The Effect of Dietary Selenium Source and Vitamin E Levels on Performance of Male Broilers

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ABSTRACT : Selenium and vitamin E are micronutrients essential for normal health and maintenance in poultry. They are necessary in preventing free radical damage to phospholipid membranes, enzymes and other important molecules. Two experiments were conducted in a semi-commercial environment to examine the effect of Se source and vitamin E level in diet on broiler performance and meat quality. Increasing vitamin E from 50 IU to 100 IU did not affect growth performance of broilers although the 24 h drip-loss was tended to be reduced (p=0.06). There was an interaction between vitamin E and the source of Se in glutathione peroxidase activity (GSH-Px) and Se concentration in excreta. Increasing vitamin E from 50 IU to 100 IU elevated GSH-Px and Se concentration in excreta by 42 IU/g Hb and 0.9 ppm for the organic Se group, respectively, but reduced GSH-Px and Se concentration in excreta by 16 IU/g Hb and 1.3 ppm for inorganic group, respectively. Vitamin E played no role in the feather coverage of the birds when scored on day 37. Organic Se is more effective in improving feather score and 24 h drip-loss, with a markedly higher deposition rate in breast muscle and a lower excretion rate in the excreta (p<0.05) compared to the inorganic Se source. Both vitamin E and the source of Se did not affect (p>0.05) the energy utilisation by birds. *(Asian-Aust. J. Anim. Sci. 2004. Vol 17, No. 7 : 1000-1006)*

INTRODUCTION

Selenium (Se) is involved in membrane integrity and numerous selenoproteins, and is required by poultry for the maintenance of optimal health and meat quality. In the previous study, Naylor and Choct (2003) found that increased dietary Se levels markedly improved feed conversion ratio (FCR) as a result of lower feed intake of birds while maintaining the same weight gain. Selenium supplementation increased feathering with organic Se (selenized yeast) being superior to inorganic Se (sodium selenite). Birds receiving organic Se in their diets had improved eviscerated weight, breast yield and reduced drip loss. It is well understood that selenium has a strong interaction with vitamin E in the protection of cells from free radical damage and improvement of bird health and immune status, and both vitamin E and Se are antioxidants essential for cell survival in environments containing peroxides. Feeding vitamin E to animals improves the quality of meat products by preventing lipid oxidation and minimises drip-loss in meat during storage (Mitsumoto et al., 1995; Surai, 2002). More importantly, vitamin E supplementation enhances the ability of the immune system by prolonging the functional life of phagocytes preventing excess hydrogen peroxide activity. Alpha-tocopherol and glutathione peroxidase protect tissues from oxidative damage associated with the free radicals generated during the respiratory burst of macrophages and neutrophils (experienced during immune response). Inadequate vitamin E status lowers corticosteroid synthesis and thus reduces the animal's ability to cope with stress (Aseltine, 1992).

Because of the overlap of the biological actions of vitamin E and Se, a deficiency of Se results in an increase in the animal's requirements for vitamin E. Most of the vitamin E responsive disorders expressed in animals also respond to Se treatment. Diseases such as exudative diathesis and pancreatic fibrosis in poultry, nutritional liver necrosis and mulberry heart disease in pigs and white muscle disease in sheep could be prevented by dietary Se supplementation (Sunde, 1994). Se is between 50 and 100 times more effective an antioxidant than vitamin E (Mervyn, 1985). While a Se supplementation rate of 0.1-0.5 ppm is satisfactory for most animals including poultry, there is a difference between inorganic and organic sources in meeting the requirements of poultry. Organic sources are more effective in increasing tissue Se levels as they are deposited along with its sulphur analogue than inorganic Se. The response of broilers to Se supplementation could vary greatly with the level of vitamin E in the diet. Two experiments were conducted to assess the effect of different Se sources and the level of vitamin E on the performance of male broilers.

