Nutrient digestibility of broiler chicken fed diets supplemented with probiotics phytase-producing

A S Anggraeni, A E Suryani, A Sofyan, A A Sakti, L Istiqomah, M F Karimy, I N G Darma

Research Division for Natural Product Technology, Indonesian Institute of Sciences, Indonesia

Corresponding author’s email: ayus001@lipi.go.id/ayu.anggraeni07@gmail.com

Abstract. Phytic acid is an anti-nutrition substance due to its ability to bind minerals such as Mg, Fe, Zn, Mn, Ca, and enzyme proteins resulted in decrease in mineral solubility. Phytic acid levels can be reduced by phytase [myo-inositol hexacyrophosphate phosphohidrolase]. Microbial phytase can come from fungi, bacteria, and yeast sources. This research was conducted with the aims to evaluate the effect of probiotics phytase-producing supplementation as feed additive on nutrient digestibility of broiler. This study was performed in a completely randomized design for the environmental design, which consist of five treatments. Negative control (without probiotics phytase-producing), LAB (Lactobacillus plantarum A1-E) phytase-producing, yeast (Candida tropicalis TKD-3) phytase-producing, probiotic consortium (L. plantarum A1-E and C. tropicalis TKD-3) and positive control (commercial probiotic). The observed variables are feed intake, energy intake, excreta weight, energy excretion, nitrogen intake, nitrogen excretion, nitrogen retention, apparent metabolizable energy (AME), true metabolizable energy (TME), apparent metabolizable energy corrected for nitrogen (AMEn) and true metabolizable energy corrected for nitrogen (TMEn). Data were analyzed by using analysis of variance (ANOVA) and followed by Duncan’s multiple range test to distinguish the effect of different treatment mean. The results of variation analysis on AMEn and TMEn showed significant different while another parameter showed no significant difference between treatments. The use of C. tropicalis TKD-3 as probiotics phytase-producing on broiler chicken diet increase nutrient digestibility, especially apparent metabolic energy corrected nitrogen, and true metabolic energy corrected nitrogen.

1. Introduction

Poultry generally consumes grains such as corn, rice processing waste and other high-fiber agro-industrial wastes with low digestibility, phytic acid and high phytate salts which disrupt the absorption of minerals [1]. Phytic acid (myo-inositol hexakhiridihydrogenphosphate, IP6) is a polyanionic molecule with six phosphate groups and is capable of forming insoluble complexes with divalent cations, starch and protein, reducing their availability for poultry [2]. Phytic acid is an anti-nutrient substance because it can bind to minerals such as Mg, Fe, Zn, Mn, Ca and enzyme proteins which results in decreased mineral solubility so that the availability of minerals becomes low [3]. Minerals deficiency cause present of phytates can result in instability and fragility of the bone, which can reduce productivity in broiler chickens.
Phytic acid levels can be reduced by the phytase [myo-inositol hexaphosphate phosphohydrolase], an enzyme that catalyzes sequential phytate hydrolysis of phosphate and inositol from Penta- to mono-phosphate to reduce or eliminate the anti-nutritional effects and produce divalent cationic bioavailability of essential minerals in the feed [4]. Poultry can produce some endogenous phytase [5] but this is insufficient for the effective hydrolysis of dietary phytates compounds in feed ingredients, so resulting in low absorption of minerals in poultry. The addition of microbial phytases will ameliorate the detrimental effects of phytates in the poultry diets [2].

