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The Effect of Butterfly Pea Flower Extract on Broilers Raised in High-Density Environments (Impact on Performance, Blood Profile and Gut Microbes)

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ABSTRACT

Background: High-density farming systems can lead to oxidative stress and negatively impact broiler performance. Antibiotic growth promoters (AGPs) were used to improve broiler performance under stress conditions. However, long-term use of AGPs can lead to antibiotic resistance in humans as consumers. Therefore, the use of AGPs is now banned in broiler production. This study aimed to evaluate the effects of butterfly pea flower extract on the performance, blood profile and gut microbes of broilers raised in high-density environments.

Methods: After 15 days of rearing, 370 unsexed Lohmann broiler chicks were assigned to five treatments in a completely randomized design, consisting of $CONT^{+}$, $CONT^{-}$, BPE0.5, BPE1.0 and BPE1.5. Sampling was conducted on day 35.

Result: The results indicated that the inclusion of butterfly pea flower extract in the diet of broilers raised at a high density (16 birds/ m^2) significantly ($p<0.05$) improved performance, lowered the pH in the ileum and cecum, increased lactic acid bacteria and reduced coliform bacteria in both the ileum and cecum. There was no significant effect ($p>0.05$) on the blood profile of broilers.

Key words: Blood profile, Broiler, Butterfly pea, Gut microbes, High-density environments, Performance.

INTRODUCTION

Broiler farming in Indonesia is one of the key industries as it fulfills the community's need for animal protein, particularly in the form of chicken meat, which is an affordable and accessible source of protein. The efficiency of broiler farming is crucial for achieving maximum production outcomes with minimal operational costs. Implementing high stocking density in broiler farming is one strategy to increase meat production per square meter, thereby maximizing profits. However, high-density farming systems can lead to oxidative stress and negatively impact broiler performance and health (Abudabos *et al.,* 2013). Previously, antibiotic growth promoters (AGPs) were used to improve broiler performance under stress conditions (Haque *et al.,* 2020). However, long-term use of AGPs can lead to antibiotic resistance in both livestock and humans as consumers. Therefore, the use of AGPs is now banned in broiler production worldwide, including in Indonesia. Without AGPs, broiler farming under high stocking density has been reported to result in performance issues (Altaf *et al.,* 2019). For sustainable broiler production, it is crucial to find safe alternatives to AGPs. Efforts have been made to address performance and health issues in broilers reared under high-density conditions during the ban on antibiotic use (Magnuson *et al.,* 2020, Sugiharto *et al.,* 2022).

Modern knowledge suggests that plant extracts contain phenolic compounds that can function as antioxidants (Oso *et al.,* 2021). Butterfly pea flower (*Clitoria ternatea* L.) is one such plant with a high antioxidant content, making it a potential source of natural antioxidants (Niranjan *et al*., 2020; Sapsuha *et al*., 2023). Butterfly Pea Flower belongs to the *Papilionaceae* or *Fabaceae* family (legumes). Previous research has shown that butterfly pea flowers contain bioactive compounds such as flavonoids, phenols and terpenoids, which can function as antibacterial, antifungal and antioxidant agents (Seftiono *et al.,* 2021, Sapsuha *et al.,* 2023). These compounds have the ability to fight free radicals in the body, which can cause cell damage and various diseases. Therefore, the use of butterfly pea flower extract is expected to replace the role of AGPs, which have been banned from use.

Our previous study showed an improvement in broiler performance when butterfly pea flower extract was included in feed mixtures (Sapsuha *et al.,* 2023). The bioactive compounds and antioxidant capacity found in butterfly pea flowers can effectively improve physiological conditions, resulting in better broiler growth performance (Sapsuha

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et al., 2023). To our knowledge, there is no prior documentation on the use of butterfly pea flower extract in broilers under stress conditions. Therefore, we investigated the impact of butterfly pea flower extract on the performance, blood index and gut ecology of broilers reared under stress conditions caused by high stocking density. We hypothesize that butterfly pea flower extract can improve the performance, blood profile and gut microbes of broilers raised in high-density conditions.

