# Potential of Nanoparticels Dayak Onion (*Eleutherine palmifola*) as an Immunomodulator for Supporting Spleen Tissue Growth and Broiler Performance

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**Abstract.** The aim is to analyse the potential of nanoparticles in Dayak Onion as an immunomodulator in supporting spleen tissue growth and improving broiler production performance. The research material is nanoparticles Dayak Onion (NPDO) and broiler. The treatment was administered of NPDO 1%, 2%, 3%, positive and negative (control). The research method is experimental. The variable are immune response, spleen tissue and broiler production performance. The Research design is completely randomized. Data were analysed using Analysis of Variance and followed by the Duncan Test (5%). The treatment had a significant effect ($P < 0.05$) on the immune response (Ig G and Ig Y). The highest concentration of Ig G was in the Negative Control (36.64 g/L), while the highest concentration of Ig Y was in NPDO 3% (22 g/L). The best increase in spleen weight was observed at NPDO 3% (2.81g), and the lowest tissue damage score was recorded at NPDO 3% (1.7%). NPDO does not affect broiler performance, as measured by average weight gain (AWG) and index performance (IP). The conclusion is that NPDO provide a good response to immunity and supporting spleen tissue, but not to broiler performance production. NPDO as a feed additive for immunomodulators' broilers is recommended to be given at 3% of the basal feed.

**keywords**: broiler; dayak onion; immunomodulator: nanoparticles, production performance

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# 1 Introduction

Herbal feed additives or additional feed derived from medicinal plants have begun to be recognized by the poultry farming industry since the ban on the use of Antibiotic Growth Promoters (AGP). AGP is used to increase livestock productivity. AGP is a feed additive antibiotic given to livestock, especially poultry, to eliminate pathogenic bacteria in the digestive tract to obtain good body weight and feed conversion ratio. In fact, AGP is administered at a therapeutic dose in the hope that it does not enter the meat or egg tissue. Cases of antibiotic resistance and cross-resistance in poultry have strengthened the prohibition on the use of AGP. AGP is considered to cause antibiotic resistance in consumers in the future [1].

The use of herbal feed additives as a Natural Growth Promoter (NGP) is an alternative solution to replace AGP in the poultry farming industry, both laying hens (layers) and broilers. Herbal plants as NGP have been provided so far in the form of simplicia or extracts, yielding varying results in terms of production performance. Preparations in the form of simplicia cannot provide accurate dosage, while the weakness of the extract form is its low bioavailability. The alternative to increasing solubility and maintaining the functional properties of extracts is to formulate extracts in nanoparticle preparations, thereby enhancing the delivery capacity of herbal bioactive compounds, increasing compound solubility, and improving particle absorption [2].

Dayak onion (*Eleutherine palmifolia*) is one of the medicinal plants being studied as a substitute for AGP. Dayak onions are an indigenous plant from Central Kalimantan that is widely distributed. Dayak onions are wild plants commonly found in tropical forests. Dayak onions contain antioxidants, phenols, polyphenols and their derivatives [3]. Research on the effects of simplicia and Dayak Onion extract on poultry production performance has been carried out with varying results.

Dayak Onion Flour at a concentration of 2.5% in broiler feed aged 15-35 days increased carcass percentage by 66.97% and reduced abdominal fat by 0.76%, improved broiler production performance at a concentration of 2% [4]. The addition of 0.3% Dayak Onion extract and Lactobacillus acidophilus to broiler feed rations improved protein digestibility, meat protein mass, and final broiler weight. However, it did not affect protein intake. Additionally, it increased crude fibre digestibility and cumulative body weight gain, despite similar digestion rates and energy utilization [5].

Research on Dayak Onions as an immunomodulator is still limited, despite their high antioxidant activity, with an IC50 of 52.38 ppm [6]. Research on the immunogenic properties of Dayak Onions only examines the blood profile, not the immunogenic profile or tissue anatomical pathology of broiler immune organs, resulting in incomplete information regarding their use as an immunomodulator. Holistic research is necessary to determine the potential of Dayak Bawang extract nanoherbal feed additive as an immunomodulator to support spleen tissue growth and enhance broiler production performance. The research aims to analyze the potensial of feed additive nano particles Dayak Onion as an immunomodulator in supporting spleen tissue growth and broiler production performance

# 2. Methods

Research on the potential of Nano Particles Dayak Onion (NPDO) feed additive as an immunomodulator employed experimental methods with a completely randomized design.

