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Effect of Low Tannin Sorghum Based Feed on Physical and Nutritional Quality of Layer Chicken Eggs

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Abstract

This study was carried out to evaluate the effect of replacing maize with low tannin sorghum (LTS) in layer feed on the physical and nutritional quality of eggs of layers at peak egg production stage. One hundred and twenty, 30-weeks old hens were grouped into three blocks of forty birds per block and were subjected to three different diets: 100%Maize, 50%Maize50%LTS and 100%LTS for 8 weeks. Eggs were collected on the 4th and 8th week of feeding trial. The results showed that eggs had statistically similar weights, amounts of protein, fat, Vitamin E and Vitamin A. The colour of egg yolks increased in lightness and reduced in hue and Chroma significantly across all the dietary blocks with increase in LTS while cholesterol content decreased. The saturated fatty acid content in the albumin and yolk oil extract remained constant irrespective of the period of feeding or variation of Maize and LTS content. The Monounsaturated fatty acid content decreased significantly (P=0.0003) during the whole trial diet period. The Polyunsaturated fatty acid content remained constant for the albumin (P=0.4095) while the yolk showed an increase (P=0.1162) from the initial 15.82±0.57 g/100g on the 4th week to 24.05±7.25 g/100g on the 8th week for diets with 100%Maize. 50%M50%LTS increased from 14.59±0.16 to 21.48±4.19 g/100g and 100%LTS had its Polyunsaturated fatty acid content decline from 13.36±0.31 to 10.71±0.32g/100g. This study indicates that LTS can replace Maize as a source of energy in chicken feeds with no adverse effects on the quality of the eggs**.**

Keywords: albumin, cholesterol, colour, Haugh unit, vitamins, yolk

1. Introduction

Feed is an integral part of chicken production enterprise. It is one of the key expenditures in an intensive chicken production system (Narrod, Tiongco, & Costales, 2012). Energy in feed is an important aspect of the feed quality as it fuels chicken physical and reproductive activities (Bryden et al., 2009). In Kenya maize has been used as the key source of energy in the feed industry. However, maize is a commodity in high demand as it acts as both human food and animal feed ingredient. There is therefore a need to use alternative sources of energy which perform just as well as maize and this can be well achieved by use of LTS grains (Ulrich et al., 2000).

Sorghum is the fifth most popular cereal crop in the world (USDA, 2017). It can grow in arid and semi-arid regions with minimal water, labour and farm input requirement to produce good yield. This makes it suitable for Kenya since the climate is favourable. The problem with high tannin sorghum varieties when used as chicken feed is that they reduce the feed intake, the live weight gain, egg production and size and therefore reduced feed efficiency per dozen of eggs per kg mass (Issa, 2009; Ka & Mukhtar, 2015). Low Tannin sorghum (LTS) is a variety of sorghum with low tannin content of as low as 1% which enables it to have minimal deleterious effects when compared to the High tannin sorghum varieties.

In egg production, the productivity of the hens as well as the quality characteristics are of key importance in the consumer acceptability of the eggs(Choi, Park, & Kim, 2010). The physical quality of the eggs are influenced by the hen breed, housing system, hen age and environmental conditions(Akhtar et al., 2007; Brand, Parmentier, &

Kemp, 2004; Mugnai et al., 2016; Parmar et al., 2006). The feed can be used to manipulate some of the quality attributes of the eggs but this is limited as the feed can't perform a versatile role as in meat (Griffin, 1992; Karges & Corzo, 2010). The protein quality and content of the eggs are highly regulated by the chicken systems and are hard to manipulate based on diets. The saturated fatty acid content of the eggs cannot also be manipulated by the feed ,however, the polyunsaturated fatty acids and the monounsaturated fatty acids are more pliable to be changed by the feed (Meluzzi et al., 2000).

Egg yolk colour is of great importance in the determination of the quality of the yolk as the consumers prefer a deep yellow colour (Beardsworth & Hernandez, 2004; Jeffrey A. Coutts, 2007). The colour of egg yolk is the most pliable to dietary manipulation as the chicken are not able to synthesize the colouring pigments on their own. The colour is brought about by presence of carotenoids in the chicken diet. LTS has no carotenoids and has detrimental effect on the colour of the egg yolk. It is therefore very important to introduce this component to the layer chicken diets if LTS is to be used in the feed formulation. Preferably use of plant leaves and pure carotenoids like xanthophyll could be exploited for this purpose (Hasin, Ferdaus, Islam, Uddin, & Islam, 2006; Liu et al., 2012 & Bonilla et al., 2017).

The objective of this study was to evaluate the effect of feed on the quality of eggs as affected by feed whose source of energy are maize and Low tannin white sorghum in varying quantities.

2. Methodology

2.1 Layer Chicken Feeding and Performance

The chicken were reared at a commercial poultry farm at Happy Valley Estate, Thika, Kiambu County with geographical coordinates 1.0679 °S, 37.1500 °E

One hundred and twenty, thirty-week old, Isa brown layer chicken at peak egg production period feeding on a basal commercial diet were randomly selected and placed into cages/blocks of 40 hens per cage. The chicken were subjected to the trial feed with formulations as shown in Table 1 for 8 weeks. The eggs were collected on the 4th and 8th week of trial for analysis.

