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CHEMICAL COMPOSITION AND SENSORY EVALUATION OF MEAT OF *Tswana* HENS FED DIFFERENT PROTEIN SOURCES: MOPANE WORM, BAMBARA GROUNDNUT AND MORAMA BEAN

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ABSTRACT: This study investigated the chemical composition and sensory evaluation of meat of *Tswana* hens fed diets containing mopane worm (*Imbrasia belina,* Westwood) or Bambara groundnut (*Vigna subterranea* (L) Verde) or morama bean (*Tylosema esculentum,* Burchell A. Schreiber) as different protein sources under intensive management system. Sixty Tswana hens (25 weeks old) were bought from a local farmer and reared up to 38 weeks of age on diets containing *T. esculentum* or *V. subterranea* or *I. belina* and a commercial layer diet as a control. Experimental diets met the nutritional composition of control diet. Whole thighs, drumsticks obtained from the carcasses at week 28, 33 and 38 were deboned. The thigh, drumstick meat and bone were analysed for phosphorus (P), calcium (Ca) and potassium (K) using AOAC methods. Furthermore, boiled 2 x 5 cm portions of drumsticks, breasts and thighs were organoleptically assessed for flavour, odour, juiciness, tenderness and firmness by untrained panelists (15 males and 15 females) using a 5-point Likert scale. Bone P and Ca were high in all treatment diets at 28 weeks compared to 38 weeks of age. The meat DM, CP, P, Ca and K and bone P, Ca and K contents were not significantly affected by treatment diets. Organoleptic quality from all treatment groups and across different ages received moderate (3.52) to good rating (4.13). In conclusion, *Imbrasia belina* (Mopane worm) or *Vigna subterranea* (Bambara groundnut) or *Tylosema esculentum* (Morama bean) can replace soybean meal in *Tswana* hens without compromising meat and bone chemical composition and also organoleptic qualities.

Keywords: Crude protein, Minerals, Organoleptic Attributes, Protein Sources, *Tswana* hens

INTRODUCTION

The main constituents of layer meat are water, protein and fat (FAO, 2003). Increases in collagen and elastin, the proteins forming the connective muscle tissues are related to the toughness of the meat of layer birds. Age and sex of a bird influence the fat content, as does a high caloric diet. Layer meat provides a good source of high quality protein, iron and phosphates and the B vitamins (riboflavin and niacin). The vitamin content of the meat is influenced to a large degree by the vitamin content of the feed consumed (FAO, 2003).

Meat quality is a generic term used to describe properties and perception of meat that includes attributes such as carcass composition and the eating quality (Maltin et al., 2003). Consumer's sensory evaluation of eating quality takes into account tenderness, juiciness and flavour of meat as the most important elements. Tenderness and juiciness contribute to meat texture and form the basis for poultry meat quality (Dransfield, 1995). The quality of meat is changed from birth and through the growth period until the meat is mature enough for consumption.

Diet is an important factor in meat quality since it determines the quantity of fat that in turn affects meat flavour (Sokołowicz et al., 2016). Feed costs per unit increase in body weight are of great practical and economic importance in assessing the productivity of poultry meat. The major challenge of producing poultry meat goes to the cost of dietary protein (Rezaeipour et al., 2016); hence reduction of feed costs is a major concern for poultry producers. Poste (1990) stated that replacement of component of a maize soybean diet with a locally available alternative requires the assessment not only of the poultry performance and carcass chemical characteristics but also of the resulting meat quality. Since *Imbrasia belina* (Mopane worm) or *Vigna subterranea* (Bambara groundnut) or *Tylosema esculentum* (Morama bean) are consumed by human being, it could be assumed that these protein sources should also be an ingredients that could be included successfully in Tswana hen's diets.

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The protein content of Morama bean is 31.4 % while the oil content is twice as high as that of soya bean and is comparable to that of groundnut (Bower et al. 1988). Bambara groundnut is an indigenous African crop grown primarily for its seeds which are eaten fresh when semi ripe, as a pulse when dry and mature, or ground into a flour. Its crude protein is 17-25% (Belewu et al. 2008). Madibela et al. (2009) reported protein content of mopane worm to be about 56.8%. There is little information on chemical composition and organoleptic attributes of meat from *Tswana* hens fed diets formulated using local ingredients as protein sources under intensive system in Botswana. Therefore, a study was undertaken to investigate the composition and sensory evaluation of meat of hens fed diets with *Imbrasia belina* (mopane worm) or *Vigna subterranea* (Bambara groundnut) or *Tylosema esculentum* (Morama bean) as different protein sources.

