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Effects of a Vitamins D and C Supplement on Performance, Hatchability, and Blood Profiles of Broiler Breeders

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ABSTRACT

Vitamin D is a fat-soluble vitamin that plays a crucial role in controlling Calcium and Phosphor homeostasis, bone mineralization, and modulation of immune responses. Vitamin C is a cofactor of enzymatic reactions with anti-inflammatory and antioxidant properties to prevent and repair damage to cells in the body from exposure to free radicals and the immune system. The current study aimed to investigate the effects of dietary supplementation of 25(OH)D3 with vitamin C at different doses on broiler breeders' blood profile, egg quality, and hatchability. The adaptation process before collecting the data was 2 weeks. A total of 6200 females and 620 male broiler breeders in the laying period aged 32-46 weeks were divided into 4 treatment groups with 5 replicates (each peach contained 310 female and 31 male breeders). The treatments included control as T0 (0 g/ton Nutricell HyC®), T1 (100 g/ton Nutricell HyC®), T2 (200 g/ton Nutricell HyC®), T3 (400 g/ton Nutricell HyC®) supplemented in feed. The observed variables were performance in breeding farms and hatcheries. The treatments with experimental doses indicated significant differences in the performance of broiler breeders, including feed intake, body weight, egg weight, egg mass, hen day production, hen house production, feed conversion ratio, and parameters of blood profile. The results showed a significant difference between the treatments and the control group in terms of hatch performance, clear eggs, exploding eggs, hatchability eggs, fertile eggs, salable chicks, and hatching of fertile eggs. However, no significant effects on fertility, culling of chicks, and embryonic mortality in the treatment groups were indicated. In conclusion, Nutricell HyC® with a dose of 400 g/ton in feed has indicated the best result in breeding farm and hatchery performance of broiler breeders in the laying period.

Keywords: Blood profile, Broiler breeder, Calcidiol (25(OH)D3), Nutricell HyC®, Performance, Vitamin C

INTRODUCTION

Broiler breeder operations aim to optimize the hatchability rate as well as saleable chicks and high-quality chicks. The success of a hatchery depends on the number of high-quality day-old chicks (DOC). Fertility and hatchability are the main factors that could affect the supply of DOC and are influenced by two factors, including breeder and hatchery management (Cobb, 2021). According to Lare et al. (2021), feed quality plays a vital role in broiler breeder performance, and it could affect egg quality, embryo growth, fertility, and egg hatchability.

Egg yolk provides a nutrient supply for embryo development during incubation and is a secondary nutrient resource for DOCs (Romanoff, 1960; Dayan et al., 2020). Meanwhile, eggshells provide minerals and trace elements supply for embryo development and become a major contributor of calcium (Ca) to support the skeletal mineralization of the embryo during the second half of the incubation or development phase (Yair and Uni, 2011; Halgrain et al., 2022). One of the egg criteria related to the incubation process and time to hatch is the porosity of the eggshell, which affects the exchange of oxygen (O_2), carbon dioxide (CO_2), and dihydrogen monoxide (H_2O) vapor in the shell (Narushin et al., 2002). The amount and size of the pores determine the eggshell quality. Eggshell thickness affects the activity of the embryo and the loss of

moisture conditions in the external environment of the egg (Bergoug et al., 2013). Extreme porosity and shell thickness have the potential to inhibit embryo development through pathogenic bacteria contamination or might be lethal to embryo development even with little contamination of pathogenic bacteria (Dzoma, 2010).

Vitamin D has an important role in the absorption of Ca and phosphorus (P), regulation of parathyroid hormone (PTH), immune system, mineralization, bone mobilization, and control concentrations of Ca in the blood and muscles (Garcia et al., 2013). The Ca and P storage depends on the availability of minerals in the diet that affects bone development and mineralization (Regassa et al., 2015). The formation of eggshells and maintenance of plasma Ca levels in chickens depend on the availability and use of Ca in the diet and minerals in bone, especially the medullary bone of breeders (Kerschnitzki et al., 2014).

The body response that is not specific and adaptive to any condition is a symptom of stress in chickens that is influenced by various factors, from external and internal pressure, such as climate change, transportation, as well as changes in drinking water and types of feed and infections (Etim et al., 2013). Stress conditions in chickens might activate the pituitary-adrenal axis and cause the secretion of adrenocorticotropin hormone (ACTH), which affects the blood homeostasis system (Scanes, 2016). The ratio of heterophile to lymphocytes (H/L) describes the body's response to various stress conditions as a marker to assess poultry's welfare and health status (Huth and Archer, 2015).

Vitamin C is a water-soluble vitamin synthesized by liver cells, functions as a cofactor for several enzymes, is an antioxidant, and can improve immune function (Abidin and Khatoon, 2013). Ascorbic acid is a powerful natural antioxidant that could reduce stressful conditions' adverse effects and improve performance (Rao et al., 2011; Whitehead and Keller, 2019). Under normal conditions, poultry could synthesize AA in their liver cell to meet their needs, but if under stress conditions, supplementation in feed was required (Van Hieu et al., 2022). when chickens are under stress, supplementation of AA in the feed could improve health and oxidative status (Gouda et al., 2020). Fletcher et al. (2021) reported that intake of AA during stressful conditions could control many brain activities due to the increased production of neurotransmitters and norepinephrine. The purpose of this study was to evaluate the effect of combined supplementation of 25(OH)D_3 with vitamin C in Nutricell HyC® products at different doses on broiler breeder performance, blood profile, egg quality, and hatchability.

MATERIALS AND METHODS

Formulation of experimental diets

The experimental diets are formulated and presented in Table 1.

