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EFFECT OF α-TOCOPHEROL AND ASCORBIC ACIDS ON PERFORMANCE AND BLOOD IMMUNITY PROFILE OF MALE NATIVE MUSCOVY DUCK

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ABSTRAK

Penelitian ini bertujuan mengkaji pengaruh α-tokoferol dan asam askorbat terhadap performan dan profil imunitas darah entok. Materi penelitian menggunakan 84 ekor entok jantan umur sembilan minggu. Penelitian dilaksanakan berdasarkan rancangan acak lengkap dengan 7 perlakuan yaitu E0C0: pakan basal tanpa diberi α-tokoferol dan asam askorbat, E400: pakan basal+400 IU α-tokoferol, E600: pakan basal+600 IU α-tokoferol, C400: pakan basal+400mg asam askorbat, C600: pakan basal+600mg asam askorbat, E200C200: pakan basal+200 IU α-tocopherol+200mg asam askorbat dan E300C300: pakan basal+300 IU α-tokoferol+300mg asam askorbat. Tiap perlakuan diulang sebanyak 4 kali dan setiap unit percobaan terdiri 3 ekor. Variabel yang diamati adalah bobot badan, konsumsi pakan, konversi pakan, jumlah leukosit, packed cell volume (PCV,) total protein plasma (TPP) , basofil, eosinofil, heterophyl, monosit, limfosit, dan rasio heterofil/limfosit (H/L). Hasil penelitian mengindikasikan bahwa perlakuan E0C0, E400, E600, C400, C600, E200C200 dan E300C300 berpengaruh tidak nyata terhadap bobot badan, konsumsi dan konversi pakan, berpengaruh nyata terhadap nilai PCV, tetapi tidak berpengaruh terhadap TPP. Perlakuan E0C0, E400, E600, C400, C600, E200C200 dan E300C300 berpengaruh nyata terhadap jumlah leukosit, persentase heterofil dan limfosit, tetapi tidak berpengaruh terhadap persentase eosinofil, monosit dan rasio H/L. C400 menghasilkan persentase PCV, heterofil dan rasio H/L tertinggi. C600 menghasilkan jumlah leukosit tertinggi. Suplementasi asam askorbat 400 – 600 mg/kg pakan dapat memperbaiki profil imunitas tetapi tidak memperbaiki performan entok.

Kata kunci: daya tahan tubuh, Entok, tocopherol, asam askorbat

ABSTRACT

The purpose of the research was to assess the effect of α-tocopherol and ascobic acids on the performance and immunity blood profile of male native Muscovy duck. The materials used were 84 male Muscovy ducks at 9 weeks old. The research used completely randomized design with 7 treatments. The treatments were E0C0 : basal diet without α-tocopherol and ascobic acids, E400: basal diet+ α-tocopherol 400IU, E600: basal diet+α-tocopherol 600IU, C400: basal diet+ascorbic acid 400mg/kg, C600: basal diet+ascorbic acid 600mg/kg, E200C200: basal diet+α-tocopherol 200IU+ ascorbic acid 200mg/kg, and E300C300: basal diet+α-tocopherol 300IU+ ascorbic acid 300mg/kg. Each treatment was repeated 4 times and each replication consisted of 3 ducks. The observed variables were body weight, feed consumption, feed conversion ratio, packed cell volume (PCV), total of plasma protein (TPP), leucocyte count, basophil, heterophil, eosinophil, monocyte, lymphocytes

count and heterophil/lymphocyte (H/L) ratio of Muscovy duck. The result indicated that E0C0, E400, E600, C400, C600, E200C200 and E300C300 had no significant difference in body weight, feed intake and feed conversion. There were significant effects on PCV, leucocyte count, percentage of heterophil and lymphocytes, but had no significant effect on eosinophil, monocyte and the H/L ratio. The C400 resulted a higher PCV count, percentage of heterophil, and H/L ratio. The C 600 produced the highest leucocyte count. In conclusion, the supplementation of ascorbic acid at 400 - 600 mg/kg feed ascorbic acid could improve the immune profile, but could not improve the performance of Muscovy duck.

