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Feed preference of grower ostriches consuming diets differing in *Lupinus angustifolius* inclusion levels

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Abstract

Feed costs contribute the largest proportion of the input costs of slaughter birds in an intensive ostrich production unit. Alternative, cheaper feedstuffs, such as lupins (sweet and bitter cultivars), were therefore evaluated to determine the optimal lupin inclusion level in ostrich rations without affecting feed preference and intake. Sixty South African Black ostriches were randomly divided into ten paddocks of six birds per paddock. Three trials, with five different experimental diets, were conducted to investigate the diet preference of grower ostriches in a free-choice system. Feed and water were supplied *ad libitum*. The position of the diets in the successive paddocks was varied by rotating the five feed troughs in a clockwise direction, but within each paddock the position of each feeder and diet stayed the same throughout the three trials. In the first two trials, sweet (trial 1) or bitter (trial 2) lupins replaced soybean oilcake meal to have 0, 7.5, 15, 22.5, and 30% lupin inclusion levels in the diet. In trial 3 the soybean oilcake meal was replaced with either sweet or bitter lupins to have dietary inclusion levels as follows: 0% lupins, 15% sweet, 15% bitter, 30% sweet, or 30% bitter. The daily intake per group for each diet was monitored over a period of five days each. The average initial body weight of the birds was 73.6 ± 0.5 kg. No interaction was found between day and diet for the three trials and dry matter intake (DMI) did not differ between the five treatments for any of the three trials. In the second trial the birds tended to show a preference for the 7.5% bitter lupin inclusion level and discriminated against the 15% and 30% bitter lupin inclusion levels. Regression analysis of DMI on lupin inclusion rates revealed no significant trends. In conclusion, the study revealed that soybean oilcake meal can be replaced in the diets of grower ostriches by sweet lupin inclusion levels up to 30%, without any significant detrimental effect on diet preference and feed intake.

Keywords: Alkaloids, dry matter intake, feed palatability, lupins, visual appearance of the feed

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Introduction

Feed costs make the largest proportion (*ca*. 75%) of the input costs of slaughter birds in an intensive ostrich production unit (Brand *et al.*, 2000; Brand & Gous, 2006; Jordaan *et al*., 2008). Volatile feed prices, seasonal droughts, exchange rates, and market trends (consumer preference and economic cycles) have a large effect on the profitability of ostrich production. It is thus of cardinal importance to optimise the aspects of the ostrich production unit which can be controlled by the producer, such as nutrition (Carstens, 2013). Least-cost diet formulations and the use of alternative protein sources are two ways of decreasing feeding costs; however it is important that this does not have a detrimental effect on the quality of the end products.

Protein sources are becoming scarcer and more expensive, especially for use in animal feeds (Brand *et al*., 2004a; Laudadio & Tufarelli, 2011). Depending on the feeding phase (pre-starter, starter, grower or finisher), protein composes up to 22.5% of a balanced diet (Brand & Gous, 2006). Ostrich producers are therefore looking for alternative, locally-produced protein sources that are less expensive but still deliver acceptable production yields. Lupins have been identified as one possibility. It has already been successfully included in the diets of both monogastric (Brand *et al*., 1995) and ruminant animals (Brand *et al*., 1992; Brand *et al*., 1997), and in some cases have effectively replaced previously-used protein sources such as

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soybean oilcake meal. However, it is important to note that due to the presence of anti-nutritional factors, such as alkaloids, lupins can only be included at certain levels for efficient utilisation and to prevent undesirable effects (Brand & Brandt, 2000; Tufarelli *et al*., 2015). This problem is to some extent eliminated by the presence of sweet (low in alkaloids, <0.1%) and bitter (alkaloid-rich, 0.1 – 4.0%) varieties within the species. To reduce the risk of toxicity an alkaloid level of less than 0.6 g/kg is deemed suitable for animal feeds (McDonald *et al*., 2011). The low alkaloid content of the sweet lupin varieties makes their use in diets with prolonged intakes of little concern regarding toxicity for the animal (Laudadio & Tufarelli, 2011).

According to Ferguson *et al.* (2002), under certain circumstances, commercial farm animals can make a rational choice between feeds according to their nutritional needs. Brand *et al.* (2004b) provided five types of lupins to young ostriches in a free-choice system and noted that in addition to smell and taste, colour and previous exposure to a certain type of feed may have an influence on feed preference. It has been found in the industry that young growing and finisher birds in feedlots refuse to eat feed when the composition or physical characteristics are suddenly changed. This phenomenon is generally observed when feed with a relatively green colour is changed to a feed that is less green in colour. Birds therefore need to be gradually exposed to a new feed to avoid a decrease in intake and consequently a drop in production (Brand, 2008).

Milton *et al*. (1994) found during a field study on food selection by ostriches in Southern Africa that ostriches did not feed on toxic plants. The authors assumed that the birds identified these species by sight but also suggested that taste and smell may also play a role in determining the palatability of the feed, as while the ostriches were foraging, they occasionally dropped plucked plant material. It therefore seems that when ostriches check the quality of a feed they conduct a preliminary visual inspection, as well as utilising taste and smell. Chemoreceptive events in the mouth and olfactory epithelium are responsible for the final recognition and selection of the feed. These structures trigger the emotional experience of acute pleasure or displeasure (Kruger, 2007).

The aim of this study was to determine to what degree lupins (sweet and bitter cultivars) can be included in ostrich rations without affecting feed preference and intake. A regression analysis of the mean DMI per bird per treatment diet was performed over the different lupin inclusion levels (%) to establish the feed intake (kg/day) of ostriches when diets containing different levels of lupins (sweet and bitter cultivars) were fed. The time that the birds were exposed to the different diets was also taken into consideration to determine if the time of feeding had any effect on their feed preference. The colour of the feed was determined by the CIE Lab-System in to determine whether the diets in the respective trails were similar in colour.

