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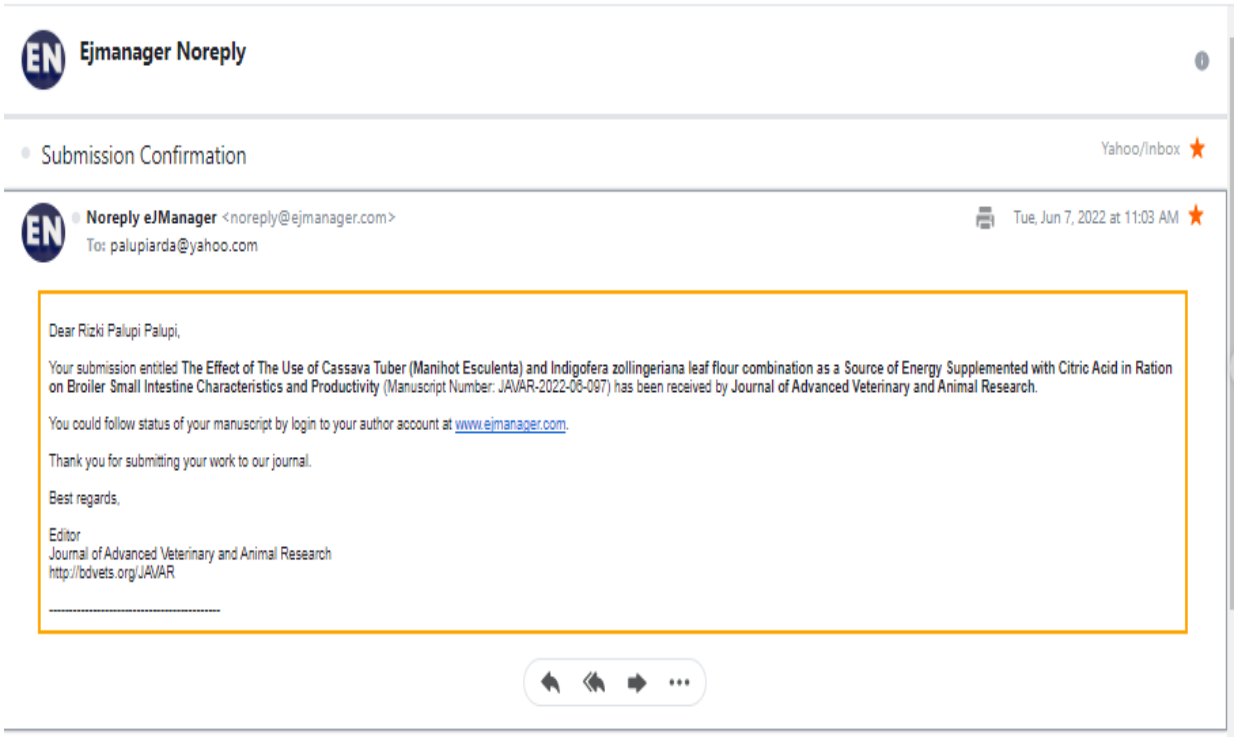

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Your submission entitled **The Effect of The Use of Cassava Tuber (Manihot Esculenta) and Indigofera zollingeriana leaf flour combination as a Source of Energy Supplemented with Citric Acid in Ration on Broiler Small Intestine Characteristics and Productivity** (Manuscript Number: JAVAR-2022-06-097) has been received by **Journal of Advanced Veterinary and Animal Research**.

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The Effect of The Use of Cassava Tuber (Manihot Esculenta) and Indigofera zollingeriana leaf flour combination as a Source of Energy Supplemented with Citric Acid in Ration on Broiler Small Intestine Characteristics and Productivity

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Abstract : The aim of this study is to determine the effect of using a combination of cassava tuber (Manihot esculenta) and Indigofera zollingeriana leaf flour as an energy source supplemented with citric acid in the ration on performance, carcass quality, digesta pH, viscosity and number of villi in the intestines of broiler. This study used a Completely Randomized Design (CRD) with 5 treatments and 5 replications, each replication consisted of 4 broilers. The treatment was a substitution of corn in the ration with a combination of cassava tuber and Indigofera zollingeriana leaf (CIF): T0 (without CIF), T1 (5% CIF substitute for corn + 0.2% citric acid), T2 (10% CIF substitute for corn + 0.2% citric acid), T3 (15% CIF corn substitute + 0.2% citric acid) and T4 (CIF corn substitute + 0.2% citric acid). Each treatment ration was supplemented with 0.2% citric acid. The observed variables were ration consumption, body weight gain, feed conversion, live weight, carcass percentage, abdominal fat percentage, digesta pH, viscosity and number of villi of broilers. The results of this study showed that the combination of cassava tubers and Indigofera zollingeriana leaf flour supplemented with 0.2% citric acid had a significant effect ($P<0.05$) on body weight gain, ration conversion, live weight, carcass percentage and number of villi in broiler intestines. However, it had no significant effect ($P>0.05$) on ration consumption, percentage of abdominal fat, digesta pH and viscosity of broilers. The conclusion of this research is that the combination of cassava tubers and Indigofera zollingeriana leaf flour at the level of 10% supplemented with 0.2% citric acid can be used as an energy source to replace corn without giving a bad effect on production performance, carcass quality and small intestine characteristics of broilers.

Keywords : Broiler, carcass, performance, Indigofera zollingeriana flour, cassava tuber flour.

ORIGINAL ARTICLE,

The Effect of The Use of Cassava Tuber (Manihot Esculenta) and Indigofera zollingeriana leaf flour combination as a Source of Energy Supplemented with Citric Acid in Ration on Broiler Small Intestine Characteristics and Productivity

Statement of novelty: The combination of cassava tuber and Indigofera zollingeriana leaf flour at a level of 10%, supplemented with 0.2% citric acid can be used as an energy source to replace corn without negatively impact on broilers performance.

The Effect of The Use of Cassava Tuber (Manihot Esculenta) and Indigofera zollingeriana leaf flour combination as a Source of Energy Supplemented with Citric Acid in Ration on Broiler Small Intestine Characteristics and Productivity

ABSTRACT

The aim of this study is to determine the effect of using a combination of cassava tuber (Manihot esculenta) and Indigofera zollingeriana leaf flour as an energy source supplemented with citric acid in the ration on performance, carcass quality, digesta pH, viscosity and number of villi in the intestines of broiler. This study used a Completely Randomized Design (CRD) with 5 treatments and 5 replications, each replication consisted of 4 broilers. The treatment was a substitution of corn in the ration with a combination of cassava tuber and Indigofera zollingeriana leaf (CIF): T0 (without CIF), T1 (5% CIF substitute for corn + 0.2% citric acid), T2 (10% CIF substitute for corn + 0.2% citric acid), T3 (15% CIF corn substitute + 0.2% citric acid) and T4 (CIF corn substitute + 0.2% citric acid). Each treatment ration was supplemented with 0.2% citric acid. The observed variables were ration consumption, body weight gain, feed conversion, live weight, carcass percentage, abdominal fat percentage, digesta pH, viscosity and number of villi of broilers. The results of this study showed that the combination of cassava tubers and Indigofera zollingeriana leaf flour supplemented with 0.2% citric acid had a significant effect ($P<0.05$) on body weight gain, ration conversion, live weight, carcass percentage and number of villi in broiler intestines. However, it had no significant effect ($P>0.05$) on ration consumption, percentage of abdominal fat, digesta pH and viscosity of broilers. The conclusion of this research is that the combination of cassava tubers and Indigofera zollingeriana leaf flour at the level of 10% supplemented with 0.2% citric acid can be used as an energy source to replace corn without giving a bad effect on production performance, carcass quality and small intestine characteristics of broilers.

Keywords: Broiler, carcass, performance, Indigofera zollingeriana flour, cassava tuber flour.

INTRODUCTION

Corn is the primary energy source in broiler rations, but its use as broiler feed material competes with the needs of people who consume corn for energy. This will have an impact on corn availability and may cause corn prices to rise, necessitating the use of alternative feed to replace corn as an energy source in the ration. One of the feed ingredients that can be used as an energy source to replace corn is cassava tuber because cassava tuber contains relatively the same energy source as corn. According to Wahyudi et al. **[1]**the metabolic energy content of corn is 3350 kcal/kg, whereas cassava tubers have a metabolic energy of 3519 kcal/kg **[2]**.

The use of cassava tuber as an energy source has a certain weakness, namely the protein content of cassava tuber is lower than that of corn. Ngiki et al. **[3]**stated that the protein content in cassava tubers is 1.3%. Protein deficiency necessitates the addition of protein-rich feed ingredients. *Indigofera zollingeriana* leaf flour can be categorized as a protein source. According to Palupi et al. **[4]**, *Indigofera zollingeriana* shoot flour contains 28.98% crude protein, 8.49% crude fiber, and 3.30% crude fat. In addition to its high protein content, *Indigofera zollingeriana* has many advantages, one of which is the presence of antioxidants because it contains carotenoids in the form of beta-carotene. Yadav et al. **[5]** reported that cassava tubers can be used as an energy source to replace maize by 25% in starter phase broiler rations and 37.5% in finisher phase broiler rations.

Efforts can be made to maximize the efficiency of using alternative feed for livestock by adding an *acidifier* to increase the digestibility of the nutrients in the feed. As for the type of *acidifier* that can be used as a feed additive is citric acid. Citric acid has the ability to lower the pH in the digestive tract, resulting in the formation of an acidic atmosphere and affecting the rate of digestion and the population of pathogenic bacteria in the digestive tract **[6,7]**. Deepa et al. **[8]** declared that the addition of 2% citric acid was able to increase feed consumption, increase body weight and improve feed conversion in broiler. Saputra et al. **[9]** stated that the administration of

a citric acid *acidifier* increased protein and calcium deposition in meat as well as production efficiency in broiler farms.

Based on the description above, it is necessary to conduct research on the utilization of cassava tubers and *Indigofera zollingeriana* leaf flour combination as an energy source to replace corn supplemented with citric acid in the ration on broiler production performance, carcass quality, and small intestine characteristics.

MATERIALS AND METHODS

Methods and Sample Preparation

An animal feeding experiment was conducted at the experimental station, Department of Animal Science, Faculty of Agriculture, Universitas Sriwijaya. The birds were cared for according to the Animal Welfare Guidelines of the Indonesian Institute of Sciences. The approval of the experiment was granted from Universitas Sriwijaya.

This research used 100 DOC broilers. The broilers were placed in 25 postal cages measuring 70 cm x 70 cm x 70 cm. Four broilers were placed in each cage. The cages are equipped with feeders, drinking water containers, and 60-watt incandescent lamps as lighting and heating sources during brooding.

The equipments required to create a combination of cassava tuber and Indigofera zollingeriana are grater, bucket, knife, plastic, rope, oven, tarpaulin, scales, measuring tape and stationery. A stereo microscope, pH meter, and viscocytometer were used in the laboratory observations.The feed ingredients used to prepare the ration in this study were cassava tuber, Indigofera zollingeriana leaf, concentrate, corn, fish flour and rice bran. Subsequently, additional feed ingredients in the form of citric acid were added.

