ISSN 1682-8356
ansinet.org/ijps

INTERNATIONAL JOURNAL OF

POULTRY SCIENCE

ANSInet
308 Lasani Town, Sargodha Road, Faisalabad - Pakistan
Mob: +92 300 3008585, Fax: +92 41 8815544
E-mail: editorijps@gmail.com

International Journal of Poultry Science 14 (10): 584-588, 2015
ISSN 1682-8356

Effects of *Pluchea indica Less* Leaf Extract and Chlorine to Hematological Profiles of Broiler Chickens

Harvey Febrianta, Vitus Dwi Yunianto and Bambang Sukamto
Faculty of Animal Science and Agricultural Education, Diponegoro University,
Semarang, 50275, Central Java, Indonesia

Abstract: *Pluchea indica Less* is one of the medicinal plants which can be used as a natural feed additive to improve poultry production, especially broilers, while chlorine is used to inhibit bacteria growth. The purpose of this study was to assess and evaluate the effect of *Pluchea indica Less* leaf extract and chlorine to the hematological profile of broilers. The experimental design applied in this study was completely randomized design (CRD) with 5 treatments and 4 replications. The treatments were as follows T0: Basal feed without adding *Pluchea indica Less* leaf extract and chlorine; T1: Basal feed + 2% *Pluchea indica Less* leaf extract + 30 ppm chlorine; T2: Basal feed + 4% *Pluchea indica Less* leaf extract + 20 ppm chlorine; T3: Basal feed + 6% *Pluchea indica Less* leaf extract + 10 ppm chlorine; T4: Basal feed + 8% *Pluchea indica Less* leaf extract + 0 ppm chlorine. Data were analyzed using analysis of variance (ANOVA) at the level of 95%, when the result was significant, means were separated by Duncan's multiple range tests. The values were considered significant if $P<0.05$. The parameters measured were: number of erythrocytes, hematocrit, hemoglobin levels, number of leukocyte, and difference in leukocytes (lymphocytes, monocytes, and eosinophils) in broilers. Increased level of *Pluchea indica Less* leaf extract along with decreased level of chlorine showed significant result ($P<0.05$) to leukocytes, lymphocytes, monocytes, hemoglobin. On the other hand, the results for eosinophils, erythrocytes, and hematocrit were not significant ($P>0.05$). In conclusion, *Pluchea indica Less* leaf extract at the level of 8% was able to replace chlorine and also able to increase the number of leukocytes, hemoglobin levels, percentage of lymphocytes, and monocytes in broilers.

Key words : *Pluchea indica Less* leaf extract, chlorine, blood profiles, broilers

INTRODUCTION

Broiler has more advantages than other types of chicken. The advantages include rapid growth with a high body weight in relatively short period, low feed conversion, and fibrous meat quality. These advantages make broiler become a solution in handling public demand for chicken meat.

Beside these advantages, many farmers inevitably face many obstacles in management of broiler. One of them is disease. Disease caused by pathogenic bacterial infection may lower the immune system and this can decrease the growth in broiler chickens. Moreover, the disease may also cause a high mortality level which can lead to economic loss for the farmers.

Water disinfection program is commonly used by farmers to inhibit bacterial growth. The program is done by adding some chlorine in drinking water (known as chlorination). Unfortunately, this process leaves harmful *trihalo methane* (THM) residue which is carcinogenic (Stern *et al*., 2002). For this reason, it is important to identify alternative feed addictive to replace chlorine. Feed addictive can be extracted from medical plants including *Pluchea indica Less*.

Pluchea indica Less has active compounds such as alkaloids (0.316%), essential oils, tannins (2.351%), and flavonoids (4.18%). They serve as antibacterial compounds which are also able to affect the blood profile in broiler chicken.

Other than transporting metabolism result, blood can be used as an indicator to determine the normal function of the immune system. Proper functioning immune system of broilers will lead to maximum production through efficient digestion and absorption of nutrients. The purpose of this study is to evaluate *Pluchea indica Less* leaf extract and chlorine to hematological profiles of broiler chickens.

MATERIALS AND METHODS

Experimental birds: A total of 140 male broiler chickens (MB - 202 Platinum Sexing). The treatments started when the broilers reached 15 days old, with 494.20 ± 16.99 g average body weight. Each placement was randomly performed through a lottery. The treatments were performed for around 15 to 35 days.

