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Full Length Research

Effect of *Imbrasia belina* (Westwood) or *Vigna subterranea* (L) Verde or *Tylosema esculentum* (Burchell) Schreiber as protein sources in diets fed to Tswana hens on egg quality

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ABSTRACT: A 90 days study was conducted to compare the effects of feeding Tswana hens reared under intensive system and fed diets formulated from *Imbrasia belina* (Westwood), *Vigna subterranea* (L) Verde and *Tylosema esculentum* (Burchell) Schreiber as sources of protein on egg quality. Four isonitrogenous and isocaloric diets were formulated by totally replacing soybean meal (SBM) with *I. belina*, *V. subterranea* and *T. esculentum* as protein sources while commercial layer diet was used as control diet. Twelve eggs from each pen were collected at 28, 33 and 38 weeks of age for egg quality analysis in a completely randomized design. Egg weight (EW), shell weight (SW), shell thickness (ST), shell index (SI), shell percentage (SP), egg surface area (ESA), egg volume (EV), egg contents (EC) and Haugh units (HU) were measured. Repeated measure analysis showed significant ($P<0.05$) week × diets interaction effect on EW and SW. At week 28, hens on *T. esculentum* diet had the highest ($P < 0.05$) EW while those on control diet attained the highest ($P < 0.05$) EW at week 33. Hens fed *I. belina* diets had the highest ($P < 0.05$) SW at week 28. However, at weeks 33 and 38, hens on *I. belina* diets had the smallest ($P < 0.05$) SW. Hens fed *V. subterranea* and *T. esculentum* diets had significantly lower overall SI compared to those on control and *I. belina* diets. *Tylosema esculentum* diets did not promote ($P<0.05$) ESA, EV and EC. However, no influence ($P>0.05$) of dietary treatments was observed on overall ST, SP and HU. The inclusion of *I. belina* and *V. subterranea* on layer diet had no adverse effects on egg quality while the totally replacement of SBM with *T. esculentum* as protein source impaired egg quality. These results, suggest that *I. belina* and *V. subterranea* could completely replace SBM as protein source in the layer diets without any detrimental effects on egg quality.

Keywords: Anti-nutritional factors, egg quality, legumes, protein, Tswana chicken.

INTRODUCTION

Morama bean (*Tylosema esculentum* (Burchell) Schreiber) seeds, Bambara groundnuts (*Vigna subterranea* (L) Verde) seeds and Phane (*Imbrasia belina* (Westwood)) can be important soybean meal (SBM) substitutes in poultry diets in Botswana. The use of *I. belina* (Westwood) and legumes (Abdelnour et al., 2018) as source of protein for the poultry feed industry is expected to increase further in the near future. This is as a result of Bovine Spongiform Encephalopathy (BSE) outbreak in the United Kingdom in 1986, which was thought to be spread by the inclusion of animal by-products in animal feeds. The incident resulted in many livestock feed industries either choosing to or being banned from using animal by-products as sources of protein (Farrell, 1997). In Botswana, protein sources (i.e., feather meal, blood meal and meat-on-bones) generated by the poultry industry are by law not allowed to be included in poultry diets. As a result, plants are the major sources of protein available for poultry feeding. Although some of these plant proteins contain sufficient amount of protein (~40%) as well as, an essential amino acid profile closely matching that of SBM (Anjos, 2014), the presence of several anti-nutritional factors (ANFs), which include glucosinolates, sinapine, phytic acid, polyphenolic compounds, erucic acid, protein inhibitors and the indigestible non-starch polysaccharides, may negatively influence egg quality in poultry production (Wickramasuriya et al., 2015).

Legumes are low in sulphur amino acids. Therefore, to improve nutritive value and to reduce adverse effects of legume seeds, most researches have centered on supplementation of methionine. A number of feeding trials with some legumes have been carried out with poultry species. For example, Ciurescu and Pană (2017) studied the effects of untreated field pea (*Pisum sativum* L.) as substitute for SBM and enzymes supplementation on egg production and quality of laying hens. The authors concluded that field pea could be included in laying hen diets at dietary level up to 350 g/kg. However, other studies showed that addition of alternative legume seeds such as peas and faba beans in the layer diet significantly affected yolk colour (Laudadio and Tufarelli, 2012).

