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Performance and Heterophil to Lymphocyte (H/L) Ratio Profile of Broiler Chickens Subjected to Feeding Time Restriction

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Abstract: The objective of the current study was to evaluate the effect of feeding time restriction in alternate day on performance and Heterophil to Lymphocyte (H/L) ratios as indices stress of broiler chickens. A total 180 seven day-old unsexed broiler chicks were randomly allocated to three treatments: chicks fed *ad libitum* (P-0); chicks had free access to feed from 07:00 to 09:00, 12:00 to 13:00 and 17:00 to 18:00 (P-1) and from 07:00 to 09:00 and 17:00 to 18:00 (P-2). The feeding time restriction was given at 7, 9, 11, 13, 15, 17, 19 and 21 days of age and chicks was access feed *ad libitum* on alternate days (8, 10, 12, 14, 16, 18 and 20 days of age). The chicks were fed *ad libitum* during 22 to 42 days of age. Body weight gain, feed intake and feed conversion ratio in feed restricted broilers were lower ($p<0.05$) than *ad libitum* broilers during 7 to 21 days of age. There were significantly increases heterophil and Heterophil/Lymphocyte (H/L) ratio. During realimentation period from 22 to 42 days of age, there were no statistically significant differences between feed restricted and *ad libitum* broilers on performance and differential leucocyte count. It was concluded that although feeding time restriction led to induce stress during starter periods, but there were not influenced on the performance and differential leucocyte cells of broilers during realimentation periods.

Key words: Feeding time restriction, performance, H/L ratio, broiler

INTRODUCTION

Continuous genetic selection and improvement in nutrition have led to a very fast growth rate in modern broiler chicken strains. The early-life fast growth rate is accompanied by a number of problem, such as incidence of metabolic disorders and incidence of skeletal diseases (Julian, 1993; Olkowski *et al*., 2008). For these reason, early-life fast growth rate of broiler has been blamed for welfare concerns and then the broiler industry has attempted to find the solutions to these concerns. Thus numerous researches have been conducted to find the appropriate ways of solutions such as controlling feed intake by ways of controlling optimal growth rate. In particular, feed restriction in broiler has been commonly adopted to control body weight, metabolic disorder and reduce mortality. Several researches has shown that chickens subjected to feed restriction for short periods during the early growth phase show improvement of feed efficiency and reach normal weight to that chickens fed *ad libitum* at market weight (Zubair and Leeson, 1994; Tolkamp *et al*., 2005; Zhan *et al*., 2007). A number of variations of feed restriction including limiting the time of daily access to feed (Khetani *et al*., 2009; Onbasilar *et al*., 2009; Mohebodini *et al*., 2009) and skip-a-day feed removal (Khajali *et al*., 2007; Benyi *et al*., 2009; Ghazanfari *et al*., 2010), have been evaluated but results have been conflicting (Navidshad *et al*., 2006; Cornejo *et al*., 2007; Khetani *et al*., 2009; Ghazanfari *et al*., 2010). Such inconsistencies may relate to different feeding strategies applied which may affect the birds' response.

Time feeding restriction is a feed restriction schedule which chicks have daily free access to feed in specific time. A number of variations on this ways have been evaluated, ranging from the removal of feed for up to 8 hours a day, allowing birds to feed only once/hour and feeding once every other day (Demir *et al*., 2004; El-Fiky *et al*., 2008; Benyi *et al*., 2009, 2010; Boostani *et al*., 2010; Saffar and Khajali, 2010). However, Mohebodini *et al*. (2009) reported that although intermittent feeding through free access to feed during four periods of 2 hours (06:00-08:00, 12:00-14:00, 18:00-20:00 and 00:00-02:00) from 7-21 days of age had lower on body weight gain than control (436,3 vs 495,3 g), but had no effect on weight gain during refeeding from 22 to 42 days of age. That might be due to the lower intensity of early feed restriction. However, Khetani *et al*. (2009) concluded that feed restriction by limiting the time to eat was not successful.

Duration of feed restriction to broiler chickens has now become an important issue of animal welfare because feed restriction can act as a stressor on the animal. The main welfare problems of broiler are related to the severe feed restriction method. Gross (1983) indicated that stresses that occur early in life, while many systems of the chicks are still developing, may have long-lasting impact and could possibly modify the expression of their genetic potential. Indicator that can be used to identify symptoms of stress through changes in differential of leucocyte with increasing heterophil and lymphocyte ratios (Gross and Siegel, 1983, 1986; Maxwell, 1993). It has been demonstrated that Heterophil to Lymphocyte

(H/L) ratios in the blood are elevated in feed restricted birds up to 16 weeks of age but that a certain amount of adaptation occurs to feed restriction (Maxwell *et al*., 1990, 1992; Hocking *et al*., 1993).

