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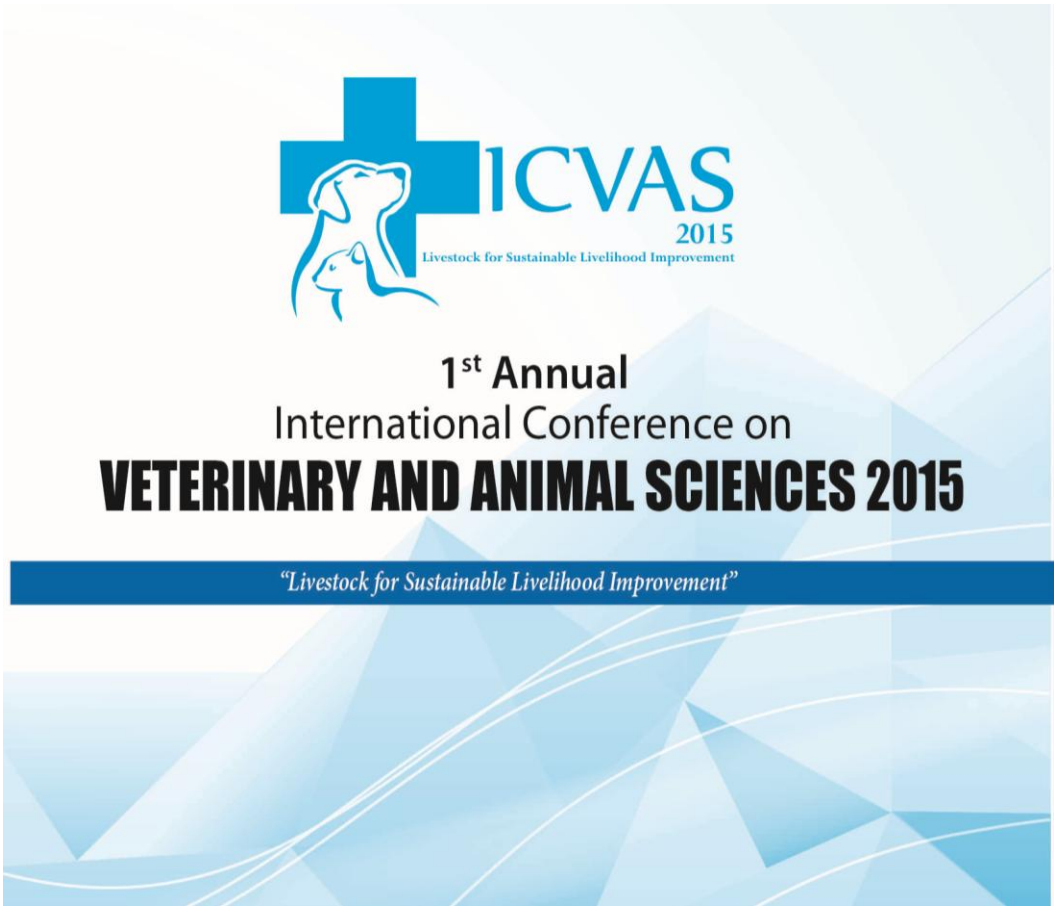

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EFFECT OF GROUDNUT HAULM AND COTTON SEED CAKE SUPPLEMENTATION ON DRY MATTER INTAKE, NUTRIENT DIGESTIBILITY AND LIVE WEIGHT CHANGE OF GRAZING SHEEP IN THE SEMI-ARID REGION OF NIGERIA

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Abstract

The study was designed to assess the effect of cotton seed cake, groundnut haulm and wheat offal supplementation on dry matter intake, live weight gain, nutrient digestibility on grazing rams. Sixteen (16) mixed breeds ofsheep of Balami andUdaRams aged between nine and ten months with an average live weight of 28.05 ± 3.18kg were used for the study. The rams were randomly allocated into four treatment groups with four (4) animals per treatment in a Completely Randomized Block Design. The research lasted for three (3) months apart from ten (10) days of adaptation period. The rams were allowed to go for grazing daily and were supplemented as follows: Treatment 1 (62.5% wheat offal plus 37.5% groundnut haulm), Treatment 2(62.5% wheat offal plus 25% groundnut haulm plus 12.5% cotton seed cake), Treatment 3(62.5% wheat offal plus 12.5% groundnut haulm plus 25% cotton seed cake) and Treatment 4(62.5% wheat offal plus 37.5% CSCrespectively. The highest dry matter intake 2700g was recordedin treatment 1 and thehighest live weight gain 2400g was obtained in treatment 3 for the period of three months. Nitrogen retention was recoded to be highest in T4 (333.00g/animal/day) .The cost of feeding was lowest in treatment 1(12.19) and highest in treatment 4(12.78). It can therefore be recommended to agro- pastoralist that grazing rams can be supplemented with wheat offal and groundnut haulm at 62.5% plus 37.5% /head/day to maintain their body weight during the dry season.

Keywords: Digestibility, Dry matter intake, Grazing, Supplementation, Wheat offal

INTRODUCTION

As feed represents the greatest cost in livestock production, its availability is affected by seasonal variation in both quality and quantity and this equally affects animal productivity (Formyanyan and Mbomi, 1987). It is estimated that 40% of the dry season grazing time is spent by local sheep on crop residues (Powell, 1983). Despite the availability of these crop residues in large quantities and their potentials as substantial feed resources, they are poorly utilized due to protein limitation during the long dry season (Formyanyan and Mbomi, 1987).

Efforts to improve intake and utilization of these poor quality residues by animals have been through treatment (Sundstol, 1981) and supplementation with protein sources (Keihaway and Leibholz, 1983). Treatments of crop residues with chemicals like alkali and urea, biologically by treatment with enzymes or inoculate are expensive and corrosive (White *et al.* 1981). Supplementation with conventional concentrate rich feeds in protein has not been economically feasible due to its high cost and demand for use by the increasing population and feeding monogastric. Therefore, there is need to explore the

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potentials of alternative sources of supplementary feeds rich in nitrogen which are inexpensive, available and can improve the nutritive value of poor quality crop residues for ruminants. Groundnut haulms are generally used to fatten animals in the northern part of Nigeria (Adu and Lakpini, 1983). Ikhatua and Adu (1984) reported that groundnut haulm is better quality roughage than *Digitatiasmutsii* hay and that it contained adequate protein to maintain ruminant without any form of supplementation during the period of feed scarcity. The objective of the study was to evaluate the effect of feeding groundnut haulm, cotton seed cake and wheat offal supplements on dry matter intake, nutrient digestibility and live weight change of grazing sheep in the semi-arid zone of Nigeria.

MATERIALS AND METHODS

Sixteen (16) mixed breeds of sheep of Balami and UdaRams aged between nine and ten months within average live weight 28.0 ± 53.18kg were used for the study. The animals were divided into four groups based on similarity in live weight. They were taken for grazing daily. The animals were randomly allocated to four treatments with four animals per treatment. The 4 treatments were: T_1 wheat offal 250g/head/day plus groundnut haulm 150g/head/day; T_2 wheat offal 250g/head/day plus groundnut haulm l00g/head/day plus cotton seed cake 50g/head/day; T_3 wheat offal 250g/head/day plus groundnut haulm 50g/head/day plus cotton seed cake 200g/head/day and T_4 wheat offal 250g/head/day plus cotton seed cake 150g/head/day. The rams were randomly allocated into four treatment groups with four animals per treatment in a Completely Randomized Block Design. Each group was offered water and minerals lick *ad libitum*. Prior to the experiment, the animals were de-wormed against endo-parasite with albendazole bolus and were allowed a 10 day adjustment period during which the experimental diet was offered to the animals. They were housed in pens with concrete walls and floor. Dry matter intake was recorded daily. Feeds refusal was collected and weighed daily before the next feeding. Growth performance was determined on weekly basis. The feeding trial lasted for three months apart from 10 days adaptation period. Nutrient digestibility was conducted after the feeding trial which lasted for14 days; seven days adaptation and seven days collection period. Sub-samples of feed offered and faeces were collected daily for chemical analysis.

Chemical Analysis

Feeds and fecal samples collected during the research were analyzed for dry matter (DM), crude protein (CP), crude fiber (CF), either extracted (EE), Nitrogen free extract (NFE) and total ash according to the method of AOAC (1996).

Statistical Analysis

All data collected were subject to analyses of variance using the General Linear Model (GLM) of SPSS (2001) at 5% probability.

RESULTS AND DISCUSSION

The result of the chemical composition of the experimental diets used in this study was summarized in Table 1. The dry matter content of the formulations was 95.00%, 91.20%, 93.00% and 97.20% for treatments T_1, T_2, T_3 and T_4 respectively. The crude protein was 13.70%, 15.80%, 17.90% and 20.0%. The highest dry matter and crude protein (97.20 and 20.0%) was recorded in T_4 and the lowest dry matter in T_2 (91.20%); the crude protein was lowest in T_1 (13.70%). This is in line with the report of Fabian *et al*. (2015). The crude fiber values ranged from 14.90% to 18.0% with T_2 (18.0%) being the highest and T_1 (14.90%) the lowest; these figures fall within 21.0% as reported by Fabian *et al*. (2015).Ash content ranged from 6.00 to 7.50% with the highest value in T_4 and the lowest in T_1. The values were much higher than 2.5% revealed Fabian *et al*. (2015). The ether extract varied from 2.60% to 4.00% which fall much below (17.8%) reported Calhoun *et al*. (1995). The figures for NFE varied from 47.80 to 50.40% with the highest value being recorded in T_3 and T_1 the lowest.

Table 1: Chemical composition of experimented diets (% Dry matter)

Parameters	T1	T2	T3	T4
Dry matter	95.00	91.20	93.00	97.20
Crude protein	13.70	15.80	17.90	20.10
Crude fiber	14.90	18.00	15.20	15.80
Ash	6.00	6.60	6.50	7.50
Ether extract	2.60	3.60	3.00	4.00
Nitrogen free extract	47.80	48.20	50.40	49.80

Table 2: Dry matter intake, live-weight changes, feed conversion efficiency and feed cost

Parameters	T1	T2	T3	T4	SEM
Dry matter intake (kg)	2.70	2.26	1.94	2.12	0.16
Initial live-weight (kg)	32.10	31.90	31.80	32.40	0.13
Final live-weight (kg)	34.30	34.18	34.20	34.10	0.15
Total live weight gain(g)	2200	2280	2400	1700	0.17
Feed conversion ratio	1.23	0.99	0.81	1.25	0.06
Feed cost(₦/Kg)	12.19	12.39	12.58	12.78	0.13

The summary of the dry matter intake, live weight gain, feed conversion ratio and cost are summarized in Table 2. There were significant difference ($P<0.05$) in the dry matter intake among treatment groups. The highest dry matter intake was obtained in T_1 (2.70kg) and the lowest in T_3 (1.94kg).The increase in dry matter intake was due to supplementation as supported by the report of Ammorman *et al*. (1992) who stated that inclusion of nitrogen and energy sources supplementation lead to higher dry matter intake, increased digestion of the low quality roughage and body weight gain. Treatment T_3 (2,400g) recorded the highest live-weight gain for the whole research while the lowest value was obtained in T_4 (1,700g).This result coincides with the report of (Formyanyan and Mbomi, 1987) who opined that supplementation of cotton seed cake to maize and rice stalk, for grazing sheep and goats in the dry season increases dry matter intake and live-weight gain. This finding is in harmony with the works of Nyako *et al* .(2012) who supplemented cowpea husk 300g with 1000g *Gmelina* leaves and obtained a higher live weight gain and an increased in dry matter intake.

The feed conversion ratio (FRC) of the rams is shown in Table 2 in which significant difference ($P<0.05$) in feed efficiency between the treatments was recorded. Treatment T_4 (1.25) showed the highest FCR while T_3 revealed the lowest 0.81, indicating that the feed was better utilized in T_3 than the remaining treatments. This result is in consonance with the report of Doma (1999) who reported that the lower the FCR of a diet the better the diet. The result also reflected the ability of the animals on diet T_3 to convert the feed consumed to weight gain (Ososanya *et al*; 2013). The

higher growth rate of the animals on T_3 in this study could therefore be ascribed to more efficient utilization of the feed by the animals as shown by the lower FCR.

Table 2 shows the feed cost in naira per kilogram with the highest cost per kilogram weight gain in T_4 (12.78) and the lowest cost per kilogram weight gain was recorded in T1 (12.19). The result implied that feed cost per kilogram increased linearly across the treatments, the increase in the cost is therefore attributable to the relative cost of cotton seed cake used as the supplement, thus total feed cost raised with increase in cotton seed cake supplementation. This is however contrary to report of Tamburawa *et al*. (2012) who recorded a decrease in feed cost with increase in supplementation.

Table 3: Nutrient digestibility and nitrogen balance

Parameters	T1	T2	T3	T4	SEM
Digestibility (%)					
Dry matter (%)	97.78	96.86	97.37	97.89	0.23
Crude protein (%)	98.36	97.86	96.42	99.06	0.56
Crude fiber (%)	96.98	95.42	94.65	96.66	0.54
Nitrogen balance					
Nitrogen intake (g/day)	681.00	526.00	576.00	803.00	61.35
Nitrogen in faeces (g/day)	463.00	468.00	510.00	456.00	12.17
Nitrogen in urine (g/day)	37.00	37.00	41.00	20.00	4.68
Nitrogen retention (g/day)	180.00	210.00	240.00	333.00	33.10

The results of the nutrient digestibility are summarized in Table 3. There was significant difference ($P<0.05$) of DM digestibility between treatments. The highest value was obtained in T_4 (97.89%) and the lowest digestibility was recorded in T_2 (96.86%); the highest digestibility might be attributed to presence of adequate energy/protein in the treatment diet, that is, lower fiber level in the diet. There was significant difference ($P<0.05$) of crude protein digestibility between treatments. The highest figure was obtained in T_4 (99.06%) and the lowest in T_3. The higher digestibility obtained in T_4 was in agreement with Preston and Leng (1987) who reported that concentrates such as cotton seed cake in the diet of sheep and goats enhance protein digestibility. This result recorded was also in agreement with Mosleyane (1983) who reported higher digestibility in groundnut haulm supplemented to sheep and goats.

Nitrogen retained was higher in T_4 (333g) followed by T_3 (240.00g), T_2 (210.00) and T_1 (180.00g). Significant differences existed in nitrogen intake in rams in treatments T_1 and T_4 as compared to rams in Treatments T_2 and T_3. N- Balance and retention which were a function of nitrogen ingested and digested also differ significantly ($p<0.05$) with T_4 (333.00g) having recorded the highest, while T_1 being the lowest (180.0g). The low N- balance and retention observed in T_1 might have been influenced by the absence of cotton seed cake in the treatment. The study posted the best positive N-balance for T_4, suggesting that the protein requirements for rams to improve rumen microbial activity were adequately met by the diets (Ososanya and Akinlade, 2015).The differences in nitrogen intake could also be due to the slight variation in the amount of DM consumed and N concentration of the different diets. Therefore, it can be seen from Table 3 that the highest retained nitrogen was obtained in animals (T_4) that were

supplemented with more quantity of cotton seed cake as compared to other treatments. The result also shows that there was positive nitrogen balance between the treatment groups which indicates that the nitrogen was well utilized by the animals.

CONCLUSION

This research shows that locally available feed ingredients (crop residues) and industrial byproducts can be combined together in appropriate ratios to meet the nutritional requirements of ruminant animals and at the same time will reduce the cost of buying conventional feeds by Agro-pastoralist. It can be deduced from this study, higher dry matter intake and live weight gain were recorded in $T_1$and T_3 respectively, and the cost of feeding was lowest in Treatment T_1. Nitrogen retention was recoded to be highest in T4 (333.00g/animal/day). Looking at the cost of feeds, nutrient composition and digestibility, it is better to use Treatment T_1 in the Semi-arid region of Nigeria which has short duration of rainfall. Therefore, it is recommended that Agro-pastoralist who are grazing sheep (Balami and Uda rams) in the Semi-arid region should supplement wheat offal and groundnut haulm at 62.5% plus 37.5%/head/day to maintain their body weight during the dry season.

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HATCHING EGG WEIGHT AND LIVING AREA IN CLOSED TUNNEL VENTILATED EVAPORATIVE COOLING POULTRY HOUSE ON SUBSEQUENT BROILER PERFORMANCES

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Abstract

This study was conducted to evaluate the effect of hatching egg weight (HEW) and living area of Cobb500 broiler strain in closed tunnel ventilated evaporative cooling poultry house (CTVECPH) on subsequent broiler meatiness performances. Three hundred chicks representing each HEW classes (Heavy: 50g-56g, Medium: 57g-63g, Light: 64g-70g) were randomly selected. Hundred chicks from each HEW class were positioned into three separated living areas inside CTVECPH (Distance from air inlet- Front: 0ft-133ft, Middle: 133ft-266ft, Back: 266ft-400ft). Live weight was measured maintaining 7 days intervals. Data on pre-slaughter live weight, dressed weight, muscle weight of breast, upper thigh and lower thigh and length of keel, femur and tibia were collected from representative 5 birds of each HEW class reared in each living area at 35 days of age. Average Live weight, relative live weight gain, dressing percentage and meatiness of breast, upper thigh and lower thigh were calculated. Both HEW and Living area were affected on broiler meatiness performances. The pre-slaughter live weight, Dressing percentage and meatiness of breast, upper thigh and lower thigh were highest in heavy HEW class and front living area of CTVECPH. Study results conclude that, birds reared in front living area in CTVECPH and originated from heavy hatching eggs have taken the performance advantage than the birds lived in back living area in CTVECPH and originated from light hatching eggs. Flock uniformity, high efficiency of poultry houses and ultimately high profit can be achieved by manipulating this behavior as required.

Keywords: Broiler, Meatiness, Hatching egg, Live weight, Dressing percentage

INTRODUCTION

Broiler industry is a one of the best fast growing industry in Sri Lankan livestock sector. Broiler (*Gallus gallus domesticus*) is a young chicken (usually 6-8 weeks of age) of their sex that weights 3-5 lbs., that is tender meated and that has a soft pliable smooth textured skin and a flexible breast bone cartilage (Ensminger, 1992). As a developing country, it provides higher amount of protein to the nation to minimize the protein malnutrition in Sri Lankan people with comparatively lower price than other protein sources.

Broiler industry gained higher profit in a short time period is the most important fact for its fast improvement (Smith, 1990). Annual chicken production of Sri Lanka is 150.32MT (Department of Animal Production and Health, Sri Lanka, 2015). It has provided higher value in the GDP among the livestock sector in Sri Lankan economy. Contribution to Sri Lankan GDP by Broiler industry is 7.29 (Provisional data, Department of Animal Production and Health, Sri Lanka, 2015). The current per capita consumption of chicken in Sri Lanka, approximately 7.2 kilograms (Department of Animal Production and Health, Sri Lanka, 2015).

The demand for poultry is expected to continue growing in developing economies, reflecting population increases, improved disposable incomes and consumer taste preferences. The demand for poultry products in Sri Lanka is envisaged to grow in tandem with the expected rise in per capita income. Chicken production has increased by 4.5 percent to 150,980 metric tons in 2014. Increased chicken

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production was mainly driven by the increased contribution of large chicken manufacturers in the country encouraged by the measures introduced by the government. Despite the increase in chicken production, chicken prices also increased in 2014 (Annual Report 2014, Central Bank of Sri Lanka). Throughout last decades, customer demand for chicken has varied from whole chicken to separated chicken parts. Now the industry focus on improving qualities of separate chicken parts to satisfy the emerging customer demand.

