

Welfare Assessment Of Broiler Chickens Subjected To Feed Restriction And Fed Enzyme Supplemented Diet

Rabie Hassan Fayed; Abeer Hamada Abdel Razek; and Bassma Mohamed Baghwish

Animal & poultry Behaviour and Management, Department of Veterinary Hygiene and Management, Fac. Vet. Med., Cairo University, Giza 12211, Egypt
rhfayed@hotmail.com

Abstract: This study was conducted to evaluate the strategy of feed restriction as well as enzyme supplementation on the performance, behaviour, and physiology as indicators of welfare in broilers. The experiment carried out for 6 weeks. One hundred and eighty day old Cobb chicks were equally divided into 4 groups each of 3 replicates. Group 1 (control group) were fed ad libitum with no enzyme supplementation, Group 2, fed ad libitum with enzyme supplementation, Group 3 (restricted group) supplied with 75 % of quantity of feed consumed by the birds fed ad libitum on the previous day from 7 to 17 day old with no enzyme supplementation and Group 4 (restricted with enzyme supplementation) supplied with enzyme supplementation from 7 to 17 day old .The average weekly body weight and weight gain, Feed intake and feed conversion rate (FCR) dressing percentage and giblet weight (heart, liver, and gizzard) were calculated as physical indicators. The following behavioural parameters were measured: feeding, drinking and resting behaviour as focal sampling, where comfort and agonistic behaviour as scan sampling. Determination of H/L ratio, glucose and corticosterone hormone level as physiological parameters of welfare was recorded. Data obtained in this experiment revealed that, at the age of 6 weeks, (G4) which fed restricted diet supplemented with enzyme showed significantly ($p < 0.05$) heavier final body weight , body weight gain and had significantly ($p < 0.05$) the lowest daily feed intake ,the best feed conversion throughout the entire rearing period and highest dressing yield %. Feed restricted groups (G3, G4) showed significant ($p < 0.05$) decrease in the number of approach to feeder and drinker while spent more time in feeding and drinking especially during the restriction period at 2nd and 3rd weeks than those fed ad libitum, however, feed restriction increased significantly ($p \leq 0.05$) the resting frequency with lower time spent resting than birds fed ad libitum. Regarding the physiological responses, birds subjected to feed restriction without enzyme supplementation (G3) had a marked heterophilia, and lymphocytopenia consequently with higher H/L Ratio ;had the highest overall mean of blood glucose level and Highest overall mean of blood corticosterone hormone level than the other groups. The practice of feeding exogenous enzymes to feed-restricted chickens could be a desirable feeding strategy that might offer an economic advantage over a continuous ad libitum feeding regimen.

[Rabie Hassan Fayed; Abeer Hamada Abdel Razek; and Bassma Mohamed Baghwish. **Welfare Assessment Of Broiler Chickens Subjected To Feed Restriction And Fed Enzyme Supplemented Diet.** *J Am Sci* 2012;8(12):36-42]. (ISSN: 1545-1003). <http://www.jofamericanscience.org>. 5

Key words: Broiler chickens; Enzymes; Feed restriction; Performance; Welfare indicators.

1. Introduction

Welfare depends on how the individual may perceives its living environment, taking into account not only the physical aspects of the environment, but also the social aspects . In the last decades there has been a great improvement in poultry production based on the careful control of several aspects, among which nutrition and management, Nowadays, the search for good welfare conditions is a global tendency in animal production; (Moura, et al. 2006).

Poultry Welfare is good when all needs associated with the maintenance of good health and needs to show that certain behaviours are met so welfare varies from very poor to very good and can be scientifically assessed. In general, minimum mortality, low morbidity, little or no risk of injury, good body condition, the ability to express species-specific activities including social interactions, exploration, and play, and the lack of abnormal

behaviour and of physiological signs of stress, including alterations of immune responses, indicate that there are no major welfare problems and welfare is good (SCAHAW, 2000).

Several welfare indicators may be used to assess welfare, such as health (mortality, mobility, and level of injuries) (Estevez, 2003); management (which type of rearing is offered to the flock); physiological responses to stress (respiratory rate, body temperature, variation in cortisol levels (Craig et al. 1986), or immune status (Patterson and Siegel, 1998) and ultimately meat quality (Chevillon, 2000). Behaviour is frequently used by experienced farmers to determine potential problems in birds (Dawkins, 1999 and Duncan, 2002) Improvements in genetics and nutrition over the last 20 years have led to increase of growth rates in modern broiler strains which consume feed ad libitum; unfortunately this high growth rate is associated with increased body fat

deposition, high mortality and high incidence of metabolic and skeletal disorders which have negative impact on broiler welfare (Zubair and Leeson, 1996). So there is a critical need to increase efforts to reduce some of these problems and also reducing feed cost which ranged from 60-70% without compromising the final productivity. (Sarvestani et al. 2006).

