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EFFECTS OF SELENIUM AND VITAMIN C SUPPLEMENTED WITH HIGH ENERGY DIET ON THE PERFORMANCE OF BROILERS IN COLD (15 ^{o}C) ENVIRONMENT

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Summary

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One hundred and twenty one-day-old, Ross 308 broiler chicks were randomized into 1 control and 3 experimental groups each containing 30 birds. Each group was further divided into one main and two replicate subgroups. Group I (control) was fed the basal diet, group II (HE) was fed a high energy diet (3250 kcal/kg as starter diet; 3300 kcal/kg as grower diet), group III (HE+Se) was fed the HE diet supplemented with 1 mg/kg Se as sodium selenite, group IV (HE+Vit C) was fed the HE diet supplemented with 250 mg/kg vitamin C as ascorbic acid. The highest feed intake values were observed in the HE+vitamin C (P<0.01) during 4–40 days of age. Performance of the animals was positively affected by the supplementation of vitamin C and selenium, and the crude protein retention increased only in Group IV. Relative liver weight was the highest in group II (P<0.05) and relative thyroid weight – in the control group (P<0.05), but the relative weights of heart, lung, spleen and gizzard did not differ significantly among groups. With these results, we may suggest the supplementation of vitamin C and selenium in broilers exposed to cold environment instead of feeding them only with high energy.

Key words: broiler, cold-conditioning, high energy; performance, selenium, vitamin C

INTRODUCTION

Nutrient requirements have been commonly established in an environment protected from climatic extremes. For that reason, they are most relevant during optimum environmental conditions and less appropriate when animals are exposed to environmental stress (NRC, 1981**)**. Stress declines the production and moreover, may increase the death rate in poultry (Filizciler *et al*., 2002; Cerci *et al*., 2003). Cold environment as a physical stressor, has been shown to have variable modulatory effects on cells of the immune system in animals (Tatli Seven & Seven, in press). Despite the general awareness that energy demands are increased by cold and that the magnitude of those demands is moderated by total body insulation, few quantitative data exist relating to environment, nutrient needs, and productive efficiency (NRC, 1981). Low ambient temperature results in increased feed intake and decreased performance in poultry (Spinu & Degen, 1993; Stillman *et al*., 1998). Animals acclimated to cold were shown to achieve higher maximum sustained energy intake rate than non-acclimated ones (Liu *et al*., 2002).

Factors as feed quality and feed supplements have an impact on the potential for acclimatization in poultry. Selenium (Se) is an essential trace element which is involved in thyroid metabolism. Thyroid function is known to be altered by many environmental factors, such as energy intake and dietary composition in addition to ambient temperature (Goehring *et al*., 1984; Gerloff, 1992; Chang *et al*., 2005). Se is essential for appropriate thyroid hormone synthesis, activation and metabolism as the three isoenzymes of iodothyronine 5'-deiodinase are selenoenzymes (Behne *et al*., 1990).

Vitamin C (Vit C) is a small, water-soluble anti-oxidant molecule which acts as a primary substrate in the cyclic pathway for enzymatic detoxification of hydrogen peroxide. In addition, it acts directly to neutralize superoxide radicals, singlet oxygen or superoxide and as a secondary anti-oxidant during reductive recycling of the oxidized form of α-tocopherol, another lipophilic anti-oxidant molecule (Shalata & Neumann, 2001). The dietary supplementation of Vit C reduced the negative effects of environmental stress in poultry because of the reported benefits of the vitamin on poultry reared under heat or cold stress (Tatli Seven *et al*., 2008; Tatli Seven & Seven, in press). Pardue & Thaxton (1986) have documented that particular environmental stressors can alter ascorbic acid use or synthesis in avian species. A combination of the antioxidant Vit C and a diet of high energy (HE) could have a positive effect on broiler's performance reared in a cold environment.

This study was planned to determine the effects of Se and Vit C supplements to a high energy diet on feed intake, body weight gain, feed conversion ratio, mortality, crude nutrients retention, relative organ weights of broilers reared in a cold environment (15 ^{o}C).

MATERIALS AND METHODS

In this study, 120 one-day-old, Ross 308 broiler chicks were included. They were randomly divided into 1 control and 3 experimental groups of 30 birds each. Each group was further divided into one main and two replicate subgroups.