To face this increasing market demand, genetic improvement on specific characters of commercial broiler strains is being performed continuously. There are a few number of different broiler strains are grown all over the country like Cobb 500, Hubbard Classic, Hubbard Flex, Hubbard F15 and Indian River. In large scale broiler farming companies like New Anthoney's Farms, Bairaha, Maxies, Crysbro, Prima, Nelna, Weehena, Delmo, chickens are reared in CTVECPHs. Even though there are so many techniques to maintain same environmental conditions throughout the house, there can be significant variations in ventilation, temperature and relative humidity like important environmental conditions and those can affect to broiler performances directly.

Previous researchers have identified the factors which are affecting the performances of the broilers (Moran, 1999). The genetic contribution of the broiler chicken (Rondelli *et al*., 2003) as well as non-genetic factors such as age of slaughter (Marcato *et al.*, 2006), sex (Elazeem and Shahin, 2005), nutrition (Toledo *et al*., 2004) and environment (Singh, 1996) were reported in the previous studies which significantly affect to birds performances. Their researches have shown the clear evidence how these factors affect to the dressing percentage, the breast, thigh, muscle parts, bones, skin, fat and abdominal offal development of the bird. (Moran, 1999), (Nikolova and Pavlovski, 2009).

As a common practice, large scale commercial hatcheries perform hatching egg grading according to weight. They only focus on chick quality when marketing. Vieira and Moran (1998) have stated that broiler chicks from heavy hatching eggs generally had a live performance advantage over those from light weight. But there are less evidences on how hatching egg weight effect on final broiler performances related meatiness.

In commercial broiler farms, over stocking and early thin out is a common practice. They stock bird as allowing $0.5ft^2$ of floor space per bird and at the age of 28 days thin out few thousands of birds as each remaining bird is given $0.6ft^2$ of floor space as recommended. Industry has increased CTVECPH efficiency by performing this practice throughout many years. As the internal environment of CTVECPH is created condition gradients by tunnel ventilation and evaporative cooling system, broiler performances can be vary in different living areas even inside a poultry house. If the effect can be identified, stocking and early thin out can be manipulated to gain higher efficiency of poultry house and high profitability.

Keeping the above conditions in view, the present study was undertaken to identify the influence of hatching egg weight and living area in CTVECPH on live broiler and post slaughter performances.

METHOD

Experimental Site

The study was carried out at New Anthoney's Farm's commercial broiler hatchery, Aththanagalla, Sri Lanka, commercial broiler farm, Lunugama, Sri Lanka and in poultry processing plant, Thittapattara, Sri Lanka.

Treatments

The experiment contains two treatments. The treatment combinations are;

Table 1: Treatment Combinations

W1A	Light weight (51g – 56g) + A (Distance from air inlet = 0 - 133ft)
W1B	Light weight (51g – 56g) + B (Distance from air inlet = 133 - 266ft)
W1C	Light weight (51g – 56g) + C (Distance from air inlet = 266 - 400ft)
W2A	Medium weight (57g – 63g) + A (Distance from air inlet = 0 - 133ft)
W2B	Medium weight (57g – 63g) + B (Distance from air inlet = 133 - 266ft)
W2C	Medium weight (57g – 63g) + C (Distance from air inlet = 266 - 400ft)
W3A	Heavy weight (64g – 70g) + A (Distance from air inlet = 0 - 133ft)
W3B	Heavy weight (64g – 70g) + B (Distance from air inlet = 133 - 266ft)
W3C	Heavy weight (64g – 70g) + C (Distance from air inlet = 266 - 400ft)

Experimental Design

100 day old chicks from each treatment combination were reared in Chore-Time closed tunnel ventilated evaporative cooling poultry house for 35 days.

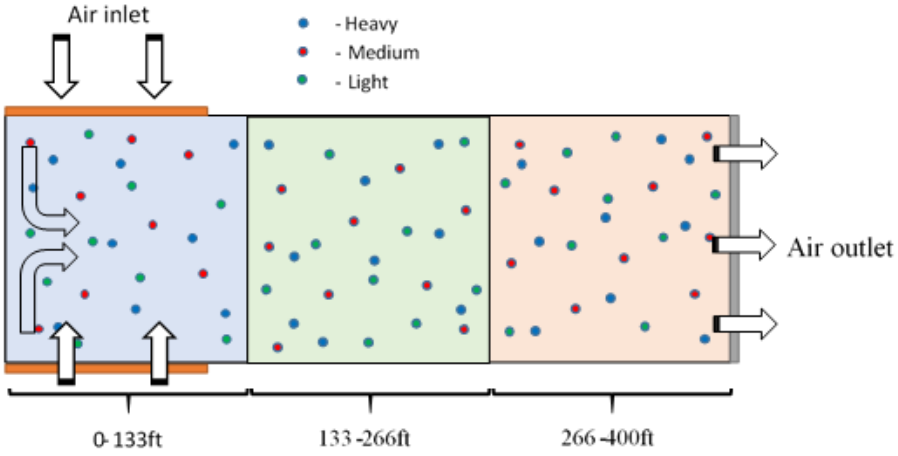

Figure 1: Experimental Design (chick placement)

Table 2:Chick Distribution among Treatments

Chore-Time™ Closed tunnel ventilated evaporative cooling poultry house		
Living area A	Living area B	Living area C
Light weight 100 chicks Medium weight 100 chicks Heavy weight 100 chicks	Light weight 100 chicks Medium weight 100 chicks Heavy weight 100 chicks	Light weight 100 chicks Medium weight 100 chicks Heavy weight 100 chicks

Aims and Objectives

Aims

1. To identify whether living area in CTVECPH or HEW effect to the final performances of broiler production.
2. To increase the efficiency of poultry houses by practicing over stocking and early thin out of high yielding birds.
3. To achieve these aims following objectives have set.

Objectives

1. Define the final broiler performances from three different areas in CTVECPH.
2. Define the final broiler performances from three different HEW classes.

Hatching Eggs

Hatching eggs were obtained from 37 weeks old parent flock of Cobb500. Hatching eggs were weighed and classified into three weight classes before place in setter. Eggs from three weight classes were incubated separately in Pas Reform SmartSetPro™ incubation system.

Chick Placement

Three hundred chicks from each hatching egg weight class were randomly selected. 100 chicks from each hatching egg weight class were placed in each living area in poultry house which were completely separated by placing partitioning net frames in between living areas.

Identification

Chicks in different hatching egg weight class were given three different easily identifiable permanent color markings on their neck area. Neck area was selected because plumage on neck remain comparatively for long time.

Just before catching randomly selected birds from each treatment combination were tagged using numbered cable tie on their leg just after the hock joint.

Feeding Schedule

Birds were fed commercial formulated ration, 1-7 days Broiler pre-starter 200C (Gold Coin Feed Mills, Colombo 15, Sri Lanka), 7-21 days Broiler starter 201C (Gold Coin Feed Mills, Colombo 15, Sri Lanka), 21-35 days Broiler finisher 202P (Gold Coin Feed Mills, Colombo 15, Sri Lanka). Birds were totally fed average of 3250g of feed per bird.

Slaughtering

Labeled birds were weighed before hanging on semi-automated slaughtering line. Birds were slaughtered and readings were taken throughout the processing line.

Data Collection

Data was collected throughout the rearing period and end of the rearing period at the processing plant. During rearing period, live weight of each and every treatment were measured in 7 days intervals. At slaughtering live weight, net weight, weight of breast, thigh and drumstick and length of keel, tibia and femur of randomly selected three birds from each treatment combinations will be measured. Weighing was done at slaughtering using digital lab scale (Mettler Toledo, Greifensee, Switzerland). Using collected data, weight gain, dressing percentage and meatiness of breast, upper thigh and lower thigh were calculated.

Data Analysis

The individual and joint analysis of variance and the averages were compared by the Duncan's Multiple Range Test at the level of 5% of probability with aid of the statistical software SAS™ (version 9.0). Standard error of means were computed using Microsoft Excel 2013.

RESULTS AND DISCUSSION

In order to achieve the objectives, the weights and lengths were measured and meatiness and dressing percentage were calculated. The results pertaining to the experimental investigation are presented below.

Live Weight

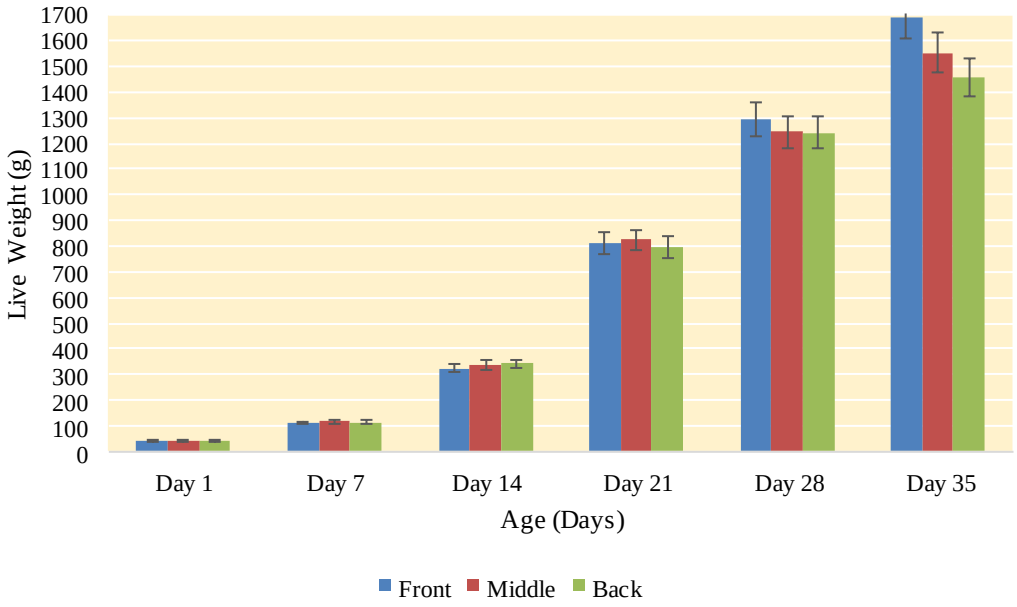

Figure 2: Live Weight According to Living Area

Live weight according to three different living areas in CTVECPH are illustrated in Figure 1. During the early life stages of broilers (Day 1-14), chicks in back area have gained higher weight than other two areas. It was significantly high in day 14. Chicks in front living area have gained less weight than other two areas during that period. Those can be resulted by sudden temperature drops caused by winchill effect from air inlet. After 21st day, chicks in front living area have started to gain higher weight until they slaughter. It was significantly higher than others after 28th day of age.

On 21st day chick's diet was changed to commercial broiler finisher pellets. Windusari *et al*., (2014) proved that metabolic activity of broilers increased concurrently with the increasing age. Also there are evidences on pellets feeding increases the growth rate of broilers (Zohair *et al*., 2012). Those may cause to increasing body heat generation. As their body temperature goes high, they take the advantage of living near the air inlet which supplies cooled humidified air into the house and effect of winchill effect (Czarick and P. Lacy, 1996) over chicks in back living area.

Figure 3 shows the live weight of broilers during their entire life. According to results which were obtained, chicks which were originated from heavy hatching eggs have gained higher weight than chicks from other two HEW classes. The results for live weight observed in chicks hatched from the three different weight classes agree with those of Ulmer-Franco *et al*, (2010) who considered hatching egg weight an accurate predictor of broiler live weight.

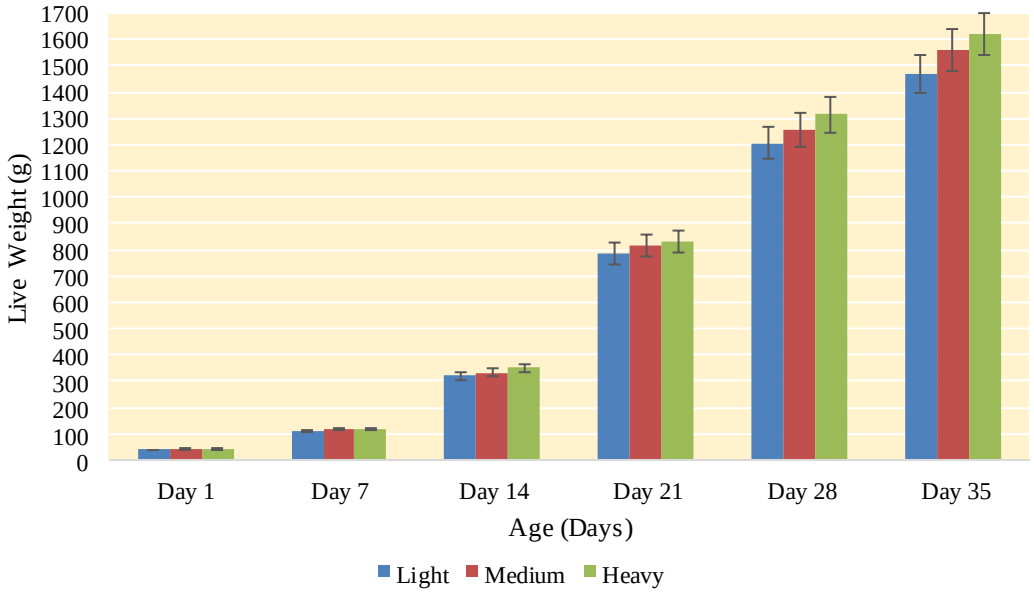

Figure 3: Live Weight According to Hatching Egg Weight

Relative Live Weight Gain

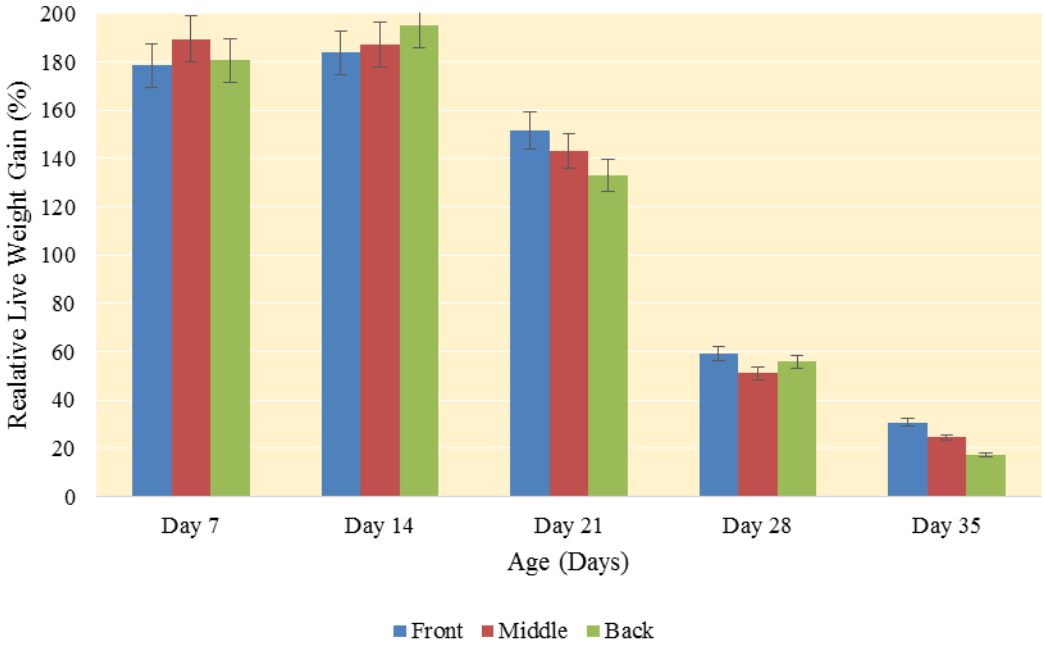

Figure 4: Relative Live Weight Gain According to Living Area

Figure 5 shows the relative live weight gain throughout the bird's life. As previously identified, chicks in front living area may face to stress of sudden temperature drops and it cause to their less relative weight gain than other two areas during early stages of life. But later part of their life they have gained significantly higher daily live weight gain than chicks lived in other two areas.

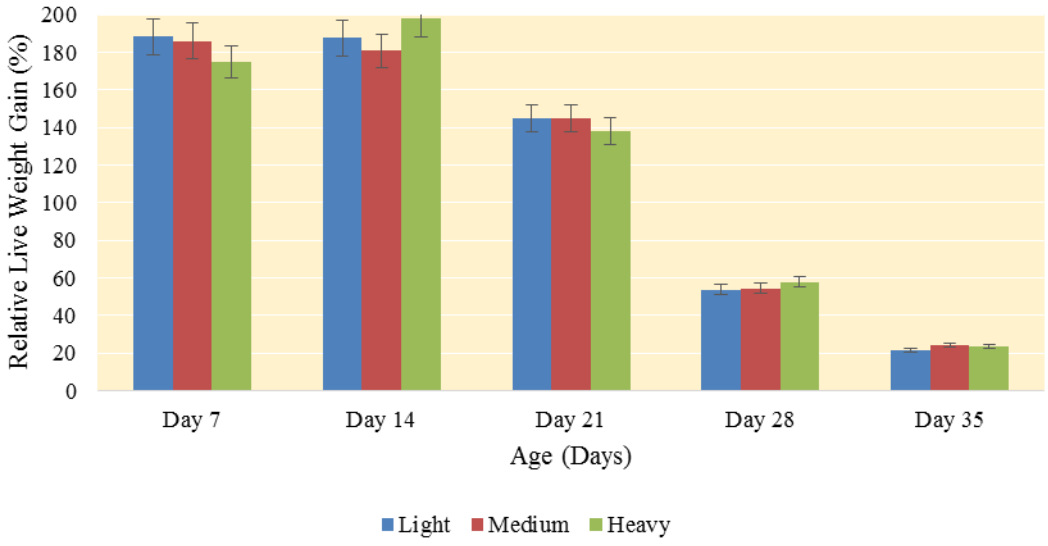

Figure 5: Relative Live Weight Gain According to Hatching Egg Weight

Results obtained by present study have shown in figure 5. In first week of chick's life, chicks from light and medium hatching eggs were gained higher relative live weight gain than others. During second week chicks from heavy hatching eggs have gained higher relative live weight gain than others. But rest of life, relative live weight gain of all three treatments were not shown significant differences.

Table 2: Final Measurements of Broilers after 35 Days of Rearing in Three Different Living Areas in CTVECPH.

Measurements and Calculations		Living Area			Hatching Egg Weight Class		
		Front (0-133ft)	Middle (133-266ft)	Back (266-400ft)	Heavy (64-70g)	Medium (57-63g)	Light (51-56g)
Live Weight(g)		1689.6± 42.25^{a}	1550± 26.38^{b}	1457.2± 25.63^{b}	1622.8± 50.06^{a}	1558.8± 18.12^{ab}	1467.6± 36.77^{b}
Dressing Percentage		82.01± 0.30^{a}	81.46± 0.20^{ab}	80.95± 0.25^{b}	81.96± 0.18^{a}	81.02± 0.23^{b}	80.75± 0.26^{b}
Meatiness	Breast	22.83± 0.62^{a}	19.91± 0.56^{b}	18.99± 0.87^{b}	21.6± 0.79^{a}	20.12± 0.32^{ab}	19.28± 0.49^{b}
	Upper Thigh	11.55± 0.55^{a}	11.14± 0.15^{a}	9.24±0.42^{b}	11.54± 0.21^{a}	10.86± 0.26^{ab}	10.6± 0.21^{b}
	Lower Thigh	5.04± 0.12^{a}	4.81± 0.07^{a}	4.34±0.18^{b}	4.88± 0.09^{a}	4.80±0.09^{a}	4.49± 0.12^{b}

* $p < .05$.