Table 1. The analysis of experimental diets of broiler breeders Cobb strain aged 32-46 weeks

	T0	T1	T2	T3
Raw material (%)				
Yellow corn	45	45	45	45
Wheat flour	15	15	15	15
Soybean meal	16.6	16.6	16.6	16.6
Rice bran	4	4	4	4
Wheat pollard	2	2	2	2
Full fat soya	5	5	5	5
Salt	0.3	0.3	0.3	0.3
Limestone grit	7.1	7.1	7.1	7.1
Monodicalcium phosphate	2	2	2	2
Vitamin E	0.2	0.2	0.2	0.2
Choline chloride	0.5	0.5	0.5	0.5
Vitalink	0.3	0.3	0.3	0.3
Crude Palm Oil	0.2	0.2	0.2	0.2
Methionine	0.1	0.1	0.1	0.1
Lysine	0.2	0.2	0.2	0.2
Threonine	0.1	0.1	0.1	0.1
Calculation in formulation				
Metabolizable Energy (kcal/kg)	2835	2835	2835	2835
Vitamin D3 (IU/g)	0	3750	4000	4500
Vitamin C (g)	0	95	190	380
Nutrition composition (%)				
Crude protein	16.54	16.88	16.46	16.37
Ash	11.14	11.12	10.30	10.43
Crude fat	6.05	5.92	5.83	5.79
Crude fiber	3.42	3.48	3.63	3.54
Moisture	11.01	10.98	10.90	11.20
Starch	34.29	33.40	34.85	36.69
Calcium	2.79	3.04	2.95	2.61
Phosphor	0.41	0.44	0.46	0.42

T0/Control: 0 g/ton Nutricell HyC®; T1:100 g/ton Nutricell HyC®; T2:200 g/ton Nutricell HyC®; T3:400 g/ton Nutricell HyC®

Ethical approval

The study was conducted under the supervision of the animal health manager in PT Peternakan Ayam Manggis, Indonesia. Following the SOP and Guidelines of Cobb, so no need the ethical approval.

Experimental design

The study was carried out from July to October 2021 in a broiler breeder farm and hatchery of PT Peternakan Ayam Manggis in Tenjo Ayu, Sukabumi, West Java, Indonesia. Feed was produced at the feed mill PT Megah

Prayasa Sentosa, Cikupa, Tangerang, Banten, Indonesia. The DOCs were purchased from the hatchery PT Hybro Indonesia, Cianjur, Indonesia. The study was conducted using 6200 female broiler breeders Cobb strain aged 32-46 weeks. The chickens were distributed randomly into 20 pens (310 female and 31 male broiler breeders were added). The trial feed adaptation period was performed in two weeks. The treatments included basal diet without Nutricell HyC® supplementation as control (T0), basal diet with 100 g/ton (T1), 200 g/ton (T2), 400 g/ton (T3) of Nutricell HyC®. Each treatment consisted of five replication (Cobb, 2021). Nutricell HyC® contains a combination of 25(OH)D3 and vitamin C to improve broiler breeder performance.

Trial feed was produced by mixing Nutricell HyC® supplied by PT Nutricell Pasific with soybean meal in a premix mixer. Mixed additives were then transferred into the main mixer to be mixed with the whole formulation, then pelleted and crumbled under feed mill standard operation. The formulation included HyD 62.500 µg, vitamin C 950.000 mg, and filler.

The cages used were openside with size 8 x 9 (m), lined with fibrocement tiles, had a concrete floor with rice husk litter, and were equipped with polypropylene side curtains, feeder tubs for females and tube feeders for males, and nipple drinking holder. Drinking water was given *ad libitum* and fed once daily at 05.45 am. The light was supplied by natural light from the sun and artificial light by lamps for 17 hours from 03 pm until 08 am with lux 15. The ambient temperature was around 25-29°C, and relative humidity was around 72-75%.

Blood profile analyses were conducted at IPB University Bogor, Indonesia, and proximate analysis was conducted at PT Nutricell Pasific, Jakarta, Indonesia.

Production performance

Eggs were collected four times a day, and the number of released and rejected eggs from each pen was recorded every day until the end of the experiment. Random sampling was performed weekly for each cage to monitor the body weight (BW) of broiler breeders. Briefly, BW of broiler breeder in the laying period was evaluated twice a week to determine the broiler breeder restricted feeding based on its BW relative to its target BW. Broiler breeder performance parameters, such as egg mass, feed conversion ratio (FCR), hen day production (HDP), and hen house production (HHP) were calculated as follows:

$$\text{Egg mass (g)} = \text{HDP (\%)} \times \text{Average egg weight (g)} \quad \text{(Formula 1)}$$

$$\text{FCR (per g egg mass)} = \frac{\text{Feed intake (g)}}{\text{Egg production (g)}} \quad \text{(Formula 2)}$$

$$\text{HDP (\%)} = \frac{\text{Number of egg produced during the period}}{\text{Number of hens at the early egg produced}} \times 100 \quad \text{(Formula 3)}$$

$$\text{HHP (\%)} = \frac{\text{Number of egg produced during the period}}{\text{Number of hens at the early egg produced}} \times 100 \quad \text{(Formula 4)}$$

Incubation responses

The hatching process lasted for 21 days, where the incubation process was 18 days in the setter machine and 3 days hatching in the hatcher machine. Eggs were collected 3-4 times daily and transferred to the hatchery when graded a satisfactory quality. In the hatchery, eggs were classified into two groups, namely indubitable and non-indubitable. The indubitable eggs were fumigated at 25°C with 70% humidity for 15 minutes. After that, they were stored in an environmentally controlled room (18-21°C and 60-70% relative humidity) for a maximum period of 5 days. Indubitable eggs were transferred into a pre-heating room for 8-12 hours at a temperature of 25-26°C before being transferred into the setter machine. The non-indubitable eggs, such as cracked, shell-less, double yolk, abnormally shaped, were recorded and separated.

A total of 9900 eggs from broiler breeders within the age range of 34 to 46 weeks were set for incubation twice a week. The eggs were set with the same temperature, humidity, and turning conditions in the setter machine. The temperature was 37.5°C, relative humidity was 83%, and turning occurred automatically every hour at a 90° angle. After ten days of incubation, several eggs from each treatment were candled to determine fertility and embryonic mortality. The unclear eggs were returned to the setter machine to follow incubation. Clear eggs were separated and recorded. A random sampling of each treatment (< 7 days) was done to determine early embryo mortality and infertility. The candling process was carried out after 18 days of incubation to determine clear eggs and unclear eggs. The clear eggs were then separated and recorded. The unclear eggs detected during the candling process were then transferred to the hatch basket and kept in the hatchery machine at a temperature of 36.5°C and relative humidity of 85% until 21 days of incubation.

