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Performance, Yolk Lipid, Egg Organoleptic Properties and Haematological Parameters of Laying Hens Fed Cholestyramine and Garlic Oil

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Abstract: This study investigated the effect of dietary garlic oil and cholestryamine at varying concentrations on performance, yolk lipids, cost-benefit ratio, organoleptic assessment of eggs and haematological parameters of laying hens. A 2x2 factorial arrangement of the two supplements under completely randomised design was used for the experiment. The inclusion levels of garlic oil were 100 and 200mg/kg, whereas cholestryamine were at 50 and 100mg/kg. The results showed that laying performance was not compromised except for the reduced egg weight for hens fed supplemented diets. Addition of garlic oil and cholestryamine to layer diet did not significantly influence shell weight and shell thickness. Garlic oil significantly ($P<0.02$) reduced yolk cholesterol with hens fed 100mg/kg garlic oil had lower yolk cholesterol than those fed 200mg/kg garlic oil. Significant interactive influence of both supplements reduced yolk triacylglycerol. Inclusion of the supplements in layer diet increased feed cost, but lowered profit in terms of naira per kilogramme and naira per tray. Improved egg organoleptic evaluation was observed by consumers fed eggs of hens containing the supplements. White blood cells of hens fed garlic oil were significantly increased.

Key words: Cholestryamine · Feed cost · Garlic oil · Profit · White blood cells · Olk cholesterol

INTRODUCTION

Cholestyramine is a bile acid sequestrant which binds bile in the gastrointestinal tract to prevent its reabsorption [1], thereby eliminating bile in faeces. The faecal bile excretion enables plasma cholesterol to be converted to bile acid by liver which lower plasma cholesterol. However, bile acid sequestrants induce extreme hypertriglyceridemia [2, 3]. Cholestyramine is commercially available Garlic is another health beneficial crop possessing hypotensive, hypocholesterolemic and anticarcinogenic properties. The potential health benefit of many processed garlic products such as garlic oil, powder produced varying responses. Garlic oil significantly decreased serum cholesterol and blood pressure in human subjects [4]. However, garlic oil preparation did not lower serum triacylglycerol, total-and low density lipoprotein (LDL)- cholesterol in 25 human subjects [5]. Furthermore, garlic oil fed at 0.02% to laying hens had no effect on plasma and yolk cholesterol based on strain [6]. Processed dried garlic paste incurred much cost and time consuming [7], although it showed strong beneficial effect on serum lipids and abdominal fat pad of broiler chickens [8]. Reports on the use of cholestyramine and commercially available garlic oil on laying performance and yolk cholesterol are very few. The inconsistency in responses of animals and humans to dietary garlic oil needs further re-evaluation to assess their benefits on yolk cholesterol and triacylglycerol accumulation in eggs. Hence, this study evaluated performance, yolk lipids, cost-benefit effect, h aematological parameters and egg organoleptic characteristics of laying hens fed both garlic oil and cholestyramine at varying dietary concentrations.

MATERIALS AND METHODS

Sources of Garlic Oil and Cholestyramine: Garlisules® is a herbal neutraceutical supplement made of pure steam distilled oil of raw garlic (*Allium sativum*) which weighed 250mg per capsule. It is manufactured by Asoj Soft Caps PVT limited, India and it is marketed in Nigeria by Kimco Group of Companies limited, Lagos, Nigeria.

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Table 1: Ingredient Composition of Experimental Diets

Ingredients	Control	T1	T2	T3	T4
Maize	43.20	43.20	43.20	43.20	43.20
Groundnut cake	14.10	14.10	14.10	14.10	14.10
Fish meal	2.00	2.00	2.00	2.00	2.00
Wheat offal	20.00	20.00	20.00	20.00	20.00
Palm Kernel meal	10.00	10.00	10.00	10.00	10.00
Bone meal	3.00	3.00	3.00	3.00	3.00
Oyster shell	7.00	7.00	7.00	7.00	7.00
Vit. Premix[a]	0.25	0.25	0.25	0.25	0.25
Salt	0.25	0.25	0.25	0.25	0.25
Methiomine	0.10	0.10	0.10	0.10	0.10
Lysine	0.07	0.07	0.07	0.07	0.07
Garlic oil[b]	—	+	+	++	++
Cholestryamine[c]	—	*	**	*	**
Total	100.00	100.00	100.00	100.00	100.00
Calculated energy (kcal ME/kg)	2505.50	2505.50	2505.50	2505.50	2505.50
Determined crude protein (%)	16.00	16.00	16.00	16.00	16.00
Determined calcium (%)	3.60	3.60	3.60	3.60	3.60
Determined phosphorus (%)	0.50	0.50	0.50	0.50	0.50
Determined crude fibre (%)	6.30	6.30	6.30	6.30	6.30

