Ciência Rural, Santa Maria, v.45, n.11, p.2050-2055, nov, 2015 http://dx.doi.org/10.1590/0103-8478cr20140564
ISSN 0103-8478 ANIMAL PRODUCTION

Inclusion of canthaxanthin and 25-hydroxycholecalciferol in the diet of broiler breeders on performance and incubation parameters

Inclusão de cantaxantina e 25-hidroxicolecalciferol na dieta de matrizes de corte sobre desempenho e parâmetros de incubação

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ABSTRACT

The objective of this experiment was to evaluate the effects of a diet containing canthaxanthin and 25-hydroxycholecalciferol (25-OH-D3) on the production and reproductive performances of Cobb 500 broiler breeders aged 53 to 61 weeks. The study included 36,000 Cobb 500 female and 3,600 male broiler breeders aging 51 weeks. The birds were housed in three poultry houses divided into four plots, totaling 12 experimental units each containing 3,000 females and 300 males. The birds received the following treatments: control diet (without the studied additives) or the control diet with the addition of 60mg kg^{-1} feed of 25-(OH)-D3 and canthaxanthin feed (6mg of canthaxanthin and 2,760,000IU of 25-(OH)-D3 per kg of feed) per the supplier's recommendations. The experimental design used was a randomized complete block design with two treatments and six replicates. The results were subjected to analysis of variance followed by the F-test. The treatments had no effect on egg production, usability of eggs and number of incubatable eggs per bird housed. The inclusion of canthaxanthin and 25-(OH)-D3 in the diet reduced embryonic mortality while increasing egg yolk pigmentation, hatching percentage, and number of viable chicks produced per bird. Therefore, the use of 60mg kg^{-1} of 25-(OH)-D3 plus canthaxanthin is recommended in the diet of Cobb 500 broiler breeders aging 53 to 61 weeks to improve important reproductive traits that have great economic impacts on poultry farms.

Key words: *carotenoids, vitamin D, broiler breeders, hatching, egg production.*

RESUMO

O objetivo deste experimento foi avaliar dietas contendo Cantaxantina e 25-hidroxicolecalciferol (25-OH-D3) sobre o desempenho produtivo e reprodutivo de matrizes de corte da linhagem Cobb 500, de 53 até 61 semanas de idade. Foram utilizadas 36.000 fêmeas e 3.600 machos com 51 semanas de idade, reprodutores de corte Cobb 500. As aves foram alojadas em três aviários divididos em 4 parcelas, totalizando 12 unidades experimentais, cada uma contendo 3.000 fêmeas e 300 machos. As aves foram submetidas aos seguintes tratamentos: dieta controle (sem os aditivos em estudo) ou dieta com adição de 60mg kg^{-1} de ração de 25-(OH)-D3 e Cantaxantina (6mg de cantaxantina e 2.760.000UI de 25-(OH)-D3 por kg de ração), conforme recomendações do fornecedor. O delineamento experimental utilizado foi em blocos casualizados, com dois tratamentos e seis repetições. Os resultados foram submetidos à análise de variância e posteriormente aplicou-se o teste F. Não houve efeito dos tratamentos sobre as variáveis produção de ovos, aproveitamento de ovos e número de ovos incubáveis por ave alojada. A inclusão de Cantaxantina e de 25-(OH)-D3 na dieta reduziu a mortalidade embrionária e aumentou a pigmentação da gema dos ovos, o percentual de eclosão e o número de pintos viáveis produzidos por ave. Portanto, recomenda-se a utilização de 60mg kg^{-1} de 25-(OH)-D3 mais Cantaxantina na dieta de matrizes de corte da linhagem Cobb 500, de 53 a 61 semanas de idade, para melhorar importantes características reprodutivas que têm grande impacto econômico para as empresas avícolas.

Palavras-chave: *carotenoides, vitamina D, matrizes pesadas, eclosão, produção de ovos.*

INTRODUCTION

To maximize the profitability of the broiler breeder production chain, it is necessary to increase egg production and hatching rates to obtain more viable offspring. The inclusion of substances with antioxidant properties in the diet of broiler breeders aids the enzymatic defense system in controlling the damage caused by free radicals in cells.

