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Impact of Dietary Supra-Nutritional Levels of Vitamins A and E on Fertility Traits of Broiler Breeder Hens in Late Production Phase

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Abstract: This study aimed to evaluate the effect of dietary supplementation with supra-nutritional levels of vitamins A and E on fertility and productivity traits of Ross-308 broiler breeder hens during the late production phase. The trial was conducted for nine weeks, from 61 to 69 weeks of age, and designed to test four levels of vitamin A (100, 125, 150 and 200% above the Ross catalogue recommendations) and four levels of vitamin E (100, 200, 300 and 400% above Ross catalogue instructions), maintaining constant the other rearing conditions. Vitamins were combined in 16 treatments, with four replicates per treatment each including seven females, and one rooster broiler was used in every two replicates. A total of 448 hens and 32 roosters were used in the experiment. Fertility parameters were weekly evaluated. According to the results, egg-related parameters (number and weight of eggs, non-conform eggs and hatchability) were not affected by treatment, but dietary treatments increased and extended the hens' productivity for an additional six weeks in most experimental groups. The chick-related parameters (number and weight of produced chicks) and chick yield differed significantly among groups ($p < 0.05$). The best economic index was found in birds fed basal-diet plus 100% of vitamin A and 200% of vitamin E. In conclusion, the present study showed that a combination of dietary supra-nutritional levels of vitamins A and E allowed to extend the laying period and to reduce the decline of fertility in older breeder hens at the end of the laying stage; in particular, among the 16 tested treatments, feeding of supra-nutritional level of 100% vitamin A and 200% vitamin E lead to the best results.

Keywords: broiler breeder; vitamin A; vitamin E; nutrition

1. Introduction

More than 50 billion chickens are annually raised as a low-cost source of both meat and eggs [1,2]. In 2016, the world's broiler meat production amounted to about 89.5 million metric tons [3], while poultry

meat consumption in Organization for Economic Cooperation and Development (OECD) member countries reached 43.7 million tons (equivalent to 29.1 kg per capita) [4].

Poultry meat and egg production is strictly dependent on the availability of good hatching eggs. The number of eggs produced by a hen is largely determined by genetics, whereas egg quality and hatchability depends on various factors including the age of bird, diet and housing conditions. Daily egg production (hen-day production) is an important trait in commercial flocks, whether the eggs are used for consumption as shell or derived products, or for hatching. Therefore, the breeders' fertility is of vital importance for egg producers and breeding companies to achieve maximum number of eggs per hen and maximum return per amount invested.

Vitamins and minerals are crucial nutrients for metabolic and physiological processes, and thereby for animal productive and reproductive performance [5]. Fat soluble-vitamins like A and E positively influence egg production (i.e., number, weight or egg quality) and hatchability. Vitamins also improve the immune status in birds and promote the normal metabolism in order to defend against disease and stress. Recent findings on the role of vitamin A [6–8] and E [9,10] on biological systems presented new viewpoints for requirements of both humans and animals to these nutrients. Vitamin A has been associated with the health and integrity of cell membranes, while vitamin E is often associated with the synthesis of various hormones, and in livestock species, it was a direct effect on gonadal function was reported [11,12]. Moreover, vitamin E was also associated to a redistribution of cholesterol among the lipoproteins (favoring the high-density lipoprotein) and to a decrease in plasma total cholesterol level [13]. Through the years, considerable research has been carried out to determine the appropriate level of vitamins A and E in poultry diet, adjusting them in relation to the different stages of production [14–18]. Nevertheless, the nutritional requirement for vitamins proposed for commercial flocks may not support the actual needs of broiler breeders. Moreover, the improvement of genetic, management and environment of breeder flocks lead to changes in breeders vitamins requirements [19], which draw the attention of researchers into the role of vitamins on the reproductive performance of broiler breeders [13,20–28]. However, additional research on some vitamins may contribute to clarify the optimum vitamin allowance needed for particular strains of parent stock or to maintain a high production level.

Therefore, the present study aimed to test the effect of supra-nutritional levels of vitamins A and E on the reproductive performance of broiler breeder hens during the late production phase (from week 61 to 69), focusing on the main reproductive traits related to the profitability in hatchery industry.