Experimental diets: Chickens were fed (CP 511) commercial feed for the starter phase at 1 - 11 days old,

Corresponding Author: Harvey Febrianta, Faculty of Animal Science and Agricultural Education, Diponegoro University, Semarang, 50275, Central Java, Indonesia

and at 12 - 14 days old, broilers were fed with the basal feed as an adaptation period. When the broilers reached 15 day old, chickens were given the combination of basal feed + *Pluchea indica Less* leaf extract + chlorine. The ingredients for basal feed are corn, rice bran, soybean meal, fish meal, premix. The composition of ingredients and analysis of diets are shown in Table 1.

Table 1: Ingredients and chemical composition of the experimental diets

Ingredients	Chemical composition (%)
Corn	59.50
Rice bran	5.54
Soybean meal	26.40
Fish meal	7.56
Premix	1.00
Total basal feeds	100.00
Nutrient composition	
Metabolizable energy (kcal/kg)	3132.46
Crude protein (%)	21.15
Crude fat (%)	6.65
Crude fiber (%)	3.77
Calcium (%)	0.58
Phosphorous (%)	0.41

The materials used in the treatments were *Pluchea indica Less* leaf extract and chlorine. The treatments of *Pluchea indica Less* leaf extract and chlorine were given every morning with 5 treatments (T0, T1, T2, T3, T4) and 4 replicates (R1, R2, R3, R4). Each experimental unit contained 7 broilers. The treatments tested are as follows :

T0 = Basal feed without adding *Pluchea indica Less* leaf extract and chlorine
T1 = Basal feed + 2% *Pluchea indica Less* leaf extract + 30 ppm chlorine
T2 = Basal feed + 4% *Pluchea indica Less* leaf extract + 20 ppm chlorine
T3 = Basal feed + 6% *Pluchea indica Less* leaf extract + 10 ppm chlorine
T4 = Basal feed + 8% *Pluchea indica Less* leaf extract + 0 ppm chlorine

Parameters measured: Blood sampling was performed at the end of the study, respectively - each chicken per replicate, the chickens were not fed for 2 hours prior to taking the blood samples. Blood was collected from brachial vein situated in the chicken wings using 3 ml syringe. The blood samples were then put into plastic tubes containing anticoagulant *ethylene diamine tetra acetic acid* (EDTA). Blood samples were used to examine the following parameters: haemoglobin (Hb), packed cell volume (PCV), number of red blood cells (erythrocytes), number of white blood cells (leukocytes), and differentiation of leukocytes (lymphocytes, monocytes, and eosinophil).

Statistical analysis: Data were analyzed using Random Complete Design (CRD). If any significant difference was found in the treatments, the Duncan's Mean Range Test (DMRT) was taken as the follow-up test.

RESULTS AND DISCUSSION

The results of this study for average haematological status in broiler chickens treated with *Pluchea indica Less* leaf extract and chlorine are presented in Table 2.

Erythrocytes: Based on the analysis of variance, it is found that the *Pluchea indica Less* leaf extract and chlorine were not significant (P>0.05) to erythrocytes of broiler chickens. Average number of broiler's erythrocytes treated with *Pluchea indica Less* leaf extract and chlorine ranged around 2.55 x 106/mL. Meanwhile, according to Sugito (2009), the number of normal erythrocytes of broiler chickens is 2.5 ± 0.2 106/mL. This indicates that the number of erythrocytes in this study remained within the normal limits.

The addition of *Pluchea indica Less* leaf extract and chlorine did not significantly affect the number of erythrocytes. Flavonoids as compounds of *Pluchea indica Less* leaf extract that reduce free radicals did not affect the total number of erythrocytes, so the number of erythrocytes were not significantly changed. Flavonoids prevent free radicals by inhibiting the release of peroxidase and reduce the absorption of Fe (ferrum/ iron). Low Fe may affect the erythrocyte (Nijveldt *et al*., 2001).

Tannins have the ability to bind the proteins (Francis *et al*., 2002). The presence of tannins cause protein binding and it coats the intestinal wall, of which interfere with the absorption of protein. This can cause delays in the manufacture of erythropoietin hormones and reduce the formation of erythrocytes.

The process of red blood cells formation occurs in the bone marrow (Altan *et al*., 2000). The first cells that can be recognized as a series of proerythroblast, which will split several times before reaching 8-16 mature red blood cells. The first generation of cells is called *basophilic erythroblast* because it can take a base colour and gather a small amount of haemoglobin. At the later stage, the cells develop into *polychromatic erythroblast* which begins to contain haemoglobin. Blood colour will become red because of the haemoglobin. This phase is called *orthochromatic erythroblast*, of which during this cycle haemoglobin will turn into reticulocytes and around 34% of haemoglobin-nuclei concentration condenses and shrinks in size. Within 1 to 2 days, reticulocytes will turn into mature erythrocytes (Moura *et al*., 2004).

