British Poultry Science (2000) **41:** 664–666

SHORT COMMUNICATION

Effects of continuous supplementations of ascorbic acid, aspirin, vitamin E and selenium on some haematological parameters and serum superoxide dismutase level in broiler chickens

B. TRAŞ, F. INAL, A.L. BAŞ, V. ALTUNOK, M. ELMAS AND E. YAZAR

Faculty of Veterinary Medicine, University of Selçuk, Konya, Turkey

Abstract 1. This study was conducted using male broiler chickens to determine the effects of ascorbic acid, aspirin, ascorbic acid+aspirin, vitamin E+selenium and ascorbic acid+aspirin+vitamin E+selenium supplementations on haematological parameters and serum superoxide dismutase concentration.
2. One hundred and twenty day-old male *Hubbunt* broiler chicks were randomly divided into 6 experimental groups of 20 chicks each and placed in different pens. Groups 2, 3, 4, 5 and 6 were given a diet supplemented with ascorbic acid, aspirin (in water), ascorbic acid+aspirin, vitamin E+selenium and ascorbic acid+aspirin+vitamin E+selenium, respectively for 45 d while group 1 was given a commercial broiler diet.
3. There was no significant effect of ascorbic acid, aspirin, ascorbic acid+aspirin, vitamin E+selenium supplementations on any of the haematological parameters (red blood cell, haemoglobin, haematocrit, mean corpuscular volume, mean corpuscular haemoglobin concentration, mean corpuscular haemoglobin) in broilers but ascorbic acid+aspirin+vitamin E+selenium supplementation significantly decreased the white blood cell counts.
4. In addition to this, ascorbic acid, aspirin, ascorbic acid+aspirin and ascorbic acid+aspirin+vitamin E+selenium supplementations had no significant effect on the serum superoxide dismutase level, but vitamin E+selenium supplementation increased the serum superoxide dismutase level.

INTRODUCTION

In poultry, vitamin, mineral and drug supplementations as a single agent or in combination have been suggested to improve performance (weight gain, food conversion, egg production, weight, and quality) and immune response to diseases and also to decrease the economic losses related to high temperature, stocking density and food quality (Smart *et al.*, 1985; Stilborn *et al.*, 1988; Cheng *et al.*, 1988; Latshaw, 1991; Bollengier-Lee *et al.*, 1998). Although many studies have been conducted to determine the influence of different supplementations of these substances on performance, survival during heat stress and immune response, there is little information concerning the effects of these substances on haematological parameters and antioxidant enzymes. There was no significant effects of vitamin E (Vit E) and selenium (Se) on haematocrit (PCV), haemoglobin (Hb), red blood cell (RBC) and white blood cell (WBC) counts in other animals (Fontaine *et al.*, 1977; Ji *et al.*, 1990; Romaniuk *et al.*, 1995). On the other hand, Men-kin *et al.* (1994) reported that ascorbic acid (AA) supplementation increased Hb concentrations and RBC counts. It has been reported also that vitamin C decreased iron absorption in the jejunum and caeca in chickens (Nagorna-Stesiak and Leckowski, 1994). Salicylates do not ordinarily alter the WBC or platelet counts, and the PCV or Hb levels, but high doses of aspirin (ASP) decrease the plasma iron concentration and shorten the erythrocyte survival time in humans (Insel, 1996).

Free radicals and peroxides play a significant role in physiological phenomena and in the pathogenesis of various diseases and are thought to participate in ageing, damaging oxidative tissues and increasing stress (Guemouri *et al.*, 1991). The biological effects of these highly reactive substances are controlled *in vivo* by an endogenous antioxidant system, consisting of Vit E and C, carotenoids, histamine containing dipeptides and glutathione peroxidase, superoxide dismutase (SOD) and catalase-like antioxidant enzymes (Chan & Decker, 1994). Non-steroidal anti-inflammatory agents, Se, folic acid, acetylcysteine, and DMSO also have antioxidant effects (Booth & McDonald, 1988). Several factors such as toxic diet contaminants, vitamin and mineral deficiencies, rapid growth hight-emperature stocking density and inflammatory diseases cause depression of antioxidant enzymes (Stocker *et al.*, 1986; Levander *et al.*, 1989; Rizzo *et al.*, 1994; Bottje *et al.*, 1995). Hill (1992) and Ogo *et al.* (1996) stated that Vit E and Vit E+Se were effective in SOD enzymes of rat and pig.

The aim of this study was to determine the effects of continuous supplementations of AA, ASP, Vit E and Se on haematologic parameters and serum SOD level in broilers.

Correspondence to: Associate, Professor Dr Bünyamin Traş, Department of Pharmacology and Toxicology, Faculty of Veterinary Medicine, University of Selcuk, 42031, Konya, Turkey. E-mail: btras@selcuk.edu.tr
Accepted for publication 13th June 2000.

