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Mining the Feed Effects on Five Weeks Old Broiler Chicken

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Abstract: *Fattening the broiler chicken at lower age for better profit is the main focus of farmers who resort to various feeds to reach their goal. Any adverse effect in chicken's body through feed might produce health hazard to the consumers. Therefore, it is important to discover the concentration level of bio-chemical parameters in chicken's body due to different types of feeds available in the market. The study was conducted on seventy chickens which were randomly allocated to provide two types of feeds. Blood samples were taken at 35thdayand readings for lipid profiles, liver enzyme parameters along with calcium and creatinine were determined through lab experiment. Analysis and graphs indicated that the feed provided by the hatcher produces significantly higher weight. Nevertheless, multivariate analysis of variance revealed that lipid profile levels and liver functioning were significantly different for two types of feed. A reflective mode partial least squares path model (PLSPM) indicates health status (as measured by weight and calcium) has significant effect on lipid profile level, although the other paths representing lipid profile and health status to liver functioning were not found to be significant. Moreover, we observed different PLS coefficients for two types of feeds but possessing same pattern of relationship.*

Keywords: *Broiler, Lipid profile, Liver function, Multivariate analysis of variance and PLS Path Modelling.*

I. Introduction

In Bangladesh poultry industry is one of the major livestock sub-sectors which can ensure to supply cheap sources of good quality nutritious animal protein to the nation. Poultry meat contributes approximately 37 percent of the total animal protein in Bangladesh [1]. Improving body weight and feed efficiency of the birds is the main objective of the poultry meat industry since many years. There are other parameters, such as low cholesterol and improved fatty acid profile, which need to be taken into consideration [2].

A high level of toxic elements of blood and also lipid profile (i.e., total cholesterol) level in the chickens' body can eventually lead to serious consequences on consumers' health. The concentration levels of these blood parameters are changeable due to different types of poultry feeds. Lipid profile is the risk indicators of coronary heart disease [3].Lowering blood cholesterol concentration dietary monounsaturated fatty acids (e.g. oleic) were very effective and may be important in preventing coronary heart disease [4].

The effect of feeding garlic powder (GP) on the performance, digestibility, digestive organs and lipid profile of broilers were inspected [5] and found that GP could provide positive advantages in broilers performance as it significantly decreased total cholesterol (COL), triglycerides (TG), low density lipoprotein (LDL) and increased high density lipoprotein (HDL) levels compared to control birds and these result supported the findings of [6]. The effects of alpha lipoid acid on the performance and serum lipid profile in broiler chicken was found to have no effect on serum cholesterol, triglycerides and VLDL-cholesterol levels, but reduced the LDL-cholesterol level and increased the HDL-cholesterol level of broilers compared to control group [7]. The effect of high diet feed on lipid profile levels on chickens was studied by [8]. Poultry species, age and breeding condition is known to affect cholesterol deposition ([9], [10], and [11]).

The food which is be free from all types of health hazards and have good taste and necessary nutritional diet for human growth and development is healthy food. Nowadays food security and food safety is the top most public health concern worldwide. Important aspects of food quality on health safety are destroyed by entering the hazardous type of pollution in the food chain. Increasing the LDL and total cholesterol level the risk of serious problem of human like as stroke, heart attack and atherosclerosis also increases. Since the poultry fattening at the lower age for better profit, it is important to know the concentration level of these above parameters at different types of feed of chickens as well as identify the available better feed in the market. Bearing in mind lack of available information of poultry feed effects on serum bio-chemical parameters, this study was undertaken to investigate the effect of feed on lipid profile, liver function and others parameters in chicken.

Lipid profile and liver function's readings were considered as group of variables while other measurements were regarded as univariate. As the data did not violate classical assumptions, simple t-tests were performed for univariate tests, whereas multivariate analysis of variance (MANOVA) was resorted for grouped-variables. Chickens' weight and some biochemical parameters were found to differ significantly for two types of feeds. PLSPM is a non-parametric approach resorted to investigate any simultaneous dependency of group of

variables [12]. A reflective mode PLSPM revealed that health status has significant effect on lipid profile level; whereas lipid profile and health status do not play significant role on liver functioning. We observed different PLS coefficients for two types of feeds; however, the pattern of relationship was similar.

