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Research Article
Effects of Antioxidants (Selenium, Vitamin C and Vitamin E) and Feed Allocation on Broiler Breeder Egg Production, Fertility and Hatchability

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Abstract
Background and Objective: Optimum feed allocation and antioxidant levels are important for sustained fertility of broiler breeder flocks. This study investigated effects of different antioxidant sources and levels on male fertility. **Materials and Methods:** In Experiment 1, males were divided assigned to a large comb (LC) or small comb (SC) height group at 20 week and fed a layer diet with either organic high selenium yeast [(HiSe Yeast) Selplex®] or inorganic selenium [sodium selenite (NaSe)]. In Experiment 2, all birds were randomly assigned to receive a High (Vitamins C, E and HiSe) or Normal antioxidant layer diet. **Results:** Experiment 1: LC males receiving NaSe exhibited reduced fertility that was related to a significantly heavier BW relative to the feed allocation. Similarly, the SC males that received more ME relative to BW responded with improved fertility but significantly increased BW at 40 week of age. An interaction between comb size and selenium source suggested that when higher BW males (LC) were underfed, the HiSe Yeast maintained high fertility irrespective of gain in BW. Experiment 2: Overall, High antioxidant levels increased fertility. Further, after 38 week of age, there was a decrease in fertility due to an inadequate feed allocation for males that had a reduced effect on the High antioxidant group. However, when male feed allocation was increased at 54 week of age the differences due to antioxidant level were diminished. **Conclusion:** Consistent feed increments that maintained a consistent male BW gain and elevated antioxidant levels is important for maintaining fertility.

Key words: Broiler breeder, fertility, selenium, Vitamin C, Vitamin E

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Data Availability: All relevant data are within the paper and its supporting information files.

INTRODUCTION

The detrimental effects of oxidative damage on male poultry fertility have been described in the literature[1]. In mammals, the seminal plasma has been shown to contain antioxidant machinery composed of a number of enzymes [superoxide dismutase, catalase, glutathione peroxidase (GSH-Px)] and free radical scavengers such as Vitamins C and E, hypotaurine, taurine and albumin[2-5]. For instance, the effects of selenium on fertility and reproductive performance have generally been attributed to its antioxidant function as a component of the GSH-Px system[6]. In a similar manner, Vitamins C and E have been shown to protect semen against oxidative damage[7]. Therefore, several researchers have tried to demonstrate the beneficial effect of elevated levels of dietary antioxidants on birds[1,8].

It has also been indicated that organic selenium from a high selenium yeast source could improve fertility when compared to inorganic selenium, because selenium from yeast was taken up more efficiently by tissues[9,10]. However, the commercial field responses have not been consistent[11], suggesting that the effect of this antioxidant may be dependant upon other factors, such as the male feeding program and male sexual development. In fact, literature[12] had previously demonstrated that female fertility was dependent upon cumulative nutrition prior to photostimulation and, under commercial conditions, improper feeding relative to BW was a common problem. Since the effect of organic antioxidants could be influenced by the feed allocation program and sexual maturity, the objective of these experiments was to evaluate the effect of selenium source alone and apparent sexual maturity, as evidenced by comb development (height), in the presence of a more than normally limited male feed allocation and the effect of a combination of supplemental organic antioxidants.

MATERIALS AND METHODS

Experiment 1: An experiment was conducted to evaluate the effects of the interaction between male apparent sexual maturity, as indicated by comb height and two selenium sources, a high selenium yeast [HiSe Yeast (Selplex®, Alltech, Nicholasville, KY 40356)] *versus* sodium selenite (NaSe), in the presence of a more than normally limited male feed allocation (Fig. 1) on broiler breeder fertility. A group of 200 Ross 344 males and 1,080 females (Ross 308 slow-feather) was reared under the same conditions as generally described[13]. Briefly, day-old chicks were placed in a 20-pen growing house with

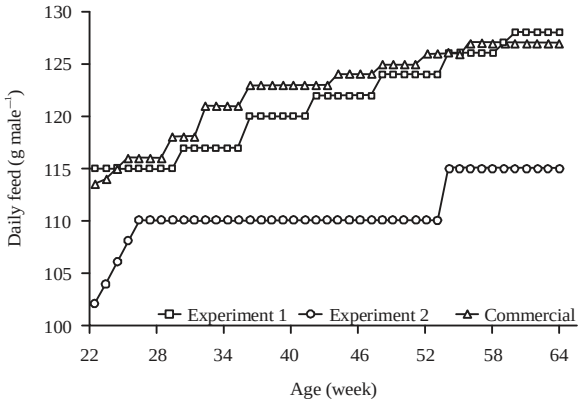

Fig. 1: Broiler breeder male feeding programs during the production period in Experiment 1 and Experiment 2
A typical commercial feeding program is shown for comparison. The feeding program employed in Experiment 1 was meant to be slightly more restrictive than normal

