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Effects of Qualitative Dietary Restriction on Performance, Carcass Characteristics, White Blood Cell Count and Humoral Immune Response of Broiler Chicks

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Abstract: Three hundred and twenty unsexed Ross 308 broiler chicks were distributed between 16 battery pens and each of four experimental dietary treatments were assigned to 4 pens (n=20) to evaluate the effect of dietary energy and protein restriction during early growing period on performance of broiler chicks. In order to formulate restricted diets, 100, 200 and 300 g/kg ground wood charcoal (WC) were added to the basal control diet which formulated based on NRC, 1994 and included 0 g/kg WC. The results indicated that diet restriction decreased BW of chicks as comparing to BW of chicks fed control diet on days 15, 21 and 42 of age ($P \leq 0.05$) but there was no significant different between BW of chicks fed control and 10% restricted diet on days 15, 21 and 49. On days 9-15 of age, chicks fed restricted diets did have deceased FI comparing to chicks fed on control diet; however, the difference between control and 10% restricted groups was not significant. There was no significant difference in the overall FCR (9-49 d) between chicks fed the restricted and non-restricted control diet, except for chicks fed on 20% restricted diet that had the highest FCR during the experiment. It was concluded that dietary inclusion of WC up to 10% to restrict broiler diets would not have deleterious effect on performance of broiler chicks with no adverse effect on Ab response against NDV and visceral and carcass measurements.

Key words: Diet restriction · Immunity · Wood charcoal · Broilers

INTRODUCTION

Feed restriction, which is denying the fast growing birds a full access to nutrients that are required for their normal growth and development, is categorized into quantitative and qualitative feed restriction. In a quantitative feed restriction, birds are physically declined access to the feed during certain times of the day, while in a qualitative feed restriction birds are declined full access to particular nutrients through the provision of a feed diluted with inert fibres (such as rice hulls) or WC [1-4]. Restriction of feed is one of the primary management tools currently used to reduce the incidence of metabolic disorders [5-12]. Feed restriction is associated with improvement in arterial oxygenation mainly by reducing metabolic demands during the critical periods of the life span of a bird [13-14]. However, prolonged feed restriction diminishes the potential of compensatory growth [9, 15] and on the relative weight of breast muscle [8]. There have also been reports of negative effects on thyroid gland activity [16] and on the plasma triiodothyronine concentration [17]. The objective of this study was to examine the effectiveness of early-age growth limitation, achieved through qualitative feed restriction (energy and protein dilution by dietary inclusion of WC) to improve performance of broiler chicks.

MATERIALS AND METHODS

Birds and Experimental Diets: A total number of 320 unsexed Ross 308 broiler chicks was obtained from a commercial hatchery and fed with a commercial starter diet from 1 to 8 days of age. At d 9 of age, chicks were weighed and randomly distributed among 16 battery pens. Diet dilution was achieved by substitution of WC for the major ingredients in the corn-soybean meal diet formulated based on NRC 1994. The experimental diets (Table 1), containing 0, 100, 200 and 300 g ground WC per kg diet were fed to chicks between 9 to 15 days of age. After this period, chicks were fed regular starter, grower and finisher diets between 16-21, 21-42 and 42-49 days of age, respectively. Ground WC used in this experiment contained 93.5 % DM, 14.5 % ash, 77.2 % CF, 1.91 % CP and 1.98 % EE. During the experiment, feed intake (FI) and

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Table 1: Composition of experimental diets (%)

	Starter					
	Ground wood charcoal (g/100g)					
	0	10	20	30	Grower	Finisher
Corn	58.98	55.65	49.39	43.19	67.36	66.95
Soybean meal	35.55	30.21	26.15	22.13	28.43	26.54
Soybean oil	1.57	-	-	-	0.65	3.37
Limestone	1.26	1.25	1.23	1.22	1.03	0.83
Dicalcium phosphate	1.42	1.51	1.59	1.68	1.33	1.25
Wood charcoal	-	10.03	20.07	30.00	-	-
Common salt	0.50	0.50	0.50	0.50	0.50	0.50
Vit. & Min. Premix[1]	0.50	0.50	0.50	0.50	0.50	0.50
Lysine-HCL	0.09	0.19	0.36	0.53	0.14	0.05
DL-Met	0.13	0.17	0.21	0.25	0.06	0.02
Calculated analysis						
ME (Kcal/kg)	2900	2553	2262	1974	2950	3100
Crude protein	20.85	18.35	16.27	14.19	18.44	17.44
Lysine	1.18	1.10	1.10	1.10	1.04	0.92
Methionine	0.45	0.45	0.45	0.45	0.35	0.30

body weight was measured and body weight gain (BWG) as well as feed conversion ratio (FCR) were calculated weekly. Mortality was measured throughout the experiment.

