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Modelling the Evolution of Serum Cholesterol Level of Broiler Chickens

M. Alam[1,2], M. Ohid Ullah[1], M.S. Islam[1]

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ABSTRACT

Background: With the demand of the growing population, the broiler industry has grown up rapidly over the last few decades and it plays as an affordable source of good quality nutritious animal protein. This broiler industry focuses mainly on optimizing the profit through improving body weight and feed efficiency but the health issues of consumers are not taken into consideration seriously. It is important to know the changing pattern of concentration level of the biochemical parameter (total cholesterol) due to different feeds as well as different ages of chicken.

Methods: This experimental study through longitudinal data was conducted using repeated measurements from each of seventy randomly selected broilers, partitioned into two groups according to two types of feed, at four-time points. Since measurements from the same subject were taken at four time periods, traditional approach of analysis may not be appropriate as it ignore the correlation between repeated measurements. Therefore, linear mixed model was adopted for the analysis of our obtained dataset.

Result: Linear mixed effect model did not reveal any significant difference of standard and hatcher's supplied feeds over time on the evolution of total cholesterol level. This might be due to little difference in different compositions of both feeds. However, both exploratory data analysis and modelling confirmed that irrespective of the available feed types, total cholesterol level of broiler serum increased significantly over time (age) which leads to a recommendation for the consumers to eat younger age (lower weight) broiler chicken.

Key words: Broiler, Cholesterol, Evolution, Longitudinal study.

INTRODUCTION

Due to the growing population throughout the globe, there has been an increased demand for safe, nutritious, continuous and diversified food supply. Nevertheless, most of the developing countries are suffering from malnutrition due to inadequate production of food (Shakila *et al*. 2017). Moreover, livestock production plays a foremost social role in terms of providing food, generating employment, income and thus contributions to rural development (Wirsenius *et al*. 2010). The broiler industry is one of the major livestock sub-sector and is committed to supplying a cheap source of good quality nutritious animal protein (Simons, 2009). The farmers mainly focus on optimizing the profit through improving body weight and feeds efficiency. The traditional feeding of broiler chickens is based on diets for three age groups, *i.e.* prestarter, starter and finisher diets. Study for increasing body weight through multiple phase feeding rather than traditional feeding approach was conducted by Tikate *et al*. (2021). Changing the nutrition level of feed in every week could be a better choice. Cicek and Tendogan (2016) described a mathematical function for optimizing slaughter age of broiler chickens in terms of commercial benefit.

Native indigenous chicken, broiler and layer are commercially produced for consumption in Bangladesh (Miah *et al.,* 2016). The national share of commercial strain of chickens to indigenous chicken (local chicken) in terms of egg production is almost equal (50:50) and that of meat production is 60:40 in Bangladesh, although the growth rate of indigenous chicken is slower than the commercial broiler when raised under the same commercial conditions (Bhuiyan, 2011). In Bangladesh, 89% of the rural household rear poultry and the average flock size per household is 6.8. Poultry meat alone contributes 29% of the total meat production in Bangladesh (BBS, 2001). Lipids are a group of fats and fat-like substances that are important constituents of cells and sources of energy. Different plasma lipids vary greatly in various populations due to differences in geographical, cultural, economic and social, genetic conditions and dietary habits (Hart *et al*. 1997; Vartiainen *et al*., 1998; Abubakar *et al*. 2009). Lipid profile is mainly used to assess the risks of developing cardiovascular diseases and to monitor the management of the afflicted (Castelli *et al*. 1992; NCEP, 2002; Dauqan *et al*. 2011). A typical lipid profile includes total cholesterol (TC), triglyceride

[1]Department of Statistics, Shahjalal University of Science and Technology, Sylhet-3114, Bangladesh.
[2]Department of Agricultural Statistics, Sylhet Agricultural University, Sylhet-3100, Bangladesh.

