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Evaluation of the effect of a modified proprietary poultry feed on cholesterol excretion in laying birds

Chris Oche IKESE*,[1] Simon Terver UBWA,[1] Sunday Ogakwu ADOGA,[1] Stephen Inegedu AUDU,[2] and Michael AKOR[1]

[1]*Department of Chemistry, Benue State University, Makurdi, Nigeria*
[2]*Department of Chemistry, Nasarawa State University, Keffi, Nigeria*

Abstract. The effect of modified proprietary poultry feed on cholesterol excretion in laying birds was evaluated. The study sort to lower the cholesterol content of eggs at the point of their physiological formation via a modification of the proprietary feed fed to laying poultry birds. This was with a view to lower the net cholesterol content in their eggs, thereby lowering the cholesterol levels available in such eggs for intake upon consumption. A 20% rice bran modification of the proprietary feed was made. Animal subjects were divided into experimental and control groups and the mean total cholesterol in their faecal droppings where determined before and after varying their feeding programs. Whereas the experimental group was fed with the modified feed, the control group was maintained on the unmodified feed. Enzymatic colorimetric method was used for the determination of mean total cholesterol in the dried faecal droppings of each group of birds under investigation. The results showed that the mean total cholesterol excretion before and after the modified feed regimen were 5.97±0.16 mg/g and 9.99±0.47 mg/g respectively and were found to be significantly different when compared using a *t*-test at $p > 0.05$ and 49 degree of freedom The results also showed that the modified proprietary poultry feed increased total cholesterol excretion in the faecal droppings of laying birds fed with the modified feed by 67.3%, and this was found to be statistically significant at $p > 0.05$. Hence, a 20% rice-bran modification of the proprietary feed yields a modified feed with a proven potency in elevating total cholesterol excretion while producing no drastic deviation from the proximate composition of the unmodified proprietary feed and is thus likely to have no adverse effect on the productivity of laying birds.

Keywords: cholesterol; cholesterol excretion; laying birds; modified poultry feed; rice bran.

1. Introduction

Although chicken eggs are a rich source of protein and other vital nutrients, they contain very large cholesterol deposits in their yolks and this has been implicated in promoting cardiovascular and coronary heart diseases [1, 2]. However, avoiding or advising against the consumption of eggs is impracticable and unrealistic to say the least as they are among the cheapest sources of high-quality protein for low income households. However, this calls for a need to regulate cholesterol intake via food choices, and perhaps, research tailored towards lowering the cholesterol content of eggs right at the point of their physiological formation. In this regard, it is thought that if dietary cholesterol intake of egg-laying birds from proprietary poultry feeds can be lowered via feed modification, then it might be possible to lower the level of cholesterol available in eggs and other body tissues of the poultry bird. The fraction of dietary cholesterol absorbed have been reported to vary between 15% to 75% and is about 50% on average, with the remainder being excreted in feaces [3]. Hence the amount of cholesterol remaining in bird fecal droppings depends on the amount of cholesterol that has been absorbed. A high cholesterol absorption would result in a faecal dropping containing low level of cholesterol [3].

Rice bran, a known natural source of phytosterols [4, 5], has been known to have great nutritional and therapeutic values. It has also been reported to lower cholesterol levels in humans [6]. Phytosterols have been found to decrease intestinal absorption of cholesterol thereby lowering cholesterol levels [3, 7]. Although, no harmful effects have been observed with the phytosterol in rice bran, even after prolonged administration of excessive amounts to humans and animals [8], care must be taken during food modifications with phytosterol sources to avoid excessive lowering of the cholesterol of the target animals - a condition called hypocholesterolemia [9]. An increased cholesterol excretion in birds will lower their physiological cholesterol levels and thus decrease the amount of cholesterol available for incorporation in eggs and other tissues of laying birds [3].

This study is an attempt to evaluate the effect of a rice bran-modified proprietary poultry feed on cholesterol excretion in laying birds. The study presents poultry farmers with a potential new strategy for lowering the amount of cholesterol available in birds for incorporation into eggs right before they are formed, thus sparing consumers the more cumbersome alternative, of a cholesterol control diet regimen. With reduced cholesterol levels in eggs, the study would have

* Corresponding author. *E-mail address*: chrisjieng@gmail.com (Chris Oche Ikese)

contributed positively to stemming the tide of coronary heart diseases in society.

2. Experimental

2.1. Materials and reagents

Vita® layer mash, a brand of proprietary poultry feed, was procured. Rice bran was then collected from a rice-mill in Makurdi-Nigeria. Also, 50 healthy laying birds were procured from a poultry farm in Makurdi metropolis. These were then separated into a control group and an experimental group comprising 25 birds each. Both the control and experimental groups were acclimatized to their living environment [10] by sheltering them in a cage where they were provided with adequate unmodified Vita® layer marsh-called the unmodified feed (UMF) and portable drinking water ad libitum for 7 days prior to the commencement of the experiment.

