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Feeding dietary inclusion of inulin on immune status, protein metabolism, and growth performance of Kedu chicken

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ABSTRACT

Indonesian Indigenous poultry originated from the central part of Java Island, called Kedu chicken, given diet added with inulin of dahlia tuber extract (IDTE) to evaluate body resistance and protein deposition. The experimental animals were 150 birds of 14-day-old Kedu chicken (initial body weight was 148 ± 16.4 g) until 12 weeks old. Dietary treatments were T1: farmer's diet with protein concentrate; T2: T1 + 1.2% inulin of IDTE, and T3: modified diet with common protein sources + 1.2% IDTE. A completely randomized design was assigned with 3 treatments (5 replications with 10 birds each). Parameters observed were rate of passage, protein and fiber digestibilites, Nitrogen (N) and Calcium (Ca) retentions, body resistance (plasma concentration of corticosterone/CTC, level of immunoglobulin A/IgA, and heterophyl-lymphocyte/H/L ratio), protein metabolism (fecal N^{τ}–methylhistidine/ N^{τ}–MH and muscle protein mass), feeds consumption, feed conversion ratio (FCR), and body weight gain (BWG). Analysis of variance and Duncan's test were applied to analyze data. Rate of passage, N and Ca retentions, BWG, IgA level, and muscle protein mass increased; in contrast, FCR, fecal N^{τ}–MH, and plasma CTC decreased due to the feeding effect of the T3 diet. All parameters, except IgA level, between treatment T1 and T2 indicated similar values. It can be concluded that a modified diet added with IDTE (T3) can modulate the increase in N retention and immune status, with the decrease in muscle protein degradation indicated by low fecal N^{τ}–MH excretion, and finally, brings about a better growth rate, with higher muscle protein deposition.

Keywords: Corticosterone, Fecal N^{τ}–methylhistidine, Immunoglobulin A, Kedu chicken, Modified diet, Protein deposition

INTRODUCTION

One of the Indonesian indigenous poultry breeds, known as Kedu chicken, originated from Kedu village in Central Java Indonesia. They are suspected to have higher body resistance and productivity than other native chickens . However, the advantages of Kedu chicken must be elucidated to clarify the real condition. Feed quality improvement with sufficient nutrient content is absolutely necessary to pay attention to clarify the productive performance capability, which is supported by body resistance condition. A high-quality diet is relevant with the supply of important nutrients such as protein, which can be converted into amino acids for further utilization and distribution into immune responsible-organs and target tissue for muscle protein deposition. A healthier animal is considered more effective in nutrient utilization, especially protein, for biological production processes. Nutrients supply and utilization strengthen production improvement can be supported by the supplementation of natural additives, such as inulin, that environmentally and healthy friendly.

Natural additive used in the present study was inulin derived from dahlia tuber in an extract form. The effectiveness of inulin is strongly dependent on the type and dose of supplementation. Previous report indicated that inulin derived form of dahlia tuber in an extract from fed to the crossbred native chickens was more effective than that in a powder form, with the best level at 1.17% (Krismiyanto *et al*., 2014). Inulin in modulating intestinal bacteria was by favoring a quick growth of beneficial bacteria, such as *Lactobacillus* sp. and *Bifidobacterium*, and inhibiting the growth of pathogens (Bucław, 2016). Poultry, especially broiler, given a diet supplemented with inulin extract from dahlia tuber was reported to have contribution to the improve of intestinal microbes balance due to the increase in lactic acid bacteria population and the reduce in *E. coli* count **(**Suthama *et al*., 2023). This physiological change was an indication of better intestinal health that bring about the enhance protein and some essential amino acids digestibility, and finally improve growth performance.

The increased protein deposition can be compared with the result of a previous study (Suthama *et al*., 2021) that broiler given additive of natural acidifier was indicated by low fecal excretion of N^{τ}–methylhistidine with higher Immunoglobulin A (IgA) concentration and thus resulted in higher growth rate. The improved protein deposition, in terms of muscle protein mass, was due to the increased synthesis and low degradation rates of muscle protein found in broiler chickens fed inulin or *Lactobacillus acidophilus* (Suthama *et al*., 2023). The protein deposition rate not only depends on the availability of substrate(s), such as protein supply but is also affected by the healthy condition or immune status of the animals. Acceleration of protein or amino acid metabolisms in relation to protein turnover can be mediated to be more pronounced when the birds have high body resistance. The increased immune status (Jamilah *et al*., 2013) supported by the availability of protein and/or amino acids (Ma'rifah *et al*., 2013) for protein synthesis is a reliable determinant and plays an important role in producing higher muscle protein deposition or muscle protein mass.