Corn and soybean meal-based ration was formulated according to the requirements suggested by NRC (1994). Diets were formulated as starter and grower diets (Table 1). The groups were fed as followed: group I (control) was fed a basal diet, group II (HE) was fed a high-energy diet (3250 kcal/kg as starter diet; 3300 kcal/kg as grower diet); group III (HE+Se) was fed the HE diet supplemented with 1 mg/kg Se as sodium selenite; group IV (HE+Vit C) was fed the HE diet supplemented with 250 mg/kg Vit C as ascorbic acid. Mortalities were determined. A photoperiod of 23 h light and 1 h dark was maintained. Chicks were warm-room reared at 33.20±2.50 ^{o}C during the first week and at 26.20±1.85 ^{o}C for the second week. Starting at day 14 and through week 6, birds were cold-stressed at an average room temperature of 14.90 ±1.98 ^{o}C.

Liver, heart, lung, kidney, spleen, gizzard and thyroid organs were obtained from 10 birds of each group by the 40th day and weighed. The relative organ weights (organ weight per 100 g live body weight) were determined. Feed intake (FI), body weight (BW), body weight gain (BWG), feed conversion ratio (FCR) were determined at experimental days 21, 28, 35 and 40. At the end of experiment, 5 hens per treatment were individually caged to determine retention of

Table 1. Composition of experimental diets, %

Ingredients	Control group		High energy groups	
	Starter	Grower	Starter	Grower
Corn	56.50	60.81	54.93	58.63
Soybean meal	32.10	30.65	26.50	31.80
Fish meal	5.00	–	9.00	–
Soybean oil	3.00	5.00	6.60	6.60
Limestone	1.30	1.50	1.10	1.60
Dicalcium phosphate	1.00	0.95	0.80	0.30
L-lysine hydrochloride	0.20	0.04	0.10	0.10
Vitamin-mineral premix[1]	0.35	0.50	0.50	0.50
DL-methionine	0.30	0.20	0.22	0.22
Sodium chloride	0.25	0.25	0.25	0.25
Calculated nutrient content[2]				
ME, kcal/kg	3036	3190	3250	3300
CP, %	22.40	19.20	22.40	19.20
Calcium, %	1.00	0.90	1.00	0.80
Total phosphorus,%	0.48	0.54	0.60	0.42
Selenium ppm (analysed)	42.20	39.65	44.10	39.20

[1] Vitamin and mineral premix provided per kg diet: vitamin A, 12000 IU; cholecalciferol, 1500 IU; vitamin E, 30 mg; vitamin K, 3.5 mg; vitamin B1, 3 mg; vitamin B2, 6 mg; vitamin B6, 5 mg; vitamin B12, 30 µg; Ca-D-pantothenate, 10 mg; Folic acid, 0.75 mg; D-biotin, 0.08 mg; Mn, 80 mg; Zn, 60 mg; Fe, 40 mg; Cu, 5 mg; Se, 0.15 mg; Co, 0.1 mg; I, 0.4 mg; [2] based on NRC (1994) feed composition tables.

dietary nutrients at the same low temperature.

Excreta of hens were collected for 3 days. Chemical analyses of the diet and excrement samples were analyzed according to procedures of AOAC (1995). The basal and high energy diets were analysed for Se (Brown & Watkinson, 1977). For nutrient retention studies, chicks in cages were fed finisher diets containing 0.25% chromium oxide as an indigestible marker at 35 days of age. Faeces were dried in a forced-air drying oven at 60 °C for 3 days and stored. Dry matter, organic matter, crude ash, crude protein and crude fat retention were determined according to the method of Petry & Rapp (1971). Mortality during the experiment was recorded.

Data were subjected to analysis of variance, and where significant differences were observed, Duncan's multiple range test was run as a *post hoc* test. The results were considered as significant at P<0.05.

RESULTS

There were no significant differences between groups in BW and BWG values except at 40th days and 4–40 days, respectively (Table 2). These parameters for HE +Vit C group were significant higher vs the other groups (P<0.05). The FI values were highest at days 4–40 in the group that consumed the HE + Vit C diet (P<0.01; Table 2). FCR was the worst in the control group at 4–40 days (P<0.05).

However, the HE+Vit C group exhibited the highest mortality rate (three deaths total, 1 for each of periods 4–21, 29–35 and 36–40 days). One chicken from controls died as well. There were not deaths in HE and HE+Se groups.

The ash and fat retentions of control and experimental groups were not significantly different (Table 3). Protein retention was significantly higher in the HE+Vit C group compared to the other three groups (P<0.05).

The relative weight of liver was the highest in the HE group (P<0.05) (Table 4). The relative weights of heart, lung, spleen and gizzard however were not statistically significant between groups. Thyroid relative weight was the highest in control chicks (P<0.05).

