

USE OF CANTHAXANTHIN AND/OR 25-OH D3 FOR IMPROVED HATCHABILITY IN POULTRY

FIELD OF THE INVENTION

- 5 The present invention relates to the use of canthaxanthin and/or at least one vitamin D metabolite, preferably 25-hydroxy vitamin D3 (25-OH D3), for improving breeder hatchability. More particularly the invention relates to the use of canthaxanthin and/or 25-hydroxy vitamin D3 in the manufacture of a feed or veterinary composition for improving hatchability in poultry.

BACKGROUND

- 10 To maximize the fertility of the egg and the hatchability of the embryo during the breeding phase, optimal nutritional status of breeders is essential for the effective transfer of nutrients to the embryo.

In accordance with the present invention it has been found that problems in chicken breeding can be eliminated or substantially ameliorated by administering to the animals an effective

- 15 amount of Canthaxanthin or 25-OH-D3, optionally a combination of both nutrients.

Applicant now surprisingly found that relatively high concentrations of essential nutrients in the embryo such as canthaxanthin or 25-OH-D3 supplied via maternal supplementation are related to improved hatchability, fertility and lower embryo mortality during the first phase of embryo development.

- 20 Tritsch *et al.* (US 2003/0170324) disclose a feed premix composition of at least 25-OH D3 in an amount between 5% and 50% (wt/wt) dissolved in oil and an antioxidant, an agent encapsulating droplets of 25-OH D3 and oil, and a nutritional additive (e.g., Vitamin D3). The premix may be added to poultry, swine, canine, or feline food. This composition stabilizes 25-OH D3 against oxidation.
- 25 Simoes-Nunes *et al.* (US 2005/0064018) discloses adding a combination of 25-OH Vitamin D3 and Vitamin D3 to animal feed. In particular, about 10 µg/kg to about 100µg/kg of 25-OH Vitamin D3 and about 200 IU/kg to about 4,000 IU/kg of Vitamin D3 are added to swine feed. This addition improves the pig's bone strength.

Stark *et al.* (US 5,695,794) disclose adding a combination of 25-OH Vitamin D3 and Vitamin D3 to poultry feed to ameliorate the effects of tibial dyschondroplasia.

Borenstein et al US 5,043,170 discloses the combination of Vitamin D3 and either 1-alpha-hydroxycholecalciferol or 1alpha, 25-dihydroxycholecalciferol to improve egg strength and leg strength in laying hens and older hens.

Fleshner-Barak (WO 03/007916) discloses administration of bisphosphonate compound and natural vitamin D derivative such as 1,25-dihydroxyvitamin D3 or 24,25-dihydroxyvitamin D3, or 25-OH vitamin D3.

Daifotis *et al.* (WO 03/086415) disclose inhibiting bone resorption by a combination of at least one bisphosphonate compound and from about 100 IU to about 60,000 IU of a no activated metabolite of vitamin D2 and/or vitamin D3.

The aforementioned documents did not teach or suggest that the use of canthaxanthin and 25-OH D3 or a combination thereof would be surprisingly beneficial to improve hatchability.

DETAILED DESCRIPTION OF THE INVENTION

As used throughout the specification and claims, the following definitions apply:

"Vitamin D metabolite" means any metabolite of Vitamin D as for example 25-hydroxy vitamin D3, 1,25-dihydroxy vitamin D3 or 24,25-dihydroxy vitamin D3.

"25-OH D3" refers specifically to 25-hydroxy vitamin D3.

"Poultry" is meant to include turkeys, ducks and chickens (including but not limited to broiler chicks, layers, breeders).

Canthaxanthin and 25-OH D3 may be obtained from any source, and a composition thereof may be prepared using convenient technology.

In a first aspect, one or more feed compositions suitable for poultry use are provided to administer canthaxanthin or 25-OH D3 and combinations thereof as nutrients to improve hatchability, fertility and lower embryo mortality during the first phase of embryo development.

In a second aspect, a poultry feed is provided which comprises from about 10 µg/kg to about 100 µg/kg of 25-OH D3 and/or from about 2 to 100 ppm canthaxanthin, preferably 2 to 10 ppm.

In another aspect, a method of administering canthaxanthin and/or 25-OH D3 to poultry breeders is provided to improve hatchability, fertility and lower embryo mortality during the first phase of embryo development.

The method for improving hatchability in poultry comprises administering to the animal in need of such treatment an amount of about 2 ppm to 100 ppm of canthaxanthin, preferably 2 to 10 ppm, and/or about 10 µg/kg to about 100 µg/kg of 25-OH D3.

