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Research Paper

Addition of acidifier with inulin and papain enzyme in the ration towards leukocyte differential in bottle growth period

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Abstract
The aim of the study was to examine the effect of adding acidifier with inulin and papain enzymes in the ration on the differential leukocytes in turkeys during the growth period. The research material consisted of 80 male turkeys aged 12 weeks with an average weight of 1165 ± 62.24 g, an acidifier consisting of a mixture of lactic acid, propionic acid, and formic acid, and inulin sourced from dahlia tuber extract and the enzyme papain. The feed ingredients used in this study were corn, soybean meal, fish meal, rice bran, premix, $CaCO_3$, lysine, and methionine. The research design used a completely randomized design with 5 treatments and 4 replications. The treatments applied included T_0 (control diet/RK), T_1 (RK + 1% acidifier), T_2 (RK + 1% acidifier + 1.2% inulin), T_3 (RK + 1% acidifier + 0.5% papain enzyme), and T_4 (RK + 1% acidifier + 1.2% inulin + 0.5% papain enzyme). Parameters measured included heterophiles, lymphocytes, leukocytes, and total plasma protein. Data were analyzed using variance at 5% level and Duncan's test of significant differences at 5% level. The results showed that the addition of acidifier with inulin and papain enzymes had a significant effect ($p < 0.05$) on the differential leukocytes (heterophiles, lymphocytes, leukocytes, and total plasma protein). The conclusion is that the addition of 1% acidifier + 1.2% inulin + 0.5% papain enzyme (T_4) can maintain the health condition of the body seen from the differential leukocytes in male turkeys during the growth period.

Keywords
Acidifier, Leukocyte differential, Papain enzyme, Inulin, Turkey

1. INTRODUCTION

Indonesia is a country with a very large number and population growth. This has an impact on the need for animal protein, especially poultry meat. Poultry meat provides a higher source of animal protein than other meats. According to data from the Central Statistics Agency, consumption of poultry meat in 2021 is 0.142 kg. Several types of poultry that have the potential to be bred as a provider of animal protein needs is turkey. The demand for turkey meat is increasing every year, especially in large restaurants or hotels, so an effort is needed to increase productivity. Based on data from the Central Statistics Agency, it shows that the turkey population in Indonesia is still relatively low, at approximately 25,000 birds. A common problem that often occurs in the field is turkey productivity which has not been considered. One of the efforts that can be made to increase turkey productivity is to modify the ration by adding feed additives.

Organic acids are included in one type of acidifier which functions to lower intestinal pH thereby inhibiting the growth of pathogenic bacteria in the body of livestock (Tajudin et al., 2021). The addition of acidifier mixture in livestock rations can increase livestock productivity. A ration containing acidifier can suppress the growth of certain species of bacteria, especially acid intolerant species such as *E. coli*, *Salmonella* sp., and *Campylobacter* spp. Types of acidifier that can be used as a mixture of rations are citric acid, lactic acid, propionic acid, acetic acid, formic acid, or a mixture of organic acids (Handajani and Hastuti, 2013). Prebiotic inulin is found in several plants such as chicory root extract (*Chicoryum inthybus L*). The addition of inulin as a prebiotic can increase the growth of beneficial bacteria in the intestine and can increase endurance in livestock (**?**). The addition of the papain enzyme as a source of protease enzymes can improve the quality of low crude protein rations and can increase turkey ration consumption (Fitasari, 2011).

Health indicators to determine success in turkey rearing can be seen through the leukocyte differential assessment. In general, differential leukocytes can provide an overview

of the level of immunity in livestock (Purnomo et al., 2015). Leukocytes are blood cells that play a role in protecting the body's defense against various disease attacks by phagocytes and producing antibodies Wulandari et al. (2016). The increase and decrease in the number of leukocytes describe the response of white blood cells in preventing the spread of disease in livestock. Leukocyte differential is an indicator to show the health status of livestock against various kinds of infections and diseases that attack. Leukocyte differential consists of two groups, namely granulocytes including heterosinophils, eosinophils, and basophils, and the agranulocyte group including lymphocytes and monocytes (Saputro et al., 2016). Several factors can affect the leukocyte differential, namely environmental conditions, age, and nutritional content of the ration, especially protein, which has a very important role in the process of leukocyte formation (Ulupi and Ihwantoro, 2014). Nutritional factors, especially protein, have an important role in the process of leukocyte formation because it is a component of blood. Based on these results, it is expected that the addition of acidifier, inulin, and papain enzymes in the ration has a positive effect on the health condition of the body seen from the differential leukocytes in turkeys of the growth period.