Experimental Design

This study used a Completely Randomized Design (CRD) with 5 treatments and 5 replications, each replication consisted of 4 broilers. The treatment was a substitution of corn in the ration

with a combination of cassava tuber and Indigofera zollingeriana leaf (CIF): T0 (without CIF), T1 (5% CIF substitute for corn + 0.2% citric acid), T2 (10% CIF substitute for corn + 0.2% citric acid), T3 (15% CIF corn substitute + 0.2% citric acid) and T4 (CIF corn substitute + 0.2% citric acid).

Making Cassava Tuber Flour

Cassava tuber flour was made by peeling and washing the cassava skin. The cassava was then grated to aid in drying. Cassava was dried by first drying it in the sun and then drying it in an oven at a temperature of 50oC for 24 hours. The process of reducing the particle size of dried cassava was done by breaking the cassava as crumble.

Making Indigofera zollingeriana leaf flour

Harvesting Indigofera zollingeriana leaves and separating the leaves from the twigs to dry the Indigofera zollingeriana leaves was done in order to make Indigofera zollingeriana leaf flour. Indigofera zollingeriana leaves are dried by first drying them in the sun and then drying them in an oven at a temperature of 50oC until they are dry enough to be grinded. The flour mill machine is used to process the dried Indigofera zollingeriana leaves into flour.

Ration

The feed ingredients used to prepare the treatment ration consisted of: commercial feed in T0 treatment, concentrate, milled corn, fish flour, rice bran, a combination of cassava tubers and Indigofera zollingeriana in which the composition had been arranged according to the treatment. After the treatment ration was mixed according to the composition of the ration, then it was supplemented with 0.2% citric acid in the treatment using CIF. The nutritional composition of the ingredients of the ration is shown in Table 1 and the composition of the ingredients of the ration and the nutrient content of the research ration is shown in Table 2.

Cage Preparation

Before use, cages must be cleaned of any dirt attached to the cage and liming evenly with the provided disinfectant. To eliminate germs and microorganisms that cause disease, a disinfectant

was sprayed evenly throughout the cage. The cage was then left for 1 or 2 weeks. Clean feed and drinking stations, as well as other cage equipment, were placed in each cage. Each cage unit was labeled with treatment and replication.

Rearing

Broiler DOC that has just arrived was given drinking water mixed with brown sugar with a concentration of 50 grams per liter of water for the first 4 hours as an energy source to restore the condition of the broiler DOC due to the stress of traveling from the hatchery to the rearing cage. Rearing was carried out for 4 weeks. The chicken ration was given in accordance with the treatment. Provision of feed and drinking water was done adlibitum or continuously. Every day in the morning, the cage is cleaned. DOC data collection for broilers was weighed once a week.

Research Data Collection

Data on broiler chicken performance was collected once a week by weighing the weight gain of the chickens. Then the amount of feed given was weighed as well as the remainder of the feed.

Observed Variables

Feed consumption: The ration consumption was calculated by weighing the ration given and the remaining ration every week. The following formula was used to calculate ration consumption per head per week [10].

Body weight gain: Weekly body weight gain was measured by weighing the chickens at the end of the week. Weekly body weight gain can be calculated by using the following formula [10].

Feed conversion: Feed conversion was calculated by dividing the average feed consumption in one week by the weekly average body weight gain. The ration conversion calculation was done by using the following formula [10].

Live weight: Live weight is obtained by measuring the weight of chicken that had been fasted for 6 hours to obtain an empty live weight before slaughtering [11].

Carcass percentage: Carcass percentage is the ratio between carcass weight and live weight multiplied by one hundred percent [12].

Percentage of abdominal fat: Abdominal fat percentage is obtained by comparing the weight of abdominal fat with live weight multiplied by one hundred percent [13].

The number of small intestine villi: Samples of the small intestine of the ileum are 4-5 cm long, cut, and the contents were then removed. After that, the ileum was cleaned with NaCl solution and later stored in formalin solution with a concentration of 10%. Subsequently, the lumen of the small intestine was cut 4 μm thick using a microtome and placed on a slide for staining with the Haematoxylin-eosin method. The specimens were then observed under a microscope with a magnification of 40x and the number of all villi (unit/transverse cut) was counted [14]

Small Intestine Digesta pH: Measurement of digesta pH was carried out after the broilers were 28 days old, then fasted for 6 hours and then taken out. After that, the small intestine was separated from the ileum, then the contents of the small intestine from the ileum were removed and put into a container for pH observation, using a pH meter [15].

Digesta Viscosity: After removing the digesta ileum, 1 gram of digesta was diluted with aquadest to a volume of 10 ml. The solution was centrifuged at 300 rpm for 5-10 minutes. A viscocytometer is used to measure the viscosity of the centrifuged supernatant liquid [16].

Data Analysis

The data obtained will be processed using Statistical Product and Service Solutions (SPSS) software ver. 20 based on the design used. If there are differences between treatments, Duncan New Multiple Range Test (DNMRT) will be tested.

RESULTS AND DISCUSSION

The results of the study of giving a combination of cassava tuber and *Indigofera zollingeriana* leaf flour supplemented with citric acid in the ration on the performance of broilers is shown in Table 3.

Feed Consumption

The analysis of variance results revealed that using a combination of cassava tuber and *Indigofera* leaf flour supplemented with citric acid in the ration had no significant effect (P>0.05) on ration consumption. The average ration consumption in this study was 54.91-56.99 g/head/day. Feed consumption in the study was higher when compared to the research results of Chang`a et al. **[18]** who reported that the average consumption of cassava flour feed with the addition of the *Ronozyme* enzyme in broiler feed was 44.94-46.77 g/head/day. The results of the treatment that had no significant effect on ration consumption were in accordance with the results of the research of Yadav et al. **[5]** who stated that the consumption of ration using cassava tubers as a substitute for corn at the level of 50% which had a metabolic energy content of 2878 kcal/kg and a protein content of 20.61% gave the same effect on the control ration.

This proved that the combination of cassava tubers and *Indigofera zollingeriana* leaf flour had a high palatability and nutritional quality, allowing broilers to respond well to treatment rations. Treatments that had no significant effect on ration consumption revealed that using cassava tubers and *Indigofera zollingeriana* leaf flour combination at 5%, 10%, 15%, and 20% did not cause physical, taste, and odor differences so that broilers liked it and did not cause a decrease in palatability. According to Akhadiarto **[19]**, the amount of feed consumption is influenced by the palatability of the feed, palatability itself depends on the smell, taste, and shape of the ingredients that make up the ration. Furthermore, the nutritional quality of all ration treatments was the same, ensuring that the protein and energy balance consumed by broilers was adequate. This is consistent with the statement of Ahmed **[20]** which states that giving rations with relatively the same energy content will have the same effect on ration consumption.

Cassava tuber feed ingredients contain anti-nutritional substances in the form of HCN, while *Indigofera zollingeriana* leaf flour contains anti-nutritional substances in the form of tannins and saponins. These anti-nutritional substances have no effect on ration consumption when a combination of cassava tubers and *Indigofera zollingeriana* leaf flour is used up to 20% in broilers rations during rearing. According to Jayanegara et al. **[21]**, tannins and saponins contain

antinutrients with astringent taste. The astringent taste of antinutrients in treatment rations was tolerated by broilers and did not reduce palatability of the treated rations.

Live Weight Gain

The results of the analysis of variance showed that using a combination of cassava tuber and *Indigofera zollingeriana* leaf flour supplemented with citric acid in the ration had a significant effect ($P<0.05$) on broiler body weight gain. In this study, the average body weight gain of broiler chickens was 36.60-43.91 g/head/day. Body weight gain in this study was slightly higher than that described by Hossain et al. **[22]**, who reported an average of 40.44-41.07 g/head/day with cassava provision in broiler rations. In line with the research results of Ojewola et al. **[23]**, using 10% cassava tubers as a substitute for corn has the same effect on body weight gain in broilers, but giving 20%-100% cassava tubers can have a negative effect on body weight gain.

The use of cassava tubers and *Indigofera zollingeriana* leaf flour combination 5-10% as a substitute for corn had no significant effect ($P>0.05$) on body weight gain, despite the fact that both T1 and T2 contained anti-nutritional substances. T1 contained tannins up to 0.04 g/kg, saponins 0.00054 mg/kg, and 0.14 mg/kg HCN. Meanwhile, T2 contained 0.08 g/kg tannins, and 0.0010 mg/kg saponins, and 0.28 mg/kg HCN. Even though there were anti-nutritional substances in T1 and T2, 0.2% citric acid supplementation improved broiler digestion and had the same effect on broiler body weight gain as the control treatment. *Acidifier* serves to accelerate the condition of the digestive tract to become acidic so that protein-digesting enzymes can work more quickly and become active **[24]**. The use of cassava tuber and *Indigofera zollingeriana* 15-20% leaf flour combination as a substitute for corn had a significant effect ($P<0.05$) on body weight gain in broilers.

The decrease in broiler chicken body weight gain was caused by an increase in the content of anti-nutritional substances, specifically tannins, saponins, and HCN at T3 and T4. *Indigofera zollingeriana* leaf flour contributed 0.13-0.17 g/kg tannins, 0.0016-0.0021 mg/kg saponins, and 0.42-0.56 mg/kg HCN at T3 and T4. Tannins can bind to proteins, reducing protein digestibility.

However, the complex bonds of tannins with proteins can be released at low pH in the digestive tract, allowing protein to be degraded by digestive enzymes and the amino acid content to be utilized by livestock. Furthermore, saponins are considered to have an inhibitory effect on livestock growth because they inhibit the activity of a number of digestive enzymes such as trypsin and chymotrypsin. Additionally, the HCN in the ration can inhibit the production of ATP, resulting in a lack of energy in the livestock **[21]**. Citric acid supplementation with a dose of 0.2% at 15-20% substitution using corn had also not been able to work properly due to an increase in anti-nutritional substances, resulting in less-than-optimal absorption of ration nutrients in the digestive tract.

Ration Conversion

The analysis of variance results revealed that using a combination of cassava tuber and *Indogofera zollingeriana* leaf flour in the ration supplemented with citric acid in the ration had a significant effect ($P<0.05$) on the conversion value of the ration. The average ration conversion value in this study ranged from 1.29 to 1.50. The results of the ration conversion in this study were lower than those of Rahmadani et al. **[25]** who reported that the ration conversion value had an average of 1.88-1.96 with the addition of cassava with *isoamylase* added in broiler rations. In addition, the results of Yadav et al. **[5]** stated that the conversion of broiler rations had an average of 1.59-1.82 in the use of cassava tuber as a substitute for corn to 50% in broiler rations. This is consistent with the findings of Ojewola et al. **[23]**, who argued that broilers fed with cassava tuber rations at a 5% level as a substitute for corn has no negative effect on the on the conversion value of the ration.

