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Modulation Of Intestinal Microstructure And Morphometric Parameters In Arabian Chickens Supplemented With Probiotic And Fermented Gracilaria Sp.

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Abstract:
Background*: The cultivation Gallus turcicus is a business with high potential as a supplier of eggs and meat in Indonesia. The high productivity of chickens, growth and relatively easy maintenance process are also the reasons why chickens are in great demand by the community, especially the people of Lombok. The main problem that becomes a challenge in the process of chickens cultivation is the emergence of diseases that attack the health of chickens, especially the health of the digestive system that can interfere with the absorption of nutrients, feed consumption, growth and productivity of chickens. Many disease controls utilize antibiotics as an alternative, but excessive use of antibiotics can cause antibiotic resistance and residues in livestock products. An alternative that can be used is to utilize probiotic consortium and fermented Gracilaria sp extract as supplementation in improving macro and microanatomy of the small intestine.*
Materials and Methods*: The study used a completely randomized design with four groups, P0 group that did not receive probiotic and Gracilaria sp supplementation; P1 group that was supplemented with 1% probiotic alone; P2 group that was supplemented with 1% probiotic + 5% Gracilaria sp without fermentation; and P3 group that was supplemented with 1% probiotic + 5% fermented Gracilaria. Parameters observed included digestive enzyme activities (amylase, lipase, and protease) and total digestive tract weight of chickens..*
Results*: The results showed that probiotic supplementation and natural prebiotic fermented Gracilaria spsignificantly improved the microanatomical structure (villi height, villi basal and apical width and crypta depth) and macroanatomy (intestinal weight and length).*
Conclusion: *Improvement in the structure of the chicken digestive tract due to the combination of probiotic supplementation and Gracillaria fermentation will have an impact on optimizing digestion and absorption of nutrients, resulting in increased body weight of Gallus turcicus.*
Key Word*: Gallus turcicus; Gracilaria sp.; probiotics; prebiotics; Microanatomy; Macroanatomy.*

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I. Introduction

Gallus turcicus belongs to a group of free-range (non-breed) chickens that play an important role as meat and egg producers in Indonesia. These chickens start laying eggs at 4.5 to 5.5 months of age, with peak production occurring at 8 to 9 months of age. In one year, Arabian chickens can produce about 250 to 280 eggs with an average weight of 40 to 45 grams per egg [1], [2]. Genetic factors and the provision of quality feed in sufficient quantities play a role in increasing the productivity of Arabian chickens.

The relatively high level of egg production and fast growth, Arabian chickens are a cultivation option that is in great demand by the community. In the process of Arabian chicken culture, the main challenge that must be faced is the emergence of diseases that attack health, especially the health of the penning system in chickens. Many diseases that attack the digestive system of farm animals are caused by bacterial infections, protozoa, parasites and pathogenic microflora that live in the intestines of farm animals. The susceptibility of poultry to potentially pathogenic microorganisms such as *Escherichia coli*, *Salmonella sp.* and *Clostridium perfringens* as well as other pathogenic microflora established in the small intestine, thus competing with the host for nutrients and reducing the digestion of fats, as well as fat-soluble vitamins due to the deconjugating effect of bile acids [3].

The control of various diseases due to pathogen infections utilizes antibiotics that can be mixed in the feed and drinking of livestock, but routine antibiotic administration can cause antibiotic resistance problems and antibiotic residues in livestock products [4]. An alternative that can be done to overcome this problem is to provide additional probiotics and seaweed extract *(Gracilaria sp)* in chicken feed.

Probiotics are nonpathogenic microorganisms that when consumed in sufficient quantities can benefit the health of the digestive system of livestock. *Lactic Acid Bacteria* (LAB) such as *Lactobacillus sp., Streptococcus hermophilus, and Bifidobacterium sp*. are a group of bacteria that have been widely used as probiotics that are able to maintain beneficial microflora in the intestine, help nutrient absorption and maintain the health of tissues in the digestive system of poultry [5], [6]. LAB is able to maintain the health of the organ structure of the digestive system can increase the activity of enzymes that help the process of enzymatic digestion of food such as amylolytic, proteolytic, lipolytic and cellulolytic [7].