2. Materials and Methods

The current research was performed at a commercial broiler breeder farm in Eshpala (Bandar-e Anzali, Guilan province, Iran; 37°28'39'' N, 49°17'11'' E; 22 m below sea level) and at a commercial hatchery in Mashatuk (Shaft, Guilan province, Iran; 37°16'35'' N, 49°28'18'' E; 7 m below sea level). The experimental design was previously approved by the Ethics Committee of the Islamic Azad University (Rasht Branch, Rasht province, Iran), in accordance to the Experimental Animal Care and International Regulations (directive No. 2007/43/EEC, received by D.L. 1999/74/EC).

2.1. Animals and Management

A total of 480 Ross-308 broiler breeders (448 hens and 32 roosters) were randomly assigned to one of the 16 dietary treatments for nine weeks (from 61 to 69 weeks of age). Each experimental unit was composed by seven hens and 0.5 roosters (one rooster per two replicates). The dietary treatments were replicated four times each. Hens at end of the production cycle (61-week-old hens) and roosters entered the trial period with an average body weight (BW) of 4.24 ± 0.1 and 4.96 ± 0.1 kg, respectively; the initial BW was similar among experimental diets and replicates. Before the trial, birds were fed standard diets to cover their nutrient requirements.

The broiler breeders farm was wired with fences and nets. Birds were housed in cages (2.0 × 1.5 × 1.0 m) kept on the ground. The cages were placed in a row, each cage opening by a door to the outside. In each cage, there was a coop for hens, one for the rooster and a two-floor laying trap. A nipple watering system was positioned around the cages, the water being supplied by a central repository. The poultry facility had thermostatically controlled curtains and cross-ventilation. The lighting schedule consisted of 16 h of light and 8 h of dark during the entire experimental period.

Eggs were manually collected six times per day, placed in setter trays (144 eggs per tray), transported in a closed refrigerated vehicle and subsequently incubated in a commercial hatchery. There, eggs were screened and non-conform eggs were removed. The eggs were disinfected on the farm and in the hatchery. After eggs identification, they were weighed and placed on incubator trollies to allow air circulation around the eggs, and fumigated for 20 min with formaldehyde gas. Thereafter, the eggs were set in the electronically controlled, multi-stage incubators (James Way Co., Cambridge, Canada) and care was taken to avoid the influences of positioning during incubation. After a pre-warming stage that extended for 6 h at around 24 °C and 65% relative humidity, a new fumigation was performed in the day of setting, inside the incubation chamber. Eggs were then set to incubate under the conventional temperature and humidity (37.5 °C and 65–70%) as described by Reis et al. [29], and monitored automatically. The eggs were turned hourly through 90° through the incubation process. On incubation day 19, the eggs were individually candled in the transfer-room (around 24 °C and 65% relative humidity), using a hand candling lamp. Only fertilized eggs with living embryos were transferred to hatching baskets and randomly distributed in the front part of the same trolley. All chicks were removed after 21 days of incubation. The numbers of saleable chicks and culls were determined as practiced in commercial operations [30].

2.2. Experimental Design and Treatments

This study was designed to test the effects of the dietary supplementation with supra-nutritional levels of vitamin A and vitamin E on fertility of hens and hatchability of eggs, over a standard broiler breeder diet designed to meet or exceed birds nutrient requirements [31], as follows:

Group 1 (control)—standard basal-diet supplying 100% of vitamin A (3 mg/kg) and vitamin E (37 mg/kg) requirements;

Group 2—basal-diet plus 125% of vitamin A and 100% of vitamin E;

Group 3—basal-diet plus 150% of vitamin A and 100% of vitamin E;

Group 4—basal-diet plus 200% of vitamin A and 100% of vitamin E;

Group 5—basal-diet plus 100% of vitamin A and 200% of vitamin E;

Group 6—basal-diet plus 125% of vitamin A and 200% of vitamin E;

Group 7—basal-diet plus 150% of vitamin A and 200% of vitamin E;

Group 8—basal-diet plus 200% of vitamin A and 200% of vitamin E;

Group 9—basal-diet plus 100% of vitamin A and 300% of vitamin E;

