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Effect of *Imbrasia belina* (westwood), *Tylosema esculentum* (Burchell) Schreiber and *Vigna subterranea* (L) Verde as protein sources on growth and laying performance of *Tswana* hens raised under intensive production system

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A B S T R A C T

A feeding trial was conducted to determine growth and laying performance of indigenous *Tswana* hens fed diets formulated with four different protein sources consisting of either *Tylosema esculentum* (Burchell) or *Vigna subterranea* (L) Verde or *Imbrasia belina* (Westwood) and commercial diets under intensive system in Botswana. Sixty 25 weeks old normal feathered *Tswana* hens were bought from a local farmer and reared up to 38 weeks on diets containing *T. esculentum* (Burchell) or *V. subterranea* (L) Verde or *I. belina* (Westwood) and a commercial diet (control). The hens were then randomly allocated to four treatment groups of 15 hens, with each treatment having three replicates of five hens each. *Tylosema esculentum*, *V. subterranea* and *I. belina* diets were formulated to meet the nutritional composition of commercial layer diet using *T. esculentum* (Burchell) Schreiber beans, *V. subterranea* (L) Verde beans and *I. belina* (Westwood), respectively as sources of protein before the start of egg production. Data were analyzed using PROC GLM of SAS (2002-2008) as a completely randomized design with initial body weight as a covariate. Hens fed control diet had high (P<0.05) feed intake (84.6±7.9 g) and those fed *T. esculentum* diet the lowest feed intake (54.6±7.9 g). Hens fed *I. belina*, control and *V. subterranea* diets had the highest (P<0.05) average daily gain (ADG) per hen of 4.77±1.31g, 3.27±1.16 g and 3.13±1.51 g, respectively. On the other hand, hens fed *T. esculentum* diet had the lowest (P<0.05) ADG/hen (-0.82±1.60 g). The FCR was highest (P<0.05) for control diet with 1.76±0.16 g and lowest for hens fed *I. belina* diet with 1.10±0.16 g. Hen day egg production was highest (P<0.05) for control diet (90±0.87%) and lowest (41±0.87%) for *T. esculentum* diet. These results suggest that *I. belina* (Westwood) and *V. subterranea* (L) Verde are potentially good protein sources in poultry diets.

1. Introduction

Poultry industry in developing countries contributes to the sustainability of rural economies and towards gender equity (Gueyé, 2000). In Botswana, indigenous *Tswana* chickens serve as a source of protein to rural households and are usually sold to purchase groceries, pay school fees, transport fees and medication (Moreki *et al.,* 2010). Family chicken production is one of the most important poultry enterprises in low income communities (Saina *et al*., 2005) in that they can convert feed available around a house or village into highly nutritious, well appreciated products (Alders *et al.,* 2009). Okitoi *et al.* (2009) reported that nutrient intake from scavengeable feed resource was below the requirement of free-ranging local hens; hence limitation of scavengeable feed resource in terms of nutrients (energy, proteins and essential amino acids) supply was evident. Therefore, supplementation is essential to increase nutrient intake for optimum production.

Energy and high quality protein are the two most expensive nutrients to supply in practical poultry rations and are of greatest concern to poultry nutritionists (Rose, 1997). High quality protein is usually supplied by soybean meal, fish meal and meat meal in commercial poultry diets in both developed and developing countries. In Botswana, the quantity of soybean and fish available are not adequate for human consumption and are therefore unavailable for the formulation of poultry rations. Consequently, poultry feed manufacturers are forced to seek alternative but cheaper protein resources which can reasonably supplement or replace the inadequate supply of the more expensive soybean.

The imported soybean meal, make the poultry feeds expensive for the small-scale poultry. It is therefore not only important to search for locally available protein source replacements but also to investigate their composition, nutritive value as well as, bird performance of using such replacements over the imported ingredients.

