ASEAN Journal of Scientific and Technological Reports

Research article

Evaluation of Nutrient Digestibility and Metabolizable Energy of Yeast-Fermented *Acacia mangium* Leaf by Japanese Quails

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Citation:
Lerdsuwan, S.; Pisuttharachai, D.; Nalinanon, W. Evaluation of Nutrient Digestibility and Metabolizable Energy in Yeast-Fermented *Acacia mangium* Leaf by Japanese Quails.. *ASEAN J. Sci. Tech. Report.* **2023**, *26*(2), 11-19. https://doi.org/10.55164/ajstr.v26i1.247548

Article history:
Received: October 10, 2022
Revised: December 26, 2022
Accepted: January 20, 2023
Available online: March 21, 2023

Abstract: The nutrient digestibility and the metabolizable energy (AME) of *Acacia mangium* leaf (AM) and yeast-fermented AM (YFAM) by Japanese quails were investigated. Fifty-four of 4-weeks-old Japanese quails were divided into 3 groups with six replications of three quails. Each quail was randomly fed with an experimental diet composed of dextrose (protein-free diet for determining endogenous excretion) 40 % AM and 40% YFAM. The quails were raised individually in a metabolic cage, where feed and water were provided *ad-libitum*. Both feed intake and feces weight were recorded. Experimental diets and excreta were sampled and subjected to proximate analysis for gross energy. The results of nutrient composition indicated that the fermentation AM with yeast highly significantly increased (P<0.01) dry matter (DM), ash, and nitrogen-free extractives (NFE) content but decreased (P<0.01) crude protein (CP), crude fiber (CF), and gross energy (GE) content. In addition, the birds fed dietary YFAM compared with AM showed significantly increased (P≤0.01) DM, organic matter (OM), CP, and GE digestibility but decreased (P≤0.01) EE and CF digestibility. The protein utilization of birds fed dietary YFAM showed significantly greater (P≤0.01) FI, protein retained, protein intake, net protein utilization (NPU), and AME than those fed dietary AM. In conclusion, the fermentation of AMLM with yeast improved nutrient composition and enhanced the digestibility of nutrients, protein utilization, and AME of AMLM in Japanese quail.

Keywords: *Acacia mangium* Leaf; Japanese Quail; Amino Acid; Digestibility; Metabolizable Energy

1. Introduction

Japanese quail also is the smallest avian species farmed for meat and egg production. Several aspects account for the utility of this bird. First, it has attained economic importance as an agricultural species producing eggs and meat that are enjoyed for their unique flavor. Second, the low maintenance cost associated with its small body size (80-300 g) coupled with its short generation interval (3-4 generations per year), disease resistance, and high egg production. Egg production is important in Japan and Southeast Asia, while meat is the main product in Europe, India, and Nigeria [1].

An accurate feed formulation is essential for optimizing feed efficiency and minimizing feed costs for poultry production. Because energy and amino acid (AA) account for the major cost of poultry diets, determining the availability of energy and AA in feedstuffs is essential for accurate diet formulations [2]. In contrast, feed cost is approximately 65-70% of the total cost of poultry production [3]. High feed costs and competition between humans and poultry for food/feed resources [4,5] hinder the growth of the poultry production industry in most developing countries, Hence the necessity to explore the use of non-convention feed resources, especially those that do not attract competition between humans and monogastric animal [6]. Leaf meal from *the Acacia mangium* tree is one resource. An exotic and fast-growing tree can be used for furniture, sawn wood, pulp, and paper [7]. The waste, such as residual wood, branches/twigs, leaves, and bark, accounts for more than 60 % of the total biomass [8]. *Acacia mangium* leaves contain 14% CP, 2.15 % EE, 39.81 NFE, and 26.4 % CF [9], which can be used for ruminant animals [10,11]. It's possible to support broiler growth, optimum inclusion levels of *Acacia mangium* leaf meal (AMLM) in support growth without adverse effects on growth performance have been established at 5% in starter chicks and 10% in grower-finisher chicks [12]. The utilization of AMLM in poultry diets must be limited due to its high fiber content and anti-nutritional factors. For this reason, processes for upgrading the nutrient value of AMLM using microbial fermentation by-products may be a substitute to improve the nutrient availability of by-products [13].