Visceral Measurements: On day 47 of age, one bird of each replicate was randomly selected, weighed after feed deprivation for 12 h and killed by cervical dislocation. The carcasses were then opened and the thymus, spleen, bursa, abdominal fat pad, liver, gizzard, heart, lung and pancreas removed and weighed. Relative organ weights were calculated as [organ weight (g)/ 100 g carcass weight].

Relative Percentage of White Blood Cells: On days 21 and 28 of age, blood samples were taken from one randomly selected bird from each replicate and white blood cells counted. Relative percentage of the various groups of white blood cells as well as the ratio of heterophiles to lymphocytes per each blood sample was calculated.

Measurement of Antibody to Newcastle Disease Virus (NDV): Antibody response to inactivated NDV vaccine was used to examine the humoral immunity of chicks. At 14 and 28 days of age, all 320 chicks were vaccinated against NDV. Blood samples were withdrawn from the wing vein 7 and 14 d after first- and 7, 14 and 21 d after second-vaccination (booster) for determination of primary and secondary antibody (Ab) responses. The sera were applied to Hemagglutination Inhibition (HI) test to determine Ab to NDV, expressed as reciprocal log2 values for the highest dilution that displayed HI test [18].

Statistical Analysis: Data of this experiment were analyzed by analysis of variance using General Linear Models (GLM) procedures of SAS. When necessary, the means were compared by Duncan's Multiple Range Test. The level of significance was reported at $P \leq 0.05$.

RESULTS

Birds' Performance: As it is shown in Tables 2 to 5, diet restriction decreased BW of chicks on days 15, 21 and 42 of age comparing to BW of chicks fed control diet; however, on days 15, 21 and 49 there was no significant different between BW of chicks fed control and 10% diluted diet. On days 9-15 of age, chicks fed on diluted diet did have deceased FI comparing to chicks fed on control diet, but the difference between control and 10% diluted groups was not significant. Diet dilution on early life (9-15d) of chicks did not significantly affect FI after it (day 16 till market age or day 49). There was no significant difference in the overall FCR (9-49 d) between chicks fed on diluted and non-diluted control diets, except for chicks fed on 20% diluted diet that had the highest FCR during the experiment.

Visceral Measurements: The relative weights of visceral organs (g/ 100 g carcass weight) are presented in Tables 6 and 7. Dietary dilution did not have significant effect on the relative weight of liver, pancreas, lung, breast muscle, thigh, drumstick and Bursa of Fabrecious. Chicks fed diluted diets had increased relative weight of gizzards and the difference between chicks in

Table 2: Body weight of broiler chicks fed on control and diluted (ground wood charcoal-included) diets

	Body weight (g/chick)				
	Days of age				
	9	15	21	42	49
Control	154.26	333.00[a]	624.11[a]	2223.61[a]	2922.64[a]
100 g/kg Charcoal	149.98	320.14[a]	593.41[a]	2081.36[b]	2708.77[ab]
200 g/kg Charcoal	157.71	286.45[b]	550.76[b]	1804.63[b]	2359.14[c]
300 g/kg Charcoal	149.94	293.63[b]	542.18[b]	2003.94[c]	2622.32[b]
SEM	1.820	6.184	9.781	42.622	62.682
P values	0.39	<0.01	<0.01	<0.01	<0.01

Table 3: Body weight gain of broiler chicks fed on control and diluted (ground wood charcoal-included) diets

