The effect of Butterfly Pea (*Clitoria ternatea* L.) on the relative weight and length of digestive organs in broiler chicken

Iis Yuanita[1*], *Lisnawaty* Silitonga[1], *Hastin* Ernawati Nur Chusnul Chotimah[2], *Ellen* Christ Tambunan[3], *Indah* Gebyana Gultom[1], *Nopia* Permata Sari Malau[1], *Frans* Novaber Simanullang[1], *Uhfi* Halituja[1], *Anita* Theresia Br Gultom[1], *Melisa* Yuniarti[1], *Asgar* Juli Silaban[1], *Pirnindi* Julyarti Br Simamora[1], and *Junita* Naibaho[1]

[1]Animal Science Study Program, Faculty of Agriculture University of Palangka Raya, Palangka Raya, Jl. Yos Sudarso Palangka Raya 73111, Indonesia
[2] Agrotechnology Study Program, Faculty of Agriculture University of Palangka Raya, Palangka Raya, Jl. Yos Sudarso Palangka Raya 73111, Indonesia
[3] Agricultural Industrial Technology Study Program, Faculty of Agriculture University of Palangka Raya, Pangka Raya, Jl. Yos Sudarso Palangka Raya 73111, Indonesia

Abstract. The goal of this investigation was to check a diet enriched with butterfly pea flower (*Clitoria ternatea* L.) flour on the relative weight and length of the tracts of poultry digestive system. The study employed a Completely Randomized Design (CRD), consisting five dietary treatments with five replications. Each replication used four-day old chicks (CP707 strain). The treatments were as follows: P0 (basal diet without butterfly pea flower flour), P1 (basal diet + 1% butterfly pea flower flour), P2 (basal diet + 2% butterfly pea flower flour), P3 (diet + 3% butterfly pea flower flour), and P4 (diet + 4% butterfly pea flower flour). Various parts of the digestive system such as the crop, proventriculus, gizzard, small intestine (duodenum, jejunum, ileum), cecum, and colon were used for experiments. The finding discovered that feeding butterfly pea flower meal in the diet had significantly impact ($P<0.05$) on the relative weight of duodenum, but it did not significantly affect ($P>0.05$) the relative weight of crop, proventriculus, gizzard, length of duodenum, relative weight and length of jejunum, ileum, cecum, and colon. To conclude, the use of butterfly pea flower flour in the diet can enhance duodenal function without any negative effects on the relative weight of the crop, proventriculus, and gizzard, or the length of the duodenum.

[1]* Corresponding author: iis.yuanita@pet.upr.ac.id

1 Introduction

The changes in consumption patterns are also influenced by their increased purchasing power. Among the various sources of animal protein, broiler chicken meat is one of the most widely consumed commodities. Broiler chicken has a great potential for meat production, but its production highly relies on the availability of feed with nutritional quality standards and quantity. Feed consumption is associated with the digestive organs' role in breaking down and absorbing feed nutrients.

Broiler chicken producers have employed various strategies to improve productivity and digestive tract healthfulness by including additives such as antibiotics [1]. The addition of antibiotic growth promoters (AGP) at low dietary levels aims to enhance the efficiency of nutrient uptake in the digestive tract, thereby stimulating broiler chicken growth. Nevertheless, AGP use has raised concern, especially the creation of pathogenic bacteria resistance and antibiotic residues in animal products, that has led to its use being prohibited by the government. As a substitute, the use of natural materials such as herbal plants that also have a function as phytobiotics has begun to be developed to replace AGP. Phytobiotics are safer as they leave no residues and are still efficient in promoting growth performance, for example, the gastrointestinal tract of broiler chickens.

Phytobitics are a plant extract origin feed additive that contain active substance which adds to improving livestock welfare and productivity, specifically by suppressing the growth of bacteria causing disease in the gut. Optimal performance of the digestive apparatus is paramount because the organ is involved in nutrient absorption from the diet to be circulated around the body later on, thereby allowing maximum growth to be achieved [2]. Feed has a direct influence on the structure and performance of the digestive organs, and enhancing the activity of these organs can potentially impact their weight and dimensions. The level of crude fiber in feed has been shown to improve nutrient intake efficiency, evidenced by an increase in the size and number of intestinal villi and by increasing the volume of the digestive organs [3].