### 2.1. Research Preparation

Preparations were made for materials, tools, and laboratories, both in the field laboratories of Universitas Islam Malang, Indonesia, where experimental animals, namely broilers, were raised, and in the integrated laboratories of Universitas Islam Malang for the preparation and manufacture of NPDO. Provided 100 Day Old Chick (DOC) broilers to be reared for 35 days. The cages in the field laboratory were disinfected before DOC entered (i.e., when the chicks arrived). DOC grouping was carried out at 10 days of age. Grouping based on treatment, namely K+ = Control with vitamins, K- = negative control without vitamins, P1 = Giving NPDO at 1% of basal feed, P2 = 2% and P3 = 3%.

### 2.2. DOC maintenance

Chick-in was carried out after the preparation of the brooder, curtains, cage equipment, and feed were available. During maintenance, the vaccine and vitamin administration schedule was carried out according to the Standard Operating Procedure. B2 feed was given when broilers were 15-35 days old.

### 2.3. Preparations of NPDO

Dayak onion extract was formulated into nanoparticles using the ionic gelation method with varying concentrations of chitosan polymer (0.1%, 0.2%, 0.3%, and 0.4%) and tripolyphosphate. Test parameters included determining the size and polydispersity index of nanoparticles using a particle size analyzer.

### 2.4. Treatment of Experimental Animals (Broilers)

NPDO was given to broilers aged 14 days at the dose according to the treatment. NPDO was mixed into the feed.

### 2.5. Data Collection

Production performance measurements (Weight Gain and Index Performance) were carried out at the end of the study, namely, broilers aged 35 days. Blood and spleen tissue samples were also taken at the end of the study. Blood was taken to see the Immunoglobulin Y and G molecular profile and spleen tissue (size and PA of the spleen).

### 2.6. Data Analysis

The data obtained were analyzed using Analysis of Variance and subsequently analyzed using the Duncan Test to determine the differences between each treatment.

## 3. Result and Discussion

### 3.1. Respon Immune

NPDO Feed Additive has a significant effect ($P<0.05$) on the immune response of broilers. The immune response measured is IgG and IgY. Dayak Onion Extract in nanoparticle form has high bioavailability, allowing the bioactive components to be maximally absorbed by body cells. The average of Ig and Ig Y can be seen in Table 1.

**Table 1**. The average of Ig and Ig Y

| Concentration (g/L) | NPDO Treatments | | | | |
|---|---|---|---|---|---|
| | C+ | C- | 1% | 2% | 3% |
| Ig G | 10,10[a] | 36,64[d] | 12,57[a] | 18,91[b] | 24,97[c] |
| Ig Y | 20,72[b] | 25,53[c] | 18,94[a] | 21,57[b] | 26,22[d] |

[a,b]Values with different superscript within the same row were significantly different (P<0,05). Control+= broilers receiving vitamin without NPDO ; Control-= broilers receiving nor vitamin and NPDO, NPDO =Nano Particles Dayak Onion Dayak , Ig= Immunoglobulin.

NPDO affects broiler's immunity. IgG and IgY are part of the immune system that is formed in response to microbes. Immunoglobulins in poultry consist of three classes: IgY, IgA, and IgM. IgY, which stands for immunoglobulin yolk, is an antibody produced in egg yolk (yolk) from poultry. IgY is the primary antibody (75%), primarily found in serum and egg yolk. IgY was previously referred to as immunoglobulin G (IgG) due to similarities in function and concentration in serum. However, it turns out that the two classes of antibodies have fundamental differences in molecular weight, structure and biochemical function [7].

Broilers without NPDO and Vitamins, or negative controls, have yielded the highest IgG results, with levels below NHBD 3%. IgG is a response to the vaccination program, which must be carried out in accordance with the broiler maintenance Standard Operating Procedures. The response to the vaccine results in an increase in the amount of protein, enhanced cellular defence mechanisms, and a high production of antibodies. In this situation, the number of T lymphocytes, B lymphocytes, plasma cells and antibodies will increase. The increase in protein due to the vaccination response can lead to an increase in the number of Immunoglobulin G and lymphocytes [8].