The objectives of this study were to determine the growth and laying performance of *Tswana* hens fed *I. belina* (Westwood) or *T. esculentum* (Burchell) Schreiber or *V. subterranea* (L) Verde as sources of protein under intensive rearing system. The specific objectives of this part of the study are:

- To determine and compare feed intake, average daily gain and feed conversion ratio of *Tswana* hens fed either *I. belina* (Westwood) or *V. subterranea* (L) Verde or *T. esculentum* (Burchell) Schreiber as sources of protein compared to those fed a commercial layers diet.
- To evaluate and compare the laying performance and mortality rate of *Tswana* hens fed either *I. belina* (Westwood) or *V. subterranea* (L) Verde or *T. esculentum* (Burchell) Schreiber as sources of protein as compared to those fed a commercial layers diet.

2. Material and methods

2.1. Study area

The experiment was carried out from February to May 2010 at Botswana College of Agriculture (BCA), Content Farm; Sebele located 10 km north of Gaborone (the capital city of Botswana). The study site was located on 25^{o} 56’ 29.1” east and 24^{o} 35’18.8” south at an altitude of 983 metres above sea level. According to Aganga and Omphile (2000), the average annual rainfall for Sebele is 450 mm and the mean daily temperature 30 °C.

2.2. 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 was lit continuously for 16 hours.

2.3. Preparation of experimental diets

2.3.1. Chemical analysis of the feedstuff

Prior to diet formulation, proximate analysis of *I. belina* (Westwood), *V. subterranea* (L) Verde and *T. esculentum* (Burchell) Schreiber were determined by the method of AOAC (1996). 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 1996). Table 1 presents the chemical composition of the protein sources used in the diet formulations (%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's mash) 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.

2.3.2. Diet formulation

Three experimental diets containing either roasted *I. belina* (Westwood) or dehulled *T. esculentum* (Burchell) Schreiber or dehulled *V. subterranea* (L) Verde 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 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.

2.4. 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 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 (4x3x5). 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 Equation 1: 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.
T is the treatment degrees of freedom.

2.5. Measurement of parameters

Diets offered to the hens and the leftovers were weighed daily using an electronic balance (Adam 6010 model). Feed intake was determined by subtracting leftover feed from feed offered to the hens. The hens were weighed at the beginning of the experiment and subsequently on weekly basis. Average daily weight gain was determined as the difference between initial and final weights divided by the period of study (90 days). Number of eggs laid was recorded daily. Feed conversion ratio (FCR) was determined by dividing feed intake with weight gain on a weekly basis (Mwale *et al.*, 2008). Mortality in percentage was recorded as and when it occurred.

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

Ingredients	Treatment diets			
	T. esculentum	*V. subterranean*	*I. belina*	*Commercial*
Yellow maize (%)	59	19	78	*
T. esculentum (%)	37	00	00	
V. subterranea (%)	00	77	00	
I. belina (%)	00	00	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				
[1]Met. energy (MJ/KG)	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

2.6. Statistical analysis

The statistical model for the design was: $Y_{ijk} = \mu + Ţ_i + b\,(x_{ijk} - \bar{X}) + \varepsilon_{ij} + \sigma_{ijk}$

Y_{ijk} = $_{ijk}$ Response variable (Fi, ADG, FCR, H.E.D.P and mortality)
μ = Population mean
$Ţ_i$ = $i^{th}_{(1,2\ldots,4)}$ Treatments effects
$_j$ = (control, *V. subterranea* (L) Verde*, T, esculentum* (Burchell) Schreiber and *I. belina* (Westwood) diets)
b = regression coefficient

X_{ijk} = covariate (initial weight)
ε_{ij} = ij^{th} experimental error (variation within pens)
σ_{ijk} = $_{ijk}$ Sampling error (variation within hens)

Data were analyzed using PROC GLM of SAS (2002-2008) as a completely randomized design with initial body weight as a covariate according to the above model. Differences among treatment means were determined using the PDIFF option in the LSMEANS statement of the GLM procedure of SAS (2002-2008).The level of significance was set at P<0.05**.**

3. Results

Feed intake for the control hens was highest (P<0.05) at 84.6±7.9 g while feed intake for the treatment diets did not differ (Table 3). Hens fed *T. esculentum* had the lowest (P<0.05) ADG of -0.82±1.60 g compared to control, *I. belina* and *V. subterranea* diets which did not differ significantly. Hens on control and *T. esculentum diets* had the highest (P<0.05) FCR of 1.76±0.16 g and 1.61±0.16 g, respectively. Percentage hen day egg production was highest (P<0.05) for hens on control diet and lowest for hens fed *T. esculentum* diet. Mortality rate for hens on *T. esculentum*, *V. subterranea,* control and *I. belina* was 13.33%, 13.33%, 6.67 and 0%, respectively.

