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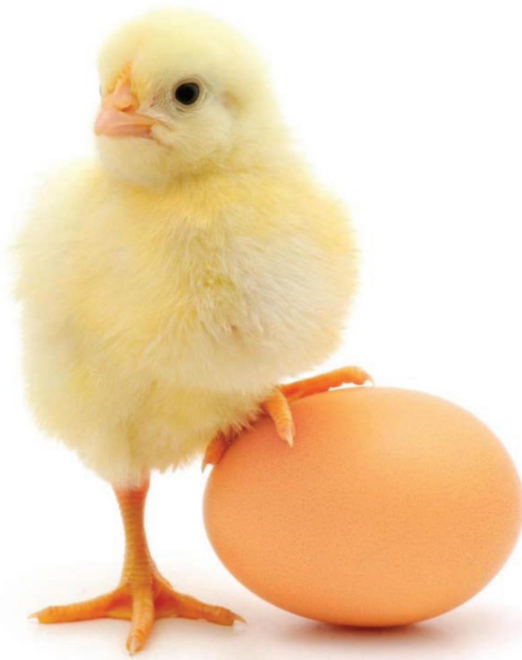

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Research Article
Development of a Natural Oil Blend Formulation to Replace Antibiotics and Growth Promoters in Broiler Chicken Feed

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Abstract
Background and Objective: There has been continued effort to find an alternative to antibiotic growth promoters (AGPs) to improve broiler chicken performance. Several studies have been conducted on the use of herbal active compound for development of natural oil blend formulation (NOBF). This study aimed to explore the effectiveness of NOBF on the productivity, health and safety of broiler chickens. **Materials and Methods:** The parameters observed were productivity (feed and water consumption, weight gain, final weight, feed conversion ratio (FCR), mortality and meat proximate test), health (red blood cell features-red blood cell count, hematocrit and hemoglobin; white blood cell features-white blood cell count, white blood cell differentiation into lymphocytes, monocytes, heterophils, eosinophils, basophils and the heterophil/lymphocyte ratio; and Newcastle disease antibody titer), safety, liver function [serum glutamate pyruvate transaminase (SGPT) and serum glutamate oxalate transaminase (SGOT)] and kidney function (urea and creatinine). A total of 320 day-old chickens (DOC) were divided in a complete randomized design into 2 groups: one group was given standard feed without AGP or essential oil (control) and the other group was given feed (without AGP) with NOBF added at a dose of 2 kg t^{-1} feed, with 160 repetitions and dietary treatments were administered for 30 days. Blood samples were collected at the beginning (H0) and end (H35) of the research. **Results:** The NOBF significantly improved the final weight by week 4 compared to the control ($p<0.05$), showed lower mortality and did not influence the physiological function, liver function, or kidney function of broiler chickens. **Conclusion:** The administration of NOBF at a dose of 2 kg t^{-1} feed to broiler chickens was proven effective in increasing performance and was safe to use (nontoxic).

Key words: Broiler chicken, natural oil blend formulation, poultry feed, broiler health, Productivity

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Competing Interest: The authors have declared that no competing interest exists.

Data Availability: All relevant data are within the paper and its supporting information files.

INTRODUCTION

Poultry is a developing sector in Indonesia. Poultry-derived food products are experiencing an increase in demand along with an increase in the Indonesian population[1]. This situation has positively influenced the poultry industry and improve the broiler chickens productivity. However, broiler chicken management is prone to viral, bacterial, fungal and parasitic diseases that are massively contagious[2-6]. A high rate of poultry disease causes a high number of deaths and morbidities, which can decrease production and affect meat quality[7].

Previously, efforts were made by farmers to improve broiler chicken performance by using antibiotic growth promoters (AGPs). The use of AGP in animal feed reduce the population of pathogenic microorganisms, which can increase feed efficiency and allow optimum livestock growth[8]. However, massive overuse of antibiotics can cause microorganism resistance and antibiotic residues[9,10]. Thus, an alternative solution is needed to maintain chicken productivity at its optimum level without the use of AGPs.

Several studies have been performed to increase broiler chicken performance through the use of herbal materials such as garlic, ginger, curcumin, Javanese ginger and galanga[11-14]. Pine is an essential oil and an active compound found in plants that increases broiler chicken performance[15]. Essential oils can be utilized as antibacterials, antifungals, antivirals and antioxidants[16]. It is anticipated that the antibacterial properties of essential oil blend will inhibit pathogenic bacteria that cause diseases in broiler chickens.

Natural oil blend formulations (NOBFs) contain several essential oils, including pine oil (*Pinus* sp.), lavender oil (*Lavandula* sp.) and eucalyptus oil (*Eucalyptus* sp.). Pine oil contains active compounds that act as antibacterial and anti-inflammatory compounds[17,18]. Linalool essential oil generated from lavender has antibacterial effects[19]. Eucalyptus oil contains a cineol active compound that is antibacterial[20]. We hypothesized that the essential oil content in NOBF will show synergy in increasing the productivity of broiler chickens.

Objective: This study aimed to explore the safety administration and effectiveness of NOBF on the productivity and health of broiler chickens.

MATERIALS AND METHODS

Experimental design: This study was conducted from April to July 2019 in the poultry cage of the Animal Laboratory of Management Unit (Unit Pengelola Hewan Laboratorium-UPHL) in the Faculty of Veterinary Medicine, IPB University (FKH-IPB). This study used 320 day-old chicks (DOCs), code CP707. The birds were divided in a complete randomized design with 2 groups: first group of chicken was given standard feed without an AGP or essential oil (control) and second group was given feed (without an AGP) supplemented with NOBF at a dose of 2 kg t^{-1} feed (treatment). Each group consisted of 160 repetitions. The chickens were acclimatized for 5 days and dietary treatment was administered for 30 days.

Preparation, caging and feeding stage: Chicken cages, feeding equipment and cage equipment were disinfected by soap water and disinfectant (a combination of glutaraldehyde, ammonium chloride and isopropanol) before the research was performed. The cage used was a colony cage layered with sterile husk litter. The feeds used were commercial feed (without additional compounds) and feed supplemented with NOBF. Feed was divided according to chicken age, which consisted of starter feed (1-14 days) with feed code Booster 500, grower feed (15-21 days) with feed code S11 and finisher feed (22-38 days) with feed code S12. All feed used in this study was produced at Feed mill, Surabaya of Charoen Pokphand Indonesia. The proximate analysis of feed used is presented in Table 1.