Table 1. Feed formulation for layer hens

Ingredient	Amounts (kg)		
	100%Maize Diet 1 (control)	50%Maize+ 50%Sorghum Diet 2	100%Sorghum Diet 3
Maize	65.0	32.5	-
Sorghum	-	32.5	65.0
Soybean meal	24.5	24.5	24.5
Vitamin Premix*	0.25	0.25	0.25
Iodized salt	0.25	0.25	0.25
Methionine	0.085	0.12	0.155
Lysine Hcl	0.05	0.05	0.05
Bone meal	2.30	2.30	2.30
Limestone	7.50	7.50	7.50
Total (kg)	100	100	100
Calculated nutrient value			
Nutrient	*Value*		
Metabolisable energy	235Kcal/kg		
Crude protein	16.5%		
Calcium	3.25%		
Phosphorus	0.4%		
Lysine	0.72%		
Methionine	0.35%		

* Vitamin premix(kg^{-1}): Vitamin A (I.U) 6250000,Vitamin D3 (I.U) 1000000,Vitamin E (I.U) 15000,Vitamin K3 (Mg) 1000, Vitamin B1 (Mg) 500,Vitamin B2 (Mg) 2500, Vitamin B6 (Mg) 2500, Vitamin B12 (Mg) 10, Pantothenic acid (Mg) 600, Nicotinic acid (Mg) 15000, Folic acid (Mg) 500, Biotin (Mg) 35, Choline chloride (Mg) 150000, Iron (Mg) 20000, Copper (Mg) 2500, Zinc (Mg) 25000, Manganese (Mg) 15000, Iodine (Mg) 600, Cobalt (Mg) 400, BHT (Anti-oxidant, Mg) 125000.

The eggs were then transported from the experimental farm in Happy Valley to JKUAT Food Biochemistry Laboratories for physical and chemical analysis.

2.1.1 Sample Preparation

Prior to analysis, twenty eggs from each trial diet were broken and physical characteristics determined before separating the components into albumin and yolk. The samples were stored in the fridge at 0 °C until analysis.

2.2 Determination of Proximate Composition

Proximate composition (moisture, crude protein, and total lipid) of the egg yolk and egg albumin was carried out using the AOAC 2014 method.

2.3 Determination of Fatty Acid Profile of the Lipid Extracts

Fatty acid profile was determined according to the procedure described by Almeida et al., (2006). Total lipids were extracted from the albumin and yolk samples using a modified Bligh and Dyer method (1957). After extraction the lipids were methylated using methanolic HCl (5%). The fatty acid methyl esters (FAME) were then determined using Gas Chromatography (Shimadzu GC-14B) equipped with Flame Ionization Detector (FID) and fused capillary column (Omegawax^{TM} 5530) of 30M×0.53mm×0.5µm dimensions. The fatty acids were identified by comparing with the retention times of standards of known methyl esters (Sigma Aldrich 37 components FAME; linoleic acid methyl ester mix (cis/trans); trans-11-vaccenic acid methyl ester; conjugated linoleic acid methyl ester), and the esterified samples. The fatty acids were quantified in g per 100g of the sample.

2.4 Determination of Cholesterol Content

This was done using High-Pressure Liquid Chromatography (HPLC) by a method described by Almeida et al. (2006). This method involves two main steps: saponification and cholesterol measurement. Saponification involved the addition of 2ml of 50% potassium chloride 50% potassium hydroxide and 5ml of 95% and 5% ethanol absolute and distilled water respectively to the sample 2g of sample. The mixture was heated in a water bath (SHA-C water bath shaker) for complete solubilization at 60 ℃ for 10 min. This was followed by an addition of 5 ml distilled ice-cold water and the sample was then cooled in an ice bath. The non-saponifiable fraction was extracted three times using 10 mL of hexane. Aliquots of hexane extracts (3 mL) were dried under a vacuum. The extract obtained from saponification was dissolved in 3 mL of acetonitrile-isopropanol solution (70:30, v/v) and analysed with normal phase HPLC using unmodified silica gel as a stationary phase. HPLC apparatus which consisted of a ternary solvent The HPLC apparatus consists of a SHIMADZU® system including a ternary solvent delivery system (LAD 10); a Rheodyne 20 mL loop injector with column temperature of 30 ℃; ultraviolet detector; and software (CLAS-VP 10) for data processing. The mobile phase consisted of acetonitrile and isopropanol (70:30, v/v). Cholesterol identification was done by co-chromatography and by comparing sample retention times with standard retention times (Sigma and Polyscience, U.S.A. ® C8667). Quantification for each sample was achieved by using a standard curve for the standard solutions.

2.5 Determination of Vitamin E Content

This was carried out as described by Ponte et al. (2008). Two grams of albumin and yolk samples of each was mixed separately with hexane to dissolve all the vitamin E. The hexane phase was extracted and analysed using HPLC. The HPLC apparatus consists of a SHIMADZU® system including a ternary solvent delivery system (LAD 10); a Rheodyne 20 mL loop injector with column temperature of 30 ℃; ultraviolet detector. The conditions for HPLC were mobile phase consisting of 98%hexane and 2% isopropanol, a normal phase column consisting of silica 60, the oven temperature was set at 25^0 C, the flow rate of the mobile phase was 1 to 1.5ml/minute and the injection volume was 20 µl.