High productivity, including protein deposition, can be achieved when the animal is avoided from suffering unfavorable conditions of either nutritional or environmental stressors. There are many stress sources (stressors), as reported previously (Akinyemi and Adewole, 2021), that physiologically weaken immune status, lower the efficiency of nutrient utilization, and interfere growth and/or productivity. Blood parameters were very sensitive and important indicators of the physiological response in chickens against stress sources (Farag and Alagawany, 2018). Response of body resistance or immune system in poultry to environmental conditions can be indicated by the change in hematological conditions, such as heterophil-lymphocyte ratio (H/L ratio), plasma concentration of corticosterone (CTC), and immunoglobulin level (IgA, IgM, and IgY).

The immune response of Kedu chicken in connection with the feeding status of diet quality had never been elaborated yet. However, it was previously reported that the high adaptability of Kedu hens, indicated by lower blood CTC, was due to feeding diet with a common protein source since the early period (Wahyuni *et al*., 2011). It was reported that Indonesian crossbred native chicken given diet supplemented with prebiotic inulin, increased body resistance and nutrient utilization, and improved growth (Fajrih *et al*., 2014). Further evaluation using the same birds fed with supplemental probiotic *Lactobacillus* sp. and prebiotic inulin resulted in the increased meat antioxidant activity and muscle protein mass (Abdurrahman *et al*., 2016[a]), and reduced either meat fat or cholesterol content (Abdurrahman *et al*., 2016[b]).

It has been previously described that the blood profiles were the anticipated parameters to evaluate the adaptive mechanism of the animal to the environmental condition. In some animals, especially chickens, the protein metabolism adaptability to changing conditions and feeding status can be evaluated by specific determinants through fecal excretion of N^{τ}–methylhistidine (N^{τ}–MH). N^{τ}–methylhistidine (N^{τ}–MH) is an intermediary amino acid derived from the degradation of biological cells, mainly muscle protein, which cannot be reutilized by the chickens. Therefore, fecal excretion of N^{τ}–MH is a validation tool of the protein deposition index and reflects an indicator of adaptability response as expressed by muscle protein mass and growth rate. The rate of fecal N^{τ}–MH excretion can be associated with corticosterone (CTC) level, a stress-induced hormone, and immune status such as immunoglobulin A (IgA). As a comparison, poultry suffered protein/amino acid deficiency under hot climates, increased corticosterone and cortisol, and reduced protein deposition and growth (Qaid and Al-Garadi, 2021).

Immune status and efficiency of nutrient utilization are the two aspects that become the main attention of the present study to support the development of Kedu chickens in the original habitat (in situ at Kedu village, Temanggung district). The population of Kedu chickens in Temanggung district and the surrounding was very small, with approximately 13,500 birds from the total native chicken population of 164,901 birds (Central Bureau of Statistics of Temanggung Districts, 2020). Data are not available yet for the following year until present, so there is a special concern about the population. The decreasing population of indigenous poultry, especially Kedu chicken, is somehow apprehensive about the endangering population to be extinct breed in the near future. Therefore, it is important to pay attention to improve management and feed quality in relation to immune response and protein deposition or growth of Kedu chicken. The experiment conducted was at least to provide a contribution to the local governmental program, especially the conservation of indigenous poultry breeds.

MATERIALS AND METHODS

The experimental protocol used in the present study was approved by the Institutional Animal Care and Use Committee of Diponegoro University, Semarang, Central Java, Indonesia. The experiment was conducted *in situ* at the original habitat of the birds in collaboration with the officially formal Kedu chicken Farmer Group.

Experimental Animal and Diet

One hundred and fifty (150) birds of Kedu chicken as experimental animals were reared at the original habitat (in situ) until 12 weeks old. The birds were given a commercial standard diet for 2 weeks prior to providing treatment. The homogenous body weight of 14-day-old chickens (average body weight was 148±16.4 g) were divided into 3 groups of experimental diet (Table 1) until 3 months old. One week at the last period of the treatment, half of the chickens (75 birds) were separated and moved to the individual cage for excreta collection (digestibility trial) and blood sampling.

