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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

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Abstract

This study aimed to evaluate protein quality of top leaf meal of *Indigofera zollingerina* based on its net protein utilization (NPU) as well as to evaluate its metabolizable energy. Fifty broiler chicks were used in determination of NPU while fifteen broiler strain Cobbs chickens aged 5 weeks were used for metabolizable energy measurement. This study used descriptive method in which data obtained were analyzed and compared to NPU and metabolizable energy of soybean meal. The results showed that top leaf meal of *Indigofera zollingerina* had NPU of 38.58 – 46.98, which is 70.14% - 85-42% of that NPU value of soybean meal is 55. Its metabolizable energy was 2791.12 kcal/kg, 9.46% higher than that of soybean meal. As the NPU and metabolizable energy were close to those of soybean meal, top leaf meal of *Indigofera zollingerina* can be used to substitute protein from soybean meal in poultry feed.

Keywords: metabolizable energy, NPU, top leaf meal of Indigofera zollingerina

Abstrak (Indonesian)

Penelitian ini bertujuan untuk mengevaluasi kualitas protein melalui pengukuran *Net Protein Utilization* (NPU) dan mengevaluasi kandungan energi metabolis tepung pucuk *Indigofera zollingerina*. Materi yang digunakan untuk mengukur NPU tepung pucuk *Indigofera zollingeriana* adalah anak ayam broiler sebanyak 50 ekor sedangkan untuk mengukur energi metabolis tepung pucuk *Indigofera zollingeriana* digunakan 15 ekor ayam broiler *strain Cobb* yang berumur 5 minggu. Penelitian ini menggunakan metode deskriptif, data yang diperoleh dianalisis dengan membandingkan NPU dan energy metabolis tepung pucuk *Indigofera zollingeriana* dengan NPU dan energy metabolis bungkil kedelai. Hasil penelitian diperoleh NPU tepung pucuk *Indigofera zollingeriana* sebesar 38,58 sampai 46,98, sedangkan NPU bungkil kedelai adalah 55. Nilai NPU tepung pucuk *Indigofera zollingeriana* 70.14 % sampai 85,42 % dari NPU bungkil kedelai. Kandungan energi metabolis 2791,12 kkal/kg. Energi metabolis ini lebih tinggi 9,46 % dari energi metabolis bungkil kedelai. Berdasarkan nilai NPU dan kandungan energi metabolis tepung pucuk *Indigofera zollingeriana* yang mendekati nilai NPU bungkil kedelai, tepung pucuk *Indigofera zollingeriana* dapat digunakan sebagai pakan substitusi protein bungkil kedelai dalam ransum ternak unggas.

Kata-kata kunci: Energi metabolis, NPU, tepung pucuk *Indigofera zollingeria*

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INTRODUCTION

Evaluation on protein and nutrient content suggested that top leaf meal of *Indigofera zollingeriana* can be used to partly substitute protein and other nutrients of soybean meal. Top leaf meal of *Indigofera zollingeriana* contains 28.98% of protein and 8.49% of crude fiber (Palupi *et al*., 2014). The effectiveness of protein of top leaf meal of *Indigofera zollingeriana* compared to that of soybean meal in poultry feed needs to be assessed to determine the quantity needed in the feed.

It takes more than just information about nutrient content (e.g. carbohydrate, fat, protein, vitamins, minerals) of the feedstuff to properly prepare a feed, but also the information on their biological evaluation. Hence, the quality of the protein and metabolizable energy of top leaf meal of *Indigofera zollingeriana* are necessary to know. Protein quality of a feed depends on the types and proportion of its amino acids, the protein digestibility, and the amount of protein retained from feed. Protein of top leaf meal of *Indigofera zollingeriana* composed of a fairly complete amino acid composition, namely histidine 0.67%, Threonine 1.14%, Arginine 1.67%, methionine 0.43%, tyrosine 1.05%, valine 1.56%, phenylalanine 1.60%, isoleucine 1.35%, leucine 2.26% and lysine 1.57% [1].

