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Effect of Different Levels of Fennel Extract and Vitamin D_3 on Performance, Hatchability and Immunity in Post Molted Broiler Breeders

Research Article

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ABSTRACT

A 3 × 3 factorial experiment with three levels of fennel extract (FE) (0, 50, and 100 mg/kg of diet) and three levels of added vitamin D_3 (Vit D_3) (0, 3500 and 4200 IU/kg of diet), was carried out to evaluate reproductive performance and immune response of post molted broiler breeders (76-84 weeks). Broiler breeders were weighed at 10 week after molting (74 weeks) then randomly distributed into 36 pens in order to have a similar mean pen body weight (BW). Each pen consisted of 10 hens and 1 rooster. The birds received experimental diets at 74 weeks of age. Performance data were recorded daily, whereas egg quality traits were determined every 4 weeks. To determine the settable hatch, fertile hatch, embryonic mortality, real hatch, chick quality, chick sexing, chick weight and relative chick weight, eggs from each pen were collected three times a week, marked and incubated in a commercial hatchery for 21 days. Two blood samples were taken from two hens in each pen to assay Newcastle and Influenza antibody titers every two weeks. Dietary fennel extract supplementation significantly increased egg production, chick quality and improved Newcastle antibody titer over time ($P<0.022$). Furthermore, the addition of FE to diet significantly decreased double yolk and pewee eggs ($P<0.001$). Yolk color was affected by the addition of vitamin D_3 in the whole experimental period. Significant interactions between FE and vitamin D_3 on embryonic mortality were observed. The results of this study showed that supplementation of diet with FE exhibits beneficial effects on egg production, double yolk eggs, peewee eggs, chick quality, and Newcastle antibody titer. Dietary supplementation of vitamin D_3 improved yolk color with no adverse effect on productive performance.

KEY WORDS broiler breeder, embryonic mortality, Fennel extract, hatchability, vitamin D_3.

INTRODUCTION

The World Health Organization has estimated that 80% of the earth's inhabitants rely on tradition medicine for their primary health care needs, and most of these therapies involve the use of plant extracts or their active components (Mehmet *et al.* 2005). Plants (specially herbs) have been used as food for medicinal purposes for centuries and some of them have played a significant role in maintaining human health and improving the quality of human life for thousands of years (Osman *et al.* 2005). Aromatic plants have been used traditionally in therapy against some diseases for a long time in the world. In different herbs, a wide variety of active phytochemicals, including the flavonoids, terpeniods, lignans, sulfides, polyphenolics, carotenoids, coumarins, saponins, plant sterols and phthalides have been

identified (Craig, 1999). Feed additives were used for broiler breeders to increase utilization of the limited feed allowance and, in turn, improve egg production performance, fertility, and hatchability. The addition of aromatic plants to feeds and water has been shown to improve feed intake, feed conversion ratio and carcass yield (Hertrampf, 2001). Som studies stated that fennel (*Foeniculum vulgaris*) is one of the aromatic plants containing a high percentage of linolenic and stearic acids. In addition, fennel is characterized by the presence of 16.81% trans anethole and 47.20% estragole with 64.01% of total sweeting components in essential oil. Eldeek *et al*. (2003) reported that the use of fennel in diets increases body weight and improves feed conversion ratio. Egg production, shell quality and bone strength, decrease with aging, whereas, as hens mature sexually, plasma estrogen concentration slowly increase (Madison, 2002). It is generally assumed that estrogen decrement over the production cycle, drops slowly during molt (Hoshino *et al*. 1988), and estrogen level increases again with the beginning of egg production cycle (Johnson, 1986). These changes underlie the egg production patterns of commercial layers, where a gradual decline in egg number from the peak reached shortly after sexual maturity, is witnessed. Hansen *et al*. (2003) confirmed the dramatic decrease in blood estrogen concentration in hens at 70 week compared to those at peak production (~29 weeks). The changes in egg production, eggshell and bone quality are attributable to modified hormone profiles, decreased sensitivity of tissues to hormone action, and diminuished ability of hens to the transport of calcium in the duodenum (Hansen, 2002). Calcium and estrogen are needed for production and secretion of luteinizing hormone and progesterone (Onagbesan and Peddie, 1989). The complex interactions between calcium and estrogen also include estrogen-activation of vitamin D and enhancement of calcium transport from the gut (Bar and Hurwitz, 1979). This study was conducted to investigate the effect of different levels of FE and vitamin D_3 on performance, hatchability and humoral immunity of post-molted broiler breeder hens.

MATERIALS AND METHODS

Fennel extraction method

In this study, the decoctionwas (the process of boiling a substance in a liquid to extract its active ingredients) used to preserve the active ingredients of the herb without any increase in temperature. Twenty gram of fennel seeds was mixed in 200 ml of 70% ethanol.

The mixtures were then left in refrigerator overnight to release all active components from the herb and then filtered through gauze and evaporated under vacuum conditions at 40ºC using a rotary evaporator (Rotavapor R-114, Buchi Labortechnik AG, Flawil and Switzerland) (Saeedi *et al*. 2010).

Husbandry and experimental design

All procedures used in this experiment were approved by the Animal Care Committee of the Ferdowsi University of Mashhad. An experiment characterized by a 3 × 3 factorial arrangement of three levels of fennel extract (0, 50, and 100 mg/kg of diet) and three levels of added vitamin D_3 (0, 3500 and 4200 IU/kg of diet), was carried out with Ross 308 post molted broiler breeder hens (76-84 weeks). At 74 week of age, hens were selected on the basis of their egg production and BW and assigned to feeding regimens for two weeks, prior to trail to ensure that the egg production and weight profile in each group was similar. There were no significant differences between replicates. The experiment then, started at 76 weeks of age and lasted for 8 weeks. A total of 360, 76-weeks-old Ross 308 post molted broiler breeders were randomly allocated into 9 treatments, with 4 replicates of 10 hens each. Breeders were distributed into 36 pens in such order to have a similar mean body weight. In each pen 10 hens and 1 rooster (2×1 m^2) were assigned, with 16 L:8 D lighting program and a temperature maintained close to 21˚C. All hens were fed with experimental diets from 74 to 84 weeks of age. They were supplied with feed and water restriction. Access to water was limited by a time clock and a solenoid system sufficient to control litter moisture. Each pen was equipped with a hanging feeder for rooster, a tube feeder for hens, a bell drinker and one 2-hole nest box. The floor of breeder pens was covered with pine shaving as litter material. With the exception of hand feeding, the housing condition was comparable with commercial standards. A premix of FE and natural Zeolite as carrier was used to prepare 0, 50 and 100 mg FE per kg of diets. Three levels of vitamin D_3 were added to vitamin premixes. The experimental diets were formulated to have similar AME, crude protein, amino acids and minerals (Table 1) according to the Ross 308 broiler breeder Inc. recommendation (Ross Breeders Ltd, Newbridge, Midlothian and UK).

Performance traits and egg quality

Eggs were manually collected 6 times a day. Egg production and egg weight were recorded daily. Actual egg production, egg weight and settable eggs were calculated every 2-weeks from the daily egg counts. Numbers of abnormal doubled-yolk eggs, peewee eggs, cracked and soft-shell eggs were recorded daily. Total eggs from each pen were collected during the last 2 days of each 4 weeks interval, weighed and graded as indicated by the European Council Directive (2006). The four categories registered for egg size were extra large (>73 g), large (73 to 63 g), medium (63 to 53 g) and small (<53 g).