Table 3
Growth and laying performance of *Tswana* hens fed three protein sources *(I. belina, T. esculentum* and *V. subterranea* from 25 to 38 weeks of age

Parameters	Treatment diets			
	Control	***T. esculentum***	***V. subterranea***	***I. belina***
FI/hen /day(g)	84.6±7.9[a]	54.9±7.9[b]	59.3±7.9[b]	59.6±7.9[b]
FCR	1.76±0.16[b]	1.61±0.16[b]	1.23±0.16[a]	1.10±0.16[a]
ADG/hen(g)	3.27±1.16[a]	-0.82±1.60[b]	3.13±1.51[a]	4.77±1.31[a]
HDEP (%)	90±0.87[a]	41±0.87[d]	67±0.87[c]	75±0.87[b]
Mortality(%)(n)	6.67(1)	13.33(2)	13.33(2)	0.00(0)

[abcd]: Means with different superscript on the same row differ significantly (P<0.05)
FI/hen /day= feed intake per hen per day; FCR= feeds conversion ratio; HDEP= hen day egg production; ADG/hen= average daily gain per hen: n= number of hens died; n= number of birds which died per treatment.

4. Discussion

The observation that hens fed control diet had the highest feed intake could be due to the fact that the control diet had higher digestibility compared to other treatment diets. Higher digestibility would have led to faster passage rate, thus increasing feed intake and more nutrients becoming available to the birds for metabolic utilization. On the contrary, Nobo *et al*. (2012a) fed guinea fowl keets diet containing *I. belina* and found that inclusion of *I. belina* in guinea fowl diet did not influence feed intake of guinea fowl keets. The differences in results could be attributable to age and species difference.

The FCR for hens fed control diet was 1.76, whereas birds on diets containing *T. esculentum*, *V. subterranea* and *I. belina* had FCR of 1.61, 1.23 and 1.10, respectively. The higher FCR in hens fed *I. belina* and *V. subterranea* diets could be indicative of higher nutrient utilization by *Tswana* layers. The two diets were highly converted to gain 1 g in body weight; hence had comparable average daily gains (ADG) but were greater than control and *T. esculentum* diets. This finding suggested that hens fed *I. belina* diet utilized protein more efficiently than those fed the control diet. Madibela *et al*. (2006) reported that *I. belina* (Westwood) is superior in essential amino acids content compared to soybean meal and fishmeal but ranked moderately when compared to blood meal. Nobo *et al.* (2012a, 2012b) reported high FCR of 3.09 to 4.29 when feeding guinea fowl growers diets containing *I. belina* as a protein source compared to FCR of 3.83 to 3.96 for control diet. The difference in the present and previous results could be ascribable to age and species difference.

The lower ADG for hens fed *T. esculentum* diet compared to other treatment diets could be attributable to possible poor digestibility of protein in *T. esculentum* (Burchell) Schreiber beans due to the presence of trypsin inhibitors. Bower *et al.* (1988) reported trypsin inhibitor of 20% of the seed protein in *T. esculentum* beans. Trypsin inhibitor is known to bind protein within the ileal epithelium, thus disrupting the digestive and absorption processes of proteins and consequently lowering body weight. Extrapolating on this fact, it also inhibits the activities of the proteolytic enzyme trypsin, which results in lower activities of other proteolytic enzymes that require trypsin for activation. The lowering of proteolytic activity of such enzyme results in a slow release of essential amino acids leading to depressed growth. This observation is supported by Ogbonna and Oredein (1998) who reported depressed growth in cockerel chicks due to trypsin inhibitor in *Mucuna esculanta.*

There could also be high level of non-digestible carbohydrates that depress the digestibility of protein by binding the amino acids (Tshovhote *et al*., 2003). Another reason could be that *T. esculentum* diet had more fibre (Table 1) than the recommended level. *Tylosema esculentum* (Burchell) Schreiber could also have imparted unpalatable taste to the feed resulting in birds consuming less feed. The above factors may have contributed to loss of weight in the hens fed *T. esculentum* diet. The results of this study are consistent with Tulenu *et al.* (2008) who found lower (6.65%) body weight gain for hens fed raw Velvet beans (*Mucuna utilis)* compared to 18% for control.