In vivo protein evaluation, for example by evaluating the nitrogen balance, can assess the biological value of a feed more accurately than the *in vitro* one. Nitrogen is chosen as this compound is present in protein but not in any other main nutrient needed by body (carbohydrate and fat). Measurement on the proportion of absorbed protein being retained by the body is known as NPU (Net Protein Utilization). NPU indicates the percentage of protein from feed/food that can be converted into body protein [2][3]. NPU value depends on nitrogen retention of the feed, while the nitrogen retention depends on the content and quality of protein of the feed [4]. Given the same nitrogen consumption, increasing nitrogen retention will increase NPU [5].

Beside its protein biological value, information on energy value of the feed is also important. A feedstuff can be better utilized when its metabolic energy is known. This information is most applicable in the formulation of poultry feed, as a guideline in determining the proportion of a certain feedstuff in the feed. Apart from the nutrient content (carbohydrate, fat, protein, vitamins, and minerals), the energy content of the feed needs to be considered when formulating poultry feed, as the energy level of a feed would determine the portion of consumption [6]. Therefore, the metabolic energy of top leaf meal of *Indigofera zollingeriana* needs to be known before being utilized as soybean meal substitute in poultry feed.

Metabolizable energy of a feedstuff is calculated as the difference in the gross energy of the feedstuff consumed and of the excreta. Metabolizable energy needs to be corrected for nitrogen retention as the ability of the poultry in utilizing gross energy of the feed protein greatly varies. Changes in the protein level of feed given to poultry can lead to the difference in the amount of retained protein, hence resulting in different metabolizable energy [7].

MATERIALS AND METHODS

Materials

Fifty Day-old-chick (DOC) broiler strain Cobb chicks were used to measure NPU of top leaf meal of *Indigofera zollingeriana* while fifteen of the same type of chicken at 5 weeks old were used for metabolic energy determination. Feed for NPU determination was prepared according to [8], consisting of starter diet, very low protein diet, and the diet which will be tested for its protein quality (test diet). Protein of the test diet was prepared from top leaf meal of *Indigofera zollingeriana.*

Equipment used to measure NPU of top leaf meal of *Indigofera zollingeriana* were colony cage equipped with feed and water container, scale, cleaning equipment, and lamp as the source of light and heat for the chicken, especially during the night.

Before given the real treatment, chickens were allowing to adapt with the diet. The adaptation was done as follow:

1. Starter diet was administered to the chickens at the start of the study for 7 days (since DOC to 8 days old) to provide nutrition for their growth and to reduce death risk during their early life.
2. Adaptation diet was administered to the chickens for 8 days following the starter diet (8-16 days old). Chicken were given test diet to let them adapt with it.
3. Test diet was administered to the chickens for 15 days (17-32 days old), consisted of feed with very low protein content and feed containing top meal leaf of *Indigofera zollingeriana* as protein source.
4. During administration of test diet, the growth and feed consumption by the chicken were recorded.

Chickens were grouped into 2 diet treatments; each had 5 replicates containing 5 individual chickens. Feed and water were given at *ad libitum* basis, as shown in Figure 1. The diets were:

R0: very low protein diet

R1: diet in which its 10% protein came from that of top leaf meal *Indigofera zollingeriana* (test diet)


Figure 1. Diet treatments

The ingredients of the feed with very low protein content (R0) were tapioca flour, sugar powder, DCP, premix, and palm oil. In the feed containing protein of top leaf meal of *Indigofera zollingeriana*, tapioca flour was replaced with top leaf meal of *Indigofera zollingeriana*. Feed was prepared to meet metabolizable energy of 3100 kcal/kg. The composition and nutritional value of the feeds were shown in Table 1.

Variable being observed was:

NPU (Net Protein Utilization) according to Leeson and Summers [8], calculated as follow:

$$NPU = \frac{Bf - Bk}{If} x\ 100$$

where:

Bf = N of chicken's carcasses with test diet (g)

Bk = N of chicken's carcasses with N-free diet (g)

If = N intake by chicken with test diet (g)

Determination of Metabolizable Energy

Determination of metabolizable energy was done on 15 broiler strain Cobb chickens at 5 weeks of age. They were placed in 15 metabolic cages. Equipment used in this test were metabolic cages of 50cm x 30cm x 56cm, scale, excreta container, plastic bag, aluminum foil, freezer, oven to dry the excreta, and mortar for excreta grinding (sample).