To determine percentages of early embryo mortality (1-7 days), middle embryo mortality (8-14 days), and late embryo mortality (15-21 days), the eggs which could not be hatched were sampled. The clear eggs were assumed

infertile, and the rest were recorded separately as fertile eggs with the early dead embryo and dead embryonic eggs.

Fertility data were collected, and fertility percentage was obtained from the number of fertile eggs to the number of eggs set in the incubator and was calculated as Formula 5.

$$\text{Fertile eggs (\%)} = \frac{\text{Number of fertile eggs}}{\text{Number of eggs incubated}} \text{x}100$$

(Formula 5)

After 21 days of incubation, DOCs were pulled out from the incubator and visually selected according to commercial hatchery standards. Abnormal DOCs, including missing eyes, legs with cuts, splayed legs, cross beak, and poor feathering, as well as chicks that could not flip over in less than 10 seconds, were counted as culling chicks and separated according to the Cobb hatchery management guide (Cobb, 2021). All unhatched eggs were subjected to random sampling to predict estimated days of embryo mortality.

Hatchability data were collected, and hatchability percentage was obtained from the number of hatched eggs to the number of eggs set in the incubator and was calculated as Formula 7.

$$\text{Hatchability (\%)} = \frac{\text{Number of chick hatched}}{\text{Number of eggs incubated}} \text{x}100$$

(Formula 6)

The percentage of the hatch of fertile is an indicator of the effectiveness of the hatchery. The calculation method is indicated in Formula 7.

$$\text{Hatch of fertile (\%)} = \frac{\text{Percentage of hatchability}}{\text{Percentage of fertile}} \text{x}100$$

(Formula 7)

The embryo mortality was observed and divided into three groups, namely early embryo mortality (1-7 days), middle embryo mortality (8-14 days), and late embryo mortality (15-21 days) incubation. The calculation of the percentage of embryonic mortality is indicated in Formula 8.

$$\text{Embryonic mortality (\%)} = \frac{\text{Number of embryo mortality}}{\text{Number of fertile eggs}} x100$$

(Formula 8)

Hematology determination

The process of taking blood samples was carried out on broiler breeders aged 45 weeks on the underside of the wings. The blood sample was taken using a syringe of as much as 2 ml and put in an anticoagulant tube. The collecting blood sample was at 06.00 am, and the anticoagulant tube was included in the ice flask, after finished direct sent to the laboratory of Bogor Agriculture University (IPB), Bogor, Indonesia. For analysis of Ca and P, blood plasma was separated in a tube containing fresh blood spiked with anticoagulant substances. Centrifuged until the red blood cells fell to the bottom of the tube, the white blood cells were on top and formed a layer of buffy coat, and the blood plasma was on top of the layer yellowish. Hematological analysis of blood consisted of the hemoglobin, hematocrit, leukocyte, erythrocyte, eosinophil, basophil, monocyte, heterophile, lymphocyte, Ca, and P.

The erythrocyte measurement method used was microvisual. Accordingly, the blood was diluted in an erythrocyte pipette with an isotonic solution, then put into the counting chamber. The number of erythrocytes was calculated in a certain volume using a conversion factor. The leukocyte measurement was the same with erythrocyte, and differential white blood cell (WBC) counts were made on monolayer blood films, fixed and stained with erythrocyteGiemsa-Wright's stain. The total WBC count was determined by a manual method using a hemacytometer. The method used to measure hemoglobin was Sahli's method (Van Lerberghe et al., 1983). According to Sahli's method, hemoglobin was converted into acid hematin with the help of 0.1 N HCL solution then the resulting color was compared visually with the standard color. The tool used is Sahli's hemoglobinometer. The method used to measure hematocrit was a microtube. Therefore, when the blood was centrifuged, heavier cells (erythrocytes) fell to the bottom of the tube, while the lighter cells (leukocytes and platelets) were above the heavier cells. The tool used in this method was a microcapillary tube with heparin and a microhematocrit centrifuge (Sastradipradja et al., 1989).

Statistical analysis

The IBM statistical SPSS version 26 was used for data analysis. The observed data showed as mean ± standard deviation in the table. One-way analysis of variance (ANOVA) was used to analyze the data. The differences among treatments at a significant level of ($p < 0.05$) followed by the Tukey test. All percentage data were perform as quadratic transformation for statistical analysis.

RESULTS AND DISCUSSION

Broiler breeder performance fed Nutricell HyC® are shown in Table 2. Supplementation of Nutricell HyC® at various levels (T1-T3) significantly decreased feed intake of broiler breeder in comparison to control group ($p < 0.05$,

Table 2). With regard to body weight, egg weight, egg mass, HDP and HHP, only T3 was significantly higher than that of T0 ($p < 0.05$). Similarly, T3 decreased FCR of the broiler breeders significantly as compared to T0 ($p < 0.05$). Meanwhile, a non-significant effect was observed on the parameter of broiler breeder depletion. It could be seen that the BW of broiler breeders is still within the standard range, and they were not overweight. The older brooders need to reduce their feed intake to minimize reproductive problems during the egg-laying phase; therefore, it is necessary to limit feed because body weight gain in broiler breeders must be limited throughout production (Richards et al., 2010). Fritts and Waldroup (2003) observed that male broiler strain cobb fed 25(OH)D3 could better support body weight gain than vitamin D_3. The supplementation of Nutricell HyC® increased the egg weight, compared to control feed, but all treatments showed egg weight in the range required for hatching. Standard egg weight at the age of 34-46 weeks was 60.7-66.5 g. According to Ummer-Franco et al. (2010), egg weight was not affected by fertility, early and middle embryo death, culling of chicks, feed intake, or FCR. Wang et al. (2020) indicated that 25(OH)D3 increased the relative weight and thickness of the eggshells. The corticosterone hormone increases energy supply when chickens experience stress, where vitamin C has a vital role in the biosynthesis of this hormone (Siegel and Kampen, 1984). Heat stress conditions at various levels significantly affect egg production, egg weight, eggshell quality, weight gain, and mortality (Lin et al., 2006). The supplementation of Nutricell HyC® in this study might increase feed efficiency. According to Araujo et al. (2019), supplementary feed canthaxanthin + 25(OH)D3 increased egg production and FCR but did not affect feed intake.