One possible nutritional strategy of reducing feed cost is to restrict feed intake of the birds in the early stage of life which show improvement in feed efficiency and reach a weight similar to that of birds fed ad libitum at the time of slaughter (Novel et al. 2008 and 2009) The other nutritional strategy is using feed additives as enzyme supplementation which usually did not contain a single enzyme but they are enzymatic preparations containing a variety of enzymes as amylase, xylanase, protease, galactosidase, pectinase, cellulose and lipase which have been used successfully on poultry performance improvement (Knudsen, 1997 and Pinheiro et al. 2004). Non starch polysaccharide (NSP)-degrading enzymes usually result in numerous beneficial effects, such as increased utilization of nutrients (e.g., fat and protein), improved values, increased growth rate, improved feed: Gain, decreased viscosity of intestinal digesta, reduced incidence of sticky excreta, improved litter conditions and reduced environmental pollution due to a decreased output of manure and gases such as ammonia (Costa et al. 2008).

Therefore the objective of this study was to clarify the impact of feed restriction and enzyme supplementation on the welfare of broiler chickens.

2. Material And Methods

2.1. Birds and Housing

This study was conducted at the Department of Veterinary Hygiene and Management, Faculty of Veterinary Medicine, Cairo University. Day old Cobb broiler chicks were purchased from a local hatchery. A starter diet (23 % CP and 3029.84 kcal/kg ME) were supplied from 0–3 weeks while finisher diet (20 % CP and 2949.69 kcal/kg ME) were fed from 3 – 6 weeks (end of trials). The basal broiler starter and finisher diets were formulated to meet the NRC (1994) nutrient requirements for broilers.

The experimental birds were kept on floor litter system in a poultry research unit including separate symmetrical pens each of (2.25 x 2m), each pen divided into equal 3 subgroups. The pens were thoroughly cleaned, washed and disinfected before chicks arrival. The floor of all pens was covered by a uniform layer of finely chopped wheat straw. Fresh clean water was available all the time through bell shaped drinking devices. Birds were fed through plastic pan feeders. All the birds were provided with the same management conditions as temperature,

relative humidity, ventilation and light. Continuous photoperiod lighting program was used throughout the experimental period providing light intensity of 10 lux/ sq.m. The chicks were brooded at 35°C during first week and thereafter; the temperature was reduced by 3°C every week until the temperature reached to the room temperature (22±1°C). The relative humidity was ranged from 50-70 % during the trial period.

2.2. Experimental design

This study was conducted to evaluate the strategy of feed restriction as well as enzyme supplementation on the performance, behaviour, and physiology as indicators of welfare in broilers. The experiment carried out for 6 weeks. One hundred and eighty day old Cobb chicks were equally divided into 4 groups each of 3 replicates. Group 1 (control group) were fed ad libitum with no enzyme supplementation, Group 2, fed ad libitum with enzyme supplementation, Group 3 (restricted group) supplied with 75 % of quantity of feed consumed by the birds fed ad libitum on the previous day from 7 to 17 day old with no enzyme supplementation and Group 4 (restricted with enzyme supplementation) supplied with enzyme supplementation from 7 to 17 day old (Santoso et al. (1993); Pinheiro et al. (2004) and Novel et al. (2008)). The enzyme preparation used known as NUTRI-ZYM Dry produced by INVE TECHNOLOGIES NV, Belgium, European Union and used in 0.5 kg / ton Dose. The composition of Enzyme used is tabulated in table 1.

Table 1. Composition of the enzyme used in the trial

Ingredients	concentration
Alpha- Amylase	0.40 %
β -Glucanase preparation (Endo- 1, 3 (4) - β- glucanase E.C.3.2.1.6), (Endo-1, 4 β-glucanase E.C.3.2.1.4)	0.40 %
Endo-xylanase preparation	0.07 %
Pectinase preparation	0.40 %
Endo-proteinase preparation	2.58%
Dried yeast (inactive <i>Saccharomyces cerevisi</i>)	25 %
Limestone as carrier up to	100 %
With the following activities on product basis	
Endo- β –Glucanase	6800 BU/g
Endo-xylanase	8760 BXU/g
Proteinase	14.06 Uhg /g
Endo- cellulase	1300 ECU/g
Pectinase	1.04 PA/g
Alpha- Amylase	50 U /g