Metabolic cages and other equipment were cleaned and sterilized with disinfectant beforehand to prevent contamination by diseases from previous usage. Lamps of 100 Watt were used and turned on during the night only. Chickens were placed randomly in cages marked with the type of treatment.

Table 1. Composition and nutritional value of the treatment feeds for determination of Net Protein Utilization (%).

Ingredient	Very low protein diet (R0)	Test diet (R1)
Tapioca flour	63.6	0
Sugar powder	27	58
Palm oil	4.8	2.85
Top leaf meal of *I.zollingeriana*	0	36.1
DCP	3.1	1.75
Premix	1.5	1.3
total	100	100
Nutritional value:		
Met. energy(kcal/kg)	3100	3100
Crude protein (%)	0.69	9.9
Fat (%)	5.14	1.51
Crude fiber (%)	0.74	3.96
Calcium (%)	0.9	0.52
Phosphorus (%)	0.88	0.41
Lysin (%)	0.45	0.96
Methionine (%)	0.45	0.54

Procedures of the Research

Adaptation Phase.

Prior to being caged, chickens were weighed to know their initial weight before given treatment. They were then force-fed with 30 g of top leaf meal paste of *Indigofera zollingeriana*.

Fasting Phase.

Chickens were prohibited on feed for 24 h (but water was still provided) to clean the digestion tract of the chicken from previous feed [9].

Treatment Phase.

Chickens were weighed to know their weight after fasting phase. Feed (30 g/head) was force-fed to 10 chickens. Excreta were collected 24 h after feeding. Five chickens were re-fasted for 24 h (water was given *ad libitum*) to measure endogenous nitrogen and energy. The implementation of the treatment was illustrated in Figure 2.

(1) (2) (3)

Figure 2. Research implementation to measure metabolic energy

Note: (1) Weighing down the initial weight; (3) Force feeding of top leaf meal paste of *Indigofera* *zollingeriana*; (4) Placement of chickens in metabolic cages for excreta collection.

Excreta for endogenous measurement were collected once after chickens being fasted for 24 h. During collecting process, excreta was sprayed with low concentration of H_2S0_4 (0.01 N) to bind nitrogen and prevent evaporation. Excreta samples were stored in freezer for 24 h to prevent decomposition by microorganisms. They were taken out of freezer and let to melt prior to drying in oven (50^{0}C for 48 h). Dried excreta were ground and analyzed for its nitrogen content and gross energy. Measurement of nitrogen concentrations in excreta by proximate analysis and measuring of gross energy used a bomb calorie meter.

Metabolizable energy of top leaf meal of *Indigofera zollingeriana* was calculated according to [10]:

a. Apparent metabolizable energy(AME) (kcal/kg)

$$AME = \frac{(EBxK) - (EBexE)}{K} x\ 1000$$

b. True metabolizable energy (EMM) (kcal/kg)

$$TME = \frac{(EBxK) - [(EBexE) - (EBkxEE)]}{K} x\ 1000$$

c. Apparent metabolizable energy corrected for nitrogen (AMEn) (kcal/kg)

$$AMEn = \frac{(EBxK) - [(EBexE) + (8.22\ xRN)]}{K} x\ 1000$$

d. True metabolizable energy corrected for nitrogen (EMMn) (kcal/kg)

$$TMEn = \frac{(EBxK) - [(EBexE) - (EBkxEE) + (8.22xRN)]}{K} x1000$$

where:

EB = gross energy of feed (kcal/kg)
EBe = gross energy of excreta (kcal/kg)
EBk = gross energy of excreta endogenous(kcal/kg)
K = feed intake (gram)
E = mass of excreta sample (gram)
EE = mass of excreta endogenous (gram)
RN = nitrogen retention (gram)
= (feed consumption x N of feed) – (mass of excreta x N of excreta)
8.22 = corrected number as uric acid (kcal/g RN)

Data Analysis

Data of net protein utilization and metabolic energy of top leaf meal of *Indigofera zollingeriana* were descriptively analyzed by comparing them with those of soybean meal.

