PHYSIOLOGICAL RESPONSES OF BLOOD AND IMMUNE ORGANS OF BROILER CHICKEN FED DIETARY BLACK CUMIN POWDER (*Nigella sativa*) DURING DRY SEASONS

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ABSTRAK

Penelitian ini bertujuan untuk mengetahui respon fisiologi darah dan organ limfoid ayam broiler akibat pemberian jintan hitam selama musim kemarau. Materi yang digunakan dalam penelitian ini adalah 100 ekor DOC ayam broiler *unsex* strain CP 707, serta ransum broiler fase *starter* dan *finisher* yang ditambahkan tepung jintan hitam. Penelitian menggunakan rancangan acak lengkap dengan 5 perlakuan dan 4 ulangan, dan tiap ulangan terdiri atas 5 ekor ayam. Perlakuan yang diterapkan adalah T0 (Ransum basal); T1 (Ransum basal dan antibiotik dan multivitamin); T2 (Ransum yang ditambahkan jintan hitam 20 g/kg ransum); T3 (Ransum yang ditambahkan jintan hitam 40 gram/kg ransum); T4 (Ransum yang ditambahkan jintan hitam 60 g/kg ransum). Hasil penelitian menunjukan bahwa pemberian tepung jintan hitam (*Nigella sativa*) dalam pakan sebagai *feed additive* tidak dapat meningkatkan ketahanan tubuh pada ayam broiler, tetapi dapat meningkatkan persentase berat organ limpa pada level jintan hitam 60 g/kg ransum dimana dapat mengurangi dampak infeksi penyakit yang masuk ke dalam darah.

Kata kunci : jintan hitam, respon fisiologi, organ limfoid, ayam broiler

ABSTRACT

This study was designed to determine the physiological response of blood and immune organs of broiler chickens fed on various concentration of dietary black cumin powder (BCP) during the dry season. A total number of 100 unsexed one-day old Cobb broiler chicks were used and distributed to 5 treatments (control, antibiotics and without BCP, 20 g/kg BCP, 40 g/kg BCP and 60 g/kg BCP) and 4 replications (5 birds for each). Physiological responses of blood and immune organs were measured at 30 day of age. Addition of BCP to broiler ration did not significantly effects on physical properties of blood (leukocytes count, erythrocytes count, haemoglobin, hematocrit, monocytes, and eosinophils) and relative weights of thymus and bursa of fabricius, but significantly ($P<0.05$) increased relative weights of spleen when compared to control. It was concluded that the black cumin grinds (Nigella sativa) as a feed additive could not change the physical properties of blood, relative weights of thymus and bursa of fabricius, but it increased the relative weight of spleen at the level of 60 g/kg BCP, which could reduce adverse effects of infectious diseases in broiler chicken.

Keywords: Nigella sativa, physiological response, lymphoid organs, broilers

INTRODUCTION

The ambient temperature may influence the immune response and performance of broilers. High temperature as heat stress increase the level of glucocorticoid hormones, which cause suppression of cell proliferation factor, or interleukin II then can be disrupt the formation of the immune system on broilers (Siegel and Latimer *et al*, 1984; Mashaly *et al*., 2004). Various strategies have been suggested to overcome the negative effect of heat stress in broilers by addition antibiotics as feed additive. Most retrospective and prospective studies showed that after the introduction of an antibiotic not only the level of resistance of pathogenic bacteria, but also of commensally bacteria increases (Van den bogaard and Stobberingh,

2000). Therefore, it needs an alternative antibiotic feed additive natural replacement that can increase the formation of the immune system on broiler and safety for human health. Broiler immunity against a variety of disease agents by improving the function of immune system cells and a disruption of the immune system can be determined by physiological conditions such as hematologic conditions and biochemical values in the blood and also lymphoid organ weights (Togyani *et al*., 2011). Isroli *et al*. (2009) reported that the level of immunity in chickens can be determined using the level of blood of erythrocytes, leukocytes and differential of leukocytes. Phagocytosis process in leukocyte will protect against the disease in the body (Guyton and Hall, 2006). The number of blood leukocytes in broiler chickens depends on the conditions of stress, physiological activity, nutrition, age; and an abnormal blood leukocytes reflects some disease conditions in broiler chickens (Delman and Brown, 1989). Immune system disorders as a result of physiological response also can be determined using the relative weight of lymphoid organs such as bursa Fabrisius, thymus, and spleen.

