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Original Research Article

Performance, Egg Quality and Some Blood Biochemical Parameters in Laying Hens Exposed to Chronic Heat Stress Affected by Feed Rationing

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ABSTRACT

This study was performed to investigate the effects of feed rationing on laying performance, egg quality and some blood biochemical parameters in laying hens exposed to chronic heat stress. A total of 640 (Lohmann LSL-Lite White egg layers, 50 weeks of age) were placed into the cages as 4 feeding rationing treatments with 4 replicates (n = 40 hens/cage). The dietary treatment groups were assigned as control feeding (*ad-libitum*, CF), daily once morning feeding (102 g feed, DMF), daily once evening feeding (102 g feed, DEF), daily twice feeding (morning and evening 51 g feed, DTF). The egg production was the highest in DMF and DEF groups during the experimental period. Higher egg weight was observed in CF (62.8 g) and DMF (61.1 g) between 51-55 weeks, also egg weight was the highest in CF (63.2 g, P<0.05) between 56-60 weeks. The best feed conversion rate was observed in the DTF group (1.66 between 51-55 weeks and 1.80 between 56-60 weeks of age, P<0.01). At 60 weeks of age, the highest level of total cholesterol, AST, ALT, and H/L ratio were higher in the control group. It could be concluded that feed rationing could be beneficial to alleviate the negative impacts of heat stress on laying performance, egg quality and stress level of hens in comparison with *ad-libitum* feeding.
Keywords: Heat stress, Feed rationing, Laying performance, Egg quality, Blood biochemistry

INTRODUCTION

In layer hen management, the quality of diet and feeding regimes are important issues to maximize egg production and eggshell quality, subsequently profitability in egg production (Sozcu and Ipek 2017). Because it is known that feed cost accounts for approximately 65 to 70% of the total cost of production (Bell and Weaver 2002; Godfray *et al.* 2010). Inaccurate feeding strategies could cause economic losses and serious welfare problems due to reduced egg production, producing smaller egg, reduced eggshell quality, overfeeding and fattening, skeletal problems, and mortality (Bryden *et al.* 2021). Therefore, in commercial layer industry, many practical adjustments for feeding have been made depending on many factors, for example production type, genotype, age, egg weight, environmental conditions, production system, etc. (Sakomura *et al.* 2019).

One of the most important factors affecting the feeding of laying hens is environmental conditions, indeed the environmental temperature has critical importance affecting the feed consumption (Yoshida *et al.* 2011). At that point, heat stress is an important environmental stress factor that has the potential to damage the health status and performance parameters of birds (Daghir 2008). It is known that heat stress begins when the environmental temperature rises above the comfort zone between 16°C-25°C for poultry species (Diarra and Tabuaciri 2014; Akbarian *et al.* 2016). Recent studies have shown that high-yield broiler and layer genotypes have become more susceptible to chronic heat stress in modern poultry production (Abo Ghanima *et al.* 2020b; Ibrahim *et al.* 2021). When laying hens are continuously exposed to higher environmental temperature over 30°C, they are exhibiting some behavioral responses against stressful condition to increase the heat loss, for example higher respiratory rate, panting, loss of appetite, and altered metabolism. As a result of acute or chronic heat stress, egg quality parameters could show a deterioration with a decline of egg production (Deng *et al*. 2012; Ebeid *et al.* 2012; Saleh *et al.* 2020b) that finally results in the increment of mortality and morbidity (Abo Ghanima *et al.* 2020a; Quinteiro-Filho *et al.* 2012) and serious economic losses (Abo Ghanima *et al.* 2020b).