RESULTS AND DISCUSSION

Net Protein Utilization of Top Leaf Meal of Indigofera zollingeriana

Prior to measurement of the nitrogen content in chickens' carcasses, their weight gain and feed intake were recorded (Table 2). In Table 2, it can be seen that the weight of chickens fed with very low protein diet (R0) declined by -2.75 g/head/day in average. It indicates that the nutritional value in the diet was too low to suffice basic daily requirement, leading to catabolism of body fat and protein for energy supply. Meanwhile, chickens fed with protein-containing diet (R1) gained their body weight by 7.84 g/head/day in average. Their body weight was increased by around 29.31% in 15 days. It gives an indication that protein of top leaf meal of *Indigofera zollingeriana* can be retained by broiler chickens for synthesis of protein tissue. The absence of protein in feed cuts the protein supply that helps in growing new tissues [11].

Table 2. Average weight gain and feed intake of chickens with very low protein diet (R0) and with test diet containing top leaf meal of *I. zollingeriana* (R1) during 15 days of treatment (g/head/day).

Replicate	Weight gain/reduction R0	Weight gain/reduction R1	Feed intake R0	Feed intake R1
1	-3.47	5.73	25.60	20.27
2	-2.93	13.73	22.93	24.53
3	-0.93	10.80	27.33	24.00
4	-3.07	4.67	22.13	21.67
5	-3.33	4.27	24.33	19.47
Average	-2.75±1.04	7.84±4.20	24.46±2.08	21.98±2.23

The average feed intake in R0 group was 24.46 g/head/day, while that in R1 group was 21.98 g/head/day. The higher feed intake in R0 group was to fulfill the needs for other nutrients, such as energy. An increase in muscle mass is only possible when protein is available at higher amount than what needed for tissue maintenance and repair [12]. Lack of protein supply in R0 group allowed no formation of new tissues.

The N content of the carcasses, N intake of the test diet group, and NPU of top leaf meal of *Indigofera zollingeriana* are presented on Table 3. The average NPU of top leaf meal of *Indigofera zollingeriana* was 40.79% (Table 3), indicating the amount of nitrogen out of total protein content in the feed that can be utilized by the chickens for their growth. NPU of soybean meal is 55% [8]. It means that NPU of top leaf meal of *Indigofera zollingeriana*

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is lower than that of soybean meal, only 74.16% of it. The low NPU of top leaf meal of *Indigofera zollingeriana* is accounted for its lower amino acid score compared to that of soybean meal, 10.49 and 16.34 respectively. Amino acid score describes protein biological quality of a feedstuff as protein is composed of amino acids [11].

Table 3. Average N carcasses, N intake, and NPU of top leaf meal of *Indigofera zollingeriana*

Replicate	N carcasses R1 (g)	N carcasses R0 (g)	N intake R1 (g)	NPU
1	55.66	44.05	30.09	38.58
2	65.01	50.08	36.43	40.98
3	61.55	45.01	35.64	46.43
4	57.02	43.58	32.17	41.78
5	51.67	41.22	28.91	36.16
Average	58.18±5.19	44.79±3.27	32.65±3.32	40.79±3.84

Nevertheless, top leaf meal of *Indigofera zollingeriana* still has higher NPU when compared to other non-conventional feedstuffs, such as sunflower seed and sesame seed. The NPU of sunflower seed and sesame seed are 37.92% and 37.03% respectively [13]. NPU indicates percentage of protein in feed/food that can be transformed into body protein [2]. In this study, the amount of nitrogen in carcasses of R1 group was higher than that in R0 group. It was because the protein in top leaf meal of *Indigofera zollingeriana* is of good biological value. Its amino acids can be utilized to make new tissues, indicated by the increase in body weight at the end of the experiment. High biological value means that feed contains proper amount of amino acids needed by the body [14]. Protein deposition rate increases with additional supply of amino acids in feed, when amino acids are limited, energy supply in feed does not affect protein deposit in broiler chickens [15].

Metabolizable Energy of Top Leaf Meal of Indigofera zollingeriana

The average Apparent Metabolizable Energy (AME), True Metabolizable Energy (TME), Apparent Metabolizable Energy corrected for nitrogen (AMEn), and True Metabolizable Energy corrected for nitrogen (TMEn) of top leaf meal of *Indigofera zollingeriana* are presented on Table 4.

