	431.0
Ash (g/kg)	39.0	62.0
Ether extract (g/kg)	415.0	15.0
Crude Fibre (g/kg)	99.0	66.0
Neutral detergent fibre (g/kg)	203.0	140.0
Acid detergent fibre (g/kg)	154.0	87.0
Minerals		
Calcium (g/kg)	4.2	3.0
Phosphorous (g/kg)	6.7	6.5
Magnesium (g/kg)	2.4	3.3
Copper (mg/kg)	3.0	18.0
Manganese (mg/kg)	35.0	50.0
Zinc (mg/kg)	40.0	46.0
Iron (mg/kg)	82.0	373.0
Amino acids (g/kg)		
Lysine	10.9	26.7
Methionine	4.0	6.0
Cysteine	4.9	6.5
Arginine	12.0	31.9
Threonine	8.7	16.8
Tryptophan	2.6	5.6
Tyrosine	6.1	16.0
Histidine	5.5	11.6
Valine	10.1	20.7
Phenylalanine	8.1	22.0
Isoleucine	7.7	19.8
Leucine	13.8	33.2
Fatty acids (%)		
C12:0	0.2	0.0
C14:0	0.2	0.2
C16:0	5.0	11.0
C16:1	0.4	0.2
C18:0	2.0	4.0
C18:1	56.0	22.0
C18:2	22.0	54.0
C18:3	9.0	8.0

2.6.3 Anti-nutritional factors in full-fat canola

Although canola is a rapeseed cultivar with very low levels of anti-nutrients such as glucosinolates (GLS) and erucic acid (Bell, 1982), when included at excessively high levels, these can affect animal performance, as found by various studies. This was attributed to the anti-nutrients and the consequence of palatability of canola (Summers *et al*., 1988; Shaw *et al*., 1990). There are factors, other than glucosinolates and erucic acid, which affect the nutritional value of canola. These factors include sinapine, phytic acid, tannins and high dietary fibre (Khajali & Slominski, 2012), with the last mentioned being less of a concern with regards to ostrich nutrition.

Glucosinolates (GLS) are large sulphur-containing secondary plant metabolites that can be found in all major varieties of Brassica plants. Due to alteration of the side-chain structure, a large amount (120) of different glucosinolates exists (Chen & Andreasson, 2001). Some of the more important glucosinolates found in canola meal include progoitrin, gluconapin, gluconapoleiferin, glucobrassicin, glucobrassicanapin and 4-hydroxyglucobrassicin (Khajali & Slominski, 2012). These GLS molecules are not toxic as is, however, when the canola seeds are in the presence of moisture and undergo rupturing, the enzyme myrosinase, which is also be found in the seed, starts to hydrolyse the GLS to produces unstable aglucones. These aglucones are then broken down into products such as, nitriles, goitrin, thiocyanates and isothiocyanates. These substances affect the function of thyroid glands causing thyroid and liver enlargement, haemorrhagic liver syndrome (laying hens) which depresses growth performance, decreases egg production and ultimately can lead to mortalities (Ibrahim & Hill, 1980; Fenwick, 1982; McCurdy, 1990; Tripathi & Mishra, 2007) The toxic breakdown products of aglucones not only have a direct physiological effect on the animals, but can also decrease feed consumption. This is due to the unpleasant taints and odours of these substances, that result in a bitter taste and indirectly decreases growth performance (Ibrahim & Hill, 1980; Khajali & Slominski, 2012). McNeill *et al*. (2004) fed double zero rapeseed meal to broiler chickens and found that the birds on the higher inclusion diet (200g/kg feed) had slower weight gain and lower FCR from 0-21 days of age than the birds receiving the lower rapeseed meal inclusion (100g/kg feed). This reduction in performance was attributed to the higher levels of GLS in the higher rapeseed inclusion diet.

During crushing and pelleting (steam) of canola seed, heat is applied, which inactivates myrosinase. The breakdown of GLS however still continues to some extent, as a result of thermal decomposition which produces breakdown products similar to that of enzymatic breakdown by myrosinase (Campbell & Slominski, 1990). Studies by Slominski *et al*. (1987) and Campbell & Slominski (1989) found that GLS can also be broken down in the lower intestine of poultry through microbial degradation with the hydrolytic activity that mainly takes place in the cecae.

Concentrations of GLS in canola, though, have been declining at a steady pace. In Canada the GLS concentrations in canola have been reported to be ca. 10 µmol/g (Newkirk, 2009), with Brand *et al*. (2007) reporting on average, the GLS concentrations in South African canola cultivars is found to be 17.83 µmol/g. Leeson *et al*. (1987) found that broilers have a GLS tolerance at levels of 11.6 µmol/g of feed, although Tripathi & Mishra (2007) reported that GLS concentrations above 8.0 µmol/g of feed will result in depression of growth. Mawson *et al*. (1994) reported that minimal growth depression in broilers will result from GLS concentrations of 4.0 µmol/g of feed, however, when these concentrations were increased to between 6.0 - 10.0 µmol/g of

feed, a degree of growth depression was observed, with severe growth depression resulting when concentrations were elevated to over 10.0 µmol/g of feed.

It is clear that caution should be taken when formulating canola into animal feeds to ensure that GLS levels within the feed are not too high, preventing negative effects on production. However, with the double zero cultivars this is of less a concern.

Erucic acid is a fatty acid contained in the oil of canola/rapeseed seeds (Bell & Keith, 1982; Bell & Shires, 1982; Guil *et al*., 1997). It is considered to be an anti-nutrient and can be toxic when excessive amounts are ingested, this may result in growth depression, increased mortality, myocarditis and fat accumulation in the heart (Guil *et al*., 1997; Dingyuan & Jianjun, 2007; Kramer, 2012). Although erucic acid is an anti-nutrient with detrimental effects on animal health and performance, the development of double zero canola cultivars renders erucic acid less of a concern (Breytenbach, 2005), as these cultivars contains very low levels of erucic acid (Bell, 1993; DeBonte *et al*., 2001).

Sinapine is a choline ester of sinapic acid (Butler *et al*., 1982), which is mainly found in the seed embryo (Bell & Shires, 1982) and normally makes up 1 – 4% of air-dried oil-free canola meal (Blair & Reichert, 1984). Sinapine is associated with a fishy taint found in certain brown-shelled eggs (Butler *et al*., 1982), due to a genetic defect in laying hens of Rhode Island Red breeding. These hens cannot convert trimethylamine which causes the fishy taint in the yolk to odourless N-oxidase using liver or kidney trimethylamine-oxidase (Khajali & Slominski, 2012). Sinapine also has a bitter taste (Blair & Reichert, 1984) that may affect feed consumption, although it is rarely found to be detrimental in pig diets (Bell, 1984).

Phytic acid is the primary form in which phosphorus, and possibly inositol, is stored in most grains and seeds. The precise function of phytic acid in animal nutrition is not yet fully understood (Khajali & Slominski, 2012). However, it is known that phytic acid forms insoluble complexes with a number of minerals (Ca, Fe, Zn, Mn, Mg) and proteins, making them biologically unavailable to the animal and thus it is considered an anti-nutrient (Cabahug *et al*., 1999). Phytate (phytic acid in salt form) can also alter sodium (Na) separation and subsequently affect the ability of the gastrointestinal tract to carry nutrients with Na-dependent transport, which includes glucose and peptides (Cowieson *et al*., 2009). Phytic acid can however be broken down to yield inositol and inorganic phosphorus by an enzyme called phytase, which results in improved utilization of phosphorus and growth performance of monogastric animals (Khajali & Slominski, 2012). Józefiak *et al*. (2010) found that the growth performance and insulin liver receptor sensitivity in broilers was improved by the addition of phytase and carbohydrase to the wheat-based feed containing full-fat canola.

Tannins are complex polyphenolic compounds that can be divided into hydrolysable and condensed fractions (Yapar & Clandinin, 1972), with condensed tannins being present in rapeseed hulls (Khajali & Slominski, 2012). These condensed tannins are mainly found in rapeseed with brown hulls (Durkee, 1971) and can amount to 1.9 – 6.2 g/100 g of oil-free canola/rapeseed hulls (Naczk *et al*., 2000). The majority of tannins in canola and rapeseed hulls are insoluble and make up 70 – 96% of the total tannins found in the hulls. Tannins give an unattractive, dark colour to meals and milled seed and can create insoluble complexes with proteolytic enzymes, proteins and fibre in the gastrointestinal tract, influencing protein digestion (Naczk *et al*., 2000; Khajali & Slominski, 2012). Water-soluble tannins (tannic acid) may be partially responsible for poor growth performance in broilers. However, the anti-nutritional effect of tannins in canola is of less a concern

with regards to inclusion of canola into animal feeds, this is due to the fact that the tannins in canola are located in the cell walls and are mostly water-insoluble (Khajali & Slominski, 2012).

2.6.4 The use of full-fat canola in animal feeds

Pigs

Full-fat canola has been incorporated in to pig rations for many years. Castell & Falk (1980) included FFCS in pig diets at levels of 3 to 15% and found no differences regarding growth performance between all the canola inclusion diets or the control diet (0% canola, containing soybean oilcake meal). However, they concluded that because FFCS is rich in linolenic acid, it might lead to a faster rate of autoxidation in the feed or meat products. The increase in unsaturated fatty acids (contained in FFCS) also results in softer fat on the carcasses, which may have negative effects on the grading of the carcasses. When FFCS incrementally replaced full-fat soybeans at inclusions of 8, 16 and 24% of the total diet in weaner and grower-finisher pigs rations, Brand *et al*. (1999) found that there were no differences between 0% inclusion of FFCS and any of the FFCS included diets. They did however, find that the grower-finisher pigs showed a decrease in dry matter (DM) digestibility as the FFCS inclusion increased and it was recommended to include 16% FFCS into the diets of grower-finisher pigs for maximum efficiency. Busboom *et al*. (1991) fed a control diet with no FFCS, a diet containing 20% intact FFCS and a diet containing 20% ground FFCS to finisher pigs and found that there were no differences between diets regarding ADG and FCR, however, the 20% ground FFCS diet resulted in lower feed consumption. They speculated that the reduced feed consumption might have been due to the unpleasant taste of ground canola seed. Busboom *et al*. (1991) concluded that the higher dietary fat concentration resulting from the inclusion of FFCS did not have any negative effects on carcass characteristics and at the inclusion rates fed to the pigs in their study would not result in rancid pork.

Poultry

Full-fat canola seed has been used to great extent in poultry diets. Talebali & Farzinpour (2005) found that inclusion of FFCS of up to 12% in broiler diets had no detrimental effect on end body weight. Although, FFCS inclusion of 9 - 12% did result in higher feed consumption, resulting in higher FCR's of the birds fed the 12% inclusion diet. However, the FCR's between the 3 and 9% inclusion diets did not differ from one another. Lee *et al*. (1991) indicated that there were no differences between a control diet containing soybean oilcake meal and 10 or 20% full-fat canola containing diets with regards to end body weight, FCR, carcass yield or mortality rate of broilers.

Józefiak *et al*. (2010) reported on the importance of complete seed rupturing of full-fat oilseeds when fed to poultry, as nutrient-encapsulation by cell walls occur due to incomplete seed rupturing, reducing the availability of nutrients such as phosphorous, energy (lipids) and protein. The nutritional value of oilseeds can be enhanced by incorporating cell wall-degrading enzymes into the feed (Józefiak *et al*., 2010). Meng *et al*. (2006) found that the FCR, dry matter and non-starch polysaccharides digestibility and apparent metabolisable energy content can be improved by using cell wall-degrading enzymes in poultry diets.

Ruminants

In a study by Rule *et al*. (1994) to evaluate growth performance and fatty acid composition of bovine tissue when fed canola, soybean and products of the two seeds, bulls and steers were presented with feed containing 17.6 and 15.3% ground full-fat canola, respectively. Dry matter intake, ADG and FCR of both the

bulls and steers did not differ between diets containing ground canola and diets containing soybean meal (7.7% of the total diet). The conclusion with regards to fatty acid composition, was that dietary full-fat canola transformed the fatty acid composition of the muscle, liver, kidney and adipose tissue, by decreasing 16:0 and 16:1 fatty acids and increasing 18:0, 18:1, 18:2, and 20:1 fatty acids. When canola seed (8% of dietary DM) was used to supplement low-quality hay, either as ground seed or whole seeds, no differences were found between the two canola supplemented diets or the control (only hay) with regards to DMI (Leupp *et al*., 2006). The supplementation of canola (whole or ground) in the diets resulted in higher apparent and true ruminal crude protein digestibility and the processing of canola resulted in higher *in situ* disappearance rate of DM and fibre, therefore it was concluded that the processing of canola seed increases the degradation of the seeds, which is beneficial.

Beauchemin *et al*. (2009) determined whether enteric methane production can be reduced by adding a variety of long-chain fatty acid sources to the diets of lactating dairy cows. Compared to sunflower and flax seed, crushed full-fat canola seed (9.3% inclusion) had the best potential in reducing methane production without having negative effects on feed digestibility and milk production. Stanton *et al*. (1997) investigated whether conjugated linoleic acid (CLA) levels in bovine milk can be manipulated through dietary inclusion of rapeseed. They found that when full-fat rapeseed levels in dairy cow rations were increased from 825 g/cow/day to 1650 g/cow/day, cis-9,trans-11 octadecadienoic acid (CLA) levels in the milk will increase from 5.23 to 7.89 mg/g of milk fat.

Petit *et al*. (1997) evaluated the performance of growing Outaouais lambs receiving a grass silage/barley based diet supplemented with either raw canola and soybean or extruded canola and soybean. The results of their study revealed that there were no differences between raw canola and raw soybean supplemented diets concerning DMI, ADG, FCR and end weight. The findings were similar with regards to extruded canola and soybean supplementation, with no differences observed between these two diets. However, differences were observed between raw canola and soybean supplemented diets, with the raw soybean supplemented diets resulting in a higher dressing percentage than the raw canola supplemented diets. No differences for dressing percentage were observed between the extruded canola and soybean diets. Dry matter digestibility and total PUFA concentrations also showed no differences between any of the diets. In the study by Petit *et al*. (1997), it was concluded that extrusion of oilseeds increased the ADG of lambs and decreased the ruminal N degradability of the feed. In another study by Brand *et al*. (2001), South African Mutton Merino finishing lambs were fed diets in which full-fat canola incrementally (0, 6, 12 and 18% inclusion of total feed) replaced fishmeal and barley as a protein and energy source. Results showed no differences with regards to DMI between the diets, however, it was found that the 0% canola inclusion diets resulted in slower growth rates (14% slower than the 6% canola inclusion diet) and lower FCR's (21% lower than the 6, 12 and 18% canola inclusion diets). Even though these differences were observed regarding growth performance, no differences were observed between diets concerning dressing percentage. Brand *et al*. (2001) concluded that FFCS is a very good energy and protein source that can be utilized in finishing lamb diets with great success.

2.7 Conclusion and objectives

Feed costs are considered as the highest input costs of an intensive ostrich production unit. Protein and energy sources making up the largest portion of any animal ration, have the highest contribution to the cost of

the feed. With the increasing global population, the demand for food is growing, placing more pressure on agriculture to meet this demand. With this growing population, protein sources for use in animal feeds are becoming scarce and more expensive due to the competition between direct human consumption and the incorporation of protein sources into animal feeds. The ostrich industry is ever growing, but it has a very cyclic nature that is currently evident and emphasised by the outbreak of AI, which dampens growth of the industry. In order to aid the ostrich industry in reducing feed cost, alternative locally produced protein sources need to be evaluated. These alternative protein sources can enable home feed mixers to grow the source on the farm and incorporate it into diets without further processing. This will also reduce importation and transport costs, which will contribute to lower feeding costs. Full-fat canola seed has the potential to be utilised as a protein source in ostrich diets, replacing soybean oilcake meal to a certain extent. The research done to compile this review will provide insight and aid the intended research to evaluate the use of full-fat canola seed in ostrich diets and the effects it may have on production and slaughter traits.

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Chapter 3

Feeding preferences of grower ostriches towards diets containing increasing levels of full fat canola seed

Abstract

The largest expense of an intensive ostrich production unit is feed cost (*ca.*75%). Protein makes up a great portion of any animal feed and this expense can be lowered by utilizing locally produced feedstuffs such as canola, although it is not clear whether ostriches will readily consume canola due to its anti-nutritional or other known factors. To evaluate the feeding preference of ostriches towards canola, 60 South African Black ostriches (82.2 ± 1.06 kg in live weight) were placed in ten camps of six birds per camp. Each camp had five identical feed troughs each containing diets where full-fat canola seed (FFCS) incrementally (0, 25, 50%, 75 and 100% of protein source) replaced the soybean oilcake meal (9.8% of the total diet composition in control diet) as protein source. Feed and water were made available *ad libitum*. Dry matter intake (DMI) was measured on a daily basis and feed colour characteristics were measured based on L*, a* and b* colour attributes. Only the 25%FFCS (25% soybean oilcake meal replacement diet) showed a higher DMI (817.4 ± 81.98 g/bird/day) than the other diets (average of 488.8 ± 81.98 g/bird/day). Although there were slight differences between some colour attributes, it is believed to have had no effect on DMI. Based on the results of this study, FFCS can be used to replace 25% soybean oilcake meal without any negative effect on DMI; resulting in an inclusion level of 6.8% FFCS in ostrich diets.

3.1 Introduction

Ostrich farming is a relatively new practice compared to cattle, sheep and other livestock. Smit (1964) noted that ostrich farming only started between 1857 and 1864, considering that in England and Wales in the year 1086, farmers were already rearing livestock with numbers of over 100 cattle and 9000 sheep on one farm alone (Thirsk, 1989). Owing to the relative young age of the ostrich industry there is still room for improvement as pertaining to production practices.

Ostriches are multi-purpose animals producing feathers, skins (leather) and meat. Initially ostriches were mainly reared for their feathers, which were incorporated in the fashion industry (Osterhoff, 1979). Intensive ostrich farming only started in the 1960's after which the industry began to change with leather becoming the predominate commodity in 1975 and later on in the 1980's saw the increase in popularity of ostrich meat (van Zyl, 2001).

Food products consumption of animal origin has increased at a very high rate, it is predicted that *per capita* demand for these products will increase even more in developing countries (Bradford, 1999). With population growth, the increase of higher living standards and urbanization, the demand for higher quality protein grows. China and other Asian countries already show an increase in demand for animal products (Bradford, 1999; Cao & Li 2013). Between 1997 and 1999 worldwide, total meat production was 218 million tons with predictions that it will rise to 376 million tons in 2030 (Gardner, 2013). In order to meet the fast growing demand for animal food products, production systems on the farms need to be optimized and input

costs reduced. Ostrich meat is considered to be healthier than other meats due to the low fat content and composition of fatty acids (Harris *et al*., 1994; Sales & Hayes, 1996; Girolami *et al*., 2003) and can make a contribution to supplying animal protein to the local populations and international markets.

The largest expense of an intensive ostrich production system is feed cost (Aganga *et al*., 2003; Brand & Jordaan, 2004), this can account for ca. 75% of total input costs (Brand & Jordaan, 2004). The profitability of an intensive ostrich production unit can be improved by lowering the feeding costs (Brand & Jordaan, 2004; Jordaan *et al*., 2008). There are several factors other than feed cots influencing profitability that the farmer cannot control, thus it is of key importance to optimize factors such as nutrition which can be to some degree controlled by the producer (Carstens, 2013). Brand & Jordaan (2004) stated that feed costs can be reduced by making use of well formulated least cost diets and incorporating the use of locally produced feedstuffs. Engelbrecht (2016) reported on similar feed cost reduction solutions, emphasizing that as long as these solutions are not detrimental to end product quality it can be utilized to reduce feed cost.

Protein is essential for growth and optimal production of animals. According to Brand & Gous (2006), depending on the feeding phase the ostriches are in, protein can constitute up to 22.8% of a diet. With global population growth, protein is becoming scarcer, more expensive and especially less available to be utilised in animal feed which necessitates the research in finding alternative protein sources (Brand *et al*., 2000a; Brand *et al*., 2004; Sridhar & Bhat, 2007). Canola is locally produced in the Western Cape province of South Africa and has promising potential to be utilised as a protein source in animal rations, replacing soybean oilcake meal (Brand *et al*., 2007). Brand *et al*. (2000) evaluated FFCS as a potential protein and energy source for ostriches and found that the FFCS contained 192 g crude protein (CP) per kilogram of feed and 11.2 g lysine/kg of feed. Canola has long been used in other animal feeds such as poultry (Ajuyah *et al*., 1991; Lee *et al*., 1991), pigs (Busboom *et al*., 1991) and ruminants (Mir *et al*., 1984). However, no information is available about the suitability of canola in ostrich diets. The use of rapeseed in animal feed was very limited due to high levels of glucosinolates and erucic acid (Bell, 1993), however the development of double zero canola cultivars (a rapeseed cultivar containing lower levels of these anti-nutrients) made it possible to include higher levels into diets (Dale, 1996).

The aim of this study was therefore to determine the optimal and ideal FFCS inclusion level for grower ostrich diets based on animal preference, without reducing DMI.

3.2 Materials and method

The trial was conducted in May 2016 on the Oudtshoorn Research farm (22°25’E, 33°63’S, at altitude of 307 m) in the Klein Karoo region of South Africa. Ethical clearance for this study was obtained from the Elsenburg ethical committee (R14/108). The trial ran for a total of 10 days with the first part being conducted over five days with a seven day rest interval followed by another five day trial period. In this trial, 60 ostriches at an age of 233 days, weighing 82.2 ± 1.06 kg, were divided into 10 camps (32 m x 30 m) containing six animals per camp, in a randomised block design.

Each camp had five feeding troughs (46 cm x 23 cm x 20 cm) in a fixed position, containing a different diet based on the FFCS inclusion level to evaluate if there were a preference towards certain diets. Feeding trough allocation switched in the camps so that only two camps had the same order. Throughout the trial, feed and water troughs (lastly mentioned with dimensions 29 cm x 20 cm x 15 cm) were monitored three times daily

to ensure animals had *ad libitum* feed and water supply. The feed in all the troughs were mixed twice a day by hand to stimulate feed intake. Daily feed supply was recorded as well as refusals weighed back the next day at the same time for the whole trial period. Refusals were subtracted from feed supplied to determine daily feed intake for each diet.

Mixit2+ software (Agricultural Software Consultants Inc., San Diego, USA) was used to formulate the five iso-nutritional diets with different FFCS inclusion levels. The optimization model to predict nutritional requirements of ostriches developed by Gous & Brand (2008) aided in formulating balanced diets. The control diet contained only soybean oilcake meal (9.8% of the total diet composition) as the main protein source and the four treatment diets were formulated so that FFCS incrementally replace the soybean oilcake meal in the diets (0, 6.9, 13.8, 20.6 and 27.5% FFCS inclusion, respectively) as seen in Table 3.1. The diet formulations and nutrient compositions for each of the five diets are presented in Table 3.2.

The control diet with 0% FFCS included will be referred to from here on out as the 0%FFCS. The maximum FFCS inclusion (27.5%) diet will be referred to as the 100%FFCS as the FFCS replaced all of the soybean oilcake meal in the diet. The remaining three FFCS inclusion levels are expressed as a percentage of 27.5 (100%FFCS), thus referring to them as the 25%FFCS, 50%FFCS and 75%FFCS respectively (Table 3.1).