Table 2. Effect of Nutricell HyC® supplementation supplementation added in feed on broiler breeder performance at the age of 34-46 weeks

Treatments / Production Performance	T0 (control)	T1	T2	T3	p-value
Feed intake (g/day)	159.07 ± 0.04^{b}	158.96 ± 0.04^{a}	158.90 ± 0.07^{a}	158.93 ± 0.02^{a}	0.001
Body weight (g)	3727.45 ± 4.81^{a}	3729.37 ± 4.82^{ab}	3734.26 ± 4.12^{ab}	3736.34 ± 4.03^{b}	0.021
Egg weight (g)	65.63 ± 0.53^{a}	65.89 ± 0.33^{ab}	66.03 ± 0.37^{ab}	66.58 ± 0.24^{b}	0.009
Egg mass (g)	49.35 ± 0.91^{a}	49.62 ± 0.41^{a}	50.13 ± 0.35^{ab}	50.81 ± 0.44^{b}	0.005
HDP[1] (%)	75.18 ± 0.86^{a}	75.31 ± 0.53^{ab}	75.92 ± 0.24^{ab}	76.31 ± 0.56^{b}	0.026
HHP[1] (%)	71.85 ± 0.82^{a}	71.96 ± 0.50^{a}	72.65 ± 0.23^{ab}	73.02 ± 0.54^{b}	0.013
Depletion[1] (%)	0.28 ± 0.02	0.25 ± 0.04	0.25 ± 0.02	0.23 ± 0.03	0.065
FCR	3.23 ± 0.07^{b}	3.21 ± 0.02^{ab}	3.17 ± 0.07^{ab}	3.14 ± 0.03^{a}	0.012

[1:] The data were performed as quadratic transformation; [a,b:] Value in the same row with different superscript letters significantly differ ($p < 0.05$). T0/Control: 0 g/ton Nutricell HyC®; T1:100 g/ton Nutricell HyC®; T2:200 g/ton Nutricell HyC®; T3:400 g/ton Nutricell HyC® ; HDP: Hen day production, HHP: Hen house production; FCR: Feed conversion ratio.

Table 3. Effect of Nutricell HyC® supplementation in feed on broiler breeder eggshell porosity at the age of 34-46 weeks[1]

Treatments / Eggshell porosity	T0 (control)	T1	T2	T3	p-value
Score 1 (%)	0	0	0.22 ± 0.31	0.11 ± 0.25	0.261
Score 2 (%)	0.11 ± 0.25^{a}	1.56 ± 0.82^{b}	4.22 ± 1.45^{c}	6.22 ± 2.16^{c}	<0.001
Score 3 (%)	8.11 ± 3.01^{a}	22.22 ± 4.76^{b}	34.00 ± 3.08^{c}	40.11 ± 3.25^{c}	<0.001
Score 4 (%)	91.78 ± 2.87^{d}	76.11 ± 5.42^{c}	61.55 ± 4.55^{b}	53.56 ± 3.82^{a}	<0.001

[1:] All data were performed as quadratic transformation. [a,b,c:] Value in the same row with different superscripts letters a significantly differ ($p < 0.05$); T0/Control: 0 g/ton Nutricell HyC®; T1: 100 g/ton Nutricell HyC®; T2: 200 g/ton Nutricell HyC®; T3: 400 g/ton Nutricell HyC®; Score 1 : Excellent eggshell quality; Score 2: Good eggshell quality; Score 3: Average eggshell quality; Score 4: Poor eggshell quality.

The Nutricell HyC® supplementation in broiler breeder diet generally demonstrated an improvement in the eggshell quality (Table 3). The supplementation of Nutricell HyC® could significantly decreased the poor eggshell quality (score 4), increased the average eggshell quality (score 3), and also enhanced the good eggshell quality (score 2) compared to control group ($p < 0.05$). However, Nutricell HyC® supplementation did not cause a significant effect on the excellet eggshell quality (score 1, $p > 0.05$). The combination of 25(OH)D3 with vitamin C contained in Nutricell HyC® might increase Ca and P absorption in the small intestine and Ca deposition at

bones which played a role in the formation of $CaCO_3$, during the formation of the eggshell inside the uterus which lasts for 20 to 21 hours (Fritts and Waldroup, 2003). Calcium and P intake could not decrease eggshell quality, ascorbic acid availability also play an important role in the regulation of Ca metabolism, and eggshell formation, which is required for the conversion of 25(OH)D3 to $1.25(OH)2D_3$ was limited (Bains, 1995; Lohakare et al., 2005). Nutricell HyC® contains vitamin C formed as sodium ascorbate, which is very important for synthesizing the eggshell's organic matrix. The calcified eggshell play as a barrier of eggs and embryos against physical damage and contamination from pathogenic bacteria. The eggshell serves as a Ca resource in embryo development and the exchange of water and gases between the embryo and environment during extra-uterine development through the eggshell structure (Nys et al., 2004). Saunders-Blades and Korver (2014) mentioned Vitamin D_3 is very important for the absorption of Ca and P from the intestine during eggshell formation. Although Ca be absorbed passively from the intestine, vitamin D3 allows chickens to absorb Ca in sufficient quantities to maintain eggshell formation and normal medullary bone reserves. Wang et al. (2020) observed that supplementation of 25(OH)D3 in a laying hen diet increased serum 25(OH)D3, the content of carbonic anhydrase (CA) serum, which is an enzyme that plays a major role in the deposition of calcium carbonate in eggshell formation. Supplementation of vitamins C and E in feed can prevent the deterioration of eggshell quality and reduce the effects of stress due to high environmental temperatures in broiler breeders (Chung et al., 2005).