2. EXPERIMENTAL SECTION

2.1 Livestock, rations, and equipment

The livestock used were 12 weeks old male turkeys of 80 heads with an average weight of $1,165 \pm 62.24$ g. The feed ingredients that make up the ration can be presented in Table 1. The acidifier used consists of a mixture of lactic acid, propionic acid, and formic acid, as well as inulin sourced from dahlia tuber extract and the enzyme papain. The tools used in research activities are grinders, digital scales with an accuracy of 0.01 g, places to feed and drink.

2.2 Mixture of Acidifier, Inulin and Papain Enzyme Preparation

The preparation stage before preparing the treatment ration begins with the preparation of an acidifier made from a mixture of lactic acid, propionic acid, and formic acid with a ratio of 1:1:1 each. Making inulin from bitter palm root extract follows the research method of Krismiyanto et al. (2020), and the commercial papain enzyme comes from the production of Chinese herbs.

2.3 Turkey Rearing

Turkey maintenance was carried out for 68 days consisting of 14 days of adaptation and 54 days of treatment. This stage begins with the adaptation phase in turkeys, which is carried out for 14 days before the turkeys are treated with a control ration. From 15 to 54 days old, they are given a treatment ration. For 1 to 14 days, the turkeys are adapted to the ration in stages, namely:

- 1-7 days old: 100% commercial ration
- 8 days old: 75% commercial ration + 25% homemade ration (T0)
- 9 days old: 50% commercial ration + 50% homemade ration (T0)
- 10 days old: 25% commercial ration + 75% homemade ration (T0)
- 11-14 days old: 100% homemade ration (T0)

From 15 to 68 days old, they are given treatment rations. Feeding turkeys treated with an acidifier method consisting of a mixture of lactic acid, propionic acid, and formic acid with a ratio of 1:1:1 in the amount of 1 ml, 1.2% inulin, and 0.15% papain enzyme mixed with a small amount of ration (approximately 20 g) given every morning and waited until finished. Then, rations without acidifier, inulin, and papain enzymes are added to meet the daily ration requirements.

Table 1. Rations Composition and Nutrient Content

Rations Material	Composition (%)
Yellow Corn	55.31
bran	13.64
Soybean meal	20.00
Fish flour	10.00
$CaCO_3$	0.50
Premix	0.25
Lysine	0.10
methionine	0.20
Total	100.00
Nutrient Content* (%)	
Metabolic Energy** (kcal/kg)	3,086.46
Proteins (%)	25.67
Fat (%)	4.75
Fiber (%)	6.92
Ca (%)	1.35
P Total (%)	1.00

*Results of ration analysis at the Laboratory of Nutrition and Feed Science, Faculty of Animal Husbandry and Agriculture, Diponegoro University (2021).
**Based on the calculation results of the Bolton formula (1967).

2.4 Research Design

The study was arranged using a completely randomized design (CRD) with 5 treatments and 4 replications, each unit containing 4 turkeys. The treatments given to turkeys are as follows:

- T0 = control ration
- T1 = control ration + 1% acidifier
- T2 = control ration + 1% acidifier + 1.2% inulin
- T3 = control ration + 1% acidifier + 0.5% papain enzyme
- T4 = control ration + 1% acidifier + 1.2% inulin + 0.5% papain enzyme

Table 2. Differential mean leukocytes in male turkeys during the growth period due to the addition of acidifier with inulin and papain enzymes in the ration

Parameters	Treatment				
	T0	T1	T2	T3	T4
Leukocytes (× 103/ml)	9.98b	10.71b	11.08b	11.06b	12.38a
Heterophile (%)	31.50c	35.50ab	36.75ab	34.50bc	38.50a
Lymphocytes (%)	60.75a	60.00a	55.00ab	56.75b	55.50b
Total Plasma Protein (g/dl)	2.55b	2.75bc	3.05ab	2.85abc	3.10a

Note: a, b, c Different superscripts in the same line show significant differences (p <0.05).

2.5 Research Parameters

Parameters measured were differential leukocytes, including heterophils, lymphocytes, leukocytes, and total plasma protein. Blood samples for leukocyte differential analysis were taken on day 35 before carcasing by cleaning the inner wings with alcohol. Then, a syringe was injected into the brachial vein and approximately 3 ml of blood was taken. The blood was put into a tube that had been given an anticoagulant in the form of EDTA to avoid blood clots and stored in a cooling box until analysis. Leukocyte differential data collection was carried out at the Microbiology Laboratory, Faculty of Agriculture, Gadjah Mada University, Yogyakarta.