Yeast is mainly used in single-cell protein (SCP) production [14], such as *Saccharomyces cerevisiae*, one of the most widely commercialized types of yeast. This is used in the diet of poultry because of its rapid growth rate and high efficiency in converting carbon sources into protein [15]. Fermentation of AMLM with Baker's yeast (Saccharomyces cerevisiae) could be one of the best solutions to enriching its nutritional value. During fermentation yeast requires some nutrients (carbon, nitrogen, trace mineral, and vitamins) to increase in the fermentation medium. The lack of these nutrients hinders their activity which hampers the beneficial effects of the fermentation technique [16]. Starch and molasses could serve as a carbon source in the fermentation system, while urea could supply nitrogen for yeast cell synthesis, which helps the yeast to perform a proper function in the fermentation system [17-18]. Yeast and yeast products produced from agro-industrial by-products are rich in protein contents [19]. Furthermore, live yeast addition to animal feed has been known to improve the nutritional quality of feed, nutrient digestibility [20], and performance of animals [21]. However, there is no information about the effects of AMLM and fermented AMLM on Japanese quails' digestive and metabolic processes. Thus, this study aimed to determine the chemical composition, amino acid profile, and metabolizable energy of *Acacia mangium* leaf meal (AM) and fermented *Acacia mangium* leaf meal (YFAM) in Japanese quail.

2. Materials and Methods

2.1 Sample preparation

Fresh *acacia mangium* leaves were harvested from one-year-old *acacia mangium* tree stands in the KMITL PCC forest in Chumphon province, Thailand. Whole fresh leaves were separated from a branch about 60 cm above. The Leaves (weight 10 kg) were cut and spread out on a clean concrete floor well-ventilated room for 7 days until they became crispy (weight 3.5 kg) [22]. Dried *Acacia mangium* leaves were ground through a 3-horse-power locally built Hammermill (NPT, Thailand) equipped with a 2 mm screen (weight 3.1 kg). Sample AM was taken and analyzed for proximate analysis [23], Tannin [24], amino acid [25], and Gross energy (GE) according to Analytical methods for oxygen bombs. Total carbohydrate content was determined by subtracting the percentage of moisture, crude ash fiber, protein, and fat from 100%.

The yeast used to be Pakmaya® baker's yeast, a commercial yeast culture (*Saccharomyces cerevisiae*) manufactured by the Pakmaya brand in Izmit, Turkey. That was purchased from a store at Pathiu market in Chumphon province, Thailand.

2.2 The preparation of solid-state AMLM yeast fermented

Dried *Acacia mangium* leave meal (1 kg) was weighed into a mixing bowl, and moisture content was adjusted to 60% by adding the calculated amount of distilled water. Adding nutrient solution with yeast (*Saccharomyces cerevisiae)* (50 g.), urea (10 g.), molasses (300 g), Tapioca starch (200 g), $MgSo_4.2H_2O$ (7 g) and KH_2PO_4 (13 g) mixed, collected in a plastic bag, and then allowed to incubate and fermentation in temperature room (27 ^{0}C) for 6 days. The sample was terminated by oven drying at 60 ^{o}C for 24 hours, cooled to a temperature room, milled, and packaged in air-tight containers. The procedure described by Oboh and Akindahunsi [26] and Aruna et al. [27].

2.3 Digestibility and metabolizable energy

Fifty-four Japanese quail, 4 weeks of age, were used to test the metabolizable energy value of AMLM. The birds were assigned to 32 width x 41 length x 13 high cm individual metabolic cages and allotted to 3 groups with 6 replicates of 3 birds/ cage. A reference diet (protein-free diet for determining endogenous excretion) was used as a basal diet (control diet) to calculate the metabolizable energy, which was determined using the substitution method according to Scott *et al.* [28]. Dextrose in the basal diet was replaced by 40 % *Acacia mangium* leaf meal (AM) and 40 % Yeast fermented AM (YFAM) to make up test diets (Table 1).

Table 1. Ingredients composition of experimental diets.

Ingredients	Control diet	AM	YFAM
Dextrose	98.81	58.81	58.81
AMLM	-	40.00	-
Yeast fermented AMLM	-	-	40.00
Dicalcium phosphate (P17)	0.50	0.50	0.50
salt	0.19	0.19	0.19
Vitamins & trace mineral	0.50	0.50	0.50
total	100.00	100.00	100.00

1/ This premix provided the following microelements (μg/kg): vitamin A 4,500,000 IU; vitamin D 750,000 IU; vitamin E 10,000, vitamin K_3 750; vitamin B_1 1,100; vitamin B_2 3,000; vitamin B_3 10,000; vitamin B_6 2,000; vitamin B_{12} 12.5; pantothenic acid, 7,000; folic acid 425; biotin 100; Cu 5,000; Fe 4,800; I 500; Mn 30,000; Se 100; Zn 50,000.

