Effect of Deep Litter System with or without Access to Grass or Legume Pastures on Egg Fatty Acids and Proximate Composition of Laying Hens.

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ABSTRACT

A study was carried out to examine the effects of rearing systems {deep litter system (DL), deep litter with access to legumes pasture (LP) and deep litter with access to grasses (GP)} on egg fatty acids and proximate composition of ISA Brown layer strain of chicken. Eighty birds were assigned to each treatment, replicated four times with 20 birds each. Fatty acids were analyzed in the eggs at early and peak production phases. Eggs from each group were collected randomly to determine their fatty acid profiles and proximate composition. Lipids were extracted from the eggs and the fatty acid methyl esters were prepared from oil samples and also, fatty acids were separated and identified Fatty acid values were reported as percentages. Results indicated that the levels of docosahexanoic fatty acids was higher ($P<0.05$) in LP (1.29) than those of GP (0.55) and DL (0.06). The level of oleic acid was also higher ($P<0.05$) in the eggs of LP (41.9) than those of GP (37.51) and DL (32.71). The difference in rearing systems did not have significant influence on the proximate composition in the early and peak production phase. However, at the late production phase (60 weeks of age), the moisture content was significantly higher in LP than those of the DL and GP. It was concluded that access to LP enhanced egg fatty acid profile than those of the DL and GP as indicated by the parameters measured.

(Keywords: eggs, pasture, deep litter, fatty acid, proximate composition)

INTRODUCTION

The global trend towards food products with organic or animal care certification indicates increasing consumer attention to the manner in which food is produced. The traditional housing of egg-type chickens in conventional cages, long perceived as the most efficient method of housing laying hens, is now widely considered to have a negative effect on the welfare of hens (Craig and Swanson, 1994). Concerns about the welfare of laying birds prompted an industry-wide search for a better system of housing including free range system.

Throughout Europe, concern for the welfare of laying hens in conventional cages has prompted changes in housing (Savory, 2004). Cage-based systems are being phased out and those that are retained must meet high welfare standards (Keeling and Svedberg, 1999). A laying hen directive approved in 1999 by the European Union, prohibits new investment in conventional cages beyond 2003 and banned their use from January 1, 2012 (European Commission, 1999). This directive stated that all existing cages must meet the 750 cm^2/bird space requirements and that each cage must be enriched with facilities that will allow birds to express normal behaviors.

Pastured poultry depends on raising chickens directly on pasture. This practice has been developed over the last twenty years and allows the birds to receive a significant amount of pasture forage as feed. The birds are kept on fresh pasture, which allows the birds to be raised in a cleaner, healthier environment. The practice of pasture-raised poultry provides chickens with fresh pasture and small quantities of insects, and worms (Glatz *et al*., 2005), which in turn can lead to enhanced quality of eggs. In the last two decades, the quality of chicken egg has been an area of primary consumer concern, due to the connection between specific dietary lipids and the development of coronary heart disease and some forms of cancer (Simopoulos and Salem, 1992). Due to the health benefits associated with the consumption of polyunsaturated fatty acids (PUFA), much research has been carried out to enrich eggs with these fatty acids (Van Elswyk *et al*.,1992). Inclusion of pasture species in the feed of chicken provides a unique method of manipulating the content of some micronutrients with a view to improving the nutrient intake of consumers or improving their overall health.

Yannakopoulos *et al.* (2005) reported that feeding flaxseed to laying hens increases the omega-3 fatty acid in the egg by 6 to 8 times, making one egg equal to 114 g of cold water fish as source of the omega-3 fatty acids. The beneficial effects of omega-3 fatty acids include reducing heart disease, reducing circulating cholesterol levels and suppressing inflammation. The current recommended ratio of omega-6 to omega-3 intake is 1 to 4, while the Western diet provides a ratio approximately 20 to 1. This imbalance is being linked to such problems as heart attacks, diabetes, cancer, etc. in humans and animals (Klatt, 1986). By eating various grasses and herbs, free-range layers enrich their food and assimilate valuable natural nutrients that influence some egg quality traits (Nys, 2000; Van Den Brand *et al.,* 2004).

