Review Article

Analysis of Nutrient Digestibility, Hematological Status, and Blood Malondialdehyde Levels in Broiler Chickens Given a Combination of Cassava Tuber and *Indigofera zollingeriana* Leaves Flour as a Source of Energy in the Ration

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Abstract | This study aims to determine the effect of using a combination of cassava (*Manihot esculenta*) tuber and indigofera (*Indigofera zollingeriana*) leaves meal in rations on nutrient digestibility, hematological status and malondialdehyde levels in broiler chicken blood. The study was conducted experimentally with a completely randomized design, which consisted of 5 treatments and 5 replications. The treatment was the use of a combination of cassava tuber and indigofera leaves meal (70%+30%; CCI) in the ration, namely: T0 (0% CCI in the ration), T1 (5% CCI in ration), T2 (10% CCI in ration), T3 (15% CCI in ration) and T4 (20% CCI in ration). The material used was 100 day-old broiler chick (Cobb strain), which were placed in 25 postal cage units. The feed used was commercial broiler chicken feed: a mixture of concentrate, corn, rice bran and fishmeal, and CCI according to the treatment. The observed variables were nutrient digestibility (dry matter, crude protein and crude fiber), hematological status and blood malondialdehyde levels in blood of broiler chickens. Data were analyzed by ANOVA and further tested by Duncan Multiple Range Test (DMRT). The results of this study indicated that the CCI had a significant ($P<0.05$) effect on nutrient digestibility, hematological status and malondialdehyde levels in blood of broiler chicken. The CCI at a level of 10% in the ration increased hemoglobin levels by 17.09% and reduces malondialdehyde levels by 14.28% in broiler blood compared to commercial feed (control group). The results suggested that combination of cassava tubers and indigofera leaves (CCI) at a level of 10% could be used in broiler production without deteriorating the health of birds.

Keywords | Cassava, Broiler chickens, Indigofera, Malondialdehyde, Nutrient digestibility

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INTRODUCTION

Broiler chickens have a relatively fast harvest period and can guarantee the availability of meat that meet the nutritional needs of consumers. The main objective of raising broiler chickens is to gain body weight and carcass quality which is high and safe for human consumption. The demand for broiler chickens is relatively high because the meat is safe and tender, the body size is large, the chest shape is wide, dense and filled, the efficiency of the feed is relatively high, and the body weight gain is very fast (Juniarti and Ngitung, 2019). A good broiler carcass is linked with the management of feed supply, both in quality and quantity, so it is necessary to carry out a proper feeding program according to their needs. Efforts to produce good and quality broiler carcasses require feed containing nutrients and energy suitable for broiler chicken growth. One source of energy in the ration that can be used is cassava root.

Cassava (*Manihot esculenta*) is a tropical plant suitable for animal feed, especially for broiler chickens as an alternative energy source to replace corn. The metabolic energy content of maize and cassava tubers is almost the same. According to Wahyudi et al. (2017) that corn contains metabolic energy of 3350 Kcal/kg, and cassava tubers have metabolic energy of 3519 Kcal/kg (Abdlebasit et al., 2021). Cassava can be used as an energy source to replace corn up to 50% without affecting growth performance of broiler chicken (Chang'a et al., 2020). However, one of the obstacles in using cassava root is the low crude protein content which is 1.10%. This needs to be combined with protein source feed ingredients, one of the feed ingredients that can be used is *Indigofera zollingeriana*. According to Palupi et al. (2014) *Indigofera zollingeriana* shoot meal 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 507.6 mg/kg of beta-carotene, which is one of the precursors of vitamin A.

The presence of beta-carotene in indigofera can be used as functional feed in rations. Beta-carotene is a source of antioxidants in rations that reduce malondialdehyde levels in the blood of broiler chickens. A decrease in blood malondialdehyde levels is a condition indicating that there is an increase in antioxidant levels in the blood. The high content of antioxidants in broiler chicken blood can interfere with metabolism. This can be overcome by giving functional feed containing antioxidants (Palupi et al., 2014; Palupi et al., 2018). Based on the description above, it is necessary to research the proportion of the combination of cassava tuber and indigofera leaves (CCI) which can be used as an energy source to replace corn in rations and its effect on reducing malondialdehyde levels in broiler blood.