Group 10—basal-diet plus 125% of vitamin A and 300% of vitamin E;

Group 11—basal-diet plus 150% of vitamin A and 300% of vitamin E;

Group 12—basal-diet plus 200% of vitamin A and 300% of vitamin E;

Group 13—basal-diet plus 100% of vitamin A and 400% of vitamin E;

Group 14—basal-diet plus 125% of vitamin A and 400% of vitamin E;

Group 15—basal-diet plus 150% of vitamin A and 400% of vitamin E;

Group 16—basal-diet plus 200% of vitamin A and 400% of vitamin E;

The ingredient composition and nutrient content of the basal-diet fed to birds was similar for all groups (Table 1), and the vitamin supplementation was maintained constant through the 9-week trial period.

Table 1. Experimental basal-diet fed to broiler breeder hens and roosters.

Ingredients, %	Hens	Roosters
Maize	69.64	66.00
Soybean meal (44% CP)	18.0	11.0
Wheat bran	2.47	18.37
Vitamin-Mineral mix	0.5	0.5
Calcium carbonate	6.5	1.5
Oysters shell	0.5	0.5
Dicalcium phosphate	1.4	1.4
DL-methionine	0.4	0.08
L-lysine HCL	0.01	0.07
Salt	0.2	0.2
Sodium bicarbonate	0.15	0.15
Natuzyne-P *	0.03	0.03
Toxin binder *	0.1	0.1
Formycine Gold *	0.1	0.1
Nutritional analysis		
ME, kcal kg ⁻¹	2800	2700
Crude protein, %	14.0	13.5
Calcium, %	3.0	1.1
Available Phosphorus, %	0.4	0.4
Sodium, %	0.16	0.16
Chloride, %	0.16	0.18
Lysine, %	0.6	0.6
Methionine, %	0.03	0.3
Ether extract, %	2.9	3.15
Fibre, %	2.9	4.2
Linoleic acid, %	1.65	1.80

* Natuzyne-p, containing phytase: 1,000,000 U/g, β glucanase: 700 U/g, α -amylase: 700 U/g, cellulose: 6000 U/g, pectinase: 700 U/g, xylanase: 10,000 U/g, lipase: 30 U/g and protease: 3000 U/g; Toxin binder contains natural hydrated sodium calcium aluminium silicates; Formycine Gold is a broad spectrum disinfectant feed additive that includes ammonia, formaldehyde, propionic acid and sodium bentonite. The basal-diet (control) supplied per kg: 3 mg of vitamin A and 37 mg of vitamin E.

2.3. Data Collection

Hens and roosters were weighed individually before feed distribution, at the beginning and at the end of the experiments (Big Dutchman, Vechta, Germany), and BW changes were calculated accordingly. Weekly, the egg production and weight were recorded. At the hatchery, eggs were evaluated by a trained operator for eggshell defects and screened, at day 12 were assessed the unhatched eggs, and at the end of incubation, the number of live chicks was counted. Chicks presenting physical abnormalities, including weakness, red hocks or unhealed navels, were culled and counted as chick losses. Data from both the breeder farm and the hatchery were used to estimate the fertility and hatchability indexes. The effects of supra-nutritional levels of vitamins A and E were assayed using the following parameters, assessed weekly and per female, as follows: hatchable egg production (number of hatchable eggs); total weight of hatchable eggs (weight of all the eggs); average weight of hatchable eggs (Olba BV, Coevorden, Holland); number of non-conform eggs (dirty and/or eggshell defects); total weight of hatchable eggs at transfer to the hatcher (corresponding to the ratio between total weight of all eggs and number of eggs), average weight of hatchable eggs at transfer into the incubator (corresponding to the ratio between the average weight of all eggs and the weight of each egg); percentage of non-conform eggs; number of chick and the total and average chick weight; the chick yield (representing the ratio between the weight of chick at hatch as percentage of egg weight at setting); hatchability index (representing the ratio between the number of healthy chicks hatched and the number of fertile eggs set); and the economic index (sum of the weekly chick number and half of the dirty eggs).