Due to the negative impacts of heat stress on laying performance, health and welfare status of laying hens, some feeding strategies have gained importance and have been applied under commercial conditions (Daghir 2008). These strategies include various treatments as feed additives supplementation into feed or water for example, natural antioxidants, organic acids, vitamins, minerals, electrolytes, prebiotics, probiotics, phytobiotics, feeding rationing, feed restriction, changing of feed form, drinking cold water (Abdel-Moneim *et al.* 2021; Attia *et al.* 2016; Cramer *et al.* 2018; Jahromi *et al.* 2016; Saleh *et al*. 2018, 2020). Changing in feed portioning as partial

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feed restriction or controlled feeding regime especially under heat stress conditions could be helpful to alleviate the harmful effects of stress (MacLeod and Hocking 1993).

The best laying performance of hens could be provided by controlling feed intake during a day, especially under stressful conditions. It is well known that feed consumption is linked with diurnal rhythm and ovulation time (Sauveur and Mongin 1974). Some studies focused on broiler breeders demonstrated that feeding three times a day caused an increment in egg production (Cave 1981) and feeding twice a day increased both laying performance and egg weight (Spradley *et al.* 2008). This study aims to evaluate the effects of the feed rationing (daily feed requirement given with portioning during day and *ad libitum* feeding) on egg laying performance, egg quality parameters, serum biochemical parameters in laying hens exposed to chronic heat stress.

MATERIALS AND METHODS

A total of 640 beak-trimmed pullets (Lohmann LSL-Lite White egg layers, 50 weeks of age) were placed into the enrichment cages as 4 feeding rationing treatments and with 4 replicates (n = 40 hens/cage) in each treatment group. The study was performed between 50 and 60 weeks of age. At 50 weeks of age, all hens were individually weighed with a precision of ±0.1 g digital scale and then randomly placed into treatments.
The dietary treatment groups were assigned as.
1- Control feeding (*ad libitum* feeding, CF),
2- Daily once morning feeding with an amount of 102 g feed (DMF)
3- Daily once evening feeding with an amount of 102 g feed (DEF)
4- Daily twice feeding with an amount of 102 g feed (morning 51 g feed and evening 51 g, DTF)

The hens were kept in an enrichment cage system which was equipped according to the optimum standards explained by the EU Directive 1999/74/EC for laying hens kept in cage systems. In the galvanized wire enrichment cage, each hen was provided a total floor area of 750 cm^2. Each cage compartment was comprised of nipple drinkers (8 nipples/cage compartment), through type galvanized feeders (12 cm of feeder space/hen), an egg belt, a manure belt, nail rasps (8 rasps/cage compartment), perches (18 cm/hen), a nesting area that was surrounded by an orange curtain (102 cm^2/cage compartment), and a scratching pad area with green artificial turf (5.92 cm^2/cage compartment). The lighting program was constantly applied as 16 hours' light and 8 hours' dark period during the experimental period. Standard commercial layer diets were used between 50 and 60 weeks of age (17.35% CP and 2815 ME kcal/kg, Table 1). The nutrient level of feed was analyzed according to the AOAC procedures (2000). Water was offered ad libitum to all hens during the experimental period.

During the experimental period, the amount of feed (daily 102 g) was determined according to the optimum feed consumption value given in the management guide (Lohmann LSL-Lite Layers Management Guide). In the control group, an auto-feeding system was used and feeding was made twice during the day (10.00 am and 10:00 pm). Feed was provided by hand to hens at the same time all days (morning feeding at 08.00 am, evening feeding at 6.00 pm). This experiment was performed under heat stress during the summer season (between June and September). During the experimental period, the temperature and humidity measurements were daily performed (Figure 1). The mean value of inside temperature and relative humidity were observed as 33.2ºC and 76.3%, respectively.