MATERIALS AND METHODS

Two experiments were conducted to examine the effects on health and performance of male broilers of inorganic and organic Se sources and the level of vitamin E. Experiment 1 was conducted under a commercial situation at a research

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Table 1. Experimental design-selenium and vitamin E levels of the diets

Diet ID.	Se level	Product	Se Source	Vitamin E
A	0.1 ppm	Na Selenite	Inorganic	50 IU
B	0.1 ppm	Sel-Plex 50	Organic	50 IU
C	0.1 ppm	Na Selenite	Inorganic	100 IU
D	0.1 ppm	Sel-Plex 50	Organic	100 IU

farm at Bartter Enterprises Pty Ltd in Griffith, NSW, Australia and Experiment 2 was conducted at The University of New England, Armidale, NSW, Australia.

Experiment 1

Husbandry : A total of 2,400 day old male broiler chicks (Bartter strain) were divided into 24 floor pens (approximately 100 birds per pen). The floor was covered in rice hulls to absorb excreta and spilt water. Each wire pen contained two Bell automatic waterers and galvanized metal feed hoppers both suspended from the roof. On day one feed was made available to the chicks in 30 cm aluminium pie trays (2 per pen) and starter crumble was also spread on scratch paper. Bell water holders were regularly scrubbed to prevent slime build up and both water supply and the feeders were raised to an optimal height as the birds grew with age.

Upon placement of chicks all pens were counted and weighed to gain an average day-old bird weight. Birds were then weighed weekly. Birds showing obvious sickness or weaknesses (such as tibial dischondroplasia) were euthanised by cervical dislocation. As the trial was conducted in an old broiler shed, the temperature inside the test area was manually regulated to be around 30-32°C during the first 3 days, then it was decreased by 2°C per week to a final 18-22°C at the end of the growing period. The birds were constantly monitored to maintain them in a tight ambient temperature range by using thermostatically controlled gas brooders above the birds (one for every 6 pens) and side ventilation shutters on the shed.

The automatic brooders were constantly adjusted over the first four weeks according to the bird comfort level. If the birds were warm they gained a characteristic pose with wings spread out and beaks open to suck in cool air (sometimes panting) this is when the thermostat had to been turned down. If, however, the birds began huddling and fluffing up their plumage, they were cold and the thermostat on the brooders had to be turned up.

From day one to day 21 the brooders required attention all day as young chicks were not capable in maintaining their core temperature. As the days warmed up and cooled down the gas thermostat was adjusted accordingly. In the later stages of the trial the major concern was ventilation of the shed to remove excess heat and any ambient ammonia which constrains production.

Table 2. Ingredient composition of Bartter control diets

Diet Ingredient	Starter control (%)	Grower control (%)
Wheat (11.5%)	50.00	61.36
Soybean meal (48%)	15.00	9.00
Barley (10%)	10.00	10.00
Meat meal (30%)	10.00	8.13
Rice pollard (13%)	6.00	4.00
Cotton (41%)	4.00	4.00
Tallow	3.00	1.83
Limestone (36.5%)	0.56	0.40
Lysine	0.31	0.23
DL-methionine	0.23	0.18
Sodium bicarbonate	0.18	0.14
Salt	0.09	0.15
Choline Cl (50%)	0.08	0.02
Starter premix	0.50	0.50
Total	100	100

Experimental design

The experiment is a 2×2 factorial design in randomised complete blocks, including two sources of Se and two levels of vitamin E. Inorganic Se was supplied as sodium selenite and organic Se as Sel-Plex 50 (Alltech Biotechnology Australia Pty Ltd). Overall there were four diets and each diet was provided to six pens containing 100 birds per pen (Table 1).

Feed

Both starter and finisher diets were formulated by Bartter Enterprises Pty Ltd using its commercial specifications. The rations differed only in the source and level of Se and vitamin E content as shown in Table 2. A xylanase (Bio-Feed Wheat, 1,000 XU per kg diet as part of the premix) was included in the premix to assist in energy utilization of the wheat. Organic Se was supplied by Alltech Biotechnology Australia Pty Ltd, as a Se yeast product Sel-Plex 50. Hoffmann La-Roche Australia specially formulated vitamin and mineral premixes to contain no selenium or vitamin E. Birds were fed a starter crumble for the first three weeks. At 21 days of age the birds were transferred onto the grower pellets until processing at day 38.