Table 3.1 Percentage full-fat canola seed and soybean oilcake meal inclusion in treatment diets

Protein source (%)	Diets expressed as the percentage full-fat canola seed replacing soybean oilcake meal				
	0%FFCS	25%FFCS	50%FFCS	75%FFCS	100%FFCS
Full-fat canola seed	0.0	6.9	13.8	20.6	27.5
Soybean oilcake meal	9.8	7.4	4.9	2.5	0.0

Feed were milled and pelleted (8mm ø) on the Oudtshoorn Research farm. Approximately 1.5 kg of feed was sampled for each one ton batch of every diet when pelleted. Samples were ground with the Retsch TM ZM200 sample mill (Haan, Germany) using a 1.5 mm screen for further chemical analyses. Ground samples were analysed to verify that the diets contained the formulated nutrient levels.

The following fractions and components were determined; dry matter (DM), crude fibre (CF) acid detergent fibre (ADF), neutral detergent fibre (NDF), ether extract (EE), ash, crude protein (CP), metabolisable energy (ME), calcium (Ca) and phosphorous (P) and amino acids. Analyses were based on the methods of the Association of Official Analytical Chemists (AOAC, 2012) (refer to page 40 for specific method numbers) and presented in Table 3.2.

Table 3.2 Ingredients and chemical composition of diets containing incremental levels of full-fat canola seed that were used in a preference study (as fed basis)

Ingredients kg/ton	Diets expressed as the percentage full-fat canola seed replacing soybean oilcake meal				
	0%FFCS	25%FFCS	50%FFCS	75%FFCS	100%FFCS
Full-fat canola	0.00	68.79	137.57	206.36	275.14
Maize (Yellow grain)	492.19	419.19	346.19	273.18	200.18
Lucerne meal, 17% CP	246.09	227.11	208.12	189.14	170.15
Oats hulls	18.44	66.13	113.82	161.51	209.20
Soybean oilcake meal, 44% CP	98.44	73.83	49.22	24.61	0.00
Molasses meal	80.00	80.00	80.00	80.00	80.00
Limestone, ground	26.30	26.86	27.42	27.98	28.54
Kynofos 21/ MCP[1]	22.44	21.77	21.10	20.42	19.75
Common salt/NaCl[2]	9.84	9.88	9.93	9.97	10.01
Vitamin & mineral premix*	5.00	5.00	5.00	5.00	5.00
Bentonite	20.00	20.00	20.00	20.00	20.00
Synthetic lysine (L-lysine 95%)	1.34	1.49	1.65	1.80	1.95
Nutrients					
ME[3] MJ/kg feed	11.50	11.50	11.50	11.50	11.50
Dry matter (g/kg)	872.95	862.33	861.18	862.38	855.23
Crude protein (g/kg)	132.50	134.22	127.97	123.44	116.88
Ash (g/kg)	95.03	98.63	102.28	106.03	120.55
Crude fat (g/kg)	25.88	39.13	64.18	85.98	95.30
Crude fibre (g/kg)	126.23	124.70	123.83	143.75	136.05
Acid detergent fibre (g/kg)	152.58	155.95	154.21	179.99	168.44
Neutral detergent fibre (g/kg)	225.50	232.53	231.68	258.03	249.90
Calcium (g/kg)	29.30	21.70	22.70	21.90	21.45
Phosphorous (g/kg)	8.35	7.90	9.37	7.10	9.10

*Refer to Annexure A for full vitamin and mineral premix composition.

[1]Monocalcium phosphate

[2]Sodium chloride

[3]Metabolisable energy (as formulated)

Dry matter determination included drying a 2 g sample for 24 hours at 100 °C (AOAC, 2012) (method 934.01). Crude fibre (Goering & Van Soest, 1970), ADF and NDF (Van Soest *et al*., 1991) were obtained by making use of the ANKOM A200 Fibre Analyzer (ANKOM Technology Corporation, New York, USA). Ether Extract determination was done using a Tecator Soxtec system HT 1043 (Tectator, Höganas, Sweden) and the extraction fluid diethyl-ether (AOAC, 2012) (AOAC method 2003.06). Ash was determined with the Labcon Muffle furnace RM7 (Labcon, Johannesburg, South Africa) (AOAC method 942.05). Crude protein was calculated from the nitrogen content determined with a LECO TruMac N Nitrogen Determinator, version 1.3X (LECO Corporation, Michigan, USA) and using the following factor N x 6.25 (AOAC, 2012) (AOAC method

990.03). Amino acid content (Table 3.3) was determined using a method developed by the Central Analytical Facilities (CAF) at Stellenbosch University, this includes hydrolysis of milled feed samples in hydrochloric acid and Waters Acquity Ultra Performance Liquid Chromatography (UPLC) with UV or fluorescence detection after derivatization with 6-aminoquinolyl-N-hydroxysuccinimidyl carbamate (AQC) (Taylor, M.J.C., Pers. Comm., Central Analytical Facilities, Stellenbosch University, Stellenbosch, 7600, South Africa, 4th October 2017).

Table 3.3 Amino acid composition of diets in which soybean oilcake meal was gradually replaced by full-fat canola seed to determine the effect on the feed preference of grower ostriches

Amino acids (g/kg)	Diets expressed as the percentage full-fat canola seed replacing soybean oilcake meal				
	0%FFCS	25%FFCS	50%FFCS	75%FFCS	100%FFCS
Lysine	0.77	0.86	0.88	0.88	0.79
Methionine	0.12	0.14	0.14	0.13	0.20
Threonine	0.45	0.49	0.50	0.45	0.46
Arginine	0.90	0.98	0.92	0.90	0.76

Samples were analysed for Ca and P with the dry ashing method of the Agri Laboratory Association of South Africa (AgriLASA, 1998) (method 6.1.1). The Thermo Electron iCAP 6000 Series Inductively Coupled Plasma (ICP) Spectrophotometer (Thermo Electron Corporation, Milan, Italy) with a vertical quartz torch fitted with a Cetac ASX-520 auto sampler was used to measure Ca and P concentrations (Table 3.2). Merck Titrisol standards (1000 ppm) (Merck, Darmstadt, Germany) and iTEVA Analyst software were used to calculate the Ca and P concentrations.

Glucosinolate concentrations in the FFCS that was used in the trial were determined based on an adapted method of liquid chromatography–mass spectrometry (LC-MS) described by Sasaki *et al*. (2012). The Waters Synapt G2 system, ESI probe, ESI negative, Cone Voltage 15 V (Waters Corporation, Milford, USA) with a Waters BEH C18 column at 55 °C (17 µm, 2.1x100mm, Milford, USA) and 0.1% NH4OH and acetonitrile as solvents were used for the analysis. After the glucosinolate content of the FFCS were determined it was multiplied by the percentage FFCS in each diet to obtain the concentration of glucosinolates in each diet (Table 3.4).

Table 3.4 Glucosinolate content of full-fat canola seed (as is basis) and diets (calculated) in which soybean oilcake meal was gradually replaced by full-fat canola seed to determine the effect on the feed preference of grower ostriches

Glucosinolates (µmol/g)	Diets expressed as the percentage full-fat canola seed replacing soybean oilcake meal					
	Full-fat canola	0%FFCS	25%FFCS	50%FFCS	75%FFCS	100%FFCS
Progoitrin	0.947	0.00	0.065	0.131	0.195	0.260
Sinigrin	0.035	0.00	0.002	0.005	0.007	0.010
Glucobrassicin	0.186	0.00	0.013	0.026	0.038	0.051
Gluconapin	1.072	0.00	0.074	0.148	0.221	0.295
4-hydroxyglucobrassicin	2.141	0.00	0.148	0.295	0.441	0.589
Epiprogoitrin	2.065	0.00	0.142	0.285	0.425	0.568
Gluconapoleiferin	0.089	0.00	0.006	0.012	0.018	0.024
Glucobrassicanapin	0.209	0.00	0.014	0.029	0.043	0.057
Gluconasturtin	0.145	0.00	0.010	0.020	0.030	0.040
Total	6.889	0.00	0.475	0.951	1.419	1.894

Feed colour was measured to establish if the five diets differed in colour which may also have an effect on 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 measure surface colour of unground (as fed) feed samples of each diet, according to the three CIE Lab-System colour attributes L* (white-black), a* (green-red) and b* (blue-yellow). Calibrations were done using standards provided (BYK-Gradner). For the measurements, samples were evenly spread out in a deep circular flat bottomed container before taking five readings on the surface in different locations of each diet.

Data were statistically analysed to determine if there were significant differences at the $P \leq 0.05$ level between diets. Analysis of variance (ANOVA) was performed on the CIE Lab-System colour attributes to establish if there were significant colour differences between the five diets by using Fisher's least significant difference (LSD) t test. Randomised block ANOVA with the GLM procedure of SAS Enterprise Guide (Version 9.4, SAS Institute Inc., Cary, NC, USA) aided in verifying if there were any significant differences for DMI/bird/day and percentage dry matter intake (%DMI)/bird/day between diets. Percentage DMI for a specific diet was calculated as follows, DMI/day of a specific diet in a certain camp divided by total DMI/day of all the diets in that camp multiplied by 100. Analyses were performed on average intake/bird/camp/day for each of the diets over the 10 trial days. In the case of significant differences, Fisher's LSD was used to determine which diets differed from each other.

3.3 Results

The colour of the various diets differed slightly (Table 3.5). The light reflectance (L*) differed ($P \leq 0.05$) between diets, with the 0%FFCS, 50%FFCS and 75%FFCS having the lighter colour (although not differing from each other) than the 25%FFCS and 100%FFCS, except for the 50%FFCS that did not differ ($P > 0.05$) from the 100%FFCS. Nor did the 25%FFCS and 100%FFCS differ from each other. There were no significant differences among the diets for a* whilst the b* for the 100%FFCS was lower than all the rest, which did not differ from each other.

Table 3.5 Colour attribute differences between diets, in which soybean oilcake meal was gradually replaced by full-fat canola seed (Least square means ± standard error)

Surface colour attributes	Diets expressed as the percentage FFCS replacing soybean oilcake meal				
	0%FFCS	25%FFCS	50%FFCS	75%FFCS	100%FFCS
Mean L*	58.57^{a}±1.13	54.61^{c}±3.14	57.03^{ab}±1.16	58.08^{a}±1.46	55.04^{bc}±1.51
Mean a*	2.81±0.55	3.61±0.42	3.35±0.65	3.26±0.52	3.08±0.88
Mean b*	21.60^{a}±0.33	21.67^{a}±0.83	21.66^{a}±0.87	20.80^{a}±0.51	19.66^{b}±0.86

^{a,b,c} Row means with different superscripts differ significantly ($P \leq 0.05$)

As pertaining to feed intake, no differences ($P > 0.05$) were found for DMI/bird/day or %DMI/bird/day between any of the camps. However, differences ($P \leq 0.05$) were observed between diets for DMI/bird/day and %DMI/bird/day (Table 3.6); the 25%FFCS had the highest ($P \leq 0.05$) DMI/bird/day (577.61 ± 81.98 g/bird/day) (41.5% higher than the 0%FFCS) and %DMI/bird/day (20.5 ± 2.9 %/bird/day) (43.7% higher than the 0%FFCS) compared to all the other diets. There were no differences ($P > 0.05$) in feed intake between any of the other diets.

Table 3.6 The effect of soybean oilcake meal replacement with full-fat canola seed (FFCS) on the mean dry matter intake (DMI) and %DMI of five ostrich grower diets, presented as least square means ± standard error of the mean

	Diet	FFCS inclusion level (%)	Soybean replacement level (%)	Mean DMI/Bird/Day (g)	Percentage DMI/Bird/Day (g)
Treatment	1	0	0	577.61^{b} ± 81.98	20.55^{b} ± 2.95
	2	6.9	25	817.38^{a} ± 81.98	29.53^{a} ± 2.95
	3	13.8	50	414.38^{b} ± 81.98	14.90^{b} ± 2.95
	4	20.6	75	493.23^{b} ± 81.98	18.02^{b} ± 2.95
	5	27.5	100	469.97^{b} ± 81.98	17.00^{b} ± 2.95

^{a,b} Column means with different superscripts differ significantly ($P \leq 0.05$)

3.4 Discussion

Most animals will consume feed primarily to satisfy their nutritional requirements (Rose & Kyriazakis, 1991; Forbes & Shariatmadari, 1994), thus DMI of a poultry ration will firstly be determined by the energy content of the feed. Ostriches receiving feed with higher energy levels have a lower feed intake than those receiving lower energy diets (Brand *et al*., 2000a; Brand *et al*., 2000b; Brand *et al*., 2002; Brand *et al*., 2006). As the diets in this investigation were formulated to be iso-nutritional (Table 3.2), the preference towards the 25%FFCS (Table 3.6) could not be due to differences in energy content. This validates closer inspection into the assumption that the preference may be due to sensory aspects such as feed taste and colour or anti-nutrients.

Hill (1979) reported that poultry have poorly developed smell and visual senses which renders taste, smell and colour of less importance when it comes to diet selection. However, Fischer *et al*. (1975) found the colour pecking preferences of White Leghorn chicks when placed in a dark surrounding with different illuminated colour, were towards blue-violet and orange-red colours. When chicks were placed in a light surrounding, they had preferences towards the violet and yellow-orange colours. In both the dark and light conditions, the animals discriminated against green which received the lowest peck count. Green is a prominent background and habitat colour where most animals feed which led Fischer *et al*. (1975) to postulate that the preference pecking at violet and yellow-orange might be due to the strong contrast these colours have with green and not towards the colours themselves. This was similarly explained by Hailman (1968). However, the findings of Khosravinia (2007) contradicts those of Fischer *et al*. (1975) and Hailman (1968), as it was found that overall, chickens prefer green feed opposed to orange, red, white and yellow feed. These findings were in accordance with that of Cooper (1971), who found that five out of seven pens of turkey poults consumed more green feed than yellow, red, blue and natural coloured feed. Khosravinia (2007) did however find that significantly more of the orange feed was consumed at a low light intensity.

According to Bubier *et al*. (1996), ostrich chicks prefer green colour over yellow, blue, black and red. In their study they placed ostrich chicks in a basket and presented the five different colours to them in the form

of strips of insulation tape. The pecks over 30 min were counted as well as the frequency of pecks at a colour calculated. Green was pecked at the most, although green and white had no significant difference in pecking frequency. Bubier *et al*. (1996) went on stating that chicks were more prone to investigate feeding trays and consume pellets when there were a thin layer of feed creating a contrast between feed and tray colour, this validates the assumption of Fischer *et al*. (1975). Janse van Vuuren *et al*. (2007) performed similar trials where they placed different coloured flags together with ostrich chicks and found that the most pecks were at the green flag. Some studies however, came to the conclusion that ostrich chicks had no definite preference towards green coloured feed (Janse van Vuuren *et al*., 2007; Kruger *et al*., 2008).

Examining results from the present trial, it is clear that the L* value for the 50%FFCS and 25%FFCS (Table 3.5) were higher than the rest of the diets, indicating that these rations were of a slightly whiter colour. In the case of b* values, the 100%FFCS had a lower value than all the other diets and it is expected that it would have a slightly more blue appearance. Despite the differences in these two attributes, to the naked eye, one cannot see any obvious differences between the five diets. It must also be considered that the differences were too small to contribute to feed preference, keeping in mind that the 25%FFCS had the highest DMI but did not differ from the 100%FFCS for L*, only differing from the 100%FFCS for b*. Based on the findings of other studies it is not clear if ostriches have a feed colour preference although they do have a peck preference towards green. It is also more likely that it is a contrast between colours attracting the attention and leading to higher feed consumption and not a specific feed colour. Although feed colour did not have an influence on DMI in the current study, it would be of great value and interest to further investigate to what extent feed colour may have an effect on DMI. Other factors such as feed flavour can also be influential on diet selection and warrants attention.

Taking a variety of flavours into account, Sizemore & Lillie (1956) as well as Romoser *et al*. (1958) found that it had no effect on intake nor feeding efficiency of chickens. Kare & Pick (1960) suggested that due to poultry having restricted saliva production, the animals have limited ability to taste and that DMI is very stable even if taste is altered, furthermore it is stated that the taste of feed needs to be altered to a great extent before it can influence feed intake. In contradiction to this, Kare *et al*. (1957) stated that a fowl has an acute ability to taste. Brand *et al*. (2008) found that ostriches did not have any taste buds in their beaks. However in a study of the oropharyngeal opening, it was found that on a dark semi-circular part of the posterior third part of the hard palate there were several delicate papillae that may function as taste sensors (Tadjalli *et al*., 2008). These finding makes it very likely that ostriches do have to some extent the ability to taste. There is also the possibility that the taste buds are lower down in the throat of the bird.

In this trial, the ostriches favoured the 25%FFCS over the control which is the standard commercial diet that they received before the trial began. Kare & Pick (1960) mentions that poultry will frequently avoid the unaccustomed feed and prefer the feed they are used to. However, the influence of FFCS on feed intake is not clear. Talebali & Farzinpour (2005) observed that chickens in a group which received a high (12%) FFCS inclusion diet had a higher feed intake (4016g/bird) over a 42 day period than a group that received the control diet with no FFCS (3583g/bird). Contradicting these findings, Roth-Maier *et al*. (1988) found that an increase of FFCS in the diet of chickens reduced the performance of the birds. Summers *et al*. (1988) stated that although a precise cause for the decrease in feed intake has not been found, the lowering of feed intake may be due to the presence of phytic acid in FFCS. Phytic acid reduces the ability to absorb calcium and thereby

reducing feed intake. However, the results from the current study are not in agreement with any of the three last mentioned studies and statements, only the 25%FFCS was favoured over the rest of the diets which did not differ in DMI (P >0.05). There were no trends of increased or decreased DMI as FFCS inclusion increased, which rules out phytic acid as an influence seeing that the 100%FFCS had a much higher FFCS inclusion (27.5%) and thus a higher phytic acid concentration than the 0%FFCS, with no difference in DMI between the two diets.

Canola contains glucosinolates (Paul *et al*., 1986) and α-linolenic acid (Galliard, 1980). Although canola is a variety of rapeseed containing low levels of these compounds (DeBonte *et al*., 2001), it may affect the taste of the feed and influence DMI. Fenwick *et al*. (1983a) and Fenwick *et al*. (1983b) found that glucosinolates and other naturally produced pesticides and toxins have a bitter, sour and caustic taste. Similarly, van Doorn *et al*. (1998) and Mithen *et al*. (2000) noted that glucosinolate metabolites in food will cause a bitter taste. Hill (1991) further reported that glucosinolates leads to reduced DMI in ruminants. Fenwick *et al*. (1983a) supported this and went on stating that glucosinolates, namely sinigrin and progoitrin in the feed are the causes for bitterness and thus reduced DMI. Quinsac *et al*. (1994) conducted nutritional trials using rapeseed meal (*Brassica napus L*.) as a basis to determine to what extent glucosinolates effects production of chickens. In their trial, the diet containing 300 g/kg of rapeseed meal, contained 9.2 µmol/g progoitrin and had no effect on DMI, the same was found for the diet containing 9.5 µmol/g progoitrin. Full-fat canola seed has been tested in various studies to establish concentrations of glucosinolates that affects animal performance. Quinsac *et al*. (1994) fed diets to broilers containing a total glucosiolate content of 15.8 µmol/g, with no difference in feed intake compared to diets containing no glucosinolates. These and other studies showed that glucosinolate contents needs to be much higher that the values shown in Table 3.4 to effect animal performance (Roth-Maier *et al*., 2004; Opalka *et al*., 2001). There are also other anti-nutritional factors that may influence animal feed intake, α-Linolenic acid is a very unstable fatty acid that can oxidise rapidly (Galliard, 1980), and develops unpleasant aromas and a rancid taste during storage (Hawrysh, 1990). Erucic acid is another anti-nutrient contained in rapeseed which can have a toxic effect on animals if excessive amounts are consumed (Dingyuan & Jianjun, 2007). However, low levels of erucic acid are found in canola (< 2% of total fatty content of a seed) (DeBonte *et al*., 2001). Ferguson *et al*. (2002) found that pigs have the ability to detect toxins/anti-nutrients in feed and avoid intake of the feed containing these harmful components. These findings regarding unpleasant tastes and toxins of canola do not explain the results obtained from the present trial. The ostriches consumed more of the 25%FFCS than the rest of the diets, but did not discriminate against the 100%FFCS when compared to the 0%FFCS. According to the above mentioned studies this should have been the case if the canola contained high levels of anti-nutrients. This might justify the assumption that these anti-nutrient levels were too low (Table 3.4) to influence DMI, seen that canola were developed for this precise reason; to have lower levels of these compounds that influence taste (Stefansson & Kondra, 1975; Lee *et al*., 1991; DeBonte *et al*., 2001). Based on the results of other research, Talebali & Farzinpour (2005) concluded that feed intake inhibition does not result from the undesirable taste canola might have.

In a study to improve DMI of newly hatched ostrich chicks, feed flavour was altered by adding artificial flavourings (Kruger *et al*., 2008). Salty, sweet, bitter and sour flavours were added to a standard feed and placed in four different feeding troughs, respectively. The chicks showed a preference towards the salty taste. Janse van Vuuren (2008) ran a similar trial where seafood, meat, citrus, mint, lucerne, and aniseed flavours

were provided to ostrich chicks in a free choice feeding system. The chicks tended to favour the seafood flavour in two consecutive trials. These findings support the idea that ostriches do have the ability to distinguish between flavours and have preferences towards salty and seafood flavours. However, Brand *et al*. (2018) investigated feed preference of ostriches towards sweet, bitter and a combination of sweet and bitter lupins respectively that replaced the soybean oilcake meal in ostrich grower diets. There were no preference (P >0.05) between sweet and combination of sweet and bitter lupin inclusion levels. It was only found that there was a tendency (P =0.11) for birds to prefer a 7.5% bitter lupin inclusion level and discriminate to some small extent against the 15% and 30% inclusion levels. Considering the large difference between alkaloid content of sweet and bitter lupins, the fact that the birds had access to both types of lupins in the trial and that there were no differences for DMI between the two types (Brand *et al*., 2018), leads to the speculation that ostriches have a limited ability to taste. It is therefore assumed based on the trials conducted by Brand *et al*. (2018) including the current trial, that the taste did not stimulate a reduced DMI response and that the diet flavour and aroma needs to be altered to a greater extent to induce reduced DMI.

3.5 Conclusion

Considering that the 25%FFCS was the only diet favoured by the ostriches and that the 0%FFCS, 50%FFCS, 75%FFCS and 100%FFCS had no differences in DMI, it can be ruled out that the taste of or anti-nutrient content in FFCS are the factors responsible for these results. A similar conclusion can be drawn regarding feed colour, as feed colour attribute differences did not explain the higher DMI of the 25%FFCS; the ostriches preferred a small amount (6.8%) of FFCS in their diets. It can be considered that for some reason the ostriches favoured the combination and ratio of the other raw materials and FFCS in the 25%FFCS. Further research needs to be conducted to establish why the 25%FFCS was favoured. At this point of time it is clear that FFCS can be include in diets up to a level of 25%, without any detrimental effects on feed intake. Growth trials also need to be conducted to ensure that the performance of the birds is not influenced negatively by the FFCS inclusion.