Research chicken management: DOCs were given sugar water on day 1 to rejuvenate the chickens post-transport. During rearing, chickens were vaccinated with ND-I, IBD and ND II consecutively when they reached the age of 4, 10 and 17 days, respectively. Drinking water was given *ad libitum* and replaced every day.

Observed parameters: The parameters observed to test the effectivity of NOBF on the performance of broiler chickens were broiler productivity, broiler health and the safety of NOBF.

Observation of broiler chicken productivity: Broiler chicken productivity was observed by measuring feed consumption, water consumption, weight gain, final weight, mortality and feed conversion ratio (FCR), as well as meat quality (thigh, breast and wing) was tested by proximate analysis. Meat proximate analysis was performed on day 35 after treatment. Proximate analysis was performed on the water content, crude protein, crude fat, carbohydrate or nitrogen-free extract (NFE) and ash.

Observation of broiler chicken health: Broiler chicken health was observed through red blood cell features (red blood cell count, hematocrit and hemoglobin), white blood cell features

Table 1: The feed proximate analysis of feed given during research

	Feed types					
Parameters	Booster 500K	Booster 500T	S11 K	S11 T	S12 K	S12 T
L-histidine (mg kg^{-1})	6181.73	8589.68	6716.24	5384.80	6096.50	6567.56
L-threonine (mg kg^{-1})	10467.17	12899.95	10543.63	9908.83	10017.09	10574.90
Phosphorus (mg kg^{-1})	6559.82	5604.71	6513.73	4428.14	6914.02	4882.50
Water Content (%)	10.98	11.05	10.70	11.04	10.92	10.49
Protein (%)	22.81	23.43	20.22	19.89	18.75	18.74
Ash (%)	6.12	6.42	6.04	5.52	6.55	7.22
Energy from Fat (kcal 100 g^{-1})	52.11	50.22	50.22	53.46	54.36	57.33
Total Fat (%)	5.79	5.58	5.58	5.94	6.04	6.37
Total Energy (kcal 100 g^{-1})	360.55	358.02	360.94	363.46	360.32	361.01
Carbohydrate (%)	54.30	53.52	57.46	57.61	57.74	57.18
Calcium (mg 100 g^{-1})	1460.75	664.24	1871.27	1179.52	1599.06	794.04
Natrium (mg 100 g^{-1})	300.01	124.04	298.57	218.30	314.17	563.62
Potassium (mg 100 g^{-1})	1275.23	1072.92	1412.05	876.83	1350.01	947.79
Total Aflatoxin (mcg kg^{-1})	4.23	7.93	5.73	11.80	9.98	6.09
L-serine (mg kg^{-1})	11952.31	14860.72	12462.54	11207.11	10753.04	10466.73
L-glutamic acid (mg kg^{-1})	41077.10	43812.67	32728.94	42564.36	32021.70	30666.03
L-phenylalanine (mg kg^{-1})	12217.00	18926.94	14453.77	11417.93	11484.92	13183.18
L-Isoleucine (mg kg^{-1})	9109.51	9929.11	7353.67	8107.43	7629.92	7052.55
L-valine (mg kg^{-1})	10209.96	11168.01	8963.53	9580.34	9018.85	8465.21
L-alanine (mg kg^{-1})	9645.96	10412.46	9382.94	10416.01	9052.76	8563.30
L-arginine (mg kg^{-1})	13949.49	18862.13	14432.00	13485.37	13421.17	13305.51
glycine (mg kg^{-1})	9660.12	11519.98	9904.24	9079.32	9410.37	8719.60
L-lysine (mg kg^{-1})	10895.28	10212.13	8316.12	11166.86	9049.81	7568.65
L-aspartic acid (mg kg^{-1})	17706.22	19912.26	15133.32	20496.05	14089.07	13615.68
L-leucine (mg kg^{-1})	17950.71	19191.89	16001.96	16664.04	16242.31	15364.24
L-tyrosine (mg kg^{-1})	6136.55	9799.44	7548.03	6135.94	6167.99	6701.27
L-proline (mg kg^{-1})	12546.82	13168.20	11305.50	12108.37	11020.93	10336.72

(white blood cell count and differentiation), blood chemistry (triglyceride, total cholesterol, high density lipoprotein (HDL), low density lipoprotein (LDL), total protein and albumin and antibody titer tests. The analysis of red and white blood cell features and blood biochemistry of broiler chickens were performed on day 0 (before treatment) and day 35 (after treatment). An antibody titer test was performed by sampling blood serum twice, 7 days after ND I vaccination and 7 days after ND II vaccination (booster).

Observation of the safety of NOBF: The safety of NOBF administration was observed through liver function [serum glutamate pyruvate transaminase (SGPT) and serum glutamate oxaloacetate transaminase (SGOT)], kidney function (urea and creatinine) and observation of gross pathology (GP) and histopathology (HP) of the liver and kidney. GP and HP examinations of the liver and kidney were performed on day 35 after treatment.

Data analysis: Data were analyzed using Student's t-test with the statistical software program Minitab 18.

Table 2: The effect of NOBF administration on broiler chicken productivity

	Groups	
	Control	NOBF
Feed consumption (g $chicken^{-1}$ day^{-1})	68.80 ± 6.00^a	70.55 ± 3.33^a
Water consumption (mL $chicken^{-1}$ day^{-1})	190.01 ± 16.72^b	224.27 ± 10.80^a
Weight gain (g)	1457.00 ± 8.15^b	1555.50 ± 3.24^a
Final weight (g)	1496.20 ± 7.94^b	1594.70 ± 3.16^a
Mortality (%)	10.00	5.63
FCR	1.65 ± 0.14^a	1.59 ± 0.07^a

Annotation: Different superscripts in the same row indicate significantly different results ($p<0.05$)

RESULTS AND DISCUSSION

Broiler chicken productivity: Weight, food and water efficiency, mortality: The effect of NOBF administration on chickens was observed from several indicators: weight gain, final weight, mortality and FCR. The effectiveness of NOBF administration on the productivity of broiler chickens are presented in Table 2. Feed consumption was not statistically different between chickens fed diet supplemented with NOBF and the control group. Feed consumption of chickens in treatment group was 70.55 g $chicken^{-1}$, while control group chickens had a feed consumption value of 68.80 g $chicken^{-1}$.