Keywords: immunity, muscovy duck, tocopherol, ascorbic acid

INTRODUCTION

Indonesia climate with 20.4-36.8^{o}C day temperature, 18.4 – 4.2^{o}C of evening temperature and 55.3 – 85.8% of relative humidity (Badan Pusat Statistik, 20015) is not optimum for duck farming because this may induce stress in animal (El-Badry *et al.*, 2009). High temperature resulted in restraint of the development of poultry immune organs. Heat stress stimulates the release of corticosterone and catecholamines and initiate lipid peroxidation in cell membranes. α-Tocopherol supplementation is very effective for poultry because this may reduce the negative effects of corticosterone induced stress (Puthpongsiriporn *et al*., 2001). α-Tocopherol and ascorbic acid supplementations in diet, particularly in a combination, alleviate the counterproductive effects of high ambient temperature and humidity on the birds (Ajakaiye *et al*., 2010). They are protective agent that deactivates reactive oxygen species (ROS) and significantly prevent oxidative damage (Ramnath *et al*., 2008; Prisyanto *et al*., 2014). α-Tocopherol and ascorbic acid as an external antioxidant enhances an enzymatic and non-enzymatic antioxidant in preventing the free radical formation (Chevion *et al*., 2003).

α-tocopherol prevents the formation of free radicals by donating hydrogen ions, thus forming vitamin E radical. Furthermore enadiol and hydrogen at C2 and C3 of ascorbic acid serve as an electron donor to vitamin E radical, thus vitamin E in turn can function as an antioxidant. Ascorbic acids supplies electron to intracellular and extracellular biochemical activity and removes reactive oxygen in cell. Ascorbic acid deficiency results in decreased immune response, depressed response to mitogenic, disturbed immunoglobulin metabolism, depressed T lymphocyte responses and antibody production (Ramnath *et al*., 2008).

Accordingly, a welfare disorder in the duck is observable from three indicators - physiology, production and immune system (Mcgrath *et al*., 2010; Lawrence and Stott, 2009). Body weight, feed intake and feed conversion are the visible growth performance indicator in Muscovy duck (Ogunwole *et al.*, 2013). Blood is an important component to organize body physiology and serves as a fowl health indicator (Syahruddin *et al.*, 2013; El-Badry *et al.*, 2009). A good blood profile will support better physiology. Sejian *et al.* (2011) stated that animal welfare involves adaptations of normal physiology and behaviour leading to health status that ultimately increases productivity. Leucocyte is part of the immune system against several infectious diseases, while erythrocyte determines physiology. Leucocyte can move freely, interact and capture cellular debris, foreign material or intruding microorganism. Total plasma protein in blood is 7.2 - 8 g/dL or 7% of whole blood and only 2-3% of total body protein (Ismoyowati *et al.,* 2006) having multi-function, such as circulating lipid molecule, hormone, vitamin and zinc, enzyme, complement components, protease inhibitor and regulating activity of non cellular functional in the immune system (Moyes and Schulte, 2008). Packed cell volume (PCV) is the percentage of cell content in blood. PCV measures erythrocyte percentage in whole blood volume. PCV is erythrocyte fraction described in percent of all blood (Gandasoebrata, 2004). The purpose of this research was to assess the effect of α-tocopherol and ascobic acids on performance and immunity blood profile of native male Muscovy duck.

MATERIALS AND METHODS

A total of eighty four male Muscovy ducks aged at 8 weeks old were given feed with 21% protein and 3100 kcal/kg ME (Table 1). The birds were assigned in 28 litter cage sized 2x1m^2. The cage was provided with feeders and drinkers, scale and thermometer. All birds had regular medicine and vaccines.

Table 1. Composition and Nutrients Content of Basal Treatment Diet

Feed ingredient	%	Calculated Nutrient	Amount
Corn	30.00	Crude protein (%)*)	21.02
Soybean Meal	7.00	ME (kcal/kg)*)	3103
Rice bran	38.20	Extrat ether (%)*)	5.73
Poultry Meat Meal	17.00	Crude fiber (%)*)	5.53
Oil	6.10	Ca (%)	0.94
CaCO3	1.00	P_{av} (%)	0.56
Topmix	0.20	Lysine (%)	1.11
NaCl	0.10	Metionine (%)	0.64
L-lysine HCl	0.10	Methionine + cystine (%)	0.65
DL-Methionine	0.30		
Total	100.00		

The experimental research was allocated to completely randomized design. Seven treatments were E0C0 : the basal diet without α-tocopherol and ascorbic acids, E400: the basal diet + α-tocopherol 400IU, E600: the basal diet + α-tocopherol 600IU, C400: the basal diet + ascorbic acid 400mg/kg, C600: the basal diet + ascorbic acid 600mg/kg, E200C200: the basal diet + α-tocopherol 200IU + ascorbic acid 200mg/kg, and E300C300: basal diet + α-tocopherol 300IU + ascorbic acid 300mg/kg. The muscovy ducks were randomly placed in four replicates with 3 ducks of each group in litter cage. Data were tested using analysis of variance followed by honestly significant difference test. The observed variables were body weight, feed consumption, feed conversion ratio, packed cell volume (PVC), leucocyte count, heterophil percentage, eosinophils percentage, lymphocytes percentage and monocytes percentage, heterophil/lymphocyte (H/L) ratio. In each bird, body weight was measured weekly and feed intake was recorded daily.

