

Full Length Research Paper

Heat stress amelioration and production performance in layers supplemented with herbal liquid anti-stressor product

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As the body temperature of birds rise, feed consumption, growth rate, feed efficiency, survivability, egg production and egg quality tends to decline. In order to address the problem of heat stress, an experiment was conducted to determine the effect of AV/LAP/19 liquid (Supplied by M/s Ayurvet Ltd. Baddi, HP, India) containing natural vitamin C with bioflavonoids and selenium, and synthetic ascorbic-acid (vitamin C) supplementation on the performance of commercial layers. 120 birds, 55 weeks of age were divided into 4 equal groups of 30 birds each. They were exposed to a heat stress of $39\pm 8^{\circ}\text{C}$ temperature and temperature humidity index (THI) of 81.33 ± 1.20 . All bird groups were offered basal diet deficient in vitamin C. Negative effect of high ambient temperature and relative humidity was evidenced from the high serum cortisol level (mg/L) (3.42 ± 0.19 to 3.67 ± 0.20) in all groups. It was significantly optimized and lowered down in all treated groups. Concomitantly, increased alkaline phosphatase and alkaline transaminase levels were also lowered in treated birds. The total protein, albumin and globulin were having numerically higher levels in treated birds. It was concluded that the herbal liquid AV/LAP/19 at both dosages ameliorated the heat stress in layer birds as well as resulted in better feed efficiency and immunomodulation. However, significantly better egg production (%) was recorded in the birds supplemented with AV/LAP/19.

Key words: AV/LAP/19, heat stress, herbal products, egg production, vitamin C.

INTRODUCTION

Heat stress remains a perpetual challenge for the poultry enterprises in tropical climate of India. It results from a negative balance between the net amount of energy flowing to its surrounding environment and the amount of heat energy produced by the layers (Ajakaiye et al., 2011). High temperatures, especially when coupled with high humidity, impose severe stress on layer birds and

lead to reduced performance (Ajakaiye et al., 2011). High environmental temperatures stimulate the hypothalamo hypophyseal-adrenocortical axis which increases corticosteroid secretion in response to stress (Ramnath et al., 2008). Higher levels of circulating corticosteroids have a catabolic effect through increase in the free radicals by altering oxidative metabolism, causing impairment of cellular

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functions and thus damage to the cell membrane, muscle wasting and retarded growth (Sujatha et al., 2010). During the periods of heat stress, most of the production energy is diverted to thermoregulatory adaptations which results in oxidative stress induced immunosuppression, predisposing birds to various infectious diseases and high mortality rates (Maini et al., 2007). In laying hens, heat stress suppresses body weight, egg production, egg weight, shell quality and is generally accompanied by suppression of feed intake (Mashaly et al., 2004). Therefore, it remains a prime issue to re-evaluate the poultry management in hot weather, so that heat stress is minimized. There are various strategies to minimize heat stress in layer hens either by changing their environmental condition or by modification of their diets (Ajakaiye et al., 2010). Nutritional strategy being more viable during heat period is based on diet balancing in order to cover the needs of stressed birds for amino acids (protein), energy and electrolytes (Balnave, 2004; Dagher, 2009). The body requirement of ascorbic acid during heat stress in poultry is greater than the amount synthesized by normal tissues and its administration to broilers during heat stress has been shown to be beneficial to the body (Balogun et al., 1996). Non enzymatic antioxidants vitamin C and vitamin E are used and are being explored extensively in the poultry diet, because of their antioxidant effect in the neutralization of the free radicals generated during heat stress (MUSA-Azara et al., 2012; Ajakaiye et al., 2010, 2011; Ramnath et al., 2008; Bell and Marion, 1990; Orban et al., 1993; Zapata and Gernat, 1995).

In the past few decades, a number of Ayurvedic herbal preparations have been extensively used in poultry industry (Ramnath et al., 2008). Polyherbal products containing different immunomodulator (*Withania somnifera*), antistressor (*Phyllanthus emblica*, *Mangifera indica*) and adaptogenic (*Ocimum sanctum*, *W. somnifera*) herbs have been used to protect tissues from superoxide radicals and enhance cell survival by stimulating antioxidative enzymatic systems (Sujatha et al., 2010). The objective of this study was to determine the comparative beneficial effects of dietary incorporation of feed additive-synthetic vitamin C and polyherbal liquid supplement AV/LAP/19 (M/s Ayurved Ltd. Baddi, HP, India) containing natural vitamin C with bioflavonoids and selenium, on feed consumption, egg production, weight and mortality in laying hens exposed to a chronic heat stress.

