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Effects of Feed Type, Sex and Plumage Condition on Tonic Immobility and Blood Parameters in Broilers

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Abstract: The aim of this study was to determine the effects of feed type, sex and plumage condition on tonic immobility and blood parameters in broiler birds. Eighty broiler birds of Anak (2000) breed were raised from day-old to 8 weeks of age. They were randomly divided into 4 groups of 20 birds each. A different type of feed was given to each group from day-old to 8 weeks of age. The birds were raised on deep litter in the first 4 weeks, and later they were raised in battery cages for the remaining 4 weeks. Tonic immobility of the birds was determined between 0 and 600 seconds. Blood parameters were also determined. Results obtained indicated that generally, tonic immobility was averagely good (less than 300 seconds) with birds fed with feed type 4 having the best (shortest) tonic immobility. Also, tonic immobility was influenced by plumage condition. The normal ranges of Packed Cell Volume (PCV), Haemoglobin (Hb) concentration and total plasma protein were used to arrive at the fact that feed type and plumage condition influenced tonic immobility and blood parameters. Males had better (shorter) tonic immobility than females.

Key words: Feed type, sex, plumage condition, tonic immobility, blood parameters, broiler

Introduction

Poultry industry has been identified as one of the most important economic sectors in Nigeria due to numerous contributions made in terms of employment opportunities, supply of daily protein requirements of the people, manure production, and overall sustainable development. However, maximum benefits can only be derived from poultry birds that are reared in conducive environment with minimum or no stress factors. This is because, the effect of stress in poultry production is so important that so many experiments have been conducted so as to assess the degree of impairment of production and profitability of the business. Scot (2002) described stress as the state manifested by a specific syndrome, which consists of all the non-specifically induced changes within a biological system. Tonic immobility is an indicator of fear and stress (Knowles and Broom, 1990; Frazer and Brown, 1990; Jones, 1992). According to Scot (2002), when birds have their feathers ruffled up or not disposed the way they are supposed to be, it is simply a sign of stress, fear or disease. Similarly, the level of stress in the bird can be determined by the packed cell volume and the haemoglobin concentration (Bush, 1991a,b). The packed cell volume, haemoglobin concentration and total plasma protein are strongly related to the quality of feed fed to birds (Coles, 1986). The author further stated that, they are good indicators as a means of evaluation while the production is in progress. The objective of this study was to determine the effect of feed type, sex and plumage condition on tonic immobility and blood parameters in broiler.

Materials and Methods

Site description: The experiment was conducted in one of the poultry pens of the Animal Science Department of Ahmadu Bello University, Zaria which is located at Samaru village in Zaria Area of Kaduna State; at about 75km away from Kaduna. Zaria is located on a plateau at a height of about 22,100 ft above sea level in the centre of Northern Nigeria. It is located on latitude 11^0 33" N and 7^o 42" E and having a tropical continental climate with marked periods of rainfall ranging from 1102 mm to 1904 mm. The mean temperature fluctuates from 31°C (88°F) maximum in dry season to 18°C (65°F) minimum in wet season.

Experimental design and procedures: Eighty broilers were raised from day-old to 8 weeks of age. The experimental design was a completely randomized design with 4 groups of 20 birds each. A different type of feed was given to each group from day-old to 8 weeks of age. The proximate composition of the feed types is given in Table 1. Within the first 4 weeks, the birds were raised on deep litter and in the last 4 weeks, they were raised in battery cages. There was mortality during the experiment and this led to the following final population sizes: Treatment 1; 19 birds; treatment 2; 18 birds; treatment 3, 14 birds; treatment 4, 17 birds. Three birds per treatment were sampled for tonic immobility measurements and determination of Packed Volume Cell (PVC) and haemoglobin concentration. Plumage condition was arrived at using a scoring system at selected sites (breast, back, wings, tails and neck). The feathering scores used were: 1 = poor, 2 = average and 3 = good.