Corresponding Author: M.S. Islam, Department of Statistics, Shahjalal University of Science and Technology, Sylhet-3114, Bangladesh. Email: shahed-sta@sust.edu

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(TG), high-density lipoprotein (HDL), low-density lipoprotein (LDL), very-low-density lipoprotein (VLDL) and ratios derived from the above values (Egwurugwu *et al*. 2013). Animal protein can be hypercholesterolemic that causes heart and artery diseases of consumers, which is possible to control through modifying certain components from the animal diet. The reduction of fat in poultry birds has become one of the prime focuses of nutritional research. High protein diets influence growth performance (Malden *et al*. 1997). However, too much or too poor protein can be harmful to health because it is associated with degenerative diseases such as obesity and cancer (Veith, 1998). Ahmad *et al*. (2017) experimented the performance of fermented rice bran on broiler chickens and recommended this as feed for broilers.

We know that total cholesterol is an important component of the lipid profile. Since the broiler chicken fattening is being resorted to at lower age for better profit, it is important to know the concentration level of total cholesterol at different ages of chickens to identify its optimum level. There exists no substantial longitudinal study that evaluates the evolution of biochemical components in chickens' bodies over time. For exploring the latent pattern, we conducted this longitudinal study and applied a linear mixed model approach as a proper statistical analysis. The specific objective of this study was to discover, how the serum cholesterol of broiler evolve (change over age of broiler) and how these evolutions depend on the broiler feed.

MATERIALS AND METHODS

A longitudinal study was conducted on a broiler farm of Sylhet Sadar, Bangladesh according to the direction of the Poultry Science Department of Sylhet Agricultural University, Bangladesh. The experiment was carried out following the U.K. Animals (Scientific Procedures) Act, 1986 and associated guidelines, EU Directive 2010/63/EU for animal experiments. The field experiment was conducted in the summer season from 15th April 2016 to 19th May 2016 for a total of 35 days and the laboratory experiment was carried out in the Department of Physiology, Sylhet Agricultural University, Bangladesh.

Selection of sample size

Using the sample size selection procedure for a longitudinal study (Hedeker *et al*. 1999), we decided 35 as the required sample size for both group where we considered time points four, power of the test 0.80, significance level 0.05, attrition rate 0.05, standard deviation (SD) 0.70 and correlation coefficient (Rosário *et al*. 2007) (ρ) 0.80 as the parameters for sample size calculation.

Experimental design

In this study, 70 (Cobb-500) broiler chicks were randomly chosen from 600 one-day-old chicks from a hatchery belonging to the same batch and same breeding stock. As the chicks were collected from the same hatchery, we assumed that the variation in weight would be only due to random factor and therefore no other local control in the design of the experiment was performed. Chicks were randomly distributed in two groups of equal size (35 chicks) and were provided Standard feed (Feed-A) and Hatcher supplied feed (Feed-B) respectively. Water was supplied ad libitum during the entire experimental period. Feed was adapted to the three main phases of the rearing period: starter period (1-14 days of old), grower period (15-25 days old) and the finisher period (26-35 days old). The composition of the two diets and their nutrient components were analyzed using proximate analysis and are presented in Table 1.

Repeated measurements were performed for each chicken at the age of 14th day, 21st day, 28th day and 35th day (Fig 1). A blood sample of 1 ml/bird was collected from the wing vein into the EDTA tube in the early morning and transferred to the Laboratory of the Physiology Department at Sylhet Agricultural University for analyses of total cholesterol within two hours of collection. After centrifugation (3000 g for 10 min at room temperature) plasma was harvested and stored in Eppendorf tubes at -20°C until

Table 1: Ingredients and nutrient compositions of experimental diets.