The flower helps enhance the health of the digestive system due to the presence of diverse bioactive compounds like tannins, flavonoids, saponins, alkaloids, triterpenoids, phenols, anthocyanins, and steroids. As for the chemical and phytochemical value, the butterfly pea flower flour has a 24.02% crude protein, 2.91% crude fat, 57.91% carbohydrates, 1096.8 mg/ml triterpenoids, 87.6 mg/ml, and 0.96 mg/g anthocyanins [2]. Moreover, butterfly pea flowers could be utilized as a supplemental feed for broiler chickens due to its higher nutritional value and bioactive compounds which in return will help maintain the performance as well as the gut health of the broiler chickens.

Flavonoids, antioxidants in butterfly pea flowers, were related to human health and extensively used as functional food and food ingredient drinks [4, 5]. Nonetheless, the scope of using butterfly pea flower flour as an additional feed for broiler chickens is still limited, and therefore, requires further exploration. Research is needed to examine the changes in the structure and physiology of the broiler chicken's digestive organs when fed with butterfly pea flower flour, along with the investigation of the possible bioactive role of this component in the gastrointestinal tract.

2 Materials and Methods

This research received the approval from the Ethics Committee at the Faculty of Medicine, University of Palangka Raya, under the protocol number 108/UN24.9/LL/2024.

2.1 Formulating butterfly pea flower flour

The study began with the removal of the flour made from the butterfly pea flowers. The butterfly pea flowers were indirectly sun-dried to achieve a moisture content of approximately 5%, following the procedure outlined by Yuanita et al. [2]. After that, the butterfly pea flowers were ground into a powder after the drying process. The powder formed was sifted to obtain flour of consistent texture.

2.2 Trial feeding

This study incorporated progressive levels of butterfly pea flower flour into basal rations and fed them to broiler chickens raised for 35 days. One hundred-day old chicks (DOCs) were divided into 5 treatment groups with 5 replications each. Each replication comprised 4 DOCs with a mean initial weight of 42.78±3.5 grams. During the maintenance period, feeding and drinking water were supplied ad libitum. The treatments included P0: basal ration without the addition of butterfly pea flower flour; P1: basal ration + 1% butterfly pea flower flour; P2: basal ration + 2% butterfly pea flower flour; P3: basal ration + 3% butterfly pea flower flour; and P4: basal ration + 4% butterfly pea flower flour. The nutrient content of the basal diet used conformed to the Indonesian National Standard (SNI) 8173:2015 and was derived from commercial feed by PT Wonokoyo Jaya Corp, as shown in Table 1.

Table 1. Nutritional value of basal rations.

Nutritional content	Composition (%)	
	Starter (<21 days) *	Finisher (22-42 days)*
Metabolic energy, Kcal/kg**	3.000	3.100
Water content, %	Max. 14	Max. 14
Crude Protein, %	21-23	18-20
Crude Fiber, %	Max. 5	Max. 5
Crude fat, %	Min. 5	Min.6
Ash content, %	Max. 7	Max. 8
Calcium, %	0.8-1.1	0.8-1.1
Phosphorus, %	Min. 0.5	Min. 0.45

Notes: * Based on analysis from Wonokoyo Jaya Corp., ** Based on SNI 8173:2015.

2.3 Sampling and measurement of variables

This research covered the relative weight of the crop, proventriculus, ventriculus, relative weight, and length of small intestine subdivided into its parts (duodenum, jejunum and ileum), cecum and large intestine. The chickens were slaughtered at 35 days of age, with a sample chicken taken from each replication. The chickens were fasted for 12 hours before slaughtering to ensure that the digestive tract was clear of feed residues. Slaughtering was performed following Islamic rules, by severing the four major channels: the jugular vein, carotid artery, esophagus, and trachea. After slaughter, an incision was made by slashing the abdomen from the left and right, extending to the front chest. The region was then incised open, exposing the internal organs. The digestive organs analyzed were the esophagus, crop, proventriculus, gizzard, small intestine, ceca, and colon.

The measurement of the digestive organs was done with the methods that have been tested previously. The weight of the digestive organ was measured in grams (g). The relative organ weight was calculated based on the formula: (organ weight/live weight) × 100%. The digestive organ length was directly measured upon slaughtering of the chicken using a measuring tape. The organ length was recorded in centimeters (cm) as done by Icharoen [6].

2.4 Data analysis

Data were tested for analysis of variance (ANOVA) to assess the effect of treatments on the observed characteristics. In cases where the analysis indicated significant differences, Duncan's Multiple Range Test (DMRT) was applied for a more accurate determination of the differences between treatments [7].