In addition to probiotics, seaweed extract can also be used as an additional source of nutrients in chicken feed. Seaweed containing prebiotics is often used as a complementary ingredient and an additional source of vitamins, minerals and antioxidants in poultry feed. Prebiotics can be a substrate for good bacteria in the gut, which will increase the growth and activity of good bacteria, and inhibit the growth of pathogens in the colon [8], [9]. Several types of seaweed with high availability in the Indonesian region are often used as additional mixtures in poultry feed, one of which is *Gracilaria sp.*

The high crude fiber content in seaweed cannot be digested directly, so it needs to be fermented first before being used for chicken feed mixture. Fermentation is one of the biological processing technologies of feed ingredients that involve the activities of microorganisms to improve the nutrition of an ingredient [10], [11]. The results of previous research stated that feed fermented with *Effective Microorganisms-4* (EM-4) which contains yeast, photosynthetic bacteria and lactic acid bacteria which can increase enzyme activity can hydrolyze organic compounds such as proteins into polypeptide compounds, oligopeptides and amino acids [10], [12]. Recent research has explained that fermentation of seaweed in chicken feed mixtures has an impact on the microanatomy of the digestive tract. This is because lactic acid bacteria that play a role in the fermentation process can increase the villi of the small intestine so that the absorption surface area and weight of the small intestine will increase and the absorption of nutrients can be more optimal [13].

II. Material And Methods

This research is an experimental study conducted using the *Completely Randomized Design* (CRD) method in four treatment groups. The research was conducted for approximately 60 days and consisted of three main stages, namely: probiotic starter making, *Gracilaria sp.* extraction, and livestock rearing stage. Probiotic starter was made from pure isolates of *Lactobacillus sp*., *Bifidobacterium sp*., and *Streptococcus sp*. *Gracilaria sp.* extract was prepared through a controlled fermentation process and without fermentation as a comparison. After the rearing period, digesta and small intestinal tissues of chickens were taken for analysis and observation of the microanatomical and macroanatomical structures of the digestive tract of *Gallus turcicus*.

Study Location: The rearing process was carried out at BTN Pagutan, Mataram, Indonesia, The process of making histology preparations of chicken intestines was carried out at the Lombok Marine Fisheries and Aquaculture Center, Sekotong, NTB. Micro and macroanatomy observations were conducted at the Integrated Laboratory of UIN Mataram, Lombok, NTB.

Subjects and selection methods: *Gallus turcicus* used in this study were 7 days old with the same body weight. Before the treatment, the chickens were given the same basal feed in the form of BR 1 pellets as much as 30% of the weight of the chicken. In chicken drinking water, kinamycin antibiotic 1 gram/liter was added ad libitum to reduce the number of bacteria in the digestive tract of chickens. Chicken preparation was carried out for one week.

Maintenance

The treatment was carried out for approximately 35 days, until the chickens had experienced more rapid growth, so that enzyme activity tests could be carried out using the small intestine digesta of chickens. Feed is given in the amount of 30% of the weight of the chicken. Feeding was done twice a day, precisely at 07:00 and 17:00 WITA. The treatment consisted of:

P0: BR feed without supplement
P1: BR feed + 5% probiotic
P2: BR feed + 5% probiotic + *Gracilaria sp.* extract without fermentation
P3: BR feed + 5% probiotic + *Gracilaria sp.* extract controlled fermentation

Preparation of bacterial starter

Pure isolates of Lacrobacillus sp., *Bifidobacterium sp*. and *Streptococcus sp*. in MRSA media were taken as much as one ose and put into a tube containing 9 mL of MRSB media. Then the sample was homogenized using a vortex and incubated for 1-2 x 24 hours at 37°C. After incubation, the starter of *Lactobacillus sp.* bacteria was taken as much as 1 mL using a sterile micropipette and put into an erlenmeyer containing 99 mL of MRSB media, then vortexed to make it homogeneous. The sample was incubated at 37°C for 1 x 24 hours, then the standard density of *Lactobacillus sp.* 107 CFU/mL was measured by comparing it to Mc Farland 0.5 solution.