16 pens for females and 4 pens for males. Each 3.96×3.96 m pen was equipped with the equivalent of 750 cm of linear feeder space (six tube feeders) and two automatic drinkers. After 23 h of light per day for one week all birds were reared to 21 week of age with 8 h of light per day at an average intensity of 15 lux. At 21 week of age an average of 55 females and 6 males were allocated to each of the 16 pens and photostimulated with 14 h of light. The day length was increased to 15 h 14 day later and then to 15.5 and 16 h at 5% and 50% rate of lay, respectively. Natural light entered the house through open or translucent curtains during normal daylight hours. Supplemental light provided an average intensity of 35 lux when natural light was not present. Feed was provided daily during the first week of age and then a 4/3 feed allocation program was used until 21 week of age after which a daily feeding program was employed. Access to water was limited by a time clock and solenoid system sufficient to control litter moisture and allow the birds to have unlimited access to water until one hour after all feed was consumed and a similar amount on non-feed days during rearing. Water was limited to 8 h per day during the production period. From 200 males, 96 males were classified according to their comb height. The males with large sized combs (LC) and small sized combs (SC) were allocated to 8 pens each in such a manner as to ensure a similar male BW distribution within each comb height group among all of the pens in the respective treatments. Males and females were fed the same breeder diet (Table 1) with either 500 mg kg^{-1} of HiSe Yeast or 1 g kg^{-1} of NaSe. Both diets were formulated to supply 0.3 ppm of elemental selenium. The male feed allocations were intended

Table 1: Composition of broiler breeder diets in experiment 1

			Breeder diet (Selenium source)	
Ingredient and analysis	Starter diet	Grower diet	HiSe Yeast	NaSe
	(%)			
Corn	65.10	68.00	69.60	69.60
Soybean meal (48% CP)	22.20	17.00	20.10	20.10
Wheat bran	7.64	9.88	-	-
Potassium carbonate	-	-	0.15	0.15
Dicalcium phosphate	1.62	1.60	1.60	1.60
Limestone	1.24	1.28	6.10	6.10
Mineral premix[1]	0.20	0.20	0.20	0.20
Vitamin premix[2]	0.10	0.10	0.10	0.10
Salt	0.45	0.58	0.58	0.58
Coccidiostat	0.05	0.05	0.05	0.05
Methionine	0.08	0.03	0.03	0.03
Sodium selenite (NaSe)	0.10	0.10	-	0.10
HiSe Yeast (Selplex ®)	-	-	0.05	-
Sand	-	-	0.05	-
Lysine HCl	-	0.008	-	-
Choline chloride	0.20	0.20	-	-
Poultry fat	1.00	1.00	1.16	1.16
Antibiotic	0.025	0.025	0.025	0.025
Total	100.00	100.00	100.00	100.00
Calculated analysis[3]				
Crude protein (%)	17.00	15.00	15.50	15.50
ME, kcal g^{-1}	2,925	2,925	2,925	2,925
Lysine (%)	0.88	0.75	0.80	0.80
Methionine+cystine (%)	0.70	0.80	0.75	0.75
Calcium (%)	0.90	0.90	2.70	2.70
Available phosphorus (%)	0.45	0.45	0.40	0.40
Selenium (mg kg^{-1})	0.30	0.30	0.30	0.30

[1]Mineral Premix contained the following in milligrams per kilogram of diet; Manganese: 120, Zinc: 120, Iron: 180, Copper: 10, Iodine: 2.5, Cobalt: 1.0. [2]Vitamin premix contained the following per kilogram of diet: Vitamin A: 13,200 IU; Cholecalciferol: 4,000 IU; Vitamin E: 66 IU, Vitamin B12: 34.6 µg, Riboflavin: 13.2 mg, Niacin: 110 mg, Pantothenic acid: 22 mg, Vitamin K: 4 mg, Folic acid: 2.2 mg, Thiamine: 4 mg, Pyridoxine: 8 mg and Biotin: 252 µg, Ethoxyquin: 66 mg. [3]Data expressed on a percentage of dry matter basis. Formulations were confirmed by proximate analysis

to be slightly more restrictive than might be normal in commercial practice (Fig. 1) in order to create a marginal ME deficiency during the early production period.

Male BW was measured at 4, 12, 21, 24, 26, 27, 29, 32, 36, 40, 48, 56 and 64 week of age. Male shank length was measured from the tibiotarsal articulation to the foot pad after the latter was bent as if the foot were in contact with the ground at 21, 24, 28, 32 and 36 week of age. The comb height was measured from the top of the head to the highest point of the comb at the same ages that shank length was measured. Eggs were collected twice daily from the nests and stored in an egg cooler at 18-20°C and 60% RH until incubated. Eggs laid on the floor and slats were collected separately but not incubated. Analysis of percentage fertility, hatchability and embryo mortality was conducted weekly from 28-36 week of age and biweekly from 38-64 week of age by macroscopic examination of all unhatched eggs from sets of 60 eggs per pen on the respective weeks. Additionally, the internal egg quality was investigated by measuring albumen height[14] and yolk weight at 48 and 60 week of age. Male and female mortality were recorded daily and feed allocations adjusted appropriately. At 64 week of age, all remaining males from 3 pens per treatment combination were necropsied and the testes excised and weighed.