Materials and methods

The study was conducted in July 2015 at the Oudtshoorn Research Farm in the Klein Karoo region of South Africa (situated at longitude 22°15’ E and latitude 33°37’ S at an altitude of 300 m above sea level). The experimental design included 60 South African Black ostriches of 43 weeks of age that were randomly divided into 10 paddocks with six birds per paddock. The average initial body weight of the birds was 73.6 ± 0.5 kg. Ethical clearance (R14/108) for this study was granted by the Elsenburg ethical committee.

Three trials with five experimental diets per trial were conducted to investigate the diet preference of grower ostriches in a free-choice system. In Trials 1 and 2, soybean oilcake meal was replaced by sweet (Trial 1) or bitter (Trial 2) lupins in a step-wise manner to produce five experimental diets with lupin inclusion levels of 0%, 7.5%, 15%, 22.5%, and 30%. Trial 3 differed from Trial 1 and 2 in that soybean oilcake meal was replaced by alternatively sweet or bitter lupins to provide diets with 0% lupins, 15% sweet, 15% bitter, 30% sweet, and 30% bitter lupin inclusion levels. The positions of the feeders containing each diet in the successive paddocks were altered by rotating the five feed troughs in a clockwise direction, but within each paddock the specific position of each feeder and diet stayed the same throughout the three trials. The feed troughs were spread more or less evenly within each paddock and provided sufficient feeding space per bird. The approximate dimensions of the feed troughs were 46 cm x 23 cm x 20 cm and of the paddocks, 32 m x 30 m. Both feed and water were provided *ad libitum,* with the dimensions of the water buckets being 29 cm x 20 cm x 15 cm.

The daily intake of each diet per group was monitored over a period of five days for each trial. This was done by weighing back the refusals of the day and subtracting it from the amount of feed offered during the day. The feed in the feed troughs was mixed twice daily (early in the morning and at midday) by hand to stimulate feed intake. The recording of feed provided and feed refused occurred at the same time every day. Despite inclement weather conditions on certain days of the trial periods, data capturing was completed without disruptions.

The treatment diets were formulated using Mixit2+ feed formulation software® (Agricultural Software Consultants Inc., San Diego, USA). The raw material composition of the sweet and bitter lupins and soybean oilcake meal used in the diet formulations of this study can be found in Table 1. The raw material samples

were grounded using a RetschTM ZM200 sample mill (Haan, Germany) with a 1.5 mm screen to create a meal with a consistent particle size. Thereafter the raw materials were analysed using the methods of the Association of Official Analytical Chemists (AOAC, 2002) for dry matter (DM) (method 934.01), ash (method 942.05), crude protein (CP) (method 976.05), crude fibre (CF) (method 962.09), ether extract (EE) (method 920.39), acid detergent fibre (ADF) (Goering & van Soest, 1970), neutral detergent fibre (NDF) (Robertson & van Soest, 1981). The calcium (Ca) and phosphorous (P) values were analysed using method 6.1.1 (Dry Ashing) of the Agri Laboratory Association of Southern Africa guidelines (ALASA) (ALASA, 1998).

Table 1 Raw material composition of the sweet and bitter lupins and soybean oilcake meal used in the diet formulations of this study

Nutrient component (g/kg)	Sweet lupins	Bitter lupins	Soybean oilcake meal
Dry matter	902.5	898.7	910.8
Ash	29.5	26.2	62.5
Crude protein	309.4	313.8	463.1
Crude fibre	154.0	156.3	32.0
Ether extract	48.9	42.2	10.3
Neutral detergent fibre	244.8	231.9	81.9
Acid detergent fibre	196.9	189.4	44.3
Calcium	2.60	2.60	2.90
Phosphorous	4.90	4.50	8.30

The formulations of the diets are presented in Tables 2 - 4. The experimental diets were mixed, milled and pelleted at the Kromme Rhee Research Farm (situated 18°50' E, 33°37' S with an altitude of 177 m above sea level) and transported to the Oudtshoorn Research Farm. Tables 2 - 4 also provide the nutritional compositions of the experimental diets. These values were determined for samples randomly collected during the feed mixing and pelleting process. The samples were ground using a RetschTM ZM200 sample mill (Haan, Germany) with a 1.5 mm screen to create a meal with a consistent particle size. Thereafter, all samples of the same experimental diet were pooled and analysed for dry matter (DM), crude protein (CP), ether extract (EE), ash, crude fibre (CF), acid detergent fibre (ADF), neutral detergent fibre (NDF) and *in vitro* organic matter digestibility (IVOMD) using a Bran + Luebbe InfrAlyzer 500 near infrared reflectance spectrometer (IA-500) (NIRS). The samples (*ca.* 6.0 g) were individually presented in closed cups and scanned in the reflectance mode at between 1100 - 2500 nm in the near-infrared region with 2 nm intervals, acquiring 701 data points for each sample. The spectroscopic measurements were interpreted using Bran + Leubbe SESAME Version 2.00 software (Bran + Luebbe GmbH, Norderstedt, Germany). The ME (MJ/kg feed) was calculated using the following equation: ME = 0.015 x IVOMD (g/kg DM) (Van der Honing & Alderman, 1988). The IVOMD was determined by an adaptation of the method of the two-stage rumen fluid-pepsin technique described by Tilley and Terry (1963). It involves firstly 48 hour fermentation by rumen micro-organisms in a buffer solution, followed by 48 hour pepsin-hydrochloric acid digestion. The residue represents the indigestible part of the sample.