MATERIALS AND METHODS

The research was carried out in the poultry house of the Technology and Livestock Industry Department. The animals were cared according to the Indonesian Institute of Sciences Animal Welfare Guidelines. The approval of the experiment was granted from Universitas Sriwijaya with approval number KPPHP-2021-1. This research was designed in 2 stages of research activities within 1 year. The first stage of the study was to measure the digestibility of food substances from a combination of cassava tuber and *Indigofera zollingeriana* leaves as a substitute for corn in broiler rations. The nutrient composition of cassava tubers and Indigofera leaves was made to match the nutrient content of corn, especially its energy and protein content. The composition that approaches or equals the energy and protein content of corn was 70% cassava tubers and 30% indigofera leaves. In the second stage, measurements of broiler production performance and broiler carcass quality were carried out in vivo (Palupi et al., 2022), as well as measuring malondialdehyde levels in broiler blood.

Research Materials

This study used 100-day-old chicks (DOC) of broiler chickens (Cobb strain). The chickens were placed in experimental cages (postal cages). The size of each postal cage unit was 100cm long x 100cm wide x 60cm high, totaling 25 units. Each cage unit was a replicate that is occupied by 4 DOC of broiler chickens.

The study was conducted experimentally using a completely randomized design (CRD) consisting of 5 treatments and 5 replications. DOC chicks immediately after arrival at farm were given drinking water mixed with brown sugar with a concentration of 2% for the first 4 hours as a source of energy to restore the condition of DOC broiler chicks due to the stress of traveling from the hatchery to the rearing pen. Maintenance was carried out for 4 weeks. Feeding and drinking water were given ad libitum. The cages were cleaned twice a day, morning and evening.

Before use, the cages were cleaned of dirt adhering to the cage and disinfected and liming evenly. Disinfectants were sprayed evenly throughout the cage with the aim of eliminating germs and disease-causing microorganisms. Then the cage was left for 1 or 2 weeks. Equipment for food and drinkers as well as other cage equipment were washed, and then placed in each cage unit. Each cage was labeled with treatment and repetition to facilitate recording.

Research ration

The feed ingredients used to prepare the ration were ground corn, concentrate, rice bran and combination of cassava and *Indigofera zollingeriana* leaves 5 to 20% according to the treatment (Table 1). The rations were prepared and stirred every 1 week by stirring with a mixer. The rations were given ad libitum with a controlled frequency of administration 3 times a day, 50% in the morning, 30% in the afternoon and 20% in the afternoon (Palupi et al., 2022). The nutritional content of the rations used is listed in Table 1 and the composition of the rations used as a mixture of treatment rations is presented in Table 2. The ration was composed of iso protein and iso energy with an average protein content of 21% and a metabolic energy content of 2900 Kcal.

The composition of the treatment ration at T1 consisted of 40% corn and 5% CCI (a combination of cassava tubers and *Indigofera zollingeriana* flour), which consisted of 70%

Table 1: Nutrient content of feed ingredients for treatment rations

Raw material	Nutrient Content (%)							Metabolic energy Kcal/ kg
	Crude Protein	Crude fat	Crude fiber	Calcium	phos-phorus	Ly-sine	Methio-nine	
Commercial feed (BR1 comfeed)[a]	21.00	5.00	5.00	0.80	0.50	0.16	0.10	3000
Rice bran [b]	12.00	13.00	12.00	0.20	0.20	0.59	0.26	2580.63
Corn [b]	8.60	3.90	2.00	0.20	0.10	0.26	0.18	3370.00
Concentrate (KLK super comfeed) [c]	33.22	3.37	5.40	2.72	1.45	1.70	0.80	2276.99
Fish meal[d]	40.53	5.64	2.20	5.00	2.50	4.69	1.91	2665.58
Cassava [e]	01.10	0.55	2.30	0.32	0.71	1.03	0.39	3519
Indigofera zollingeriana [f]	28.98	3.30	8.49	0.52	0.34	1.57	0.43	2791.12
CCI [g]	9.41	1.37	4.15	0.37	0.59	1.19	0.40	3300.6

[a] Pt. Japfa Comfeed, [b] NRC (1994), [c] Pt. Cargill, [d] Utomo *et al.* (2013), [e] Abdlebasit *et al.* (2021), [f] Palupi *et al.* (2014), [g] Palupi et al., 2022.