Table 1: Proximate composition of feed types

	Broiler Starter					
Feed type	% Dry Matter	% Crude protein	% Crude Fibre	% Oil	% Ash	% NFE
1 Mash	97.72	24.44	6.64	6.90	8.36	53.73
2 Mash	97.76	24.06	4.98	6.11	8.12	56.73
3 Mash	98.61	23.13	4.63	5.47	8.00	58.77
4 Pelleted	97.67	25.94	4.62	6.24	7.85	55.35
Broiler Finisher						
1 Mash	97.67	22.44	4.97	5.80	9.44	57.35
2 Mash	97.54	21.69	4.15	6.56	9.73	57.89
3 Mash	97.78	20.94	5.68	5.86	8.59	58.13
4 Pelleted	97.47	24.68	4.13	5.89	8.27	56.03

Table 2: Effect of feed type on Tonic Immobility, Packed Cell Volume, Haemoglobin Concentration and Total Plasma Protein

	Feed types						
	1	2	3	4	Mean	SEM	P Level
Tonic Immobility (Seconds)	241.20±56.28[a] (33-600)	239.80±56.75[a] (51-554)	217.65±52.54[a] (17-600)	188.07±52.99[a] (23-600)	221.68	54.64	NS
Packed Cell Volume (%)	22.48±2.24[a] (7-37)	24.58±2.60[a] (14-31)	23.71±2.09[a] (19-34)	25.21±2.11[a] (16-38)	24.00	2.26	NS
Haemoglobin Concentration (g dL^{-1})	7.49±0.75[a] (2.33-12.33)	8.19±0.75[a] 4.66-10.33)	7.90±0.70[a] (6.33-11.33)	8.40±0.70[a] (5.33-12.66)	8.00	0.73	NS
Total Plasma Protein (g dL^{-1})	4.22±0.23[a] (3.50-6.00)	4.24±0.23[a] (3.60-5.60)	4.39±0.22[a] (4.00-5.80)	4.50±0.22[a] (3.50- 6.00)	4.34	0.23	NS

Figures in parentheses are ranges for the various characteristics measured; NS = p>0.05 a: Means along the same row with the same superscripts are not significantly different from each other. SEM: Overall standard error of the mean.

The tonic immobility was determined using a U-shaped wooden cradle and a stop watch. Each time, the individual bird was placed on its back with the head hanging in the U-shaped wooden cradle (Jones and Faure, 1981). The bird was restrained for 10 seconds. Eye contact was completely avoided between the bird and the experimenter after the experimenter removed his hands from the cradle. A stop watch was used to record latencies until the bird righted itself. If the bird righted itself in less than 10 seconds, then it was considered that tonic immobility had not been induced. Then the restraining procedure had to be repeated. If the bird did not show righting response over the 10 seconds test period, then a maximum score of 600 was given for righting time.

Materials used for Packed Volume Cell (PCV) determination include; needle and syringes, blood specimens, micro-haematocrit/capillary tubes, micro-haematocrit centrifuge, micro-haematocrit reader and flame of Bunsen burner. A venous (wing vein) blood sample was taken into a test-tube containing anti-coagulant agent (EDTA). The blood was mixed well and gently for 2 min. The mixed blood was then drawn up a 75 x 1.5 mm capillary tube for ¾ of its length and the other end of the capillary tube was sealed using the flame of the Bunsen burner. The capillary tube was placed in the haematocrit centrifuge with the sealant at the outer end and the centrifuge lid was closed. The tubes were centrifuged at 15,000 revolutions per min (rpm) for 3 min. The Packed Volume Cell (PCV) was later read using the micro-haematocrit reader. The reading was then expressed as a percentage of packed red cells in the total volume of the whole blood.

The haemoglobin concentration was determined by dividing PCV value by 3 (Schwalm *et al.*, 1975). It was expressed in g/dl. The total plasma protein was determined using a refractometre. The face of the refratometre was cleaned and the capillary tube was broken at the level of the buffy coat. The plasma was emptied into the refractometre and the reading was recorded. The total plasma protein was expressed in $g100mL^{-1}$.

Statistical analysis: The general linear model procedure of the statistical analysis system (SAS, 1989) was used to analyze the effect of feed type, sex and plumage condition on tonic immobility and blood parameters in broiler birds.

Results

Table 1 Proximate composition of feed types. Table 2 presents data on the effect of feed type on tonic immobility, packed cell volume, haemoglobin concentration and total plasma protein. Feed type did not significantly (p>0.05) influence tonic immobility, packed cell volume, haemoglobin concentration and total plasma protein in broiler birds.