Experiment 2: An evaluation of the effects of an additional antioxidant package containing Vitamin C, Vitamin E and HiSe Yeast during the broiler breeder production period on BW, egg production, fertility and hatchability was conducted. Day-old chicks were placed in a 24-pen litter floored growing house with 12 pens for females and 12 pens for males. At placement, there were 220 females and 24 males in each female and male pen, respectively. The feed management and lighting program was similar as described for Experiment 1 above. An average of 200 females and 20 males were moved to two-thirds slat laying quarters at 21 week of age and photostimulated as described for Experiment 1 above. Male and female mortality were recorded daily and feed allocation adjusted accordingly. Male feed allocation was maintained during the production period at 110 g $male^{-1}$ day^{-1} until

Table 2: Composition of broiler breeder diets in Experiment 2

			Breeder diet (Antioxidant level)	
Ingredient and analysis	Starter diet	Grower diet	High	Control
	(%)			
Corn	65.10	68.00	64.13	64.23
Soybean meal (48 % CP)	22.20	17.00	19.10	19.10
Wheat middlings	7.64	9.88	5.00	5.00
Corn gluten meal	-	-	2.00	2.00
Dicalcium phosphate	1.62	1.60	1.47	1.47
Limestone	1.24	1.28	6.10	6.10
Mineral premix[1]	0.20	0.20	0.05	0.05
Vitamin premix[2]	0.10	0.10	0.10	0.10
Salt	0.45	0.58	0.41	0.41
Coccidiostat	0.05	0.05	0.05	0.05
Methionine	0.08	0.03	0.07	0.07
Sodium selenite (NaSe)	0.10	0.10	-	0.10
Antioxidant package[3]	-	-	0.20	-
Mold inhibitor	-	-	0.05	0.05
Lysine HCl	-	0.08	0.05	0.05
Choline chloride	0.20	0.20	0.12	0.12
Beef tallow	1.00	1.00	1.10	1.10
Total	100.00	100.00	100.00	100.00
Calculated analysis[4]				
Crude protein (%)	17.00	15.00	16.02	16.03
ME (kcal g^{-1})	2,925	2,925	2,912	2,918
Lysine (%)	0.88	0.75	0.82	0.82
Methionine+cystine (%)	0.70	0.80	0.63	0.63
Calcium (%)	0.90	0.90	2.70	2.70
Available phosphorus (%)	0.45	0.45	0.42	0.42
Vitamin E, I.U kg^{-1}	-	-	114.25	20.25
Vitamin C (mg kg^{-1})	-	-	120.00	0.00
Selenium (mg kg^{-1})	0.30	0.30	0.30	0.30

[1]Mineral Premix contained the following in milligrams per kilogram of diet: Manganese: 120, Zinc: 120, Iron: 180, Copper: 10, Iodine: 2.5, Cobalt: 1.0. [2]Vitamin premix contained the following per kilogram of diet; Vitamin A: 13,200 IU, Cholecalciferol: 4,000 IU, Vitamin E: 66 IU, Vitamin B12: 34.6 µg, Riboflavin: 13.2 mg, Niacin: 110 mg, pantothenic acid: 22 mg, Vitamin K: 4 mg, Folic acid: 2.2 mg, Thiamine: 4 mg, Pyridoxine: 8 mg and Biotin: 252 µg. [3]Antioxidant package contained the following per kilogram: vitamin E (106 IU); vitamin C as Stay-C 35%; HiSe Yeast (Selplex®) 0.1%; Ethoxyquin: 66, 127 mg. Stay-C dry mixture is a fine powder containing mono-, di- and triphosphate esters of L-ascorbic acid in a suitable carrier. It provides a minimum of 35% of ascorbic acid by weight equivalent to 150 g of ascorbic acid per kg of dry mixture. [4]Data expressed on a percentage of dry matter basis. Formulations were confirmed by proximate analysis

54 week of age and then increased by 5 g $male^{-1}$ day^{-1} (Fig. 1) after fertility had been observed to decline. The normal antioxidant diet used during the production period was amended with additional quantities of Vitamins C and E and HiSe Yeast replaced sodium selenite (NaSe) (Table 2). Four 12-hole conventional nests were provided in each breeding pen. Twelve tube feeders for females were placed over the slat area while there were two tube feeders in the pine shavings litter area for the males. Separation of sexes was insured by special grills on the female feeder that prevented the non-dubbed males from accessing the female feeders. There were four bell-type drinkers in each of 12 breeding pens. Male BW was measured individually at 21, 24, 26 and after 32 week of age at the same ages indicated in Experiment 1. Eggs were collected and handled as described in Experiment 1, except that 180 eggs per pen were set during each respective week. At 28, 34, 40, 46, 52 and 58 week of age individual eggs were weighed to the nearest 0.1 g, with the contents removed and shells dried to constant weight and weights were recorded.

