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The potential of lemon cui (*Citrus microcarpa*) from Ternate Island as a natural feed additive for broiler chickens reared at high density

Yusri Sapsuha[a] | **Suryati Tjokrodiningrat**[b] ✉ | **Yusnani Yusnani**[a] | **Sri Lestari**[a] | **Eka Kusuma Dewi**[c] | **Nur Sjafani**[a]

[a]Department of Animal Science, Faculty of Agriculture, Universitas Khairun, Ternate, North Maluku, Indonesia.
[b]Department of Agriculture, Faculty of Agriculture, Universitas Khairun, Ternate, North Maluku, Indonesia.
[c]Department of Agribusiness, Faculty of Agriculture, Universitas Khairun, Ternate, North Maluku, Indonesia.

Abstract This study aimed to determine the potential effects of lemon cui from Ternate Island on the performance of broiler chickens reared at high density. The study used 360 broiler chicks (14 days old) in a completely randomized design with the following treatments: T0 (chicks reared at a normal density of 8 birds/m² without lemon cui juice), T1 (chicks reared at a high density of 16 birds/m² without lemon cui juice), T2 (chicks reared at a high density of 16 birds/m² with 0.5 ml/L lemon cui juice), T3 (chicks reared at a high density of 16 birds/m² with 1.0 ml/L lemon cui juice), and T4 (chicks reared at a high density of 16 birds/m² with 1.5 ml/L lemon cui juice). The results showed that the administration of lemon cui juice in the drinking water of broiler chickens reared at high density significantly ($p<0.05$) improved performance. The thymus and bursa of Fabricius were smaller ($p<0.05$) in broiler chickens reared at high density without lemon cui juice. In conclusion, the administration of lemon cui juice from Ternate Island can improve performance and enhance the development of immune organs in broiler chickens reared at high density.

Keywords: lemon cui, performance, broiler chickens, high density, feed additive

1. Introduction

Broiler chickens are a significant source of animal protein in Indonesia's livestock industry. Efficiency is key to successful broiler production. To increase meat production per square meter, broilers are typically reared at high density (Costa et al., 2021). However, high-density rearing can cause stress, negatively impacting growth, physiological conditions, and the balance of the gut microflora (Li et al., 2019). Various health and performance issues, including oxidative stress, which is a major cause of cell damage and disruption of the antioxidant balance in broilers, can occur under high-density conditions (Simitzis et al., 2012). Previously, synthetic antioxidants such as butylated hydroxytoluene (BHT) and butylated hydroxyanisole (BHA) were commonly used to mitigate growth retardation under stress conditions (Salami et al., 2015). Without synthetic antioxidants, high-density broiler rearing has been reported to cause growth and health problems (Sapsuha et al., 2023). The ban on synthetic antioxidants in feed has spurred research into the use of natural feed additives for sustainable broiler farming.

The utilization of organic acids in broilers has become a topic of interest in the livestock industry in recent years. Organic acids can serve as natural antioxidants that are beneficial for broiler health and productivity, including improving nutrient digestibility, maintaining the gut microflora balance, enhancing the immune system (Pratama et al., 2021), and helping reduce environmental stress (Pearlin et al., 2020). Recent studies have shown that high-density rearing can reduce broiler performance, making organic acids a promising alternative solution to natural antioxidants (Sugiharto et al., 2022). Ascorbic acid, or vitamin C, is an organic acid that can be used as a feed additive. Previous research has shown that ascorbic acid at 50 ppm in feed is effective in achieving beneficial effects in broilers (Imik et al., 2013). Furthermore, Adeyemi et al. (2015) reported that ascorbic acid supplementation in feed can improve the performance of broilers reared at high density.

Lemon cui (*Citrus microcarpa*) (Figure 1) is a local citrus variety widely grown on Ternate Island, North Maluku Province. It is used by the local community to remove the fishy smell from fish and as a seasoning in traditional cuisine to enhance the aroma and appetite. Lemon cui has good nutritional value and includes antioxidants such as vitamin C, flavonoids, and phenolic compounds (Cheong et al., 2012). Research has shown that lemon cui from Ambon Island contains flavonoids, vitamin C, and other phenolic compounds with potential as natural antioxidants (Munadi et al., 2023). To our knowledge, the antioxidant and bioactive compounds of lemon cui from Ternate Island have

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not yet been identified. Sugiharto (2016) reported that the antioxidant and bioactive contents of various plants vary depending on geographic location, soil conditions, climate, and cultivation methods, thereby affecting plant effectiveness. Therefore, we investigated the bioactive compounds and IC50 antioxidant capacity of lemon cui from Ternate Island and their effects on broiler chickens reared under high-density stress conditions. It is hypothesized that the acidic and bioactive compounds in lemon cui from Ternate Island can improve the performance of broiler chickens reared at high density.