Table 1 Nutrients in diets for post molted broiler breeder hens, from 76 to 84 weeks of age

Item / Ingredient (%)	0 vitamin D_3 IU/kg			3500 vitamin D_3 IU/kg			4200 vitamin D_3 IU/kg		
	0 mg/kg FE[1]	50 mg/kg FE	100 mg/kg FE	0 mg/kg FE	50 mg/kg FE	100 mg/kg FE	0 mg/kg FE	50 mg/kg FE	100 mg/kg FE
Corn	52.45	52.45	52.45	52.45	52.45	52.45	52.45	52.45	52.45
Wheat	19.50	19.50	19.50	19.50	19.50	19.50	19.50	19.50	19.50
Soybean meal	18.55	18.55	18.55	18.55	18.55	18.55	18.55	18.55	18.55
Wheat bran	0.60	0.60	0.60	0.60	0.60	0.60	0.60	0.60	0.60
Calcium carbonate	6.75	6.75	6.75	6.75	6.75	6.75	6.75	6.75	6.75
Mono calcium phosphate	1.03	1.03	1.03	1.03	1.03	1.03	1.03	1.03	1.03
NaCl	0.25	0.25	0.25	0.25	0.25	0.25	0.25	0.25	0.25
NaHCo3	0.25	0.25	0.25	0.25	0.25	0.25	0.25	0.25	0.25
Vitamin premix[2]	0.25	0.25	0.25	0.25	0.25	0.25	0.25	0.25	0.25
Mineral prmix[3]	0.25	0.25	0.25	0.25	0.25	0.25	0.25	0.25	0.25
DL-methionine	0.02	0.02	0.02	0.02	0.02	0.02	0.02	0.02	0.02
Zeolite[3]	0.10	0.10	0.10	0.10	0.10	0.10	0.10	0.10	0.10
Total	100	100	100	100	100	100	100	100	100
Calculated analyzed									
Metabolized energy	2751	2751	2751	2751	2751	2751	2751	2751	2751
Crude protein	14.51	14.51	14.51	14.51	14.51	14.51	14.51	14.51	14.51
Available phosphorus	0.35	0.35	0.35	0.35	0.35	0.35	0.35	0.35	0.35
Calcium	2.85	2.85	2.85	2.85	2.85	2.85	2.85	2.85	2.85
Methionine	0.26	0.26	0.26	0.26	0.26	0.26	0.26	0.26	0.26
Crude fiber	3.09	3.09	3.09	3.09	3.09	3.09	3.09	3.09	3.09

FE: fennel extract.
[2] Vitamin premix (per kg of diet) supplied: vitamin A: 12000 IU; vitamin E: 100 IU; vitamin K3: 7 mg; vitamin B1: 3 mg; vitamin B2: 12 mg; vitamin B3: 12 mg; Nicotinic acid: 40 mg; vitamin B6: 4 mg; vitamin B9: 1.5 mg; vitamin B12: 0.04 mg; vitamin B19: 0.25 mg; Choline chloride: 200 mg.
Vitamin premix: vitamin D_3 was added at the rate of 0, 3500, and 4200 IU/kg of diet to provide three vitamin D_3 diets.
[3] Mineral premix provided (mg/kg of diet): Mn: 60; Fe: 60; Zn: 100; Cu: 10; Co: 0.2; I: 0.5 and Se: 0.4.
[4] Zeolite was used as carrier at the rate of 0.1% of diet, but contained 0, 50, and 100 mg to provide three FE diets.

Fresh selected eggs were assessed for specific gravity as described by Hempe *et al*. (1988) as follows:

Specific gravity= weight in air (g) / [weight in air (g) - weight in water (g)]

A random sample of total eggs per replicate was taken from the collection of the last 2 days of each 28 d interval. After the specific gravity determination, the eggs were weighed, broke and the yolks were separated from the albumen.

Before determining yolk weight, the chalaza was removed by forceps. Each yolk was rolled on a blotting paper towel to remove adhering albumen. The shells were cleaned from any adhering albumen and dried at room temperature for 48 hours. Albumen weight was calculated by subtracting the weight of yolk and shell from the whole egg weight. Shell weight per unit of surface area (SWUSA) was calculated via dividing the shell weight (mg) by the egg surface area (cm^2). Egg surface area was figured according to Carter (1975) applying the equation: 3.9782 × egg weight $(g)^{0.7056}$.

Shell thickness was defined as mean among measurements at three different parts of the egg (air cell, equator, and sharp end) using a shell thickness digital measuring gauge (Seri 500, Mitutoyo, Tokyo and Japan).

Egg sample that had been collected for specific gravity was used for yolk color with an egg multi tester (EMT-5200, Robotmation Co. Ltd, Tokyo and Japan). Haugh units were calculated from the data of albumen height and egg weight through the formula:

$HU= 100 \log_{10} (H -1.7 W^{0.37}+7.56)$.

Where:
HU: haugh unit.
H: height of the albumen (mm).
W: egg weight (g).

Moreover, shell strength was measured with an egg force gauge (Sanovoeng Co. Ltd., Tokyo and Japan).

All of the hens were weighed on weekly intervals early in the morning before offering the feed. Daily feed allowances were adjusted weekly to maintain a targeted weekly body weight gain as recommended by the Ross Breeders Ltd, Newbridge and Midlothian UK. Daily feed was adjusted to provide a similar nutrient intake for all birds.

Hatch characteristics

Egg production and settable eggs were recorded daily. Percentage of settable eggs per hen was defined as ratio of total settable eggs to total laid eggs per pen. Settable eggs were

weighed to determine the average daily egg weight. Mortality was recorded daily. Settable eggs were separated from dirty, deformed, broken, cracked, excessively small (peewee), or double yolked and stored in a cold room. Eggs were collected for six consecutive days every other week for all replicates, and weighed individually. Within 2 hours after hatching, each chick was weighed. The relative body weights of chicks were determined using a universally accepted formula:

Relative body weight of a chick (%)= [individual body weight (g) / mean egg weight group (g)] × 100.

Thirty-six settable eggs per pen were set for incubation biweekly between 76-84 weeks of age. Eggs were incubated in Jamesway model Micro Pt- 100 commercial incubator. Incubator was set at 37.15 ˚C dry bulb and 29.62 ˚C wet bulb temperatures (0-19 days). Eggs were candled on day 10 of incubation for monitoring infertile eggs. All infertile eggs were opened and examined macroscopically for evidence of embryonic mortality. All unhatched eggs were analyzed for developmental stage of dead embryos. The time of embryonic death was assigned to one of four categories: early dead (≤7 days), mid-dead (8-16 days), late dead (17-21 days), and pips. Fertility was expressed as the rate of fertile eggs to total eggs set. On day 19, eggs were transferred to baskets and the baskets were placed randomly into the hatcher cabinets. Hatcher was set at 36.44 ˚C dry bulb and 32.18 ˚C wet bulb temperatures.