Meal feeding has been used and shown to be an effective feed restriction program in broiler production. The advantage of meal feeding compared to skip-a-day feed restriction program is that it is less stressful (Susbilla *et al*., 2003). Meal feeding through feeding time restriction is an alternative of feed restriction that is mild and easily applied. There is a little information available concerning on growth performance and profile Heterophil to Lymphocyte (H/L) ratios of broiler chickens subjected feeding time restriction. Therefore, the aim of this study was evaluate the effect of feeding time restriction in alternate day on performance and profile Heterophil to Lymphocyte (H/L) ratios as indices stress of broiler chickens.

MATERIALS AND METHODS

Chickens and experimental design: A total of 180 seven days old unsexed commercial broiler chicks strain Lohmann were randomly allotted to 18 cages (100 x 100 x 60 cm) with wire floor. The 18 cages of experimental unit were divided into 3 treatments with 6 replicate cages of 10 chicks each per cage. The cages were located in a conventional open-sided house with natural cyclic temperatures (minimum, 20°C; maximum, 34°C). A Completely Randomized Design (CRD) was used to evaluate 3 different treatments, included: chicks fed *ad libitum* as control (P-0); chicks had free access to feed from 07:00 to 09:00, 12:00 to 13:00 and 17:00 to 18:00 (P-1) and from 07:00 to 09:00 and 17:00 to 18:00 (P-2). The feeding time restriction was given at age 7, 9, 11, 13, 15, 17, 19 and 21 days and chicks had access to feed *ad libitum* on alternate days at age 8, 10, 12, 14, 16, 18 and 20 days. The chicks were fed *ad libitum* (realimentation period) during 22 to 42 days of age. All chicks were fed standard broiler starter crumble (21% CP) and finisher pellet (19% CP) diets from 1 to 21 and 22 to 42 days, respectively. Water was available at all times. The chicks were reared under continuous lighting.

Blood sampling: At 21 and 42 days of age, direct blood smears were taken from 2 chicks by a wing vein of each unit cage. The smears were stained using May-Grunwald-Giemsa stain and heterophils and lymphocytes were counted to a total of 60 cells (Gross and Siegel, 1983).

Variables measure and statistical analysis: Variables measured included feed intake, body weight and differential leucocyte cells (heterophil and lymphocyte). All data were analyzed based on a completely randomized design using SAS software (SAS Institute, 2001). Data were presented as mean±std. The significant differences between treatment means were determined by Duncan's multiple range test. All statements of significance are based on testing at $p<0.05$.

RESULTS AND DISCUSSION

Growth performance: The response of performance of broilers to early feeding time restriction is presented in Table 1. Body weight gain, feed intake and feed conversion ratio in feed restricted broilers were significantly lower ($p<0.05$) than *ad libitum* broilers during 7 to 21 days of age. These finding indicated that feed restriction during the starter period resulted in decrease of feed intake and subsequently in reduce of body weight gain. Feed restricted broilers in P-1 and P-2 had about 6.50 and 10.93% less body weight and also, consumed 15.54 and 19.98% less feed, respectively. The reduction in body weight was obviously due to reduced feed intake as feed intake was partially or completely ceased. However, these study indicate that the feeding time restriction could improve feed efficiency. Improvements in feed utilization have also been reported that fed intermittently had better feed efficiency than those given fed *ad libitum* (Svihus *et al*., 2010). During realimentation period from 22 to 42 days of age, there were no statistically significant differences between feed-restricted and *ad libitum* broilers when body weight, weight gain, feed intake and feed conversion ratio were determined at 42 days of age. Although feed restriction was applied on the hatching day, near full recovery was attained. That might be due to the lower intensity of early feed restriction. The restricted broilers were able to adapt quite quickly to feeding time restriction, as indicated by the similar body weight gain as *ad libitum* fed birds after an adaptation period and no significant reduction in body weight at the termination of the experiment. Feed intake in the compensatory growth period (22-42 days) did not differ between the *ad libitum* and feed restricted groups. This implies that catch-up growth occurred by improved feed efficiency in birds experienced feed restriction. In other words, catch-up growth was not achieved by enhanced feed consumption. The reductions in body weight as affected by the feed restriction programs overcame by the end of experiment indicating a successful catch-up growth occurred. Saffar and Khajali (2010) reported that meal feeding for a week by allowing birds to feed in two 4 h intervals was reduced about 20.8% in body weigh relative to the full-fed control but retarded growth caught up to 42 days of age. Khetani *et al*. (2009) studied the effect of limited time feeding on growth performance of broiler chickens and found that body weight at 42 days of age, weight gain, feed intake and feed conversion ratio were not affected by treatment. These results are similar with previous reports (Zhan *et al*., 2007; Mohebodini *et al*., 2009).