Effect on Pre-Slaughter Live Weight by Living Area and Hatching Egg Weight

The living area had significantly affected to pre-slaughter live weight. The highest mean live weight was resulted front living area (1689.6g) and other two areas live weight were middle living area (1550g), back living area (1457.2g). Front living area which had highest mean significantly differ from middle and back living area. But there was not a significant difference between middle and back living areas which having low pre-slaughter live weight.

The hatching egg weight had significantly affected to pre-slaughter live weight. The highest mean live weight was resulted heavy hatching egg weight class (1622.8g) and other two hatching egg weight classes live weight were medium hatching egg weight class (1558.8g), light hatching egg weight class (1467.6g). Heavy hatching egg weight class which had highest mean significantly differ from light hatching egg weight class. But there were no significant difference between heavy hatching egg weight class and medium hatching egg weight class and medium hatching egg weight class and light hatching egg weight class which having low pre-slaughter live weight. There are evidences which support this statement. Vieira and Moran (1998) states that broiler chicks from heavy eggs generally had a live performance advantage over those from small eggs.

Effect on Dressing Percentage by Living Area and Hatching Egg Weight

The living area had significantly affected to dressing percentage. The highest mean dressing percentage was resulted front living area (82.0163%) and other two areas dressing percentage were middle living area (81.4601%), back living area (80.9544%). Front living area which had highest mean significantly differ from back living area. But there were no significant difference between front living area and middle living area and middle living area and back living area which having low dressing percentage.

Birds which lived in back living area might subjected to a heat stress during later part of their life as they can't access to fresh cooled air from air inlets. That may cause to reduced dressing percentage. But present study results do not agree with those results of Skomorucha *et al*, (2010) who proved that broilers gain higher dressing percentage in elevated temperatures. But there are evidences on reduced dressing percentage is one of the consequences of exposing birds to high air temperature (Sokołowicz *et al*., 1996).

Concerning the dressing percentage, there was influence of hatching egg weight on the dressing percentage of broilers. The increase was from 80.758% (Light hatching egg weight) to 81.964% (Heavy hatching egg weight).

The Hatching egg weight had significantly affected to dressing percentage. The highest mean dressing percentage was resulted heavy hatching egg weight class (81.964%) and other two hatching egg weight classes dressing percentage were medium hatching egg weight class (81.022%), light hatching egg weight class (80.758%). Heavy hatching egg weight class which had highest mean significantly differ from medium and light hatching egg weight classes. But there was not a significant difference between medium and light hatching egg weight classes which having low dressing percentage. Disanayaka, (2013) has proven that, birds which were reared in CTVECPH show high dressing percentage than which were reared in open house. That means housing conditions significantly effect on dressing percentage.

Effect on Meatiness by Living Area and Hatching Egg Weight

Meatiness of Breast

The living area had significantly affected to meatiness of breast. The highest mean meatiness of breast was resulted front living area (22.8356) and other two living areas meatiness of breast were middle living area (19.9115), back living area (18.9968). Front living area which had highest mean significantly differ from middle and back living areas but there was not a significant difference between middle and Back living areas which having low meatiness of breast. There is a probability to create temperature gradients inside the CTVECPH and it may cause to reduce meatiness of breast of birds lived in back living area. But according to Gornowicz *et al*., (2007) there was not a significant effect of higher temperature on yield of breast muscle.

The hatching egg weight had significantly affected to meatiness of breast. The highest mean meatiness of breast was resulted heavy hatching egg weight class (21.604) and other two hatching egg weight classes live weight were medium hatching egg weight class (20.12), light hatching egg weight class (19.282). Heavy hatching egg weight class which had highest mean significantly differ from light hatching egg weight class. But there were no significant difference between heavy hatching egg weight class and medium hatching egg weight class and medium

hatching egg weight class and light hatching egg weight class which having low meatiness of breast.

Meatiness of Upper Thigh

The living area had significantly affected to meatiness of upper thigh. The highest mean meatiness of upper thigh was resulted front living area (11.5503) and other two living areas meatiness of upper thigh were middle living area (11.1401), back living area (9.2421). Front living area which had highest mean was not significantly differ from middle living area. But there were a significant difference between front living area and back living area and middle living area and back living area which having low meatiness of upper thigh.

The data pertaining to the average meatiness of upper thigh of three hatching egg weight classes are in figure 13. The hatching egg weight had significantly affected to meatiness of upper thigh. The highest mean meatiness of upper thigh was resulted heavy hatching egg weight class (11.548) and other two hatching egg weight classes meatiness of upper thigh were medium hatching egg weight class (10.862), light hatching egg weight class (10.506). Heavy hatching egg weight class which had highest mean significantly differ from light hatching egg weight class. But there were no significant difference between heavy hatching egg weight class and medium hatching egg weight class and medium hatching egg weight class and light hatching egg weight class which having low meatiness of upper thigh.

Meatiness of Lower Thigh

The living area had significantly affected to meatiness of lower thigh. The highest mean meatiness of lower thigh was resulted front living area (5.0424) and other two living areas meatiness of lower thigh were middle living area (4.815), back living area (4.341). Front living area which had highest mean was not significantly differ from middle living area. But there were a significant difference between front living area and back living area and middle living area and back living area which having low meatiness of lower thigh.

The hatching egg weight had significantly affected to meatiness of lower thigh. The highest mean meatiness of lower thigh was resulted heavy hatching egg weight class (4.884) and other two hatching egg weight classes meatiness were medium hatching egg weight class (4.8), light hatching egg weight class (4.492). Heavy hatching egg weight class which had highest mean significantly differ from light hatching egg weight class. But there were no significant difference between heavy hatching egg weight class and medium hatching egg weight class and medium hatching egg weight class and light hatching egg weight class which having low meatiness of lower thigh. When comparing open poultry houses and CTVECPH, birds from CTVECPH perform higher in pre-slaughter live weight, dressing percentage and meatiness parameters (Disanayaka *et al*., 2013). However this study proved that there is a significant variations of environment conditions inside a CTVECPH and ultimately broiler performances are affected by those condition variations.

While present study results do not agree with Gornowicz *et al*., (2007) who showed that there is no significant effect of high temperature on leg meat yield, Sosnówka-Czajka and Herbut, (1999) has examined high leg muscle yield in birds subjected to air temperature decrease. Those evidences are enough to endorse the results of present study.

CONCLUSION

Live and final performances of Cobb500 broilers were affected by living area in CTVECPH and HEW. Live weight, relative live weight gain, pre-slaughter live weight, Dressing percentage, Meatiness of breast, Meatiness of upper thigh and Meatiness of lower thigh were significantly increased in chicks which lived in front living area and originated from heavy hatching eggs.

To gain higher uniformity inside a CTVECPH it is recommended to place chicks originated from heavy hatching eggs in back area of CTVECPH and chicks originated from light hatching eggs in front area of CTVECPH. Chicks which are living near the cooling pads or front area of poultry house gain higher weight in less time duration than other middle and back area of poultry house. It is recommended to farmers to perform early thin out from front area of poultry house to gain higher live weight yield and profitability.

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ASSESSMENT OF COBB500 BROILER GRANDPARENT'S BODY WEIGHT ON EGG FERTILITY, BEHAVIOR AND TESTICULAR WEIGHT

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Abstract

Female broiler grandparent fertility is more important as the male fertility because the prime objective is to achieve around 25,000 broiler chicks from one female grandparent breeder. Broiler breeder (Cobb500 fast feather) grandparents (male : female=50:500) from three different female body weights (underweight, middleweight and overweight) with middleweight males at different ages (34, 38, 42 and 46 weeks) were studied for egg fertility with 219 eggs (n=657) . Three groups based on the body weight (underweight, middleweight and overweight) of 3 males (n=9) at three different age levels (34, 40 and 46 weeks) were studied for testicular weight. Middleweight male behaviors were observed at three different ages (34–37, 38-41 and 42–45 weeks) towards underweight, middleweight and overweight females. Middleweight females showed the highest egg fertility at each age levels. Egg fertility was reduced with age of females. There was a significant ($P<0.05$) effect of male body weight and age on testicular weight. Testicular weight was reduced with age. Testicular weight reduction was higher in underweight and overweight. At age of 42–45 weeks, there was a significant ($P<0.05$) effect in mounting without mating behavior towards female body weight of underweight and overweight. At age of 38–41 weeks, there was a significant effect ($P<0.05$) in lying behavior at female body weight levels of underweight and middleweight. Female broiler breeder body weight deviation (±10%) from standard body weight is directly affected on the egg fertility in initial part of the production (34-46 weeks). Male broiler breeder body weight has to be maintained in middleweight (within ± 10% from standard weight) in production period to keep optimum testicular weight.

Keywords: Age, behavior, body weight, broiler breeder grandparents, egg fertility, testicular weight

INTRODUCTION

The genetic selection of broiler breeder lines (*Gallus gallus domesticus*) for the production of meat is primarily focused on producing broiler chickens that can obtained high slaughter weight with a reduced feed conversion ratio. These production successes of the final product (the broiler) are contrasted with the reproductive rates of the breeding stock as seen in a decline in fertility in the last weeks of the life (Vizcarra *et al*., 2010; Kirk *et al*., 1980).

Many known factors play a role in broiler breeder fertility, including the physical condition of the birds (Bowling *et al*., 2003; McDaniel *et al*., 1981), the ratio of males to females (Hazary *et al*.,2001), nutrition, the growth curve, and environmental factors such as disease and temperature (McDaniel *et al*.,1995). Siegel *et al.* (1985) observed a negative relationship between breast yield and fertility, whereas Hocking and Duff (1989) found that a decline in fertility was caused by a reduction of

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overall reproductive fitness. Bowling *et al*. (2003) also reported a negative relationship between sperm mobility and body weight.

Uniformity is measure of the amount of body weight variation in a flock. Attainment of the breeder target body weight at any specific age and flock uniformity are the two most important criteria of pullet quality. Flock uniformity is the percentage of birds that are within ±10% of the average flock body weight recommended for a particular age. The goal of the producer is to have 80% of the pullets within these specified ranges. Flocks with high uniformity will reach peak egg production earlier, and have higher peaks than those of low uniformity values. Poor uniformity is associated with variation in the degree of sexual maturity of hens, where underweight pullets have delayed onset of egg production and overweight ones have an early start (Yuan *et al*., 1994).

Ross (2004) observed the body weight of the roosters affect the fertility values, not only for the influence on different spermatic parameters, for the effect on the extremities of these birds because some of the excessive weight on the males can result in injuries (dyschondroplasia and destruction of articular cartilage that leads to rupture of tendons and ligaments), and can therefore negatively impact the hatch percentage for the flock. This correlation of fertility with the integrity of the limbs of the males to be true in poultry considered to be obese and in poultry that was slightly overweight. In contrast, at the other extreme, it has also been widely reported that animals with a low body weight often have under developed testes and are considered sub fertile from the point of a certain threshold.

Bilcik *et al*. (2005) have observed that the fertility level and the number of matings were greater in individual males being with females than other males, which indicates the importance of competition between roosters and consequences that this can have on fertility.

The daily number of copulations is influenced by the libido of the roosters which in turn is determined by their hormone levels, especially testosterone. Although the maximum values were found between roosters 33 to 40 weeks of age, a gradual decrease was seen until reaching minimum vales at the end of the life (Vizcarra *et al*., 2010).

Ross (2008) reported that fertility was peaked between 30 and 38 weeks of age and fertility declined at the 45 weeks. Testes size is highly correlated with fertility, poor fertility often being associated with small testes. It is therefore vital to ensure that management does not inhibit the development of the testes at any stage.

The body weight of the broiler breeder males normally doubles during the life of the flock, starting at approximately 2500g (from 6 weeks of age as consequence of the broilerization program used for grandparent selection) to approximately 5000g at 55 weeks of age. The testicular weight undergoes an explosive increase in size between 19 and 23 weeks of age, and this increase in testicular mass (total weight of both testes) will be from approximately 1g to more than 50g. Later with the occurrence of testicular regression, approximately 44% of the weight can be lost between 36 and 55 weeks of age, resulting in a final total testicular weight of 29.5g at the end of the rooster life. The overweight poultry with good testicular development, sperm production and libido, and they may be unable to perform a complete mating with the hen because of the excessive body weight. On other extreme, roosters with lower body weight, poor testicular development and low sperm production may exist within the flock and have a low level of sexual activity because of their unfavorable social position and subordination. As a result, roosters in either subcategory (overweight or underweight) can lead to a decreased fertility on the farm (Fragoso *et al*., 2012).

Many studies have conducted to relate to identify the impact of broiler male fertility, but the impact of female broiler on the egg fertility is less identified and studied.

This study is conducted to achieve the following objectives to identify the effect of female broiler breeder body weight on egg fertility at different age levels, to determine the broiler breeder male sexual and activeness behaviours towards different female breeder's body weight levels and to determine the effect of male broiler breeder's body weight and age levels on testicular weight.

METHODOLOGY

Stocks and management

All observations took place at the Fortune G-P Lanka (LTD) Farm, Galewela, Matale. Cobb500 fast feather (grandparent stock) broiler breeder male (n=50) along with Cobb500 fast feather females (n = 500) were used for this study.

Three treatment groups were used based on the body weight of birds and designated as underweight (UW), middleweight (MW) and overweight (OW). Each treatment was consisted with the age levels of as 34 week, 38 week, 42 week and 46 week and weight.

Housing and Feeding

Birds used in this trial were reared in the dark-out production house in a production facility that was a closed house with concrete floor and evaporative cooling system. There were eight ventilation fans in the building. The house was used negative pressure mechanical air flow system. The speed of air flow was 2–3 m/s and the speed of fans were 650–700 rpm. The average internal house temperature was 28^0C and the average pressure was 60Pa. The average relative humidity was 60–75%. The length of a house was 133m and width is 13m, central height was 3.65m and side height was 26m. Lighting schedule was maintained during the experimental period based on Cobb500 broiler breeder management guide (Table 1).

Table 1: Recommended lighting program in dark-out production house

Age (weeks)	Age (days)	Light hours	Light intensity (lux)
1 to 3	Day old to 21	Decreasing from 24 hours a day 1 to 8 hours by 14-21 days	Days 0-2 maximum light (>20 lux) reducing to 20 lux by day 7
3 – 20	21 – 140	8	5 – 10
20 – 21	140 – 147	11	40 – 60
21 – 22	147 – 154	13	40 – 60
22 – 23	154 – 161	14	40 – 60
23 - 60	161 - 420	15	40 – 60

The house contained separate feeders for the males and females to control body weight of male and female, feed was provided ad libitum (Table 2), and nests were provided to facilitate egg collection.

Table 2: Recommended nutrients level (% per 1000 kcal/kg metabolize energy) in given feed

Phase	Units	Starter	Grower	Pre-breeder	Breeder 1	Breeder 2
Age						
(days)		0 – 28	29 – 128	127–154	155 –280	281+
(weeks)		0 - 4	5 - 19	19 - 22	23 - 40	41+
Crude protein	%	18.96	16.01	15.99	5.99	14.99
Calcium	%	0.358	0.383	0.524	1.048	1.119
Phosphorus	%	0.160	0.156	0.157	0.157	0.139
Potassium	%	0.215	0.232	0.227	0.227	0.209
Sodium	%	0.067	0.075	0.066	0.066	0.066
Chloride	%	0.067	0.075	0.066	0.066	0.066
Linoleic acid	%	0.436	0.430	0.419	0.454	0.349
Lysine	%	0.359	0.230	0.260	0.262	0.255
Methionine	%	0.158	0.104	0.117	0.123	0.120
Tryptophan	%	0.079	0.058	0.065	0.066	0.064
Threonine	%	0.251	0.191	0.216	0.199	0.194
Arginine	%	0.377	0.230	0.260	0.236	0.230
Valine	%	0.241	0.173	0.195	0.210	0.204

Isoleucine	%	0.251	0.191	0.216	0.199	0.194
Leucine	%	0.424	0.299	0.338	0.293	0.286
Histidine	%	0.115	0.076	0.086	0.089	0.087
Phenylalanine	%	0.233	0.150	0.169	0.173	0.168

Experimental Design

There were three pens based on the body weight of female breeder parents; underweight, middleweight and overweight. Each pen was contained 500 females and 50 males. The female to male ratio was maintained at 10:1.

Only the female birds were categorized weight, as underweight, middle weight and overweight at the age start of 25 weeks. The male birds in each pen were maintained at middleweight (Table 3).

Standard body weight at 25 weeks, male is 3770g and female is 3140g (Cobb500 breeder management guide, 2008).

Table 3: Cobb500 broiler breeder body weight levels at 25 weeks

Male	Female		
MW (in between ±10% from standard weight)	UW (below -10% from standard weight)	MW (in between ±10% from standard weight)	OW (above +10% from standard weight)
3393g – 4147g	Below 2826g	2826g – 3454g	Above 3454g

Body Weight Measurement

Birds were weighed individually of the final grading at 25 weeks of age. Female birds were divided to underweight, middleweight and overweight pens according to the Table 3 weight levels. Middle weight males were kept in each pen according to the Table 4.

Table 4: Average body weight at each age levels under each body weight categories

Age (weeks)	Male standard body weight (Cobb guide, 2008)	Body weight categories			
		Male	Female		
		Middleweight	Underweight	Middleweight	Overweight
34	4320g	4431g	3548g	3654g	3928g
38	4420g	4222g	3778g	3810g	3934g
42	4520g	4524g	4037g	4062g	4149g
46	4620g	4612g	4106g	4190g	4226g

Average female weight at 34, 38, 42 and 46 weeks of age were obtained by using 20% sample of population. During the study, 100 females were weighed from each body weight levels (Table 4). Average male weight at 34, 38, 42 and 46 weeks of age were obtained by using total number of males in each pen (50 males).

Weight was measured by using UWE scale (serial number: hs0022566). Birds were weighed before feeding at the afternoon (13:30).

Egg Samples Collection and Storage

The egg samples were collected at each 34, 38, 42 and 46 weeks of age. Egg sample size for the replicate was 219. Three replicates from each age (34, 38, 42 and 46 weeks) under the each body weight levels were collected within first three days of the given age weeks. 219 eggs were randomly collected from each pen (UW, MW and OW pens). Collected eggs were cleaned with wet cotton cloth to remove debris. Cleaned egg samples were fumigated by formaldehyde gas with 20 minutes by using 20ml Formalin and 60g Potassium Permanganate. Fumigated egg samples were stored in cool room at 19^0C temperature. Stored eggs were labeled before send to the hatchery to identify the each sample clearly at hatchery. Eggs were transported to hatchery [Hatchery, Fortune GP Farms Lanka (LTD)] on fourth day of the week at 08:30 from the farm. Egg transporting lorry arrived to hatchery within 45 minutes. Driving speed of the vehicle was maintained at 20 – 30 km/hour. Eggs were fumigated for 20 minutes by Formaldehyde gas before stored few hours under 16^0C temperature until placed in the setter. Eggs were transferred to pre heating room (27^0C) for 30 minutes before send to setter machine (Capacity: 14,112 hen eggs, Number of egg trays: 112, Overall dimensions in mm: 2385 x 2185 x 2030 (L x W x H), Floor space: 5.2 sq. meter, Water requirement: 5 litres per hour)

Composite sampling technique was used to prepare the replicates to give same egg age condition for each three replicates. Eggs were placed in setter trays (150 eggs containing trays) in normal procedure.

