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Influence of Early Skip-a-day Feed Withdrawal on the Haematological Indices, Serum Protein and Nutrient Digestibility of Broilers

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Abstract: In a study to determine the influence of early skip-a-day (SAD) feed withdrawal on the heamatology and nutrient digestibility of broiler chickens, two hundred and twenty-five (225) Marshall broilers were, at day old, randomly assigned to 5 treatments of 45 chicks each, with each treatment having three replicates of 15 birds per replicate as follows: A full-fed control group (AL) and groups with feed withdrawn for 24 hours for 3 days (SAD-3), 4 days (SAD-4), 5 days (SAD-5) and 6 days (SAD-6) from 8th to 18th day of age after feeding them *ad libitum* for the first 7 days. Feed was provided *ad libitum* to all the chickens from 19 to 56 days of age. The birds were raised on deep litter. Heamatological variables measured or calculated on the 56th day included WBC, RBC, PCV, Hb, MCHC, MCH, MCV and total serum proteins. A metabolism trial was also carried out to determine the effect of feed restriction on nutrient digestibility/retention by the broilers. SAD feed withdrawal significantly ($p<0.05$) effected mean Packed Cell Volume (PCV), Red Blood Cell Counts (RBC), Haemoglobin content (Hb), Mean Corpuscular Haemoglobin Concentration (MCHC) and total serum protein, while total leucocyte count (WBC), Mean Corpuscular Haemoglobin (MCH) and Mean Corpuscular Volume (MCV) were unaffected. Each of the treatments to which the skip-a-day feed withdrawal was applied generally had a mean percent PCV value that was not significantly different from that of the control treatment group (AL). Birds feed-restricted beyond 4 days (SAD-5 and SAD-6) had significantly ($p<0.05$) lower mean RBC, Hb and MCHC values than birds in the control treatment group (AL). Feed restriction beyond 3 days significantly ($p<0.05$) lowered mean Hb and MCHC contents in relation to birds fed *ad libitum* thus indicating that the birds were not under stress from early SAD feed restriction imposed on them but were in a good state of health. Mean MCV was numerically higher at the most severe levels (SAD-5 and SAD-6) of early feed withdrawal compared with the control treatment, which is suggestive of increase in haemoglobin production at the more severe levels of feed withdrawal. The differences were, however, not significant ($P>0.05$). Serum protein was significantly lower for broilers feed-restricted for 5 and 6 days (5.90 and 5.60 g/dL respectively) than for the control, SAD-3 and SAD-4 treatments (6.40, 6.23 and 7.30 g/dL respectively). Variations in mean differential counts between treatments were not significant ($p>0.05$). However, basophil counts increased slightly while eosinophils decreased slightly in feed-restricted birds in relation to the control birds. Early feed withdrawal had no significant ($p>0.05$) effect on apparent dry matter and crude fibre digestibility. Nitrogen retention tended to increase as the severity of SAD feed withdrawal increased while mean fat retention for broilers subjected to 4, 5 or 6 days of SAD feed withdrawal were not significantly different from that of birds fed *ad libitum*. It was concluded that early skip-a-day feed withdrawal for up to 6 days did not lead to severe stress, or adversely alter nutrient digestibility and retention in the broilers.

Key words: Feed restriction, haematology, nutrient retention, skip-a-day, feed withdrawal

INTRODUCTION

The challenge of growing broiler chickens efficiently in resource-poor countries involves maintaining a high body weight of about 1.8-2.5 kg at market age, avoiding lowered feed efficiency, reducing excessive fat deposition, lowering disease susceptibility and mortality and at the same time, devising ways of reducing the cost of production. The ideal practice of feeding broiler chickens *ad libitum* with balanced rations in order to achieve rapid growth has sometimes not been sustained in the tropics due to high cost of finished commercial poultry feeds. Consequently, some commercial poultry farmers in these countries indulge in indiscriminate restriction of feed to broilers as a way of reducing feed costs and coping with periodic feed shortages.