[b]+ = 100mg garlic oil/kg
++ = 200mg garlic oil/kg
[c]* = 50mg cholestryamine/kg
** = 100mg cholestryamine/kg

[a]Vitamin premix supplied the following vitamins and trace elements per/kg diet: Vit A 6250IU; Vit D_3 1250IU; Vit E 14.38mg; Vit K_3 1.25mg; Vit B 1.88mg; Niacin 31.25mg; Calcium pantothenate 6.25mg, Vit B_6 3.13mg; Vit B_{12} 0.02mg; Choline Chloride 250mg; Folic acid 0.63mg; Biotin 0.03mg; Mn 75mg; Fe 62.5mg, Zn 50mg; Cu 5.31mg, I 0.94mg; Co 0.19mg; Se 0.08mg and Antioxidant 75mg

Each sachet of Questran® weighed 9g containing 4g of cholestyramine. It is manufactured by Bristol-Myers Pharmaceuticals, Uxbridge UB8 IDH, England and Swords Co. Dublin, Ireland. It is marketed in Nigeria by DANA pharmacy, Ibadan, Nigeria.

Management of the Experimental Birds: A total of 180 laying hens of Black Harco strain were distributed into five dietary treatment groups. Each treatment group contained 36 birds of 4 replicates. Feed and water were supplied *ad libitum* to the hens. The experimental hens were kept in two-tier battery cage system for a period of 8 weeks of study. The birds were individually housed in a single cell. Appropriate vaccinations and medications were carried out as at when due.

Formulation of Experimental Diets: A total of five experimental diets were formulated. All the diets contained the same levels of nutrients (Table 1). Basal control diet neither contains garlic oil nor cholestyramine. A 2x2 factorial arrangement was adopted for the supplements. The two factors were garlic oil and cholestyramine, each at two dietary concentrations. Diets in T1 and T2 contained 100mg garlic oil/kg, whereas diets in T3 and T4 contained 200mg garlic oil/kg. Cholestyramine was added to diets in T1 and T3 at 50mg/kg, while T2 and T4 diets contained 100mg cholestyramine/kg.

Chemical Analysis: Three blood samples per replicate were collected in EDTA bottles on the 42nd day of the study for determination of haematological parameters of the hens. Three eggs per replicate were also used for assessment of yolk cholesterol and triacylglycerol using enzymatic kits of Sigma Diagnostics.

Organoleptic Assessment of Eggs: This was carried out on a nine point hedonic scale (1 for extremely dislike to 9 extremely like) for palatability traits such as colour, flavour, juiciness, tenderness and overall acceptability using a 20 member panel.

Statistical Analysis: Data collected were analyzed by 2x2 factorial analysis of variance under one way ANOVA using General Linear Model of [9]. Duncan option of the software was used for separation of significant means. A probability of 5 percent was considered significant ($P<0.05$).

RESULTS

Dietary supplementation of garlic oil and cholestyramine significantly influenced laying performance of experimental hens such as feed intake (P<0.0009), egg weight (P<0.004) and feed conversion (P<0.0002) (Table 2). Garlic oil and cholestyramine interactively (P<0.015) decreased egg weight as dietary supplements concentrations increased. However, increased feed intake and poorer feed conversion were experienced by hens fed supplemented diets. Both garlic oil and cholestryamine contributed to the poor feed conversion. Hens fed diet containing 100mg garlic oil/kg and 100mg cholestryamine/kg (T2) had the worst feed conversion. Dietary treatment did not significantly affect hen day production and egg qualities (data not shown). Both garlic oil and cholestyramine did not adversely affect shell weight and shell thickness. Supplemental garlic oil significantly reduced (P=0.002) yolk cholesterol with hens fed 100mg garlic oil/kg had lower yolk cholesterol than those fed 200mg garlic oil/kg. Synergistic action of garlic oil and cholestyramine was responsible for the lowering effect (P<0.047) on yolk triacylglycerol (Table 3).