Egg Fertility Checking

Egg candling was practiced 18th day of the incubation period. The manual Farm Innovators Model 3300 Egg Candler Machine was used for the process. Fertile and infertile eggs were recorded according to following observations, fertile eggs as (i) the embryo was located in the large end of the egg, where the blood vessels radiate under the surface of the egg shell, (ii) the embryo appears as a dark spot that becomes larger, eventually only a dark mass and the aircell were seen and infertile eggs as (iii) brightly transmits light.

Testicular Measurement

Testicular weight was measured to the nearest 0.0g. Birds were sacrificed by using cervical dislocation method. Birds (n=9) as 3 birds from each weight category at each age level as recorded at 34, 40 and 46 weeks old birds at underweight, middleweight and overweight males. Weight was measured by digital electrical scale (power 2xAAA batteries, tare full capacity). Standard body weight at 34 weeks, 40 weeks and 46 weeks were according to the Cobb guide, (2008). Overweight males were taken +10% deviations from standard weight. Underweight males were taken -10% deviations from standard weight (Table 5).

Table 5: Male body weight for testicular weight measurements

Age (weeks)	Body weight level	Replicate number	Body weight (g)
34	Underweight	01	3880
		02	3880
		03	3880
	Standard weight	01	4320
		02	4320
		03	4320
	Overweight	01	4750
		02	4750
		03	4750
40	Under weight	01	4020
		02	4020
		03	4020
	Standard weight	01	4470
		02	4470
		03	4470
	Over weight	01	4920
		02	4920
		03	4920
46	Under weight	01	4160
		02	4160
		03	4160

Standard weight	01	4620
	02	4620
	03	4620
Over weight	01	5080
	02	5080
	03	5080

Behavior Observation of Males

Male behaviors such as mating, mounted without mating, moving and lying were observed by using an ethogram (Table 6). Scan sampling technique at every 10 minutes interval was practiced. All behavioral observations took place at the same time of day in the late afternoon because mating behavior peaks in the late afternoon to early evening hours (Lake *et al*., 1956; Moyle *et al*., 2010). Behavior observations were taken in morning at 06:30 to 08:00 and in evening at 17:00 to 18:30 with every 10 minutes frequencies. Behavioral parameters were observed weekly, at 34 weeks and continuing to 46 weeks.

Table 6: Ethogram used to observe the male behavior

Behaviour	Description
Mating	The male mounted, gripped, and trod a female and appeared to achieve cloacal contact. The female ruffled her feathers following the male's dismount.
Mounted without mating	The male approached a female and placed one or more foot on her back. The female avoided the male, and no further elements of the copulatory sequence were observed.
Moving	Birds who are running here and there and walking around the pen
Lying	Birds lying on litter, feeders
Pecking, stirring up litter, scratching feathers etc.	All the other behaviours than mentioned above

Statistical Analysis

The experimental data were analyzed by using ANOVA (SAS 9.0). Means of fertility were separated by Duncan's Multiple Range Tests. Means of the behavior were separated by Tukey's Studentized Range Test.

RESULTS AND DISCUSSION

Egg Fertility

Table 7: The effect of body weight levels on egg fertility at the different age levels

Age levels (weeks)	UW	MW	OW	P value
34th week	88.58c ±0.27	96.04a ±0.15	92.23b±0.27	< 0.0001
38th week	88.89c ±0.40	93.61a±0.27	91.93b ±0.15	< 0.0001
42nd week	84.17c ±0.15	90.41a ±0.27	88.58b ±027	< 0.0001
46th week	83.56b ±0.27	85.84a±0.27	81.28c ±0.27	< 0.0001

Different letters show statistically significant ($P < 0.05$) differences among the treatments. Means with the same letter are not significantly different. (UW - Underweight, MW – Middleweight, OW – Overweight)

When considering the egg fertility in 34, 38, 42 and 46 weeks of age under the UW, MW and OW body weight levels, there was a significant difference among ($P < 0.05$) the treatments.

At the 34^{th,} 38th and 42nd weeks' egg fertility % was highest in the MW group and the lowest egg fertility % was observed in UW group (Table 7).

At the 46th weeks the egg fertility % was highest in the MW group, lower egg fertility % was observed in the OW group, while the UW group egg fertility was higher than the OW and lower than the MW groups (Table 7).

Considering the egg fertility mean values (Table 7), MW group was the highest fertility % shown at 34, 38, and 42 and 46 weeks of age, OW group was shown the lower egg fertility % than 34, and 42 weeks of age levels but at 46th week's OW group shown the lowest egg fertility % (Table 7), UW group was shown lowest egg fertility % at 34, 38 and 42 weeks of age levels, at the 46th weeks of age level UW group was shown lower egg fertility % than MW group and higher than OW group (Table 7). Fertility% had been reduced from 34th, 38th, 42nd and 46th weeks of age in the all three body weight level groups (UW, MW and OW).

Table 8: The effect of age levels on egg fertility at the body weight levels

Body weight levels	34th week	38th week	42nd week	46th week	P value
UW	88.58[a] ±0.27	88.88[a] ±0.40	84.17[b] ±0.15	83.56[b] ±0.27	< 0.0001
MW	96.04[a] ±0.15	93.61[b] ±0.27	90.41[c] ±0.27	85.84[d] ±0.27	< 0.0001
OW	92.23[a] ±0.27	91.93[a] ±0.15	88.58[b] ±0.27	81.28[c]±0.27	< 0.0001

Different letters show statistically significant ($P < 0.05$) differences among the treatments. Means with the same letter are not significantly different. (UW - Underweight, MW – Middleweight, OW – Overweight)

When considering the UW group there wasn't a significant deference in between 34th week of age and 38th week of age and in 42nd week of age and 46th week of age. There was a significant difference ($P < 0.05$) among each age levels of the MW and OW body weight groups.

Robinson *et al.* (1993) found, the egg fertility was reduced in overweight female broiler breeders due to reduced mating success (which limits sperm transfer to the female), by a reduction in the duration of fertility, and possibly by impaired sperm transport to the site of fertilization (because the normal passage of developing eggs is more random than in egg-type hens). The production of settable eggs was limited by poor shell quality as a result of a lack of coordination of the shell calcification process, and by a high incidence of multiple-yolked eggs. Early embryonic mortality was high in the eggs of overweight hens, as such eggs were often poorly calcified, which results in increased shell porosity and egg weight loss. Overweight hens exhibit short laying sequences.

Laying high incidence of multiple-yolked eggs reduce the egg quality and poor shell quality as a result of a lack of shell calcification process cause for early embryonic deaths (Robinson *et al.*, 1993). It was very difficult to identify the small undeveloped embryos at candling date because of spoilage. Therefore, those were calculated as infertile eggs.

Renema *et al.* (1999) observed, the both oviduct and ovary weight correlated with body weight indicating an effect of body size or body weight on reproductive tract development. Underweight breeders had a poor developed reproductive tract and it cause to reduce the fertility.

Age has an adverse effect on the reproductive success of birds (Mather and Laughlin, 1979; Noble *et al*., 1986; O'Sullivan *et al*., 1991; Latour *et al*., 1996). The age-related decrease in avian fertility is due, in part, to a decline in egg production (Bahr and Palmer, 1989; Etches, 1990; Robinson *et al*., 1990). Lerner *et al*. (1993) reported a decline in fertility occurs once the species-specific maximum for egg production has been reached. While fertility over 95% can be achieved at the beginning of the reproductive period, fertility declines with increasing age after 45-50 week of age in broiler breeder flocks (Hocking and Bernard, 2000). Peak egg production of breeders occur at 32nd – 33rd weeks of ages (77% or 78%) normally at that period the egg fertility is optimum (90%) and slowly increase further and when near to the 40th weeks of age it going to be reduced [according to Fortune GP Farms Lanka (LTD) Farm and Hatchery records (2015)].

Testicular Weight

Table 9: The effect of age levels on testicular weight in three different body weight levels

Body weight levels	34th week	40th week	46th week	P value
UW	28.22[a] ±0.23	23.56[b] ±0.45	18.18[c] ±0.22	< 0.0001
MW	40.41[a] ±0.34	37.40[b] ±0.26	35.10[c] ±0.16	< 0.0001
OW	52.74[a] ±0.37	28.63[b] ±0.50	18.67[c] ±0.18	< 0.0001

Different letters show statistically significant ($P < 0.05$) differences among the treatments. Means with the same letter are not significantly different. (UW - Underweight, MW – Middleweight, OW – Overweight)

The effect of age (34th, 40th and 46th weeks) on testicular weight there was a significant difference ($P < 0.05$) among the age levels at the UW, MW and OW levels.

Testicular weight reduced of UW, MW and OW body weight groups from 34th week to 46th week. Testicular weight reduction was higher in OW group than MW and UW group, testicular weight reduced slowly in MW males than UW and OW males.

Table 10: The effect of body weight levels on testicular weight at age levels

Age levels (weeks)	UW	MW	OW	P value
34th week	28.22^{c} ±0.23	40.41^{b} ±0.34	52.74^{a} ±0.37	< 0.0001
40th week	23.56^{c} ±0.43	37.40^{a} ±0.26	28.63^{b} ±0.50	< 0.0001
46nd week	18.18^{b} ±0.22	35.10^{a} ±0.16	18.66^{b} ±0.18	< 0.0001

Different letters show statistically significant ($P < 0.05$) differences among the treatments. Means with the same letter are not significantly different. (UW - Underweight, MW – Middleweight, OW – Overweight)

Considering the effect of the body weight on testicular weight there was a significant difference ($P < 0.05$) among UW, MW and OW body weight groups at the three age levels of 34th, 40th and 46th weeks.

When considering the 34th week of the age, OW group showed the highest testicular weight and MW group showed the testicular weight higher than UW group. At the 40th and 46th weeks of age highest testicular weight was observed in the MW group and the lowest testicular weight was observed in the UW group. OW group's testicular weight was higher than UW group and lower than MW group. At the age of 46th week the testicular weight of the OW group and was more or less similar to the UW group. Powley (2008) observed significant growth of the testes occurs in the first three weeks after the first light stimulation and he further mentioned testes weight peaks around 28-30 weeks, beyond 35 weeks there a natural decline in testes size and infertility occurs, the rate of this decline is accelerated if management is poor, after 30-35 weeks of age there is a natural reduction in testes weight and sperm production, and a decline in fertility, there is a clear link between bodyweight, testes weight and fertility and indeed, as has been shown, heavy over fleshed males often have sub-optimal testes development.

Hocking (1990) found low bodyweights or a loss of body condition/weight is associated with low testis weight. This may have a negative impact on fertility as smaller testis is associated with poorer fertility. Mcgary *et al*. (2005) found the levels of testosterone and corticosterone determine the testicular development of the roosters and their behavior. Body weight and conformation, testicular development and spermatogenesis, hierarchal relationships were established between roosters and their hormonal levels. The roosters that with the extremely low testicular weights corresponded to animals that possibly never reached their optimal body weight at any stage of their development. Testosterone and corticosterone levels increased progressively from week 24 to week 33 of age and remained at this higher level until approximately 40 weeks of age. From this point, and began to decline significantly. Regression in testicular weight of 44.54%, which was accompanied by a decrease in sperm production and an increase in body weight between 36 and 55 weeks of age (Fragoso *et al*., 2012).

Male Behavior

Table 11: Effect of body weight level on male behavior at different age limits

Age limit (weeks)	Behavior	UW	MW	OW	P value
34 - 37	Lying	11.86±0.74	12.72±0.76	13.36±1.23	0.55
38 - 41	Lying	17.56^{b}±0.53	19.86^{a}±0.73	18.78^{ab}±0.36	0.05
42 - 45	Lying	20.22±0.36	21.44±0.22	20.47±0.46	0.09
34 - 37	Moving	14.66±0.46	16.06±1.40	14.58±0.70	0.49
38 - 41	Moving	15.00±0.58	15.28±0.85	15.67±0.73	0.81
42 - 45	Moving	14.67±0.65	14.78±0.49	14.64±0.51	0.98

34 - 37	Mating	5.14±0.17	5.41±0.20	5.36±0.25	0.63
38 - 41	Mating	5.14±0.16	5.47±0.16	5.25±0.19	0.42
42 - 45	Mating	4.63±0.12	4.69±0.09	4.61±0.14	0.88
34 - 37	Mounting without mating	4.50±0.32	4.91±0.31	4.61±0.13	0.55
38 - 41	Mounting without mating	3.56±0.07	3.61±0.10	3.78±0.12	0.33
42 - 45	Mounting without mating	3.08^{b}±0.05	3.27^{ab}±0.05	3.50^{a}±0.09	0.008

Different letters show statistically significant ($P < 0.05$) differences among the treatments. Means with the same letter are not significantly different. (UW - Underweight, MW – Middleweight, OW – Overweight)

Considering the lying behavior of the 34 – 37 and 42 – 45 weeks of age limits there wasn't significant difference ($P > 0.05$) among the body weight levels (UW, MW, OW). At the 38 – 41 weeks of age level there was a significant difference ($P < 0.05$) between UW and MW groups of males.

Considering the moving behavior of the 34 – 37, 38 - 41 and 42 – 45 weeks of age limits there wasn't significant difference ($P > 0.05$) among the body weight levels (UW, MW, OW).

Considering the mating behavior of the 34 – 37, 38 - 41 and 42 – 45 weeks of age limits there wasn't significant difference ($P > 0.05$) among the body weight levels (UW, MW, OW).

Considering the mounting without mating behavior of the 34 – 37 and 38 - 41 weeks of age limits there wasn't significant difference ($P > 0.05$) among the body weight levels (UW, MW, OW). However, at the 42 – 45 weeks of age level, there was a significant difference ($P < 0.05$) between UW and OW groups of males.

CONCLUSION

Middleweight grandparent females show the highest egg fertility % ($P<0.05$) than overweight and underweight female grandparents in all four age levels. Egg fertility was reduced with the age ($P<0.05$). There is a significant ($P<0.05$) effect of grandparent male body weight on testicular weight at all age level. Testicular weight reduce with age ($P<0.05$). There is a significant ($P<0.05$) effect in the mounting without mating behavior of males towards female body weight of underweight and overweight levels of 42 – 45 weeks of age limit. There is no effect from the female body weight levels to mating and moving behaviors of males. There is a significant ($P<0.05$) effect in the lying behavior of males towards female body weight levels of underweight and middleweight at the age limits of 38 – 41 weeks. Female broiler breeder body weight deviation (±10%) from standard weight during end of the rearing period (25 weeks) is directly affect for the egg fertility in initial part of the production (34 to 46 weeks). Male broiler breeder body weight has to be maintained in middle weight (within ± 10% from standard weight) in production period to keep optimum testicular weight.

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PREVALENCE OF RODENT INTESTINAL PARASITES AND RISK PERCEPTION OF ZOONOTIC INFECTION IN RESIDENTS OF GARHWAL, UTTRAKHAND, INDIA

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Abstract

An investigation was undertaken on sanitary risk due to prevalence of parasites in commensal rodents, living in close proximity of the human population. The study was conducted from April 2011 to March 2014 in Garhwal region of Uttrakhand, India. A total 749 murine rodents sampled from various habitats of two major cities Dehradun and Hardwar. Three hundred residents of the two cities completed a questionnaire survey on some aspects of rodent's parasitism such as parasite species; mechanisms of infection, awareness of zoonotic diseases caused by rodents. Overall prevalence of infection was (63.7%), and rodent Species contribution was 80.7% (*Rattus rattus)*, 11.6% (*Rattus norvigicus)*, and 7.7% (*Mus musculus*).There was a significant difference in the overall prevalence of infection in rats between the two cities ($P<0.05$). Prevalence % of infection was associated with specific rat species ($P<0.05$). In the sampled rats a total 13 taxonomic group of parasites (6 Cestodes, 5 nematodes, 1 acanthocephalan, and 1 species of protozoa) were obtained. A high prevalence of the *Cysticercus faciolaris, Taenia taeniaeformis* and *Giardia* was observed and the majority of the rats were poly parasitized. The predominant zoonotic species in rodent were *Hymenolepis nana, Hymenolepis diminuta, Strongyloides,* and *Giardia*. It was concluded that the risk of zoonotic infection by rodent intestinal parasite was sufficient to have the potential to cause an outbreak in human population.

Keywords: Rodents, prevalence, zoonotic, poly parasitized

INTRODUCTION

Rodents are key mammalian group and are highly successful in adapting many environments throughout the world. Rats belonging to the family muriadae harbor a number of helminthes parasites, which can be transmitted to man and other vertebrates. They are major vectors of human and domestic animal diseases worldwide. Studies have also implicated several species of rat as transmission agents of some helminth parasites of man and domestic animals (Alicata, 1969; Malhotra, 1986; Udonsi, 1989).

They may harbour micro-organisms that can be transmitted either through contact with infected rodent urine or faeces, The two dominant commensal rat species, the brown rat (*Rattus norvegicus*) and the black rat (R. *rattus*), are distributed worldwide (Macdonald *et al.,* 2005).

The eggs of parasites are passed out in rodent droppings in fields, grain stores and amongst food stuff in houses, and are responsible for disease spread (Khatoon *et al.,* 2004). The objective of the present study was to monitor sanitary risk due to prevalence of parasites in commensal rodents, living in close proximity of the human population in Dehradun and Haridwar, Uttarakhand, India.

MATERIALS & METHODS

Study Area: Dehradun is the capital of new state, Uttrakhand. Now a days, construction is going on and trees are cut off as a result rats are moving towards the city structure for their intellectual nourishment, Whereas, Hardwar is a populous

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district and is a holy place wherein peoples visit throughout the year .

Rat sampling: Rat sampling was conducted during the period of three consecutive years from April 2011to January 2014. Both wooden and metal cages (17 x 19.5) were used to trap rats. The trapped rats were kept individually in laboratory cages for 4-5 days for fecal examination.

Parasites examination: Direct smear and Flotation or Concentration method were used for the helminths egg identification. Infected animals were anaesthetized with diethyl ether and dissected. The viscera was exposed via a mid-ventral incision and inspected microscopically. The liver, small and large intestine was examined for parasites. Helminthes were fixed in 70% alcohol /AFA and identified with the help of (Yamaguti, 1959, Soulsby, 1982). The data was analyzed by Chi- square test for prevalence of infection.

CROSS-SECTIONAL STUDY

A questionnaire based cross-sectional study design was employed to look on the perception of the public on zoonotic infection. The 300 respondents were stratified into three groups, namely, lower class, middle class & upper middle class. A specifically designed questionnaire on health risk awareness was handed out between the residents of both the city. All data collected were subjected to Chi-square test (χ^2). The level of significance was set at P<0.05.