3 Results and Discussion

3.1 Relative weight of crop, proventriculus and ventriculus

The average relative weights of the crop, proventriculus, and gizzard (ventriculus) of the broiler chickens treated with butterfly pea flower flour are presented in Table 2.

Table 2. Relative weight of crop, proventriculus, and ventriculus of broiler chickens.

Treatments	Crop (%)	Proventriculus (%)	Ventriculus (%)
P0	0.40 ± 0.15	0.47 ± 0.04	1.44 ± 0.19
P1	0.37 ± 0.08	0.50 ± 0.08	1.60 ± 0.19
P2	0.37 ± 0.14	0.50 ± 0.06	1.46 ± 0.18
P3	0.40 ± 0.07	0.50 ± 0.08	1.46 ± 0.20
P4	0.49 ± 0.17	0.58 ± 0.17	1.46 ± 0.29

Notes: P0 (basal ration without the including of butterfly pea flower flour), P1 (basal ration + 1% butterfly pea flower flour), P2 (basal ration + 2% butterfly pea flower flour), P3 (basal ration + 3% butterfly pea flower flour), P4 (basal ration + 4% butterfly pea flower flour).

The result of the experiment indicated that addition of butterfly pea flower flour to the ration had no significant effect ($P>0.05$) on relative weight of the crop, proventriculus and ventriculus organs of broiler chicken. The relative weight of the crop obtained in this experiment was ranged from 0.37 to 0.49% of live body weight. Lowest relative proventriculus weight obtained was in the control treatment (P0) at 0.47%, while highest in the P4 treatment (4% butterfly pea flower flour) at 0.58%. The lightest relative ventriculus weight was also found in the control treatment (P0) at 1.44%, and the highest was found in the P1 treatment (1% butterfly pea flower flour) at 1.60%. According to Ukim et al. [8], relative weight of ventriculus under normal condition is between 0.40-0.50% of the live weight, while relative ventriculus weight is between 1.6-2.3%. Based on these references, the relative weight ratios of the digestive organs in this study were within the physiological range.

There are evidences that the higher the level of supplementation with butterfly pea flower flour in the ration tends to raise the relative weight of the broiler chicken crop, proventriculus, and ventriculus. Such a rise is quite possible because the content of bioactive compounds in butterfly pea flower flour that are from flavonoids and phenolic compounds has been the main factor behind the explanation of their high antioxidant activity and, thus, the health status of chicken is improved, and also the status and function

of digestive organs such as crop, proventriculus, and ventriculus are positively influenced. Furthermore, the use of butterfly pea flower flour as a supplement also introduces the substances of secondary metabolites such as alkaloids, terpenoids, and steroids in the ration. They proactively become contributing factors that provide support to the integrity and function of the digestive system. This result aligns with the study of Pasukamonset et al. [9], who have revealed that antioxidant substances promote healthiness and good function of the digestive system. Yuanita et al. [2] support this by claiming that bioactive compounds such as polyphenols and flavonoids are able to boosts the functioning of the digestive system. Antioxidant compounds like flavonoids can stimulate protein synthesis, which is a key function for the development of organ tissues. Hence, the thesis that the employment of butterfly pea flower flour as a source of antioxidant compounds contributes to the amelioration of nutrient utilization efficiency, in particular of protein, as well as protein synthesis and the growth of digestive organs in broiler chickens can be upheld.

3.2 Relative weight and length of small intestine segments

The average relative weight and length of the small intestine segments (duodenum, jejunum, and ileum) of the broiler chickens that received the butterfly pea flower flour are shown in Table 3. The analysis demonstrated that incorporating the butterfly pea flower flour into the feed significantly ($P<0.05$) influenced the relative duodenum weight. Nonetheless, no significant difference ($P>0.05$) was found in the relative weight of jejunum and ileum, or in the length of the duodenum, jejunum and ileum length compared to the control group.

Table 3. Relative weight (%) and length (cm) of small intestine segments (duodenum, jejunum, ileum) of broiler chickens.