Dietary treatment significantly (P=0.0001) affected cost implication of the study (Table 2). Incorporation of garlic oil and cholestyramine significantly increased feed cost, feed cost per tray of eggs. However, it significantly reduced profits in terms of naira per kilogramme and naira per tray of eggs. Furthermore, dietary treatment significantly enhanced organoleptic characteristics of eggs such as colour, flavour and general acceptability of eggs by consumers (Table 3). Eggs of hens in T2 had the richest colour and most pleasant flavour whereas eggs of hens in T3 were most accepted by consumers. Supplemental garlic oil and cholestyramine did not significantly influence haematological parameters of the experimental hens except for the single action of garlic oil on white blood cells (Table4). Hens fed 200mg/kg garlic oil had significantly (P=0.005) increased white blood cells.

Table 2: Laying performance of hens and cost implication of feeding garlic oil and cholestryamine

Parameters	Control	T1	T2	T3	T4	P.value	SEM	Gar oil	Chol.	Int.
Egg weight (g/egg)	58.16^{a}	58.29^{a}	56.64^{ab}	55.32^{b}	55.20^{b}	0.004	0.61	0.0001	0.007	0.015
Feed intake (g/bird/day)	112.48^{b}	135.00^{a}	138.58^{a}	129.63^{a}	136.04^{a}	0.0009	3.61	0.296	0.193	0.700
HDP (%)	73.22	70.07	73.45	71.94	71.88	0.149	0.97	0.871	0.146	0.135
Feed conversion (Feed / egg)	2.68^{c}	3.41^{b}	3.80^{a}	3.27^{b}	3.35^{b}	0.0002	0.12	0.042	0.093	0.270
Feed cost (N/kg)	64.65^{d}	69.67^{c}	73.67^{ab}	70.69^{bc}	74.67^{a}	0.0001	1.11	0.385	0.004	1.000
Income (N/kg)	354.57^{b}	344.75^{bc}	386.27^{a}	346.96^{b}	327.35^{c}	0.0001	5.88	0.001	0.120	0.001
Feed cost / tray (N/tray)	330.22^{c}	486.48^{a}	507.78^{a}	417.25^{b}	473.72^{a}	0.0001	11.37	0.002	0.010	0.190
Profit (N/kg)	161.82^{a}	67.86^{bc}	63.01^{c}	84.87^{b}	43.41^{cd}	0.0001	6.24	0.856	0.006	0.022
Profit (N/tray)	269.78^{a}	113.59^{bc}	92.22^{cd}	132.75^{b}	76.28^{d}	0.0001	11.36	0.901	0.010	0.191

Means along the same row with different superscripts are significantly different (P<0.05).

Table 3: Yolk lipids and organoleptic properties of eggs from hens fed garlic oil and cholestryamine

Parameters	Control	T1	T2	T3	T4	P.value	SEM	Gar oil	Chol.	Int.
Colour	7.50^{bc}	7.90^{ab}	8.40^{a}	8.20^{a}	7.20^{c}	0.0001	0.18	0.018	0.185	0.0001
Flavour	6.10^{c}	7.3^{ab}	8.10^{a}	7.70^{a}	6.7^{bc}	0.0001	0.30	0.102	0.741	0.004
Juiciness	5.60	6.30	6.10	6.70	5.50	0.194	0.40	0.796	0.074	0.200
Tenderness	6.10	5.10	5.10	5.20	5.00	0.399	0.45	1.000	0.822	0.822
Acceptance	6.00^{d}	7.60^{bc}	8.00^{ab}	8.50^{a}	6.8^{cd}	0.0001	0.29	0.577	0.018	0.0002
Yolk cholesrerol (mg/g)	18.55^{a}	14.38^{b}	15.00^{ab}	18.33^{a}	17.70^{ab}	0.032	1.06	0.002	0.797	0.476
Yolk triacyglycero (mg/g)	17.18^{a}	16.05^{ab}	15.45^{ab}	14.00^{b}	15.80^{ab}	0.018	0.61	0.065	0.149	0.047

Means along the same row with different superscripts are significantly different (P<0.05).