Extraction of *Gracilaria sp*

Gracilaria sp. extract will be added to chicken basal feed. In this study, two types of *Gracilaria sp.* extract were made, namely extracts without fermentation and with controlled fermentation. *Gracilaria sp.* extract was made by taking 1 kg of *Gracilaria sp.* which had been cleaned and cut into pieces, then dried in a dry oven at 70-80ºC for 45 minutes to remove excess salt content. The dried seaweed will be pulverized to form seaweed powder. The refined *Gracilaria* extract can be used as a feed mixture without fermentation by adding a little water to the mixture.

Gracilaria sp. extract with controlled fermentation is made by mixing fine *Gracilaria* powder and mixed with EM4 and 1% sugar in a ratio of 1:1. The mixture was put into a sterile container, covered and incubated for 3-4 days at room temperature. After the fermentation process, 5% of fermented *Gracilaria* extract was mixed into chicken feed.

Observation of Microanatomy Characteristics of Small Intestine

In macro measurements, the small intestine of chickens was taken and cleaned. The digesta was removed before measuring the total length and weight of the small intestine.

In the microanatomical measurement of the small intestine of chickens, a 4-5 cm long sample of the small intestine was cut, the contents were removed, the intestine was cleaned with 0.01% physiological NaCl solution, and then stored in 10% formalin solution. After that, the lumen of the small intestine was cut to 4 µm thick using a microtome and placed on a slide for staining with the Haemoxylin-eosin method. The preparations were then observed under a microscope with 40 x magnification and the number of all villi (unit/transverse cut) was counted. Villi height (µm) was measured using a microscope equipped with a micrometer at 40 x magnification. Measurement started from the base (lamina propria) to the top of the villi.

Data Analysis

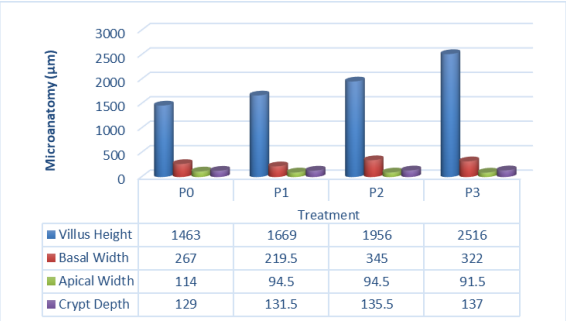
Macroanatomy and microanatomy data of the small intestine were analyzed using analysis of variance and continued with Duncan's test if there were significant differences. Observation data that are not normally distributed are tested with the Kruskal-Wallis test, while further tests are carried out with the Mann-Whitney test with a significance level of 0.05. Observation data will be analyzed with the help of SPSS 24

III. Result And Discussion

The morphological structure of the intestine, such as villus height and crypta depth, is a key indicator in assessing the efficiency of digestive function.

Table 1: Microanatomical Characteristics of the Small Intestine in *Gallus turcicus*

Parameters	Treatment (µm)			
	P0	P1	P2	P3
Villus Height	1463±333,7	1669±198	1956±111,7	2516±203,6
Basal Width	267±32,5	219,5±30,4	345±117,4	322±125,9
Apical Width	114±25,4	94,5±14,9	94,5±54,4	91,5±1,2
Crypt Depth	129±15,5	131,5±12	135,5±4,9	137±29