Pastures are assumed to be good sources of α-linolenic acid (ALA) and other bioactive compounds. It may constitute a source of energy and protein for birds raised in free-range systems as the presence of a large range of bioactive compounds in the forage, such as xanthophylls and several hypocholesterolemic and anticarcinogenic compounds, may lead to improvement in poultry meat quality (Ponte *et al*., 2008). The nutrients from the forage serve as a precursor for Omega-3 essential fatty acid. There is therefore a need to carry out a study to establish the benefits of free-range production system on the welfare, behaviour and performance of the birds. It is also desirable to establish which of the pasture species would provide greater benefits to quality of products and the welfare of the birds.

MATERIALS AND METHODS

Experimental Site

This study was carried out at the Teaching and Research Farms Directorate (TREFAD), Federal University of Agriculture, Abeokuta, Ogun State, Nigeria. It is located within the rainforest zone of south western Nigeria at latitude 7^0 13', 49^0 46'N, longitude 3^0 26', 11^0 98'E and altitude 76mm above sea. The climate is humid with a mean annual rainfall of 1037mm. The annual mean temperature and humidity are usually 34^0C and 82 % respectively (Amujoyegbe *et al.,* 2008).

Paddock Establishment

Prior to the establishment of the paddock, the land was ploughed and harrowed. *Cynodon dactylon* was established vegetatively (with the use of sprigs). The sprigs were planted at $7m^3$/ha into a well prepared seedbed. *Stylosanthes hamata* seeds were scarified using hot water treatment and then sown at the rate of 3kg of seed/ha. Irrigation was practiced during the dry season with the source of water from a borehole.

Experimental Birds, Materials and Management

A total number of 240 Isa-Brown pullets were obtained from a reputable commercial farm (Animal Care, Ogere, Nigeria). Birds were housed under three experimental groups viz. deep litter, deep litter with access to a grass-based pasture and deep litter with access to a legume-based pasture. Nesting boxes, perches, feeding and water troughs were provided in the houses. The stocking density of 7 hens/m^2 was used.

Four replicates of 20 birds (a total of 80 birds per treatment) were used for each of the experimental groups. During the experimental period, the birds with access to pasture had access to outdoor run during the day light (8.00 am-6.00 pm). Water and feed were provided *ad libitum.*

About 8-10 cm depth of wood shavings was used as bedding. Routine and occasional management practices in poultry production were carried out. Feeders and drinkers were cleaned, and litter was changed as at when due. Sanitation of the environment was maintained throughout the experimental period. The experimental diet is shown in Table 1. The proximate composition of *Stylosanthes hamata* and *Cynodon dactylon* are shown in Table 2 and 3, respectively.

Table 1: Percentage Composition of Layer Diet used in the Experiment.

Ingredient	Percentage
Maize	48
Soybeans	11
Groundnut cake	8.75
Fishmeal	1.5
Palm kernel cake	5
Wheat offal	14.00
Bone meal	2.5
Limestone	8.5
*Premix	0.25
Salt	0.25
Lysine	0.1
Methionine	0.15
Total	**100**
Calculated feed Analysis	
Me (Kcal/kg)	2,489.05
CP (%)	17.13
CF (%)	4.30
Ether extract (%)	4.30
Calcium (%)	4.11
Phosphorus (%)	0.92
Lysine (%)	0.88
Methionine (%)	0.44

*Supplied per kg diet: Biotin = 40gm; Zn = 58mg; Fe = 5800mg; Vit A = 1,000,000 i.u , Folic acid = 500mg; Se = 120mg; 1 = 60mg; Nictotinic acid = 2800mg; Cu = 700mg; Mn = 4800mg; Vit k = 1,500mg; Riboflavin = 500gm; Co = 300g