2.3. Welfare indicators

2.3.1. Physical indicators

A random sample of 10 % of each pen was weighted weekly by individual weighting of the identified random samples of each group to obtain the average weekly body weight and weight gain (Yalcin et al. 1998; Lei and Beek, 1997). Feed intake and feed conversion rate (FCR) were calculated according

to Dagaas and Claveria, (2008) where the average weekly feed intake was divided by the average weekly weight gain. At the end of experiment, five birds from each replicate were picked up randomly and slaughtered for their dressing percentage and giblet weight (heart, liver, and gizzard). The birds were weighed after removing heads, legs and viscera to determine the carcass weight included wings and necks and the dressing yield. The heart, liver and gizzard were weighed and their percentages to live body weights were calculated. (Petek et al. 2000 and Amina et al. 2008). The mortality rate was recorded weekly throughout the experimental period. (Novel et al. 2009)

2.3.2. Behavioural indicators

Birds used in this study were observed as focal (Martin and Bateson, 1993) and scan samples (Sandilands et al. 2005) for six weeks. Birds were observed 3 days /week. Behavioural observation was 20 min / replicate/ day, in two observational periods; in the morning (8.00-11.00 am) and at afternoon (14.30-17.30 pm). The following behavioural parameters were measured: feeding, drinking and resting behaviour as focal sampling, where comfort and agonistic behaviour as scan sampling (Cornetto and Estevez, 2001).. Continuous focal animal sampling was used for feeding, drinking and resting behaviour Webster, (2000), All focal birds were chosen randomly from each pen and were identified on their body using special dyes. Five birds were observed / day / replicate for 5 min/ period/day. The number of birds performing comfort and agonistic behaviour was recorded each minute for 5 minutes / period / replicate / day.

2.3.3. Physiological indicators

Blood samples were collected weekly starting from 2nd week for determination of differential leucocytic count, serum glucose and blood corticosterone hormone level. A total of 5

randomly selected chickens from each replicate were gently removed from their pen and blood samples (0.5 ml) were taken into EDTA tubes from each bird, two blood smears from each sample were made using the 2-slide wedge method (Houwen, 2000), blood films were air dried, fixed in methanol and stained with Diff-Quick stain (Dade Behring Inc., Deerfield, IL). In the differential leucocytic counts, 100 leukocytes were counted on each slide using oil immersion microscopy 100 x magnifications for determination of heterophil, lymphocyte percentage and H/L ratio by dividing the number of heterophil by the number of lymphocyte. (Gross and Siegel, 1983). Blood samples were centrifuged at 3000 rpm for 3 min and serum was obtained and frozen at -20°C until the chemical analysis. Glucose Analysis was conducted on an automated spectrophotometer using a standard diagnostic kit. (Zulkifli et al. 2000 and Amina et al. 2008) and corticosterone hormone analysis (Schaaf et al. 2000).

2.4. Statistical analysis

Data were analyzed in a factorial arrangement with 2 levels of feed condition (ad libitum and restricted) and 2 levels of enzyme addition (with and without enzyme addition). The Statistical analysis of the obtained results was conducted by using SPSS program (statistical Package for Social Science) version 12 for windows 17 (Dytham, 2003). The experiments were arranged as a completely randomized design with groups as the experimental unit. The mean values for the performance, physiological and behavioural variables were calculated for each group. T test, non-paired of analysis of variance one way were used as a parametric tests for the data analysis (Petrie and Waston, 2006). The data were expressed as mean \pm SE. A level of significance as minimal acceptable level was assessed at ($p < 0.05$).

3. Results

Results of this study were tabulated in tables 2, 3 and 4.

Table 2: Effect of feed restriction and enzyme supplementation on Physical indicators of broiler welfare