II. Experimental Design And Method

2.1 Experimental units and treatments

Randomly selected seventy broiler chicks from 600 (Cobb-500) were randomly distributed into two groups each belongs of 35 birds. Chicks were collected for the study belonged to the same batch and the same breeding stock, reared under strict hygienic and similar conditions. Rooms, brooder battery and cages were thoroughly cleaned with 2.5% phenol and subsequently fumigated with formaldehyde gas. Sunlight and electric bulbs were used as source of heat and light, electric fan were used for controlling the room temperature. Birds of the both groups were vaccinated against disease according to the direction of the expert of the poultry science department of Sylhet Agricultural University (SAU), Sylhet, Bangladesh. Two different feed i.e., Feed A (Standard feed available in Market), Feed B (Supplied by Hatcher) for each stage (i.e., starter, grower and finisher) were supplied to the two groups from the first day to 35^{th} day of the experiment. Water was supplied ad labium during the entire experimental period. Composition of the diets and its nutrient composition for the above-mentioned phases are presented below.

Table 1. Ingredients and nutrient compositions of experimental diets

Nutrient compositions	Feed A	Feed B
Starter period (day 01 to day 14)		
Crude Protein	Min 21.50%	22.00-23.00%
Fat	Min 3.50%	5.00-6.00%
Crude Fibre	Max 5.00%	3.00-4.00%
Moisture	Max 12.00%	10.00-11.00%
Grower period (day 15 to day 25)		
Crude Protein	Min 20.00%	Min 21.00-22.00%
Fat	Min 3.00%	5.00-6.00%
Crude Fibre	Max 5.00%	3.00-4.00%
Moisture	Max 12.00%	10.00-11.00%
Finisher period (day 26 to day 35)		
Crude Protein	Min 19.00%	Min 21.00-22.00%
Fat	Min 3.00%	5.00-6.00%
Crude Fibre	Max 5.50%	3.00-4.00%
Moisture	Max 12.00%	10.00-11.00%

2.2 Collection of blood sample for the bio-chemical parameters

From the wing veins of every bird of eachgroup's3ml blood sample were collected for lipid profile, liver function and other bio-chemical parameter test at 35^{th} day age of chicken. We collected blood sample with the help of expert personnel at very early morning for fasting stage of the bird and also measured the weight of each sampled bird. Blood samples were transferred to the physiology laboratory of SAU, Sylhet within two hours of collection and serum were separated by centrifugation (3000g, for 10 minutes at room temperature) and stored in eppendorf tubes for analysis.

2.3 Studies of bio-chemical parameters of blood

The chemistry semi-auto analyzer AUTOPAK was used for the concentrations of the biochemical parameters estimation by routine methods. Commercially available kits (RANDOX, County Antrim, United Kingdom) were used to analyse the serum for total cholesterol (TC) in enzymatic endpoint method, total triglycerides (TG) in GPO-PAP method, low density lipoprotein (LDL) in enzymatic (colorimetric) method, high density lipoprotein (HDL), calcium in colorimetric method, creatinine in colorimetric method and values were expressed as mg/dL. Furthermore, aspartate amino transferase (AST) and alanine amino transferase (ALT) for liver functioning were measured and expressed as U/L.

2.4 Methodology

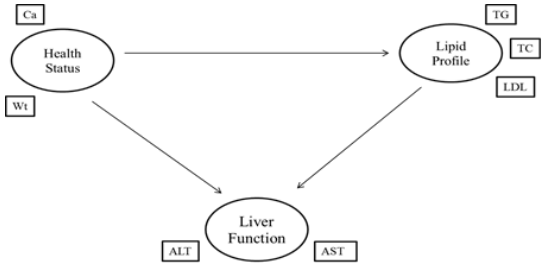
Exploratory analyses as well as inferential analysis t-test, ANOVA and MANOVA were applied using the data obtained from the laboratory experiment for the difference of two groups according to two feeds due to individual blood parameter and also clustered parameters. PLS-PM was applied to get an idea of how the set of dependent variables are systematically explained by their sets of predictors i.e., to obtain score values of latent variables for prediction purposes.

In PLSPM, we considered the observed data set X constituted in 3 (mutually exclusive) blocks as X_1=health status which contains variables weight and calcium of serum, X_2 =lipid profile which contains variables TC, TG, LDL and X_3= liver function which contains variables ALT, AST. Since the indicators HDL

plausibly generate opposite effect from all others indicators of lipid profile, we exclude this from lipid profile block.Each block X_j is assumed to be associated with a latent variable LV_j. The latent variables are estimated as a linear combination of their manifest variables (indicators of block). Moreover, an estimated LV_j is called a score, which we denoted as:

$$\widehat{LV_j} = Y_j = \sum_k w_{jk} X_{jk} \quad \text{- - - (1)}$$

where k is the number of indicators of blocks. Our overall model in structural relationship is as follows:



The Structural Model in mathematical notation can be written as:

$$LV_j = \beta_0 + \sum_{i \to j} \beta_{ji} LV_i + error_j \quad \text{- - - (2)}$$

where the subscript i of LV_i refers to all the latent variables that are supposed to predict LV_j , the coefficients β_{ji} are the path coefficients and they represent the "strength and direction" of the relations between the response LV_j and the predictorsLV_i,β_0 is just the intercept term, and the $error_j$ term accounts for the residuals.