Table 2: Composition of treatment rations during the study[1]

Raw Material	Treatments				
	T0	T1	T2	T3	T4
Commercial feed (%) Rice brand (%)	100	26	26.5	27.5	28
Corn (%)		40	35	30	25
Concentrate (%)		15	14.5	13.5	13
Fish meal (%)		14	14	14	14
Combination of Cassava and Indigofera / CCI (%)		5	10	15	20
Total	100	100	100	100	100
Crude Protein (%)	21.00	20.70	20.62	20.42	20.34
Crude Fat (%)	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
Phosphorus (%)	0.50	0.68	0.68	0.68	0.59
Lysine (%)	0.16	1.22	1.26	1.30	1.34
Methionine (%)	0.10	0.48	0.55	0.56	0.57
Metabolic Energy(Kcal)	3000	2905.63	2903.68	2903.25	2901.30

[1] Palupi *et al.* (2022)

cassava tubers and 30% *Indigofera zollingeriana* flour. In each treatment there was a decrease in concentration in the composition of corn by 5% (T1 40%, T2 35%, T3 30%, and T4 25%), while in CCI there was an addition of 5% in each treatment (T1 5%, T2 10%, T3 15%, T4 20%) (Palupi et al., 2022).

Preparation of Cassava and *Indigofera* Flour

Cassava tuber flour for feed was prepared by peeling the skin of the cassava followed by the washing. While making of *Indigofera zollingeriana* leaf powder for feed ingredients was carried out by harvesting the *Indigofera zollingeriana* leaves and separating the leaves from the branches to dry the *Indigofera zollingeriana* leaves. These both plant parts were dried. The process of drying was done in the sun and then continuing to dry using an oven until completely dry for easy grinding. The grinding was done using a flour machine as suggested earlier (Palupi et al., 2022).

Feed digestibility

Excreta collection at the age of 20 days was carried out. One chicken from each experimental unit was taken randomly with a body weight close to the average weight of chickens in each treatment. Chickens were placed in individual cages and each cage was equipped with a place to feed and a place to drink water. The placement of the chickens was done randomly, then the chickens were fasted for 6 hours. After that, plastic sheets were placed under the cage as a base for excreta storage. Chickens were given rations according to treatment, then excreta were stored for 24 hours. The excreta was sprayed with 0.1 N H_2SO_4 periodically every 2 hours during storage so that the nitrogen in the excreta did not evaporate. After 24 hours, each broiler chicken from the cage unit was weighed for its remaining feed and excretion. Excreta samples were dried, milled and then excreta samples and rations were analyzed to determine crude fiber and crude protein content (Tillman et al., 2005).

Digestibility of Dry matter (DM), Crude Protein (CP), Crude fiber (CF) was calculated using the following equations (Tillman et al., 2005).

Measurement of Hematological Status

Measurement of hematological status was carried out by taking blood samples from broiler chickens (n=10/group). Broiler blood samples were taken when the treatment was about to end, namely in the fifth week. The hematological status measured in this study was the number of erythrocytes, hematocrit values, and hemoglobin levels.

Erythrocyte counts were done using a set of tools called a hemocytometer. Blood staining with a special diluent, namely Hayem's solution, which is isotonic and functions as a dye for erythrocytes. The blood was diluted in the hemocytometer pipette and then put into the counting chamber and counted under a microscope.

The determination of the hematocrit value in blood was carried out by the microhematocrit method. The blood mixed with the anticoagulant was centrifuged using a centrifuge machine so that it forms layers. The layer consisting of erythrocyte granules was measured and expressed as a volume % of the total blood.

Blood with 0.1 N HCl solution will form brown hematine. The color was matched to the Sahli standard color by adding distilled water as a diluent. Determination of hemoglobin (Hb) levels was carried out using the acid Hematine method with a Sahli-Hellige Hemometer (g/dL).

Measurement of malondialdehyde

Antioxidant activity testing was carried out by observing the levels of malondialdehyde (MDA) in chicken blood plasma. Sample contains 0.25 mL of blood hemolysate was added in tube with 0.50 of 10% TCA solution. For blank 0.25 mL of distilled water was used instead of hemolysate with 0.50 mL of 10% TCA solution. Each tube was stirred (vortexed) and centrifuged using a clinical centrifuge at 4000 rpm for 1 minute. The supernatant layer from each tube was taken and put into a new tube. A total of 0.75 mL of 0.67% TBA solution was pipetted into each tube. The centrifugation tube was put into the boiling bath for 10 minutes, then cooled. The absorbance of each tube was read, namely the sample and blank on the spectrophotometer with a wavelength of 532 nm. The MDA profile was calculated by the formula: MDA profile ($nm/M^{-1}\ cm^{-1}$)= $A.\varepsilon^{-1}$

Information : A = Absorbance at a wavelength of 532 **λ.** nm = 153,000 M-1 cm -1

Data analysis

The data obtained during the study were analyzed using a completely randomized design (CRD) according to what was used. If there is a significant difference, a Duncan's Multiple Range Test was performed (Steel and Torrie, 1995).