Figure 1 Lemon cui (*Citrus microcarpa*).

2. Materials and Methods

2.1. Preparation of lemon cui juice

Lemon cui was collected from local plantations on Ternate Island, North Maluku Province, Indonesia. The harvested lemons were mature, as indicated by their green skin; at this ripening stage, the water content was relatively high, and the characteristic aroma of the lemon cui was still strong. The lemons were cleaned and washed with clean water to remove any dirt, cut in half, juiced, and filtered to obtain lemon cui juice. The juice was filtered through filter paper and stored in a refrigerator until use.

2.2. Phytochemical composition and antioxidant activity of lemon cui juice

The total phenol and flavonoid contents of the lemon cui juice were determined via modified Folin-Ciocalteu and spectrophotometric methods. The 2,2-diphenyl-1-picrylhydrazyl hydrate (DPPH) assay was used to determine the antioxidant capacity of lemon cui. The antibacterial inhibitory effect of lemon cui juice on *E. coli* growth was tested via the disc diffusion method (Sapsuha et al., 2022).

2.3. Broiler chicken experiment

A total of 400 unsexed Lohmann broiler chickens with an initial body weight of 32.7±0.09 g were reared together until day 14 on commercial starter feed. On day 14, the chicks were individually weighed, and 360 chicks (average body weight 412±4.17 g) were selected for the experiment. The experiment was arranged in a completely randomized design with five treatments and five replicates each: T0 (negative control with a normal density of 8 birds/m²), T1 (positive control with a high density of 16 birds/m²), and T2, T3, and T4, with a high density of 16 birds/m². The feed was provided in mash form and formulated (Table 1) as starter feed (days 14--21) and finisher feed (days 22--35). Lemon cui juice was added to the drinking water from day 14 at 0.5, 1.0, and 1.5 ml/L for T2, T3, and T4, respectively, whereas no lemon cui juice was added for T0 and T1. Feed and water were provided ad libitum until day 35. All chickens were vaccinated with the commercial Newcastle disease (ND) vaccine via eye drops on day 4 and in the drinking water on day 18 and with the Gumboro vaccine in the drinking water on day 12. Body weight gain, feed intake, and the feed conversion ratio (FCR) were recorded weekly. On day 35, one chicken from each replicate was randomly selected, slaughtered, and defeathered. After evisceration, the internal organs were removed and weighed. The carcass percentage and commercial carcass proportions of the broilers were also determined.

The experiment was conducted on a completely randomized design. The data obtained were analyzed via ANOVA (SPSS 25.0). Duncan's multiple range test was used to distinguish significantly different means ($p<0.05$).

3. Results

3.1. Antioxidant activity and phytochemical composition of lemon cui juice

Table 2 shows the phytochemical composition and antioxidant activity of the lemon cui juice. The analysis of

the phytochemical composition of lemon cui juice revealed a phenol content of 1.13 g/100 g and a flavonoid content of 1.07 g/100 g, with an antioxidant activity (2,2-diphenyl-1-picrylhydrazyl hydrate, DPPH) IC50 of 7,074.29 µg/mL. Lemon cui juice also exhibited antibacterial activity against *E. coli* (inhibition zone diameter of 11.9 mm).

Table 1 Feed ingredients and nutrient composition of the research rations.

Items (%, except that otherwise mentioned)	Starter (1-21)	Finisher (22-35)
Yellow corn	55.11	60.58
Soybean meal	24.85	20.17
Fish flour	12.11	11.32
Palm oil	2.3	2.3
DL-methionine	0.38	0.38
Bentonite	1.07	1.07
Limestone	1.28	1.28
MCP	1.32	1.32
Premix	1.24	1.24
Chlorine chlorite	0.06	0.06
NaCl	0.28	0.28
Nutrient content based on laboratory analysis:		
ME (kcal/kg)	2,983	3,080
Crude protein	21.18	19.34
Crude fiber	2.24	2.78
Ether extract	5.11	5.17
Ca	1.45	1.44
P	0.57	0.57

Table 2 Chemical and functional compositions and antibacterial activity of nutmeg flesh extracts.

