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RESEARCH ARTICLE

Temulawak (Curcuma xanthorrhiza Roxb.) Nanoemulsion can be Substituted as Natural Growth Promoter in Broiler Chickens

Jacyntha Orinetha[1], Jihan Kaamillia Salsabil[1], Salsabiela Milenia Putri[2] and Anggi Muhtar Pratama[3*]

[1]Faculty of Veterinary Medicine, Universitas Gadjah Mada, Fauna Street No. 2, Yogyakarta 55281, Indonesia
[2]Faculty of Pharmacy, Universitas Gadjah Mada, Sekip Utara Street, Yogyakarta 55281, Indonesia
[3]Department of Pharmacology, Faculty of Veterinary Medicine, Universitas Gadjah Mada, Fauna Street No. 2, Yogyakarta 55281, Indonesia
*Corresponding author: anggi.muhtar.p@mail.ugm.ac.id

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A B S T R A C T

The extensive use of antibiotics growth promoters leads to residues and bacterial resistance; therefore, *temulawak* nanoemulsion (TN) is expected to be a potential alternative as natural growth promoter (NGP) in broiler chickens. This study was aimed to assess the effect of TN on broiler chickens' productivity, blood profile, and antibiotic resistance. A total of 72 one-day-old broiler chicks strain MB (Lohman 202) were reared for 35 days and divided into four groups: negative control (without supplementation), positive control (AGP 50 mg/L), and administration of TN 2 mg/kg and 4 mg/kg BW. Body weight (BW), feed intake (FI), feed conversion ratio (FCR), and index performance (IP) were calculated to evaluate broiler chicken' productivity. On day 35, blood and cloacal samples were collected for hematology and antibiotic resistance tests. The data were analysed using SPSS software. The results showed that supplementation of TN 4 mg/kg BW improved broiler chickens' productivity with efficient FI (P>0.05), optimum FCR (P>0.05) during 30-34 days of trial, and the highest IP value. TN 4 mg/kg BW group also had the lowest increasing levels of WBC and eosinophil compared to other groups, while PCV, RBC, and other leukocyte differential levels were normal compared to standards (P>0.05). Moreover, this group showed a decrease in the resistance rate to Amoxicillin and Ceftriaxone compared to control and AGP treatment. It was concluded that TN 4 mg/kg BW could be used as NGP.

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INTRODUCTION

Broiler chicken's production and consumption rate in Indonesia were increased 2.27% every year due to affordable prices and obtainability of chicken meat (Etikaningrum and Iwantoro, 2017). In order to reach maximum economic advantages, farmers added subtherapeutic doses of antibiotics as feed additives to accelerate and maximize broiler chicken's production, known as Antibiotic Growth Promoter (AGP). These antibiotics are assumed to maintain health status, accelerate their growth, and increase feed efficiency in broiler chickens (Muaz *et al.,* 2018; Hamid *et al.,* 2019).

Poultry became the highest antibiotics user in animal production sector by 2019, according to Burow *et al*. (2020). The uncontrolled use of antibiotics, whether to promote health or as feed additives in broiler chickens, has led to an increase in bacterial resistance cases of antibiotics (Hamid *et al.,* 2019), including commensal bacteria such as *Escherichia coli* (*E. coli*). These bacteria are suspected of having gene mutations that led to resistance against numerous antibiotics and subsequently shared these genes with other commensal and pathogenic bacteria through the food chain. These days, *E. coli* is resistant to several antibiotics, including antibiotics from Penicillin and Cephalosporin groups which are widely used in human and animal health, such as Amoxicillin and Ceftriaxone (Roth *et al.,* 2019; Burow *et al.,* 2020). Furthermore, antibiotics are also associated with some risks of residues in broiler meat that could threaten human health, such as allergy, cancer, toxicity, and other health problems (Etikaningrum and Iwantoro, 2017; Hamid *et al.,* 2019; Iriyanti and Hartoyo, 2019).