MATERIALS AND METHODS

Study area

The experiment was conducted at the Botswana College of Agriculture (now Botswana University of Agriculture and Natural Resources), Content farm at Sebele, located 10 km north of Gaborone (the capital city of Botswana) from February to May 2010. The study site is located on 25°56' 29.1" east and 24°35'18.8" south at an altitude of 983 metres above sea level (Manyeula et al., 2018). The average annual rainfall is 450 mm and average daily temperature 30°C (Aganga and Omphile, 2000).

Birds and management

A total of 60 normal feathered *Tswana* hens (15 birds per treatment) aged 25 weeks (point of lay) were fed diets containing *I. belina*, *T. esculentum*, *V. subterranea* and a commercial layer diet (control) up to 38 weeks of age. The hens were reared intensively and supplemented with maize before the start of the study. At the beginning of the study, hens were individually weighed and tagged, then were vaccinated against Newcastle disease and infectious bursal disease (IBD). Clean water and feed were provided *ad libitum* for 90 days. Birds were raised on a deep litter system and received 16 hours of light daily throughout the experimental period.

Birds and slaughter

Three *Tswana* hens from each treatment (one from each replicate) were slaughtered at 28, 33 and 38 weeks of age. A humane slaughter method was carried out at Botswana University of Agriculture and Natural Resources (BUAN) slaughter facility, which involved rendering the birds insensible by electrical stunning and thereafter cutting jugular vein to allow for bleeding. Following dressing, the carcasses were individually packed in polythene bags and kept in the freezer at -17°C for proximate and mineral analyses and sensory evaluation.

Preparation of experimental diets

Chemical analysis of the diets: Prior to diet formulation, proximate analysis of *I. belina*, *V. subterranea* and *T. esculentum* were determined by the method of AOAC (2005). Calcium (Ca) was analysed using Inductively Coupled Plasma Mass optical Emission Spectrometer (Optimal 2100DV Model) while phosphorus (P) was determined using Kjeldahl digestion by ultraviolet spectrophotometer (Shimadzu UV 160 pc model) (AOAC, 2005). Table 1 presents the chemical composition of the protein sources used in the formulation of diets (%DM).

The protein sources were ground and mixed with other ingredients to formulate their respective mash diets. Diets were isonitrogenous (16% CP) and isocaloric (13 MJ/Kg) and were formulated to meet the nutritional value of the control diet (commercial layer diet) as recommended by National Research Council (1996). The *T. esculentum* and *V. subterranea* diets were supplemented with DL methionine and lysine to ensure that these amino acids were not limiting for egg production and quality. The feed mixer auger (pet 14, animal shredder hammer mill foliage TRF 600) was used to mix different diets homogeneously. Ingredients used in the formulation of the different diets are shown in Table 2.

Diet formulation

Three experimental diets containing either roasted *I. belina* or dehulled *T. esculentum* and also dehulled *V. subterranea* as sources of protein were formulated using the Feed Mixer Computer Software (OSUNRC2002 model). Roasted *I. belina* was purchased from local farmers in Tsetsebye in Central district, dehulled *T. esculentum* from Letlhakeng in Kweneng district and dehulled *V. subterranea* was also purchased from the Botswana Agricultural Marketing Board in Gaborone.

Bone and meat chemical analysis: The right hand thigh and drumstick portions were cut from the frozen chickens and deboned for meat and bone chemical analyses. Analyses were conducted for laboratory dry matter (DM; AOAC method no 930.15), organic matter (OM; AOAC method no 924.05) and crude protein (CP; AOAC method no

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984.13). Bone and meat P was determined using UV spectrophotometer (Shimadzu UV 160 pc Model) while, bone and meat Ca and K were analysed using Inductively Coupled Plasma Mass optical Emission Spectrometer (Optimal 2100DV model).