DISCUSSION

In general, FI increased with dietary HE during the experiment and this increase was found to be statistically significant, especially in the HE+Vit C group (Table 2). Results of the present study are in agreement with the findings of Reece & McNaughton (1982). In this study, the authors reported that FI increased as die-

Table 2. Effects of selenium (Se) and vitamin C (vit C) supplements to high energy (HE) diet on feed intake, body weight, body weight gain, and feed conversion ratio (FCR) of broilers in cold environment

	Days	Groups				P
		Control	HE	HE+Se	HE+Vit C	
Feed intake (g)	4–21	84.51±0.92[b]	81.60±3.40[b]	89.15±3.47[ab]	94.60±3.22[a]	P<0.05
	22–28	114.80±1.84[b]	116.69±1.84[b]	127.36±1.90[a]	135.47±3.22[a]	P<0.01
	29–35	146.20±2.60	151.25±3.36	148.79±2.21	148.49±1.35	NS
	36–40	160.10±4.22	170.43±3.30	165.75±2.84	171.69±3.21	NS
	4–40	126.40±1.06[b]	130.02±2.79[ab]	131.75±2.60[ab]	137.56±1.50[a]	P<0.01
Body weight (g)	4	51.55±0.10	51.93±0.15	51.68±0.25	51.87±0.70	NS
	21	662.70±30.15	675.18±23.69	633.18±32.42	694.72±17.12	NS
	28	1171.36±47.53	1146.25±35.99	1115.02±51.55	1221.10±31.67	NS
	35	1701.36±114.84	1795.93±46.05	1736.56±62.70	1870.71±52.78	NS
	40	1983.18±137.15[b]	2271.25±57.19[a]	2179.06±77.27[ab]	2418.46±68.60[a]	P<0.05
Weight gain (g)	4–21	35.95±1.77	36.66±1.39	34.20±1.90	38.21±1.10	NS
	22–28	72.66±8.94	67.29±6.29	73.67±8.04	73.78±6.34	NS
	29–35	75.71±11.99	92.81±7.50	88.79±8.54	92.41±9.93	NS
	36–40	89.28±20.77	101.13±12.56	88.43±16.79	110.69±15.00	NS
	4–40	53.65±3.80[b]	61.64±1.58[a]	59.09±2.14[ab]	65.73±1.90[a]	P<0.05
FCR (g feed/ g gain)	4–21	2.38±0.12	2.25±0.10	2.60±0.18	2.49±0.07	NS
	22–28	1.58±0.30	1.76±0.32	1.73±0.54	1.84±0.16	NS
	29–35	1.95±0.34	1.64±0.21	1.67±0.60	1.61±0.23	NS
	36–40	1.80±0.90	1.69±0.29	1.86±0.75	1.55±0.50	NS
	4–40	2.36±0.27[a]	2.10±0.50[b]	2.25±0.10[b]	2.11±0.11[b]	P<0.05

NS: non significant; [a, b]: mean values with different superscripts within a row differ significantly.

Table 3. Effects of selenium (Se) and vitamin C (vit C) supplements to high energy (HE) diet on ash, ether extract and crude protein retentions of broilers in cold environment

Digestibility, %	Groups				P
	Control	HE	HE+Se	HE+Vit C	
Ash	61.59±4.81	61.41±3.93	60.48±3.30	61.33±4.24	NS
Ether extract	65.04±2.85	64.86±2.27	63.71±2.12	65.84±3.62	NS
Crude protein	51.61±3.24[b]	52.55±2.16[b]	55.89±3.34[b]	63.69±3.89[a]	P<0.05

NS: non significant; [a, b]: mean values with different superscripts within a row differ significantly.

Table 4. Effects of selenium (Se) and vitamin C (vit C) supplements to high energy (HE) diet on relative organ weights (organ weight /100 g live body weight) of broilers in cold environment

Organ	Groups				P
	Control	HE	HE+Se	HE+Vit C	
Heart	0.59±0.03	0.63±0.03	0.61±0.05	0.61±0.01	NS
Liver	2.00±0.04[b]	2.28±0.04[a]	2.00±0.14[b]	2.10±0.07[b]	P<0.05
Lung	0.55±0.04	0.53±0.05	0.44±0.03	0.49±0.02	NS
Spleen	0.12±0.005	0.15±0.01	0.13±0.09	0.15±0.01	NS
Gizzard	2.47±0.11	2.72±0.20	2.37±0.19	2.68±0.09	NS
Thyroid gland	0.01±0.001[a]	0.009±0.0009[ab]	0.007±0.0009[b]	0.007±0.001[b]	P<0.05