These global health issues have drawn attention of developing some antibiotics alternatives; therefore, plant extracts are preferred nowadays. A potential Indonesian plant that can be used as an NGP is *temulawak*. This plant has long been used to enhance palatability and reportedly improves constipation, diarrhea, and other gastrointestinal problems (Rahmat *et al.,* 2021). Furthermore, *temulawak* has commonly used for feed additives (Rajkumari and Sanatombi, 2017). Some terpenes compounds in *temulawak* have also been reported to have antibacterial properties through some bacteria, including *E. coli* (Akarchariya *et al.,* 2017; Rahmat *et al.,* 2021). However, research by Iriyanti and Hartoyo (2019) and Nazari-Vanani *et al*. (2017) reported a hydrophobic character and a low absorption level of *temulawak* extracts inside the intestine that may decrease its effectiveness (Harwansh *et al.,* 2019).

Temulawak nanoemulsion (TN) is considered as an alternative to this problem. Nanoemulsion has been known to preserve extract stability and its bioactive compound when consumed orally, especially for insoluble drugs (Harwansh *et al.,* 2019). Moreover, according to Baskara *et al*. (2019), nanoemulsion formulation can control drugs' efficacy by decreasing oxidation and evaporation levels from the bioactive compounds.

This study was conducted to assess the effect of TN treatment on broilers chickens' productivity, blood profile, and bacterial resistance to Amoxicillin and Ceftriaxone.

MATERIALS AND METHODS

Research permission: This research has been approved by Research Ethics Commission of Faculty of Veterinary Medicine, Universitas Gadjah Mada with number: 00048/EC-FKH/Int./2021.

Plant determination and characteristics: Taxonomy and rhizome plant identification was determined in Systematic Plants Laboratory, Faculty of Biology, Universitas Gadjah Mada. Rhizomes of *temulawak* were collected from Bojonegoro (6º 59'-7º 37' S dan 112º 25'-112º 09' E) (Pratama *et al.,* 2021). Antioxidant activity was determined by the 1-1-diphenyl-2-picrylhydrazyl (DPPH) method (Rosidi, 2020), while phytochemical compounds were characterized by Gas Chromatography-Mass Spectrometry (GCMS) method (Xiang *et al.,* 2018).

Extraction and nanoemulsion formulation: *Temulawak* rhizomes were extracted using the maceration technique for 3x24 hours at a ratio of 1:5 (Simplicia:70% ethanol). Nanoemulsion was formulated by homogenization method using extract, VCO, Tween 80, PEG 400 and aquadest at a ratio of 1:3:21:10:65. All materials except aquadest were stirred using a magnetic stirrer at a speed of 1000 rpm for 10 minutes, then aquadest was added slowly while increasing the speed to 1250 rpm for 10 minutes (Kusumawardani *et al.,* 2020). *Temulawak* nanoemulsion was tested for their particle size and zeta potential using Particle Size Analyzer (PSA) with Dynamic Light Scattering method (Chan *et al.,* 2017).

In vivo experimental design: A total of 72 one-day old broiler chicks strain MB (Lohman 202, mixed sex). They were reared for 35 days and had free access to water and feed on BR-1 Comfeed® with nutrient content shown in Table 1. Chicks were randomly divided into four treatment groups with three replicates of six broilers each. Treatments were administered on day 23 for seven days by mixing AGP and TN to drinking water as follows: negative control (without any supplementation), positive control using AGP (Colistin; Bio & Green Technology®) 50 mg/L, and administration of TN at two different dose (2 mg/kg and 4 mg/kg BW).