Although early restricted feeding in modern meat-type chickens has been shown to be beneficial in reducing metabolic disorders and leg problems associated with excessive growth in the starter phase (Yu *et al*., 1990; Khan and Zafar, 2005; Dozier *et al*., 2002), feed restriction, depending on its severity, could also predispose broilers to stress and reduce productivity (Dozier *et al*., 2002). The need to conduct careful studies to determine adequate feed restriction levels that will not adversely affect the health status and nutrient utilization of broilers therefore becomes necessary. Haematological and serum biochemistry parameters have been widely used as indices of the health and physiological status of animals (Graczyk *et al.*, 2003; Uchegbu *et al.*, 2010). This study was therefore carried out to determine the response of broiler chickens subjected to *ad libitum* feeding or early skip-a-day feed withdrawal in terms of their haematological variables, serum protein and differential counts and their nutrient digestibility/retention at the finishing phase of production.

Table 1: Feed composition for the experiment (% of diet)

Feed stuffs	Starter diet	Finisher diet
Maize	40.00	44.00
SBM	20.00	15.00
GNC	10.52	7.50
PKC	10.20	11.00
Wheat offal	12.72	16.80
Fish meal	3.00	2.00
Bone meal	2.00	2.00
Oyster shell	1.00	1.00
Salt	0.02	0.25
Methionine	0.25	0.10
Lysine	0.14	0.10
Premix	0.25	0.25
Total	100.00	100.00
Determined		
Dry matter (%)	89.51	88.49
Crude protein (%)	23.20	19.96
Crude fiber (%)	5.50	4.36
Ether extract (%)	6.50	5.81
Ash (%)	8.30	5.54
NFE (%)	56.50	64.33
ME (kcal/kg)	3023.00	3204.00

SBM = Soya Bean Meal; GNC = Groundnut Cake; PKC = Palm Kernel Cake; NFE = Nitrogen-Free Extract; ME = Metabolizable Energy

MATERIALS AND METHODS

The experimental site: The experiment was carried out at the Poultry unit of the Teaching and Research Farm, Department of Animal Science, Delta State University, Asaba Campus, Asaba, Nigeria. Asaba is location is on longitude 06°45'E and latitude 06°12'N and has annual rainfall which ranges from 1800-3000 mm.

The experimental diets: Two experimental diets (detailed composition in Table 1) were formulated for the starter and finisher phases of production. The starter diet contained approximately 2900 kcal/kg ME and 23% crude protein, while the finisher diet contained approximately 3000 kcal/kg and 20% crude protein.

Experimental animals and their managements: Two hundred and twenty-five (225) unsexed day-old Marshal broiler chicks were used for this experiment. The chicks were brooded on deep litter and routinely vaccinated against Newcastle disease and *Gumboro*. Brooding lasted for four weeks. The birds were fed a starter diet *ad-libitum* for 7 days after which they were subjected to one of five different skip-a-day regimes of feed withdrawal from the 8th to the 18th day. All the chicks were fed *ad-libitum* from the 19th to the 56th day. Clean drinking water was provided *ad-libitum* throughout the experiment.

Experimental design and statistical model: The 225 birds were randomly assigned to 5 experimental treatments of 45 birds per treatment in a Completely Randomized Design (CRD). Each treatment was further divided into 3 replicates of fifteen (15) birds each. The treatments were as follows:

AL : (*Ad-libitum* feeding throughout the experiment) (control),
SAD-3 : (24-h feed withdrawal on days 8, 10 and 12),
SAD-4 : (24-h feed withdrawal on days 8, 10, 12 and 14),
SAD-5 : (24-h feed withdrawal on days 8, 10, 12, 14 and 16) and
SAD-6 : (24-h feed withdrawal on days 8, 10, 12, 14, 16 and 18).