Dietary treatments tested in the present study were T1: farmer's formulated diet, T2: T1+inulin extract of dahlia tuber extract (IDTE) at 1.2%, and T3: modified diet using common protein sources+IDTE 1.2%. The procedure of IDTE preparation and the selected level at 1.2% was based on the results of a previous study (Krismiyanto *et al*., 2014). Dahlia tubers were obtained from the waste of dahlia flower plantation at Sumowono Sub-District, Semarang Regency, Central Java. The farmer's diet consisted of yellow corn, rice bran, protein concentrate, and premix, but the modified diet was composed of commonly used ingredients for poultry (Table 1). The nutrient content of experimental diets, especially protein and energy, were the same, with approximately 16% and 2600 kcal/kg, respectively. The birds were given dietary treatments *ad libitum* and free access to drinking water.

Parameters and Measurement Procedures

Immune status observed in the present study was based on blood concentrations of immunoglobulin A (IgA) that were measured using ELISA Kit (Kamiya Biomedical Company, Japan), and blood corticosterone (CTC) was determined according to the method of Mashaly *et al*. (1994). Heterophyl and lymphocyte were measured from smeared blood samples on microscopic glass slides, and then they were air dried and continued to stain using Giemsa staining (Thiam *et al*., 2022). The number of heterophils – was divided by the number of lymphocytes to obtain the heterophil-lymphocyte ratio (H/L ratio). Performances, especially body weight gain, were closely related to protein metabolisms, included protein digestibility, Nitrogen retention, fecal N^{τ}–methylhistidine (N^{τ}–MH) excretion, and muscle protein mass. Protein digestibility was measured according to the method of Atchade *et al*. (2019), and Nitrogen retention was determined based on the total collection method combined with an indicator of Fe_2O_3 (Abdollahi *et al*.,

Table 1. Composition and Nutritional Content of Experimental Diet

Ingredients	Farmer's diet (T1)	Farmer's diet + inulin (T2)*	Modified diet + inulin (T3)*
	---------------------------- (%)	---------------------------	
Yellow corn	51.0	51.0	51,0
Rice bran	18.0	18.0	15,5
Protein concentrate	29.0	29.0	–
Fish meal	–	–	9,8
Soybean meal	–	–	23,2
Premix	2.0	2.0	0,5
Inulin dahlia tuber extract	-	1.2	1.2
Total	100	101.2	101.2
Nutritional content** (%)			
Metabolizable energy*** (kcal/kg)	2,585.8	2,555.1	2655.8
Crude protein	15.5	15.3	16.1
Ether extract	6.6	6.5	6,2
Crude fibre	9.7	9.6	8,8
Calcium	3.2	3.2	2,1
Phosphor	1.5	1.5	0,9

*Nutrient content of T2 and T3 have been adjusted to 100% unit
**By analysis
****In vivo* test values of individual feedstuff prior to formulating a diet for the feeding trial

2021). Nitrogen retention, as a percentage of N intake, was determined as follows: N retention (%) = 100 × [((Feed intake × N_{diet}) − (Excreta output × $N_{excreta}$))/ (Feed intake × N_{diet})]

Protein is known to have a tight affinity to Ca during its metabolism. Therefore, Ca absorbability or retention was calculated based on the slightly modified formula for mineral retention in general (Thomas and Ravindran, 2010). The formula was as follows: Ca retention (percentage) = [(Ca consumed – Ca excreta)/Ca consumed]/100. Muscle protein mass was obtained by multiplying the percentage of meat protein with the weight of whole-body meat (Suthama *et al*, 2018). The meat protein content was measured using the Kjeldhal method (AOAC, 2005). The fecal N^{τ}–methylhistidine (N^{τ}–MH) excretion as an indicator of muscle protein degradation rate that influenced muscle protein mass was determined firstly according to the procedure of Hayashi *et al*. (1987) and developed further for the simultaneous measurement (Shiraishi *et al*., 2023).

Experimental Design and Statistical Analysis

The present study was assigned in a completely randomized design with 3 treatments based on experimental diets tested (T1, T2, and T3) as described in the sub-section of parameters and measurement procedures. Respective treatment consisted of 3 replications with 10 birds each. Analysis of variance was applied to evaluate the effect of treatment, followed by Duncan's multiple range tests with a probability of 5% (Steel and Torrie 1991).