	Body weight gain (g/chick/day)				
	9-15	15-21	21-42	42-49	9-49
Control	25.53[a]	48.51[a]	76.16[a]	47.71	49.47[a]
100 g/kg Charcoal	24.30[a]	45.54[a]	69.10[b]	51.87	47.70[ab]
200 g/kg Charcoal	18.38[b]	44.04[ab]	59.70[c]	50.87	43.25[c]
300 g/kg Charcoal	19.74[b]	39.76[b]	68.82[b]	53.27	45.39[bc]
SEM	<0.01	0.02	<0.01	0.86	0.02
P values	0.881	1.133	1.793	2.230	0.811

Table 4: Feed intake of broiler chicks fed on control and diluted (ground wood charcoal-included) diets

	Feed intake (g/chick/day)				
	9-15	15-21	21-42	42-49	9-49
Control	62.02[a]	71.88	148.87	248.73	132.65
100 g/kg Charcoal	59.35[ab]	69.28	142.08	230.22	125.23
200 g/kg Charcoal	55.45[c]	68.51	140.71	226.63	122.82
300 g/kg Charcoal	56.51[bc]	66.67	147.07	237.47	127.18
SEM	0.823	0.781	2.774	5.251	1.754
P values	<0.01	0.27	0.73	0.50	0.24

Table 5: Feed conversion ratio of broiler chicks fed on control and diluted (ground wood charcoal-included) diets

	Feed conversion ratio (g feed: g gain)				
	9-15	15-21	21-42	42-49	9-49
Control	2.43[b]	1.48	1.95[b]	2.64	2.13[b]
100 g/kg Charcoal	2.44[b]	1.52	2.05[ab]	2.51	2.13[b]
200 g/kg Charcoal	3.05[a]	1.55	2.38[a]	2.78	2.44[a]
300 g/kg Charcoal	2.73[ab]	1.63	2.12[ab]	2.68	2.29[ab]
SEM	0.081	0.033	0.060	0.071	0.045
P values	0.02	0.46	0.12	0.67	<0.01

Table 6: The relative weight of visceral organs (g/ 100 g carcass weight) of broiler chicks fed on control and diluted (ground wood charcoal-included) diets

	Visceral organs (g/ 100 g carcass weight)				
	Liver	Gizzard	Pancreas	Fat pat	Lung
Control	2.69	1.95[b]	0.03	2.81[a]	0.65
100 g/kg Charcoal	2.85	2.42[ab]	0.25	2.22[a]	0.60
200 g/kg Charcoal	2.92	2.99[a]	0.24	2.65[ab]	0.56
300 g/kg Charcoal	3.02	2.58[ab]	0.27	1.61[c]	0.62
SEM	0.101	0.112	0.016	0.141	0.024
P values	0.76	0.05	0.38	<0.01	0.47

Table 7: The relative weight of breast, thigh, drumstick, Bursa of Fabrecious, thymus and spleen (g/ 100 g carcass weight) of broiler chicks fed on control and diluted (ground wood charcoal-included) diets

	Visceral organs (g/ 100 g carcass weight)					
	Breast	Thigh	Drumstick	Bursa	Thymus	Spleen
Control	29.34	25.51	11.94	0.11	0.29^{ab}	0.13^{b}
100 g/kg Charcoal	28.09	24.42	11.54	0.15	0.29^{ab}	0.15^{ab}
200 g/kg Charcoal	28.46	25.40	11.62	0.11	0.19^{b}	0.11^{b}
300 g/kg Charcoal	28.84	24.78	11.82	0.16	0.33^{a}	0.19^{a}
SEM	0.511	0.432	0.291	0.009	0.021	0.011
P values	0.87	0.82	0.96	0.16	0.05	0.04

Table 8: White blood cell counts (percentage of total) of on day of 21 of age broiler chicks fed on control and diluted (ground wood charcoal-included) diets

	White blood cell (% of total)					
	L	H	M	E	B	H/L
Control	57.00	41.00	0.50	1.00	0.50^{ab}	71.94
100 g/kg Charcoal	58.50	38.50	1.50	0.50	1.00^{a}	69.34
200 g/kg Charcoal	59.25	39.75	1.00	0.01	0.01^{b}	67.22
300 g/kg Charcoal	59.00	37.75	1.25	1.00	1.00^{a}	64.12
SEM	1.157	1.100	0.173	0.181	0.154	3.420
P values	0.06	0.77	0.19	0.14	0.04	0.90