Table 2: Chemical Composition of *Stylosanthes hamate.*

Moisture content (%)	81.22
Dry matter (%)	18.76
Crude protein (%)	4.16
Fibre content (%)	1.16
Carbohydrate (%)	10.01
Ash content(%)	1.07
Sodium (mg/g)	2.67
Potassium (mg/g)	4.66
Phosphorus (mg/g)	2.12
Magnesium (mg/g)	0.61
Calcium (mg/g)	0.77
Iron (mg/g)	1.89
Zinc (mg/g)	0.02

Table 3: Chemical Composition of *Cynodon dactylon.*

Moisture content (%)	76.78
Dry matter (%)	23.22
Crude protein (%)	2.10
Fibre content (%)	1.02
Carbohydrate (%)	14.36
Ash content (%)	1.18
Sodium (mg/g)	2.12
Potassium (mg/g)	6.78
Phosphorus (mg/g)	1.27
Magnesium (mg/g)	0.73
Calcium (mg/g)	0.77
Iron (mg/g)	1.24
Zinc (mg/g)	0.01

Fatty Acid Determination

Eggs from each group were collected to determine their fatty acid profiles. Lipids were extracted from eggs by the method of the AOAC (2000). The fatty acid methyl esters were prepared from oil samples according to IUPAC (1976) and from subsequent fatty acid profiles determined by gas chromatography. The gas chromatographic analysis of the sample was performed on an Agilent Technologies GC Model: 7890A interfaced with Mass Selective Detector model: 5975C (MSD). The electron ionization was at a 70v with an ion source temperature at 250 ^{o}C. Highly pure helium gas (99.9% purity) was used as carrier gas, while HP-5ms (30mm X 0.25mm X 0.320µm) was used as the stationary phase. The oven temperature was at 100 ^{o}C held for 4 minutes and ramped to 270^{o}C at the rate of 3.5 ^{o}C/minutes holding for 6 minutes. 1µ/l was auto injected. Peaks separated, were identified by comparison with standard samples of known composition. Internal standard (Sigma cat no: 189-19) was used for fatty acid quantification. Fatty acid values were reported as percentages.

Proximate Analysis

The egg white was separated from the egg yolk and the chalazae were removed. The yolk was carefully rolled on filter paper (Whatman No. 4, Whatman International Ltd., Maidstone, UK) to remove chalazae and traces of albumen adhering to the vitelline membrane. The vitelline membrane was then disrupted with a scalpel blade and yolk was collected in a beaker. The proximate composition (moisture, ash, protein, and lipid content) of whole egg, egg white, and yolk was analyzed following the AOAC methods (AOAC, 2000).

Statistical Design and Analysis

The data collected were analyzed using a completely randomized design. The model is shown below:

$Y_{ij} = \mu + T_i + \Sigma_{ij}$
Y_{ij} = Observed value of dependent variable
μ = Population mean
T_i = Effect of i^{th} rearing system
Σ_{ij} = Residual error.

RESULTS

Table 4 shows the effects of rearing system on egg fatty acid composition during early production. There was no significant difference in the levels of myristic, palmitic, margaric, stearic, butyric and pamitoleic, and eicosenoic acids. Oleic acid was more abundant ($P<0.05$) in LP and GP than DL. The level of myristoleic acid in GP and DL was comparable but significantly lower than that of LP. Eicosadienoic acid level in LP and GP were comparable but significantly lower than that of DL. In contrast, arachidonic acid was significantly lower in DL than those of GP and LP. Docosapentenoic acid level in LP was significantly higher than that of GP and the level in GP was significantly higher than that of the DL. Docosahexanoic acid also followed similar trend in all the rearing system.

Table 5 shows the effects of rearing system on egg fatty acid composition at peak production. Myristic acid was similar in LP and GP but significantly higher than that of DL. However, stearic, palmitoleic, eicosadienoic acids were comparable in LP and GP but lower than that of the DL. The difference in the rearing systems did not have significant difference on Palmitc, margaric and arachidonic acids levels. The level of oleic and myristoleic, docosapentenoic, docosahexanoic acid in LP was higher that of GP while the level in GP was significantly higher than that of DL. Eicosenoic acid level in GP was similar to that of DL but significantly higher than that of LP. The level in DL and LP were also comparable. Moreover, eicosapentenoic acid level was similar in DL and GP but significantly lower than that of LP.