2.6 Determination of Vitamin A (retinol) Content

This was determined as described by Zahar, M., & Smith, (1990). Briefly, to a 50ml glass stoppered centrifuge tube, 2 g of sample was added and then 5ml of absolute ethanol containing 0.1% (wt/vol) ascorbic acid followed by 2ml of 50% (wt/vol) KOH. Centrifuge tubes were closed tightly, agitated carefully and placed in a water bath at 80^0 C for 20 mins. During this period, tubes were agitated periodically to ensure complete digestion of fat. After saponification, the tubes were cooled with running water and then placed in ice water bath 20mls of hexane containing 0.01% (weight /vol) BHT was then added. Centrifuge tubes were mixed vigorously with a vortex for 1 min, allowed to stand for 2 minutes and again vortexed for 1 min. 5mls of cold water (1^0 C) was added to each sample and the tubes were inverted 10 times. Centrifugation was done at 1000 × g for 10 minutes. 10 ml of the upper, organic layer was accurately removed by pipette into a rotary flask and the solvent was evaporated under vacuum at 40^0 C using a rotary evaporator. The residue was immediately dissolved in 1ml of methanol. The samples and the standards were then injected into HPLC, SHIMADZU® system including a ternary solvent delivery system (LAD 10); a Rheodyne 20 mL loop injector with column temperature of 30 ℃; ultraviolet detector, with the following conditions; the mobile phase comprised of 95%methanol and 5% distilled water, the flow rate of the mobile phase was 0.8ml/min, the detector used was the ultra violet detector at 325nm wavelength and the injection volume was 20 µl.

2.7 Physical Measurements

2.7.1 Determination of Haugh unit

This was determined by the method illustrated by Menezes et al. (2012). The Haugh unit was used to measure protein quality based on height of the egg white. Eggs sampled for each dietary treatment was weighed individually, their widths and lengths measured then broken on a white and smooth surface. The average measurements of albumin height and egg weight was used to compute Haugh unit score for each egg as:

$$Haugh\ Unit = 100 \times log(H + 7.57 - 1.7W^{0.37}) \tag{1}$$

Where: H = albumen height (mm) and W = egg weight (g).

2.7.2 Determination of Yolk Index

This was done as illustrated by Heiman and Wilhelm, (1937). Yolk height was determined using a tripod micrometre while yolk diameter was measured using steel Vernier callipers. The Yolk index was calculated as:

$$yolk\ index = \left(\frac{yolk\ height}{yolk\ diameter}\right) \times 100 \tag{2}$$

2.7.3 Determination of Albumin Index

This was carried out as illustrated by Rath et al., (2015). After measuring the height and width of albumen of each broken egg, the albumen index was calculated as:

$$Albumin\ Index = \left(\frac{albumin\ height(mm)}{albumin\ length\ (mm) \times albumin\ width(mm)}\right) \times 100 \tag{3}$$

2.7.4 Colour Measurement

This was carried out using the CIELAB method where the colour values were determined as illustrated by Qiao et al. (2001). The eggs were broken and yolk separated from the albumin. The yolk was put into a transparent glass bottle that was covered using an opaque, black plastic bag and the torch shone for measurement using the CIE Minolta colorimeter. The L*, a* and b* values were recorded for each yolk by taking the colour from three arbitrary places on the glass bottle bottom. The values read were then computed to determine the hue angle and Chroma.

2.8 Statistical Analysis

The quantitative data was analysed by STATA/IC 12.0 statistical software in which the evaluation of residual homogeneity was checked using the Shapiro-Wilk test ($p<0.05$). Further due to inherent limitations of ANOVA in describing difference in progression of variables over time, the analysis of covariance (ANCOVA) which combines features of both ANOVA and regression was also applied to test effects of diet and age on egg quality, and the interaction effects. When the coefficient of the interaction term was significant ($P < 0.05$), it was concluded that there was a significant difference between diets and age of hens. One-way ANOVA was performed where treatment outcomes at a specific point in storage time needed to be compared. Means were separated using Bonferroni adjustment at 95% confidence level.

3. Results and Discussion

3.1 Proximate Composition

The Table 2 indicate the proximate composition of the albumin and the yolk. The results show that the yolk was generally richer in fat and protein than the albumin. There was no significant (P=0.9383) difference in the moisture content due to interaction of diet and age in the albumin. There was also no significant difference observed when the main effect was diet (P=0.9065) or age (P=0.9805).

Layers fed on diets with higher maize content laid eggs with less variation in protein content ranging between 26.91±2.81 to 26.69±3.51 % against 23.94±1.20 to 22.59±4.19% for 50%M50%LTS and 28.49±2.40 % to

23.34±1.11 % protein for 100%LTS. There was no significant difference in relation to interaction between diet and age (P=0.9968) in the albumin of the eggs and the diets and age had no significant effect independently as their P-values were 0.0925 and 0.6161 respectively. The fat content of the albumin was generally low compared to the yolk with a range of between 0.83 to 4%. There was no significant difference caused by effect of interaction between diet and age (P=0.4879). Diet had a P-value of 0.5203 while age had a P-value of 0.2444 as the main variables. This shows that the proximate composition of the egg albumin were not affected by the diet or age of the birds as this is an inherent property of the chicken body to regulate these properties.

Table 2. Proximate composition of the eggs

Period	Diet	Yolk			Albumin		
		Fat (%)	Protein (%)	Moisture (%)	Fat (%)	Protein (%)	Moisture (%)
4 weeks	100% Maize	3.25±0.18^{a}	26.91±2.81^{a}	54.78±0.34^{a}	0.91±0.02^{a}	20.36±0.95^{a}	87.34±0.05^{a}
	50%M50%LTS	8.07±1.77^{a}	23.94±1.20^{a}	56.56±1.09^{a}	1.22±0.39^{a}	17.20±0.95^{a}	87.08±0.07^{a}
	100% LTS	7.89±1.46^{a}	28.49±2.40^{a}	55.22±0.91^{a}	0.74±0.06^{a}	18.71±1.66^{a}	87.03±0.01^{a}
8 weeks	100% Maize	21.49±2.95^{b}	26.69±3.51^{a}	55.64±1.34^{a}	0.84±0.12^{a}	19.72±1.80^{a}	88.13±0.04^{a}
	50%M 50%LTS	21.04±2.60^{b}	22.59±4.19^{a}	56.18±0.61^{a}	4.00±1.60^{a}	16.75±0.56^{a}	87.42±0.05^{a}
	100% LTS	6.57±0.08^{a}	23.34±1.11^{a}	54.75±0.14^{a}	1.38±0.84^{a}	18.20±1.22^{a}	86.04±2.19^{a}
P-Value	A& D	0.0006	0.6578	0.6888	0.4879	0.9968	0.9383

A; age, D; diet

Means with different superscripts on the same column are significantly different p<0.05.