The feed consumption in the treatment group was higher than that of the control group. The final weight of the treatment group was higher than that of the control group.

Table 2 indicates that broiler chickens fed diet supplemented with NOBF had higher water consumption than that of the control chickens. Water consumption in the NOBF-treated group was 224.27 mL chicken^{-1} and in the control group was 190.01 mL chicken^{-1}. Results showed that NOBF-treated chickens had higher feed and water consumption than that of the controls.

The final weight of NOBF-treated chickens was significantly higher ($p<0.05$) than that of the control chicken (Table 2). The average final weight of NOBF-treated chickens was 1594.70 g chicken^{-1}, while that in the control group was 1496.20 g chicken^{-1}. The addition of NOBF to feed improved weight gain by 6.76% compared to that of control chickens. This measurement showed that the addition of NOBF to feed can increase the weight gain and final weight of broiler chickens.

Weight gain, final weight and efficiency in this study can be observed from the feed conversion ratio (FCR). The NOBF-treated chickens showed lower FCR value (3.63%) than that of the control (Table 3). The FCR value of the NOBF-treated chickens was 1.59, while that of the control group was 1.65. Mortality percentage (death) is used to evaluate the advisability of tested material on chickens. High morbidity in chickens causes massive losses to farmers. Administration of NOBF to broiler chickens resulted in a mortality percentage of 5.63%, while mortality in the control group was 10.00%. Chicken mortality also decreased in the NOBF-treated group by 43.70% compared to controls (Table 2). Table 3 shows the weekly growth rate of broiler chickens after 4 weeks.

The results showed that at weeks 3 and 4, weight gain was higher in NOBF-treated chickens than that of the control. The average weekly weight gain in the treatment group was 124.19, 286.71, 404.01, 369.99 and 370.65 g chicken^{-1}. The average weekly weight gain for the control group was only 132.53, 272.23, 363.53, 353.97 and 334.74 g chicken^{-1}. Water and feed consumption, when related to the observed chicken final weight, showed that NOBF-treated chickens had a higher final weight than that of the control group.

Proximate analysis was performed to determine the effectiveness of NOBF on the productivity of broiler chickens. Proximate analysis measuring water content, crude protein, crude fat and carbohydrates in meat (thigh, breast and wing) in both the control and treatment groups showed relatively similar values (Table 4). The proximate analysis of the thigh, breast and wing of NOBF-treated chickens at the end of research is shown in Table 4. The ash content in the thigh meat of NOBF-treated chickens was lower (1.10%) than that of the control (1.23%). In contrast, the ash content in the breast and wing in all treatment groups had the same value.

Table 3: The effect of NOBF administration on broiler chicken weight gain within 4 weeks

Weeks No.	Groups: Control	NOBF
1	132.53±8.44^{a}	124.19±7.88^{a}
2	272.23±13.63^{a}	286.71±5.94^{a}
3	363.53±14.92^{b}	404.01±8.02^{a}
4	353.97±10.13^{b}	369.99±16.49^{ab}
5	334.74±22.92^{a}	370.65±15.15^{a}

Annotation: Different superscripts in the same row indicate significantly different results ($p<0.05$)

Table 4: Proximate meat on the breast, thigh and wing meat area of broiler chickens given NOBF treatment at the end of research

	Treatments: Control	NOBF
Breast		
Water content (%)	75.32±1.11^{a}	74.70±0.52^{a}
Crude protein (%)	18.40±1.15^{a}	19.95±0.43^{a}
Crude fat (%)	0.43±0.18^{a}	0.60±0.24^{a}
Carbohydrate (%)	4.29±0.80^{a}	3.38±0.62^{a}
Ash (%)	1.57±0.11^{a}	1.37±0.13^{ab}
Thigh		
Water content (%)	75.64±1.58^{a}	76.87±1.11^{a}
Crude protein (%)	16.15±0.07^{a}	15.32±0.79^{a}
Crude fat (%)	2.20±0.29^{a}	2.57±1.35^{a}
Carbohydrate (%)	4.79±1.33^{a}	4.14±2.23^{a}
Ash (%)	1.23±0.02^{a}	1.10±0.03^{b}
Wing		
Water content (%)	72.83±0.55^{a}	73.92±0.21^{a}
Crude protein (%)	18.96±0.92^{a}	17.71±0.83^{a}
Crude Fat (%)	1.47±0.37^{a}	2.56±0.99^{a}
Carbohydrate (%)	5.51±0.90^{a}	4.48±1.54^{a}
Ash (%)	1.23±0.13^{a}	1.33±0.17^{a}

Annotation: Different superscripts in the same row indicate significantly different results ($p<0.05$)

Broiler chicken health examination

Hematology examination: The results showed that the red blood cell of the NOBF-treated chickens was not significantly different ($p>0.05$) from that of the control group and was still within the normal range (Table 5). The results showed that the red blood cell counts in both the control ($3.43\times10^{6}/mm^{3}$) and NOBF-treated group ($3.50\times10^{6}/mm^{3}$) were within the normal range in China. According to Al-Nedawi[21] the normal range of chicken red blood cells is within 2.40-$3.90\times10^{6}/mm^{3}$. The observed red blood cell and white blood cell features in NOBF-treated chickens before and after treatment are presented in Table 5.