Physical indicators	Experimental groups			
	Group1	Group2	Group3	Group4
Body weight (gm)	934.7 \pm 9.76 ^{ac}	986.86 \pm 11.76 ^b	924.57 \pm 13.05 ^{ac}	1000.14 \pm 18.64 ^d
Daily feed intake (gm)	87.00 \pm 5.56 ^{ac}	85.45 \pm 7.45 ^b	84.38 \pm 4.45 ^{ac}	82.33 \pm 6.87 ^d
Feed conversion rate (FCR)	1.59 \pm 0.08 ^{abc}	1.52 \pm 0.12 ^{abc}	1.52 \pm 0.06 ^{abc}	1.38 \pm 0.10 ^d
Slaughtering	2225.0 \pm 7.04 ^{ac}	2346.00 \pm 8.04 ^b	2254.00 \pm 11.01 ^{ac}	2474.00 \pm 10.47 ^d
Weight after slaughtering	1620.00 \pm 7.80 ^{ac}	1795.80 \pm 13.61 ^b	1645.80 \pm 9.22 ^{ac}	1890.60 \pm 26.84 ^d
Dressing yield %	72.65 \pm 0.28 ^{ac}	75.26 \pm 0.37 ^{bd}	72.82 \pm 0.44 ^{ac}	76.25 \pm 0.95 ^{bd}
Gizzard weight	46.80 \pm 0.40 ^{ac}	30.28 \pm 1.66 ^{bd}	47.96 \pm 2.90 ^{ac}	30.44 \pm 2.74 ^{bd}
Gizzard %	2.10 \pm 0.02 ^{ac}	1.27 \pm 0.07 ^{bd}	2.12 \pm 0.13 ^{ac}	1.23 \pm 0.11 ^{bd}
Liver weight	50.32 \pm 0.92 ^{ac}	41.44 \pm 1.32 ^{bd}	50.72 \pm 3.60 ^{ac}	42.20 \pm 1.19 ^{bd}

Table 3: Effect of feed restriction and enzyme supplementation on Behavioural indicators of broiler welfare

Behavioural indicators	Experimental groups			
	Group1	Group2	Group3	Group4
Feeding frequency	4.12±0.08 ^{ab}	4.33±0.04 ^{ab}	2.78±0.09 ^{cd}	2.66±0.05 ^{cd}
Time spent feeding	78.49±7.22 ^{ab}	76.55±6.86 ^{ab}	91.22±3.39 ^{cd}	90.14±2.87 ^{cd}
Drinking frequency	3.11±0.05 ^{ab}	3.35±0.04 ^{ab}	1.89±0.05 ^{cd}	1.99±0.08 ^{cd}
Time spent drinking	49.75±6.21 ^{ab}	48.81±8.88 ^{ab}	63.86±4.27 ^{cd}	60.22±2.86 ^{cd}
Resting frequency	2.96±0.04 ^{ab}	2.88±0.05 ^{ab}	4.56±0.06 ^{cd}	4.14±0.06 ^{cd}
Time spent resting	160.45±3.12 ^{ab}	165.68±4.43 ^{ab}	148.00±7.3 ^{cd}	145.73±5.76 ^{cd}

a, ,b, c, d: means within the raw having different superscripts are significantly different at $p \leq .05$.

G 1: (control group) fed ad libitum

G 2: (group fed ad libitum with enzyme supplementation)

G 3 : (restricted group with no enzyme supplementation)

G 4 :(restricted group with enzyme supplementation)

Table 4. Effect of feed restriction and enzyme supplementation on Physiological indicators of broiler welfare

Physical indicators	Experimental groups			
	Group1	Group2	Group3	Group4
Heterophil %	29.67±2.44 ^{abd}	28.20±6.09 ^{abd}	32.25±3.96 ^c	28.80±4.50 ^{abd}
lymphocyte %	65.33±8.71 ^{abd}	66.76±5.62 ^{abd}	61.94±5.34 ^c	66.48±6.11 ^{abd}
H/L ratio	0.46±0.08 ^{abd}	0.42±0.04 ^{abd}	0.52±0.03 ^c	0.44±0.09 ^{abd}
Glucose level (mg/dl)	239.60 ± 25.98 ^{abd}	234.5 ± 19.75 ^{abd}	250.0±15.97 ^c	235.20±24.09 ^{abd}
Corticosterone level (ng/ml)	4.36 ± 0.07 ^{abd}	3.84 ± 0.09 ^{abd}	7.05 ± 0.04 ^c	3.98 ± 0.03 ^{abd}

4. Discussions

4.1. Physical indices of broiler welfare

Data obtained in this experiment revealed that, at the age of 6 weeks, (G4) which fed restrict diet supplemented with enzyme showed significantly ($p < 0.05$) heavier final body weight (1000.14 ± 18.64 g) and body weight gain (405.17 ± 9.48) then (G2) which fed ad libitum supplemented with enzyme, which recorded (986.86 ± 11.76) for body weight and (384.00 ± 11.93) for weight gain compared to other groups which gained relatively similar body weight and body weight gain (Table 2). The increasing of bodyweight and weight gain with enzyme supplementation may be attributed to the improvement of the overall digestion which observed more by feed restriction due to the compensatory growth as mentioned by Onbaşilar et al. (2009) which found that chickens subjected to feed restriction attained complete compensation in live weight and weight gain at 42 day of age as the ad libitum group and Pinheiro et al. (2004) which showed that the exogenous enzyme was responsible for increased live weight and body weight gain in broiler. While Attia et al. (1998); Shariatmadari and Vaez Torshizi (2004) and Lazaro et al. (2004) found that feed restriction enzyme supplementation lower the weight in broilers.