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Chapter 4

Production and slaughter performance of ostriches fed full-fat canola seed

Abstract

In an effort to reduce feeding costs, which make up the largest expense (*ca.* 75%) in an intensive ostrich production system, alternative protein sources are regularly explored to replace more expensive protein sources. Full-fat canola seed (FFCS) is a locally produced alternative protein source that has potential for inclusion in the diets of ostriches. In this investigation chicks that was 84 days of age, weighing 24.7 ± 0.36 kg were randomly allocated to 15 groups (9 - 12 animals per group), with three replications per treatment diet, and fed five iso-nutritional diets with varying levels of FFCS. Birds were reared according to standard practises and fed through three different feed phases until being slaughtered at 309 days of age (93.2 ± 1.82 kg). Within each feeding phase, FFCS incrementally (0%, 25%, 50%, 75% and 100% of protein source) replaced soybean oilcake meal as protein source and were supplied *ad libitum*. Dry matter intake (DMI), average daily gain (ADG), feed conversion ratio (FCR) and end weights were recorded within each phase and over the entire trial period. Carcass/slaughter trait data were collected at slaughter. No differences were observed regarding production traits during the starter and finisher growth phases. Dry matter intake during the grower phase was lowest (P =0.01) (1.52 kg/bird/day) for the 100%FFCS (100% replacement of soybean oilcake meal), the rest of the diets with a mean DMI of 1.80 kg/bird/day did not differ from each other. The 100%FFCS also showed the slowest growth (P =0.01) (152.0 g/bird/day) during the grower phase, as did the 25%FFCS (208.9 g/bird/day) and 75%FFCS (209.5 g/bird/day) diets. The 0%FFCS (236.2 g/bird/day) and 50%FFCS (267.8 g/bird/day) diets resulted in higher ADG. End weights during the grower phase for the 0%FFCS, 25%FFCS, 50%FFCS and 75%FFCS (74.8, 72.2, 76.8 and 72.5 kg respectively) did not differ from each other. The 100%FFCS resulted in lower end weights (67.4 kg), although not differing from the 25%FFCS and 75%FFCS. For the overall trial period, the only differences were within ADG, with the 0%FFCS, 50%FFCS and 75%FFCS replacement diets showing the fastest growth and the 100%FFCS, although not differing from the 25%FFCS and 75%FCCS diets, resulting in the slowest growth. The fat pad weights were the only slaughter trait revealing differences between diets, with the 50%FFCS resulting in the heaviest fat pad weights. Based on these results, it is recommended that a maximum of 20.6% inclusion of FFCS be used in diets during the grower phase as it may lead to reduced performance when exceeded, although in the other phases, FFCS can be included up to the maximum levels evaluated (100% replacement of soybean oilcake meal) without any detrimental effects.

4.1 Introduction

Ostrich diet formulation were initially based on the knowledge and standards of poultry energy and amino acid requirements as there were limited information available regarding ostrich requirements. This led to inaccurate formulation causing an over- or undersupply of metabolisable energy (ME) to ostriches, increasing costs or reducing animal performance, leading to lower profitability (Angel, 1996; Brand *et al*., 2014). Mellett (1993) reported that poor feed conversion ratios (FCR) were observed amongst ostriches in South Africa, which may have been the result of the type of feed or poor management. However, pioneering work by Swart (1988), Cilliers *et al*. (1994) and Cilliers (1995) led to a better understanding of the metabolism of

ostriches and established true ME values, specifically for ostriches. Since then, more research on ostrich nutrition has been conducted (Ullrey & Allen, 1996; Cilliers *et al*., 1998; Brand *et al*., 2000c; Glatz *et al*., 2003; Gous & Brand, 2008; Carstens, 2013; Brand *et al*., 2014; Viviers, 2015; Engelbrecht, 2016).

With the ostrich industry experiencing a decline during 1997 and 1998, the focus of nutritional studies shifted to cost efficient diet formulation and feeding in order to lower the input cost of feeding, which is the largest expense (ca. 75%) of an intensive ostrich production unit (Brand *et al*., 2002). The use of mathematical simulation models, developed by Gous & Brand (2008), are very effective tools in determining the exact nutritional requirement of ostriches and formulating least cost diets. Brand & Jordaan (2004) noted that by incorporating locally produced feedstuffs in well formulated least cost diets, the feeding cost of intensive ostrich production units can be reduced. Bearing in mind that the protein source in an ostrich diet can make up 22.8% of the diet (Brand & Gous, 2006), together with protein becoming scarcer and more expensive (Brand *et al*., 2000a; Brand *et al*.,2004a), one can reduce feed cost by finding alternative locally produced protein sources to incorporate in ostrich diets.

Soybean oilcake meal is currently used as the main protein source in ostrich diets, however full-fat canola seed has the potential to replace soybean oilcake meal and is produced locally in the Western Cape region of South Africa, the major ostrich production region in the world (Brand *et al*., 2007). Due to high levels of glucosinolates and erucic acid, rapeseed inclusion in animal rations has been restricted in the past (Bell, 1993). Although double zero canola cultivars were developed with much lower anti-nutrients than rapeseed, making its use in animal feed more suitable (Dale, 1996), little information is available on the use of canola in ostrich diets and the effect it may have on production and slaughter performances of the birds.

Therefore, the aim of this study was to evaluate to what extent full-fat canola seed can be included in slaughter ostrich diets as a protein source replacing soybean oilcake meal, without having any detrimental effect on the health, production and slaughter traits of the birds.

4.2 Materials and methods

The trial was conducted at the Oudtshoorn Research farm (-33.631811, 22.257171, at altitude of 307 m) from February 2016 to September 2016. Ethical clearance was granted by the Western Cape Department of Agriculture's ethics committee (R14/108). In total 187 day old South African Black ostrich chicks that were hatched on the farm were randomly divided into 15 groups of 9 to 12 chicks per group. The groups were allocated to one of 15 identical paddocks (10 m x 5 m) with adequate shaded shelter and indoor housing (5 m x 3 m) during the evening for protection against the elements. At 84 days of age the growth trial started and the chicks were relocated to larger camps of 25 m x 6 m, five treatment diets were then randomly allocated to the groups with three replications per treatment diet. At 154 days of age the birds were again moved to larger camps (40 m x 30 m) to accommodate growth and prevent skin damage. At 105 days of age the chicks were vaccinated against Newcastle disease.

Slaughter ostriches are fed according to four phases based on their age; namely the pre-starter (0-60 days), starter (60-135 days), grower (135-210 days) and finisher (210-300 days) phases (Brand & Gous, 2006; Brand & Olivier, 2011). During the pre-starter phase all the groups were reared on a standard commercial pre-starter diet (Table 4.1). Feed and water were available *ad libitum* throughout the entire trial period.

Table 4.1 Pre-starter diet ingredients that were fed to ostrich chicks leading up to the production trial period

Ingredients	Amount (kg/ton)
Maize (Yellow grain)	504.36
Lucerne meal, 17% CP	100.87
Soybean oilcake meal, 44% CP	172.82
Fish meal	75.65
Canola oilcake meal	50.44
Canola oil	50.44
Limestone, ground	24.31
Kynofos 21/MCP[1]	4.01
Common salt/NaCl[2]	10.09
Vitamin & mineral premix*	5.04
Synthetic lysine (L-lysine 95%)	1.97
Nutrients (as formulated)	
ME[3] MJ/kg feed	14.36
Dry matter (g/kg)	907.40
Crude protein (g/kg)	205.68
Ash (g/kg)	27.54
Crude fat (g/kg)	78.46
Crude fibre (g/kg)	54.31
Calcium (g/kg)	15.18
Phosphorous (g/kg)	6.03

*Refer to Annexure A for full vitamin and mineral premix composition.
[1]Monocalcium phosphate
[2]Sodium chloride
[3]Metabolisable energy

Mixit2+ software (Agricultural Software Consultants Inc., San Diego, USA) was used to formulate five iso-nutritional diets with different levels of FFCS replacing soybean oilcake meal as protein source for the starter, grower and finisher phase. The diet formulations were based on the optimization model predictions developed by Gous & Brand (2008).The control diets contained no FFCS and only soybean oilcake meal as the main protein source. Full-fat canola seed incrementally replaced the soybean oilcake meal in each of the four treatment diets to ultimately replace all the soybean oilcake meal. A detailed illustration of the FFCS inclusion and soybean oilcake meal replacement can be seen in Table 4.2. The nutrient composition of the FFCS and soybean oilcake meal are presented in Table 4.3. Diet formulation and nutrient composition for the different trial phase diets are presented in Tables 4.4 - 4.6.

The diets containing no FFCS will be referred to from here on as the 0%FFCS. The diets with maximum FFCS inclusion will be abbreviated as 100%FFCS, as soybean oilcake meal was completely replaced with FFCS. The three FFCS inclusion diets remaining are expressed as a percentage of the maximum FFCS inclusion level (100%FFCS) in each production phase, hence 25%FFCS, 50%FFCS and 75%FFCS, respectively. Using the starter phase diets as an example, the highest FFCS inclusion was 31.3% thus referred

to as the 100%FFCS. The lowest FFCS inclusion was 7.8%, which is 25% of 31.3% and thus will be referred to as the 25%FFCS. The remaining two diets had inclusions of 15.6% and 23.5% FFCS and will therefore be referred to respectively, as the 50%FFCS and 75%FFCS.

Milling and pelleting (8 mm ø) of feed took place on the Oudtshoorn Research farm. In order to chemically analyse feed, approximately 1.5 kg feed samples were collected of every batch of feed pelleted. The Retsch TM ZM200 sample mill (Haan, Germany) was used to grind the samples to a particle size of 1.5 mm for analysis. Ground feed samples as well as ground FFCS and soybean oilcake meal samples, that were used in the trial, were analysed based on the methods set by the Association of Official Analytical Chemists (AOAC, 2012), determining the nutrient composition of the feeds, which are presented in Tables 4.3 - 4.6. The following fractions were determined with the specific methods: Dry matter (DM) (AOAC method 934.01), crude fibre (CF) (Goering & Van Soest, 1970), acid detergent fibre (ADF) and neutral detergent fibre (NDF) (Van Soest *et al*., 1991), ether extract (EE) (AOAC method 2003.06), ash (AOAC method 942.05), crude protein (CP) (AOAC method 990.03). The metabolisable energy (ME) (MJ/kg feed) of the feeds are presented as formulated, however, the ME of the two protein sources (Table 4.3) were calculated using the formula ME(ostrich) = 6.35 + 0.645 x ME(poultry) (Brand & Gous, 2006). Metabolisable energy for poultry was calculated based on the formulas of the Centraal Veevoerderbureau (2004):

AME = (18 x dCP x CP) + (38.8 x dFat x Fat) + (17.3 x dNFE x NFE)/1000

Digestibility Coefficients; dCP = 0.85, dFat = 0.83, dNFE = 0.75

Nitrogen free extract (NFE) were calculated using the formula of Davie (1988);

NFE = 100 – (%moisture + %Ash + %CP + %Fat + %CF)

Table 4.2 Percentage full-fat canola seed and soybean oilcake meal inclusion in treatment diets fed to slaughter ostriches in each production phase

Phase	Full-fat canola seed and soybean oilcake meal inclusion levels				
	0%FFCS	25%FFCS	50%FFCS	75%FFCS	100%FFCS
Starter					
Full-fat canola seed (%)	0.0	7.8	15.6	23.5	31.3
Soybean oilcake meal (%)	18.7	15.4	12.0	8.6	5.2
Grower					
Full-fat canola seed (%)	0.0	6.9	13.8	20.6	27.5
Soybean oilcake meal (%)	9.8	7.4	4.9	2.5	0.0
Finisher					
Full-fat canola seed (%)	0.0	6.3	12.5	18.8	25.1
Soybean oilcake meal (%)	8.1	6.1	4.1	2.0	0.0

Table 4.3 Nutrient composition and glucosinolate concentrations of full-fat canola seed and soybean oilcake meal that were used in the diets of slaughter ostriches during the growth trial, on an as fed basis

Nutrients	Full-fat canola seed	Soybean oilcake meal
ME[1] MJ/kg (Ostrich)	17.99	14.14
Dry matter (g/kg)	939.55	905.85
Crude protein (g/kg)	204.06	460.63
Ash (g/kg)	39.15	62.80
Crude fat (g/kg)	410.70	24.25
Crude fibre (g/kg)	154.75	30.80
Acid detergent fibre (g/kg)	183.10	87.30
Neutral detergent fibre (g/kg)	217.80	108.50
Calcium (g/kg)	49.70	3.40
Phosphorous (g/kg)	13.20	7.40
Glucosinolates (µmol/g)		
Progoitrin	0.947	0.00
Sinigrin	0.035	0.00
Glucobrassicin	0.186	0.00
Gluconapin	1.072	0.00
4-hydroxyglucobrassicin	2.141	0.00
Epiprogoitrin	2.065	0.00
Gluconapoleiferin	0.089	0.00
Glucobrassicanapin	0.209	0.00
Gluconasturtin	0.145	0.00
Total	6.889	0.00

[1]Metabolisable energy

Calcium (Ca) and phosphorous (P) were determined by the dry ashing method developed by the Agri Laboratory Association of South Africa (AgriLASA) (AGRILASA, 1998) (method 6.1.1). Amino acid content (Tables 4.4 – 4.6) was determined using a method developed by the Central Analytical Facilities (CAF) at Stellenbosch University; this includes hydrolysis of milled feed samples in hydrochloric acid and then running the hydrolysed samples through a Waters Acquity Ultra Performance Liquid Chromatography (UPLC) with UV or fluorescence detection after derivatization with 6-aminoquinolyl-N-hydroxysuccinimidyl carbamate (AQC) (Taylor, M.J.C., Pers. Comm., Central Analytical Facilities, Stellenbosch University, Stellenbosch, 7600, South Africa, 4th October 2017).

Glucosinolate concentrations in the FFCS that were used in the trial were determined based on an adapted liquid chromatography–mass spectrometry (LC-MS) method, as described by Sasaki *et al*. (2012). The Waters Synapt G2 system, ESI probe, ESI negative, Cone Voltage 15 V (Waters Corporation, Milford, USA) with a Waters BEH C18 column at 55 °C (17 µm, 2.1x100mm, Milford, USA) and 0.1% NH_4OH and acetonitrile as solvents were used for the analysis. After the glucosinolate concentrations of the FFCS were determined, the concentrations were multiplied by the percentage of FFCS in each diet to obtain the concentration of glucosinolates in each diet (Table 4.7).

Table 4.4 Ingredients and chemical composition of starter diets fed to slaughter ostrich chicks from 84-146 days of age containing incremental levels of full-fat canola seed as protein source (as fed basis)

Ingredients (kg/ton)	Diets expressed as the percentage full-fat canola seed replacing soybean oilcake meal				
	0%FFCS	25%FFCS	50%FFCS	75%FFCS	100%FFCS
Full-fat canola seed	0.00	78.18	156.35	234.53	312.70
Maize (yellow grain)	597.23	526.10	454.97	383.83	312.70
Lucerne meal, 17% CP	129.40	164.80	200.21	235.61	271.01
Soybean oilcake meal, 44% CP	187.48	153.64	119.80	85.96	52.12
Canola oil	24.88	18.66	12.44	6.22	0.00
Limestone, ground	23.00	21.65	20.30	18.95	17.60
Kynofos 21/ MCP[1]	22.39	21.23	20.07	18.91	17.75
Common salt/NaCl[2]	9.95	10.07	10.19	10.30	10.42
Vitamin & mineral premix*	3.48	3.52	3.57	3.61	3.65
Synthetic lysine (L-lysine 95%)	2.18	2.15	2.13	2.10	2.07
Nutrients					
ME[3] MJ/kg feed	13.50	13.50	13.50	13.50	13.50
Dry matter (g/kg)	875.35	880.25	886.85	870.03	856.98
Crude protein (g/kg)	157.19	156.56	161.25	166.41	163.28
Lysine	1.00	1.08	1.18	1.27	0.95
Methionine	0.11	0.23	0.32	0.12	0.19
Threonine	0.50	0.57	0.58	0.52	0.58
Arginine	1.06	1.12	1.11	1.12	1.02
Ash (g/kg)	84.15	86.25	91.08	99.15	100.08
Crude fat (g/kg)	41.48	63.23	89.58	118.50	137.15
Crude fibre (g/kg)	62.18	65.50	87.58	101.05	110.00
Acid detergent fibre (g/kg)	77.20	86.85	104.04	134.13	136.23
Neutral detergent fibre (g/kg)	137.11	137.13	155.25	190.48	197.78
Calcium (g/kg)	18.75	21.00	20.55	25.70	22.90
Phosphorous (g/kg)	8.95	9.75	8.70	9.80	10.00

*Refer to Annexure A for full vitamin and mineral premix composition.

[1]Monocalcium phosphate

[2]Sodium chloride

[3]Metabolisable energy (as formulated)

Table 4.5 Ingredients and chemical composition of grower diets fed to slaughter ostriches from 147-230 days of age containing incremental levels of full-fat canola seed as protein source (as fed basis)

Ingredients (kg/ton)	Diets expressed as the percentage full-fat canola seed replacing soybean oilcake meal				
	0%FFCS	25%FFCS	50%FFCS	75%FFCS	100%FFCS
Full-fat canola seed	0.00	68.79	137.57	206.36	275.14
Maize (yellow grain)	492.19	419.19	346.19	273.18	200.18
Lucerne meal, 17% CP	246.09	227.11	208.12	189.14	170.15
Oats hulls	18.44	66.13	113.82	161.51	209.20
Soybean oilcake meal, 44% CP	98.44	73.83	49.22	24.61	0.00
Molasses meal	80.00	80.00	80.00	80.00	80.00
Limestone, ground	26.30	26.86	27.42	27.98	28.54
Kynofos 21/ MCP[1]	22.44	21.77	21.10	20.42	19.75
Common salt/NaCl[2]	9.84	9.88	9.93	9.97	10.01
Vitamin & mineral premix*	5.00	5.00	5.00	5.00	5.00
Bentonite	20.00	20.00	20.00	20.00	20.00
Synthetic lysine (L-lysine 95%)	1.34	1.49	1.65	1.80	1.95
Nutrients					
ME[3] MJ/kg feed	11.50	11.50	11.50	11.50	11.50
Dry matter (g/kg)	872.95	862.33	861.18	862.38	855.23
Crude protein (g/kg)	132.50	134.22	127.97	123.44	116.88
Lysine	0.77	0.86	0.88	0.88	0.79
Methionine	0.12	0.14	0.14	0.13	0.20
Threonine	0.45	0.49	0.50	0.45	0.46
Arginine	0.90	0.98	0.92	0.90	0.76
Ash (g/kg)	95.03	98.63	102.28	106.03	120.55
Crude fat (g/kg)	25.88	39.13	64.18	85.98	95.30
Crude fibre (g/kg)	131.75	124.70	123.83	143.75	136.05
Acid detergent fibre (g/kg)	152.58	155.95	154.21	179.99	168.44
Neutral detergent fibre (g/kg)	225.50	232.53	231.68	258.03	249.90
Calcium (g/kg)	29.30	21.70	22.70	21.90	21.45
Phosphorous (g/kg)	8.35	7.90	9.37	7.10	9.10

*Refer to Annexure A for full vitamin and mineral premix composition.

[1]Monocalcium phosphate

[2]Sodium chloride

[3]Metabolisable energy (as formulated)

Table 4.6 Ingredients and chemical composition of finisher diets fed to slaughter ostriches from 231-294 days of age containing incremental levels of full-fat canola seed as protein source (as fed basis)

Ingredients (kg/ton)	Diets expressed as the percentage full-fat canola seed replacing soybean oilcake meal				
	0%FFCS	25%FFCS	50%FFCS	75%FFCS	100%FFCS
Full-fat canola seed	0.00	62.63	125.26	187.88	250.51
Maize (yellow grain)	382.81	312.16	241.51	170.85	100.20
Lucerne meal, 17% CP	191.40	168.60	145.80	123.00	100.20
Oats hulls	216.57	266.83	317.09	367.35	417.61
Soybean oilcake meal, 44% CP	81.48	61.11	40.74	20.37	0.00
Molasses meal	80.00	80.00	80.00	80.00	80.00
Limestone, ground	11.05	11.78	12.51	13.24	13.97
Kynofos 21/ MCP[1]	22.89	22.51	22.12	21.74	21.35
Common salt/NaCl[2]	9.57	9.68	9.80	9.91	10.02
Vitamin & mineral premix*	4.79	4.85	4.90	4.96	5.01
Bentonite	20.00	20.00	20.00	20.00	20.00
Synthetic lysine (L-lysine 95%)	0.52	0.66	0.80	0.94	1.08
Nutrients					
ME[3] MJ/kg feed	10.50	10.50	10.50	10.50	10.50
Dry matter (g/kg)	864.78	871.50	874.90	874.50	870.50
Crude protein (g/kg)	120.63	112.50	115.16	107.19	110.78
Lysine	0.55	0.69	0.76	0.69	0.65
Methionine	0.15	0.18	0.18	0.18	0.18
Threonine	0.39	0.39	0.40	0.38	0.40
Arginine	0.78	0.77	0.89	0.73	0.77
Ash (g/kg)	110.80	91.58	106.93	102.60	93.73
Crude fat (g/kg)	21.18	38.98	75.85	100.50	110.23
Crude fibre (g/kg)	129.88	147.73	144.98	151.43	158.75
Acid detergent fibre (g/kg)	150.20	172.73	184.65	196.65	198.60
Neutral detergent fibre (g/kg)	261.33	286.10	289.50	318.50	330.90
Calcium (g/kg)	14.50	11.40	13.90	13.90	11.45
Phosphorous (g/kg)	8.80	8.25	9.65	9.75	8.40

*Refer to Annexure A for full vitamin and mineral premix composition.

