

## Effect of *Azolla microphylla* and Pellets Combination on Meat Quality and Ileal Villi in Muscovy Duck (*Cairina moshata*)

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**Abstract.** This study aims to evaluate the combination of *Azolla microphylla* and pellets on meat quality, and ileal villi in muscovy duck (*Cairina moshata*). The material used was 60 male and female DOD (unsex). The method in this study was an in vivo experiment using a completely randomized design (CRD) with 3 treatments and 5 replicates. The treatment consisted of giving basal feed in the form of pellets and *Azolla* as much as 0% (P0), 5% (P1), and 10% (P2). The variables measured in this study included meat pH, cooking loss, water holding capacity, tenderness, number of villi, villi length, crypta depth, and villi surface area. The data obtained were then analyzed using Analysis of Variance (ANOVA) and continued with the smallest real difference (BNT). The results showed that the treatment had no significant effect ( $p>0.05$ ) on meat pH, cooking loss, tenderness, number of villi, villi length, crypta depth, and villi surface area but had a significant effect (stated) on water holding capacity. The addition of *Azolla microphylla* in the basal feed in the form of pellets to the carcass characteristics of ducks (*Cairina moshata*) up to 10% level can increase the water holding capacity.

**Keywords:** azolla, muscovy Duck, meat quality, ileal villi

**Abstrak.** Penelitian ini bertujuan untuk mengevaluasi kombinasi *Azolla microphylla* dan pelet terhadap kualitas daging, dan vili ileum pada entok (*Cairina moshata*). Materi yang digunakan yakni berupa 60 ekor DOD jantan dan betina (unsex). Metode pada penelitian ini adalah percobaan in vivo menggunakan Rancangan Acak Lengkap (RAL) dengan 3 perlakuan dan 5 ulangan. Perlakuan terdiri dari pemberian pakan basal berupa pelet dan *Azolla* sebanyak 0% (P0), 5% (P1), dan 10% (P2). Variabel yang diukur pada penelitian ini antara lain pH daging, cooking loss, water holding capacity, keempukan, jumlah vili, panjang vili, kedalaman kripta, dan luas permukaan vili. Data yang diperoleh kemudian dianalisis menggunakan Analisis Varians (ANOVA) dan dilanjutkan dengan Beda nyata terkecil (BNT). Hasil penelitian menunjukkan bahwa perlakuan tidak berpengaruh nyata ( $p>0.05$ ) terhadap pH daging, cooking loss, keempukan, jumlah vili, panjang vili, kedalaman crypta, dan luas permukaan vili akan tetapi berpengaruh nyata ( $p<0.05$ ) terhadap *water holding capacity*. Penambahan *Azolla microphylla* pada pakan basal berbentuk pelet terhadap karakteristik karkas entok (*Cairina moshata*) hingga level 10% dapat meningkatkan *water holding capacity*.

**Kata kunci:** azolla, entok, kualitas daging, vili ileum

### Introduction

Increasing public awareness of the nutrients needed by the body, one of which is the fulfillment of protein sources, the fulfillment of protein sources can be fulfilled from vegetable protein sources and animal protein, where the fulfillment of animal protein can be fulfilled from the poultry sector, which has recently been fulfilled from broilers, even though there are still many poultry products that can be used as a source of animal protein, one of which is the duck. This livestock animal is a waterfowl that should get more attention, this is because ducks and ducks are livestock that are resistant to the

environment, and poor feed and resistant to disease. Muscovy duck has been domesticated in Indonesia for a long time, so that its distribution is almost in all regions in Indonesia (Kamelia, and Fathurrohman, 2017).

Muscovy duck can be used as a source of animal protein that can be utilized for meat and eggs. Muscovy duck have a larger body weight when compared to ducks, so the resulting meat product will be higher than that of ducks. Muscovy duck meat has a low-fat content and a savory taste (Lase and Lestari, 2020). With the various advantages possessed by muscovy duck,

it is still unfortunate that the maintenance of muscovy duck is still traditional and thus implied to low productivity. Increasing the productivity of muscovy duck can be done by providing appropriate feed and livestock needs, perfect feed has a balanced content of nutrients so as to provide maximum results. Nutrients needed in addition to protein and energy content are amino acids (Resnawati and Bintang, 2014). Nutrient absorption is carried out in the small intestine with the presence of villi in the intestine, so that nutrients in the ration can be digested and converted for meat and bone formation (Bagun et al., 2013). So the villi in the small intestine must have a good number, length, depth, and surface area, and will result in better meat products. A good meat product can be tested through the physical quality of the meat. In order to get a good product, the feed given to ducks must be of good quality, cheap, and always available, one of the alternative feeds that can be utilized is *Azolla microphylla*.

*Azolla* is an aquatic plant that floats on the surface of the water, has small leaves, thin stems, and taproots that hang in the water. *Azolla* can grow quickly, it is often considered a pest by farmers, even though *Azolla* has a high nutritional content, which consists of 20-35% protein, 10-15% minerals, 7-10% amino acids, bioactive substances and biopolymers based on dry weight. *Azolla* also contains relatively low carbohydrates and oil, but *Azolla* is easily digested by livestock that consume it, due to its high protein content and low lignin content (Bhatt et.al., 2020). *Azolla* can grow naturally in stagnant water, in waterways, ponds, rivers, including in swamps. *Azolla* cultivation does not require extensive land, and does not require nitrogen fertilizers that can pollute the environment, even *Azolla* can produce nitrogen so that it can be used as a natural fertilizer in addition to being used for animal feed (Ting et.al., 2022). *Azolla* has various types of nutritional content that are good for livestock

but there is a fairly high crude fiber content of around 27.10%, which can affect feed consumption, especially in poultry considering that poultry do not have the ability to digest high enough root fiber (Adzman et al., 2021), added by Melita et al., 2018 stated that the addition of fermented *azolla* to crossbred native chicken feed by 10% can increase body weight and protein consumption.