MATERIALS AND METHODS

Preparation of butterfly pea flower extract

Butterfly pea flowers were obtained from gardens around the campus of Universitas Khairun in Ternate, North Maluku Province, Indonesia. Before use, the butterfly pea flowers were air-dried and ground into powder. One kilogram of butterfly pea flower powder was extracted using the maceration technique by soaking it in 4 liters of 96% ethanol solution for 3 × 24 hours. During the maceration process, stirring was done twice a day, in the morning and evening. The resulting filtrate was then filtered and evaporated using a rotary evaporator to obtain butterfly pea flower extract (Sapsuha *et al.,* 2023).

In vivo experiment

The Animal Ethics Committee of the Faculty of Agriculture, Universitas Khairun (No. 15/KEPH/PH/2024) approved this study. The trial was carried out on May to June 2024 in the experimental cage Department of Animal Science, Faculty of Agriculture, Universitas Khairun. A total of 370 unsexed Lohmann broiler chickens were reared collectively with normal stocking density from day 0 to day 14. On day 14, the broilers (with a body weight of 425.65±2.63 g) were randomly assigned to five treatments, with each treatment replicated five times: $CONT^+$ (positive control with normal stocking density of 10 birds/m²), $CONT^-$ (negative control with high stocking density of 16 birds/m²) and BPE0.5, BPE01.0 and BPE1.5 with high stocking density of 16 birds/m². The feed provided was in mash form and formulated (Table 1) as starter feed (days 1-21) and finisher feed (days 22-35). Butterfly pea flower extract was added to the feed starting on day 14 at 0.5, 1.0 and 1.5 ml/kg for BPE0.5, BPE01.0 and BPE1.5 respectively, while no butterfly pea flower extract was added to the feed in $CONT^+$ and $CONT^-$ treatments. During the rearing period, the broilers were housed in ventilated broiler cages with sawdust as bedding.

Data collection and analysis

Body weight gain, feed intake and feed conversion ratio (FCR) were recorded weekly. On day 35, blood samples were collected from male chicks representing the average body weight of each pen via the brachial vein in the wing. Blood samples were collected from each bird for every replicate (five birds per treatment). To avoid physiological differences related to sex and body weight, male chicks with body weight reflecting the average weight of each pen were used for blood sampling.

For practical reasons, the chickens from which blood samples had been previously taken were slaughtered and their internal organs were subsequently removed. The internal organs of the experimental chickens were collected and weighed using an analytical balance. To measure the gut bacterial population, digesta was taken from the ileum and cecum and placed in sterile sample containers. Digesta was also collected from the duodenum, jejunum, ileum and cecum to measure pH values (using an electronic pH meter; Thermo Fisher Scientific Inc.). The complete blood profile was determined using the Prima Fully-Auto Hematology Analyzer (PT Prima Alkesindo Nusantara, Jakarta, Indonesia) following the manufacturer's protocol.

The study was conducted based on a Completely randomized design. The obtained data were analyzed using ANOVA (SPSS version 16.0) and Duncan's Multiple Range Test was employed to differentiate statistically significant means ($p<0.05$).

RESULTS AND DISCUSSION

Broiler performance

Table 2 presents the performance of broilers raised under high-density conditions and given butterfly pea flower extract. Broilers raised under normal stocking density ($CONT^+$) had

Table 1: Feed compositions of broilers as starter (days 1-21) and finisher (days 22-35).

Items	%, unless otherwise noted	
	Starter (days 1-21)	Finisher (days 22-35)
Yellow corn	56.87	63.12
Soybean meal	36.37	30.3
Palm oil	2.22	2.22
DL-methionine	0.26	0.26
Bentonite	1.16	0.98
Limestone	1.42	1.42
Monocalcium phosphate	1.4	1.4
Premix[1]	0.22	0.22
Chlorine chlorite	0.08	0.08
NaCl	0.30	0.30
Nutrient contents		
Metabolizable energy (kcal/kg)[2]	2,964	3,042
Crude protein (%)	21.68	19.96
Crude fiber (%)	3.23	3.12
Crude fat (%)	4.22	4.57