There is little information on the egg quality responses of Tswana hens to the use of *T. esculentum* seeds, *V. subterranea* seeds and *I. belina* as dietary protein sources. This study was conducted to investigate the effect of totally replacing SBM with graded levels of *T. esculentum* seeds, *V. subterranea* seeds and *I. belina* in diets for Tswana hens on egg quality traits. It was hypothesised that replacing SBM with *T. esculentum*, *V. subterranea* and *I. belina* in layer diets would have no adverse effects on egg quality characteristics.

MATERIAL AND METHODS

Study area

The experiment was carried out from February to May 2010 at Botswana College of Agriculture (now Botswana University of Agriculture and Natural Resource), Content Farm in Sebele. The farm is located 10 km north of Gaborone, the capital city of Botswana. During the study period the temperature ranged between 19 and 25 °C.

Birds and their management

A total of 60 normal feathered Tswana hens aged 25 weeks (point of lay) were used in the study. The hens were raised under semi-intensive system supplemented with maize before the start of the study. At the beginning of the study, birds were individually weighed and tagged. All the hens were vaccinated against Newcastle disease and infectious bursal disease (IBD) and were raised on a deep litter system. Clean water was provided *ad libitum* for 90 days and the house lit continuously for 16 hours.

Chemical analysis of the feedstuff

Prior to diet formulation, proximate analysis of *I. belina* (Westwood), *V. subterranean* (L) Verde and *T. esculentum* (Burchell) Schreiber were determined by the method of AOAC (2005). Calcium (Ca) was analyzed using Inductively Coupled Plasma Mass Optical Emission Spectrometer (Optical 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 diet formulations (%DM). *I. belina*, *T. esculentum* and *V. subterranea* were ground and mixed with other ingredients to formulate *I. belina* diet, *T. esculentum* diet and *V. subterranea* diet respectively in a mash form. 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

Four experimental diets containing either roasted *I. belina* or dehulled *T. esculentum* or *V. subterranea* and soybean meal as sources of protein were formulated using the Feed Mixer Computer Software (OSUNRC 2002 model). Roasted *I. belina* was purchased from local farmers in Tsetsebye in the Central District while dehulled *T. esculentum* (Burchell) Schreiber was obtained from Letlhakeng in Kweneng District and dehulled *V. subterranea* (L) Verde from the Botswana Agricultural Marketing Board in Gaborone.

Experimental design

Four experimental diets consisting of *V. subterranea* (L) Verde, *T. esculentum* (Burchell) Schreiber, *I. belina* (Westwood), and commercial layer diet (mash) were used

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

Parameters	Ingredients			
	Z. mays	***T. esculentum***	***V. subterranea***	***I. belina***
Dry matter (%)	79.3±1.4	90.1±4.2	89.3±5.7	91.4±8.5
CP (%)	7.0±0.1	30.0±1.6	18±0.7	54±3.7
Crude fibre (%)	1.0±0.1	17.0±3.2	4.1±0.0	8.1±0.0
Crude fats (%)	4.1±0.1	40.0±2.3	5.9±0.5	13.9±2.1
Ash (%)	0.7±0.1	1.2±0.2	1.1±0.2	5.4±0.1
Calcium (%)	0.6±0.0	0.6±0.0	0.40±0.1	0.7±0.0
Phosphorus (%)	0.3±0.0	0.5±0.0	0.5±0.0	0.5±0.0
Gross energy (MJ/kg)	1.638±0.0	1.993±0.0	2.440±0.0	1.713±0.0

Table 2. Ingredients and nutrient composition of experimental diets formulated from *T. esculentum, V. subterranea, I. belina* and commercial diets.