Table 1. The composition and nutrient level of laying hen diet (between 50 and 60 weeks of age)

Ingredient	**(%)**
Corn	65.21
Soybean meal (48% CP)	22.42
Dicalcium phosphate	1.47
Limestone	10.07
Salt	0.35
Premix[1]	0.30
DL-Methionine	0.15
L-Lysine-HCL	0.03
Calculated chemical analysis	
ME, kcal/kg	2,825
Available phosphorus, %	0.37
Chemical analysis	**(%)**
Dry matter	91.3
Crude ash	7.3
Crude fiber	3.2
Crude protein	17.45
Calcium	3.86
Total phosphorus	0.75

[1] 1 kg of premix contains: Vitamin A 8.000 IU; Vitamin D3 2.000IU; Vitamin B2 4 mg; Vitamin B12 10 mg; Vitamin E 15 mg; Vitamin K32 mg; Vitamin B1 3 mg; Niacin 30 mg; Cal-D-pantothenic acid 10 mg;Vitamin B6 5 mg; Folic acid 1 mg; D-biotin 0.05 mg; Vitamin C 50 mg;Choline Chloride 300 mg; Mn 60 mg; Zn 50 mg; Fe 60 mg; Cu 5 mg; Co0.5 mg; Iodine 2 mg; Se 0.15 mg.

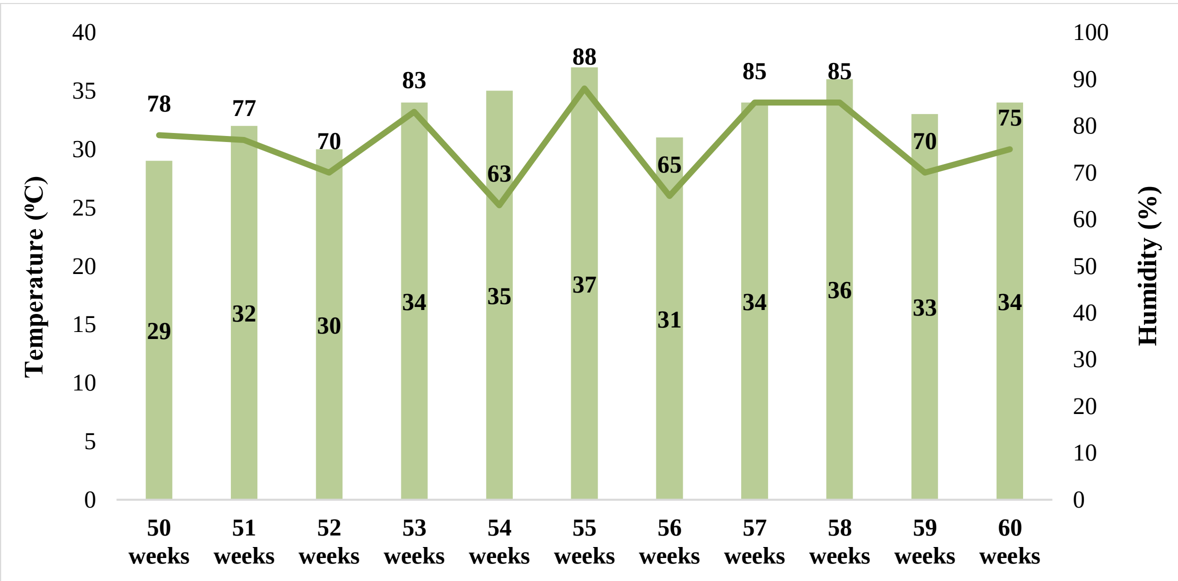


Figure 1. The mean values of temperature and relative humidity between 50 and 60 weeks of age.

The hens were individually weighed weekly from 50 weeks of age to 60 weeks of age. Feed intake and egg weight were recorded weekly. All eggs were collected daily from each group, and the hens were monitored daily until the end of the experiment. Egg production values and mortality were recorded daily. Egg production was calculated by dividing the number of daily eggs by the number of hens on the same day. The efficiency of feed utilization was calculated as a ratio between weekly feed intake and multiplication weekly egg production and egg weight. Data for body weight, egg production, egg weight and feed efficiency were given as the mean of each 5-wk period between 50 and 60 weeks of age. The mortality was daily monitored and recorded until the end of the experiment.

