

The blood haematological profile on laying hens that treated by different levels of yeast supplementation

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Abstract: The study was conducted to evaluate the effect of different dose of yeast probiotics on hematological profile of hemoglobin, erythrocytes, hematocrit, MCV, MCH, MCHC, RDW- CV, leucocyte, eosinophil, basophil, neutrophil, lymphocytes in blood of laying hens before and after received a Newcastle disease (ND) vaccine. Total of 80 laying hens (18-week) were randomly divided into four treatments. Each treatments consisting 4 replicate with 5 hens each. The first treatment was fed a basal feed as a control, and the second, third and fourth treatment were basal feed that supplemented with the yeast that contains 1×10^9 CFU/g at the level of 0.5, 1.0 and 1.5 g/per kg of feed during 2 weeks observation. Layers were vaccinated with ND vaccine strain LaSota on 7 days after yeast supplementation treatment. Blood samples were collected on 24 h before and 5 h after vaccination. Results indicated that treated with yeast showed not significant effect on MCV, MCH, MCHC, RDW-CV, leucocyte, basil, neutrophils, lymphocytes. Yeast supplementation had significantly ($p < 0.05$) increase in the count of the hemoglobin, erythrocytes, and hematocrit, particularly at before vaccine administration. The result provides a new information that yeast supplementation has a beneficial effect on under low challenge or stress condition.

1. Introduction

Feed is the largest source of costs in poultry farming production systems, accounting for more than 70%. Several strategies can applicate to improved feed efficiency in a poultry farm. One of the strategies is to use antibiotics to improved productivity through reduced feed conversion and disease protection. Despite the well documented beneficial effects of antibiotics in increasing poultry growth, reducing mortality and increasing resistance to disease, but antibiotic use also is associated with several disadvantages and challenges.

Severals studies showed that there might a correlation between the use of antibiotics with resistance to certain microorganisms [1] [2]. Regarding the use of antibiotic massively as a driver of growth and demand for products antibiotics free from consumers, the alternative products need to be developed. Yeast is a feed additive material that has been widely used for ruminants and poultry as a natural growth promotor [3]. Yeast walls containing prebiotic compounds include mannan oligosaccharides, beta glucans, and amino acids [4] [5] which can function as agents to increase immunity to disease. In addition, yeast is a compound that can stimulate bacteria in the digestive tract [5] improve the health and immune system and as a bio control agent for reducing stress [3] [6].



The immune system in poultry can be identified from the hematological profile of livestock blood profiles. Hematological testing is not only used for diagnosis for medicinal purposes, but also for health monitoring of treatment responses [7]. In addition, the hematological analysis is used as a guide in diagnosing stress levels caused by vaccine administration that can cause stress and pressure on poultry. Therefore this study was aimed to determine the condition of hematology profiles in layer hens fed with the addition of yeast before and after vaccination treatment.

2. Materials and Methods

2.1. Animal and diets

The experimental was conducted in farm of Politeknik Negeri Jember, Indonesia. A total 80- 18 weeks-old Hi-sex Brown layer (body weight (BW)=1530±31 g) that provided from local pullet farm were allocated in the individual battery system for the layer. The hens were fed *ad-libitum* as a standard mixed of mash diet which has been formulating according to the manual guideline for layers (**Table 1**).

The chicken's experiments were divided into four treatments. Each treatments consisting 4 replicates with 5 hens in each replicate. The first treatment was fed a basal feed as a control, and the second, third and fourth treatment were fed the basal that supplemented with the yeast *Saccharomyces cerevisiae* that contains 1×10^9 CFU/g at the dose of 0.5, 1.0 and 1.5 g/per kg of diets, respectively during 2 weeks observation. The feed was free from antibiotics and other feed supplements. Water was provided *ad-libitum* during the experiments periods. The Layer hens were vaccinated using ND (New castle disease) vaccine strain La-Sota via nasal drops on 7 d after yeast supplementation treatment.

2.2. Blood sampling and analysis

The blood sampled were taken in week 19 at 24 h before and 5 h after vaccine administration. The samples were collected as soon as possible from the wing vein (three/treatment) using a 1 mL syringe. One mL was carefully moved into heparinized tubes (BD Vacutainer). An automated hematology analyzer using XS series XS-1000i/XS-800i (Sysmex Corporation) according to ISO standard ISO 9001:2015 was used to analysis the concentrations (cells/ μ l) of WBC and RBC. The leukocytes and lymphocyte were determined by separation under an optical microscope.

The data were analyzed by one-way ANOVA using the General Linear Model (GLM) in MINITAB procedure (Minitab inc USA) with the following models $Y_{ij} = \mu + \alpha_i + \varepsilon_{ij}$. Where Y= dependent variables, μ : overall mean, α_i : the effect of treatment, ε_{ij} : residual, The significant differences among the treatment groups were analyzed by Duncan's multiple range test at 0.05 significant level.