Blood sample of Muscovy duck was taken at 14 weeks old for hematology analysis. Body weight was recorded weekly and feed conversion was calculated daily. Blood sampling was carried by a veterinarian and in conformity with that taken from the brachial vein. The blood sampling was in accordance with the ethics code of Indonesian Veterinarian and the Laws of the Republic of Indonesia No. 18, 2009 on Animal Husbandry and Animal Health those obtained a certificate of eligibility of animal conduct (Animal Ethical Clearence) No 282540009/3/2013. PCV analysis was conducted by aspirating blood up to 1cm from the top end using Hawkslay. Capillary tube was clogged with crystal seal, wax or soap, then placed in micro centrifuge in open capillary end center-faced and centrifuged for 5 minutes at 12.000 RPM, after which the capillary pipe was taken out to read PVC value using hematocrit reader. Refractometer hand was used to count blood Total Plasma Protein. One or two drops of the separated/centrifuged serum or blood plasma was put on the plate and pressed with a plastic lid to see the amount of total plasma protein in gram at certain scales.

Leucocyte count started by blood dilution with a Turk solution in leucocyte pipe then put in counting chamber (Nugroho, 2013). The investigation was as follows: a) capillary blood, EDTA blood or oxalate blood was aspirated to 0,5 bars; b) blood droplets were discarded on pipette tip; c) The pipette tip was dipped into the Turk solution at 45^{o} angle and hold at 0.5 bars. The Turk solution was aspirated to 11 bars to avoid air bubble; d) pipette tip was covered by finger, then the rubber bulb was removed; e) shaking for 15-30 seconds; f) storing with lid on horizontally on the table inside the counting chamber;

g) Pipette was shaken for 3 min to keep the fluid from spilling out; h) all fluids in capillary pipe were discarded (3-4 drops) and quickly tap pipette tip on counting chamber by touching the glass cover rim with 30^{o} angle. The fluid was filled with counting chamber with capillary pressure; i) leucocyte was left for 2-3 minutes until settle; j) objective microscope lens was used with 10x magnification, focusing on the bisecting lines; k) leucocyte was counted in four big area from upper left to right, bottom to left, bottom to left and so on. Cells in line were counted from left and upper lines; l) total leucocyte per µL blood was–cell count.

RESULTS AND DISCUSSION

Muscovy Duck Performance

The high and low of duck meat production was significantly affected by physiological performance. The observed performance indicator was fed intake, body weight and feed conversion. The treatments of E0C0, E400, E600, C400, C600, E200C200 and E300C300 resulted in higher body weight than the control. The highest body weight of Muscovy duck in the research (2357.50 ± 85.78g) was reached by C400, 10 analysis of variance result showed that they did not significantly affect (P>0.05) on body weight, feed intake and feed conversion of 14 week old male Muscovy duck (Table 2). These results agree with studies of In agreement with results of Schiavone *et al.*, (2010) and Marzoni *et al.*, (2014). They found that the performance 42 of Muscovy duck (live body weight at 63 days, feed consumption and feed conversion ratio) were not influenced by dietary treatments of 20 kg soybean oil + 30-230 mg/kg α -tocopheryl acetate and natural antioxidant supplementation.

Surai *et al.* (2003) reported that α-tocopherol and ascorbic acid and its combination improve body vitality as proven by zero mortality or disease occurance in duck during research period and the increased hemoglobin compared to control, however this could not trigger hunger center in hypothalamus so that Muscovy duck consumed more feed.

Packed Cell Volume (PCV) and Total protein Plasma (TPP)

The observed physiological indicator was PCV and TPP. Blood is essential for physiological process in fowl, mainly for enzyme activity and hormone synthesis, and hormone concentration in plasma will affect productivity (Ismoyowati *et al.,* 2006).