Taste panel evaluation

A day after slaughter of chickens, drumsticks, breasts and thighs were obtained, cut and cooked for one hour by boiling in water (Lewko et al., 2017). Thereafter, meat portions were cut into pieces of about 2 x 5 cm cubes and given to untrained panelists (15 males and 15 females) from BUAN to evaluate. The panellists were given guidelines on meat attributes and were asked to assess cooked meat sample for the six organoleptic attributes (i.e., odour, odour intensity, flavour, juiciness, tenderness and firmness) on Likert scale of 1 to 5, where odour was ranked from offensive to natural; odour intensity from odourless to strong; flavour from tasteless to very good; juiciness from very dry to very juicy, tenderness from very tough to very tender and firmness from very soft to very firm (Levie, 1979).

Table 1 - Chemical composition of *Zea mays (Z. mays)*, *T. esculentum, V. subterranea* and *I. belina* used in the present study.

Parameter	*Z. mays*	*T. esculentum*	*V. subterranea*	*I. belina*
Dry matter (%)	79.32±1.4	90.12±4.2	89.32±5.7	91.36±8.5
Crude protein (%)	7±0.14	30±1.6	18±0.7	54±3.7
Crude fibre (%)	1.0±0.14	17±3.2	4.1±0.02	8.1±0.01
Crude fats (%)	4.1±0.07	40±2.3	5.9±0.5	13.9±2.1
Ash (%)	0.7±0.07	1.2±0.2	1.1±0.2	5.4±0.1
Calcium (%)	0.6±0.01	0.61±0.03	0.40±0.07	0.7±0.01
Phosphorus (%)	0.3±0.02	0.46±0.01	0.65±0.002	0.45±0.02
Gross energy (MJ/kg)	1.638±0.02	1.993±0.04	2.440±0.01	1.713±0.009

Table 2- Ingredients and nutrients composition of experimental diets formulated from *T. esculentum, V. subterranea* and *I. belina* used in the present study

Ingredients	*T. esculentum*	*V. subterranea*	*I. belina*	Control
Yellow maize (%)	59	19	78	*
T. esculentum (%)	37	0	0	*
V. subterranea (%)	0	77	0	*
I. belina (%)	0	0	18	*
Dicalcium phosphate (%)	1.2	1.1	1.2	*
Limestone (%)	1.1	1.2	1.1	*
Iodized salt (%)	0.4	0.4	0.4	*
Vat. Mineral premix (%)	0.1	0.1	0.1	*
DL-methionine (98%)	0.6	0.6	0.6	*
Lysine HCL (%)	0.6	0.6	0.6	0.6
Calculated analysis				
Met. energy (MJ/kg) [1]	13.68	13.38	13.18	13
Crude protein (%)	16.01	16.09	16.1	16
Calcium (%)	3.54	3.51	4.21	4.5
Phosphorus (%)	0.91	0.82	0.99	0.5
Determined analysis				
Dry Matter (%)	93.80±4.2	93.84±3.9	93.84 ±5.1	84±2.5
Crude protein (%)	15.96±1.02	16.00±2.0	16.80±1.4	16±0.8

*= Company confidential information. [1]Estimated using a method by Oduguwa et al. (2000) that is: ME (Kcal/kg) = 37 x % CP + 81.8 x % EE + % NFE. 1 caloric = 4.184 joules

Statistical analysis

Chemical composition and sensory evaluation data were analysed using PROC GLM procedure of SAS (2010) as a completely randomized design. Bone and meat chemical composition data were analysed according to the following model: $Y_{ijk} = \mu + D_i + W_j + (D \times W_{ij}) + E_{ij} + S_{ijk}$

Where Y_{ijk} = response variables, μ = population mean, D_i = treatments effects, W_j = week of age, $(D \times W)_{ij}$ = interaction of age and diets, E_{ij} = experimental error, S_{ijk} = Sampling error. Differences among treatment means were determined using the PDIFF option in the LSMEANS statement of the GLM procedure of SAS (2010). Sensory evaluation data were analysed using non-parametric multivariate analysis. The level of significance for both chemical, proximate composition and sensory evaluation was set at $P<0.05$.