Table 4: Haematological parameters of hens fed garlic oil and Cholestryamine

Parameters	Control	T1	T2	T3	T4	P.value	SEM	Gar oil	Chol.	Int.
Packed cell volume (%)	21.00	22.25	21.50	20.50	19.25	0.662	1.45	0.182	0.500	0.866
Heamoglobin (g/dl)	7.00	7.43	7.18	6.85	6.43	0.660	0.48	0.193	0.492	0.858
White blood cells (/mm^{3})	8825.00	8400.00	8450.00	9100.00	8876.00	0.091	210.16	0.005	0.642	0.467
Red blood cell (x10^{3}/mm^{3})	3500.00	3699.00	3575.00	3375.00	3191.3	0.636	242.46	0.160	0.535	0.904
Neutrophils (%)	39.00	36.00	41.50	38.50	36.25	0.340	2.07	0.520	0.453	0.114
Lymphocytes (%)	54.25	55.50	52.00	52.75	55.33	0.385	1.96	0.601	0.861	0.079
Monocytes (%)	1.75	2.25	1.25	2.00	1.75	0.100	0.25	0.601	0.013	0.124
Eosinophils (%)	5.00	6.25	5.25	6.75	6.67	0.384	0.64	0.173	0.310	0.496

Means along the same row with different superscripts are significantly different (P<0.05).

DISCUSSION

Reduced eggs weight of hens fed supplemented diets could be due to antilipidemic effects of the supplements. Laying performance was not negatively affected except for eggs weight .This response was quite different from the observation of [6] who reported that egg production, egg weight, feed intake and feed efficiency were not affected when 0.02 % garlic oil was fed to hens of Babcock B-300 strian. Additive effect of garlic oil and cholestyramine may account for the reduced egg weight in this study. The result of yolk lipids showed that 100mg garlic oil/kg and 50mg choletryamine/kg (T1) has remarkable yolk cholesterol reduction without negative effect on egg weight. This observation is in variant with the findings of [6] who reported that garlic paste did not reduce serum and yolk cholesterol on the basis of strain. Significant differences existed among strains with respect to egg production, yolk weight and yolk cholesterol concentration [10]. The potential of cholestyramine to induce high triacylglycerol in egg yolk was not shown in this study. This may be the beneficial action of garlic oil on cholestyramine for lowering effect on yolk triacylglycerol.

Cost implication for incorporating garlic oil and cholestyramine in layer diet supported the assertion of [7] that processed garlic paste incurred much cost. The increased feed cost and reduced profit for hens fed supplemented diets showed that farmers may be reluctant to adopt this method for lowering yolk cholesterol and triacylglycerol.

Dietary garlic oil and cholestyramine did not significantly affect haematological parameters of hens except for white blood cells. Earlier study had shown that 1, 1.5 and 2% dried garlic paste produced reduced packed cell volume, haemoglobin and red blood cells [8]. The low dietary concentration of garlic oil and non-absorptive nature of cholestyramine into the vascular system may be responsible for non significant effect on red blood cells and haemoglobin. Cholestyramine bound bile acids in the intestine and formed a non-absorbable complex that is excreted in faeces [11]. This showed that cholestyramine could be used as hypolipidemic agent in laying hens without any adverse effect on blood components and cells. The significant influence of garlic oil on white blood cells implied that garlic could improved immuno-competence of the birds and confirmed the observation of . Iranloye [12] that rats fed garlic juice (200mg/kg) for 30 days had increased total white blood cells, neutrophils, lymphocytes and monocyte counts. Earlier study had shown that garlic extract stimulated immune functions [13].

The preservation of the intestinal bile acid pool is critical for the absorption of essential lipids and fat soluble vitamins [14]. These authors showed that female mice fed cholestyramine had better intestinal lipid absorptive capacity than male mice fed cholestyramine. This was because cholesterol 7 alpha-hydroxylase activity (which is responsible for endogenous bile acid synthesis) and mRNA were greater in females than in males. A similar intestinal lipid absorptive capacity may exist in laying hens and this need further research to elicit information on how cholestyramine interfere with lipid metabolism in laying hens. The fact that garlic oil and cholestyramine did not significantly affect egg internal and external qualities particularly egg shell weight and shell thickness in this study need further clarification. It had been reported that bile acid sequestrants have non-specific binding effect on a number of drugs [15] and fat soluble vitamins [16, 17]. Cholestyramine (2%) fed rats had a net negative balance for calcium and a lower net positive balance for magnesium, iron and zinc than the control [18]. The low dietary cholestyramine in this study may account for non-adverse effect on shell weight and shell thickness.

The improved organoleptic characteristics of eggs disagreed with the observation of [19] that there were no difference in flavour and colour of eggs from hens consuming up to 3% dietary garlic paste. The synergistic influence of garlic oil and cholestyramine may account for the variation. Furthermore, low dietary garlic oil concentration may be responsible for the varying responses in this study.