Table 1: Mean (±std) body weight gain, feed intake and feed conversion ratio in broiler chicken subjected to feeding time restriction

	Treatments		
Performance	P-0	P-1	P-2
Body weight (g/chick)			
7 d	124.35±1.02	123.99±1.12	123.59±0.95
21 d	702.93±59.97[a]	657.25±35.16[ab]	626.13±11.36[b]
42 d	1807.11±99.97	1783.52±62.22	1765.22±72.65
Body weight gain (g/chick)			
7 to 21 d	578.58±59.65[a]	533.25±34.87[ab]	507.04±16.16[b]
22 to 42 d	1104.18±83.78	1126.27±75.98	1134.59±72.25
7 to 42 d	1682.76±108.74	1659.53±69.38	1641.63±79.52
Feed intake (g/chick)			
7 to 21 d	938.41±18.74[a]	792.61±36.10[b]	750.94±24.75[c]
22 to 42 d	2445.59±141.81	2387.52±95.88	2519.35±218.76
7 to 42 d	3383.94±160.91	3180.18±136.69	3270.30±249.11
Feed conversion ratio (g:g)			
7 to 21 d	1.62±0.20[a]	1.49±0.12[b]	1.48±0.15[b]
22 to 42 d	2.22±0.15	2.12±0.08	2.22±0.11
7 to 42 d	2.01±0.08	1.92±0.07	1.99±0.09

[a,b]Mean within a row with no common superscripts differ at p<0.05. Chicks fed *ad libitum* (P-0); chicks had free access to feed from 07:00 to 09:00, 12:00 to 13:00 and 17:00 to 18:00 (P-1); from 07:00 to 09:00 and 17:00 to 18:00 (P-2) at age 7, 9, 11, 13, 15, 17, 19 and 21 days and had access feed *ad libitum* on alternate day at 8, 10, 12, 14, 16, 18 and 20 days of age

Table 2: Mean (±std) of heterophil, lymphocyte cells and Heterophil:Lymphocyte (H/L) ratio in broiler chicken subjected to feeding time restriction

	Treatments		
Differential counts	P-0	P-1	P-2
Heterophil (%)			
21 d	27.33±1.91[c]	44.17±2.11[b]	53.83±3.34[a]
42 d	27.30±1.97	25.83±2.54	24.17±2.41
Lymphocyte (%)			
21 d	55.17±2.41[a]	39.00±2.52[b]	29.50±1.71[c]
42 d	57.83±3.34	57.67±2.49	59.67±4.78
H/L ratio			
21 d	0.51±0.05[c]	1.14±0.12[b]	1.85±0.17[a]
42 d	0.47±0.05	0.45±0.06	0.43±0.05

[a,b,c]Mean within a row with no common superscripts differ at p<0.05. Chicks fed *ad libitum* (P-0); chicks had free access to feed from 07:00 to 09:00, 12:00 to 13:00 and 17:00 to 18:00 (P-1); from 07:00 to 09:00 and 17:00 to 18:00 (P-2) at age 7, 9, 11, 13, 15, 17, 19 and 21 days and had access feed *ad libitum* on alternate day at 8, 10, 12, 14, 16, 18 and 20 days of age

