Performance of Layer Birds Supplemented with Herbal Antistress Product Ayucee and Synthetic Vitamin C under Physiological Heat Stress

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Abstract

Heat stress exerts high deteriorating impact on the poultry industry which could be ameliorated by dietary incorporation of synthetic vitamin C. Certain herbs either alone or in combination thereof are also a rich source of ascorbic acid in natural form. Thus, a 12-week experiment was conducted to determine the effects of high temperature and relative humidity on production performance in layer chickens supplemented with herbal antistress product and synthetic vitamin C. A total of 90 layer birds of 55 weeks age were divided in 3 equal groups (T0, T1 and T2) of 30 birds in each. They were exposed to a heat stress of daily temperature 39±8℃, and temperature humidity index (THI) of 81.33±1.20. To curb the losses incurred due to heat stress, birds in group T1 and T2 were treated respectively with synthetic vitamin C @ 100 g/tonne and herbal premix Ayucee containing natural Vitamin C and bioflavonoids @ 100 g/tonne of feed on daily basis for a period of seven weeks. Group T0 was not treated by any supplementation and kept as control. High ambient temperature and relative humidity resulted in high serum cortisol level (mg/L) (3.47±0.20 to 3.64±0.15) in all groups. It was significantly optimized and lowered down in both the treated groups. A significant difference existed between the initial and after treatment values in both the treatment groups while serum cortisol level was on a continuous rise in untreated T0 control group. Treatment with herbal Ayucee premix and synthetic vitamin C successfully optimized the increased ALT and AST levels and total protein, albumin and globulin in treated birds. Better growth and performance in terms of higher body weight, egg production % and egg weight was resulted from dietary incorporation of antistressor product containing vitamin C. Thus considering the cost benefit ratio herbal natural product Ayucee premix can replace synthetic vitamin C.

Keywords: Ayucee, heat stress, bioflavonoids, egg weight, Vitamin C

Introduction

Poultry industry has developed in several areas such as nutrition, genetics, management to maximize the efficiency of growth performance, egg and meat yield (Munj *et al.*, 2010). Although it acts as a driving force in the development of livestock sector in India alongside it suffers many constraints hindering its overall growth. Heat stress is one amongst such and a serious problem for poultry industry in many parts of the world (Ali *et al.*, 2010).

Animals have known zones of thermal comfort that vary by the species, physiological status, relative humidity, the velocity of ambient air and the degree of solar radiation (NRC, 1981). When the environmental temperature exceeds upper critical temperature of the thermo neutral zone, thermoregulatory reactions are limited and animal is considered to be heat stressed (Sahin *et al*,. 2009). High ambient temperatures compromise performance and productivity through reducing feed intake and decreasing nutrient utilization, growth rate, egg production, egg quality, and feed efficiency, which lead to economic losses in layer poultry (Sahin *et al.*, 2009; Mashaly *et al.*, 2004). High temperatures, especially when coupled with high relative humidity, impose severe stress on layer birds (Ajakaiye *et al.*, 2011). These adverse environmental conditions lead to oxidative stress associated with increased oxidative damage and lowered plasma concentrations of antioxidant vitamins (Sahin *et al.*, 2009). Ascorbic acid synthesis is decreased at elevated environmental temperature making it an essential dietary supplement during the summer (Naqvi and Ezeji, 2012). The role of dietary supplements such as vitamin C for alleviating the effect of heat stress in poultry either by reduced rectal temperature, increased Hb and PCV %, reduced plasma concentration of cholesterol and overall growth performance has been illustrated by many investigators (M Usa-Azara *et al.*, 2012; Ajakaiye *et al.*, 2010; Ajakaiye *et al.*, 2011; Ramnath *et al*, 2008; Abdel-Fattah, 2006; Sahin and Kucuk, 2003; Abou-Zeid *et al.*, 2000; Abou-Egla, 2001; Bell and Marion, 1990; Orban *et al*, 1993; Zapata and Gernat, 1995). Its dietary supplementation reduces the respiratory quotient in heat-stressed birds by emphasizing an increase in fatty acid oxidation over the increase in protein-derived gluconeogenesis (McKee *et al.*, 1997). Ascorbic acid supplementation improves carcass quality and produces higher carcass weight and CP content, while reducing