MATERIALS AND METHODS

The experiment was conducted at the Department of Livestock Production Management, College of Veterinary Sciences, Bidar, Karnataka State, India. Due approval for conducting the experiment was taken from 'Committee for the Purpose and Control and Supervisions on Experimental Animals' (CPCSEA), India. The experimental setup was arranged during the extreme hot summer to early monsoon period (May 2012 to July 2012), where mean

temperature-humidity index was 81.33 ± 1.20 . c was calculated as per the formula proposed by Kelly and Bond (1971):

$$\text{Temperature humidity index (THI)} = (\text{Tdb}) - (0.55 - 0.55 \text{ RH}) \times (\text{Tdb} - 58)$$

where Tdb is dry bulb temperature (°F) and RH is relative humidity expressed as fraction of 1.

Bidar is located at 17.9°N 77.55°E with an average elevation of 615 m from seashore. The climate of this place is characterized by general dryness throughout, where summer is the driest part of the year. May is observed as the hottest month with average daily maximum temperature of 38.8°C and the relative humidity in the afternoon is between 30 and 40%.

Experimental design

Total of one hundred and twenty (n=120) commercial layers of 55-week old age were reared in cages. The layers were divided into 4 groups, namely T0, T1, T2 and T3 with 3 replicates in each group comprising of 10 birds per replicate. The separation of groups was vertically arranged in 2 tier reverse cages to nullify tier effect. All the groups were managed under identical management and environmental conditions except the nutritional treatment given to them. All the birds were exposed to environmental and physiological stress. Commercial layer mash without vitamin C was fed *ad libitum* to all the groups except group T1 where vitamin C at 100 g/tonne of feed was supplemented. Groups T2 and T3 were supplemented with liquid AV/LAP/19 through water for 7 weeks at 3 and 5 ml/100 birds/day, respectively. Thus supplementation of herbal product AV/LAP/19 and synthetic Vitamin C in treatment groups was immediately commenced from 56th week and continued up to 62nd week. The assessment of performance continued for another 5 weeks, that is, up to 67th week. Group T0 was not given any treatment and served as control group in the study. Growth attributes in terms of daily/weekly egg production, egg weight, hen-day egg production (%), and weekly feed consumption were calculated.

Laboratory analysis

In order to determine the level of heat stress and relevant treatment efficacy, biochemical and hormonal estimation were carried out on 56, 60 and 67th week. Blood from individual birds was drawn and serum separated to estimate the serum cortisol measured by radio immune assay (RIA). Total protein, albumin, serum glutamic oxaloacetic transaminase (SGOT) and serum glutamic-pyruvic transaminase (SGPT) levels were determined by using Autospan clinical chemistry diagnostic kits (SPAN diagnostics Ltd, Surat, India). The hemagglutination inhibition (HI) titers were estimated as per the method of Allan and Gouch (1974). The data from the study was pooled and was subjected to suitable statistical analysis as described by Snedecor and Cochran (1994). Significance was set at $P < 0.05$.

RESULTS AND DISCUSSION

Record of temperature was maintained on daily basis where mean maximum daily temperature of $39 \pm 8^\circ\text{C}$ was recorded throughout the experiment. The combination of daily temperature of $39 \pm 8^\circ\text{C}$ and THI of 81.33 ± 1.20 being above the threshold established for poultry (Tao and Xin, 2003; Karaman et al., 2007) indicates that the layer birds

were subjected to heat stress. All respective birds groups except control were treated with synthetic vitamin C and natural vitamin C with bioflavonoids and selenium containing liquid AV/LAP/19 effect as the following.

Growth performance

Environmental stress to layers resulted in declined growth performance in terms of average body weights, total body weight gain, weekly egg production percent, feed efficiency and egg weights. Results have been summarized in Tables 1 and 2.

Body weight

Polyherbal liquid supplement AV/LAP/19 at 3 and 5 ml/100 birds/day, respectively in group T2 and T3 had numerically higher body weight (g) (1609.42 and 1608.28) than that of control group T0 (1512.71) as well as synthetic vitamin C supplemented group T1 (1518.86). Supplementation of synthetic as well as herbal antistressor vitamin C has been scientifically well proven to increase body weight gain as well as improve growth and performance of birds during summer (Sahin et al., 2003; Sapkota et al., 2006; Maini et al., 2007; Sujatha et al., 2010).