During the trial period feed was supplied *ad libitum* via the automatic hopper feeders and the scratch paper and trays provided during the first few days. Water was also provided *ad libitum* throughout the trial. The diets were a wheat and soybean meal based ration (Table 2), also containing protein as meatmeal, and an enzyme to assist in energy utilization of the wheat. The diet specifications provided by Bartters are; metablosiable energy (ME)=12.94 MJ/kg, crude protein (CP)=24.07% for starter, and ME=13.37 MJ/kg and CP=22.36% for grower.

The diets were mixed at Bartter Enterprises Pty Ltd, Griffith in their commercial feed mill. Twelve different rations were formulated and mixed-six starter crumble diets and six grower pellet batches. Special starter and finisher

premixes were formulated at Roche. Each five tonne feed batch was supplemented with 35 kg of the required premix. This premix was formulated and prepared without any selenium or vitamin E. The micronutrients were weighed out and sequentially mixed through the premix. It was diluted through the feed as it passed through a flow-veyer, which distributed the micromix evenly over the entire batch. The premix, Se, vitamin E and raw materials were mixed, pelleted and stored in a holding bin.

Five tonnes of feed were mixed for each diet, the commercial batch size for the mill. The required amount of feed for these experiments (1.5 tonnes starter crumble and 2 tonnes grower pellets) was captured in a holding bin and manually bagged out. The diets were placed in 40 kg feed bags, stitched and individually weighed and labelled. Pallets of feed were delivered to the test shed by truck, 120 kg of each diet were sent to UNE for Experiment 2. The excess feed not required for the experiment from each batch was diverted into a storage bin and used by Bartters on their commercial broiler farms.

Measurements

Feed efficiency and mortality : Average chick weight was recorded on day one when the trays of chicks were being placed. All 2,400 chicks were weighed on days 7 and 14. On day 21 and day 28, 50 birds from each pen were weighed and pen averages calculated. Feed intake was recorded at day 21 when the ration was changed from starter crumble to grower pellets and on day 37 when the feed was withdrawn prior to processing. Daily mortality, temperature range, feed intake and any observations were reported. Dead birds were removed and counted each morning. Any sick chicken was culled and these were recorded separately.

Feather scoring : Feather coverage was determined using the method described by Edens (1996). Ten birds from each pen were randomly chosen at day 37 and feathering measured against a scorecard. Sections of the bird including back, breast and legs were examined for feather size and coverage.

Body parameter measurements : On day 37 feed was withdrawn and birds fasted overnight prior to processing. On day 38 all birds were weighed and a subset of 55 birds from each diet were leg tagged before slaughter. The flock was commercially processed at Bartter's (Processing Plant Griffith, NSW). The tagged birds were hung with the whole batch and traced through the abattoirs. They were weighed at different stages as they passed through the processing line and were selectively processed. They birds were hung by their legs and electrically stunned before death by automatic throat cutting. The flock was bled for a approximately five minutes, then automatically plucked and eviscerated.

The eviscerated weight of the test chickens was reported in this stage of the processing. The carcasses were then placed in a spin chiller (at about 3°C) for 30 minutes, then retrieved and reweighed. This weight is the dressed weight of the birds-the final product for the whole chicken market. The dressed chickens were stored at 3°C overnight and reweighed the next day. The 24 h drip-loss was determined as the amount of weight loss from each carcass after storage. The breast fillets and marylands (thigh and drumstick portions) were dissected and weighed.

Experiment 2

Husbandry : Male broiler chickens (200) were supplied by Baiada Poultry, Tamworth. Upon arrival at The University of New England (UNE), 120 healthy day old chicks were divided into 20 cages. The birds were housed in electrically heated brooders. These brooders consisted of wire mesh floors with galvanized cage partitions, slide-in excreta trays and external feeders and water troughs. At day 21, four birds from each pen were moved to apparent metabolisable energy (AME) cages. These wire mesh cages were also fitted with external galvanized feeders and water trough, each having a separate galvanized slide-in excreta tray.

Brooders and cages were located in an insulated room with heat provided by two electrical, oil-filled bar heaters. The floor was occasionally wetted to increase the room's humidity. Except for the 4 day AME trial period, the excreta trays were cleaned out every 3-5 days depending on odours and the amount of excreta eliminated. Dead and sick birds were removed daily.

Experimental design and feed : The same experimental design and feed as Trial 1 were used in this experiment. The diets were stored for approximately four months at the UNE Animal House between experiments (from April to July).