[1]Monocalcium phosphate

[2]Sodium chloride

[3]Metabolisable energy (as formulated)

Table 4.7 Glucosinolate concentrations (calculated) of diets containing incremental levels of full-fat canola meal as protein source fed to slaughter ostriches in three production phases

	Diets expressed as the percentage full-fat canola seed replacing soybean oilcake meal				
Glucosinolates (µmol/g)	0%FFCS	25%FFCS	50%FFCS	75%FFCS	100%FFCS
Starter					
Progoitrin	0.00	0.074	0.148	0.221	0.296
Sinigrin	0.00	0.003	0.005	0.008	0.011
Glucobrassicin	0.00	0.015	0.029	0.043	0.058
Gluconapin	0.00	0.084	0.167	0.250	0.335
4-hydroxyglucobrassicin	0.00	0.167	0.334	0.499	0.670
Epiprogoitrin	0.00	0.161	0.322	0.481	0.646
Gluconapoleiferin	0.00	0.007	0.014	0.021	0.028
Glucobrassicanapin	0.00	0.016	0.033	0.049	0.065
Gluconasturtin	0.00	0.011	0.023	0.034	0.046
Total	0.00	0.537	1.075	1.605	2.156
Grower					
Progoitrin	0.00	0.065	0.131	0.195	0.260
Sinigrin	0.00	0.002	0.005	0.007	0.010
Glucobrassicin	0.00	0.013	0.026	0.038	0.051
Gluconapin	0.00	0.074	0.148	0.221	0.295
4-hydroxyglucobrassicin	0.00	0.148	0.295	0.441	0.589
Epiprogoitrin	0.00	0.142	0.285	0.425	0.568
Gluconapoleiferin	0.00	0.006	0.012	0.018	0.024
Glucobrassicanapin	0.00	0.014	0.029	0.043	0.057
Gluconasturtin	0.00	0.010	0.020	0.030	0.040
Total	0.00	0.475	0.951	1.419	1.894
Finisher					
Progoitrin	0.00	0.060	0.118	0.178	0.238
Sinigrin	0.00	0.002	0.004	0.007	0.009
Glucobrassicin	0.00	0.012	0.023	0.035	0.047
Gluconapin	0.00	0.068	0.134	0.201	0.269
4-hydroxyglucobrassicin	0.00	0.135	0.268	0.403	0.537
Epiprogoitrin	0.00	0.130	0.258	0.388	0.518
Gluconapoleiferin	0.00	0.006	0.011	0.017	0.022
Glucobrassicanapin	0.00	0.013	0.026	0.039	0.052
Gluconasturtin	0.00	0.009	0.018	0.027	0.036
Total	0.00	0.434	0.861	1.295	1.729

The growth trial commenced at 84 days of age at the onset of the starter phase with 161 chicks weighing 24.7 ± 0.36 kg. The first day of the grower phase started at 147 days of age, with the finisher phase starting at 231 days and ending at 294 days of age marking the end of the growth trial. During the starter phase, feed

refusals were weighed back and recorded on a weekly basis on the same day, while the chicks were weighed every three weeks. During the grower and finisher phase, feed refusals were weighed back every three weeks on the same day the chicks were weighed. Recorded animal weights were used to calculate ADG, feed refusals were subtracted from feed provided to calculate DMI of each group for every three-week interval. Feed conversion ratio for each treatment diet was determined by dividing DMI through the ADG in each phase. The production traits were calculated over the whole trial period as well as for each feeding phase. Contrary to standard procedures, feathers were not clipped at six to seven months of age, as the strenuous effect would influence feed intake and growth of the birds at that time. Mortalities were recorded and if the cause of death were not obvious (leg injuries, culling due to leg abnormalities etc.) post-mortems were performed by the state veterinary to establish if mortalities were nutrition related.

The 15 experimental groups were sorted into their five respective treatment groups at 294 days of age and relocated to quarantine camps. Animals were weighed and treated for external parasites. Blood samples were drawn for avian influenza (AI) testing. The birds were then placed in five different quarantine camps, according to their treatment groups, for 14 days as obligated by the European Union (EU) meat quality standards (DAFF, 2014). During preparation for quarantine, blood was also drawn and sent to the Onderstepoort Veterinary laboratory for analysis to determine tetraiodothyronine (T4) hormone levels and thyroid functionality. The birds tested negative for AI and so after 14 days in quarantine, the birds were transported (7 km) by private contractor to the registered Klein Karoo International abattoir (-33.605797, 22.231377) in Oudtshoorn to be slaughtered at 309 days of age.

Hoffman (2012) describes the standard slaughtering procedures which was used for exsanguination and slaughtering of the animals in this trial making use of the Divac Ostrich Stunning box® (Divac, Knysna, South Africa). After exsanguination, before evisceration commenced the bled out carcass weights (bled out with feathers removed and skin still attached) were recorded. Feathers were plucked and skins flayed off each bird and kept separate for processing and later analyses. The carcasses were then eviscerated and the livers inspected for any defects and abnormalities by the local ostrich veterinarian. Liver and thyroid gland weights of all the animals were recorded to determine if the diets (containing potential anti-nutritional factors: glucosinolates) had an influence on the development and function of these organs. Fat pads (abdominal fat) were also removed and weights recorded. After evisceration the clean empty carcass consisting of thighs, chest, neck and wings were chilled in a cold room (2 °C) for 20 hours. When deep muscle temperature decreased to < 4 °C, carcasses were weighed and cold carcass weights recorded before deboning commenced. Dressing percentages were calculated dividing the cold carcass weights by bled out weight of the bird multiplied by 100. Cold carcass pH in the big drum muscle (*Muscularis gastrocnemius*) was measured (±24 hrs post mortem) and noted. The right thigh of each carcass was weighed. The big drum muscle was removed from the right thigh during deboning and its weight expressed as a percentage of the thigh weight (big drum muscle weight/right thigh weight x 100).

The GLM (general linear model) procedure of SAS Enterprise Guide (Version 9.4, SAS Institute Inc., Cary, NC, USA) was used to statistically analyse the production and slaughter data, testing for significant differences. Significance was determined at $P \leq 0.05$ and Fisher's least significant difference (LSD) t-test was performed on data that differed, to establish detailed treatment differences. Camps were used as the experimental units in this trial and thus the randomised replicates of treatment diets.

Mortalities during the trial period meant that animal numbers in each camp were not constant during the whole period or between each weighing interval. A given period (bird x days) was calculated to correct for the number of animals that consumed feed in a set period. Bird x days were calculated for each camp as the sum of (days animal numbers were constant x the number of birds during the period) divided by the days between weighing intervals. Dry matter intake per bird between each weighing interval was calculated by dividing total DMI with the bird x day of each camp. The slopes of linear regression models for growth over age (in days) of each camp were taken as the ADG for every feeding phase as well as the whole trial period.

Dry matter intake and live weights were analysed using analysis of variance (ANOVA) and regression models. Analysis of variance was also used in analysing mortality rates to establishing if there were differences between treatments. For the overall trial period, the starting weight of the birds were used as a covariate for the analysis of the production traits, whilst within a feeding phase, the end weights of the previous feeding phase were used as covariates. Analyses of variance for complete randomised designs were performed to analyse ADG and FCR, as well as for the analyses of slaughter traits, liver and thyroid gland weights and T4 hormone concentrations. Further analysis on the production traits were done by fitting relevant regression functions over FFCS inclusion levels in the diet to the data, describing observed trends within each phase and over the whole trial period. Four single degree of freedom polynomial contrasts were used to split up treatment sum of squares, determining which polynomial function (linear, quadratic, cubic and quartic) best fitted the data. Significant polynomials ($P \leq 0.05$) will be reported on. In the case of no polynomial being significant, a linear regression was used to indicate that there was no trend.

The Gompertz growth model was fitted to the data, illustrating and describing growth patterns of the birds over the whole trial period for each treatment diet. Although the growth trial only started at 84 days of age the hatching weight, 15 days of age, 29 and 42 days of age weights were brought in consideration when fitting the Gompertz growth models:

$$\text{Body Weight (BW)} = a*\exp(-\exp(-b*(\text{Age}-c)))$$

Where: a = mature weight (kg)

b = rate of maturing parameter

c = age of maximum growth (days)

4.3 Results

Observing the mortality rates that occurred over the entire trial period (from onset of starter phase up until slaughter) reveals that there were differences (P =0.032) between diets (Table 4.8). The 0%FFCS, 25%FFCS and 100%FFCS did not differ and resulted in lower mortalities, with the 100%FFCS also not differing (P >0.05) from the 50%FFCS and 75%FFCS resulting in the higher mortality rates.

Table 4.8 The effect of full-fat canola inclusion on mortality rates occurring within each treatment diet group over the entire trial period (84 – 294 days of age) presented as means ± standard error

	Diets expressed as the percentage full-fat canola seed replacing soybean oilcake meal					*P*
	0%FFCS	25%FFCS	50%FFCS	75%FFCS	100%FFCS	
Mortality rate (%)	2.78^{b} ± 2.79	3.33^{b} ± 2.79	15.76^{a} ± 2.79	12.54^{a} ± 2.79	9.14^{ab} ± 2.79	0.032

$^{a.b.c}$Row means with different superscripts differed significantly (*P* ≤0.05)

During the starter phase there were no differences (*P* >0.05) between diets for all of the production traits; differences only occurred during the grower phase and the overall trial period (Table 4.9). Differences (*P* ≤0.05) were noted during the grower phase between diets for DMI, ADG and end weight. The 100%FFCS had the lowest DMI (1.52 ± 0.05 kg/bird/day, *P* =0.01), which was 12.2% lower than the combined average DMI (1.80 kg/bird/day) of the other treatment diets, which did not differ from each other. Differences (*P* =0.01) regarding ADG, shows the 100%FFCS (152.0 ± 18.3 g/bird/day) resulting in the slowest growth rate during the grower phase, not differing from the 75%FFCS (209.5 ± 18.3 g/bird/day) and 25%FFCS (208.9 ± 18.3 g/bird/day). The 50%FFCS (267.8 ± 18.3 g/bird/day) showed the best ADG but did not differ from the 0%FFCS (236.2 ± 18.3 g/bird/day), with the 0%FFCS, 25%FFCS and 75%FFCS not differing from each other.

The 25%FFCS, 75 and 100%FFCS end weights did not differ from each other, although the 100%FFCS (67.4 ± 1.72 kg) did differ from the 0%FFCS (74.8 ± 1.75 kg) and the 50%FFCS (76.8 ± 1.73 kg). The 25%FFCS (72.2 ± 1.74 kg) and 75%FFCS (72.5 ± 1.72 kg) end weights did not differ from the 0%FFCS or the 50%FFCS. The only other differences regarding production traits, were observed within ADG for the overall trial period (*P* =0.03). Slowest growth could be seen in the animal groups receiving the 100%FFCS (269.5 ± 11.9 g/bird/day), 25%FFCS (297.4 ± 11.9 g/bird/day) and 75%FFCS (301.6 ± 11.9 g/bird/day) which did not differ from each other. The 0% FFCS (309.2 ± 11.9 g/bird/day), 75%FFCS and 25%FFCS did not differ from one another and the 50% (338.4 ± 11.9 g/bird/day) only differed significantly from the 25%FFCS and 100%FFCS.

Table 4.9 The effect of replacing soybean oilcake meal with increasing levels of full-fat canola seed in slaughter ostriches diets on the production traits in different production phases and overall trial period, presented as Least square means ± standard error (LSM ± SE)

Production Traits	Phase	Diets expressed as the percentage full-fat canola seed replacing soybean oilcake meal					*P*
		0%FFCS	25%FFCS	50%FFCS	75%FFCS	100%FFCS	
Start Weight[1] (kg)	Starter	24.70	24.70	24.70	24.70	24.70	-
	Grower	53.36	53.36	53.36	53.36	53.36	-
	Finisher	72.75	72.75	72.75	72.75	72.75	-
	Overall	24.70	24.70	24.70	24.70	24.70	-
Dry Matter Intake (kg/bird/day)	Starter	1.82 ± 0.11	1.64 ± 0.11	1.70 ± 0.11	1.70 ± 0.11	1.64 ± 0.11	0.79
	Grower	1.84^{a} ± 0.06	1.71^{a} ± 0.06	1.91^{a} ± 0.06	1.73^{a} ± 0.06	1.52^{b} ± 0.05	0.01
	Finisher	2.68 ± 0.12	2.80 ± 0.11	2.82 ± 0.12	2.78 ± 0.11	2.94 ± 0.14	0.72
	Overall	2.18 ± 0.10	2.03 ± 0.10	2.20 ± 0.10	2.10 ± 0.10	1.90 ± 0.10	0.30
Average Daily Gain (g/bird/day)	Starter	471.3 ± 30.7	442.9 ± 30.7	445.4 ± 30.7	466.5 ± 30.7	444.2 ± 30.7	0.93
	Grower	236.2^{ab} ± 18.3	208.9^{bc} ± 18.3	267.8^{a} ± 18.3	209.5^{bc} ± 18.3	152.0^{c} ± 18.3	0.01
	Finisher	330.6 ± 19.44	336.9 ± 19.44	336.4 ± 19.44	284.7 ± 19.44	339.9 ± 19.44	0.30
	Overall	309.2^{ab} ± 11.9	297.4^{bc} ± 11.9	338.4^{a} ± 11.9	301.6^{abc} ± 11.9	269.5^{c} ± 11.9	0.03
Feed Conversion Ratio (feed in kg/weight gain in kg)	Starter	3.85 ± 0.20	3.69 ± 0.20	3.87 ± 0.20	3.65 ± 0.20	3.77 ± 0.20	0.92
	Grower	8.08 ± 0.75	8.25 ± 0.75	7.10 ± 0.75	8.34 ± 0.75	10.14 ± 0.75	0.15
	Finisher	8.78 ± 0.69	8.10 ± 0.69	9.15 ± 0.69	9.80 ± 0.69	7.64 ± 0.69	0.27
	Overall	7.06 ± 0.20	6.82 ± 0.20	6.50 ± 0.20	6.94 ± 0.20	7.06 ± 0.20	0.31
End Weight (kg)	Starter	54.75 ± 2.15	52.56 ± 2.16	52.76 ± 2.15	53.94 ± 2.15	52.77 ± 2.18	0.93
	Grower	74.84^{a} ± 1.75	72.21^{ab} ± 1.74	76.83^{a} ± 1.73	72.46^{ab} ± 1.72	67.40^{b} ± 1.72	0.03
	Finisher	94.08 ± 1.25	93.43 ± 1.17	94.79 ± 1.30	91.19 ± 1.15	92.68 ± 1.47	0.36
	Overall	96.62 ± 2.24	92.47 ± 2.26	97.92 ± 2.24	91.27 ± 2.24	87.90 ± 2.27	0.07

[1]End weights of previous phase used as covariate for start weights

[a.b.c]Row means with different superscripts differed significantly ($P \leq 0.05$)

Regression models that had the best fit on the production trait data, describing trends with increase of FFCS in the diets are presented in Table 4.10. In the grower phase, the trends between FFCS inclusion levels for DMI, ADG and end weight are best described by quadratic models. The model fitted (P =0.015) to DMI, shown in Figure 4.1 describes 61.51% (R^2) of the variation among the data. Only 47.75% (R^2) of the variation in ADG is explained by the model fitted to the data (P =0.020) as seen in Figure 4.2. With the R^2 =0.59, little more than half of the variation is explained by the model fitted (P =0.017) to the end weight data as shown in Figure 4.3.

Only DMI and end weight in the finisher phase had significant trends describing the data as presented in Figure 4.4 and 4.5. For both DMI (P =0.0005) and end weight (P <0.0001), linear trends have the best fit, explaining 67.16% (R^2) of the variation in DMI and 84.66% (R^2) of the variation in end weight. In the overall trial period only ADG can be described with a significant (P =0.045) quadratic model (R^2 =0.4040) as seen in Figure 4.6.

Table 4.10 Regression models and their equations fitted to the data of production traits of slaughter ostriches to describe trends due to the change in full-fat canola seed inclusion in the diets within each production phase and the overall trial period (x = full-fat canola inclusion as percentage of protein source)

Production trait	Production phase	Function	Equation	R^2 (%)	P Model
Dry Matter Intake (kg/bird/day)	Starter	Linear	$y = -0.0012x + 1.7616$	11.75	NS
	Grower	Quadratic	$y = -0.00006x^2 + 0.004x + 1.7842$	61.51	0.015
	Finisher	Linear	$y = 0.0018x + 2.714$	67.16	0.0005
	Overall	Linear	$y = -0.002x + 2.1801$	15.98	NS
Average Daily Gain (g/bird/day)	Starter	Linear	$y = -0.10x + 460.20$	0.93	NS
	Grower	Quadratic	$y = -0.0203x^2 + 1.3578x + 223.08$	47.75	0.020
	Finisher	Linear	$y = -000.1x + 332.4$	1.91	NS
	Overall	Quadratic	$y = -0.0135x^2 + 1.0509x + 301.36$	40.40	0.045
Feed Conversion Ratio (feed in kg/weight gain in kg)	Starter	Linear	$y = -0.0008x + 3.8039$	0.91	NS
	Grower	Quadratic	$y = 0.0006x^2 - 0.0476x + 8.3457$	38.57	NS
	Finisher	Linear	$y = -0.0023x + 8.8073$	0.43	NS
	Overall	Quadratic	$y = 0.0002x^2 - 0.0165x + 7.0654$	25.95	NS
End Weight (kg)	Starter	Linear	$y = -0.0106x + 53.883$	15.45	NS
	Grower	Quadratic	$y = -0.0016x^2 + 0.1035x + 73.635$	58.82	0.017
	Finisher	Linear	$y = -0.0178x + 94.123$	84.66	< 0.0001
	Overall	Linear	$y = -0.0743x + 96.949$	30.48	NS

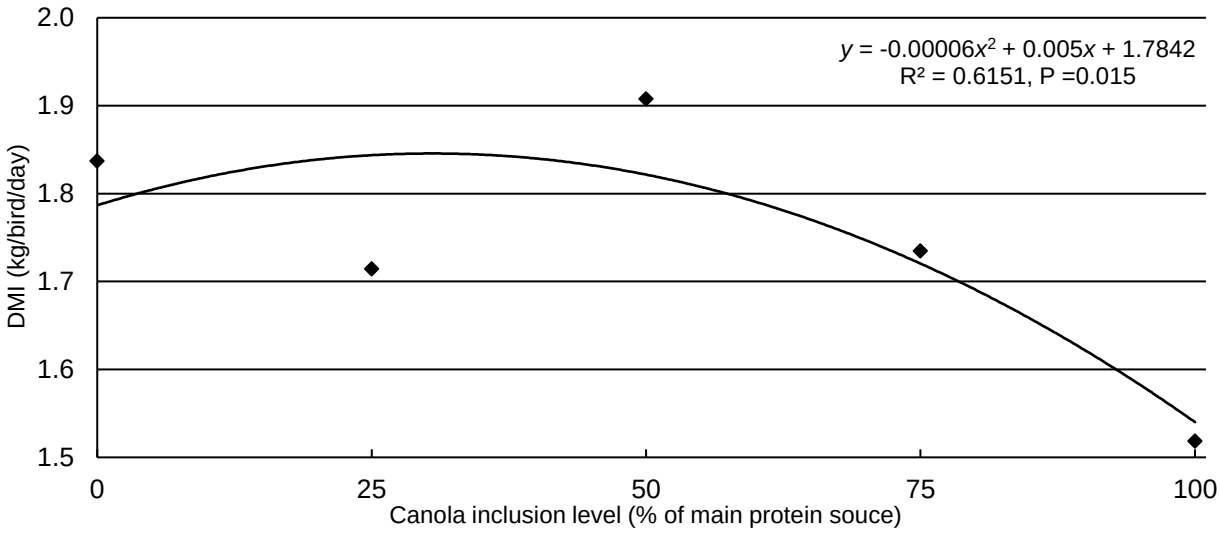

Figure 4.1 Quadratic function fitted to the LS mean dry matter intake of slaughter ostriches for the grower phase (147-230 days) with varying levels of full-fat canola in the diets

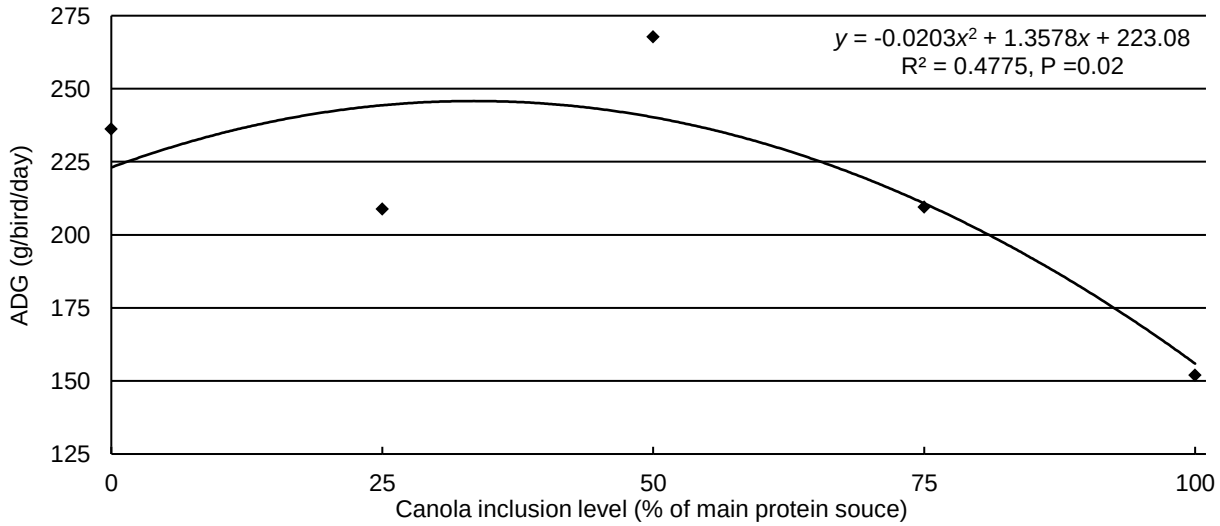

Figure 4.2 Quadratic function fitted to the LS mean average daily gain of slaughter ostriches for the grower phase (147-230 days) with varying levels of full-fat canola in the diets

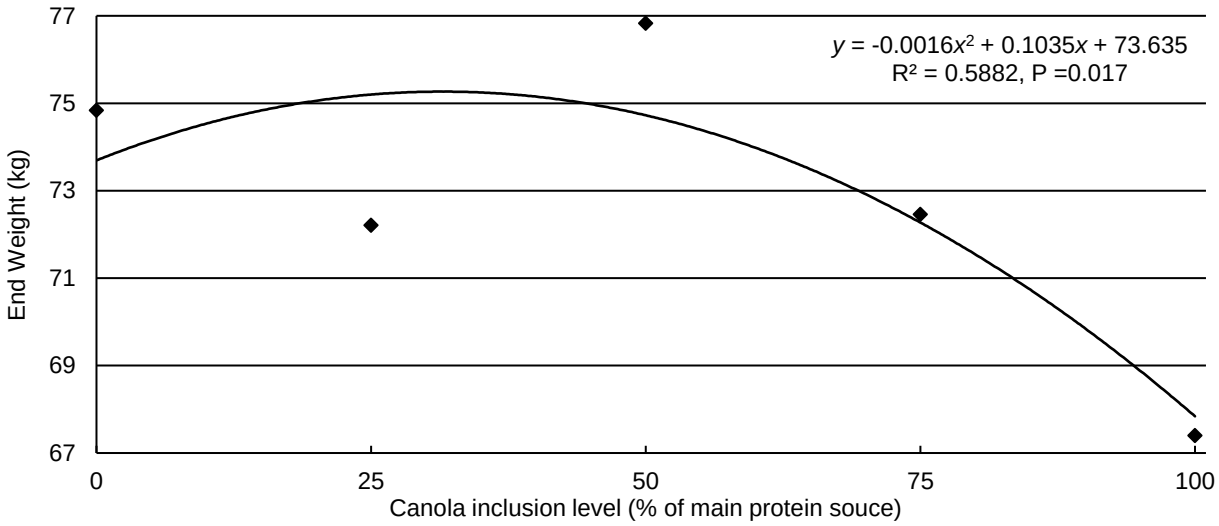

Figure 4.3 Quadratic function fitted to the LS mean end weight of slaughter ostriches for the grower phase (147-230 days) with varying levels of full-fat canola in the diets