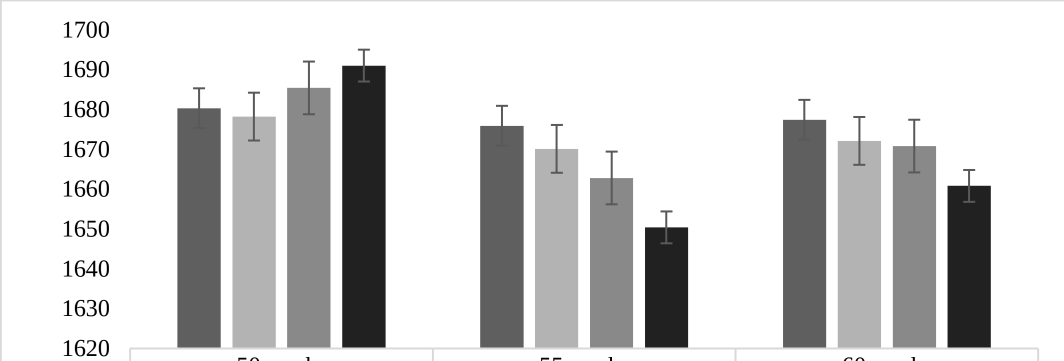
A total of randomly sampled 30 eggs from each treatment group were processed to egg quality parameters at 60 weeks of age. The measurements were performed 24 h after eggs were laid. The eggshell breaking strength (kg/cm^2) was measured by an eggshell force reader machine (Egg Force Reader, Orka Food Technology, Ramat HaSharon, Israel). The eggs were weighed and then broken to separate yolk and albumen. The chalazae were carefully removed from the yolk, and the yolk weight was measured with a ±0.01 g precision. The eggshells were carefully washed and dried for 24 h in a drying oven at 105°C (Nüve FN-500, Ankara, Turkey) and then weighed with a precision of ±0.01 g. Albumen weight was calculated by subtracting the weights of yolk and eggshell from the total egg weight. The ratios of eggshell, albumen, and yolk were calculated as a percentage of total egg weight. Eggshell thickness was measured using a caliper with a ±0.01 mm precision at 3 different points (air cell, sharp end, and equator region) of the eggshell. The eggshell thickness was given as an average of these three eggshell regions' measurement values.

At 60 weeks of age, 4 ml blood samples were collected in nonheparinized tubes from the jugular veins of 20 hens per treatment group. Serum was collected by the method of Calneck et al. (1992) and stored at -20°C for future analysis. The serum samples were analyzed for the activity of aspartate amino transferase (AST) and alanine amino transferase (ALT) using an automatic analyzer (Roche Cobas 6000 C501, Roche Diagnostics, Regensburg, Germany). Plasma levels of immunoglobulins (IgA, IgG, and IgM), triiodothyronine (T_3) and thyroxine (T_4) concentrations, and total cholesterol were measured by a commercial kit (Roche Cobas 6000 series C501 and E601 modules, Roche Diagnostics, North America) according to the analysis techniques described by Carew *et al.* (1997) and Mountzouris *et al.* (2010). One milliliter of blood samples was used for the smearing process on slides of blood to count the heterophils (H) and lymphocytes (L). After air drying, the slides were stained with May Grünwald and Giemsa stain (Clark *et al.* 2009). Each slide was counted for the number of H and L using a microscope with 100×magnification and oil immersion. The means of two slides were calculated for each hen. The H/L ratios were calculated using H/L= number of heterophils / number of lymphocytes.

In the study, data of laying performance, egg quality parameters, and serum biochemical parameters laying hens exposed to chronic heat stress by feed rationing were analyzed using the GLM procedures of SAS statistical software (Version 9.1., SAS Institute Inc, 2003). Each replicate was considered an experimental unit for performance parameters (n = 4 cages/treatment), egg quality parameters (n = 30 eggs/treatment), serum biochemical parameters (n = 20 hens/treatment). Significant differences among the means were compared using the Tukey test and were considered statistically different at a level of $P < 0.05$. The total mortality data were analyzed using chi-square tests to determine the differences among the treatment groups. Data are presented as the means ± SE in all the tables.