The results of this research revealed that replacing corn up to 10% with a combination of cassava tuber and *Indigofera zollingeriana* leaf flour had the same effect on broiler ration conversion. This happened because T1 and T2 had the same effect on body weight gain and ration consumption. In addition, 0.2% citric acid supplementation was able to provide a maximum effect on the

digestive tract, so that the use of cassava tuber up to 10% could be digested well by broilers. The reason for that was because both T1 and T2 had the same effect on body weight gain and ration consumption. Moreover, 0.2% citric acid supplementation was able to provide the best effect on the digestive tract, allowing broiler chickens to digest cassava tubers up to 10%. Saputra et al. **[9]** discovered that *step down* feeding with 0.4% natural citric acid from lime juice can improve feed conversion in broilers.

The high value of ration conversion in T3 and T4 treatments was due to the low body weight gain of broilers despite consuming the same amount of ration. Furthermore, 0.2% citric acid supplementation did not improve nutrient digestibility in the T3 and T4 treatment rations due to an increase in anti-nutritional substances. According to Allama et al. **[26]**, a low ration conversion value indicates that the broiler feed efficiency is good because the more efficient the broilers use feed in producing meat.

Live Weight

The results of the analysis of variance showed that the use of a combination of cassava tuber and *Indigofera zollingeriana* leaf flour supplemented with citric acid in the ration showed significant results ($P<0.05$) on live weight. The average live weight of broiler chickens in this study was 1153.6-1368 g/head. The live weight value in this study was higher than the research results of Unigwe **[27]** in which the average live weight of 28-day-old broilers fed with 12%-36% substitute for corn with cassava tuber flour as the main source of energy was 1195.8-1044.7 g/head.

Starch makes up the majority of the nutrients found in cassava tubers. Starch is a carbohydrate which is a glucose polymer composed of amylose and amylopectin **[28]**. The high content of amylopectin in cassava tubers causes the starch content to be easily digested by broilers, even better than corn. The use of a combination of cassava tuber and *Indigofera zollingeriana* leaf flour as much as 20% in the ration will cause an increase in antinutrients that decreases live weight. The live weight of broiler is influenced by the ability of the broiler to convert the ration into meat which is hampered by the presence of cyanide in the ration. Of course, this does not optimize the

nutrient absorption process, so the absorbed nutrients are not optimally converted into meat, affecting broiler live weight **[29]**.

The use of *Indigofera zollingeriana* leaf powder at T1 and T2 contributed 0.14-0.28 mg/kg of HCN. T3 and T4 contributed 0.42-0.56 mg/kg of HCN. The content of antinutrients such as cyanide acid (HCN) in cassava tuber is around 17.5 mg/kg, to avoid cyanide poisoning in cassava, processing is necessary in reducing toxic content **[30]**. The addition of 20% cassava tubers and *Indigofera zollingeriana* leaf flour supplemented with 0.2% citric acid did not increase broiler live weight. This is because the addition of 0.2% citric acid was unable to tolerate antinutrition in the treatment ration.

Carcass Percentage

The results of the analysis of variance showed that the use of cassava tubers and *Indigofera zollingeriana* leaf flour combination supplemented with citric acid in the ration showed significant results ($P>0.05$) on the percentage value of broiler carcasses. In this study, the average percentage of broiler carcasses was 68.90-72.3%. Khempaka et al. **[31]** reported that the average percentage of broiler chicken carcasses aged 28 days fed with 4%-20% fermented cassava tuber feed *Aspergillus oryzae* was 66.10%-67.42%. According to Kana et al. **[32]**, the average percentage of broiler carcasses fed cassava tuber with a combination of oil palm and cocoa husks as an alternative energy source to replace corn as much as 50-100% was 68.79-73.71%.

The content of metabolic energy in the ration is expected to stimulate broiler growth, resulting in a high live weight and a high carcass weight. This is consistent with the research of Solikin et al. **[33]** which states that carcass weight is closely related to the final weight of the chicken, the greater the weight of the chicken, the greater the carcass weight. However, the percentage of carcass decreased with the use of a combination of cassava tubers and *Indigofera zolllingeriana* leaf flour with different compositions for each treatment ration even though the metabolic energy of all rations was made the same. Nevertheless, the provision of cassava tubers in poultry feed is limited due to its low protein content, unbalanced amino acid profile, high fiber content and the

297 presence of antinutrients, especially cyanogenic glucosides (HCN). Additionally, there are other
298 antinutrients such as tannins and saponins discovered in *Indigofera zollingeriana* leaf flour. The use
299 of *Indigofera zollingeriana* leaf flour in treatments T1 and T2 contributed 0.04-0.08 g/kg tannins and
300 0.00054-0.0010 mg/kg saponins. Meanwhile, the use of *Indigofera zollingeriana* leaf flour in
301 treatments T3 and T4 contributed 0.13-0.17 g/kg tannins and 0.0016-0.0021 mg/kg saponins.
302 Furthermore, Palupi et al. **[4]**mentioned that there are 0.29% tannins and 0.036 ppm saponins in
303 Indigofera zollingeriana shoot flour.
304 As a result, the presence of citric acid in the treatment ration aided in the absorption of *Indigofera*
305 *zollingeriana* leaves. Citric acid accelerates the acidification of the digestive tract, allowing protein-
306 digesting enzymes to work more quickly and actively **[24]**. The feeding of 15-20% cassava tubers
307 and *Indigofera zollingeriana* leaf flour supplemented with 0.2% citric acid did not increase the
308 percentage of broiler carcasses. This was because the addition of 0.2% citric acid did not increase
309 nutrient absorption from the treatment ration.
310 **Abdominal Fat Percentage**
311 The outcome of the analysis of variance showed that the use of cassava tuber and *Indigofera*
312 *zollingeriana* leaf flour combination supplemented with citric acid in the ration showed no
313 significant effect (P>0.05) on the percentage of abdominal fat in broiler chickens. The average
314 percentage of broiler abdominal fat in this study was 0.76-1.29%. The percentage value of
315 abdominal fat in this study was higher than that of Khempaka et al. **[31]** in which the average
316 percentage of abdominal fat of 28-day-old broiler chickens fed fermented cassava tuber *Aspergillus*
317 *oryzae* with 4%-20% feeding is 0.73%-1.09%. Cabel et al. **[34]** reported that the percentage of
318 abdominal fat in broiler carcasses is normally between 0.73%-3.78%. This shows that the
319 treatment of cassava tubers could not reduce abdominal fat deposits, because the energy content
320 of each ration was the same so that the level of energy accumulation in the broiler body in the
321 form of body fat was the same between treatments. Subekti et al. **[11]** stated that the formation
322 of abdominal fat in the body of chicken occurs due to excess energy obtained by the feed they

consume. Abdominal fat can also be formed from the high crude fat content in the ration. The nutritional requirements of broiler chickens must contain 3-4% crude fat in the ration, while in the combination of cassava tubers and *Indigofera zollingeriana* leaf flour in the crude fat treatment ration it is 5-6%. Fouad et al. **[35]** also stated that abdominal fat is related to total carcass fat, with the higher the abdominal fat content, the higher the carcass fat content. Fat in in the body of chicken is derived from feed and is produced through the process of fat synthesis in the feed. According to the findings of this study, the combination of cassava tubers and *Indigofera zollingeriana* leaf flour supplemented with 0.2% citric acid in the treatment ration on the abdominal fat of broilers, which was given 5-20%, did not result in excess metabolic energy in the ration. The addition of 0.2% citric acid to the treatment ration increased the percentage of abdominal fat. This is in contrast to the research of Fik et al. **[36]**, which found that 0.5%-1.5% citric acid in feed can reduce the percentage of broiler abdominal fat because of the differences in ration energy content and dosage in broiler rations.

Small Intestine Villi Number

The results of the analysis of variance showed that the use of a combination of cassava tuber and *Indigofera zollingeriana* leaf flour supplemented with citric acid in the ration showed a significant effect ($P<0.05$) on the number of ileum villi of broilers. Based on the results of further tests, the average number of ileum villi in broiler chickens in treatment T0 was significantly different ($P<0.05$) with treatments T1 and T2. This is because for the T1 and T2 treatments, 0.2% citric acid supplementation were added, although the pH of the ileum digesta obtained was the same as the T0 treatment (control). Nevertheless, the addition of citric acid was able to increase the number of small intestinal ilium villi in broilers. Hidayat et al. **[37]** stated that *acidifier* (citric acid) in general can replace the role of antibiotics and is able to increase the absorption of food extracts in the small intestine.

The results of further tests on T0 treatment were not significantly different ($P>0.05$) with T3 and T4 treatments. This is because in the T3 and T4 treatments there was an increase in the amount

of CIF (a combination of cassava tuber and *Indigofera zollingeriana* leaf flour) by 15-20%, which means an increase in the concentration of antinutrients in the ration, namely HCN (cyanic acid), tannins, and saponins. Even though the T3 and T4 treatments contained 0.2% citric acid supplementation, the supplemented citric acid did not increase the number of villi as in T1 and T2 treatments.

Treatments at T1 and T2 had different effects ($P<0.05$) with treatments T3 and T4. This was due to the increasing concentration of antinutrients at T3 and T4, although the concentration of antinutrients (HCN, tannins and saponins) in this study was still within the limits that could be tolerated by broilers. Jayanegara et al. **[21]** stated that the maximum limit of HCN content in poultry is 10 mg HCN/kg feed, but a low concentration of antinutrients could affect the number of villi in the ileum of the small intestine of broilers in this study. The presence of antinutrients in the ration caused the number of small intestinal villi to be lower when compared to the research of Kusuma et al. **[38]** which reported that the lowest number of intestinal villi in the research of alternative feeds using a total of 60% palm kernel cake and onggok fermented food in the ration, is 386.60±19.91 μm.

Digesta pH

The outcome of the analysis of variance showed that the use of a combination of cassava tubers and *Indigofera zollingeriana* leaf flour supplemented with citric acid in the ration showed no significant effect ($P>0.05$) on the pH value. The average pH value in this study ranged around 5.56-5.86. Puspasari et al. (2016) stated that the normal digestive pH in each part of the small intestine is different, in the duodenum it is approximately 4.17-5.68, the jejunum around 5-6 and the ileum roughly 5.8-6. Partial replacement of corn as an energy source in the ration with a combination of cassava tuber and *Indigofera zollingeriana* did not affect the pH value of the small intestine digesta until the feeding of 20% combination of cassava tubers and *Indigofera zollingeriana* in the ration. However, the pH value result in the ileum was relatively low, which was 5.56-5.86, when compared to the ileum which generally had a high pH value which was in the range of 6-7.

Based on research of Mabelebele et al. **[39]**, it is reported that ideally the pH value in broiler chickens ranges from 3.47 in the (gizzard) to 6.43 in the (small intestine) digestive tract.

Organic acid has a role in increasing the activity of proteolytic enzymes in the digestive tract (Kim et al., 2015) and is also able to increase the natural immune response in poultry **[40]**. The pH value and viscosity value have important factors in influencing the flow rate of nutrients in the digestive tract, this causes the digesta rate to be fast and allows a decrease in the digestive process and the absorption of nutrients becomes less effective, resulting in the decrease of the availability of nutrients for the synthesis of body tissues. According to Cahyaningsih et al. **[41]** a low degree of acidity in the digestive system (stomach and intestines) is able to optimize the absorption of nutrients in the stomach and intestines because it can slow down the rate of digestion, resulting in the optimization of feed nutrients absorption, so it does not interfere with the digestive process and nutrients utilization, or the growth of harmful bacteria will be inhibited **[42]**.