The active component of plant herbs as feed additive can substitute the function of dietary antibiotic supplementation. Mutia *et al*. (2012) showed that addition of plant herbs can lead to increase relative weight of bursa Fabrisius. Bursa of fabricius serves as primary lymphoid organs that are able to capture the antigen which produces antibodies and B-lymphocytes (B-cell) (Subowo, 1993). The spleen serves as an immunological response against the antigen (Delman and Brown, 1989). Thymus is a source of the factor in the blood (blood-borne), which stimulates the differentiation of lymphoid precursors or stem cells as it participate in immune reactions.

Active substance of herbal plants can increase the endurance of chicken which also substituting for antibiotic feed addititive. Black cumin as a herbal plant can increase endurance of broilers. Tymoquinone, the active component of black cumin, serves as an anti-oxidant, anti-infection, anti-tumour and anti-inflammatory (Ragheb *et al*., 2009). Supplementation of black cumin in ration can cause a significant increase in leukocyte count, hemoglobin and alanine aminotransferase (Al-Homidan *et al*., 2002; Hermes *et al*., 2010). Nasir (2009) reported that the active component of black cumin (thymoquinone, dithymoquinone, thymol and carvacrol) can improve the digestibility of food and absorption of nutrients by stimulating digestive enzymes. Therefore black cumin as feed additive can increase endurance as seen from the physiological condition of the body of broiler chicken. This study was designed to determine the physiological response of blood and immune organs of broiler chickens fed on various concentration of dietary black cumin during the dry season.

MATERIALS AND METHODS

Experimental Design

One day old unsex Cobb's type broiler chicks (100) provided by the Charoen Pokphand Poultry Public Co., East Java, were housed at the experimental farm station of the Faculty of Animal and Agricultural Sciences, Diponegoro University. Products of antibiotics (Oxytetracyclin Therapy), vitamin (Multivitamin vitachick and vitastress) and vaccines (vaksin Medivac ND, Medivac Gumboro A, Medivac ND-Clone 45) were provided by Medion Farm Jaya Company, Bandung West Java. Feed and drinking water were offered *ad-libitum* for 28 days of the experimental period. All chicks were kept under same managerial, hygienic and environmental conditions and were maintained on a 24 hours/day artificial light. Ambient temperature (°C) and relative humidity (%) inside experimental room were recorded 3 times daily throughout experimental period, and the average values were ranged from 29 to 35 °C and 56 to 91%, respectively.

Starter chicken diet was replaced by the finisher diet at 3 weeks. Feeding on the control and test diets continued for 4 weeks. The experimental diets were formulated to contain adequate levels of nutrients for broiler chicks according to the strain manual recommendation. Chicks were fed two types of diets, starter (2994 Kcal ME / kg and 22.91% CP from 0-21 days-old and finisher (3054 Kcal ME / kg and 20.01% CP from 21- 28 days-old. At 1 d, the chicks were assigned randomly to 5 treatments and 4 replications (5 birds for each treatment). Treatment 1 was the control diet. Treatment 2 was the control diet supplemented with antibiotics, and treatment 3 was control diet supplemented with 20 g/kg BCP. Treatment 4 was control diet supplemented with 40g/kg black cumin powder

(BCP) and Treatment 5 was control diet supplemented with 60 g/kg BCP. Broiler chicks were fed on the experimental diets from 1 to 28 days of age; Chicks were individually weighed at one and 28 days of age.