It was noticed that, (G4) which fed restricted diet supplemented with enzyme had significantly ($p < 0.05$) the lowest daily feed intake (82.33 ± 6.87) and better feed conversion (1.38 ± 0.10) throughout the entire rearing period than the other groups (Table 2). As observed by Mahmood et al., (2007) who mentioned that, although birds fed ad libitum were consumed more feed compared to the birds kept under

restricted feeding programme, the restricted birds utilized their feed more efficiently than controls, and with Samarasinghe et al. (2000) Wang et al. (2005); Balamurugan and Chandrasekaran (2010) which said that addition of feed enzymes to poultry diet decreased the feed intake with improvement in feed conversion rate, Contrarily Junqueira et al (2003); Alam et al. (2003) and Shirzadi, et al. (2009) found that feed restriction or addition of exogenous enzymes in broiler resulted in increased feed intake and Khetani et al. (2008) and Malayolu et al.(2010) found no significant effects on feed conversion by feed restriction or enzyme supplementation, The decreasing of feed intake by feed restriction and enzyme supplementation may be attributed to the decreased of the maintenance requirement as the birds able to fulfill their nutrient requirement by taking less amount of feed,

In the current study the highest dressing yield % was observed in (G4) (76.25 ± 0.95) which fed restricted diet supplemented with enzyme followed by (G2) (75.26 ± 0.37) which fed ad libitum supplemented with enzyme than birds fed non supplemented diet and this explained by the highest final live weight observed in these groups. While in relation to visceral organ weight and percentage, enzyme supplementation reduced gizzard, liver, heart weight and percentages. These results are in agreement with Khan et al. (2006); Nadeem et al. (2005) and El-Deek and Al-Harhi (2004) who reported that enzymes supplementation reduced gizzard liver and heart weight of broiler.

4.2. Behavioural Indices

Feed restriction (G3, G4) decreased significantly ($p < 0.05$) the number of approach to feeder (2.78 ± 0.09) for G3 and (2.66 ± 0.5) for G4 and

drinker (1.99 ± 0.08) for G4, (1.89 ± 0.05) for G3 while spent more time in feeding (91.22 ± 3.39) for G3 and (90.14 ± 2.87) for G4 and drinking (3.86 ± 4.27) for G3 and (60.22 ± 2.86) for G4 especially during the restriction period at 2nd and 3rd weeks than those fed ad libitum and this may related to the less amount of food which offered during this period so birds are hungry so spent more time to take more feed particles. These results agreed with those reported by Kubíková et al. (2001) who found that feed restricted birds spent more time in food intake than ad libitum groups. with Hocking et al. (1993) and Dagaas and Claveria (2008) which observed that food-restricted birds showing increased drinking over time compared to birds fed ad libitum. However, feed restriction increased significantly ($p \leq 0.05$) the resting frequency as observed in (4.56 ± 0.06) for G3 and (4.14 ± 0.06) for G4 but with lower time spent resting (148.00 ± 7.32) for G3 and (145.73 ± 5.76) for G4. Similar results were obtained by Savory et al (1992) and Ayn et al. (2009) who found that restricted birds showed less time resting than birds fed ad libitum.

4.3. Physiological indices

4.3.1. Heterophil / lymphocyte ratio

Regarding the physiological responses, birds subjected to feed restriction without enzyme supplementation (G3) had a marked heterophilia (32.25 ± 3.96), and lymphocytopenia (61.94 ± 5.34) consequently with higher H/L Ratio (0.52 ± 0.03) than the other groups, the increased heterophil: lymphocyte ratio in feed restricted group revealed that quantitative feed restriction act as a stressor for broiler chicks. Maxwell et al. (1992) and De Jong et al. (2002) reported that restricted fed broiler showed increases in heterophil together with a corresponding decrease in lymphocyte % with elevated H / L ratio on the other hand Onbaşıl et al. (2009) found higher Heterophil: Lymphocyte ratio in broilers fed ad libitum than the fed restricted

4.3.2. Glucose level

In his experiment where (G3) which subjected to feed restriction without enzyme supplementation had the highest overall mean of blood glucose level (250.63 ± 15.97) which may be attributed to the stress which occurred to birds by feed restriction, These result support the view of Puvadolpirod and Thaxton (2000) and Onbaşıl et al. (2009) which explained that by the higher circulating glucocorticoid levels which associated with stress in chickens, On the other hand, Dewil et al. (1999); Kubíková et al. (2001) and Rajman et al. (2006) reported that quantitative feed restriction did not affect blood glucose level.