Table 4. Effect of Nutricell HyC® supplementation in feed on performance hatchability of broiler breeders at the ages of 34-46 weeks[1]

Treatments / Performance	T0 (control)	T1	T2	T3	p-value
Clear eggs (%)	4.07 ± 0.11^{c}	3.87 ± 0.12^{ab}	3.92 ± 0.06^{bc}	3.72 ± 0.12^{a}	0.001
Exploding eggs (%)	0.49 ± 0.05^{c}	0.42 ± 0.02^{b}	0.29 ± 0.03^{a}	0.28 ± 0.02^{a}	<0.001
Dis eggs (%)	4.82 ± 0.05^{b}	4.72 ± 0.16^{ab}	4.81 ± 0.11^{b}	4.54 ± 0.18^{a}	0.014
Hatchability eggs (%)	90.62 ± 0.13^{a}	90.99 ± 0.12^{b}	90.97 ± 0.16^{b}	91.46 ± 0.11^{c}	<0.001
Fertile eggs (%)	95.48 ± 0.11^{a}	95.79 ± 0.13^{b}	95.81 ± 0.07^{b}	96.01 ± 0.12^{c}	<0.001
Infertile egss (%)	1.62 ± 0.54	1.29 ± 0.16	1.49 ± 0.16	1.27 ± 0.37	0.353
Culling chicks (%)	0.55 ± 0.07	0.58 ± 0.10	0.57 ± 0.10	0.48 ± 0.05	0.236
Chicksalable (%)	90.13 ± 0.15^{a}	90.51 ± 0.26^{ab}	90.41 ± 0.2^{b}	91.03 ± 0.16^{c}	<0.001
Hatching of fertile eggs (%)	94.91 ± 0.08^{a}	94.99 ± 0.16^{a}	94.96 ± 0.12^{a}	95.27 ± 0.18^{b}	0.005
Early embryo mortality (%)	2.22 ± 0.48	1.76 ± 0.22	2.22 ± 0.39	1.88 ± 0.46	0.200
Intermediate embryo mortality (%)	0.55 ± 0.24	0.68 ± 0.28	0.56 ± 0.33	0.60 ± 0.35	0.902
Late embryo mortality (%)	3.15 ± 0.82	3.22 ± 0.64	4.12 ± 0.43	2.98 ± 0.70	0.063

[1]: All data were performed as quadratic transformation. [a,b,c]: Value in the same row with different superscripts letters a significantly differ ($p < 0.05$); T0/Control: 0 g/ton Nutricell HyC®; T1: 100 g/ton Nutricell HyC®; T2:200 g/ton Nutricell HyC®; T3:400 g/ton Nutricell HyC®

The treatments (T1, T2, and T3) showed higher ($p < 0.01$) hatchability, fertile eggs, hatching fertile, chicksalable, and reduce clear eggs, exploding eggs, and dis eggs compared with T0 ($p < 0.05$). However, Nutricell HyC® supplementation had no significant effect on infertile, culling chicks, early, intermediate, and late embryonic death ($p > 0.05$). The supplementation with Nutricell HyC®, which contains 25(OH)D3 could repair the quality of eggshell pores so the rate of O_2 and CO_2 transport during the incubation period becomes better, which might reduce clear eggs, exploding eggs and dis eggs (Akbari Moghaddam et al., 2019). Coto et al. (2010) and Saunders-Blades and Korver (2015) observed an improvement in eggshell thickness and hatchability of fertile eggs fed 25(OH)D3 supplementation. The result of the present study showed a significant difference in increasing hatchability of eggs, fertile eggs, chick salable, and hatching of fertile eggs ($p < 0.05$). Dietary supplementation of Nutricell HyC® contains 25(OH)D3, metabolically active vitamin D3, which does not need to be hydroxylated in the liver. Then, it enters into the bloodstream and bounds to Vitamin D protein (BDP), and undergoes hydroxylation in kidneys, epithelial cells, and immune cells are converted into $1,25\ (OH)_2D_3$ (calcitriol), which works like a hormone (Pande et al., 2015). According to Brown et al. (1999) and Brandi (2008), active metabolite synergizes with parathyroid hormone (PTH) to increase intestinal Ca absorption and Ca deposition in bone, decreases Ca excretion in the excreta and maintain bone homeostasis, cell differentiation and

proliferation, central nervous system, and modulates immune response so that it can influence on hatchability, eggshell thickness, bone structure, and DOC immunity. Vitamin C affects many tissues because of its primary function as an important component of collagen synthesis and an antioxidant. Ascorbit acid is involved in the synthesis of collagen by influencing the function of prolyl hydroxylase domain (PHD) protein as an antioxidant and important cofactor for catalyzing many biochemical reactions (Aghajanian et al., 2015). In bone formation, Vitamin C increases the production of hydroxyproline, which is required for collagen synthesis. A network of collagen fibrils is necessary for proper bone and eggshell formation Lohakare et al. (2005). Vitamin D3 supplementation in the form of 25(OH)D3 metabolism involved in Ca and P metabolism could increase bone growth, egg-laying rate, shell quality, and reproduction (Torres et al., 2009; Rosa et al., 2012). Feeds supplemented with canthaxanthin + 25(OH)D3 showed an increase in hatchability, hatchability of fertile and decreased early embryo death (Araujo et al., 2019). However, the current study showed that the results were not significantly different ($p > 0.05$) for infertile eggs, culling chicks, and early, intermediate, and late embryo mortality.

Table 5. Effect of Nutricell HyC® supplementation in feed on broiler breeder blood profile in laying period (32-46 weeks of age)

Performance \ Treatments	T0 (control)	T1	T2	T3	p-value
Hemoglobin (g/dl)	11.76 ± 1.01[ab]	10.71 ± 0.45[a]	10.64 ± 1.05[a]	12.08 ± 0.76[b]	0.035
Hematocrit[1] (%)	30.00 ± 2.83	30.20 ± 1.92	30.00 ± 3.32	33.00 ± 1.87	0.218
Leukocyte[1] (x 10^3/mm^3)	23.10 ± 4.76	23.78 ± 2.70	20.60 ± 3.12	22.04 ± 3.88	0.607
Erythrocyte (x 10^6/mm^3)	2.35 ± 0.59	2.25 ± 0.78	2.72 ± 0.95	2.37 ± 0.60	0.765
Eosinophil[1] (%)	4.79 ± 1.17	5.62 ± 2.90	8.14 ± 2.51	6.52 ± 3.54	0.268
Basophil[1] (%)	3,83 ± 1,89	3.39 ± 1.03	5.29 ± 1.45	3.55 ± 1.07	0.167
Monocyte[1] (%)	1.84 ± 0.77	1.13 ± 0.41	2.45 ± 1.50	2.03 ± 1.15	0.280
Heterophile[1] (%)	34.39 ± 6.52	38.59 ± 3.66	35.89 ± 4.22	38.64 ± 3.43	0.406
Lymfocyte[1] (%)	55.16 ± 4.58	51.28 ± 3.75	48.23 ± 2.49	49.25 ± 5.33	0.081
Heterophil/Lymphocyte[1] (%)	0.69 ± 0.17	0.77 ± 0.13	0.79 ± 0.14	0.69 ± 0.081	0.500
Calcium (mg/dl)	10.76 ± 1.48	9.88 ± 0.83	10.89 ± 0.11	9.78 ± 0.97	0.197
Phosphor (mg/dl)	6.53 ± 1.80	4.56 ± 0.67	5.50 ± 0.46	5.43 ± 1.49	0.138