Items	Values
Moisture (%)	98.92
Ash (%)	0.08
Fat (%)	7.34
Proteins (%)	0.67
Carbohydrates (%)	18.74
Total phenolics (g/100 g)	1.13
Flavonoids (g/100 g)	1.07
DPPH radical scavenging activity (IC_{50} [µg/mL])	7,074.29
Antibacterial activity against *E. coli* (mm)	11.9

3.2. Growth performance of broiler chickens

Table 3 presents the performance of broiler chickens reared at high density and given lemon cui juice. Table 3 shows that broilers reared at a high density of 16 birds/m² (T1) had significantly ($p<0.05$) lower body weight gain (BWG) than broilers reared at a normal density of 10 birds/m² (T0). Adding 0.5 ml/L lemon cui juice to the drinking water of broilers reared at high density (T2) significantly restored BWG to the same level as T0. The addition of 1 ml/L lemon cui juice (T3) increased the BWG significantly more than T0 did but was not significantly ($p>0.05$) different from the addition of 1.5 ml/L lemon cui juice (T4).

The lowest feed intake was observed in broilers reared at high density without lemon cui juice. The highest feed intake was observed in birds reared at a normal density and given 1 ml/L lemon cui juice in their drinking water (T3). Compared with chicks reared at a normal density, the highest FCR was found in birds reared at a high density without lemon cui juice (T1).

3.3. Relative weights of internal organs in broiler chickens

The relative weights of the internal organs of the broiler chickens during the study are presented in Table 4. With respect to the abdominal fat content, broilers reared at normal density (T0) and those reared at high density with 1 ml/L lemon cui juice in their drinking water (T3) had significantly lower ($p<0.05$) abdominal fat than broilers in the other treatment groups. A significant effect on the relative weight of the thymus was also detected. Compared with that of other birds, the relative weight of the thymus was significantly lower ($p<0.05$) in chicks reared at high density without lemon cui juice (T1). The bursa of Fabricius was significantly greater ($p<0.05$) in broilers given 1 ml/L lemon cui juice in their drinking water than in those given other treatments.

Table 3 Performance of broiler chickens (days 14 - 35).

Items	Treatment groups					SE	p value
	T0	T1	T2	T3	T4		
Initial body weight (g)	412.65	412.08	411.97	412.24	412.04	2.14	0.32
Final body weight (g)	1,717.22^{b}	1,582.56^{a}	1714.87^{b}	1,749.32^{bc}	1,784.07^{c}	83.57	<0.01
Weight gain (g)	1,304.57^{b}	1,170.48^{a}	1,302.90^{b}	1,337.08^{c}	1,372.03^{c}	79.76	<0.01
Feed consumption (g)	2,127.18^{b}	2,112.43^{a}	2,098.43^{a}	2,138.43^{b}	2,151.32^{b}	67.93	0.02
Conversion ration	1.63^{a}	1.80^{b}	1.61^{a}	1.60^{a}	1.57^{a}	0.07	<0.01

^{a,b,c}In the same row, different superscripts indicate a significant variation (p<0.05), SE: standard error.

Table 4 Organ weights of broiler chicks fed treatment diets.

Items (% live weight)	Treatment groups					SE	p value
	T0	T1	T2	T3	T4		
Liver	2.63	2.64	2.35	2.46	2.53	0.24	0.72
Heart	0.52	0.51	0.49	0.51	0.53	0.02	0.46
Proventriculus	0.48	0.46	0.44	0.48	0.43	0.06	0.21
Gizzard	1.65	1.58	1.41	1.53	1.52	0.14	0.30
Pancreas	0.31	0.29	0.30	0.32	0.28	0.05	0.25
Duodenum	0.52	0.54	0.48	0.49	0.53	0.07	0.28
Jejenum	1.18	1.17	1.16	1.15	1.18	0.18	0.46
Ilium	0.68	0.72	0.64	0.62	0.70	0.32	0.41
Cecum	0.74	0.72	0.68	0.76	0.70	0.16	0.56
Spleen	0.07	0.08	0.11	0.07	0,08	0.04	0.26
Thymus	0.18^{b}	0.11^{a}	0.16^{b}	0.17^{b}	0.18^{b}	0.10	<0.01
Bursa Febricus	0.08^{a}	0.07^{a}	0.07^{a}	0.12^{b}	0.08^{a}	0.09	<0.01
Abdominal fat	1.31^{a}	1.77^{b}	1.82^{b}	1.28^{a}	1.79^{b}	0.31	0.02

$^{a, b}$In the same row, different superscripts indicate significant variation (p<0.05), SE: standard error.