Carotenoids are an example of this type of substance because they have antioxidant, pigmenting, provitaminic and immunomodulatory activities

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Received 04.12.14 Approved 04.04.15 Returned by the author 07.16.15
CR-2014-0564.R2

(SURAI et al., 2003). Carotenoids play an important antioxidant role because they remove free radicals, absorb and dissipate the excess energy and recycle vitamin E (ROCHA et al., 2011). Studies have shown that canthaxanthin can help effectively reduce lipid peroxidation in various tissues and in avian embryos (SURAI et al., 2003). When present in the egg, canthaxanthin is transferred to the embryo and can protect it against oxidative damage during the incubation and post-birth periods (KARADAS et al., 2005).

The peroxidation of lipids stored in fertile egg yolk results in reduced available energy for embryonic development and in the formation of toxic compounds, resulting in increased embryonic mortality and consequently decreased egg hatchability. Embryonic tissues also have high concentrations of polyunsaturated fatty acids (PUFAs); therefore, they too are subject to peroxidation (CHERIAN et al., 2007).

The effect of canthaxanthin on fertility may be due to the antioxidant effect of this carotenoid on both the hen and the rooster. The improvement in fertility on the part of the rooster may be due to two factors: antioxidant protection of the spermatozoa and an increase in vitamin A. The increase in fertility in hens may be caused by improvement of the antioxidant mechanism of the sperm storage glands (RUTZ et al., 2007).

The supplementation of vitamin D3 in the form of its 25-(OH)-D3 metabolite may be related to improvements in birds such as skeletal growth, laying rate, shell quality and reproduction, as this vitamin is involved in the metabolism of calcium and phosphorus. The embryo also metabolizes vitamin D3 present in the egg yolk, using it for skeletal formation (TORRES et al., 2009; ROSA et al., 2012).

In this context, the present study aimed to evaluate the effects of diets containing canthaxanthin and 25-OH-D3 on the production and reproductive performances of broiler breeders aged 51 to 63 weeks.

MATERIALS AND METHODS

Facilities

The experiment was conducted at the BRF-Brasil Foods S/A poultry breeding farm and hatchery, in the city of Rio Verde, located in the southwest region of the state of Goiás, Brazil. The trial took place between October and January and encompassed the productive period of the breeders from 51 to 61 weeks of age.

The study included 36,000 female and 3,600 male Cobb breeders aged 51 weeks. The birds were housed in three poultry houses divided into four plots, totaling 12 experimental units that each contained 3,000 females and 300 males. The poultry houses used negative pressure ventilation and were equipped with Isotelha® roofing, pad-cooling, automatic nests, automatic channel feeders, and drinking nipples.

The environmental conditions for bird raising were determined according to the Breeder Management Manual (COBB, 2008). When the temperature reached the upper limit, evaporation plates and exhausts were triggered, keeping the birds in the thermal neutral zone.

To determine the reproductive parameters, the eggs were incubated in multi-stage machines, and the environmental conditions of the machines were determined by the breed's incubation manual. During incubation, the eggs were kept at a temperature of 37.4ºC and 55% humidity in the incubators and at 36.9ºC and 60% humidity in the hatchers.

Treatments

The birds received the following treatments: control diet or the standard diet with the addition of 25-(OH)-D3 and canthaxanthin, by including the product ROVIMIX® MaxiChick (DSM Nutritional Products Ltd) in the diet at a dose of 60mg kg^{-1} (6mg of canthaxanthin and 2,760,000IU of 25-(OH)-D3 per kg of feed). The diet of the experimental period, from the 51st to the 61st week of age, was labeled Laying II (Table 1). The bird's nutritional and energy requirements were determined by the recommendation of Rostagno et al. (2005).

Variables assessed

The production variables analyzed were laying rate, percentage of usable eggs, egg yolk pigmentation and number of incubatable eggs per bird housed. The reproductive variables analyzed were hatching, hatchability, fertility, embryonic mortality and number of viable chicks per bird housed.

The eggs produced were selected and classified into incubatable eggs and non-incubatable eggs. The incubatable eggs were those in perfect conditions for incubation. The non-incubatable eggs were those eggs with shell deformations, thin shells, irregular calcium deposits, and those that were long, rounded, or cracked.