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RESULTS

Repeated measures analysis showed significant (P<0.05) week × diet interaction effect on bone P and Ca with the exception of bone K (Table 3). Hens fed *V. subterranea* diet had the lowest (P<0.05) bone P concentration in all ages. At week 28, there was no dietary effect (P>0.05) on the concentration of P in the bone. Hens fed *V. subterranea* diet had lowest bone P contents in week 33. However, on week 38, hens fed *T. esculentum* and *I. belina* diets had higher (P<0.05) bone P content compared to other diets. Age (weeks) did not significantly affect bone Ca contents. Treatment diets did not significantly affect bone Ca contents at week 28 and 38. However, highest bone Ca contents was observed on hen fed *T. esculentum* diets at week 33. For bone K contents, there were neither age (weeks) nor treatment diet effects.

Dietary treatments and age (weeks) did not influence (P>0.05) DM, CP, P, Ca and K contents of the meat. Meat from hens fed *V. subterranea* diet had significantly lower odour than those fed control diet but there was no significant difference in odour in meat from hens fed control diet, *T. esculentum* and *I. belina* diet at 28 weeks of age (Figure 1). At 33 and 38 weeks, diets did not influence (P>0.05) odour. Similarly, age (weeks) also did not affect odour in all diets. Generally, consumer panellists ranked the meat odour between 3.52 and 4.13 (i.e., moderate to good) in all treatment diets and across the ages. Dietary treatments and age (week) did not influence (P>0.05) meat odour intensity, juiciness and tenderness for the duration of the study (Figures 2 to 4). Generally, consumer panellists ranked the meat odour intensity, juiciness and tenderness moderate (3.52) in all treatment diets and across the ages. There was no difference (P>0.05) in flavour between hens fed treatment diets at 28 and 38 weeks of age. However, at week 33 meat from hens fed *I. belina* diet had significantly moderate (low) flavour compared to hens on control and *V. subterranea* diets.

Also, no differences (P>0.05) in flavour between hens fed *T. esculentum* and *I. belina* diets were observed (Figure 5). Similarly, there were no significant (P>0.05) differences in meat flavour in all weeks from hens fed control, *V. subterranea* and *T. esculentum* diets. However, meat from hens fed *I. belina* diet had significantly (P<0.05) higher flavour at week 28 compared to 33 and 38 weeks.

Diets did not influence (P<0.05) firmness at week 28 and 38. However, at week 33 meat from hens fed *V. subterranea* diets was more (P<0.05) firm compared to that of hens on control diet. Hens fed control, *T. esculentum* and *I. belina* diets had similar (P<0.05) firmness at week 33 (Figure 6). Age (weeks) also did not affect firmness in the entire study periods. Generally, consumer panellists ranked the meat firm to slightly firm in all treatment diets and across the ages.

Table 3 - Mineral composition (%) of bone of *Tswana* hens fed three protein sources (*I. belina, T. esculentum and V. subterranea*) raised under intensive system from 28 to 38 weeks of age

Parameters	Age (weeks)	Control	*V. subterranea*	*T. esculentum*	*I. belina*
Phosphorus	28	19.5±0.6^{ax}	14.3±0.6^{bx}	19.7±0.6^{ax}	18.0±0.6^{ax}
	33	17.3±0.6^{ay}	15.0±0.6^{bx}	16.3±0.6^{by}	16.3±0.6^{by}
	38	15.7±0.6^{ax}	14.7±0.6^{bx}	16.3±0.6^{ay}	16.3±0.6^{ay}
Calcium	28	45.1±2.0^{ax}	42.7±2.0^{ax}	45.4±2.0^{ax}	41.7±2.0^{ax}
	33	37.4±2.0^{bx}	36.8±2.0^{bx}	41.7±2.0^{ax}	39.8±2.0^{bx}
	38	37.0±2.0^{ax}	38.0±2.0^{ax}	48.7±2.0^{ax}	37.0±2.0^{ax}
Potassium	28	2.70±0.6	1.50±0.6	2.20±0.6	2.50±0.6
	33	1.50±0.6	1.90±0.6	2.60±0.6	2.40±0.6
	38	3.00±0.6	2.10±0.6	2.90±0.6	2.10±0.6