RESULTS AND DISCUSSION

Rate of Passage, Nutrients Digestibility, and Retention

The feeding effect of inulin derived from dahlia extract tuber (IDTE) added to the farmer's diet (T2) and modified diet (T3) resulted in a slower rate of passage, higher protein digestibility and Ca retention. The three parameters were higher ($p<0.05$) than those of group fed farmers' diets without IDTE (Table 2). The other two parameters, fiber digestibility and nitrogen retention, were statistically the same as compared to those of either T1 or T3, but numerically indicated

Table 2. Nutrient Digestibility and Retention in 3-month-old Kedu Chickens Fed Dahlia Inulin Extract

Parameter	Dietary Treatment			SEM	*p*-value
	T1	T2	T3		
Rate of passage (min)	258.8±15.5^{b}	270.0±14.8^{b}	274.1±15.2^{a}	19.8	0.03
Protein digestibility (%)	79.9±3.9^{b}	83±4.8^{a}	85.8±4.2^{a}	12.2	0.02
Fiber digestibility (%)	34.2±1.7^{b}	37.5±1.9^{ab}	39.1±1.2^{a}	1.84	<0.001
Nitrogen retention (g/g diet)	0.038±0.02^{b}	0.041±0.02^{b}	0.050±0.03^{a}	0.004	<0.001
Ca retention (%)	51.1±2.6^{b}	57.9±1.5^{a}	56.7±1.9^{a}	1.90	0.02

$^{a\text{-}b}$ Means in the same row with different superscripts differ significantly ($p<0.05$)

the tendency of better values. Kedu chicken kept in an open house system under the original habitat condition having slightly high temperature can be overcome with additive supplementation, such as the inulin source in the present study. The slower digesta passage rate, due to the addition of IEDT as an inulin source, can increase nutrient availability, such as protein digestibility and Ca retention (Table 2).

All parameter improvements in T3 (Table 2), were closely correlated with growth performances (Table 4), which will be further discussed. Calcium (Ca) retention and protein digestibility are two nutrients that can be able to form a Ca-binding protein important for growing chickens. Current experimental reports indicated that Ca-binding protein (peptide) derived from chicken blood have a high stability (Yang *et al.*, 2024). However, all parameters in T1 were the lowest because there was no supporting effect of inulin dahlia tuber (IEDT), except fiber digestibility and nitrogen retention, which were similar to those in T2 and T3.

It was reported that inulin derived from dahlia tuber significantly brought about the slower digesta rate of passage in crossbred native chicken (Krismiyanto *et al.*, 2014) and improved protein and most essential amino acids digestibility in broiler (Alzueta *et al.*, 2010; Suthama *et al.*, 2023). The increased Ca retention in either T2 or T3 due to feeding IEDT (Table 2) was consistent with the report of Kozłowska *et al.* (2016) that inulin affects more than one target such as improving health condition, increases Ca absorption, and bone mineral content. Dahlia inulin functions as a “feed source” for endogenous beneficial bacteria, such as LAB, and it can be fermented to produce short chain fatty acid/SCFA (Cholis *et al.*, 2018; Suthama *et al.*, 2023). The production of SCFA leads to the reduction of intestinal pH and increased Ca absorption via the dissolution Ca and brought about the highly available for organ targets (Varvara and Vodnar, 2024). Production of SCFA also improved intestinal bacterial balance, since high LAB population and low pathogen, and given an impact on gut health as a factor that increased protein digestibility. The increase in protein digestibility has been proved previously in broiler fed inulin that higher SCFA production can be correlated with the conducive condition for LAB growth but inhibit *E. coli* (Suthama *et al.*, 2023).

Extract dahlia tuber supplementation is closely related to the dietary fiber content because it affects the ability of endogenous LAB to ferment inulin as an indigestible carbohydrate for host animal. The report of Zhang *et al.* (2023) indicated that crude fiber digestibility increase in broiler fed diet containing 7-9% crude fiber. This previous finding supported the present result by considering that the fiber content of the T3 diet, in particular, was the lowest (8.8%), and this condition is assumed to be a great possibility to maximize the activity of LAB in lowering intestinal pH. The lower the pH, the higher the concentration of H ion to penetrate to the fiber, that bringing about the change in crude fiber form from crystalline to amorphous and become easier to digest, although poultry does not possess fiber digestive enzyme. It was also consistent with the previous report that crude fiber cannot be hydrolyzed by intestinal digestive enzymes but it can be fermented by bacteria in the gut (Williams *et al.*, 2017; Jha and Mirsha, 2021). Inulin dahlia tuber extract (IDTE) supplementation as an inulin source in the present study was especially beneficial for the improvement of nutrients digestibility, including fiber. The increase in fiber digestibility can be stated as another side that causes nutrient digestibility improvement, such as protein and Ca retention.