The egg albumin had generally very low fat content of between 0.83±0.01% to 4.00±1.60 %, low protein content of between 16.75±0.56% to 20.36±0.95% and high moisture content of between 86.04±2.19 percent to 88.13±0.04 %. This is to signify that the albumin is made up of very high water content and is very lean compared to the yolk. The results from this study gave similar findings as those from previous research work that proved that the protein content and the proximate content of eggs are not altered by dietary interventions as they are very tightly controlled in the avian systems (La et al., 2001; Meluzzi et al., 2000; Milinsk et al., 2003).

Egg yolk had higher fat content ranging between 3.25±0.18% to 21.49±2.95% across all the ages and diets, this variation was significantly (P<0.0001) different in relation to interaction between the diets and age of the birds. The protein content of the egg yolks ranged between 20.39±1.96 to 28.49±2.40 %. There was no significant (P=0.6212) difference based on the effect of variation in diet and age of the hen on the protein content. The protein content of the yolk was higher than that of the albumin while the moisture content was lower than that of the yolk with the values ranging between 54.74±0.43 to 56.56±1.09 %. There was however no significant (P=0.9383) difference caused by interaction of both diet and age of the layers on the moisture content of the yolks.

The lack of variability in the proximate components of the egg albumin and egg yolks from chicken fed on various amounts of maize and LTS in the feed of the chicken backs up previous finding that these components are not affected by diets but by other factors including the strain and age of the layers (Minelli et al., 2007).

3.2 Cholesterol, Vitamin E and Vitamin A Content

The results in Table 3 show the response of the eggs in relation to the cholesterol, vitamin E and vitamin A content. The albumin had significantly lower contents of these parameters in comparison to the yolk irrespective of the period from the introduction of the trial diet. There was no significant (P=0.7930) difference by the effect of the interaction of age and variation of amount of maize and LTS in the diet on the cholesterol content of the egg albumin. Diet alone had no significant (P=0.7930) effect on the cholesterol content while the age produced a significant difference (P=0.035) on the cholesterol content.

Table 3. Cholesterol, Vitamin E and Vitamin A Content

Period.		Cholesterol		Vitamin E		Vitamin A	
	Diet	Albumin	Yolk	Albumin	Yolk	Albumin	Yolk
4 weeks	100% Maize	167.19±8.30[a]	222.06±9.97[a]	12.07±1.20[a]	21.13±2.26[a]	0.19±0.13[b]	0.21±0.05[a]
	50%M50%LTS	156.33±11.14[a]	215.75±6.52[a]	11.03±1.46[a]	18.96±1.58[a]	N.D.[a]	0.12±0.01[a]
	100%LTS	162.79±8.79[a]	188.54±3.29[a]	12.23±2.31[a]	18.67±2.79[a]	N.D.[a]	0.12±0.01[a]
8 weeks	100% Maize	134.27±6.06[a]	167.54±12.02[a]	11.98±1.12[a]	20.16±0.85[a]	0.14±0.02[ab]	0.29±0.14[a]
	50%M50%LTS	154.35±19.93[a]	167.04±10.76[a]	12.09±1.14[a]	18.88±1.14[a]	0.16±0.02[ab]	0.27±0.12[a]
	100% LTS	132.98±3.52[a]	128.78±10.85[a]	12.48±0.89[a]	18.56±1.21[a]	0.13±0.01[ab]	0.14±0.03[a]
P-value	A& D	0.3307	0.8432	0.1287	0.4562	0.1725	0.7839

A; age, D; diet

Means with different superscripts on the same column are significantly different by Bonferroni posthoc test p<0.05.

3.2.1 Cholesterol Content

Eggs laid by hens fed on 100% Maize had the highest cholesterol content of 222.06±9.97mg/100g and 167.54±12.02mg/100g for the 4th and 8th week collection respectively in the yolk as compared to the rest of the eggs. The cholesterol content for the other treatments were 162.79±8.79 and 156.33±11.14mg/100g for the 50%M50%LTS and 100%LTS respectively. The cholesterol content of the egg yolk differed considerably between the eggs collected on the 4th and 8th week. The egg samples from hens fed on 100% LTS had the least cholesterol in the yolks. There was a significant (P=0.001) difference due to interaction between diet and age on the cholesterol content. Diet as the main effect also caused a significant (P=0.0041) difference on the cholesterol content of the egg yolks. The reports on manipulation of feed studies and reduction cholesterol content concluded that the eggs have 200-250mg/100g of cholesterol (Griffin, 1992 & Singh, Cheng, & Silversides, 2009). This agrees with the results found as the egg samples that were analysed had similar amounts of cholesterol.