[1]Premix contained (per kg of diet) of vitamin A 7,750 IU, vitamin D3 1,550 IU, vitamin E 1.88 mg, vitamin B1 1.25 mg, vitamin B2 3.13 mg, vitamin B6 1.88 mg, vitamin B12 0.01 mg, vitamin C 25 mg, folic acid 1.50 mg, Ca-d-pantothenate 7.5 mg, niacin 1.88 mg, biotin 0.13 mg, Co 0.20 mg, Cu 4.35 mg, Fe 54 mg, I 0.45 mg, Mn 130 mg, Zn 86.5 mg, Se 0.25 mg, L-lysine 80 mg, Choline chloride 500 mg, DL-methionine 900 mg, CaCO3 641.5 mg, Dicalcium phosphate 1500 mg.

significantly higher body weight ($p<0.05$) compared to broilers raised under high-density conditions without butterfly pea flower extract ($CONT^-$). Throughout the rearing period, higher body weight gain ($p<0.05$) was observed in the $CONT^+$, BPE0.5, bpe1.0 and bpe1.5 groups compared to the negative control ($CONT^-$), while no significant differences ($p>0.05$) were observed between the positive control ($CONT^+$; normal stocking density of 10 birds/m²) and the butterfly pea flower extract treatments (BPE0.5 and BPE1.0).

Our study showed that raising broilers in high-density conditions reduced final body weight, weight gain and feed intake. Several studies have shown that high stocking density is associated with impaired growth and reduced feed efficiency in broilers (Yin *et al.,* 2017, Gholami *et al.,* 2020). Other research has also demonstrated a significant reduction in weight gain with increasing stocking density (Son, 2013, Adeyemo *et al.,* 2016, Esmaeili-Fard *et al.,* 2022). The decline in broiler growth performance under high-density conditions can be attributed to several factors, including increased temperature and reduced air circulation, increased stress and restricted space in the pen, which limits growth and feed access, potentially leading to nutritional deficiencies (Shakerı *et al.,* 2014, Sugiharto, 2022).

The current study shows that butterfly pea flower extract can mitigate the negative impact of high stocking density on broiler growth performance. To date, no research has been available in the literature explaining the effects of butterfly pea flower extract on weight gain improvement in broilers raised under high-density conditions. However, the effectiveness of butterfly pea flower extract is likely attributable to the synergistic action of various phytochemicals present in the flower (Lijon *et al.,* 2017, Sapsuha *et al.,* 2023) which in turn may improve the physiological conditions of the chickens. These improved conditions enhance feed utilization and efficiency, leading to better growth performance in broilers. Phytochemicals such as flavonoids, phenols and saponins have been reported to promote higher growth rates and better feed efficiency in broilers due to their ability to scavenge/neutralize free radicals and maintain the integrity of the intestinal mucosa (Zhou *et al,.* 2019; Essien and Udoh, 2021, Chen *et al.,* 2021). Additionally, previous studies have shown that butterfly pea flower extract has antibacterial properties (Nguyen *et al.* 2016, Sapsuha *et al.* 2023) that can stimulate the growth of beneficial bacteria, deactivate pathogenic bacteria and enhance nutrient metabolism and absorption in the digestive tract, ultimately improving broiler growth performance.

Regarding feed consumption, it was observed that the inclusion of butterfly pea flower extract resulted in higher feed intake ($p<0.05$) during the rearing period. The addition of butterfly pea flower extract led to a lower feed conversion ratio ($p<0.05$) compared to the negative control ($CONT^-$). However, the inclusion of 0.5 ml/kg butterfly pea flower extract in the diet (BPE0.5) did not result in a significant difference in feed conversion ratio ($p>0.05$) compared to the negative control ($CONT^-$).