Chloroform and acetic anhydride from Sigma diagnostics were procured and used without a further purification.

2.2. Modification of proprietary feed

8.0 kg of the Vita® layer mash was thoroughly blend with 2.0 kg of rice bran to obtain a 4:1 Vita® feed to Rice bran feed-mix, corresponding to a 20% rice bran modification. This was packaged in a plastic bag and labelled as modified feed (MF).

2.3. Chemical characterization of feeds

Both the modified and unmodified feeds were characterized by proximate analysis using Association of Official Analytical Chemists (AOAC) methods of analysis [11]. Moisture content was determined by oven-drying method, crude protein by micro Kjeldahl method, crude fat by Soxhlet method, ash content by using a muffle furnace, crude fiber by acid and alkaline hydrolysis, and nitrogen free extract (total carbohydrate) was determined by difference as reported by Ikese et al. [12]. Each proximate parameter in both feeds were determined in triplicates and the mean calculated.

2.4. Determination of mean total cholesterol in faecal dropping

Total cholesterol was first determined for both experimental and control groups for 4 consecutive days and the mean calculated in each case before the commencement of variation in their feed type. This was to ascertain the baseline total cholesterol level in the faecal droppings of both groups, and thereafter total cholesterol was again determined for 28 days at 4 days interval following commencement of variation in the feed types fed to each group. The experimental group was fed with the modified feed and the control group maintained on the unmodified feed. The colorimetric methods described by Sperry [13] and Ikese et al. [14] were modified for use in the determination. Dried faecal droppings of birds in the group under investigation were ground into fine powder and 1 g of the ground faecal dropping was dissolved in 10 mL chloroform. The resulting mixture was agitated thoroughly and allowed to stand for 2 h with further shaking before centrifugation at 35000 rpm for 5 min. 20 µL of the clear supernatant, distilled water and 200 mg/dL cholesterol standard were pipette separately into test tubes marked as sample, blank and standard respectively. 1 mL each of analytical grade acetic anhydride was separately added to each tube as color-forming reagent, which was then agitated and incubated for 15 min at 37 ºC in a thermostatic water bath for color development. The absorbance of the sample and standard solution was read against the blank at 500 nm using a Lovibond™ 180270 colorimeter. The amount of cholesterol in each sample was calculated using Equation 1. The total cholesterol excretion in the faeces of each group on a given day was then obtained by calculating the mean of faecal total cholesterol of all birds in the group. Also, the change in mean total cholesterol excretion and percent increase in total cholesterol excretion for control and experimental groups at the end of the experiment (day 28) were calculated using Equations 2 and 3 respectively [14]:

$$Cholesterol\ in\ faecal\ sample = \frac{Absorbance_{sample}}{Absorbance_{standard}} \times Conc.standard\ (mg/g) \quad (1)$$

$$Change\ in\ mean\ total\ cholesterol\ \left(\frac{mg}{g}\right) = C_{28} - C_0 \quad (2)$$

$$Percent\ increase\ in\ cholesterol\ excretion\ (\%) = \frac{C_{20} - C_0}{C_0} \times 100 \quad (3)$$

where: C_{28} = mean total cholesterol excretion in dropping of group on day 28; C_0 = mean total cholesterol excretion in dropping of group on day 0 (baseline).

2.5. Statistical analysis

Results are expressed as mean ± standard deviations of triplicate determinations. The mean total cholesterol (MTC) excretion of the experimental group was compared with that of the control group on day 28 using *t*-test. Also, the MTC in the droppings of the experimental groups on day 0 and day 28 were compared by *t*-test.

3. Results and discussion

Table 1 shows that the 4:1 modification of the proprietary poultry feed with rice bran increased the fiber content by 1.47% thus, increasing the potential of the modified feed to absorb more intestinal cholesterol for increased excretion in faeces as reported by a similar study [10].

Table 1. Proximate composition of modified (MF) and unmodified (UMF) proprietary poultry feed.

Sample	Moisture content (%)	Ash (%)	Crude protein (%)	Crude fat (%)	Crude fibre (%)	Total carbohydrate (%)
MF	11.16±0.11	3.21±0.32	10.90±0.08	12.50±0.28	9.32±0.34	52.850±0.12
UMF	11.62±0.13	3.90±0.14	10.16±0.07	9.96±0.21	7.85±6.35	56.62±0.51

The feed modification appears to have had only a marginal but notable effect on the carbohydrate, crude fat and fiber compositions of the resulting modified feed when compared with the composition of the unmodified proprietary poultry feed. However, the protein moisture and ash contents essentially remained unaltered. The implication is that this feed modification will ensure increased cholesterol excretion without having adverse effects on poultry productivity (especially egg production) and farmers will be more favorably disposed to adopt this strategy for lowering cholesterol in poultry products.

Table 2. Mean total cholesterol (MTC) excretion in faecal droppings of experimental and control birds.