5 In another aspect, a premix composition for poultry feed comprising 25-hydroxy vitamin D3 and canthaxanthin is provided.

Canthaxanthin and 25-hydroxy vitamin D3 are suitably administered together with the food. The term food as used herein comprises both solid and liquid food as well as drinking fluids such as drinking water. Particularly, inventive ingredients can be added as a formulated powder to a premix containing other minerals, vitamins, amino acids and trace elements which is added to
10 regular animal food and thorough mixing to achieve even distribution therein.

In the manufacture of poultry feed in accordance with the invention, from about 2 ppm to 100 ppm, preferably 2 – 10 ppm of canthaxanthin and, if required, from about 10µ/kg to about 100µg/kg of 25-hydroxy vitamin D3 are added to regular poultry food. Alternatively, a food premix may be prepared on the basis of regular food components by adding these active
15 ingredients to such food components in higher concentration.

According to the present invention the canthaxanthin compound is available under the Trademark ROVIMIX® Hy-D® 1.25 % and canthaxanthin under the Trademark CAROPHYLL®Red.

20 According to the present invention it is further advantageous if the composition also contains one or more of the following ingredients: Vitamin A, Vitamin E, Biotin, copper (e.g. as CuSO₄), zinc (e.g. as ZnSO₄), cobalt (e.g. as CoSO₄), selenium (e.g. as Na₂SeO₃), iodine (e.g. as KI), manganese (e.g. as MnSO₄) and/or calcium (e.g. as CaSO₄).

The following non-limiting Examples are presented to better illustrate the invention.

25 **Example 1: Effect of Carophyll Red (Canthaxanthin) on the productive and reproductive development of broiler chickens**

Material & Methods

In this study 360 females and 36 male broiler chickens were used, all 45 weeks of age, and of Cobb 500 lineage. The birds were housed together in their respective treatment groups according to body weight and the uniformity of the batch.

30 Pre-experiment phase – 37th to 45th week:

In the pre-experiment phase, the birds underwent the recommended handling and feeding practices in the breeders' guide. To assess the fertility of the birds in each box, an incubation session was carried out for one week, and using embryo diagnostics on the eggs that did not hatch, the percentage of fertility in each box was determined. The level of fertility was taken into

consideration when distributing the treatments to each of the boxes, so that all treatments had the same level of fertility at the beginning of the study.

Period of Experimentation – 46th to 66th weeks:

- At the start of this period, all the birds were weighed and this was repeated every 28 days during the period that the birds received the treatment (Table 1). During the experiment, information was collected on the daily production of eggs. Sample of the birds were weighed on a weekly basis.

Feeding

- The diet given to the birds was a standard feed for broiler chickens with the addition of the products that were tested. The feed met all nutritional requirements in relation to the developmental stage of the birds and the recommendations in the breeders' guide. The feed was entirely vegetable-based, using corn and Soya bran (Appendices 1, 2 and 3).

- TABLE 1. Treatments used in the experiment on broiler chickens (lineage - Cobb 500) for a period of six months.

Treatments	Carophyll Red (ppm)
1	0
2	60

Experimental Design

The experimental design was entirely random, with two treatments and six groups of 30 female and 3 male chickens.

20 Methodology

- The laying rate was calculated weekly. To assess the weight of the eggs, specific gravity, average weight of eggs, yolk weight, albumen weight and the coloration of the yolk, all the eggs that were not considered suitable for incubation, collected on any given day, were used. Specific gravity was determined through the emersion of the eggs in saline solutions with densities of 1065; 1070; 1075; 1080; 1085; 1090 and 1095. The weighing of the eggs, yolks and albumen were carried out using a precision weighing scale (0.001g). The coloration of the yolks was determined using the color fan from DSM Nutritional Products®.

- To evaluate hatching, hatchability, fertility and embryonic mortality the eggs were collected daily. They were then classified and marked with the number of the corresponding box. Those that were not considered suitable for incubation were stored for a maximum period of seven

days in an air-conditioned room with temperature and humidity control. Incubation was carried out in a multi-stage incubator and on day 18, the eggs were transferred to a brooder. On day 21 the chicks were taken out of the brooder, vaccinated and classified. The eggs that did not hatch then underwent embryo diagnostics to evaluate fertility and the phase of embryonic mortality.

5 Statistical Analysis

After the data was obtained, a variation analysis was carried and standard deviation was calculated. These statistical procedures were carried out with the help of the statistical program SAS.