[1:] Data were performed as quadratic transformation. [a,b,c;] Value in the same row with different superscripts letters a significantly differ ($p < 0.05$); T0/Control: 0 g/ton Nutricell HyC®; T1: 100 g/ton Nutricell HyC®; T2:200 g/ton Nutricell HyC®; T3:400 g/ton Nutricell HyC®

Table 5 showed that Nutricell HyC® supplementation had no significant effect on most of blood parameters ($p > 0.05$). However, the amount of hemoglobin for T3 was significantly higher than that of T1 and T2 ($p < 0.05$). In this research, the hemoglobin levels among treatments ranged from 10.64 g/dl to 12.08 g/dl, where the hemoglobin level was still within normal limits. According to Weiss et al. (2010), the amount of hemoglobin in chickens (*gallus domesticus*) ranged from 7 to 13 g/dl. This is similar to the research conducted by Roy and Mishra (2011) examining the effects of antistress agents on broiler breeders. The findings indicated that hemoglobin levels in different groups ranged from 9 to 10.13 g/dl.

The findings of the current study showed the ratio of heterophile/lymphocyte (H/L) in broiler breeders ranged from 0.69% to 0.79%, indicating that the levels were still normal. Hachesoo et al. (2011) reported that H/L ratio of indigenous broiler breeders was 0.70 %, and H/L ratio of Ross-308 was 0.71%. According to Gross and Siegeh (1983), the level of stressors in poultry could be indicated by the ratio of Heterophils/Lymphosytes, about 0.2% (low level), 0.5% (normal level), and 0.8% (high level) to environmental adaptation. Therefore, it can be concluded that the levels of blood parameters were in normal ranges and H/L ratio of broiler breeders in the production phase obtained in this research can be interpreted as appropriate health and immune status. This shows that the supplementation of Nutricell HyC® with different levels can maintain the H/L ratio of broiler breeders in the production phase in the normal range. Table 5 shows that the supplementation of Nutricell HyC® in the feed has no significant difference in Ca and P levels of the broiler breeders' blood plasma ($p > 0.05$). In this research, blood plasma Ca levels ranged from 9.78 to 10.76 mg/dl, and P levels in blood plasma ranged from 4.56 to 6.53 mg/dl.

The results of the present study are in the same line with the findings of Hachesoo et al. (2011), who reported Ca and P levels in the blood plasma of indigenous chickens were 9.36 mg/dl and 4.23 mg/dl, respectively, while Ca and P levels for Ross-308 chickens were 9.28 mg/dl and 4.44 mg/dl, respectively.

CONCLUSION

In conclusion, the obtained results of the current study indicated that Nutricell HyC® supplementation with doses of 400 g/ton (T3) gave the best results in increasing egg production, eggshell porosity, hatchability, and immunity of broiler breeders aged 34-46 weeks. Future studies can investigate the effect of supplementation of Vitamin D3 more than 4500 IU/g on broiler breeder performance.

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Competing interests

The authors declare no conflict of interest for this manuscript

Authors' contributions

Nanik Setiyaningsih conducted the study in a breeding farm and hatchery contributed to data collection, analyzed data, processed statistical data, and prepared manuscripts. Sumiati, Anuraga Jayanegara, Wira Wisnu Wardani contributed to the supervision of the study, the analysis of the result, and the writing of the manuscript. All authors confirmed the statistical result and approved the final version of the manuscript.

Ethical consideration

The authors declare that they have checked the manuscript for plagiarism, double publication, and no data fabrication or redundancy.

Availability of data and materials

The data relating to the article will be sent by the corresponding author according to reasonable requests.

REFERENCES

Abidin Z and Khatoon A (2013). Heat stress in poultry and the beneficial effect of ascorbic acid (vitamin C) supplementation during periods of heat stress. World's Poultry Science Journal, 69(1): 135-152. DOI: https://www.doi.org/10.1017/S0043933913000123

Aghajanian P, Hall S, Wongworawat MD, and Mohan S (2015). The roles and mechanisms of actions of vitamin C in bone: New development. Journal of Bone and Mineral Research, 30(11): 1945-1955. DOI: https://www.doi.org/10.1002/jbmr.2709

Akbari Moghaddam Kakhki R, Heuthorst T, Mills A, Neijat M, and Kiarie E (2019). Interactive effects of calcium and top-dressed 25-hydroxy vitamin D3 on egg production, egg shell quality, and bones attributes in aged Lohmann LSL-lite layers. Poultry science, 98(3): 1254-1262. DOI: https://www.doi.org/10.3382/ps/pey446

Araujo LF, Araujo CSS, Pereira RJG, Bittencourt LC, Silva CC, Cisneros F, Hermes RG, Sartore YGA, and Dias MT (2019). The dietary supplementation of canthaxanthin in combination with 25(OH)D3 results in reproductive, performance, and progeny quality gains in broiler breeders. Poultry Science, 98(11): 5801-5808. DOI: https//www.doi.org/10.3382/ps/pez377

Bains BS (1995). The role of vitamin C in stress management. World's Poultry, 12(4): 38-41.