2.4. Statistical Analysis

Data recorded during the trial were organized in Microsoft Excel spreadsheet for subsequent statistical analysis. The IBM SPSS Statistics 23.0 (IBM Corp., New York, NY, USA) was used to perform the statistical analysis. Confirmed the principles of normal distribution of data by the Shapiro-Wilk's test, the effect of treatments on each parameter were estimated using ANOVA, and the group means were compared using Tukey's test at 5% probability. To compare the weekly variations of tested parameters among groups, the GLM procedure was used to establish the main effects and interactions of treatment (fixed factor) and time (co-variable). To account for multiple testing, we used the Bonferroni correction and considered significant at $p < 0.05$.

3. Results

Overall, the egg-related parameters, such as the number of hatchable eggs, total and average weight of eggs, non-conformed eggs, total and average weight of eggs at transfer to the hatchery and the egg losses did not differ among experimental treatments (Table 2).

Table 2. Effect of dietary treatments on egg-related parameters.

Item		Hatchable Eggs/Hen (pcs)	Total of Hatchable Eggs Weight/Hen (g)	Hatchable Egg Weight (g)
Vitamin A *	100	21.73	1538.9	70.94
	125	21.21	1518.8	71.73
	150	21.04	1496.4	70.88
	200	21.40	1515.7	70.90
	SEM	2.05	202.1	1.11
<i>p</i> -value		0.972	0.984	0.398
Vitamin E *	100	21.79	1557.1	71.41
	200	20.54	1465.7	71.42
	300	20.74	1466.8	70.56
	400	22.30	1580.2	71.04
	SEM	2.64	211.5	1.33
<i>p</i> -value		0.599	0.959	0.413
Treatment 1 **		21.96	1574.6	71.80
Treatment 2		22.25	1590.0	71.44
Treatment 3		21.61	1556.6	71.81
Treatment 4		21.36	1507.1	70.61
Treatment 5		20.82	1489.6	71.48
Treatment 6		19.36	1402.2	72.41
Treatment 7		23.32	1643.2	70.48
Treatment 8		18.64	1327.9	71.32
Treatment 9		21.14	1493.0	70.77
Treatment 10		22.07	1557.1	70.60
Treatment 11		15.82	1108.5	69.38
Treatment 12		23.93	1708.5	71.49
Treatment 13		23.00	1598.4	69.69
Treatment 14		21.14	1525.9	72.48
Treatment 15		23.39	1677.1	71.83
Treatment 16		21.68	1519.4	70.18
SEM		2.98	174.5	1.25
<i>p</i> -value		0.572	0.532	0.250

* % relative to the standard catalogue; ** See Material and Methods section for the detailed treatments.

At the end of the feeding experiment (week 69), the BW of hens and roosters were similar among treatment (data not shown). During the entire period, the number of hatchable eggs produced was similar among groups ($p = 0.572$), though a numeric difference was recorded (from 15.82 to 23.93 eggs/hen). A similar trend was found in regards to the total and the average weight of hatchable

eggs ($p = 0.532$ and $p = 0.250$, respectively), and the total and average weight of hatchable eggs at the transfer into incubator ($p = 0.156$ and $p = 0.490$, respectively) (Table 3).

Table 3. Effect of dietary treatments on egg-related parameters.