The total alkaloid contents of the finely-ground pooled feed samples containing either the sweet (Eureka) or bitter (SSL 10) *Lupinus angustifolius* cultivars were determined as described by Boschin *et al*. (2008), with minor modifications. The sample preparation method was modified by extracting the total alkaloid content directly using a 50:50 methylene dichloride:methanol mixture (MDC:MeOH). GC-MS with a 30 m x 0.25 mm, internal diameter 0.25 μm, AT-Wax capillary column was then used to analyse the total alkaloid content. The temperature program was as follows: 150 °C for 5 minutes increased by 5 °C per minute up to 300 °C then maintained at 300 °C for 15 minutes. Analyses were performed in split mode with a split ratio of 1:25. The injection volume was 1 μL, injection temperature 250 °C, interface temperature 300 °C and the acquisition was from *m*/*z* 50 to 450. The source operated in EI mode at eV. The total alkaloids were identified using Mass library (Agilent) and the detection limit for quantifying the total alkaloids was 100 g/ml. However, no alkaloids were found in the respective feed samples at this detection limit. Therefore, the sweet and bitter lupin cultivars used in this study were the same cultivars (sweet *L. angustifolius* and bitter *L. angustifolius*) used by Smith (2005). The spectrophotometry method described by Von Baer *et al*. (1978) was

used to determine the total alkaloid content of these cultivars in the study by Smith (2005). This method is a quantitative determination of total alkaloids with bromocresol purple at 405 nm. The total alkaloid content of the sweet and bitter lupin cultivars in the study by Smith (2005) was 49.1 mg/kg and 15 204.5 mg/kg respectively. These values were used to calculate the estimated amount of total alkaloids of the five dietary treatments of this study for each of the three trials (Tables 2 - 4).

Table 2 The formulation and nutritional composition (as fed basis) of five treatment diets containing different sweet lupin inclusion levels fed to grower phase slaughter ostriches (Trial 1)

Raw materials (kg/ton)	Diet number and percentage lupin inclusion level				
	1 (0%)	2 (7.5%)	3 (15%)	4 (22.5%)	5 (30%)
Maize meal	590.6	544.9	499.2	453.5	407.8
Soybean oilcake meal	149.3	111.0	74.7	37.3	0.00
Sweet lupins	0.00	76.5	152.9	226.5	300.0
Lucerne meal	186.4	193.5	200.5	210.6	220.7
Molasses powder	25.0	25.0	25.0	25.0	25.0
Monocalcium phosphate	17.4	17.2	16.6	15.9	15.2
Limestone	14.5	14.8	15.0	15.3	15.5
Salt	10.0	10.0	10.0	10.0	10.0
Synthetic lysine	0.87	0.76	0.65	0.53	0.42
Synthetic methionine	0.41	0.43	0.45	0.46	0.48
Mineral and vitamin premix*	5.00	5.00	5.00	5.00	5.00
Nutrient component					
DM[1] (g/kg)	893.9	911.2	900.1	911.8	901.5
ME MJ/kg feed[2]	13.4	13.7	13.5	13.7	13.5
IVOMD[3] (g/kg)	851.2	838.1	841.3	825.5	827.2
CP[4] (g/kg)	160.3	163.5	169.9	159.9	175.8
Ash (g/kg)	90.3	99.3	99.5	111.1	100.8
EE[5] (g/kg)	22.8	28.0	26.8	28.2	33.1
CF[6] (g/kg)	67.1	89.2	74.7	88.9	88.1
ADF[7] (g/kg)	97.6	124.3	108.1	130.5	119.7
NDF[8] (g/kg)	166.4	206.5	173.0	204.2	188.0
Total alkaloid content (ppm)	0.00	3.68	7.37	11.0	14.7

*Refer to APPENDIX 1 for the composition of the vitamin and mineral premix for grower ostriches
[1]Dry matter
[2]Metabolisable energy
[3]*In vitro* organic matter digestibility
[4]Crude protein
[5]Ether extract
[6]Crude fibre
[7]Acid detergent fibre
[8]Neutral detergent fibre

Table 3 The formulation and nutritional composition (as fed basis) of five treatment diets containing different bitter lupin inclusion levels fed to grower phase slaughter ostriches (Trial 2)

Raw materials (kg/ton)	Diet number and percentage lupin inclusion level				
	1 (0%)	**2 (7.5%)**	**3 (15%)**	**4 (22.5%)**	**5 (30%)**
Maize meal	590.6	544.9	499.2	453.5	407.8
Soybean oilcake meal	149.3	112.0	74.7	37.3	0.00
Bitter lupins	0.00	76.5	152.9	226.5	300.0
Lucerne meal	186.4	193.5	200.5	210.6	220.7
Molasses powder	25.0	25.0	25.0	25.0	25.0
Monocalcium phosphate	17.4	17.2	16.6	15.9	15.2
Limestone	14.5	14.8	15.0	15.3	15.5
Salt	10.0	10.0	10.0	10.0	10.0
Synthetic lysine	0.87	0.76	0.65	0.53	0.42
Synthetic methionine	0.41	0.43	0.45	0.46	0.48
Vitamin and vitamin premix*	5.00	5.00	5.00	5.00	5.00
Nutrient component					
DM[1] (g/kg)	893.9	902.6	898.0	908.0	902.0
ME MJ/kg feed[2]	12.8	12.6	12.6	12.2	12.3
IVOMD[3] (g/kg)	851.2	839.0	839.7	810.5	822.2
CP[4] (g/kg)	160.3	170.2	172.1	168.3	185.3
Ash (g/kg)	90.3	94.5	102.9	98.3	103.8
EE[5] (g/kg)	22.8	26.9	28.6	31.0	29.5
CF[6] (g/kg)	67.1	84.4	79.1	102.9	96.1
ADF[7] (g/kg)	97.6	115.7	112.4	134.8	126.6
NDF[8] (g/kg)	166.4	192.5	186.7	210.0	199.4
Total alkaloid content (ppm)	0.00	1 140.3	2 280.7	3 421.0	4 561.4

*Refer to APPENDIX 1 for the composition of the vitamin and mineral premix for grower ostriches
[1]Dry matter
[2]Metabolisable energy
[3]*In vitro* organic matter digestibility
[4]Crude protein
[5]Ether extract
[6]Crude fibre
[7]Acid detergent fibre
[8]Neutral detergent fibre

Table 4 Formulation and nutritional composition (as fed basis) of five treatment diets containing sweet or bitter lupins at different inclusion levels fed to grower phase slaughter ostriches (Trial 3)