Viscosity

The analysis of variance results showed that the combination of cassava tubers and *Indigofera zollingeriana* leaf flour supplemented with citric acid in the diet showed no significant effect ($P>0.05$) on digesta viscosity. The average digesta viscosity in this study was at (1.91 d.Pas$\pm$0.35) to (2.29 d.Pas$\pm$0.42). The viscosity in this study was higher than that of Emma et al. **[43]** where the lowest viscosity value is (0.10$\pm$0.004 d.Pas) and the highest viscosity (0.23$\pm$0.008 d.Pas).

The viscosity value in the digestive tract is directly proportional to the high or low pH value. Negative effects will appear if the viscosity of the small intestine increases, which will then reduce the efficiency of digestion by slowing the diffusion rate of endogenous enzymes to react with nutrients, as well as improving blood biochemistry in quail and broilers **[44]**. The value of viscosity is influenced by the level of viscousness, the higher the viscousness level, the worse the viscosity value will be, inversely if the viscousness level is low or watery, then the viscosity value is adequate **[45]**.

Viscosity has a significant impact on the value of performance data for poultry, with high or low viscosity values affecting the value of feed digestibility and feed flow rate while in the digestive tract. Furthermore, changes in the viscosity value can occur as a result of the type of feed consumed, such as feed with a high solubility value, which can result in a low viscosity value in a treatment. This occurrence is thought to be caused by the influence of *Non-Strach Pollysacharides* (NSP) solubility value in feed ingredients. According to Saputro et al. **[46]**, most types of carbohydrates that reach the colon without hydrolysis include *Non-Starch Polysaccharides* (NSP), *Resistant Starch* (RS), and *Short Chain Carbohydrates* (SC).

CONCLUSION

The use of a combination of cassava tuber and Indigofera zollingeriana leaf flour at a level of 10% supplemented with 0.2% citric acid can be used as an energy source to replace corn in the ration without negatively impacting ration consumption, body weight gain, ration conversion, live weight, percentage carcass, percentage of abdominal fat, digesta pH, viscosity, and number of villi in the small intestine of broilers.

CONFLICT OF INTERESTS

The authors report no conflict of interest.

AUTHORS' CONTRIBUTION

All authors contributed to the work, discussed the results and contributed to the final manuscript.

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561

562 **Table 1.** Chemical Compostion

Item	Chemical Composotion (%)							ME (cal/kg)
	CP	EE	CP	Ca	P	Lisin	Metionin	
Pakan komersil[a]	21.00	5.00	5.00	0.80	0.50	0.98	0.38	3000
Dedak Padi[b]	12.00	13.00	12.00	0.20	0.20	0.46	0.21	2580.63
Jagung giling[b]	8.60	3.90	2.00	0.20	0.10	0.26	0.19	3370.00
Konsentrat[c]	33.22	3.37	5.40	2.72	1.45	0.9	0.4	2276.99
Tepung Ikan[g]	40.53	5.64	2.20	5.00	2.50	2.08	0.76	2665.58
Umbi Singkong[d]	01.10	0.55	2.30	0.32	0.71	0.08	0.04	3519
Indigofera Zollingeriana[e]	28.98	33.30	8.49	0.52	0.34	1.75	0.43	2791.12
CIF[f]	9.41	1.37	4.15	0.37	0.59	0.58	0.16	3300.6

563 Note: CP, Crude Protein; EE, Extract Ether; CF, Crude Fiber; Ca, Calcium; P, Phosfor; ME, Metabolisme Energy.
564

565 **Table 2.** Treatment Ration Compostion

Item	Treatments				
	T0	T1	T2	T3	T4
Comercial Feed (%)	100	26	26,5	27,5	28
Rice Bran (%)		40	35	30	25
Corn (%)		15	14,5	13,5	13
Concentrate (%)		14	14	14	14
Fish Flour (%)		5	10	15	20
Total	**100**	**100**	**100**	**100**	**100**
Crude Protein (%)	21,00	20,70	20,62	20,42	20,34
Extract Ether (%)	5,00	6,40	6,32	6,29	6,21
Crude Fiber (%)	5,00	5,17	5,31	5,48	5,62
Calcium (%)	0,80	1,19	1,21	1,21	1,22
Phosfor (%)	0,50	0,68	0,68	0,68	0,59
Lisin (%)	0,16	1,22	1,26	1,30	1,34
Metionin (%)	0,10	0,48	0,55	0,56	0,57
Metabolisme Energy (Ccal)	3000	2905,63	2903,68	2903,25	2901,30

566 Note: T0, without CIF; T1, corn substitution with 5% CIF; T2, corn substitution with 10% CIF; T3, corn
567 substitution with 15% CIF; and T4, corn substitution with 20% CIF.
568

569 **Table 3.** Effect of feed treatment on performance, carcass and quality of the digestive tract

Variabels	Treatments				
	T0	T1	T2	T3	T4
Consumption (g/days)	54.91±2.29	56.86±2.22	56.99±1.33	55.17±1.69	54.96±1.61
ADG (g/days)	42.44[a]±3.42	43.91[a]±2.35	41.61[a]±3.03	37.36[b]±2.60	36.60[b]±2.63
FCR	1.301[a]±0.122	1.297[a]±0.074	1.375[a]±0.112	1.480[b]±0.070	1.508[b]±0.119
Live Weight (g)	1368[a]±98.40	1316.8[ab]±49.39	1227.8[ab]±146.66	1175.2[bc]±123.45	1153.6[c]±226.64
Carcass (%)	72.36[a]±2.37	71.57[ab]±1.50	70.67[ab]±0.73	67.72[c]±4.29	68.90[c]±1.90

Abdominal Fat (%)	1.22±0.36	1.16±0.46	0.76±0.27	1.16±0.17	1.29±0.30
Number of villi	244.4^{a}±10.96	286.4^{b}±10.21	273.0^{b}±11.29	240.6^{a}.±8.87	241.8^{a}±2.86
pH Digest	5.56±0.15	5.56±0.32	5.80±0.14	5.66±0.11	5.86±0.11
Viscosity	1.93±0.42	1.95±0.52	1.91±0.35	2.29±0.42	2.15±0.20

570 Note: ADG, Average Daily Growth; FCR, Feed Conversion Ratio; T0, without CIF; T1, corn substitution with 5%
571 CIF; T2, corn substitution with 10% CIF; T3, corn substitution with 15% CIF; and T4, corn substitution with 20%
572 CIF. Means with different superscripts letters in the same line differ significantly ($P<0.05$).
573

574

2. Bukti konfirmasi review dan hasil review pertama (27 Oktober 2018)

Article Revision Letter for Authors - (JAVAR-2022-06-097)

From: Noreply eJManager (noreply@ejmanager.com)

To: palupiarda@yahoo.com

Date: Saturday, July 16, 2022 at 07:21 PM GMT+7

Dear Rizki Palupi Palupi,

Your manuscript entitled \"The Effect of The Use of Cassava Tuber (Manihot Esculenta) and Indigofera zollingeriana leaf flour combination as a Source of Energy Supplemented with Citric Acid in Ration on Broiler Small Intestine Characteristics and Productivity\" (Ms.Nr. JAVAR-2022-06-097) was reviewed by expert reviewers of the Journal of Advanced Veterinary and Animal Research. As an initial decision, your manuscript was found interesting but some revisions have to be made before it can reach a publishable value.
Please answer all the comments below point-by-point in an accompanying response letter to your revised submission.

You should send your revised manuscript via the online system of ScopeMed on my.ejmanager.com.

Sincerely yours,

Nazmul H. Nazir, PhD
Editor-in-Chief
Journal of Advanced Veterinary and Animal Research

COMMENTS for Authors:

=> Reviewer # 1

Use of cassava tuber and indigenous zollingeriana leaves, as a source of energy for broiler - good choice.

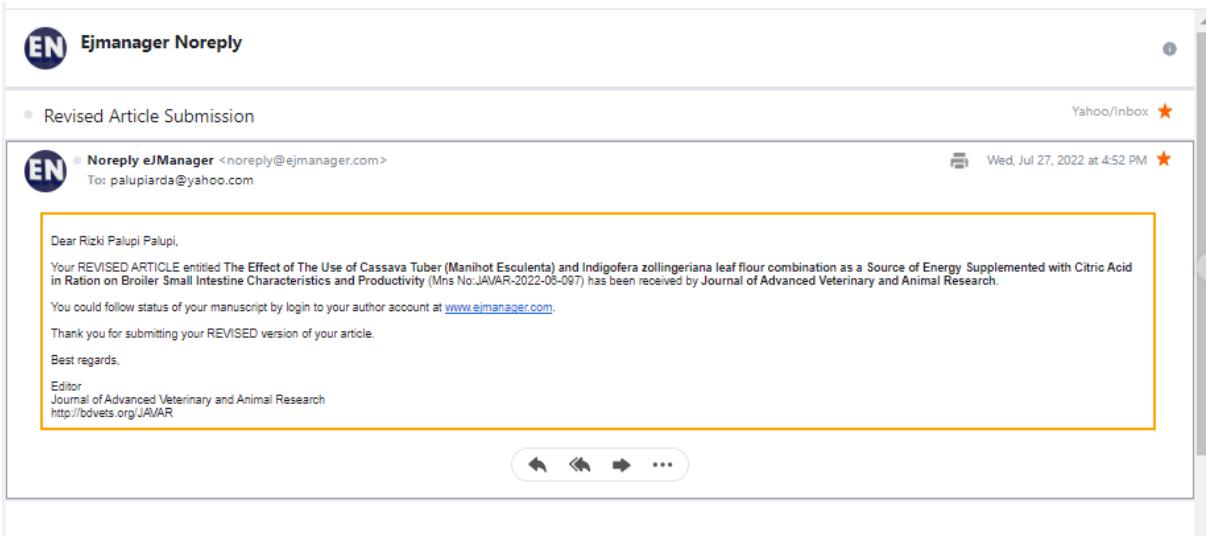
1. Knowledge gap is weakly written in the Introduction section. The knowledge gap will depend on the latest reference citation. Several more recent references should be cited in the introduction section.
2. Novelty should be focused on the writing of the objective.
3. Ethical approval with approval number is required for this study.
4. Discussion section should be revised focusing on the novelty, along with citations of several recent references that are needed to make the DIscussion section comprehensive.
5. Check the references carefully. For example, reference no. 3 has no Journal name mentioned.

Good luck.

=> Reviewer # 2

a. Abstract is not compatible with the style of JAVAR. The abstract should be sub-divided into Objective, Materials and Methods, Results, and Conclusion.
b. List of abbreviations and Acknowledgment sections are missing. Check the latest issue of JAVAR for the style.
c. Reference style is not compatible with JAVAR. Check the style carefully and arrange the references accordingly.