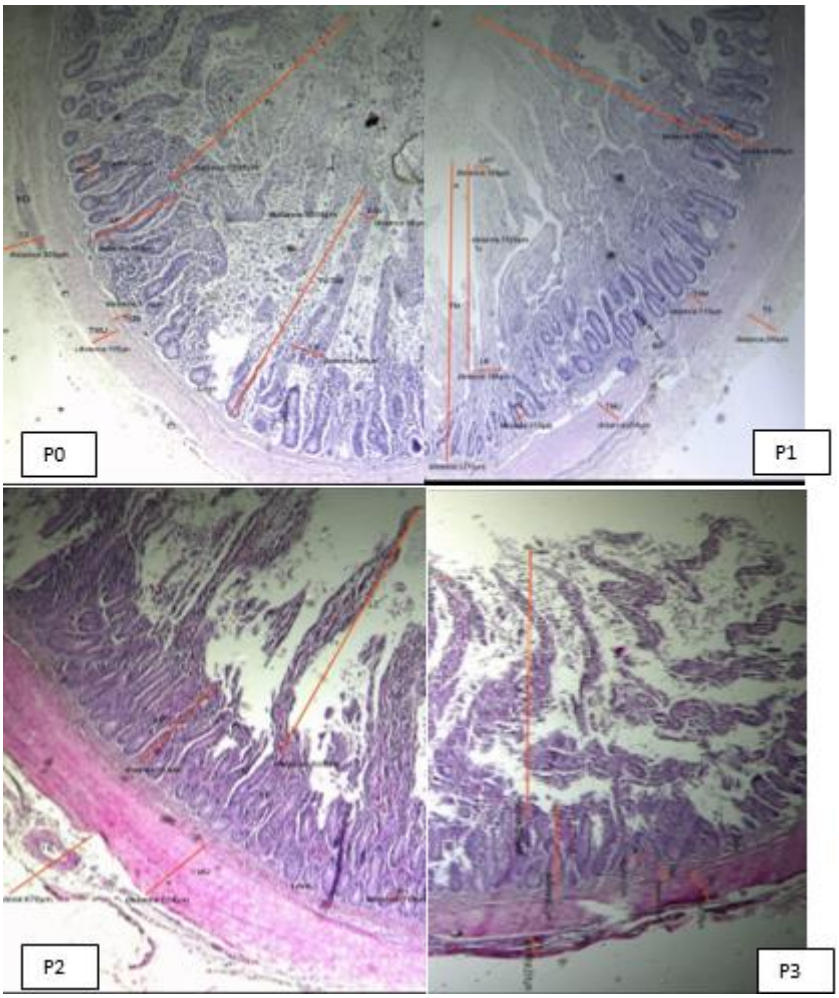


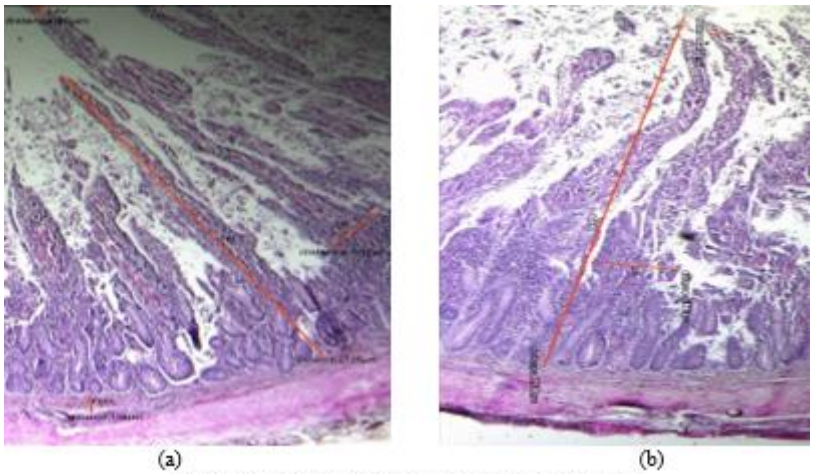
Figure 1: Microanatomical characteristics in treatment groups P0, P1, P2, and P3 at 10x magnification