Table 1: Nutrient content in broiler feed days 1-35 using BR-1 Comfeed®

Nutrient	Parameter
Water Content	Max.12.00%
Ash	Max. 7.00%
Crude Protein	Min. 21.00%
Crude Fat	Min. 5.00%
Crude Fibre	Max. 5.00%
Calcium (Ca)	0,8-11%
Phosphor (P)	Min. 0.50%
Enzyme	Phytase ≥ 400 μ FTU/kg (min)
Urea	-
Aflatoxin Total	Max. 50 μ/kg
Lysin	Min. 1.20%
Methionine	Min. 0.45%
Methionine + Cysteine	Min. 0.80%
Tryptophan	Min. 0.19%
Threonine	Min. 0.75%

Broiler productivity: Body weight (BW) and feed intake (FI) were calculated every day during maintenance. Feed conversion ratio (FCR) was calculated every week to analyse the feed amount to reach 1 kg meat. A smaller FCR value shows a smaller amount of feed needed to reach 1 kg of meat (Haryanto *et al.,* 2016). Index performance (IP) was calculated at the end of the rearing period to see the efficiency of productivity. The higher of the IP number, the more efficient of the poultry business. (Szollosi *et al.,* 2021).

Hematology test: Blood profile tests can be an indicator of health status in broiler chickens. Blood samples were collected from 6 broiler chickens of each group from the brachialis vein on day 35 and homogenized immediately. The samples were analysed using a hemacytometer to calculate the composition of blood compounds.

***Escherichia coli* resistance test to amoxicillin and ceftriaxone:** Cloacal samples were collected on day 35 from 6 broiler chickens from each group, then cultured using Mac Conkey Agar. *Escherichia coli* that has been isolated were further tested using Mueller Hinton Agar (MHA) (Herawati *et al.,* 2020).

Statistical analysis: Productivity were calculated using this formula (Haryanto *et al.,* 2016):

$$\text{FCR} = \frac{\text{FI (kg)}}{\text{total weight gain(kg)}}$$

Productivity efficiency in this study was measured using the formula (Szollosi *et al.,* 2021):

$$\text{IP} = \frac{[(100\text{-mortality}) \times \text{average BW}]}{(\text{FCR} \times \text{harvest age})} \times 100$$

Productivity and productivity efficiency were calculated and compared to each group using SPSS software with One-Way ANOVA and Kruskal-Wallis methods. Hematology data were analysed qualitatively through observation and comparing to standards by Weiss and Wardrop (2010), while the bacterial resistance test was analysed by calculating the inhibition zone to determine the resistance ratio (Herawati *et al.,* 2020).

RESULTS

Plant characteristics: The result of *temulawak* determination and characterization was described as below:

Division : Tracheophyta
Sub-Division: Spermatophyta
Class : Magnoliopsida
Super Ordo : Lilianae
Ordo : Zingiberales
Familia : Zingiberaceae
Genus *: Curcuma*
Species : *Curcuma xanthorrhiza* Roxb.
Local Name : *Temulawak*

The antioxidant activity of *temulawak* was 64.49 and 64.36%. Phytochemical screening of rhizome characterized the presence of 2,4,6-trimethylphenol (Mesitol), a-humulene, a-curcumene (curcumin), and trans-caryophyllene.

Extraction and nanoemulsion production: A total of 2 kg of Simplicia produced 137 g extract, which had 6.86% extract yield. Nanoemulsion formulation produces 2 L TN, with the nanoparticle size of 232.90 nm and zeta potential was -53.70 mV.

Broiler productivity: Broiler productivity in terms of BW, FI, and FCR were found non-significantly different (p>0.05) during the trial (Table 2), while highest IP was observed for group TN 4 mg/kg BW (329.27) as shown in Fig 1. On 18-22 day of trial, highest BW (718.12 g) was recorded for group TN 4 mg/kg BW, better FI (1713.60 g) were recorded for AGP group and optimal FCR was recorded for group supplemented with TN 4 mg/kg BW (1.47). On 23-29 day of trial, higher BW was observed for TN 2 mg/kg BW group (1117.20 g), while FI was higher for control group (2053.30 g) and better FCR noted for group supplemented with TN 2 mg/kg BW (1.50). On 30-34 day of trial, greater BW (1631.10 g) and FI (2225.80 g) was observed for control group and better FCR was recorded for TN 4 mg/kg BW group (1.33).