4.3.3. Blood corticosterone hormone level

Highest overall mean of blood corticosterone hormone level observed in G3 which subjected to feed restriction without enzyme supplementation

(7.05 ± 0.04) than the other groups as limiting feed intake during the rapid growth period considered stress on meat type chickens, these result are in coinciding with Kubíková et al. (2001); De Jong et al. (2002) and Rajman et al. (2006) who found that feed restriction elevated the blood corticosterone levels in broilers. It can be concluded that, the practice of feeding exogenous enzymes to feed-restricted chickens could be a desirable feeding strategy that might produce birds with maximum final body weight, best feed conversion with a minimum feed intake; In addition, this practice might offer an economic advantage over a continuous ad libitum feeding regimen.

5. Acknowledgments

Authors are thankful for the collaboration of people without whom this work would not have been possible: Professor Dr. Mohamed Y. Mattoock, for the support of the statistical analysis; Professor. Dr. Moustafa Bashandy, Head of Clinical Pathology Department, for help with blood analyses.

Corresponding author:

Prof. Dr. Rabie H. Fayed,
Fac. Vet. Med., Cairo University,
Giza, 12211,
Egypt.
E-mail: rhfayed@hotmail.com

References

1. Alam MJ, Howlader MAR, Pramanik MAH, Haque MA. Effect of Exogenous Enzyme in Diet on Broiler Performance. *Inter. J. Poult. Sci.* 2003; 2:168-73.
2. Amina A Salem, Enaiat MM EL Anwer, Eman Abo-Eita M, Namra MMM. Productive and Physiological performance of Golden Montazah male chickens as affected by feed restriction and antzyme supplementation. *Egypt. Poult. Sci.* 2008; 28 (IV):1137-64.
3. Attia FM, Alsobayel AA, Aldabiby AAS. The effect of feed restriction on performance and abdominal fat content of broilers. *Agric. Sci.* 1998; 10 (1): 19-31.
4. Ayn I, Ali K, Umran S, Onder C, Bilgehan Y. Effect of Different Diets on the Behaviour of Slow-growing Broiler Genotype, *J. Appl Anim. Res.* 2009; 35(1): 87.
5. Balamurugan R, Chandrasekaran D. Effect of multienzyme supplementation on weight gain, feed intake, feed efficiency and blood glucose in broiler chickens. *Indian J. Sci. Technol.* 2010; (2): 193-5.
6. Chevillon P. Obem-estar dos suínos Durante o pré-abate e o atordoamento. I Conferência Virtual sobre Qualidade de Carne Suína. 2000; Available from: www.embrapa.gov.br.
7. Cornetto T and Estevez I. Behavior of the Domestic Fowl in the Presence of Vertical Panels, *Poultry Science* .2001; 80: 1455–62.