The usability of the eggs was determined by the percentage of incubatable eggs in relation to the total number of eggs produced. The determination of egg yolk pigmentation was made with the help of a DSM® color fan, using pigmentation scores from 1 to 15, which fall between opaque yellow and intense orange. The greater the value was that was obtained from the fan, the greater the degree of yolk

Table 1 - Proximate composition and nutritional profile of the diets of females and males.

Ingredients	Laying I	Laying II
Corn	68.22	68.52
Soybean Meal	21.22	21.57
Wheat Bran	14.50	0.11
Dicalcium Phosphate	1.90	1.64
Limestone	6.26	7.21
Salt	0.40	0.40
Vitamin and Mineral Premix[1]	0.50	0.50
DL-methionine	0.004	0.004
---------- Calculated Composition ----------		
Metabolizable Energy (kcal/kg)	2860	2850
Crude Protein (%)	16.00	15.96
Arginine Total (%)	0.87	0.94
Lysine Total (%)	0.75	0.80
Methionine Total (%)	0.46	0.35
Methionine + Cystine (%)	0.72	0.55
Threonine Total (%)	0.51	0.59
Tryptophan Total (%)	0.17	0.17
Isoleucine Total (%)	0.68	0.63
Leucine Total (%)	1.48	1.46
Valine Total (%)	0.70	0.72
Histidine Total (%)	0.45	0.42
Phenylalanine Total (%)	0.79	0.77
Calcium (%)	3.00	3.30
Available Phosphorus (%)	0.45	0.40

1 – Vitamin and Mineral Premix: Levels per kg of product: Vit. A 2,090,000IU; Vit. E 7,600mg; Vit. D3 332,500IU; Vit. K3 950mg; Nicotinic Acid 8,500mg; Vit. B1 475mg; Vit. B12 1,900mg; Vit. B6 950mg; Folic Acid 237.5mg; Biotin 38mg; Choline 72,000mg; Pantothenic Acid 3,800mg; Copper 12,400mg; Iron 12,000mg; Iodine 160mg; Manganese 14,000mg; Selenium 108mg and Zinc 14,000mg.

pigmentation. To assess the pigmentation, 100 eggs from each treatment were evaluated weekly.

To evaluate the reproductive variables, 20,640 eggs were incubated weekly per treatment group. After being counted, the hatched chicks were classified according to the quality of their umbilical scarring, legs and beaks. Each week, incubation residues were analyzed to determine the fertility and hatchability percentages and the phase of embryonic mortality through embryo diagnosis.

Statistical analysis

The experimental design used was a randomized complete block design, with each of the three poultry houses considered one block, with two treatments, and six replicates of 3,000 females and 300 males each. To evaluate the reproductive parameters, 20,640 eggs were incubated weekly per treatment, equating to six replications of 3,440 eggs per treatment. To evaluate the pigmentation of egg yolks, 100 eggs from each treatment group were analyzed weekly. For the statistical analysis, weeks 51 and 52 were considered periods of adaptation of the birds to the diet; therefore, they were not considered in the data analysis. For the analysis, the data were grouped every three weeks, consisting of weeks 53 to 55, 56 to 58, and 59 to 61, as well as the total period from 53 to 61 weeks. The results underwent analysis of variance using the program SISVAR 5.3 (FERREIRA, 2010), and the F-test was applied at a significance level of 5% probability to determine the significant differences between the parameters ($P \leq 0.05$).

RESULTS AND DISCUSSION

Production performance of broiler breeders

No treatment effects were observed on the variables egg production percentage, egg usability percentage, and number of incubatable eggs per bird housed (Table 2). Similarly, ZHANG et al. (2011) fed breeders a basic diet and a basic diet supplemented with 6 mg of canthaxanthin/kg over 24 weeks. The authors found no effect of the treatment on laying rate, number of eggs produced per hen, or egg weight.

GARCIA et al. (2002) evaluated the inclusion of different amounts of canthaxanthin (0, 12, 24, 36, 48 and 60mg kg^{-1} of feed) in the diet of laying hens and concluded that the inclusion of canthaxanthin in the diet did not affect production parameters, except for the pigmentation of egg yolks. TORRES et al. (2009) supplemented breeders with 25-(OH)-D3 and observed no effect on egg production during the period of 32-67 weeks. However, egg shell quality, measured by specific gravity, improved with 25-(OH)-D3 supplementation in breeders aged 60 weeks.