The hematocrit and hemoglobin values presented in Table 5 did not show significant differences between the control group and treatment group ($p>0.05$). Hematocrit

Table 5: The features of red blood cell, white blood cell and white blood cell differentiation before and after treatment

	Groups	
	Control	NOBF
Before treatment (day 0)		
Red blood cell count (10^6/mm^3)	2.10±0.22[a]	1.88±0.06[a]
Hematocrit (%)	21.78±4.73[a]	20.18±1.08[a]
Hemoglobin (g%)	9.33±1.38[a]	9.55±1.39[a]
White blood cell count (10^3/mm^3)	16.28±1.53[a]	12.36±1.93[b]
Lymphocyte (%)	55.80±5.63[a]	61.40±10.21[a]
Monocyte (%)	2.400±0.55[a]	2.20±0.44[a]
Heterophil (%)	41.80±5.21[a]	36.40±9.81[a]
Eosinophil (%)	0.00±0.00[a]	0.00±0.00[a]
Basophil (%)	0.00±0.00[a]	0.00±0.00[a]
H/L	0.76±0.18[a]	0.63±0.31[a]
After treatment (day 30)		
Red blood cell count (10^6/mm^3)	3.43±0.21[a]	3.50±0.22[a]
Hematocrit (%)	25.74±1.95[a]	26.16±3.01[a]
Hemoglobin (g%)	11.22±0.27[a]	10.80±0.59[a]
White blood cell count (10^3/mm^3)	13.12±3.75[b]	15.72±5.17[ab]
Lymphocyte (%)	55.17±1.59[b]	58.00±3.23[ab]
Monocyte (%)	3.20±1.03[a]	2.70±0.67[a]
Heterophil (%)	44.40±5.66[a]	44.00±7.79[a]
Eosinophil (%)	0.20±0.426[a]	0.60±0.84[a]
Basophil (%)	0.00±0.00[a]	0.00±0.00[a]
H/L	0.81±0.11[a]	0.76±0.15[a]

Annotation: Different superscripts in the same row indicate significantly different results ($p<0.05$)

Table 6: Blood biochemistry features of broiler chickens given NOBF by the end of research

	Groups	
Parameter	Control	NOBF
Triglyceride (mg dL^{-1})	134.50±19.10[a]	121.00±49.50[a]
Total cholesterol (mg dL^{-1})	149.00±18.40[a]	150.50±4.95[a]
HDL (mg dL^{-1})	93.50±4.24[a]	93.50±0.71[a]
LDL (mg dL^{-1})	31.50±4.95[a]	26.50±0.70[a]
Total protein (g dL^{-1})	2.84±0.59[a]	3.13±0.73[a]
Albumin (g dL^{-1})	1.00±0.13[a]	1.13±0.24[a]

Annotation: Different superscripts in the same row indicate significantly different results ($p<0.05$)

shows the percentage of red blood cells against total blood volume. The normal hematocrit value ranged from 22-45% for chicken, while the normal hemoglobin value ranged from 5.58-15.14 g%[21]. The NOBF- treated group had a hematocrit value of 26.16% and hemoglobin value of 10.80 g%, while the control group had a hematocrit value of 25.74% and hemoglobin value of 11.22 g%. The administration of NOBF did not lead to significantly different hematocrit and hemoglobin values compared to the control group.

White blood cell (leucocyte) feature examination consisted of white blood cell count and its differentiation percentage (lymphocytes, monocytes, heterophils, eosinophils and basophils), as well as heterophil and lymphocyte ratio percentage (H/L). Based on the results presented in Table 5, the average white blood cell counts and differentiation in all groups before and after treatment showed no significant difference and were still within the normal range. According to Arfah[22], normal leucocyte count was within 12.00-30.00×10^3/mm^3. The leucocyte counts after treatment in the control group and NOBF-treated group were 13.12×10^3/mm^3 and 15.72×10^3/mm^3, respectively.

The lymphocyte percentages after treatment in the control group and NOBF-treated group were 55.17 and 58.00%, respectively. The monocyte percentages after treatment in the control and NOBF-treated groups were 3.20 and 2.70%, respectively. The heterophil percentages after treatment in the control and NOBF-treated groups were 44.40 and 44.00%, respectively. The normal range of heterophil count in chickens is within 30.4-52%[23]. The eosinophil percentages after treatment in the control and NOBF-treated groups were 0.20 and 0.60%, respectively. The basophil percentage was 0% or not found in all chicken groups (Table 5). Broiler chicken stress index measurements showed that chickens experienced a decrease in the stress index in the NOBF-treated group compared to the control ($p>0.05$) both before and after treatment.

Blood biochemistry and antibody titer: Blood biochemistry of chickens showed the triglyceride levels, total cholesterol, HDL, LDL, total protein and albumin in blood serum. The triglyceride level, total cholesterol, HDL, LDL, total protein and albumin values in research chickens before treatment were 103.00-184.60, 185.60-217.80, 111.40-124.50, 13.20-27.25, 1.50-2.23 and 0.54-0.68 g dL^{-1}, respectively. The results showed no significant difference between the control group and NOBF-treated group after treatment. Blood biochemistry feature analysis of NOBF-treated broiler chickens at the end of the research is presented in Table 6.

The triglyceride values in the control and NOBF-treated groups after treatment were 134.50 and 121.00 mg dL^{-1}, respectively. The total cholesterol level of broiler chicken in the control and treatment groups was not significantly different. According to Mide[24] the normal range of cholesterol in chickens is within 125-200 mg dL^{-1}. The total cholesterol in the control and NOBF-treated groups was 149.00 and 150.50 mg dL^{-1}, respectively and was still within the normal range. This result showed that the administration of NOBF did not disturb body cholesterol metabolism.

According to Medah *et al.*[25] the HDL levels of chicken did not show a significant difference between the control and treatment groups. The normal HDL level in chicken is >22 mg dL^{-1}. The HDL levels in the control and NOBF-treated groups were 93.50 and 93.50 mg dL, $^{-1}$ respectively. The chicken LDL levels did not show a significant difference between the control and treatment groups. Medah *et al.*[25] reported that the normal LDL level in chicken is <130 mg dL^{-1}. The chicken LDL levels in the control and NOBF-treated groups after treatment were 31.50 and 26.50 mg dL^{-1}, respectively. These values are still within the normal range. The control and NOBF-treated chickens had total protein values of 2.84 and 3.13 g dL^{-1}, respectively. Thrall *et al.*[26] stated that the normal range of total protein for broiler chicken is from 2.5-4.5 g dL^{-1}. The results showed that the total protein and albumin levels in the chicken blood serum of the control and treatment groups were not significantly different. The broiler chicken albumin levels in the control and NOBF-treated groups were 1.00 and 1.13 g dL^{-1}, respectively. The next health examination involved an antibody titer test against one of the most frequent diseases to attack broiler chickens and cause high mortality: Newcastle disease (ND). The ND antibody titers in broiler chickens in the control and NOBF-treated groups are presented in Table 7.

One of the most frequent serological tests used to determine antibody titers is the hemagglutination inhibition (HI) test. A protective titer of ND antibody is ≥4 log 2 HI unit[27]. The results showed that antibody titers against ND based on the HI method in the control and treatment groups were not significantly different and were within the protective titer level.