Results

10 TABLE 2. Laying rate during the periods 46th – 55th, 56th- 66th weeks and total period (21 weeks)

Treatments	Laying rate (%)		
	46th to 55th weeks	56th to 66th week	Total period
Control	58.19 ± 3.87	48.75 ± 3.19	53.25 ± 2.68
Carophyll Red	59.66 ± 2.97	51.35 ± 3.66	55.31 ± 2.22
Arithmetic Mean	58.93	50.05	54.28
C.V. (%)	5.86	6.87	4.54
P	0.4781	0.2207	0.1793

15 TABLE 3. Hatchability in the periods between the 46th–56th and 56th-66th weeks and the total period (21 weeks)

Treatments	Hatchability (%)		
	46th to 55th week	56th to 66th week	average of the 21 weeks
Control	92.48 ± 0.98 b	93.42 ± 0.51 b	92.97 ± 0.54 b
Carophyll Red	94.33 ± 0.76 a	95.96 ± 0.53 a	95.18 ± 0.56 a
Arithmetic Mean	93.41	94.69	94.08
C.V. (%)	0.94	0.55	0.59
P	0.0047	0.0001	0.0001

(a>b Duncan's test)

TABLE 4. Hatching, hatchability, fertility, and embryonic mortality during the observed period (21 weeks)

Treatments	Hatching	Hatchability	Fertility	Embryonic Mortality
	(%)			
Control	83.03 ± 0.89 b	92.97 ± 0.54 b	90.98 ± 0.81 b	5.46 ± 0.75 a
Carophyll Red	86.03 ± 0.42 a	95.18 ± 0.56 a	92.11 ± 0.48 a	3.72 ± 0.86 b
Arithmetic Mean	84.53	94.08	91.54	4.59
C.V. (%)	0.83	0.59	0.75	16.77
P	0.0001	0.0001	0.0171	0.0029

(a>b Duncan's test)

TABLE 5. Effect of the treatments on the average embryonic mortality rate over the observed period

Treatments	Embryonic Mortality (%)			
	M1	M2	M3	M4
Control	1.80 ± 0.45 a	0.89 ± 0.70	0.69 ± 0.28	2.07 ± 0.23 a
Carophyll Red	1.04 ± 0.41 b	0.66 ± 0.33	0.58 ± 0.33	1.44 ± 0.58 b
Arithmetic Mean	1.42	0.77	0.64	1.76
C.V. (%)	28.02	40.41	54.07	21.86
P	0.0083	0.2225	0.5980	0.0171

(a>b Duncan's test)

- 5 M 1- Embryonic Mortality in the first 48 hours of incubation
M 2- Embryonic Mortality occurring between day 3 and day 7 of incubation
M 3- Embryonic Mortality occurring between day 8 and day 14 of incubation
M 4- Embryonic Mortality occurring between day 15 and day 21 of incubation

TABLE 6. Embryonic Mortality between the 19th and 21st weeks and for all 21 weeks of the study

Treatments	Embryonic Mortality (%)			
	Week 19	Week 20	Week 21	21 weeks
Control	5.32 ± 3.24	4.57 ± 1.57	5.18 ± 2.72	5.46 ± 0.75 a
Carophyll Red	3.33 ± 4.12	3.51 ± 2.56	3.12 ± 2.12	3.72 ± 0.86 b
Arithmetic Mean	4.33	4.04	4.15	4.59
C.V. (%)	57.93	52.75	54.88	16.77
P	0.0127	0.4081	0.1490	0.0029

(a>b Duncan's test)

TABLE 7. Number of eggs, incubable eggs and chicks per bird housed during the observed period (21 weeks)

Treatments	Production of eggs/bird	Incubatable eggs/bird	Chicks/bird
Control	78.27 ± 3.95	71.71 ± 4.46	5952 ± 3.18 b
Carophyll Red	81.30 ± 3.40	75.25 ± 3.46	64.73 ± 2.82 a
Arithmetic Mean	79.79	73.48	62.13
C.V. (%)	4.55	5.36	4.76
P	0.1793	0.1503	0.0122