The number of eggs that hatched was recorded at 21.5 days of incubation. Hatchability of fertile eggs was expressed as the rate of hatching chicks to fertile eggs, and cumulative hatchability was expressed as percentage of hatching chicks to the total eggs set. At the end of 21.5 days of incubation, pipped eggs were recorded and real hatch was expressed as:

Real Hatch= total hatched chicks / total egg - (fertile eggs+pipped eggs).

Real hatch parameter include some of the eggs recorded as "pipped", which survived through incubation but did not hatch; therefore, they were not included in the analysis. Such eggs were counted as if they hatched, thus causing the estimate of failure to hatch to be biased downward. Chick quality was defined as normal and abnormal chick, already described by Dziaczkowska (1980). After hatching, broiler chickens were feather-sexed for gender rate.

Newcastle and influenza immunity

Two blood samples (2 to 3 mL) were collected from the left brachial vein of marked hen in each pen at 4, 6 and 8 weeks after administration of the dead vaccine. All blood samples were centrifuged at 1000 × g for 5 min to separate sera and then the sera was harvested and stored at -20 ˚C until analysis.

The presence of Newcastle and Influenza antibodies in sera samples were measured using the hemagglutination inhibition (HI) method.

Statistical analysis

All data were analyzed according to a completely randomized design with a 3 × 3 factorial arrangement. The General Linear models of SAS (SAS, 2003) were used to analyze all the data. The effects of dietary FE level on different variables were separately analyzed applying linear and quadratic contrasts. Newcastle and Influenza antibody titers data were analyzed using the repeated measurement procedure of the SAS in PROC MIXED procedure (SAS, 2003). Differences among treatment means were measured by Duncan's multiple range test and considered significant at $P<0.05$.

RESULTS AND DISCUSSION

Productive performance

The effect of different dietary treatments on egg production and egg weight are shown in Table 2. Hens fed 50 mg/kg FE diet had significantly ($P<0.05$) higher egg production (Table 2) than those fed 0 and 100 mg/kg FE (54.72 *vs.* 51.57 and 51.25%, respectively) during 76 to 84 weeks of age. The interaction of vitamin D_3 and FE on egg production was observed ($P<0.05$) during 78-80 weeks of age. Fennel extract had quadratic effect ($P=0.006$) on egg production. In agreement with our study, Sahin *et al*. (2007) reported that isoflavone (phytoestrogen) supplementation improved egg production in quails.

Fennel extract have oestrogen-like compounds which induce egg production. In another study, phytoestrogen supplementation increased performance and improved egg quality variables in quails (Akdemir and Sahin, 2009). Such improvement might be due to the antibacterial and antifungal properties of fennel (Hodgson *et al*. 1998). Furthermore, these investigators reported that bacteria or yeasts are susceptible to fennel or in combination with Propyl paraben that is the major component of the fennel oil. Other compounds of fennel oil like fenchone, methyl chavicol and anethole, may exhert some biological function. Birds that are lying regularly will have continuously high oestrogen, whereas birds laying very few eggs are likely to have low levels of oestrogen (Whitehead, 2004). These findings are in accordance with the results of Bar and Hurwitz (1987). They reported that old birds (20 month) produced significantly fewer but heavier eggs.

Table 2 Effect of fennel extract (FE) and vitamin D_3 on broiler breedes egg production and egg weight from 76 to 84 weeks of age

Treatment / FE (mg/kg)	Egg production (%)					Egg weight (g)				
	76-78 wk	78-80 wk	80-82 wk	82-84 wk	76-84 wk	76-78 wk	78-80 wk	80-82 wk	82-84 wk	76-84 wk
0	21.42	49.91	65.66	69.30	51.57^{b}	66.64	68.45	69.50	69.91	68.63
50	21.59	54.57	69.80	72.92	54.72^{a}	68.01	68.60	68.66	68.97	68.56
100	20.93	49.25	65.68	69.12	51.25^{b}	68.01	68.80	69.34	69.69	68.96
SEM	0.291	1.49	1.29	1.04	0.661	0.616	0.442	0.401	0.381	0.381
Vitamin D_3 (IU/KG)										
0	21.18	45.31^{b}	69.03	71.04	51.64	67.42^{b}	68.69	69.03	69.46	68.65
3500	21.21	54.80^{a}	66.78	69.39	52.97	66.66^{b}	68.28	69.24	69.39	68.40
4200	21.55	53.92^{a}	65.32	70.90	52.92	67.58^{a}	68.88	69.23	69.72	69.10
SEM	0.291	1.49	1.29	1.04	0.661	0.616	0.442	0.401	0.381	0.381
FE × vitamin D_3										
0 × 0	21.85	45.46^{bc}	68.66	67.67	50.91	67.61	69.06	69.93	70.67^{ab}	69.32
0 × 3500	21.21	49.76^{ab}	61.27	70.47	50.68	65.14	68.41	69.62	69.77^{ab}	68.28
0 × 4200	21.20	54.52^{ab}	67.06	69.76	53.13	67.17	67.89	68.88	69.20^{ab}	68.29
50 × 0	22.15	54.52^{ab}	73.84	74.04	56.14	68.23	69.17	68.92	69.47^{ab}	68.95
50 × 3500	21.01	57.09^{a}	71.67	71.52	55.32	67.15	67.06	68.56	69.12^{ab}	68.12
50 × 4200	21.63	52.09^{ab}	63.88	73.19	52.69	68.65	69.00	68.50	68.32^{b}	68.62
100 × 0	19.55	35.95^{c}	64.60	71.42	47.88	66.41	67.85	68.24	68.25^{b}	67.69
100 × 3500	21.41	56.66^{ab}	67.42	66.19	52.92	68.70	68.80	69.48	69.19^{ab}	68.79
100 × 4200	21.83	55.14^{ab}	65.04	69.76	52.94	69.93	69.74	70.32	71.64^{a}	70.41
SEM	0.530	2.98	2.56	2.06	1.31	1.065	0.765	0.695	0.660	0.660
Effect	Probability									
FE	0.366	0.143	0.171	0.114	0.021	0.594	0.892	0.258	0.314	0.791
Linear	0.313	0.814	0.992	0.928	0.794	0.377	0.628	0.998	0.728	0.597
Quadratic	0.331	0.053	0.064	0.040	0.006	0.664	0.971	0.089	0.143	0.671
Vitamin D_3	0.690	0.006	0.324	0.646	0.488	0.014	0.707	0.948	0.863	0.530
FE × vitamin D_3	0.065	0.028	0.142	0.571	0.071	0.157	0.403	0.404	0.034	0.188

FE: fennel extract.
SEM: standard error of the means.
The means within the same column with at least one common letter, do not have significant difference (P>0.05).

Exogenous estradiol would increase the hepatic production of yolk precursors, leading to hypertrophy of the liver (Julian and Williams, 1999).

Treatment with estradiol elevates plasma concentrations of vitellogenin and VLDL, possibly through increased production, but has no effect on liver mass, egg production or clutch completion.

Dietary effects of aromatic plant extracts on performance of laying hens from 32 to 40 weeks of age were investigated (Botsoglou *et al*. 2005).

No significant differences in egg production among the treatment groups were reported. Very limited data are available regarding the effect of dietary plant extract on the performance of broiler breeders. Bozkurt *et al*. (2009) observed that egg production was not affected by essential oil premixes containing fennel throughout 22 to 45 weeks of laying periods. Egg weight was not affected by FE during the study (Table 2).