RESULTS AND DISCUSSION

The study aimed investigate the effects of feed restriction on the body weight gain, laying performance, egg characteristics and egg weight, mortality, and blood biochemical parameters under chronic heat stress in laying hens. The effects of feed rationing on body weight of laying hens under chronic heat stress are presented in Figure 2. The mean body weight between 50 and 60 weeks was found to be similar among the treatment groups. The body weight of hens was not significantly affected by feed rationing because the birds stabilized their body weight at this age period. This finding is similar to previous results indicated by Oyedeji et al. (2007).



	50 weeks	55 weeks	60 weeks
CF	1680,3	1675,9	1677,4
DMF	1678,2	1670,1	1672,1
DEF	1685,4	1662,8	1670,8
DTF	1691	1650,4	1660,8

Figure 2. Body weight of laying hens exposed to feed rationing under chronic heat stress conditions.

The effects of feed rationing on egg performance, egg weight and feed efficiency in laying hens under chronic heat stress conditions are shown in Table 2. The mean value of egg laying performance was found to be the highest in DMF and DEF groups between 51 and 55 weeks of age (82.4% and 82.7% respectively) and between 56-60 weeks of age (79.8% and 80.2% respectively, P<0.05). The changes in egg weight differed between the periods among the treatment groups. Higher egg weight was observed in CF (62.8 g) and DMF (61.1 g) between 51-55 weeks of age, whereas egg weight was found to be the highest in CF (63.2 g, P<0.05) between 56-60 weeks of age. In each of the 5-weeks interval, lower feed efficiency was observed in the DTF group (1.66 between 51-55 weeks and 1.80 between 56-60 weeks of age, P<0.01). Table 3 presents that any significant differences were observed for mortality among treatment groups under chronic heat stress (P>0.05).

Table 2. The effects of feed rationing on egg laying performance and egg weight in laying hens under chronic heat stress conditions.

Treatment group	**Egg laying performance (%)**		**Egg weight (%)**		**Feed efficiency**	
	51-55 weeks	**56-60 weeks**	**51-55 weeks**	**56-60 weeks**	**51-55 weeks**	**56-60 weeks**
CF	80.8 ± 1.4^{b}	77.4 ± 1.4^{b}	62.8 ± 2.3^{a}	63.2 ± 1.8^{a}	2.17 ± 0.27^{a}	2.25 ± 0.21^{a}
DMF	82.4 ± 1.9^{a}	79.8 ± 1.5^{a}	61.1 ± 2.4^{a}	60.8 ± 2.1^{b}	1.97 ± 0.16^{b}	2.04 ± 0.10^{b}
DEF	82.7 ± 2.1^{a}	80.2 ± 1.8^{a}	59.4 ± 2.1^{ab}	58.6 ± 1.7^{c}	1.79 ± 0.11^{c}	1.87 ± 0.05^{c}
DTF	78.9 ± 1.6^{c}	74.8 ± 1.7^{c}	58.7 ± 1.9^{b}	57.4 ± 2.0^{c}	1.66 ± 0.08^{d}	1.80 ± 0.06^{d}
P value	*	*	*	*	**	**

CF: Control feeding (ad libitum feeding), DMF: Daily once feeding with an amount of 120 g feed in the morning, DEF: Daily once feeding with an amount of 120 g feed in the evening; DTF: Daily twice feeding with an amount of 120 g feed (morning 60 g feed and evening 60 g)
[a-d] Means bearing different superscripts within the same line are significantly different.
n: 4 replicate per treatment group (40 laying hen/pen).
* *P*<0.05, ** *P*<0.001