The results showed that the treatment with a combination of probiotics and fermented *Gracilaria* (P3) had the most significant value of villi height and depth of chicken intestinal crypts compared to other treatments, including the treatment with a combination of probiotics and non-fermented *Gracilaria*. The increase in villus height and crypta depth is thought to occur due to improvements in the structure of the intestinal mucosa which has implications for increased nutrient absorption. The results of Verawati's research (2023), showed that probiotic supplementation acts as an effective alternative feed additive in maintaining the health of the chicken digestive tract and increasing the efficiency of the digestive process [14]. Probiotics, especially lactic acid bacteria such as *Lactobacillus*, *Bifidobacterium*, and *Streptococcus*, have a positive impact on the balance of gastrointestinal microbiota. The presence of these beneficial microflora can suppress the growth of pathogenic bacteria and support the health of chicken digestive organs [7], [15].

An increase in the population of beneficial microflora in the gut due to probiotic administration has a direct impact on increasing enzymatic activity, feed digestibility, and improving the structure of the digestive tract. Probiotics such as *Lactobacillus* produce *short-chain fatty acids* (SCFAs), including butyrate, acetate and propionate. SCFAs, especially butyrate, are the main source of energy for intestinal epithelial cells (enterocytes) and stimulate mucosal cell proliferation. This process contributes to an increase in villi height, which expands the absorption surface area and improves the absorption efficiency of nutrients such as proteins, amino acids, glucose and fatty acids. Taller villi allow more enterocytes (clumnar cells in the intestine) to capture and absorb nutrients in the intestinal lumen [16], [17], [18].

In supporting the improvement of intestinal villi structure and function, probiotics are also able to reduce inflammation and mucosal damage due to invasion of pathogenic bacteria. This leads to an increase in the apical width and basal width of the villi, reflecting a healthy and optimally functioning gut. Probiotics also trigger the production of digestive enzymes, either through secretion of the intestinal mucosa or from enzymes produced directly by microbes [19], [20]. The increased apical and basal width of the villi strengthens the overall structure of the villi, making them more resistant to damage from food friction or pathogenic microorganisms. This width also indicates good cell proliferation capacity, which means that the intestinal

epithelium is able to renew itself faster. Fermented *Gracilaria sp.* produces bioactive compounds such as florotanin and oligosaccharides that have antioxidant effects and strengthen intestinal tissue integrity [21], [22].



(a) (b)

Figure 2: (a) Villus width in P2; (b) Villus width in P3

In addition to the effects of probiotics, the administration of *Gracilaria* extract that has been fermented by lactic acid bacteria also shows a significant role in improving the structure of the intestinal mucosa which causes an increase in the thickness of the small intestinal crypta. Crypta is a regenerative area of the intestinal mucosa, where new epithelial cells proliferate. Increased crypta thickness indicates more active cell regeneration activity, in compensation to the demand for tissue repair or to maintain the rapid growth of villi. This is important to maintain the long-term length and integrity of the villi. Probiotics were shown to stimulate the expression of epithelial growth genes such as IGF-1 and EGF, which accelerate regeneration of the intestinal mucosa [23].

Through fermentation, the crude fiber in *Gracilaria* sp extract will be lowered, thus accelerating the availability of nutrients and increasing the absorption process in the digestive tract. This is in line with research by Sugiharto (2018) which states that fermentation by lactic acid bacteria in the intestine can convert the crude fiber component in *Gracilaria* into SCFA, which not only strengthens the integrity of epithelial cells, but also improves the anatomical structure of the digestive tract and increases the absorption of water and nutrients from feed [24].

Furthermore, the fermented SCFA production also provides energy for intestinal epithelial cells, which directly stimulates villi elongation, crypta deepening, and intestinal mucosal thickening [25]. This process is important to strengthen the intestinal barrier function and maximize the efficiency of nutrient absorption. Surtina (2020) also emphasized that SCFA can stimulate epithelial cell proliferation and differentiation, which contributes to the overall integrity and resilience of the chicken digestive tract [26].