Item	Non-Conform Eggs (<i>n</i>)	Total of Hatchable Eggs Weight at Transfer to Incubator (g)	Hatchable Egg Weight at Transfer to Incubator (g)	Egg Weight Loss (%)	
Vitamin A *	100	1.58	1369.1	63.02	11.11
	125	1.30	1358.0	64.12	10.61
	150	2.00	1338.3	63.38	10.83
	200	1.32	1351.1	63.20	11.08
	SEM	0.56	198.5	1.22	0.47
<i>p</i> -value	0.184	0.242	0.633	0.710	
Vitamin E *	100	1.73	1390.6	63.78	11.01
	200	1.51	1307.0	63.67	10.87
	300	1.51	1303.8	62.69	11.26
	400	1.46	1415.2	63.59	10.49
	SEM	0.74	207.3	1.24	0.50
<i>p</i> -value	0.880	0.201	0.370	0.466	
Treatment 1 **	1.82	1396.6	63.60	11.40	
Treatment 2	1.14	1429.6	64.27	10.18	
Treatment 3	2.86	1395.5	64.42	10.31	
Treatment 4	1.11	1340.8	62.82	12.15	
Treatment 5	0.86	1331.0	63.86	10.66	
Treatment 6	1.36	1242.8	64.18	11.24	
Treatment 7	2.25	1472.0	63.22	10.48	
Treatment 8	1.57	1182.1	63.41	11.08	
Treatment 9	2.07	1321.8	62.56	11.46	
Treatment 10	0.89	1383.1	62.68	11.19	
Treatment 11	1.54	989.7	61.81	11.74	
Treatment 12	1.54	1520.5	63.70	10.66	
Treatment 13	1.57	1427.1	62.08	10.92	
Treatment 14	1.82	1376.7	65.36	9.81	
Treatment 15	1.36	1496.0	64.06	10.80	
Treatment 16	1.07	1360.9	62.86	10.45	
SEM	0.51	202.5	1.01	0.48	
<i>p</i> -value	0.386	0.156	0.490	0.661	

* % relative to the standard catalogue; ** See Material and Methods section for the detailed treatments.

The average egg weight was similar among the groups; through the trial, most of groups presented an egg weight that would allow its grading as large (L; 63–73 g, according to the European Union size grading system). No differences were observed among treatments for the number of non-conform eggs ($p = 0.386$). The worst performance was found when birds were fed basal-diet plus 150% of vitamin A and 300% of vitamin E (group 11), where it the smaller number and lighter eggs were produced, even though the number of non-conform eggs and the percentage of eggs loss were similar compared to the other groups. Hens in groups 7, 12, 13 and 15 laid a higher number of hatchable eggs compared to different groups, showing also a higher total weight of hatchable eggs at transfer into the incubator.

The hatchability was not different among treatments ($p = 0.100$); in particular, treatments 3, 11 and 15 presented lower hatchability values compared to other groups, whereas the birds in treatment 7 showed the higher hatchability. At the end of the feeding period, the chick-related parameters were significantly different by treatment, in terms of number of chicks produced ($p = 0.029$), total and average chick weight ($p = 0.024$ and $p = 0.003$, respectively), and chick yield ($p = 0.013$) per hen. In general, although not significant, supplementing 200% of vitamin A was more appropriate compared to 150%, while no differences were found when comparing the different levels of vitamin E, except for the chick yield and hatchability index that resulted slightly superior when vitamin E supplementation was used at 200%. Birds in treatments 1, 7 and 12 produced the higher number of chicks per hen, as well as a higher number of total chicks weight (Table 4), where treatment 12 had a higher value of chick weight.

Table 4. Effect of dietary treatments on chick-related parameters.

Item	Number of Chicks (n)	Total Chicks Weight (g)	Average Chick Weight (g)	Chick Yield (%)	
Vitamin A *	100	14.56	695.69 ab	47.80 ab	67.39 b
	125	12.83	615.62 ab	47.64 ab	66.51 ab
	150	11.61	537.57 a	45.18 a	63.27 a
	200	14.80	712.1 b	48.11 b	67.09 b
	SEM	4.12	133.2	2.02	2.18
<i>p</i> -value	0.120	0.019	0.014	0.037	
Vitamin E *	100	13.55	645.36	46.99	65.72 a
	200	14.84	712.02	48.12	67.54 b
	300	12.18	579.12	46.71	65.09 a
	400	13.24	624.54	46.91	65.91 a
	SEM	3.95	174.2	2.13	2.05
<i>p</i> -value	0.443	0.559	0.391	0.028	
Treatment 1 **	16.71 ab	813.78 ab	48.85 ab	68.00 ab	
Treatment 2	12.39 ab	597.15 ab	48.18 ab	67.78 ab	
Treatment 3	9.79 a	437.64 ab	43.08 a	60.08 a	
Treatment 4	15.32 ab	732.88 ab	47.84 ab	67.02 ab	
Treatment 5	14.57 ab	711.23 ab	48.91 ab	68.45 ab	
Treatment 6	13.71 ab	671.00 ab	48.96 ab	67.99 ab	
Treatment 7	18.61 b	880.65 a	47.47 ab	67.35 ab	
Treatment 8	12.46 ab	585.20 ab	47.14 ab	66.37 ab	
Treatment 9	11.71 ab	541.59 ab	46.27 ab	65.30 ab	
Treatment 10	13.50 ab	654.74 ab	48.46 ab	68.64 b	
Treatment 11	7.07 a	311.55 b	43.00 a	59.95 a	
Treatment 12	16.43 ab	808.61 ab	49.11 b	66.47 ab	
Treatment 13	15.25 ab	716.17 ab	47.17 ab	67.78 ab	
Treatment 14	11.71 ab	539.61 ab	44.97 ab	61.64 ab	
Treatment 15	11.00 ab	520.44 ab	47.16 ab	65.71 ab	
Treatment 16	15.00 ab	721.92 ab	48.34 ab	68.51 b	
SEM	3.87	147.8	1.58	1.79	
<i>p</i> -value	0.029	0.024	0.003	0.013	