Measurement

AME determination : A 4 day AME trial was conducted beginning at day 28. Feed intake was recorded for the four day trial period and all excreta collected. Excreta were dried for 48 h at 80°C then weighed and ground. Dry matter (DM) of pelleted and ground diets was determined by drying overnight at 105°C. The gross energy (GE) content of each ration was determined with a DDS isoperibol calirometer (DDS Instruments, Johannesburg, South Africa). The GE of the dried excreta samples was also measured and the AME values for each diet calculated.

Performance and blood measurements : Birds were kept until 42 days of age in this experiment in order to aid the collection of blood samples from bigger birds. Thus, at day 42 one bird from each pen was selected and a blood sample taken from the neck vein. A 4.5 ml sample was extracted and stored on ice with prepared acid citrate dextrose

Table 3. Effect of selenium source and vitamin E level (IU/kg diet) on feed intake, feed conversion ratio (FCR) efficiency, mortality and feathering in male broilers

Selenium source	Se level (ppm)	Vitamin E level (IU/kg)	Intake (g/38 d)	38 day live weight (g)	38 day FCR	Mortality (%)	Feather score
Inorganic	0.1	50	3,985	2,181	1.805	4.5	2.53
Inorganic	0.1	100	3,888	2,181	1.767	3.5	2.42
Organic	0.1	50	3,913	2,171	1.777	4.5	3.21
Organic	0.1	100	3,848	2,209	1.729	3.5	3.28
Pooled SE			58	26	0.026	0.8	0.05
P value							
Se source			0.176	0.714	0.219	1.000	0.000
Vitamin E			0.345	0.465	0.104	0.246	0.596
Se source×vitamin E			0.787	0.469	0.857	1.000	0.062

Table 4. Effect of selenium source and vitamin E level on 38 day weight, eviscerated weight, dressed weight, breast and maryland yield and 24 h drip-loss in broilers

Selenium source	Se level (ppm)	Vitamin E level (IU/kg)	Dressed weight (g)	Eviscerated weight (g)	Breast weight (g)	Maryland weight (g)	24 h drip-loss (%)
Inorganic	0.1	50	1,569	1,525	411	528	1.37
Inorganic	0.1	100	1,592	1,554	414	537	1.14
Organic	0.1	50	1,568	1,534	401	526	1.01
Organic	0.1	100	1,600	1,558	406	523	0.90
Pooled SE			21	20	6.5	7.3	0.07
P value							
Se source			0.182	0.197	0.168	0.278	0.001
Vitamin E			0.860	0.759	0.605	0.746	0.063
Se source×vitamin E			0.834	0.888	0.882	0.378	0.476

anticoagulant. Blood samples were analyzed for red blood cell GSH-Px activity and plasma haemoglobin levels in the Physiology Department, UNE. After blood collection the birds were euthanised by cervical dislocation and weighed to gain an average 42 day liveweight.

Selenium deposition and excretion : The bird selected for the blood sampling was also used in the dissection for body parts. A sample of breast muscle (approximately 50 g) and both testes were removed and stored on ice until frozen. Breast muscle tissue, testis and ground excreta samples were sent to State Chemistry Laboratory, Victoria, for Se analysis.

Data analysis

The experiments were a 2×2 factorial design. The main treatment factors were the source of Se and level of vitamin E in the diet. The data were analysed using ANOVA in Statgraphics Package (Manugistica Inc., MD, USA).

RESULTS

Performance of broilers

Broilers on all treatments performed very well in both trials. Increasing vitamin E had no significant effect ($p>0.05$) on the final liveweight in both trials. Se source or vitamin E level (Table 3) did not influence feed intake at day 37, FCR and mortality. No interactions between Se source and vitamin E level were detected. The increase in vitamin E in diets did not improve feather coverage of the birds when scored on day 37, but organic Se significantly ($p<0.001$) improved the feather scores (Table 3). There tended ($p=0.06$) to be an interaction in feather score between Se source and the level of vitamin E.

Vitamin E level and Se source at 0.1 ppm had no significant influence ($p>0.05$) on eviscerated weight, dressed weight, breast fillet yield or average maryland weight at processing (Table 4). No interactions between vitamin E level and Se source existed.