3. Bukti konfirmasi submit revisi pertama, respon kepada reviewer, dan artikel yang diresubmit (27 Juli 2022)



Ejmanager Noreply

Revised Article Submission

Yahoo/Inbox

Noreply eJManager <noreply@ejmanager.com>
To: palupiarda@yahoo.com

Wed, Jul 27, 2022 at 4:52 PM

Dear Rizki Palupi Palupi,

Your REVISED ARTICLE entitled **The Effect of The Use of Cassava Tuber (Manihot Esculenta) and Indigofera zollingeriana leaf flour combination as a Source of Energy Supplemented with Citric Acid in Ration on Broiler Small Intestine Characteristics and Productivity** (Mns No:JAVAR-2022-06-097) has been received by **Journal of Advanced Veterinary and Animal Research**.

You could follow status of your manuscript by login to your author account at www.ejmanager.com.

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Best regards,

Editor
Journal of Advanced Veterinary and Animal Research
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Original Article

The Effect of The Use of Cassava Tuber (Manihot Esculenta) and Indigofera zollingeriana leaf flour combination as a Source of Energy Supplemented with Citric Acid in Ration on Broiler Small Intestine Characteristics and Productivity

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ABSTRACT

Objective: The study was aimed ~~The aim of this study is~~ to determine the effect of using a combination of cassava tuber (Manihot esculenta) and Indigofera zollingeriana leaf flour as an energy source supplemented with citric acid in the ration on performance, carcass quality, digesta pH, viscosity and number of villi in the intestines of broiler.
Materials and Methods: The research design used was Completely Randomized (CRD) with 5 treatments and 5 replications, each replication consisting of 4 broilers.~~This study used a Completely Randomized Design (CRD) with 5 treatments and 5 replications, each replication consisted of 4 broilers.~~ The treatment was a substitution of corn in the ration with a combination of cassava tuber and Indigofera zollingeriana leaf (CIF): T0 (without CIF), T1 (5% CIF substitute for corn + 0.2% citric acid), T2 (10% CIF substitute for corn + 0.2% citric acid), T3 (15% CIF corn substitute + 0.2% citric acid) and T4 (CIF corn substitute + 0.2% citric acid). Each treatment ration was supplemented with 0.2% citric acid. The observed variables were ration consumption, body weight gain, feed conversion, live weight, carcass percentage, abdominal fat percentage, digesta pH, viscosity and number of villi of broilers.
Results: this study showed that the combination of cassava tubers and Indigofera zollingeriana leaf flour supplemented with 0.2% citric acid had a significant effect ($P<0.05$) on body weight gain, ration conversion, live weight, carcass percentage and number of villi in broiler intestines. However, it had no significant effect ($P>0.05$) on ration consumption, percentage of abdominal fat, digesta pH and viscosity of broilers.
Conclusions: the combination of cassava tubers and Indigofera zollingeriana leaf flour at the level of 10% supplemented with 0.2% citric acid can be used as an energy source to replace corn without giving a bad effect on production performance, carcass quality and small intestine characteristics of broilers.

Keywords: Broiler, carcass, performance, Indigofera zollingeriana flour, cassava tuber flour.

INTRODUCTION

Corn is the primary energy source in broiler rations, but its use as broiler feed material competes with the needs of people who consume corn for energy. This will affect corn accessibility and may make corn costs rise, requiring the utilization of elective feed to supplant corn as an energy source in the proportion ~~This will have an impact on corn availability and may cause corn prices to rise, necessitating the use of alternative feed to replace corn as an energy source in the ration~~ **[1,2]**. One of the feed ingredients that can be used as an energy source to replace corn is cassava tuber because cassava tuber contains relatively the same energy source as corn. According to Wahyudi et al. **[3]**the metabolic energy content of corn is 3350 kcal/kg, whereas cassava tubers have a metabolic energy of 3519 kcal/kg **[4]**. The tuber of cassava will be cut up and dried up, and milled or pelletized and incorporated in broilers diet and capable to replaced 50% maize in broilers ration with no adverse effect on their performances; and addition of 40% cassava flour or addition of 20% cassava peel meal in layer's ration was pleasing for laying performance ~~The tuber of cassava will be cut up and dried up, and milled or pelletized and incorporated in chickens' diet and it can also be included in broilers diet and capable of substituting 50% maize in broilers ration with no adverse effect on their performances and addition of 40% cassava flour or addition of 20% cassava peel meal in layer's ration is pleasing for laying performance of chickens~~ **[5]**.

The use of cassava tuber as an energy source has a certain weakness, namely the protein content of cassava tuber is lower than that of corn. Ngiki et al. **[6]** stated ~~that the protein content in cassava tubers is~~cassava tubers protein content was 1.3%, while the latest results showed a lower value of 1.14% **[7]**. Protein deficiency necessitates the addition of protein-rich feed ingredients. *Indigofera zollingeriana* leaf flour can be categorized as a protein source. According to Palupi et al. **[8]**, *Indigofera zollingeriana* shoot flour contains 28.98% crude protein, 8.49% crude fiber, and 3.30% crude fat. In addition to its high protein content, *Indigofera zollingeriana* has many advantages, one of which is the presence of antioxidants because it contains carotenoids in the form of beta-carotene. Yadav et al. **[9]** reported ~~that~~ cassava tubers can be used as an energy ~~source~~ to replace maize by 25% in starter phase broiler rations and 37.5% in finisher phase broiler rations.

Efforts can be made to maximize the efficiency of using alternative feed for livestock by adding an *acidifier* to increase the digestibility ~~of the nutrients in the feed~~. As for the type of *acidifier* that can be used as a feed additive is citric acid. Citric acid has the ability to lower the pH in the digestive tract, resulting in the formation of an acidic atmosphere and affecting the rate of digestion and the population of pathogenic bacteria in the digestive tract **[10–14]**. Deepa et al. **[15]** declared that the addition of 2% citric acid was able to increase feed consumption, increase body weight and improve feed conversion in broiler, while in other studies reported that lower citric acid supplementation of 1.5% had an impact on body weight, body weight gain, and carcass characteristics of broiler chickens **[16]**.

In view of the depiction above, it is important to lead research on the usage of ~~Based on the description above, it is necessary to conduct research on the utilization of~~ cassava tubers and *Indigofera zollingeriana* leaf flour combination as an energy source to replace corn supplemented with citric acid in the ration on broiler production performance, carcass quality, and small intestine characteristics.

MATERIALS AND METHODS

Animal Ethics

An animal feeding experiment was conducted at the experimental station, Department of Animal Science, Faculty of Agriculture, Universitas Sriwijaya. The birds were cared for according to the Animal Welfare Guidelines of the Indonesian Institute of Sciences. The approval of the experiment was granted from Universitas Sriwijaya with approval number KPPHP-2021-1.

Methods and Sample Preparation

This research used 100 Day Old Chick (DOC) broilers. The broilers were placed in 25 postal cages measuring 70 cm x 70 cm x 70 cm. Four broilers were placed in each cage. The cages are equipped with feeders, drinking water containers, and 60-watt incandescent lamps as lighting and heating sources during brooding.

The equipments required to create a combination of cassava tuber and Indigofera zollingeriana are grater, bucket, knife, plastic, rope, oven, tarpaulin, scales, measuring tape and stationery. A stereo microscope, pH meter, and viscocytometer were used in the laboratory observations.The feed ingredients used to prepare the ration in this study were cassava tuber, Indigofera zollingeriana leaf, concentrate, corn, fish flour and rice bran. Subsequently, additional feed ingredients in the form of citric acid were added.

Experimental Design

This study used a Completely Randomized Design (CRD) with 5 treatments and 5 replications, each replication consisted of 4 broilers. The treatment was a substitution of corn in the ration with a combination of cassava tuber and Indigofera zollingeriana leaf (CIF): T0 (without CIF/Control Diet), T1 (5% CIF substitute for corn + 0.2% citric acid), T2 (10% CIF substitute for corn + 0.2% citric acid), T3 (15% CIF corn substitute + 0.2% citric acid) and T4 (CIF corn substitute + 0.2% citric acid).

Making Cassava Tuber Flour

Cassava tuber flour was made by peeling and washing the cassava skin. The cassava was then grated to aid in drying. Cassava was dried by first drying it in the sun and then drying it in an oven at a temperature of 50℃ for 24 hours. The process of reducing the particle size of dried cassava was done by breaking the cassava as crumble.

Making Indigofera zollingeriana leaf flour

Harvesting Indigofera zollingeriana leaves and separating the leaves from the twigs to dry the Indigofera zollingeriana leaves was done in order to make Indigofera zollingeriana leaf flour. Indigofera zollingeriana leaves are dried by first drying them in the sun and then drying them in an oven at a temperature of 50℃ until they are dry enough to be grinded. The flour mill machine is used to process the dried Indigofera zollingeriana leaves into flour.

Ration

The feed ingredients used to prepare the treatment ration consisted of: commercial feed in T0 treatment, concentrate, milled corn, fish flour, rice bran, a combination of cassava tubers and Indigofera zollingeriana in which the composition had been arranged according to the treatment. After the treatment ration was mixed according to the composition of the ration, then it was supplemented with 0.2% citric acid in the treatment using CIF. The nutritional composition of the ingredients of the ration is shown in Table 1 and the composition of the ingredients of the ration and the nutrient content of the research ration is shown in Table 2.

Cage Preparation

Before use, cages must be cleaned of any dirt attached to the cage and liming evenly with the provided disinfectant. To eliminate germs and microorganisms that cause disease, a disinfectant was sprayed evenly throughout the cage. The cage was then left for 1 or 2 weeks. Clean feed and drinking stations, as well as other cage equipment, were placed in each cage. Each cage unit was labeled with treatment and replication.

Rearing

Broiler DOC that has just arrived was given drinking water mixed with brown sugar with a concentration of 50 grams per liter of water for the first 4 hours as an energy source to restore the condition of the broiler DOC due to the stress of traveling from the hatchery to the rearing cage. Rearing was carried out for 4 weeks. The chicken ration was given in accordance with the treatment. Provision of feed and drinking water was done ad-libitum or continuously. Every day in the morning, the cage is cleaned. DOC data collection for broilers was weighed once a week.

Research Data Collection

Data on broiler chicken performance was collected once a week by weighing the weight gain of the chickens. Then the amount of feed given was weighed as well as the remainder of the feed.

Observed Variables

Feed consumption: The ration consumption was calculated by weighing the ration given and the remaining ration every week. The following formula was used to calculate ration consumption per head per week [10].

Body weight gain: Weekly body weight gain was measured by weighing the chickens at the end of the week. Weekly body weight gain can be calculated by using the following formula [10].

Feed conversion ratio (FCR): Feed conversion was calculated by dividing the average feed consumption in one week by the weekly average body weight gain. The ration conversion calculation was done by using the following formula [10].

Live weight: Live weight is obtained by measuring the weight of chicken that had been fasted for 6 hours to obtain an empty live weight before slaughtering [11].
Carcass percentage: Carcass percentage is the ratio between carcass weight and live weight multiplied by one hundred percent [12].