The birds were fed a diet twice daily at 7.00 and 19.00, at a near maintenance level, and they were weight before and after each collection period. After 7 days adaptation period, a 7-day collection period was started with adding 0.5% chromium oxide in experimental diets as an initial and a marker. As a finishing marker, 0.5% chromium oxide was added to each experimental diet on the 6th day of the collection period. A collection of excreta (mixed feces and urine) was started when the chromium oxide appeared in the excreta and kept until the appearance of chromium oxide in the excreta. Total excreta were collected during the collection period. Every day, the excreta samples were dried in a drying oven at 60oC for 72 h and collected in plastic containers. At the end of each experimental period, total excreta were grounded to 1 mm in the blender mill grinder. Diet and excreta samples were taken for chemical analysis [23], consisting of dry matter (DM), ether extract (EE), crude protein (CP), crude fiber (CF), and gross energy GE). The apparent digestibility coefficients, protein utilization, and apparent metabolizable energy (AME) were computed.

The nutrient digestibility can be calculated according to kong and Adeola [29] as follows:

$$\text{Digestibility (\%)} = \left[\frac{Cinput - C\ output}{Cinput}\right] \times 100$$

Cinput and *Coutput* are the amounts of components ingested and voided via the feces, respectively.

The protein utilization can be calculated according to kong and Adeola [29] as follows:

Protein intake (g/bird) = the concentration of CP in diet (g/kg DM diet) X Feed intake

Net protein utilization (NPU; g/g) = Protein retained / Protein intake

The ME content of AM and YFAM were calculated according to the equation developed by Scott et al. [28] as follows:

ME per gm diet = Energy per gm diet – (excreta energy per gm diet + 8.22 x gm N retained per gm diet)

To compute the ME of material substituted for glucose, the following equation applies:

$$\text{ME per gm substitute} = 3.64 - \left[\frac{\text{ME per gm referent diet} - \text{ME per gm diet with substitute}}{\text{Proportion of substitute}}\right]$$

(3.64 = experimentally established ME per gm of glucose dry matter)

2.4 Statistical analysis

All data were analyzed through analysis t-test design with 6 replications in each treatment, using the GLM procedure of SAS [30] with the main effect of 2 experimental diets were AM and YFAM.

3. Results and Discussion

3.1. Results

3.1.1 Amino acid of AMLM

The amino acids of AM are evaluated and listed in Table 2. The sample contains 1) essential amino acids /100 g: Arginine 801 mg, Histidine 460 mg, Isoleucine 393 mg, Leucine 1,150 mg, Lysine 810 mg, Methionine 417 mg, Phenylalanine 742 mg, Threonine 680 mg, Tryptophan 31 mg and Valine 624 mg respectively. 2) non-essential amino acids/100 g: Alanine 642 mg, Aspartic acid 1,266 mg, Cystine 594 mg, Glutamic acid 1,672 mg, Glycine 879 mg, Proline 447 mg, Serine 771 mg and Tyrosine 540 mg respectively.

Table 2. Chemical analysis of amino acid content (% dry matter) of *AM*.

Amino acids	Concentration (mg/100g sample)	Amino acids	Concentration (mg/100g sample)
Essential amino acids		**Non-essential amino acids**	
Arginine	801	Alanine	642
Histidine	460	Aspartic Acid	1,266
Isoleucine	393	Cysteine	594
Leucine	1,150	Glutamic Acid	1,672
Lysine	810	Glycine*	879
Methionine	417	Proline	447
Phenylalanine	742	Serine*	771
Threonine	680	Tyrosine	540
Tryptophan	31		
Valine	624		

* Glycine and serine represent poultry diets' first-limiting non-essential amino acids [27].