Ingredients	***T. esculentum***	***V. subterranea***	***I. belina***	**Commercial**
Yellow maize (%)	59.0	19.0	78.0	*
T. esculentum (%)	37.0	0	0	*
V. subterranea (%)	0	77.0	0	*
I. belina (%)	0.0	0	18.0	*
Dicalcium phosphate	1.2	1.1	1.2	*
Limestone (%)	1.1	1.2	1.1	*
Iodized (%)	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)	13.7	13.4	13.8	13.0
Crude protein (%)	16.0	16.1	16.0	16.0
Calcium (%)	3.5	3.5	4.2	4.5
Phosphorus (%)	0.9	0.8	0.1	0.5
Determined analysis				
Dry matter (%)	93.8±4.2	93.5±43.9	93.5±15.1	84.0±2.5
Crude protein (%)	16.0±2.0	16.0±2.0	16.0±0.8	16.0±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. Caloric = 4.184 joules.

in this study. Twelve pens each measuring 1.2 m by 2 m and 60 Tswana hens were used. Five chickens were randomly allocated to each pen. The diets were then randomly allocated to the 12 pens such that each diet was replicated three times. The design of the experiment was therefore a completely randomized design (CRD) with four treatments, three replications and five sampling units per replication. The number of replications was determined using a resource equation (Mead, 2000) to ensure minimum degrees of freedom of error. The resource equation is given as:

$$E = N – T$$

Where E is the error degrees of freedom and should be between 10 and 20, N is the total degree of freedom and T is the treatment degrees of freedom.

Measurement of parameters

Thirty-six eggs (12 from each replicate) from each treatment were collected at 28, 33 and 38 weeks of age for egg quality analysis. The eggs were weighed using an electronic scale sensitive to 0.001 g. The height, width of eggs and shell thickness were measured using Micrometer screw gauge sensitive to 0.001 mm. Thereafter, the eggs were cracked open on a white tile and shell thickness was measured according to Alkan et al. (2008). The shells were washed under slow flowing water to remove adhering albumen (Kuhl and Seker, 2004) and then wiped with a paper towel to remove excessive moisture. Egg shell thickness (ST) was estimated in the three locations (sharp, blunt and equator). Egg shape index (%) was calculated as [egg width (cm)/egg length (cm)*100]. Egg surface area (ESA) was expressed in centimeter squared (cm^2) using formula of Carter (1975), $3.9782W^{0.7056}$, where W is the egg weight (EW) in grams. Egg content (EC) was calculated by subtracting shell weight (SW) from EW while egg volume (EV) was measured using the formula of Carter (1975). Shell percentage (SP) was expressed as SW divided by ESA multiplied by 100 whereas Haugh unit (HU) was calculated using the formula 100log ($H+7.57-1.7W^{0.37}$), where H is the albumen height and W is the EW (Haugh, 1937). For clarity purpose, the formulae are given below:

$$\text{Shape index (\%)} = \frac{\text{Egg width(cm)}}{\text{Egg length(cm)}} \times 100$$

Egg Surface Area (mm^2) = $3.9782W^{0.7056}$

Where W = egg weight (g)

$$\text{Egg Content (g)} = \text{Egg weight(g)} - \text{Shell weight (g)}$$

$$\text{Shell Percentage (\%)} = \frac{\text{Egg weight(g)}}{\text{Egg surface area(mm}^2)} \times 100$$

Haugh unit = 100 log ($H + 7.57 - 1.7W^{0.37}$)

Where: H = albumen height and W = egg weight.

Statistical Analysis

Data on egg quality were analysed using repeated measures procedure of SAS (2010) according to the following general linear model:

$$Y_{ijk} = \mu + D_i + W_j + (D \times W_{ij}) + E_{ijk}$$

where Y_{ijk} = response variable, μ = overall mean, D_i = fixed effects of diet, W_j = effects of week (age of chicken), $(D \times W)_{ij}$ = effects of interaction between diets and week and E_{ijk} = experimental error (variation within the pens), ijk = sampling error (variation within the hens).

The probability of difference (PDIFF) option in the LSMEANS statement of the GLM procedure of SAS (2010) was used to separate means. Statistical significance was declared at $P \leq 0.05$.