Raw materials (kg/ton)	Diet number and percentage lupin inclusion level				
	1 (0%)	2 (15% Sweet)	3 (15% Bitter)	4 (30% Sweet)	5 (30%Bitter)
Maize meal	590.6	544.9	544.9	407.8	407.8
Soybean oilcake meal	149.3	112.0	112.0	0.00	0.00
Sweet lupins	0.00	76.5	0.00	300.0	0.00
Bitter lupins	0.00	0.00	76.5	0.00	300.0
Lucerne meal	186.4	193.5	193.5	220.7	220.7
Molasses powder	25.0	25.0	25.0	25.0	25.0
Monocalcium phosphate	17.4	17.2	17.2	15.2	15.2
Limestone	14.5	14.8	14.8	15.5	15.5
Salt	10.0	10.0	10.0	10.0	10.0
Synthetic lysine	0.87	0.76	0.76	0.42	0.42
Synthetic methionine	0.41	0.43	0.43	0.48	0.48
Mineral and vitamin premix*	5.00	5.00	5.00	5.00	5.00
Nutrient component					
DM[1] (g/kg)	893.9	900.1	898.0	901.5	902.0
ME MJ/kg feed[2]	12.8	12.6	12.6	12.4	12.3
IVOMD[3] (g/kg)	851.2	841.3	839.7	827.2	822.2
CP[4] (g/kg)	160.3	169.9	172.1	175.8	185.3
Ash (g/kg)	90.3	99.5	102.9	100.8	103.8
EE[5] (g/kg)	22.8	26.8	28.6	33.1	29.5
CF[6] (g/kg)	67.1	74.7	79.1	88.1	96.1
ADF[7] (g/kg)	97.6	108.1	112.4	119.7	126.6
NDF[8] (g/kg)	166.4	173.0	186.7	188.0	199.4
Total alkaloid content (ppm)	0.00	7.37	2 280.7	14.7	4 561.4

*Refer to APPENDIX 1 for the composition of the vitamin and mineral premix for grower ostriches
[1]Dry matter
[2]Metabolisable energy
[3]*In vitro* organic matter digestibility
[4]Crude protein
[5]Ether extract, [6]Crude fibre
[7]Acid detergent fibre
[8]Neutral detergent fibre

The mineral compositions and amino acid profiles of the diets are presented in Tables 5 - 7. To determine the mineral content of the pooled reference samples the finely ground (passed through a 1.5 mm sieve) feed samples were analysed using method 6.1.1 (Dry Ashing) as described in ALASA (ALASA, 1998). A 1 – 3 g sample of each diet was weighed and placed in a porcelain crucible. The crucibles were placed in a muffle furnace and left to ash overnight at 460 - 480 °C. Once the samples had cooled down, 5 ml of 1:1 hydrogen chloride (HCl) was added to each crucible to dissolve the samples. The crucibles were then placed in an oven for 30 minutes at 60 °C for evaporation to take place. The samples were left to cool and then filled to a total volume of 40 ml with deionized water and mixed thoroughly before being filtered into an amber bottle. The mineral concentrations was measured using a Thermo Electron iCAP 6000 Series Inductively Coupled Plasma (ICP) Spectrophotometer (Thermo Electron Corporation, Milan, Italy) fitted with a vertical quartz torch and Cetac ASX-520 autosampler. Concentrations were determined using Merck Titrisol standards with concentrations of 1000 ppm (Merck, Darmstadt, Germany) and calculated using iTEVA Analyst software.

The amino acid profiles were determined using the method described by Grace Davison (2008), through hydrolysis of the samples in hydrochloric acid and high pressure liquid chromatography (HPLC). In a hydrolysis tube, 6 ml of 6 N HCl and 15% Phenol solution was added to 0.1 g of feed sample. Nitrogen was then added and the samples were placed under vacuum. This was done by flushing the hydrolysis tube with the nitrogen to remove oxygen and create an anaerobic environment. The sealed hydrolysis tubes were then placed in an oven for 24 hours at 110 °C to allow complete protein hydrolysis. The samples were left to cool and then filtered using a hydrophylic polyvinylidene difluoride syringe filter (PVDF - 0.45 µm, 33 mm) before being transferred into 1.5 ml Eppendorf tubes. Amino acids were derivatised with *o*-phthalaldehyde and 3-mercaptopropionic acid in borate buffer (Agilent Technologies, Waldbronn, Germany). Reverse-phase Dionex HPLC (Dionex Corporation, California, USA) was used to separate the amino acids on a 3.9 x 150 mm C18 Nova-Pak column (Waters, Ireland) at a 1.1 ml/minute flow rate. L-Amino acid standards (2.5 *µ*mol/ml in 0.1 N HCl) (Thermo Scientific, Illinois, USA) were used to identify the amino acids.

Table 5 The mineral and amino acid composition (as fed basis) of feeds containing five inclusion levels of sweet lupins fed to grower phase slaughter ostriches (Trial 1)

	Diet number and percentage lupin inclusion level				
	1 (0%)	**2 (7.5%)**	**3 (15%)**	**4 (22.5%)**	**5 (30%)**
Minerals					
Calcium (g/kg)	12.2	15.2	14.9	12.2	14.4
Phosphorous (g/kg)	6.00	7.60	7.40	6.40	7.25
Magnesium (g/kg)	2.30	2.60	2.50	2.50	2.50
Sodium (g/kg)	3.88	4.11	5.01	3.56	4.86
Manganese (mg/kg)	210.9	279.8	257.8	345.6	273.6
Copper (mg/kg)	14.3	16.8	16.8	20.3	19.3
Iron (mg/kg)	333.5	332.4	393.4	357.8	268.6
Zinc (mg/kg)	119.0	151.4	137.7	170.2	147.8
Amino acids (g/kg)					
Lysine	9.80	14.0	17.2	14.7	24.6
Methionine	0.30	0.60	0.20	0.10	0.10
Arginine	6.50	8.20	10.0	8.60	13.5
Threonine	4.90	5.50	6.10	5.20	7.30
Tyrosine	5.10	5.80	6.50	5.70	7.90
Aspartic acid	13.0	14.9	16.1	13.6	19.2
Glutamic acid	19.5	23.3	26.4	22.8	33.3
Serine	6.10	7.00	7.90	6.70	9.70
Histidine	2.30	2.60	2.70	2.20	3.10
Glycine	5.30	6.10	6.80	5.80	8.30
Alanine	6.10	6.80	7.40	6.50	8.70
Valine	6.30	7.10	7.60	6.50	8.90
Phenylalanine	6.60	7.40	7.90	6.80	9.20
Isoleucine	5.20	6.00	6.50	5.50	7.80
Leucine	11.2	12.6	13.7	12.1	16.2