Bergoug H, Burel C, Guinebretiere M, Tong Q, Roulston N, Romanini CEB, Exadaktylos V, Mcgonnell IM, Demmers TGM, Verhelst R, et al. (2013). Effect of pre-incubation and incubation conditions on hatchability, hatch time and hatch window, and effect of post-hatch handling on chick quality at placement. World's Poultry Science Journal, 69(2): 313-334. DOI: https://www.doi.org/10.1017/S00043933913000329

Brandi L (2008). 1alpha (OH)D_3 One-alpha-hydroxy-cholecalciferol-an active vitamin D analog. Clinical studies on prophylaxis and treatment of secondary hyperparathyroidism in uremic patients on chronic dialysis. Danish Medical Bulletin, 55(4): 186-210. Available at: https://pubmed.ncbi.nlm.nih.gov/19232159/

Brown AJ, Dusso A, and Slatopolsky E (1999) Vitamin D. American Journal of Physiology, 277, F157-F175. DOI: https://www.doi.org/10.1152/ajprenal.1999.277.2.f157

Cobb (2021). Breeder management guide. pp. 1-153. Available at: https://www.cobbafrica.com/wp-content/uploads/Cobb-500-Breeder-Managment-Guide.pdf

Coto C, Cerate S, Wang Z, Yan F, Min Y, Costa FP, and Waldroup PW (2010). Effect of source e level of vitamin D on the performance of breeder hens and the carryover to the progeny. International Journal of Poultry Science, 9(7): 623-633. DOI: https://www.doi.org/10.3923/ijps.2010.623.633

Chung MK, Choi JH, Chung, and Chee KM (2005). Effects of dietary vitamin C and E on egg shell quality of broiler breeder hens exposed to heat stress. Asian-Australasian Journal of Animal Sciences, 18(4): 545-551. DOI: https://www.doi.org/10.5713/ajas.2005.545

Dayan J, Reicher N, Melkman-Zehavi T, and Uni Z (2020). Incubation temperature affects yolk utilization through changes in expression of yolk sac tissue functional genes. Poultry Science, 99(11): 6128-6138. DOI: https://www.doi.org/10.1016/j.psj.2020.07.037

Dzoma BM (2010). Some factors affecting fertility and hatchability in the farmed ostrich: A review. Journal of Animal and Veterinary Advances, 9(2): 229-239. DOI: https://www.cabdirect.org/cabdirect/abstract/20103053159

Etim NN, Enyenihi GE, Williams ME, Udo DM, and Offiong EE (2013). Haematological parameters: Indicators of the physiological status of farm animals. British Journal of Science, 10(1): 33-45.

Fletcher BD, Flett JAM, Wickham SR, Pullar JM, Vissers MCM, and Conner TS (2021). Initial evidence of variation by ethnicity in the

relationship between vitamin C status and mental states in young adults. Nutrients, 13(3): 792. DOI: https://www.doi.org/10.3390/nu13030792

Fritts CA and Waldroup PW (2003). Effects of source and level of vitamin D on live performance and bone development in growing broilers. Journal of Applied Poultry Research, 12(1): 45-52. DOI: https://www.doi.org/10.1093/japr/12.1.45

Garcia AFQM, Murakami AE, do Amaral Duarte CR, Rojas ICO, Picoli KP, and Puzolti MM (2013). Use of vitamin D3 and its metabolites in broiler chicken feed on performance, bone parameters and meat quality. Asian-Australasian Journal of Animal Sciences, 26(3): 408-415. DOI: https://www.doi.org/10.5713/ajas.2012.12455

Gouda A, Amer SA, Gabr S, and Tolba SA (2020). Effect of dietary supplemental ascorbic acid and folic acid on the growth performance, redox status, and immune status of broiler chickens under heat stress. Tropical Animal Health and Production, 52(6): 2987-2996. DOI: https://www.doi.org/10.1007/S11250-020-02316-4

Gross WB and Siegel HS (1983). Evaluation of the heterophil/lymphocyte ratio as a measure of stress in chickens. Avian Disease, 27(4): 972-979. DOI: https://www.doi.org/10.2307/1590198

Hachesoo BA, Talebi A, and Rezai SA (2011). Comparative study on blood profiles of indigenous and Ross-308 broiler breeders. Global Veterinaria, 7(3): 238-241. Available at: https://www.idosi.org/gv/GV7(3)11/6.pdf

Halgrain M, Bernardet N, Crepean M, Meme N, Narcy A, Hincke M, and Godbert SR (2022). Eggshell decalcification and skeletal mineralization during chicken embryonic development: Defining candidate genes in the chorioallantoic membrane. Poultry Science, 101(2): 101622. DOI: https://www.doi.org/10.1016/j.psj.2021.101622

Huth JC and Archer GS (2015). Comparison of two LED light bulbs to a dimmable CFL and their effect on broiler chicken growth, stress, and fear. Poultry Science, 94(9): 2027-2036. DOI: https://www.doi.org/10.3382/ps/pev215

Kerschnitzki M, Zander T, Zaslansky P, Fratzl P, Shahar R, and Wagermaier W (2014). Rapid alterations of avian medullary bone material during the daily egg-laying cycle. Bone, 69: 109-117. DOI: https://www.doi.org/10.1016/j.bone.2014.08.019

Lohakare JD, Kim JK, Ryu MH, Hahn TW, and Chae BJ (2005). Effects of vitamin C and vitamin D interaction on the performance, immunity, and bone characteristics of commercial broilers. Journal of Applied Poultry Research, 14(4): 670-678. DOI: https://www.doi.org/10.1093/japr/14.4.670

Lare L, Talaki E, Dzogbema KF, Sodjedo C, and Lombo Y (2021). Influence of dietary protein on embryonic development and hatching traits of indigenous quinea fowls (*numida meleagris*) breeders in Togo. Journal of World Poultry Research, 11(4): 422-430. Available at: http://eprints.science-line.com/id/eprint/236/1/JWPR%2011%284%29%20422-430%202021.pdf

Lin H, Jiao HC, Buyse J, and Decuypere E (2006). Strategies for preventing heat stress in poultry. World's Poultry Science Journal, 62(1): 71-86. DOI: https://www.doi.org/10.1079/WPS200585

Narushin VG and Romanov MN (2002). Egg physical characteristics and hatchability. World's Poultry Science Journal, 58(3): 297-303. DOI: https://www.doi.org/10.1079/WPS20020023

Nys Y, Gautron J, Garcia-Ruiz JM, and Hincke MT (2004). Avian eggshell mineralization: Biochemical and functional characterization of matrix proteins. Comptes Rendus Palevol (Palevol Reports), 3(6-7): 549-562. DOI: https://www.doi.org/10.1016/j.crpv.2004.08.002