In terms of feed intake, the administration of butterfly pea flower extract appears to increase feed consumption, which in turn can lead to weight gain. The inclusion of plant extracts has been reported to enhance the palatability and flavor of feed, thereby increasing feed intake and weight gain (Hussein *et al.,* 2020; Tavangar *et al.,* 2021). The improved feed conversion observed in broilers given butterfly pea flower extract provides evidence of better feed utilization. The use of plants as phytogenics in broiler feed can improve intestinal digestibility, ultimately promoting broiler growth (Mahanta *et al.,* 2017, Huang *et al.* 2018).

Relative weight of broiler internal organs

The results showed that raising broilers under high-density conditions had no significant effect ($p>0.05$) on the weight of internal organs, abdominal fat and carcass traits of broilers (Table 3 and Table 4). Our study shows that the weight of internal organs and abdominal fat of broilers raised under high-density conditions is relatively similar to those raised under normal stocking density. This aligns with reports from several researchers indicating that stocking density does not have a significant impact on the weight of internal organs and abdominal fat in broilers (Yin *et al*., 2017, Sugiharto *et al.,* 2022). Similarly, Toghyani *et al.,* (2011) and Mareta *et al*., (2020) found no effect of cinnamon, garlic and bilimbi on the relative weight of internal organs in broilers.

Broiler blood profile

Table 5 presents the blood profile of broilers raised under high-density conditions. The results indicated that the administration of butterfly pea flower extract to broilers raised

Table 2: Performance of broilers chickens.

Items	Treatment groups					SE	p value
	$CONT^+$	$CONT^-$	BPE0.5	BPE1.0	BPE1.5		
Initial BW (g)	427.63	425.87	425.25	424.42	425.07	12.25	0.15
Final BW (g)	1,853.27^c	1,425.43^a	1,575.70^b	1,725.82^c	1,725.64^c	153.55	<0.01
Weight gain (g)	1,425.64^c	999.56^a	1,150.45^b	1,300.75^c	1,300.57^c	151.61	<0.01
Accumulative FI (g)	2,637.43^c	2,187.61^a	2,262.57^b	2,362.42^b	2,300,35^b	0.18	0.04
FCR	1.85^b	2.18^c	1.96^c	1.81^b	1.76^a	1.73	<0.01

^{a,b,c}On the same row, different superscripts indicated a significant variation ($p<0.05$). SE: Standard error, FI: Feed intake, BW: Body weight, FCR: Feed conversion ratio.

Table 3: Organs weight of broiler chicks fed treatment diets.

Items (% live weight)	Treatment groups					SE	p value
	$CONT^+$	$CONT^-$	BPE0.5	BPE1.0	BPE1.5		
Liver	2.24	2.23	2.32	2.27	2.53	0.21	0.72
Heart	0.39	0.37	0.39	0.38	0.40	0.06	0.81
Proventriculus	0.41	0.41	0.39	0.40	0.42	0.07	0.53
Gizzard	1.46	1.42	1.47	1.48	1.42	0.15	0.30
Pancreas	0.21	0.22	0.22	0.24	0.13	0.02	0.41
Spleen	0.09	0.10	0.09	0.08	0.09	0.07	0.26
Thymus	0.14	0.12	0.13	0.12	0.12	0.08	0.65
Bursa Febricus	0.07	0.07	0.09	0.09	0.08	0.04	0.56
Duodenum	0.46	0.54	0.47	0.43	0.52	0.06	0.11
Jejenum	1.16	1.18	1.16	1.17	1.14	0.18	0.64
Ilium	0.64	0.70	0.57	0.69	0.62	0.42	0.78
Cecum	0.74	0.72	0.75	0.75	0.73	0.29	0.76
Abdominal fat	1.75	1.78	1.65	1.49	1.43	0.82	0.54

SE: Standard error.

Table 4: Carcass traits of broiler chicks fed treatment diets.