Phytase activity has been detected in plants, animal tissues, and from several microbial species. Microbial phytases activity could be hydrolyzed phytate more efficiently than phytase derived from plants. Microbial phytase can be derived from fungi, bacteria, and yeast sources [6]. Lactic acid bacteria (LAB) is one source of microbial phytase. Extracellular phytase activity of several LAB isolated from various sources has been reported such as L. amylovorus, L. plantarum, Enterococcus faecium, Leuconostoc gelidum, and Bacillus subtilis [7-10]. Intracellular phytase activity is found in L. sanfranciscensis, L. fructivorans, L. lactis, and L. alimentarius [11], Bifidobacterium dentium, L. reuteri, and L. salivarius [4], L. plantarum and L. fermentum from the Pennisetum americanum L. (Leeke) plant [12], and Pediococcus pentoaceus [13]. In addition, yeast has been reported as a microorganism that is useful for phytase production [14-21]. The phytase secretion can be intracellular, periplasmatic, directly to the culture medium or as bound to the cell wall. The genes encoding yeast phytases have so far been described for Debaryomyces castelli (PHYDe), Kodamaea ohmeri (PHY1), Hansenula fabianii (Hphytase), Pichia anomala (PPHY), Schwanniomyces occidentalis, Pichia stipitis (PHO5), Pichia guilliermondii (PGUG), Kluyveromyces marxianus, and Saccharomyces cerevisiae (PHO3, PHO5, PHO11, PHO12) [22]. Pichia anomala is reported to have a high activity of intracellular phytase, and an insignificant extracellular phytase activity [23,24]. The main site of phytase degradation by microbial phytase in poultry relatively in the distal gastrointestinal tract [25]. Application of microbial phytase in poultry has been reported in the form of enzyme as feed additive. Omojola et al. [26] stated that the addition of phytase improved the broiler performance, feed intake, FCR, and increase the apparent retention of nitrogen. Dietary supplementation of phytase in diet improved the average daily gain, increased the levels of serum calcium (Ca), tibia Ca and P, AID of AA and apparent digestibility of energy and Ca in starter stage [27]. However the use of probiotics with phytase activity specifically in the form of a consortium of LAB and yeast to increase poultry performance has not been explored yet. Probiotic application on poultry diet has an impact on changes in activity microbiota in the digestive tract by working directly or indirectly and possible changes in the composition of microorganisms. This change will potentially affect feed digestibility in poultry consume it. This research aims to evaluate the effect of phytase-producing probiotics supplementation as feed additive on nutrient digestibility of broiler.

2. Materials and methods

2.1. Preparation of probiotics phytase-producing

*Lactobacillus plantarum* A1-E with phytase activity was isolated from intestine of Indonesian native chicken used as probiotic candidate for poultry [28]. Probiotic candidate of LAB was carried out using the spray drying method according to Barbosa-Canovas et al. [29] while yeast according to Chandraleka et al. [30]. LAB isolate was grown in de Man Rogosa Sharpe (MRS) Agar media and incubated at 37 °C for 18 h, then cultivated in MRS Broth media. LAB was propagated in MRS Broth media and incubated at 37 °C for 24 h. *Candida tropicalis* TKD-3 strain HNJ-1 was isolated from soybean *Tempeh* as a traditional fermented food from Indonesian. *Candida tropicalis* isolate was grown in Choloramphenicol Yeast Glucose (CYG) Agar media and incubated at 30 °C for 48 h, then was cultured on CYG Broth media and incubated at 30°C for 48 hours. Each probiotic culture was then centrifuged to obtain the biomass (pellets). The biomass was mixed with filling material consist of 20% (w/v) sterile skim solution for LAB and sterile maltodextrin solution (40%, w/v) for yeast to improve its viability during the microencapsulation process. The mixture was homogenized using a
plate stirrer. Encapsulation using spray dryer was carried out according to operating condition as follows: 110 °C inlet air temperature, exhaust 68 °C, and the speed of pump 3. Dry culture of LAB adjusted to 6x10⁸ CFU/g, while yeast was obtained a population of 4x10⁶ CFU/g of cell density.

2.2. Birds and experimental design

*In vivo* assay was conducted in the field laboratory of BPTBA LIPI. This study had fulfilled the ethical clearance requirement (Certificate No. 00097/04/LPPT/VIII/2018). Probiotic with phytase activity was supplemented in finisher diet — maintenance of broiler chickens in a closed house enclosure measuring 10x10 meters. A total of 100 broiler chickens (Cobb strain) 28 days old (finisher period) were used in this experiment. The experimental design used a completely randomized design with 5 treatments, 4 equal replicates, and 5 broilers each. The treatments were: YA: negative control, YB: *L. plantarum* A1-E (6x10⁸ CFU/g), YC: *C. tropicalis* TKd-3 (4x10⁶ CFU/g), YD: consortium of *L. plantarum* A1-E and *C. tropicalis* TKD-3, YE: commercial probiotics (10¹¹ CFU/g) contained *Bacillus subtilis*, *L. acidophilus*, *S. cerevisiae*, and *Aspergillus oryzae* produced by Han Poong Industry C0., Ltd, Korea.