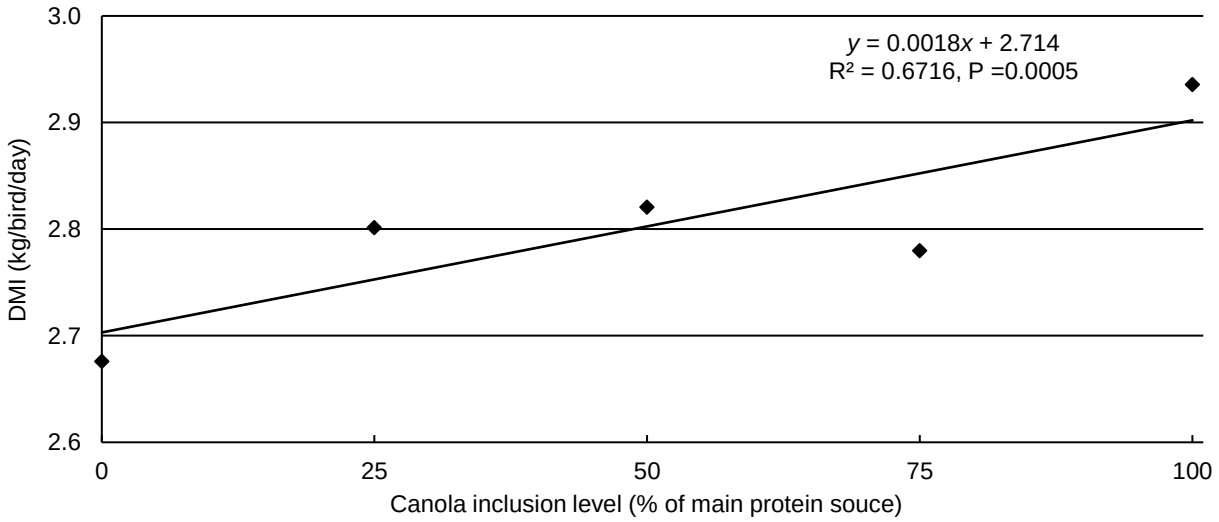

Figure 4.4 Linear function fitted to the LS mean dry matter intake of slaughter ostriches for the finisher phase (230-294 days) with varying levels of full-fat canola in the diets

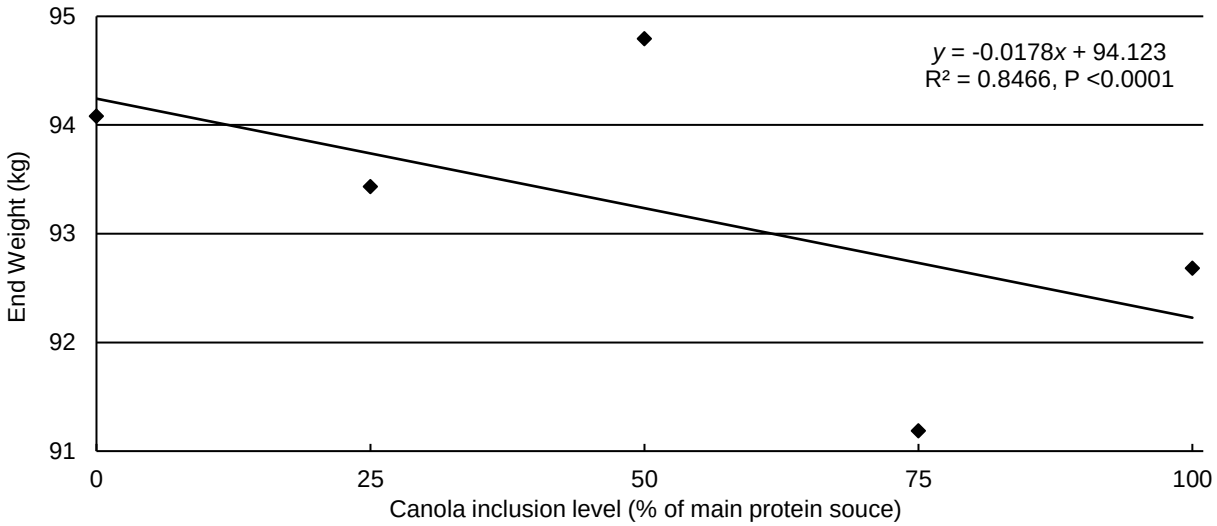

Figure 4.5 Linear function fitted to the LS mean end weight of slaughter ostriches for the finisher phase (230-294 days) with varying levels of full-fat canola in the diets

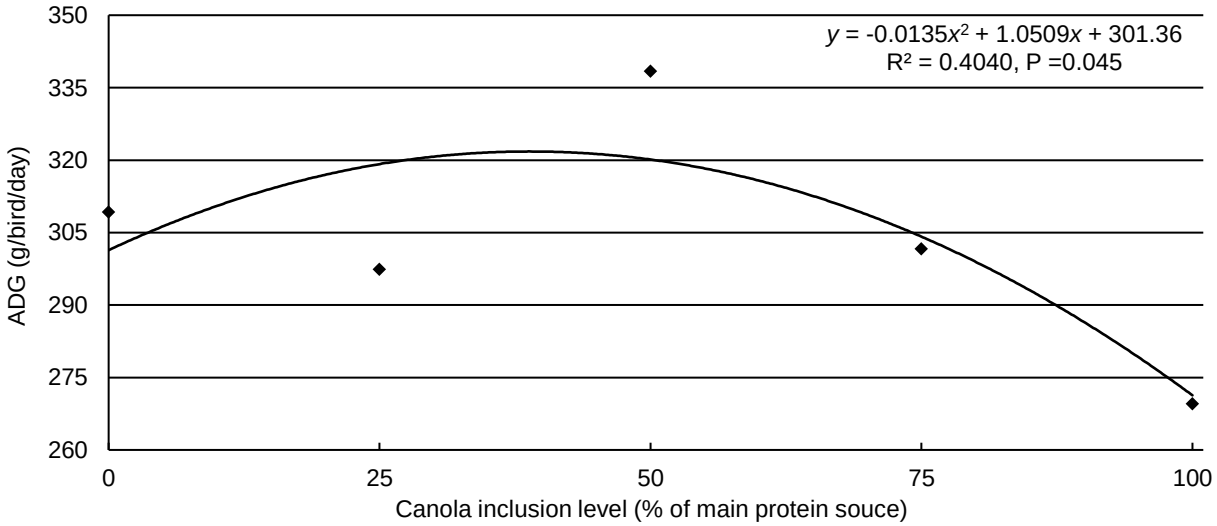

Figure 4.6 Linear function fitted to the LS mean average daily gain of slaughter ostriches for the overall production period (84 – 294 days) with varying levels of full-fat canola in the diets

The Gompertz growth curve fitted to the growth data for the overall trial period illustrates the ideal sigmoidal growth pattern for all the diets (Fig. 4.7) with the equations fitting the data very well, explaining most (R^2 = 99%) of the variation as seen in Table 4.11. The only differences (P =0.01) regarding the parameters of the growth curve were within *a* (mature weight) with the 0%FFCS, 25%FFCS and 50%FFCS showing the heaviest mature weights, although not differing from the 0%FFCS (95.5 ± 2.42 kg) and 25%FFCS (96.4 ± 2.42 kg), the 50%FFCS (102.1 ± 2.42 kg) resulted in the heaviest mature weights. The 75%FFCS (93.3 ± 2.42 kg) and 100%FFCS (85.8 ± 2.42 kg) did not differ, with the 100%FFCS resulting in low mature weights.

Furthermore, the 75%FFCS also did not differ (*P* >0.05) from the 0%FFCS and 25%FFCS but did differ (*P* ≤0.05) from the 50%FFCS.

Table 4.11 Predicted growth parameters (± standard error) of slaughter ostriches fed diets with varying levels of full fat canola seed (FFCS) incrementally replacing soybean oilcake meal (SOM) as protein source (0%FFCS / 100%SOM, 25%FFCS / 75%SOM, 50%FFCS / 50%SOM, 75%FFCS / 25%SOM, 100%FFCS / 0%SOM) based on the Gompertz growth curve

Treatment diet	Gompertz growth parameters			R^2 (%)	*P**
	a	*b*	*c*		
0%FFCS	95.49^{ab} ± 2.42	0.0139 ± 0.001	107.74 ± 4.36	99.36	<0.0001
25%FFCS	96.39^{ab} ± 2.42	0.0132 ± 0.001	107.92 ± 4.36	99.37	<0.0001
50%FFCS	102.10^{a} ± 2.42	0.0129 ± 0.001	116.97 ± 4.36	99.72	<0.0001
75%FFCS	93.27^{bc} ± 2.42	0.0141 ± 0.001	105.24 ± 4.36	99.50	<0.0001
100%FFCS	85.77^{c} ± 2.42	0.0147 ± 0.001	101.03 ± 4.36	99.06	<0.0001
P - Value	0.01	0.63	0.21	-	-

*Correlation significance

a = Mature weight (kg), *b* = Rate of maturing parameter, *c* = Age at maximum growth (days)

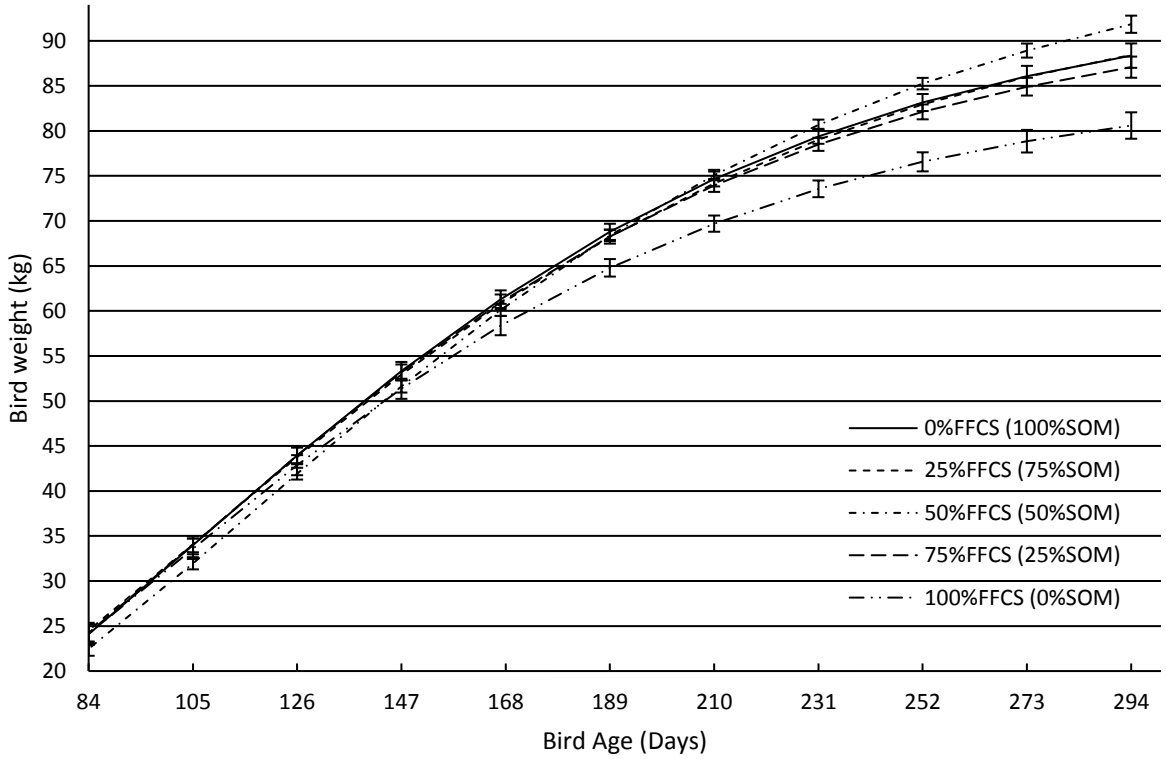

Figure 4.7 Gompertz growth curve fitted to live weight increase of slaughter ostriches from hatching to 294 days of age (trial starting at 84 days of age) consuming diets with varying levels of full-fat canola seed (FFCS) incrementally replacing soybean oilcake meal (SOM) as protein source

Assessing the slaughter traits shown in Table 4.12, the only differences found between FFCS inclusion levels are for the fat pad (abdominal fat) weight. The highest fat pad weights were observed in birds that received the 50%FFCS (4.62 ± 0.22 kg). Fat pad weights from the 0%FFCS (3.93 ± 0.22 kg), 25%FFCS (3.79 ± 0.22 kg) and 100%FFCS (3.70 ± 0.22 kg) did not differ from one another. The 75%FFCS (3.23 ± 0.22 kg) did not differ from the 100%FFCS or the 25%FFCS and all had low fad pad weights.

No differences were observed regarding liver weight, thyroid gland weight or T4 hormone concentrations due to diet (glucosinolate content in the diets) (Table 4.13).

Table 4.12 The effect of increasing full-fat canola inclusion levels on the slaughter traits of ostriches (309 days of age), presented as Least square means ± standard error (LSM ± SE)

Slaughter Traits	Diets expressed as the percentage full-fat canola seed replacing soybean oilcake meal					*P*
	0%FFCS	25%FFCS	50%FFCS	75%FFCS	100%FFCS	
Live weight (kg)	91.55 ± 2.58	85.29 ± 2.58	92.40 ± 2.58	88.29 ± 2.58	85.65 ± 2.58	0.25
Bled out weight (kg)	85.61 ± 2.04	80.87 ± 2.04	87.90 ± 2.04	83.15 ± 2.04	81.58 ± 2.04	0.17
Cold carcass weight (kg)	43.37 ± 1.33	40.64 ± 1.33	43.87 ± 1.33	40.93 ± 1.33	39.52 ± 1.33	0.17
Dressing percentage (%)	50.71 ± 0.81	50.23 ± 0.81	49.96 ± 0.81	49.27 ± 0.81	48.42 ± 0.81	0.37
Right thigh weight (kg)	16.01 ± 0.48	14.92 ± 0.48	16.27 ± 0.48	15.39 ± 0.48	14.99 ± 0.48	0.25
Big drum muscle (*Muscularis gastrocnemius*) weight (kg)	1.08 ± 0.033	1.00 ± 0.033	1.11 ± 0.033	1.07 ± 0.033	1.04 ± 0.033	0.27
Big drum muscle contribution to right thigh weight (%)	6.75 ± 0.083	6.71 ± 0.083	6.83 ± 0.083	6.92 ± 0.083	6.95 ± 0.083	0.23
Fat pad (abdominal fat) weight (kg)	3.93^{b} ± 0.22	3.79^{bc} ± 0.22	4.62^{a} ± 0.22	3.23^{c} ± 0.22	3.70^{bc} ± 0.22	0.01
Cold carcass pH	5.92 ± 0.052	5.98 ± 0.052	5.94± 0.052	5.87 ± 0.052	5.98 ± 0.052	0.54

$^{a.b.c}$Row means with different superscripts differed significantly ($P \leq 0,05$)

Table 4.13 Liver weight, thyroid gland weight and T4 hormone concentrations of slaughter ostriches fed diets with increasing full-fat canola levels, presented as Least square means ± standard error (LSM ± SE)

Organ weight	Diets expressed as the percentage full-fat canola seed replacing soybean oilcake meal					*P*
	0%FFCS	25%FFCS	50%FFCS	75%FFCS	100%FFCS	
Liver weight (kg)	1.51 ± 0.047	1.39 ± 0.047	1.62 ± 0.047	1.51 ± 0.047	1.44 ± 0.047	0.06
Thyroid gland weight (g)	55.82 ± 4.00	57.31 ± 4.00	69.76 ± 4.00	59.61 ± 4.00	50.82 ± 4.00	0.07
Hormone concentration						
T4 (nmol/L)	1.81 ± 0.19	1.37 ± 0.18	1.16 ± 0.24	1.16 ± 0.22	1.16 ± 0.22	0.13

[a.b.c]Row means with different superscripts differed significantly ($P \leq 0,05$)

4.4 Discussion

Knowledge about the nutritional potential of raw materials in ostrich diets are limited and must be evaluated so that accurate and cost efficient diet formulation can be improved, to attain optimal animal production. Alternative protein sources need to be investigated, especially for use in animal feeds due to these sources becoming scarcer and more expensive as the animal feed industry has to compete with the human food industry for protein sources (Brand *et al*., 2000a, Brand *et al*., 2004b; Sridhar & Bhat, 2007).

Although there were differences with regards to mortality rates between diets during the current study (from onset of starter to slaughter) (Table 4.8), it is believed not to be the result of the inclusion levels of FFCS in the treatment diets fed to the birds or any other nutrition related reasons. Brand (2016) noted that mortalities of ostrich chicks can be as high as 40% under commercial production conditions. Schoon (2012) reported that the mortality rates can even be as high as 50%. In a study by Carstens (2013), a mortality rate of 50% was reported. It is therefore clear that the mortality rates of the current study (Table 4.8) is relatively low compared to the other studies mentioned. Most of the mortalities were due to leg abnormalities or other injuries during the earlier stages of the trial period that necessitated culling and were not visibly correlated with diets. Glucosinolates are the main anti-nutrients within the treatment diets that could have been suspected to cause bird fatalities if consumed in excess. However, the 100%FFCS diets during this trial contained the highest concentration of glucosinolates (Table 4.7), yet the mortality rates of the 100%FFCS did not differ from the rest of the diets with lower glucosinolate concentrations.

Double zero canola cultivars development in recent years has made it possible to incorporate rapeseed into animal feeds at higher inclusion levels (Dale, 1996). The advance in the use of rapeseed is due to the lower anti-nutrient concentrations, such as glucosinolates and erucic acid in canola seed (Bell, 1993). Quinsac *et al*. (1994) evaluated a diet with glucosinolate concentration of 15.8 µmol/g of feed fed to broiler chickens and found no detrimental effect on DMI when compared to rations with no glucosinolates. In the current study, the 100%FFCS during the starter phase had the highest total glucosinolate concentration of 2.156 µmol/g of feed (Table 4.7), with no differences regarding DMI, ADG, FCR or end weight between the diets being observed (Table 4.9). As there were no performance differences between the birds receiving different FFCS inclusion levels during the starter phase, it could be concluded that the glucosinolate concentrations were too low to have an effect on animal performance. The same conclusions have been reached in other studies where

it was found that glucosinolate concentrations needed to be much higher than the values shown in Table 4.7 in order to affect animal performance (Opalka *et al*., 2001; Roth-Maier *et al*., 2004).

Ostriches like many other animals (Rose & Kyriazakis, 1991) will consume feed to satisfy their energy requirement as shown by Brand *et al*. (2004c). In their study, ostriches exhibited a higher DMI for feed of a lower energy concentration; the higher DMI compensated for the lower energy concentration and thus satisfied the bird's energy requirement, resulting in no differences regarding ADG between low and high energy diets. In order to prevent differences in feed intake because of different dietary energy concentrations, the diets in this investigation were formulated to be iso-nutritional (Tables 4.4 - 4.6) and it can thus be assumed that any differences were not due to energy or other nutrient differences between diets.

During the starter phase of this trial, no differences were observed for any of the production traits and it seems that the FFCS inclusion of up to 31.3% of the diet had no effect on the production performance of the birds. Taking a closer look at the specific production traits during the starter phase (Table 4.9) and comparing the results of the current study to previous studies, it is clear that the birds in this trial performed in accordance with what others have reported (Brand *et al*., 2000b; Brand *et al*., 2014; Viviers, 2015; Engelbrecht, 2016).

When examining the production trait results during the grower phase, it is clear that most of the differences occurred in this phase. Birds receiving the 100%FFCS had the lowest DMI with the rest of the diets not differing from each other. This is conflicting with the findings of Roth-Maier *et al*. (2004) and Brand *et al*. (1999); in their study they fed diets with different amounts of canola (0%, 8.6%, 17.3% and 25.9% in Roth-Maier *et al* and 0%, 8%, 16% and 24% in Brand *et al*) to growing and finishing pigs with no differences in feed intake. Talebali & Farzinpour (2005) however did find differences between diets with varying levels of canola (0%, 3%, 6%, 9% and 12%) with regards to DMI when fed to broiler chickens. Although the results were inversely related to the findings of the current trial, as the diet with the higher canola inclusion had the higher DMI; it may be considered that the lower DMI of the 100%FFCS may be due to factors such as taste or smell or the fact that the 100%FFCS had a higher crude fibre concentration. However referring back to Chapter 3, it is clear that there were no differences between the 100%FFCS and the other diets with regards to intake preference and that only the 25%FFCS was consumed in higher amounts. Carstens (2013) found that during the grower phase, dietary bulk density only resulted in lower dry matter intake when crude fibre concentrations were higher than 30% of the total diet (158 g/kg) and that there were no differences for DMI between diets with 88 g/kg and 158 g/kg crude fibre concentrations. The highest crude fibre concentration during the grower phase of the current study was that of the 75%FFCS (143.75 g/kg) and not that of the 100%FFCS (Table 4.5), which is lower than the critical concentration Carstens (2013) found. Therefore dietary bulk density was not the factor that caused lower DMI of the 100%FFCS. Although it must also be considered that the longer trial period during the grower phase might have an influence on the taste preference of the birds and that the 100%FFCS may have had an unpleasant taste build-up over longer periods. Birds receiving the 100%FFCS also showed the slowest growth rate, although not significantly different from the 25%FFCS and 75%FFCS. The lower ADG obtained by the birds receiving the 100%FFCS is likely due to the lower DMI. Roth-Maier *et al*. (2004) found that higher levels of canola in the feed led to decreased ADG in especially finisher pigs, which they attributed to the glucosinolate concentration in the feed. Brand *et al*. (1999) found that the canola inclusion had no influence on ADG of pigs, which contradicts the findings just mentioned. However, as mentioned the glucosinolate concentrations in the current trial diets were very low and unlikely to have affected the ADG. This

is supported by the fact that the 25%FFCS did not differ from the 100%FFCS in terms of ADG, although the 25%FFCS had a much lower glucosinolate concentration. Considering that during the grower phase differences were observed for both the DMI and ADG, it is reasonable to assume differences must occur between the FCR as well, but this is not the case and differences did not occur for FCR between the diets. Brand *et al*. (2000b) reported similar findings, where DMI and ADG showed differences between diets with varying levels of fibre but no differences for FCR. At the end of the grower phase the weights of the birds differed significantly, with the 100%FFCS, 25%FFCS and 75%FFCS resulting in the lighter live weights, bearing in mind that the 25%FFCS and 75%FFCS did not differ from the 0%FFCS and 50%FFCS which resulted in the heavier end weights. Seeing that the 100%FFCS had the lowest DMI accompanied by a low ADG resulting in light end weights, it would be wise to consider not including more than 20.6% full-fat canola seed (75%FFCS) in the diets (replacing 75% of the soybean oilcake meal) during the grower phase. Although the 75%FFCS and 100%FFCS did not differ in regards to ADG and end weight, these two diets did have differences for DMI and it needs to be kept in mind that the 75%FFCS did not differ from the 0%FFCS which showed better performance results than the 100%FFCS.

Comparing the grower phase of the current trial to the studies of Brand *et al* (2004c), Viviers (2015) and Engelbrecht (2016) (similar nutrient specifications) it is clear that the birds had lower production performance during the current trial. This however seems to be only in the grower phase, as during the finisher phase that followed the birds of the current trial out-performed or had equal production performance to that of the mentioned studies. Compensatory growth during the finisher phase may be the reason for the improved performance of the birds. Viviers (2015) postulated that due to compensatory growth during the finisher phase, the birds in his study were able to achieve the same slaughter weights at the end, regardless of the groups fed a lower protein level having lower performance capability during the grower phase. Compensatory growth is the phenomena that occurs when the current level of nutrition is increased to higher levels during or between growth phases (Lawrence & Fowler, 2002). It is not implied that during the current trial the nutritional planes were higher during the finisher phase or different among diets, in fact the five treatment diets throughout each phase were formulated to be iso-nutritional (Tables 4.4 – 4.6). It is however possible that the diets during the finisher phase was more favourable to the birds, resulting in the compensatory growth. Within the finisher phase no differences were observed for any of the production traits which is in accordance with what Brand *et al*. (1999) found when feeding different locally produced canola inclusions to pigs.