In contrast to the P3 treatment, the results of the P2 treatment showed relatively low averages of chicken digestive tract microanatomy. This is because *Gracilaria* extract without fermentation has a high crude fiber content that can suppress the process of breakdown and absorption of nutrients contained in feed, so that nutrient digestibility will be inhibited [27]. Crude fiber that is not digested properly will lead the chicken's digestive tract to work harder, resulting in thickening of the mucosal walls which causes inhibition of the absorption process of nutrients in the feed, such as carbohydrates / glucose, protein and fat [28]. Nutrients in feed that cannot be digested properly, will certainly reduce the production of digestive enzymes in the intestine and have an impact on reducing feed consumption. As a result, chickens will feel full more easily

Chicken Digestive Tract Weight

Probiotic supplementation in feed can also affect the anatomical structure of the chicken digestive tract. As a result, the nutrient absorption area is more complex and wider followed by increased enzyme activity that supports feed digestion [26]. The wider nutrient absorption area will have an impact on increasing the length and weight of the chicken's digestive tract. The small intestine of chickens, which consists of the duodenum, jejunum and ileum, is a part of the digestive tract that plays an important role in the process of digestion and absorption of nutrients.

Table 2: Digestive Tract Weight of Arabian Chickens

Parameter	Sample Code			
	P0	P1	P2	P3
Weight of Small Intestine (grams)				

Duodenum	10,42	10,96	11,34	12,3
Jejenum	14,5	14,91	15,43	17,13
Ileum	12,71	13,18	12,8	13,82
Length of Small Intestine (cm)				
Duodenum	21,65	22,45	22,95	24,8
Jejenum	55	52,35	52,3	56,7
Ileum	47,35	43,1	45,15	47,45

Table 2 shows that the small intestine weight in treatment group P3, which received additional dietary fiber through controlled fermentation, had the highest value. The small intestine, particularly the jejunum, plays a crucial role in the absorption of nutrients, especially carbohydrates, amino acids, vitamins, and minerals. Supplementation with probiotics and fermented *Gracilaria sp* in poultry feed contributes to the improvement of the microanatomical structure of the digestive tract and positively affects its macrostructure. Wijoyo (2024) reported that probiotic supplementation in poultry could increase the length and relative weight of the small intestine. This increase is attributed to the thickening of the intestinal mucosa, which causes anatomical changes (including the length and width of villi and the depth of the crypts), thereby expanding the absorption area and supporting digestive efficiency and poultry growth [29].

The crude fiber in fermented *Gracilaria* not only supports the growth of beneficial gut microbiota but also improves the anatomical structure of the chicken digestive tract. Improvements in the structure and function of the digestive system result in increased size and weight of digestive organs such as the small intestine, *gizzard*, and cecum [25], [30]. When the structure of the small intestine is improved, including thickening of the mucosal wall, epithelial cell proliferation, villi compaction, and crypt deepening occur. These changes enhance the mucosal tissue mass, especially in the nutrient absorption area (jejunum), which explains why group P3 showed the highest length and weight of the jejunum. The thickening indicates high functional activity of the small intestine, particularly in the jejunum, reflecting more efficient nutrient absorption, increased enzymatic activity, and improved resistance to intestinal stressors or pathogens [31].

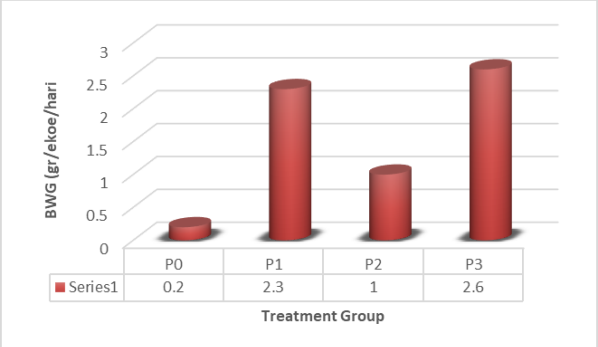
Treatment P2 exhibited a higher small intestine weight compared to P0 and P1. Although unfermented *Gracilaria* still contains natural bioactive compounds such as sulfated polysaccharides, which function as natural prebiotics to support probiotic activity and the growth of beneficial gut microflora [23]. Its effectiveness is still lower than that of fermented *Gracilaria* (P3). This is because the high crude fiber content in unfermented *Gracilaria* can irritate the intestinal mucosa, leading to thickening as a defensive response to micro-inflammation in the intestinal wall. If left unchecked, this may hinder nutrient absorption, reduce feed intake, and ultimately decrease the productivity of Arab chickens.