ISSN 0007-1668(print)/ISSN 1466-1799(online)/00/050664-03 © 2000, British Poultry Science Ltd
DOI: 10.1080/00071660020009225

MATERIALS AND METHODS

One hundred and twenty day-old male *Hubbunt* broilers were randomly divided at random into 6 groups of 20 chicks each. Each group was placed in a different pen and food and water were provided *ad libitum*. Animals were kept under the same conditions throughout the experimental periods of 45 d. Group 1 served as control and was fed on a commercial broiler basal diet (Table 1). Group 2 was fed on a basal diet and ASP (300 mg/l water, Bayer); group 3 was given a basal diet supplemented with coated AA granules (100 mg/kg diet, Roche); group 4 was given a basal diet supplemented with coated AA (100 mg/kg diet) plus ASP (300 mg/l water); group 5 was given a basal diet supplemented with Vit E (60 mg/kg diet, Roche) plus Se (sodium selenite 0·1 mg/kg diet, Roche); and group 6 was given a basal diet supplemented with Vit E (60 mg/kg diet), Se (0·1 mg/kg diet), coated AA (100 mg/kg diet) and ASP (300 mg/l water) for 45 d.

When the chicks reached 45 d of age, the feeding trial was terminated and 12 broilers were selected at random from each treatment group and bled by cardiac puncture. The white blood cell (WBC) counts were obtained with a haemocytometer. Red blood cell (RBC) counts, haemoglobin (Hb), haemotocrit (PCV), mean corpuscular volume (MCV), mean corpuscular haemoglobin concentration (MCHC) and mean corpuscular haemoglobin (MCH) in animals were measured by haemocell-counter (Medonic-Biobac, Sweden). Superoxide dismutase (SOD) levels were measured in serum samples by a commercially available kit (Randox-Ransod, sd 125) using a spectrophotometer (Shimadzu UV).

One-way ANOVA and least significant difference test using a computer programme (SPSS for Windows, Release 6·0, 1993) was used to test the significance of differences. Statements of statistical significance are based on $P< 0·05$.

Table 1. *Composition of basal diet*

Ingredients	Starter, g/kg	Grower, g/kg
Yellow maize	439	512
Barley	80	68
Soyabean meal	300	240
Sunflower meal	55	48
Fish meal	40	30
Vegetable oil	50	67
Limestone	12	12
Dicalcium phosphate	13	10
Salt	3·0	3·5
Vitamin premix[1]	2·5	2·5
Mineral premix[2]	1·0	1·0
DL methionine	2·5	3·0
Lysine HCl	2·0	3·0
Calculated nutrient content		
Crude protein, g/kg	220·5	190·7
True metabolisable energy, MJ/kg	12·60	13·41
Calcium, g/kg	10·2	8·6
Available phosphorus, g/kg	3·7	4·0
Lysine, g/kg	12·0	12·6
Methionine, g/kg	5·9	6·6

[1]Supplied per kg of diet: retinol, 900 µg; cholecalciferol, 15 µg; menadione sodium bisulphite, 2 mg; thiamine, 2 mg riboflavin, 5 mg; calcium pantothenate, 15 mg; niacin, 30 mg; pyridoxine, 3·5 mg; biotin, 0·2 mg; folic acid, 0·6 mg; vitamin B_{12}, 0·02 mg; choline chloride, 200 mg.
[2]Supplied per kg of diet: MnO, 333 mg; $ZnSO_4.4H_2O$, 220 mg; ferric citrate, 450 mg; $CuSO_4.4H_2O$, 35 mg; KIO_3, 2 mg; $CoSO_4.8H_2O$, 1 mg; Na_2SeO_3, 0·35 mg.

RESULTS AND DISCUSSION

Results are summarised in Table 2. No changes were observed in the haematological parameters in any group except group 6. The WBC counts of broilers in group 6 fed on a diet supplemented with AA+Vit E+Se and water containing 300 mg/l ASP were statistically lower ($P< 0·05$) than group 1 and 2. Vit E+Se supplementation caused a statistically significant increase ($P< 0·05$) in the serum SOD level.

No significant changes were observed in the haematological parameters of broilers with AA, ASP, AA+ASP and Vit E+Se supplementation during the period of 45 d. On the other hand, AA+ASP+Vit E+Se supplementation reduced them ($P< 0·05$). While these results seem to be in agreement with reported studies that Vit E and Vit E+Se had no effect on the Hb, PCV and RBC counts in horse, pig and sheep (Fontaine *et al.*, 1977; Ji *et al.*, 1990; Shlosberg *et al.*, 1996), high dietary concentrations of Vit E cause a decrease in Hb, PCV, RBC and MCHC in broiler chicks (Ayed *et al.*, 1989). We observed that vitamin C supplementation had no effect on the haematological parameters although increased RBC counts and Hb and PCV levels were reported by Al-Taweil and Kassab (1990) and Men-Kin *et al.* (1994).