| Table 1. Feed composition (%) and nutrient content of the basal feed |
|---------------------------------------------------------------|
| Ingredients                                                   | Composition (%) |
| The feed of broiler finisher stage                            |                 |
| Corn                                                         | 55.00           |
| Rice bran                                                    | 6.00            |
| Soybean meal                                                 | 29.50           |
| Meat bone meal                                               | 3.00            |
| Palm oil                                                     | 2.50            |
| Premix                                                       | 0.50            |
| Di-Calcium Phosphate (DCP)                                   | 0.80            |
| Salt (NaCl)                                                  | 0.10            |
| Limestone                                                    | 1.70            |
| L-Lisin                                                      | 0.60            |
| DL-Metionin                                                  | 0.30            |
| Total                                                        | 100.00          |
| Nutrient content                                             |                 |
| Moisture (%)                                                 | 10.82           |
| Crude protein (%)                                            | 19.14           |
| Ether extract (%)                                            | 6.90            |
| Crude fiber (%)                                              | 6.91            |
| Ash (%)                                                      | 6.73            |
| Calcium (%)                                                  | 1.76            |
| Chloride (%)                                                 |                 |
| Total phosphorus (P)                                         | 1.17            |
| Aflatoxin (ppb)                                              | 46.71           |
| Metabolic energy (kcal/kg)                                   | 2,775.2         |
| Amino acid (%)                                               |                 |
| - Lysine                                                     | 0.96            |
| - Methionine                                                 | 0.25            |
| - Methionine + Cysteine                                      | 0.71            |

Chickens are given access to drinking water and ad libitum feed, the composition of feed presented in table 1. Chickens were vaccinated with ND-IB vaccines on the first day of age, IBD vaccine at 12 days of age, and ND Lasota vaccine at 20 days of age. Experiment was performed during 7 days, started at 28 up to 35 days old. Nutrition of feed was analyzed by the method described in the Association of Official Analyzing Chemists [31].
2.3. *In vivo assay and collection of samples*

Measurements of chicken digestibility refers to the modified methods [32] when the chicken entered the finisher period (28 days of age). Five chickens from each experimental unit separated randomly into a metabolic cage to measure energy digestibility. The metabolic cage was provided with plastic pads to accommodate chicken excreta. Each probiotic treatment was given to 20 chickens divided into four replication groups. Before treatment, chickens were adapted for three days without probiotic administration and fed to spend ± 30 g/head for 24 hours then fastened from feed for 24 hours, while water provided *ad libitum*. Each phytase-producing probiotic was given according to the administration of additive dose (1% of feed consumption). Excreta from chickens is accommodated to ensure there is no feed in the digestive tract. Excreta was carried out for 36 hours since the probiotic treatment was stopped. Collected excreta immediately sprayed with H$_2$SO$_4$ 0.01 N to bind the nitrogen and avoid evaporation. Excreta samples dried at 50 °C oven for 48 hours, then milled and analysed for moisture content, crude protein, and gross energy based on the [31] method.

The value of energy retention consists of energy consumption and energy excretion, while nitrogen retention includes nitrogen consumption, excretion nitrogen based on [33] calculations methods and for nitrogen retention based on [34] as follows:

Energy consumption

\[ \text{Energy consumption} = GE \times Fi \]  

(1)

Energy Excretion

\[ \text{Energy excretion} = GEk \times Ek \]  

(2)

Consume Nitrogen

\[ \text{Consume nitrogen} = \frac{Fi \times CP}{6.25} \]  

(3)

Nitrogen excretion

\[ \text{Nitrogen excretion (NE)} = \frac{Ek \times CPk}{6.25} \]  

(4)

Endogenous Nitrogen Excretion

\[ \text{Endogenous nitrogen excretion (ENe)} = \frac{E \times CPe}{6.25} \]  

(5)

Nitrogen retention (RN)

\[ RN = Fi \times Nf - (Nf - Ek) \]  

(6)

Information:

GE = Gross energy of feed (kcal kg$^{-1}$)

Fi = Feed intake (gram)

GEk = Gross energy excreta of treated chicken (gram)

Ek = excreta weight of treated chicken (gram)

CP = crude protein on feed (%)

CPk = crude protein excreta of treated chicken (%)

Ee = excreta weight of control/endogenous chicken (gram)

CPe = crude protein excreta control/endogenous chicken (%)

Nf = nitrogen on feed (%)

Calculation of metabolic energy based on [33] calculations methods as follows:

Apparent Metabolizable Energy (AME) (kcal/kg)

\[ \text{AME} = \frac{(GE \times Fi) - (GEk \times Ek)}{Fi} \times 1000 \]  

(7)

True Metabolizable Energy (TME) (kcal/kg)

\[ \text{TME} = \frac{(GE \times Fi) - [(GEk \times Ek) - (GEe \times Ee)]}{Fi} \times 1000 \]  

(8)
Apparent metabolizable energy corrected to zero nitrogen balance (AMEn) (kcal/kg)

\[ \text{AMEn} = \frac{(\text{GE} \times \text{FI}) - [(\text{GEEk} \times \text{Ek}) + (8.22 \times \text{RN})]}{\text{FI}} \times 1000 \]  

(9)