Table 6 The mineral and amino acid composition (as fed basis) of feeds containing five inclusion levels of bitter lupins fed to grower phase slaughter ostriches (Trial 2)

	Diet number and percentage lupin inclusion level				
	1 (0%)	**2 (7.5%)**	**3 (15%)**	**4 (22.5%)**	**5 (30%)**
Minerals					
Calcium (g/kg)	12.2	15.2	14.9	12.2	14.4
Phosphorous (g/kg)	6.00	7.60	7.40	6.40	7.25
Magnesium (g/kg)	2.30	2.60	2.50	2.50	2.50
Sodium (g/kg)	3.88	4.11	5.01	3.56	4.86
Manganese (mg/kg)	210.9	262.8	262.9	241.2	321.2
Copper (mg/kg)	14.3	15.8	18.1	15.8	17.2
Iron (mg/kg)	333.5	322.2	385.2	371.8	304.3
Zinc (mg/kg)	119.0	137.7	145.6	120.6	161.8
Amino acids (g/kg)					
Lysine	9.80	13.1	16.4	13.5	23.0
Methionine	0.30	0.24	0.18	0.20	0.06
Arginine	6.50	8.10	9.70	8.78	12.9
Threonine	4.90	5.26	5.63	4.72	6.35
Tyrosine	5.10	5.61	6.13	5.20	7.15
Aspartic acid	13.0	14.2	15.3	13.3	17.6
Glutamic acid	19.5	22.7	25.8	21.9	32.1
Serine	6.10	6.82	7.54	6.36	8.98
Histidine	2.30	2.41	2.51	1.99	2.72
Glycine	5.30	5.87	6.44	5.46	7.59
Alanine	6.10	6.38	6.66	5.56	7.21
Valine	6.30	6.65	7.01	6.08	7.72
Phenylalanine	6.60	7.15	7.71	7.40	8.82
Isoleucine	5.20	5.66	6.11	5.22	7.02
Leucine	11.2	12.0	12.7	10.5	14.2

Table 7 The mineral and amino acid composition (as fed basis) of feeds containing five inclusion levels of either sweet or bitter lupins fed to grower phase slaughter ostriches (Trial 3)

	Diet number and percentage lupin inclusion level				
	1 (0%)	**2 (15% Sweet)**	**3 (15% Bitter)**	**4 (30% Sweet)**	**5 (30% Bitter)**
Minerals					
Calcium (g/kg)	12.2	14.3	14.9	12.9	14.4
Phosphorous (g/kg)	6.60	7.20	7.40	6.90	7.25
Magnesium (g/kg)	2.30	2.50	2.50	2.40	2.50
Sodium (g/kg)	3.88	4.89	5.01	5.31	4.86
Manganese (mg/kg)	210.9	257.8	262.9	273.6	321.2
Copper (mg/kg)	14.3	16.8	18.1	19.3	17.2
Iron (mg/kg)	333.5	393.4	385.2	268.6	304.3
Zinc (mg/kg)	119.0	137.7	145.6	147.8	161.8
Amino acids (g/kg)					
Lysine	9.80	17.2	16.4	24.6	23.0
Methionine	0.30	0.20	0.18	0.10	0.06
Arginine	6.50	10.0	9.70	13.5	12.9
Threonine	4.90	6.10	5.63	7.30	6.35
Tyrosine	5.10	6.50	6.13	7.90	7.15
Aspartic acid	13.0	16.1	15.3	19.2	17.6
Glutamic acid	19.5	26.4	25.8	33.3	32.1
Serine	6.10	7.90	7.54	9.70	8.98
Histidine	2.30	2.70	2.51	3.10	2.72
Glycine	5.30	6.80	6.44	8.30	7.59
Alanine	6.10	7.40	6.66	8.70	7.21
Valine	6.30	7.60	7.01	8.90	7.72
Phenylalanine	6.60	7.90	7.71	9.20	8.82
Isoleucine	5.20	6.50	6.11	7.80	7.02
Leucine	11.2	13.7	12.7	16.2	14.2

The CIE Lab-System describes colour according to three surface colour attributes namely L* (lightness), a* (redness), and b* (yellowness). The L* coordinate represents the lightness (reflection) of the sample, where 0 = black and 100 = white. The a* coordinate signifies the red/green spectrum, where a positive value indicates the degree of redness and a negative value indicates that green pigments are being detected. The b* coordinate characterises the yellow/blue range, with a positive value indicating the degree of yellowness and a negative value indicating the degree of blueness (BYK-Gardner GmbH). The surface colour of the finely ground feed samples was measured using a colour-guide 45°/0° colorimeter with an aperture size of 20 mm and an illuminant/observer ratio of D65/10° (Catalogue number 6805, BYK-Gardner GmbH, Geretsried, Germany). Calibration of the colorimeter was done using the standards provided (BYK-Gardner). The finely ground sample was spread evenly in a petri dish and five repeats were taken per measurement per sample.

Statistical analysis was performed using Statgraphics Centurion (Version 15; Statpoint, Inc., Virginia, USA), SAS Enterprise Guide (Version 9.2; SAS Institute Inc., Cary, USA) and Microsoft Office Excel 2010 (Version 14.0, Microsoft Corporation by Impressa Systems, Santa Rosa, California). Descriptive statistics were performed on the respective CIE Lab-System colour attributes (L*, a* and b*) per diet for each trial to determine whether changes in colour could explain the differences observed in fed intake. A multifactor analysis of variance (ANOVA) was done for all three trials separately to determine which of the two main effects, day and diet, had a statistically significant effect on the mean dry matter intake (DMI) per bird per

treatment diet. The multifactor ANOVA was also used to test whether there was a significant interaction between the two main effects. A one-way ANOVA was conducted to evaluate the mean DMI as well as the %DMI per bird per day by diet. A regression analysis of the mean DMI per bird was done per treatment diet over the different lupin inclusion levels (%) for Trial 1 (sweet lupins) and 2 (bitter lupins). Statistical differences were declared at P <0.05.