Hens fed a diet supplemented with 4200 IU/kg vitamin D_3 had significantly (P<0.05) heavier eggs than those fed diets containing 0 or 3500- IU/kg, from 76 to 78 weeks. Fennel extract at 50 mg/kg of diet level decreased peewee eggs compared to control hens (P<0.05, 0.196 *vs*. 0.039). Older studies show that, pullets from commercial layer strains, reach sexual maturity much earlier than their counterparts (Summers and Leeson, 1993).

As a result, pullets have lighter body weight at the onset of egg production. A positive correlation exists between body weight of pullets at the age of housing, and egg weight during the egg production cycle (Keshavarz, 1995). The selection process aimed at early sexual maturity has resulted in the production of high percentages of small and peewee-sized eggs during the early stages of the egg production cycle (Keshavarz, 1995). In the present study, dietary FE significantly affected broiler breeder body weight gain (Table 3).

Table 3 Effect of fennel extract (FE) and vitamin D_3 levels, on settable eggs and egg grading of broiler breeders from 76 to 84 weeks of age

Treatment / FE (mg/kg)	Settable eggs (%)					Egg characteristics		Egg grading (%)			BWG (g)
	76-78 wk	78-80 wk	80-82 wk	82-84 wk	76-84 wk	DY (%)	PE (%)	Extra	Large	Medium	
0	96.39	98.98	98.20	98.30	97.97	0.382^{a}	0.196^{b}	26.66	64.44	8.88	79.63^{b}
50	96.43	98.60	99.03	96.93	97.75	0.164^{b}	0.039^{a}	13.32	76.66	10.00	96.30^{ab}
100	96.05	98.64	99.11	98.16	97.99	0.283^{ab}	0.186^{b}	21.11	64.44	14.44	126.6^{a}
SEM	1.002	0.465	0.791	0.609	0.384	0.091	0.077	3.335	5.187	3.852	10.78
Vitamin D_3 (IU/kg)											
0	97.65	99.11	99.34	98.23	98.58	0.195	0.086	15.55	75.55	8.880	111.5
3500	95.91	99.13	99.36	97.13	97.63	0.256	0.226	25.55	62.22	12.22	105.5
4200	95.30	97.98	99.36	98.04	97.49	0.378	0.110	20.00	67.77	12.22	84.45
SEM	1.002	0.465	0.791	0.609	0.384	0.091	0.077	3.335	5.187	3.852	10.78
FE × vitamin D_3											
0 × 0	96.91	99.55	99.08	98.17	98.43	0.198	0.260	20.00	73.33	6.670	104.1
0 × 3500	94.80	100	98.00	97.22	97.50	0.148	0.148	26.66	66.67	6.670	80.56
0 × 4200	97.45	97.40	97.52	99.51	97.97	0.798	0.181	33.33	53.33	13.33	54.17
50 × 0	100	99.06	100	99.04	99.52	0.000	0.000	16.66	83.33	0.000	116.6
50 × 3500	96.55	97.90	97.60	95.69	96.93	0.354	0.119	16.66	66.67	16.67	75.00
50 × 4200	92.75	98.84	99.51	96.08	96.79	0.138	0.000	6.66	80.00	13.33	97.22
100 × 0	96.05	98.71	98.95	97.49	97.80	0.386	0.000	10.00	70.00	20.00	113.8
100 × 3500	96.39	99.51	99.49	98.48	98.47	0.264	0.411	33.33	53.33	13.33	161.1
100 × 4200	95.75	97.70	98.90	98.52	97.70	0.198	0.048	20.00	70.00	10.00	104.8
SEM	1.735	0.805	1.370	1.055	0.665	0.158	0.134	5.770	8.975	6.665	18.6
Effect	Probability										
FE	0.482	0.433	0.128	0.317	0.976	0.001	0.001	0.073	0.274	0.652	0.020
Linear	0.957	0.309	0.073	0.921	0.969	0.199	0.240	0.321	1.000	0.029	0.006
Quadratic	0.234	0.428	0.329	0.136	0.831	0.231	0.386	0.038	0.112	0.929	0.611
Vitamin D_3	0.272	0.590	0.429	0.640	0.066	0.121	0.010	0.211	0.309	0.830	0.226
FE × vitamin D_3	0.159	0.743	0.273	0.132	0.073	0.240	0.594	0.225	0.634	0.481	0.134

FE: fennel extract; DY: doble yolk; PE: peewee egg and BWG: body weight gain during 76-8 weeks.
SEM: standard error of the means.
The means within the same column with at least one common letter, do not have significant difference (P>0.05).

As body weight at the onset of egg production cycle increases, peewee eggs decreases (Keshavarz, 2003), therefore body weight might be one of the important factors determining the number of peewee eggs, and the main factor that controls egg size.

Increasing FE level in the diet significantly decreased double-yolk eggs. Taherkhani *et al*. (2010) showed that by increasing fat pad weight, double-yolk eggs increased.

They also indicated that feed-satiated hens had significantly (P<0.05) higher abdominal fat pad weights than restricted hens.

Interestingly, restricted broiler breeder hens stored less (P<0.05) fat in the abdominal cavity, compared with hens having free access to diet. The results of this study are in agreement with Taherkhani *et al*. (2010). When dietary FE level is increased to 50 mg/kg, a reduction is witnessed in extra-large sized eggs, whereas medium-sized eggs are increased linearly (P<0.05). Nasra *et al*. (2010) reported that there is a reverse relationship between egg production and egg size.

Egg quality

The specific gravity of eggs from hens fed diets with different vitamin D_3 or FE were similar (Table 4). Many factors could influence egg shell quality, such as an increased presence of sulfate groups in the shell matrix which in turn, significantly increases the Ca binding ability, and both shell percentage and specific gravity, as well as the overall shell quality (Simkiss and Taylor, 1957). In this study, all nutrients in the diets were similar, and therefore this may account for the absence of effect of both FE and vitamin D_3 on shell quality. Fennel extract and vitamin D_3 supplementation in broiler breeder diets had no detrimental effects on SWUSA, shell weight and shell strength. In contrast, shell thickness in hens fed diets supplemented with 50 mg/kg FE, tended to be thicker than birds fed control diet (0.312 vs. 0.37 mm, P=0.063), and FE resulted in a quadratic effect (P=0.038) on shell thickness. Keshavarz (2003) reported that by increasing egg size, shell quality was decreased. This is due to constant distribution of shell over a larger egg.