CONCLUSION

- Garlic oil at 100mg /kg with 50mg cholestryamine/kg (T1) could be included in layer diet to lower yolk cholesterol and triacylglycerol without any adverse effect on egg weight.
- The beneficial effect of garlic oil eliminated the nature of cholestryamine to induce hypertriacylglycerol as shown in reduced yolk triacylglycerol.
- Garlic oil and cholestryamine did not negatively affect red blood cells and haemoglobin of the laying hens. This implied that garlic oil and cholestryamine could be included in layer diet without affecting blood essential components.

- Improved organoleptic indices of the eggs from hens fed supplemented diets assessed by consumers was shown in the study.

REFERENCES

1. Anonymous, 2008. Cholestyramine, http://www. en. wikipedia. org, accessed on 05-12-08
2. Crouse, J.R., 1987. Hypertriglyceridemia: A contraindication to the use of bile acid binding resins. American J. Med., 83: 243-248.
3. Garg, A. and S.M. Grundy, 1994. Cholestrymine therapy for dyslipidemia in non-insulin- dependent diabetes mellitus: Ashort term, double blind, crossover trial. Annals of Internal Med., 121: 416-422.
4. Barrie, S.A., J.V. Wright, J.E. Pizzorno, 2008. Effects of garlic oil on platelet aggregation, serum lipids and blood pressure in humans. www.orthomed.orj accessed on 26/02/08
5. Berthold, H.K., T. Sudhop and K. Bergmann. 1998. Effect of a garlic oil preparation on serum lipoproteins and cholesterol metabolism. J. American of Medical Association, 279: 1900-1902.
6. Reddy, R.V., S.F. Lightsey and D.V. Maurice 1991. Effect of feeding garlic oil on performance and egg yolk cholesterol concentration. Poultry Sci., 70: 2006 - 2009.
7. Lawal, S., 2007. Growth performance, organs and carcass measurement of broilers fed three dietary protein concentrations and a mixture of garlic and ginger. Bachelor of Techn. Thesis, Dept of Animal Prod. and Health, Ladoke Akintola Univ. of Tech. Ogbomoso, Nigeria.
8. Ademola, S.G., G.O. Farinu and G.M. Babatunde, 2009. Serum lipids, growth and haematological parameters of broilers fed garlic, ginger and their mixtures. World J. Agricultural Sci., 5(1): 99-104.
9. SAS. 1999. SAS User's Guide: Statistics, version 8th edn. (Cary, NC, SAS Institute).
10. Han, C.K. and N.H. Lee, 1992. Yolk cholesterol content in eggs from major domestic strains of breeding hen. Asian -Australasian J. Animal. Sci., 5: 461-464.
11. Parr pharmaceutical companies Inc. 2005. Questran(R) powder (Cholestyramine for oral suspension) and Questran (R) Light (Cholestyramine suspension) prescribing information, Spring Valley, New Yolk,
12. Iranloye, B.O., 2002. Effect of chronic garlic feeding on some haematological parameters. African J. Biochemical Res., 5: 81-82.
13. Sumiyoshi, H., 1997. New pharmacological activity of garlic and it constituents (review). Folia Pharmacologica Japonica. 110(Supplement 1): 93- 97.
14. Schwarz, M., D.W. Russell, J.M. Dietschy and S.D. Turley, 2001. Alternate pathways of bile acidsynthesis in the cholesterol 7 -alpha hydroxylase knockout mouse are not unregulated by either cholesterol or cholestyramine feeding. J. Lipid Res., 42: 1594-1603.
15. Farmer, J.A. and A.M. Gotto, 1994. Antihyperlipidaemic agents. Drug interactions of clinical significance. Drug. Safety. 11: 301-309.
16. Knodel, L. and C.R.L. Tablert 1987. Adverse effects of hypolipidaemic drugs. Medical Toxicol., 2: 10 -12.
17. Elinder, L.S., K. Hadell, J. Johansson, J. Molgaard, I. Holme, A.G. Olsson, G.Walldius 1995. Probucol treatment decreases serum concentrations of diet - derived antioxidants. Arteriosclerosis Thombosis and Vascular Biol., 15: 1057-1063.
18. Watkin, D., W.R. Khalafi, M.M. Cassidy, G.V. Vahouny 1985. Alterations incalcium, magnesium, iron and zinc metabolism by dietary cholestyramine. Digestive Diseases and Sci., 30: 477-482.
19. Birrenkott, G., G.E. Brockenfelt and Owens, E. Halphin, 2000. Yolk and Blood cholesterol levels and organoleptic assessment of eggs from hens fed a garlic supplemented diet. Poultry Sci., 79(Supplement): 75(Abstract).