The result shows that feed type did not significantly (p>0.05) influence tonic immobility, packed cell volume, haemoglobin concentration and total plasma protein of

Table 3: Effect of sex on Tonic Immobility, Packed Cell Volume, Haemoglobin Concentration and Total Plasma Protein of Broiler Birds

	Sex	
	Male	Female
Tonic Immobility (Seconds)	183.8±43.43[a]	259.56±49.72[a]
Packed Cell Volume (%)	23.21±1.73[a]	24.78±1.98[a]
Haemoglobin Concentration (g dL^{-1})	7.73±0.58[a]	8.25±0.66[a]
Total Plasma Concentration (g $100mL^{-1}$)	4.25±0.18[a]	4.44±0.20[a]

a:Means along the same row with the same superscripts are not significantly different from each other

broiler birds. Since all sampled birds were subjected to the same stress factor, their reaction is that there is no significant ($p>0.05$) influence of feed type on tonic immobility. For the Packed Volume Cell (PCV) and the haemoglobin concentration, the mean value in each treatment is lower than the normal range value. The total plasma protein value of all the treatments is within the range.

Table 3 presents results of the effect of sex on tonic immobility, Packed Volume Cell (PCV), haemoglobin concentration and total plasma protein of broiler birds. Sex did not significantly ($p>0.05$) affect tonic immobility, Packed Volume Cell (PCV), haemoglobin concentration and total plasma in broilers. But considering the absolute values of these parameters, we noticed that males had higher values of tonic immobility, packed cell volume, haemoglobin concentration and total plasma protein.

Table 4 presents the data on the effect of plumage condition on tonic immobility, Packed Volume Cell (PCV), haemoglobin concentration and total plasma protein of broiler birds. There was no significant effect of plumage condition on the measured parameters. However, on the basis of absolute values, birds having good plumage condition had a lower mean value for tonic immobility, followed by birds having a fair plumage condition and lastly by birds having a poor plumage condition (higher mean value of tonic immobility). The Packed Volume Cell (PCV) and haemoglobin concentration mean values are higher with birds having a good plumage condition, followed by birds with fair plumage condition and those with poor plumage condition (lower mean values of packed cell volume and haemoglobin concentration). The total plasma protein mean value is higher in birds with good plumage condition, followed by birds with fair and poor plumage.

Table 5 presents data on the correlated relationship between tonic immobility, packed cell volume, total plasma protein and haemoglobin concentration of broiler birds.

From the observed relationships, an increase in tonic immobility will cause an increase in the total plasma protein. The larger the tonic immobility value, the more stressed are the birds and the lower the immune system. An increase of the total plasma protein above 6g dL^{-1} will be detrimental to the birds as they could face dehydration occurring with hyperglobulinemia and hyperalbuminemia (Bush, 1991a,b).

An increase in the length of the tonic immobility will cause a decrease in the value of the packed cell volume and the haemoglobin concentration. This result could be affected by the haemolysis of the red blood cells which affects negatively the values of packed cell volume and haemoglobin concentration.

Packed cell volume and haemoglobin concentration had a perfect relationship. This confirmed the method used to determine the haemoglobin concentration from the packed cell volume (Schwalm *et al.*,1975).

Discussion

The results generally showed that tonic immobility was averagely good. There was no significant difference in the tonic immobility of birds fed different feed types. But, considering the absolute values of the tonic immobility, it could be said that bird fed with feed type 4 (shorter tonic immobility) performed better. And only, birds fed with feed type 4 had a tonic immobility value shorter (better) than the mean tonic immobility value of all the birds. The relatively good tonic immobility performance of birds fed with feed type 3 can be judged from the fact that from the beginning of the experiment, the birds from this group had been stressed due to poor quality feed. So, they had more or less become used to the stress which no more represented a problem to them than it could be in other treatments.

The result also showed that the values of the mean packed cell volume and the haemoglobin concentration of birds fed with the different feed types are less than the normal range value of 35% to 55% and 11.678g dL^{-1} to 18.33g dL^{-1} respectively. this observation under abnormal situations would have indicated a state of anemia in the birds. But the averagely good conditions of the birds lead to exploring other reasons. One of the reasons could be haemolysis due to either clinical disorder or due to faults in collection or storage of the blood samples (Bush, 1991a,b). Another reason could be hypothermia which causes haemo-concentration. However, birds fed with feed type 4 had an absolute packed cell volume value that was better than that of birds fed with other feed types. This indicated that birds in this group were better conditioned than the rest fed with other feed types.