Table 4 Effect of fennel extract (FE) and vitamin D_3 levels on specific gravity, shell weight per unit of surface area (SWUSA), shell%, egg shell thickness and shell strength of molted broiler breeders from 76 to 84 weeks of age

Treatment / FE (mg/kg)	Specific gravity			SWUSA (mg/cm^2)			Shell (%)			Egg shell thickness (mm)	Shell strength (kg force)
	80 wk	80 wk	Overall	80 wk	80 wk	Overall	80 wk	80 wk	Overall		
0	1.081	1.083	1.082	81.82	79.56	80.70	9.22	9.18	9.20	0.307	3.321
50	1.083	1.084	1.084	81.07	78.95	80.00	9.27	9.15	9.21	0.312	3.420
100	1.082	1.082	1.082	80.27	81.08	80.68	9.38	9.13	9.25	0.298	3.171
SEM	0.0005	0.0008	0.0005	1.000	1.230	0.904	0.108	0.155	0.099	0.006	0.136
Vitamin D_3 (IU/kg)											
0	1.081	1.081	1.081	81.51	80.13	80.81	9.18	8.88	9.03	0.306	3.361
3500	1.083	1.084	1.083	82.11	78.13	80.13	9.43	9.29	9.36	0.300	3.211
4200	1.083	1.084	1.083	79.54	81.33	80.44	9.26	9.28	9.27	0.310	3.342
SEM	0.0005	0.0008	0.0005	1.000	1.230	0.904	0.108	0.155	0.099	0.003	0.136
FE × vitamin D_3											
0 × 0	1.080	1.079	1.080	80.27	78.66	79.46	9.04	8.46	8.84	0.305	3.291
0 × 3500	1.082	1.086	1.084	84.06	77.29	80.70	9.36	9.41	9.38	0.304	3.243
0 × 4200	1.082	1.085	1.083	81.15	82.72	81.93	9.26	9.48	9.37	0.310	3.440
50 × 0	1.083	1.083	1.083	81.29	78.39	79.85	9.48	9.03	9.25	0.312	3.840
50 × 3500	1.082	1.084	1.083	82.16	80.67	81.41	9.19	9.32	9.26	0.287	3.220
50 × 4200	1.085	1.085	1.085	79.75	77.78	78.76	9.15	9.10	9.12	0.294	3.190
100 × 0	1.080	1.082	1.081	82.96	83.35	73.12	9.02	8.99	9.01	0.301	2.962
100 × 3500	1.084	1.083	1.084	80.12	76.42	78.29	9.75	9.13	9.44	0.308	3.171
100 × 4200	1.081	1.082	1.082	77.72	83.48	80.62	9.37	9.26	9.31	0.327	3.382
SEM	0.001	0.001	0.001	1.730	2.140	1.565	0.188	0.268	0.172	0.006	0.237
Effect	Probability										
FE	0.344	0.735	0.460	0.642	0.564	0.867	0.354	0.982	0.937	0.063	0.543
Linear	0.498	0.636	0.951	0.353	0.460	0.988	0.157	0.852	0.735	0.398	0.494
Quadratic	0.197	0.538	0.219	0.987	0.443	0.599	0.875	0.990	0.916	0.038	0.390
Vitamin D_3	0.316	0.255	0.116	0.283	0.302	0.900	0.488	0.218	0.145	0.215	0.769
FE × vitamin D_3	0.466	0.644	0.535	0.508	0.235	0.292	0.247	0.715	0.515	0.084	0.371

FE: fennel extract.
SEM: standard error of the means.

Consequently, limiting the egg size should also prevent the loss of shell thickness. At 74 to 76 weeks of age and during the whole period of experiment, hens consuming FE diet quadratically affected albumen weight (Table 5).

The differences between dietary treatments were not significant over the entire period of the experiment. Yolk weight was not influenced by dietary supplementation of FE and vitamin D_3.

It is possible that these changes of albumen weight might be attributed to a higher concentration of fat. Estrogen affects fat metabolism (Bird, 1946) therefore the increase in fat metabolism leads to an increase in both yolk and albumen weight (Safaa *et al*. 2008), although some studies have reported an improvement proportionally greater for the albumen than for the yolk (Grobas *et al*. 1999). Whitehead (1995) suggested that the effect of fat on albumen weight was due to the influence of certain unsaturated fatty acids on estrogen production, which is mainly responsible for albumen secretion.

Ewan (1991), hypothesized that the increase in fat content may lead to increase digestibility of nutrients such as protein and amino acids, through a slower passage rate.

Yolk color (Table 5) was affected by vitamin D_3 treatment ($P<0.01$). These results are consistent with a previous report (Park *et al*. 2005). There were competitive interactions between vitamin D and vitamin A absorption (Aburto and Britton, 1998). Therefore addition of vitamin D_3 to diet my affect vitamin A absorption, which in turn has an influence on the yolk color index.

Hatch characteristics

Fennel extract and vitamin D_3 had a significant effect ($P<0.05$) on the settable hatch at 78-79 weeks (Table 6), although it was not significantly influenced during the whole period under investigation. Hens fed diet supplemented with 100 mg/kg FE had significantly ($P<0.05$) higher fertile hatch than those fed the control diet at 76-77 weeks.

Table 5 Effect of fennel extract (FE) and vitamin D_3 on the egg fractions of molted broiler breeder hens from 76 to 84 weeks of age

Treatment / FE (mg/kg)	Albumin (%)			Yolk (%)			Haugh unit	Yolk color
	78 wk	84 wk	Overall	78 wk	84 wk	Overall		
0	59.83	61.81	60.26	30.94	29.13	30.03	77.41	6.25
50	65.53	61.60	63.57	30.86	29.36	30.11	79.18	6.09
100	61.07	61.31	61.19	30.90	29.29	30.09	77.74	6.18
SEM	1.543	0.435	0.435	0.395	0.377	0.267	1.427	0.089
Vitamin D_3 (IU/kg)								
0	61.69	61.89	61.79	30.59	29.34	29.97	75.33	5.90[b]
3500	61.78	61.36	61.57	31.48	28.95	30.21	78.64	6.25[a]
4200	69.96	61.49	62.23	30.62	29.49	30.05	80.37	6.37[a]
SEM	1.543	0.435	0.435	0.395	0.377	0.267	1.427	0.089
FE × vitamin D_3								
0 × 0	60.60	62.13	61.45	30.34	29.04	29.69	76.14	6.00
0 × 3500	59.44	61.77	60.60	31.19	28.81	30.00	76.36	6.11
0 × 4200	59.45	61.37	60.41	31.28	29.55	30.41	79.74	6.66
50 × 0	64.46	61.54	63.00	30.49	29.80	30.14	75.91	5.83
50 × 3500	66.96	61.16	64.06	31.94	29.51	30.72	80.90	6.22
50 × 4200	65.16	62.12	63.64	30.16	28.77	29.46	80.75	6.22
100 × 0	60.01	61.82	60.91	30.96	29.18	30.07	73.95	5.88
100 × 3500	58.93	61.15	60.04	31.31	28.52	29.92	78.65	6.44
100 × 4200	64.28	60.98	62.63	30.42	30.15	30.29	80.62	6.22
SEM	2.670	0.753	0.753	0.685	0.653	0.462	2.470	0.154
Effect	Probability							
FE	0.219	0.782	0.165	0.993	0.931	0.983	0.725	0.338
Linear	0.968	0.491	0.888	0.951	0.805	0.897	0.890	0.259
Quadratic	0.086	0.948	0.061	0.925	0.781	0.899	0.436	0.342
Vitamin D_3	0.493	0.743	0.811	0.322	0.667	0.851	0.118	0.008
FE × vitamin D_3	0.901	0.887	0.867	0.750	0.567	0.482	0.900	0.345

FE: fennel extract.
SEM: standard error of the means.
The means within the same column with at least one common letter, do not have significant difference (P>0.05).