Table 1. Ingredients and chemical composition of the layers diets

Item	%
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Ingredient (% of DM)	
Corn	49.5
Mix concentrate feed	34.5
Rice Bran	15.0
Top mix minerals ¹	0.94
DL-Methionine	0.05
L Lysin	0.01
Total	100
Calculated composition	
Metabolism energy (Kcal/kg of DM)	2917.0
Crude Protein (%)	17.2
Fiber (%)	3.9
Fat (%)	3.0
Calcium (%)	3.2
Phosphor (%)	0.7
Lysine (%)	1.13
Methionine (%)	0.56

¹)Minerals (%), P (0.25), Ca (2.0), Mg (0.45), Na (0.35); trace elements (mg/kg):

Cu (15) vitamins (IU/kg): vitamin A (6,000), vitamin D3(1,250), and vitamin E (10 mg/kg).

3. Results and discussions

3.1 Feed consumption

Body weight and feed intake were measured weekly on week 18 to week 20. In the current study, the chicken reached of BW 1540 g at 18 weeks. This weight result was in accordance with the manual standard guideline of layers chickens. From the statistical analysis, there was no significant difference in feed consumption (Table 2), but yeast consumed by chickens increases according to the high level of treatment. The major yeast cell wall is oligosaccharide, glycoprotein, peptides, minerals, and vitamins [8] [9] which have been proposed to produce positively affect response the chickens that consume them in broiler chicks [10]. In agreement to [11] reported that yeast supplementation in feed can increase body weight as a growth promoter in starter chickens. However, the data from the addition of yeast level did not affect feed consumption. Another study reported that the addition of probiotics did not cause differences in feed consumption in broiler chickens [12]. Differences in animals response may be due to the formulation of yeast in the feed which is difficult in the comparison of yeast effects

Table 2. Effects of yeast supplementation on feed intake and body weight

Parameter	Yeast supplementation (g/kg of diets)			
	0	0.5	1	1.5
Live BW (g)	1542± 126	1547±90	1553 ± 96	1563±84
Average FI g /d/hen	108.5±3	106.3±8	107.3±5	102.4±8

BW= body weight, FI = feed intake

3.2. Blood hematology profile

This study aims to evaluate blood hematological profiles in daily conditions compared to high-stress condition (after vaccination treatment). Hematological blood profiles in the pre-vaccination and 5 h after vaccination are shown in Table 3.

In the pre-vaccination condition, statistical analysis showed that the addition of yeast did not show significant differences in parameters, MCV, MCH, MCHC, RDW CV, leucocyte, eosinophil, basophil, neutrophil, lymphocyte, except for hemoglobin (Hb), erythrocyte and hematocrit in sampling before

vaccination. From the data shows that with increasing levels of consumption of yeast can increase Hb content. Comparison of laying hens fed with 1.5 g / kg yeast was higher than without addition of yeast on Hb content of (7.7 vs 6.7 g / dl), erythrocyte (2.56 vs 2.25) and Haematocrit (32.7 vs 29%). At the addition of 1.5 g / kg significantly increase the Hb content, the comparison of the results shows the influence of nutrients contained in yeast, with increasing yeast nutrients consumed. Blood Hb content is affected by Fe content, with more and more available elements of Fe will potentially increase hemoglobin. Hb content in yeast treatment 1.5 g / kg that is equal to 7.73 is still in the normal range [13]. According to reported that the normal Hb content in laying hens ranged from 7.3-10.9 g / dl.