True metabolizable energy corrected to zero nitrogen balance (TMEn) (kcal/kg)

\[ \text{TMEn} = \frac{(\text{GE} \times \text{FI}) - [(\text{GEEk} \times \text{Ek}) - (\text{GEEe} \times \text{Ee}) + (8.22 \times \text{RN})]}{\text{FI}} \times 1000 \]  

(10)

Information:

GE = Gross energy of feed (kcal kg\(^{-1}\))

K = Feed consumption (gram)

GEEk = Gross energy excreta of treated chicken (grams)

Ek = Excreta weight of treated chicken (gram)

GEEe = Gross Energy excreta chicken control (gram)

Ee = excreta weight of chicken control (gram)

RN = Nitrogen retention (gram)

8.73 = Corrected coefficient as uric acid (kcal g\(^{-1}\) RN)

2.4. Statistical analysis

The normality of the data and homogeneity of the variance were verified. Data will be analysed using variance analysis or analysis of variance (ANOVA) using the CoSTAT [35]), and subsequent Duncan Multiple Range Test [36].

3. Results and discussions

Energy feed intake can be defined as the amount of energy available in a feed or diet that enters the digestive system of poultry [37]. Energy feed intake was influenced by the amount of feed intake from livestock, besides that, gross energy from feed also affected energy consumption. Energy feed intake in broilers can be varied when the gross energy of feed differs between treatments. In this study showed that high feed intake resulted in high energy feed intake, hence the results of the variance analysis on ration consumption values with a value range of 79.91-90.54 g/head and energy feed intake with a value range of 59.57-81 kcal/g/head. Therefore the feed intake and feed energy intake did not show significant differences between treatments (table 2).

### Table 2. Body weight, feed intake, feed energy intake, weight of excreta, and energy excretion

| Treatments | Body weight (g/head) | Feed intake (g/head) | Feed Energy intake (kcal/g/head) | Weight of excreta (g/head) | Energy excretion (cal/g/ head) |
|------------|----------------------|----------------------|---------------------------------|--------------------------|-----------------------------|
| YA         | 1457.70±58.56        | 90.54±11.00          | 81.36±16.47                    | 16.80±3.65               | 15.24±4.45                  |
| YB         | 1449.96±56.93        | 79.91±8.19           | 60.74±10.89                    | 15.48±6.26               | 11.87±5.14                  |
| YC         | 1472.70±91.45        | 82.75±17.13          | 59.57±17.94                    | 14.81±4.12               | 10.65±3.89                  |
| YD         | 1447.44±164.08       | 80.01±13.48          | 63.16±5.79                     | 18.77±5.26               | 15.02±4.32                  |
| YE         | 1494.08±57.22        | 85.60±12.31          | 64.75±13.07                    | 15.97±2.96               | 11.88±1.67                  |

YA: negative control; YB: *L. plantarum* A1-E (6x10^8 *CFU* / g); YC: *C. tropicalis* TKd-3 (4x10^6 *CFU* / g); YD: A1-E and TKD-3 dry culture consortium; YE: commercial probiotics (10^11 *CFU* / g) contain *B. subtilis, L. acidophilus, S. cerevisiae*, and *A. oryzae*.

This result indicated that probiotics addition didn’t influence feed intake [38]. This condition happen because in this study, the raw material for dietary was similar for all treatments therefore that
gross energy had less effect on energy feed intake. Poultry consume their feed intake to accommodate different diets with various energy contents at different ages and in response to dietary energy. Feed intake is increase as dietary energy intake decreases and vice versa [39]. Dietary factors particularly dietary nutrient composition had significant effect, with dietary energy intake had the most predictable effect on feed intake in poultry [40]. Low feed energy intake in LAB and yeast treatment is not an indicator that the treatment given is not good for chicken. Another indicator factors is the excreta weights that influence the feasibility of the treatment in broiler chicken. Weights of excreta and feed intake will affect the digestibility of chickens calculated in the form of metabolic energy [37].

Excreta is a remnant of the digestive process in the body that cannot be reused. The results of the excreta collection in this study are shown in table 2. Excreta weights ranged from 14.81-18.77 gram/head (table 2). The highest excreta weight was found in the treatment of the consortium of LAB A1-E and yeast TKD-3 of 18.77 g/head. The lowest excreta weight was generated by treatment with probiotics sourced from yeast TKD-3 of 14.81 grams/head. The low weight of excreta indicates that only small of the diet is not digested by the body. However, excreta not only consist of non-digestible diets, but can be a mucosa resulting from the decay of worn out digestive devices, microbes, and digestive enzymes [41]. Calculation of the excreta weight was done because excreta weight has directly affected on energy excretion. Besides excretion weight, gross energy in the excreta is the factor that influences energy excretion. The relationship between excreta weight, excreta gross energy, and energy excretion is linear. Results range from 10.65-15.24 kcal. This result indicated that treatment YC (yeast TKD-3 probiotics) give the best optimum feed digested, because the lower energy excretion indicates that the feed is digested more.