It is known that nutrient requirement for egg formation is not uniform during the day, after 8 hours of laying the nutrient requirement shows an increment (Morris and Nelbendov 1961). In this study, feeding in the morning or evening provided an increment in egg production compared to the control feeding or daily twice feeding under heat stress. Contrarily, egg weight tended to be heavier in the control group, which could be related to higher body weight in this group. When regarding the feed efficiency, a significant deterioration was observed in the control. These results could indicate that feeding method is comparable for egg laying performance, egg weight and feed efficiency. In a previous study, Oyedeji *et al.* (2007) compared different feed rationing methods in laying hens and found a higher egg production (67.2% and 66.2% respectively) and a more feed efficiency (1.85 and 1.87 respectively) in *ad-libitum* feeding and feeding at the morning (with an amount of 150 g feed), the lowest egg weight (50.60 g) in three times feeding (50 g feed per each feeding) in laying hens at 40 weeks of age.

Table 3. The effects of feed rationing on mortality in laying hens under chronic heat stress conditions (between 50 and 60 weeks of age).

Treatment group	Dead of hens	Mortality (%)
CF	6	15.00
DMF	1	2.50
DEF	2	5.00
DTF	1	2.50
Chi-square	6.908	
P value	0.075	

CF: Control feeding (ad libitum feeding), DMF: Daily once feeding with an amount of 120 g feed in the morning, DEF: Daily once feeding with an amount of 120 g feed in the evening; DTF: Daily twice feeding with an amount of 120 g feed (morning 60 g feed and evening 60 g).

The effects of feed rationing on egg quality parameters in laying hens under chronic heat stress conditions are shown in Table 4. At 60 weeks of age, the egg weight was found to be the highest in the CF group (63.5 g) and the lowest in the DEF (58.8 g) and DTF (57.2 g) groups (P<0.05). Eggs obtained from the CF group had the highest percentage for albumen and the lowest percentage for eggshell (67.4% and 9.0% respectively, P<0.05). Eggshell breaking strength and eggshell thickness were found to be the lowest in the CF groups (2.34 kg/cm^2 and 0.302 mm respectively, P<0.05). The decline in eggshell weight in the control group is similar to other previous studies reported by Backhouse and Gous (2005) and Kontecka *et al.* (2012). These researchers found enhanced eggshell weight which could be related to more feed utilization regarding with increased feed frequency and timetable in broiler breeder hens (Taherkhani *et al.* 2010). As a result of lower eggshell weight, both eggshell breaking strength and eggshell thickness showed a deterioration in the control group under heat stress conditions.

Table 4. The effects of feed rationing on egg quality parameters in laying hens under chronic heat stress conditions at 60 weeks of age.

Treatment group	Egg weight (g)	Yolk ratio (%)	Albumen ratio (%)	Eggshell ratio (%)	Eggshell breaking strength (kg/cm^2)	Eggshell thickness (mm)
CF	63.5±1.8^a	23.6±1.4	67.4±1.8^a	9.0±0.04^c	2.34±0.14^b	0.302±0.03^b
DMF	60.4±1.3^b	24.0±1.3	65.7±2.0^b	10.3±0.06^b	3.14±0.16^a	0.398±0.03^a
DEF	58.8±1.4^c	24.1±1.1	64.5±1.9^b	11.4±0.10^a	3.15±0.14^a	0.414±0.04^a
DTF	57.2±1.6^c	23.7±1.4	64.9±1.5^b	11.4±0.08^a	3.00±0.12^a	0.439±0.04^a
P value	*	NS	*	**	*	**

CF: Control feeding (ad libitum feeding), DMF: Daily once feeding with an amount of 120 g feed in the morning, DEF: Daily once feeding with an amount of 120 g feed in the evening; DTF: Daily twice feeding with an amount of 120 g feed (morning 60 g feed and evening 60 g)
$^{a-d}$ Means bearing different superscripts within the same line are significantly different.
n: 30 eggs/treatment group.
* *P*<0.05, ** *P*<0.001