Formulation safety

Liver and kidney function: Chicken blood serum examination at the levels of SGPT, SGOT, urea and creatinine has the objective of observing the status of liver and kidney function in research chickens. SGPT, SGOT, urea and creatinine levels in chickens before treatment were 151.90-227.20, 1.80-3.58, 1.10-2.22 and 0.26-0.29 mg dL^{-1}, respectively. Liver and kidney function tests in NOBF-treated broiler chickens at the end of the study are presented in Table 8.

SGPT value of 1-2 U/L [28] is said to be normal and a normal SGOT content is 70-279 U/L[29] for broiler chicken. The concentrations of SGPT in both the control and NOBF-treated groups were 1.41 and 1.14 U L^{-1}, respectively. The SGOT concentration in the treatment group and the control group showed no significant difference and was still within the normal range. The SGOT concentrations in the control and NOBF-treated groups were 242.00 and 237.50 U L^{-1}, respectively. Overall, SGOT concentrations in the NOBF-treated groups were within the normal range.

The urea level was not significantly different between the control and treatment group and was still within the normal range (Table 8). The urea levels of the control and NOBF-treated groups were 1.14 and 1.05 mg dL^{-1}, respectively. Harrison and Lightfoot[30] found that the normal creatinine level in broiler chickens is 0.1-0.4 mg dL^{-1}. Results showed that, the creatinine levels were not significantly different between the control and treatment group (Table 8). The creatinine levels in the control and NOBF-treated groups were 0.33 and 0.30 mg dL^{-1}, respectively. These results showed that the creatinine levels in all groups were within the normal range.

Table 7: Newcastle disease antibody titer by the HI method (Log[2])

	Groups	
Parameters	Control	NOBF
D+7 ND I	5.00±0.71[a]	5.33±0.41[a]
D+7 ND II	5.33±1.08[a]	5.67±0.41[a]

Annotation: Different superscripts in the same row indicate significantly different results ($p<0.05$)

Table 8: Liver and kidney function examination of broiler chickens given treatment by the end of research

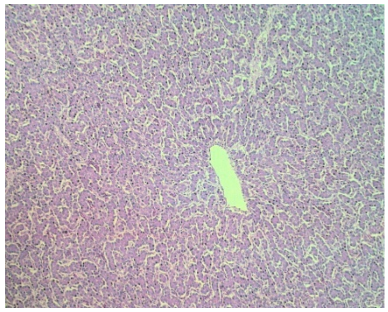
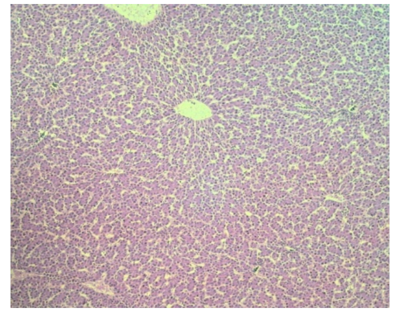
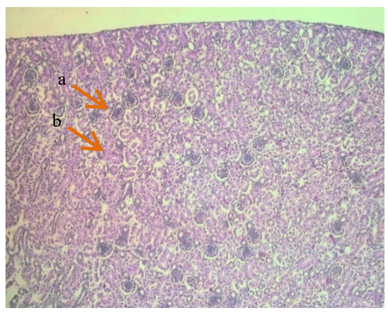
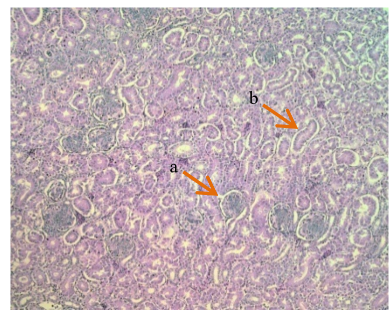
	Groups	
Parameters	Control	NOBF
SGOT (U L^{-1})	242.00±129.90[a]	237.50±70.00[a]
SGPT (U L^{-1})	1.41±0.79[a]	1.14±0.72[a]
Urea (mg dL^{-1})	1.14±0.36[a]	1.05±0.70[a]
Creatinine (mg dL^{-1})	0.33±0.05[a]	0.30±0.02[a]

Annotation: Different superscripts in the same row indicate significantly different results ($p<0.05$)

Gross pathology and histopathology: Macroscopic changes in the liver and kidney may indicate the presence of disorder. Histopathological examination of the liver and kidney of NOBF-treated broiler chickens at the end of the research is presented in Table 9. The results showed that there were no changes in the macroscopic features of the liver in either the control or treatment group. Based on histopathological examination, no lesion was found in liver or kidney cells. This result indicated that NOBFs are safe to use because they do not cause damage to liver and kidney cells.

Broiler chicken productivity: Feed consumption is the main requirement for obtaining energy to increase activity and weight gain of broiler chicken. The feed consumption in broilers can be influenced by the palatability level or fondness level in terms of taste[31]. Feed and water consumption values that were not significantly different in the NOBF-treated group produced higher final weight gain than those of the control group. This finding showed that NOBF administration through feed can increase the efficiency of feed intake, thus causing a higher final weight. Budiansyah[32] reported that the final weight of broiler chickens harvested at 4-5 weeks of age is 1.2-1.9 kg $chicken^{-1}$ and the broiler chicken weight gain may reach 48.65 g $chicken^{-1}$ day^{-1}. An optimal feed intake is probably caused by the active compounds in NOBF, which can function as antibacterial agents. NOBF contains

Table 9: Histopathology examination results of liver and kidney from broiler chickens given NOBF by the end of research (Hematoxylin Eosin Staining, 100× magnification)

	Groups	
Organ	Control	NOBF
Liver		
	 No damage or lesion was found in hepatocytes	 No damage or lesion was found in hepatocytes
Kidney		
	 No degeneration or necrosis in the (a) glomerulus and (b) tubules	 No degeneration or necrosis in the (a) glomerulus and (b) tubules

three main components: pine oil, lavender oil and eucalyptus oil. The active compounds contained in the NOBF are linalool, isobornyl acetate and sineol.