Supplementation of C400 in feed tended to increase the values of PCV and TPP. The lowest PCV was reached by E0C0 and the lowest of TPP was reached by E200C200 (Table 3). The result of variance analysis showed that E0C0, E400, E600, C400, C600, E200C200 and E300C300 significantly affected (P<0.05) the PCV percentage, but had no significant effect on protein plasma level of a 14 week old male duck (Table 3). There was a different (P<0.05) in PVC

Table 2. Body Weight, Feed Consumption, FCR of Muscovy Duck Aged 14 Weeks Old Feed α-Tocopherol and Ascorbic Acid

Treatments	BW(g) ^{ns}	FC (g) ^{ns}	FCR^{ns}
E0 C0	2210.42 ± 79.68	9018.50 ± 157.12	4.08 ± 0.23
E 400	2280.76 ± 144.32	9396.75 ± 280.15	4.12 ± 0.22
E 600	2264.69 ± 45.07	9398.50 ± 114.51	4.15 ± 0.17
C 400	2357.50 ± 85.78	9170.68 ± 240.37	3.89 ± 0.11
C 600	2288.68 ± 264.19	9360.75 ± 198.29	4.09 ± 0.18
E200C200	2358.46 ± 81.29	9481.00 ± 134.67	4.02 ± 0.30
E 300C 300	2248.70 ± 94.84	9039.75 ± 159.55	4.02 ± 0.22

BW = body weight, FC= Feed consumption 37 CR= Feed Conversion Ratio (9-14 weeks old), ^{ns} Means without superscripts in the same column is not differ significantly (p>0.05)

Table 3. PVC and TPP level of Muscovy Duck Aged 14 Weeks Old Feed α-Tocopherol and Ascorbic Acid

Treatments	PCV (%)*	TPP(d/dl) ns
E0 C0	36.25 ± 0.50[a]	3.70 ± 0.38
E 400	42.00 ± 4.08[ab]	3.80 ± 0.23
E 600	41.00 ± 4.24[ab]	3.75 ± 0.10
C 400	44.25 ± 2.22[cb]	4.50 ± 1.18
C 600	42.50 ± 2.38[ab]	4.15 ± 0.86
E200C200	41.50 ± 2.65[ab]	3.55 ± 0.53
E 300C 300	40.25 ± 3.59[ab]	3.7 0± 0.80

PCV= Packed Cell Volume,TPP = Total Protein Plasma, ns Means without superscripts in the same column is not differ significantly (p>0.05); * Means with different superscripts in the same column is differ significantly (p<0.05)

value between control and C400 treatments. The PCV value of Muscovy duck in the research was lower than Muscovy duck in southeastern Nigerian (46.00 ± 1.73) from Okeudo *et al.* (2003). Ismoyowati *et al.* (2012) stated that PCV and total plasma protein level during dry season was lower than that in the wet season. This indicated that environmental factors, namely temperature and humidity strongly influenced the duck's physiology..

Supplementation of ascorbic acid at 400 mg/kg feed attributed to the increased absorption of Fe and Cu as the main components of red blood cell. Ascorbic acid inhibits hemosiderin formation that is less mobilezable to free Zn when needed. Ascorbic acid in feed would induce an acidic condition, thus reducing ferric to more absorbable ferrous in the small intestines. Absorption of non heme iron is quadruple owing to ascorbic acid (Adriani and Wirjatmadi, 2012).

PCV or hematocrit highly depends on erythrocyte count, because erythrocyte is the biggest cell mass of blood. PCV value rise and fall affects blood viscosity, the higher PCV percentage more red blood cell produced, and ascorbic acid boosts iron sufficiency and absorption, so that animals are free from anemia (Winarsi *et al.*, 2005). Plasma proteins consist of albumin, globulin and fibrinogen and they play a crucial role in maintaining homeostasis. These proteins have multiple functions; albumin is the most abundant and osmotically active plasma protein, and it is an important carrier of many substances in the peripheral circulation. Globulins are classified on the basis of their electrophoretic mobility as alpha-, beta- and gamma-globulins. While fibrinogen is important in blood clot formation, thereby preventing loss of blood from a ruptured blood vessel. The decreasing total protein concentration is due to the progressive albumin increase and globulin decrease (Harvey, 2001; Nelson and Cox, 2008). This indicated the well-functioning transport of bilirubin, thyroid hormone, cytoskeletal components, extracellular compounds and as protein carrier.

The use of plasma in biochemistry and hematology is for monitoring the health conditions of birds. It is also useful for distinguishing pathogenic processes from those that might be purely physiological (Ortizo *et al.*, 2014). Albumin serves as the major amino acid pool, the catabolism of albumin provides protein precursors needed for growth or other physiological needs. The high performance of production has a low of total plasma protein (Wang *et al.*, 2013). Plasma proteins are the key components of plasma and they play a crucial role in maintaining homeostasis (Yaqub *et al.*, 2013).

Immune Profile

Hematological and plasma biochemical profiles provide reliable information on the health status of poultry (Ismoyowati *et al.*, 2012). Table 4 showed that supplementing α-tocopherol, ascorbic acid and their combined did not significantly affected on monocyte and eosinophil percentage and H/L ratio of duck., but significantly affected total leucocyte count, heterophil percentage, and lymphocyte percentage.