The effects of feed rationing on blood biochemical parameters in laying hens under chronic heat stress conditions at 60 weeks of age are shown on Table 5. At 60 weeks of age, the total cholesterol level was higher in the control group (166.7 mg/dL), whereas the highest level of AST and ALT were observed in the control group (177.5 IU/l and 0.80 IU/l respectively, P<0.01). H/L ratio was the highest in the control group (0.91%) compared to the other treatment groups (P<0.01). The observed changes in blood biochemical parameters clearly showed that feed rationing affected different trends under heat stress conditions. It is known that the stress factors could increase the cholesterol level of layer hens (Turk and Barnett 1971, Scholtyssek 1992). A higher level of cholesterol in the control group could be related to *ad-libitum* feeding under heat stress which could cause extra stress due to feed metabolism in addition to heat stress. This indication is supported by deterioration in eggshell properties including the ratio of eggshell weight, eggshell breaking strength, and thickness in the control group.

Table 5. The effects of feed rationing on blood biochemical parameters in laying hens under chronic heat stress conditions at 60 weeks of age.

Treatment group	CF	DMF	DEF	DTF	P value
Total cholesterol (mg/dL)	166.7±10.1[a]	146.8±8.4[a]	120.1±13.2[b]	142.5±10.4[b]	*
T_3 (ng/ml)	0.55±0.12	0.50±0.15	0.68±0.15	0.63±0.20	NS
T_4 (μg/dl)	0.74±0.21	0.75±0.14	0.84±0.17	0.74±0.21	NS
AST (IU/l)	177.5±15.1[a]	141.9±13.4[b]	150.4±12.8[b]	132.2±16.4[b]	**
ALT (IU/l)	0.80±0.15[a]	0.53±0.21[b]	0.30±0.15[b]	0.35±0.18[b]	**
IgG (mg/dl)	119.3±21.1	126.0±18.7	131.8±22.4	129.0±24.2	NS
IgA (mg/dl)	32.0±3.4	31.1±2.8	33.4±2.4	32.6±3.1	NS
IgM (mg/dl)	8.60±2.4	9.80±1.8	9.35±3.5	8.70±3.3	NS
Heterofil (%)	27.5±2.1[a]	23.4±3.2[ab]	24.2±2.2[b]	18.3±2.4[c]	*
Lenfosit (%)	30.4±3.2[c]	40.2±2.8[a]	41.2±3.1[a]	36.0±2.1[b]	*
H/L	0.91±0.05[a]	0.61±0.07[b]	0.59±0.09[b]	0.52±0.07[b]	**

CF: Control feeding (ad libitum feeding), DMF: Daily once feeding with an amount of 120 g feed in the morning, DEF: Daily once feeding with an amount of 120 g feed in the evening; DTF: Daily twice feeding with an amount of 120 g feed (morning 60 g feed and evening 60 g)
[a-d] Means bearing different superscripts within the same line are significantly different.
n: 20 hens/treatment group.
* $P<0.05$, ** $P<0.001$

It is known that hepatic enzyme activity including AST and ALT is an indicator for function and health status of the liver in birds (Cheesborough 1987). Both enzymes are released when some degenerative detriments occur in the liver and muscles (Johnston 1999). The observed higher level of AST and ALT levels in the control group could be accepted an indicator for a higher stress level of hens with *ad-libitum* feeding under heat stress. These results could signify that feed rationing under heat stress could provide some protecting effects against the negative effects of heat stress. Furthermore, a higher H/L ratio could be accepted as a stress indicator in the control group compared to the others. Gross and Siegel (1983) indicated that the H/L ratio could be accepted as a reliable parameter for the stress evaluation in birds.

CONCLUSIONS

The current study exhibits the importance of feeding strategies in layer hen nutrition, especially under chronic heat stress conditions, regarding performance parameters and stress level in birds. Observations suggest that *ad-libitum* feeding provided an increment in egg weight, on the other hand caused a deterioration laying performance, feed efficiency, eggshell quality traits, and an increment stress level of birds. It could be suggested that feed rationing could be effective to reduce feed wastage and increase profitability in comparison with *ad-libitum* feeding.

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