When considering the effect of canola inclusion levels on production traits over the entire trial period, only the ADG showed significant differences. This may be due to the differences observed in the grower phase which is emphasized by the low ADG shown by the 100%FFCS fed group. Considering that the DMI and FCR of the 100%FFCS were not higher than that of the other diets for the overall trial period. Together with the ADG and the end weights (although slightly lower) of the 25%FFCS and 75%FFCS not differing from the 100%FFCS, the 100%FFCS cannot be regarded as the lowest performing diet. Further research is required before such an assumption can be made. Although differences were observed during the grower phase and the overall trial period, the growth of the birds during the grower, finisher and overall trial period were in accordance with what other researchers reported (Brand *et al*., 2000b; Brand *et al*., 2000c, Brand *et al*., 2004c; Dalle Zotte *et al*., 2013; Viviers, 2015; Engelbrecht, 2016).

When examining Figure 4.1, a clear quadratic trend can be observed between DMI and the FFCS inclusion in the diets, during the grower phase. Although there are no differences (P >0.05) between most diets, except for the 100%FFCS differing from all the other diets (Table 9), the trend shows a slight increase in DMI as the FFCS inclusion increased from 0% (100%SOM) to 50% (50%SOM) followed by a sharper decrease in DMI as the FFCS inclusion increased more. On further examination of Figures 4.2 and 4.3, the same quadratic trend is observed during the grower phase. Again not differing from all the diets, the 50%FFCS shows higher ADG (Fig. 4.2) during the grower phase with slower growth resulting as FFCS inclusion increases from 50% to 100% and to a lesser extent also decreases as FFCS inclusion decreases from 50% to 0% inclusion. Figure 4.3 illustrates how the quadratic trend for DMI and ADG during the grower phase also resulted in this trend being followed in regards to end weight. Considering that there were no differences between diets regarding FCR during the grower phase as well as no significant trend, feed intake is the only factor affecting the performance of the birds. Although it was found in Chapter 3 that the 25%FFCS was preferred, the grower trial period being longer than the preferences trial may reveal that the 100%FFCS, due to anti-nutrients, is discriminated against during this phase resulting in lower DMI, slower growth and lighter end weights. Glucosinolates are regarded as biological insecticides which have a natural occurrence in some plants. It has been found that these biological insecticides may accumulate in fish and become harmful as it increases (Sabra & Mehana, 2015).This may warrant further research to establish whether glucosinolates may have a bio-accumulation effect when the birds are raised on diets containing low concentrations of these anti-nutrients, which may affect animal performance.

Even though there were no significant differences between diets during the finisher phase regarding DMI and end weight, both production traits had significant linear trends fitting the data. As shown in Figure 4.4, DMI increased with an increase in FFCS inclusion in the diet and the inverse effect can be observed regarding end weight, with a decrease in end weight as the FFCS inclusion increased (Fig. 4.5).

During the overall trial period, only ADG could be fitted with a significant trend. Figure 4.6 shows the quadratic trend, with growth rate increasing as FFCS inclusion increases to 50% of the protein source, followed by a sharper decrease similar to what was seen in the grower phase as inclusion levels increased towards 100% replacement of soybean oilcake meal.

When examining Figure 4.7 it is clearly shown by the Gompertz curve that the birds in the different diet groups grew in a similar sigmoidal pattern, however the 100%FFCS and 75%FFCS resulted in lighter mature weights (Table 4.11). Based on the growth pattern, the birds receiving the 50%FFCS had a very favourable growth performance resulting in heavier mature weights. Viviers (2015) found that birds receiving different levels of protein showed different rates of maturity but at the end, had the same mature weight and reached their maximum growth rates at the same age. He went on postulating that this may be the result of compensatory growth. However, during the current trial all the diets were iso-nutritionally formulated and some other factors must be responsible for the results obtained. Although the end weight at the end of this trial showed no significant differences, the predicted mature weights do show differences (Table 11). This may raise the question that if the trial were to be longer, would there not have been differences between end weights for the different diets? This however is of less a concern seeing that it is standard practice to slaughter ostriches at 10 to 11 months of age, as it is the most cost efficient age to slaughter (Deeming *et al*., 1999; Girolami *et al*., 2003), thus the trial was constructed to simulate standard commercial practices.

When considering the effect that canola inclusion may have on slaughter traits of ostriches, it is clear that the inclusion levels evaluated in this trial had little effect (Table 4.12). This can be expected as the different inclusion levels had little effect on the production traits, which directly influence the slaughter traits. However, the differences found between diets regarding fat pad weights need to be evaluated.

Hoffman *et al*. (2005) made the assumption that excess energy is stored in the abdominal fat depots of the ostrich. Regardless of the differences found between diets, the fat pad weights recorded in the current study are lower than most previous trials conducted. This indicates that energy was well balanced in the diets, with the animals still performing in accordance with these previous studies. The 50%FFCS had the heaviest fat pad weights (Table 4.12), this can be expected seeing that the 50%FFCS showed some of the fastest growth rates during the overall trial period (Table 4.9). Fat is a late maturing tissue (Brant *et al*., 2012) and increased deposition in most animals only occurs after maturity is reached (Owens *et al*., 1993); during this period protein deposition slows down and fat deposition increases and occurs at a faster rate than protein deposition (Deeming *et al*., 1999). It seems that the animals receiving the 50%FFCS diets may have reached maturity earlier than the other treatment groups due to their higher growth rates. Owens *et al*. (1993) noted that an animal's growth rate is one of the factors that influences the time that the animal will reaches maturity. The growth rates of the birds receiving the 50%FFCS did not differ from that of the 0% nor the 75%FFCS for the overall trial period, although when ranking these three growth rates, the 50%FFCS had the higher ADG followed by the 0%FFCS and then the 75%FFCS (Table 4.9). Keeping the growth rates in mind and observing the fat pad weights in Table 4.12, it is clear that besides the 50%FFCS having the highest fat pad weights, the 0%FFCS not differing from the 75%FFCS, resulted in the second highest fat pad weights and the 75%FFCS resulted in lower weights. The birds receiving the 50%FFCS and reaching maturity earlier is beneficial, as these birds can be slaughtered at a younger age, with the producer saving on feed cost. Care should however be taken not to feed these animal longer than needed as producers may sometimes be penalised for excessive amounts of fat on the carcass, this can also have a negative effect on feed cost as birds become less efficient after maturity when they start laying down fat (Deeming *et al*., 1999). The higher fat accumulation of the birds on the 50%FFCS is of less of a concern regarding this study. Considering that no differences between diets were observed regarding cold carcass and thigh weights, and that the overall slaughter performance of the birds is in line with what other researchers found in their studies (Brand *et al*., 2004a; Hoffman *et al*., 2007; Hoffman *et al*., 2012; Brand *et al*., 2014; Viviers, 2015; Engelbrecht, 2016). Further research to establish what led to the higher fat pad weights is however warranted.

In studies (Ibrahim & Hill, 1980; Butler *et al*., 1982; Opalka *et al*., 2001; Maroufyan & Kermanshahi, 2006) where canola meal and rapeseed meal was fed to chickens and pigs it was found that high levels of glucosinolates had caused enlargement of the liver and thyroid gland as well as decreased T4 hormone concentrations. Campbell & Smith (1979) as well as Ibrahim & Hill (1980) however, came to the conclusion that meals containing lower concentrations of glucosinolates had a reduced effect on the organs mentioned above. This may explain the findings of Roth-Maier *et al*. (2004) where different levels of canola meal was fed to pigs with no difference in thyroid gland weights observed. The findings in the current study (Table 4.13) is in accordance with the lastly mentioned study and validates the statements of Ibrahim & Hill (1980) and Campbell & Smith (1979), as there were no differences between diets for liver weight, thyriod weight and T4

hormone concentrations. This further emphasises that the glucosinolate concentrations in diets used in this trial were too low to have noticable effects on animal performance.

4.5 Conclusion

From the results gathered, it is concluded that canola can be included in slaughter ostrich diets within the ranges evaluated in this trial, while still achieving high performance that can compete with that of the presently fed standard commercial diets. One concern however, is that during the grower phase caution needs to be taken when more than 75% (20.6% total inclusion in diet) of the protein source are replaced with canola. This may lead to reduced performance and it is therefore recommended not to exceed this inclusion level during the grower phase until further research is done to better understand why the birds showed a decrease in production. Canola is known to have high fibre content, and thus a possible explanation for this negative influence could be that the high fibre concentration within the higher FFCS inclusion diets depressed DMI and thus other production parameters, as fibre can also be considered as an anti-nutritional factor. The other uncertainty is the fact that the 50%FFCS resulted in more abdominal fat accumulation. Although the 50%FFCS had higher fat pad weights, the animals obtained the same cold carcass weights (after fat was trimmed), dressing percentage and right thigh weights, which makes the fat accumulation less of a concern. Birds may have a high ADG on this diet, which can lead to maturity being reached earlier and birds being slaughtered earlier leading to savings on feeding cost. Further research to gain more knowledge on the factors causing this need to be undertaken, so that the economic impact can be better understood and profit margins elevated. With the grower phase as an exception, canola can be utilised as the sole plant protein source (100%FFCS) in slaughter ostrich diets up to the levels included during this study (31.3% for starter phase and 25.1% for finisher phase) with no detrimental effects on production or slaughter performance. Further research needs to be undertaken to evaluate what the effects of canola inclusion may be on end product (skin, muscle and feathers) quality of slaughter ostriches.

4.6 Reference

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Chapter 5

The effect of varying full-fat canola seed inclusion levels in slaughter ostrich diets on the feather yields, leather traits and meat characteristics

Abstract

Ostriches are multi-purpose animals, with feathers, leather and meat being derived from the birds to contribute to the income generated from slaughter ostrich production. The aim of this study was to evaluate the effect of different dietary full-fat canola seed (FFCS) inclusion levels on the feathers, leather and meat composition of slaughter ostriches. The trial was conducted on 187 South African Black ostriches that were randomly divided into 15 groups of 9 – 12 birds in each group. The groups were fed one of five treatment diets, resulting in three replications per treatment. They were fed from 84 days of age (24.7 ± 0.36 kg) through the starter, grower and finisher phases and were slaughtered at 309 days of age (93.2 ± 1.82 kg). Within each phase the treatment diets were formulated to be iso-nutritional, with FFCS incrementally replacing soybean oilcake meal (0%, 25, 50, 75 and 100% replacement with FFCS) as the protein source. Feed and water were supplied *ad libitum* throughout the trial. The only differences between diets regarding feather yields were within chick body short (P =0.021) and unmarketable feathers (P =0.011). The 50%FFCS resulted in the heavier yields of chick body short feathers (283.2 ± 14.84 g) than the100%FFCS group (202.3 ± 14.84 g), which resulted in the lighter yields. The 50%FFCS had the lightest yield (97.1 ± 13.18 g) of unmarketable feathers, differing from all the other diets which yielded a combined average of 161.2 g. Skin thickness was the only leather trait showing differences (P =0.038) between diets. With the 0%FFCS presenting thicker skins (0.65 ± 0.027 mm) than the 100%FFCS and 75%FFCS with combined average thickness of 0.54 mm. Moisture and protein concentration of the meat were the only chemical components showing differences (P =0.008 and P =0.004, respectively) between treatments. Meat from the 100%FFCS was found to have higher moisture concentrations (77.0 ± 0.29%) than the 25%FFCS (75.2 ± 0.24%), 0%FFCS (75.6 ± 0.24%) and 50%FFCS (75.8 ± 0.42%). The inverse trend was seen with regards to protein concentration, with 25%FFCS resulting in the highest concentration (22.6 ± 0.19%) while the 100%FFCS had the lowest (20.9 ± 0.23%) protein concentration. Within the fat tissue, dietary FFCS inclusion decreased the total SFA concentration, increased the MUFA and PUFA concentrations, increased the PUFA:SFA ratio and decreased n-6:n-3 ratio. Overall it is recommended that full-fat canola seed can be used to replace 75% (not exceeding inclusion levels used within each phase in this trial) of the soybean oilcake meal in slaughter ostrich diets, without affecting the end products.

5.1 Introduction

The ostrich is a multi-purpose animal, producing feathers, leather and meat to the market (DAFF, 2016). At first, ostrich feathers were the most favoured product with leather also becoming a popular product in later years. Ostrich meat only recently took to the market as a popular niche product and healthier red meat alternative in the early 1990's (ANON, 2004). Currently, the ostrich industry is under pressure due to the outbreak of avian influenza (AI) (DAFF, 2017). Over the years the industry has had some setbacks and it is

difficult to achieve the high profit margins that were once seen. During the late 1990's the ostrich industry reached a low point. This was the catalyst for an increase in ostrich nutrition research in order to improve profitability through least cost and well balanced diet formulation. Seeing that nutrition can account for ca. 75% of the input cost of an intensive ostrich production system, the research efforts were indispensable (Brand *et al*., 2002; Gous & Brand, 2008).

Full-fat canola seed (FFCS) may have the potential to be utilised in ostrich diets, replacing more expensive protein sources such as soybean oilcake meal and so aiding in lowering feeding costs. Carstens (2013) pointed out that the supply of nutrients to the birds must be carefully managed as an over- or undersupply may lead to unnecessary high feed costs or less than optimal production of the birds. However, to optimise profit margins, consideration should not only be given to the effect of nutrition on growth performance. The focus should also be placed on the effect of nutrition on all three end products produced by ostriches (Engelbrecht, 2014).

Although feathers have the lowest contribution to the market (DAFF, 2016), it remains a valuable end product in aiding the industry through tough times, such as the recent outbreak of AI (DAFF, 2017) which is threatening raw meat exportation (Viviers, 2015). Past studies have investigated the influence of dietary energy and protein concentrations on the yield and quality of ostrich feathers (Brand *et al*., 2004a; Brand *et al*., 2014; Viviers, 2015). However, there is limited literature available on the influence that specific raw materials may have on ostrich feathers. One exception is the study of Brand *et al*. (2018), where the effect of lupin inclusions on end products were evaluated, with the treatments having no effect on the feather quality or production.

The ostrich leather industry is the one end product sector that has been the most stable with regards to income generated, and is regarded as the strongest pillar on which the ostrich industry depends (Engelbrecht *et al*., 2005). Ostrich leather is a very sought after product in the fashion industry due to its unique quill patterns, suppleness and durability. To optimise yield and quality of the leathers, various factors, such as nutrition, need to be considered (Cooper, 2001). Thus, it is of high importance to evaluate to what extent FFCS may affect the production of ostrich skins, ensuring that it has no detrimental effects on leather quality traits.

Ostrich meat is considered as a healthier red meat option (Sales & Hayes, 1996), with lower intramuscular fat concentration, favourable fatty acids profile, high protein concentration and it is also high in iron and vitamin E (Mellett, 1992; Hoffman *et al*., 2005; Majewska *et al*., 2009; Dalle Zotte *et al*., 2013). Consumers have become more concerned with nutritional quality of their food and are moving towards healthier living, insisting to be better informed of the nutritional value of the products they consume (Sales & Hayes, 1996; Horbańczuk & Sales, 1998). It is thus important to evaluate the chemical composition of the meat and how FFCS may affect it.

The aim of this study therefore, was to evaluate what the effect of incremental inclusion of FFCS in the diets of slaughter ostriches may be on the feather and skin yields and quality as well as the chemical composition of the meat and fatty acid profile of the fat being produced by slaughter ostriches.

5.2 Materials and methods

The ostriches used in this study were reared at the Oudtshoorn Research farm (-33.631811, 22.257171, at altitude of 307 m) with animals being fed trial diets from February 2016 to September 2016. Ethical clearance for the study was granted by the Western Cape Department of Agriculture's ethics committee (R14/108). In

total, 187 day old South African Black ostrich chicks that were hatched on the farm were randomly divided into 15 groups of 9 to 12 chicks per group. The groups were allocated to one of 15 identical paddocks (10 m x 5 m) with adequate shaded shelter, and indoor housing (5 m x 3 m) during the evening for protection against the elements. During the pre-starter phase, the chicks were reared on the same diet, with the treatment diets being presented to the birds from the onset of the starter period. At 84 days of age, the growth trial started and the chicks were relocated to larger camps of 25 m x 6 m. Five treatment diets were then randomly allocated to the groups with three replications per treatment diet. At 154 days of age the birds were again moved to larger camps (40 m x 30 m) to accommodate growth and prevent skin damage. The current trial used the feathers, skins and muscles of the same birds that were used in the production trial which is elaborated on in Chapter 4. Thus the management, slaughter and feeding procedures are the same as what is explained in the previous chapter.

During this trial feathers were not harvested (clipped or plucked) at six months of age, as the harvesting of blood/green (immature) feathers is not permitted based on welfare aspects (Engelbrecht, 2014) and it has a negative effect on leather traits as the nodules are stretched (Engelbrecht *et al*., 2009). Feather harvesting also results in stress that could have had an impact on growth, resulting in reduced growth performance and undesirable growth curves.

As mentioned in Chapter 4, the birds were fed five treatment diets, according to standard practises in the starter, grower and finisher phases. The diets were formulated with increasing levels of FFCS incrementally replacing soybean oilcake meal as the main protein source, as depicted in Table 4.2, with diet compositions shown in Tables 4.1 as well as in Tables 4.4 to 4.6. Diet formulations were based on the optimization model predictions developed by Gous & Brand (2008), making use of Mixit2+ software (Agricultural Software Consultants Inc., San Diego, USA).

The diets containing no FFCS will be referred to from here on as the 0%FFCS. The diets with maximum FFCS inclusion will be abbreviated as 100%FFCS, seeing that soybean oilcake meal was completely replaced by FFCS. The three FFCS inclusion diets remaining are expressed as a percentage of the maximum FFCS inclusion level (100%FFCS) in each production phase, hence 25%, 50% and 75%FFCS respectively. Using the starter phase diets as an example, the highest FFCS inclusion was 31.3% and is referred to as the 100%FFCS. The lowest FFCS inclusion was 7.8%, which is 25% of the maximum 31.3% inclusion and thus will be referred to as the 25%FFCS. The remaining two diets had inclusions of 15.6% and 23.5% FFCS and will therefore be referred to as the 50%FFCS and 75%FFCS diets respectively.

At the end of the growth trial, the birds from the 15 experimental groups were separated and allocated to their five respective treatment groups at 294 days of age for relocation to quarantine camps. Birds were then weighed and treated for external parasites. Blood samples were also drawn for avian influenza (AI) testing. The birds were then placed in five different quarantine camps, according to their treatment groups where they still received their experimental diets for 14 days, as obligated by the European Union (EU) meat quality standards (DAFF, 2014). The bird were cleared for slaughter after 14 days in quarantine camps, as tests results for AI came back negative. The birds were transported (7 km) by private contractor to the registered Klein Karoo International abattoir (-33.605797, 22.231377) in Oudtshoorn to be slaughtered at 309 days of age. The standard slaughtering producers were followed, as described by Hoffman (2012), making use of the Divac Ostrich Stunning box®.

After exsanguination, the feathers of each bird were plucked and skins removed. The feathers of each bird were kept separate in bags before being transported to Klein Karoo International's feather department. After the skins had been removed, each skin received an identification tag for traceability before being taken to the tannery on the same premises as the abattoir.

Feather processing as described by Viviers (2015) and Brand *et al*. (2018), included drying of the feathers upon arrival for 48 hours at 50 °C and at 70 °C for the last 30min, in order to sterilize the feathers. After drying the feathers, qualified graders sorted and classed the feathers into their respective economical classes (Table 5.1). Dried feather weights of each class were recorded for statistical analysis. Feathers of each bird were kept separate during the whole process to enable linkage of feathers to each individual bird. The feather class weights of each bird were added together to determine the total dry feather yield of a bird.

Table 5.1 Economical feather classes of ostrich feathers found in this study, originating from different body regions as described in industry

Feather classes	Description and body region
Chick wing	White plumes at the edge of the wings.
Drab silver floss	Soft, shorter body feathers from the bird's ventral side.
Chick body floss	Softer, short body feathers.
Chick blondene floss	The only soft feather found on the wings.
Back feathers	Body feathers found on the back of the bird.
Chick body short	Body feathers found between the thighs and the tail section.
Chick tail	The body feathers found on the tail section of the bird.
Unmarketable	Feathers from all body regions not meeting grading specifications.

After the raw skins were received by the tannery, the subcutaneous fat was removed and the skins underwent a tanning process similarly to that described by Brand *et al*. (2018), resulting in the transformation of skins to the chrome-crust form. After the tanning process was completed, the dry skin size (dm^2) was measured and leather was graded on a subjective evaluation by qualified skin graders. The chrome-crust skins were then transported to Elsenburg Research Institute for further evaluation. The number of nodules in a 10 cm^2 area was counted at five different locations on the skin as shown in Figure 4.1. Five random nodule diameters were then measured in the same 10 cm^2 cardboard square at each of the five locations on the skins. Pinholes were counted in a 5 cm^2 square within the 10 cm^2 square at the same locations. Leather thickness on the right hand side, outside of the crown area, between location 3 and 4 (Fig. 4.1) was measured using a calliper. Dry skin size, leather grading, average nodule and pinhole count (within 10 and 5 cm^2), average nodule diameter and leather thickness for each skin were compared statistically.

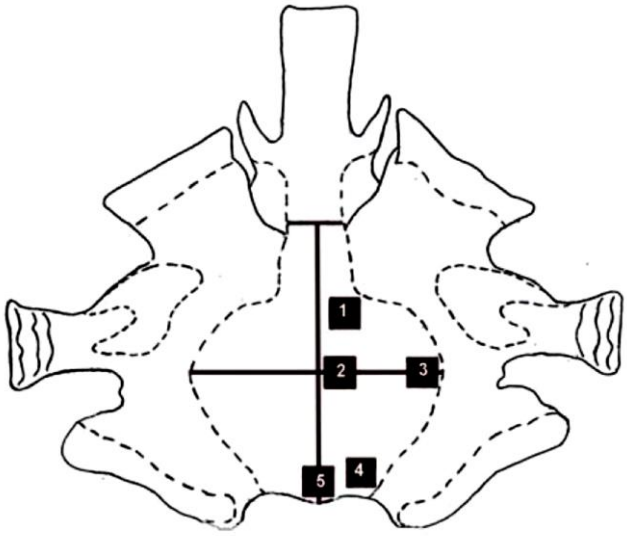

Figure 5.1 The five evaluation locations on the ostrich skin, where measurements and counts were taken during the current trial, *viz*. 1 – neck, 2 – mid-crown, 3 – upper leg, 4 – lower flank, 5 – butt (Cloete *et al*., 2004)

After evisceration, the clean empty carcasses consisting of thighs, chest, neck and wings were chilled in a cold room for 20 hours. When deep muscle temperature decreased to under 4 °C, carcasses were weighed and cold carcass weights recorded before deboning commenced. Based on the last weight of the birds recorded on the farm, the individual ostriches in each of the five treatment groups were sorted in ascending order in each of the five treatment groups. Ten birds around the median in each of the five treatment groups were then identified for fat and muscle sample collection. After the fat pads (abdominal fat) were removed, the 10 samples in each treatment group were collected, vacuumed and frozen. During the deboning process, the big drum muscles (*Muscularis gastrocnemius*) (Fig. 5.2) were removed from the right thighs, weighed and the identified 10 muscles within each treatment group collected and vacuumed. The 50 selected fat samples and 50 big drum muscles were then transported to the Department of Animal Sciences at the University of Stellenbosch, where they were kept frozen and stored at -20 °C for proximate and fatty acid analysis.