Based on the description above, it is necessary to conduct research on the effect of *Azolla microphylla* and basal feed in the form of pellets on the quality of meat and ileal villi of duck (*Cairina moshata*). *Azolla microphylla* added at a certain level is expected to affect the quality of meat and ileal villi in ducks (*Cairina moshata*).

## Materials and Methods

The material used was 60 male and female (unsex) ducks (*Cairina moshata*) that were reared for 60 days. The cages used were insulated cages measuring 120×60×40 cm. The experiment had three treatments and five replications. Each partition was filled with 4 ducks. Feeding was restricted twice a day in the morning and evening at 90 g/head/day. The basal feed given was in the form of pellets, at the age of 1-20 days in the form of concentrates and at the age of 15 days began to be tried in the form of pellets. The composition of the basal feed in the form of pellets was corn, kejung, bran, premix, tapioca starch and concentrate. The nutritional content of the basal feed in this study is presented in Tables 1 and 2.

Pellet making begins with preparing all feed ingredients (Concentrate, corn, kejung, premix, tapioca flour and bran), then put into a mixer for mixing, after all mixed evenly added water gradually to print the pellets, then gradually put into the pellet molding machine, after forming then aerated, then put into sacks, then before storage is done drying for 3-5 days so that the water content is not too high.

Table 1. Nutrient content of basal diet

Substance	Composition (%)
Dry matter	87.82
Ash	9.07
Crude Protein	13.74
Crude Fat	7.39
Crude Fiber	8.69
Gross Energy <sup>1</sup> (kcal/kg)	3540.95

Source: Central Laboratory, University of Muhammadiyah Malang \*1Adzman et al., 2021

Table 2. Nutrient content of *Azolla microphylla*

Substance	Composition (%)
Dry matter	62,42
Ash	1,67
Crude Protein	28,00
Crude Fat	0,33
Crude Fiber	2,59
Gross Energy <sup>1</sup> (kcal/kg)	2440,65

Source: Central Laboratory, University of Muhammadiyah Malang \*1Adzman et al. 2021

This experiment used the in vivo method using a completely randomized design (CRD) consisting of three treatments and five replicates so that there were 15 experimental units. Each experimental unit contained 4 ducks. The treatments in this experiment were:

- P0: Basal feed without the addition of *Azolla*
- P1: Basal feed +5% fresh *Azolla*
- P2: Basal feed +10% fresh *Azolla*

The variables observed in this experiment were meat pH, cooking loss, water holding capacity, tenderness, number of villi, villi length, crypta depth, and villi surface area.

Sampling is done by weighing the weight of the ducks before slaughter. Slaughter was carried out using a sharp knife and as much as possible must pay attention to animal welfare,

by cutting the trachea, oesophagus, carotid artery, and jugular vein (Abdullah et al., 2019). One duck was taken per experimental unit. Visceral organs were removed, ileal small intestine was taken, and thigh meat was taken.

All data were analyzed using ANOVA analysis of variance to determine any differences between treatments. If there is a difference, then further analysis will be carried out using the smallest real difference test (BNT).

## Results and Discussion

The results of nutritional evaluation research on the combination of pellets and *Azolla microphylla* on meat quality include meat pH, cooking loss, water holding capacity, and tenderness shown in Table 3.

Table 3. Effect of pellets and *Azolla microphylla* on meat quality in Muscovy duck

Variable	Treatment		
	P0	P1	P2
Meat pH	5.34±0.15	5.36 ±0.27	5.26 ±0.11
Cooking Loss (%)	43.34± 2.09	41.97±2.99	42.09±2.88
Water Holding Capacity (%)	43.98 ±3.83 <sup>a</sup>	51.14 ±3.61 <sup>b</sup>	44.35±4.06 <sup>a</sup>
Tenderness mm/g/10 secon	30.82 ±5.25	28.78 ±5.64	38.08 ±7.34

Notes: Notation (a-b) different lowercase superscripts in the same row indicate significant effect (p<0.05)

### **Effect of Treatment on the pH of Muscovy duck Meat**

The results of the study on meat pH are shown in Table 3, which shows the lowest to highest average values in order, namely P2 ( $5.26 \pm 0.11$ ), P0 ( $5.34 \pm 0.15$ ), and P1 ( $5.36 \pm 0.27$ ). The results of observations and analysis of variance showed that the provision of pellets and *Azolla microphylla* in ducks on meat pH did not have a significant effect ( $p > 0.05$ ). The results in this study are still within normal limits, this is in accordance with the opinion of Anas et al., (2019) stated that animal muscle tissue before cutting has a pH value between 5.1 to 7.2 and will decrease after cutting, this is due to the process of glycolysis and lactic acid is produced which will affect the pH value, the normal pH range of postmortem meat is around 5.5. The pH value can also affect the tenderness of the meat. Soeparno et al., (2011) stated that the ideal meat pH standard ranges from 5.3 to 5.9. Factors that can affect the pH value include stress before slaughter, hormone/drug injection, species, individual livestock, muscle type, electrical stimulation, enzyme activity and glycolysis. The ideal pH level also depends on the rate of glycolysis after slaughter and is influenced by the presence of glycogen reserves in the muscle. The lower the glycogen content, the slower the glycolysis process so that the resulting pH value will be higher. Glycogen in muscles can be influenced by the condition of the livestock before and shortly before slaughter such as due to stress experienced by livestock. Low temperatures can inhibit the rate of pH decrease, and vice versa if high temperatures can accelerate the rate of pH decrease (Roswandono et al., 2021). The change in temperature during the study can also affect the pH value of meat, where the higher the ambient temperature during maintenance, the lower the pH value, this is in accordance with the opinion of Rini et al., (2019) stated that temperature differences during maintenance have a significant effect on the pH value of meat, where

during maintenance high ambient temperatures ( $35-36^{\circ}\text{C}$ ) produce lower pH values (acidic), and vice versa at maintenance temperatures of  $23-24^{\circ}\text{C}$ , resulting in higher pH values, this can be related to the concentration of lactic acid in different muscle tissues after cutting. Livestock reared at high ambient temperatures will experience heat stress which can result in the release of hormones, accelerate the breakdown of muscle glycogen by anaerobic glycolysis enzymes and the accumulation of lactic acid which causes high lactic acid production. High lactic acid in meat will result in a rapid decrease in pH after cutting so that the final pH of the meat will be low.