Percentage of abdominal fat: Abdominal fat percentage is obtained by comparing the weight of abdominal fat with live weight multiplied by one hundred percent [13].

The number of small intestine villi: Samples of the small intestine of the ileum are 4-5 cm long, cut, and the contents were then removed. After that, the ileum was cleaned with NaCl solution and later stored in formalin solution with a concentration of 10%. Subsequently, the lumen of the small intestine was cut 4 μm thick using a microtome and placed on a slide for staining with the Haematoxylin-eosin method. The specimens were then observed under a microscope with a magnification of 40x and the number of all villi (unit/transverse cut) was counted [14].

Small Intestine Digesta pH: Measurement of digesta pH was carried out after the broilers were 28 days old, then fasted for 6 hours and then taken out. After that, the small intestine was separated from the ileum, then the contents of the small intestine from the ileum were removed and put into a container for pH observation, using a pH meter [15].
Digesta Viscosity: After removing the digesta ileum, 1 gram of digesta was diluted with aquadest to a volume of 10 ml. The solution was centrifuged at 300 rpm for 5-10 minutes. A viscocytometer is used to measure the viscosity of the centrifuged supernatant liquid [16].

Data Analysis

The data obtained will be processed using Statistical Product and Service Solutions (SPSS) software ver. 20 based on the design used. If there are differences between treatments, Duncan New Multiple Range Test (DNMRT) will be tested.

RESULTS AND DISCUSSION

Table 3 shows the findings of the investigation into the effects of feeding broilers a mixture of cassava tuber and Indigofera zollingeriana leaf flour supplemented with citric acid in the ration. ~~The results of the study of giving a combination of cassava tuber and *Indigofera zollingeriana* leaf flour supplemented with citric acid in the ration on the performance of broilers is shown in Table 3.~~

Feed Consumption

According to the results of the analysis of variance, adding citric acid to a flour made from cassava tubers and Indigofera leaves did not significantly affect ration consumption (P>0.05). In this study, the average daily ration consumption ranged from 54.91 to 56.99 g/head/day.~~The analysis of variance results revealed that using a combination of cassava tuber and *Indigofera* leaf flour supplemented with citric acid in the ration had no significant effect (P>0.05) on ration consumption. The average ration consumption in this study was 54.91-56.99 g/head/day~~. Feed consumption in the study was higher when compared to the research results of Chang`a et al. **[17]** who reported that the average consumption of cassava flour feed with the addition of the *Ronozyme* enzyme in broiler feed was 44.94-46.77 g/head/day. The treatment's outcomes, which had no significant influence on ration consumption, were consistent with the study's findings by~~The results of the treatment that had no significant~~

~~effect on ration consumption were in accordance with the results of the research of~~ Yadav et al. **[9]** who stated that the consumption of ration using cassava tubers as a substitute for corn at the level of 50% which had a metabolic energy content of 2878 kcal/kg and a protein content of 20.61% gave the same effect on the control ration. Another study also reported the substitution of 50% cornstarch in broiler rations without adverse effects on chicken appearance and the expansion~~addition~~ of 40% cassava flour or the expansion~~addition~~ of 20% cassava peel flour in laying hens rations could improve the performance of laying hens **[5]**.

This proved that the combination of cassava tubers and *Indigofera zollingeriana* leaf flour had a high palatability and nutritional quality, allowing broilers to respond well to treatment rations. Treatments that had no significant effect on ration consumption revealed that using cassava tubers and *Indigofera zollingeriana* leaf flour combination at 5%, 10%, 15%, and 20% did not cause physical, taste, and odor differences so that broilers liked it and did not cause a decrease in palatability. According to Akhadiarto **[18],** the palatability of the feed, which is regulated by the smell, taste, and form of the components that make up the ration, affects how much feed being consumed.~~the amount of feed consumption is influenced by the palatability of the feed, palatability itself depends on the smell, taste, and shape of the ingredients that make up the ration~~. Furthermore, the nutritional quality of all ration treatments was the same, ensuring that the protein and energy balance consumed by broilers was adequate. This is consistent with the statement of Ahmed **[19]** which states that giving rations with relatively the same energy content will have the same effect on ration consumption.

HCN is an anti-nutritional compound found in cassava tuber feed components, whereas tannins and saponins are anti-nutritional compounds found in indigofera zollingeriana leaf flour.~~Cassava tuber feed ingredients contain anti-nutritional substances in the form of HCN, while *Indigofera zollingeriana* leaf flour contains anti-nutritional substances in the form of tannins and saponins~~. These anti-nutritional substances have no effect on ration consumption when a combination of cassava tubers and *Indigofera zollingeriana* leaf flour is used up to 20% in broilers rations during rearing. According to Jayanegara et al. **[20],** tannins and saponins contain antinutrients with astringent taste. The astringent taste of antinutrients in treatment rations was tolerated by broilers and did not reduce palatability of the treated rations.

Live Weight Gain

According to the results of the analysis of variance, adding citric acid to a ration that included cassava tuber and Indigofera zollingeriana leaf flour had a significant effect (P 0.05) on broiler body weight gain.~~The results of the analysis of variance showed that using a combination of cassava tuber and *Indigofera zollingeriana* leaf flour supplemented with citric acid in the ration had a significant effect (P<0.05) on broiler body weight gain~~. In this study, the average body weight gain of broiler chickens was 36.60-43.91 g/head/day. Body weight gain in this ~~study~~ experiment was slightly higher than that described by Hossain et al. **[21],** who reported an average of 40.44-41.07 g/head/day with cassava provision in broiler rations. In accordance with the findings~~In line with the research results~~ of Ojewola et al. **[22],** using 10% cassava tubers as a substitute for corn has the same effect on body weight gain in broilers, but giving 20%-100% cassava tubers ~~can have a negative effect on~~could reduce body weight gain. H~~h~~owever, the results of other studies reveal that the use of cassava tuber can be increased up to 50 – 75% if through the fermentation process **[2]**.

The use of cassava tubers and *Indigofera zollingeriana* leaf flour combination 5-10% as a substitute for corn had no significant impact~~effect~~ (P>0.05) on body weight gain, despite the fact that both T1 and T2 contained anti-nutritional substances. T1 contained tannins up to 0.04 g/kg, saponins 0.00054 mg/kg, and 0.14 mg/kg HCN. Meanwhile, T2 contained 0.08 g/kg tannins, and 0.0010 mg/kg saponins, and 0.28 mg/kg HCN. Even though there were anti-nutritional substances in T1 and T2, 0.2% citric acid supplementation improved broiler digestion and had the same effect on broiler body weight gain as the control treatment. *Acidifier* serves to accelerate the ~~condition~~ status of the digestive tract to become acidic so that protein-digesting enzymes can work more quickly and become active **[12–14]**. The use of cassava tuber and *Indigofera zollingeriana* 15-20% leaf flour combination as a substitute for corn had a significant ~~effect~~ impact (P<0.05) on body weight gain in broilers.

An increase in the content of anti-nutritional substances, specifically tannins, saponins, and HCN at T3 and T4, was the cause of the decreased body weight gain in broiler chickens.~~The decrease in broiler chicken body weight gain was caused by an increase in the content of anti-nutritional substances, specifically tannins, saponins, and HCN at T3 and T4~~. *Indigofera zollingeriana* leaf flour contributed 0.13-0.17 g/kg tannins, 0.0016-0.0021 mg/kg saponins, and 0.42-0.56 mg/kg HCN at T3 and T4. Tannins can bind to proteins, reducing protein digestibility. However, the complex bonds of tannins with proteins can be released at low pH in the digestive tract, allowing protein to be degraded by digestive enzymes and the amino acid content to be utilized by livestock **[23,24]**. Furthermore, saponins are considered to have an inhibitory effect on livestock growth because they inhibit the activity of a number of gastrointestinal enzymes ~~digestive enzymes~~ such as trypsin and chymotrypsin **[25]**. Additionally, the HCN in the ration can inhibit the production of ATP, resulting in a lack of energy in the livestock **[20]**. Citric acid supplementation with a dose of 0.2% at 15-20% substitution using corn had also not been able to work properly due to an increase in anti-nutritional substances, resulting in less than ideal nutrition absorption in the digestive system.~~resulting in less-than-optimal absorption of ration nutrients in the digestive tract.~~

Ration Conversion

The findings of the analysis of variance showed that adding citric acid to a ration that contained cassava tuber and Indogofera zollingeriana leaf flour had a significant impact (P< 0.05) on the ration's conversion value. In this investigation, the range of the average ration conversion value was 1.29 to 1.5.~~The analysis of variance results revealed that using a combination of cassava tuber and *Indogofera zollingeriana* leaf flour in the ration supplemented with citric acid in the ration had a significant effect (P<0.05) on the conversion value of the ration. The average ration conversion value in this study ranged from 1.29 to 1.50~~. The results of the ration conversion in this ~~study~~ experiment were ~~lower than~~less encouraging than~~those of~~ Rahmadani et al. **[26],** who reported that the ration conversion value had an average of 1.88-1.96 with the addition of cassava with *isoamylase* added in broiler rations. In addition, ~~the results of~~ Yadav et al. **[9]** stated that the use of cassava tuber as a substitute for corn to 50% in broiler rations had an average conversion rate of 1.59-1.82.~~stated that the conversion of broiler rations had an average of 1.59-1.82 in the use of cassava tuber as a substitute for corn to 50% in broiler rations~~. This is in line with what Ojewola et al. found~~This is consistent with the findings of Ojewola et al~~. **[22],** who argued that broilers fed with cassava tuber rations at a 5% level as a substitute for corn has no negative effect on the on the conversion value of the ration.

According to the results of a study,~~The results of this research revealed that~~ replacing corn up to 10% with a combination of cassava tuber and *Indigofera zollingeriana* leaf flour had the same effect on broiler ration conversion. This happened because T1 and T2 had the same ~~effect~~ impact on body weight gain and ration consumption. In addition, 0.2% citric acid supplementation was able to provide a maximum effect on the digestive tract, so that the use of cassava tuber up to 10% could be digested well by broilers. The reason for that was because both T1 and T2 had the ~~same~~ similar impact~~effect~~ on body weight gain and ration consumption. Moreover, 0.2% citric acid supplementation was able to provide the best effect on the digestive tract, allowing broiler chickens to digest cassava tubers up to 10%. The results showed that citric acid supplemented in the ration ranging from 0.25 - 1% could increase feed conversion in broilers **[27,28]**.

The high value of ration conversion in T3 and T4 treatments was due to the low body weight gain of broilers despite consuming the same amount of ration. Furthermore, 0.2% citric acid supplementation did not improve nutrient digestibility in the T3 and T4 treatment rations due to an increase in anti-nutritional substances. According to Allama et al. **[29],** A low ration conversion value means that the feed efficiency of the broilers is good since the more effectively the broilers use feed to produce meat.~~a low ration conversion value indicates that the broiler feed efficiency is good because the more efficient the broilers use feed in producing meat~~.