Lavender contains 26.5-34.7% linalool, which functions as an antibacterial agent that can suppress the growth of *Staphylococcus aureus*, *Escherichia coli* and *Pseudomonas aeruginosa*[19]. Essential oil contains terpenoids that contain bicyclic molecules, including isobornyl acetate with antimicrobial and anti-inflammatory activities[33]. According to a study by Yang *et al.*[34], isobornyl acetate has anti-inflammatory and antioxidant activities. One active compound contained in eucalyptus that inhibits bacterial growth is sineol. Eucalyptus essential oil is effective against bacteria that are resistant to β-lactam antibiotics and thus can be used as alternative therapeutic preparations[20].

The FCR is the comparison between feed consumption and weight gain within a certain period. A high feed conversion ratio shows that the amount of feed needed to increase weight per weight unit is high. According to Alfian *et al.*[35], the lower the FCR value is, the higher the feed use efficiency. The FCR value can be used to measure livestock productivity, economy evaluation indicators and the success of broiler chicken farming[36].

Water is an important aspect in determining performance and productivity of chickens. Moreover, chicken habits and consumption patterns influence meat quality. The lack or excess of water consumption can cause issues, such as disturbing health and body performance, causing dehydration and lowering feed consumption, which ultimately lower performance and productivity and eventually cause death[37]. Proximate analysis was performed to determine the water content and crude protein, crude fat, carbohydrate and ash levels in the breast, thigh and wing regions in broiler chicken meat. The water and fat content in meat shows the opposite pattern. This means that meat with a high-water content contains a low-fat content. Rutkowska *et al.*[37] stated that the water content in the body of a zebra finch bird is inversely proportional to the fat content, as the breakdown of fat produces CO_2 and H_2O (water). NOBF administration also decreased the ash content in the thigh region of broiler chicken meat. The lower ash content in meat showed the higher concentration of organic compounds. Organic compounds include important food compounds, such as proteins and fats[38]. The results also proved that NOBF administered to broiler chickens through feed can lower the mortality percentage.

Broiler chicken health: Blood feature examination was performed to determine the effect of NOBF administration through feed on chicken health status. Hematology values have a role in livestock health status diagnosis[39]. Results showed that differentiation of red blood cell and white blood cell did not show any significant difference between the NOBF-treated group and the control group either before or after treatment. This result showed that NOBF did not cause disorders in red blood cell or white blood cell count or their differentiation. Normal erythrocyte count is one of the indicators of protein and amino acid balance in chickens, as it allows red blood cells to be produced in normal amounts[40].

White blood cells defend body against viral, bacterial, parasitic infections and tumor cell proliferation[41]. White blood cell differentiation consists of lymphocytes, monocytes, heterophils, eosinophils and basophils. Lymphocytes are a part of white blood cells that function in the humoral- and cellular-mediated immunity[42]. Normal lymphocyte percentages in chickens ranged from 55-66% out of total white blood cells[43]. Monocytes are precursors of myelocyte cells that are phagocytic and change into macrophages once they move into the tissue. Monocytes play a role in chronic infection[41]. The normal monocyte percentage in chickens ranged from 2–9% of total white blood cells[43]. Heterophils are phagocytic cells that counter invading bacteria and viruses[44]. Eosinophils work when parasitic infection occurs in chickens. The number of eosinophils increase during parasitic infection, such as during worm infestation[41]. Basophils are white blood cells that play a role during allergic reaction[44]. The percentage ratio of heterophils and lymphocytes (H/L) showed the broiler chicken stress index. The higher the percentage ratio between heterophils and lymphocytes is, the higher the stress level of the chicken.

Based on blood biochemistry examination, the administration of NOBF did not affect the levels of triglycerides, cholesterols, HDL, LDL, total protein, or albumin in chicken blood serum before or after treatment and all were still within the normal range.

The safety of NOBF administration: Safety measurement of the administration of NOBF can be observed from liver and kidney function. Liver function was assessed by measuring the levels of SGPT and SGOT enzymes. SGPT and SGOT enzymes are used as indicators of liver damage. SGOT is normally found in several organs, such as the heart, kidney, brain, muscle and liver (the most abundant), while SGPT is normally concentrated in the liver. When liver damage occurs, these enzymes will be released by the liver into the blood vessels, causing an increase in the enzyme level in the blood. NOBF-treated chickens showed non-significant difference in SGPT and SGOT enzyme levels compared with the control group. The SGPT and SGOT levels did not exceed normal levels, showing that the preparations were safe for the liver.

Urea and creatinine concentrations showed the kidney function of chickens . Joshi *et al.*[45] observed that the normal range of urea levels in chickens are within 0-5 mg dL^{-1}. Creatinine is a byproduct of muscle metabolism excreted by the kidney. Creatinine serum originates from muscle mass, is unaffected by diet or activities and is entirely secreted by the glomerulus[46]. An increase in the level of serum creatinine signifies a lowering glomerulus filtration rate and caused creatinine, which is supposed to be excreted, to return into the blood vessels[46].

The macroscopic and microscopic features of the liver and kidney were observed to determine the safety of the preparations at the end of the research . In gross anatomy, no lesions were found in the liver or kidney of any of the groups. Histopathological examination of the liver showed similar features between the control and treatment groups, with no lesions observed in either group. The structure and the conformation of liver lobules did not show any damage and lesions. The results showed that no necrosis or degeneration was found on the liver cells. Histopathological examination of the kidney showed similar microscopic features and no damage was found in any group. The results also did not show necrosis or degeneration due to glomerular structures or kidney tubules. Based on histopathological examination, the NOBF preparation was safe because it did not cause damage to liver or kidney cells.

CONCLUSION

The administration of NOBF at a dose of 2 kg t^{-1} feed to broiler chickens was proven to be effective in improving the productivity and health of broiler chickens, signified by the increase in final weight gain and lower mortality than that of the control group. The administration of NOBF at a dose of 2 kg t^{-1} feed was proven safe (nontoxic to chickens) based on liver function (SGPT and SGOT) and kidney function (urea and creatinine), as well as the gross pathology and histopathology of the liver and kidney.

RECOMMENDATIONS

The administration of NOBF liquid at a dose of 2 kg t^{-1} feed can be used to improve the productivity and health of broiler chickens, with a lower mortality rate. These results showed that NOBF can be applied in commercial broiler chickens.