The supplementation of vitamin D_3 significantly affected fertile hatch at 81-82 weeks (Table 6). As dietary FE increased, the hatchability of the fertile egg tended to decrease linearly (P=0.052). Johan *et al.* (1950) reported a trend toward lower hatchability with increasing estrogen. Estrogen may affect hatchability by influencing calcium metabolism during egg formation in the oviduct (Common *et al.* 1947) or through its effect on fat metabolism (Bird, 1946). Bernier (1947), found a positive correlation between egg production and hatchability that is in agreement with the results of our study. Some authors (Munro and Kosin, 1943) have shown that the genital tracts of young female fowls are enlarged by estrogen therapy. Since sperm cells in fowls are subjected to the oviduct environment for relatively long periods and since fertilization is believed to occur in the infundibulum (Olsen and Neher, 1948), it has been considered that estrogenic treatment might not significantly influence fertility. Bozkurt *et al.* (2009) findings on fertility traits, confirm earlier work that showed no beneficial effects of supplemented estrogenic compounds in the diets of broiler breeder. Supplementation of fennel extract at 100 mg/kg significantly increased embryonic mortality at mid stage of eggs laid at 81-82 weeks (Table 7).

The interaction among dietary vitamin D_3 and FE was significant on late embryonic mortality, but not significant for the whole period of incubation. Whitehead (1995) suggested that the effect of fat on albumen weight was due to the influence of certain unsaturated fatty acids on oestrogen production, which is mainly responsible for albumen secretion.

Thin watery albumen will also reduce hatchability, and therefore phytoestrogen might affect this mechanism and consequently embryonic mortality. Husseiny *et al.* (2002) reported that the addition of phytoestrogen to broiler breeder diet, decreases total lipid in liver and yolk. In addition, most of the energy needed during embryonic development is taken from the fat stores in the yolk (Tona *et al.* 2001), and therefore FE can affect embryonic development in incubation by reducing yolk fat deposition. Interactions between FE × vitamin D_3 on late embryonic mortality at 83 to 84 weeks and the whole period under study, were reported. The highest value of late embryonic mortality was observed in hens fed a diet without vitamin D_3 supplementation. The effect of vitamin D_3 on late embryonic mortality was more evident in hens fed control diet than those fed any amount of vitamin D_3 supplemented diet.

Table 6 Effect of fennel extract (FE) and vitamin D_3 on hatchability of molted broiler breeders from 76 to 84 weeks of age

Treatment / FE (mg/kg)	Settable hatch (%)				Fertile hatch (%)				Real hatch (%)			
	78-79 wk	81- 82 wk	83-84 wk	Overall	78-79 wk	81- 82 wk	83-84 wk	Overall	78-79 wk	81- 82 wk	83-84 wk	Overall
0	89.29[a]	85.86	84.51	85.77	90.57[a]	91.09	89.75	90.48	93.28[b]	91.77	95.05	93.16
50	87.34[a]	87.14	84.79	86.42	89.83[a]	89.56	91.23	90.21	94.29[a]	92.28	97.16	94.58
100	80.64[b]	82.94	81.66	81.52	83.67[b]	87.71	86.86	86.48	97.64[a]	95.39	95.62	96.04
SEM	1.572	2.095	2.203	1.797	1.656	1.601	2.132	1.208	0.791	1.427	1.291	0.841
Vitamin D_3 (IU/kg)												
0	82.74[b]	84.45	82.34	82.83	86.38	86.60[b]	89.09	87.17	94.98	91.31	97.35	94.37
3500	89.10[a]	81.79	81.34	83.30	90.65	87.88[b]	87.43	88.66	95.76	93.07	97.25	94.58
4200	86.26[ab]	89.70	87.28	87.59	87.81	93.89[a]	91.32	90.97	94.59	95.06	95.23	94.82
SEM	1.572	2.095	2.203	1.797	1.656	1.601	2.132	1.208	0.791	1.427	1.291	0.841
FE × vitamin D_3												
0 × 0	85.10	83.58	81.48	83.39	88.51	83.58	86.06	86.05	97.81	91.42	94.27	94.50
0 × 3500	98.33	81.48	81.32	83.68	98.33	93.46	87.94	92.41	100	96.70	94.59	96.29
0 × 4200	87.45	92.54	90.74	90.24	87.45	96.24	95.26	92.98	95.91	98.06	98.01	97.33
50 × 0	85.18	91.31	87.96	88.15	87.72	92.13	93.89	91.25	94.81	93.17	100	96.04
50 × 3500	87.03	80.55	79.39	82.32	90.30	85.25	89.39	88.29	94.81	91.52	95.50	93.94
50 × 4200	89.81	89.55	87.03	88.80	91.48	91.31	90.74	91.09	93.24	92.15	95.83	93.75
100 × 0	75.55	78.46	77.59	76.94	81.19	84.09	87.33	85.35	90.95	89.34	97.65	92.55
100 × 3500	85.02	83.33	83.33	83.89	85.87	84.94	85.09	85.28	93.89	90.98	95.65	93.51
100 × 4200	79.14	87.03	84.07	83.73	82.84	94.11	88.23	88.83	94.64	94.97	91.85	93.40
SEM	2.720	3.625	3.810	3.110	2.865	2.770	3.690	2.090	1.370	2.470	2.235	1.455
Effect	Probability											
FE	0.007	0.468	0.635	0.220	0.039	0.448	0.457	0.097	0.011	0.267	0.874	0.139
Linear	0.002	0.403	0.438	0.165	0.016	0.212	0.416	0.053	0.005	0.136	0.939	0.052
Quadratic	0.348	0.368	0.590	0.288	0.327	0.944	0.344	0.316	0.299	0.527	0.613	0.986
Vitamin D_3	0.031	0.088	0.236	0.229	0.162	0.027	0.544	0.224	0.409	0.296	0.696	0.946
FE × vitamin D_3	0.336	0.399	0.503	0.493	0.547	0.117	0.690	0.378	0.486	0.624	0.295	0.622

FE: fennel extract.
SEM: standard error of the means.
The means within the same column with at least one common letter, do not have significant difference (P>0.05).

Hens fed diet supplemented with 50 mg/kg FE and 3500 IU/kg vitamin D_3 had lower late embryonic mortality than those fed without supplementation during the whole trial. Effect of FE on embryonic mortality was evident with aging.

A widely held hypothesis suggests a cascade mechanism with the intent of explaining the decrease of intestinal calcium absorption, starting with low estrogen levels causing increased bone resorption. The released calcium, increases its presence in the extracellular space, suppressing parathyroid hormone (PTH) secretion and further decreasing $1,25(OH)_2D_3$ production and plasma content, resulting finally in decreased intestinal calcium absorption (Gallagher *et al*. 1979).

These findings support Narbaitz *et al*. (1987), who showed that vitamin D metabolites are required by the embryo in order to mobilize calcium from the shell, and that decreased hatchability in vitamin D-deficient embryos is related to a defect in calcium mobilization from the shell. Salable chicks (Table 8) were significantly affected by FE treatment (P<0.05). Specifically, the addition of 100 mg/kg fennel extract significantly increased salable chicks compared to those fed diet with zero FE (P<0.05).

A significant interaction was observed between FE × vitamin D_3 on salable chicks for the whole period of study (Table 8).