The pH value produced between P0 (without the addition of *Azolla microphylla*) with P1 and P2 (the addition of *Azolla microphylla* 5% and 10%) does not produce a significant effect this is due to the presence of crude fiber content in *Azolla* and high basal rations, in total containing 11.28% crude fiber whose levels of each treatment are not much different so as to produce a pH value of meat that is not much different. This is in accordance with the opinion of Prantika et al., (2014) stated that the presence of crude fiber content can cause pH levels in meat to be the same / not significantly different. Added by Tugiyanto and Heriyanto (2018) stated that the antioxidant properties of active compounds and proteins in *Azolla microphylla* flour can maintain the balance of hydrogen ions. Shebis et al. 2013; Tugiyanto and Heriyanto (2018) stated that the presence of antioxidant compounds can work by contributing hydrogen atoms in the metabolic process, so this can prevent changes in pH caused by the process of glycolysis and lactic acid production. Added by Suryadi et al., (2019) who stated that the same pH value in broiler meat was caused by the condition of the chickens in this study having a healthy condition, even after being given probiotics. In healthy chickens, metabolic processes such as glycolysis will continue to produce glycogen, so the pH will not change

significantly. Anaerobic glycolysis metabolism results in the accumulation of fatty acids in the muscle so that it can change the pH.

### **Effect of Treatment on Cooking Loss of Muscovy duck**

The results of the study on cooking loss are shown in Table 3 which shows the lowest to highest average value in order, namely P1 ( $41.97 \pm 2.99\%$ ), P2 ( $42.09 \pm 2.88\%$ ), and P0 ( $43.34 \pm 2.09\%$ ). The results of observations and analysis of variance showed that giving pellets and *Azolla microphylla* to ducks on cooking loss did not give a significant effect ( $p > 0.05$ ). The cooking loss value in this study is not much different from Rahmat et al., (2021) who stated that the provision of tempe and tape bran to chicken resulted in cooking loss values between 37.22% and 43.14%. Cooking loss describes the value of meat mass that is reduced due to heating or cooking processing. The value of cooking loss is related to water binding capacity. The higher the water binding capacity, the less water and liquid nutrients are released or wasted during heating, resulting in less meat mass. Low cooking loss has good quality because the possibility of the release of meat nutrients during cooking is low (Santoso, 2021). Added by Ardiansyah et al., (2021) who stated that when the cooking process takes place, fewer nutrients are lost, as for the high loss of nutrients during the cooking process can be caused by several factors, such as the method used, cooking time and temperature. High temperatures can cause protein denaturation and result in a decrease in water binding capacity. The increase in cooking loss value can be caused by a decrease in the pH of the meat after cutting which results in many damaged myofibril proteins, it is followed by a loss of protein's ability to bind water which ultimately increases the cooking loss. The lower the pH of the meat, the higher the cooking loss value.

The value of cooking loss is closely related to the pH of the meat, if the pH value of the meat

is low it produces a high cooking loss value, in this study the pH of the meat ranged from 5.26 to 5.36 which resulted in cooking loss values between 41.97% to 43.34%, this is in accordance with the opinion of Lawrie 1995; Irmayani et al., (2023) stated that meat that has a high final pH value above 6.0 produces a low cooking loss value of around 20%, while meat that has a low final pH below 5.9 produces a high cooking loss of around 40-50%. Cooking loss is the amount of weight lost during cooking. The smaller the cooking loss, the less water and water-soluble nutrients are lost and vice versa. The greater the value of cooking loss, the more water lost and water-soluble nutrients.

Cooking loss in the provision of *Azolla microphylla* in feed (P1 and P2) gives lower results than the control feed (P0) this means that the provision of *Azolla microphylla* can reduce the cooking loss of duck meat caused by the loss of nutrients during cooking less. This is in accordance with Swain et al., (2022) stated that the value of cooking loss of breast meat decreases with the addition of 5-10% azolla flour into the feed. Added by Tulanggalu et al., (2017) stated that meat that has a low cooking loss value has good quality because the loss of nutrients during cooking will be less than meat with greater cooking loss. Cooking loss describes meat juice and is a function of temperature and length of heating time. The factors that affect cooking loss are pH value, sarcomere length of muscle fibers, length of muscle fiber pieces, myofibril contraction status, sample size and weight, meat cross section, cooking loss is greater in smaller or shorter fiber lengths, heating, meat fat, feed consumption, protein-energy, and age in relation to WHC (Soeparno et al., 2011).

The addition of *Azolla microphylla* to the feed did not affect the cooking loss but succeeded in reducing the cooking loss value when compared to the control feed, this is due to the high protein and energy content in the feed, so that it can affect the cooking loss value of duck meat. On the other hand, the age and nation of livestock in this study are the same so that it does not

have a significant effect, this is in accordance with the opinion of Dewayani et al., (2015) stated that the value of cooking loss is influenced by several factors including pH value, myofibril contraction status, sample size and weight, meat cross section, heating, length of muscle fiber pieces, livestock nation related to meat fat, sarcomere length of muscle fibers, age, and energy consumption in feed.

### **Effect of Treatment on Water Holding Capacity of Muscovy duck Meat**

The results of the study on the water holding capacity of meat are shown in Table 3, which shows the lowest to highest average values in order, namely P0 ( $43.98 \pm 3.83$ %), P2 ( $44.35 \pm 4.06$ %), and P1 ( $51.14 \pm 3.61$ %). The results of observations and analysis of variance showed that giving pellets and *Azolla microphylla* to ducks on water holding capacity gave a real influence ( $p < 0.05$ ). These results are still in normal conditions, this is in accordance with the opinion (Dewayani et al., 2015) stated that the use of onggok and tofu dregs as a substitute for corn on water holding capacity produces values between 39.80% to 50.33%. Added by Abdia, et al (2017) stated that WHC of broiler meat given feed based on rice bran fermented rumen fluid produces a value of 54.48% to 62.05%. The water holding capacity (WHC) of meat is the ability of meat protein to bind water in the meat, so that WHC can describe the level of damage to meat protein. Therefore, a high WHC illustrates that the meat has good quality. The presence of protein denaturation can reduce the ability of muscle protein to bind water and result in poor water holding capacity.