Immune Status and Growth Performance

The blood level of corticosterone (CTC) and heterophyl-lymphocyte ratio (H/L ratio) in T2 and T3 were lower (P<0.05) than those in T1, but the concentration of immunoglobulin A (IgA) in T2 as well as T3 were higher (P<0.05) than tat in T1 because the addition of IDTE (Table 3). Inclusion of IDTE to the farmer's formulated diet (T2) and modified diet (T3) significantly (P<0.05) reduced fecal N^{τ}–methylhistidine (N^{τ}–MH) excretion (Table 4). The increased body weight gain (BWG) and muscle protein mass (MPM) were closely related to the amount of Nτ–MH excretion. Both BWG and MPM of T2 and T3 treatment were higher than those of the T1 treatment; on the other hand, the feed conversion ratio (FCR) was significantly (p<0.05) decreased with no change in feed consumption (Table 4). The improved body weight gain in T2 and T3 was also supported by the significantly (p<0.05) lower CTC and H/L ratio with higher IgA concentrations (Table 3). Amount of Nτ–MH excretion is an intermediary amino acid released from the degradation of muscle cell (actin and myosin) that cannot be reutilized by the body of host animal. It can be used to validate protein breakdown on growth rate as an end point of production.

A similar amount of feed consumption, and since BWG significantly (p<0.05) increased, it brought about a decrease in FCR (Table 4). The FCR value found in the present study was much higher than that of in modern poultry. However, it can be categorized well enough for Indonesian native poultry, considering that Kedu chickens are reared with an open house system under farmer conditions. Higher body weight gain (BWG) in T2 and T3 could be strongly affected by the increase in muscle protein mass (MPM) due to the supporting effect of protein digestibility and Ca retention. Calcium (Ca) and protein bond in the form of Ca-binding protein, in which protein functions as a substrate to accelerate muscle growth through the increased in protein synthesis (Suthama *et al.*, 2021), and Ca supports bone growth (Kozłowska *et al*., 2016).

There were similar results to the previous report that BWG increased in broiler given protein microparticle with acidifier due to enhanced protein digestibility and reduced N^{τ}–MH excretion (Suthama *et al*., 2021). N^{τ}–methylhistidine (N^{τ}–MH) excretion can be used to assess the rate of protein degradation (Eid *et al*., 2001; Suthama *et al*., 2021) and positively correlated with meat protein deposition that further determined growth rate in poultry. A similar amount of feed consumption, and since BWG was significantly (p<0.05) increased, it brought about a decrease in FCR (Table 4). The FCR value found in the present study was much higher than that of modern poultry. However, it can be categorized well enough for Indonesian native poultry, considering that Kedu chickens are reared with an open house system under farmer conditions. Higher body weight gain (BWG) in T2 and T3 could be strongly affected by the increase in muscle protein mass (MPM) due to the supporting effect of protein digestibility and Ca retention. Calcium (Ca) and protein bond in the form of Ca-binding protein, in which protein functions as a substrate to accelerate muscle growth through the increased in protein synthesis (Suthama *et al*., 2021), and Ca supports bone growth (Kozłowska *et al*., 2016).

Excretion of N^{τ}–MH is a valid index of muscle protein deposition in particular and growth in general for either broiler or layer. It has been found that the higher growth rate of broilers than that of layer was due to a lower rate of protein degradation in broilers. The validation

Table 3. Immune Status Indicator of 3-Month Old Kedu Chickens Fed Dahlia Inulin Extract

Parameter	Dietary Treatment			SEM	*p*-value
	T1	T2	T3		
Plasma CTC (ng/l)	6.84±0.20[a]	5.91±0.19[b]	5.02±0.18[c]	0.45	0.005
Plasma IgA (µg/ml)	279.5±10.3[b]	301.4±11.4[a]	316.2±12.5[a]	15.6	0.03
H/L ratio	0.71±0.03[a]	0.63±0.02[b]	0.65±0.02[b]	0.08	<0.001