Nutrient compositions	Amount (g/100 g) for standard feed available in market (Feed-A)	Amount (g/100 g) for feed supplied by hatcher (Feed-B)
Starter period (day 01 to day 14)		
Crude protein	21.50	22.00-23.00
Fat	3.50	5.00-6.00
Crude fiber	5.00	3
Moisture	12.00	10.00-11.00
Grower period (day 15 to day 25)		
Crude protein	20.00	21.00-22.00
Fat	3.00	5.00-6.00
Crude fiber	5.00	3.00-4.00
Moisture	12.00	10.00-11.00
Finisher period (day 26 to day 35)		
Crude protein	19.00	21.00-22.00
Fat	3.00	5.00-6.00
Crude fiber	5.50	3.00-4.00
Moisture	12.00	10.00-11.00

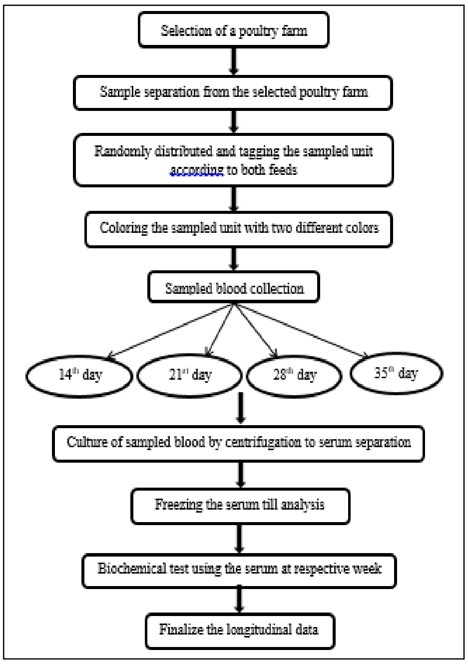


Fig 1: Steps of the research.

assayed. Total cholesterol was measured by the chemistry semi-auto analyzer AUTOPAK, according to a standardized protocol using the commercial analyzing kit supplied by Bio-trade International (BD). Serum total cholesterol was estimated by using the enzymatic (Cholesterol Esterase, Cholesterol Oxidase and Peroxidase) method (Prasad *et al*. 2009).

Statistical analysis

Longitudinal data are typically composed for investigating the changes in an outcome variable over time, with the aim of compare these changes among groups (Patrick and Vetter, 2018). Commonly used statistical techniques assume independence of the observations or measurements, but the values comes from the repeatedly measured in the same individual are usually more similar to each other than values from different individuals so that they are not independent (Fitzmaurice and Ravichandran, 2008). Overlooking this correlation between repeated measurements can result in biased estimates, as well as incorrect, so appropriate analysis of repeated-measures data desires specific statistical techniques that account for such within-subject correlations (DeLivera, Zaloumis, Simpson, 2014). In a longitudinal study with more than two time periods, the well-known t-test or F-test approach is not applicable due to correlation within the measurements of the same subject. Therefore, the data was statistically analyzed using linear mixed models (LMM) (Gibbons and Hedeker, 2000; Goldstein *et al*. 2002; Anupama and Chandrashekara, 2014).

RESULTS AND DISCUSSION

Exploratory data analysis (EDA)

Individual profile

The selected broiler's total cholesterol value (mg/dl) at age (14th day, 21st day, 28th day and 35th day) were plotted against time (Fig 2) which exposed the subject-specific pattern of individual profile plot of cholesterol level for each category of feed. The two individual profile plots exposed that there may exist within-subject variability, as well as between-subject variability. These figures suggested that the changing pattern of individuals' total cholesterol levels of broiler is different over time and their starting point is not same. So, we might consider random intercepts and random slopes in the statistical model for the total cholesterol of broiler chicken.

Mean profile

To perceive the average change, the average cholesterol level of broiler serum at each time point due to feed was plotted in Fig 3(a). The average change for standard feed (A) and hatcher's supplied feed (B) was observed to be quite similar (approximately linear over time) for the total cholesterol level of broilers. At some point, the mean cholesterol level due to two feeds overlapped which indicates that there exists an interaction between total cholesterol level and time (age).