The 24 h drip-loss : Water loss over a 24 h period for the dressed birds tended ($p=0.06$) to be reduced as the dietary vitamin E level was increased from 50 IU to 100 IU. Increasing the vitamin E content of the feed reduced average drip-loss from 1.19 to 1.02%. Se source also significantly ($p=0.001$) influenced drip-loss, with the organic source reducing the postmortem fluid loss. Vitamin E level and Se source, however, did not interact to improve drip-loss (Table 4).

Glutathione peroxidase activity (GSH-Px), haemoglobin and energy utilization : Se source and vitamin E level of the diets significantly ($p<0.05$) interacted, affecting the GSH-Px activity in the red blood cells (Table 5). When sodium selenite was the source of the mineral in the feed, increasing the vitamin E levels from 50 IU to 100 IU reduced the GSH-Px activity from 207.6 IU/g Hb to 191.3 IU/g Hb. Increasing the vitamin E in the organic Se rations, however, increased the erythrocyte GSH-Px status from 144.5 IU/g

Table 5. Effect of selenium source and vitamin E level on 42 day weight, apparent metabolisable energy (AME), haemoglobin concentration and glutathione peroxidase activity in broilers

Selenium source	Se level (ppm)	Vitamin E level (IU/kg)	42 day weight (g)	AME (MJ/kg dry matter)	GSH-Px (IU/g Hb)	Haemoglobin (g/dL)
Inorganic	0.1	50	2,408	14.85	207.6	7.45
Inorganic	0.1	100	2,554	14.78	191.3	7.75
Organic	0.1	50	2,565	14.52	144.5	7.46
Organic	0.1	100	2,570	14.39	186.2	7.04
Pooled SE			90.5	0.18	12.6	0.23
P value						
Se source			0.326	0.071	0.018	0.808
Vitamin E			0.449	0.592	0.349	0.169
Source×vitamin E			0.481	0.866	0.042	0.162

Table 6. Effect of Se source and vitamin E level on Se excretion and deposition in the breast muscle and testis of broilers

Selenium source	Se level (ppm)	Vitamin E level (IU/kg)	Breast Se (ppm)	Testis Se (ppm)	Excreta Se (ppm)
Inorganic	0.1	50	0.210	0.567	4.39
Inorganic	0.1	100	0.218	0.558	3.04
Organic	0.1	50	0.254	0.461	1.04
Organic	0.1	100	0.234	0.424	1.94
Pooled SE			9.9	38.9	0.54
P value					
Se source			0.006	0.005	0.000
Vitamin E			0.559	0.566	0.677
Source×vitamin E			0.179	0.726	0.050

Hb to 186.2 IU/g Hb. However, both Se source and vitamin E level of the ration had no influence (p>0.05) on the plasma haemoglobin levels (Table 5) and the apparent metabolisable energy content in the diet.

Selenium deposition and excretion : Increasing vitamin E in the diet from 50 IU to 100 IU did not affect the Se content of the breast muscles (Table 6). Se source influenced the Se content of the breast with organic Se being a better supply for reserve accumulation in the muscle. Sodium selenite at the 0.1 ppm increased (p<0.01) Se deposition into the testes at day 42. Vitamin E level in the ration had no effect on Se concentration in the testes.

There was a significant interaction in the Se concentration in excreta between vitamin E level and the Se source in the diet (p=0.05). Increasing the vitamin E supplementation in birds receiving inorganic Se reduced Se excretion from 4.39 ppm to 3.04 ppm, but increased Se excretion from 1.04 ppm to 1.94 ppm for the organic Se group.

DISCUSSION

Increasing vitamin E level in the diets from 50 IU to 100 IU did not influence important production parameters such as 37 day feed intake, feed efficiency, mortality, eviscerated weight, dressed weight or part yield (breast or maryland), but improved water retention of dressed birds. These results are not surprising because the vitamin E level in the current study is well over the minimum requirement in broiler rations (10 IU) recommended by NRC (1994), with the lowest supplementation level of vitamin E being 5 times of above requirement, which did not include the contribution by other feed ingredients. This clearly demonstrated that increasing vitamin E over 50 IU is not beneficial for the chicken meat production unless the improvement in meat quality as shown by the reduction of drip-loss is substantial. Many studies have confirmed that the supplementation of vitamin E can improve meat quality because vitamin E prevents damage to cells throughout the body by maintaining cell membrane integrity. Tocopherol is a component of the cellular defense mechanism, protecting against free radicals in both the cell membrane and in sub-ellular membranes. It protects important enzymes, polyunsaturated fatty acids and transport proteins from free radical attack. It is believed that maximum benefits are gained in broilers fed 100 IU/kg α-tocopherol (Mitsumoto et al., 1995).