* % relative to the standard catalogue; a,b: Values within the same column followed by different letters differ significantly ($p < 0.05$). ** See Material and Methods section for the detailed treatments.

In contrast, treatments 11 presented the lowest values for the number of chicks produced per hen as well as for total and average chick weight (Table 4). Considering the economic index for the entire experimental period, no significant differences were found among groups ($p = 0.530$), even though the poorest indices were detected in treatment 11, whereas treatment 7 had the best economic index (Table 5).

Table 5. Effect of dietary treatments on chick-related parameters.

Item	Hatchability(%)	Female Weight Gain (g/d)	Male Weight Gain (g/d)	EconomicalIndex	
Vitamin A *	100	67.44	0.10	15.35	
	125	60.68	0.09	13.48	
	150	55.78	0.10	12.62	
	200	69.89	0.10	15.40	
	SEM	9.55	0.03	0.09	3.77
<i>p</i> -value	0.251	0.915	0.695	0.991	
Vitamin E *	100	62.74 b	0.10	0.24	14.42
	200	73.02 a	0.11	0.24	15.59
	300	59.74 b	0.11	0.30	12.93
	400	58.29 b	0.08	0.26	13.97
	SEM	14.51	0.02	0.08	3.66
<i>p</i> -value	0.479	0.091	0.556	0.540	

Table 5. Cont.

Item	Hatchability(%)	Female Weight Gain (g/d)	Male Weight Gain (g/d)	EconomicalIndex
Treatment 1 **	75.40	0.09	0.19	17.63
Treatment 2	57.56	0.09	0.25	12.96
Treatment 3	47.41	0.11	0.28	11.21
Treatment 4	70.59	0.10	0.24	15.88
Treatment 5	72.64	0.10	0.25	15.00
Treatment 6	71.21	0.10	0.20	14.39
Treatment 7	79.41	0.11	0.28	19.73
Treatment 8	68.82	0.12	0.25	13.25
Treatment 9	58.79	0.11	0.38	12.75
Treatment 10	59.84	0.12	0.30	13.95
Treatment 11	47.96	0.09	0.25	7.84
Treatment 12	72.38	0.10	0.29	17.20
Treatment 13	62.94	0.09	0.38	16.04
Treatment 14	54.11	0.07	0.25	12.63
Treatment 15	48.34	0.09	0.18	11.68
Treatment 16	67.78	0.09	0.23	15.54
SEM	9.25	0.03	0.10	2.59
p-value	0.100	0.845	0.884	0.530

* % relative to the standard catalogue; a,b: Values within the same column followed by different letters differ significantly ($p < 0.05$). ** See Material and Methods section for the detailed treatments. Hatchability = number of healthy chicks from fertile eggs.