L: Lymphocytes, H: Heterophiles, M: Monocytes, E: Eosinophils, B: Basophils, H/L: Heterophiles: Lymphocytes ratio

Table 9: White blood cell counts (percentage of total) of on day of 28 of age broiler chicks fed on control and diluted (ground wood charcoal-included) diets

	White blood cell (% of total)					
	L	H	M	E	B	H/L
Control	61.00	36.00	1.75	0.75	0.75	61.00
100 g/kg Charcoal	61.00	37.00	0.50	0.75	0.75	60.93
200 g/kg Charcoal	59.00	39.00	0.50	0.75	0.75	66.25
300 g/kg Charcoal	61.33	36.33	1.33	0.66	0.66	59.30
SEM	0.973	1.053	0.242	0.144	0.150	2.801
P values	0.88	0.81	0.20	0.99	0.99	0.86

L: Lymphocytes, H: Heterophiles, M: Monocytes, E: Eosinophils, B: Basophils, H/L: Heterophiles: Lymphocytes ratio

Table 10: Primary and secondary antibody response (Log2) against Newcastle Disease Virus of broiler chicks fed on control and diluted (ground wood charcoal-included) diets

	Antibody response (Log2) against Newcastle Disease Virus				
	Primary Ab response				
	7 days after 1st vaccination	2nd vaccination	Secondary Ab response		
Days of age	21	28	35	42	49
Control	0.75	1.00	1.25	1.00	2.25^{ab}
100 g/kg Charcoal	0.50	0.75	1.00	1.00	1.50^{bc}
200 g/kg Charcoal	0.25	1.05	1.25	0.50	0.50^{c}
300 g/kg Charcoal	0.25	1.05	1.25	0.50	3.00^{a}
SEM	0.128	0.143	0.100	0.111	0.305
P values	0.49	0.33	0.81	0.16	0.01

control and 20% diluted groups was significant ($P \leq 0.05$). Relative percentage of fat pad was the lowest in chicks fed on 30% diluted diets. The relative weights of thymus and spleen were affected from diet dilution ($P \leq 0.05$).

White Blood Cells: The white blood cell counts of birds which were bled at 21 and 28 days of age are shown in Tables 8 and 9, respectively. Diet dilution had no significant effect on the white blood cell counts and the ratio of heterophiles to lymphocytes on sampling days of

21 and 28, except for basophils on sampling day of 21 which was significantly lower in chicks fed on 20% diluted diets (P=0.04).

Antibody Response to NDV: Primary and secondary Ab response against Newcastle Disease virus (NDV) are presented in Table 10. There was no significant effect of diet dilution on early age on Ab response to NDV on days 21, 28, 35 and 42 of age; however, on day 49 of age the highest and lowest Ab titers were seen in chicks fed on 30%- and 20%-diluted diets, respectively.

DISCUSSION

It has been demonstrated that a period of slow growth, followed by compensation to regular market weight, reduces maintenance costs as well as improved feed efficiency [19,20]. There is a transient decrease in basal metabolic rate of feed restricted birds, leading to less energy required for maintenance [20,21]. The success of feed restriction programs in improving feed efficiency and allowing full BW recovery has been attributed to a number of factors. The energy that supports growth compensation may come from the reduced requirement for maintenance energy related to a lower body weight and metabolic adaptation [13]. Greater feed intake relative to BW and its associated digestive adaptations may also be contributing factors to growth compensation [22]. Most studies of early feed restriction in broilers have focused on nutritional conditions during the feed restriction period. Model calculations by Plavnik and Hurwitz [23] based on expected growth rate and body composition showed higher requirements for most of the essential amino acids by the restricted-refed birds. However, studies by Jones and Farrell [24] also showed that dietary supplementation with lysine or methionine during the refeeding period resulted in inconsistent responses for final body weight and carcass composition. The fibrous nature of WC used to dilute diets as well as decreased BW might have been the reasons for increased relative weight of gizzard in chicks fed on diluted diets.

In conclusion, dietary inclusion of WC up to 10% to restrict broiler diets would not have deleterious effect on performance of broiler chicks with no adverse effect on Ab response against NDV and visceral and carcass measurements.

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