The Measurement Model: Mathematical notation of the outer model in reflective mode as:

$$X_{jk} = \lambda_{0jk} + \lambda_{0jk} LV_i + error_j \quad \text{- - - (3)}$$

The structural coefficients were estimated by ordinary least squares in multiple regression of Y_j on theY_i's andloadings are preferably calculated as correlations between a latent variable and its indicators as $\widehat{\lambda_{jk}} = cor(X_{jk}, Y_j)$. All of these analyses were done using statistical software R and SPSS.

III. Results And Discussion

3.1 Exploratory data analysis

We resorted to boxplots of all the variables for exploratory data analysis. The boxplot in Figure 1(a) shows that different median levels of weights were produced by two types of feeds. Figure 1(b) shows that the calcium levels are almost similar for two feeds, although there are couple of outlying observations for feed A.

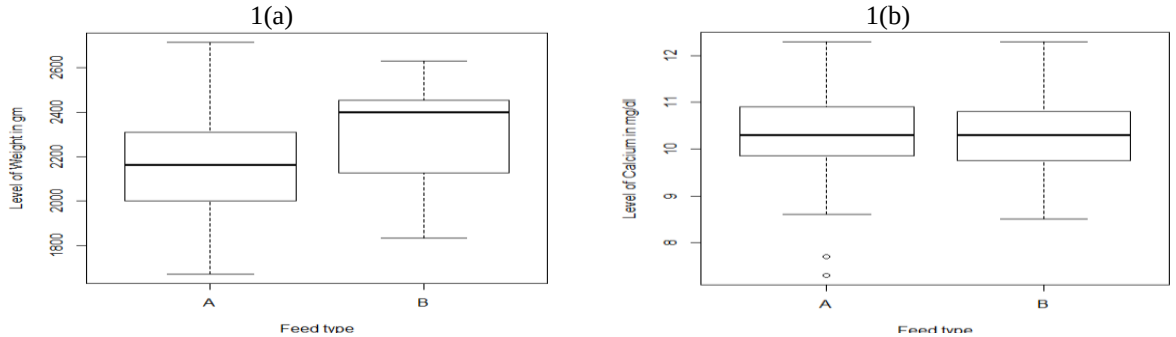


Figure 1. Box-plot for distribution of weight and calcium levels for two types of feed.

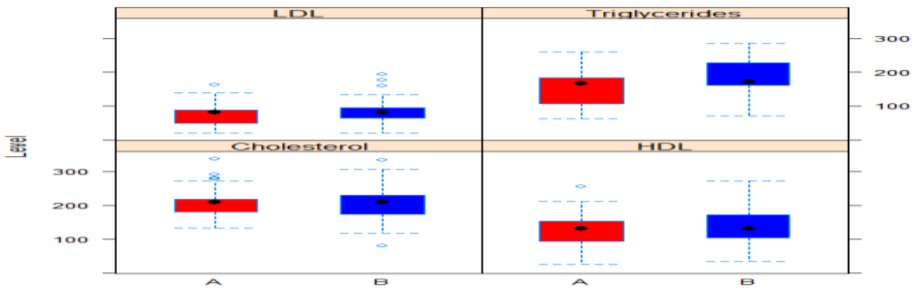


Figure 2. Box-plot for distribution of chicken's serum lipid profile levels for two types of feed. Color blue correspond to feed A and red to feed B.

The boxplot in Figure 2 compares four indicators of lipid profile. These levels are not quite same for both type of feed, especially the boxplot for triglycerides demonstrated that feed A has apparently lower level. The boxplot of liver function parameters (Figure 3) shows that different types of feeds produce different median levels of ALT and AST. Moreover, there is much less variation in reading for ALT than in AST.