3.2.2 Vitamin E Content

The vitamin E content of the yolks was within a range of between 18.96±1.21 to 21.13±2.26mg/100g for both the 4th and 8th week of collection. Samples of eggs collected from hens fed on 100% Maize had the highest vitamin E contents of 21.13±2.26 and 20.16±0.85mg/100g for 4th and 8th week collection respectively. Maize grains have been cited to have between 0.07 to 0.11mg/100g Vitamin E content (Rouf Shah, Prasad, & Kumar, 2016) while sorghum grains have 0.174 to 0.552mg/100g vitamin E content (Afify, El-Beltagi, Abd El-Salam, & Omran, 2012). The diets had no significant effect on the vitamin E content of both the yolk and the albumin giving P-values of 0.7075 and 0.0677 respectively. Aljamal (2011) reported on the effect of varying the level of vitamin E in the diet on the egg vitamin E content. However, this study varied only the source of energy by reducing the amount of maize while increasing the amount of LTS, since maize has considerably higher vitamin E content than sorghum, the lower Vitamin E of the egg albumin and yolk could be explained by this since eggs from chicken fed on pure maize diets had higher vitamin E content than those with sorghum grains.

3.2.3 Vitamin A Content

Vitamin A content of the egg yolks ranged from 0.21±0.05 to 0.12±0.01mg/100g on the 4th week of the trial feeding and 0.29±0.04 to 0.14±0.03mg/100g on the 8th week. This was a slight increase in the amounts, however, no significant (P=0.3215) difference was observed on the interaction of variation of diet and age, and no significant (P=0.6424) difference was also observed on the variation of amount of maize and LTS in the diets. It has been reported that carotenoids are the colouring compounds of egg yolks. Since they are pro-vitamin A forms which are converted to active vitamin A as retinol the values obtained from the colour test combined with the results on vitamin A content reveal that the diets were devoid of this vitamin in its active or inactive form. (Liu et al., 2012; Bonilla et al., 2017). For the albumin there was however a significant (P=0.008) difference caused by effect of interaction of diet and age on vitamin A content. Diet as the main effect produced no significant (P=0.7171) difference while age had a significant (P=0.0001) difference in the yolk retinol content. Albumin samples showed significantly lower vitamin A content as compared to yolks with the values ranging from none detectable to 0.19±0.13mg/100g. LTS has considerably low vitamin A content (Bonilla et al., 2017) and this resulted to lower vitamin A content in the albumin and the yolk of the eggs from hens fed on LTS than those of hens fed on 100% Maize.

The lower nutrient quality of the albumin in relation to the yolk could be explained by the role of the yolk in embryo formation and growth in the egg before the chick is hatched. The yolk has to have adequate or even extra content of the nutrients to enable the formation of the embryo. It, therefore, has to be richer than the albumin in the nutrient content (Griffin, 1992).

3.3 Fatty Acid Profile of Egg

The results in Table 4 show the fatty acid profile of the albumin samples from the different trial diets. There was no significant effect of interaction of age and diet on the palmitic (P=0.1304), stearic (P=0.3908), linoleic (P=0.7960), linolenic (P=0.7297) acid contents of the albumin. Diets had a significant effect for most of the fatty acids present (P≤0.05).

Table 4. Fatty Acid Profile of Albumin in % of Lipid Extract

	4 weeks			8 weeks			P-values
	100% Maize	50%M5%LTS	100% LTS	100% Maize	50%M5%LTS	100% LTS	A&D
Palmitic (C16:0)	19.68±0.50[a]	19.42±0.82[a]	20.78±0.80[a]	23.61±2.08[a]	20.69±0.52[a]	26.89±1.06[a]	0.1304
Palmitoleic (C16:1)	1.21±0.77[a]	0.69±0.24[a]	3.10±0.16[b]	1.58±0.84[a]	1.18±0.10[a]	0.96±0.13[a]	0.0314
Stearic (C18:0)	7.61±0.27[ac]	6.21±1.03[a]	5.94±0.23[a]	8.74±0.96[a]	9.06±0.04[a]	9.13±0.06[a]	0.3908
Oleic (C18:1)	14.77±0.51[a]	29.59±0.08[b]	27.08±1.68[b]	21.13±4.09[c]	20.51±0.48[c]	27.19±0.39[b]	0.0039
Linoleic (C18:2)	10.47±0.24[a]	13.88±0.11[a]	15.18±0.76[a]	9.45±1.92[a]	11.70±0.62[a]	13.28±0.04[a]	0.796
Linolenic (C18:3)	1.51±0.02[b]	0.49±0.01[a]	ND[a]	1.71±0.77[a]	0.30±0.04[a]	0.29±0.19[a]	0.7297
Arachidonic(C20:4)	1.13±0.68[ab]	4.29±0.08[c]	3.29±0.18[c]	0.55±0.32[b]	1.74±0.25[a]	1.96±0.08[a]	0.037
EPA (C20:5)	1.76±0.01[a]	1.24±0.00[a]	3.94±0.20[c]	2.95±0.32[b]	2.59±0.07[ab]	2.28±0.04[a]	<0.0001
DHA (C22:6)	ND	ND	ND	ND	ND	ND	N/A
SFA	43.13±1.40[c]	32.45±1.73[a]	35.68±0.44[ab]	40.35±1.85[bc]	32.57±0.29[a]	35.78±3.42[ab]	0.0171
MUFA	17.11±0.44[a]	34.59±0.07[b]	33.48±2.02[b]	23.27±4.57[a]	23.43±0.33[a]	30.83±0.44[b]	0.0044
PUFA	13.74±0.26[a]	15.63±0.12[a]	19.13±0.96[a]	14.09±3.01[a]	14.59±0.57[a]	15.86±0.18[a]	0.4095
n6/n3 ratio	6.77±0.13[c]	11.56±0.11[d]	3.85±0.03[ab]	3.68±0.51[a]	4.66±0.39[c]	5.94±0.05[c]	<0.0001

A; Age, D; diet.

Means with different superscripts on the same row are significantly different p<0.05.

SFA= saturated fatty acids (C14:0, C15:0, C16:0, C17:0, C18:0, C20:0).