Table 4: Effects of Rearing System on Egg Fatty Acid Composition (as % of total fatty acids) at 24 Weeks Old.

Parameters	Rearing systems			SEM
	Deep litter	Legume pasture	Grass pasture	
Myristic	0.05	0.13	0.08	0.013
Palmitic	27.92	26.41	25.95	0.51
Margaric	0.17	0.15	0.19	0.01
Stearic	10.70	9.95	9.89	0.53
Palmitoleic	0.23	0.18	0.22	0.015
Oleic	30.49[b]	37.33[a]	34.13[a]	1.01
Myristoleic	0.27[b]	1.34[a]	0.29[b]	0.15
Eicosenoic	0.15	0.34	0.17	0.06
Eicosadienoic	11.31[a]	9.82[b]	9.75[b]	0.22
Arachidonic	2.75[b]	3.27[a]	3.19[a]	0.07
Eicosapentenoic	0.00[b]	0.04[a]	0.01[b]	0.01
Docosapentenoic	0.00[c]	0.12[a]	0.05[b]	0.02
Docosahexanoic	0.04[c]	0.82[a]	0.12[b]	0.11

[ab] Means within rows with different superscripts are significantly different ($P<0.05$)

Table 5: Effects of Rearing System on Egg Fatty Acid Composition (as % of total fatty acids) at 60 Weeks old.

Parameters	Rearing systems			SEM
	Deep litter	Legume pasture	Grass pasture	
Myristic	0.07[b]	0.19[a]	0.14[a]	0.02
Palmitic	28.20	26.70	26.00	0.45
Margaric	0.15	0.13	0.15	0.01
Stearic	11.20[a]	10.20[b]	10.40[b]	0.15
Palmitoleic	1.30[a]	0.21[b]	0.30[b]	0.16
Oleic	32.71[c]	41.90[a]	37.51[b]	37.37
Myristoleic	0.75[c]	1.07[a]	0.87[b]	0.42
Eicosenoic	0.18[ab]	0.11[b]	0.23[a]	0.02
Eicosadienoic	11.9[a]	9.95[b]	10.20[b]	0.30
Arachidonic	3.31	3.47	3.52	0.07
Eicosapentenoic	0.00[b]	0.07[a]	0.02[b]	0.01
Docosapentenoic	0.00[c]	0.14[a]	0.07[b]	0.02
Docosahexanoic	0.06[c]	1.29[a]	0.55[b]	0.15

[abc] Means within rows with different superscripts are significantly different (P<0.05)

Proximate Analysis of Eggs

Table 6 shows the effects of rearing systems on the proximate analysis of eggs. The difference in rearing systems did not have significant influence on the proximate analysis in the early and peak production phase. However, at the late production phase (60 weeks of age), the moisture content was significantly higher in LP than those of the DL and GP. DL and GP were similar. The dry matter content was similar in the DL and GP and significantly higher than that of the LP. The fat and ash content also followed the same trend as in the dry matter content. There was no difference in the crude protein percentage. The carbohydrate in the DL and GP were similar but DL was significantly higher than that of LP.

DISCUSSION

The synthesis, digestion and degradation of fatty acids is carried out under complicated enzymes systems inside the cell and the activity of these enzymes is affected by many factors such as temperature, substrate (fatty acid) concentration, pH, enzyme concentration and the end-product concentration (Michael *et al*., 2000). It has been shown that the fatty acid composition of animal products can easily be manipulated by nutrition (Jarosław *et al*., 2003). Thus, apart from genetic factors, fatty acid composition of eggs can be manipulated by what is offered to the birds.