^{ab}Values on the same row within the same parameter and different superscripts are significantly (P<0.05) different. ^{xy}Values on the same column within the same parameter and with different superscripts are significantly (P<0.05) different

Table 4 - Mineral and proximate composition (%) of meat of *Tswana* hens fed three protein sources (*I. belina, T. esculentum and V. subterranea*) raised under intensive system from 28 to 38 weeks of age

Parameters	Age (weeks)	Control	*V. subterranea*	*T. esculentum*	*I. belina*
Dry matter	28	29.5±6.3	30.6±6.3	30.4±5.1	31.5±6.3
	33	28.4±5.1	28.8±5.1	26.2±5.1	30.6±5.1
	38	28.1±5.1	30.5±5.1	30.9±5.1	30.6±5.1
Crude protein	28	82.7±7.7	80.4±7.7	83.1±7.7	82.0±7.7
	33	78.0±7.7	73.0±7.7	56.7±7.7	78.0±7.7
	38	81.2±7.7	75.8±7.7	76.0±7.7	76.2±7.7
Phosphorus	28	3.1±0.6	3.1±0.6	2.7±0.6	3.2±0.6
	33	3.0±0.6	3.0±0.6	2.7±0.6	2.9±0.6
	38	3.7±0.6	4.2±0.6	3.5±0.6	3.1±0.6
Calcium	28	4.2±0.7	2.8±0.3	2.5±0.1	2.9±0.1
	33	3.2±1.3	3.0±2.2	3.0±1.7	3.1±0.6
	38	3.9±1.5	4.2±1.7	3.6±1.0	2.7±0.4
Potassium	28	3.1±0.6	3.1±0.6	2.7±0.6	3.2±0.6
	33	3.0±0.6	3.0±0.6	2.7±0.6	2.9±0.6
	38	3.7±0.6	3.1±0.6	3.5±0.6	3.1±0.6

Values in the same column within the same parameter and with different superscripts are significantly (P<0.05) different. Values in the same row within the same parameter and with different superscripts are significantly (P<0.05) different

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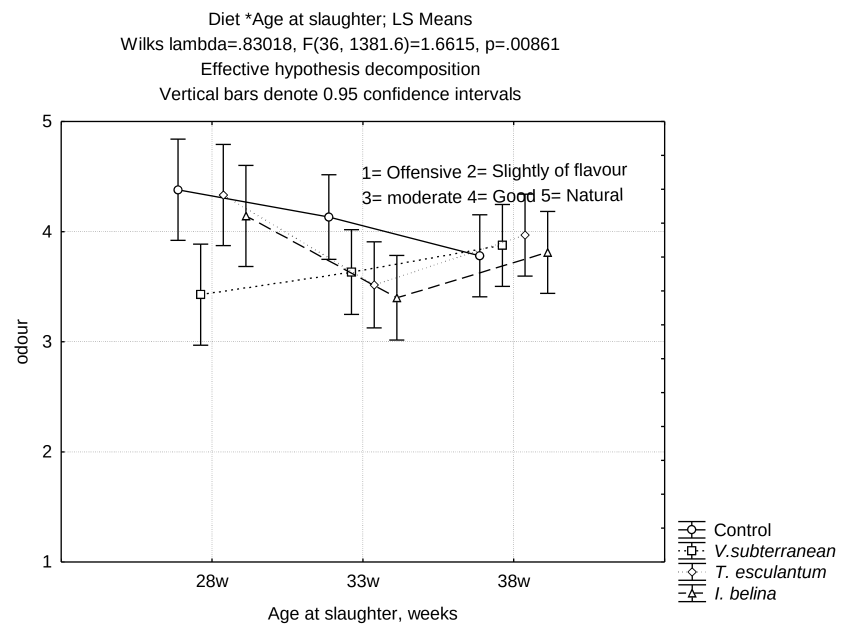

Figure 1 - Odour of meat of *Tswana* hens fed treatment diets at different ages at slaughter