Items	Treatment groups					SE	p value
	$CONT^+$	$CONT^-$	BPE0.5	BPE1.0	BPE1.5		
			(% live weight)				
Carcass	68.32	68.06	67.32	67.55	67.43	2.43	0.87
			(% eviscerated carcass)				
Breast	38.40	38.39	37.29	38.14	38.13	2.43	0.62
Upper thigh	14.75	14.82	14.69	14.80	14.07	0.54	0.92
Lower Thigh	13.77	13.54	14.70	13.56	13.21	1.45	0.76
Wings	7.40	7.52	7.60	7.32	7.33	0.76	0.54
Back	20.58	21.43	21.18	20.87	21.66	1.48	0.80
Abdominal fat	2.27	2.18	2.23	2.42	2.26	0.65	0.42

SE: Standard error.

Table 5: Blood profile of broiler chickens.

Items	Treatment groups					SE	p value
	$CONT^+$	$CONT^-$	BPE0.5	BPE1.0	BPE1.5		
Erythrocytes (10^{12}/L)	3.43	2.89	3.23	2.98	3.04	0.53	0.26
Leukocytes (10^9/L)	28.32	27.76	20.46	21.78	23.68	5.12	0.34
Haemoglobin (g/dL)	11.43	13.43	12.78	12.65	11.84	2.32	0.28
Haematocrits (%)	37.51	42.31	39.98	33.71	36.43	4.98	0.38
Thrombocytes (10^9/L)	23.34	24.83	26.64	20.17	25.42	11.09	0.56
Lymphocytes (10^9/L)	114.11	165.21	123.31	136.27	120.43	38.41	0.28
Heterophils (10^9/L)	11.42	10.31	11.28	9.67	11.47	10.98	0.82
MCV (10^{-15} L)	125.48	125.38	123.48	122.70	123.28	3.21	0.28
MCH (10^{-12} g)	32.76	34.02	33.08	32.54	32.98	2.24	0.74
MCHC (g/L)	30.28	31.38	31.10	31.62	30.98	1.46	0.80
RDW-SD (10^{-15} L)	42.69	44.89	43.05	43.79	43.31	4.21	0.64
RDW-CV (%)	10.77	10.63	11.03	11.27	10.91	0.74	0.43
MPV (10^{-15} L)	10.18	8.88	8.34	9.70	9.52	1.68	0.27

MCV: Mean corpuscular volume, MCH: Mean corpuscular haemoglobin, MCHC: Mean corpuscular haemoglobin concentration, RDW-SD: Red blood cell distribution width-standard deviation, RDW-CV: Red blood cell distribution width-coefficient variation, MPV: Mean platelet volume, SE: Standard error.

Table 6: pH and bacterial population of broiler chickens fed treatment diets.

Items	Treatment groups					SE	p value
	CONT+	CONT-	BPE0.5	BPE1.0	BPE1.5		
pH							
Duodenum	6.68	6.89	6.75	6.56	6.58	0.41	0.83
Jejunum	5.56	5.67	5.54	5.31	5.31	0.34	0.46
Ileum	6.25^{b}	6.22^{b}	5.64^{a}	5.34^{b}	5.33^{b}	0.64	0.03
Cecum	7.62^{c}	7.51^{c}	6.57^{ab}	6.36^{a}	6.28^{a}	0.45	<0.01
Lactic acid bacteria (log cfu/g)							
Ileum	8.88^{a}	9.81^{b}	10,60^{bc}	10.63^{bc}	10.72^{c}	0.68	p<0.01
Cecum	9.06^{a}	10.04^{b}	10.63^{c}	10.65^{c}	10.68^{c}	0.64	p<0.01
Coliform (log cfu/g)							
Ileum	7.40^{b}	6.31^{b}	5.31^{a}	5.22^{a}	5.07^{a}	1.28	<0.01
Cecum	8.17^{b}	7.19^{b}	7.02^{b}	7.01^{ab}	6.39^{a}	1.21	0.03

^{a,b,c} Means on the same row with different superscripts differ at $p<0.05$).

under high-density conditions had no significant effect (p>0.05) on the blood profile of broilers. Our study also shows that raising broilers under high-density conditions did not affect their blood profile. The exact reason for this condition is unknown, but raising broilers in high-density conditions from day 14 to 35 may have allowed them to adapt to the environment, thereby compensating and adjusting their physiological mechanisms to cope with the stress. Our previous research (Sapsuha *et el.,* 2021) also showed no effect of nutmeg flesh extract on broilers raised under high-density conditions.