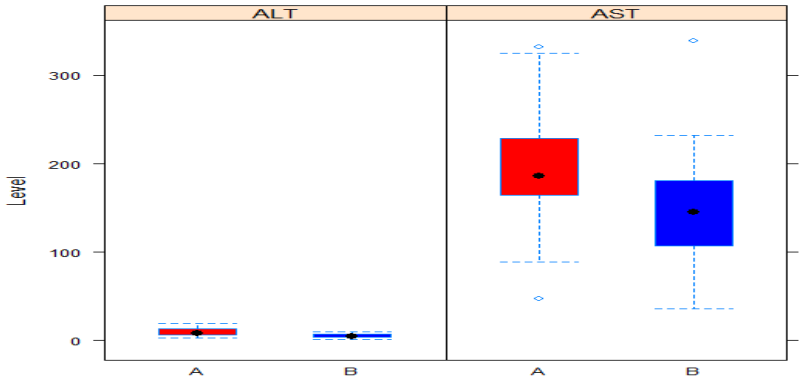


Figure 3. Box-plot for distribution of liver function parameters (ALT, AST) concentration of broilers serum for two types of feed.

3.2 Inferential data analysis

In inferential data analysis to facilitate the significance difference of biochemical parameters and their clusters due to two poultry feed we performed t-test and MANOVA.

Table 2. Effects of dietary supplementation on weight and biochemical composition (calcium and creatinine) of broiler chickens at finisher period

Blood Parameters	Feed	Mean±SE	T	p-value	Mean difference	95% Confidence Interval for mean difference	
						Lower Bound	Upper Bound
Weight (gm)	A	2159.77±44.05	-2.631	0.011	-150.650	-264.910	-36.410
	B	2310.43±36.58					
Calcium (mg/dl)	A	10.31±0.18	-0.095	0.924	-0.020	-0.500	0.460
	B	10.34±0.15					
Creatinine	A	0.43±0.01	1.900	0.062	0.030	-0.002	0.064
	B	0.39±0.01					

The mean weight of chickens was 2159.77gm and 2310.43gm for feed A and B respectively. Independent samples t-test in Table2 shows that the mean difference is significant for these two feeds. However, we did not find significant difference for the mean levels of calcium and creatinine.

Table 3. Effects of dietary supplementation on blood Lipid profile and Liver function of broiler chickens at finisher period (Multivariate Tests using Pillai's Trace)

Effect	Response	F	p-value	Observed Power
Feed	Lipid profile (TC,TG,HDL & LDL)	3.50	0.012	0.840
	Liver function (ALT & AST)	15.18	0.000	0.990

Using the Pillai's Trace test of the multivariate analysis of variance (MANOVA), the lipid profile levels and the liver function level of poultry chickens were significantly different for two types of feeds. We were also interested in finding out the specific components of lipid profile and liver function that varied significantly and performed ANOVA in this regard.

Table 4. The influence of dietary supplementation on serum lipid profile and liver function of broilers

Blood Parameters	Types of Feed	Mean±SE	F	p-value	Mean difference	95% Confidence Interval for mean difference	
						Lower Bound	Upper Bound
Cholesterol (mg/dl)	A	209.46±7.63	0.00	0.995	-0.09	-24.84	24.67
	B	209.54±9.78					
Triglycerides (mg/dl)	A	151.14±9.21	7.87	0.007	-34.74	-59.46	-10.03
	B	185.89±8.28					

HDL(mg/dl)	A	126.14±8.47	0.66	0.419	-10.34	-35.73	15.04
	B	136.49±9.49					
LDL(mg/dl)	A	77.51±6.38	0.94	0.337	-8.86	-27.13	9.41
	B	86.37±6.57					
ALT(U/L)	A	9.89±0.83	25.21	0.000	4.66	2.81	6.51
	B	5.23±0.42					
AST(U/L)	A	198.06±11.29	12.99	0.001	55.51	24.77	86.26
	B	142.54±10.48					

The results of the analysis of variance (Table4) revealed that only mean triglycerides was significantly different among the lipid profile parameters for two feeds. This also confirms the result [13] serum triglyceride level is important indicator of fat metabolism.On the other hand, both parameters observed in serum liver function i.e., mean ALT and mean AST level were significantly different for two feeds.

3.3 Results of Partial Least Squares Path Modelling

After performing PLSPM of reflective mode, we found the following structural relationship with significance values. In the inner model regression results demonstrated that the effect of health status is significant (t=3.520; p=0.001) on the lipid profile but the effect of health status and lipid profile was not significant (p=0.931 and 0.108 respectively) on the liver function. The inner model path co-efficient (Figure4) revealed that the relationships between health status and lipid profile are positive while the relationship between other latent variable pairs are negative.