Water holding capacity in this study produces a real influence where P0 and P2 do not have a significant effect but P1 has a significant effect on P0 and P2, this can be caused by several factors including because the pH of the meat tends to drop, the same species and age of livestock, and the factor of feeding which contains high protein so that it causes an

influence on water holding capacity. This is in accordance with the opinion of Soeparno et al., (2011) stated that factors that affect water holding capacity include (1) low pH of meat, resulting in water holding capacity down to the isoelectric point of the protein. (2) If the pH value is above the isoelectric point, the charge (+) is free, the charge (-) is surplus, there will be a rejection of myofilaments, forming space for water molecules so that there is an increase in water binding capacity, (3) if the pH is below the isoelectric point, due to access to the + charge, there will be a rejection of myofilaments, forming space for water molecules so that the water binding capacity increases, (4) the period of lactic acid formation causes the water binding capacity to decrease, (5) the formation of myofilaments (actin and myosin), the water binding capacity will decrease and is related to rigor mortis, (6) withering will cause WHC to increase, this is due to  $K^+$  absorption and  $Ca^{++}$  liberation, or changes in the structure of Z and I pathways, (7) cooking will cause protein solubility so that WHC will decrease, and acidic groups will be lost, due to increased pH, (8) species, age and muscle function can cause WHC to differ among muscles, (9) feed factors, transportation, temperature, humidity, storage, sex, health, pre- and post-cutting treatment and the presence of intramuscular fat can affect WHC. Water binding capacity, cooking loss, juiciness, marbling and flavor can affect the value of each other.

The protein content of the feed will affect the water binding capacity, the crude protein content of the basal feed is 13.74%, and the added protein in *Azolla microphylla* is 28.00%, that the provision of basan feed in the form of pellets and *Azolla microphylla* can affect the water binding capacity of the muscovy duck meat. This is in accordance with the opinion of Meisaroh et al., (2020). stated that the presence of amino acids and protein content can cause meat protein groups to bind more water so that the value of water holding capacity will also

increase. Added by Abdia et al., (2017) stated that the presence of crude fiber content in high feed can result in decreased digestibility so that poultry are less able to utilize food substances, so that little fat will be formed. Meat with high fat content will have a high WHC value, and vice versa, meat with low fat content has a low WHC value as well.

### **Effect of Treatment on the Tenderness of Muscovy duck Meat**

The results of the study on meat tenderness are shown in Table 3, which shows the lowest to highest average values in order, namely P1 ( $28.78 \pm 5.64$ ) mm/g/10 secon, P0 ( $30.82 \pm 5.25$ ) mm/g/10 secon, and P2 ( $38.08 \pm 7.34$ ) mm/g/10 secon. The results of observations and analysis of variance showed that the provision of pellets and *Azolla microphylla* in ducks on the tenderness gave no real effect ( $p > 0.05$ ). This result is still in normal conditions, this is in accordance with the opinion of Haikal et al., (2021) stated that the provision of guava extract produces tenderness in laying hens between 21-98 - 32.94 mm/g/10 seconds. Added by Herawati et al., (2016) stated that the soaking of strawberry juice in the meat of afkir laying hens produces a tender value of 22.84 to 38.77 mm / g / 10 seconds, the tenderness of the meat is influenced by protein denaturation which is the breakdown of proteins into smaller units that cause the meat to become more tender.

Meat tenderness in ducks with several levels of addition of *Azolla microphylla* P0 (without Azolla), P1 (5% Azolla), and P2 (10% Azolla) has no real influence, this is due to the same age of ducks, and the age of ducks is still relatively young that the connective tissue in duck meat is still small, resulting in high tenderness. This is in accordance with the opinion of Soeparno et al., (2011) stated that factors that affect tenderness in meat include antemortem factors (genetic, physiological, age, management, sex and stress) and postmortem factors, namely chilling,

refrigeration, withering, freezing, length and temperature of storage, including cooking and tenderizing. Santoso (2021) added that meat tenderness is related to meat composition, which is related to the connective weave, meat fibers, and fat cells between meat fibers. Connective tissue proteins affect meat tenderness and result in a more clayey meat structure. Meat tenderness is largely determined by meat components in the form of myofibril structure, connective tissue content, level of cross-linking, and water binding capacity by meat proteins and meat juice. Herawati and Widiarso (2021) stated that the more connective tissue in meat, the lower the meat tenderness. Meat tenderness is influenced by the content of connective tissue, where the older the age of the livestock, the more connective tissue composition and the more clayey the meat will be.

Another factor that can affect the meat tenderness value is the crude fiber content of the feed. In this study, the crude fiber contained in the feed was quite high where the crude fiber in the basal feed was 8.69%, and in *Azolla microphylla* was 2.59%, so the crude fiber contained in the feed was high. This is in accordance with the opinion of Taran et al., (2015) stated that crude fiber can reduce fat contained in the chicken body. Tenderness is one of the quality standards associated with the degradation of meat proteins and hydrolysis can make the microstructure of meat loose and with the hydrolysis of meat connective tissue proteins into shorter fragments.

### **Effect of giving pellets and *Azolla microphylla* on ileal villi in Muscovy duck**

The results of the nutritional evaluation of the combination of pellets and *Azolla microphylla* on ileal villi, consisting of the number of villi, villi length, crypta depth, and villi surface area are shown in Table 4.