In this study, the production of total unsaturated fatty acid was better on the pasture than in the hens without access to pasture in the deep litter. The greater amount of poly unsaturated fatty acids in eggs from the hens raised on the pasture may also be attributed to more suitable conditions in this housing for rearing hens than condtions in the deep litter without access to pasture. This agrees with the findinggs of Carmon and Huston (1965) who reported that a suitable rearing condition enhances fatty acids secretion into eggs. The difference in the fatty acids profile of the eggs may also be due to the additional nutrients obtained from the forage by the hens on the pasture which may have affected the production of certain fatty acids.

The findings in this trial conforms with the observation of Hussein *et al.* (2012) who observed a higher fatty acids synthesis in a more suitable housing system. Lopez-Bote *et al*. (1998) also reported that eggs in the free range system had higher omega-3 (2.6 fold) and lower omega-6 contents compared to those in the conventional system. The production of these unsaturated fatty acids can be attributed to the available pasture the hens on the pasture had access to. Karsten *et al.* (2003) found eggs from chickens raised on legume pasture had more omega-3 fatty acids than eggs from chickens raised indoors.

Table 6: Effects of Rearing Systems on the Proximate Analysis of Eggs.

Parameters	Rearing systems		
	Deep litter	**Legume pasture**	**Grass pasture**
(24 weeks old)			
Moisture content (%)	74.78±0.54	75.99±0.68	76.17±1.32
Dry matter content (%)	25.48±0.98	24.01±0.66	23.83±1.32
Fat (%)	8.90±0.72	7.88±0.64	7.80±0.76
Ash (%)	0.89±0.04	0.85±0.03	0.82±0.04
Crude Protein (%)	15.17±0.26	14.93±0.15	14.77±0.53
Carbohydrate (%)	0.52±0.05	0.45±0.04	0.44±0.03
(38 weeks old)			
Moisture content (%)	78.47±0.65	78.73±0.72	77.81±0.64
Dry matter content (%)	21.54±0.65	21.27±0.72	22.200.64
Fat (%)	6.23±0.43	5.87±0.39	6.60±0.35
Ash (%)	0.76±0.03	0.74±0.03	0.77±0.04
Crude Protein (%)	14.11±0.27	14.29±0.37	14.390.30
Carbohydrate (%)	0.43±0.03	0.37±0.02	0.43±0.04
(60 weeks old)			
Moisture content (%)	75.70±0.51^{b}	77.84±0.34^{a}	76.36±0.61^{b}
Dry matter content (%)	24.23±0.51^{a}	22.16±0.34^{b}	23.65±0.61^{a}
Fat (%)	7.10±0.22^{a}	6.02±0.12^{b}	6.79±0.17^{a}
Ash (%)	0.80±0.02^{a}	0.74±0.00^{b}	0.80±0.02^{a}
Crude Protein (%)	15.79±0.28	14.91±0.20	15.49±0.41
Carbohydrate (%)	0.61±0.03^{a}	0.49±0.03^{b}	0.55±0.04^{ab}

^{ab} Means within rows with different superscripts are significantly different (P<0.05)

The differences in the fatty acid composition of the eggs of the hens raised on grass and legume pasture could be explained by the differences of the nutrient composition of the plants (grass and legume). Karsten *et al.* (2003) compared the amount of unsaturated fatty acids in three types of pasture: alfalfa and grass; red clover, white clover, and grass; and mixed grass. They concluded that eggs from hens consuming legumes and grasses contained more omega-3 fatty acids and vitamins than eggs from hens foraging on grass alone. This study showed that legumes contain more unsaturated fatty acids and the leafier the plant, the more fatty acids it contains.

The similarity in the proximate composition of the eggs in all the housing types in first and second phases of production suggests the nutritional adequacy of the eggs from the hens on the pasture. The slight variation observed at the third phase may be due to variation in the nutrient compostion of the forage plants. The higher level of energy observed in the eggs from deep litter in the late phase conforms with the report of Radu-Rusu *et al.* (2012).

CONCLUSION

Overall, as a result of the findings in the present study, it may be concluded that eggs enriched with poly-unsaturated fatty acid can be produced by practicing pastured poultry production. Moreover, there is slight advantage in regard to the fatty acids profile of eggs produced by the birds raised in legume pasture over those raised in grass pasture.

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