Results

Regarding the colour attributes in Trial 1, the L* value of diet 2 differed (P <0.05) from that of diets 3 and 4. The value of diet 1 did not differ from the rest of the diets (P >0.05). No differences were observed for the a* attribute between the respective diets. The positive values are an indication that more red pigments than green pigments were detected in these feed samples. The b* attribute of diet 5 differed (P <0.05) from diets 1, 2, 3 and 4, while diet 4 also differed (P <0.05) from diets 2 and 3 (Table 8). Because all the values are positive, it is an indication that there are more yellow pigments than blue pigments present in the feed.

In Trial 2, the L* attribute of diet 5 differed (P <0.05) from that of diets 2, 3 and 4. The a* attribute of diets 4 and 5 did not differ significantly from each other, but they differed (P <0.05) from the a* value of diet 2. There was no difference between the a* attributes of diet 1, 4 and 5, and the a* values of diets 3, 4 and 5 did not differ significantly. The a* values of diets 1 and 2 did not differ significantly. The b* attribute of diet 5 differed (P <0.05) from the remaining four diets (Table 8).

In Trial 3, the L* attribute of diets 4 and 5 differed (P <0.05) from diets 1, 2 and 3, while the a* attribute of diet 1 differed (P <0.05) from diets 3, 4 and 5. The b* attribute of diet 1 and 3 did not differ (P >0.05) from each other, but did differ (P <0.05) from diets 2, 4 and 5 The b* attributes of diets 2, 4 and 5, differed (P <0.05) from each other and also from diets 1 and 2 (Table 8).

However, while statistically significant differences were observed between the diets for the colour attributes, these differences were small, making little visual difference in the appearance of the feeds.

Table 8 Descriptive statistics (mean ± standard error) of the CIE Lab-System colour attributes (L*, a* and b*) for diets with varying sweet and bitter lupin inclusion levels

Lupin inclusion levels	CIE Lab-System colour attributes per diet and percentage lupin inclusion level		
	L*	a*	b*
Trial 1: Sweet lupins			
0%	57.0^{ab} ± 0.90	3.1^{a} ± 0.20	19.7^{cd} ± 0.14
7.5%	60.0^{a} ± 1.51	3.2^{a} ± 0.25	21.0^{b} ± 0.51
15%	56.4^{b} ± 0.50	3.3^{a} ± 0.09	20.7^{bc} ± 0.04
22.5%	55.0^{b} ± 1.72	3.3^{a} ±0.26	19.3^{d} ± 0.43
30%	60.4^{a} ± 1.14	3.6^{a} ± 0.21	23.5^{a} ± 0.45
Trial 2: Bitter lupins			
0%	57.0^{ab} ± 0.90	3.1^{bc} ± 0.20	19.7^{b} ± 0.14
7.5%	56.3^{b} ± 1.32	2.6^{c} ± 0.30	20.3^{b} ± 0.34
15%	55.5^{b} ± 0.54	4.1^{a} ± 0.09	19.9^{b} ± 0.17
22.5%	55.1^{b} ± 1.29	3.6^{ab} ± 0.29	19.7^{b} ± 0.70
30%	59.4^{a} ± 0.26	3.7^{ab} ± 0.11	22.8^{a} ± 0.29
Trial 3: Sweet and bitter lupins			
0%	57.0^{b} ±0.90	3.1^{c} ± 0.20	19.7^{d} ± 0.14
15% sweet	56.4^{b} ± 0.50	3.3^{bc} ± 0.09	20.7^{c} ± 0.04
15% bitter	55.5^{b} ± 0.54	4.1^{a} ± 0.09	19.9^{d} ± 0.17
30% sweet	60.4^{a} ± 1.14	3.6^{b} ± 0.21	23.5^{a} ± 0.45
30% bitter	59.4^{a} ± 0.26	3.7^{ab} ± 0.11	22.8^{b} ± 0.29

$^{a-d}$ column means with different superscripts differ significantly (P <0.05)

Regarding feed intake, no interaction was found between the two main effects (day and diet) in any of the three trials (P =0.45, 0.88 and 0.99 for trials 1, 2, and 3 respectively) (Table 9).

Table 9 Effect of day on feed intake for the three different feed preference trials containing either sweet or bitter lupins.

Day	Feed intake (kg)		
	Trial 1 Sweet	Trial 2 Bitter	Trial 3 Sweet & Bitter
1	635.57^{ab} ± 23.83	865.17^{a} ± 41.44	884.53^{a} ± 43.31
2	567.00^{bc} ± 23.83	654.70^{b} ± 41.44	739.33^{abc} ± 43.31
3	537.90^{c} ± 23.83	643.37^{b} ± 41.44	820.37^{ab} ± 43.31
4	582.10^{abc} ± 23.83	735.07^{ab} ± 41.44	661.73^{bc} ± 43.31
5	670.57^{a} ± 23.83	727.87^{ab} ± 41.44	633.13^{c} ± 43.31

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

The main effects were therefore investigated individually and one-way ANOVA's were conducted to evaluate the effect of diet on the mean DMI and %DMI per bird per day. The results for both mean DMI and %DMI indicated that feed intake did not differ between the five diets for any of the three trials (Table 10).