The lowest salable chicks were obtained from broiler breeders fed diet with no added FE and vitamin D_3. Chicks not salable were classified as culls due to: splayed legs, unhealed navels, unabsorbed yolk sac, among others. Therefore, vitamin D_3 deficiency may affect healthy legs and navels and reduce salable chicks. Fennel extract has phytoestrogenic activity and could enhance $1,25(OH)_2D_3$ synthesis.

The quality of hatching eggs is imperative because eggs provide both physical protection and all the needed nutrients for the growing embryo. Shell quality (shell thickness and pore number) determines gas exchange and moisture loss during incubation Wangensteen *et al*. 1971). Poor shell quality has been associated with a higher percentage of egg moisture loss during incubation (Peebles *et al*. 2001). Indeed, shell quality is one of the major effective components that influence chick quality. Fennel extract tended to increase quadratically shell thickness, therefore by increasing shell thickness, the number of salable chicks was significantly increased.

Table 7 Effect of fennel extract (FE) and vitamin D_3 on embryonic mortality of broiler breeder hens from 76 to 84 weeks of age

Treatment	Embryonic mortality (%)															
	Early				Mid				Late				Pipped eggs			
FE (mg/kg)	78-79 wk	81- 82 wk	83-84 wk	Overall	78-79 wk	81- 82 wk	83-84 wk	Overall	78-79 wk	81- 82 wk	83-84 wk	Overall	78-79 wk	81- 82 wk	83-84 wk	Overall
0	1.58	1.93	2.19	0.838	1.87	1.58^{b}	1.24^{a}	1.46	5.81	5.98	7.46	6.42	5.81	5.67	6.84	5.57
50	2.28	0.943	0.617	2.08	1.23	0.63^{b}	1.82^{ab}	1.85	4.93	4.08	5.79	4.93	4.14	3.13	5.48	4.42
100	1.85	0.308	2.16	1.708	1.55	3.13^{a}	2.46^{b}	2.07	7.09	4.67	8.36	6.71	7.14	3.42	8.36	6.72
SEM	0.268	0.244	0.271	0.249	0.258	0.252	0.240	0.268	0.269	0.257	0.202	0.226	0.257	0.256	0.201	0.244
Vitamin D_3 (IU/kg)																
0	1.85	1.2	1.85	1.61	0.925	3.14	3.08	1.45	5.00	5.66	9.92	6.81	4.69	5.34	9.61	6.88
3500	2.32	0.00	2.18	1.54	2.16	1.57	1.21	1.75	4.37	3.43	6.11	4.64	4.19	2.48	5.80	6.07
4200	1.54	1.91	0.935	1.46	1.58	0.626	1.23	2.19	8.47	5.64	5.59	6.57	8.21	4.40	5.28	3.82
SEM	0.268	0.244	0.271	0.249	0.258	0.252	0.240	0.268	0.269	0.257	0.202	0.226	0.257	0.256	0.201	0.244
FE × vitamin D_3																
0×0	0.926	1.96	1.85	1.58	0.926	2.88	1.85	1.58	5.74	10.4	16.7^{a}	10.9^{a}	5.74	10.40	15.8^{a}	8.76
0×3500	2.88	0.00	2.85	0.617	0.277	0.926	0.952	1.23	3.86	4.63	4.6^{bc}	4.3^{bc}	3.86	4.63	3.7^{bc}	3.50
0×4200	0.926	3.85	1.87	0.309	1.93	0.926	0.926	1.58	7.82	2.92	0.92^{c}	3.8^{bc}	7.82	2.00	0.92^{c}	4.47
50×0	1.85	1.87	0.926	1.28	1.85	1.90	2.77	1.51	6.48	3.81	5.5^{bc}	5.2^{abc}	5.55	2.85	5.5^{bc}	4.60
50×3500	3.14	0.00	0.926	2.78	1.85	0.00	0.855	1.23	3.70	1.85	4.4^{bc}	3.3^{c}	3.14	0.926	4.4^{bc}	5.86
50×4200	1.85	0.952	0.00	2.16	0.00	0.00	1.85	2.81	4.63	6.58	7.4^{bc}	6.2^{abc}	3.73	5.63	6.4^{abc}	2.78
100×0	2.77	0.00	2.77	1.97	0.00	4.63	4.63	1.25	2.77	2.77	7.4^{abc}	4.3^{bc}	2.77	2.77	7.4^{ab}	7.27
100×3500	0.926	0.00	2.77	1.23	1.85	3.81	1.85	2.78	5.55	3.81	9.2^{ab}	6.2^{abc}	5.55	1.90	9.2^{ab}	8.68
100×4200	1.85	0.926	0.926	1.91	2.80	0.952	0.926	2.19	12.9	7.43	8.4^{ab}	9.6^{ab}	13.09	5.58	8.4^{ab}	4.21
SEM	0.465	0.423	0.470	0.431	0.447	0.437	0.415	0.464	0.466	0.446	0.34	0.392	0.445	0.444	0.348	0.423
Effect	Probability															
FE	0.186	0.342	0.101	0.071	0.250	0.033	0.026	0.323	0.782	0.641	0.202	0.376	0.403	0.881	0.101	0.264
Linear	0.076	0.149	0.087	0.092	0.120	0.017	0.007	0.139	0.560	0.587	0.663	0.547	0.526	0.928	0.069	0.462
Quadratic	0.771	0.946	0.178	0.100	0.564	0.262	0.906	0.935	0.709	0.454	0.243	0.210	0.237	0.626	0.237	0.206
Vitamin D_3	0.500	0.082	0.933	0.793	0.227	0.623	0.384	0.280	0.307	0.291	0.094	0.148	0.156	0.201	0.112	0.173
FE × vitamin D_3	0.771	0.435	0.901	0.388	0.617	0.911	0.694	0.758	0.460	0.279	0.010	0.009	0.544	0.273	0.016	0.289

FE: fennel extract.
SEM: standard error of the means.
The means within the same column with at least one common letter, do not have significant difference (P>0.05).

Table 8 Effect of fennel extract (FE) and vitamin D_3 on chick characteristics of broiler breeder hens from 76 to 84 weeks of age