In the present study, the addition of canthaxanthin and 25-(OH)-D3 also improved the coloration of the yolk through increased deposition of pigment in the egg yolk (Table 2).

Reproductive performance of broiler breeders

There was an observed effect of the treatments on hatching percentage but not on the fertility or hatchability of the eggs. For hatching percentage, when evaluating the total period from 53 to 61 weeks of age, the inclusion of MaxiChick increased the hatching percentage by 2.59% (Table 3).

In their experiments, SOUZA et al. (2008) and SCHER et al. (2009) added 6mg/kg of canthaxanthin to the diet of broiler breeders and observed a reduction in the number of infertile eggs and embryonic mortality as well as an increase in hatching rates. ROCHA (2011) concluded that the

Table 2 - Production performance of broiler breeders aged 53 to 61 weeks receiving diets containing canthaxanthin and 25-(OH)-D3.

Treatments	Production Period (Weeks)			
	53 - 55	56 - 58	59 - 61	53 - 61
Egg Production (%)				
MaxiChick[1]	63.18	60.41	57.60	60.40
Control	63.56	60.35	55.94	59.95
Pr > Fc	0.5567	0.8811	0.0643	0.6844
CV (%)	1.73	1.18	2.36	5.41
Usability of Eggs (%)				
MaxiChick[1]	98.25	97.92	97.75	97.98
Control	98.20	97.90	97.64	97.91
Pr > Fc	0.6269	0.8185	0.0992	0.5183
CV (%)	0.18	0.15	0.11	0.29
Number of Incubatable Eggs/Bird/Week				
MaxiChick[1]	4.35	4.14	3.94	4.14
Control	4.37	4.14	3.82	4.11
Pr > Fc	0.6003	0.8598	0.0606	0.6712
CV (%)	1.77	1.19	2.42	5.61
Egg Yolk Pigmentation (Colorimetric Evaluation)				
MaxiChick[1]	14.50	13.83	14.83	14.39
Control	8.83	8.17	7.83	8.28
Pr > Fc	0.0023	<0.0001	<0.0001	<0.0001
CV (%)	19.21	6.56	4.93	12.55

[1]Canthaxanthin 10% + 25-(OH)-D3.

addition of canthaxanthin to the diet of breeders increased the concentration of canthaxanthin and vitamin A and reduced the concentration of vitamin E in the egg yolk, improved fertility and hatching, and reduced embryonic mortality occurring within 15 days of incubation.

With respect to fertility, no significant differences were found between the treatment groups

Table 3 - Reproductive performance of broiler breeders aged 53 to 61 weeks receiving diets containing canthaxanthin and 25-(OH)-D3.

Treatments	Production period (Weeks)			
	53 - 55	56 - 58	59 - 61	53 – 61
Hatching (%)				
MaxiChick[1]	86.63	85.40	86.82	86.28
Control	82.49	85.21	84.59	84.10
Pr > Fc	0.0937	0.8786	0.2656	0.0315
CV (%)	4.45	2.49	3.76	3.42
Hatchability (%)				
MaxiChick[1]	91.33	90.60	93.47	91.80
Control	87.96	90.30	91.41	89.89
Pr > Fc	0.2511	0.8370	0.3175	0.1184
CV (%)	5.25	2.76	3.62	3.93
Fertility (%)				
MaxiChick[1]	94.77	94.32	92.38	93.82
Control	93.93	93.25	92.60	93.26
Pr > Fc	0.4387	0.1510	0.6439	0.2961
CV (%)	1.89	1.24	0.88	1.69

[1]Canthaxanthin 10% + 25-(OH)-D3.

in the present study. These data are in line with those obtained by ZHANG et al. (2011), who found no effect of canthaxanthin on the fertility and hatching of fertile eggs from heavy breeders.

However, other studies show effects of canthaxanthin on the fertility of eggs. ROCHA et al. (2011) added 6mg kg^{-1} of canthaxanthin to the diet of Cobb 500 roosters aged 46 to 60 weeks. The addition of canthaxanthin to the diet minimized the adverse effects of aging on the fertility of the roosters studied. ROSA et al. (2012) found that the addition of canthaxanthin improves fertility and decreases embryonic mortality, resulting in increased hatchability. This effect has been attributed to reduced oxidant formation during the early stages of incubation and in stored eggs.