RESULTS AND DISCUSSION

Effect of treatment on the digestibility of feed substances

The effect of the treatments on the digestibility of dry matter, crude protein, and crude fiber was calculated and results were presented in Table 3.

Dry matter digestibility

The study's results using a combination of CCI as a substitute for corn as an energy source in rations did not differ significantly in the digestibility of dry matter rations (Table 3). This is because the nutrient composition of the combination of cassava and *Indigofera zollingeriana* is not different from that of corn as an energy source. Feed ingredients' are organic matter and inorganic matter. One of the constituent of the organic matter is carbohydrates which are the most dominant in corn feed ingredients and the combination of CCI. According to Wahyudi et al. (2017) corn contains metabolic energy of 3350 Kcal/kg and cassava tubers have metabolic energy of 3519 Kcal/kg (Abdlebasit et al., 2021).

Crude protein digestibility

The results exhibited that corn substitution with a combination of cassava and Indigofera (CCI) had a significant effect on the protein digestibility (Table 3). Significant decrease in digestibility was recorded in T3 and T4 groups. This is due to the presence of anti-nutritional substances in the combination of cassava and indigofera which can inhibit the absorption of protein in the digestive tract of broiler chickens. The decrease in the digestibility of crude protein in the digestive tract of broiler chickens was due to an increase in the content of anti-nutritional substances, namely tannins, saponins and hydrogen cyanide (HCN) at T3 and T4 diets. *Indigofera zollingeriana* leaf flour contained 0.13 – 0.17 g/kg of tannins, 0.0016 – 0.0021 mg/kg of saponins and 0.42 – 0.56 mg/kg of HCN (Jayanegara et al., 2019). The tannins are known to bind with proteins, resulting in a decrease in the digestibility of amino acids

Table 3: Average digestibility of dry matter, crude protein and crude fiber of treatment rations

Treatments[1]	Dry matter Digestibility (%)	Crude protein digestibility (%)*	Crude fiber digestibility (%)
T0	89.14 ± 2.45	78.38 ± 3.12 [a]	76.89 ± 0.22
T1	88.68 ± 2.22	78.31 ± 2.56 [a]	77.74 ± 0.47
T2	88.93 ± 1.93	77.19 ± 3.14 [a]	76.55 ± 0.11
T3	88.57 ± 2.69	74.86 ± 2.33 [b]	77.08 ± 0.27
T4	87.61 ± 2.61	75.30 ± 2.24 [b]	75.93 ± 0.31

[1] T0(Control), T1(Combination of cassava tuber and Indigofera leaf meal at the rate of 5%), T2(Combination of cassava tuber and Indigofera leaf meal at the rate of 10%), T3(Combination of cassava tuber and Indigofera leaf meal at the rate of 15%) and T4(Combination of cassava tuber and Indigofera leaf meal at the rate of 20%).

*Different letters in the same column indicate significant difference (P<0.05).

Table 4: Erythrocyte, hematocrit, hemoglobin and MDA levels of broiler chickens

Treatments[1]	erythrocyte level (million/mL)	Hematocrit value (%)	Hemoglobin level (g/dL)*	MDA level (nmol/mL)*
T0	2.01 ± 0.40	19.60 ± 2.61	7.37 ± 0.61[a]	3.22 ± 0.36[a]
T1	2.18 ± 0.39	21.17 ± 1.10	8.38 ± 0.36[b]	2.86 ± 0.46[b]
T2	2.09 ± 0.66	20.71 ± 3.43	8.63 ± 0.25[b]	2.76 ± 0.27[b]
T3	2.24 ± 0.45	20.22 ± 2.19	9.78 ± 0.57[b]	2.16 ± 0.17[b]
T4	2.16 ± 0.64	20.90 ± 2.61	9.92 ± 0.02[b]	2.29 ± 0.30[b]

[1] T0(Control), T1(Combination of cassava tuber and Indigofera leaf meal at the rate of 5%), T2(Combination of cassava tuber and Indigofera leaf meal at the rate of 10%), T3(Combination of cassava tuber and Indigofera leaf meal at the rate of 15%) and T4(Combination of cassava tuber and Indigofera leaf meal at the rate of 20%).