It is well understood that vitamin E and Se work together to minimize free radical stress in cells through their action in GSH-Px. Membrane damage cannot be completely prevented with vitamin E. However, the effect of vitamin E on GSH-Px is largely dependent on whether Se is supplied as organic or inorganic form in the feed. In the current study, increasing the vitamin E supplement of birds consuming the sodium selenite treatment reduced their GSH-Px activity from 207.6 IU/g Hb to 191.3 IU/g Hb, but increasing vitamin E in the organic selenium treatments, improved the enzyme's activity from 144.5 IU/g Hb to 186.2 IU/g Hb.

The reason for this interaction is unknown, but it may associate with the Se concentration in the excreta. A similar interaction between vitamin E level and the source of Se in Se concentration in the excreta occurred, where increasing vitamin E level reduced the Se concentration in excretion by 1.4 ppm in the broilers receiving inorganic Se and increased by 0.9 ppm in those receiving organic Se. In the previous studies, sodium selenite increased GSH-Px activity in red blood cells significantly more than the organic Se (Cantor et al., 1975; Whanger and Butler, 1988; Mahan and Parrett, 1996; Choct et al., 2003). However, GSH-Px activity is not considered a good measure of an animal's Se status because it is believed that the majority of Se in the blood is not associated with GSH-Px, but with other selenoproteins (Smith and Picciano, 1987).

It has been well documented that variations exist among different chemical forms of Se in their capacity to meet the Se requirements of poultry. Feeding organic Se can increase the yield for drumsticks and thighs compared to birds receiving ration containing inorganic Se (Edens, 1996). In the previous trials, Choct et al. (2003) also found that birds receiving organic Se in their diets had improved eviscerated weight, breast yield and reduced drip loss. However, in the current study, the source of Se did not affect the performance of broilers. In consistency with previous study by Choct et al. (2003), feeding organic Se improved the feathering score, but this was not reflected in the growth and feed conversion ratio of birds, although it is believed that better feathering combined with the effect of Se on thyroid hormone activation will reduce the energy requirement for maintenance, consequently improve feed utilisation efficiency (Naylor and Choct, 2000).

Organic Se has a better utilisation efficiency than inorganic Se, as indicated by the high Se concentration in breast muscle and low Se concentration in the excreta in the current study. Mahan and Parrett (1996) also reported that inorganic Se (sodium selenite) is retained at a much lower concentration in muscle tissue, is less efficiently absorbed and is excreted at a higher rate than organic Se due to their different metabolic pathways. Inorganic Se crosses the intestinal lumen by passive diffusion (Pherson, 1993). Organic Se in the current study was supplied as Se-yeast, a most commonly used source of organic Se in animal diets. This product is derived from the fermentation of specific strains of yeast incubated in high selenium levels during their growth phase. Being biochemically similar to sulfur, Se replaces the sulfur molecule in the normal biosynthetic pathways of the yeast cell and is absorbed actively across the intestine by the same amino acid carrier (Kim and Mahan, 2003). Selenomethionine passes the brush border membrane via the same amino acid route as its sulfur analogue, methionine. Amino acids and small peptides are absorbed very efficiently by the small intestine, thus the organic mineral is effectively "smuggled" across the intestinal lumen. This higher utilisation efficiency may also explain the more significant improvement of drip-loss by organic Se in the current and previous study by Choct et al. (2003).

In conclusion, vitamin E's influence on broiler growth and performance is limited to its antioxidant role. Se is a more diverse nutrient that affects other important systems. The influence of Se on feathering and thyroid activation could improve feed efficiency and enhance growth based on previous studies. Organic Se is a better resource which can be utilized more efficiently by broilers with a reduced output from excreta.

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