Differential leucocytes: Variations in the mean differential counts of heterophil and lymphocyte at 21 and 42 days of age of the broilers subjected to early feeding time restriction are presented in Table 2. There were significant (p<0.05) difference between treatments in mean heterophil, lymphocyte and H/L ratio during feed restriction. Broiler chickens had free access to feed during 3 or 4 hours/day (P-2 and P-1) maintained higher heterophil cells, lower lymphocyte cells and higher H/L ratio during feed restriction than broilers fed *ad libitum* (P-0). It was indicated that the early feeding time restriction applied in this study show any significant stress condition in the broilers. The present study confirms earlier evidence that feed restriction can induce stress physiologically. As measured by H/L ratio, stress responses to the feed restriction occurred at the end of the restriction period. 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. Heterophil cells as a non-specific immunological defense provider and emergence of heterophil cells into the circulation comes as a consequent event to any stressful condition (Maxwell and Robertson, 1998). These findings are in agreement with Mahmoud and Yaseen (2005) who reported that broiler has a tendency to lower the physiological response to feed withdrawal subjected to six hours (10:00-16:00) three time a week until 22 days of age. However, there were no significant differences in mean heterophil, lymphocyte and H/L ratio at the end realimentation period. This finding is in accordance with Bratte (2011) who reported that there were no significant differences between the full fed and feed restricted groups in mean lymphocyte (40.36%) and neutrophil (23.21%) counts at 56 days of age. Furthermore, it was concluded that skip-a-day feed withdrawal for as much as 6 days during the starter period did not induce stress reactions in broilers at 56 days of age.

Conclusion: The results of this study showed that although feeding time restriction can induce stress during starter periods, but any influence was not observed on the performance and differential leucocyte cells of broilers during realimentation periods.

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REFERENCES

Benyi, K., O. Acheampong-Boateng, D. Norris, M. Mathoho and M.S. Mikasi, 2009. The response of Ross 308 and Hybro broiler chickens to early and late skip-a-day feed restriction. Trop. Anim. Health Prod., 41: 1707-1713.

Benyi, K., O. Acheampong-Boateng, D. Norris and T.J. Ligaraba, 2010. Response of Ross 308 and Hubbard broiler chickens to feed removal for different durations during the day. Trop. Anim. Health. Prod., 42: 1421-1426.

Boostani, A., A. Ashayerizadeh, H.R. Mahmoodian Fard and A. Kamalzadeh, 2010. Comparison of the effects of several feed restriction periods to control ascites on performance, carcass characteristics and hematological indices of broiler chickens. Braz. J. Poult. Sci., 12: 171-177.

Bratte, L., 2011. Influence of early skip-a-day feed withdrawal on the haematological indices, serum protein and nutrient digestibility of broilers. Pak. J. Nutr., 10: 831-835.

Cornejo, S., A.C. Gadelha, J. Pokniak and G. Villouta, 2007. Qualitative feed restriction on productive performance and lipid metabolism in broiler chickens. Arg. Bras. Med. Vet. Zootec., 59: 1554-1562.

Demir, E., S. Sarica, A. Sekeroglu, M.A. Ozcan and Y. Seker, 2004. Effects of early and late feed restriction or feed withdrawal on growth performance, ascites and blood constituents of broiler chickens. Acta Scandinavica, Saction A-Animal Science, 54: 152-158.

El-Fiky, A., M. Soltan, F.H. Abdou, S. El-Samra and B. El-Neney, 2008. Effect of light regime and feeding frequency on some productive, physiological traits and hormonal profiles in broiler chicks. Egypt. Poult. Sci., 28: 711-743.

Ghazanfari, S., H. Kermanshabi, M.R. Nassiry, A. Goliann and A. Salehi, 2010. Effect of feed restriction and different energy and protein levels of the diet on growth performance and growth hormone in broiler chickens. J. Biol. Sci., 10: 25-30.

Gross, W.B., 1983. Chicken-environment interactions. In: Miller, H.B. and W.H. Williams (Eds). Ethics and Animals. Humana Press, Clifton, NJ, pp: 329-337.

Gross, W.B. and P.B. Siegel, 1983. Evaluation heterophil/limphocyte as a measurement of stress in chickens. Avian Dis., 27: 972-979.

Gross, W.B. and P.B. Siegel, 1986. Effect of initial and second period of fasting on heterophil and lymphocyte ratio and body weight. Avian Dis., 30: 345-346.

Hocking, P.M., M.H. Maxwell and M.A. Mitchell, 1993. Welfare assessment of broiler breeder and layer females subjected to food restriction and limited access to water during rearing. Br. Poult. Sci., 34: 443-458.

Julian, R.J., 1993. Ascites in poultry. Avian Pathol., 22: 419-454.

