Heterophil / Lymphocyte Response and Performance of Feed and Water Restricted Broiler Chickens under Tropical Conditions

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ABSTRACT : The effects of restricting feed [60% of *ad libitum* (AL) intake] (FR) or water provided from 0900 h to 1600 h, daily) (WR) or both (FWR) from 14 to 42 days of age on heterophil/lymphocyte (H/L) response and performance in broiler chickens under the hot humid tropical conditions were determined. Feed and/or water limitation retarded growth, but had no adverse effect on overall feed conversion ratio and survivability. The trend for total feed and water consumption was similar to body weight pattern with AL>WR>FR>FWR. The nutritional regimens had significant effect on overall water:feed ratios with FR>(AL=WR)>FWR. Restriction of feed and/or water resulted in marked elevation of H/L ratios. As measured by H/L ratios, the effect of adapting to FR dissipated between 16 to 21 days after the onset of feed restriction. The H/L ratios of WR and FWR birds remained elevated throughout the duration of the experiment. (*Asian-Aus. J. Anim. Sci. 1999. Vol. 12, No. 6 : 951-955*)

Key Words : Feed and Water Restriction, Stress, Heterophil to Lymphocyte Ratios, Broilers

INTRODUCTION

Feed and water restriction or deprivation are routine husbandry practices in commercial poultry production to induce molt in laying hens and to circumvent obesity in meat-type chickens. When feed or water consumption is limited, animals may not fully satisfy their metabolic and behavioural needs. Intakes of feed and water are not only to meet the animal's requirements but they also derive 'a positive source of pleasure' through the actions of feeding and drinking (Kyriazakis and Savory, 1997). Rises in plasma concentration of corticosterone attributed to feed (e.g. Nir et al., 1975; Freeman et al., 1981; Harvey and Klandorf, 1983) or water (e.g. Beuving and Vonder, 1978; Scott et al., 1983) limitation have been well documented. Evidence is accumulating to show that chickens readily habituate to fasts of moderate duration. Working with White Plymouth Rock chickens, Zulkifli et al. (1993) reported that stress response attributed to 60% feed restriction lasted only 12 days. However, information on the period required for habituation to water restriction, and concurrent feed and water restriction is fragmentary.

Water intake increases when chickens are subjected to high ambient temperatures and survivability during heat stress is positively correlated to amount of water consumption (Fox, 1951). The availability of water to heat-stressed birds is essential to support vaporization of water from respiratory surfaces (Smith and Oliver, 1971). Under the hot and humid tropical conditions, the general recommendation for poultry is to provide water *ad libitum*. Despite the cardinal importance of adequate water intake in cooling heat-stressed chickens, studies (Abdelsamie and Yadiwilo, 1981; Ramlah and Tengku Azhariyah, 1994) in the tropics indicated that water restriction had no adverse effect on livability of broilers. The author is not aware of any reports on the effect of such practice on the physiological stress response of broilers under the hot and humid tropical conditions.

A growing body of evidence has accumulated on the inconsistency and inadequacy of plasma corticosteroid concentrations as a biological index of stress (Rushen, 1991). Gross and Siegel (1983) compared leucocytic and hormonal responses to environmental insults and exogenous corticosterone. They concluded that heterophil (H)/lymphocyte (L) ratios were a more reliable indicator of the perceived magnitude of stress than plasma corticosterone values in avian species. In this study, H/L ratios were determined in broiler chicks exposed to feed restriction, water restriction or concurrent feed and water restriction under tropical conditions. Data were also obtained on the performance of birds under these regimens.