The statistical model was:

$$X_{ij} = \mu + T_i + e_{ij}$$

Where:
X_{ij} = The observation made on the jth bird belonging to the ith treatment group
μ = Overall estimate of the population mean
T_i = Effect of the ith treatment group and
e_{ij} = Random error associated with each treatment

Data collection: At the end of the 8th week of the experiment, three birds were chosen at random from each replicate for blood evaluation. Five milliliters (5 mL) of blood was gently drawn out from a right wing vein of each of the birds with the aid of a 5 mL hypodermic syringe. Three milliliters (3 mL) of each of the blood samples was put into a labeled blood collection vial containing Ethylene Diamine Tetra-acetic Acid (EDTA) as anticoagulant and the rest of the blood (approximately 2 mL) put into a vial that contained no anticoagulant to facilitate clotting of the blood and serum collection. Serum samples were stored in a deep freezer until required for serum protein determination. Parameters determined or calculated on replicate basis were the Packed Cell Volume (PCV), Red Blood Cell Count (RBC), White Blood Cell Count (WBC), Heamoglobin (Hb), Mean Corpuscular Heamoglobin (MCH), Mean Corpuscular Volume (MCV), Mean Corpuscular Heamoglobin Count (MCHC), differential counts and serum protein. PCV was determined through the Wintrobes microhaematocrit technique. Some quantity of uncoagulated blood was allowed to flow by capillarity into capillary tubes sealed at one end and centrifuged at approximately 3000 rpm for 5 min to separate the blood into its cell and non-cell components. The height occupied by the blood cells was expressed as a percentage of the column of the whole blood. A Coulter Electronic Counter (Model ZF by Coulter Electronics Ltd., London) was used for RBC determination. WBC was determined with a Neubauer haemocytometer under a light microscope, using physiological saline for dilution. Haemoglobin (Hb) content was determined with a Cecil colorimeter (Model CE 400 by Cecil Instruments, Cambridge) at a wavelength of 625 nm after blood had been mixed with Drabkin's solution in a ratio of 1:250 (blood: Drabkin's solution). MCV was calculated as 10PCV/RBC femcoLitres. MCH was computed 10Hb/RBC pictogram. MCHC was computed 100Hb/PCV (g/100mL). Serum protein was determined with a refractometer.

Metabolic trial: Also at the end of the 8th week, three other broilers were selected at random from each replicate for a metabolism trial. The birds were housed singly in metabolism cages for a 7-day trial period. Feed of known weight was supplied daily and the left-over feed collected the following morning, air-dried, weighed. After the first 4 days of feeding, which served as an adjustment period, the broilers were fed known weights of feed for the next 3 days. Left-over feed per replicate was collected the following morning, air-dried, weighed and the weight subtracted from the initial weight of feed offered to determine daily feed intake. Total faecal droppings were collected from polythene placed under the metabolic cages. Samples of feed and faecal droppings were dried at 70°C in an oven for 72 h and ground before proximate analysis was carried out in line with AOAC (1990) procedures. Apparent nutrient digestibility/retention was computed as 100 x (Nutrient intake-Nutrient excreted)/Nutrient intake (%).

Data analysis: Data collected were subjected to one-way Analysis of Variance (ANOVA) while the Duncan's Multiple Range test (Duncan, 1955) was used to separate mean values where ANOVA showed significant effect.