Hematocrit: Based on the analysis of variance, it shows that the treatments did not significantly affect the hematocrit values in broilers (P>0.05). The haematocrit average amount of all treated broiler chickens was 29.94%. Sugito (2009) stated that the hematocrit

Table 2: Haematological profiles in broiler chickens

Hematological Profiles	Treatments of basal feeds + *Pluchea indica Less* leaf extract + chlorine				
	T0	T1	T2	T3	T4
Erythrocytes (10^6/µL)	2.60±0.22	2.68±0.15	2.50±0.14	2.50±0.14	2.52±0.09
Hemoglobin(g/100 mL)	8.88±0.22^{ab}	9.05±0.19^{a}	8.75±0.24^{b}	8.85±0.10^{ab}	8.90±0.08^{ab}
Hematocrit (PCV) (%)	29.85±0.79	30.20±1.08	29.90±0.90	29.52±0.38	30.20±0.84
Leukocytes (10^3/µL)	30.65±3.37^{b}	35.28±2.80^{a}	31.08±1.38^{b}	27.32±0.86^{c}	31.90±1.46^{b}
Lymphocytes (%)	60.70±2.48^{b}	66.05±2.10^{a}	62.02±1.18^{b}	57.02±1.23^{c}	61.02±0.74^{b}
Monocytes (%)	8.15±0.70^{bc}	9.42±0.26^{a}	8.28±0.30^{b}	7.42±0.41^{c}	8.15±0.50^{bc}
Eosinophils (%)	2.80±0.14	3.15±0.13	2.85±0.06	3.12±0.54	2.88±0.17

Description: Different superscript in the same row indicate there were differences at (p<0.05)

average in normal broiler chickens was 29.5±1.8 %. This indicates that the hematocrit percentage in this study is still within the normal limit.

Hematocrit value is associated with a number of erythrocytes although in this study, the treatment had no significant effect on hematocrit values. The treatment with 8% *Pluchea indica Less* leaf extract and 0 ppm chlorine value was associated with an increased production of pre-erythrocytes. Hematocrit value is the percentage of grain erythrocytes (red blood cells) to the total blood and positively correlated with the number of erythrocytes (Soeharsono *et al.*, 2010). Hematocrit values in this study were still in the normal range. This indicates that the *Pluchea indica Less* leaf extract and chlorine did not interfere with the hematocrit of broilers.

Hemoglobin: The results of variance analysis show that the treatment was significantly against hemoglobin levels of broiler chickens (P<0.05). The average value of hemoglobin levels for each treatment was 8.88 g/100 ml. On the other hand, according to Sugito *et al.* (2007) normal hemoglobin levels of broiler chicken is 8.73±0.64 g/100 ml. This indicates that the hemoglobin levels in this study are within normal limit. Hemoglobin levels on T1 treatment produced the highest value among the other treatments with 9.05±0.19 g/100 ml. It is assumed that the broilers in T1 treatment had healthier physiological conditions compared to those in other treatments

The results of analysis of variance showed that hemoglobin levels were within the normal range. The number of erythrocytes, hematocrit and hemoglobin in the blood are all interrelated. The high average value of erythrocytes is followed by the high average value of hemoglobin (Soeharsono *et al.*, 2010). Normal hemoglobin levels indicate the adequacy of oxygen to be circulated to all body tissues. If the erythrocytes, hematocrit, and hemoglobin values are in the normal state, then it can be inferred that the animal is physiologically in good health. Regression analysis of correlation between hemoglobin (Y) and erythrocytes (X) (Fig. 1) shows that the correlation between the increasing level of hemoglobin by erythrocytes will form a linear line with equation Y = 1.067X + 6.1536 (R^2 76.6%), meaning that 76.6% erythrocyte is influenced by hemoglobin.

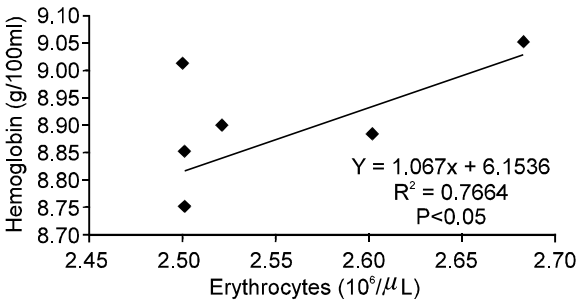

Fig. 1: Correlation Between Erythrocytes and Hemoglobin

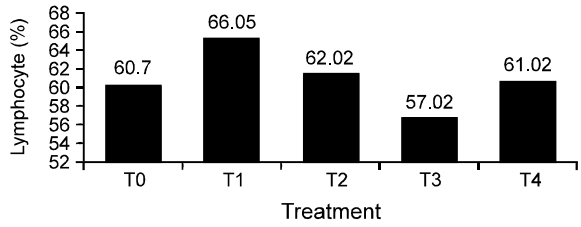

Fig. 2: Graphs effect of *Pluchea indica Less* leaf extracts and chlorine to lymphocytes