MATERIALS AND METHODS

Birds, husbandry and traits measured

Six hundred day-old straight-run broiler chicks (Arbor Acre) were wingbanded and randomly assigned in groups of 50 to 12 floor pens (10.42 m^2 area/pen) with wood shavings as litter in a conventional open sided house with cyclic temperatures (minimum, 25℃; maximum, 34℃). Relative humidity was between 70 to 90%. Broiler starter and finisher diets (mash form) (table 1) were provided from 1 to 20, and 21 days of age (DOA) onwards, respectively. Lighting was continuous. At 14 DOA, chicks were assigned to one

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of four nutritional regimens with three pens per treatment group. The regimens were as follows; (1) *ad libitum* access to feed and water (AL), (2) 60% feed restriction based on the mean feed intake for the previous day of their respective AL pens and *ad libitum* access to water (FR), (3) *ad libitum* access to feed and provision of water from 0900 h to 1600 h (WR) and, (4) 60% feed restriction based on the mean feed intake for the previous day of their respective WR pens and provision of water from 0900 h to 1600 h (FWR). Each pen contained two suspended tubular feeders with a minimal feeding space of 6.7 cm per bird. Plastic bottle drinkers with a capacity of 8 liters were used (10 birds per drinker). For estimation of evaporative water loss, 3 similar drinkers were placed at various locations in the house which were inaccessible to the birds.

Table 1. Composition of the starter and finisher diets

Ingredients	Starter (%)	Finisher (%)
Corn	57.62	65.88
Soybean meal	34.40	25.40
Fish meal	4.00	4.00
Dicalcium phosphate	1.20	1.40
Limestone	1.10	1.00
Salt	0.30	0.30
DL-methionine	0.18	0.12
Choline chloride	0.10	0.10
Palm oil	1.00	1.70
Vitamin-mineral premix[1]	0.10	0.10
Calculated analysis		
Crude Protein, %	21	19
Calcium, %	1.03	0.90
Available phosphorus, %	0.54	0.45
ME, kcal/kg	2,950	3,050

[1] Content per kg : iron, 100 g; zinc, 100 g; manganese, 110 g; copper, 20 g; iodine, 2 g; cobalt, 0.6 g; selenite, 0.2 g; folic acid, 0.33 g; vitamin B1, 0.83 g; vitamin B6, 1.33 g; biotin 2%, 0.03 g; vitamin B2, 2 g; vitamin K3, 1.33 g; vitamin B12, 0.03 g; D-calcium Pant., 3.75 g; niacin feedgrade, 23.3 g; vitamin A, 6,666,666.66 IU, vitamin D, 1,000,000 IU; vitamin E, 23,000 IU.

At 14, 20, 25, 30, 35 and 40 DOA (i.e. prior to treatment, 6, 11, 16, 21 and 26 days after onset of feed and water restrictions), prior to provision of feed and water, blood samples (0.3 mL) were collected (from the wing vein) from 9 randomly selected chicks from each treatment group with ethylenediaminetetra-acetate (EDTA) as anticoagulant. Blood smears were prepared using May-Grunwald-Giemsa stain and heterophils (H) and lymphocytes (L) were counted to a total of 60 cells (Gross and Siegel, 1983), Chicks that were bled were marked for identification. Blood was collected from different chicks on each occasion. Chicks were individually weighed at 14 DOA and weekly thereafter. Weekly feed conversion ratio (FCR) and water:feed ratio were calculated.

Statistical analyses

Body weight, weight gain, feed conversion ratios (feed/gain) and water:feed ratios data were analyzed with nutritional regimen as the main effect. Counts of H and L were converted to a ratio H/L. H/L ratios data were analyzed within age with nutritional regimen as the main effect. Mortality data were subjected to chi-square analysis. Data were subjected to analysis of variance in a fixed effect model. When effects were significant, separation of multiple means was by Duncan's multiple range test. All analyses were conducted with the aid of General Linear Models procedure (SAS® Institute, 1982). Statistical significance was considered as $p \leq 0.05$ throughout the paper.

RESULTS

Results of body weight, feed consumption, feed conversion ratios, water consumption, water:feed ratios, and mortality rate are presented in table 2. By Day 21, one week following imposition of feed and/or water restriction, the body weight of FR, WR and FWR birds was reduced as compared to those fed AL. Body weight of FR, WR and FWR birds were about 63%, 75% and 55% of that of the AL chicks, respectively. The trend for total feed and water intake was similar to body weight pattern with AL>WR>FR>FWR. From Day 21-27, WR birds had poorer FCR than their AL, FR and FWR counterparts. The AL and WR regimens resulted inferior FCR from day 35-41 with (AL=WR)>(FR=FWR). Irrespective of feed and/or water restriction, overall FCR and mortality rate were similar. There were significant differences among the treatment groups for weekly water:feed ratios. Birds subjected to FWR had the lowest water:feed ratios throughout the duration of study. Similarly, the overall water:feed ratios were affected by nutritional regimen with FR>(AL=WR)>FWR.