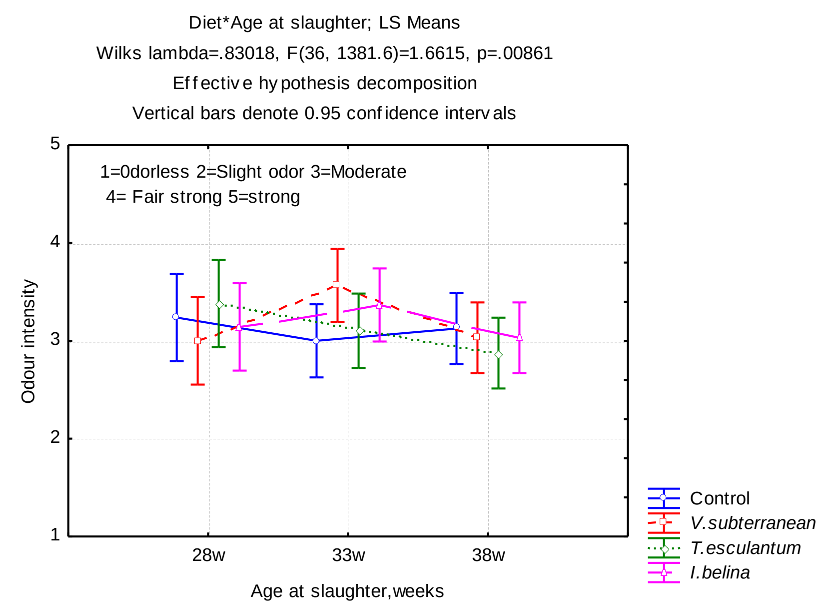

Figure 2 - Odour intensity of meat of *Tswana* hens fed treatment diets at different ages at slaughter

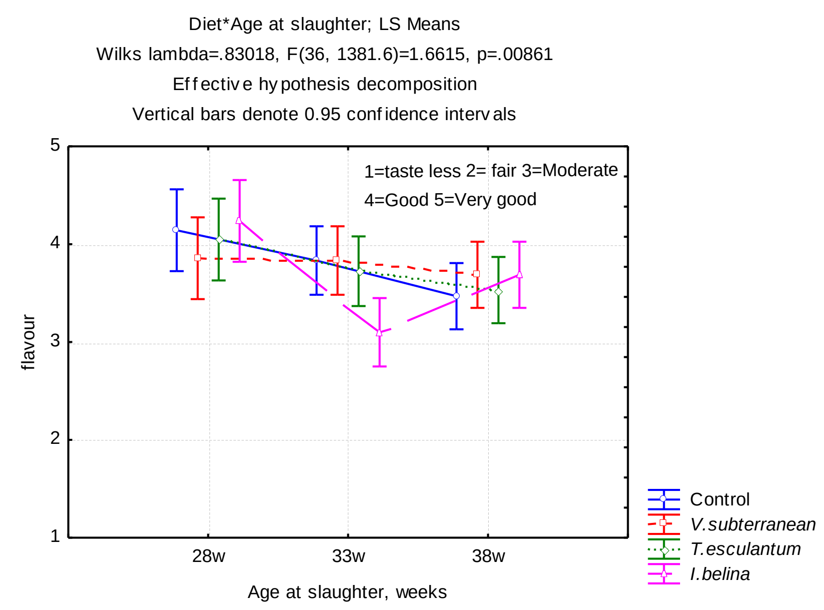

Figure 3 - Flavour of meat of *Tswana* hens fed treatment diets at different ages at slaughter