4. Discussion

This trial described the effect of a nine weeks period of dietary supplementation with supra-nutritional levels of vitamin A and E in broiler breeders during the late production cycle, when fertility traits tend to decline. Both vitamins A and E are considered important factors related to the maintenance of fertility in mammals as well as in birds [11,23,32,33]. At the beginning of the experiment, hens in all groups showed similar laying ability even though the number of produced eggs was smaller than those proposed by Aviagen [34] for performance objectives of Ross-308 breeders (≤ 2 eggs/bird/week for the majority of the experimental groups vs. 3.60 eggs/bird/week for birds aged of 61 weeks). Similarly, the hatchability index and the number of chicks resulted below to the expected values for the present breed and age ($< 80\%$ in all the groups vs. 85.6% and < 1.6 vs. 2.53, respectively, for hatchability and number of chicks, as estimated by Aviagen for 61-wk old Ross-308 hens); however, the individual egg weight was closed to the expected values [34]. These results supported the previous findings reported by Zaghari et al. [13] testing different levels of vitamin E dietary supplementation in Ross-308 breeders between 62 and 72 week of age.

Vitamin A has a beneficial effect on the integrity of epithelia of the male and female reproductive tract, and it integrates an important signaling pathway in gametogenesis [35]. Further, vitamin E plays a major role in protecting the cell against oxidative damage [33], and therefore it is essential for male and female fertility. When associated, despite their different roles, vitamins A and E may act together to promote fertility in broiler breeders. Conversely to a previous study showing that dietary supplementation of laying hens with levels of vitamin A above the National Research Council [36] recommendations had no effect on egg production [37,38], our study showed that it was possible to maintain or improve the egg production of hens under standard rearing conditions. Moreover, Yuan et al. [23] failed to find any beneficial effect in using high levels of vitamin A in diet on the reproductive performance of broiler breeder hens. However, it is worth remembering that the mentioned previous studies used longer periods of supplementation covering the entire laying period, whilst in the current study were used hens at the end of the production phase.

Insufficient levels of vitamin A are often accompanied by increased atresia [39] and the ovulation of degenerated eggs, and also with an abnormal development of the embryo [40]. On the other hand, excessive vitamin A intake leads to a decrease in egg production and egg, as well as in an increase of congenital abnormalities in embryos [23], and under-supply of vitamin E supplementation is related to

a decrease of fertile eggs and egg hatchability. In the present study, the average egg weight remained relatively unchanged through the experiment. Conversely, Iqbal et al. [41] showed in Cobb broiler breeders, that large eggs present lower rate of egg mass loss at different incubation time, even though an acceptable hatchability of fertile eggs, and a slightly increase in the number of culled chicks, which could be negatively affected by the age effect in breeders older than 60 weeks.

In the case of imbalance of vitamins A or E, the study of the productive traits per se may not allow to distinguish between an excess or insufficiency supplementation. Nevertheless, imbalance in vitamins A and E was not found in the current study for most of groups, even though the tested combinations were not equally successful on the hens productivity or fertility. The exception was the group fed basal-diet plus 150% of vitamin A and 300% of vitamin E, which showed the worst laying results.

The more notorious differences between groups were observed in chick-related parameters, in particular for the number of chicks produced. Only in birds fed basal-diet plus 150% of vitamin A and 200% of vitamin E an increase of 2 units in number of birds/hen was observed. This reflected on the total and average chick weight, which showed similar variations among groups. This is in contrast with the results reported by Hossain et al. [16], who found no influence of different levels of vitamin E supplementation on chick weight and yield in laying breeders. Additionally, the final chick yield was affected by treatment. An optimum chick yield (67–69%) was obtained for most of groups, but it was low (below 62%) for chicks in groups 3, 11 and 14, which may interfere with chick transfer to a farm and its survival. Considering that the hatching conditions were identical for all groups, the differences should be related to the combination of vitamins A and E. When comparing the different levels of vitamin A supplementation, birds supplemented with 150% above the recommended levels showed lower chick yield, while no difference was found for vitamin E supplementation, the latter agreeing with previous reports by Hossain et al. [16] and Urso et al. [26].

5. Conclusions

In conclusion, the present study showed that a combination of dietary supra-nutritional levels of vitamins A and E allowed to extend the laying period and to reduce the decline of fertility in older breeder hens at the end of the laying stage, with acceptable productive and fertility parameters; in particular, among the tested treatments, feeding of supra-nutritional level of 100% vitamin A and 200% vitamin E lead to the best results. Thus, supplementing diet with a combination of supra-nutritional levels of vitamins A and E could be used to positively manipulate egg and chick production in older broiler breeders.

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