RESULTS AND DISCUSSION

Haematological indices, plasma protein and differential counts: The response of the haematological indices and total serum protein of the broilers to early Skip-a-day (SAD) feed withdrawal is as presented in Table 2. SAD feed withdrawal had a significant ($p<0.05$) effect on mean Packed Cell Volume (PCV), Red Blood Cell Counts (RBC), Haemoglobin content (Hb), Mean Corpuscular Haemoglobin Concentration (MCHC) and on total serum protein, while total leucocyte count (WBC), Mean Corpuscular Haemoglobin (MCH) and Mean Corpuscular Volume (MCV) were unaffected. Each of the treatments to which the skip-a-day feed withdrawal was applied generally had a mean percent PCV value that was not significantly different from that of the control treatment group (AL) which received *ad libitum* feeding. However, broilers to which SAD feed withdrawal for 6 days (SAD-6) was applied had a significantly lower mean percent PCV (34.33%) than those feed-restricted for 3 days (SAD-3) (39.67%). PCV, regarded as the most reliable measure of blood cell status (Anosa and Isoun, 1978; Iheukwumere *et al.*, 2006) was, in this study, not drastically depressed by early SAD application. Values obtained (34.33-39.67%) compared favourably with those from Anak broilers fed fluted pumpkins by Nworgu *et al.* (2007). Mean RBC, Hb and MCHC generally decreased as the severity of early SAD feed withdrawal increased. Birds feed-restricted beyond 4 days (SAD-5 and SAD-6) had significantly ($p<0.05$) lower mean RBC, Hb and MCHC values than birds subjected to *ad libitum* feeding (control treatment group, AL). Feed restriction beyond 3 days significantly ($p<0.05$) lowered mean Hb and MCHC contents in relation to birds fed *ad libitum*. The decreases in these haematological indices at the higher levels of feed restriction are an indication that the birds were not under stress from early SAD feed restriction imposed on them at 8-18 days of age and were in a good state of health. Mean RBC values fell within normal range for adult chickens published by Mitruka and Rawsley (1977). Hb, which ranged from 8.00-11.50 g/dL, was consistent with values reported by Iheukwumere and Herbert (2003) (6.0-13.0 g/dL). Swason (1951) explained that excitement is a major factor which may cause an unusual increase in the

Table 2: Effect of early SAD feed withdrawal on heamatological indices and serum protein of broilers at 8 weeks of age

	Treatments					
Indices	AL	SAD-3	SAD-4	SAD-5	SAD-6	SEM
PCV (%)	37.67^{ab}	39.67^{a}	35.67^{ab}	38.33^{ab}	34.33^{b}	0.74
RBC (x10^{6}/mL)	3.10^{a}	2.87^{ab}	2.66^{ab}	2.44^{ab}	2.20^{b}	0.12
WBC (x10^{3}/mL)	5.42	5.71	5.26	5.21	4.41	0.79
Hb (g/dL)	11.50^{a}	10.67^{ab}	7.33^{c}	8.50^{bc}	8.00^{bc}	0.53
MCH (pg)	37.30	37.26	27.72	34.99	37.35	1.60
MCHC (g/100 mL)	31.11^{a}	26.88^{ab}	20.65^{b}	22.24^{b}	23.51^{b}	1.30
MCV (fl)	122.13	142.28	134.84	157.15	159.31	5.68
Serum Prot. (g/dL)	6.40^{ab}	6.23^{ab}	7.30^{a}	5.90^{b}	5.60^{b}	0.20

^{abcd}Means on the same row with identical superscript are not significantly different ($p>0.05$). SAD = Skip-a-day.
SEM = Standard error of means

Table 3: Effect of early SAD feed withdrawal on differential counts of broilers at 8 weeks of age

Differential Counts (%)	Treatments					
	AL	SAD-3	SAD-4	SAD-5	SAD-6	SEM
Basophils	3.46	8.25	3.39	6.94	4.76	0.88
Eosinophils	13.02	14.68	10.81	11.13	11.45	0.73
Lymphocytes	42.71	35.94	43.99	44.60	40.36	1.92
Monocytes	17.29[ab]	19.58[a]	14.53[b]	15.67[ab]	16.60[ab]	0.65
Neutrophils	23.52	21.54	21.89	21.05	23.21	0.59

[abcd]Means on the same row with identical superscript are not significantly different (p>0.05). SAD = Skip-a-day.
SEM = Standard error of means

Table 4: Percentage nutrient digestibility/retention

Nutrients (%)	Treatments					
	AL	SAD-3	SAD-4	SAD-5	SAD-6	SEM
Dry matter digestibility	87.83	88.50	86.83	86.00	87.67	0.41
Nitrogen retention	76.51[b]	74.99[b]	85.73[a]	81.55[ab]	88.16[a]	0.76
Crude fiber digestibility	43.56	45.56	45.76	44.63	41.01	0.40
Fat retention	69.31[a]	45.72[b]	68.78[a]	65.00[ab]	60.24[ab]	1.16