Probiotics can stimulate appetite and increase nutrition through the production of vitamins, detoxification of compounds in food, and breakdown of compounds that cannot be digested into simpler compounds [42]. Suggested by [43] that the increase in feed intake was due to the secretion of various enzymes (amylolytic, proteolytic, and lipolytic) by probiotic microbiota in the digestive tract. However, some studies show probiotics do not affect dietary feed intake [38]. These varied results are due to microbial ecology in the livestock digestive tract, the ability of microbes adhere to mucus epithelium, different species or strains of probiotics and gift methods of probiotics [44,45]. Angel et al. [46] stated that administration methods of probiotics would have a significant effect when livestock are kept by giving low nutrient rations. No significant effect on this study probably causes the provision of rations in broilers had fulfilled nutrient requirements so that probiotics were not working optimally.

Table 3. Feed nitrogen intake, nitrogen excretion, and nitrogen retention

| Treatments | Feed nitrogen intake (g) | nitrogen excretion (g) | nitrogen retention (%) |
|------------|---------------------------|------------------------|------------------------|
| YA         | 278.57±33.83              | 118.36±29.58           | 57.50±9.26             |
| YB         | 245.86±25.21              | 101.81±45.60           | 59.57±14.73            |
| YC         | 254.59±52.69              | 98.41±25.74            | 59.77±3.90             |
| YD         | 246.17±41.49              | 123.29±41.91           | 49.13±16.37            |
| YE         | 263.38±37.87              | 86.84±17.58            | 60.57±5.09             |

YA: negative control; YB: L. plantarum A1-E (6x10⁶ CFU / g); YC: C. tropicalis TKd-3 (4x10⁶ CFU / g); YD: A1-E and TKD-3 dry culture consortium; YE: commercial probiotics (10¹¹ CFU / g) contain B. subtilis, L. acidophilus, S. cerevisiae, and A. oryzae.

The treatment of this study didn’t give a significant influence on feed nitrogen intake (table 3). Feed nitrogen intake ranges from 219.26-248.43 grams for all treatments. The highest feed nitrogen intake found in control treatment and commercial treatments produce the lowest feed nitrogen intake. Application of microbial phytase in poultry has been reported in the form of enzyme as feed additive. Omoljola et al. [26] stated that the addition of phytase improved the broiler performance, feed intake, FCR, and increase the apparent retention of nitrogen. Feed nitrogen intake will affect the value of
nitrogen retention and dietary metabolic energy, but an increase in dietary metabolic energy is not always followed by an increase in nitrogen retention [37]. The highest nitrogen excretion was produced in the treatment of probiotic LAB A1-E and TKD-3 yeast consortiums while the lowest nitrogen excretion was produced in commercial probiotic treatments. Nitrogen excretion shows the amount of nitrogen that is unutilized by the livestock. If more nitrogen is retained in the animal body, there will be decreasing on nitrogen wasted in the excreta [45]. Nitrogen consumption and excretion directly affect nitrogen retention. Nitrogen retention is the difference between the value of feed nitrogen intake and the value of nitrogen excreted after correction with the value of endogenous nitrogen excretion [48]. The results showed that nitrogen retention was not affected by probiotics treatment (P> 0.05). The highest nitrogen retention was found in TKD-3 yeast probiotic treatment (59.77%). Increased nitrogen retention indicates nitrogen utilized by the body of livestock [37].

Digestive efficiency can be increased through microbiota digestion. Good microbiota help digestive processes by producing secondary metabolites that against bad microbiota on digestive tract or produce enzymes that help digestive processes [49]. Increased nitrogen retained in the digestive tract is also influenced by antimicrobial substances produced by probiotics. The high antagonistic activity by probiotics results in a decrease in the colonization of pathogenic bacteria that interfere with the digestive tract, so cell regeneration occurs on probiotics effect on increasing probiotic colonization. Other than that, Toxic components produced by pathogenic bacteria also run to decrease and modulation of the immune system. These results increase the small intestine health so that there is an increase in enzyme activity and nutrients absorption [50].