MUFA = monounsaturated fatty acids (C18:1trans-11, C18:1trans-9, C18:1n9c).

PUFA=polyunsaturated fatty acids (C18:2n6c9t12, C18:2n6t9c12, C18:2n6c, C18:3n6, C18:3n3, C18:2n9c11t, C20:2n6, C20:2n6, C20:3n6c, C20:3n3, C20:4n6, C22:2n6, C20:5n3, C22:5n-3, C22:6n3).

n-6 (C18:2n6t9t12, C18:2n6c9t12, C18:2n6t9c12, C18:2n6c, C18:3n6, C20:2n6, C20:2n6, C20:3n6c, C20:4n6, C22:2n6).

n-3 (C18:3n3, C20:3n3, C20:5n3, C22:5n-3, C22:6n3).

ND= Not Detected

The fatty acid profile of the egg albumin showed that the most abundant fatty acid class was the saturated fatty acids followed by the monounsaturated fatty acids and the least concentration was of the polyunsaturated fatty acids. The stearic acid content was very stable irrespective of the diet or period from the introduction of the diets and ranged from 5.94±0.23 g/100g to 9.13±0.06g/100g of lipid extract. The palmitic acid was the most dominant fatty acid while oleic acid was the most dominant monounsaturated fatty acid. Linoleic acid occurred in more abundance in comparison to the linolenic acid which occurred in low to undetectable quantities. It was also observed that the eicosanoids EPA and DHA occurred in very low quantities while DHA occurred in non-detectable quantities.

The n6/n3 ratio occurred in a range between 3.68±0.51 to 11.56±0.11. This showed that the lipid quality could be good since it is recommended to be between 6 and 10 for a wholesome nutritional quality (Högberg et al., 2003). Since feeding did not involve modification of the fatty acid profile of the feed, the fatty acid classes responded within a range and this was able to show that the hen systems were able to regulate the fatty acid profile of the eggs they laid. Meluzzi et al. (2000) stipulated that total proteins are not affected by dietary treatments but mineral, fat-soluble vitamins, lipids and fatty acid profile are modified by feed manipulation. However, the saturated fatty acids are almost unresponsive to dietary strategies. This concept was particularly true for the results obtained from this research hence the nutritional quality of chicken egg albumin can conclusively not be easily altered by replacing one component of the feed but by enriching or having a complete overhaul in the nutritional quality of the feed.

Table 5 shows the effect of the feed in relation to the fatty acid profile of the yolks. There was a significant effect of interaction of age of hens and diet on most of the fatty acids present in the yolk except for the stearic (P=0.5729), linoleic (P=0.1944), arachidonic (P=0.6353) and EPA (P=0.1389) acids of the lipid extracts. Fatty acid profile of the yolk showed that the highest values obtained were from palmitic acid which ranged from 28.71±0.45 g/100g to 31.19±0.62g/100g.

Table 5. Fatty Acid Profile of Yolk in % of Lipid Extract

	4 weeks			8 weeks			P-value
	100% Maize	50%M50%LTS	100%LTS	100% Maize	50%M50%LTS	100%LTS	A&D
Palmitic (C16:0)	28.71±0.45^{a}	31.19±0.62^{c}	30.02±0.12^{b}	29.45±0.14^{ab}	29.14±0.35^{ab}	29.03±0.33^{ab}	0.0097
Palmitoleic (C16:1)	0.34±0.16^{ab}	0.09±0.01^{a}	0.23±0.14^{a}	1.33±0.00^{c}	1.60±0.13^{c}	0.56±0.06^{b}	0.0004
Stearic (C18:0)	16.3±0.02^{a}	15.08±0.12^{a}	13.79±0.26^{a}	14.01±2.03^{a}	11.13±0.01^{a}	10.03±0.01^{a}	0.5729
Oleic (C18:1)	25.04±0.23^{c}	27.91±0.05^{cd}	30.02±0.04^{d}	10.23±0.11^{a}	15.12±0.39^{b}	11.03±0.01^{a}	<0.0001
Linoleic (C18:2)	13.50±0.31^{a}	10.61±0.08^{a}	12.85±0.16^{a}	21.27±7.26^{a}	19.03±3.82^{a}	9.69±0.05^{a}	0.1944
Linolenic (C18:3)	0.02±0.01^{a}	0.07±0.01^{a}	0.14±0.05^{a}	0.02±0.00^{a}	0.33±0.11^{b}	0.004±0.00^{a}	0.0059
Arachidonic(C20:4)	5.91±0.03^{a}	3.64±0.31^{a}	5.04±0.01^{a}	2.05±1.19^{a}	0.59±0.02^{a}	2.19±0.57^{a}	0.6353
EPA (C20:5)	1.35±0.22^{a}	1.95±0.20^{a}	1.04±0.12^{a}	1.52±0.01^{a}	1.36±0.07^{a}	0.96±0.29^{a}	0.1389
DHA (C22:6)	0.95±0.03^{bc}	0.73±0.04^{b}	0.67±0.08^{b}	1.24±0.01^{c}	0.76±0.33^{b}	0.05±0.00^{a}	0.0179
SFA	45.31±0.39^{a}	46.78±0.50^{a}	44.19±0.34^{a}	43.78±1.94^{a}	40.60±0.37^{a}	39.19±0.34^{a}	0.0503
MUFA	31.28±0.09^{b}	31.622±0.26^{b}	35.29±0.12^{c}	13.62±1.29^{a}	17.32±0.44^{ab}	13.79±0.61^{a}	0.0003
PUFA	15.82±0.57^{a}	13.36±0.31^{a}	14.69±0.16^{a}	24.05±7.25^{a}	21.48±4.19^{a}	10.71±0.32^{a}	0.1662
n6/n3 ratio	6.01±0.52^{a}	4.09±0.34^{a}	7.68±0.15^{a}	7.76±2.67^{a}	9.11±0.88^{a}	11.51±3.56^{a}	0.683

A; Age, D; diet.