Blood profile: Table 3 showed that the supplementation of *temulawak* (TN 2 and 4 mg/kg BW) had no significant effect on the blood profile (P>0.05) compared to the control group. Elevated WBC and eosinophil levels were reported in all group treatments; however, TN 4 mg/kg BW group had the lowest increasing levels compared to other groups. PCV, RBC, and other leukocyte differential levels were normal compared to the standards.

Decreased resistance of *E. coli* to amoxicillin and ceftriaxone antibiotics: Administration of TN 4 mg/kg BW showed a decreased in the rate of *E. coli* resistance to Amoxicillin from the Penicillin group compared to control and AGP treatment (Fig. 2). Furthermore, Fig. 3 showed that TN of 2 mg/kg BW and 4 mg/kg BW also decreased the resistance rate of Ceftriaxone from the Cephalosporins group when compared with the control group and AGP treatment.

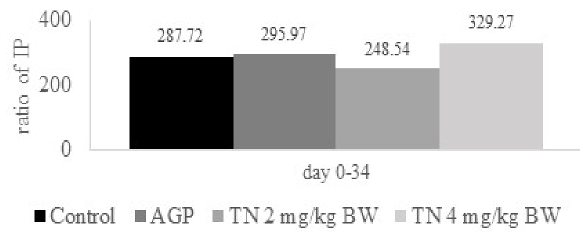

Fig. 1: Index Performance of Broilers.

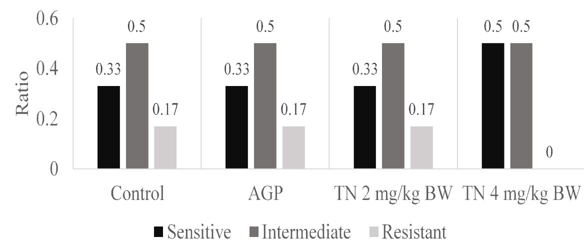

Fig. 2: Graphic of *E. coli* resistance rate to Amoxicillin.

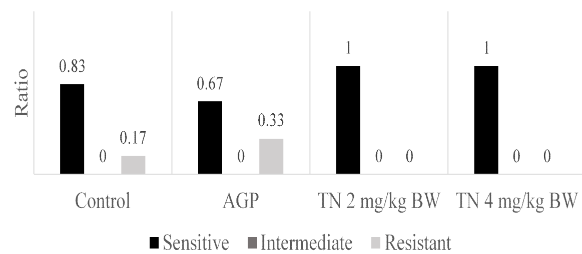

Fig. 3: Graphic of *E. coli* resistance rate to Ceftriaxone.

DISCUSSION

Antioxidant assay was determined on its compound ability to inhibit radical scavenging; therefore, *temulawak* extract could inhibit around 64.36-64.49% radical scavenging activity (Rosidi, 2020). The antioxidant ability of *temulawak* is influenced by curcumin (Moniruzzaman and Min, 2020).

The most common compounds in *temulawak* are monoterpene and sesquiterpene (Akarchariya *et al.,* 2017), curcuminoid, and terpenoid (Rahmat *et al.,* 2021). That difference can be influenced by the harvest period, *temulawak* variety, and geographical location of the *temulawak* plant (Pratama *et al.,* 2021). The most common compounds based on phytochemistry screening of *temulawak* extract through GCMS were α-curcumene, α-humulene, and trans-Caryophyllene from sesquiterpene group. This group is commonly found in *temulawak* and has good antibacterial activity (Akarchariya *et al.,* 2017; Rahmat *et al.,* 2021). A-humulene has potential as an anti-inflammatory, anti-tumor, antimicrobial, and analgesic (Leite *et al.,* 2021); while α-curcumene acts as antioxidants, antimicrobials, and antiulcer (Yousfi *et al.,* 2021).