RESULTS

Repeated measure analysis showed significant ($P < 0.05$) week × diets interaction effect on EW and SW (Table 3). At week 28, hens on *T. esculentum* diet had the highest ($P<0.05$) EW while control, *V. subterranea* and *I. belina* diets had the lowest. Also, at week 33, hens on control diet had the highest ($P < 0.05$) EW while the lowest EW was found in hens on *V. subterranea*, *T. esculentum* and *I. belina* diets. However, no differences in EW were detected at week 38 in all diets. Hens on *T. esculentum* diet had the lowest ($P < 0.05$) EW in all diets and weeks, whereas hens on *I. belina* diets had the highest ($P < 0.05$) EW at week 33 and 38. At weeks 33 and 38, hens on *I. belina* diets also had the smallest ($P < 0.05$) SW. Repeated measure analysis showed no significant ($P >0.05$) diet × week interaction effect on ST, SI, SP, ESA, EV, EC and HU (Table 4) while experimental diets had effects on overall ST, SI, SP, ESA, EV, EC and HU. Hens fed *V. subterranea* and *T. esculentum* diets had significantly lower overall SI than those on control and *I. belina* diets. However, hens on control, *V. subterranea* and *I. belina* diets had similar ($P >0.05$) overall ESA, EV and EC while those on *T. esculentum* diets had the lowest ESA, EV and EC. No influence ($P >0.05$) of dietary treatment on overall ST, SP and HU was observed.

DISCUSSION

One of the most important consideration in diet formulation is the availability and nutritional value of amino acids (Kim et al., 2011), which depend to a large extent on the nutrient composition and concentration of ANFs in the feedstuff. Previous study by Manyeula et al. (2013) reported that replacement of SBM with *I. belina, V. subterranea* and *T. esculentum* may ensure adequate digestible amino acids. In this study, replacement of SBM with *T. esculentum* was seen to reduce EW, which could be a result of low feed intake reported by Manyeula et al. (2013) and the presence of ANFs. Ferket and Gernat (2006) stated that feed intake is the single-most important factor that will determine the efficiency of nutrient utilization. As chickens decrease feed intake, their nutrient intake also decreases resulting in lighter weight. Anti-nutritional factors found in *T. esculentum* are known to suppress nutrient digestibility in chickens (Madzimure et al., 2017). In addition, Jackson et al. (2010)

Table 3. Eggs and shell weight of Tswana hens raised under intensive systems and fed diets containing *I. belina, T. esculentum, V. subterranea* and a commercial layer diet at different ages in weeks.

Parameter	Age	Control	*V. subterranea*	*T. esculentum*	*I. belina*
Egg weight (g)	28	53.3±0.7^{ay}	53.9±0.7^{ay}	48.8±0.7^{bx}	51.8±0.7^{ay}
	33	54.2±0.7^{ay}	53.9±0.7^{ax}	49.6±0.7^{bx}	53.6±0.7^{ax}
	38	55.4±0.7^{ax}	53.7±0.7^{bx}	50.2±0.7^{bx}	55.2±0.7^{ax}
Shell weight (g)	28	6.4±0.4^{bx}	6.0±0.4^{bx}	6.8±0.4^{bx}	7.8±0.4^{ax}
	33	6.9±0.4^{ax}	6.0±0.4^{ax}	6.7±0.4^{ax}	5.9±0.4^{by}
	38	6.6±0.4^{ax}	6.3±0.4^{ax}	6.9±0.4^{ax}	5.7±0.4^{by}

[abc] Means in the same row with a common superscript do not differ (P > 0.05). [xyz] Means in the same column with a common superscript do not differ (P > 0.05). EW = egg weight, SW= shell weight.

Table 4. Effects of diets containing *I. belina, T. esculentum and V. subterranea* and control diet on egg quality of Tswana hens.

Egg quality parameters	Control	*V. subterranea*	*T. esculentum*	*I. belina*	SE
Shell thickness (mm)	0.6	0.5	0.4	0.6	0.2
Shell index (%)	76.9^{a}	73.7^{c}	72.6^{c}	76.1^{b}	0.8
Shell Percentage (%)	12.3	11.3	13.4	11.4	0.8
Egg shell area (mm^2)	66.6^{a}	66.2^{a}	62.4^{b}	66.0^{a}	0.6
Egg volume (mm^3)	43.3^{a}	42.8^{a}	39.6^{b}	42.4^{a}	0.6
Egg content (g)	47.7^{a}	47.6^{a}	42.7^{b}	46.7^{a}	0.8
Haugh unit	99.8	99.1	99.5	100.1	1.8

[abc]Means in the same row with a common superscript do not differ (P > 0.05).