Table 4. Effect of giving pellets and *Azolla microphylla* on ileal villi in muscovy duck

Variable	Treatment		
	P0	P1	P2
Number of villi (per transversal cut)	2,8 ±0,81	2,28 ±0,96	3,24 ±1,11
Length of villi (µm)	318,814±151,57	330,818±78,91	508,904 ±138,36
Crypta depth (µm)	114,188 ±22,84	126,77 ± 25,17	150,05± 13,79
Villi surface area (µm <sup>2</sup> )	1398,832±494,92	1122,48 ±255,57	812,01 ±249,09

Notes: Notation (a-b) superscript different lowercase letters on the same line indicates a significant effect (P < 0.05)

#### Effect of Treatment on the Number of Ileal Villi

The results of the study on the number of villi are shown in Table 4 which shows the lowest to highest mean values in order, namely P1 (2.28 ± 0.96 per transverse cut), P0 (2.8 ± 0.81 per transverse cut), and P2 (3.24 ± 1.11 per transverse cut). The results of observations and analysis of variance showed that giving pellets and *Azolla microphylla* to ducks on the number of ileal villi gave no significant effect ( $p > 0.05$ ). This is still within the normal range in accordance with the opinion of Souza, Cicero, Costa et al., (2021) stated that the number of villi with Bouin solution fixation produces the number of villi using the intestinal strips technique is 2.33 per transverse cut, using the transverse section technique is 2.40 per transverse cut, and with the swiss roll technique is 2.80 per transverse cut. While the villi fixed using formalin produced the number of villi each using the intestinal strips technique was 2.13 per transverse cut, using the transverse section technique was 1.80 per transverse cut and with the swiss roll technique was 2.95 per transverse cut.

The number of ileal villi did not have a significant effect, but each additional level of *Azolla* tended to increase the number of villi. P2 is the highest treatment of the number of villi given pellet feed and *Azolla microphylla*, this is due to the crude fiber content contained in the feed can still be tolerated by ducks so that the feed can still be digested. This is in accordance with the opinion of Kusuma et al., (2020) stated that if the feed that can be digested by livestock is increasing, it will cause the cells in the villi to develop actively. Feed that has high digestibility can affect the feed flow rate to run slower and

result in a longer time available for digestive enzymes to degrade nutrients, so that it can be used to activate intestinal villi cells. Villi have a function in expanding the surface of the small intestine which affects the process of food absorption (Jafriati, 2020). A reduction in the number of villi can result in a reduction in the surface area of the epithelium to absorb and digest nutrients (Purnasari, 2020).

The number of intestinal villi used uses the ileum because the ileum has many goblet cells and lieberkuhn cells when compared to the jejunum and duodenum. In the ileum, nutrient absorption also occurs, this is in accordance with the opinion of Sariati et al., (2019) stated that the lieberkuhn gland is housed in the crypta contained in the intestinal villi, the number of goblet cells and lieberkuhn glands in the ileum is the most when compared to the duodenum and jejunum, this is due to the thicker tunica mucosa of the ileum. The number of goblet cells and Lieberkuhn's glands in the ileum is due to the position of the ileum at the end of the small intestine which plays a role in the process of absorption of nutrients, and the absorption of the largest nutrients occurs in the ileum. Added by Fitasari (2012) stated that the small intestine is the main place for the absorption of nutrients, which begins with (1) digesta passing through the small intestine epithelium on the surface then will be carried to the bloodstream. (2) the small intestine must be high enough to support the surface area (3) the large number of villi in the small intestine epithelial mucosa. The villi in the duodenum and jejunum are denser and will decrease in the ileum. In the periphery of the villi around the columnar epithelial cells that have



absorption capacity, the area there are hundreds of microvilli at the edge of the enteric epithelial cavity, which is between 1 to 1.5  $\mu\text{m}$  in height, and 0.1  $\mu\text{m}$  in area, thus causing the absorption area to increase hundreds of times. After the food substance is absorbed into the villi, it will then go to the circulation system in the spleen and blood.

#### **Effect of Treatment on the Length of Ileal Villi**

The results of the study on meat tenderness are shown in Table 4 which shows the lowest to highest average values in order, namely P0 ( $318.814 \pm 151.57 \mu\text{m}$ ), P1 ( $330.818 \pm 78.91 \mu\text{m}$ ), and P2 ( $508.904 \pm 138.36 \mu\text{m}$ ). The results of observations and analysis of variance showed that giving pellets and *Azolla microphylla* to ducks on the length of ileal villi gave no significant effect ( $p > 0.05$ ). This is still in the normal range in accordance with the opinion of Fitroh et al., (2019) stated that the length of the villi given banana peel as a substitute for corn in hybrid ducks produces a villi length of 376.70  $\mu\text{m}$  to 574.30  $\mu\text{m}$ , while in quail given probiotic lactic acid bacteria produces a villi length of 288.45-390.8030  $\mu\text{m}$  (Hidayat, et al, 2016). The increase in the length of the intestinal villi illustrates that the function of the small intestine works well in digesting feed, on the other hand the condition of livestock that is free from disease causes the villi to develop better (Fitroh et al., 2019).

Giving pellets and *Azolla* gives results that have no effect, but numerically giving *Azolla* can increase the length of the muscovy duck villi, the best treatment is in P2 (*Azolla* 10%), this is due to the presence of coarse sert content in feed that can still be tolerated by muscovy duck so that it can still increase the length of the villi and nutrient absorption is still going well. This is in accordance with the opinion of Kusuma et al., (2020) stated that the nutritional content of the feed can still meet the needs of livestock, despite the high crude fiber content. The higher the villi and the depth of the crypts will lead to an increase in the area of absorption of nutrients by

the small intestinal wall so as to increase livestock growth.

Crude fiber content that is too high will cause livestock to feel full, so consumption will decrease and livestock do not get enough nutrients for growth and development, thus disrupting the development process of cells that play a role in increasing villi height. The increase in villi height is closely related to the increase in the number of epithelial cells around them. The higher the size of the villi and the depth of the crypta will expand the field of absorption of nutrients carried out by the small intestinal wall so that it has an effect on increasing livestock growth.