Statistical analyses: The fertility and hatchability data were analyzed on either a weekly or biweekly basis. Additionally, these data were analyzed on a cumulative and age-based quartile time period basis. For Experiment 1, a completely randomized design with a factorial (2×2) arrangement of treatments was used. The main factors were comb height (LC or SC) and selenium source (HiSe Yeast or NaSe). The treatments were randomly distributed among 16 pens with 4 replicate pens per interaction cell. For Experiment 2, a completely randomized design with two treatments and six replicates per treatment was used. The general linear model (GLM) procedure with the repeated statement of SAS[15] was used to analyze the continuous variables. Percentage data was analyzed after arcsine transformation. The fertility data were

analyzed as categorical data where each individual egg was taken as a binomial event, either fertile or infertile, using the general model (GENMOD) procedure of SAS[15]. To test the time effect and its interaction with the treatments a split plot design with time and its interactions in the subplot was conducted using PROC MIXED of SAS[15]. Orthogonal contrasts were used to compare treatments probabilities[16]. Means were partitioned using LSMEANS and statements of statistical significance were based upon $p<0.05$ unless otherwise indicated.

RESULTS

Experiment 1: The effect of selenium source and comb height classification on male BW is shown in Fig. 2. The LC group exhibited significantly heavier BW from 21-32 week of age but the differences thereafter decreased. No significant effect was observed for selenium source or the interaction with comb height. The correlation between BW and comb height was 63%, based upon data from 21-36 week of age. The effect of selenium source and comb height classification on shank length and comb height is shown in Fig. 3a and 3b, respectively. Comb height followed a growth pattern that mirrored BW from 21-36 week of age. No significant difference was observed for shank length, although the SC and HiSe Yeast combination group exhibited the lowest value from 21-36 week of age. No significant difference was found for absolute or relative weights of the testes at 64 week of age (data not shown). There was high variability in the weekly fertility data so the effect of selenium source and comb height classification on percentage fertility was reported only on a quartile time period basis (Table 3) for clarity. Percentage fertility exhibited consistently high values (>90%) during the complete laying period in any given treatment. Significant interactions between the comb height classification, selenium source and flock age were observed. During the first quartile time period (28-35 week of age), fertility was significantly lower with NaSe and a significant interaction revealed decreased fertility in the LC male group fed NaSe. No significant differences were observed during the second and third quartile time periods for percentage fertility. During the fourth quartile time period (56-64 week of age) the NaSe group exhibited the highest fertility, while no significant interaction between comb height and selenium source was observed (Table 3). Conversely, HiSe Yeast increased

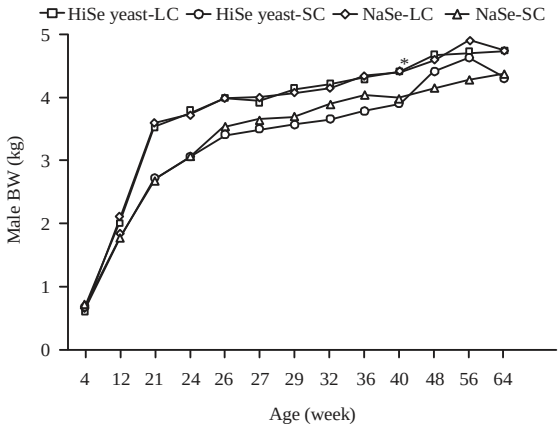

Fig. 2: Male body weight (BW) during the rearing and production period as affected by male comb height classification and selenium source during the production period in Experiment 1

Selenium was supplied as organic selenium (HiSe Yeast), or as sodium selenite (NaSe). Males were classified as either large comb (LC) or small comb (SC) at 21 week of age

Table 3: Broiler breeder fertility as affected by male comb height classification and selenium source during the laying period in Experiment 1[1]. Selenium was supplied as organic selenium (HiSe Yeast) or as sodium selenite (NaSe). Males were classified as either large comb (LC) or small comb (SC) at 21 wk of age

		Weeks of age quartile[2]				
		28-35	36-45	46-55	56-64	Cumulative
Comb height classification	Selenium source	(%)				
LC		96.6	94.9	95.8	94.2	95.3
SC		97.6	95.5	95.1	94.3	95.6
	HiSe Yeast	98.1[a]	95.2	95.0	92.4[B]	95.1
	NaSe	96.1[b]	95.1	95.9	96.1[A]	95.7
Interactions						
LC	HiSe Yeast	98.6	95.6	95.5	92.8	95.5
LC	NaSe	94.6	94.2	96.2	95.7	95.1
SC	HiSe Yeast	97.6	94.9	94.6	92.0	94.7
SC	NaSe	97.5	96.0	95.6	96.6	96.4
P-value[3]		0.001	0.001	0.96	0.08	0.001