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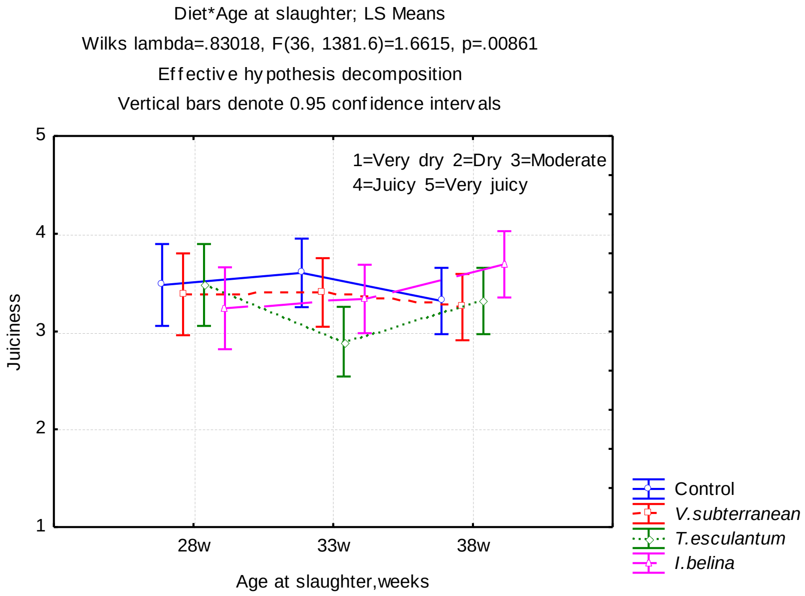

Figure 4 - Juiciness of meat of *Tswana* hens fed treatment diets at different ages at slaughter

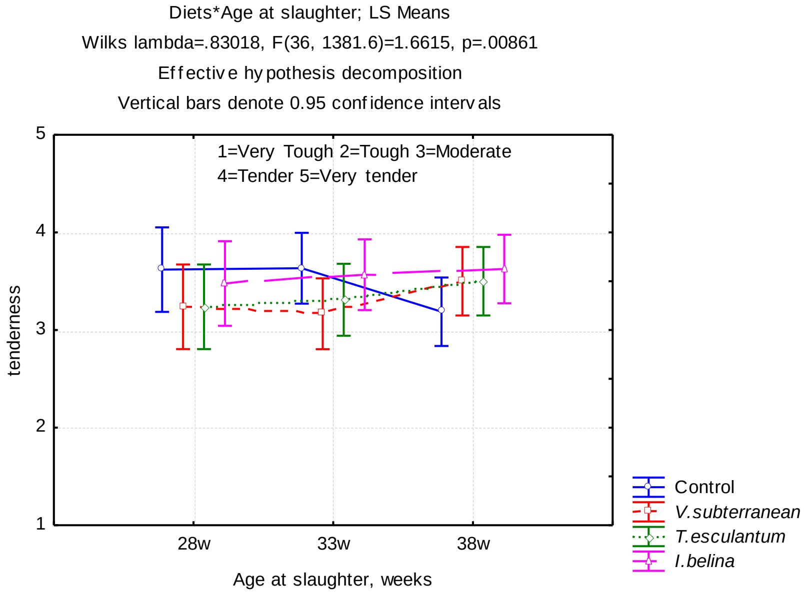

Figure 5 - Tenderness of meat of *Tswana* hens fed treatment diets at different ages at slaughter

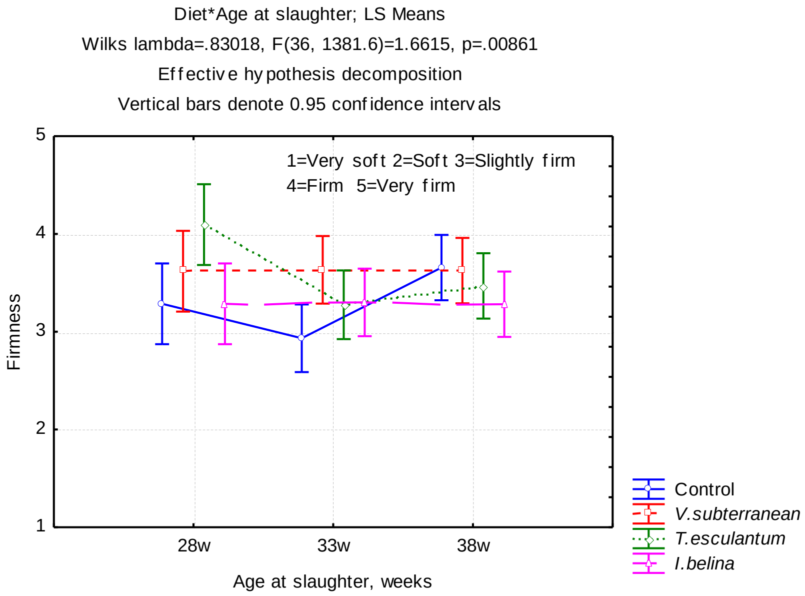

Figure 6 - Firmness of meat of *Tswana* hens fed treatment diets at different ages at slaughter