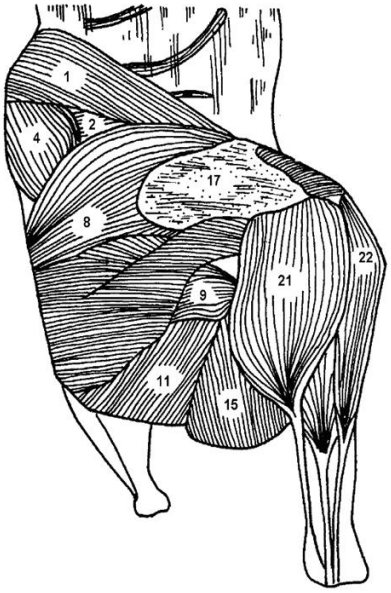

Figure 5.2 Outer muscle layer of the pelvic limb with number 21 being the big drum muscle (*Muscularis gastrocnemius*) used in the current trial for proximate analysis (Mellett, 1994)

Before analysis were performed on the meat, muscles were thawed, a representative sample from the middle of the muscle for each muscle was taken and homogenized, after which it was placed in a vacuum bag and frozen (-20 °C) again for storage, until proximate analysis commenced.

Proximate analysis of the muscle samples were done according to the Association of Official Analytical Chemists (AOAC) methods to determine the moisture, ash, crude protein and crude fat content of each thawed homogenized meat sample (AOAC, 2002). Moisture content (%) was determined by method 934.01 and ash was determined using the moisture free sample by method 942.05. Crude protein (N x 6.25) was determined by making use of the Dumas combustion method (method 992.15) and crude fat content was determined with method 920.39, making use of chloroform/methanol (1:2 vol:vol) extraction.

The thawed homogenised fat samples were analysed to determine the fatty acid profiles. Samples of the 0%FFCS (control) and 100%FFCS (highest inclusion of FFCS) of the finisher diets were also analysed to determine the change in the fatty acid profile of the feed (Table 5.2). Due to unforeseen circumstances the meat sample's fatty acid profile data could not be analysed.

Fatty acid analysis were based on the sample extraction methods described by Folch *et al*. (1957), also described in more detail by Neethling *et al*. (2014). The process started by weighing of 1 g of each feed sample and 0.1 g of each fat sample respectively. After transmethylation and extraction, 50 µL of hexane was added to the dried fatty acid methyl esters (FAME) samples and 1 µL injected into the gas chromatograph.

The Thermo Trace 1300 gas chromatograph (GC) (Thermo Electron Corporation, Milan, Italy) with a flame-ionisation detector was used to analyse the FAMEs. A Thermo ZB-FAME (30 m, 0.25 mm internal diameter, 0.25 µm film thickness) capillary column was used to perform separation. Oven temperature was incrementally increased to and held at 260 °C, which was the injection temperature, using hydrogen as a carrier gas at 1.0 mL/min rate of flow. Initially, the oven was heated to 100 °C which was held for 2 min, thereafter it was increased to 140 °C at 10 °C/min, from there it was increased to 190 °C at 3 °C/min and finally

it was increased to 260 °C at 30 °C/min and held for 5 min. Detector temperature was set at 260 °C. A standard FAME mixture (Supelco™ 37 Component FAME mix, Cat no. 47885-U, Supelco, USA) was used to identify the FAMEs in the total lipids of each sample (mg fatty acid/g of fat) by comparing the FAME's retention times to that of the standard.

In order to determine treatment differences, statistical analysis was performed on feather class weight, leather traits, chemical composition of the meat and fatty acid profile of the abdominal fat tissue. This was done with one-way analysis of variances (ANOVAs) using the GLM (general linear model) procedure of SAS Enterprise Guide (Version 9.2; SAS Institute Inc., Cary, USA). Fisher's least significant difference (LSD) t-test was used in the case of statistical differences to determine which treatments had differed significantly at the $P \leq 0.05$ level. In the case of data showing indication of possible trends, Microsoft Office Excel 2010 (Version 14.0.7163.5000, Microsoft Corporation by Impressa Systems, Santa Rosa, California) was used to perform regression analysis on the means of the data, to describe the observed trends.

Table 5.2 The fatty acid profile (% of identified fatty acids) of the control (0%FFCS) and highest full-fat canola seed inclusion diet (100%FFCS) that was fed to the ostriches in the finisher phase

Fatty acid concentration (%)	Diets expressed as the percentage full-fat canola replacing soybean oilcake meal	
	0%FFCS	100%FFCS
SFA[1]		
C10:0	0.00	2.17
C11:0	0.00	0.94
C12:0	5.56	1.88
C13:0	1.38	0.39
C14:0	2.28	0.67
C15:0	0.86	0.21
C16:0	35.82	15.06
C18:0	10.74	6.75
C20:0	2.65	1.81
C21:0	1.88	0.00
C22:0	1.03	0.77
C23:0	4.60	1.15
C24:0	2.48	0.88
Total SFA	69.27	32.67
MUFA[2]		
C16:1	0.13	0.13
C20:1	0.00	1.69
C18:1n9c	8.57	48.26
C22:1n9	0.16	0.04
C24:1	0.31	0.53
Total MUFA	9.17	50.65
PUFA[3]		
C18:2n6c	9.28	11.41
C18:3n3	1.40	2.34
C20:2n6	2.24	1.24
C20:3n6	2.48	0.61
C20:5n3	3.37	0.40
C22:2n6	1.80	0.47
C22:6n3	0.99	0.22
Total PUFA	21.56	16.68
PUFA:SFA	0.31	0.51
Total n-6 fatty acids	15.80	13.72
Total n-3 fatty acids	5.76	2.96
n-6:n-3	2.74	4.64

[1]Saturated fatty acids
[2]Monounsaturated fatty acids
[3]Polyunsaturated fatty acids

5.3 Results

For the feathers, diet only had significant effects on the chick body short (*P* =0.021) and unmarketable (*P* =0.011) feather classes (Table 5.3). When examining the results regarding chick body short it is observed that the 0%FFCS, 25%FFCS, 50%FFCS and 75%FFCS yielded the heaver weights (average yield of 261.34 g) and did not differ (*P* >0.05) from each other. The 75%FFCS also did not differ (*P* >0.05) from the 100%FFCS (202.27 ± 14.84 g) which yielded the lighter weights. The 100%FFCS however, differed significantly from the rest of the diets. When observing unmarketable feathers it is evident that the 50%FFCS yielded the lowest weights (97.11 ± 13.18 g) and differed (*P* =0.011) from all the other diets (average yield of 161.17 g) which did not differ (*P* >0.05) from each other.

With respect to leather quality traits, the only differences observed between diets were observed for the thickness of the skins (*P* =0.038), as depicted in Table 5.4. The 0%FFCS, 25%FFCS and 50%FFCS (average thickness of 0.62 mm) did not differ significantly from one another and resulted in the thickest skins, the 50%FFCS, 75%FFCS and 100%FFCS (average thickness of 0.55 mm) also did not differ (*P* >0.05) from each other and resulted in thinner skins. A linear model was fitted to the mean skin thickness data shown in Table 5.4 to best describe difference in skin thickness with dietary inclusion of FFCS. The model (y=-0.132x+0.654, where y represents skin thickness and x represents FFCS inclusion rate) (*P* =0.003), was fitted to the data describing 96.5% (R^2) of the variation among the data.

Table 5.5 reveals that the only differences regarding chemical composition of the ostrich meat were within moisture (*P* =0.008) and protein (*P* =0.004) content of the meat. The 100%FFCS had the highest moisture content (77.0 ± 0.29%) and differed significantly from all the other diets. While the moisture content of the 25%FFCS samples was lower (75.2 ± 0.24%) than that of the 100%FFCS and the 75%FFCS (76.1 ± 0.29%), although it did not differ (*P* >0.05) from that of the 0%FFCS (75.6 ± 0.24%) and 50%FFCS (75.8 ± 0.42%). The inverse effect can be observed regarding differences between diets for protein content. The 25%FFCS resulted in the higher (22.6 ± 0.19%) protein content not differing (*P* >0.05) from the 0%FFCS (22.2 ± 0.19%) or 50%FFCS (22.0 ± 0.33%), but differing significantly from the 75%FFCS (21.7 ± 0.23%) and 100%FFCS (20.9 ± 0.23%). The 100%FFCS had the lowest protein content, and differed (*P* ≤0.05) from all the other treatments.

Table 5.3 Least square means ± standard error (LSM ± SE) of feather class weights and feather income affected by the replacement of soybean oilcake meal with different levels of full-fat canola seed (FFCS) in the diets of slaughter ostriches

Feather classes (g)	Diets expressed as the percentage full-fat canola seed replacing soybean oilcake meal					*P*-value
	0%FFCS	25%FFCS	50%FFCS	75%FFCS	100%FFCS	
Total dry feather weight	865.80 ± 28.50	877.17 ± 28.50	861.27 ± 28.50	874.75 ± 28.50	846.92 ± 28.50	0.942
Dry commercially valuable feather weight	717.23 ± 34.74	725.67 ± 34.74	764.16 ± 34.74	712.93 ± 34.74	664.15 ± 34.74	0.426
Chick wing	152.64 ± 12.56	146.10 ± 12.56	145.71 ± 12.56	144.95 ± 12.56	159.35 ± 12.56	0.908
Drab silver floss	104.71 ± 23.93	87.09 ± 23.93	103.15 ± 23.93	80.56 ± 23.93	110.39 ± 23.93	0.883
Chick body floss	94.35 ± 26.82	114.20 ± 26.82	126.45 ± 26.82	122.67 ± 26.82	79.44 ± 26.82	0.701
Chick blondene floss	37.48 ± 2.11	38.16 ± 2.11	41.65 ± 2.11	38.55 ± 2.11	38.22 ± 2.11	0.673
Back feathers	47.65 ± 10.01	38.91 ± 10.01	25.88 ± 10.01	41.16 ± 10.01	27.60 ± 10.01	0.522
Chick body short	249.90^{a} ± 14.84	274.90^{a} ± 14.84	283.24^{a} ± 14.84	237.33^{ab} ± 14.84	202.27^{b} ± 14.84	0.021
Chick tail	14.79 ± 10.71	26.31 ± 10.71	38.08 ± 10.71	47.71 ± 10.71	44.44 ± 10.71	0.245
Unmarketable feathers	148.57^{a} ± 13.18	151.49^{a} ± 13.18	97.11^{b} ± 13.18	161.83^{a} ± 13.18	182.78^{a} ± 13.18	0.011
Total feather income (ZAR)	183.37 ± 9.39	182.08 ± 9.39	187.74 ± 9.39	175.91 ± 9.39	167.88 ± 9.39	0.625

$^{a.b}$Row means with different superscripts differ significantly ($P \leq 0.05$)

Table 5.4 Least square means ± standard error (LSM ± SE) of leather traits and grade affected by the replacement of soybean oilcake meal with different levels of full-fat canola seed (FFCS) in the diets of slaughter ostriches

Leather traits	Diets expressed as the percentage full-fat canola seed replacing soybean oilcake meal					*P*-value
	0%FFCS	25%FFCS	50%FFCS	75%FFCS	100%FFCS	
Crust size (dm^2)	139.70 ± 1.46	138.13 ± 1.46	138.69 ± 1.46	138.03 ± 1.46	137.03 ± 1.46	0.771
Thickness (mm)	0.65^{a} ± 0.027	0.63^{a} ± 0.027	0.59^{ab} ± 0.027	0.54^{b} ± 0.027	0.53^{b} ± 0.027	0.038
Nodule diameter (mm)	3.38 ± 0.087	3.45 ± 0.087	3.43 ± 0.087	3.44 ± 0.087	3.39 ± 0.087	0.979
Nodule count	41.42 ± 1.18	41.21 ± 1.18	42.34 ± 1.18	43.86 ± 1.18	41.42 ± 1.18	0.515
Pinhole count	26.93 ± 3.53	20.3 ± 3.53	9.84 ± 3.53	13.54 ± 3.53	18.13 ± 3.53	0.052
Leather grades						
Average grade	2.44 ± 0.16	2.16 ± 0.16	2.55 ± 0.16	2.25 ± 0.16	2.04 ± 0.16	0.254
Grade 1 (%)	21.43 ± 10.64	21.98 ± 10.64	11.11 ± 10.64	19.44 ± 10.64	32.80 ± 10.64	0.717
Grade 2 (%)	26.19 ± 10.82	46.15 ± 10.82	35.83 ± 10.82	40.28 ± 10.82	30.08 ± 10.82	0.711
Grade 3 (%)	39.68 ± 8.63	25.75 ± 8.63	40.00 ± 8.63	36.11 ± 8.63	37.12 ± 8.63	0.768
Grade 4 (%)	12.7 ± 3.96	6.11 ± 3.96	13.06 ± 3.96	4.17 ± 3.96	0.00 ± 3.96	0.168

$^{a.b}$Row means with different superscripts differed significantly ($P \leq 0.05$)

Table 5.5 Least square means ± standard error (LSM ± SE) for the chemical composition of ostrich meat affected by the replacement of soybean oilcake meal with different levels of full-fat canola seed (FFCS) in the diets of slaughter ostriches on an as is basis

Chemical component (%)	Diets expressed as the percentage full-fat canola seed replacing soybean oilcake meal					*P*-value
	0%FFCS	25%FFCS	50%FFCS	75%FFCS	100%FFCS	
Moisture	75.6^{bc} ± 0.24	75.2^{c} ± 0.24	75.8^{bc} ± 0.42	76.9^{b} ± 0.29	77.0^{a} ± 0.29	0.008
Protein[1]	22.2^{ab} ± 0.19	22.6^{a} ± 0.19	22.0^{ab} ± 0.33	21.7^{b} ± 0.23	20.9^{c} ± 0.23	0.004
Fat	2.1 ± 0.16	2.0 ± 0.16	2.0 ± 0.28	1.8 ± 0.20	1.6 ± 0.20	0.474
Ash	1.2 ± 0.033	1.2 ± 0.033	1.2 ± 0.058	1.2 ± 0.041	1.2 ± 0.041	0.707

$^{a.b}$Row means with different superscripts differed significantly ($P \leq 0.05$)
[1]Defatted

Concerning the full fatty acid profiles of the fat tissue (Table 5.6), there will only be elaboration on the fatty acids that were predominantly found within the fat tissues.

With regards to the total saturated fatty acid (SFA) concentrations contained within the fat tissue, a regression analysis revealed a significant linear trend (y=-18.16x+36.80, R^2 =0.963, P =0.003, where y represents SFA concentrations and x represents FFCS inclusion rate). As FFCS inclusion increased, the total SFA concentrations decreased. The 0%FFCS (37.8 ± 0.77%) presented the highest total SFA concentrations, subsequently followed by the 25%FFCS (31.6 ± 0.76%) and the 50%FFCS (27.7 ± 0.97%) (Table 5.6). The 75%FFSC and 100%FFCS diets did not differ (P >0.05) and resulted in the lowest concentrations of total SFA, yielding a combined average of 20.8% total SFA of the total identified fatty acids. Palmitic (C16:0) and Stearic (C18:0) acid were the predominant SFAs found within the fat tissue. Fatty acid C16:0 concentrations decreased as the dietary FFCS inclusions increased, with the 0%FFCS (29.0 ± 0.63%) resulting in the highest and the 100%FFCS (13.6 ± 0.68%) in the lowest concentrations. The 100%FFCS, however, did not differ from the 75%FFCS (14.3 ± 0.69%) with regards to C16:0 concentrations. C18:0 concentrations decreased as the dietary FFCS concentrations increased. The 0%FFCS (4.9 ± 0.20%) and 25%FFCS (4.4 ± 0.18%) resulted in the higher concentrations and did not differ significantly from each other. The 50%FFCS (4.0 ± 0.25%) resulted in the intermediate concentrations of C18:0, however, it did not differ (P >0.05) from the 25%FFCS or the 75%FFCS (3.73 ± 0.22%). Although not differing significantly from the 50%FFCS and 75%FFCS, the 100%FFCS (3.30 ± 0.21%) resulted in lower C18:0 concentrations.

Total monounsaturated fatty acid (MUFA) concentrations within the fat tissue increased as dietary FFCS inclusions increased, revealing a significant linear trend (y=11.76x+39.87, P =0.001, where y represents MUFA concentrations and x represents FFCS inclusion rate) that explained 98.6% (R^2) of the variation amongst the data. The 100%FFCS and 75%FFCS did not differ significantly from each other, resulting in the highest total MUFA concentrations, with a combined average of 50.2% of total fatty acids identified. The 0%FFCS resulted in the lowest MUFA concentrations (39.7 ± 0.67), followed by the 25%FFCS (42.5 ± 0.62%) and 50%FFCS (46.2 ± 0.85%), all differing significantly from one another (Table 5.6). Predominant MUFAs that were observed within the fat tissue were palmitoleic (C16:1) and oleic (C18:1n9c) acid. The C16:1 concentrations decreased as dietary FFCS inclusions increased. The 0%FFCS (7.2 ± 0.36%) resulted in the highest concentrations of C16:1, followed by the 25%FFCS (5.0 ± 0.33%) and 50%FFCS (2.9 ± 0.45%). The 75%FFCS and 100%FFCS

did not differ significantly and resulted in the lowest C16:1 concentration (combined average of 1.5%). The C18:1n9c concentrations on the other hand increased as the dietary FFCS inclusions increased. The 0%FFCS (31.1 ± 0.61%), 25%FFCS (36.1 ± 0.65%) and 50%FFCS (42.5 ± 0.77%) all differed significantly from one another, with the 0%FFCS resulting in the lowest C18:1n9c concentrations. The 75%FFCS and 100%FFCS did not differ (P >0.05) from each other and resulted in the highest C18:1n9c concentrations with a combined average of 47.5% (of the total identified fatty acids), which is significantly higher than the rest of the treatments.

The total polyunsaturated fatty acid (PUFA) concentrations within the fat tissue showed a significant increase as the dietary FFCS inclusions increased (Table 5.6). This increase can also be explained with a linear trend (y=7.02x+22.63, P =0.024, where y represents PUFA concentrations and x represents FFCS inclusion rate) that described most (R^2 =0.86) of the variation amongst the data. The 75%FFCS and 100%FFCS did not differ significantly and resulted in the highest total PUFA concentrations, with a combined average of 29.1% of total identified fatty acids. The 0%FFCS (22.05 ± 0.74%) resulted in the lowest total PUFA concentrations, differing from all the other treatments. The 25%FFCS (25.24 ± 0.67%) and 50%FFCS (25.25 ± 0.97%) not differing significantly, yielded intermediate total PUFA concentrations. Linoleic (C18:2n6c), α-linolenic (C18:3n3) and eicosapentaenoic (C20:5n3) acid were the most abundant PUFAs observed within the fat tissue. The C18:2n6c concentration increased as the dietary inclusion of FFCS increased. The 75%FFCS (19.3 ± 0.48%) resulted in the highest concentration of C18:2n6c, although not differing significantly from the 100%FFCS (18.5 ± 0.47%). The 50%FFCS (16.9 ± 0.56%) resulted in the intermediate concentrations and did not differ (P >0.05) from the 100%FFCS. The 0%FFCS and 25%FFCS did not differ significantly and resulted in the lowest C18:2n6c concentrations with a combined average of 14.2% of the total identified fatty acids. The C18:3n3 concentrations also increased as dietary FFCS inclusions increased. The 100%FFCS (7.5 ± 0.25%) resulted in the highest concentrations, although not differing (P >0.05) from the 75%FFCS (6.9 ± 0.25%). The 50%FFCS (6.0 ± 0.29%) and 75%FFCS did not differ significantly from each other and resulted in intermediate C18:3n3 concentrations. The 0%FFCS (2.1 ± 0.23%) and 25%FFCS (4.0 ± 0.21%) differed significantly from each other and the rest of the treatments, resulting in the lower concentrations of C18:3n3. In contrast to the other PUFAs, the concentration of C20:5n3 was seen to decrease as the dietary FFCS inclusions increased. The 0%FFCS (1.9 ± 0.45%), 25%FFCS (2.1 ± 0.40%) and 50%FFCS (1.2 ± 0.58%) did not differ significantly and resulted in the higher C20:5n3 concentrations. The 50%FFCS, 75%FFCS (0.3 ± 0.53%) and 100%FFCS (0.5 ± 0.47%), also did not differ from each other and resulted in the lower concentrations of C20:5n3.

A significant linear trend (y=0.91x+0.57, R^2 =0.93, P =0.009, where y represents PUFA:SFA ratio and x represents FFCS inclusion rate) was observed for the PUFA:SFA ratio, with the ratio increasing as the dietary FFCS inclusions increased. The 100%FFCS and 75%FFCS did not differ significantly and resulted in the highest ratios, with a combined average of 1.42. The 0%FFCS and 25%FFCS also did not differ (P >0.05) and resulted in the lowest ratios, with a combined average of 0.70.

The concentrations of total n-6 fatty acids as well as total n-3 fatty acids within the fat tissue, both followed significant linear trends (y=3.73x+16.85, R^2 =0.82, P =0.034 and y=3.29x+6.07 R^2 =0.80, P =0.040, where y represents n-6 or n-3 concentrations respectively and x represents FFCS inclusion rate), with the increase in dietary FFCS resulting in an increase in both n-6 and n-3 fatty acids. The 75%FFCS (20.8 ± 0.50%) resulted in the highest concentration of n-6 fatty acids, although not differing significantly from the 100%FFCS (19.9 ± 0.49%). The 0%FFCS (16.8 ± 0.46%) and 25%FFCS (17.7 ± 0.42%) resulted in the lowest

concentration of n-6 fatty acids. The 50%FFCS (18.4 ± 0.58%) yielded intermediate concentrations of n-6 fatty acids, which in turn did not differ (P >0.05) from the 0%FFCS, 25%FFCS or the 100%FFCS. The only differences (P =0.013) between treatments for n-3 fatty acids were between the 0%FFCS (5.3 ± 0.72%) which resulted in the lowest concentrations and the rest of the treatments which did not differ from each other, yielding a combined average of 8.32%. The ratio of n-6:n-3 fatty acids also differed (P =0.024) between diets. The 0%FFCS (3.2 ± 0.15) resulted in the highest ratios and differed from the rest of the treatments (2.42 combined average ratio), which in turn did not differ significantly from each other.