Egg production

Exposure of hens to high temperatures resulted in a significant decrease in egg production and quality. The mean egg production (%) recorded for the present experiment was significantly ($P < 0.05$) higher for the liquid AV/LAP/19 supplemented group T2 (82.21) and T3 (79.30) followed by synthetic vitamin C supplemented group T1 (78.84). Lowest egg production was noticed in untreated control group T0 (76.36). The vitamin C supplementation in layer birds improved ($P < 0.05$) the mean egg production, egg weight and egg shell thickness (Khan and Sardar, 2005), egg length, egg width, albumen weight, albumen height and yolk height were obtained for birds given vitamin C supplementation (MUSA-Azara et al., 2012).

Egg weight

The mean egg weight of layer birds in groups T2 and T3 (59.10 and 58.59) was improved. However, synthetic vitamin C supplementation in group T1 (56.82) could not mark its influence over the egg weight as mean egg weight in the untreated control group T0 (55.02) did not differ significantly from it. Thus herbal liquid antistressor formulation AV/LAP/19 does not only ameliorated the

heat stress in group T2 and group T3, but also helped the treated birds to achieve the higher production performance.

Feed efficiency

Higher growth and production performance resulted in better feed efficiency in AV/LAP/19 supplemented group T2 (1.79) and T3 (1.84). However, the feed efficiency in vitamin C supplemented group T1 (1.86) differed non-significantly from untreated control group T0 (1.92). The average feed consumption of layers was improved ($P < 0.05$) with supplementation of vitamin C (Khan and Sardar, 2005).

Biochemical analysis

The results of biochemical estimations (60 and 67th week, 56th week-base reading) are tabulated in Table 3. Before treatment in all birds, higher levels of serum cortisol (mg/L) content was found indicating the effect of high temperature and relative humidity. High ambient temperature induces production and release of corticosteroids (Siegal, 1980), exerts catabolic effects (mobilization of proteins and lipids) through muscle wasting and reduces growth rate (Sujatha et al., 2010; Odedra et al., 1983; Hayashi et al., 1994). Similar results were obtained in the present study, where serum cortisol levels (Table 3) were significantly ($P \leq 0.05$) higher in the control as compared to the treatment groups. After treatment serum cortisol level (mg/L) significantly reduced in all treated groups T1, T2 and T3 (1.97, 1.06, 1.09) as compared to control T0 (3.50). However, liquid AV/LAP/19 supplementation at both dose levels (3 and 5 ml) had even lower concentration ($P < 0.05$) of serum cortisol to those of birds offered synthetic vitamin C. The lowered concentration of cortisol signified the amelioration of heat stress in treated birds. The findings of the present experiment are in corroboration with that of Sujatha et al. (2010) where polyherbal premix (Stresroak at 1 kg/tonne of feed) was used to minimize heat stress in broilers during summer months. The amelioration action was also noted for SGPT (IU/l) and SGOT (IU/l) where AV/LAP/19 supplementation significantly improved and optimized their levels as compared to control group T0 as well as synthetic vitamin C supplemented group T1. Similarly, concentrations of blood enzymes (alkaline phosphatase (ALP), SGPT and SGOT) were lowered ($P < 0.01$) with vitamin C supplementation in all layer birds (Khan and Sardar, 2005). Similarly, decreased serum enzymes levels after vitamin C supplementation was also reported by Chakraborty and Sadhu (1983) and Takeda and Hara (1985).

The total protein, albumin and globulin levels (Table 3) were having numerically higher levels in treated birds;

Table 1. Effect of AV/LAP/19 and synthetic vitamin C anti-stressor on body weights and total weight in laying hens (g).

Age (weeks)	Group			
	T0	T1	T2	T3
56	1442	1431	1491	1496
57	1473	1439	1542	1540
59	1490	1492	1579	1586
61	1506	1525	1612	1617
63	1517	1554	1639	1646
65	1553	1581	1688	1671
67	1608	1610	1715	1702
Total weight gain	166	179	224	206
Change over control	-	13	58	40
Mean	1512.71	1518.86	1609.42	1608.28
Standard error (SE)	22.10027	25.93897	30.37442	27.59461

Results non-significant.

Table 2. Effect of feeding herbal and synthetic antistressor on weekly egg production (%) and average egg weight (g) in laying hens.