Nutritional regimen had a dramatic influence on H/L ratios (figure 1). Following 6 (14 DOA), 11 (20 DOA) and 16 (25 DOA) days of imposing FR, WR and FWR, birds responded similarly with significant rises in H/L ratios as compared to AL. At 21 DOA, FWR birds had higher ratios than their other counterparts. Among the FR birds there was a rapid decline of H/L ratios to AL levels between 16 to 21 days after onset of restriction. The H/L ratios of WR and FWR, however, did not return to the level of AL following 26 days of restriction.

DISCUSSION

As expected, the FR and FWR regimens adopted in this study resulted in adverse effects on growth. The

Table 2. Effect of feed and water restriction on performance of broilers at various ages

Parameter	Treatment			
	AL	FR	WR	FWR
Body weight (g/b)				
Day 14	326 ± 3.2	323 ± 3.6	320 ± 3.6	330 ± 2.8
Day 21	651 ± 6.5^{a}	468 ± 5.8^{c}	546 ± 6.0^{b}	444 ± 3.6^{d}
Day 28	953 ± 10.3^{a}	657 ± 9.7^{c}	752 ± 9.6^{b}	608 ± 9.0^{d}
Day 35	1,353 ± 14.4^{a}	895 ± 13.5^{c}	1,089 ± 11.8^{b}	788 ± 12.7^{d}
Day 42	1,752 ± 17.4^{a}	1,171 ± 15.2^{c}	1,392 ± 16.0^{b}	1,010 ± 15.5^{d}
Feed Consumption (g/b)				
Day 14-20	507 ± 17.9^{a}	305 ± 0.0^{c}	398 ± 28.7^{b}	238 ± 0.0^{d}
Day 21-27	654 ± 27.7^{a}	392 ± 0.0^{b}	561 ± 56.6^{a}	337 ± 0.0^{b}
Day 28-34	873 ± 15.4^{a}	524 ± 0.0^{c}	701 ± 37.0^{b}	421 ± 0.0^{d}
Day 35-41	1,026 ± 21.0^{a}	615 ± 0.0^{c}	825 ± 42.8^{b}	495 ± 0.0^{d}
Total	3,060 ± 25.8^{a}	1,836 ± 0.0^{c}	2,485 ± 163.7^{b}	1,491 ± 0.0^{d}
Feed conversion ratio (feed/gain)				
Day 14-20	1.56 ± 0.06	2.10 ± 0.16	1.76 ± 0.27	2.09 ± 0.01
Day 21-27	2.16 ± 0.19^{b}	2.07 ± 0.24^{b}	2.72 ± 0.35^{a}	2.05 ± 0.37^{b}
Day 28-34	2.18 ± 0.14	2.20 ± 0.11	2.45 ± 0.26	2.34 ± 0.20
Day 35-41	2.57 ± 0.02^{a}	2.23 ± 0.11^{b}	2.72 ± 0.14^{a}	2.23 ± 0.03^{b}
Overall	2.15 ± 0.04	2.17 ± 0.08	2.32 ± 0.94	2.19 ± 0.15
Water consumption (g/b)				
Day 14-20	957 ± 50.1^{a}	491 ± 5.0^{b}	514 ± 31.6^{b}	282 ± 15.7^{c}
Day 21-27	1,457 ± 85.1^{a}	890 ± 9.3^{b}	1,063 ± 80.1^{b}	511 ± 19.7^{c}
Day 28-34	2,074 ± 70.2^{a}	1,255 ± 35.3^{c}	1,587 ± 110.2^{b}	773 ± 42.5^{d}
Day 35-41	2,487 ± 67.7^{a}	2,031 ± 155.4^{b}	2,236 ± 31.7^{ab}	1,044 ± 46.6^{c}
Total	6,975 ± 269.5^{a}	4,667 ± 192.3^{c}	5,400 ± 194.1^{b}	2,610 ± 86.2^{d}
Water:feed ratio				
Day 14-20	1.88 ± 0.05^{a}	1.61 ± 0.02^{b}	1.30 ± 0.05^{c}	1.18 ± 0.07^{c}
Day 21-27	2.23 ± 0.04^{a}	2.27 ± 0.02^{a}	1.90 ± 0.06^{b}	1.52 ± 0.06^{c}
Day 28-34	2.38 ± 0.10^{a}	2.40 ± 0.07^{a}	2.26 ± 0.11^{a}	1.83 ± 0.10^{b}
Day 35-41	2.43 ± 0.10^{bc}	3.30 ± 0.25^{a}	2.73 ± 0.14^{b}	2.11 ± 0.09^{c}
Overall	2.28 ± 0.07^{b}	2.54 ± 0.10^{a}	2.18 ± 0.07^{b}	1.75 ± 0.06^{c}
Mortality (%)	7.3	8.7	10.0	9.3