Body Weight Gain of *Gallus turcicus*

Efficient nutrient absorption in the intestine enhances feed intake, which contributes to increased body weight and supports the growth and productivity of *Gallus turcicus* (Arab chickens). Supplementation with probiotics and fermented *Gracilaria sp.* is expected to improve digestive system health by enhancing both the microanatomical and macroanatomical structures of the small intestine, thereby optimizing digestion and nutrient absorption. The more optimal the structure of the small intestine, the more effective digestion and absorption will be, resulting in improved growth and development of the chickens. Enhanced growth and development directly support the productivity of Arab chickens, including increased body weight.

Table 3: Average Body Weight and Weight Gain of *Gallus turcicus*

Sample Code	Initial Body Weight	Final Body Weight	Body Weight Gain
P0	965	972	0,245
P1	904,5	968	2,267857143
P2	931,5	960,5	1,035714286
P3	931	1004,5	2,625



Treatment group P3, which received a combination of probiotics and fermented *Gracilaria*, showed the highest body weight gain compared to other groups. This indicates a synergistic effect between the probiotics and fermented *Gracilaria* in enhancing digestive efficiency and nutrient absorption, contributing to greater growth performance. Improvements in the microanatomical characteristics of the small intestine, along with increased weight and length of the digestive tract, serve as crucial factors in supporting the body weight gain of chickens. Enhanced microanatomical features, such as increased villus height and width as well as crypt depth, expand the nutrient absorption surface area and increase absorption efficiency. Additionally, the increase in weight and length of the digestive organs—such as the duodenum, jejunum, and ileum—reflects greater digestive capacity, promoting optimal growth in Arab chickens. Effective digestion and nutrient absorption also stimulate feed intake, which directly contributes to increased body weight.

This finding is in line with research by Ananda (2023), which showed that supplementation with probiotics and fermented fiber improved the weight and length of the digestive organs in Arab chickens, including the proventriculus, ventriculus, small intestine, cecum, and colon [32]. The development of these macroanatomical structures enlarges the digestive system's capacity for breaking down and absorbing nutrients, thereby supporting body weight gain. In another study, it was reported that probiotic fermentation supplementation in drinking water improved feed intake and significantly increased body weight gain in chickens. This was attributed to the enhanced population of beneficial microflora that support mucosal layer repair, thereby optimizing nutrient absorption and preventing gastrointestinal disorders that could hinder growth [33], [34]. These findings indicate that probiotic and prebiotic supplementation from fermented *Gracilaria sp* can serve as an effective feed additive option in poultry production.

Overall, the combination of probiotic and fermented *Gracilaria* supplementation offers significant benefits in improving the growth performance of Arab chickens by enhancing the micro- and macroanatomical structures of the intestine. This strategy represents a sustainable and effective alternative in feed formulation aimed at improving the production performance of *Gallus turcicus*.

IV. Conclusion

The supplementation of probiotics combined with fermented *Gracilaria sp* in the diet of *Gallus turcicus* significantly enhances both microanatomical and macroanatomical structures of the small intestine, including increased villus height, crypt depth, and intestinal length and weight. These improvements lead to better digestion and nutrient absorption, which ultimately contribute to higher feed intake and increased body weight. The synergistic effect of probiotics and fermented *Gracilaria* not only supports gut health but also provides a sustainable alternative to antibiotic growth promoters (AGPs). This strategy has the potential to enhance the growth performance and productivity of Arab chickens in a more natural and health-oriented manner, making it a promising feed additive for sustainable poultry farming

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