Table 3. Effects of yeast supplementation on blood hematological profile

<i>Parameter</i>	<i>Yeast supplementation (g/kg of diets)</i>			
	0	0.5	1.0	1.5
<i>Before (24 h) ND vaccine administration</i>				
Haemoglobin (HGB) (g/dl)	6.76 ± 0.85 ^a	6.13±0.40 ^a	7.37±0.93 ^{ab}	7.73±0.61 ^b
Erythrocytes (RBC) (x 10 ⁶)	2.25±0.11 ^a	2.25±0.04 ^a	2.53±0.17 ^b	2.56±0.06 ^b
Haematocrit (%)	29.00±0.95 ^a	28.63±0.25 ^{ab}	31.53±2.40 ^b	32.7±0.90 ^b
MCV (fl)	128.93±1.94	127.43±1.76	124.60±1.91	127.90±3.92
MCH	30.07±1.48	27.30±1.74	29.03±1.98	30.17±11.57
MCHC (g/dl)	23.30±1.15	21.40±1.54	23.30±1.55	23.60±1.39
RDWCV (%)	9.67±0.78	8.93±0.38	8.73±0.85	9.43±1.67
Leucocyt (WBC) (x 10 ³ /μl)	180.±11	186.±20	215.±23	202±22
Basophil (%)	1.23±0.61	3.07±2.89	0.97±0.57	0.50±0.10
Neutrophil (%)	82.63±3.10	72.20±15.59	84.07±5.63	82.10± 1.57
Lymphocyte (%)	14.87±2.50	23.43±13.18	14.30±5.27	16.43±1.19
Monocyte (%) *	1.27±0.15	1.27±0.46	0.67±0.21	0.97±0.31
Thrombosit (PLT)	1000±577	2000±1000	1333±577	2333±1527
<i>After (5 h) ND vaccine administration</i>				
Hemoglobin (HGB) (g/dl)	6.57 ± 0.29	7.07±0.55	6.63±0.57	6.77±0.74
Erythrocytes (RBC) (x 10 ⁶)	2.24±0.08	2.40±0.17	2.37±0.14	2.32±0.16
Haematocrit (%)	29.30±0.70	30.50±2.34	29.37±1.86	30.00±2.07
MCV (fl)	131.10±5.38	126.90±2.79	123.73±2.59	129.11±3.07
MCH	29.33±0.47	29.40±0.66	27.9±1.03	29.10±1.31
MCHC (g/dl)	22.43±0.90	23.17±1.03	22.6±0.51	22.50±0.87
RDWCV (%)	9.63±1.1	8.93±0.68	8.4±0.64	9.43±1.64
Leucocyt (WBC) (x 10 ³ /μl)	185±11	203±17	184±19	180±16
Basophil (%)	1.37±0.21	1.70±0.61	1.67±1.11	1.03±0.55
Neutrophil (%)	78.27±2.23	79.23±3.21	83.73±7.9	81.73±4.46
Lymphocyte (%)	18.63±1.69	18.27±2.80	13.87±0.89	15.70±4.31
Monocit(%)	1.73±0.46	0.80±0.10	0.73±0.03	1.53±0.35
Thrombosit (PLT)	1333±577	4333±2516	1666±577	1666±577

^a means in a row with no common superscript differ significantly (p<0.05).

MCV: mean corpuscular volume, MCH: mean corpuscular hemoglobin, MCHC: mean corpuscular hemoglobin concentration, RBC: red blood cell, WBC: white blood cell, MCV: Mean corpuscular volume, RDW-CV: red blood cell distribution width coefficient variation

Hemoglobin is a molecule and consists of four subunits. Each subunit contains one heme group which is conjugated by a polypeptide (globin). Heme is a porphyrin derivative containing iron [14]. Thus, it is suspected that the addition of yeast increases mineral supply, such as Fe, but is still in the normal amount of hemoglobin [9]. Hemoglobin functions to carry oxygen in red blood cells to be transported to all parts of the body. An increase in the amount of hemoglobin is influenced by oxygen levels and erythrocytes so that with the increase in the number of erythrocytes will be accompanied by an increase in the amount of Hb.

The value of erythrocyte in chickens given yeast was higher than the control. However, the amount of erythrocyte is still in normal condition. According to [15] reported the content of erythrocyte in chickens as big as 2.0-3.5x ($10^6/\text{mm}^3$). Increased of erythrocyte contributes to improving the body's defense system against disease [16]. Erythrocyte can act as a defense system for birds that produce cytokinin. The number of erythrocytes is strongly influenced by its formation. An important factor influencing the formation of erythrocytes is tissue oxygenation. If the amount of oxygen transported to the tissue decreases it will cause an increase in erythrocyte production. Haematocrit content also increased along with the addition of yeast.

Hemocytes value is influenced by erythrocyte so that if the erythrocyte increases the hematocrit value also increases. Hematocrit values will decrease if animals are under stress conditions, one of which is caused by high temperatures. In this study the hematocrit value increases with the addition of yeast, this indicates that the chicken is under lower pressure compared to without yeast.

Values, Hb, Erythrocyte and Hematocrit showed that they still had a positive effect on chicken performance. While the hematological profile of blood in chickens with the addition of yeast in the feed did not show a significant difference in hemoglobin parameters: erythrocyte, hematocrit MCV, MCH, MCHC, RDW CV, leucocyte, eosinophil, basophil, neutrophil, lymphocyte. However, the content of each parameter shows a normal value. Normally the value of blood constituents is influenced by several factors such as genotypical physiological conditions and macro and micro climatic conditions. No significant of blood constituents by the addition of yeast treatment is likely due to the body still trying to balance its metabolic processes due to stress during vaccine administration. This is because the time for blood collection is 5 hours after vaccination. According to [17] stress conditions cause unstable blood constituents, this can be seen in Table 3, the content of blood constituents shows a less consistent trend and blood sampling after vaccination was done 1 week after vaccination to determine the level of body titers from poultry.

4. Conclusion

It is concluded in the current study that the addition of yeast has a beneficial effect on under low challenge or stress condition but yeast supplementation failed to maintain stress pressure after vaccine administration.

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