Immune Organs and Haematological Analyses

At the end of the experimental period, birds from each treatment were individually weighed and slaughtered. Weight of immune organs (bursa Fabrisius, Spleen and thymus glands) were separately recorded and expressed as a percentage of live body weight (LBW). Blood samples from slaughtered birds were collected into heparinzed tubes. Blood sample of each bird was used to determine the physiological response namely red blood cells count (RBCs), white blood cells count (WBCs), differentiation of leukocytes (monocytes and eosinophils), packed cell volume (PCV), according to the standard techniques of Jain (1986). Haemoglobin content was measured by a colorimetric method.

Statistical Analysis

Data were statistically analyzed using the procedure of general linear model of SPSS 16.0. Least square means were calculated for each parameter, and differences among treatment means were tested using a multiple range test (Steel and Torrie, 1980).

RESULTS AND DISCUSSION

Effect of Dietary *Nigella sativa* Grinds on Physiological Response of Blood

Erythrocytes (RBCs) are synthesised in bone marrow and they contain haemoglobin (Hb) and their main function is oxygen transport. The proportion of the blood marked by RBCs is called as hematocrit. Leucocytes (WBCs) are part of immune system; they destroy and remove old or aberrant cells and cellular debris, as well as attack infectious agents and foreign substances (Nasir, 2009). The effect of dietary *Nigella sativa* on physiological responses of bloods were observed in this study such as erythrocytes, leukocytes, differentiation of leukocytes (eosinophils and monocytes), haemoglobin and hematocrit taken during the finisher period (Table 1).

As shown in Table 1, black cumin (T2-T4) as a feed additive had no effect on RBCs and WBCs counts, hematocrit and Hb throughout experiment ($P>0.05$) compared to control (T0) and antibiotics (T1) treatmens. Similar result was reported by Miraghaee *et al*. (2011) about the effect of black cumin powder on the RBCs and WBCs counts in broiler chickens. Al-Homidan *et al*. (2002) also showed that the supplementation of black cumin of 20 g/kg and 100 g/kg in a diet did not affect erythrocytic, Hb, and hematocrit of chicks, which were observed throughout 7 weeks of period. In contrast, Toghyani *et al.* (2010) reported that supplementation of black cumin significantly increased ($P<0.05$) RBC count, hemoglobin concentration and hematocrit percentage of birds. Our results showed that supplementation of black cumin at levels of 20 g/kgBCP (T2), 40 g/kgBCP (T3) and 60 g/kgBCP (T4) did not affect RBCs and WBCs counts, hematocrit and Hb of chicknes (Table 1). Hermes *et al.* (2010) also reported that supplementation of *Nigella sativa* meal at levels of 10 g/kg and 20 g/kg had no effect on RBCs and WBCs counts, hematocrit and Hb throughout the experimental period to overcome the deleterious effects of high temperature or any stressful conditions. This may be due to the high environmental temperature that may cause the active component of black cumin in improperly worked. Lee *et al.* (2003) reported that well-nourished, healty chicks do not respond to antibiotic supplements because they were housed under clean and disinfected condisions. Thus, it is possible in present trial that the treatments had no any beneficial effect on birds performances due to hygienic status of trial (Al-Ankari and Homeida, 1996). However, more trials are needed to clarify the effect of black cumin on physiological responses of bloods on broilers with regard to varied management conditions, including different stress factors, herbal extracts and their optimum dietary inclusion levels, dietary ingredients and composition.

Table 1 showed that average percentage of eosinophils is 3.35±1.09%. The supplementation of black cumin as a feed additive had no significant effect on percentage of eosinophils and monocytes in broiler chickens. Khalaji *et al.* (2011) also showed that the addition of black cumin seeds had no effect on percentage of eosinophils but it affected the percentage of monocytes in broiler chickens. These studies indicated that black cumin had the same effect on immune system for attacking the infection sources during dry season. This was due to very low intake of the active component to increase the number of eosinophils in the blood during the summer. Throughout the experimental period, average ambient temperature, relative humidity,

Table 1. Effect of Dietary *Nigella sativa* Grinds on Physiological Response of Blood of Broiler Chickens at 28 Days of Age with SEM