carcass crude fat content (Kutlu, 2001). It also improves the growth, feed efficiency, egg production, egg weight, number of eggs hatched, shell quality and livability during heat stress (Naqvi and Ezeji, 2012). This improved performance is associated with the suppressed stress responses indicated by the reduction in plasma corticosterone level (Mahmoud *et al.*, 2004; Mckee and Hurrison, 1995; Pardue *et al.*, 1985), adrenocorticotropic hormone (Sahin *et al.*, 2003) and increased serum insulin, T3 and T4 (thyronine) concentrations (Sahin *et al.*, 2002). In addition ascorbic acid synthesized in avian kidneys has been demonstrated to improve disease resistance in birds by optimizing the function of the immune system (Lohakare *et al.*, 2005). Thus sticking to the emergency for dietary incorporation of vitamin C, it is being constantly added to poultry diet in synthetic form. However nowadays, the poultry industry has focused more attention towards addressing public concern for environmental and food safety (Munj *et al.*, 2010). For this the ascorbic acid in organic and herbal form is being preferred by poultry producers. Ayucee (*Supplied by M/s Ayurvet Ltd. Baddi, HP, India*) a natural Vitamin C and bioflavonoids containing product owes its beneficial properties to the constituent herbs *Phyllanthus emblica, Ocimum sanctum, Terminalia chebula* and *Withania somenifera* (Rindhe *et al.*, 2012). Its efficacy for improving haematobiochemical profile (Korde *et al.*, 2011a), thyroid hormone status, growth performance (Korde *et al.*, 2011b) and meat quality attributes (Rindhe *et al.*, 2012) has been proved for broiler birds. However its potency in the form of heat stress amelioration for layers and improving the egg production parameters wasn't backed by any scientific validation. Hence the present investigation was done to assess the performance of layer birds supplemented with synthetic and herbal vitamin C under heat stress condition.

Materials and Methods

The current study was conducted during the extreme hot summer to early monsoon period (May 2012 to July 2012) at Department of Avian Production and Management, College of Veterinary Sciences, Bidar, Karnataka state, India. Bidar being located at 17.9°N 77.55°E with an average elevation of 615 meters from seashore and the climate of this place remains dry throughout the year. Here summer is the driest part of the year and May is observed as the hottest month with average daily maximum temperature of 38.8 °C and the relative humidity in the afternoon between 30 and 40%.

During the experiment daily temperature and relative humidity records of poultry house were kept and THI was calculated as per the formula proposed by Kelly and Bond (1971). THI = Tdb - (0.55-0.55RH) x (Tdb-58) where Tdb =Dry Bulb Temperature (°C) RH = Relative humidity expressed as fraction of 1.

Experimental design

Total ninety (n=90) 55-week old commercial layers (BV_{300}) were divided into 3 groups viz. T0, T1, and T2 with 3 replicates in each group comprising of 10 birds per replicate. Layer birds while exposed to environmental and physiological stress were fed *ad libitum* with commercial layer mash (Table 1) and water. Birds in groups T1 and T2 were supplemented respectively with synthetic vitamin C at the rate of 100 g/ton and Ayucee premix a natural Vitamin C and bioflavonoids at the rate of 100 g/tonne of feed for 7 weeks commencing from 56th week and continued up to 62nd week. Group T0 was not given any treatment and served as control group in the study. Growth and production performance was estimated in terms of daily/weekly egg production, egg weight, egg production (%) and weekly feed consumption for the experimental trial were calculated.

Table 1: Ingredients and chemical composition of diets

Ingredients	Diet		
	T0	T1	T2
Maize	56	56	56
Rice polished	4	4	4
Soya meal	20.5	20.5	20.5
Ground nut cake	7	7	7
Deoiled rice bran	0.95	0.95	0.95
Dicalcium phosphate	1.3	1.3	1.3
Limestone powder	4	4	4
Shell grit	5.3	5.3	5.3
Trace min.	0.50	0.50	0.50
Salt	0.25	0.25	0.25
Sodium bicarbonate	0.1	0.1	0.1
Vit mix.	0.025	0.025	0.025
Phytase PMX	0.1	0.1	0.1
Toxin binder	0.1	0.1	0.1
Trace mineral mix.	0.1	0.1	0.1
Liver tonic	0.025	0.025	0.025
DLM g/100kg	200.00	200.00	200.00
Choline chloride g/100kg	20	20	20
Energy (Kcal/kg)	2600	2600	2600
Protein %	18.00	18.00	18.00
ME:CP	154.76	154.76	154.76
Crude fat %	3.26	3.26	3.26
Crude fiber %	3.62	3.62	3.62
Calcium %	3.71	3.71	3.71
Ava. Phos. %	0.41	0.41	0.41

Laboratory analysis

Level of heat stress and relevant treatment efficacy was determined from biochemical and hormonal estimation carried out on 56th, 60th 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, AST and ALT levels were determined by using Autospan clinical chemistry diagnostic kits (SPAN Diagnostics Ltd, Surat, India). The H.I. titers were estimated as per the method of Allan and Gouch (1974).