Variance profile

Variance profile of the broiler's total cholesterol level (Fig 3b) indicates the increasing variability pattern according to time

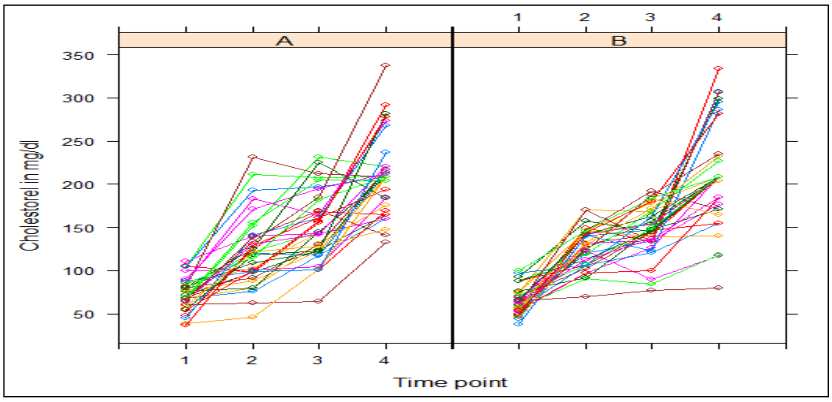


Fig 2: Individual profile of total cholesterol according to feed category.

(age) but this increasing pattern is unstable and quite different for two feed, hence it might assume non-stationary. It means that there is some remaining systematic structure in the residual profile. So we might use to select one or more random effects additional to random intercept to build the model.

Correlation structure

As the correlation structure of total cholesterol level measurements between different time points (age of broiler) didn't show any logical pattern among the measurements of different time points, so unstructured (UN) might be considered as a covariance structure in the model.

Classical analysis: Linear mixed models (LMM)

Covariance structure selection

Since the covariance structured (UN) model had the smallest AIC, AICC and BIC values (Table 2) than that of others, so we considered the unstructured (UN) as the covariance structure for the total cholesterol of broiler chicken.

Table 2: Covariance structures for total cholesterol of broiler.

Covariance structure	AIC	AICC	BIC
Model 1:CS	2781.194	2781.238	2788.435
Model 2:UN	2705.415	2706.245	2741.619
Model 3:UNR	2705.415	2706.245	2741.619
Model 4:AR(1)	2777.913	2777.957	2785.154
Model 5:ARH(1)	2708.802	2709.024	2726.904

Model selection

The log-likelihood ratio tests (*i.e.*, mixture of chi-square test) have shown that random slopes were significant in the model. As the mixed effect model is hierarchical; the selected random-effects model might include both random intercepts and slopes. Therefore, applying available fixed effects and selected random effects, we were considered the final model for total cholesterol of broiler chickens as follows:

$$Y_{ij} = (\beta_0 + b_{0i}) + (\beta_1 + b_{1i})\, t_{ij} + \beta_2\, Feed + \beta_3\, Feed \times t_{ij} + \varepsilon_{ij}$$

Where,

$b_i = (b_{0i} + b_{1i})' \sim N(0, D)$; D is a 2×2 covariance matrix and $\varepsilon_{ij} \sim N(0, \sigma^2 I_n)$,