8. Costa FGP, Goulart CC, Figueiredo DF, Oliveira CFS, Silva JHV. Economic and environmental impact of using exogenous enzymes on poultry feeding. *Int. J. Poult. Sci.*, 2008; 7: 311-4
9. Craig JV, Vargas Vargas J, Milliken GA. Fearful and associated responses of white leghorn hens: effects of cage environments and genetic stocks *Poultry Sci.*, 1986; 65: 2199.
10. Dagaas CT, Claveria JN. On farm performance of broilers subjected to 40 % level of feed restriction on the third week of age. *Philippine J.Vet. Anim. Sci.*, 2008; 34(2): 177-186.
11. Dawkins MS. The role of behaviour in the assessment of poultry welfare. *World's Poultry Science Journal*. 1999; 55: 295-303.
12. De Jong IC, Van Voorst S, Ehlhardt DA, Blokhuis HJ. Effects of restricted feeding on physiological stress parameters in growing broiler breeders. *Br. Poult. Sci.*, 2002; 43: 157-68.
13. Dewil E, Darras VM, Spencer GSG, Lauterio TJ, Decuypere E. The regulation of GH dependent hormones and enzymes after feed restriction in dwarf and control chickens. *Life Sci.*, 1999; 64: 1359-1371.
14. Duncan I J H. Poultry welfare: science or subjectivity? *Brit. Poult. Sci.*, 2002; 43: 643-52.
15. Dytham Clavin Choosing and Using Statistics: A Biologist's Guide. 2003; 2nd edition. Blackwell Publishing, Oxford, UK.
16. El-Deek AA, Al-Harhi MA. Responses of modern broiler chicks to stocking density, green tea, commercial multi enzymes and their interactions on productive performance, carcass characteristics, liver composition and plasma constituents. *International Journal of Poultry Science*. 2004; 3(10): 635-645.
17. Estevez I. addressing poultry welfare proactive strategies and voluntary regulations ,Ph.D. Department of Animal and Avian Sciences, University of Maryland, College Park, MD 20742 ,Multi-State Poultry Meeting ,May 2003; 20-22.
18. Gross B, Siegel HS. Evaluation of the Heterophil / lymphocyte ratio as a measure of stress in chickens, *Avian Diseases*. 1983; 27 (4).
19. Hocking PM, Maxwell M H, Mitchell MA. Welfare assessment of broiler breeder and layer females subjected to food restriction and limited access to water during rearing. *Br. Poult. Sci.* 1993;34 (3): 443-58.
20. Houwen B. Blood film preparation and staining procedures. *Lab. Haematol.* 2000; 6: 1-7.
21. Junqueira OM., Fonseca LEC., Araújo LF, Duarte KF, Araújo CSS., Rodrigues EAP. Feed restriction on performance and blood parameters of broilers fed diets with different sodium levels, *Rev. Bras. Cienc. Avic.* 2003;5 (2): 99-104.
22. Khan SH, Sardar R, Siddique B. Influence of enzymes on performance of broilers fed sunflower-corn based diets. *Pakistan Vet. J.* 2006; 26(3): 109-114.
23. Khetani. T, Nkukwana T, Chimonyo, M, Muchenje V. Effect of quantitative feed restriction on broiler performance, *Tropical Animal Health and Production* 2008; 41(3): 379-84.
24. Knudsen KEB. Carbohydrates and lignin contents of plant materials, *Anim. Feed Sci. Technol.* 1997; 67: 319-38.
25. Kubíková L, Výboh P, Košťál L. Behavioural endocrine and metabolic effects of food restriction in broiler breeder hens, *Acta Vet. Brno .* 2001; 70: 247-257.
26. Lazaro R, Latorre MA, Medel P, Gracia M, Mateos GG. Feeding Regimen and Enzyme Supplementation to Rye-Based Diets for Broilers, *Poultry Science* 2004;83: 152.
27. Lei S, Van Beek G. Influence of activity and dietary energy on broiler performance, carcass yield and sensory quality. *Br. Poult. Sci.* 1997; 38:183-9.
28. Mahmood S, Mehmood S, Ahmad F, Masood A, Kausar R. Effects of feed restriction during starter phase on subsequent growth performance, dressing percentage, relative organ weight and immune response of broilers. *IJAVMS*. 2007; 0(1), Supl 1: 13 Proc.
29. Malayolu HB , Baysal S, Misirliolu Z , Polat M, Yilmaz H, Turan N. Effects of oregano essential oil with or without feed enzymes on growth performance, digestive enzyme, nutrient digestibility, lipid metabolism and immune response of broilers fed on wheat-soybean meal diets. *Br. Poult. Sci.* 2010; 51(1): 67- 80.
30. Martin P, Bateson P. Measuring Behavior: An Introductory Guide. Cambridge University Press, Cambridge, U.K. 1993.
31. Maxwell, M.H., Hocking, P.M and Robertson.G.W. (1992): Differential leucocyte responses to various degrees of food restriction in broilers. *Turkeys and ducks, British poultry science* 33 : 177-187.
32. Moura, D.J, Naas, I.A., Pereira, D.F, Silva, R.B.T.R., Camargo,G.A (2006): *Animal Welfare Concepts and Strategy for Poultry Production: A Review .Brazilian Journal of Poultry Science* 8 (3) :137 – 148.
33. SCAHAW (Scientific Committee on Animal Health and Animal Welfare). *The Welfare of Chickens Kept for Meat Production . European Commission, Health and Consumer Protection Directorate-General.* 2000
34. Nadeem MA, Anjum MI, Khan AG, Azim A. Effect of dietary supplementation of non starch polysaccharides degrading enzymes on growth performance of broiler chicks. *Pakistan Vet. J.* 2005; 25(4):
35. Novel DJ, Ng'Ambi JW, Norris D, Mbajjorgu CA. Effect of sex, level and period of feed restriction during the starter stage on productivity and carcass characteristics of Ross 308 broiler chickens in South Africa .*International Journal of Poultry Science* 2008;7 (6): 530-7.