3.1.2. Nutrient content of solid-state fermented AM by yeast (Saccharomyces cerevisiae)

The results from the nutrient content of AM and YFAM are presented in Table 3. Yeast fermentation highly significantly affected dry matter, crude protein, crude fiber, ash, nitrogen-free extract, and gross energy of AM ($P<0.01$). Dry matter, ash, and nitrogen-free extract content significantly increased ($P<0.01$) in YFAM compared to AM. YFAM tended to have a higher ($P=0.0513$) crude fat content than AM. Additionally, there were a significantly decreased crude protein, fiber, and gross energy content in YFAM compared to AM ($P<0.01$). However, yeast fermentation did not affect the ether extract, calcium, phosphorus, and tannin content of AM ($P>0.05$).

Table 3. The nutrient composition of AM and YFAM (% dry matter).

Nutrient composition (%)	AM	YFAM	P-value
Dry matter	91.44 ± 0.06 [b]	93.29 ± 0.05 [a]	0.0018
Crude protein	15.97 ± 0.14 [a]	14.47 ± 0.14 [b]	0.0088
Ether extract	2.25 ± 0.07	2.55 ± 0.07	0.0513
Crude fiber	25.00 ± 0.07 [a]	20.63 ± 0.03 [b]	0.0129
Ash	4.05 ± 0.08 [b]	5.97 ± 0.03 [a]	0.0011
Nitrogen free extract	44.17 ± 0.95 [b]	49.67 ± 0.28 [a]	0.0158
Calcium	0.54 ± 0.03	0.56 ± 0.01	0.4655
Phosphorus	0.27 ± 0.04	0.29 ± 0.01	0.5918
Tannin	3.45 ± 0.14	3.44 ± 0.01	0.9298
Gross energy (Kcal/kg)	4,963.14 ± 7.11 [a]	4,720.64 ± 28.45 [b]	0.0072

[a,b] Mean values ± SD in the same row with different superscripts were significantly different. (P<0.05)

3.1.3. Digestibility and Metabolizable energy

The effects of dietary treatment on the digestibility coefficients of the nutrients such as dry matter, organic matter, ether extract, crude protein, crude fiber, nitrogen-free extract, and gross energy are shown in Table 4. The data revealed significant differences between dietary treatments in the digestibility coefficients for all nutrients of the diets except nitrogen-free extract. It could be noticed that birds fed dietary YFAM showed significantly greater (P≤0.01) dry matter, Organic matter, crude protein, and gross energy digestibility than fed dietary AM. In contrast to ether extract and crude fiber digestibility, there was a decrease (P≤0.01) in birds fed dietary YFAM.

The protein utilization of birds fed dietary YFAM showed significantly greater (P≤0.01) feed intake, retained protein intake, and net protein utilization than those fed dietary AM. Additionally, the birds fed dietary YFAM had greater (P≤0.01) apparent metabolizable energy (AME) and apparent metabolizable energy corrected nitrogen balance (AME_n) compared with those fed dietary AM.

Table 4. Digestibility and metabolizable energy of AM and YFAM in Japanese quail.

Items	AM	YFAM	P-value
Apparent digestibility (%)			
Dry matter	66.36 ± 1.95 [b]	70.42 ± 1.81 [a]	0.0039
Organic matter	68.14 ± 2.07 [b]	72.00 ± 1.81 [a]	0.0064
Ether extract	23.34 ± 3.02 [a]	12.00 ± 2.34 [b]	0.0030
Crude protein	65.72 ± 2.09 [b]	74.93 ± 1.23 [a]	0.0001
Crude fiber	28.55 ± 4.34 [a]	19.08 ± 2.45 [b]	0.0109
Nitrogen free extract	86.48 ± 1.05	87.03 ± 0.94	0.3550
Gross energy	60.15 ± 2.47 [b]	65.31± 2.47 [a]	0.0048
Protein utilization			
Feed intake (g/bird)	69.09 ± 14.91 [b]	91.41 ± 8.68 [a]	0.0001
Protein intake (g/bird)	3.10 ± 0.67 [b]	4.47 ± 0.42 [a]	0.0018
Protein retained (g/bird)	2.04 ± 0.45 [b]	3.35 ± 0.36 [a]	0.0002
Net protein utilization (g/g)	0.66 ± 0.02 [b]	0.75 ± 0.01 [a]	0.0001
Metabolizable energy (Kcal/kg)[1/]			
- AME	1428.08 ± 228.60 [b]	1993.03 ± 232.23 [a]	0.0017
- AME_n	1424.95 ± 229.45 [b]	1992.96 ± 232.57 [a]	0.0017

[a,b] Mean values ± SD in the same row with different superscripts were significantly different. (P<0.01)

[1/] AME = Apparent metabolizable energy

AME_n = Apparent metabolizable energy corrected by nitrogen balance

3.2. Discussion

Studies using Acacia mangium leaf meal (AM) as a feed ingredient mainly were focused on ruminant nutrition., and showed that using AM as a protein source diet had a positive effect on production in dairy cows [10]and Sheep [11]. However, no information is available about using AM in poultry diets.