Table 10 Least square means ± standard error (LSM ± SE) for the effect of sweet and bitter lupin inclusion levels on the mean DMI and %DMI of grower phase slaughter ostriches

Lupin variety	Treatment diet	Lupin inclusion level (%)	Mean DMI/bird/day (g)	Percentage of DMI/bird/day (%)
Sweet Trial 1	1	0	541.13 ± 35.43	18.11 ± 1.13
	2	7.5	646.20 ± 35.43	21.52 ± 1.13
	3	15	628.07 ± 35.43	20.92 ± 1.13
	4	22.5	583.70 ± 35.43	19.62 ± 1.13
	5	30	594.03 ± 35.43	19.83 ± 1.13
Bitter Trial 2	1	0	776.10^{ab} ± 78.27	21.30^{ab} ± 2.18
	2	7.5	890.60^{a} ± 78.27	24.46^{a} ± 2.18
	3	15	606.57^{b} ± 78.27	16.73^{b} ± 2.18
	4	22.5	695.53^{ab} ± 78.27	19.55^{ab} ± 2.18
	5	30	657.37^{b} ± 78.27	17.97^{b} ± 2.18
Sweet & Bitter Trial 3	1	0	893.00 ± 90.07	23.60 ± 2.34
	2	15 (Sweet)	628.23 ± 90.07	16.77 ± 2.34
	3	15 (Bitter)	672.00 ± 90.07	17.84 ± 2.34
	4	30 (Sweet)	736.97 ± 90.07	19.87 ± 2.34
	5	30 (Bitter)	808.90 ± 90.07	21.92 ± 2.34

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

Regression analysis of DMI per bird per day on lupin inclusion level for trials 1 and 2 revealed no significant trend (Figure 1). A polynomial regression was fitted to both the trial 1 and 2 DMI data, but the quadratic function was non-significant in both cases (P =0.47 and 0.62, respectively). The regression equation for the first trial accounted for 52.56% of the variance while the regression equation for the second trial accounted for 38.45%. These regression models therefore did not fit the data closely and do not describe the effect that lupin inclusion level has on DMI very accurately.

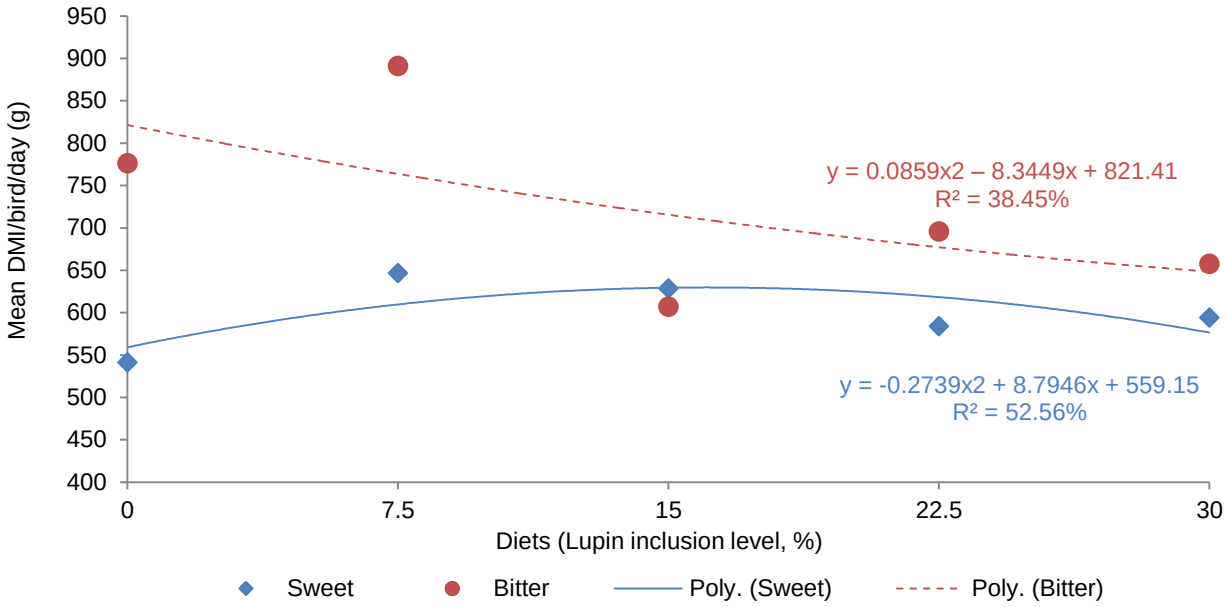

Figure 1 Quadratic functions fitted to the DMI of grower phase slaughter ostriches fed diets containing different inclusion levels of sweet and bitter lupins

Discussion

The results of this study indicated that the feed preference and intake of ostriches is not influenced by the inclusion of sweet lupins in the diet up to 30%. This is unexpected as alkaloids are bitter-tasting compounds, reducing the palatability of the feed (Smith, 2005). Previous studies on poultry and pigs found that lupin inclusion tended to reduce palatability, which result in poor acceptance and growth rates (Petterson & Fairbrother, 1996). The results found in Trial 1 of this study may be due to the low alkaloid content of the sweet lupin diets used. The prolonged consumption of the sweet lupin diets should therefore be acceptable for ostriches of the age groups tested.

Kruger *et al*. (2008) fed a conventional pre-starter mash diet (as formulated by Brand, 2005) artificially flavoured with four different commercially-produced non-toxic food flavourants (sweet, bitter, salty and sour) to ostrich chicks in a free-choice setup with no previous exposure to any of the feeds. It was concluded that the chicks preferred salty feed (34.0%), then sweet (17.9%), control (17.1%), bitter (15.7%), and sour (15.4%). This result could be attributed to the evolution of ostriches in deserts where the availability of good quality water is limited, for ostriches are able to utilise water with high salt levels due to their salt-excretory nasal glands (Kruger, 2007). It was not considered likely that the choice of salty food was directly related to flavour as Brand *et al.* (2008b) found that there were no conventional taste buds present in either two-month-old chicks or adult ostriches. If it is likely that ostriches cannot taste, and that the tendency for the ostriches to prefer the 7.5% bitter lupin inclusion level while discriminating to some extend against the 15% and 30% inclusion levels (Trial 2; Table 10) could therefore be attributed to the small sample size used rather than any particular pattern of feed-choice. It is thus possible that some other factor of the feed apart from its visual appearance, palatability thereof or the birds' previous experience may have played a role in producing this trend, for the bitter lupin diets had a much higher alkaloid content compared to the sweet lupin diets. Discrimination against the bitter lupin diets would thus almost be expected, but during Trial 3 both the mean DMI and %DMI indicated that feed intake did not differ between the five diets.