[ab]Means with same superscript within a row are not significantly different (p>0.05). SEM = Standard Error of Means

quantity of Hb in the blood. An increase in Hb content is associated with higher resistance to disease and stress (Frandson, 1981) while a decrease is an indication of reduced concentration of circulating heamoglobin (aneamia) (Allison, 1999). Mean MCHC values of 20.65-31.11 g/100 mL were similar to those reported by MVM (1986) and Ajakaiye *et al.* (2010) but lower than those published by Iheukwumere and Herbert (2003). These variations may be due to differences in heredity and other environmental factors relating to the birds used. According to Jean (1993), Talebi *et al.* (2005) and Emenalom *et al.* (2009), haematological indices of animals in good health vary between species, age, sex, diets and clinical conditions of the animals. Mean MCV was numerically higher at the most severe levels (SAD-5 and SAD-6) of early feed withdrawal compared with the control treatment, which is suggestive of increase in haemoglobin production at the more severe levels of feed withdrawal. The differences were, however, not significant (p>0.05) and values obtained (122.13-159.31fl) were within the range of 116.9-161.4fl reported for broilers by Bogado *et al.* (2010) and higher than the values of 114 -136fl reported by Alikwe *et al.* (2010) and 8.30-41.00fl for broilers subjected to various levels of water restriction (Iheukwumere and Herbert, 2003).

Serum protein was significantly lower for broilers feed-restricted for 5 and 6 days (5.90 and 5.60 g/dL respectively) than for the control, SAD-3 and SAD-4 treatments (6.40, 6.23 and 7.30 g/dL respectively).

Variations in the mean differential counts at 8 weeks of age of the broilers subjected to early SAD feed withdrawal are presented in Table 3. There were no significant (p>0.05) differences between treatments in mean basophil, eosinophil, lymphocyte and neutrophil counts. However, basophil counts increased slightly while eosinophils decreased slightly in feed-restricted birds in relation to the control birds. Maxwell *et al.* (1991) observed similar changes in differential count indices in seven-week-old broilers subjected to early feed restriction for 6, 10 and 14 days from 6 days of age. Although differences between treatments in monocyte counts were significant (p<0.05), mean monocyte count for each of the feed-restricted treatment groups was not significantly different from that of the control group. This is in agreement with the reports of Maxwell *et al.* (1992) and Hassan *et al.* (2003) in which no significant differences in monocyte counts were observed between feed-restricted birds and their full-fed controls. Sharp increases in leucocytes and its components are indicative of infection. The general similarity in differential counts between the control diet and the various feed-restricted groups indicates that the levels of feed early feed restriction applied in this study did not create any significant stress condition in the birds.

Table 4 contains mean values of dry matter and nutrients digested/retained at 8 weeks of age following full feeding or early SAD feeding. Early feed withdrawal had no significant (p>0.05) effect on apparent dry matter and crude fibre digestibility. This is in consonance with the findings of Novele *et al.* (2008) that providing 50 and 75% of *ad libitum* feeding to broiler chickens had no significant effect (p>0.05) on apparent dry matter digestibility. Nitrogen retention tended to increase as the severity of SAD feed withdrawal increased in line with the report of Novele *et al.* (2008) that chickens on 50 and 75% *ad-libitum* feeding had higher (p<0.05) nitrogen retention values than those on *ad-libitum* feeding. Mean fat retention for broilers subjected to 4, 5 or 6 days of SAD feed withdrawal were not significantly different from that of birds fed *ad libitum*.

Conclusion: Skip-a-day feed withdrawal for as much as 6 days during the starter phase of production did not induce stress reactions or appreciably alter nutrient digestibility or retention in broilers at 56 days of age.

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