*Different letters in the same column indicate significant difference (P<0.05).

which should be absorbed by the intestinal villi and utilized for growth and development of tissues in the animal's body, however, the complex bonds of tannins with proteins can be released at low pH in the digestive tract. so that protein is degraded by digestive enzymes and the content of amino acids can be utilized by livestock. Furthermore, the inhibitory activity of saponins on livestock growth is suspected because these compounds inhibit the activity of a number of digestive enzymes such as trypsin and chymotrypsin. In addition, the HCN in the ration can inhibit the production of ATP, so that livestock can experience a lack of energy (Jayanegara et al., 2019). Furthermore, cassava root starch has a relatively high amylopectin content, making it difficult for broilers to digest. This is in line with Rahmadani et al. (2021) those stated that the high concentration of amylopectin in rations can make the starch found in cassava difficult to digest because it has a relatively long chain. The molecular weight of amylopectin can reach 104-106 kDa, this value is quite high when compared to amylose compounds which are only around 100 kDa (Rakshit and Wang, 2016). Amylose involves 10–30 glucose units having α-1,4-linkages enabling self-association into a helix. Amylopectin is assembled from a large number of helices interconnected by α-1,6-branching to dominate each granule. Granule digestion by α-amylase depends on attachment along the helix length with release of maltose and maltotriose which includes α-limit dextrins from amylopectin (Moran Jr. 2019). This causes amylopectin to have a long branchedchain and is less soluble in water so that it will result in limited digestibility of cassava starch by poultry. As for citric acid supplementation with a dose of 0.2% in substitution of 15-20% of corn is also not able to work well because of an increase in anti-nutrients so that the absorption of nutrients in the digestive tract is less than optimal.

Digestibility of crude fiber

The results of analysis of variance of corn substitution as an energy source with a combination of cassava and Indigofera had no significant effect (P>0.05) on the digestibility of crude fiber (Table 3). This is because the crude fiber component of the combination of cassava and indigofera is the same as corn, so the ability of broiler chickens to digest the crude fiber component of the research rations is the same in all treatments. In line with the results of Moningkey et al. (2019) that the presence of pumpkin waste components in the ration did not affect the digestibility of the ration in the digestive tract of broiler chickens with various components of pumpkin waste in the ration. Prawitasari et al. (2012) also reported that the digestibility of crude fiber rations of Arabic chickens had no significant effect with the addition of *Azzolla microphilla* up to 9% in the ration.
Crude fiber consists of cellulose, hemicellulose and lignin, most of which cannot be digested by poultry and are bulky (Wahyu, 2004). Crude fiber can help intestinal peristalsis, prevent clumping of rations and speed up the rate of di-

gestion (Anggorodi, 1994). Crude fiber levels high more than 7%, will take longer time for digestion and the value of productive energy will be lower (Tillman et al., 1991). High crude fiber causes poultry to feel full, so it can reduce consumption because crude fiber is voluminous (Amrullah, 2003). Rations with high crude fiber content make them less palatable, resulting in lower consumption (North and Bell, 1990). Digestion of crude fiber in poultry occurs in the caecum with the help of microorganisms because birds do not have cellulose enzymes that can break down crude fiber (Wahyu, 2004). Digestion of crude fiber in poultry that occurs in the cecum reaches 20-30% (Suprijatna, 2010).

Effect of treatment on hematological status and antioxidant levels of broilers

The effect of substituting corn as an energy source with a combination of cassava (*Manihot esculenta*) and indigofera on the hematological status and antioxidant levels in broiler can be seen in Table 4.

Erythrocyte levels of broiler chickens

Based on the results of analysis of variance, the erythrocyte level of broiler chickens of various groups at the end of the study, had no significant difference ($P>0.05$; Table 4). This indicates that the replacement of corn up to 40% in the broiler ration does not have a negative impact on the formation of red blood cells of broiler chickens.

Amino acids are precursors to the formation of erythrocytes (erythropoiesis). Indigofera as part of CCI has a complex amino acid composition (Palupi et al., 2014). Indigofera's amino acids are components of protein. Indigofera's protein is dominated by pure protein with low NPN, making it easy to digest and absorb. This condition led to a smooth process of formation of broiler chicken erythrocytes in all treatments.

Blood hematocrit levels of broiler chickens

Corn substitution with a combination of cassava and indigofera up to 40% in the broiler ration did not affect ($P>0.05$) the hematocrit level of the broiler chickens (Table 4). Hematocrit is the percentage of blood volume containing red blood cells; the hematocrit value is influenced by the number and size of red blood cells. Factors that affect the hematocrit value are erythrocyte damage (erythrocytosis); decreased erythrocyte production are influenced by the number and size of erythrocytes (Gordeuk et al., 2019). The greater the number of blood erythrocytes, the hematocrit value will also increase. This is in accordance with Winarsih's statement (2005), that the hematocrit level is highly dependent on the number of erythrocyte cells, because erythrocytes are the largest mass of cells in the blood.