Means with different superscripts on the same row are significantly different p<0.05.

SFA= saturated fatty acids (C14:0, C15:0, C16:0, C17:0, C18:0, C20:0).

MUFA = monounsaturated fatty acids (C18:1trans-11, C18:1trans-9, C18:1n9c).

PUFA=polyunsaturated fatty acids (C18:2n6c9t12, C18:2n6t9c12, C18:2n6c, C18:3n6, C18:3n3, C18:2n9c11t, C20:2n6, C20:2n6, C20:3n6c, C20:3n3, C20:4n6, C22:2n6, C20:5n3, C22:5n-3, C22:6n3).

n-6 (C18:2n6t9t12, C18:2n6c9t12, C18:2n6t9c12, C18:2n6c, C18:3n6, C20:2n6, C20:2n6, C20:3n6c, C20:4n6, C22:2n6).

n-3 (C18:3n3, C20:3n3, C20:5n3, C22:5n-3, C22:6n3).

ND= Not Detected

The fatty acid content of the eggs collected after 4 weeks of feeding on the trial diet were higher than those obtained after 8 week feeding for most of the attributes as age was shown to have a significant effect as shown in Table 5. The second most abundant fatty acid was oleic acid ranging from 10.23±0.11 to 15.12±0.39g/100g on the 8th week and 25.04±0.23 to 30.02±0.04g/100g on the 4th week. Younger hen eggs had higher levels of oleic acid than eggs produced by older chicken. The third most abundant fatty acid in the yolk lipid extract was linoleic acid which ranged from 9.69±0.05 g/100g to 21.27±1.26g/100g. EPA and DHA were found in very low quantities of between 0.96±0.2 to 1.95±0.20g/100g and 0.05±0.00 to 1.24±0.00 g/100g respectively. Diets with 100% LTS sorghum had the worst performance in relation to the presence of these two eicosanoids.

The most abundant fatty acid group in the yolk was the SFA that was found in a range of between 46.78±0.50 g/100g to 39.19±0.34 g/100g followed by MUFA at between 13.79±1.29 g/100g to 35.29±0.12 g/100g and the least was the PUFA which was between 10.71±0.32 to 24.05±7.25g/100g. These results show that the PUFA regulation was more controlled in all blocks than the MUFA. The n6/n3 ratio ranged from 4.09±0.34 g/100g to 11.5 ±3.56g/100g with eggs from hens fed on 100%LTS giving higher ratios than 50%M50%LTS and 100%Maize. These results are supported by the work of Meluzzi et al. (2000) who concluded that the Saturated Fatty acid profile of the eggs are very hard to manipulate using dietary interventions. However, the MUFA content and the PUFA content could be altered by dietary intervention. The albumin was more responsive to dietary intervention since 100% LTS higher MUFA and PUFA content when compared to the yolk. For the yolk the 100% Maize diet had the highest MUFA and PUFA content for both the 4th and 8th week eggs.

3.4 Physical Characteristics

Results in Table 6 show the response of the chicken to the different diet in terms of egg physical characteristic. The layer hens produced heavier eggs on the 8th week compared to on the 4th week. This change was however not significantly different (P=0.0910). Diet had no significant effect on the weight of the eggs (P=0.7918), Albumin Index (P=0.5112), Yolk Index (P=0.3115) and Haugh Unit (P=0.3726).

Table 6. Physical attributes of eggs

Period	Diet	Egg weight (g)	Albumin index	Yolk index	Haugh Unit
4 weeks	100% Maize	50.30±2.85[a]	280.08±32.50[a]	27.65±0.10[a]	55.87±1.82[a]
	50%M50%LTS	54.93±5.12[a]	279.17±32.55[a]	30.96±4.32[a]	52.25±3.64[a]
	100% LTS	53.47±1.65[a]	297.64±28.61[a]	32.13±1.51[a]	56.67±3.11[a]
8 weeks	100% Maize	59.12±0.76[a]	336.35±53.90[a]	34.25±5.73[a]	55.42±7.63[a]
	50%M50%LTS	56.02±2.72[a]	397.92±51.14[a]	39.43±1.67[a]	64.13±2.90[b]
	100% LTS	58.62±1.20[a]	341.95±24.61[a]	34.33±0.98[a]	59.43±1.49[a]
P-value	A& D	0.6316	0.8209	0.7978	0.4982

A; age, D; diet

Means with different superscripts on the same column are significantly different $p<0.05$.

The eggs had a constant weight range of between 50g to 60 g for all the dietary formulations for the 8 week trial period. Eggs from layers fed on 100% Maize had lower weight of 50.30±2.85 g on the 4th week of the feeding trial. However, this changed on the 8th week where they recorded the highest mean weight of 59.12±0.76 g compared to the other 2 diets. There was no significant difference (P=0.6316) caused by interaction of the age of chicken and diet on the weight of the eggs. Egg weight has been reported to be affected by the age of the chicken. Brand et al. (2004), reported a vast variation in age of more than ten weeks can affect the weight of the eggs. This could however not be achieved by the hens in the feeding trial as the age group was the same at the start of the trial and the feeding program lasted for 8 weeks with sample collection being four weeks apart.