#### **Effect of Treatment on Crypta Depth**

The results of the study on the depth of crypta are shown in Table 4 which shows the lowest to highest average value in order, namely P0 ( $114.188 \pm 22.84 \mu\text{m}$ ), P1 ( $126.77 \pm 25.17 \mu\text{m}$ ), and P2 ( $150.05 \pm 13.79 \mu\text{m}$ ). The results of observations and analysis of variance showed that the provision of pellets and *Azolla microphylla* in ducks on the length of the ileum gave no significant effect ( $p > 0.05$ ). This is still in the normal range in accordance with the opinion of Gamboa et al., (2022) stated that broilers given organic acids provide a value into the crypta between 104.5  $\mu\text{m}$  to 116.9  $\mu\text{m}$ . When compared with the results of the study, the results of the study are still high. Widjastuti et al., (2023) stated that the addition of noni fruit extract to sentul chicken resulted in crypta depth between 92.35  $\mu\text{m}$  to 117.33  $\mu\text{m}$ .

The depth of crypta in this study did not have a significant effect, but numerically gave an increase, the highest treatment was P2 ( $150.05 \pm 13.79 \mu\text{m}$ ), this is due to the nutritional content of the feed that can meet the needs of ducks, that the absorption of nutrients that occur in the villi will run well, good absorption causes the depth of crypta to deepen. This is in accordance with the opinion of Kusuma et al., (2020) stated that the nutritional content that has met the

needs of livestock can result in the growth of cells in the intestinal villi developing optimally, as to increase the size of the crypta depth. The size of the depth of the crypta that is getting deeper will increase the digestive ability in the absorption of feed nutrients in the intestine, so that it can affect the growth of livestock. Livestock that experience good growth will lead to good body weight and production performance. Added by Jahanian et al., (2021) stated that the depth of crypta consists of many specialized cells, more specifically goblet cells that play an important role in cell replacement, that the deeper the crypta, the more goblet cells, on the other hand, the mucus layer synthesized and secreted by goblet cells serves to cover the gastrointestinal epithelium.

The depth of crypta that does not have a significant effect is caused by the presence of crude fiber contained in the feed so that the ducks cannot digest crude fiber that is too high, so that the ducks will stop their feed consumption faster, and as a result the nutrients absorbed by the livestock's body are not absorbed completely. This is in accordance with the opinion of Kusuma et al., (2020) stated that too high crude fiber in feed, will result in the feed leaving the digestive tract faster so that digestive enzymes do not take longer in the process of digesting food. So that livestock do not get enough nutrients so that the impact on intestinal villi that do not develop optimally. Feed is the main factor that can affect the development of cells in the animal's body. Feeding with good nutritional content and according to the needs of livestock can optimize the work of cells in the animal's body.

#### **Effect of Treatment on Villi Surface Area**

The results of the study on the surface area are shown in Table 4 which shows the lowest to highest average values in order, namely P0 ( $1398.832 \pm 494.92 \mu m^2$ ), P1 ( $1122.48 \pm 255.57 \mu m^2$ ), and P2 ( $812.01 \pm 249.0957 \mu m^2$ ). The results of observations and analysis of variance

showed that the provision of pellets and *Azolla microphylla* in ducks on the villous surface area gave no significant effect ( $p > 0.05$ ). This is still within the normal range in accordance with the opinion of Kusuma et al., (2020) stated that the provision of fermented palm kernel cake mixture and onggok as a substitute for corn produces a surface area between  $641.08 \mu m^2$  to  $1035,48 \mu m^2$ . The villous surface area does not have a significant effect, this is due to the presence of crude fiber content in the feed which is high enough so that the digestibility of the duck will decrease, the duck will stop eating if the cache is full, even though its nutritional needs have not been met, so that the absorption of nutrients in the intestine does not go well, and the cells in the body do not develop properly. This is in accordance with Kusuma et al., (2020) stated that excess crude fiber in feed will still be tolerated by livestock. Crude fiber that is still within normal limits can still help in the digestive process in the digestive tract and can activate intestinal villi cells to increase their size, thus having an effect on the higher size of intestinal villi. Feed nutrition plays an important role for the growth of cells in organs. Providing the right nutrients and according to the needs of livestock can increase the growth of cells, and vice versa if the provision of nutrients is not appropriate it will inhibit the growth of cells. The level of digestibility of feed nutrients can be caused by the surface area of the intestinal villi. The wider the villous surface in the small intestine illustrates the ability of the intestine to digest the feed that has been digested. The ability to digest and absorb feed nutrients can be caused by the surface area of the villi, the number of villi, microvilli that can increase absorption, and the number of folds. Added by Apriliani et al., (2016) stated that the length and width of the cross-sectional area of the small intestine can affect the digestion and absorption of nutrients. Weight gain, the length and size of the cavity in the small intestine, and the increase in the surface area of the small intestine also affect the

process of nutrient absorption. When the size of the intestinal villi shortens, the surface area will decrease, and the goblet cells on the surface will decrease (Permana et al., 2016). The large content of crude fiber will cause the digesta rate to be slow, this is due to the high crude fiber in the feed. A slow digesta rate results in digestive enzymes taking longer to hydrolyze food substances, this causes the absorption of food substances to be more effective and feed digestibility will increase (Kusmayadi et al., 2019).

## Conclusions

The effect of *Azolla microphylla* addition on the quality of meat and ileal villi of duck (*Cairina moschata*) up to 10% level can affect the water holding capacity, but has no effect on meat pH, cooking loss, tenderness, number of villi, villi length, crypta depth, and villi surface area.