4. Discussion

The use of herbal plants in feed aims to obtain various biological activities, such as antioxidant, antibacterial, antiparasitic, antiviral, and antifungal effects (Sapsuha et al., 2022). Our research results indicate that lemon cui juice from Ternate Island does not have potential as an antioxidant, as the analysis revealed an IC50 value above 1000 ppm. The high antioxidant capacity is due to the high water content in lemon cui juice, resulting in a very low concentration of antioxidant compounds (Anagnostopoulou et el., 2006). The antioxidant capacity is categorized as very strong with an IC50 value < 50 ppm, strong with an IC50 value between 50–100 ppm, weak with an IC50 value of 100–150 ppm, moderate at 150–200 ppm, and very weak with an IC50 value > 200 ppm (Molyneux, 2004). Our findings are not very different from those of previous studies (Munadi et al., 2023), which revealed the antioxidant capacity of lemon cui juice from Ambon Island to be 9,094.24 (µg/mL).

Lemon cui from the ternate island contains bioactive compounds such as phenols and flavonoids and has the ability to inhibit *E. coli* bacteria. Recent studies have shown that lemon cui juice from Ambon Island contains alkaloid, flavonoid, terpenoid, and saponin compounds (Munadi et al., 2023). The antibacterial activity test of lemon cui juice against *E. coli* via the disk diffusion method (Kirby-Bauer) revealed an inhibition zone of 11.9 mm, indicating that lemon cui juice has moderate inhibitory effects on *E. coli* growth. Research has shown that Pontianak orange extract (*Citrus nobilis* L. Var *Microcarpa*) at a 35% concentration has an inhibitory effect on *Streptococcus mutans* bacteria of 11.3 mm (Mardiah et al., 2017).

Our research results indicate that rearing broiler chickens at high density reduces their final body weight, weight gain, and feed intake. Several studies have reported that high stocking density can disrupt chicken growth and feed efficiency (Sapsuha et al., 2022). Our current research shows that lemon cui juice in drinking water can mitigate the negative impacts of rearing broilers at high density (16 birds/m²). This study revealed that providing lemon cui juice in drinking water can also improve the FCR of broilers reared at high density. These results indicate that lemon cui juice can enhance digestive performance in the intestines, ultimately improving nutrient utilization. In this context, lemon cui juice can act as an acidifier and a source of probiotics, ultimately enhancing the health and function of the digestive tract (Sigiharto., 2016; Pratama et al., 2021).

Until now, no research has explained the effects of using lemon cui juice from Ternate Island on improving the performance of broiler chickens available in the literature. However, the effectiveness of lemon cui juice is likely due to the synergistic action of various phytochemicals present in lemon cui juice, such as phenols and flavonoids (Table 2), which in turn can improve the physiological condition of chickens. This condition leads to increased feed utilization

and efficiency, thus enhancing broiler performance. Bioactive compounds such as flavonoids and phenols have been reported to increase growth rates and feed efficiency in broilers because of their ability to scavenge/neutralize free radicals and maintain the integrity of the intestinal mucosa (Ouyang et al., 2016; Kishawy et al., 2019).

Our findings indicate that broilers reared at high density (16 birds/m²) have greater abdominal fat content than broilers in other treatment groups. Research has shown that rearing broilers at high density leads to increased abdominal fat deposition due to reduced activity in broilers (Beg et al., 2011). This study revealed that lemon cui juice can reduce fat deposition in broilers reared at high density, suggesting a fat-lowering effect of lemon cui juice in broilers. Owing to its acidic content, lemon cui juice can inhibit fatty acid synthesis (Peng et al., 2018), thereby reducing fat accumulation in broiler abdomens.

In this study, the relative weight of the thymus was lower in broilers reared at high density. Interestingly, providing lemon cui juice in drinking water could maintain a thymus weight comparable to that of broilers reared at a normal density. Despite the impact of high density, lemon cui juice in drinking water increased the relative weight of the Bursa of Fabricius in broilers. In this context, the bioactive components in lemon cui juice (Table 2) may be responsible for supporting the development of immune organs in broilers reared at high density. To support our findings, Sugiharto et al. (2022) reported that fermentation of starfruit extract with shrimp paste could increase the relative weights of immune organs such as the thymus and bursa of Fabricius.

6. Conclusions

Lemon cui (*Citrus microcarpa*) juice from Ternate Island contains bioactive compounds such as phenols and flavonoids. Its administration to broiler chickens can improve performance and enhance the development of immune organs in broilers reared at high density.

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Ethical Considerations

This study was approved by the Animal Research Ethics Committee of the Faculty of Agriculture, Universitas Khairun No. 08/KEPH/PH/2024.

Conflicts of Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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