Table 4. Feed metabolic energy with the addition of probiotics phytase-producing to broiler chickens

| Treatments | AME (kcal/kg) | AMEn (kcal/kg) | TME (kcal/kg) | TMEn (kcal/kg) |
|------------|---------------|----------------|---------------|----------------|
| YA         | 4245.58 ± 45.18 | 4097.24±24.65<sup>b</sup> | 4331.27±47.15 | 4182.93±28.40<sup>b</sup> |
| YB         | 4268.26 ± 52.58 | 4112.32±20.67<sup>b</sup> | 4365.08±60.79 | 4219.14±27.45<sup>ab</sup> |
| YC         | 4287.53 ± 21.28 | 4134.64±15.95<sup>a</sup> | 4383.60±39.82 | 4230.71±36.96<sup>a</sup> |
| YD         | 4219.16 ± 72.74 | 4103.75±31.39<sup>b</sup> | 4317.55±59.57 | 4202.15±15.43<sup>ab</sup> |
| YE         | 4274.48 ±7.60 | 4127.33±13.83<sup>b</sup> | 4365.66±12.58 | 4218.51±22.98<sup>b</sup> |

YA: negative control; YB: L. plantarum A1-E (6x10<sup>8</sup> CFU / g); YC: Candida tropicalis TKd-3 (4x10<sup>8</sup> CFU / g); YD: A1-E and TKD-3 dry culture consortium; YE: commercial probiotics (10<sup>11</sup> CFU / g) contain Bacillus subtilis, L. acidophilus, S. cerevisiae, and Aspergillus oryzae.

Different superscript in column show a significant difference (P<0.05)

Metabolic energy is the result of a reduction between gross energy intake and gross energy excreted by livestock. Metabolic energy is influenced by the amount of intake and excretion of energy and protein. Also, the ability of livestock to metabolize rations in their bodies will also affect metabolic energy [33]. Table 4 shows the results of metabolic energy calculations in broiler chickens consisting of apparent metabolizable energy (AME), true metabolizable energy (TME), apparent metabolizable energy corrected by nitrogen (AMEn) and true metabolizable energy corrected nitrogen (TMEn). Dietary supplementation of phytase in diet improved the average daily gain, increased the levels of serum calcium (Ca), tibia Ca and P, AID of AA and apparent digestibility of energy and Ca in starter stage [27]. In many experiments, the relationship between feed intake and AME was negative or insignificant [51].

TME values greater than AME values are caused by the presence of a correction factor of endogenous energy in TME. Endogenous energy is energy derived from the residual from body's tissues metabolic processes, ruined digestive tracts, and end products containing nitrogen [38]. According to Wolynetz [48] and Zarei [34], endogenous energy produced by livestock consists of endogenous urinary and metabolic fecal. Determination of metabolic energy corrected nitrogen necessary to do because there are the different ability of livestock to utilize gross energy and crude protein from feed and dietary [52]. Apparent Metabolizable Energy corrected for nitrogen (AMEn) is accepted for poultry, although True Metabolizable Energy corrected for nitrogen (TMEn) is better than
it for some limitations, AMEn is common now [48,34]. Correction of nitrogen retention results in smaller AMEn and TMEn compared to AME and TME values.

Also, nitrogen retention can reduce the variety of values obtained. There were no significant differences between treatments (P>0.05) on AME and TME parameters but showed significant differences between treatments (P <0.05) in AMEn and TMEn parameters (table 4). Treatment with supplementation probiotic Yeast TKD-3 produce higher metabolic energy compared to other treatments of 4287.53 kcal g\(^{-1}\) (AME), 4383.60 kcal g\(^{-1}\) (TME), and significantly different in AMEn and TMEn parameters compared to other treatments 4134.64 kcal g\(^{-1}\) (AMEn) and 4230.71 kcal g\(^{-1}\) (EMMn). Higher metabolic energy probably causes probiotics Yeast TKD-3, improve the performance of small intestine in the nutrients absorption process. This is accordance with the opinion of Kompiang [53], probiotics as living microbes or spores that can live and develop in the digestive tract, especially in the small intestine and can benefit their hosts both directly and indirectly from their metabolites. Probiotics can affect the density, length, and surface area of small intestine villi so that they absorb more nutrients — the stimulation mechanism of increasing digestion by probiotics in various ways. Probiotics can increase the production of volatile fatty acids (VFA). VFA is absorbed directly in the back of the digestive tract and is used as an energy source for body tissues so nutrients availability will be increase. VFA also improve intestinal health and integrity by directly stimulating epithelial cell proliferation [54].