Statistical analysis

The data from the study were pooled and subjected to suitable statistical analysis as described by Snedecor and Cochran (1994). Significance was set at $p < 0.05$.

Results and Discussion

Normothermia zone of 22-28℃ (Donkoh, 1989) or 18-24℃ (Holik, 2009) and the temperature humidity index, THI threshold of 70, were established for poultry in the tropical regions (Bouraoui *et al.*, 2002., Karaman *et al.*, 2007). For the current experimental observations of temperature on daily basis, 39±8℃ with temperature humidity index (THI) 81.33±1.20, were above the threshold established for poultry (Tao and Xin, 2003., Karaman *et al.*, 2007) indicated that the layer birds were exposed to heat stress (Ajakaiye *et al.*, 2010). In order to relieve heat stressed birds and improving the performance they were supplemented with synthetic vitamin C and Ayucee premix containing natural vitamin C with bioflavonoids and results of which are described below.

Growth performance

Table 2 depicts the losses and concomitant amelioration from heat stress as determined from average body weights, total body weight gain, weekly egg production %, feed efficiency and egg weights.

Body weight: Though there was no significant difference observed, polyherbal premix Ayucee supplementation at the rate of 100g/tonne of feed in group T2 had numerically higher body weight (1580.57g) than that of control group T0 (1512.71g) as well as synthetic vitamin C supplemented group T1 (1518.86g). In another study broilers under extreme heat stress and receiving vitamin C improved body weight gain and feed conversion ratio (Talebi and Khademi, 2011; Celik and Ozturkcan, 2003). Supplementation of Ayucee premix to poultry birds has been proved to increase the body weight of birds (Korde *et al.*, 2011b). Perusal of literature reveals many authors had reported that dietary incorporation of synthetic and herbal antistressor vitamin C to increase body weight gain as well as improve growth and performance of birds during summer (Sahin *et al.*, 2003; Sapcota *et al.*, 2006; Maini *et al.*, 2007; Sujatha *et al.*, 2010; Jaffar and Blaha, 1996).

Table 2: Mean body weight, egg parameters and feed efficiency of laying hens fed synthetic vitamin C (T1) and herbal antistress product Ayucee (T2)

Parameter	Treatment		
	T0	T1	T2
Body weight (g)	1512.71±22.10	1518.86±25.94	1580.57±29.75
Mean egg production (%)	76.36^{a}±1.20	78.84^{a}±0.97	80.84^{b}±1.49
Mean egg weight (g)	55.02^{a}±0.47	56.82^{a}±0.32	58.60^{b}±0.68
Feed efficiency	1.92^{a}±0.03	1.86^{a}±0.04	1.79^{b}±0.03

^{ab}Means within row with different superscripts differ significantly at $p < 0.05$.

Egg production: High ambient temperature has a highly detrimental effect on egg production in laying hens (Smith, 1974). In the present study production losses mainly in terms low egg production occurred due to high temperatures and relative humidity. The mean egg production (%) was recorded to be significantly lower in untreated control group T0 (75.88 %) in contrast to significantly higher ($p<0.05$) egg production noticed in Ayucee supplemented group T2 (80.84 %). It was also improved over synthetic vitamin C supplemented group T1 (78.18 %). Earlier vitamin C supplementation in layer birds improved ($p<0.05$) the mean egg production, egg weight and egg shell thickness (Khan and Sardar, 2005) and egg length, egg width, albumen weight, albumen height and yolk height (M Usa-Azara *et al.*, 2012).

Egg weight: Several authors (Khan and Sardar, 2005; Lazar *et al.*, 1983; Slinger 1985) reported that vitamin C supplementation increased egg weight under high temperature and relative humidity. In the present study, significantly higher ($p<0.05$) mean egg weight was attained by Ayucee supplemented group (58.60 g) followed by synthetic vitamin C supplemented group T1 (56.82 g) than untreated control group T0 (55.02 g).

Feed efficiency: Perusal of literature reveals (Pardue *et al.*, 1985; Njoku, 1986; Njoku and Nwazota, 1989; Khan and Sardar, 2005) vitamin C supplementation in feed improved feed efficiency of layers under stress conditions. Similarly, herbal supplement for vitamin C, Ayucee premix, had improved feed efficiency in group T2 (1.79). These finding are in corroboration with Korde *et al.* (2011b) where supplementation of Ayucee premix to the broilers had significantly increased ($p<0.05$) live body weight and weight gain and shown a better feed efficiency compared with control birds. However the feed efficiency in synthetic vitamin C supplemented group T1 (1.86) differed non significantly from untreated control group T0 (1.92).