RESULTS

Total 749 rats were sampled from 4 city structures: grain stores, departmental store / vegetable shops, slum areas and posh residences from both the cities. The sampled rats comprised of three species, *R. rattus* accounted for 71.7% *R. norvegicus* 12.1% and *M. musculus* 16.2%.

Table 1: Rat species and % prevalence of infection

Rat spp	Total rats N=749	Infected N=477	Not infected	% prevalence of infection N=477(63.7%)
Rattus rattus	537	385	152	71.7%
Rattus norvegicus	91	55	36	60.4%
Mus musculus	121	37	84	30.6%

There is a significant difference in the overall prevalence of infection in rats between the two cities (P<0.05). Prevalence % of infection is statistically significant at p<.05 x2=6.67 p=.0098.

Table 2

City	Rat spp.	Sampled rats	infected	Not infected
Dehradun	*Rattus rattus*	235	150(63.8)	85(36.2)
	Rattus norvegicus	91	55(60.4)	36(39.6)
	Mus musculus	54	20(37.0)	34(63.0)
Hardwar	*Rattus rattus*	302	235(77.8)	67(22.2)
	Mus musculus	67	17(25.4)	50(74.6)
	Total	749	477(63.7)	272(36.3)

Four zoonotic genera of helminthes (*H. diminuta, H.nana, Taenia taeniaeformis, Strongyloides*) & one protozoan (Giardia) was observed in sampled rats (Table-3).Trichuris was the prevalent group in posh localities in Hardwar. *H.dimunata* was in 67% Rats. The endo-parasitic infestation also varied with respect to city structure.

High intensity of capillaria spp. eggs seen in the pathological section of liver in the rats (52%) captured from three city structures except posh locality of both the city. The commonest nematode was *Trichurus muris* (68.3%) fallowed by Capillaria hepatica in the rats sampled rats from hardwar city. Multiple cystic structures of *C. faciolaris* (62.9%) and eggs deposition of capellaria was frequently seen in the rats captured from hardwar city. The following parasites and their respective prevalence were detected: strongyloides (64.4%), Giardia (44%), Toxocara (43%), *Trichuris muris* (68%), *H.diminuta* (67%), *T.taeniaformis* (46%), H nana (35.6%) and Capellaria eggs (+++). Rats trapped from haridwar were found polyparasitized and presented higher prevalence of *C.faciolaris, T. taeniaformis* and *Giardia.*

Table 3: Prevalence of Intestinal Parasites examined in sampled Rats

	Name of parasite	Positive hosts (N=477)	% prevalence
1	**H.nana*	170	35.6
	**H.diminuta*	320	67.0
	T.taeniaformis	220	46.1
	C.faciolaris	300	62.9
	**Rallitina*	100	20.9
	Diphyllobothrium	50	10.5
	Syphacia muris	178	37.3
	**Toxocara muris*	206	43.1
	**Strongyle*	307	64.4
	**Capellaria*	254	53.2
	**M.Moniliformis*	240	50.3
	Trichuris muris	326	68.3
	**Giardia*	210	44.0

*Zoonotic species of parasites

Results from filled-in forms showed that 17% of them had knowledge of common rat species and their parasite. Only 47% people were aware about the disease, Plague caused by rodents. Whereas, 22% correctly answered the transmission route of intestinal parasites, that is faecal or urine contamination of food materials. While 7% of them thought that direct contact between healthy and infected animal triggers infection, 52% totally ignored the way of transmission of intestinal parasites. When asked about human health risks due to rat intestinal parasites, 22% showed awareness of the occurrence, 50% answered that no risk is given, and 57 % declared they had no idea of leptospirosis.

Table 4: Risk perception answered by respondents

Knowledge of commensal Rats species &their parasites			
	Yes %	No%	No idea%
	17	28	55
Knowledge and awareness about the common Zoonotic diseases caused by Rats			
Disease	Yes%	No%	No idea%
Hymenolepiasis	12	33	55
Plague	47	8	46
Leptospirosis	14	29	57
Scrub typhus	18	50	32
Mode of transmission perceived by respondents			
Mechanism	Yes%	No%	No idea%
Fecal droppings	20	28	52
Urine	13	46	41
Biting	22	26	52
Contact	7	57	36

The predominance of zoonotic species in rodents in the studied region were *Hymenolypis nana, Hymenolypis diminuta, Capellaria hepatica, Strongyloides*, & *Giardia*. The answers of people residing in slum area, posh locality, vegetable market, & grain market to the questionnaire showed that the majority does not know about the species of rodents intestinal parasites, the mechanisms of transmission, the risk factors for zoonotic infections, and specific prophylactic measures.

DISCUSSION

Higher prevalence (44.18 %) of *H. diminuta* in rats followed by H. nana (39.53 %) also was been noticed in rats by Gudissa *et. al.* (2011). Presently,67% prevalence of *H.diminuta* and 35.4% of *H.nana* is in our record .Total 13 taxonomic group of parasites (6 Cestodes, 5 nematodes, 1 acanthocephalan, and 1 species of protozoa) were obtained in present studies. Similarly, (Webster and MacDonald, 1995) reported rats were harbored with 13 zoonotic species. Whereas, 24 species of helminthes (11 trematodes, 4 cestodes & 10 nematodes) were identified by (Elshazly AM *et. al*; 2008),among the 271 rodents. Nama and Parihar (1976) reported only 8 helminth species in trapped rats in India. The World Health Organization recognizes 31 common food-borne human pathogens and rodents have been implicated in the transmission of many of them. (WHO; 2000).

A survey of a wide range of parasites of brown rats on United Kingdom farms with a range of 2-9 simultaneously per rat was examined by (Webster and MacDonald, 1995). Five species of nematodes were identified in the sampled rats whereas, Seven species of nematodes were recorded in the studied rodents in Indore by (S. Gaherwal *et al*., 2011). Udonsi (1989) recovered 13 helminth species in *R.rattus.* Whereas, 12 taxonomic group of helminthes are in our record.

With regards to the prevalence of 6 species of cestodes probably was the indication of the level of

exposure and the degree of susceptibility of the rats hosts to this helminth. The ability of cesticerci and strobilocerci of *T.taeniaeformis* to survive in the rat host may be attributed to a number of possible factors including the immunological competence of rat host (Ivoke Njoku; 2009). Recently, Greater infection of *H. diminuta* 19 (44.18 %) followed by H. nana 17 (39.53 %) was seen in rats (Deepesh Sharma *et. al* 2013) present studies are in agreement of Deepesh *et al.* (2013). High prevalence and intensity of protozoan Giardia are recorded in sampled rats from slum areas (44%). Webster and Macdonald (1995) study demonstrated that the zoonotic prevalence range and intensity were high among the most densely rat-populated farms. Similarly, prevalence of zoonotic parasites in rats from slum areas was high in agreement of Macdonald *et al*. (1995).

CONCLUSION

Studies indicate that the risk of zoonotic infection by rodents intestinal parasite may be high, even in one of the most developed city, Dehradun in Uttrakhand. The results suggested that the range of various parasites recovered from the fecal droppings of rodents collected from domestic habitats can make human population more vulnerable to parasitic zoonosis and may thus be of significant public health importance. The results of this study suggest that there is a real requirement for widespread education and extension regarding zoonotic disease risks.

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FIELD SURVEY ON THE INCIDENCE OF SOME IMMUNOSUPPRESSIVE VIRUSES AMONG BROILER FARMS IN EASTERN PROVINCE OF SAUDI ARABIA

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Abstract

Incidence of infection with reovirus and chicken infection anemia virus (CIAV) were determined in birds of 13 broiler's farms located in Eastern Province of Saudi Arabia. A total of 802 serum samples were collected to detect positive ELISA titers. Moreover, tissues from suspected birds were examined for histopathological lesions. On the other hand, trials to detect viral antigens in such samples were conducted using immunohistostain. Isolation trials of reovirus and CIAV from suspected birds were carried out. Results indicated that the ratio of reovirus-positive ELISA serum samples were 70% while it was 79% for CIAV. Clinical signs, necropsy findings as well as histopathological lesions were fully illustrated. Reovirus antigen was detected in intestinal tissues using immunohistostain. Reovirus could be isolated from examined birds but not CIAV. It has been concluded that reovirus is still representing a major problem threatening broiler production in Eastern Province of Saudi Arabia.

Keywords: Chickens, immunosuppression, reovirus, CIAV, immunohistostain, KSA

INTRODUCTION

Vaccination process is of great concern in poultry production as it has a critical role in biosecurity programs. The impaired immune response of vaccinated birds against natural field challenge, defined as vaccination failure, could be referred to many immunosuppressive viruses which maximize the risk of field infection with many other viral and bacterial diseases. Reovirus and chicken infectious anemia virus (CIAV) infections are two of the most important immunosuppressive agents affecting cell-mediated immune system of chickens beside their significant effect on the overall performance. Moreover, they constitute a serious economic threat, especially to the broiler industry, due to increased mortality and morbidity rate and complications with other disease conditions leading to magnify culling rates.

Avian reoviruses are members of genus Orthoreovirus of Reoviridae family (Mathews, 1982) that involved in many disease conditions as arthritis / tenosynovitis, stunting syndrome, respiratory disease, enteric disease, immunosuppression and malabsorption syndrome (Rosenberger, 2003; Sterner *et al.,* 1989; Van der Heide, 2000). Moreover, Bains *et al.,* (1974) observed a high mortality rate in broiler chicks, accompanied with hydropericardium and mild atrophied spleen due to reovirus. Furthermore, vertical transmission of reovirus has been detected (Robertson and Wilcox, 1986)

Chicken infectious anemia virus (CIAV) is a small, spherical, non-enveloped virus containing a circular single-stranded DNA genome (Pope, 1991). CIAV has been classified as genus Gyrovirus of Circoviridae family (Pringle, 1999). The chicken is the only recognized natural host for CIAV The virus has a world-wide distribution and is common in intensive poultry raising areas (McNulty, 1991) and was firstly recorded in Saudi Arabia by Al-Ankari *et al.,* (1996). CIAV is now thought to play a key role in

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several multiple etiology disease syndromes; hemorrhagic syndrome (Yuasa *et al*., 1987); aplastic anemia, gangrenous dermatitis, hemorrhagic anemia syndrome, hemorrhagic aplastic anemia syndrome, anemia dermatitis (Vielitz and Landgraf, 1988) and blue wing disease (Engstrom and Luthman, 1984). CIAV particles can be demonstrated in tissues of infected birds by immunofluorescent and immunoperoxidase techniques (McNeilly *et al.,* 1991; Zaki and El Sanousi, 1994). CIA virus was proven to be transmitted vertically (Hoop, 1992).

MATERIAL AND METHODS

Farms Locations:

Thirteen broiler's farms were included in this study. Selection of examined farms was conducted according to the geographical areas in order to represent different regions of Eastern Province of Saudi Arabia. The selected broiler's farms were located in Al-Jubayl, Ad Dammam, Al-Uqayr, Abqaiq, Al-Batalya and Al-Hasa (including Qatar road) areas.

Profile of Examined Farms:

Birds of each selected farm inspected thoroughly for detecting of any clinical signs of reo-virus or CIAV infections. The most important clinical signs traced for reo-virus infection were stunting growth, proventriculitis and feather deformity, while those for CIAV were anemia, depressed body weight gain and mortalities not exceeding 30%.

Samples Collection:

Eight hundreds and two blood samples were collected from the different assigned broiler's chicken farms. Serum samples were extracted then stored at -20^{o}C after individual labeling and coding. Such samples were used later for detecting antibodies against chicken infectious anemia virus (CIAV) and reovirus using enzyme-linked immunosorbent assay (ELISA). Tissues from the assigned flocks were collected for virus isolation and identification as well as immunoperoxidase (IP) technique. Intestine samples were collected for detection of reo-virus while liver, thymus and bursa .were examined to detect CIA virus. Tissues were divided into two halves, one kept at -80^{o}C for virus isolation and the other half fixed in 10% neutral buffered formalin for IP technique.

ELISA Kits:

ELISA Avian Reovirus Antibody test Kit (Item No. 96-6512, Lot No. 0500876) and ELISA Chicken Anemia Virus Antibody Test Kit (Item No. 96-6549, Lot No. 130008) obtained from SYMBIOTICS Corporation (11011 Via Frontera San Diego, CA 92127, USA) were used to examined serum samples for positive antibody titres.

Immunohistostain Kit:

Broad Spectrum Histostain®-Plus Kit, Zymed® 2nd Generation LAB-SA Detection System (Ref/Cat. No. 85-9843, Lot No. 50581650) obtained from ZYMED® Laboratories (561 Eccles Avenue- South San Francisco, CA 94080, USA) were used to detect virus antigen in tissues of birds.

Positive and negative control sera:

Positive anti-avian reo-virus and anti-chicken anemia virus sera as well as negative control serum were obtained from SYMBIOTICS Corporation (11011 Via Frontera San Diego, CA 92127, USA).

ELISA Technique:

The ELISA profile assay of reo-virus antibody was carried out according to methodology of Snyder et al., 1984) and the instruction of SYMBIOTICS ELISA Kit insert (Item No. 96-6512).

The ELISA profile assay of chicken anemia virus antibody was carried out according to methodology of Brewer *et al.,* (1994) and the instruction of SYMBIOTICS ELISA Kit insert (Item No. 96-6549).

Immunohistostain Technique:

Detection of antigen particles of either reo-virus or CIAV was carried out according to the methodology of McNeilly et al., (1991) and ZYMED® Broad Spectrum Histostain®-Plus Kit (Ref/Cat. No. 85-9843, Lot No. 50581650).

Reo-virus Isolation and Identification:

Intestine samples were used for isolation of reo-virus (Page *et al.,* 1982). Isolation and identification of reo-virus from suspected samples were carried out according to the methodology described by Al-Afaleq *et al.,* (1989); Rosenberger (1983) and Simmons *et al.,* (1972).

CAAV Isolation and Identification:

Liver samples were used for isolation of CAAV (McNulty, 1998) after special treatment as described by Goryo *et al.,* (1985). Isolation and identification of CAAV was carried out according to the techniques described by Al-Ankari *et al.,* (1996) and McNulty (1998).

RESULTS AND DISCUSSION

Clinical Observations of Field Cases:

The clinical signs observed in most examined flocks were detected at ages between 2-4 weeks. There were a significant number of chicks among houses appeared considerably small due to delayed growth which gave the flocks' uneven appearance with increased feed conversion ratio. The clinically affected flocks showed large number of immobile chicks huddling around the feeders and drinkers with poor abnormal feathers (Figure 1). A small number of affected chicks displayed helicopter feathers in their wings. Moreover, many other chicks were exhibited lack of pigmentation of skin, shanks and beak accompanied with diarrhea that sometimes became orange in color. Some farms which have their own slaughter house recorded increased percentage of culled chickens due to low carcass grade. Such clinical signs were highly suggestive to be malabsorption syndrome or what so-called runting-stunting syndrome (RSS) observed in cases of reovirus infection (Goodwin, *et al.,* 1993).

Necropsy Findings of Field Cases:

There were different necropsy findings that were observed in cases obtained from the different farms. The main lesions were enteritis with some times orange mucus in the lumen and diffusely enlarged proventriculus with small liver and gizzard (Figure 2). Such necropsy findings were very similar to that observed in cases of malabsorption syndrome, while no evidence was detected for any pathological changes related to chicken infectious anemia (Pass, *et al.,* 1982).

Results of Serological Examination of Serum Samples:

Total serum samples of eight hundreds and two were collected from thirteen broiler farms representing different areas of Eastern Province of Saudi Arabia. Such samples were tested with ELISA system to detect positive antibody titres against reovirus and CIAV (table 1). It has been found that percentage of reovirus positive serum samples were ranged between 13-100% with a percentage of 70% to the total examined samples. On the other hand, the percentage of positive CAAV samples was ranged between 36-100% with a total ratio of 79% from the examined samples.

Histopathological Findings:

Tissues collected from field cases were examined histopathologically to detect any changes related to either reovirus or CIAV infection. The only histopathological lesions were detected were seen in the intestine. Villus atrophy, crypt epithelial cell hyperplasia and lymphoplasmacytic inflammation of the lamina propria (figure 3&4). Moreover multiple cysts involving the intestinal crypts (cystic enteropathy) was seen that tended to be cystic enteritis (figure 5&6).

Detection of Viral Antigen by Immunohistostain:

Reovirus antigen was demonstrated consistently in intestinal tissues were it was confined to villus border of intestinal epithelium (figure 7) and this result was confirmed with control tissues stained with negative control serum (figure 8). CIAV antigen was not detected in any collected tissues on any occasion during the study.

Isolation and Identification of Virus:

Reovirus was successfully isolated from intestinal tissue samples collected from examined farms. Intestinal tissue homogenates were inoculated via yolk sac of 5 days old embryonated chicken eggs taken from reovirus free source. Embryonic death was observed 3 days post-inoculation and dead embryos exhibited a purplish discoloration. The virus isolates were identified by agar gel precipitation and virus neutralization test. On contrary, no CIAV isolates were recovered from collected liver tissue samples.

In our study, it has been found that reovirus-positive serum samples were ranged between 13-100% with total percentage of 70% of examined samples, while CIAV-positive serum samples were ranged between 36-100% with a total percentage of 79%. Clinical signs; necropsy findings and histopathological

changes observed in the intestinal tissues were highly relative to that detected in cases of so-called mal-absorption syndrome caused by reovirus infection. Furthermore, reovirus antigen was demonstrated in the tissue section of intestine by immunohistostain using specific reovirus antibodies and isolated from intestine of examined affected birds. The disease was previously recorded in Saudi Arabia by Al-Afaleq and Jones (1989), but since this time no investigations were conducted on the spread of the disease. Moreover, this study, as far as we know, considered the first one investigating the incidence of this very important economically immunosuppressive disease in the Eastern Province of Saudi Arabia. Regarding chicken infectious anemia, the disease was recorded in Saudi Arabia in 1996 by Al-Ankari *et al.*, since then no other researched were carried out to study the epidemiology of the disease. Although 79% of examined serum samples were found positive to CIAV, no virus antigen was detected or isolated from tissues of the examined birds. These findings were explained by Bulow *et al.*, (1983) and Yuasa (1994) who found that virus titres in tissues decreased greatly after antibody developed due to its neutralizing effect.

CONCLUSION

It has been concluded that reovirus infection still persisting infection of broiler's poultry farms in Eastern Province of Saudi Arabia that requires more attention to control this economically important vertically transmitted disease through massive vaccination of breeder' flocks. Nevertheless, proper biosecurity measures should be applied to minimize the risk of transmitting the disease from farm to another. Controlling the problem of reovirus infection will help greatly in overcoming vaccination failure problem among broiler flocks. It has been also concluded that although CIAV was neither detected in tissues nor isolated of examined birds, disease monitoring should be applied in order to trace any future outbreaks of anemia specially the disease was previously recorded in Saudi Arabia.

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APPENDIX

Table 1: Results of ELISA test to detect positive serum samples against reovirus and CIAV in different areas of Eastern Province of Saudi Arabia.