On the other hand, probiotics stimulate digestion by influencing regulation of the immune system. Immune system regulation leads to suppressing the negative impact of chronic immune activation. Activation of the immune system causes a transfer of the use of nutrients in the production process such as tissue formation or egg production into an immune response. Probiotics also protect epithelial cells directly and stimulate enzyme activity in the digestive tract, so there are increasing in nutrient absorption [55]. Using probiotic with encapsulated method gift significant result on AMEn and TMEn, this result accordance with study by Natsir [56] supplementation of probiotic encapsulated with maltodextrin and lactoglobulin will increase AME, AMEn and protein digestibility. It’s happening because probiotic in encapsulation will degrade in the small intestine effect on pH reducing on the tracts digestive. An acidic in the small intestine because of its addition of probiotic content of lactic acid can beneficial for development non-pathogenic bacteria so it can improve feed digestion. The dietary inclusion of phytase increases nutrient and energy bioavailability for broilers [57]. Also a study by [58] showed that addition of phytate-degrading probiotic cultures in feed could improve performance of broiler chicken. Phytate is hydrolyzed mainly in the upper gastric tract (proventriculus and gizzard), where the pH favors the action of phytase, and its substrate is more water soluble [59]. According to Sousa et al. [57], enzyme activity will increase due to a decrease in GIT pH, resulting in increased dietary nutrient absorption and improving animal performance.

4. Conclusion
The use of C. tropicalis TKd-3 as probiotic on broiler chicken diet increase nutrient digestibility, especially apparent metabolic energy corrected Nitrogen, and True metabolic energy corrected Nitrogen.

Acknowledgments
The author would like to thank to DIPA BPTBA LIPI 2018 as a source of financial support for this activity and also to Madina Nurrohmah, Melisa Ekaningrum, and Diding Ristino for their help in this activities and data collection.