[a-c] Means in the same row with different superscripts differ significantly (p<0.05)

of N^{τ}–MH excretion for protein deposition and growth was in agreement with the result previously reported (Hocking and Saunderson, 1992; Rogers dan Tomas, 2001). Some investigations indicated that either prebiotic, probiotic, or their combination had the same effect on protein accretion and growth in broilers through the regulation of N^{τ}–MH excretion. The present results were comparable with the previous reports that broiler fed *Aspergillus awamori* with or without *Saccharomyces cerevisiae* and *Aspergillus awamori* combined with or without selenium (Saleh, 2014) increased BWG and reduced abdominal fat. Both results above were due to the improvement of protein digestibility and energy utilization and the reduced amount of N^{τ}–MH excretion.

Protein utilization efficiency in T2 and T3 brought about the increasing growth rate with higher muscle protein deposition and resulted in high quality growth. The increasing level of IgA and lower H/L ratio (Table 2) were the supporting parameters. They can function as an indication of better immune response due to the effect of feeding additional IEDT in T2 as well as T3 (Table 3). Inulin is well known to have a beneficial effect on poultry production in general, and it exerts the same function for kedu chicken growth. The beneficial effect of inulin dahlia tuber compared to *Lactobacillus acidophilus* has been elucidated in broiler chicken (Suthama *et al*., 2023). Supplementation of inulin improved growth performance with high meat protein and lower meat fat mass, the same as those due to the effect of *L. acidophilus*. Higher nutrient supply because of inulin work effect in either T2 or T3, which provided a meaningful contribution to health or body defense (indicated by H/L ratio and IgA level) of kedu chickens. Thus, they can easily adapt to the environmental effect of the open house system, possibly exposure to heat stress. Blood parameters in relation to body resistance are a very sensitive and important indicator of the physiological response against stress sources (Borges *et al*., 2007; Farag and Alagawany, 2018). Sufficient nutrient supply, indicated by higher protein digestibility in T2 and T3 treatments (Table 2) during the growing period, makes it possible to overcome stressors that bring about chickens in a convenience condition. There are various stress sources (stressors) that physiologically can affect the comfortable life of birds and cause several deleterious changes, including the efficiency of nutrient utilization. It was well documented that stress impaired immune response and function, resulting in high disease susceptibility, and the accumulation effect reduces feed intake and growth (Abo-Al-Ela *et al*., 2021).

Adequate protein supply, indicated by higher digested protein, was the important nutritional factor favorably determining the rate of growth of Kedu chicken since N^{τ}–MH excretion was low. Indonesian crossbred native

Table 4. Fecal N^{τ}–methylhistidine (N^{τ}–MH) Excretion and Growth Performance of 3-Month Old Kedu Chickens Fed Dahlia Inulin Extract

Parameter	Dietary Treatment			SEM	*p*-value
	T1	T2	T3		
Fecal N^{τ}–MH (µmol/day)	9.20±0.31^{a}	8.87±0.39^{ab}	8.50±0.37^{b}	0.42	<0.001
Muscle protein mass /MPM (g/bird)	94.60±2.7^{c}	118.14±4.4^{a}	120.30±3.9^{a}	11.8	0.03
Feed consumption (g/bird/day)	64.8±2.2	64.39±2.1	63.34±3.2	2.92	0.48
Body weight gain/BWG (g/bird/day)	24.15±1.61^{b}	28.11±1.95^{ab}	31.5±1.90^{a}	1.64	0.004
Feed conversion ratio (FCR)	2.68±0.10^{a}	2.29±0.12^{b}	2.0±0.11^{b}	0.08	<0.001