Farm No.	Farm location	Number of examined houses	Total serum samples collected*	Reo-positive serum samples		CIAV-positive serum samples	
				N	Ratio	N	Ratio
1	Al-Uqayr	2	32	32	100%	31	97%
2	Al-Hasa	3	58	40	69%	33	57%
3	Ad Dammam	3	59	22	37%	54	92%
4	Al-Hasa	3	47	47	100%	47	100%
5	Al-Hasa	3	46	46	100%	43	93%
6	Al-Batalya	1	14	13	93%	14	100%
7	Al-Hasa	2	32	4	13%	28	88%
8	Al-Hasa	3	75	30	40%	64	85%
9	Al-Hasa	3	75	40	53%	65	87%
10	Al-Oyoun	6	123	82	67%	44	36%
11	Al-Hasa	4	160	145	91%	155	98%
12	Al-Jubayl	1	16	14	88%	14	88%
13	Abqaiq	2	65	44	68%	45	69%
TOTAL		36	802	559	70%	637	79%

* Statistical number representing the total number of birds in each house,

Figure 1

The clinically affected flocks showed large number of immobile chicks with poor abnormal feathers. A small number of affected chicks displayed helicopter feathers in their wings.

Figure 2

The main lesions were enteritis with some times orange mucus in the lumen and diffusely enlarged proventriculus with small liver and gizzard.

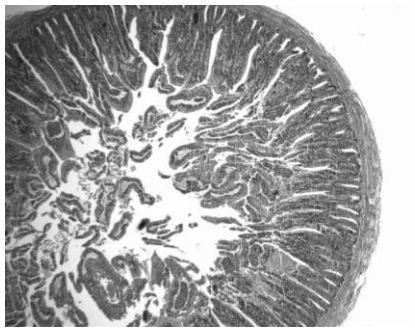

Figure 3

Increased crypt depth and cellularity in the intestinal lamina propria

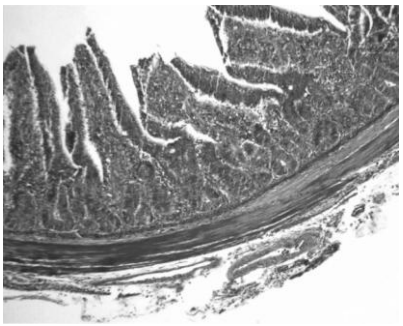

Figure 4

Villus atrophy with crypt epithelial cell hyperplasia

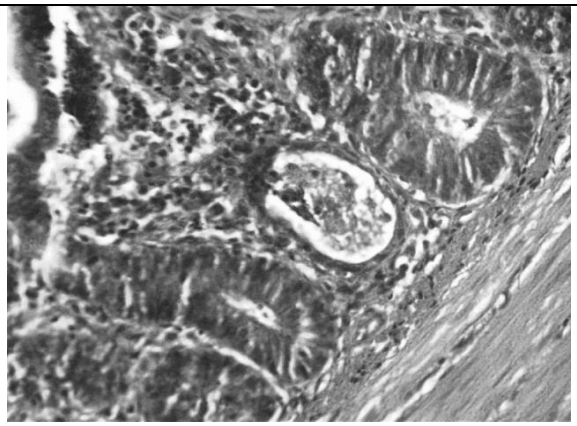

Figure 5

Large cyst in the intestinal wall with degenerated necrotic cells and mucin inside the lumen of the cysts

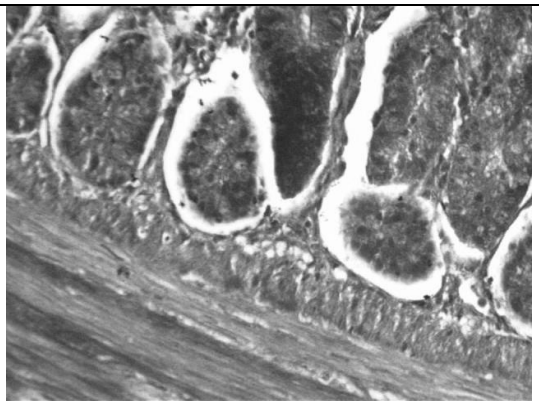

Figure 6

Sever cystic enteritis with increased depth and cellularity of intestinal lamina propria

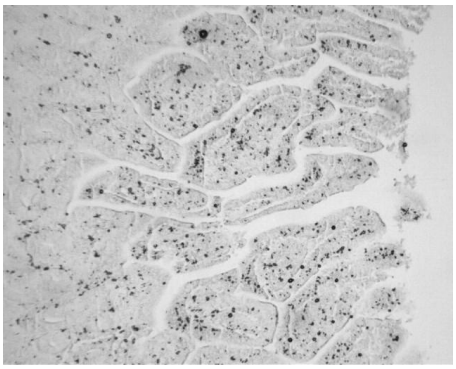

Figure 7

Reovirus antigen detected by immunohistostain as an intense brown deposit in border of villi

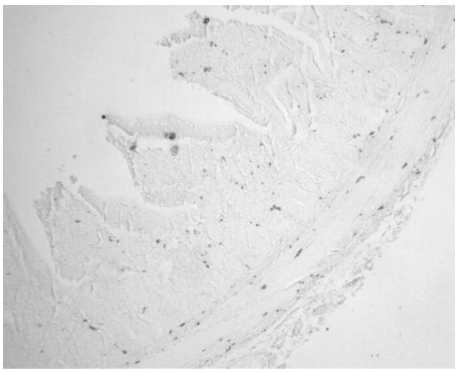

Figure 8

Immunohistosatin negative control of intestinal section

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COMPARISON OF OXIDATIVE STRESS BIOMARKERS IN FRIESIAN COWS AND EGYPTIAN BUFFALOES DURING EGYPTIAN SUMMER SEASON

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Abstract

Generally, Egypt' summer is characterized by high ambient temperature and relative humidity which results in heat stress (HS) ubiquitously affects the productive performance of livestock species. The great metabolic demands during physiological stages as well as heat stress would also be associated with oxidative stress (OS). OS designated by the increase generation of reactive oxygen species than the ability of the body antioxidant physiologic mechanisms to do safe neutralization. Twenty six healthy animals were equally enrolled in the current study aimed to compare the impact of heat stress on some physiological functions and blood oxidative stress biomarkers (OSB) between dry dairy Friesian cows and buffaloes during Egyptian summer season (July- September) . The criterion for cows and buffaloes selection and the managemental conditions were similar. Feeding system was depending on total mixed ration to meet the animal's requirements and feed intake was calculated. Atmospheric temperature, relative humidity, temperature humidity index (THI), respiration rate and rectal temperature were recorded throughout the experimental period (8wk-prepartum). Bi-weekly blood samples were collected for the malondialdehyde (MDA) and enzymatic antioxidants such as glutathione peroxidase, superoxide dismutase and catalase activities determinations. Generally the results confirmed the heat stress condition, as the THI values had ranged from 79.74 to 90.4 throughout the experimental period. In both species, HS showed a trend to increase rectal temperature and to decrease feed intake. The results revealed that there was continuous increase in MAD values as the animals come close to parturition with moderate decrements for the antioxidants activities in both cows and buffaloes. It is to be concluded that during Egyptian's summer season, HS had adversely affected feed intake and consequently animal' production performance so dietary modification to supply more antioxidants should be considered.

Keywords: Heat stress, oxidative stress, buffaloes, oxidative biomarkers, Egyptian summer

INTRODUCTION

The earth's climate has been predicted to change continuously at exceptional rates in recent decades (IPCC, 2007). Summer temperature in the Mediterranean region including Egypt is generally outside of the cow's "comfort zone" results in heat stress (HS). The term heat stress is defined as the sum of heat accumulated from the environment and the failure of the animal to dissipate heat which mostly associated with malfunction in the animal's productive or physiological metabolic process (West, 2002). Acute and chronic heat stresses exhibit different responses on animal's production and metabolism. Temperature humidity index (THI) is a suitable measure to estimate heat stress (Ravagnolo *et al.*, 2000). Armstrong (1994) classified a THI ranging from 72-78 as mild, 79-88 as moderate and 89-99 as severe. Increasing air temperature, temperature-humidity index accompanied by rising rectal temperature above critical thresholds are related to decrease dry matter intake, milk yield and reduce milk yield efficiency in dairy cows (Holter *et al.*, 1997; Ravagnolo *et al.*, 2000; Rhoads *et al.*, 2009). The magnitude of the animal's response to elevate ambient temperatures is distinct by the livestock

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species and their physiological state. Some species is reported to be the best tolerant to HS namely goats (Silanikove, 2000) while pregnant and lactating ruminants are more susceptible to heat stress than non-pregnant and non-lactating ones. Hafez et al. (1955) reported that in Egyptian buffaloes, glandular surface of sweat gland per cm2 of skin surface was 1.07 and 3.08 in cattle and that the skin thickness of buffaloes was about twice that of cattle. The sweat glands in buffaloes are underdeveloped (Koga, 1999). This indicates that buffaloes have poor heat tolerance than cattle (fewer sweat glands, black colored skin) and thus assigning buffaloes to be of poor capacity to withstand HS and so need greater alertness to compete for such hostile condition (Moran,1973). Furthermore, HS is one of the wide varieties of factors which cause oxidative stress (OS) in-vivo. Oxidative stress results from increased production of free radicals and reactive oxygen species (ROS), and a decrease in antioxidant defense mechanisms (Trevisan *et al.* 2001). The majority of the studies dealing with the effects of heat stress on OS biomarkers (cellular and molecular) response have been conducted in condition sustained moderate to severe HS under strictly defined experimental conditions (environmentally controlled chambers) on selected high yielding and intensively managed dairy cattle. Scarce studies are available for the effect of moderate to severe HS on the oxidative stress biomarkers of dairy cows and buffaloes raised under Egyptian summer environmental conditions. Therefore, the current study was planned to compare the impact of HS of the Egyptian summer conditions on the some metabolic aspects and oxidative stress biomarkers of dairy cows and Egyptian buffaloes of moderate production under field situations.

MATERIALS AND METHODS

Animals, Ration and Experimental Design:

Twenty six healthy mature animals were equally (Thirteen of Friesian cows and of Egyptian buffaloes with average weight of 725 kg ± 55 kg) were blocked on the basis of multiparous (3-5 calving), expected calving date, previous lactation yield, body weight (BW), and body condition score (BCS 3.5 to 4.5) to experimental trial during dry period (8weeks peri-parturient).The animals were raised at a private farm at the Nobaryaa province during the period of summer season (July- September). Animals were housed in shaded loose pens with adjacent outside yards supplied with evaporative mist and fan systems. The experimental animals were fed on Total Mixed Ration (TMR) which was formulated to meet or exceed the predicted requirements. Clean water was supplied all the time. Daily DMI was recorded .The ingredients and calculated analysis of ration are summarized in table (1).

Measurements and Sampling

1- Environmental temperature and humidity

Digital hygrometer-thermometer device were used for measuring relative humidity and temperature daily at (0600, 1200, and 1800 h) at fixed time. THI was calculated according to Thatcher *et al.,(* 2010).

2- Animal temperature and respiration rate

Rectal temperatures and respiration rates were obtained daily with a digital rectal thermometer and visually counting flank movements during a 15 sec interval and multiplying by 4.

3- Live Body Weight (LBW) and Body Condition Score (BCS)

Body weights and BCS measurements were done weekly by the same technician for all animals immediately at morning and before feeding.

4- Blood Sampling and Analysis

Bi-weekly blood samples were collected from all animals (cows & buffaloes) during the 8 wk pre-partum upto 2 weeks pre-partum. All blood samples were collected from the coccygeal blood vessel either into 10-mL heparin vacutainers. After proper centrifugation, the plasma samples were harvested and decanted into 1.5-mL aliquots and stored at −20°C until further analyses. Oxidative stress biomarkers assessment was done measuring different biomarkers. Plasma malondialdehyde (MDA) was determined according to the method of Draper and Hadley (1990) and plasma glutathione content (GSH) was estimated as described by Lin Hu *et al.* (1988). The catalase (CAT) and superoxide dismutase (SOD) activities in erythrocyte were determined calorimetrically by the methods described by Goth (1991) and Nishikimi *et al.,* (1972), respectively.

Statistical Analysis

The statistical analysis of the data was assesses using Minitab 17.0® (Minitab, Inc, USA) statistical program. Student's t- test for independent groups was done. Significance was considered at P ≤0.05 levels. Correlation analysis between effect of cows and buffaloes rectal temperature and DM intake were also computed.

Table 1: Ingredients percentage and calculated analysis of experimental ration

Experimental ration (8 wk-prepartum)	% DM		
Ingredient			
Corn silage	11.1		
Beet sugar pulp	7.99		
Yellow corn	19.98		
SBM 44%	3.99		
Berseem hay	52.76		
Salt	0.07		
Sodium bicarbonate	0.13		
Limestone	0.09	NE_l (Mcal/kg)	1.65
Magnesium oxide	0.05	CP	16.7
Premix Mineral	0.02	Forage NDF	13.7
Premix vitamins	0.02	Ether-Extract	2.7
Calcium bentonite	0.13	Ca	1.1
Total	100	P	0.3

Table 2: Average atmospheric temperature (0C), RH (%) and THI during 8-weeks prepartum (July-September)

Wk- prepartum	T	RH	THI
8	31.6	50.2	79.74
7	36.2	50	85.9
6	37.4	51.2	87.1
5	37.8	49.8	87.9
4	38.4	49.7	88.8
3	39.1	52.4	89.1
2	38.7	50.2	89.1
1	39.7	51.2	90.4
Average	37.7	50.2	87.2

Table 3: Average weekly rectal temperature (°c) and respiration rates (breaths/minute) of experimental animals during dry period (8weeks pre-partum)

Weeks prepartum	Rectal temperature		Respiration rate	
	Cow	Buffalo	Cow	Buffalo
8	39.1	40.5	70.5	71.8
7	38.7	39.8	69.5	68.7
6	38.5	38.7	63.4	62.8

5	39.2	40.3	67.4	67.2
4	39.4	40.8	67.8	68.1
3	38.7	40.2	65.2	66.4
2	39.8	40.7	67.9	68.4
1	40.1	41.1	71.8	72.6
t-value	0.88	1.07	2.41	3.73
Significance	*	*	*	*

* = Non-significant at P > 0.05

Table 4: Different stress biomarkers of cows and buffaloes during Egyptian summer at different weeks pre-partum

Species	Weeks Pre-partum															
	8				6				4				2			
	MAD	GSH	SOD	CAT	MAD	GSH	SOD	CAT	MAD	GSH	SOD	CAT	MAD	GSH	SOD	CAT
Caw	112.23± 10.4	38.14± 1.1	6.25 ± 0.52	26.71± 2.4	123.4 ± 8.47	37.65± 2.1 [a]	7.14 ± 0.81	26.14± 3.10	165.84± 9.7	29.4 ± 1.1[a]	6.11 ± 0.51	25.3 ± 2.6	194.7 ± 17.2.	28.7 ± 1.8[a]	5.79 ± 0.42	24.92 ± 1.7
Buffalo	125.87± 8.6	32.62± 1.8	7.48 ± 0.64	25.63± 1.4	132.33± 9.7	33.41± 1.4 [b]	6.85 ± 0.52	26.07± 1.9	185.61± 10.2	28.7 ± 0.7[b]	6.48 ± 0.32	24.6 ± 1.9	208.4 ± 16.4	27.9 ± 1.3[b]	6.26 ± 0.51	24.31 ± 1.32

Designation of the values of MAD as nmol/gHb, GSH as mol/mg Hb & SOD and CAT as U/mg Hb

Values with different superscripts at the same column are significantly differed at P< 0.05

RESULTS AND DISCUSSION

The results of atmospheric temperature (^{0}C), relative humidity (RH) and THI measurements throughout 8 weeks pre-partum are presented in table (2). The presented data revealed that the THI indices during the experimental period lasted from July to September 2013 had reached the maximum 89.1and 90.4 at the last three weeks pre- partum. However at all the times of the experimental period (Egyptian summer climate), the THI had reached more than 80 except for the first wk of the study (79.74).THI could be used as indicator of thermal climatic as indicated by McDowell *et al.*, (1976). According to Thatcher *et al.*, (2010), lactating cows are thought to experience no stress when THI is less than 72 and severe stress when THI exceeds 88. For each l0 L milk yield per day, it roughly doubles the metabolizable energy requirement of cows, and nearly 35% of this energy is dissipated as heat (Kadzere *et al.*, 2002). So, the high yielding cows suffer more than the low yielding ones, because the upper critical temperature shifts downwards as milk production, feed intake and heat production increases (Silanikove, 2000). Nevertheless, buffaloes have been reported to be more susceptible to HS than cows as the increase in temperature causes stress due to increased body heat loading and low potentiality to dissipate heat from the body surface ascribed to fewer and ill- developed sweat glands as well as black colored skin (Koga, 1999). The THI values of more than 72 is considered as stressful and THI over 78 is recognized as very severe HS to buffaloes (Payne ,1990). The degree to which an animal resists change in body temperature

varies in different species because of differences in their heat regulating mechanisms (Salah *et al.* 1995).

Rectal temperature (RT) is one of the criteria most frequently used in the literature to characterize the animals temperature follows that of ambient temperature. The results of the impact of Egyptian summer conditions on rectal temperatures and respiration rates of cows and buffaloes during 8 wk pre- partum are presented in table (3). The results demonstrated that there was no significance difference between species, although both cows and buffaloes were severely affected by HS as the recorded rectal temperature values were higher than normal values in the thermoneutral periods (Cole and Hansen, 1993; West, 2002). However, buffaloes seemed to be more affected by the hostile environmental condition of this study compared with their respective cows. Buffaloes had recorded up to one degree increase in their rectal temperatures at most of the point's period compared to cows. Similarly, Joshi and Tripathy (1991) recorded as 2.6^0C rise in rectal temperature in buffalo's calves when exposed to direct sun rays in the months of June and July. Air temperature (13-18 ˚C), RH (55-65%) and wind velocity (5-8 km/h) are the optimum conditions for buffaloes as suggested by Payne (1990) and the THI $\geq$ 77 is very stressful. On contrary, Mullick (1959) reported that the rectal temperature during summer months under high and low humid conditions were always less for buffaloes than cattle.

No one can discuss the above results away from the environmental factors dominate Egyptian summer condition including atmospheric temperature, relative humidity and THI (table 2). A rise of 1 0C or less in rectal temperature is enough to reduce performance in most livestock species (McDowell *et al,* 1976). Also, milk yield declined by 1.8kg for each 0.55 0C increase in rectal temperature (Johnson *et al,* 1963).

The relation between THI as a measure of HS and animal rectal temperature (Figure 1) demonstrated that at the peak of severe HS, the THI were 89.1and 90.4 attended during the last three weeks pre- partum. Both species suffered as much as, the animal capacity to sustain the thermoneutral response is disrupted led to an increase in rectal temperature. All the above data made rectal body temperature a sensitive indicator of physiological response to heat stress as it is nearly constant under normal condition (Kadzere *et al*, 2002).