2.6 Statistical Analysis

Data were analyzed using analysis of variance at a significance level of 5%. If there is a significant difference, then proceed with Duncan's test at the 5% level.

3. RESULTS AND DISCUSSION

Based on the research conducted, it is possible to obtain differential data on male turkey leukocytes during the growth period with the addition of an acidifier with inulin and papain enzymes in the ration presented in Table 2.

3.1 Blood Leukocyte Count

Based on the results of statistical analysis, the addition of acidifier with inulin and papain enzymes in the ration had a significant effect ($P < 0.05$) on the blood leukocyte count of male turkeys during the growth period. The results of this study indicated that the range of leukocyte counts in male turkeys during the growth period ranged from $9.98 - 12.38 \times 10^3$/ml, where the average number was in a healthy condition. The normal leukocyte count in male turkeys during the growth period is in the range of $16.0 - 25.5 \times 10^3/\text{mm}^3$ (Darwati et al., 2013).

The addition of acidifier with inulin and papain enzymes in the ration had an effect on the blood leukocyte count of male turkeys during the growth period, which showed that the T4 treatment (12.38×10^3/ml) was higher than the addition of other treatments. The higher the level of addition of the treatment, the higher the number of leukocytes. The average number of leukocytes in the male turkey's blood indicates an unhealthy condition, so efforts are needed to increase leukocytes to fight pathogenic bacteria and viruses that enter the body. An increase and decrease in white blood cells in turkeys can cause turkeys to become susceptible to disease. A decrease in the number of leukocytes can be caused by changes in the environment that will stress livestock (Saputro et al., 2016; Santi, 2018).

3.2 Heterophiles

Based on the results of statistical analysis, the addition of acidifier with inulin and papain enzymes in the ration had a significant effect ($P < 0.05$) on the number of blood heterophiles in male turkeys during the growth period. The results of this study indicated that the heterophile number of male turkeys during the growth period ranged from 31.50% - 38.50%, where the average number was in an unhealthy condition.

The addition of acidifier with inulin and papain enzymes (T4) in the ration had an effect on the blood heterophile count of male turkeys during the growth period compared to the control treatment (T0). The use of inulin as a prebiotic can increase the growth of lactic acid bacteria in the digestive tract, leading to a higher number of heterophils. Acidification can also inhibit the growth of pathogenic bacteria (Faradila et al., 2016; Mariana and Susanti, 2012).

3.3 Lymphocytes

Based on the results of statistical analysis, the addition of acidifier with inulin and papain enzymes in the ration had a significant effect ($P < 0.05$) on the blood lymphocyte count of male turkeys during the growth period. The results of this study indicated that the range of lymphocyte counts for male turkeys during the growth period ranged from 55.50% – 60.75%, where the average number was in an unhealthy condition. The normal lymphocyte count in male turkeys during the growth period is around 50.6% (Tethool et al., 2009).

The addition of acidifier with inulin and papain enzyme (T4) in the ration on the number of blood lymphocytes of male turkeys during the growth period showed higher results compared to the T1 and T0 treatments. Inulin as a prebiotic can increase the growth of lactic acid bacteria, lead-

ing to a higher number of lymphocytes. Acidification can lower the pH in the digestive tract and inhibit the growth of other bacteria. A decrease in the number of lymphocytes in turkey blood can occur because pathogenic bacteria cannot thrive in acidic environmental conditions (Sumarsih et al., 2012; Hartoyo et al., 2021).

3.4 Total Plasma Proteins

Based on the results of statistical analysis, the addition of acidifier with inulin and papain enzymes in the ration had a significant effect ($P < 0.05$) on the total blood plasma protein of male turkeys during the growth period. The results of this study indicated that the total plasma protein range for male turkeys during the growth period ranged from 2.55 - 3.10 (gr/dl), where the average number was in an unhealthy condition.

The addition of acidifier with inulin and papain enzymes (T4) in the diet to the total plasma protein of male turkeys during the growth period was higher than the T1 and T0 treatments. Acidifiers such as lactic acid can increase the response to disease attacks in livestock, resulting in an increase in plasma protein in the blood (Hartoyo et al., 2021).

4. CONCLUSIONS

Based on these results it can be concluded that the addition of acidifier, inulin and papain enzymes in the ration has a positive effect on the health condition of the body seen from the differential leukocytes in turkeys of the growth period.

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