Attribute	Group			
	T0	T1	T2	T3
Mean egg production (%)	76.36 ^a	78.84 ^a	82.21 ^b	79.30 ^b
Standard error (SE)	1.20	0.97	1.71	1.50
Mean egg weight (g)	55.02 ^a	56.82 ^a	59.10 ^c	58.59 ^{bc}
Standard error (SE)	0.473036	0.319525	0.524982	0.398569
Feed efficiency	1.92 ^a	1.86 ^a	1.79 ^b	1.84 ^a
Standard error (SE)	0.027121	0.036957	0.029244	0.037257

The values bearing minimum one common superscript in a row do not differ significantly.

however, their levels did not differ significantly in control as well as treated birds.

Immunomodulation

The results on immuno-modulatory investigations

are depicted in Table 4. The observations and analysis of antibody titers against Ranikhet disease (RD) in 59th, 63rd and 67th week revealed cognizable enhancement in antibody titers of herbal product treated layers (1:256) in comparison with non-treated as well as layers supported

supported with vitamin C (1:64, 1:128).

Conclusion

Heat stress is a major welfare problem in the

Table 3. Effect of feeding synthetic vitamin C and herbal antistressor on biochemical parameters in ameliorating heat stress in layers.

Parameter	Control		Synthetic vitamin C		AV/LAP/19 (3 ml/100 birds/day)		AV/LAP/19 (5 ml/100 birds/day)	
	Before treatment	After treatment	Before treatment	After treatment	Before treatment	After treatment	Before treatment	After treatment
Cortisol (mg/ml)	3.64 ^a ±0.15	4.21 ^a ±0.13	3.50 ^a ±0.17	1.97 ^b ±0.23	3.67 ^a ±0.20	1.06 ^d ±0.05	3.42 ^a ±0.19	1.09 ^d ±0.05
Total protein (g/dl)	5.27±0.15	5.16±0.13	5.30±0.14	5.43±0.13	5.47±0.18	5.28±0.11	5.26±0.17	5.26±0.11
Albumin (g/dl)	2.41±0.08	2.21±0.09	2.34±0.08	2.38±0.09	2.59±0.12	2.48±0.07	2.45±0.13	2.40±0.07
Globulin (g/dl)	2.86±0.09	2.95±0.07	2.96±0.11	3.05±0.06	2.89±0.08	2.79±0.08	2.81±0.07	2.86±0.07
SGPT (IU/L)	58.13 ^a ±1.70	59.83 ^a ±1.69	57.53 ^a ±2.18	46.07 ^b ±1.94	59.33 ^a ±1.69	38.80 ^b ±0.87	58.73 ^a ±2.36	38.70 ^b ±0.72
SGOT (IU/L)	61.93 ^a ±1.85	63.07 ^a ±1.11	59.73 ^a ±2.24	43.90 ^b ±1.94	58.80 ^a ±1.48	40.10 ^b ±0.75	58.07 ^a ±1.63	40.40 ^b ±0.75

Mean values with different superscripts in a row differ significantly (P<0.05).

Table 4. Antibody titre levels against RD by using HI tests in layers supplemented synthetic and herbal anti-stressor products vs. untreated control.

S/N	Sampling week	Sample no.	HI titre			
			T ₀	T ₁	T ₂	T ₃
1	59	1	1:32	1:32	1:128	1:128
		2	1:64	1:64	1:256	1:128
		3	1:32	1:64	1:128	1:256
		4	1:64	1:128	1:256	1:128
		5	1:32	1:32	1:128	1:128
2	63	1	1:64	1:128	1:256	1:256
		2	1:128	1:256	1:256	1:256
		3	1:64	1:128	1:256	1:128
		4	1:128	1:128	1:256	1:256
		5	1:128	1:128	1:256	1:256
3	67	1	1:32	1:64	1:128	1:128
		2	1:64	1:128	1:256	1:128
		3	1:64	1:64	1:128	1:128
		4	1:32	1:128	1:256	1:128
		5	1:32	1:64	1:256	1:256

poultry industry leading to huge economic losses every year, because of mortality and decreased production. Dietary supplementation of synthetic vitamin C and herbal liquid AV/LAP/19 containing

natural vitamin C with bioflavonoids and selenium ameliorated the heat stress by optimizing serum cortisol, SGPT and SGOT levels. A non-significantly different positive impact of supplementing

AV/LAP/19 at two different dose rates was reflected from improved total body weight gain, feed efficiency and egg weight in layers as well as immunopotential when compared with non

production (%) was recorded in the birds of group T2 supplemented with AV/LAP/19 at 3 ml/100 birds/day followed by T3 supplemented with AV/LAP/19 at 5 ml/100 birds/day as compared to untreated control group. Thus it may be recommended to use herbal liquid formulation to curb the losses incurred from heat stress.

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