Regarding the respiration rate (Table 3), there was no significant difference between species and both species attended the highest respiration rates at the last wk pre-partum which is compatible to the results of THI and rectal temperature. Heat stress had significantly affected respiration rate (breaths/minute) when compared to normal values (76.8 &77.8 vs. 40-50 for Cow & buffalo, respectively) as presented by other studies (Kumar, 2005& Silanikove *et al.,* 2009). Salem (1980) reported increase in respiration rate of buffaloes and crossbreds cattle during summer compared to other seasons. A higher respiration rate of 71.5/minute during summer compared to 38.8/minute in winter was recorded in lactating cows by Schwartz *et al,* (2009) and Silanikove *et al.,* (2009) Respiration rate is indicator of HS in the hot environment and has significant correlations with circulating corticoids concentration (Kumar, 2005). Normal respiration rate is approximately 10–30 breaths/minute (Hafez et al., 1955). There is a very high positive correlation between the respiration rate and ambient temperature. The increase in breathing rate (panting) sharply increases the loss of Co2 via pulmonary ventilation which upsetting the critical balance of carbonic acid to bicarbonate excretion causing respiratory alkalosis (Benjamin, 1981). In cattle under heat load, about 15% of the endogenous heat is lost directly from the body core via respiratory tract (McDowell *et al.,* 1976). Heat stress that is characterized by elevated respiration rates and rectal temperatures has been implicated in impaired metabolism (schwartz *et al,* 2009); and in poor reproduction performance in dairy cattle (Ingraham *et al.,* 1994) as well as in dairy buffaloes (Verma *et al.,* 2000) independent of any effects on feed intake.

The results of regression analysis between cows and buffaloes rectal temperatures and dry matter intakes are presented in figure 2 and 3, respectively. It was noticed that with the increase in the rectal temperature there was a decrease in the dry matter intake in both species. The increase of the rectal temperatures of the cows over 40^OC was associated with dramatic decrease in the DMI to less than 10.5 kg/ day. However, Buffaloes demonstrated higher rectal temperatures especially in the last week pre-

partum which was associated with a DMI drop to less than 9.5 kg/ day. The significant negative correlation between animal's rectal temperature and its DMI was previously reported in Holsteins (Holter *et al.*, 1996). West *et al.*, (2002) confirmed a decrement of 0.85 kg in DMI for each degree (°C) increase in the air temperature. Moreover, the same correlation was demonstrated in buffaloes as up to 40 % reduction in voluntary DMI during summer months was recorded as compared to the amount consumed during cooler months (Nangia and Gary, 1992 and Ashour *et al.*, 2007). Feed intake reduction due to the increase in temperature might be attributed to several reasons among them the direct effect of elevated temperature on the appetite center in the hypothalamus resulting in reduction of the production of VFA which are the main energy source in ruminants (Baile and Forbes, 1974) or to the inverse relationship between DMI and NEFA concentrations during the periparturient period (Overton and Waldron, 2004).

The results of the impact of HS on oxidative stress biomarkers of dry cows and buffaloes are presented in table (4). It was observed that the values of MAD (nmol/gHb) as indicator to OS were ranged from 112.12to 194.7 and from 125.87 to 208.4 in cows and buffaloes, respectively during the 8 wk pre- partum. There was insignificant difference between species; however, buffaloes exhibited the highest values especially at the last 2wk pre-partum. Moreover, the MAD concentrations in both species demonstrated marked increase toward the calving date (2 wk pre-partum) as the metabolic load increased. These results indicates that despite the metabolic stress occurs on pregnant dry animal which was aggravated by the HS, it endeavors to adjust with the environment by secreting large quantities of cortisol (Stott and Wiersma, 1971). Higher concentration of this catabolic hormone normally results in lipolysis and adipose mobilization (Rhoads *et al.*, 2009) in heat stressed cows to initiate and maintain milk production (Abilay *et al.*, 1975). Similar to our results, several studies demonstrated that the MDA concentrations increased around calving (Pathan *et al.*, 2009 & Ganaie *et al.*, 2013).

The results of the enzymatic antioxidants capacity of dry cows and buffaloes during summer season (Table 4) revealed that there was insignificant difference between species except for the GSH starting from 6 wk pre- partum. One can observe that as the animals come near to calving time, the levels of the enzymatic antioxidants exhibited modest instable decrements. The average values recorded for GSH, SOD and CAT in cows and buffaloes are within the values recorded by other studies (Bernabucci, *et al.*, 2002&2005 and Megahed *et al.*, 2008 & Allaam *et al.*, 2014), respectively. The modest decrease in the enzymatic antioxidant activities was in harmony with the confirmed HS condition observed in the current study. Megahed *et al.* (2008) examined the effects of heat stress in Egyptian buffaloes in summer and winter seasons and reported that SOD activities were significantly lower in the summer as compared to winter season. On contrary, Bernabucci *et al.* (2002) and Pathan *et al.* (2009) demonstrated increase in SOD and GPx around calving and suggested that this might be the result of a possible homeostatic control. The enzymatic antioxidants, mostly metalloenzymes are the first line of defense system counteract oxidative damage of the internal cellular constituents induced by ROS (Weiss, 2006). The discrepancy of the enzymatic antioxidant activities results might be attributed to multifactor at this particular points among them the physiological status of the animal (heifer, dry or first trimester of milk production), type of HS (experimental chambers vs. field situation) as well as the methodology used. One possible explanation that both species were subjected to long term HS at stressful metabolic and physiological status (Dried pregnant animals) which was accompanied by marked reduction in DMI. This reduction in DMI (low nutrients and antioxidants intakes) plus persisting secretion of catabolic cortisol to satisfy energy needs had resulted in lipolysis which eventually increased the production of free radicals and ROS and exhausted the antioxidant defenses.

CONCLUSION

It is to be concluded that Egyptian summer environmental condition is incriminated for HS and using THI is a good indicator. As high yielding animals, Moderate yielders are also affected by HS. RT and DMI are highly correlated to HS. Dairy buffalo seems to be more affected by HI than cow. As the field of oxidative stress in ruminant medicine is still in the early stages, the results of OS biomarkers furnish a base for such data especially for buffaloes. Effective nutritional strategies considering supplying

surplus antioxidants to the ration of heat stressed animal is a smart and effective promising path to alleviate metabolic environmental heat loads.

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APPENDIX

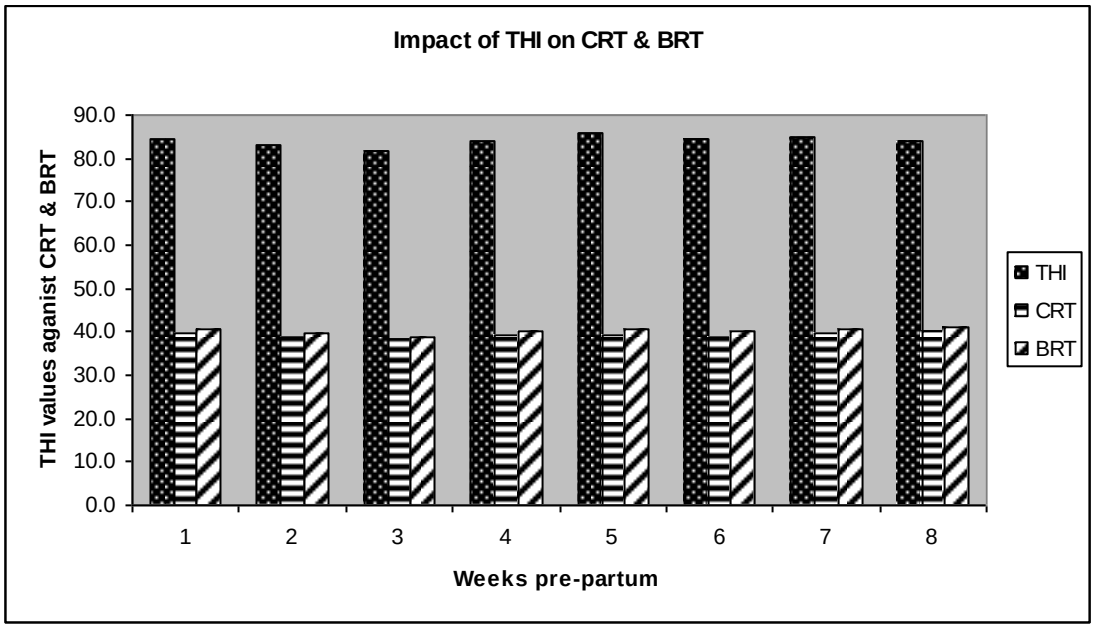

Figure 1: Impact of THI on cows and buffaloes rectal temperatures (^{0}C)

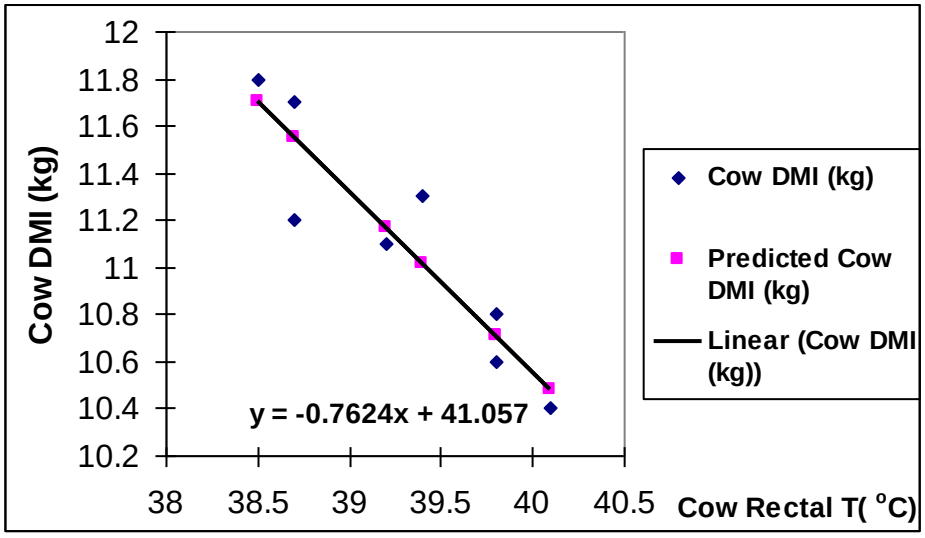

Figure 2: Regression analysis between cows' rectal temperature (^{o}c) and DMI (kg)

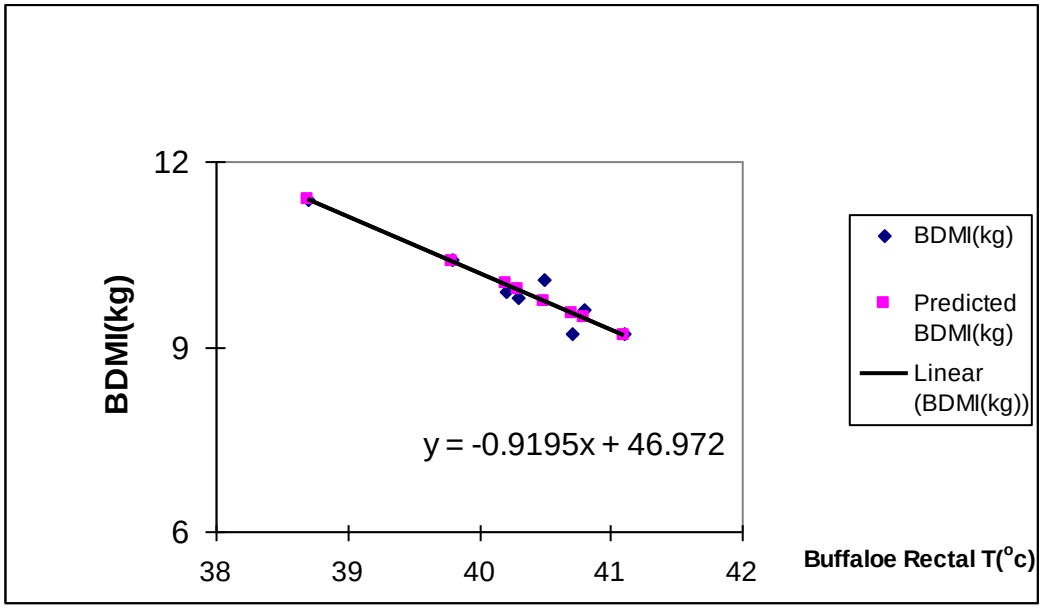

Figure 3: Regression analysis between buffaloes' rectal temperature (^{O}C) and DMI (kg)

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EFFICACY OF USING REDUCED PROTEIN AND ENERGY DIETS FORMULATED ON AMINO ACID PROFILE CONCEPT ON LAYER'S PERFORMANCE AND EGG QUALITY TRAITS

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Abstract

The current experiment was conducted to evaluate the impact of practical application of reduced dietary crude protein (16&14%) and metabolizable energy levels (2710 Kcal/Kg) in diets formulated on ideal amino acid profile basis on performance parameters such as egg weight (EW), average weight gain (AWG), hen day egg production % (HDEP) and egg mass (EM)), egg quality traits and economic efficiency of laying hens. A total of 480 commercial Bovans – Brown laying hens (24-40 weeks of age) was randomly assigned in 3X2 factorial design (three CP levels, 18, 16, 14% and two ME levels 2810, 2710Kcal/Kg diet). Six groups each of 4 replicates (20 birds / replicate) were used in a random block design. Reduced CP diets were supplemented by crystalline amino acids so as the ratio of limiting amino acids to lysine was kept constant. Statistical analysis using correlation coefficient was used to elaborate the interactions between performance parameters and egg quality traits in dietary treatments. No significant differences ($P > 0.05$) were observed among dietary treatments in some performance parameters (AWG, EW as well as EM) and egg quality traits (egg specific gravity, albumin weight and index as well as yolk weight). Moreover, the best net revenue and economic efficiency % were achieved by birds fed reduced CP diet that contained 14% CP and 2810 ME Kcal/Kg. In conclusion, reduced crude protein – amino acid balanced diets can used as a reliable tool in egg production industry and may have beneficial effects if applied probably in laying hens nutrition.

Keywords: Layers, amino acid profile, egg quality, reduced protein, reduced energy, Bovans-Brown

INTRODUCTION

The current continuous augment of feed ingredients' prices as well as the increasing concern over environmental pollution, have put great burdens for the development of a sustainable, gainful, and competitive egg-production system. Dietary energy and protein account approximately 85% of total feed cost. At present, there are wide ranges of dietary energy and protein levels have been used in egg industry during different phases of production. Practical commercial poultry feeds based on corn-soybean diets and supplemented with excessive amino acids are not only much more expensive but also lead to unnecessary ruining of the environment. Recent technological advances in crystalline amino acids (AA) manufacturing especially for other amino acids rather than methionine and lysine have encouraged nutritionists to use low-protein - AA-fortified diets for various classes of poultry. Nevertheless, there are biological limits to the amount of dietary protein which can be substituted with synthetic AA (Keshavaraz and Austic 2004). Accordingly, the concept of ideal protein profile can be applied by measuring the requirement for a single amino acid (e.g. lysine) then the ideal ratios for all other amino acids are calculated compared to it to promote maximum utilization and decrease nitrogen excretion. (Bregendahl *et al*., 2008). Therefore, the objective of the current study is to evaluate Bovans – Brown laying hens performance and egg quality traits fed formulated diets with reduced levels of CP (16% and 14%) and ME (2710 Kcal/Kg) while keeping the ratio between lysine and other limiting amino acids constant during phase 1 of egg production.

MATERIALS AND METHODS

Bird's Management, Diets and Experimental Design

A total of 480 commercial Bovans – Brown laying hens was used during phase 1 of egg production (24-40 weeks of age). Bird's management, photo-regimen and vaccination were done in accordance with protocol described by the breeder ' management guide. After 2 weeks preliminary period (24-26 weeks), laying hens were randomly assigned into six dietary treatments (80 birds / group) in a random block design and each block consisted of 4 replicates (20 birds/ replicate). All replicates were equally distributed into upper and lower cages to minimize cage level effect where the four adjoining cages represented a replicate. Based on a 3×2 factorial arrangement, birds were fed on six experimental diets consisting of 2 intakes of dietary ME levels (2810 and 2710 Kcal/kg)) and each of 3 intakes of dietary crude protein levels (18%, 16% and 14%). Birds were fed formulated corn- soybean based diets to meet their requirements. Composition, levels of commercial amino acids added and calculated analysis of the formulated diets are shown in Table (1) and (2), while ideal amino acid profile of experimental diets relative to lysine is demonstrated in Table (3).

Table 1: Ingredients (%) of experimental diets fed during experimental trial

Ingredients	CG18*	G18L**	CG16*	G16L**	CG14*	G14L**
Yellow corn	57.23	53.43	63.85	60.05	70.65	65.70
soybean meal 47%	28.97	27.76	23.28	22.07	17.18	16.93
Soybean oil	2.65	2.16	1.51	1.02	0.30	0.00
Wheat bran	0.00	5.50	0.00	5.50	0.00	5.50
Met	0.16	0.16	0.21	0.21	0.26	0.26
Lys	0.00	0.00	0.06	0.06	0.21	0.21
Thr	0.00	0.00	0.04	0.04	0.10	0.10
Trp	0.00	0.00	0.03	0.03	0.07	0.07
Val	0.00	0.00	0.00	0.00	0.09	0.09
Sodium bicarbonate	0.30	0.30	0.30	0.30	0.30	0.30
NaCl	0.17	0.17	0.17	0.17	0.17	0.17
Monocalcium	1.70	1.70	1.70	1.70	1.75	1.75
Limestone	8.32	8.32	8.35	8.35	8.42	8.42
Premix***	0.50	0.50	0.50	0.50	0.50	0.50
TOTAL	100	100.00	100	100.00	100	100.00

*****Represent the group fed the same level of CP% but with high ME (2810Kcal/kg)

****** Represent the group fed the same level of CP% but with reduced ME (2710Kcal/kg)

***LayMix-Rannim each 1 kg contains: Vit. A: 25 MIU, Vit. B_1: 2 g, Vit. B_2: 10 g, Calcium D Pantothenate: 16 g,Vit. B_6: 3 g Vit. B_{12}: 0.032 g, Vit. D: 35 MIU, Vit. E: 16 g, Vit. K: 2 g, Niacin 24 g, Folic acid: 2 g, Organic BaseQ.S.

Table 2: Calculated analysis of experimental diets fed during experimental trial

SID = Standard ilealy digestible amino acid basis

Nutrient	CG18	G18L	CG16	G16L	CG14	G14L
ME (kcal/kg)	2810	2710	2810	2710	2810	2710
CP%	18.00	18	16	16	14	14.36
EE%	5.18	4.73	4.19	3.73	3.12	2.83
CF%	2.22	2.61	2.14	2.53	2.05	2.45
SID Lys%	0.88	0.87	0.79	0.77	0.75	0.76
SID Met%	0.41	0.41	0.43	0.43	0.46	0.46
SID Met+Cys%	0.68	0.67	0.68	0.67	0.67	0.68
SID Cys%	0.26	0.26	0.23	0.23	0.20	0.21
SID Trp%	0.21	0.21	0.22	0.22	0.22	0.23
SID Thr%	0.57	0.57	0.54	0.53	0.52	0.52
SID Val%	0.78	0.77	0.69	0.68	0.68	0.69
SID Ile%	0.68	0.67	0.59	0.58	0.49	0.50
Calcium%	3.56	3.57	3.56	3.56	3.57	3.58
Phosphorus(total)%	0.74	0.78	0.73	0.76	0.72	0.76
Phosphorous (available)	0.48	0.49	0.48	0.49	0.49	0.50
Sodium%	0.16	0.49	0.17	0.16	0.16	0.16
Cl%	0.14	0.15	0.16	0.16	0.19	0.19
K%	0.79	0.82	0.71	0.74	0.63	0.67

Table 3: Ideal amino acid profile of experimental dietary treatments relative to lysine

Amino acid profile relative to lysine[1]	% to SID[2]					
	CG18[3]	G18L[3]	CG16	G16L	CG14	G14L
Lys	100	100	100	100	100	100
Met + Cys	77	76	77	76	76	77
Trp	24	24	25	25	25	26
Thr	65	65	61	60	59	59
Val	88	87.5	79	88	77	79
Ile	78	76	67	70	56	57

[1]Lysine requirements set as 100%.