pH values and gut bacterial count in broilers

Table 6 presents the pH values and gut microbiota of broilers raised under high-density conditions. The results show that the administration of butterfly pea flower extract to broilers raised in high-density conditions significantly (p<0.05) reduced the pH in the ileum and cecum, with no significant effect (p>0.05) on the pH of the duodenum and jejunum. The data in Table 6 also indicate that the administration of butterfly pea flower extract to broilers raised under high-density conditions significantly (p<0.05) increased lactic acid bacteria and significantly (p<0.05) reduced coliform bacteria in the ileum and cecum.

Our study also shows that raising broilers under high-density conditions did not affect their blood profile. The exact reason for this condition is unknown, but raising broilers in high-density conditions from day 14 to 35 may have allowed them to adapt to the environment, thereby compensating and adjusting their physiological mechanisms to cope with the stress. Raising broilers under high-density conditions can increase the pH in the cecum. Similar results were reported by Jang *et al.* (2014) and Tsiouris *et al.* (2015), who observed an increase in cecal pH in broilers raised under high-density conditions. It was further suggested that the rise in cecal pH might result from reduced litter quality, which affects the gut microbiota. The administration of butterfly pea flower extract to broilers raised under high-density conditions can lower the pH in the ileum and cecum. The carbohydrate content, especially oligosaccharides found in nearly all plants, can serve as a good substrate for fermentation processes that support the growth of beneficial microbes (Blajman *et al*., 2015).

The acidic conditions in the digestive tract, particularly in the ileum and cecum, support the balance of gut microflora, where lactic acid bacteria populations increase and pathogenic bacteria populations decrease (Dittoe *et al.,* 2019). Lactic acid bacteria produce lactic acid, which helps maintain the acidic pH of the broiler's digestive tract. Additionally, the acidic environment in the digestive tract can enhance the effectiveness of nutrient absorption by the intestinal walls, ensuring nutritional needs are met, health status is maintained and productivity is increased (Blajman *et al*., 2015, Sjofjan *et al.* 2021). Regarding stocking density, raising broilers under high-density conditions can reduce lactic acid bacteria and increase coliform bacteria in the ileum and cecum. The effects of rearing broilers at high density can cause stress (Sugiharto *et al*., 2022), characterized by changes in behavior, biochemistry and physiology, all aimed at restoring homeostasis, which ultimately alters the composition of gut microbes.

The results of this study show that the administration of butterfly pea flower extract to broilers raised under high-density conditions can increase lactic acid bacteria and reduce coliform bacteria in the ileum and cecum. The reason why butterfly pea flower extract has a positive impact on gut microbes is that it contains active components that can act as antibacterial agents (Lijon *et al*., 2017, Sapsuha *et al.,* 2023), thus supporting the growth of beneficial bacteria and inhibiting pathogenic bacteria (Nguyen *et al.,* 2016; Sapsuha *et al.,* 2023). Additionally, the carbohydrate content, particularly oligosaccharides found in nearly all plants, can serve as a good substrate for the growth of beneficial microbes.

CONCLUSION

The administration of butterfly pea flower extract up to 1.5 mL/kg of feed can improve performance, increase the total lactic acid bacteria and reduce the total coliforms without

causing negative effects on the blood profile of broilers raised under high-density conditions.

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Disclaimers

The views and conclusions expressed in this article are solely those of the authors and do not necessarily represent the views of their affiliated institutions. The authors are responsible for the accuracy and completeness of the information provided, but do not accept any liability for any direct or indirect losses resulting from the use of this content.

Informed consent

All animal procedures for experiments were approved by the Committee of Experimental Animal care and handling techniques were approved by the Animal Ethics Committee of the Faculty of Agriculture, Universitas Khairun (No. 15/KEPH/PH/2024).

Conflict of interest

The authors declare that there are no conflicts of interest regarding the publication of this article. No funding or sponsorship influenced the design of the study, data collection, analysis, decision to publish, or preparation of the manuscript.

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