The lower HEDP for hens fed *T. esculentum* diet observed in the current study could be due to anti-nutritional factors which resulted in poor digestibility of protein in *T. esculentum* (Burchell) Schreiber as reported by Bower *et al*. (1988). Anti-nutritional factors such as trypsin inhibitor inhibit the metabolism of dietary protein needed for normal egg production and might also interfere with the normal secretion of hormones (*e.g.*, follicle stimulating hormones, luteinizing hormones) and progesterone needed for yolk formation in the anterior pituitary gland (Akanji and Ologhobo, 2007). The other reason could be that *T. esculentum* (Burchell) Schreiber had high fibre content (Table 1) that resulted in nutrients required not being released to the laying hens. The reduction in HDEP for hens fed *T. esculentum* diet is in agreement with Akanji and Ologhobo (2007) who reported a decrease in HDEP in hens fed diets containing raw tropical legume seeds. The lower feed intake in hens fed *T. esculentum* diet could lead to deficiency in protein intake. Similarly, Adeyemo *et al.* (2006) recorded low HDEP in birds fed diets containing 12% crude protein (CP).

The low HDEP on hens fed *V. subterranea* diet could be due to low amino acids and minerals (P and Ca) digestibilities in *V. subterranea* diet due to the presence of anti-nutritional factors. Akanji and Ologhobo (2007) reported that the presence of phytates has the tendency to bind Ca and P thereby making them available in insufficient quantities for egg formation. This suggests that hens fed *V. subterranea* diet were in severe protein deficiency as the proteins were unavailable to hens; hence the inability of the hens to perform to their genetic potential. Therefore it is vital to improve digestibility of protein and absorptivity of minerals in *V. subterranea* (L) Verde before these can be used as protein sources in poultry diets.

Hen day egg production for hens fed *I. belina* diet (11 eggs/clutch) and control diet (14 eggs/clutch) fell within the reported range of number of eggs per clutch for *Tswana* hens. In Botswana, Aganga *et al.* (2000) reported a range of 10-13 eggs per clutch for *Tswana* hens under extensive management system, whereas Moreki (2010) reported three clutches (15.4±4.53 eggs per clutch) in a year. The reported HDEP for hens fed *I. belina* diet in the current study is indicative of a better supply of amino acids and minerals (P and Ca) in *I. belina* diet compared to other diets. The low HDEP for hens fed *T. esculentum* diet (3 eggs/clutch) and V. *subterranea* diet (8 eggs/clutch) in the current study fell below the reported range of number of eggs per clutch for *Tswana* hens. The low HDEP is likely to be due to the presence of anti-nutritional factors (*e.g.*, phytates and trypsin inhibitor) in raw *T. esculentum* (Burchell) Schreiber and *V. subterranea* (L) Verde. According to Bower *et al.* (1988), anti-nutritional factors form complexes with the dietary protein, thus resulting in low protein digestibility. Card and Nesheim (1975) concluded that anti-nutritional factors will reduce the metabolism of the dietary protein and subsequently have adverse effect on egg formation.

Post mortem examination that was conducted by the National Veterinary Laboratory at Sebele showed that the cause of mortality was coccidiosis.

5. Conclusion

The results obtained from the study suggest that treatment diets had lowest feed intake. Birds fed *T. esculentum* diet had the lowest (P<0.05) ADG, while hens fed *I. belina* and *V. subterranea* consumed less feed to produce 1 g of meat. Hen day egg production was different for all treatments with control diet having the highest HDEP while hens fed *T. esculentum* diet had the lowest HDEP. Dietary treatments did not seem to have an effect on mortality.

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