Parameter	Treatment				
	P0	**P1**	**P2**	**P3**	**P4**
Duodenum (%)	0.55 ± 0.03^{b}	0.47 ± 0.08^{ab}	0.42 ± 0.06^{a}	0.44 ± 0.02^{a}	0.50 ± 0.08^{a}
Jejunum (%)	1.20±0.14	1.07±0.11	1.14±0.25	1.01±0.04	1.04±0.22
Ileum (%)	0.92±0.09	0.88±0.09	0.83±0.08	0.66±0.36	0.88±0.17
Duodenum (cm)	30.1±2.04	26.8±3.11	26.1±6.94	27.0±2.52	27.5±2.18
Jejunum (cm)	71.0±6.27	75.6±9.02	74.7±6.80	72.1±3.42	73.5±2.50
Ileum (cm)	71.2±6.50	70.3±9.34	71.9±9.04	73.7±9.51	71.9±6.15

Notes: ^{a,b} Different superscripts in the same row indicate significant differences ($P<0.05$); P0 (basal ration without the addition of butterfly pea flower flour), P1 (basal ration + 1% butterfly pea flower flour), P2 (basal ration + 2% butterfly pea flower flour), P3 (basal ration + 3% butterfly pea flower flour), P4 (basal ration + 4% butterfly pea flower flour).

The results showed that the relative weight of the duodenum in broiler chickens fed with butterfly pea flower flour tended to be lower, and was followed by a shorter duodenal length compared to the control group. Nevertheless, when compared to other digestive organs (Tables 2 and 4), the treatment with butterfly pea flower flour actually showed a tendency to increase in relative weight. This finding indicates that although the relative weight of the duodenum decreased, the efficiency of nutrient absorption from the ration increase, which was reflected in the higher relative weight of other digestive organs. This increase in absorption efficiency is thought to be because of the content of antioxidant compounds in butterfly pea flower flour, especially flavonoids, which play a part in increasing the length of the villi of the small intestine as the main structure in nutrient absorption. Flavonoid inclusion can increase the length of the duodenal villi of broilers.

This villi elongation increases the absorptive surface within the digestive tract so that the absorption of nutrients becomes more ideal.

Based on Table 3, the relative weight of chicken duodenum is 0.42-0.55%, jejunum 1.01-1.20%, and ileum 0.66-0.92%. The value is more than the one given in Incharoen et al.'s [10] research, which reported that the relative weights of broiler chicken duodenum, jejunum, and ileum were 0.31%, 0.52%, and 0.42%, respectively. The difference in values may be because of the fact that the flour of the butterfly pea flower consists of bioactive compounds such as flavonoids, saponins, and tannins that could be used as antibacterial agents. These compounds are the main contributors if the inhibition of the disease-causing bacteria that are referring as the coliform group, is going to be achieved in the small intestine. Thus, by reducing the rate of bacteria that are the agents of the disease, the intestinal microflora will be more balanced and thus will create the space for the health-promoting bacteria, just like the lactic acid bacteria [11]. An increase in lactic acid bacterial population has been considered to be the factor responsible for the growth of small intestinal villi, thus improving the surface area of absorption and the rate of nutrient absorption efficiency.

It has been established that the increase in villi growth in the small intestine, particularly in the jejunum, provides more surface area for nutrient absorption. This increase in absorption surface naturally impacts the relative weight of the broiler chicken's digestive organs. The ileum, in addition to being the final site of nutrient absorption in the small intestine, also acts as a place for the proliferation of beneficial microbial growth. The tannin content of the butterfly pea flower flour plays a part in modulating the intestinal microflora by suppressing pathogenic microbial populations and sustaining the presence of non-pathogenic microbes that aid in nutrient absorption. Sapsuha's study [12] indicates that giving feed with antioxidant, e.g., nutmeg flesh, will suppress pathogenic bacteria and keep the count of beneficial bacterial colonies constant in the intestine. The decrease in the number of pathogenic microbes facilitates the formation of the morphology of the digestive tract, especially in the ileum, which is presumed to be a mechanism that facilitate villi development and the expansion of the absorption region of nutrients. Additionally, the flavonoid contained in butterfly pea flower flour act as antioxidant which stabilizes the pH of the small intestine. Flavonoid has the ability to decrease the pH level of the digestive system, creating an acidic state that promotes the growth of lactic acid bacteria. Similar with the previous statement, Anjani et al. [13] stated that the acidic intestinal environment is known to contribute to the balance of intestinal microorganism, especially in increasing the population of lactic acid bacteria. The increase in the population of these bacteria has an impact on the health of the digestive tract, which then support optimal regeneration and growth of the jejunal villi and expands the area of nutrient absorption. Thus, a healthy digestive tract condition promotes the efficiency of overall nutrient absorption in broiler chickens.