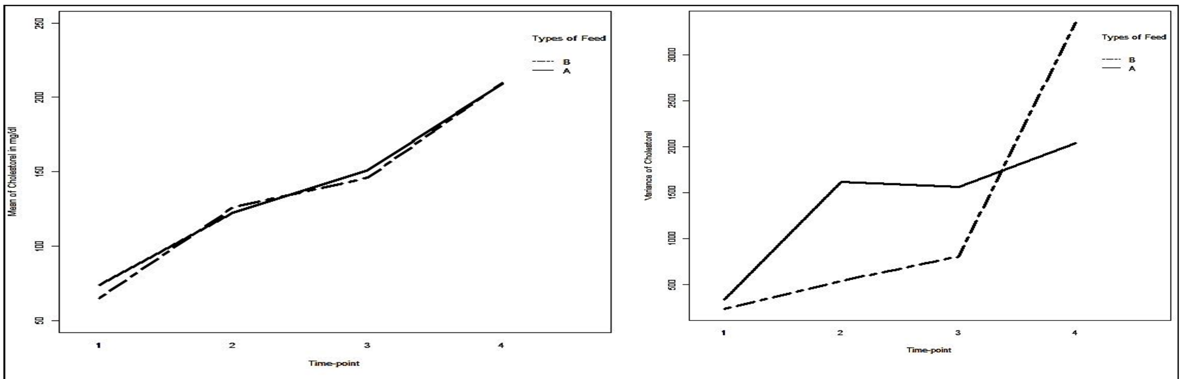
β_0, β_1, β_2 and β_3 are parameters of the fixed effects, b_{0i} and b_{1i} are parameters of the random effects (random intercepts and random slopes for the i^{th} subject respectively) and ε_{ij} are the residuals.

Table 3 demonstrated that the estimates of fixed effects and their test of significance as well as the estimates of variance components of random effects. The fixed effect intercept and time (age) were found significant on the total cholesterol level of the broiler which supports that age and gender differences affect serum lipids (Malik *et al*. 1995; Shahid *et al*. 1985). But the remaining fixed effect *i.e.,* broiler feed and also the interaction effect of feed and time had no

Table 3: Estimates of mixed effect models with random intercepts and slopes for total cholesterol.

Effect	Estimate	SE	df	t -value	Pr> |t|
Intercept	26.560	4.238	208	6.266	0.000*
Feed	8.791	5.994	68	1.466	0.147(NS)
Time	41.070	2.390	208	17.177	0.000*
Feed*Time	-0.943	3.381	208	-0.278	0.780(NS)
Covariance parameter estimates					
Var (b_{0i})	75.359				
Cov (b_{0i}, b_{1i})	-80.924				
Var (b_{1i})	108.243				
Residual	272.97				

*Feed-B (Hatcher's supplied feed) was taken as the reference category.
*Significant at P≤0.05; NS- Non Significant at P>0.05.



(a) Mean profile (b) Variance profile

Fig 3: (a) Mean profile and (b) Variance profile of total cholesterol according to feed category.

significant effect on the total cholesterol of broiler chicken. These results support the findings that fattening of the chickens should be compromised with low-fat feed and the consumers should look for low-age chickens (Prasad *et al*. 2009).

We know the total cholesterol is the single indicator/ parameter of lipid profile which was studied in this research. There remains the scope to extend the study for all indicators of lipid profile separately (using LMM Approach) and combindly (Joint Modelling Method). We applied only two commercial feeds as treatment but there remain opportunity to study more than two custom processed feeds with different nutritional compositions. Furthermore, there is huge opportunity to apply this repeated measures study for the different animal study experiments.

CONCLUSION

This is a new dimensional repeated measures study on serum total cholesterol analysis of broiler chicken, where measurements were collected from each chicken at four equally spaced time intervals. The total cholesterol is an important tool in assessing the lipid profile of broiler serum as well as humans. This study provides the consciousness about the effect of feed on total cholesterol over the age of broiler chicken. Understanding the changing pattern of total cholesterol levels at different ages of broiler's is an important issue for the consumers because of food with high cholesterol level increases the risk of cardiovascular disease of human. From the EDA and LMM analysis, it may be concluded that the total cholesterol level of broiler significantly changes (linearly increasing) over time (age). This finding suggests the consumers to eat younger age broiler chicken due to lower cholesterol levels. On the other hand, it was found that there is no significant feed effect (*i.e.*, there is no significant difference for Feed-A and Feed-B) on the evolution of total cholesterol of broilers serum over time, which may be due to the little difference in nutrient compositions of both feeds.

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