$^{a\text{-}c}$ Means in the same row with different superscripts differ significantly ($p<0.05$)

chickens given a diet supplemented with prebiotic inulin, increased body resistance and nutrient utilization and finally improved growth (Fajrih *et al*., 2014). Further studies using the same birds fed diet supplemented with inulin and *Lactobacillus* sp. improved nutrient utilization and muscle protein mass (Abdurrahman *et al*., 2016[a]), also resulted in better growth quality because meat fat decreased and body resistance improved (Abdurrahman *et al*., 2016[b]). Lower blood CTC concentration (Table 3), a protein degradative hormone, was consistent with the reduced excretion of faecal N^{τ}–MH (Table 4), indicating the higher availability of substrate (protein) for muscle protein mass (Table 4). Muscle protein mass improved due to an increased amount of protein supply (highly digested protein) and reduced faecal N^{τ}–MH excretion, although no change in intestinal protease activity (Suthama *et al*., 2014). The increased protein supply because of protein digestibility improvement and lower faecal N^{τ}–MH excretion in T2 and T3 contributed to the higher availability of substrate (protein) for the metabolic process of protein synthesis that supports growth. It is reliable to state that the improved protein deposition and BWG in both treatments, T2 and T3, was induced by IDTE supplementation.

In contrast, were given a diet without IEDT as an inulin source (T1) resulted in significantly highest CTC and lowest IgA concentrations; it can be assumed that birds suffered an excess on the immune system because no supporting effect of inulin dahlia tuber (IDTE). The higher faecal N^{τ}–MH excretion in T1 (Table 3) also strengthens the assumption that the muscle protein degradation increased at a high rate. Hence, growth developed very slowly (Table 2). Corticosterone (CTC) is a responsible hormone for ensuring the level of body nutrient stores in furnishing the energy demand in relation to the surroundings, especially when the chickens faced on the unfavourable condition (Butcher and Miles, 2014). This CTC hormone is known to increase the use of glucose and amino acids, especially when it was lack of energy supply condition. It is also considered from the viewpoint of nutrient supply since the birds were fed a diet containing very high rice bran (Table 1) caused lower N retention and higher faecal N^{τ}–MH excretion in T1. Lower Ca and N retention in T1 can be associated with a faster rate of digesta and lower fiber digestibility (Table 2) due to the feeding effect of high dietary fiber content (Table 1). Attention is needed when feeding rice bran to poultry due to their limitation in digesting fiber. However, local chicken (Kedu chicken) used in the present study was considered to have higher capability in using rice bran. As a comparison, it was recommended that rice bran for modern poultry, such as broiler, was allowed to be maximal as much as 15%, with fermented rice bran (Supriyati *et al*., 2015). Diets for T1 and T2 in the present study were composed of the same amount of slightly high rice bran, but results were different due to the beneficial effect of inulin extract of dahlia tuber (IDTE) in T2 treatments.

The results of the T1 treatment suggested that an insufficient supply of amino acid due to low protein digestibility (Table 2) should be added with an additive as a supporting substance in order to ensure an increase in nutrient utilization efficiency. Therefore, in the case of low nutritional supply (protein and/or amino acid) without any additive supplementation, such as T1 in the present study, muscle protein synthesis did not achieve a high rate since the level of CTC was also high (Table 2). A group of CTC hormones, including glucocorticoids, can regulate skeletal muscle protein and glucose metabolism (Kuo *et al*., 2013). Birds reared under the open house system were assumed to suffer heat stress without boosting the effect of inulin derived from IEDT (T1) and brought about the increased circulating CTC, low H/L ratio, and IgA concentration (Table 2), and thus reduced immune status. Further effect, it can be associated with the decrease in the rate of protein synthesis, and on the other hand, increases degradation as indicated by the high N^{τ}–MH excretion (Table 4). This physiological condition arrived at the reduced protein deposition (low muscle protein mass) and produced poor body weight. However, it is postulated that additive supplementation is considered important for chickens kept in hot climates. Previous studies proved that higher meat products with improved breast muscle characteristics and reduced both fat mass and cholesterol content in crossbred local chicken fed diet with additional

Lactobacillus sp. and prebiotic inulin (Abdurrahman *et al*., 2016[a]; 2016[b]), although the birds were kept under open house system. It was also reported previously (Al-Aqil *et al*., 2019) that broilers kept under a closed house system suffered less stress, as indicated by lower blood CTC concentration than those in an open house.

CONCLUSION

Indigenous poultry, kedu chickens, provided farmer's formulated diet (T2) added with inulin derived from dahlia tuber extract (IDTE) produced higher immune status as indicated by high immunoglobulin A (IgA), low blood corticosterone (CTC) and H/L ratio. Therefore, it led to the improvement of growth performance with higher muscle protein mass.

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CONFLICT OF INTEREST

We certify that there is no conflict of interest with any financial, personal, or other relationships with other people or organizations related to the material discussed in the manuscript.

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