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REFERENCES

1. Badan Pusat Statistik, 2021. Produksi Daging Ayam Ras Pedaging menurut Provinsi, 2009-2019. https://www.bps.go.id/linkTableDinamis/view/id/1064/.
2. Fancher, C.A., L. Zhang, A.S. Kiess, P.A. Adhikari, T.T.N. Dinh and A.T. Sukumaran, 2020. Avian pathogenic *Escherichia coli* and *Clostridium perfringens*: Challenges in no antibiotics ever broiler production and potential solutions. Microorganisms, 8: 1-27.
3. Abdisa, T. and T. Tagesu, 2017. Review on Newcastle Disease of Poultry and its Public Health Importance. J. Vet. Sci. Technol., 10.4172/2157-7579.1000441.
4. Awad F., R. Chhabra, M. Baylis and K. Ganapathy, 2014. An overview of infectious bronchitis virus in chickens. World's Poult. Sci. J., 70: 375-384.
5. Dhama, K., S. Chakraborty, A.K. Verma, R. Tiwari and R. Barathidasan *et al*., 2013. Fungal/mycotic diseases of poultry-diagnosis, treatment and control: A review. Pak. J. Biol. Sci., 16: 1626-1640.
6. Abebe, E. and G. Gugsa, 2018. A review on poultry coccidiosis. Abyss. J. Sci. Technol., 3: 1-12.
7. Yadav, R., D. Niyogi, K.K. Tripathi, S.V. Singh and M. Kumar, 2018. The incidence, morbidity and mortality of the diseases of broiler birds in and around NDUAT, Kumarganj, Faizabad. Int. J. Curr. Microbiol. Applied Sci., 7: 5095-5105.
8. Ritter, G.D., G.R. Acuff, G. Bergeron, M.W. Bourassa and B.J. Chapman *et al*., 2019. Antimicrobial-resistant bacterial infections from foods of animal origin: understanding and effectively communicating to consumers. Ann. N. Y. Acad. Sci., 1441: 40-49.
9. Kumar, S., A.K. Sharma, D.D. Ray and S. Ghosh, 2014. Determination of discriminating dose and evaluation of amitraz resistance status in different field isolates 5 of Rhipicephalus (Boophilus) microplus in India. Exp. Appl. Acarol., 62: 413-422.
10. Lee, S., B. de Bie, J. Ngo and J. Lo, 2018. O16 antigen confers resistance to bacteriophage T4 and T7 but does not reduce T4/T7 adsorption in *Escherichia coli* K-12. J. Exp. Microbiol. Immunol., Vol. 22.
11. Andriyanto, I.M. Paramita, D.N. Aripin, P. Nugraheni and Z.U. Putri *et al*., 2016. Administration of *Jamu jahkenkun* to improve productivity and hematology profiles of broiler chickens. Int. J. Poult. Sci., 15: 126-133.
12. Andriyanto, F. Agustin, D.N. Aripin, P. Nugraheni and G.H. Suryanata *et al*., 2016. Improved productivity and health performances of broiler chickens administered with Jamu ginger, curcuma and turmeric. Int. J. Poult. Sci., 15: 487-492.
13. Karangiya, V.K., H.H. Savsani, S.S. Patil, D.D. Garg, K.S. Murthy, N.K. Ribadiya and S.J. Vekariya, 2016. Effect of dietary supplementation of garlic, ginger and their combination on feed intake, growth performance and economics in commercial broilers. Vet. World, 9: 245-250.
14. Natsir, M.H., 2016. Penggunaan kombinasi kunyit (*Curcuma domestica*) Dan jahe (*Zingiber officinale*) bentuk enkapsulasi dan tanpa enkapsulasi terhadap karakteristik usus dan mikroflora usus ayam pedaging. Buletin Peternakan, 40: 1-10.
15. Zamora, G.M., L.A.D. Meléndez, M.E. Hume and R.S. Vázquez, 2017. Performance, blood parameters and carcass yield of broiler chickens supplemented with Mexican oregano oil. Rev. Brasileria Zootecnia, 46: 515-520.
16. Ebani, V.V. and F. Mancianti, 2020. Use of essential oils in veterinary medicine to combat bacterial and fungal infections. Vet. Sci., Vol. 7, No. 4 10.3390/vetsci7040193
17. Basholli-Salihu, M., R. Schuster, A. Hajdari, D. Mulla, H. Viernstein, B. Mustafa and M. Mueller, 2017. Phytochemical composition, anti-inflammatory activity and cytotoxic effects of essential oils from three *Pinus* spp. Pharm. Biol., 55: 1553-1560.
18. Raho, G.B., 2014. Antibacterial potential of essential oils of the needles of Pinus halepensis against Staphylococcus aureus and *Escherichia coli*. J. Coastal Life Med., 2: 651-655.
19. Smigielski, K., R. Prusinowska, A. Stobiecka, A. Kunicka-Styczyñska and R. Gruska, 2018. Biological properties and chemical composition of essential oils from flowers and aerial parts of lavender (*Lavandula angustifolia*). J. Essent. Oil Bear. Plants, 21: 1303-1314.
20. Chaves, T.P., R.E.E. Pinheiro, E.S. Melo, M.J.d.S. Soares and J.S.N. Souzaa *et al*., 2018. Essential oil of *Eucalyptus camaldulensis* Dehn potentiates β-lactam activity against *Staphylococcus aureus* and *Escherichia coli* resistant strains. Ind. Crops Prod., 112: 70-74.