S/N	Sample collection day	MTC excretion in control birds (mg/g)	MTC excretion in experimental birds (mg/g)
1	Day 0	5.97±0.16	5.97±0.16
2	Day 4	6.26±0.45	7.20±1.40
3	Day 8	6.34±0.49	7.57±0.80
4	Day 12	6.57±0.57	8.36±0.64
5	Day 16	6.39±0.41	8.76±0.62
6	Day 20	6.44±0.39	8.94±0.28
7	Day 24	6.26±0.45	9.20±0.48
8	Day 28	6.50±0.57	9.99±0.47

Table 2 shows a marginal but steady increase in the MTC excretion in the faecal droppings of birds fed with the modified feed as the regimen progressed from day 4 to day 28. Importantly too, the mean total cholesterol excretion before and after the modified feed regimen (5.97±0.16 mg/g and 9.99±0.47 mg/g respectively) were found to be significantly different when compared using a *t*-test at $p > 0.05$ and 49 degree of freedom as the calculated value of *t* (9.22) was greater than the critical value of *t* (2.77) for the experimental group. This is suggestive of an increased bile acid-binding effect conferred on the proprietary feed following the 20% modification with rice bran and this is consistent with claims by other authors [15 - 19] who have reported that, binding bile acids and increasing their faecal excretion has been linked with cholesterol lowering in plasma and liver. Also the stated result trend in Table 1, corroborates a similar study [20], which showed that hamsters fed diets containing 11%, 22%, 33%, and 44% rice bran resulted in plasma cholesterol reductions of 8%, 11%, 15%, and 21%, respectively, compared with control values, thus implying that more cholesterol must have been excreted in faeces. It was however reported that plasma cholesterol reductions were significant only with the diet containing 44% rice bran.

For the control group however, the mean total cholesterol excretion on day 0 (5.97±0.16 mg/g) and day 28 (6.50±0.57 mg/g) were not significantly different as the calculated value of *t* (1.26) was lower than the critical value (2.77) at $p > 0.05$ level of probability and 4 degree of freedom. But a *t*-test at $p > 0.05$ and 4 degree of freedom show there is a significant difference between the mean total cholesterol excretion in faecal droppings of the control (6.50±0.57 mg/g) and experimental group (9.99±0.47 mg/g) at the end of the 28 day varied feed regimen seeing as the critical value of *t* (2.77) was lower than the calculated value of *t* (10.51). This implies that the feed modification prevents a high retention of cholesterol by birds, thus resulting in a voluminous excretion of the unabsorbed cholesterol in the faecal dropping of experimental birds and this is consistent with the findings of Brown and Goldstein [21].

Table 3. Change in cholesterol excretion before and after the experiment.

Group	MTC excretion on day 0/ baseline (mg/g)	MTC excretion on day 28 (mg/g)	Change in MTC excretion (mg/g)	Percent change in MTC excretion (%)
Control	5.97±0.16	6.50±0.57	0.53	8.9
Experi-mental	5.97±0.16	9.99±0.47	4.02	67.3

Table 3 shows a 67.3% gross increase in cholesterol excretion in the experimental group. This amounts to a 58.4% net increase in cholesterol excretion of birds fed with the modified poultry feed (experimental group) when corrected for the 8.9% increase in cholesterol excretion that was observed in birds fed with the unmodified feed (control group) (i.e. 67.3% minus 8.9%). Considering that rice bran is a known natural source of sitosterol (a phytosterol), the trend observed in Table 3 becomes justified, and corroborates reports by Clarenburg et al. [8] who reported that sitosterol when administered to experimental subjects lowered the levels of cholesterol and other lipids in various tissues of different animal species by interfering with intestinal absorption of dietary and enterohepatically circulating cholesterol thus, leading authors to conclude that phytosterols reduced cholesterol levels by promoting faecal excretion of sterols and their degradation products. In any case, the observed 67.3% increase in cholesterol excretion in the experimental group is significantly higher than the 8.9% increase in the control group and the latter may be due to other prevailing environmental and physiological factors that affected cholesterol excretion under the prevailing rearing conditions. From Table 3, the percent change in cholesterol excretion in the faecal dropping of experimental and control birds at the end of the feeding regimen stand at 67.3% and 8.9% respectively.

4. Conclusion

The study found that a 20% rice-bran modification of the proprietary poultry feed increases total cholesterol excretion in the faecal droppings of birds fed with the modified feed by 67.3%, and this is statistically significant at $p > 0.05$. Hence, a 20% rice-bran modification of the proprietary feed yields a modified feed with proven potency in elevating total cholesterol excretion by 67.3% and this modification produces no drastic deviation from the proximate composition of the unmodified proprietary poultry feed presently used by farmers and is likely to have no adverse effect on the productivity of laying birds. Hence, rice-bran modification of proprietary poultry holds potential as an effective strategy for lowering cholesterol content of poultry products such as chicken and eggs by increasing cholesterol excretion in their faecal dropping.

Conflict of interest

The authors declare no conflict of interest.

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