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## References

- Abdia, R., D. Rosyidi., and I. Djunaedi. 2017. Pengaruh Pemberian Pakan Dedak Padi Terfermentasi Cairan Rumen Terhadap Kualitas Fisik Daging Ayam. *Jurnal Ilmu dan Teknologi Hasil Ternak*. 12(1): 22-28.
- Abdullah, F. A. A., G. Borilova. and I. Steinhäuserova. (2019). Halal Criteria Versus Conventional Slaughter Technology. *Animals*. 9(530): 1–13.
- Adzman, N., Goh, S. J., Johari, A., Zainal Alam, M. N. H., and Kamaruddin, M. J. 2022. Preliminary Study On Azolla Cultivation And Characterization For Sustainable Biomass Source. *Journal OF Physics: Conference Series*. 2259(1): 0–9. <https://doi.org/10.1088/1742-6596/2259/1/012018>.
- Anas, M., I. D. Novieta., dan Fitriani. 2019. Konsentrasi dan Lama Perendaman Ekstrak Jahe Merah (*Zingiber Officinale* R) yang Berbeda Terhadap Nilai pH dan Susut Masak Daging Entok (*Cairina Moschata*). *Prosiding Seminar Nasional*. 2: 303-306.
- Apriliyani, N. I., Djaelani, M. A., dan Silvana, D. 2016. *Profil Histologi Duodenum Berbagai Itik Lokal Di Kabupaten Semarang*. 18(2)
- Ardiansyah, A., R. Riyanti., D. Septinova., dan K. Nova. 2021. Kualitas Fisik Daging Broiler di Pasar Tradisional Kota Bandar Lampung. *Jurnal Riset dan Inovasi Peternakan*. 5 (1): 50-56.
- Bangun, G.D.D., L.D. Mahfudz, dan D. Sunarti. 2013. Pengaruh Penggunaan Tepung Rumput Laut (*Gracilaria Verrucosa*) Dalam Ransum Ayam Broiler Terhadap Berat Dan Ukuran Tulang *Tibia* Dan *Tarsometatarsus*. *Animal Agricultural Journal*. 2(1): 489 – 496
- Bhatt, N., Singh, N. P., Singh, K., Kandpal, D., Chaudhary, P., and Patoliya, P. 2020. Azolla-A Potent Unconventional Feed And Its Effect Of Feeding On Various Livestock Species-A Review. ~ 1693 ~ *Journal Of Entomology And Zoology Studies*. 8(2): 1693–1698. <http://www.entomoljournal.com>.
- Dewayani, R.E., H. Natsir., dan O. Sjoftjan. 2015. Pengaruh Penggunaan Onggok dan Ampas Tahu Terfermentasi *Mix Culture Aspergillus Niger* dan *Rhizopus Oligosporus* Sebagai Pengganti Jagung dalam Pakan Terhadap Kualitas Fisik Daging Ayam Pedaging. *Jurnal Ilmu dan Teknologi Hasil Ternak*. 10(1): 9-17.
- Fitasari, E. 2012. Penggunaan Enzim Papain Dalam Pakan Terhadap Karakteristik Usus dan Penampilan Produksi Ayam Pedaging. *Buana Sains*. 12(1): 7-16.
- Fitroh, A. A. H., dan Adib N. R. B. 2019. Substitusi Jagung Menggunakan 3 Jenis Kulit Pisang Yang Berbeda Dalam Pakan Terhadap Histomorfologi Vili Usus Itik Hibrida. In *Agrinova: Journal Of Agriculture Innovation*. 2(2) <https://jurnal.ugm.ac.id/agrinova/>
- Gamboa, S. M. Y., Arce-Menocal, J., Ávila-González, E., López-Coello, C., Garibay-Torres, L., and Herrera-Camacho, J. 2023. Organic Acids For Broilers: Effects On Intestinal Morphology And Growth Performance. *Revista Colombiana De Ciencias Pecuarias*. 36(2): 55–65. <https://doi.org/10.17533/udea.rccp.v36n2a1>
- Haikal, M.T., L. Suryaningsih., dan E Wulandari. 2021. Pengaruh Pemberian Ekstrak Jambu Biji (*Psidium guajava*) Terhadap Daya Ikat Air, Susut Masak, Keempukan dan pH Daging Ayam Petelur Afkir. *Jurnal Teknologi Hasil Peternakan*. 2(2):75-81.
- Hermawati, G., D. S. Sutardjo dan L. Suryaningsih. 2016. Pengaruh Perendam Sari Buah Stroberi (*Fragaria vesca*) Dalam Berbagai Konsentrasi Terhadap Mutu Fisik Daging Ayam Petelur Afkir. *Student E-Journal*. 1-11
- Hidayat, C. S., M., Harimurti, S., Lies, D., and Yusiati, M. 2016. Pengaruh Suplementasi Probiotik