The average AME of top leaf meal of *Indigofera zollingeriana* was 2952.95kcal/kg, lower than its TME of 3598.43 kcal/kg (Table 4). It was because the AME has not yet been corrected with the energy in endogenous excreta which still contained energy from the residue of digestion (19.36 kcal). Part of the energy comes from catabolism of body tissues to fulfill basic needs during fasting phase, while the other comes from end products containing nitrogen [16]. According to [17], excretion of nitrogen in feces consumes energy, causing metabolizable energy to decrease.

Table 4. Average metabolizable energy of top leaf meal of *Indigofera zollingeriana* (kcal/kg)

Unit	AME	TME	AMEn	TMEn
1	2864.45	3509.92	2701.84	3347.66
2	2888.32	3533.79	2728.77	3373.75
3	2703.63	3349.10	2572.96	3218.43
4	3005.04	3650.51	2834.62	3480.09
5	2861.60	3507.07	2679.96	3325.43
6	3116.52	3761.99	2947.97	3593.44
7	3087.24	3732.71	2901.71	3547.18
8	3039.78	3685.25	2880.88	3526.35
9	2751.33	3396.80	2590.14	3235.61
10	3211.63	3857.10	3072.53	3718.00
Average	2952.95	3598.43	2791.12	3436.59

Note: AME apparent metabolizable energy
TME: true metabolizable energy
AMEn: apparent metabolizable energy corrected for nitrogen
TMEn: true metabolizable energy corrected for nitrogen.

Calculation of metabolizable energy needs to be corrected for nitrogen retention as the ability of body in utilizing gross energy from crude protein greatly varies [7], Nitrogen retention is the difference of the amount of nitrogen consumed and excreted after being corrected for endogenous nitrogen [10]. The amount of fecal nitrogen affects nitrogen retention. The more nitrogen retained, the less nitrogen being excreted with feces [18]. The average TMEn of top leaf meal of *Indigofera zollingeriana* was 3436.59 kcal/kg while its AMEn was 2791.12 kcal/kg. Its TMEn is higher than its AMEn before and after being corrected for energy excreted together with the remaining of metabolic activity, such as undigested feed, enzymes, and damaged intestinal mucosal cells. Endogenous energy consists of metabolic fecal and endogenous urinary, which are originated from catabolism of body tissues to fulfill basic life requirements during fasting phase and from nitrogenous end products [16]. Declining protein content in food and nitrogen retention in time during digestion, different growth rate and ability in retaining nitrogen lead to the need for correction factor in determination of TME [19].

The metabolizable energy of top leaf meal of *Indigofera zollingeriana* is higher than that of other legumes, such as lamtoro (*Leucaena leucocephala*) and gamal (*Gliricidia sepium*) leaf meal, 2547.10 kcal/kg and 2045.24 kcal/kg respectively. However, it is lower by 15.11% than the metabolizable energy of corn gluten meal (3500 kcal/kg). Metabolizable energy of a feedstuff is affected by several factors, including the crude fiber and antinutrient content. The higher the crude fiber content, the lower the metabolizable energy. Crude fiber negatively correlates with energy digestibility [20]. *Indigofera zollingeriana* meal has relatively low crude fiber (8.49 %) and antinutrients (0.29 % tannin and 0.036% saponin).

Tannin level higher than 5% can suppress nitrogen retention and amino acid digestibility, reducing metabolizable energy of a feedstuff [6]. Metabolizable energy of top leaf meal of *Indigofera zollingerina* was 9.46% higher than that of soybean meal (2550 kcal/kg). Based on this level of energy, top leaf meal of *Indigofera zollingerina* can be used together with soybean meal to produce quality poultry and reduce the use of soybean meal in poultry feed.

CONCLUSION

Top leaf meal of *Indigofera zollingerina* had NPU of 40.79%, which is 74.16% of that of soybean meal. Its apparent metabolizable energy corrected for nitrogen was2791.12 kcal/kg and was 9.46 % higher than that of soybean meal (2550 kkal/kg). In conclusion, this study showed that top leaf meal of *Indigofera zollingerina* can be used as a potential alternative of protein source to partly substitute protein from soybean meal.

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