21. Al-Nedawi, A.M., 2018. Reference hematology for commercial Ross 308 broilers. J. Vet. Res., 22: 566-570.
22. Arfah, N.M., 2015. Pengaruh Pemberian Tepung Kunyit Pada Ransum Terhadap Jumlah Eritrosit, Hemoglobin, PCV, dan Leukosit Ayam Broiler. Bachelor Thesis, Universitas Hasanuddin Makassar,.
23. Purnomo, D., S. Sugiharto and I. Isroli, 2015. Total leukosit dan diferensial leukosit darah ayam broiler akibat penggunaan tepung onggok fermentasi rhizopus oryzae pada ransum. J. Ilmu-Ilmu Peternakan, 25: 59-68.
24. Mide, M.Z., 2014. Pemanfaatan temulawak (*Curcuma xanthorrhiza* roxb) dalam ransum sebagai upaya menurunkan lemak abdominal dan kolesterol darah broiler. J. Ilmu Ternak Vet., 19: 572-575.
25. Medah, S.R., A.O.M. Dima and V.M. Ati, 2019. Profil lipid darah ayam broiler (*Gallus* sp.) yang diberi kombinasi perlakuan ekstrak jahe (*Zingiber officinale*) dan jeruk nipis (*Citrus aurantifolia*). Jurnal Biotropikal Sains, 16: 26-35.
26. Thrall, M.A., D.C. Baker, T.W. Campbell, D. DeNicola and M.J. Fettman *et al*., 2007. Hematologia e Bioqui´mica Cli´nica Veterina´ria. Sa˜o Paulo Roca, Brasil .
27. Kencana, G.A.Y., I.N. Suartha, N.M.A.S. Paramita and A.N. Handayani, 2016. Vaksin kombinasi newcastle disease dengan avian influenza memicu imunitas protektif pada ayam petelur terhadap penyakit tetelo dan flu burung [Combined newcastle disease (ND) and avian influenza (AI) vaccines induce protective immune esponse in commercial layer against ND and AI]. J. Vet., 17: 257-264.
28. Nanbol, D.L., B.N. Duru, H.D. Nanbol, C.A. Abiliu, D.M. Anueyegu, P.R. Kumbish and M. Solomon, 2016. Establishment of reference values for some biochemical and haematological parameters for broilers and layers in Plateau State Nigeria. Vom J. Vet. Sci., 11: 30-35.
29. Abdullah, B., E. Kusumanti and U. Atmomarsono, 2015. Pengaruh penambahan tepung temukunci (*Boesenbergia pandurata* ROXB.) Dalam ransum terhadap bobothidup, kadar SGOT SGPT dan kondisi hati ayam broiler. [The effect of fingerroot flour addition (*Boesenbergia pandurata* ROXB.) In the ration on liveweight, SGOT SGPT content and liver conditions of broiler] Anim. Agric. J., 4: 41-46.
30. Harrison, G.J. and T. Lightfoot, 2011. Clinical Avian Medicine. Spix Publishing, Inc. USA. .
31. Nuningtyas, Y.F. and Y.F. Nuningtyas, 2014. Pengaruh penambahan tepung bawang putih (*Allium sativum*) sebagai aditif terhadap penampilan produksi ayam pedaging. J. Ternak Tropika, 15: 65-73.
32. Budiansyah, A., 2010. Performan ayam broiler yang diberi ransum yang mengandung bungkil kelapa yang difermentasi ragi tape sebagai pengganti sebagian ransum komersial. Jurnal Ilmiah Ilmu-Ilmu Peternakan, 13: 260-268.
33. Kar, S., P. Gupta and J. Gupta, 2018. Essential oils: Biological activity beyond aromatherapy. Nat. Prod. Sci., 24: 139-147.
34. Yang, L., J. Liu, Y. Li and G. Qi, 2018. Bornyl acetate suppresses ox-LDL-induced attachment of THP-1 monocytes to endothelial cells. Biomed. Pharmacother., 103: 234-239.
35. Alfian, A., N. Amin and M. Munir, 2015. Pengaruh pemberian tepung lempuyang (*Zingiber aromaticum* val) dan tepung kunyit (*Curcuma domesticus*) terhadap konsumsi dan konversi ransum broiler. [Effect of flour ginger (*Zingiber aromaticum* val) and flour turmeric (*Curcuma domesticus*) to consumption and broiler feed conversion]. J. Galung Tropika, 4: 50-59.
36. Nugraha, Y.A., K. Nissa, N. Nurbaeti, F.M. Amrullah and D.W. Harjanti, 2017. Pertambahan bobot badan dan feed conversion rate ayam broiler yang dipelihara menggunakan desinfektan herbal. J. Ilmu-Ilmu Peternakan, 27: 19-24.
37. Rutkowska, J., E.T. Sadowska, M. Cichoń and U. Bauchinger, 2016. Increased fat catabolism sustains water balance during fasting in zebra finches. J. Exp. Biol., 219: 2623-2628.
38. Anggreini, R.E.A., 2015. Pengaruh Kualitas Air Terhadap Performa Ayam Broiler. Trouw Add Sci.,
39. Hastuti, D., S.N. Awami and B. Iskandar M, 2011. Pengaruh perlakuan teknologi amofer (*Amoniasi fermentasi*) pada limbah tongkol jagung sebagai alternatif pakan berkualitas ternak ruminansia. J. Ilmu-Ilmu Pertanian, 7: 55-65.
40. Napirah, A., 2013. Pengaruh penambahan tepung kunyit (*Curcuma domestica* valet) dalam pakan terhadap parameter hematologi darah puyuh (*Coturnix-coturnix* japonica) pedaging. Buletin Peternakan, 37: 114-119.
41. Wibawan, I.W.T. and D.S. Retno, 2013. Intisari Imunologi Medis. Fakultas Kedokteran Hewan Institut Pertanian Bogor, Bogor.
42. Nicholas, F.W., 2004. Pengantar Genetika Veteriner, Pustaka Wira Usaha Muda,
43. Dharmawan, N.S., I.G. Mahardika, N.N. Suryani, N.P.M. Andini and A.K.S. Dewi, 2019. Parameter biokimia dan hematologi sapi bali lepas sapih yang diberi ransum energi protein bertingkat. (Biochemical and hematological parameters of weaning bali cattle feeded with leveled protein energy ration). J. Veteriner, 20: 558-565.
44. Ulupi, N. and T.T. Ihwantoro, 2014. Gambaran darah ayam kampung dan ayam petelur komersial pada kandang terbuka di daerah tropis. Jurnal Ilmu Produksi Dan Teknologi Hasil Peternakan, 2: 219-223.
45. Joshi, N., T.G. Wandita, S. Yang, H. Park and S.G. Hwang, 2019. Effects of supplementing laying hens with purified amino acid prepared from animal blood. Trop. Anim. Sci. J., 42: 46-52.
46. Duncker, D., H. Oswald, A. Gardiwal, U. Lüsebrink and T. König, 2013. Stable cystatin C serum levels confirm normal renal function in patients with dronedarone-associated increase in serum creatinine. J. Cardiovasc. Pharmacol. Ther., 18: 109-112.