Live Weight

The findings of the analysis of variance showed ~~The results of the analysis of variance showed that the use of a~~using combination of cassava tuber and *Indigofera zollingeriana* leaf flour supplemented with citric acid in the ration had~~showed~~ significant results ($P<0.05$) on live weight. The average live weight of broiler chickens in this study was 1153.6-1368 g/head. In this investigation, the live weight value was higher than the findings of Chang'a et al. ~~The live weight value in this study was higher than the research results of Chang'a et al~~ **[17]** in which the average live weight of 24-day-old broilers fed with 25%-75% substitute for corn with cassava tuber flour as the main source of energy was 1171.3-1354.7 g/head. However, this value is lower than the cassava tuber fermentation experiment conducted by Uguru et al **[2]**.

Starch makes up the majority of the nutrients found in cassava tubers. In terms of carbohydrates, amylose and amylopectin make up the glucose polymer known as starch. ~~Starch is a carbohydrate which is a glucose polymer composed of amylose and amylopectin~~ **[30]**. The high content of amylopectin in cassava tubers causes the starch content to be easily digested by broilers, even better than corn. The use of a combination of cassava tuber and *Indigofera zollingeriana* leaf flour as much as 20% in the ration will cause an increase in antinutrients that decreases live weight. The live weight of broiler is influenced by the ability of the broiler to convert the ration into meat which is hampered by the presence of cyanide in the ration. Of course, this does not optimize the nutrient absorption process, so the absorbed nutrients are not optimally converted into meat, affecting broiler live weight **[31]**.

The use of *Indigofera zollingeriana* leaf powder at T1 and T2 contributed 0.14-0.28 mg/kg of HCN. T3 and T4 contributed 0.42-0.56 mg/kg of HCN. The content of antinutrients such as cyanide acid (HCN) in cassava tuber is around 17.5 mg/kg, to avoid cyanide poisoning in cassava, processing is necessary in reducing toxic content **[32]**. Linamarin, also known as phaseolunatin, is an illustration of a cyanogenetic glycoside and can be found in linseed, Java

beans, and cassava. Because hydrogen cyanide (HCN) is toxic and is released by the hydrolysis of the cyanogenetic glycosides, plants that contain these glycosides may be harmful to animals. Before poisoning sets in, the glycoside must be hydrolyzed because it is not dangerous by itself. However, a typically present enzyme in the plant may easily break down the glycoside to its component parts. ~~An example of a cyanogenetic glycoside is linamarin (also called phaseolunatin), which occurs in linseed, Java beans and cassava. The cyanogenetic glycosides liberate hydrogen cyanide (HCN) on hydrolysis; be- cause of the toxic nature of this compound, plants containing this type of glycoside are potentially dangerous to animals. The glycoside itself is not toxic and must be hydrolysed before poisoning occurs. However, the glycoside is easily broken down to its components by means of an enzyme that is usually present in the plant~~ **[33]**.The addition of 20% cassava tubers and *Indigofera zollingeriana* leaf flour supplemented with 0.2% citric acid did not increase broiler live weight. This is because the addition of 0.2% citric acid was unable to tolerate antinutrition in the treatment ration.

Carcass Percentage

The application of cassava tubers and Indigofera zollingeriana leaf flour combined with citric acid in the ration produced significant results (P>0.05) on the percentage value of broiler carcasses, according to the results of the analysis of variance. The average proportion of broiler carcasses in this investigation ranged from 68.90 to 72.3%.~~The results of the analysis of variance showed that the use of cassava tubers and *Indigofera zollingeriana* leaf flour combination supplemented with citric acid in the ration showed significant results (P>0.05) on the percentage value of broiler carcasses. In this study, the average percentage of broiler carcasses was 68.90-72.3%.~~ Khempaka et al. **[34]** reported that the average percentage of broiler ~~chicken~~ carcasses aged 28 days fed with 4%-20% fermented cassava tuber feed *Aspergillus oryzae* was 66.10%-67.42%. While the results of other studies showed that fermented cassava tubers given between 50-75% of the ration for 56 days gave a significant ~~effect~~ influence on the average value of carcass percentage**[2]**.

The content of metabolic energy in the ration is expected to stimulate broiler growth, resulting in a high live weight and a high carcass weight. According to study by~~This is consistent with the research of~~ Solikin et al. **[35]**~~,~~, carcass weight is directly correlated with the final weight of the chicken; the higher the chicken's weight, the higher the carcass weight.~~which states that carcass weight is closely related to the final weight of the chicken, the greater the weight of the chicken, the greater the carcass weight.~~ However, the percentage of carcass decreased with the use of a combination of cassava tubers and *Indigofera zolllingeriana* leaf flour with different compositions for each treatment ration even though the metabolic energy of all rations was made the same. Nevertheless, due to its low protein content, imbalanced amino acid profile, high fiber content, and presence of antinutrients, particularly cyanogenic glucosides, the use of cassava tubers in chicken feed is restricted (HCN).~~the provision of cassava tubers in poultry feed is limited due to its low protein content, unbalanced amino acid profile, high fiber content and the presence of antinutrients, especially cyanogenic glucosides (HCN).~~ Additionally, there are other antinutrients such as tannins and saponins discovered in *Indigofera zollingeriana* leaf flour. The use of *Indigofera zollingeriana* leaf flour in treatments T1 and T2 contributed 0.04-0.08 g/kg tannins and 0.00054-0.0010 mg/kg saponins. Meanwhile, the use of *Indigofera zollingeriana* leaf flour in treatments T3 and T4 contributed 0.13-0.17 g/kg tannins and 0.0016-0.0021 mg/kg saponins. Furthermore, Palupi et al. **[8]** mentioned that there are 0.29% tannins and 0.036 ppm saponins in Indigofera zollingeriana shoot flour. The chemical nature and dosage of tannins determine their antinutritional effects.

Tannins are heat stable, and they reduced the ability of both animals and people to digest protein. This was probably due to the fact that they either made protein partially inaccessible or inhibited digestive enzymes, which increased fecal nitrogen. The bitterness and throat-irritating properties of saponins, on the other hand, led to their recognition as anti-nutrient elements because of their negative impacts, including growth retardation and reduced food intake. The anti-nutritional properties of tannins depend upon their chemical structure and dosage. Tannins are heat stable and they decreased protein digestibility in animals and humans, probably by either making protein partially unavailable or inhibiting digestive enzymes and increasing fecal nitrogen. on other hands, saponins were recognized as anti-nutrient constituents, due to their adverse effects such as for growth impairment and reduce their food intake due to the bitterness and throat-irritating activity of saponins. Also, saponins were found to diminish the bioavailability of supplements and lessening enzym activity and it influences protein absorbability by restrain different stomach related catalysts like trypsin and chymotrypsin In addition, saponins were found to reduce the bioavailability of nutrients and decrease enzyme activity and it affects protein digestibility by inhibit various digestive enzymes such as trypsin and chymotrypsin **[25]**.

As a result, the presence of citric acid in the treatment ration aided in the absorption of *Indigofera zollingeriana* leaves. Citric acid accelerates the acidification of the digestive tract, allowing protein-digesting enzymes to work more quickly and actively **[36,37]**. The feeding of 15-20% cassava tubers and *Indigofera zollingeriana* leaf flour supplemented with 0.2% citric acid did not increase the percentage of broiler carcasses. This was because the addition of 0.2% citric acid did not increase nutrient absorption from the treatment ration.

Abdominal Fat Percentage

The outcome of the analysis of variance showed that the use of using cassava tuber and *Indigofera zollingeriana* leaf flour combination supplemented with citric acid in the ration showed no significant effect impact ($P>0.05$) on the percentage of abdominal fat in broiler chickens. The average percentage of broiler abdominal fat in this study was 0.76-1.29%. Compared to Khempaka et al. The percentage value of abdominal fat in this study was higher than that of Khempaka et al. **[34]** this study's percentage value of abdominal fat was higher, in which the average percentage of abdominal fat of 28-day-old broiler chickens fed fermented cassava tuber *Aspergillus oryzae* with 4%-20% feeding is 0.73%-1.09%. Cabel et al. **[38]** reported that the percentage of abdominal fat in broiler carcasses is normally between 0.73%-3.78%. This shows that the treatment of cassava tubers could not reduce abdominal fat deposits, because the energy content of each ration was the same so that the level rate of energy accumulation in the broiler body in the form of body fat was the same between treatments. Subekti et al. **[39]** stated that the formation of abdominal fat in the body of chicken occurs due to excess energy obtained by the feed they consume. The high crude fat content of the diet might also lead to the development of abdominal fat. Abdominal fat can also be formed from the high crude fat content in the ration. The nutritional requirements of broiler chickens must contain 3-4% crude fat in the ration, while in the combination of cassava tubers and *Indigofera zollingeriana* leaf flour in the crude fat treatment ration it is 5-6%. Fouad et al. **[40]** Also mentioned is the relationship between abdominal fat and total carcass fat, i.e., the higher the abdominal fat concentration, the higher the carcass fat content also stated that abdominal fat is related to total carcass fat, with the higher the abdominal fat content, the higher the carcass fat content. Fat in in the body of chicken is derived from feed and is produced through the process of fat synthesis in the feed.

According to the findings of this study, the combination of cassava tubers and *Indigofera zollingeriana* leaf flour supplemented with 0.2% citric acid in the treatment ration on the abdominal fat of broilers, which was given 5-20%, did not result in excess metabolic energy in the ration. The addition of 0.2% citric acid to the treatment ration increased the percentage of abdominal fat. This is in contrast to the research of Fik et al. **[16]**, which found that 0.5%-1.5% citric acid in feed can reduce the percentage of broiler abdominal fat because of the differences in ration energy content and dosage in broiler rations.

Small Intestine Villi Number

According to the results of the analysis of variance, adding citric acid to the diet along with cassava tuber and Indigofera zollingeriana leaf flour had a significant impact (P<0.05) on the number of ileum villi in broilers. The average number of ileum villi in broiler chickens in treatment T0 was significantly different from treatments T1 and T2 (P<0.05), according to the outcomes of additional tests.~~The results of the analysis of variance showed that the use of a combination of cassava tuber and *Indigofera zollingeriana* leaf flour supplemented with citric acid in the ration showed a significant effect (P<0.05) on the number of ileum villi of broilers. Based on the results of further tests, the average number of ileum villi in broiler chickens in treatment T0 was significantly different (P<0.05) with treatments T1 and T2.~~ This is because for the T1 and T2 treatments, 0.2% citric acid supplementation were added, although the pH of the ileum digesta obtained was the same as the T0 treatment (control). Nevertheless, the addition of citric acid was ~~able~~ effective to increase the number of small intestinal ilium villi in broilers. Hidayat et al. **[41]** stated that *acidifier* (citric acid) in general can replace the role of antibiotics and ~~is able~~was effective to increase the absorption of food extracts in the small intestine.