NPDO 3% yielded the highest IgY. NPDO particles are expressed within cells without altering the bioactive components, including antioxidants and antibacterials. This facilitates the transfer of antibodies into the serum. IgY provides passive immunity in broilers, while IgG provides active immunity. The bioactive substances in NPDO are better as immunomodulators by modulating immune molecules. The antimicrobial activities of herbs against various pathogens are mainly attributed to the natural bioactive molecules present in these plants, such as alkaloids, flavonoids, and terpenoids. The mechanisms by which herbs enhance the immune system and combat pathogens include disrupting bacterial cell membranes, suppressing protein synthesis, and limiting pathogen replication through the inhibition of nucleic acid synthesis. Medicinal herbs have been shown to treat various infectious diseases by modulating the immune system's components, for instance, by reducing pro-inflammatory cytokines or promoting the production of anti-inflammatory cytokines [9]. IgY has several advantages, including immunogenicity, specificity, and affinity. The transfer of IgY from serum to egg yolk is facilitated by receptors that carry out the selective transfer of antibodies from the parent serum [7].

### 3.2. Supporting Spleen Tissue

Administering the NPDO feed additive had a significant effect ($p < 0.05$) on supporting spleen tissue. Supporting spleen tissue is modelled in 2 parameters: spleen weight and spleen tissue damage scoring. The results of supporting spleen tissue can be seen in Table 2.

Giving NPDO 3% in basal feed yielded the best spleen weight results, although the difference was not statistically significant compared to the control. NPDO made Dayak Onion bioactive substances easily soluble, and functional properties were maintained despite changes. Apart from that, it will increase the delivery capacity of onion herbal bioactive compounds. Dayak thereby increases the solubility and the absorption of Dayak Onion

particles. The bioactive substances in Dayak Onions stimulate the proliferation of red pulp and white pulp cells in the spleen. This causes the weight of the spleen to increase. The Control (+) treatment resulted in the same spleen weight as the 3% Dayak onion treatment. The control (+) treatment also added vitamins, as is typical for broiler rearing. The vitamins provided had the same effect on spleen weight, although the mechanisms were different. Both treatments supported the proliferation of spleen cells, which play a crucial role in the immune system.

Primary lymphoid organs in poultry consist of the thymus and bursa of Fabricius. In contrast, secondary lymphoid organs include the spleen, tonsils, Meckel's diverticulum, Harderian glands, and mucosa-associated lymphoid tissue (MALT) in the digestive tract and respiratory tract. The role of the spleen in the defence system is related to the immunological response to antigens that have successfully reached the blood circulation, thereby preventing the invasion of organisms or toxins before they spread widely. Apart from that, the spleen functions as a place for the maturation of antibody-producing cells [10].

**Table 2.** Supporting Spleen Tissue

| Spleen | NPDO Treatments | | | | |
|---|---|---|---|---|---|
| | C+ | C- | 1% | 2% | 3% |
| Weight (g) | $2.69^{a}$ | $1.88^{b}$ | $1.70^{b}$ | $2.81^{b}$ | $2.76^{a}$ |
| Damage skoring* | $1.9^{ab}$ | $2.6^{c}$ | $2.5^{c}$ | $2.1^{b}$ | $1.7^{a}$ |

$^{a,b}$Values with different superscript within the same row were significantly different. Control+= broilers receiving vitamin without NPDO ; Control-= broilers receiving nor vitamin and NPDO, NPDO=Nanoparticles Dayak Onion *Skoring spleen tissue damage (Etriwati, 2017).

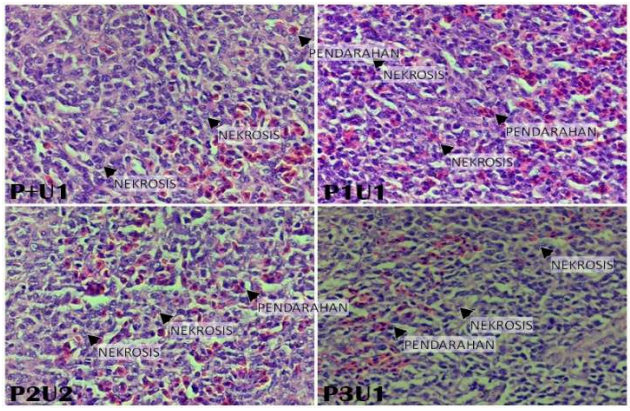


**Fig. 1.** Damage to spleen tissue by scoring the amount of necrosis, degeneration, and inflammation. NPDO 3% on mark P3U1 with a score of 1.7 indicates that necrosis, degeneration, and inflammation are only focal or some are multifocal, whereas NPDO 1% and 2% with marks P1U1 and P2U2 are diffuse. HEx400