Pande VV, Chousalkar KC, Bhanugopan MS, and Quinn JC (2015). Super pharmacological levels of calcitriol (1,25-(OH)2D3) inhibits mineral deposition and decreases cell proliferation in a strain dependent manner in chicken mesenchymal stem cells undergoing osteogenic differentiation *in vitro*. Poultry Science, 94(11): 2784-2796. DOI: http://www.doi.org/10.3382/ps/pev284

Romanoff LA (1960). Avian diseases. In: H.E. Evans (Editor), The avian embryo: Structural and functional development. American Association of Avian Pathologists., 4(4): 541-542. DOI: https://www.doi.org/10.2307/1587707

Rao PS, Kalva S, Yerramilli A, and Mamidi S (2011). Free radicals and tissue damage: Role of antioxidants. Free Radicalss and Antioxidants, 1(4): 2-7. DOI: https://www.doi.org/10.5530/ax.2011.4.2

Regassa A, Adhikari R, Nyachoti CM, and Kim WK (2015). Effects of 25-(OH)D3 on fecal Ca and P excretion, bone mineralization, Ca and P transporter mRNA expression and performance in growing female pigs. Journal of Environmental Science and Health, Part B, 50(4): 293-299. DOI: https://www.doi.org/10.1080/03601234.2015.999612

Richards MP, Rosebrough RW, Coon CN, and McMurtry JP (2010). Feed intake regulation for the female broiler breeder: In theory and in practice. Journal of Applied Poultry Research, 19(2): 182-193. DOI: https://www.doi.org/10.3382/japr.2010-00167

Roy S and Mishra SC (2011). Effect of antistress agents on haemato-biochemical profiles of broiler and breeder hen during summer. Veterinary World, 4(2): 60-63. Available at: http://www.veterinaryworld.org/Vol.4/February%20-%202011/Effect%20of%20Antistress%20agents%20on%20Haemato-Biochemical%20profiles%20of%20broiler%20and%20breeder%20hen%20druing%20summer.pdf

Rosa AP, Scher A, Sorbara JOB, Boemo LS, Forgiarini J, and Londero A (2012). Effects of canthaxanthin on the productive and reproductive performance of broiler breeders. Poultry Science, 91(3): 660-666. DOI: https://www.doi.org/10.3382/ps.2011-01582

Sastradipradja D, Sikar SHS, Widjayakusuma R, Maad A, Unandar T, Nasution H, Suriawinata R, and Hamzak K (1989). Penuntun Praktikum Fisiologi Veteriner. Pusat Antar Universitas Ilmu Hayati. Bogor.

Saunders-Blades JL and Korver DR (2014). The effect of maternal vitamin D source on broiler hatching egg quality, hatchability, and progeny bone mineral density and performance. Journal of Applied Poultry Research, 23(4): 773-783. DOI: https//www.doi.org/10.3382/japr.2014-01006

Saunders-Blades JL and Korver DR (2015). Effect of hen age and maternal vitamin D source on performance, hatchability, bone mineral density, and progeny *in vitro* early innate immune function. Poultry Science, 94(6): 1233-1246. DOI: https://www.doi.org/10.3382/ps/pev002

Scanes CG (2016). Biology of stress in poultry with emphasis on glucocorticoids and the heterophil to lymphocyte ratio. Poultry Science, 95(9): 2208-2215. DOI: https://www.doi.org/10.3382/ps/pew137

Siegel HS and Kampen MV (1984). Energy relationships in growing chickens given daily injections of corticosterone. British Poultry Science, 25(4): 477-485. DOI: https://www.doi.org/10.1080/00071668408454889

Torres CA, Vieira SL, Reis RN, Ferreira AK, Silva PXD, and Furtado FVF (2009). Productive performance of broiler breeder hens fed 25-hydroxycholecalciferol. Revista Brasileira de Zootecnia, 38(7): 1286-1290. DOI: https://www.doi.org/10.1590/S1516-35982009000700018

Ulmer-Franco AM, Fasenko GM, and O'Dea Christopher EE (2010). Hatching egg characteristics, chick quality, and broiler performance at 2 breeder flock ages and from 3 egg weights1. Poultry Science, 89(12): 2735-2742. DOI: https://www.doi.org/10.3382/ps.2009-00403

Van Hieu T, Guntoro B, Qui NH, Quyen NTK, and Al-Hafiz FA (2022). The application of ascorbic acid as a therapeutic feed additive to boost immunity and antioxidant activity of poultry in heat stress environment. Veterinary World, 15(3): 685-693. DOI: http://www.doi.org/10.14202/vetworld.2022.685-693

Van Lerberghe W, Keegels G, Cornelis G, Ancona C, Mangelschots E, and Van Balen H (1983). Haemoglobin measurement: The reliability of some simple techniques for use in a primary health care setting. Bulletin of the World Health Organization, 61(6): 957-965. Available at: https://apps.who.int/iris/bitstream/handle/10665/264977/PMC2536237.pdf?sequence=1&isAllowed=y

Yair R and Uni Z (2011). Content and uptake of minerals in the yolk of broiler embryos during incubation and effect of nutrient enrichment. Poultry Science, 90(7): 1523-1531. DOI: https://www.doi.org/10.3382/ps.2010-01283

Wang J, Qiu L, Gong H, Celi P, Yan L, Ding X, Bai S, Zeng Q, Mao X, Xu S, et al. (2020). Effect of dietary 25-hydroxycholecalciferol supplementation and high stocking density on performance, egg quality, and tibia quality in laying hens. Poultry Science, 99(5): 2608-2615. DOI: https://www.doi.org/10.1016/j.psj.2019.12.054

Schalm OW, Jain NC, and Carol EJ (2010). Schalm's veterinary hematology. In: Weiss DJ and Wardrop KJ (Editors), 6th Edition. Blackwell Publishing Ltd., Oxford. Available at: https://www.vet-ebooks.com/schalms-veterinary-hematology-6th-edition/

Whitehead CC and Keller T (2019). An update on ascorbic acid in poultry. World's Poultry Science Journal, 59(2): 161-184. DOI: https://www.doi.org/10.1079/WPS20030010