The results of further tests on T0 treatment were not significantly different (P>0.05) with T3 and T4 treatments. This is because in the T3 and T4 treatments there was an increase in the amount of CIF (a combination of cassava tuber and *Indigofera zollingeriana* leaf flour) by 15-20%, which means an increase in the concentration of antinutrients in the ration, namely HCN (cyanic acid), tannins, and saponins. Even though the T3 and T4 treatments contained 0.2% citric acid supplementation, the supplemented citric acid did not increase the number of villi as in T1 and T2 treatments.
Treatments at T1 and T2 had different effects (P<0.05) with treatments T3 and T4. This was due to the increasing concentration of antinutrients at T3 and T4, although the concentration of antinutrients (HCN, tannins and saponins) in this study was still within the limits that could be tolerated by broilers. Jayanegara et al. **[20]** stated that the maximum limit of HCN content in poultry is 10 mg HCN/kg feed, but a low concentration of antinutrients could affect the number of villi in the ileum of the small intestine of broilers in this study. The presence of antinutrients in the ration caused the number of small intestinal villi to be lower when compared to the research of Kusuma et al. **[42]** which reported that the lowest number of intestinal villi in the research of alternative feeds using a total of 60% palm kernel cake and onggok fermented food in the ration, is 386.60±19.91 µm.

Digesta pH

The outcome of the analysis of variance showed ~~that the use of~~using a combination of cassava tubers and *Indigofera zollingeriana* leaf flour supplemented with citric acid in the ration showed no significant ~~effect~~ impact (P>0.05) on the pH value. The average pH value in this

study ranged around 5.56-5.86. ~~Puspasari et al. (2016) stated that the normal digestive pH in each part of the small intestine is different, in the duodenum it is approximately 4.17-5.68, the jejunum around 5-6 and the ileum roughly 5.8-6.~~ Partial replacement of corn as an energy source in the ration with a combination of cassava tuber and *Indigofera zollingeriana* did not affect the pH value of the small intestine digesta until the feeding of 20% combination of cassava tubers and *Indigofera zollingeriana* in the ration. However, the pH value result in the ileum was relatively low, which was 5.56-5.86, when compared to the ileum which generally had a high pH value which was in the range of 6-7. Based on research of Mabelebele et al. **[43],** it is reported that ideally the pH value in broiler chickens ranges from 3.47 in the (gizzard) to 6.43 in the (small intestine) digestive tract.

Organic acid has a role in increasing the activity of proteolytic enzymes in the digestive tract and is also able to increase the natural immune response in poultry **[27,37,44]**. . The pH value and viscosity value have important factors in influencing the flow rate of nutrients in the digestive tract, this causes the digesta rate to be fast and allows a decrease in the digestive process and the absorption of nutrients becomes less effective, resulting in the decrease of the availability of nutrients for the synthesis of body tissues. According to Cahyaningsih et al. **[45]** a low degree of acidity in the digestive system (stomach and intestines) is able to optimize the absorption of nutrients in the stomach and intestines because it can slow down the rate of digestion, resulting in the optimization of feed nutrients absorption, so it does not interfere with the digestive process and nutrients utilization, or the growth of harmful bacteria will be inhibited **[37,44,46]**.

Viscosity

The outcome of the analysis of variance showed ~~The analysis of variance results showed~~ that the combination of cassava tubers and *Indigofera zollingeriana* leaf flour supplemented with citric acid in the diet showed no significant ~~effect~~ impact (P>0.05) on digesta viscosity. The average digesta viscosity in this ~~study~~ experiment was at (1.91 d.Pas±0.35) to (2.29 d.Pas±0.42). The viscosity in this study was higher than that of Emma et al. **[47]** where the lowest viscosity value is (0.10±0.004 d.Pas) and the highest viscosity (0.23±0.008 d.Pas).

The viscosity value in the digestive tract is directly proportional to the high or low pH value. Negative effects will appear if the viscosity of the small intestine increases, which will then reduce the efficiency of digestion by slowing the diffusion rate of endogenous enzymes to react with nutrients, as well as improving blood biochemistry in quail and broilers **[48]**. The value of viscosity is influenced by the level of viscousness, the higher the viscousness level, the worse the viscosity value will be, inversely if the viscousness level is low or watery, then the viscosity value is adequate **[49]**.

Viscosity has a significant impact on the value of performance data for poultry, with high or low viscosity values affecting the value of feed digestibility and feed flow rate while in the digestive tract. Furthermore, changes in the viscosity value can occur as a result of the type of feed consumed, such as feed with a high solubility value, which can result in a low viscosity value in a treatment. This occurrence is thought to be caused by the influence of *Non-Strach Pollysacharides* (NSP) solubility value in feed ingredients. According to Saputro et al. **[50]**, Non-Starch Polysaccharides (NSP), Resistant Starch (RS), and Short Chain Carbs are the most common forms of carbohydrates that pass through the colon without being hydrolyzed (SC). ~~most types of carbohydrates that reach the colon without hydrolysis include *Non-Starch Polysaccharides* (NSP), *Resistant Starch* (RS), and *Short Chain Carbohydrates* (SC).~~

CONCLUSION

In place of corn in the ration, a 10% mixture of cassava tuber and Indigofera zollingeriana leaf flour can be used as an energy source without having a negative impact on the broilers' consumption of the ration, body weight gain, ration conversion, live weight, percentage of carcass, percentage of abdominal fat, digesta pH, viscosity, or number of villi in the small intestine. ~~The use of a combination of cassava tuber and Indigofera zollingeriana leaf flour at a level of 10% supplemented with 0.2% citric acid can be used as an energy source to replace corn in the ration without negatively impacting ration consumption, body weight gain, ration conversion, live weight, percentage carcass, percentage of abdominal fat, digesta pH, viscosity, and number of villi in the small intestine of broilers.~~

LIST OF ABBREVIATIONS

CIF: cassava tuber and Indigofera zollingeriana leaf; CRD: Completely Randomized Design; DOC: Day Old Chick; C: control (basal diet); T0: without CIF/Control Diet; T1: 5% CIF substitute for corn + 0.2% citric acid; T2: 10% CIF substitute for corn + 0.2% citric acid; T3: 15% CIF corn substitute + 0.2% citric acid; T4: CIF corn substitute + 0.2% citric acid; FCR: feed conversion ratio.

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CONFLICT OF INTERESTS

The authors report no conflict of interest.

AUTHORS' CONTRIBUTION

All authors contributed to the work, discussed the results and contributed to the final manuscript.

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Table 1. Chemical Compostion

Item	Chemical Composotion (%)							ME (cal/kg)
	CP	EE	CP	Ca	P	Lisin	Metionin	
Comercial Feed	21.00	5.00	5.00	0.80	0.50	0.98	0.38	3000
Rice Bran	12.00	13.00	12.00	0.20	0.20	0.46	0.21	2580.63
Corn	8.60	3.90	2.00	0.20	0.10	0.26	0.19	3370.00
Concentrate	33.22	3.37	5.40	2.72	1.45	0.9	0.4	2276.99
Fish flour	40.53	5.64	2.20	5.00	2.50	2.08	0.76	2665.58
Cassava	01.10	0.55	2.30	0.32	0.71	0.08	0.04	3519
Indigofera Zollingeriana	28.98	33.30	8.49	0.52	0.34	1.75	0.43	2791.12
CIF	9.41	1.37	4.15	0.37	0.59	0.58	0.16	3300.6

Note: CP, Crude Protein; EE, Extract Ether; CF, Crude Fiber; Ca, Calcium; P, Phosfor; ME, Metabolisme Energy.

Table 2. Treatment Ration Compostion

Item	Treatments				
	T0	T1	T2	T3	T4
Comercial Feed (%)	100	26	26,5	27,5	28
Rice Bran (%)		40	35	30	25
Corn (%)		15	14,5	13,5	13
Concentrate (%)		14	14	14	14
Fish Flour (%)		5	10	15	20
Total	**100**	**100**	**100**	**100**	**100**
Crude Protein (%)	21,00	20,70	20,62	20,42	20,34
Extract Ether (%)	5,00	6,40	6,32	6,29	6,21
Crude Fiber (%)	5,00	5,17	5,31	5,48	5,62
Calcium (%)	0,80	1,19	1,21	1,21	1,22
Phosfor (%)	0,50	0,68	0,68	0,68	0,59
Lisin (%)	0,16	1,22	1,26	1,30	1,34
Metionin (%)	0,10	0,48	0,55	0,56	0,57
Metabolisme Energy (Ccal)	3000	2905,63	2903,68	2903,25	2901,30

Note: T0, without CIF; T1, corn substitution with 5% CIF; T2, corn substitution with 10% CIF; T3, corn substitution with 15% CIF; and T4, corn substitution with 20% CIF.

Table 3. Effect of feed treatment on performance, carcass and quality of the digestive tract

Variabels	Treatments				
	T0	T1	T2	T3	T4
Consumption (g/days)	54.91±2.29	56.86±2.22	56.99±1.33	55.17±1.69	54.96±1.61
ADG (g/days)	42.44^{a}±3.42	43.91^{a}±2.35	41.61^{a}±3.03	37.36^{b}±2.60	36.60^{b}±2.63
FCR	1.301^{a}±0.122	1.297^{a}±0.074	1.375^{a}±0.112	1.480^{b}±0.070	1.508^{b}±0.119
Live Weight (g)	1368^{a}±98.40	1316.8^{ab}±49.39	1227.8^{ab}±146.66	1175.2^{bc}±123.45	1153.6^{c}±226.64
Carcass (%)	72.36^{a}±2.37	71.57^{ab}±1.50	70.67^{ab}±0.73	67.72^{c}±4.29	68.90^{c}±1.90
Abdominal Fat (%)	1.22±0.36	1.16±0.46	0.76±0.27	1.16±0.17	1.29±0.30
Number of villi	244.4^{a}±10.96	286.4^{b}±10.21	273.0^{b}±11.29	240.6^{a}.±8.87	241.8^{a}±2.86
pH Digest	5.56±0.15	5.56±0.32	5.80±0.14	5.66±0.11	5.86±0.11
Viscosity	1.93±0.42	1.95±0.52	1.91±0.35	2.29±0.42	2.15±0.20

Note: ADG, Average Daily Growth; FCR, Feed Conversion Ratio; T0, without CIF; T1, corn substitution with 5% CIF; T2, corn substitution with 10% CIF; T3, corn substitution with 15% CIF; and T4, corn substitution with 20% CIF. Means with different superscripts letters in the same line differ significantly ($P<0.05$).

4. Bukti konfirmasi artikel accepted (27 Juli 2022)

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Journal of Advanced Veterinary and Animal Research

July 27, 2022

Dear Rizki Palupi Palupi

I am pleased to inform you that your manuscript titled "The Effect of The Use of Cassava Tuber (Manihot Esculenta) and Indigofera zollingeriana leaf flour combination as a Source of Energy Supplemented with Citric Acid in Ration on Broiler Small Intestine Characteristics and Productivity" (Manuscript Number: JAVAR-2022-06-097 was accepted for publication in the Journal of Advanced Veterinary and Animal Research.

For information, the article published by JAVAR is indexed in PubMed, PMC, ESCI (Web of Science Group), Scopus, and others.

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5. Bukti konfirmasi artikel published online (12 Oktober 2019)

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