References
[1] Rostami H and Giri A 2013 Int. J. Advanced Biotech. 4 62-71
[2] Pirgozliev V, Oduguwa O, Acamovic T and Bedford M R 2008 Brit Poultry Sci 49 144-154
[3] Boivin G and Meunier P J 2002 Calcif. Tissue Int. 70 503–511
[4] Palacios M C, Haros M, Rosell C M and Sanz Y 2008 Food Microbiol 25 169–176
[5] Applegate T J, Angel R and Classen H L 2003 Poult. Sci 82 1140–1148
[6] Shannugam G 2018 Int. J. Curr. Microbiol. App. Sci. 7 1006-1013
[7] Sreeramulu G, Srinivasa D S, Nand K and Joseph R 1996 Lett. Appl. Microbiol. 23 385-388.
[8] Zamudio M, Gonza À lez A and Medina J A 2001 Lett. Appl. Microbiol. 32 181-184
[9] Anastasio M, Pepe O, Cirillo T, Palomba S, Blaiotta G and Villani F 2010 J. Food Sci. 75 M28-M35
[10] Vohra A and Satyanarayana T 2003 Crit. Rev. Biotechnol. 23 29–60
[11] De Angelis M, Gallo A, Corbo M R., McSweeney P L H, Faccia M, Giovine M, and Gobbetti M 2003 Int. J. Food Microbiol. 87 259-270
[12] Songré-Quattara L T, Mouquet-Rivier C, Icard-Vernière C, Humblot C, Diawara B and Guyot J P 2008 Int. J. Food Microbiol. 128 395-400
[13] Raghavendra P and Halami P M 2009 Int. J. Food Microbiol. 133 129-134
[14] Wykoff D D and O’Shea E K 2001 Genetics 159 1491–1499
[15] Andlid T, Veide J and Sandberg A S 2004 Int. J. Food Microbiol. 97 157–169
[16] Nuobariene L, Hansen A S, Jespersen L and Arneborg N 2011 J. Appl. Microbiol. 110 1370–1380
[17] Olstorpe M., Schnürer J and Passoth V 2009 FEMS Yeast Res. 9 478–488
[18] Sandberg A S and Andlid T 2002 Int. J. Food Sci. Technol. 37 823–833
[19] Segueilha L, Lambrechts C, Boze H, Moulin G and Galzy P 1992 J. Ferment. Bioeng. 74 7–11.
[20] Ushasree M V, Vidya J, and Pandey A 2014 Process Biochem. 49 1440–1447
[21] Fonseca-Maldonado R, Maller A, Bonneil E, Thibault P, Botelho-Machado C, Ward R J P, Mde L 2014 Protein Expr. Purif. 99 43–49.
[22] Greppi A, Krych L, Costantini A, Rantsiou K, Hounhouigan D J, Arneborg N, Cocolin L and Jespersen L 2015 Int. J. of Food Microbiol. 205 81–89
[23] Vohra A and Satyanarayana T 2001 Biotechnol Lett 23 551–554
[24] Olstorpe M, Schnürer J and Passoth V 2009 FEMS Yeast Res. 9 478–488.
[25] Svihus B 2014 J. Appl. Poult. Res. 23 306-314
[26] Omujola A B, Otunla T A, Olusola O O, Adebiyi O A and Ologhobo A D 2014 Am. J. Exp. Agric. 4 1637-48
[27] He S, Medrano R F, Yu Q, Cai Y, Dai Q and He J 2017 Asian-Australas J Anim Sci 30 1442-9
[28] Istiqomah L, Damayanti E, Cahyanto N and Prijambada I D 2015 Bakteri asam laktat penhasil fitase sebagai suplemen pakan dan proses pembuatan suplemen tersebut Paten P00201302693
[29] Barbosa-Canovas G V E, Ortega-Rivas P J and Yan H 2005 Food Powders: Physical Properties, Processing, and Functionality. (USA, New York: Kluwer Academic/Plenum Publishers)
[30] Chandralekha A, Hrishikesh A T, Amrutha N, Umesh H. H,Raghavarao K S M S and Ramachandra G 2016 Dry. Technol. 34 1-46
[31] AOAC Association of Official Analysis Chemist 2012 Official methods of analysis. AOAC International 19th ed. (USA,Washington DCUSA: Association of Official Analysis Chemist)
[32] Farrel D J, Atamahrainda S I and Pym R A E 1978 Brit Poultry Sci. 23 375-382
[33] Sibbald I R, and Wolynetz M S 1986 Poult. Sci. 65 78-84.
[34] Zarei A 2006 Int. J. Poult. Sci. 5 627-628
[35] Cohort 2008 CoSTAT Version 6.400 Copyright 1998-2008. Cohort Software. 798 (USA: Lighthouse Ave, Montere, CA 93940)
[36] Gomez, K A and Gomez, A A 2007. Procedure of Statistics for Agricultural Research. 2nd ed (Indonesia: University of Indonesia – Press, Jakarta. Indonesian version)
[37] Wahju J. 2004. Ilmu Nutrisi Unggas. (Indonesia:Yogyakarta Gadjah Mada Univ Pr)
[38] Mountzouris K C, Tsitsikos P, Palamidi I, Arvaniti A, Mohnl M, Schatzmayr G and Fegeros K 2010 Poult. Sci. 89 58-67
[39] Albuquerque R, Faria D E, Junqueira O M, Salvador D, Faria Filho D E and Rizzo M F 2003.
Rev Bras Cienc Avic. 5 99-104
[40] Ferket P R and Gernat A G 2006 Int J Poult Sci 5 905-911
[41] Amrullah IK. 2004. Nutrisi Ayam Broiler. (Indonesia: Bogor Lembaga Satu Gunung Bogor)
[42] Hemaiswarya S, Raja R, Ravikumar R and Carvalho I S 2013 Braz Arch Biol Technol. 56 113-119
[43] Rahman A U, Khan S, Khan D, Hussain M, Ahmed S, Sohail S M, Ahmed I, Haq I U and Shah Z 2009 Sarhad J Agric. 25 469-473
[44] Fuller R. 1989. Appl Bacteriol. 66 365-378
[45] Jin L Z, Ho Y W, Abdullah N and Jalaludin S 1998 Poult Sci. 77 1269-1265
[46] Angel R, Dalloul R A and Doerr J 2005 Poult Sci. 84 1222-1231
[47] Mirnawati, Sukamto B and Yudianto V D 2013 JITP. 3 25-32.
[48] Sibbald I R. and M S Wolynetz 1985 Poult Sci. 64 127-138
[49] Meng X, Slominski B A, Nyachoti C M, Campbell L D and Guenter W 2005 Poult Sci. 84 37-47
[50] Applegate T J, Klose V, Steiner T, Ganner A and Schatzmayr G 2010 J Appl Poult Res. 19 194-210
[51] Scott T A 2005 Rec Adv Anim Nutr 15 237-44
[52] McDonald P, Edwards RA, Greenalgh JFD and Morgan CA. 2002. Animal Nutrition. 6th Ed. (London : UK Longman)
[53] Kompiang I P 2009 Pengembangan Inovasi Pertanian. 2 177-191
[54] Ajuwon J M 2016 J Appl Poult Res. 25 277-283
[55] Wang Y, Gu Q 2010 Res Vet Sci. 89 163-167
[56] Natsir, M H 2007 J. Ternak Tropika 6 13-17
[57] Sousa de J P L, Albino L F T, Vaz R G M V, Rodrigues K F, Da Silva G F, Renno L N, Barros V R S M and Kaneko I N. 2015 Rev. Bras. Cienc. Avic. 17 69-76
[58] Askelson, T E, Ashley C, Jason T L and Tri D 2014 Appl. Environ. Microbiology 80 943–950
[59] Selle P H and Ravindran V 2007 Anim Feed Sci Tech 135 1-41