Table 2: Average of BW, FI, FCR before, at, and after treatment using AGP and TN

Day	Para meter	Treatment			
		Control	AGP	TN 2 mg/kgBW	TN 4 mg/kgBW
18-22	BW(g)*	681.73±113.01	698.07±110.95	686.30±114.66	718.12±99.88
	FI(g)*	1379.80±340.27	1713.6±854.81	1334.6±432.84	1404.00±46.35
	FCR*	3.19±4.74	1.59±0.97	2.050±2.50	1.47±0.70
23-29	BW(g)*	1098.20±169.17	1075.80±139.58	1117.20±152.63	1084.7±160.35
	FI(g)*	2053.30±465.09	1972.00±574.04	1984.30±522.56	1795.30±433.61
	FCR*	1.53±0.29	1.85±0.55	1.50±0.45	1.78±1.00
30- 34	BW(g)*	1631.10±155.99	1528.5±128.38	1603.40±129.88	1607.1±155.12
	FI(g)*	2225.80±521.75	1887.0±505.22	2120.40±159.34	2117.6±430.85
	FCR*	1.61±0.96	1.47±0.45	1.84±1.26	1.33±0.20

Means±deviation standard values (P<0,05); * Non-Significant

Table 3: Hematology profile of broiler after being treated with AGP and TN

	Treatments				Standard**
	Control	AGP	TN 2 mg/kg BW	TN 4 mg/kg BW	
PCV (%)1;*	27.33±3.51	26.33±2,52	25.33±1.53	24.67±1.53	22-35
WBC (10^3/mm^3)2;*	52.62±4.47	54.30 ±18.62	54.85±7.79	50.75±6.05	16-40
RBC (10^6/mm^3)3;*	2.29±0.15	2.17±0.51	2.28±0.16	2.43±0.32	2.0-3.2
Eosinofil (%)*	8,67±416	19.00±14.79	8.33±6.11	12.33±10.97	0-7
Heterofil (%)*	14.00±8.185	19.00±5.19	15.67±1.16	16.67±7.09	9-56
Limfosit (%)*	77.00±5.29	75.00±6.56	76.00±7.21	78.00±5.29	24-84
Monosit (%)*	0.33±0.58	0.67±1.16	00.00±00.00	0.33±0.577	0-30

Means±deviation standard values (P<0,05): *Non-Significant: **Weiss and Wardrop, 2010: [1]PCV=Packed Cell Volume or haematocrit: [2]White Blood Cells; [3]Red Blood Cells.

The size of TN used in this study was 232.9 nm. Nanoemulsion size varies from 100-600 nm (Costa *et al.,* 2014); therefore, TN was in optimal size in this study. The results also showed that the zeta potential of the nanoemulsion was -53.7 mV. According to Costa *et al.* (2014), a particle with a zeta potential of more than +30 mV (positive charge) or less than -30 mV (negative charge) is considered as a stable particle; hence TN can be concluded to have good stability.

Overall BW, FI, and FCR were found non-significantly different (p>0.05) during the trial, but TN 4 mg/kg BW supplementation has shown positive impact on broiler productivity. It was indicated by efficient FI and optimal FCR during 30-34 day of trial, also highest IP value during the trial. The value of FCR indicates the amount of feed needed to reach 1 kg of meat; hence, a smaller FCR value shows a smaller amount of feed needed to reach 1 kg of meat (Haryanto *et al.,* 2016). *Temulawak* nanoemulsion 4 mg/kg BW group had the smallest FCR value of 1.33 during 30-34 day of trial; therefore, it can be determined that TN 4 mg/kg BW could optimize the FCR value and increased feed efficiency. The IP calculation aims to assess the overall efficiency of the product, higher IP shows better production efficiency (Szollosi *et al.,* 2021). The group with TN 4 mg/kg BW had the highest IP value of 329.27, hence, it could be inferred that TN 4 mg/kg BW could maximize production efficiency. Curcumin in *temulawak* may influence this good productivity performance, as it has a gastroprotective effect. This effect may increase nutrient digestibility and metabolism by stimulating the bile secretion and activating digestive enzymes to absorb nutrients better with maximum weight gain (Iriyanti and Hartoyo, 2019; Badran *et al.,* 2020). In addition, research by Kim *et al.* (2013) and Lee *et al.* (2013) showed that administration of curcumin could prevent necrotic enteritis, which are infectious diseases that often occur in broiler chickens, led to economic loss and mortality. Curcumin can maintain broiler chickens' digestive tract health, and improves the absorption of nutrients (Kim *et al.,* 2013).