The oropharyngeal cavity of the ostrich as well as its components (beak, hard palate, pharynx, tongue and the larynx) was studied by Tadjalli *et al*. (2008). It was found that the caudal third portion of the hard palate contains a semi-circular darker area that is covered by many small delicate papillae. The other two thirds of the rostral part of the hard palate, that divides it into two regions, lack papillae. The ostrich also lacks a transverse row of papillae caudal to the infundibular opening at the junction with the oesophagus. While Gentle (1971) stated that chickens have a good sense of taste, Kare and Pick (1960) noted that despite the selection against bitter components when under free-choice conditions, a very high concentration bitter-tasting substances in feed is required over long periods before any reduction in feed intake is

observed. The tendency observed during Trial 2 may therefore warrant further research, since the question of how important taste is in determining palatability in this species might rise.

Ferguson *et al*. (2002) noted that when young pigs were given a choice of diets, they instinctively avoided potentially harmful substances (toxins), anti-nutritional factors or unpalatable components in the feeds. Thereafter, they selected more nutritionally balanced feeds or feeds with more favourable amino acid profiles that would satisfy their requirements for growth and production. The iso-nitrogenous nature of the diets used in this study, together with the low alkaloid contents found in the sweet lupin diets, as well as the seemingly absence of taste in ostriches could explain for the lack of variation in DMI between the five diets during Trial 3.

During the evaluation of lupins in the diets of pigs and poultry, it was found that the maximum lupin inclusion levels for pigs are as follows: 10 - 15% in starter diets, 20 - 25% in grower diets, 30 - 35% in finisher diets and 20% in dry and lactating sow diets (Petterson & Fairbrother, 1996). Inclusion levels of up to 25% of low-alkaloid lupin-seed meal can be tolerated by broiler chickens without affecting growth unfavourably (Brenes *et al.*, 1993). Research has also shown that a maximum inclusion level of 25 - 35% of either *L. angustifolius* or *L. albus* will not affect the laying performance of hens (Edwards and van Barneveld, 1998), while in broiler chicken diets it should not exceed 10%.

Forbes and Shariatmadari (1994) stated that when a single feed is provided, the intake is determined primarily by the energy content thereof. This is supported by Rose and Kyriazakis (1991), who reported that one of the major factors determining the diet selection of poultry and pigs are their nutrient requirements. Brand *et al*. (2012) consequently assumed that male and female South African Black ostriches would select feeds under free-choice feeding conditions according to their protein and energy requirements. In this study, the treatment diets were formulated to be iso-caloric (equal ME levels), while also meeting the requirements of the birds and although the diets did differ slightly in terms of their CP, fat, and CF contents, these differences were unlikely to have been great enough to have had a significant effect on diet selection and DMI (Table 2 - 4). The results from this study, indicate that DMI and %DMI did not differ between the five treatments in any of the three trials, it also suggests that the diets provided satisfied the nutrient requirements of the birds.

Although significant differences were observed in the CIE Lab-System values between the feeds, colour of the feed not differ to any great extent under visual inspection (light yellowish-brown) (Table 8). The differences in colour therefore may have not been great enough to cause any difference in feed preference and intake. It must also be noted that the pattern of feed preference and intake observed during Trial 2 for the DMI and %DMI did not correspond with the differences observed in the colour attributes. It is therefore not clear whether the colour of the feed influenced the feed preference and intake of the birds, but it appears that some other factors apart from colour may have had a more important influence for determining feed preference. This is in contrast to the findings of Bubier *et al*. (1996), who provided strips of insulation tape of different colours (green, white, red, blue, yellow, and black) to chicks and found that the green tape produced the greatest pecking response. This can be related to the herbivorous nature of the ostrich in the wild. The second colour of preference was white, which can be related to the coprophagy of adult dung, which is usually accompanied by white urate deposits. However, the results obtained in this study correspond with the results of Brand *et al*. (2008a), who found that while chicks showed a preference for green plastic strips they did not distinguish between feeds of different colours. In addition, Kruger (2007) found that chicks preferred an untreated pre-starter diet (mash and light brown in colour) to artificially coloured feeds. The question now arises whether ostriches base their choice of feed selection on the colour of the feed and may therefore warrant further research.

According to Forbes and Covasa (1995), feed intake in a free-choice feeding system does not only depend on the metabolic requirements or physiological state of the chicken, but also on factors such as previous experience and social interactions. They advise exposing pullets to all the grains that they may be offered later in life during the rearing period in order to allow them to learn their nutritional characteristics. Rose *et al*. (1986), as well as Forbes and Covasa (1995) also suggested that the type, form and nutrient content of the feed has a profound effect on diet selection. Factors such as trough design, position of the trough, breed, sex, management, and genetics may also contribute to determining which diet is selected by the birds. In this study, lupins were the only component of the diet to which the birds had not been exposed to previously. In addition, all other possibly influential factors were kept constant and were the same for all the paddocks in order to reduce the risk of any outside influences on diet selection.

Conclusion

The results of this study indicate that soybean oilcake meal can be replaced in the diets of grower phase ostriches by sweet lupin inclusion levels of up to 30% without any significant effect on feed selection. The tendency of the birds to discriminate to some extend against higher percentages of bitter lupin diets may warrant further research. In this study the inclusion levels of sweet and bitter lupins, replacing soybean oilcake meal, were only up to 30%. Further studies will be required to evaluate the effect of higher levels of lupins on feed intake. Lupins are widely used as raw material in livestock feeds as it is cost-competitive with multiple other protein sources. Results from this study may assist in establishing a potential market for lupins as well as improving the profit margins of ostrich farmers and the local grain legume industry.

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Authors' Contributions

Concept and design: TSB; data collection and analysis: JAE; drafting of paper: JvdM; critical revision and final approval of version to be published: LCH.This statement is to certify that all the authors of this paper made substantial contributions to conception and design, and/or acquisition of data, and/or analysis and interpretation of data. All the authors have seen and approved the manuscript being submitted.

Conflict of Interest Declaration

The authors certify that they have no affiliations with or involvement in any organization or entity with financial or non-financial interest in the subject matter and materials discussed in this manuscript.

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APPENDIX 1

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

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

**RECOMMENDATION*: To make half ton of feed divide premix pack into two parts.