Similar to previous studies, the percentage of embryonic mortality was affected by the treatments in the present study. It was found that eggs originating from breeders supplemented with canthaxanthin and 25-(OH)-D3 showed lower embryonic mortality during the initial and final phases of incubation (Table 4).

The reduction in mortality in the storage and initial incubation phases is related to reduced lipid peroxidation of egg components and of the embryo (ROSA et al., 2012). In the final stages of incubation, however, the increase in the metabolism of the embryo leads to higher oxygen consumption and increased lipid oxidation in the yolk to meet the energy requirements of the embryo (LATOUR et al., 2000).

TORRES et al. (2009) found lower mortality of embryos in breeders supplemented with 25-(OH)-D3. According to the authors, the decrease in embryonic mortality may be related to the increased availability of calcium for the formation of the chick skeleton.

Analyzing the number of chicks per bird housed (Table 5), there was a significant difference between the treatment groups in the 59 to 61 week period. Over the total experimental period, a difference was observed only at 10% probability (P=0.0928). This variable has a high financial impact for the

Table 4 - Effect of canthaxanthin and 25-(OH)-D3 on embryonic mortality (EM) at different incubation stages.

Treatments	Production Period (Weeks)			
	53 - 55	56 - 58	59 - 61	53 - 61
	EM 0-4 d[2] (%)			
MaxiChick[1]	1.98	2.18	2.51	2.23
Control	4.50	3.17	2.58	3.42
Pr > Fc	0.0198	0.0094	0.9073	0.0078
CV (%)	21.03	8.59	17.02	20.25
	EM 5-10 d[3] (%)			
MaxiChick[1]	1.19	1.12	0.79	1.04
Control	1.06	0.79	1.06	0.97
Pr > Fc	0.7577	0.5783	0.4655	0.7885
CV (%)	29.01	46.86	29.35	33.24
	EM 11-17 d[4] (%)			
MaxiChick[1]	0.99	0.86	0.99	0.95
Control	0.60	0.86	0.93	0.79
Pr > Fc	0.0497	0.9976	0.8365	0.3881
CV (%)	17.05	32.33	25.47	27.61
	EM 18-21 d[4] (%)			
MaxiChick[1]	2.25	2.58	2.12	2.31
Control	4.96	3.57	3.51	4.01
Pr > Fc	0.0034	0.2203	0.0187	0.0002
CV (%)	14.39	17.74	13.23	17.32
	Total Embryonic Mortality (%)			
MaxiChick[1]	6.42	6.75	6.42	6.53
Control	11.11	8.40	8.07	9.19
Pr > Fc	0.0083	0.0751	0.1725	0.0004
CV (%)	12.13	8.41	12.00	11.68

[1]Canthaxanthin 10% + 25-(OH)-D3. [2]Embryonic mortality between 0 and 4 days of incubation. [3]Embryonic mortality between 5 and 10 days of incubation. [4]Embryonic mortality between 11 and 17 days of incubation. [5]Embryonic mortality between 18 and 21 days of incubation.

Table 5 - Effect of canthaxanthin and 25-(OH)-D3 on the number of live chicks produced per bird/week.

Treatments	Production Period (Weeks)			
	53 - 55	56 - 58	59 – 61	53 - 61
	Number of chicks/Bird/Week			
MaxiChick[1]	3.76	3.54	3.42	3.57
Control	3.60	3.52	3.23	3.45
Pr > Fc	0.1100	0.8459	0.0382	0.0928
CV (%)	4.14	2.99	3.90	5.85

[1]Canthaxanthin 10% + 25-(OH)-D3.

poultry segment, and the effect of canthaxanthin and 25-(OH)-D3, even at 10% probability, is interesting for companies in this sector.

CONCLUSION

The use of 60mg kg^{-1} of MaxiChick in the diet of Cobb 500 broiler breeders aged 53 to 61 weeks is recommended to improve important reproductive characteristics that can have a significant economic impact on poultry farms.

BIOETHICS AND BIOSSECURITY COMMITTEE APPROVAL

This study was approved by the Research Ethics Committee of the Instituto Federal de Educação, Ciência e Tecnologia Goiano on 10/09/2012, under the protocol number 24/2012.

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