Blood profile tests can be an indicator of health status in broilers. The normal PCV, RBC, and most leukocyte differential counts in TN groups may indicate curcumin's influence to promote health status in broilers. This compound is widely known for its anti-inflammation and antioxidant activities. Curcumin may cause an increase in some antioxidant enzymes level and elevate the lipid peroxidase inhibition. These mechanisms may reduce stress and inflammation activities that improve digestibility and broiler chickens' performance (NM *et al.,* 2018). Curcumin has also known to upsurge hepatoprotective effects against food toxins through its antioxidant mechanism by preventing the oxidation and production of free radicals (such as reactive oxygen species (ROS), nitric oxide, superoxide, hydrogen peroxide, peroxynitrite, and hypochlorous acid) and activating some antioxidant enzymes (for example catalase, glutathione transferase (GST), and heme-oxygenase-1). These mechanisms may act as anti-inflammatory reactions and prevent several diseases (Badran *et al.,* 2020; Rahmat *et al.,* 2021).

Nevertheless, increased leucocyte levels were also found in all treatments. According to Badran *et al.* (2020), leucocyte levels may elevate due to the curcumin immunomodulatory effect, which prevents broiler chickens from pathogens by inducing the T3 and T4 hormones of the thyroid. These hormones play an important role in stimulating lymphocytes production as antibody mechanisms from broiler chickens, thus the levels of leucocytes may be increased. Leucocytes level also depends on stress conditions, physiological activities, nutrition, aging, and the possibility of infections (Salam *et al.,* 2013).

The rate of *E. coli* resistance to Amoxicillin and Ceftriaxone decreased after being treated with TN. These findings were associated with some terpenes as most phytochemical compounds on *temulawak* that associated with bactericidal and bacteriostatic effects on *E. coli* (Akarchariya *et al.,* 2017; Iriyanti and Hartoyo, 2019). One of the terpenes compounds is curcumin, which has antimicrobial mechanisms by inhibiting cytokinesis and bacterial cell multiplication, obstructing bacterial cell membrane and wall structure, resulting in cell lysis (Rahmat *et al.,* 2021). Bacterial resistance to antibiotics in broilers may occur since day one, whether by chromosome mutation or receiving resistance genes from surrounding and other bacteria (Herawati *et al.,* 2020).

Based on the results of this study indicated that TN is a potential substitution for NGP. As the bioactive component of *temulawak*, curcumin has gastroprotective, antioxidant, and antibacterial mechanisms, improving productivity and health status in broiler chickens (NM *et al.,* 2018; Iriyanti and Hartoyo, 2019). Evidently, TN also decreased the risk of bacterial resistance to antibiotics, therefore broiler chickens' meat can be food safety requirements and positively impact in human health.

Conclusion: *Temulawak* nanoemulsion 4 mg/kg can improves broiler chickens' productivity with an efficient FI, optimum FCR, and the highest IP value. This group also showed good performance from a normal blood profile with the lowest increasing levels of WBC and reduced the *E. coli* resistance rate of Amoxicillin and Ceftriaxone antibiotics. These results might be attributed to curcumin's gastroprotective, antioxidant, and antibacterial activities which improve digestibility and promote better performance in broiler chickens. Therefore, TN 4 mg/kg BW show a promising potential alternative to NGP that may prevent the risk of residues and antibiotic resistance and leads to better health status in broiler chickens and human.

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Author's contribution: JO, JKS, SMP, and AMP planned the research and prepared the manuscript. JO, JKS, and SMP executed the research and were involved in the analysis. AMP reviewed and critically revised the final revision of the manuscript.

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