We have encountered no information regarding the effects of AA, ASP, Vit E and Se supplementations on the antioxidant enzymes in poultry. The results of this current study show that Vit E+Se supplementation increased the serum SOD levels. However, there were no significant effects of AA, ASP, AA+ASP and AA+ASP+Vit E+Se supplementations on the serum SOD levels. Our study confirms the findings of Ogo *et al.* (1996) and Hill (1992), who reported that Vit E and Vit E+Se increased erythrocyte SOD concentration in rats and pigs.

The present study suggests that AA, ASP, AA+ASP and Vit E+Se supplementations have no effects on the haematological parameters during the 45 d period but AA+ASP+Vit E+Se supplementation significantly decreased the white blood cell counts. In addition, Vit E+Se supplementation increased serum SOD levels but no significant effects of AA, ASP, AA+ASP and AA+ASP+Vit E+Se supplementations were observed in serum SOD levels.

Table 2. *Effects of aspirin, ascorbic acid, aspirin+ascorbic acid, vitamin E+selenium, aspirin+ascorbic acid+vitamin E+selenium supplementations on haematological parameters and serum superoxide dismutase concentrations of broiler chicks between 1 and 45 d of age (n = 12, mean±SE)*

Parameters	Group 1 (Control)	Group 2 ASP	Group 3 AA	Group 4 ASP+AA	Group 5 Vit E+Se	Group 6 ASP+AA+ Vit E+Se
WBC (10^3/mm^3)	18·56±18·1^a	18·83±3·8^a	16·96±2·4^{ab}	17·92±3 ·64^{ab}	16·80±2·9^{ab}	10·08±0·4^b
RBC (10^6/mm^3)	2·64±0·2 NS	2·64±0·2 NS	2·57±0·1 NS	2·46±0·1 NS	2·49±0·1 NS	2·34±0·1 NS
Hb (g/dl)	13·13±0·5 NS	13·79±0·8 NS	12·64±0·5 NS	12·18±0·3 NS	12·86±0·4 NS	11·89±0·7 NS
PCV (%)	31·40±1·6 NS	31·90±2·3 NS	30·57±0·8 NS	29·60±0·7 NS	29·86±0·9 NS	27·57±1·3 NS
MCV (fl)	119·70±2·3 NS	120·44±0·5 NS	119·37±0·9 NS	120·25±1·5 NS	119·82±1·2 NS	121·00±0·8 NS
MCH (pg)	50·071·70 NS	52·851·40 NS	49·180·53 NS	50·131·26 NS	51·721·18 NS	50·831·97 NS
MCHC (g/dl)	41·98±0·7 NS	43·49±1·1 NS	41·36±0·4 NS	41·64±0·6 NS	43·12±0·7 NS	43·06±0·8 NS
SOD (U/dl)	106·63±25^a	78·78±13^a	95·56±16^a	125·33±30^a	239·50±44^b	75·00±12^a

ASP; aspirin (in water), AA; ascorbic acid, Vit E; vitamin E, Se; selenium. WBC; white blood cell, RBC; red blood cell, Hb; haemoglobin, PCV; haematocrit, MCV; mean corpuscular volume, MCH; mean corpuscular haemoglobin, MCHC; mean corpuscular haemoglobin concentration, SOD; superoxide dismutase. Values in the some row not sharing the same letter were significantly different (P<0·05).

REFERENCES

Al-Taweil, R.N. & Kassab, A. (1990) Effect of dietary vitamin C on ascites in broiler chicks. *International Journal of Vitamin Nutrition Research*, **60:** 366–371.

Ayed, I.A., Dafaalla, R. & Adam, S.E.I. (1989) Effects of various levels of dietary vitamin E on broiler chicks. *Veterinary Human and Toxicology*, **31:** 50–53.

Bollengier-Lee, S., Mitchell, M.A., Utomo, D.B., Williams, P.E.V. & Whitehead, C.C. (1998) Influence of high dietary vitamin E supplementation on egg production and plasma characteristics in hens subjected to heat stress. *British Poultry Science*, **39:** 106–112.

Booth, N.H. & McDonald (1988) *Veterinary Pharmacology and Therapeutics* 6th Edn, pp. 153–407 (Iowa State University Press, USA).

Bottje, W., Enkvetchakul., B., More, R. & McNew, R. (1995) Effect of alpha-tocopherol on antioxidant, lipid peroxidation and the incidence of pulmonary hypertansion syndrome (ascites) in broilers. *Poultry Science*, **74:** 1356–1369.