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DISCUSSION

Bone P content of *Tswana* hens fed control, *T. esculentum* t and *I. belina* diets in the current study is consistent with the findings of Moreki et al. (2011) who reported medullary bone P content of 16.87±0.34 in broiler breeder hens at 35 weeks of age. The current results are also in line with Chiripasi et al. (2013) who fed guinea fowl diet containing *I. belina* and found that inclusion of *I. belina* in guinea fowl diet had no influence on bone Ca contents of guinea fowl. The decrease in bone P and Ca contents with age in this study is consistent with Valable et al. (2018) who reported the decline of percentage bone P and Ca with increasing age of chickens. The reduction in Ca is expected, as the hen requires Ca and P for egg shell formation during the laying period. The mean meat P content of *Tswana* chickens reported in the current study is slightly higher (3.0±0.3 vs. 2.57± 0.19) than that reported by Mareko et al. (2010) on broiler chickens fed 40% level of *Phane* as supplement. The DM value (26.1±5.1) in the current study is consistent with De Marchi et al. (2005) who reported DM of 25.90±0.78 on breast of 25 weeks old female padovada breeds of chicken in Italy. The mean meat CP contents in the current study (76.6±7.7) compares very well with the findings of Saina (2005) (75.4% CP) on guinea fowl raised under an intensive rearing system and fed 18% CP, 13MJ/kg broiler phase 2 mash diet. Similar observations were made by Nsoso et al. (2008) (80.23±0.84%) on 5 weeks old keets raised on concrete floor and fed broiler starter diets in Botswana.

As illustrated in Figures 2 to 4, the taste panel scores for meat odour, flavour and juiciness did not show much differences across diets and ages. Odour and flavour received scores that averaged 4 (good odour and flavour) while juiciness received average score of 3 (moderate juiciness). This observation is supported by the earlier work of Mareko et al. (2006) who showed that guinea fowl meat had moderate to good odour. Mareko et al. (2010) reported that a higher percentage of panellists (41.67%) said that meat from broilers fed diets containing *I. belina* had a moderate odour. The present results are in agreement with Moreki et al. (2012) who fed guinea fowl diets containing maize, millet and sorghum as energy sources and reported that inclusion of cereal grain in guinea fowl diet did not influence organoleptic attributes of guinea fowl meat. It seems that feeding diets formulated with *I. belina* (Westwood), *V. subterranea* (L) Verde and *T. esculentum* (Burchell) Schreiber as sources of protein did not influence meat odour and flavour of *Tswana* hens. The observation that *Tswana* chickens meat received a moderate juicy rating in this study is in line with Mareko et al. (2006) who showed that over 66% of respondents felt that guinea fowl meat was moderately juicy when fed growers mash and reared on concrete and earth floors. Mareko et al. (2010) also reported moderate juiciness in broiler meat of chickens fed a diet containing *I. belina.* Similar findings were reported by Okeudo et al. (2006) in broiler chickens. Taste panel scores for odour intensity and tenderness in this study did not differ across diets and age. Odour intensity and tenderness received a moderate ranking that averaged 3 implying that diet supplemented with *I. belina* (Mopane worm) or *V. subterranea* (Bambara groundnut) or *T. esculentum* (Marama bean) did not affect odour intensity and tenderness of *Tswana* hens.

CONCLUSION

The bone P, Ca and K contents of *Tswana* hens raised under intensive system were not influenced by treatment diets. However, it was observed that these minerals decreased over time. Furthermore, dietary treatment had no influence on meat DM, CP, P, Ca and K of Tswana hens. Organoleptic properties of *Tswana* hens was not influenced by the incorporation of either *I. belina* or *V. subterranea* and also *T. esculentum i*n the layer diets as protein sources.

DECLARATIONS

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Authors' contribution
All authors contributed equally to this work from starting proposal writing up to preparation of manuscript.

Competing Interests
The authors declare that they have no conflict of interest with regards to the research, authorship or publications of this manuscript.

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