Treatment	Salable chicks (%)				Male (%)				Chick weight (g)				Relative chick weight (% set weight [1])			
FE (mg/kg)	78-79 wk	81- 82 wk	83-84 wk	Overall	78-79 wk	81- 82 wk	83-84 wk	Overall	78-79 wk	81- 82 wk	83-84 wk	Overall	78-79 wk	81- 82 wk	83-84 wk	Overall
0	91.29	94.22	96.28	93.94^{b}	50.97	51.87	50.91	51.25	47.64	49.19	48.95	48.59	68.86	71.65	70.42	70.30
50	91.86	97.88	98.12	95.95^{a}	50.08	54.39	52.74	53.07	47.76	48.83	48.55	48.38	69.54	70.62	70.96	70.37
100	91.26	97.47	98.04	96.17^{a}	52.41	56.10	45.90	51.57	48.17	49.21	48.41	48.55	69.46	71.47	69.80	70.23
SEM	0.725	1.118	0.731	0.563	2.971	2.505	2.442	1.254	0.653	0.376	0.442	0.398	0.872	0.492	0.421	0.419
Vitamin D_3 (IU/kg)																
0	93.28	95.95	94.38	94.78	59.26	48.21	51.45	52.85	47.66	49.18	48.93	48.57	69.04	70.71	70.96	70.26
3500	89.63	96.75	98.82	95.14	44.5	57.22	49.27	50.64	48.51	48.71	48.52	48.54	70.14	71.12	70.21	70.43
4200	91.59	96.87	99.23	96.14	51.42	46.92	48.84	52.40	47.35	49.34	48.45	48.40	68.69	71.91	70.04	70.21
SEM	0.725	1.118	0.731	0.563	2.971	2.505	2.442	1.254	0.653	0.376	0.442	0.398	0.872	0.492	0.421	0.419
FE × vitamin D_3																
0×0	90.74	91.16	91.17	91.02^{b}	56.02	46.64	49.89	50.85	47.73	49.84	50.05	49.21	68.98	72.13	71.26	70.79
0×3500	90.99	94.64	97.67	94.50^{a}	48.09	50.00	52.85	50.63	50.20	48.53	48.53	48.79	72.53	70.86	69.87	70.64
0×4200	92.04	96.87	100	96.30^{a}	47.85	58.98	50.00	52.28	45.83	49.20	48.28	47.77	66.31	71.95	70.13	69.46
50×0	96.70	97.84	97.85	97.46^{a}	60.76	49.78	55.81	55.44	47.34	49.47	49.06	48.62	68.41	68.98	71.23	69.54
50×3500	88.34	96.77	98.81	94.64^{a}	43.17	63.39	51.15	52.57	47.89	48.32	48.32	48.18	69.58	71.35	71.06	70.66
50×4200	90.56	99.04	97.70	95.77^{a}	52.32	50.00	51.28	51.20	48.06	48.70	48.27	48.34	70.63	71.55	70.58	70.92
100×0	91.95	98.85	94.13	95.87^{a}	61.87	48.23	48.66	52.24	48.03	48.23	47.69	47.89	70.09	71.03	70.40	70.46
100×3500	90.02	98.85	100	96.29^{a}	44.10	58.28	43.80	48.72	47.99	49.28	48.72	48.67	69.11	71.15	69.69	69.99
100×4200	92.45	94.71	100	96.39^{a}	55.41	61.78	45.23	53.74	48.57	50.12	48.81	49.10	69.34	72.23	69.32	70.24
SEM	1.255	1.935	1.265	0.975	5.14	4.335	4.225	2.170	1.130	0.652	0.765	0.690	1.510	0.852	0.730	0.725
Effect	Probability															
FE	0.883	0.119	0.247	0.049	0.846	0.902	0.256	0.727	0.931	0.788	0.741	0.943	0.981	0.414	0.273	0.977
Linear	0.874	0.093	0.158	0.026	0.570	0.708	0.240	0.638	0.819	0.970	0.462	0.954	0.881	0.698	0.383	0.919
Quadratic	0.647	0.214	0.367	0.274	0.974	0.805	0.241	0.711	0.762	0.496	0.838	0.739	0.910	0.306	0.175	0.855
Vitamin D_3	0.058	0.860	0.001	0.331	0.046	0.052	0.796	0.250	0.550	0.573	0.776	0.962	0.551	0.341	0.368	0.944
FE × vitamin D_3	0.067	0.259	0.072	0.043	0.895	0.101	0.931	0.818	0.474	0.348	0.521	0.574	0.324	0.458	0.964	0.557

[1] Weight calculation of live chicks only.
FE: fennel extract.
SEM: standard error of the means.
The means within the same column with at least one common letter, do not have significant difference (P>0.05).

Immune response

When FE or were added to the diet of molted broiler breeder hens, Newcastle or Influenza antibody titers were not significantly elevated (Table 9). However, Newcastle titer was reduced as the birds aged regardless of FE addition to the diet.

Newcastle antibody titer in birds fed diet with 0.0 mg/kg FE dramatically decreased from 696.88 to 177.77 anti-$\log_2$ hemagglutination inhibition units (78 to 82 week of age). The addition of 50 mg/kg FE to diet slightly reduced Newcastle antibody titer during 78 to 80 weeks of age. This decrement was less in hens fed 50 mg/kg diet.

Therefore, the addition of FE in diet may improve antibody titer and keeps levels higher than normal for longer time.

Table 9 Effect of fennel extract (FE) and vitamin D_3 on Immune response of post molted broiler breeder hens from 76 to 84 weeks of age

Treatment / FE (mg/kg)	Newcastle titer	Influenza titer
0	414.8	445.6
50	455.1	421.9
100	446.8	502.5
SEM	4.86	6.08
Vitamin D_3 (IU/kg)		
0	463.4	421.9
3500	502.5	490.7
4200	350.8	457.5
SEM	4.86	6.08
FE × vitamin D_3		
0 × 0	696.88^{a}	711.11
0 × 3500	369.77^{def}	398.22
0 × 4200	177.77^{g}	227.55
50 × 0	568.88^{bc}	711.11
50 × 3500	468.33^{cd}	320.00
50 × 4200	327.11^{ef}	234.66
100 × 0	654.22^{ab}	810.66
100 × 3500	426.66^{de}	426.66
100 × 4200	259.55^{fg}	270.22
SEM	6.04	7.99
Effect	Probability	
FE	0.910	0.730
Vitamin D_3	0.300	0.804
FE × vitamin D_3	0.989	0.968
FE × Period	0.022	0.930

FE: fennel extract.
SEM: standard error of the means.
The means within the same column with at least one common letter, do not have significant difference (P>0.05).

Simon *et al.* (1980) reported that fennel acts as a mild expectorant, useful for coughs or bronchitis and may be effective in curing phlegmon and promoting a healthy status of liver and kidney. Also, Ahmed *et al.* (1986) reported that estrogen was known to modulate the immune system in humans and mice. Although the mode of action is unclear, it is likely that this steroid may act on its effectors target by first interacting with specific receptor proteins to form a steroid-receptor complex (Yamamoto, 1985). Gonadal steroids are known to exert their immunoregulatory effects directly on lymphocytes effectors target (Stimson, 1988), and indirectly by altering various endocrine functions via their effects on the hypothalamus, pituitary gland, gonads and thymus (Ahmed *et al.* 1985). Moreover, the relationship between the hormone and the immune system is established early in ontogeny, probably due to modifications in the thymus (Fabris, 1981; Herradon *et al.* 1991), and bursa in birds (Glick, 2009; Le-Douarin *et al.* 1980; Coulson *et al.* 1982).

CONCLUSION

In conclusion, dietary FE supplementation at 50 or 100 mg/kg increased egg production, salable chicks and maintained more Newcastle antibody titer overtime. However, only the addition of 50 mg/kg to diet decreased peewee and double yolk eggs and increased shell thickness and medium egg size numbers in molted broiler breeder hens. The addition of vitamin D_3 to diet caused a positive effect on yolk color. Furthermore, late embryonic mortality was significantly decreased with the addition of 50 mg/kg FE and 3500 IU/kg vitamin D_3 to diet. The results of this experiment show that post molted broiler breeder may need less vitamin D_3, when compared to Ross recommendation for broiler breeder hens. The addition of 50 mg/kg FE improved performance, hatchability and immune response in post molted broiler breeder hen. Further research is required to understand the mechanisms by which FE improves the overall performance.

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