Table 5.6 Least square means ± standard error (LSM ± SE) of the fatty acid profile (% of identified fatty acids) of the abdominal fat tissue of ostriches receiving diets with increasing full-fat canola seed inclusions

Fatty acid concentration (%)	Diets expressed as the percentage full-fat canola replacing soybean oilcake meal					*P*-value
	0%FFCS	25%FFCS	50%FFCS	75%FFCS	100%FFCS	
SFA[1]						
C10:0	0.99^{ab} ± 0.10	0.86^{b} ± 0.09	1.33^{a} ± 0.13	1.03^{ab} ± 0.11	1.11^{ab} ± 0.11	0.106
C11:0	0.51^{a} ± 0.07	0.54^{a} ± 0.06	0.04^{b} ± 0.09	0.00^{b} ± 0.07	0.00^{b} ± 0.07	<0.0001
C12:0	0.42 ± 0.10	0.59 ± 0.9	0.90 ± 0.13	0.66 ± 0.11	0.75 ± 0.11	0.248
C14:0	0.81^{a} ± 0.03	0.74^{a} ± 0.03	0.70^{a} ± 0.04	0.56^{b} ± 0.03	0.54^{b} ± 0.03	0.0002
C15:0	0.17 ± 0.01	0.15± 0.01	0.15 ± 0.01	0.16 ± 0.01	0.14 ± 0.01	0.133
C16:0	28.95^{a} ± 0.63	23.85^{b} ± 0.58	19.71^{c} ± 0.80	14.27^{d} ± 0.69	13.55^{d} ± 0.68	<0.0001
C18:0	4.90^{a} ± 0.20	4.40^{ab} ± 0.18	3.97^{bc} ± 0.25	3.73^{c} ± 0.22	3.30^{c} ± 0.21	0.001
C20:0	0.30 ± 0.02	0.31 ± 0.02	0.25 ± 0.02	0.27 ± 0.02	0.30 ± 0.02	0.383
C21:0	0.32^{a} ± 0.02	0.31^{ab} ± 0.02	0.25^{bc} ± 0.02	0.24^{c} ± 0.02	0.27^{abc} ± 0.02	0.034
C24:0	0.38^{b} ± 0.04	0.57^{a} ± 0.04	0.29^{b} ± 0.05	0.31^{b} ± 0.04	0.32^{b} ± 0.04	0.003
Total SFA	37.80^{a} ± 0.77	31.61^{b} ± 0.76	27.66^{c} ± 0.97	21.22^{d} ± 0.83	20.29^{d} ± 0.82	<0.0001
MUFA[2]						
C14:1	0.13^{a} ± 0.01	0.12^{ab} ± 0.01	0.10^{c} ± 0.01	0.09^{c} ± 0.01	0.10^{bc} ± 0.01	0.009
C16:1	7.15^{a} ± 0.36	5.01^{b} ± 0.33	2.89^{c} ± 0.45	1.48^{d} ± 0.39	1.47^{d} ± 0.38	<0.0001
C17:1	0.54^{a} ± 0.06	0.52^{a} ± 0.05	0.06^{b} ± 0.06	0.15^{b} ± 0.05	0.16^{b} ± 0.05	0.0001
C18:1n9c	31.14^{d} ± 0.61	36.07^{c} ± 0.56	42.47^{b} ± 0.77	46.65^{a} ± 0.66	48.36^{a} ± 0.65	<0.0001
C20:1	0.37^{c} ± 0.03	0.54^{b} ± 0.02	0.61^{b} ± 0.03	0.79^{a} ± 0.03	0.79^{a} ±0.03	<0.0001
C22:1n9	0.04 ± 0.004	0.04 ± 0.003	0.03 ± 0.01	0.04 ± 0.004	0.04 ± 0.004	0.063
C24:1	0.19^{a} ± 0.02	0.15^{b} ± 0.01	0.08^{c} ± 0.02	0.12^{bc} ± 0.02	0.08^{c} ± 0.2	0.001
Total MUFA	39.73^{d} ± 0.67	42.46^{c} ± 0.62	46.24^{b} ± 0.85	49.32^{a} ± 0.72	51.00^{a} ± 0.72	<0.0001
PUFA[3]						
C18:2n6c	13.57^{c} ± 0.44	14.78^{c} ± 0.41	16.93^{b} ± 0.56	19.28^{a} ± 0.48	18.50^{ab} ± 0.47	<0.0001
C18:3n6	1.86^{a} ± 0.16	1.35^{b} ± 0.16	0.42^{c} ± 0.19	0.43^{c} ± 0.17	0.46^{c} ± 0.16	<0.0001
C18:3n3	2.05^{d} ± 0.23	4.00^{c} ± 0.21	6.04^{b} ± 0.29	6.88^{ab} ± 0.25	7.54^{a} ± 0.25	<0.0001
C20:2n6	0.28^{ab} ± 0.01	0.30^{a} ± 0.01	0.28^{ab} ± 0.02	0.25^{b} ± 0.01	0.25^{b} ± 0.01	0.033
C20:3n6	0.40^{a} ± 0.05	0.37^{a} ± 0.05	0.29^{ab} ± 0.06	0.31^{ab} ± 0.05	0.15^{b} ± 0.05	0.045
C20:3n3	0.78^{a} ± 0.07	0.78^{a} ± 0.07	0.37^{b} ± 0.09	0.30^{b} ± 0.08	0.31^{b} ± 0.08	0.001
C20:4n6	0.66^{a} ± 0.03	0.56^{b} ± 0.03	0.48^{b} ± 0.04	0.52^{b} ± 0.03	0.50^{b} ± 0.03	0.011
C20:5n3	1.90^{a} ± 0.45	2.13^{a} ± 0.40	1.24^{ab} ± 0.58	0.25^{b} ± 0.53	0.46^{b} ± 0.47	0.020
C22:6n3	0.52 ± 0.07	0.61 ± 0.06	0.42 ± 0.08	0.50 ± 0.07	0.49 ± 0.07	0.251
Total PUFA	22.05^{c} ± 0.74	25.24^{b} ± 0.67	25.25^{b} ± 0.97	29.46^{a} ± 0.80	28.71^{a} ± 0.78	<0.0001
PUFA:SFA	0.60^{c} ± 0.07	0.79^{bc} ± 0.06	0.90^{b} ± 0.09	1.40^{a} ± 0.07	1.43^{a} ± 0.07	<0.0001
Total n-6 fatty acids	16.77^{c} ± 0.46	17.72^{c} ± 0.42	18.40^{bc} ± 0.58	20.79^{a} ± 0.50	19.90^{ab} ± 0.49	0.0002
Total n-3 fatty acids	5.27^{b} ± 0.72	7.52^{a} ± 0.66	8.29^{a} ± 0.94	8.67^{a} ± 0.78	8.81^{a} ± 0.76	0.013
n-6:n-3	3.20^{a} ± 0.15	2.41^{b} ± 0.13	2.52^{b} ± 0.18	2.47^{b} ± 0.16	2.28^{b} ± 0.15	0.002

^{a,b,c,d}Row means with different superscripts differed significantly ($P \leq 0.05$)
[1]Saturated fatty acids
[2]Monounsaturated fatty acids
[3]Polyunsaturated fatty acids

5.4 Discussion

Ostrich feathers have the lowest income contribution to the market at an estimated 10%, compared to leather contributing 50% and meat 40% of the income generate by the ostrich industry (2018, Dr. A. Olivier, Pers. Comm., South African Ostrich Business Chamber, PO Box 952, Oudtshoorn, 6620, South Africa). Research on the quality and production of ostrich feathers are limited and findings have not been fully implemented. Due to the lower importance of feather production (since 1975), chances are that research may not be focussed directly on improving feather quality of ostriches within the near future (Swart *et al*., 1984; Brand & Cloete, 2015).

When referring to Table 5.3, differences between treatment diets are only relevant within the chick body short and unmarketable feathers, feather classes. The 100%FFCS resulted in lower chick body short feather yields. Although the 100%FFCS did not differ from the 75%FFCS, it did differ from the rest of the diets. This may bring to consideration that the higher FFCS inclusions in the diet resulted in lower yields of this specific feather class. When observing the unmarketable feather class, the only difference was between the 50%FFCS, resulting in the lowest yield and the rest of the diets that did not differ from each other. In the case of the unmarketable feathers it is difficult to consider that treatment diet were the main effect of influence. The results found in the current study are conflicting with the studies of Viviers (2015), Brand *et al*. (2018) and Carstens (2013), whom in most of the cases found that diet had no effect on feather quality or production. Viviers (2015) however, did find in one of the trials that the birds receiving lower protein concentrations in the diet had lower tail feather yields. Brand *et al*. (2014) found that diets with lower energy concentration might result in lower commercially valuable feather yields. In the last two mentioned studies the treatment diets had different protein or energy concentrations, it is thus more relevant to expect that the nutritional level of the diets would have an effect on feather production. However, in the current study the diets were iso-nutritional and it is less likely that the diets will have a major influence on feather production. Although there were differences within chick body short and unmarketable feathers, there were no differences within total commercially valuable feather yields and total feather income per bird between diets. Considering that diet had no effect on the more important last mentioned traits, the differences regarding the feather traits in this study is of little concern. Knowledge on the effect of diet on feather quality and productions are very limited and does warrant further research in the future.

When observing the leather traits presented in Table 5.4 it is clear that there were only differences observed between diets within skin thickness. The 0%FFCS, 25%FFCS and 50%FFCS resulted in thicker skins. However the 50%FFCS did not differ from the 75%FFCS nor the 100%FFCS, which yielded the thinner skins. These findings are in accordance with the findings of Brand *et al*. (2018), who found that 25% dietary replacement (25LD) of soybean oilcake with sweet lupins resulted in thicker skins. This was speculated to be in correspondence with the heaver slaughter weights of the birds that received the 25LD. This might have been the case seeing that Brand *et al*. (2018) reported on thinner skins than what was found in studies conducted by Cloete *et al*. (2006) and Brand *et al*. (2004) in which cases, the birds had heaver slaughter weights. This might also be the case in the current study, seeing that although not significant, the 75%FFCS and 100%FFCS resulted in slightly lighter end weights (91.3 and 87.9 kg respectively) than the rest of the diets (average of 95.7 kg) (Chapter 4, Table 4.11). This being said, the linear regression model fitted to the data accounted for most of the variation (96.5%) between the diets and indicated that with each incremental increase of FFCS in the diet, the skin thickness decreased. Thus, the lighter end weights of the birds receiving the higher FFCS

inclusion diets might be the reason for these diets resulting in thinner skins. The findings of the present study are in line with that of other studies where it has been found that diet had little effect on leather traits and that traits such as skin size is affected more by growth and thus indirectly effected by diet (Brand *et al*., 2004a; Cloete *et al*., 2006; Engelbrecht *et al*., 2009; Brand *et al*., 2014; Viviers, 2015; Brand *et al*., 2018). Insight on the effect that diet might have on skin traits are still vague and needs to be investigated further to better understand the influence diet may have on these traits.

When examining Table 5.5 it is unexpected to find that there are differences between diets within the moisture and protein contents of the meat. The 0%FFCS, 25%FFCS and 50%FFCS resulted in the lowest moisture contents; while at the same time resulting in the highest protein contents. The 100%FFCS resulted in the highest moisture and lowest protein content. The chemical composition of ostrich meat can be influenced by the type of diet that the bird is fed, especially by the energy:protein ratio of the diet (Hoffman, 2005). The differences in moisture and protein may be the result of the energy:protein ratios of the diets in the grower and finisher phases. The crude protein concentration was somewhat higher for the 0%FFCS and slightly decreasing as the FFCS levels increased in the diets (Chapter 4, Tables 4.5 and 4.6). However, this is unlikely to be the cause, seeing that the 75%FFCS and 0%FFCS had no differences in moisture and protein concentration even though there were slight differences in crude protein concentrations of the diets. Other studies have found that diet influenced the ash and fat concentration of ostrich meat (Viviers, 2015; Brand *et al*., 2018), but there seems to be no literature that can support the findings of the current trial. In most cases studies have found that nutrition did not have an influence on the chemical composition of ostrich meat (Hoffman *et al*., 2005; Carstens, 2013; Dalle Zotte *et al*., 2013). At this stage it is unclear what the reasons are for the results obtained regarding moisture and protein concentration of the meat and further research is required on the effect FFCS may have on these chemical meat characteristics.

Raes *et al*. (2004) reported that the fatty acid profile of the diet fed to ruminants does not necessarily resemble the fatty acid profile that is reflected in the meat or fat tissues. This however, is the case in monogastric animals, as the feed they consume and the animal's tissue will have the same or very similar fatty acid profile. This is because in ruminants, the microorganisms in the rumen and their ability to hydrolyse and hydrogenate unsaturated fatty acids influence the fatty acid profile of meat and fat. Hoffman *et al*. (2005) noted that based on the results of their study, it appears that fatty acid metabolism in ostriches are similar to that in ruminants.

The fatty acids that were the most abundantly found within the fat tissue of the birds were SFA's; C16:0 and C18:0, MUFA's; C16:1 and C18:1n9c, PUFA's; C18:2n6c, C18:3n3 and C20:5n3 (Table 5.6). These fatty acids were also found in abundance in the fat tissue of birds receiving the control diet in the study by Hoffman *et al*. (2005), and is in accordance with what Hoffman *et al*. (2012) found for South African Black ostrich genotypes. One exception is the high concentrations of C20:5n3 fatty acids that were found in the current study, which is not in agreement with the above-mentioned studies. The concentration of C20:5n3 fatty acids decreased as the dietary FFCS inclusion increased, this is due to the higher FFCS inclusion diet (100%FFCS) containing a lower concentration of C20:5n3 (Table 5.2). Long chain fatty acids such as C20:5n3 is not a major fatty acid found within oilseeds; however, C18:3n3 fatty acid can be converted by animal to the longer chain C20:5n3 fatty acid (Gregory *et al*., 2014). This might be the reason why the C20:5n3 fatty acid concentrations were higher in the current study than what was reported by Hoffman *et al*. (2005) and Hoffman *et al*. (2012),

as canola seed is rich in C18:3n3 fatty acids (Centraal Veevoerderbureau, 2004; Orsavova *et al*., 2015). The decrease of total SFA concentrations in the fat tissue can be expected as canola is known for having low SFA concentrations (Orsavova *et al*., 2015), which will lead to the higher dietary FFCS inclusion feed having lower SFA concentrations (Table 5.2). The inverse is true regarding MUFA, as canola is known to have high MUFA concentrations (Orsavova *et al*., 2015), leading to higher concentrations of these MUFA's in the feed and subsequently in the tissue of the birds (Table 5.6). This however, is not the case when examining C16:1 fatty acids. There were no differences between the 0%FFCS and 100%FFCS feeds for the C16:1 concentration, although the fat tissue of the 0%FFCS fed birds had a higher concentration of C16:1 fatty acids than the 100%FFCS fed birds. When looking at the PUFA concentrations in the feed (Table 5.2), a similar phenomenon as for the C16:1 fatty acids is seen. The 0%FFCS feed had a higher PUFA concentration than that of the 100%FFCS feed, which is not reflected within the fat tissue of the birds, where the 0%FFCS fed birds had lower PUFA concentrations than that of the 100%FFCS fed birds. This might be due to the ostrich having the ability to breakdown fibre through microbial fermentation in the hindgut (Swart *et al*., 1993), which also enables them to metabolise fatty acids in a similar fashion to ruminants, as Hoffman *et al*. (2005) found. The same can be seen with regards to total n-6, n-3 fatty acids and the n-6:n-3 ratio, with feed fatty acid ratios not being reflected within the fat tissue of the birds. This is in accordance with the reports of Raes *et al*. (2004) on fatty acid metabolism in ruminants, where the feed fatty acid profile is not always reflected in the animal's tissue.

The inclusion of FFCS in the ostrich diets had a beneficial effect on the fatty acid profile of the fat tissue, which resulted in higher n-3 fatty acids, PUFA and MUFA concentrations. This led to an increase of the PUFA:SFA ratio and a decrease in the n-6:n-3 ratio. All of these changes in the fatty acid profile of the tissue is beneficial in the sense that it improves the health aspects of the tissue for human consumption, making it more favourable and healthier (Connor, 2000; Simopoulos, 2002).

An increase in n-3 fatty acids as a result of FFCS inclusion, may lead to meat and fat becoming rancid at a faster rate, due to oxidative rancidity, which will compromise shelf life and taste (Mazhar *et al*., 1990). Therefore caution should be taken when providing the birds with a feed that will increase the n-3 fatty acid concentration of tissue. It is important to find a compromise between the beneficial increase of n-3 fatty acids in the tissue and the detrimental effect of rancidity. Further research is thus warranted to establish to what extent FFCS affects the fatty acid profile of the meat of ostriches, and to what extent can FFCS be included into ostrich diets without jeopardizing shelf life and taste of the meat.

5.5 Conclusion

It can be concluded from the results of the current study that FFCS had very little influence on the end products of ostriches. When considering that there were no differences between diets for the total commercially valuable feather yields and total feather income per bird, it is recommended that FFCS can be included into the diet of slaughter ostriches up to the maximum inclusion levels evaluated in the this trial.

When considering the influence of FFCS on leather traits, it seems that higher inclusions of FFCS resulted in thinner skins, which is undesirable. This may be due to the higher FFCS inclusion diets which resulted in somewhat slower growth. Thus to prevent the undesirable effect that high levels of FFCS may have on skin thickness it should rather not be included at levels higher than that of the 75%FFCS diets evaluated in this trial, in order to ensure a more desirable skin thickness.

It would seem that the FFCS inclusion's influence on the chemical composition of the meat might lead to lower protein concentrations. Although the differences were very small, it is recommended not to exceed the inclusion levels used for the 75%FFCS diets during this trial until further research is done

Inclusion of FFCS in the diets had a beneficial effect on the fatty acid profile of the fat tissue, which may hold advantages with regards to human health. However, the inclusion of dietary FFCS might cause meat and fat to become rancid at a faster rate, which will decrease shelf life and palatability. Thus further research needs to be conducted to establish if dietary FFCS inclusion will cause an increase in the rate at which oxidative rancidity takes place.

Overall, it is recommended that full-fat canola seed can be used to replace 75% (not exceeding inclusion levels used within each phase in this trial) of the soybean oilcake meal in slaughter ostrich diets, without affecting the end products produced by ostriches and still achieve similar results as the current standard commercial diets.

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Chapter 6

General Conclusion and future prospects

The overall focus of this study was to evaluate the viability of full-fat canola seed (FFCS) inclusion in slaughter ostrich diets as an alternative locally produced protein source, replacing currently imported soybean oilcake meal. Full-fat canola seed is one of the few protein sources that are produced in the Western Cape area of South Africa, with the ostrich industry also primarily situated within this region. Therefore the effects that dietary FFCS inclusion may have on the production parameters of slaughter ostriches and the end products that the birds produce were examined.

Before a trial was conducted to investigate the influence of FFCS inclusion levels in the diets on production parameters, a preference trial (Chapter 3) was conducted. This trial was undertaken to establish if the ostriches had a palatability preference towards certain FFCS inclusion levels. This was done by measuring dry matter intake (DMI) in a free choice feeding system, with additional measurement of feed colour to investigate if feed colour contributed to the feed preference. In the preference trial it was found that the 25%FFCS (25% replacement of soybean oilcake meal) was favoured by the ostriches and that the other replacement rates showed no differences in DMI. The concern that the taste of or anti-nutrient content in FFCS are the factors responsible for these results can be ruled out, as the higher FFCS replacement rate diets had higher concentrations of these substances and did not differ from the control diet with no FFCS inclusion. A similar conclusion can be drawn regarding feed colour, as feed colour attribute differences did not explain the higher DMI of the 25%FFCS. The ostriches preferred a small amount (6.8% of total feed inclusion) of FFCS in their diets and it can be considered that for some reason the ostriches favoured the combination and ratio of the other raw materials and FFCS when 25% of the soybean oilcake meal was replaced. Further research needs to be conducted to establish why this replacement rate was favoured.

During the growth and productions trial (Chapter 4), the varying levels of FFCS in the diets were evaluated over the production phases (starter, grower and finisher) and the entire trial period. Parameters such as dry matter intake (DMI), average daily gain (ADG), feed conversion ratio (FCR), end weight and at the end, some slaughter traits such as cold carcass weight and dressing percentage were measured. From the knowledge gained during the growth and production trial it is accurate to state that canola can be included in slaughter ostrich diets within the ranges evaluated in this trial, while still achieving high performance that can compete with that of the standard commercial diets fed to birds at present. One concern however, is that during the grower phase caution needs to be taken when more than 75% (20.6% total inclusion in diet) of soybean oilcake meal is replaced with FFCS. This may lead to reduced performance and it is recommended not to exceed this inclusion level during the grower phase. One other uncertainty is that the 50%FFCS over the entire trial period resulted in more abdominal fat accumulation. Although the 50% FFCS had higher fat pad weights, the birds obtained the same cold carcass weights (after fat was trimmed), dressing percentage and right thigh weights. Birds may have a high ADG on this diet, which can lead to maturity being reached earlier and birds being slaughtered earlier bring about savings on feeding cost. Further research on the factors causing the poorer performance of animals on the high FFCS inclusion diets during the grower phase is warranted. The same goes for the influence of the 50%FFCS replacement diets on abdominal fat synthesis.

Birds used in the growth and production trial of Chapter 4 were also used to evaluate the effect of dietary FFCS inclusion on the end products that ostriches produce (Chapter 5). Dietary FFCS inclusion had very little influence on the end products of slaughter ostriches. The varying levels of FFCS in the diets had no detrimental effect on the total commercially valuable feather yields and total feather income per bird, it is thus recommended that FFCS can be included into the diet of slaughter ostriches up to the maximum inclusion levels evaluated in this trial. When considering the influence of FFCS on leather traits, it seems that higher inclusions of FFCS resulted in thinner skins which are undesirable. Thus to prevent this, caution should be taken when including FFCS at levels higher than that of the 75%FFCS replacement diets evaluated in this trial. It would seem that the FFCS inclusion's influence on the chemical composition of the meat may lead to lower protein concentrations, although the differences were small, it is recommended not to exceed the inclusion levels used for the 75%FFCS replacement diets during this trial until further research is done

Inclusion of dietary FFCS had a beneficial contribution with regards to the fatty acid profile of the fat tissue and may hold benefits with regards to human health. However, the inclusion of dietary FFCS might cause meat and fat to become rancid more rapidly, which will decrease shelf life and cause an unpleasant taste. Thus further research need to be conducted to establish if dietary FFCS inclusion will cause an increase in the rate at which oxidative rancidity takes place.

With the grower phase as an exception, canola can be utilised as the sole plant protein source (100% replacement of soybean oilcake meal) in slaughter ostrich diets up to the levels included during this study (31.3% for starter phase and 25.1% for finisher phase), with no detrimental effects on production or slaughter performance. However it is recommended that full-fat canola seed can be used to replace 75% (not exceeding inclusion levels used within each phase in this trial) of the soybean oilcake meal in slaughter ostrich diets, without affecting the end products produced by ostriches and achieving the same results as the current standard commercial diets. A possible explanation for the high FFCS inclusion diets' negative influence is that the high fibre concentration within these diets depressed DMI and thus other production parameters, as fibre can also be considered as an anti-nutritional factor. Seen that FFCS is known to have high fibre content this explanation is feasible. Nonetheless, more knowledge needs to be gained concerning why the birds performed poorer during the grower phase when receiving higher dietary FFCS inclusion levels. The effect of the FFCS inclusion levels on the leather thickness and meat composition also warrants further research. This additional research will aid in better understanding the economic impact that FFCS inclusion in diets may have on the ostrich industry and potential elevation of profit margins.

Annexure A

Vitamin and mineral premix composition mixed into the feed during the full-fat canola inclusion trial

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 2.5kg into 1 ton of feed, on an as fed basis.