Hemoglobin level of broiler chickens

Substitution of corn as an energy source with a combination of cassava and indigofera had a significant effect ($P<0.05$) on broiler blood hemoglobin levels. As compared to control group, all CCI treated groups have a significant enhancement in the blood hemoglobin level. Protein is a source of amino acids needed by broiler chickens, especially the amino acid glycine and the mineral Fe which is a vital component for hemoglobin formation, so that the combination of protein and Fe minerals can maintain the amount of hemoglobin in the blood (Sriwati et al., 2014). In recent days, cassava peel was recognized as a most effective agent to increase the Fe level of ground water (Wahdini et al., 2022). The average hemoglobin value of broilers under natural light-dark cycle ranges from 7.26 - 10.03 g/100mL (Makeri et al., 2017). The average hemoglobin level of broilers consuming various commercial feeds ranged from 6.85 to 7.27 g/100 mL (Sufirianto et al., 2018) and Ilo et al. (2019) reported the hemoglobin level of broilers consuming different forms of commercial feed was 10.00 to 10.43 g/100mL.

MDA levels of broiler chickens

Lipid peroxidation can produce single oxygen, hydroperoxides and lipid epoxides. Aldehydes that can be formed in lipid peroxidation are MDA (Franco, 2004) and 4-hydroxynonenal (4-HNE) (Domingues et al., 2013). MDA is the main metabolite of arachidonic acid (20:4). This event can cause livestock metabolism to be disrupted and the overhaul of fatty acids (lipid peroxidation) as a result of increased production of free radicals, especially in the oxidative phosphorylation pathway when ATP synthesis increases. Free radicals can increase lipid peroxidation which will then undergo decomposition into malondialdehyde (MDA) in the blood. The MDA test can be used to measure peroxidation that occurs in lipid membranes.

Table 4 shows that the substitution of corn with a combination of cassava and indigofera had a significant effect ($P<0.05$) on MDA levels in broiler chickens. This is because the indigofera contained ration also acts as an antioxidant that can counteract the free radicals formed during rearing. Palupi et al. (2014) reported that *Indigofera zollingeriana* flour contains beta-carotene of 506.7 mg/kg where beta-carotene can function as an antioxidant that can ward off free radicals during chicken rearing.

Based on Table 4 it can be seen that the MDA levels in the treatment groups had lower MDA levels in the blood as compared to CCI untreated group (control). All broiler chickens were subjected to heat and humidity stress with the same Temperature Humidity Index (THI) values during the rearing period, but these broiler chickens did not experience the same metabolic stress, so the MDA levels in blood were different in chickens. This is in accordance with the research of Mushawwir and Latipuddin (2018) that an increase in THI will lead to an increase in ROS which has an impact on the formation of MDA, even though broiler chickens can still maintain normal body temperature because they can be homeostatic. ROS are oxygen-derived compounds that are more reactive than oxygen in basic conditions. These ROS can enter the bloodstream due to the influence of temperature and humidity. The condition of excess ROS compounds will cause oxidative stress. According to Anthonymuthu et al. (2016), ROS cause peroxidation of fatty acids with proteins, cellular nucleic acids and fats, resulting in lipid peroxidation. The main target in lipid peroxidation by ROS is poly unsaturated fatty acid (PUFA) in the lipid membrane. The interest has been focused on phospholipase A2 (PLA2), cyclooxygenase, and lipoxygenases which are enzymes involved in the formation of lipid peroxidation.

The presence of indigofera combined with cassava has an impact as an antioxidant in the body of broiler chickens, so that the levels of free radicals (MDA) become low in the blood of the broiler chickens. Low levels of free radicals can improve the performance of digestive enzymes produced by pancreas in broiler chickens, one of which is the protease enzyme. The more protease enzyme activity that converts protein into amino acids in the digestive tract, the faster the synthesis of hemoglobin. The more body iron, vitamins, amino acids, the faster the synthesis of hemoglobin and the formation of erythrocytes. This is in accordance with the opinion of Hoffbrand and Petit (1996) stating that substances needed for the formation of erythrocytes include iron, manganese, cobalt, vitamins, amino acids and the hormone erythropoietin.

CONCLUSION

The conclusion of this study is that the use of a combination of cassava tubers and indigofera leaves at a level of 10% in the ration has the same protein digestibility value as commercial feed. This inclusion is beneficial to increase the hemoglobin levels by 17.09% and reduces malondialdehyde levels by 14.28% in broiler chickens.