Leukocyte: Based on the analysis of variance, it is found that the treatment of *Pluchea indica Less* leaf extract and chlorine significantly (P<0.05) affected the number of leukocytes in broilers. Treatments (T0, T2, and T4) were significantly different from T1 and T3. Treatment T1 had the highest percentage of blood leukocytes compared to other treatments. This was presumably due to the increased number of leukocytes which showed a high ability of the body to response to the infection or foreign objects. According to Soeharsono *et al.* (2010), high leukocyte indicates the ability of the body to fight infections.

The average number of leukocytes observed was 31.24 10^3/uL. Nowaczewski and Kontecka (2012) stated that the average number of leukocytes in broiler chickens is 32.60 10^3/uL. This indicates that the number of leukocytes in this study is still within the normal limits. High number of leukocytes is caused by the active compound of *Pluchea indica Less* leaves, such as flavonoid. One of the benefits of flavonoids is that the compound can reduce the immunosuppressive

leukocytes. Flavonoids can reduce the immobilization of leukocytes and has anti-inflammatory effect. Flavonoids prevent free radicals by inhibiting the release of peroxidase and reducing the absorption of Fe (ferrum/ iron). Low Fe can affect the number of leukocytes, the formation of hemoglobin, hematocrit and total erythrocyte (Nijveldt *et al*., 2001).

The number of leukocytes which was within the normal range indicates that the active substances contained in the *Pluchea indica Less* leaf extract did not interfere with broilers leukocyte content during the maintenance period. Leukocyte is an active unit to provide fast and powerful defense against any infectious material by means of chemotaxis (Aengwanich *et al*., 2003).

Lymphocytes: The average of broilers lymphocytes was 61.36%. This was in accordance with the opinion of Turcu *et al*. (2010) that the blood lymphocytes in normal condition of broilers is 68.3%. The value of blood lymphocytes in this study indicates that broilers are in good health with good endurance. This result is in accordance with the opinion of Aengwanich *et al*. (2003) who reported that the lymphocytes are the main constituents of the immune system that defense against microbial pathogens such as viruses, bacteria, and fungi.

Based on the variance analysis, it showed that the treatments were significant ($P<0.05$) to the lymphocytes of broiler chickens. Treatments (T0, T2, and T4) were significantly different from other treatments (T1 and T3). Treatment T1 had the highest percentage of blood lymphocytes in comparison other treatments. This was presumably due to the health condition of broiler chickens which got additional 2% *Pluchea indica Less* leaf extract and 30 ppm chlorine in the basal feed so that they remained in a good condition. Feed given was able to meet the nutritional needs of broiler chickens. As the result, the body's natural resilience is able to be maintained. According to Austic (2000), the nutrients contained in food such as energy, protein, vitamins, and minerals have an important role in the immune system. Protein is necessary for the development of lymphoid organs. Basal feed given to broiler chickens in this study contained 21.15% crude protein. This could affect the immune system by increasing the levels of immunoglobulin. Protein content in the feed can synthesize the immunoglobulin that is secreted through the intestinal mucosa. This is in accordance with the opinion of Altan *et al*. (2000) that the factors which can affect the chicken immune system include nutritional factors.

Blood lymphocytes values of broilers fed with *Pluchea indica Less* leaf extract and chlorine on treatments (T1, T2, T3, and T4) were in the normal range. This was presumably due to the effect of flavonoids in *Pluchea indica Less* leaves. Flavonoids reduce the immunosuppressive from leukocytes, thus increase the number of leukocytes and has anti-inflammatory effects (Nijveldt *et al*., 2001). The function of lymphocytes is to recognize and eliminate threats to the body (Scope *et al*., 2001).

Monocytes: Based on the results of analysis of variance, it is found that the treatments had significant effects ($P<0.05$) on the percentage of monocytes. T1 treatment had the highest percentage of blood monocytes compared to other treatments. This was presumably due to the health condition of broiler chickens which got additional 2% *Pluchea indica Less* leaf extract and 30 ppm chlorine in the basal feed which caused the birds to remain in good conditions. Average percentage of monocytes in this study was 8.28%. According to Smith and Mangkoewidjojo (1988), the normal range of monocytes in chicken is about 0.0 to 30%. This suggests that the *Pluchea indica Less* leaf extract and chlorine did not interfere with the percentage of monocytes.