TABLE 8. Calculated nutritional levels in the feed used during the experiment

Average Metabolic Energy (Kcal)	2850.00	Average Metabolic Energy (Kcal)	2850.00
Gross Protein (%)	15.96	Vitamin E (mg)	53.59
Arginine Total (%)	0.94	Vitamin K3 (mg)	2.50
Lysine Total (%)	0.80	Vitamin B1 (mg)	5.77
Methionine Total (%)	0.35	Vitamin B2 (mg)	10.82
Methionine + Cystine Total (%)	0.55	Vitamin B6 (mg)	10.85
Threonine Total (%)	0.59	Vitamin B12 (mcg)	19.00
Tryptophan Total (%)	0.17	Biotin (mg)	0.30
Isoleucine Total (%)	0.63	Folic Acid (mg)	1.74
Leucine Total (%)	1.46	Nicotinic Acid (mg)	65.36
Valine Total (%)	0.72	Pantothenic Acid (mg)	25.22
Histidine Total (%)	0.42	Copper (mg)	18.43
Chlorine Total (%)	0.77	Iron (mg)	122.94
Calcium (%)	3.30	Iodine (mg)	0.80
Available Phosphorus (%)	0.40	Manganese (mg)	83.10
Sodium (%)	0.19	Selenium (mg)	0.69
Chlorine (%)	0.28	Zinc (mg)	93.83
Potassium (%)	0.60		
Vitamin A (UI)	10450.00		
Canthaxanthin (UI)	1662.50		

TABLE 9. Composition of feed used during the experiment

Ingredients	Percentage
Corn	68.52
Soya Bran 46%	21.57
Limestone 38% Ca	7.21

Bicalcium Phosphate	1.64
Salt	0.4
Wheat Bran	0.112
DL-Methionine 99%	0.045
Premix	0.50

TABLE 10. Composition of the premix added to the diet of the chickens

Nutrient	Quantity per kilo of product	Unit
Folic Acid	237.5	mg
Nicotinic Acid	8500	mg
Pantothenic Acid	3800	mg
Biotin	38	mg
Copper	12400	mg
Choline	72000	mg
Sulphur	10222	mg
Iron	12000	mg
Iodine	160	mg
Manganese	14000	mg
Methionine	118800	mg
Oxytetracycline	8000	mg
Selenium	108	mg
Vitamin A	2090000	UI/Kg
Vitamin B1	475	mg
Vitamin B12	3800	mg
Vitamin B2	1900	mg
Vitamin B6	950	mg
Canthaxanthin	332500	UI/Kg
Vitamin E	7600	mg
Vitamin K3	950	mg
Zinc	14000	mg

Example 2: Study if the Supplementation of Canthaxanthin and 25-OH D3 in broiler breeder hens

Material & Methods:

The test has been executed according to Example 1, with the addition of ROVIMIX® Hy-D®.

5 The treatments in example 2 were as follows:

T1 – Control diet

T2 – Control diet + 60 ppm of Carophyll Red

T3 – Control diet + 69 ppm of ROVIMIX® Hy-D®

T4 – Control diet + 60 ppm of Carophyll Red + 69 ppb of ROVIMIX® Hy-D® and

10 with 6 replication/treatment of 40 broiler breeder and 4 cockerels per replication.

Egg production, fertility and hatchability are recorded weekly. The results are shown in Table 11. The data shows a good response for ROVIMIX® Hy-D® and canthaxanthin.

TABLE 11:

TRT	Average hatchability after 10 weeks
Control	85,26
ROVIMIX® Hy-D®	86,85
CAROPHYLL RED	87,22
CAROPHYLL RED+ ROVIMIX® Hy-D®	88,07

Claims

1. The use of canthaxanthin and/or at least one vitamin D metabolite for improving hatchability and fertility and for lowering embryo mortality in poultry.
2. The use according to claim 1, wherein the vitamin D metabolite is 25-hydroxy vitamin D3.
- 5 3. The use of canthaxanthin and or 25-hydroxy vitamin D3 in the manufacture of a food or veterinary composition for improving hatchability in poultry.
4. The use as in any of claims 1 to 3 in the manufacture of a poultry food comprising from about 10 µg/kg to about 100 µg/kg of 25-hydroxy vitamin D3 and from about 2 to 100 ppm canthaxanthin, preferably from about 2 ppm to 10 ppm.
- 10 5. A method for improving hatchability and fertility and for lowering embryo mortality in poultry, which comprises administering to an animal in need of such treatment an amount of about 2 ppm to 100 ppm, preferably 2 to 10 ppm of canthaxanthin and/or about 10 µg/kg to about 100 µg/kg of at least one vitamin D metabolite.
6. The method according to claim 5, wherein the vitamin D metabolite is 25-hydroxy vitamin
15 D3.
7. The method according to claim 6, wherein canthaxanthin and 25-hydroxy vitamin D3 are administered together.
8. A premix composition comprising 25-hydroxy vitamin D3 and canthaxanthin for use in breeder feed to improve hatchability.

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