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CONFLICT OF INTEREST

The authors have declared that no competing interest exists.

NOVELTY STATEMENT

The novelty of this research is the use of a combination of cassava and Indigofera leaves as a substitute energy source for corn in broiler chicken feed.

AUTHORS CONTRIBUTION

All authors developed the theory and supervised the research. Rizki Palupi contributed to the sample collection and analysis calculations. All authors read and approved the final version of the manuscript for publishing in the present journal.

REFERENCES

Abdlebasit B. H., Mutaz S., Babiker, Z. A. Elnour. (2021). Utilization of Cassava Roots as a Source of Energy in Broiler Chicken feed and its effects on Blood chemistry and Digestibility. Sudanese Online Res. J. 2(1): 57-64. https://doi.org/10.51527/v2i1.9me

Amrullah I.K. (2006). Broiler Chicken Nutrition. Lembaga Satu Gunung Budi. Bogor.

Anggorodi H. (1994). General Animal Feed Science. Penerbit Gramedia. Jakarta.

Anthonymuthu T. S., Kenny E. M., Bayir H. (2016). Therapies targeting lipid peroxidation in traumatic brain injury. Brain Res. 1640: Part A:57–76. https://doi.org/10.1016/j.brainres.2016.02.006

Chang'a EP, Abdallh ME, Ahiwe EU, Mbaga S, Zhu ZY, Fru-Nji F, et al. (2020). Replacement value of cassava for maize in broiler chicken diets supplemented with enzymes. Asian-Australas J. Anim. Sci. 33(7):1126–1137.

Domingues R.M., Domingues P., Melo T., Pérez-Sala D., Reis A., Spickett C.M. (2013). Lipoxidation adducts with peptides and proteins: Deleterious modifications or signaling mechanisms? J. Proteom. 92: 110–131. https://doi.org/10.1016/j.jprot.2013.06.004

Franco D.J. (2004). Effect of heat stress of production, physiological and metabolic parameters in three varieties of laying hens.PhD Dissertation, Univ. of Nebraska, Lincoln.

Gordeuk V.P., Key N.S., Prchal J.T. (2019). Re-evaluation of hematocrit as a determinant of thrombotic risk in erythrocytosis. Haematologica. 104(4): 653–658. https://doi.org/10.3324/haematol.2018.210732

Hoffbrand AV., Pettit JE. (1996). Leukemia. In: Essential Haematology (Capita Selecta Haematology). 2nd Edition. Jakarta; Medical Book Publishers. pp: 127-155.

Ilo S.U., Maduneme F.C., Ogbu O.C., Okonkwo M.N. (2019). Haematological and biochemical characteristics of broiler finisher fed different feed forms (pelleted and mash). J. Agricult. Sustain. 12 (2) : 175-184.

Jayanegara A., Ridla M., Laconi E., Nahromi. (2019). Anti-

Nutritional Components in Feed. Bogor: IPB Press.

Juniarti N., dan Ngitung R. (2019). The Effect of Giving Seaweed Flour to Broiler Chicken Rations on Fat and Cholesterol Levels. Bionature. 20(1): 64-78. https://doi.org/10.35580/bionature.v20i1.9762

Makeri H.K., Ayo J.O.A., Aluwong T., Minka N.S. (2017). Daily rhythms of blood parameters in broiler chickens reared under tropical climate conditions. J. Circadian Rhythms. 15 (1): 1 – 8. https://doi.org/10.5334/jcr.151

Moningkey A.F., Fenny R. Wolayan, Cathrien A. Rahasia, Mursye N. Regar. (2019). Digestibility of organic matter, crude fiber and crude fat in broiler feed fed pumpkin (Cucurbita moschata) waste flour. Zootec. 39 (2) : 257 – 265 https://doi.org/10.35792/zot.39.2.2019.24870.

Moran E.T. Jr. (2019). Starch: Granule, Amylose-Amylopectin, Feed Preparation, and Recovery by the Fowl's Gastrointestinal Tract. J. Appl. Poult. Res. 28 (3) : 566-586. https://doi.org/10.3382/japr/pfy046

Mushawwir, A, U.H. Tanuwiria, Kurnia Kamil, L. Adriani, R. Wiradimadja, N. Suwarno. (2018). Evaluation of haematological responses and blood biochemical parameters of heat-stressed broilers with dietary supplementation of Javanese Ginger powder (Curcuma xanthorrhiza) and garlic extract (Allium sativum). Int. J. Poult. Sci .17:452-458. https://doi.org/10.3923/ijps.2018.452.458

North MO, Bell DD. (1990). Commercial Chicken Production Manual. Ed ke-4. New York: Chapman and Hall.