[2] Standard ilealy digestible amino acid bases

[3] 18% CP diets is the reference profile regarding other dietary formulation.

Performance Parameters

Birds were weighed (5/replicate) as well as feed consumption was determined at biweekly interval then final weight gain as well as feed conversion ratio were calculated (g feed / g egg mass). Hen day egg production (HDEP) % for each treatment was recorded daily and egg production percent was calculated. Average egg weight (g) for each treatment was monthly recorded, and egg mass (g/hens) was calculated by multiplying egg weight by egg production percent. Protein intake (g), ME intake (Kcal/Kg), Lys. intake (g) and Met.+Cys. intake (g) for each dietary treatment were also calculated.

Egg Quality Analysis

Eggs were monthly collected from each treatment (15 eggs / treatment) and freshly collected eggs were weighted and the examination of external and internal egg quality traits including egg weight (g), egg specific gravity (ESG), yolk and albumin heights (YH &AH), ,yolk and albumin diameters (YD & AD) , egg shell thickness (EST) were determined in mm. Egg shell weight (ESW), yolk and albumin weights (YW &AW) were determined in g according to Shaimaa *et al.,* (2015). Haugh unit score (HU) was calculated as cited by Larbier and Leclercq (1994). The economic efficiency study was done using market price of ingredients in Egypt according to Shaimaa *et al.,* (2015).

Statistical Analysis

Minitab 17.0® (Minitab, Inc, USA) was used to perform analysis of variance (ANOVA) while, difference between means was separated using Duncan's multiple range test (Duncan, 1955) at $P \leq 0.05$ level. Correlation coefficient analysis between treatment effects and performance variables was also computed using Minitab 17.0 statistical program.

RESULTS AND DISCUSSION

Performance Parameters

Results of overall production performance parameters of layers throughout the experimental trial are shown in Table 4 .The overall results showed that neither the reduction of dietary CP level nor the reduction of ME had a significant impact on AWG and EW. Insignificant effect of higher level of dietary ME

(2810 Kcal/Kg) on overall AWG was not in agreement with Gunawardana *et al*., (2008) who suggested that there was a significant linear effect of added dietary energy on body weight gain. Moreover, some studies reported that reduced – CP diets negatively affected performance parameters but it should be noted that unlike our study, these studies were carried out under different experimental conditions such as the degree of CP reduction which was greater (16% vs 10%) or that these studies were conducted during the late stage of laying hens production (Koelkebeck *et al*., 1993 and Novak *et al*., 2006, respectively). Both diets containing 18% CP with the two energy levels 2810 and 2710 Kcal/Kg showed an increase in EW, as expected, because protein is an important component of eggs (Leeson *et al.,* 2000), and higher CP contents determine higher CP deposition in the egg. Lack of significant impact of high level of ME on EW is supported by the findings of Summers and Leeson (1993) who concluded that dietary energy levels in laying hens diet had no influence on EW (g). Nevertheless, several authors pointed out that increasing dietary energy levels produced heavier eggs (Harms *et al*., 2000; Bohnsack *et al*., 2002; Sohail *et al*., 2003& Wu *et al.,* 2005). Correlation coefficients (Table 5) were significantly positive ($P < 0.05$) among EW and PI, ME I, Lys. I, ($r = 0.84$, 0.82& 0.89, respectively). Moreover, there was a non-significant positive correlation coefficient between HDEP% and EW which is in contrast with Emsley *et al.,* (1977) and Nahashon *et al.,* (2006) who observed that there was a negative but not significant correlation between HDEP and EW.

The positive correlation between Lys. I and EW coincides with the findings of Zimmerman and Andrews (1987) who noticed that EW is improved by increasing the level of dietary lysine. Additionally, the 0.52% and 1.35% decrease in HDEP% (not demonstrated) were observed in G14 as compared to higher levels of CP (18% and 16%, respectively). Meanwhile, the combined effect of lowering both CP% and ME on HDEP% was more severe as HDEP% declined by 7.1% and 7.9% in G14L compared with CG18 and G16, respectively.

In the current study, reducing dietary ME level from 2810 to 2710 Kcal/Kg did not influence HDEP% which is in agreement with Leeson *et al.,* (1993) and Harms *et al.,* (2000). Table (5) showed that there was a weak positive correlation between FC and HDEP %.This positive correlation is explained logically as to more feed is needed to get more eggs (Olorede 1998 and Aduku 2004).

The lack of significant impact of different dietary treatments on EM may be a reflection to the same effect on EW and HDEP%. The highly significant ($P < 0.01$) positive correlation between HDEP and EM was in agreement with Nahashon *et al.,* (2006) and the positive significant ($P <0.05$) correlation between EW and EM supports these findings (Table 5).

Conversely, Almeida *et al.,* (2012) concluded that egg mass was higher when the diet contained 18% CP compared with 15%, independent to dietary ME content.

Results of overall FC (Table 4) show that differences in mean FC of laying hens fed diets containing two levels of ME (2810 vs. 2710 kcal of /kg) and the same level of dietary CP were insignificant .These results are in agreement with the findings of Nahashon *et al.,* (2006) & (2007) who reported that FC was not significantly affected by the narrow increment of dietary ME (100 Kcal/Kg), nevertheless, the increase in ME level over 100 Kcal/Kg induced significant increase in FC (Grobas *et al.,* 1999 and Nahashon *et al.,* 2006). Regarding the impact of dietary reduction of CP% overall FC, it is to be concluded that reducing dietary CP level by 4% from 18% to 14% even with the same energy level had a significant ($P < 0.05$) negative effect on FC. Moreover, the positive influence of high dietary CP level on FC was supported by highly significant ($p < 0.01$) positive correlation coefficients between FC and PI, Lys. I and Met. +Cys. I (Table 5). FCR (g feed /g EM) was insignificantly changed by the different dietary treatments. This may be as a consequence of the non-significant differences in FC (except for G14, G14L) as well as EM among different dietary treatments. Birds fed diets containing 14% CP consumed less feed than birds fed diets containing the 16 and 18% CP diets, resulted in a better FCR in birds fed on the 14% CP diets when compared with other dietary treatments. In agreement with the results of this study, several authors reported that performance parameters were not significantly affected when laying hens were fed diets containing

varying CP and/or ME levels either at different phases of the productive cycle (Summers and Leeson 1983, Sell *et al.,* 1987) or at early phases (Keshavarz ,1998) . On the opposite side, Nahashon *et al.*, (2007) showed that laying hens received diet containing 14% CP showed better performance parameters than those received diets with higher levels of CP (18% and 16%) which might be due to increased expenditure of energy in catabolism of excess dietary amino acids in diets with higher levels of CP (18% and 16%). This kind of explanation might suggest the improper use of synthetic amino acid which was unlike the main consideration in the current study as ideal amino acid profile was applied where the lysine: TSAA was kept almost constant in different dietary groups.

Table 4: Overall production performance of laying hens fed experimental diets

Group Parameter	CG18	G18L	G16	G16L	G14	G14L
Initial weight (Kg)	1.62	1.61	1.62	1.62	1.60	1.60
Final weight (Kg)	1.92	1.92	1.93	1.94	1.92	1.91
AWG (g)	0.30	0.31	0.31	0.32	0.32	0.31
EW(g)	60.10±0.4	59.80±0.1	59.35±0.5	58.32±0.53	58.40 ± 0.52	58.47 ± 0.50
HDEP %	93.56^{ab}±1.6	96.34^{a}±1.53	94.39^{ab}±1.8	91.37^{ab}±1.4	93.04^{ab}±1.6	86.49^{b}±1.2
EM (g/hens)	56.77±2.41	57.41±1.78	56.03±1.87	54.90±1.80	54.53±2.17	52.43±1.84
FC(g/hen/day)	114.98^{a}±0.29	115.8^{a}±0.70	112.23^{a}±0.44	113.89^{a}±0.30	103.37^{b}±0.10	106.75^{b}±0.12
FCR (g feed/g EM)	2.08±0.17	2.04±0.12	2.01±0.12	2.16±0.12	1.94±0.11	2.11±0.18
PI (g/hen/day)	20.69	20.84	17.95	18.22	14.47	14.94
ME I Kcal\kg	3230.93	3138.18	3153.66	3086.42	2904.69	2892.93
Lys.I* (g/hen/day)	1.01	1.00	0.89	0.88	0.77	0.81
Met + cys. I* (g/hen/day)	0.78	0.77	0.76	0.76	0.69	0.73

Values are means ± SE

[a,b,c] Values with different superscripts at the same row are significantly different at $P < 0.05$ 3- AWG= average weight gain, EW= egg weight, HDEP= hen day egg, EM= egg mass, FC= feed consumption, FCR= feed conversion ratio, PI= protein intake, Lys. I= lysine intake, Met+Cys. I= methionine+cystine

Table 5: Correlation coefficients among performance traits of laying hens fed varying CP and ME levels

* P < 0.05; **P < 0.01

Parameter	FCR (g feed /g EM)	ME I (Kcal\Kg)	PIa (g)	Lys.Ia (g)	Met.+cys.Ia (g)	HDEP%	EW (g)	EM(g/ hens)
FC (g)	0.473	0.903**	0.96**	0.92**	0.97**	0.51	0.69	0.752
FCR (g feed /g EM)		0.19	0.27	0.26	0.53	-0.48	-0.11	-0.2
CP%		0.92**	0.99**	0.98**	0.88*	0.69	0.87*	0.89**
ME(Kcal/kg)		0.23	-0.06	-0.04	-0.17	0.37	0.29	0.26
EM (g/ hen)		0.86*	0.87*	0.84*	0.65	0.93**	0.83*	
HDEP %		0.73	0.74	0.65	0.49		0.55	
EW (g)		0.82*	0.84*	0.89**	0.72			
PIa (g/hen/day)		0.93**		0.98**	0.91**			
ME I (Kcal\Kg)				0.89**	0.89**			
Lys.Ia (g/hen/day)					0.90**			

[a]Standard ilealy digestable amino acid basis

FCR= feed conversion ratio, EM= egg mass, HDEP= hen day egg production, PI= protein intake, Lys. I= lysine intake, Met+Cys. I= methionine+cystine intake.

Egg Quality Traits

Results of overall external and internal egg quality traits of laying hens fed different experimental diets are shown in Table (6), while the results of correlation coefficient statistical analysis indicate the interactions between performance parameters and egg quality traits in different dietary treatments are presented in Table (7). Regarding the external egg

quality traits (EW, ESG and ESW) no significant differences were observed among dietary treatments. The ST results demonstrated that dietary CP level has a positive impact on it rather than dietary energy level as ST was significantly higher ($P < 0.05$) in birds receiving diets containing 18% CP compared to birds receiving lower CP dietary levels .This observation was confirmed by the highly significant ($P < 0.01$) positive correlation coefficients between ST and PI, Lys. I and Met. + Cys. I (Table 8). On the contrary, Nahashon et al., (2007) observed that ST of laying hens was markedly affected by the dietary energy level and birds consuming lower levels of ME (2800 vs. 2900 Kcal/Kg) had significantly higher ST than birds consuming higher levels of ME. However, the highest energy (Kcal/Kg) level used in our study was 2810 which is barely similar to their lower level (2800).The positive correlation between TSAA and ST was early described by Simkiss and Taylor (1971) who suggested that this relationship is due to the fact that TSAA are essential for increasing calcium binding ability of egg shell protein matrix and consequently improving egg shell thickness and quality. Regarding the results of internal egg quality (Table 6), it can be concluded that neither the different levels of CP nor the two levels of ME had caused a significant differences on AI and Haugh unit values (except for G18L). These outcomes agree with Zimmermann and Andrews (1987) who found no effect on Haugh units due to reducing dietary energy and protein levels and with Mendonca and Lima (1999) who observed no effect of reducing protein level on albumen quality of eggs. Nevertheless, Almeida et al., (2012) suggested that although Haugh unit score was not influenced by dietary ME level , but higher levels of CP inversely lowered Haugh unit score compared to reduced CP diets (18% vs 15%) which might be related to the higher egg weights obtained for the birds fed 18% CP, reflected on Haugh units calculation. No significant differences ($P>0.05$) were observed among different dietary treatments in AW due to reducing CP levels this was confirmed with non significant positive ($P>0.05$) interactions between AW and PI, Lys. I as well as Met. +Cys. I Table (**8**). These positive relationships were also described by Novak et al., (2004) who suggested that increasing TSSA and lysine intake per hen daily had a significant influence on albumen weight. On the contrary, the same authors (2006) reported that egg components were influenced by protein intake but not by the TSAA: Lys. and albumen solid percentages all decreased linearly when protein intake was decreased and were probably one of the factors responsible for the reduction in egg weight. These results are in partial consistent to the finding of Novak et al., (2006) as AW had showed a linear decrease with dietary CP reduction but at the high ME level. In addition to that, highly significant positive ($r=0.89$, $P < 0.01$) correlation was observed between YW (g) and ME I (Kcal/Kg). Moreover, the reduction of dietary ME levels at any CP level had no effect on YI (Table 6), nevertheless, YI was significantly higher ($P < 0.05$) in groups fed the highest dietary CP level (18%) compared with the groups fed 16% and 14% CP .This would indicate that YI was improved linearly with the increase in CP intake. These results are supported by the presence of significant ($P < 0.05$) positive correlation coefficients between YI and PI, Lys I as well as a highly significant ($P < 0.01$) correlation coefficient between YI and Met + Cys. (Table 7).

Table (7) : Overall egg quality traits of laying hen

Group Parameter	CG18	G18L	G16	G16L	G14	G14L
EW (g)	60.10± 0.64	59.80 ± 0.61	59.35 ± 0.65	58.32 ± 0.53	58.40 ± 0.52	58.47 ± 0.50
EV	50.00	50.00	50.00	50.00	50.00	50.00
ESG (cm3)	1.20 ± 0.01	1.19 ± 0.01	1.19 ± 0.01	1.17 ± 0.01	1.17 ± 0.01	1.17 ± 0.01
YH (mm)	16.40a±0.12	16.00ab±0.11	15.97abc±0.18	15.17cd±0.27	15.25bcd±0.22	14.97d±0.22
AH (mm)	10.07b±0.11	10.80a± 0.14	10.84a ± 0.18	10.81a± 0.17	10.75a ± 0.15	10.87a± 0.11
YD (mm)	40.45b±0.14	41.13ab±0.18	41.51a ± 0.14	40.95ab±0.13	40.90ab ± 0.14	40.87ab±0.14
AD (mm)	32.77a±0.21	31.87c± 0.20	32.20bc± 0.24	33.10a± 0.20	31.57c ± 0.20	31.90c± 0.20
YW (g)	14.85± 0.28	14.77 ± 0.27	14.95± 0.22	14.72± 0.20	14.44 ± 0.22	14.04 ± 0.20
AW (g)	38.32a±0.39	38.10a± 0.35	37.29ab± 0.39	36.67b± 0.36	36.96ab ± 0.35	37.43a± 0.31
ESW (g)	6.91 ± 0.08	6.95 ± 0.07	7.12 ± 0.07	6.94 ± 0.06	6.97 ± 0.05	6.99 ± 0.06
YI	0.40a±0.003	0.39a± 0.004	0.38bc± 0.005	0.37bc±0.006	0.37bc ± 0.005	0.37c± 0.005
AI	0.32ab±0.004	0.34a± 0.005	0.34a ± 0.006	0.33ab±0.005	0.34a± 0.004	0.34a± 0.004
ST (mm)	0.33a±0.002	0.33a± 0.002	0.32cd± 0.002	0.32cd±0.002	0.31d ± 0.002	0.32b± 0.002
Haugh unit	99.00b±0.01	99.48a± 0.01	98.97b ± 0.01	98.97b± 0.01	98.97b ± 0.01	98.97b± 0.01

1-Values are means ± SE

2-^{a,b,c} Values with different superscripts at the same row are significantly different at $P < 0.05$.

3- EW= egg weight, EV= egg volume, ESG= egg specific gravity, YH= yolk height, AH= albumin height, YD= yolk diameter, AD= albumin diameter, YW= yolk weight, AW= albumin weight, ESW= egg shell weight, YI= yolk index, AI= albumin index, ST= shell thickness.

Table (8): Correlation coefficients among performance and egg quality traits of laying hens fed varying CP and ME levels

Parameter	FC (g)	FCR (g feed /g egg mass	PI (g/hen/day)	ME.I (Kcal/kg)	Lys.Ia (g/hen/day)	Met.+cys.Ia (g/hen/day)	HDEP (%)	EW (g)	EM (g/hen)
ESG (cm3)	0.68	-0.11	0.81*	0.87*	0.86*	0.73	0.55	0.98**	0.82*
YH (mm)	0.67	-0.19	0.82*	0.88*	0.85*	0.69	0.66	0.96**	0.88*
AH (mm)	-0.34	-0.07	-0.50	-0.56	-0.57	-0.41	-0.23	-0.63	-0.43
YW (g)	0.73	-0.09	0.76	0.89**	0.68	0.66	0.88*	0.64	0.88
AW (g)	0.49	0.30	0.67	0.55	0.78	0.56	0.24	0.9**	0.57
ESW (g)	-0.18	-0.35	-0.28	-0.05	-0.30	-0.11	-0.132	-0.08	-0.10
YI	0.69	-0.11	0.81*	0.87*	0.86*	0.73**	0.55	0.98**	0.82*
AI	-0.47	-0.37	-0.54	-0.60	-0.57	-0.53	0.22	-0.49	-0.35
ST (mm)	0.86*	0.42	0.89**	0.76	0.95**	0.89**	0.40	0.83*	0.64
Haugh unit	0.34	-0.34	0.30	0.44	0.27	0.34	0.39	0.42	0.48

ESG= Egg sp. Gravity, YH=Yolk height, AH=Albumin height, ESW= Egg shell weight, YI= Yolk index, AI=Albumin index, ST= Shell thickness

*P < 0.05; **P < 0.01

aStandard ilealy digestable amino acid basis

Economic Efficiency Study

Results of economic efficiency study revealed that lowering both dietary CP and ME energy reduced the price/Kg feed. However, reduced CP diets with higher ME level 2810 kg/kg (CG16 and CG14) achieved more efficient economical profits as their prices were lower than the diet with higher levels of ME and CP(18%) but with no adverse impact on either laying hen performance (egg number/hen).

CONCLUSION

Reduced CP diets formulated on ideal amino acid profile basis may replace the current commercially available diets used in laying hens nutrition in Egypt as the levels of CP found in commercially available diets usually exceeds bird's requirements as stated by NRC (1994) or even by breeds managemental guide.

Under the conditions of this study, reduced CP diet (14% CP) formulated on ideal amino acid profile basis through dietary supplementation with crystalline amino acids was able to maintain most of laying hens performance parameters and egg quality traits while minimizing feed costs and increasing net revenue as well as E.E% during the first phase of egg production.

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