Treatment	RBC (jt/µl)	WBC (10^3/µl)	Haemoglobin (g/dL)	PCV (%)	Eosinophils (%)	Monocytes (%)
T0	2.70±0.24	9.29±0.27	5.73±0.38	27.25±1.25	3.0±1.58	14.00±4.76
T1	2.78±0.24	8.48±0.77	5.18±0.34	25.75±1.11	1.5±0.87	17.00±2.45
T2	3.06±0.31	8.73±0.53	6.58±0.35	27.50±1.44	5.5±4.86	17.50±2.18
T3	2.61±0.16	9.90±1.31	5.70±0.36	26.25±0.95	4.3±2.32	15.50±3.66
T4	2.60±0.10	9.90±0.44	6.28±0.35	26.50±0.29	2.5±1.56	15.75±3.61

RBC = red blood cells; WBC = white blood cells; PCV = hematocrit; SEM = Standard Error Mean; T0 = control diet (without BCP); T1= control diet with antibiotics; T2 = diet contained 20g/kg BCP; T3 = diet contained 40g/kg BCP; T4 = diet contained 60g/kg BCP

and H/L ranged from 29 to 35 °C; 56 to 91%; and 1.68; respectively. The environmental experiment during dry season may change concentrations of blood cortisol that in turn effecting on the inflammation and immune responses. The cortisol is a glucocorticoid steroid hormone which reduces the rate of proliferation in B cells, T cells, eosinophils, and neutrophils (Padgett and Glaser, 2003). McFarlane and Curtis (1989) found that an elevation in ambient temperatures (30.4 and 34.8 °C) for 7 days caused an increase in the H/L ratio in female broiler chickens, and that the leukocyte ratio changes are less variable and more stable than plasma corticosterone levels. Altan *et al.* (2000) also reported that an increase in ambient temperature up to 39 ± 1 °C for two hours causes an increase in the heterophil/basophil ratio and the H/L ratio from 0.25 to 0.43, and it causes a decrease in the monocyte/lymphocyte ratio. Although the number of eosinophils are not affected by the increased ambient temperature.

The Effect of Black Cumin (*Nigella sativa*) on Immune Organs Weight on Broiler Chickens

The effect of dietary black cumin on immune organs weight of broiler chickens were expressed as relative weight of bursa of fabricius, spleen, and thymus taken during the finisher period (Table 2). The supplementation of black cumin and antibiotics in the diet had no significant effect on weight bursa of fabricius of chickens. These results showed the active contents of black cumin were not able to increase the lymph follicles in the bursa of fabricius so it did not increase the formation of lymphocytes. Bursa of fabricius size may change when the chickens have a disease. The more often bursa of fabricius produces antibodies so it may cause the depletion of lymphoid follicles that in turn reducing relative weight of bursa of fabricius. Continuous release of glucocorticoid may cause atrophy of the lymphoid organs such as the bursa fabricius, thymus, spleen, lymph nodes (Susilowati, 2010). The same results of also reported by Tollba and Hassan (2003), the supplementation of black cumin in the feed did not effect on the weight of the bursa of fabricius.

Table 2 shows average of spleen percentage is about 0.13±0.01%. Present results showed that supplementation of black cumin at 40 g/kg BCP (T3) and 60 g/kg BCP (T4) in the diet significantly increased ($P<0.05$) of the spleen weight per body weight of broiler, but there were no significant different among T0, T1, T2 in spleen weight of chickens. It also explained that the black cumin supplementation at level of 20 g/kg BCP and 40 g/kg BCP showed the similar effect with the supplementation of antibiotic on the relative weight of spleen.