3.3 Relative weight and length of cecum

The results indicated that dietary inclusion of butterfly pea flower flour in the diet had no significant ($P>0.05$) effect on relative weight and cecum length of broiler chickens. Numerically, however, the minimum relative cecum weight was observed in control treatment (P0) at 0.49% and maximum in the P3 treatment at 0.68%. This numerical increase is prospectively due to the content of phytochemical compounds in butterfly pea flower flour such as flavonoids and saponins, which possess anti-bacterial activity. The flavonoids and saponins function to inhibit the development of pathogenic bacteria within the digestive tract, even in the cecum. Pathogenic microbial inhibition enhances the number of beneficial bacteria, including lactic acid bacteria, which contribute an important role in

digestive tract health. The improved ceca health resulting from the increase in beneficial microorganisms therefore explain the increased cecum weight and length.

Table 4. Relative weight and length of broiler chicken cecum.

Treatment	Cecum Relative Weight (%)	Cecum Length (cm)
P0	0.49 ± 0.06	17.30 ± 1.20
P1	0.57 ± 0.08	17.60 ± 2.61
P2	0.51 ± 0.07	17.00 ± 1.87
P3	0.68 ± 0.06	17.90 ± 1.75
P4	0.53 ± 0.08	17.60 ± 2.61

Notes: P0 (basal ration without the addition of butterfly pea flower flour), P1 (basal ration + 1% butterfly pea flower flour), P2 (basal ration + 2% butterfly pea flower flour), P3 (basal ration + 3% butterfly pea flower flour), P4 (basal ration + 4% butterfly pea flower flour).

The increase in the relative cecum weight of broiler chickens here is thought to be related to the action of flavonoid compounds possessing antibacterial activity. Flavonoid present in butterfly pea flower flour is susceptible to hydrolysis by intestinal microorganism and release a few fermentation products, including an increase in lactic acid bacterial population. Flavonoids, as stated by Kawabata et al. [14], promote an increase on the population of lactic acid bacteria, which, in turn, ferment crude fiber into short-chain fatty acids (SCFA). SCFA possess the ability to promote further growth of lactic acid bacteria. This comes in agreement with Zhang et al. [15] who authored that the activity of lactic acid bacteria in cecum will support performance in the secretion of enzymes and increases the process of digestion.

3.4 Relative weight and length of the large intestine

This study found that feeding of butterfly pea flower flour supplementation within the diet had no significant ($P>0.05$) effect on the relative weight or the length of broiler chicken large intestines.

Table 5. Relative weight and length of large intestine of broiler chicken.

Treatment	Large Intestine Relative Weight (%)	Large Intestine Length (cm)
P0	0.18 ± 0.11	10.0 ± 2.26
P1	0.15 ± 0.05	10.7 ± 2.91
P2	0.17 ± 0.06	10.6 ± 3.93
P3	0.16 ± 0.04	10.2 ± 1.92
P4	0.16 ± 0.05	10.0 ± 1.00

Notes: P0 (basal ration without the addition of butterfly pea flower flour), P1 (basal ration + 1% butterfly pea flower flour), P2 (basal ration + 2% butterfly pea flower flour), P3 (basal ration + 3% butterfly pea flower flour), P4 (basal ration + 4% butterfly pea flower flour).

The length of the large intestine of broiler chickens on which this study was ranged from 10.00 to 10.6 cm. The large intestine weight in butterfly pea flower flour treatment was not significantly different, but was lower than in the control. This situation is interpreted because of the maximum utilization of nutrients in the small intestine, thus, in the large intestine, only water is absorbed, and the load is also lighter. The efficiency of absorption in the small intestine is perhaps the main factor influenced by the antioxidant compound in the butterfly pea flower flour, chiefly flavonoids, which have been reported to be able to increase the population of lactic acid bacteria [2]. The growth of these lactic acid bacteria can suppress the activity of toxic enzymes from pathogenic bacteria in the large intestine, thereby establishing an environment that supports efficient digestion and nutrient

absorption. This efficiency, in turn, is reflected in the weight and length of the large intestine of broiler chickens.

4 Conclusion

The use of butterfly pea flower flour in the diet has been shown to increase the capability of duodenum without any negative effects on the relative weight of the crop, proventriculus, ventriculus, and the length of the duodenum. Furthermore, no significant differences were found in the relative weight and length of other segments of the digestive system, including the jejunum, ileum, ceca, and colon (large intestine).

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The authors declare that they have no conflict of interest with any organization in corresponding with this study, or any other issues concerning the use of the experimental material used.

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