There was no significant (P=0.8209) difference due to interaction of both age of hens and diet on the albumin index. The albumin index of the eggs which is an indicator of egg freshness was very high at the 4th week with a range of between 279.17±32.55 to 297.64±28.61 and the 8th week ranging between 336.35±53.90 to 397.92±51.14. The yolk indices ranged between 27.65±0.10 to 32.13±1.51 for the 4th week while on the 8th week the range was between 34.25±5.73 to 39.43±1.67.this was however also not significantly different (P=0.7978). The above results show that the egg samples from chicken fed on 100% Maize had their physical quality reduce faster since they recorded the lowest values for albumin index and yolk index for both the 4th and 8th weeks of collection.

There was no significant (P=0.4982) difference caused by interaction of diet and age of hens on the Haugh unit. The diets acting independently as the main variable gave a P-value of 0.138 while the age had a 0.3726 P-value. The Haugh unit values ranged from 52.25±3.64 to 64.13±2.90. The Haugh unit is the key factor used in grading of eggs, therefore, the eggs analysed from this feeding trial could be classified as B grade except for the 50%M50%LTS on the 8th week which were A grade (USDA, 2000). The Haugh unit is predetermined by the hens at the time they are laid, other factors that influence the Haugh unit of the eggs include flock age, the temperature of storage and the coating of the egg shells before storage(Williams et al., 1992; Scott & Silversides, 2000). These factors were not taken into consideration in this research but this knowledge could be used to explain the reason for lower Haugh unit at the 8th week compared to the 4th week of collection. The increase in Haugh unit was however very little and there was very little variability observed. Diet has no effect on the Haugh unit, albumin index and yolk index of eggs hence cannot be used to control physical quality of the eggs(Aljamal, 2011; Chung & Lee, 2014; Doyon, Hamilton, Castaigne, & Randall, 1985; Lacin, Yildiz, Esenbuga, & Macit, 2008; Scott & Silversides, 2000). This was observed to be true in this research study as there was no significant difference due to the variation in LTS and Maize amounts in the diets.

3.5 Colour

Results in Table 7 show the colour attributes of the eggs in relation to alterations in amount of maize and LTS in the diets and age of hens. The L* values were observed to reduce from the 4th week to the 8th week of the dietary feeding trial with a margin of approximately seven units across all the diets. Eggs from hens fed on 100% Maize as key source of energy recorded the lightest eggs on the 4th week of the feeding trial with L* values of 68.56±0.30 compared to 50%M50%LTS and 100%LTS that had L* values of 65.38±0.20 and 66.48±0.44 respectively. Diet had a significant effect on the L* (P=0.0418) and hue ($P<0.0001$) values while the Chroma value was not significantly affected by the variation of level of LTS and maize in the diet (P=0.5544). this is to imply the exact colour of the yolks were not affected by the variation in diet but the shades and brightness of the yolks were affected by the level of variation of maize with LTS in the chicken feed.

Table 7. Colour values of egg yolks

Period	Diet	L*	Hue	Chroma
4 weeks	100%Maize	68.56±0.30[c]	36.29±0.28[c]	44.62±0.15[d]
	50%M50%LTS	66.48±0.44[b]	38.95±0.19[c]	44.09±0.21[d]
	100%LTS	65.38±0.20[b]	37.73±0.28[c]	43.04±0.18[c]
8 weeks	100%Maize	63.63±1.33[a]	-75.07±0.35[aA]	14.76±0.44[a]
	50%M50%LTS	62.96±0.22[a]	-73.99±0.34[b]	16.06±0.71[b]
	100%LTS	61.01±0.61[a]	-74.46±0.27[ab]	16.92±0.12[b]
P-value	A& D	0.0033	0.0319	<0.0001

A; age, D; diet

Means with different superscripts on the same column are significantly different $p<0.05$.

The highest L* values were recorded for eggs from hens fed on 100% Maize while the lowest values were recorded for diets with 100%LTS. The hue angle ranged from 36.29±0.28 to 38.95±0.19 on the 4th week while on the 8th week of the trial diet the hue angle of the egg yolks ranged between -75.07±0.35 to -73.99±0.34. The Chroma values ranged from 43.04±0.18 to 44.62±0.15 on the 4th week of the feeding trial while on the 8th week the range was from 14.76±0.44 to 16.92±0.12. There was no significant (P=0.5544) difference due the effect of the variation in levels of LTS and Maize in the diets. This shows that the inclusion of sorghum had no adverse effect on the colour of the yolks. The results obtained showed that all dietary formulations were devoid of elements responsible for colour and the maize samples had higher concentrations of these than the LTS. This is because it has been reported previously that the pigmenting compounds of interest in egg yolk are the β-carotenes and the β-cryptoxanthin which are naturally found in leaves and in small quantities in some cereal grains (Liu et al., 2012; Surai, Speake, & Sparks, 2001; Bonilla et al., 2017).

Low Tannin Sorghum had a negative effect on yolk colour attributes with regards to hue and L* values. Physical attributes which included egg weights, Albumin Index, Yolk Index and Haugh Unit were not significantly affected by the variation of level of LTS and Maize in the chicken diets. The vitamin E and vitamin A content were lower in eggs of hens fed on higher LTS compared to those of eggs of hens fed on 100% Maize.

4. Conclusion

Low Tannin Sorghum can be used to replace maize in layer chicken feed but the feed formulation has to be enriched using ingredients that are rich in the micronutrients especially pro-vitamin and vitamin A and vitamin E. This will result in higher contents of these in the eggs. Due to the lower content of the cholesterol in the eggs of chicken fed on 100%LTS, the feed results in more nutritious eggs compared to feed with higher maize content. 100% LTS can therefore be used to replace 100% Maize as a source of energy in feed formulations.

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