[a,b,c,d] Means (±SEM) with different superscripts within a row are significantly different at $p<0.05$.

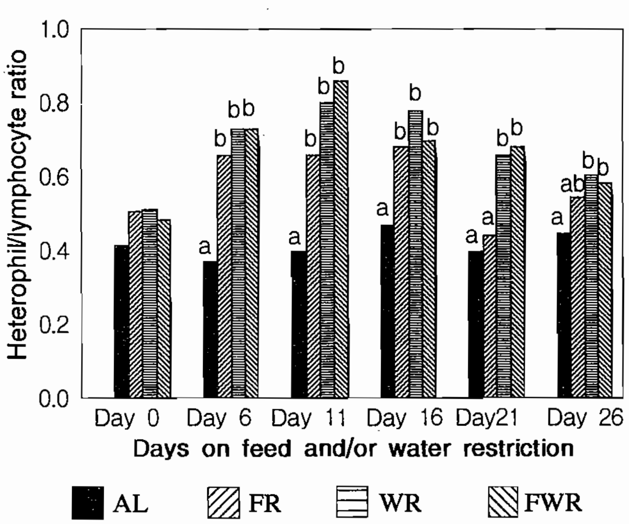

Figure 1. Effects of feed and water restriction on heterophil/lymphocyte ratios at various stages of restriction. a,b: Means with different letters within a stage of restriction are significantly different p<0.05.

retardation of growth in WR birds is consistent with previous studies in broiler chickens (Abdelsamie and Yadiwilo, 1981; Kese and Baffour-Awuah, 1982; Ramlah and Tengku Azhariyah, 1994).

The phenomenon could be attributed to limitation of water which reduced feed intake and growth, concomitantly (Bierer et al., 1966).

North and Bell (1990) indicated that chickens under feed restriction tended to over consume water which may result in undesirable wet litter conditions. Feed-restricted birds gorge themselves with water in order to feel full and also out of boredom. Data from this study, and that reported by Marks (1981), indicate that feed restriction may increase water:feed ratios in broilers. The higher overall water:feed ratios of FR birds could be attributed to either increase in absolute total amount of water intake or decrease in total feed consumption. However, in agreement with previous findings (McFarland, 1965; Bierer et al., 1966), feed restriction reduced the absolute total amount of water consumption, thus, suggesting that the elevated water:feed ratios of FR birds could be due to suppression in feed consumption. Age appears to have a profound impact on the relationship between feed and water intake. Marks (1981) reported that feed limitation during the first 14 days post-hatching was not accompanied by reduction in water intake. Despite limiting access to water, WR birds had similar overall water:feed ratios to their AL counterparts. The lack of difference in overall water:feed ratios between WR and AL birds could be attributed to the former compensated by increasing water consumption when water was before them (Kare and Biely, 1948).

Marks (1981) associated high water:feed ratios of feed-restricted birds with efficient feed utilization. In the present study, however, regardless of nutritional regimens, overall FCR were similar. Variation in degree of feed limitation could yield discrepancies in results obtained.