One of the organs that plays a role in the immune system is the spleen. Apart from functioning as a defence against microorganisms, the spleen is also the main site of destruction of old erythrocyte cells by macrophages and can react to antigens carried in the blood, immunologically filtering the blood. The spleen and mucosal-associated lymphoid tissue (MALT) are secondary lymphoid organs. The red pulp in the spleen contains many erythrocytes, plays a role in hematopoietic function, and filters the circulation of erythrocyte cells. In contrast, accumulation of lymphocyte cells is found in the white pulp. Secondary

lymphoid organs are composed of collections of lymphoid cells. The accumulation of lymphoid tissue spreads along the mucosa (MALT) and spreads naturally since embryonic development [11].

Scoring for spleen tissue damage was the smallest in broilers with NPDO 3%. NPDO with bioactive substances exhibits antioxidant and anti-inflammatory properties. This bioactive substance helps spleen cells, including those in both the white pulp and red pulp, to proliferate and develop properly. Damage to spleen tissue during chicken growth can be reduced by adding herbal feed additives [12]. A score of 1.7 indicates that necrosis, degeneration and inflammation are only focal or some are multi-focal. The low scoring of spleen damage indicates that the white pulp is a key parameter for a well-functioning immune system. The red pulp, a part of the spleen, contains a large number of erythrocytes, making it lighter in colour. In this section, numerous lymphocyte cells, particularly T lymphocytes originating from the primary lymphoid system, as well as macrophages and dendritic cells, are present. Red pulp and white pulp are separated by a membrane called the marginal zone [13]. The histopathological description of the spleen is shown in Figure 1.

### 3.3. Production Performance

NPDO had no effect ($P > 0.05$) on broiler production performance. Production performance parameters are body weight gain (BWG) and Performance Index (IP). PBB is the difference between body weights at age 35 and age 14. IP is a value that describes the production performance of broiler chickens in one maintenance period. IP reflects how efficiently the feed is used, how high the harvest weight is, how low the mortality is, and how quickly the harvest matures. The average BWG and IP can be seen in Table 3

**Table 3.** Broiler Production Performance with NPDO

| TREATMENTS OF NPDO | BWG (kg) | IP |
|---|---|---|
| C+ | 1,73 | 305,34 |
| C- | 1,75 | 321,38 |
| P1 | 1,64 | 305,78 |
| P2 | 1,57 | 280,91 |
| P3 | 1,57 | 288,38 |

The NPDO and Control treatments gave similar BWG and IP results. In this case, NPDO, a bioactive substance in Dayak Onions, works more on broiler immune cells than digestive cells. Bioactive substances in plants are secondary metabolites, such as phenols or polyphenols, saponins, flavonoids, curcumin, artemisin, tannins, and anthraquinones. The bioactive substances phenol and saponin inhibit or slow down the growth of bacteria, fungi, and parasites that target the membrane or cytoplasm. Several herbal plants, such as meniran (*Phyllanthus urinaria*), noni (*Morinda citrifolia*), ginger (*Zingiber officinale*) and bitter/Sambiloto (*Andrographis paniculate*), are reported to have immunomodulatory activities [14]. Secondary metabolite substances from herbal plants such as phenols or polyphenols, saponins, flavonoids, curcumin, artemisin, tannins, anthraquinones are more antimicrobial and anti-inflammatory [15]. The antimicrobial and anti-inflammatory properties of phenol can enhance broiler immunity. BWG and IP are primarily determined by the working mechanism of digestive cells rather than immune cells. NHBD has no effect on broiler production performance, both weight gain (BWG) and Index Performance (IP).

## 4. Conclusion

Nano Particles Dayak Onion (NPDO) feed additive provides a good response to immunity and supports spleen tissue. NPDO has potential as an immunomodulator for supporting spleen tissue growth, but not for improving broiler production performance. NPDO as a feed additive for immunomodulatory broilers is recommended to be given at 3% of the basal feed.

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