Chan, K.M. & Decker, E.A. (1994) Endogenous skeletal muscle antioxidant. *Critical Reviews in Food Science and Nutrition*, **34:** 403–426.

Cheng, T.M., Coon, C.N. & Hamre, M.L. (1988) Effect of environmental stress on the AA requirement of laying hens. *Poultry Science*, **69:** 774–780.

Fontaine, M., Valli, V.E.O. & Young, L.G. (1977) Studies on vitamin E and selenium deficiency in young pigs: III. Effect on kinetics of erythrocyte production and destruction. *Canadian Journal of Comparative Medicine*, **41:** 57–63.

Guemouri, L., Artur, Y., Herbert, B., Jeandel, C., Cuny, G. & Siest, G. (1991) Biological variability of superoxide dismutase, glutathione peroxidase and catalase in blood. *Clinical Chemistry*, **37:** 1932–1937.

Hill, M.R. (1992) Porcine stress deaths. *Veterinary Record*, **130:** 59.

Insel, P.A. (1996) Analgesic-antipyretic and anti-inflammatory agents and drugs employed in the treatment of gout, in: Hardman, J.G. & Limbird, L.E. (Eds) *Goodman & Gilmans's The Pharmacological Basis of Therapeutics*. 9th Edn, pp. 617–659 (USA, McGraw-Hill)

Ji, L.L., Dillon, D.A., Bump, K.D. & Lawrence, L.M. (1990) Antioxidant enzyme response to exercise equine erythrocytes. *Journal of Equine Veterinary Science*, **10:** 380–383.

Latshaw, J.D. (1991) Nutrition-mechanisms of immunosuppression. *Veterinary Immunology and Immunopathology*, **30:** 111–120.

Levander, O.A., Ager, A.L., Morris, V.C. & May, R.G. (1989) Qinghaosu, dietary vitamin E, selenium and coa-liver oil; effect on the susceptibility of mice to the malarial parasite *Plasmodium yoelii*. *American Journal of Clinical Nutrition*, **50:** 455–457.

Men-Kin, V.K., Khlstova, L.F., Fan-Din, T., Kryzhanovskaya, N.P. & Tkh-Am, F.D. (1994) Growth of broiler chickens fed on a mixture high in potassium nitrite and supplemented with varying amounts of vitamin C. *Izvestiya Timiryazevskai Sel'skokhazyaistvennai Akademii*, **2:** 145–152.

Nagorna-Stesiak, B & Leckowski, J. (1994) Absorption of iron and vitamin C in chickens. *Medycyna Weterynaryjna*, **50:** 2–455–457.

Ogo, Y., Kasa, T. & Kiriyama, S. (1996) Vitamin E prevents the elevation of thiobarbituric acid reactive substances but not hemolytic anemia in rats fed excess methionine. *Journal of Nutritional Biochemistry*, **7:** 77–84.

Rizzo, A.F., Atroshi, F., Ahotupa, M., Sankari, S. & Elovaara, E. (1994) Protective effect of antioxidants against free radical mediated lipid peroxidation induced by DON or T-2 toxin. *Journal of Veterinary Medicine Series A*, **41:** 81–90.

Romaniuk, K., Michalski, M., Sokol, R. & Szelagiwiecz, M.C. (1995) Influence of selenium and systamex (oxfendazole) on gastrointestinal parasites in sheep during growth and pregnancy. *Medycyna Weterynaryjna*, **51:** 462–464.

Shlosberg, A., Bellaich, M., Hanjr, V., Nyska, V., Nyska, A., Lublin, A., Shemesh, M., Shore, M., Shore, L., Perk, S. & Berman, E. (1996) The effect of acetylsalicylic acid and cold stress on the susceptibility of broilers to ascites syndrome. *Avian Pathology*, **25:** 581–590.

Smart, I.J., Embury, D.H., Barr, D.A., Sinclair, A.J., Karunajeewa, H., Ewing, I., Reece, R.L., Forsty, W.M. & Hooper, P.T. (1985) Absence of a role for selenium deficiency in the runting syndrome of broiler chickens in Australia. *Avian Diseases*, **29:** 1201–1211.

SAS Institute (1993) *SPSS for Windows* (Cary, NC, SAS Institute).

Stilborn, H.L., Harris, G.C., Bottje, W.G. & Waldroup, P.W. (1988) Ascorbic acid and acetylsalicylic acid (aspirin) in the diet of broilers maintained under heat stress conditions. *Poultry Science*, **67:** 1183–1187.

Stocker, R., Hunt, N.H. & Weideman, M.J. (1986) Antioxidant in plasma from mice infected with *Plasmodium vinckei*. *Biochemical and Biophysical Research Communications*, **134:** 152–158.