Biochemical analysis

Table 3 demonstrates the results of biochemical estimations at 56th, 60th and 67th weeks. Several studies demonstrated that heat stress causes an increased free radical production (Halliwell and Gutteridge, 1989; Sujatha *et al.*, 2010) and induces production and release of corticosteroids (Siegal, 1980). It 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). It was stated that by decreasing synthesis and secretion of corticosteroids, vitamin C alleviates the negative effects of stress such as heat or cold stress-related depression in performance of poultry (Kutlu and Forbes, 1993; McDowell, 1989). Higher levels of serum cortisol content before treatment in all birds was resultant of the high temperature and relative humidity where serum cortisol levels (Table 3) were significantly higher ($p\leq0.05$) in the control compared with the treatment groups. After treatment serum cortisol level reduced significantly in treated groups T1 and T2 (1.97±0.23 mg/L, 1.36±0.07 mg/L) as compared with control T0 (4.21±0.13 mg/L). In comparison Ayucee supplementation 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 antistressor premix (Stresroak @ 1 kg/tonne of feed) was used to minimize heat stress in broilers during summer months. The amelioration action was also noted for ALT and AST where Ayucee supplementation significantly improved and optimized their levels as compared with control group T0 as well as synthetic vitamin C supplemented group T1. Similarly concentrations of blood enzymes (ALP, ALT and AST) were lower ($p<0.01$) with vitamin C supplementation in layer birds (Khan and Sardar, 2005). Decreased serum enzyme levels after vitamin C supplementation was also reported by Chakraborty and Sadhu (1983) and Takeda and Hara (1985).

Table 3: Effect of feeding synthetic vitamin C (T1) and herbal antistress product Ayucee (T2) on biochemical parameters in ameliorating heat stress in laying hens

Parameter	Control (T0)		Synthetic Vitamin C (T1)		Ayucee (T2)	
	Before treatment	After treatment	Before treatment	After treatment	Before treatment	After treatment
Cortisol (mg/ml)	$3.64^{a} \pm 0.15$	$4.21^{a} \pm 0.13$	$3.50^{a} \pm 0.17$	$1.97^{b} \pm 0.23$	$3.47^{a} \pm 0.20$	$1.36^{c} \pm 0.07$
Total Protein (g/dl)	5.27±0.15	5.16±0.13	5.30±0.14	5.43±0.13	5.36±0.17	5.40±0.14
Albumin (g/dl)	2.41±0.08	2.21±0.09	2.34±0.08	2.38±0.09	2.49±0.14	2.50±0.09
Globulin (g/dl)	2.86±0.09	2.95±0.07	2.96±0.11	3.05±0.06	2.86±0.18	2.90±0.07
ALT (IU/l)	$58.13^{a} \pm 1.70$	$59.83^{a} \pm 1.69$	$57.53^{a} \pm 2.18$	$46.07^{b} \pm 1.94$	$58.20^{a} \pm 1.82$	$42.73^{b} \pm 1.12$
AST (IU/l)	$61.93^{a} \pm 1.85$	$63.07^{a} \pm 1.11$	$59.73^{a} \pm 2.24$	$43.90^{b} \pm 1.94$	$60.27^{a} \pm 2.00$	$41.47^{b} \pm 0.74$

[ab]Means within row with different superscripts differ significantly at $p < 0.05$.

The total protein, albumin and globulin levels (Table 3) were numerically higher in treated birds however their levels didn’t differed significantly in control as well as treated birds.

Immunoregulation

Heat stress caused a reduction in antibody production in laying hens (Mashaly *et al*., 2004). Similarly heat stressed and non-treated layers had lower antibody titres (1:64) results as shown in Table 4. Analysis of antibody titers against Newcastle disease in 59th, 63rd and 67th week’s revealed significant improvement in antibody titers of Ayucee layer birds (1:256) as compared with synthetic vitamin C (1:128).

Table 4: Average antibody titre levels against R.D. using HI test in laying hens fed synthetic vitamin C (T1) and herbal antistress product Ayucee (T2)

Sampling week	HI titer		
	T0	T1	T2
59	1:32	1:32	1:128
63	1:64	1:128	1:256
67	1:32	1:64	1:128

Conclusions

To alleviate the problem of heat stress recommendations regarding housing, ventilation, and cooling systems are possible at large commercial scale as it involves high cost. Instead, because of being practical, nutritional manipulation with its low cost is a common approach in poultry production. Thus it may be concluded that natural vitamin C and bioflavonoids supplementation in the form of Ayucee premix had beneficial effect on growth, feed efficiency, biochemical profile and egg production parameters. Simultaneously it has also shown the improved immune response in supplemented birds. Dietary incorporation of herbal Ayucee premix will be beneficial to minimize the production losses out of heat stress in layers.

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