The result indicated that the total plasma protein of birds fed with different feed types was within the normal range of 3g dL^{-1} to 6g $d^{-1}L$ implying that the immune status of all the birds in the different treatments was normal. This was confirmed by the relatively good physical condition of the birds. However, birds fed with feed type 4 had a better value of total plasma protein than birds fed with other feed types. This observation has further confirmed

Table 4: Effect of Plumage Condition on Tonic Immobility, Packed Cell Volume, Haemoglobin Concentration and Total Plasma Protein of Broiler Birds

	Plumage condition		
	Poor	Fair	Good
Tonic Immobility (Seconds)	260.20±4114.93[a]	213.46±39.28[a]	191.37±26.02[a]
Packed Cell Volume (%)	17.81±4.58[a]	26.90±1.56[a]	27.27±1.04[a]
Haemoglobin Concentration (g dL^{-1})	5.93±1.52[a]	8.96±0.52[a]	9.09±0.34[a]
Total Plasma Concentration (g 100 mL^{-1})	4.24±0.47[a]	4.25±0.16[a]	4.54±0.11[a]

a:Means along the same row with the same superscripts are not statistically different from each other

Table 5: Correlated Relationships between Tonic Immobility, Packed Cell Volume, Total Plasma Protein and Haemoglobin Concentration of Broiler Birds

	Tonic Immo-bility	Packed Cell Volume	Total Plasma Protein
Packed Cell Volume	-0.024		
Haemoglobin Concentration	-0.028	-0.018	
Total Plasma Concentration (g/100ml)	-0.024	1.00**	-0.018

** = p<0.01

the better condition and hence performance of the birds under feed type 4 compared to other feed types.

The effect of sex on tonic immobility, packed cell volume, haemoglobin concentration and total plasma protein showed that males had shorter tonic immobility than females when the absolute values were considered. The values of packed cell volume and haemoglobin concentration were below normal range values for both sexes, but the values for the females were higher. The value of the total plasma protein was normal for both sexes. This shows that their immune status was normal.

The result of the effect of plumage condition on tonic immobility, packed cell volume, haemoglobin concentration and total plasma protein showed that the better the plumage condition, the shorter the mean values of tonic immobility, packed cell volume, haemoglobin concentration and total plasma protein when their absolute values were considered. Campo *et al*, (2001) studied the plumage condition in relation with fearfulness in birds having poor or perfect plumage. They found significant difference among groups. Very poorly feathered birds were more stressful than birds with perfect plumage. They concluded that birds with very poor plumage were less fearful and more stressed than those with a perfect plumage. Their result suggested that very poor plumage was associated with indicators of fearfulness and stress. Therefore, the conclusion of Campo *et al.* (2001) agrees with what was observed in this study.

A good feather condition provides insulation and protection against scratches and blistering to the birds (El-fadil *et al.*, 1996). This makes the birds to have a well regulated heat loss and also to be less fearful (seen in lower value of tonic immobility). Definitely the values of the packed cell volume and haemoglobin concentration will be better than those of birds having fair or poor plumage condition.

In case of the plasma protein, the birds with good plumage condition had a higher value within the normal range, followed by birds with fair plumage condition and then birds with poor plumage condition. From the observed relationships, an increase in tonic immobility will cause an increase in the total plasma protein. The larger the tonic immobility value, the more stressed are the birds and the lower the immune system. An increase of the total plasma protein above 6g $d^{-1}L$ will be detrimental to the birds as they could face dehydration occurring with hyperglobulinemia and hyper-albuminemia (Bush, 1991b).

An increase in the length of the tonic immobility will cause a decrease in the value of the packed cell volume and the haemoglobin concentration. This result could be affected by the haemolysis of the red blood cells which affects negatively the values of packed cell volume and haemoglobin concentration.

Packed cell volume and haemoglobin concentration had a perfect relationship. This confirmed the method used to determine the haemoglobin concentration from the packed cell volume (Schwalm *et al.*, 1975).

Conclusion: This study showed that quality of the feed influences the length of the tonic immobility. More precautions should be taken while collecting blood samples in order to avoid haemolysis as this influences the right values of packed cell volume and haemoglobin concentration. The quality of feed also influences the packed cell volume and the haemoglobin concentration. The better the feed type, the better the plumage condition, and the shorter (better) the tonic immobility hence the stronger is the immune system of the birds.

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