[A,B]Means with different superscript are significantly different ($p<0.01$). [a,b]Means with different superscript are significantly different ($p<0.05$). [1]Categorical analysis does not generate standard errors. [2]Time by treatment (comb height and selenium source) interaction was significant ($p<0.01$). [3]Probability value for interaction term between comb height and selenium source

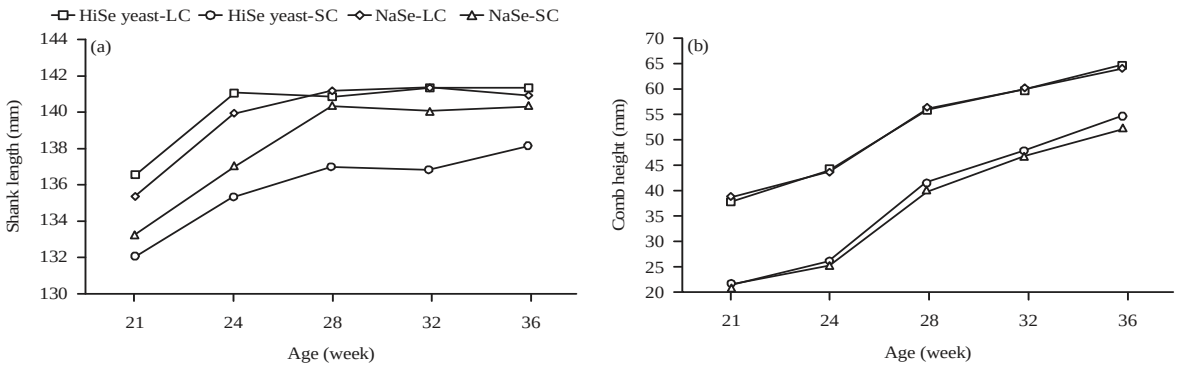

Fig. 3: Male shank length (Panel A) and comb height (Panel B) as affected by selenium source and male comb height classification in Experiment 1

Selenium was supplied as organic selenium (HiSe Yeast) or as sodium selenite (NaSe). Males were classified as either large comb (LC) or small comb (SC) at 21 week of age

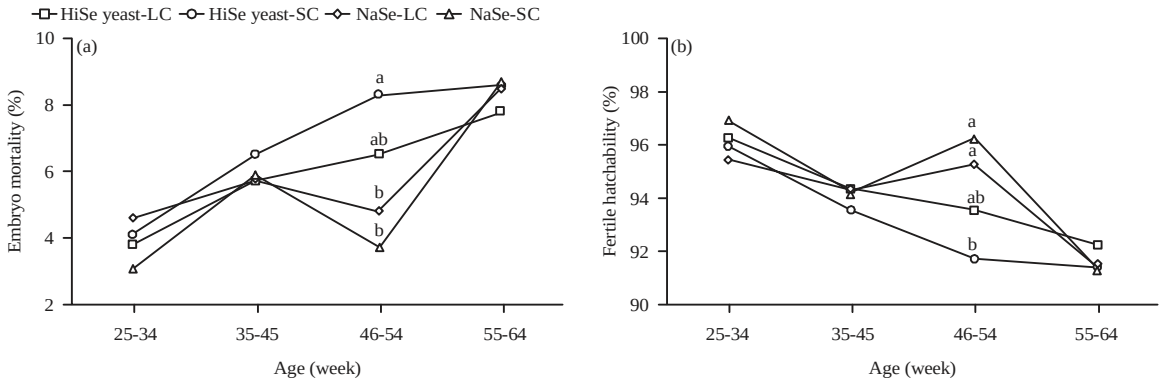

Fig. 4: Total embryo mortality (Panel A) and percentage fertile hatchability (Panel B) as affected by selenium source and male comb height classification in Experiment 1

Selenium was supplied as organic selenium (HiSe Yeast) or as sodium selenite (NaSe). Males were classified as either large comb (LC) or small comb (SC) at 21 week of age[a,b]. Means with different superscripts are significantly different ($p<0.05$). Time by treatment (comb height and selenium source) interaction was significant ($p<0.01$)

percentage total embryo mortality during the third quartile time period (46-55 week of age) (Fig. 4a). Analyses were conducted for early, late and pipped embryo mortality but only the more representative total embryo mortality was presented for the sake of brevity. There were significant interactions in the third quartile time period, which showed that HiSe Yeast increased total embryo mortality in the SC group. This embryo mortality was due mainly to late mortality and reflected in the fact that HiSe Yeast, when compared to NaSe, significantly decreased fertile hatchability in the SC group (Fig. 4b). The HiSe Yeast-LC combination group exhibited an intermediate value for embryo mortality and fertile hatchability (Fig. 4a and b).