Palupi R, Abdullah L, Astuti DA, Sumiati. (2014). Potential and utilization of Indigofera sp. shoot leaf meal as soybean meal substitution in laying hen diets. JITV. 19(3):210-219. https://doi.org/10.14334/jitv.v19i3.1084

Palupi R., Sumiati., Astuti D. A, L. Abdullah. (2018). Assessing the Effectiveness of Top Leaf Meal of Indigofera zollingeriana to Substitute Soybean Meal through Evaluation on Protein Quality and Metabolizable Energy in Poultry Feed. Indonesian J. Fundamen. Appl. Chem.. 3(2): 47-53. https://doi.org/10.24845/ijfac.v3.i2.47

Palupi R., FNL Lubis, ANT Pratama. (2022). 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. J.Adv. Vet. Anim. Res. 9 (3): 471–480. https://doi.org/10.5455/javar.2022.i616

Prawitasari R.H., V. D. Y. B. Ismadi, I. Estiningdriati. (2012). Kecernaan protein kasar dan serat kasar, serta laju digesta pada yam arab yang diberi ransum dengan berbagai level Digestibility of crude protein and crude fiber, and digesta rate in Arabic yam fed with various levels of Azolla microphylla. Anim. Agricult. J., 1 (1): 471 – 483 Online at : http://ejournal-s1.undip.ac.id/index.php/aaj

Rahmadani M., Hermana W, dan Nahrowi. (2021). Provision of cassava flour added isoamylase in feed on the performance of broiler chickens. J. Ilmu Nutrisi dan Teknologi Pakan. 19(1): 1-5. https://doi.org/10.29244/jintp.19.1.1-5

Rakshit, Sujay dan Yi-Hong Wong. (2016.) The Sorghum Genome. Springer International Publishing. Hebei. Ravindran, V. and Abdollahi, M.R. 2021. Nutrition and Digestive Physiology of the Broiler Chick: State of the Art and Outlook. Animals. 11 (10): 2795. https://doi.org/10.3390/ani11102795

Sriwati.D., Eko Widodo dan Halim Natsir. (2014). The Effect of Using Cumin Flour (Cuminum Cyminum, L) in Feed on the Blood Profile of Broilers. Fakultas Peternakan, Universitas Brawijaya. Malang.

Steel R. G. D, Torrie J.H., (1995). Principles and statistical procedures of a biometric approach. Second printing. PT. Gramedia Pustaka Utama: Jakarta.

Sufiriyanto, Iriyanti, N., E. Susanti. (2018). Haematology profiles and performance of broiler chickens fed on commercial feed. Anim. Prod. 20(3):183-190. https://doi.org/10.20884/1.jap.2018.20.3.732

Suprijatna E (2010). The development strategy of local chickens based on local resources and environmentally friendly. Proceedings of the Nasional Seminar on Local Poultry IV, pp: 55-79.

Tillman A.D., Hartadi H., Reksohadiprodjo S., Prawirokusumo S., Lebdosoekojo S. (2005). Basic Animal Feed Science. Yogyakarta : Gadjah Mada University Press.

Utomo N. B. P., Susan dan Mia S. (2013). The role of fish meal from various raw materials on the growth of the sangkuriang catfish Clarias sp. J. Akuakultur Indonesia. 12(2): 58–168.

Wahdini M., Raharja M., Syarifudin A., As Z. A. (2022). Ability of Zeolite and Cassava Peel to Improving Iron (Fe) and pH in Drilled Well Water. JURNAL KESEHATAN LINGKUNGAN: Jurnal dan Aplikasi Teknik Kesehatan Lingkungan., 19(2): 255-260. https://doi.org/10.31964/jkl.v19i2.370

Wahyu J. (2004). Poultry Nutrition Science. 5th printing. Gadjah Mada University Press. Yogyakarta.

Wahyudi, F. T., Sudrajat D., dan Malik B. (2017). Metabolic Energy of Commercial Rations and Corn in Broiler Chickens. J. Peternakan Nusantara. 3(1): 40-54. https://doi.org/10.30997/jpnu.v3i1.854

Winarsih W. (2005). The Effect of Probiotics in the Control of Subclinical Slamonellosis in Chicken Pathological Features and Performance. Thesis. Pasca Sarjana. Institut Pertanian Bogor. Bogor.