NS: non significant; [a, b]: mean values with different superscripts within a row differ significantly.

tary energy was increased in the cool environment; energy intake was less in the warm temperature and did not change appreciably as dietary energy changed. Especially, in 4–21 days, FI and BWG were determined to be higher than those reported in other studies (Tatli Seven & Seven, 2008; Tatli Seven *et al.*, 2008). This may be due to increased feed intake requirement under cold conditioning (Reece & McNaughton, 1982). BW, BWG and FCR rose with increased dietary energy (Table 2). Jackson *et al.* (1982) indicated that body weight and FCR improved with increased dietary protein or energy. However, Deaton *et al.* (1983) demostrated that FCR (g feed/g live weight) significantly decreased as dietary energy level increased.

In this experiment, the HE group and those supplemented either with Se or Vit C showed considerably higher BW and BWG as compared to control group only, but not among experimental groups. This may be mainly due to the effect of dietary HE on BW and BWG in a cold environment (Jackson *et al.*, 1982; Deaton *et al.*, 1983). It has been also reported that ascorbic acid synthesis was insufficient under stress conditions such as cold (Cheng *et al.*, 1990). Researches announced a beneficial effect of Vit C supplementation on growth rate in chickens (Tatli Seven *et al.*, 2008; Tatli Seven & Seven, in press). The synergism of dietary HE and Vit C supplementation have increased FI and BWG in this group. In this study, mortality rate was low and deaths most probably were a result of the improper thermoregulation in a low temperature environment. Acclimatization is a much stronger thermoregulatory process, although thermal conditioning by itself improves the ability to cope with cold challenge, as was demonstrated by the low mortality rate among birds (Shinder *et al*., 2002).

In this study, the retentions of ash and ether extract in groups were similar (Table 3). Protein retention was however significantly higher in the HE+Vit C group compared to other groups. With young broiler chicks, the higher intake of energy tended to increase total body protein deposition rate. An explanation for this could be the protein-sparing effect of additional energy intake. Additional intake of carbohydrates may increase blood insulin levels, which may reduce amino acid oxidation leaving more amino acids for protein deposition. Took together, at limiting amino acid intakes additional energy intake had generally no effect on protein deposition rate (Oviedo-Rondon & Waldroup, 2002**)**. In this study, protein retention of the HE+Vit C group was the highest as well as the exhibited weight gain. Vit C supplementation is reported to have a protective effect on pancreatic tissue against cold-induced oxidative damage may help the pancreas to function properly, i.e. to adequately secrete digestive enzymes, thus improving retention of nitrogen (Kaushik & Kaur, 2003). Garcia *et al*. (2004) reported that neither ambient temperature nor diet showed a significant effect on the digestibility coefficients of the ether extract, and this is in agreement with our data.

A superior effect of cold environment on the weight of internal organs, associated with thermoregulation, was reported (Shinder *et al*., 2002). Heart, lung, spleen and gizzard weights were not statistically significantly different in this study (Table 4). Liver relative weight was the highest in the HE group (Table 4). This could result from increased dietary HE during fattening (Karadas *et al*., 1999). Higher energetic diets can elicit oxidative stress probably via the enhancement of the lipoperoxidation process, while fat consumption influences oxidative stress by modifying the cellular levels of antioxidants and prooxidants, making membranes more susceptible to oxidation reactions (Moreira *et al*., 2005). Liver weights in HE+Se and HE+Vit C groups were significantly lower than those in the HE group. Antioxidant properties of Se and Vit C might have prevented liver fattening. This opinion is in agreement with the results of Eisele *et al*. (1983). However, thyroid gland weights were statistically significantly lower in experimental groups (Table 4), the higher weights being observed in controls ($P<0.05$). In this study, the higher thyroid weight in control chickens may be a result from increased demand for thyroid hormone in this group compared to experimental groups and also from deterioration of T_3 and T_4 levels in the control group in cold environment. The lower relative weights of thyroid glands of HE+Se and HE+Vit C groups might be due to the antioxidant properties of both dietary supplements as shown by Deshpande *et al*. (2002) in rats.

In conclusion, the performance of the chickens was positively affected by feeding high energy diet supplemented with Vit C and Se. The crude protein retention was increased only in the HE+Vit C group. With these results, we may suggest the supplementation of Vit C and Se in broilers exposed to cold ambient temperatures instead of feeding them only with a high energy ration.

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