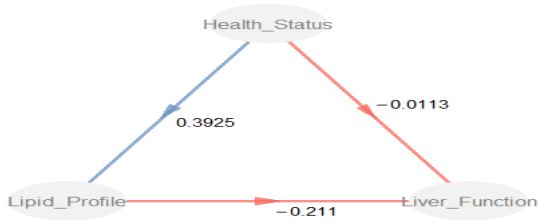


Figure 4: The PLSPM Path diagram of Lipid Profile and Health Status effect on Liver Function.

Figure5 shows that the loadings of weight in health status block, triglycerides in lipid profile block and ALT in liver function block is greater than 0.7, so these indicators considered as good/ acceptable indicators since they captured more than 50% variability by their latent construct.

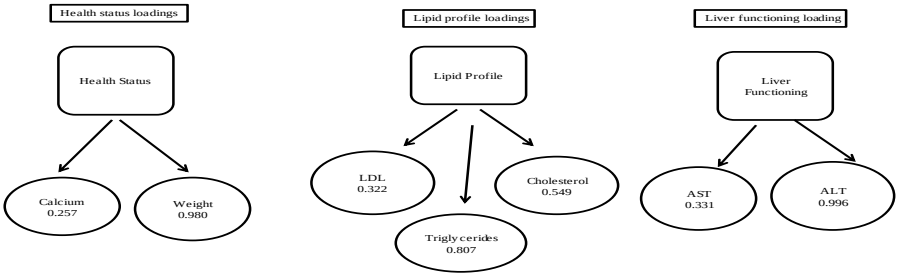


Figure 5: The outer model loadings of latent variables Health Status, Lipid Profile and Liver Functioning which shows the variability of the corresponding manifest variables.

Figure 6 reveals that the entire path coefficients for feed B were positive and path coefficients of lipid profile to liver function was negative which show inverse relationship between lipid profile and liver function for feed A. But the results of group comparisons analysis using t-test (Table 5) revealed that none of the path coefficients between the models for Feed-A and Feed-B are significantly different.

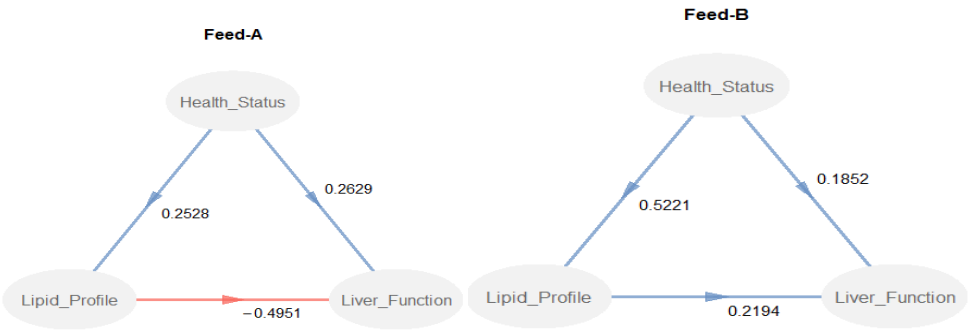


Figure 6: The PLSPM path diagram of Lipid Profile and Health Status effect on Liver Function due to Feed-A and Feed-B

Table 5. Group comparison in PLSPM for inner model coefficient using t-test (Permutation method)

Latent variables / blocks	Global	Group A	Group B	Absolute diff.	p-value
Health Status->Lipid Profile	0.393	0.253	0.522	0.269	0.644
Health Status->Liver Function	-0.011	0.263	0.185	0.078	0.901
Lipid Profile->Liver Function	-0.211	-0.495	0.219	0.715	0.109

IV. Conclusion

Experiment was conducted on seventy randomly selected broiler chickens with requisite lab test to explore the feed effect on biochemical parameter of the chickens' body and some functional relationship among the components. Statistical analyses show that the feed provided by the hatcher produces significantly higher weight than that by other balanced feed available in the market which supported the findings of [8] that birds fed high diet (Crude protein and Fat) showed higher body weight (BW). However, similar test revealed no significant difference in the calcium and creatinine levels produced by two feeds. As the variables in lipid profile levels and liver functioning are in multivariate format, MANOVA results show that although higher weight was gained through the feed from hatcher, this feed significantly increases the lipid profile parameter of triglyceride level. Nevertheless, similar test uncovers that ALT and AST levels were significantly lower due to the feed provided by hatcher. To study the structural relationship among health status, lipid profile levels and liver functioning, a reflective mode path model was conducted. The PLS-Path model revealed that the effect of health status is significant on the lipid profile. Weight, triglycerides and ALT were the influencing indicators of health status, lipid profile and liver function respectively as they captured more than half of the variability. This analysis also indicated that patterns of the influence do not vary significantly while their effect was found different for the two types of feeds.

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