Supplementation of antibiotic in diets could not improve the immune system of chickens that were reflected by the weight of spleen. Similar result was also reported by Dafwang *et al*. (1985), the supplementation of antibiotics did not effect on the weight of spleen and antibody responses of birds. In addition, there was no advantage effect of antibiotics supplementation to increase immunity, and there was no negative impact on the humoral immune system of chickens supplemented with antibiotics (Dafwang *et al*.,

Table 2. Effect of Dietary *Nigella sativa* Grinds on Relative Weight of Immune Organs of Broiler Chicks at 28 Days of Age with SEM

Treatment	BF (%)	SP (%)	TH (%)	Significance		
				BF	SP	TH
T0	0.057±0.009	0.10±0.01[a]	0.30±0.09	ns	ns	ns
T1	0.051±0.011	0.11±0.01[a]	0.24±0.03	ns	ns	ns
T2	0.059±0.017	0.14±0.02[a]	0.29±0.02	ns	ns	ns
T3	0.078±0.010	0.11±0.01[ab]	0.27±0.05	ns	*	ns
T4	0.061±0.013	0.19±0.04[b]	0.31±0.02	ns	*	ns

BF = Bursa of fabricius; SP = Spleens; TH = Thymus; T0 = control diet (without BCP); T1= control diet with antibiotics; T2 = diet contained 20g/kg BCP; T3 = diet contained 40g/kg BCP; T4 = diet contained 60g/kg BCP**;** * = significant (P<0.05); ns = non significant (P>0.05)

1985). According to the report of Kalavathy *et al*. (2008), the supplementation of oxytetracycline in diet increases feed efficiency prior to heat stress treatment, but it give no responses during heat stress treatment in chickens. In contrast, Forsgreen and Gnarpe (1973) reported that the use of antibiotics such as oxytetracycline can boost the immune system work. These differences in the response to antibiotics may be due to the type of antibiotic and broiler strains those were used in studies. According to the report of Zulkifli *et al*. (2000) the supplementation of Lactobacillus culture in the diet gave better feed efficiency than that of oxytetracycline supplementation in Hubbard and Shaver strains of broiler chickens.

Present results showed that supplementation of black cumin at 40 g/kg BCP (T3) and 60 g/kg BCP (T4) in the diet increased of the spleen weight per body weight of broiler. These results showed the active contents of black cumin were changes in the expansion around the white pulp which is a formation that will affect spleen lymphocytes. White pulp consists of lymphoid follicles those are generally composed of B lymphocytes (B-cells). Some studies showed that active contituents of black cumin exert stimulatory roles toward T cell-mediated immune responses (Swamy and Tan, 2000; Islam *et al.* 2004). The increase in relative weight of thymus and spleen may be caused by the content Nigello, and it can activate lymphoid organs and serum globulin (El-Kaiaty *et al*., 2002). Similar result was reported by Khasanah (2009), black cumin supplementation effects on the relative weight of spleen and the number of lymphoblast in mice after infection of *Salmonella typhymurium*. Black cumin increases body immunity by increasing lymphoblast which resulted in an increase in the function of T helper and NK cell functions. Therefore, black cumin can be used for increasing body immunity of chickens. Spleen will filter out foreign substances that enter in the blood. The presence of foreign objects in the spleen causes reactive processes which macroscopically looks like a swollen spleen or a large number of erythrocytes appear filled in the sinuses and pulp, as well as filled with polimort nucleated of leukocytes, lymphocytes and plasma cells (Ressang, 1984).

Supplementation of black cumin and antibiotics in the diet had no significant effect on weight of thymus. The effects of these supplements might be observed under less hygienic housing conditions. This indicated that the chicken did not indicate an acute disease in the thymus organ for all treatments, because the thymus is an indicator for the last of the chicken immune lymphoid organs. The broiler chickens in the present study were kept in clean, disinfected conditions of minimal bacterial in cages, which would possibly lead to a reduced efficacy of any dietary black cumin and antibiotic. Lee *et al*. (2003) reported that well-nourished, healty chicks do not respond to antibiotic supplements provided that they are housed under clean and disinfected condisions, thus it is possible in present trial the treatments had not any beneficial effect on performance indices due to hygienic status of trial

(Al-Ankari and Homeida, 1996).

CONCLUSION

Addition of antibiotics oxytetracyclin and black cumin powder (*Nigella sativa*) at level 2 or 4% as feed additives in the diet had the same ability to increase endurance on physiological responses of blood and immune organs of broiler chicken on dry season, but black cumin supplementation at level of 6% can improve growth of the spleen weight of broiler.

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