reported *T. esculentum* (Burchell) to be a potential source of phytonutrients including phenolic compounds such as tannin, trypsin inhibitor, phytate and oligosaccharides. In a related study, Tshovhote et al. (2003) found that tannin decreases the digestibility of protein and carbohydrates by forming insoluble enzyme resistant complexes with the dietary protein. On the other hand, trypsin inhibitor binds protein on the ileal epithelium. The presence of tannins, trypsin inhibitor and phytates in *T. esculentum* diet in this study could have reduced the metabolism of the dietary protein and subsequently had adverse effect on egg formation and bone integrity. Phytate has a tendency to bind calcium and phosphorus thereby making them unavailable in sufficient quantities for egg formation. The presence of phytate on the feed results in low EW. The reduction in EW is consistent with earlier work of Alkanji and Ologhobo (2007) who found that elevated phytate contents in raw seed legumes contribute to the reduction in EW.

The tendency of EW to increase with age in the present study agrees with Aziza et al. (2013) and Hurnik et al. (1997) who reported that egg size increases with age in layers but stabilizes within 4 to 6 weeks of laying.

Leeson and Summers (1997) and Munisi et al. (2016) observed that EW is lowest at the beginning of the production cycle but increases as age and body weight increases throughout the laying period. In the present study, EW of Tswana hens was not influenced by replacement of *I. belina*, *V. subterranea* and *T. esculentum* with SBM. This suggests that inclusion of these protein sources did not alter the physico-chemical parameters of the diet and thus did not affect its palatability and functional properties. This result is consistent with Akhatar et al. (2007) who reported mean value of 6.26±0.478 g for Fayoumi hens of Pakistan but higher than SW reported by Tuleun et al. (2008) in laying hens fed velvet beans. The tendency of SW to increase with age in this study is in agreement with Moreki et al. (2011) who reported an increase in SW of Ross broiler breeder hens from 27 to 30 weeks of age.

Repeated measure analysis showed no diet × week interaction effect on ST, SI, SP, ESA, EV, EC and HU demonstrating that the effects of diet in these parameters did not depend on the age of hens. The replacement of SBM with *I. belina*, *V. subterranea* and *T. esculentum* did not improve egg quality as indicated by lack of difference between eggs from hens fed control diet and other dietary treatment in terms of ST, SP and HU. The present results on ST falls within the mean value of 0.38±0.005 mm reported by Minh et al. (2004) in the scavenging chicken supplemented with protein. Similar results were reported by Tulenu et al. (2008) when layers were fed velvet bean.

However, Minh et al. (2004) reported a lower SP (9.3±0.15 to 10.4±0.15%) in scavenging hens supplemented with protein in Northern Vietnam. Diets and age also had no significant influence on HU. This result is in line with Gul et al. (2005) who reported HU above 72 (84.7±1.3 to 90.2±1.3) in eggs from hens fed a high level of common vetch seed (legumes) during peak period.

Eggs from hens on *T. esculentum* diet had low overall ESA, EV and EC; this is not surprising as there is a strong relationship between EW and the latter parameters. As shown in Table 3, EW was suppressed by feeding *T. esculentum* diet, indicating that ESA, EV and EC are positively correlated to EW. The formula of Carter (1975) indicates that the determination of ESA, EV and EC is dependent on EW. It is therefore, logical that a decrease in EW due to highlighted factors would result in a concomitant decrease in these parameters. In this study, the reduced EC is not surprising as there is a strong relationship between dietary protein and EC. Gunawardana et al. (2008) reported that EC (egg weight, mass, albumen and yolk weight) decreases with decreased dietary protein, which explains why eggs had a decreased EC. The tendency of ESA, EV and EC to increase with age of the hens could be due to the increase in EW and body size.

Conclusions

These results suggest that inclusion of *I. belina* (Westwood) and *V. subterranea* (L) Verde as sources of protein in layer diets had no adverse effects on egg quality. However, *T. esculentum* (Burchell) resulted in poor egg quality suggesting the need to take precautionary measures when using *T. esculentum* in place of SBM. These measures may include exogenous enzyme treatment to increase utilization and breakdown of mineral-phytic acid complexes.

Recommendation

It is recommended that *I. belina* (Westwood) and *V. subterranea* (L) Verde processed seeds can be used as alternative protein sources in layer diets for Tswana chicken hens.

CONFLICT OF INTEREST

The authors declare that they have no competing interests

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