The effect of selenium source on egg production during the production period and female BW at 48 week of age is shown in Fig. 5. NaSe resulted in slightly better egg production, that was significant at 29, 38, 43 and 51 week of age; but significant differences were not observed for eggshell weight or internal egg quality as indicated by albumen height and yolk weight (data not shown). HiSe Yeast significantly increased female BW at 48 week of age when all females in the experiment were weighed.

Experiment 2: The effect of the antioxidant concentration level on male BW from 21-64 week of age is shown in Fig. 6. The High antioxidant level produced a lower male BW at 48 week of age but no significant effect was observed for female BW. The effect of the antioxidant level on percentage fertility is shown in Fig. 7 and Table 4. Figure 7 details that percentage fertility declined after 38 week of age, while Table 4 shows the decline during the third quartile time period. However, the High antioxidant level ameliorated the

decrease in fertility. Once the daily male feed allocation was increased by 5 g at 54 week of age (about the end of the third quartile time period) fertility increased in both treatments and the difference between antioxidant levels tended to disappear. Overall, the High antioxidant level improved percentage fertility by 4.9% (Table 4). No significant difference was observed for embryo mortality. The effect of antioxidant level treatment on egg production and eggshell weight is shown in Fig. 8 and, respectively. The High antioxidant level significantly increased egg production and numerically increased ($p<0.10$) eggshell weight at 40 and 58 week of age.

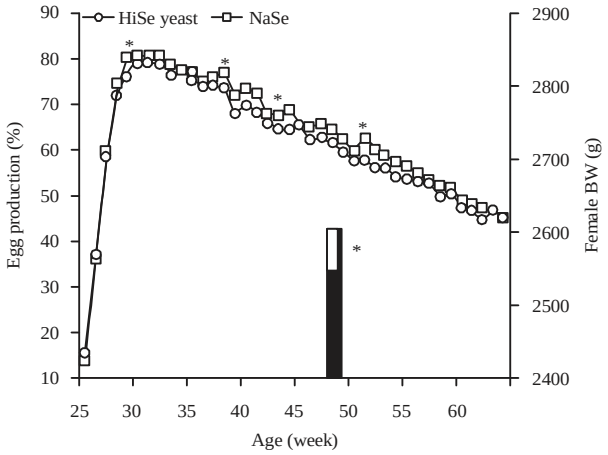

Fig. 5: Egg production and female body weight (BW) at 48 week of age, as affected by selenium source during the production period in Experiment 1

Asterisk (*) represents a significant difference ($p<0.05$) as determined by GLM procedure at each age. Selenium was supplied as organic selenium (HiSe Yeast) or as sodium selenite (NaSe)

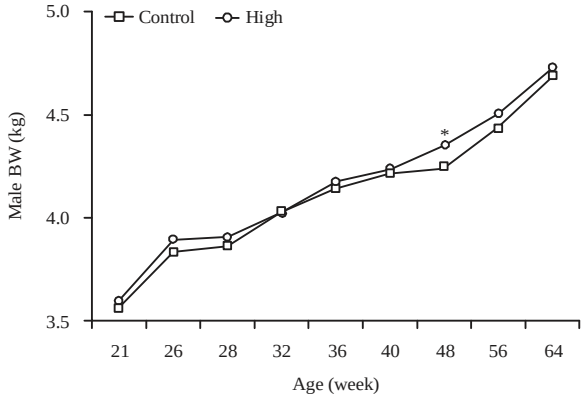

Fig. 6: Male BW as affected by antioxidant level (control or high) during the production period in Experiment 2

Asterisk (*) represents a significant difference ($p<0.05$) as determined by GLM procedure at each age

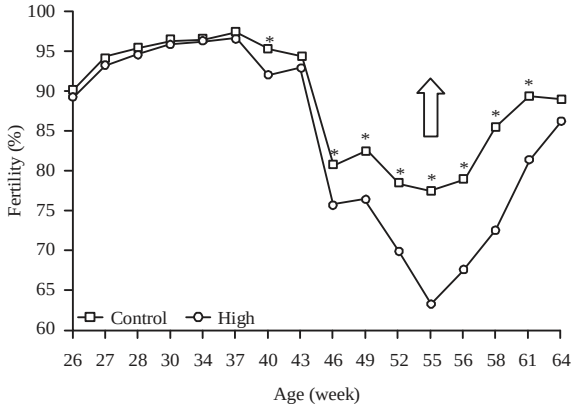

Fig. 7: Fertility during the production period as affected by antioxidant level (Control or High) in Experiment 2

The arrow indicates the age when the male feed allocation was increased by 5 g male^{-1} day^{-1}. Asterisk (*) represents a significant difference ($p<0.05$) as determined by GLM procedure at each age