Lepkovsky et al. (1960) indicated that insufficient water intake may impede rate of digestion and absorption of nutrients. The data presented here provide additional support to the findings of Ramlah and Tengku Azhariyah (1994) that while restriction had no significant effect on overall FCR, poorer FCR was noted from Day 21-27, where birds grew at the fastest rate.

Results of this experiment confirm earlier findings that restriction of feed (Nir et al., 1975; Freeman et al., 1981; Harvey and Klandorf, 1983; Zulkifli et al., 1993, 1995) or water (Beuving and Vonder, 1978; Freeman et al., 1983) or both (Scott et al., 1983) are potent stressors in poultry. The H/L ratios for FR, WR and FWR birds were dramatically elevated compared to their AL controls by 6 days of restriction. The leucocytic changes could be attributed to limitation of feed and/or water or frustration or more likely both. Animals deprived of feed or water may experience frustration if an assumed expectation of finding feed or water is thwarted. Following 21 days of restriction, H/L ratios of FR chicks were similar to their AL counterparts. The negative relationship between H/L ratios and days on feed restriction suggest that the FR birds were adapted to the limited feed allotment. Habituation, a learning process, involves the waning of an individual response to a constant or repeated insult (Fraser and Broom, 1997). According to Broom and Johnson (1993) such a waning of response could be attributed to a single gating process which reduces the efficacy of synaptic transmission in the nervous system. The effect of adapting to the 60% feed restriction which dissipated between 16 to 21 days after initiation contrast with the results of Zulkifli et al. (1993). The authors reported that the duration of H/L response lasted only 12 days when birds were restricted to 60% of *ad libitum* intake. However, since those authors used dwarf and normal White Plymouth Rocks and had a different experimental schedule, it would inappropriate to assume that the observations conflict.

The present findings provide additional support to the thesis that habituation occur more readily to some types of stressors than to others (Broom and Johnson, 1993). While habituation to FR appeared to occur following 21 days of fasting, the sustained H/L response in WR and FWR chicks suggests failure to adapt to the treatments. There is no clear explanation for the phenomenon although it is unlikely attributed to variations in perceived magnitude of stress as FR, WR and FWR birds exhibited similar H/L ratios during 6, 11 and 16 days post-treatment.

Under field conditions poultry are responding to several stressors at all times. There is the question of whether combined effect of multiple concurrent stressors would be less than the arithmetic sum of their individual effects when acting alone. The results of this study were in agreement with those of Freeman et al. (1983) that simultaneous imposition of feed and water limitation had an antagonistic effect. The H/L ratio response of birds subjected to FWR was similar to those under FR and WR following 6, 11 and 16 days post-treatment. According to McFarlane et al. (1989), the possible explanations to antagonism are; 'a) one stressor directly modifying either the intensity of another stressor or the ability of another stressor to impinge on the animal, or b) an animal's response to one stressor changing its perception to another'. On the contrary, based on production performance and H/L ratios, McFarlane et at. (1989) and McFarlane and Curtis (1989) concluded that the effects of aerial ammonia, beak trimming, coccidiosis, electric shock, heat stress and noise in broiler chickens were additive. Factors such as the nature of the stressor and duration of physiological responsiveness may have accounted for the inconsistencies.

Interestingly, despite the higher stress response (as indicated by H/L ratios) in FR, WR and FWR birds than their AL counterparts, the mortality rate of the four groups was not significantly different. Pathological state is an extreme aftermath of biological stress response and occurs when prolonged and intense physiological reactions are involved (Sapolsky, 1992). According to Moberg (1985), most stressful encounters in animals can be coped adequately without detrimental effect on well being.

In temperate countries, restricting the water intake of feed restricted broiler breeder chickens to alleviate over consumption of water, as a result of behavioural or psychogenic polydipsia, is a routine husbandry practice. There is, however, a major concern among local producers that such management practice may exacerbate heat-stress related problems. Data presented here were in consonant with those of Ramlah and Tengku Azhariyah (1994) that water restriction had no adverse effect on livability in broiler, chickens. However, because of differences in life span, management practices and physiological status between broiler and broiler breeder chickens, inferences should be made with caution.

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