Khajali, F., A. Zamani-Moghaddam and E. Asadi-Khoshoei, 2007. Application of an early skip-a-day feed restriction on physiological parameters, carcass traits and development of ascites in male broilers reared under regular or cold temperatures at high altitude. Anim. Sci. J., 78: 159-163.

Khetani, T.L., T.T. Nkukwana, M. Chimonyo and V. Muchenje, 2009. Effect of quantitative feed restriction on broiler performance. Trop. Anim. Health Prod., 41: 379-384.

Mahmoud, K.J. and A.M. Yaseen, 2005. Effect of feed withdrawal and heat acclimatization on stress responses of male broiler and layer-type chickens (*Gallus gallus domesticus*). Asian-Aust. J. Anim. Sci., 18: 1445-1450.

Maxwell, M.H., G.W. Robertson, S. Spence and C.C. McCorquodale, 1990. Comparison of haemato-logical value in restricted and *ad libitum* fed domestic fowls: White cells and thrombocytes. Br. Poult. Sci., 31: 399-405.

Maxwell, M.H., G.W. Robertson, A.I. Anderson, L.A. Dick and M. Lynch, 1991. Haematology and histopathology of seven-week-old broilers after early food restriction. Res. Vet. Sci., 50: 290-297.

Maxwell, M.H., P.M. Hocking and G.W. Robertson, 1992. Defferential leucocytes responses to variuos degrees of food restriction in broilers, turkeys and ducks. Br. Poult. Sci., 33: 177-187.

Maxwell, M.H., 1993. Avian leucocyte responses to stress. World's Poult. Sci., J. 49: 34-43.

Maxwell, M.H. and G.W. Robertson, 1998. UK survey of broiler ascites and sudden death syndromes in 1993. Br. Poult. Sci., 39: 203-315.

Mohebodini, H., B. Dastar, M. Sham-Sharg and S. Zarehdaran, 2009. The comparison of early feed restriction and meal feeding on performance, carcass characteristics and blood constituents of broiler chickens. J. Anim. Vet. Adv., 8: 2069-2074.

Navidshad, B., M. Shivazad, A.Z. Shahneh and G. Rahimi, 2006. Effects of feed restriction and dietary fat saturation on performance and serum thyroid hormones of broiler chickens. Int. J. Poult. Sci., 5: 436-440.

Olkowski, A.A., C. Wojnarowicz, S. Nain, B. Ling, J.M. Alcorn and B. Laarveld, 2008. A study on pathogenisis of sudden death syndrome in broiler chickens. Res. Vet. Sci., 85: 131-140.

Onbasilar, E.E., S. Yalcin, E. Torlak and P. Ozdemir, 2009. Effects of early feed restriction on live performance, carcass characteristics, meat and liver composition, some blood parameters, heterophil-lymphocyte ratio, antibody production and tonic immobility duration. Trop. Anim. Health Prod., 41: 1513-1519.

Saffar, A. and F. Khajali, 2010. Application of meal feeding and skip-a-day feeding with or without probiotics for broiler chickens grown at high-altitude to prevent ascites mortality. Am. J. Anim. Vet. Sci., 5: 13-19.

SAS, 2001. SAS/STAT User's Guide for Personal Computers, Release 6.12. Cary, NC, SAS Institute Inc.

Susbilla, J.P., I. Tarvid, C.W. Gow and T.L. Frankel, 2003. Quantitative feed restriction or meal-feeding of broiler chicks alter functional development of enzymes for protein digestion. Br. Poult. Sci., 44: 698-709.

Svihus, B., A. Sacranie, V. Denstadli and M. Choct, 2010. Nutrient utilization and functionality of the anterior digestive tract caused by intermittent feeding and inclusion of whole wheat in diets for broiler chickens. Poult. Sci., 89: 2617-2625.

Tolkamp, B.J., V. Sandilands and I. Kyriazakis, 2005. Effect of qualitative feed restriction during rearing on the performance of broiler breeders during rearing and lay. Poult. Sci., 84: 1286-1293.

Zhan, X.A., M. Wang, H. Ren, R.Q. Zhao, J.X. Li and Z.L. Tan, 2007. Effect of early feed restriction on metabolic programing and compensatory growth in broiler chickens. Poult. Sci., 86: 654-660.

Zubair, A.K. and S. Leeson, 1994. Effect of varying period of early nutrient restriction on growth compensation and carcass characteristics of male broilers. Poult. Sci., 73: 129-136.