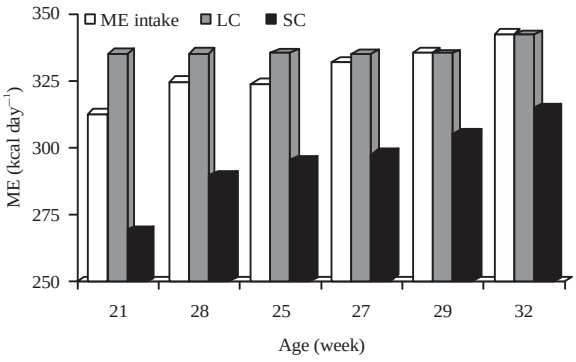

Fig. 8: Energy balance in males from 22-32 week of age

In Experiment 1, males were classified by comb height at 21 week of age as either large comb (LC) or small comb (SC) males. This figure compares ME intake with ME required for maintenance (ME_m) at experimental temperature as function of the BW as ME_m = 1.45*BW $^{0.65}$* (1.78-0.012*T) where T is average temperature in °F (Combs)[17]

Table 4: Broiler breeder fertility as affected by level of antioxidant in Experiment 2[1]

	Weeks of age quartile[2]				
Antioxidant level	28-35	36-45	46-55	56-64	Cumulative
High	94.6	95.5^{a}	79.8^{A}	85.7^{A}	88.9^{A}
Control	94.0	93.9^{b}	71.3^{B}	76.9^{B}	84.0^{B}

^{A,B}Means with different superscript are significantly different ($p<0.01$). ^{a,b}Means with different superscript are significantly different ($p<0.05$). [1]Categorical analysis does not generate standard errors. [2]Time by treatment (antioxidant level) interaction was significant ($p<0.01$)

DISCUSSION

The feeding of diets from 21-64 week of age with two different sources of selenium in Experiment 1 and with two levels of antioxidants (differed in Vitamin E, Vitamin C and source of selenium) in Experiment 2 was intended to evaluate the effect of the prolonged use of these antioxidants on male BW and reproductive performance. Because the same diets were supplied to both males and females, as would be the case commercially, it was not possible to completely separate sex effects with respect to fertility and/or hatchability. However, the present discussion has emphasized the male effect since the males were classified at 21 week of age according to their comb height as an indicator of sexual maturity (Experiment 1) and the male feed program was the only change made at 54 week of age when percentage fertility had declined (Experiment 2) while females remained the same across all treatments. Specific female data such as BW, egg production and egg-shell quality were taken to demonstrate the effect of antioxidants on overall breeder performance and to delineate obvious female effects. Experiment 2 initially showed that the High antioxidant level improved fertility. However, the effect appeared to be related to a deficient male feed allocation, as the significant difference disappeared after the male feed allocation was increased by 5 g bird^{-1} day^{-1} at 54 week of age, in the same manner that has been previously depicted[18]. At 48 week of age the Normal antioxidant group had a significantly heavier male BW and apparently required more ME to sustain reproductive activity. Although, the detrimental effects of oxidative damage on poultry male fertility has been extensively described[1,19], under commercial conditions it has been clearly demonstrated that excessive feed restriction alone decreased the intake of macronutrients such as CP and ME and depressed fertility. Furthermore, it has been observed that males that slowly but consistently gained BW showed better reproductive performance[20].

Male broiler breeders must be physiologically and behaviorally mature to successfully elicit female sexual receptivity and copulate. Although only 16% of the variability in fertility was attributed to comb area, the comb area has been thought to be a reliable indicator of male fertility in some strains[21,22]. In the present experiment the comb height was used as indicator of the comb area. Comb height has been shown to be highly correlated with comb area and this measurement, as described in Experiment 1, has been shown to be practical under both commercial and experimental conditions[23]. Previous study by Zuk *et al.*[24,25]. suggested that although large comb (LC) males tended to have better reproductive performance, there was probably a minimum acceptable comb size and once the comb reached the "minimum" size there were no reproductive differences between small comb (SC) and large comb (LC) males. Comb size has been shown to be an indicator of sexual maturity as testosterone release was shown to be highly correlated with comb development[26,27]. Testes size has also been shown to be related to comb size, although it has been shown that greater testicular weight in broiler breeders may not necessarily correspond to increased spermatogenesis[28] or higher androgen levels[29]. Furthermore, a negative relationship between testes weight and fertility has been found in some broiler breeder strains[23]. In the present experiment no significant difference was observed for testes weight at 64 week of age (data no shown), while the SC group exhibited greater comb height than those previously reported[21,22] to be subnormal. This could partially explain why grouping by comb height did not affect fertility in Experiment 1.

A significant correlation between comb height and BW (0.63) was consistent with the fact that male BW was significantly higher in the LC group, although the difference tended to disappear with age. Testosterone, the major male reproductive hormone, has been shown to be affected by the interaction of ME intake and age[30]. This study showed that testosterone decreased with age but the decline was less for diets with adequate ME levels (diets between 2,800 and 3,200 kcal kg^{-1}). In the same manner, Hulet and Brody[31] reported increased semen production in turkey toms with higher ME intakes from 34-36 week of age.