36. Novel DJ, Ng'ambi JW, Norris D, Mbajjorgu CA. Effect of different feed restriction regimes during the starter stage on productivity and carcass characteristics of male and female Ross 308 broiler chickens. *International Journal of Poultry Science* 2009 ;8 (1): 35-9.
37. Onbaşlar E, Yalçın S, Torlak E, Özdemir P. 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, *Tropical Animal Health and Production* 2009;41(7): 1513-19.
38. Patterson PH, Siegel HS. Impact of cage density on pullet performance and blood parameters of stress. *Poultry Sci.* 1998;77: 32-40.
39. Petek M. The Effects of Feed Removal during the Day on Some Production Traits and Blood Parameters of Broilers, *Turk J Vet Anim Sci.* 2000; 24: 447-52.
40. Petrie A, Waston P. *Statistics for veterinary and animal science*; 2nd edition .Blackwell science.2006.
41. Pinheiro DF, Cruz VC, Sartori JR, Vicentini Paulino MLM. Effect of Early Feed Restriction and Enzyme Supplementation on Digestive Enzyme Activities in Broilers. *Poultry Science* 2004;83:1544–50.
42. Puvadolpirod S, Thaxton JP. Model of physiological stress in chickens 1. Response parameters. *Poult. Sci.*, 2000;79 (3): 363-9.
43. Rajman M, Juráni M, Lamosová D, Mácajová M, Sedlacková M, Kost'ál L, Jezová D, Výboh P. The effects of feed restriction on plasma biochemistry in growing meat type chickens (*Gallus gallus*). *Comp Biochem Physiol A Mol Integr Physiol.* 2006;145(3): 363-71.
44. Samarasinghe K, Messikommer R, Wenk C. Activity of supplementation enzymes and their effect on nutrient utilization and growth performance of growing chickens as affected by pelleting temperature. *Arch. Anim. Nutr.*2000;53: 45-58.
45. Sandilands V, Tolkamp BJ, Kyriazakis I. Behaviour of food restricted broilers during rearing and lay--effects of an alternative feeding method. *Physiol Behav.* 2005;85 (2):115-23.
46. Santoso U, Tanaka K, Ohtani S, Youn BS. Effects of early feed restriction on growth performance and body composition in broilers. *Asian Austr. J. Anim. Sci.* 1993; 6: 401-10.
47. Sarvestani TS, Dabiri N, Agah MJ, Norollahi H. Effect of Pellet and Mash Diets Associated with Biozyme Enzyme on Broilers Performance , *International Journal of Poultry Science* 2006;5(5): 485-90.
48. Savory CJ, Seawright E , Watson A . Stereotyped behaviour in broiler breeders in relation to husbandry and opioid receptor blockade. *Applied Animal Behaviour Science* 1992;32 (4): 349-60.
49. Schaaf MJ, De Kloet ER, Vreugdenhil E. Corticosterone effects on BDNF expression in the hippocampus. Implications for memory formation. *Stress* 2000;3 :201–8.
50. Shariatmadari F, Vaez Torshizi R. Feed restriction and compensatory growth in chicks: effects of breed, sex, initial body weight and level of feeding. *British Poult Sci.* 2004; 45, Issue 2, Supplement 1: S52 - 3.
51. Shirzadi H, Moravej H, Shivazad M. Comparison of the effects of different kinds of NSP enzymes on the performance, water intake, litter moisture and jejunal digesta viscosity of broilers fed barley-based diet, *Journal of Food, Agriculture & Environment* 2009;7 (3&4) : 6 1 5 - 19
52. Wang ZR, Qiao SY, Lu WQ, Li DF. Effects of enzyme supplementation on performance, nutrient digestibility, gastrointestinal morphology, and volatile fatty acid profiles in the hindgut of broilers fed wheat-based diets. *Poultry Science* 2005; 84(6)875-81.
53. Webster A B. Behavior of White Leghorn Laying Hens after Withdrawal of Feed. *Poultry Science* 2000;79:192–200
54. Yalçın S, Özkan S, Açıkgöz Z, Özkan K. Influence of dietary energy on bird performance, carcass parts yields and nutrient composition of breast meat of heterozygous naked neck broilers reared at natural optimum and summer temperatures. *Br. Poult. Sci.* 1998; 39 :633–8.
55. Zubair AK, Leeson S. Changes in body composition and adipocyte cellularity of male broilers subjected to varying degrees of early-life feed restriction. *Poult. Sci.* 1996; 75: 719- 28.
56. Zulkifli I, Norma MTC, Israf DA, Omar AR. The effect of early age feed restriction on subsequent response to high environmental temperatures in female broiler chickens. *Poult. Sci.*, 2000; 79:1401–7.

10/21/2012