- Bakteri Asam Laktat Terhadap Histomorfologi Usus dan Performan Puyuh Jantan. 40(2).
- Irmayani., A. Savira., Munir., I. D. Novieta dan Fitriani. 2023. Pengaruh Penambahan Tepung Suruhan (*Peperomia Pellucida* L. Kunth) pada Ransum Terhadap Nilai pH dan Susut Masak Daging Itik Mojosari. *Jurnal Gallus-Gallus*. 1(2): 30-40.
- Jafriati. 2020. Praktis Belajar Biologi Untuk Mahasiswa Kesehatan. Malang: Ahlimedia Press.
- Jahanian, E., Mahdavi, A. H., and Jahanian, R. 2021. Silymarin Improved The Growth Performance Via Modulating The Microbiota And Mucosal Immunity In Escherichia Coli-Challenged Broiler Chicks. *Livestock Science*, 249. <https://doi.org/10.1016/j.livsci.2021.104529>
- Kusuma, Y. A., Sjoftan, O., dan Djunaidi, I. H. 2020. Pengaruh Fermentasi Campuran Bungkil Inti Sawit Dan Onggok (Fbiso) Sebagai Pengganti Jagung Dalam Pakan Terhadap Karakteristik Vili Usus Ayam Pedaging. *Jurnal Ilmu Ternak*. (2): 126–137. <https://doi.org/10.24198/jit.v20i2.3089>
- Kamelia D. M., dan Fathurohman. 2017. Pemanfaatan Kulit Buah Kakao Fermentasi Sebagai Alternatif Bahan Pakan Nabati Serta Pengaruhnya Terhadap Pertumbuhan Ternak Entok (*Cairina Muschata*). *Biosfer Jurnal Tadris Pendidikan Biologi*. 8(1): 66–77. <http://ejournal.radenintan.ac.id/index.php/biosfer/index>
- Kusmayadi, A., Hadi Prayitno, C., dan Rahayu, N. 2019. *Persentase Organ Dalam Itik Cihateup Yang Diberi Ransum Mengandung Kombinasi Tepung Kulit Buah Manggis Dan Tepung Kunyit The Internal Organ Percentage Of Cihateup Duck That Given Ration Containing Combination Of Mangosteen Peel And Turmeric Flour*. 5(1)
- Lase, J. A., dan Lestari, D. D. 2020. *Seminar Nasional Dalam Rangka Dies Natalis*. 4(1): 479.
- Meisaroh, T., O. Sjoftan., A. Susilo dan D. N. Adli. 2020. Pengaruh Substitusi Jagung DENGAN Bonggol Pisang Hasil *Rebinding* dalam Pakan terhadap Keempukan Daging, Whc, Kolesterol, dan Protein Itik Hibrida. *National Conference PKM Center Sebelas Maret University*. 1(1): 188-193.
- Purnasari W. P. 2020. Suplementasi Probiotik Dan Zinc Untuk Malnutrisi. In *Johc*. 1(2).
- Permana, I., Mushawwir, A., Latipudin, D., F. Profil Sel Goblet Itik Cihateup Indra Permana Profil Sel Goblet Itik Cihateup (*Anas Platyrhynchos Javanica*) Yang Diberi Fruktooligosakarida (Fos) Dalam Kondisi Pemeliharaan Minim Air Goblet Cells Profile Of Cihateup Duck (*Anas Platyrhynchos Javanica*) Which Given Fruktooligosaccharides (FOS) In Keep Of Minimum Water Condition.
- Prantika, S., W. Sarengat dan B. Dwiloka. 2014. Pengaruh Penggunaan Tepung Daun Kayambang (*Salvinia Molesta*) Dalam Ransum Terhadap Kualitas Fisikokimia Daging Ayam Broiler Lohman Jantan. *Animal Agriculture Journal*. 3(2): 300-306.
- Resnawati, H., dan Bintang, I. A. K. 2014. *Lokakarya Nasional Inovasi Teknologi Pengembangan Ayam Lokal Kebutuhan Pakan Ayam Kampung Pada Periode Pertumbuhan*. 138-141.
- Rini, S.R., Sugiharto dan L. D. Mahfudz. 2019. Pengaruh Perbedaan Suhu Pemeliharaan terhadap Kualitas Fisik Dagingayam Broiler Periode Finisher. *Jurnal Sain Peternakan Indonesia*. 14(4): 387-395
- Roswandono., A. Setyonugroho., E. H. M. Restijono., dan D. A. K. Sari. 2021. Analisis Kualitas Daging Bebek Dengan Menggunakan Uji Ph, Daya Ikat Air dan Uji Eber Di Pasar Tradisional Kabupaten Kediri. *Jurnal Vitek Bidang Kedokteran Hewan*. 11(2): 26-31.
- Santoso, H.B. 2021. Industri Ternak Unggas Petelur. Yogyakarta: Andi Offset.
- Sariati., D. Masyitha., Zainuddin., Fitriani., U. Balqis., C. D. Iskandar., dan C. N. Thasmi. 2019. Jumlah Sel Goblet dan Kelenjar Liberkuhn pada Usus Halus Sapi Aceh. *Jimvet*. 3(2):108-115.
- Soeparno., R.A. Rihastuti., Indratiningsih., dan S. Triatmojo. 2011. Dasar Teknologi Hasil Ternak. Yogyakarta: Gajah Mada University Press.
- Souza, M., Cicero, C. E., Menck-Costa, M. F., Justino, L., Gerez, J. R., Baptista, A. A. S., and Bracarense, A. P. F. R. L. (2021). Histological Evaluation Of The Intestine Of Broiler Chickens: Comparison Of Three Sampling Methods. In *Semina: Ciencias Agrarias* (Vol. 42, Issue 6, Pp. 3247–3258). Universidade Estadual De Londrina. <https://doi.org/10.5433/1679-0359.2021V42N6P3247>
- Suryadi, U., Y. R. Nugraheni., A. F. Prasetyo., and A. Awaludin. 2019. Evaluation Of Effects Of A Novel Probiotic Feed Supplement On The Quality Of Broiler Meat. *Veterinary World*. 1775-1778.
- Swain, B.K., P. K. Naik and C.K. Beura. 2022. Nutritive Value Of Azolla As Poultry Feed-A Review. *Indian J. Anim. Nutr.* 39 (1): 1-11
- Taran., V. J. Ballo, dan M. Sinlae. 2015. Pengaruh Pemberian Tepung Bonggol Pisang dan Tepung Daun Kelor Sebagai Pengganti Jagung terhadap Warna, Rasa dan Keempukan Daging Ayam Broiler. *Jurnal Nukleus Peternakan*. 2(1):67 – 74.
- Ting, J. Y., Kamaruddin, N. A., and Mohamad, S. S. S. 2022. Nutritional Evaluation OF Azolla pinnata and Azolla microphylla AS Feed Supplements for Dairy Ruminants. *Journal Of Agrobiotechnology*, 13(1s), 17–23. <https://doi.org/10.37231/Jab.2022.13.1s.314>
- Tugiyanti, E., and S. Heriyanto. 2018. Carcass Production and Meat Tenderness Characteristics OF Cull Quail Fed with *Azolla microphylla* Flour

- Supplemented Basal Feed. *Buletin Peternakan* 42 (4): 315-321.
- Tulanggalu., H. Suttedjo., dan G. Maranatha. 2017. Pengaruh Penambahan Tepung Krokot (*Portulaca oleracea Linn*) dalam Ransum terhadap Kualitas Fisik Daging Ayam Broiler. *Jurnal Nukleus Peternakan*. 4(1): 15 – 21.
- Widjastuti, T., Nurlaeni, L., Hasbuna, A., Setiawan, I., Yudaasmara, I., and Tanwiriah, W. 2023. *The Microencapsulation Of Noni Fruit Extract (Morinda Citrifolia L.) With Maltodextrin And Its Implementation As Feed Additive On Carcass Quality And Histology Of Intestinal Sentul Chicken*. 13(1).