The HiSe Yeast source used in these experiments has been reported[32] to contain 50% selenomethionine, 15% selenocystine, 15% selenocysteine, 10% selenocystathione and 10% methylselenocysteine. Obviously, these several seleno-compounds and other residues in the commercial presentation could have had unexpected effects that differed from what might be expected from selenium alone. The significant interaction between selenium source and comb height showed that LC males, which had heavier BW, failed to maintain fertility under severely feed restricted conditions. HiSe Yeast improved fertility of LC males by up to 4% but did not affect the fertility of SC males during the first quartile time period. HiSe Yeast had a greater effect when feed intake was more restricted relative to male BW. Figure 8 illustrates the ME balance in males with different comb heights relative to their maintenance requirement with the current experimental temperatures. The data showed that the LC males were heavier and the ME intake was apparently marginal. So, in such a situation an energy-sparing effect was one explanation for the apparently beneficial effect of HiSe Yeast. On the other hand, the HiSe Yeast increased female BW at 48 week of age

and reduced overall egg production by potentially a similar mechanism. Higher female BW should require more ME and egg production could have been negatively affected as a result. To illustrate this effect, the ME required daily to maintain 100 g of BW and to produce a 60 g egg each day was calculated. It was determined that 7 kcal ME day^{-1} were required to maintain each additional 100 g of BW. This extra BW demanded ~50 kcal ME $bird^{-1}$ $week^{-1}$ that represented the ME required to produce 0.25 egg $week^{-1}$. Therefore, HiSe Yeast appeared to have shifted female energy metabolism somewhat from egg production to BW gain. Selenium has been shown to be part of many selenoproteins whose functions vary widely from antioxidant capacity to energy metabolism[6]. Therefore, when males with apparently early maturity and larger BW, as for the LC group, were slightly underfed after photostimulation, HiSe Yeast appeared to have a nutrient sparing effect that allowed the LC males to maintain high fertility even when they did not gain BW in a consistent manner. A similar effect may have been responsible for the subsequently increased female BW and poorer egg production.

In contrast with the results during the early production period, NaSe produced better percentage fertility than did HiSe Yeast during the fourth quartile time period (Table 3). Additionally, HiSe Yeast increased embryo mortality (early, late and pipped) during the third quartile time period (Fig. 3a). A significant interaction showed that the negative effect of HiSe Yeast on embryo mortality occurred in the SC males. However, fertility and fertile hatchability demonstrated that the HiSe Yeast group maintained the performance above 92% (Table 3) and 91% (Fig. 4b), respectively. With such good performance it was not likely that HiSe Yeast was truly detrimental to fertility and/or embryo mortality, although a significant effect was found. However, in the light of the results, it was important to remember that organic selenium, such as selenomethionine, has been shown to be incorporated more easily into some tissues. If there was an excess in the quantity of selenium being incorporated, sub-lethal toxicity symptoms could occur[33-35]. Although in the present experiments apparent macroscopic embryo malformations were not observed, nor would the inclusion level of selenomethionine be considered toxic, it remained possible that a lower concentration may need to be considered for organic sources than for inorganic sources to obtain the same selenium status in the animal.

Although many clinical studies have demonstrated the beneficial effects of antioxidants in select cases of male infertility, some studies failed to demonstrate the same benefit. In an extensive review in human studies, Agarwal *et al.*[36] concluded that "the majority of the human studies suffered from lack of placebo-controlled, double-blind design, making it difficult to reach a definite conclusion. In addition, pregnancy, the most relevant outcome variable of fertility, was reported in only a few studies."

In Experiment 2 there was a trend towards improved eggshell weight but in Experiment 1 there was no effect. Therefore, the effect could be related to the higher vitamin concentration of the antioxidant additive package in Experiment 2. In particular, Vitamin C has been shown to play an important role in hydroxylation reactions[37] that play a role in eggshell formation[38].

The goal of the current experiments was to compare feed allocation and its interaction with selenium sources and antioxidant vitamins on broiler breeder fertility. HiSe Yeast and antioxidant vitamins (Vitamins C and E) could have had an energy sparing effect as antioxidants ameliorated fertility problems due to marginal feed allocations but when feed allocations were increased the antioxidant benefit tended to disappear. Thus, it was difficult to ascribe the beneficial effects to antioxidant properties alone. On the other hand, the data showed conflicting effects of selenium source on fertility, embryo mortality and fertile hatchability during the production period.

CONCLUSION

The results of the current experiments allowed us to conclude that HiSe yeast and elevated antioxidant vitamins (Vitamins C and E) somewhat decreased fertility problems due to marginal feed allocations. Furthermore, the interactivity of feed allocation on elevated antioxidant levels demonstrated that maintaining both optimum feed allocation and antioxidant levels might be necessary to achieve sustained fertility in broiler breeders.

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