Leucocyte count of control group was significantly differed (P<0.05) with E600, C400, E200C200 and C600 groups (Table 4). This result was in line with Selim *et al.* (2012) that 10 mg α-tocopherol and 3 mg ascorbic acid are very effective on humoral immunity and immune cells of Muscovy ducks. The immune system required antioxidants produce and maintained the balance of immune cells (haematopoesis), protects cell membranes from ROS, to fight microorganisms caused disease. Ascorbic acid affects the immune system by stimulating neutrophils and macrophage function, thus increasing chemotaxis and mobility, enhancing phagocytosis and

Table 4. Immune Profile of Muscovy Duck Aged 14 Weeks Old Feed **α**-Tocopherol and Ascorbic Acid

Treatments	Leukocyte count (cell./µl)^{ns}	Heterophyls (%)*	Eosinofil (%)^{ns}	Limfosit (%)*	Monosit (%)^{ns}	Rasio H/L^{ns}
E0 C0	9,650.00^{a}	17.25^{a}	16.50	40.75^{a}	14.50	0.42
E 400	13,312.50^{a}	28.75^{a}	14.00	64.25^{b}	14.25	0.45
E 600	15,112.50^{b}	29.75^{b}	1850	55.00^{a}	15.00	0.54
C 400	15,725.00^{b}	31.50^{b}	12.75	55.00^{a}	6.00	0.57
C 600	17,387.50^{b}	30.00^{b}	13.50	66.75^{b}	10.75	0.45
E200C200	14,925.00^{b}	30.75^{b}	9.25	69.00^{b}	12.25	0.45
E 300C 300	13,437.50^{a}	31.25^{b}	11.00	60.75^{a}	18.50	0.51

^{ns} Means without superscripts in the same column is not differ significantly (p>0.05); * Means with different superscripts in the same column is differ significantly (p<0.05)

enhance the ability of killing bacteria (Wintegrest *et al.*, 2007). α-Tocopherol has a role as an immunomodulator, increases lymphocyte proliferation and promote the cytokine production (Lee and Wan, 2010). α-Tocopherol, may also inhibit the activity of protein kinase C involved in cell proliferation and differentiation of cells in smooth muscle cells, platelets, and monocytes (Chandra, 2002). Lawhead and Baker (2005) stated that total count and type of leucocyte help to diagnose animal condition or infectious status. High leucocyte count may indicate high immunity in ducks and fast eliminating infection. Total leucocyte count in Muscovy duck in this research was 9650±1984.10 – 17387.50±835.04 (cells/µl), relatively similar with Kabir (2012).

Heterophyl percentage of C600, C400, E200C200 and E300C300 groups were significantly differed (P0<0.05) with the control and E600 groups (Table 4). Table 4 also shows that lymphocyte percentage of C600 group was significantly differed (P<0.05) with E400, E600, C400, E300C300 and control groups. Lymphocyte percentage of E200C200 group was significantly differed (P<0.01) with E400 and E600 groups, C400 and E300C300 groups. The lymphocyte percentage of E0C0 group was higher (P<0.01) than that of C600 group. These results showed that supplementing α-tocopherol and ascorbic acid improve the immune system by increasing lymphocyte count. Lymphocyte produces antibody to help disease prevention (Lawhead and Baker 2005), because α-tocopherol produces interleukin-2 that improves body immune, prevents disease and accelerates recovery, while ascorbic acid actively rejuvenates germ-killing cells, helps the production of interferon that kills the virus in the body and increases glutathione in the body (Surai *et al.,* 2003).

The H/L ratio was not affected by the supplementation treatments (Table 4). This was because α-tocopherol and ascorbic acid increased the amount of heterophil and lymphocyte that improved ducks immunity. Furthermore, α-tocopherol and ascorbic acid improve phagocyte activity by increasing lymphocyte count and preventing a decrease in hemoglobin, leucocyte and thrombocyte (Prisyanto *et al*., 2014). Ismoyowati *et al.* (2012) stated that the fowl comfort may be indicated by measuring the H/L ratio. The H/L ratio is more liable as a fowl comfort than blood corticosterone level. McGrath *et al.* (2010) stated that fowl in a good welfare or normal physiological condition is indicated with a lower H/L value than that of distress environment. Ismoyowati (2007) reported that highly productive local duck has an average H/L ratio of 0.417± 0.125

CONCLUSION

Supplementing 400-600 mg/kg feed ascorbic acid improve the blood immune profile, but could not improve the performance of Muscovy duck.

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