Monocytes work as an inflammatory response and form a second line of defense against bacterial infection. In general, the presence of monocytes in the immune system is to activate the immune response system. The presence of monocytes in the body will be high if there is a need to trigger the formation of antigen defense system because monocytes produce interleukin and interferon compounds which are used as a medium of communication between the immune cell differentiation and influence a role in the mobility of leukocyte cells (Francis *et al*., 2002).

Eosinophils: The results of analysis of variance show that the treatment was not significant ($P>0.05$) in terms of the percentage of eosinophils. The average percentage of eosinophils in the blood profiles of broilers from each treatment was 2.96%. This is consistent with Nowaczewski and Kontecka (2012) who stated that normal eosinophils in broiler chickens was 2.48%. This indicates that the percentage of eosinophils in this study remained within the normal limit.

The percentage of eosinophils was in the normal range presumably because *Pluchea indica Less* leaf extract and chlorine did not interfere with eosinophils of broilers. Eosinophil has a major function in toxification against parasites that enter the body through the lungs or gastrointestinal tract (Francis *et al*., 2002).

Conclusions: *Pluchea indica Less* leaf extract at the level of 8% can replace the function of chlorine as feed addictive, and it is able to increase leukocytes, hemoglobin, lymphocytes, and monocytes in broilers.

REFERENCES

Aengwanich, W., S. Pornchai, P. Yupin, V. Thevin, P. Parwadee, K. Suporn and S. Suchint, 2003. Effects of ascorbic acid on cell mediated, humoral immune response and pathophysiology of white blood cell in broilers under heat stress. Songklanakarin J. Sci. Technol., 25: 297-305.

Altan, O., M. Cabuk and H. Bayraktar, 2000. Effects of heat stress on some blood parameters in broilers. Turky J. Vet. Anim. Sci., 24: 145-148.

Austic, R.E., 2000. Feeding Poultry in Hot and Cold Climates. Di dalam MK Yousef, (editor). Stress Physiology in Livestock, Vol III. CRC Press, Inc. New York.

Francis, G., K. Zohar, P.S. Harinder, H.P. Makkar and K. Becker, 2002. The biological action of saponin in animal systems : A review. Br. J. Nutr., 88: 587-605.

Moura, M.A., C.H. Machado, L.C. Porfirio and R.B. Freire, 2004. Effects of ochratoxin a on broiler leukocytes. Revista Brasileira de Ciência Avícola., 6: 187-190.

Nijveldt, R.J., E. Nood, D.E.C. Hoorn and P.G. Boelens, 2001. Flavonoids: a review of probable mechanisms of action and potential applications. Am. J. Clin. Nutr., 74: 418-425.

Nowaczewski, S. and H. Kontecka, 2012. Haematological indices, size of erythrocytes and haemoglobin saturation in broiler chickens kept in commercial conditions. Animal Science Papers and Reports., 30: 181-190.

Scope, A., T. Filip, C. Gabler and F. Resch, 2001. The influence of stress from transport and handling on hematologic and clinical chemistry blood parameters of racing pigeons (*Columba livia domestica*). Avian Dis., 46: 224-229.

Smith, J.B. and S. Mangkoewidjojo, 1988. Pemeliharaan, Pembiakan dan Penggunaan Hewan Percobaan di Daerah Tropis. Universitas Indonesia, Jakarta.

Stern, N.J., M.C. Robach, N.A. Cox and M.T. Musgrove, 2002. Effect of drinking water chlorination on Campylobacter spp. colonization of broilers. Avian Dis., 46: 401-404.

Sugito., 2009. The profile of hematology and broiler daily body weight gain which treated with heat stress at different cage temperature. Agripet., 9: 10-14.

Sugito., W. Manalu, D.A. Astuti, E. Handharyani and Chairul, 2007. Heat stress effect and given of hexane extract jaloh bark (*Salix tetrasperma* Roxb) to cortisol level, triiodothyronine and hematology profile of broiler chickens. *JITV.*, 12: 175-184.

Soeharsono., L. Adriani, E. Hernawan, K.A. Kamil and A. Mushawwir, 2010. Fisiologi ternak Fenomena dan Nomena Dasar, Fungsi, dan Interaksi Organ Pada Hewan. Widya Padjadjaran, Bandung.

Turcu, D., M. Oporanu, D. Condur, P. Grigorescu and M. Roman, 2010. Contributions to the humoral immunological profile in broiler chickens treated with 50% Amoxidem. Veterinary Drug., 4: 42-45.