In Japanese quail, methionine, threonine, and lysine are the first, second, and third limiting amino acids [32]. In this experiment, AM contains significant qualities of methionine, threonine, and lysine, whose values were 417, 680, and 810 mg/100 g (0.42, 0.68, and 0.81 %), respectively. These amino acid values were near with NRC [33] recommendation. The NRC [33] recommended lysine levels of 1.3 and 1.0% and methionine levels of 0.5 and 0.45% for quails in the rearing and production periods, respectively. Furthermore, this methionine amount was higher than the values found in the Leucaena leaf meal, as reported by the Bureau of Animal Nutrition Development [34]. The amino acid profile of the studied sample suggests that its protein is of adequate nutritional value, which the feed industries can exploit for feed formulations.

Nutritional changes during the yeast fermentation in AM, dry matter, ash, and nitrogen-free extract content of YFAM were higher than in AM ($P<0.01$). This experimental result was in agreement with earlier reports. Kaewwongsa et al. [35] reported the dry matter content of the fermented cassava pulp by yeast (*Saccharomyces cerevisiae*) was higher than non-fermented ($P<0.05$). Azinnahar et al. [36] also reported that fermented De-oiled rice bran with yeast (*Saccharomyces cerevisiae*) induced increased dry matter and ash ($P<0.05$). The result shows that YFAM tended to have a higher ($P=0.0513$) crude fat content than AMLM. This was likely to Mu et al. [37] reported crude fat content of fermented rice bran was significantly higher ($P<0.05$) than those of untreated rice brans. In our experiment, crude protein, fiber, and gross energy content in YFAM were lower than in AM ($P<0.01$). Crude fiber content reduces the present experiment. Another experiment conducted by Azinnahar et al. [36] and Shi et al. [38] also reported the same result, which mentioned that microorganisms produce some fibrinolytic enzymes during fermentation, which are responsible for the reduction of fiber content.

In our study, the birds fed dietary YFAM improved dry matter, Organic matter, crude protein, and gross energy digestibility. In Addition, the protein utilization of birds fed YFAM showed significantly greater ($P\leq0.01$) feed intake, protein retained, protein intake, and net protein utilization than those fed dietary AM. These results are in congruence with early reports that supplementation with *Saccharomyces cerevisiae* improved ($P\leq0.05$) of dry matter, organic matter, crude fiber, ether extract [39,40], and crude protein [40] digestibility of Japanese quail. Afsharmanesh et al. [41] reported that the ileal digesta of broiler chickens fed with yeast in both wet and dry diets can also have enhanced absorption of nutrients, as acidification can reduce the emptying rate of the stomach and enhance the digestion process. The improvement of nutrient digestibility of the birds by fermentation of feedstuff with yeast. Kornegay et al. [42] and Abd El-Latif et al. [39] explained that live yeast, such as *Saccharomyces cerevisiae*, contains numerous enzymes that could be released into the intestine and aid existing enzymes in the digestive tract in the digestion of feed. Also, yeast contains vitamins and other nutrients that may produce beneficial production responses. The YFAM and AM have about 14.47 and 15.97% CP, showing net protein utilization results of 0.75 and 0.66, respectively. These results were agreeable with Omidiwura et al. [43], indicating that varying levels of protein in the diet (20-26% CP) of Japanese quails had a significant effect protein efficiency ratio (0.74-1.05) of the birds.

The AM's apparent metabolizable energy (AME) content for Japanese quail was analyzed to be 1,428.09 Kcal/kg. This value was lower than ME in broilers of *L. leucocephala* leaf meal (2,377 Kcal/kg) obtained by Hien et al. [44]. However, Fermentation AM with yeast significantly improved AME ($P<0.01$). The birds fed dietary YFAM was 1993.03 Kcal/kg AME. This result contradicts Nabila et al. [45] Supplementation of baker's yeast (at levels 0, 0.1, 0.2, 0.4, and 0.8 %) in broiler chicken's diet. Results showed no significant difference ($P>0.05$) was observed for metabolizable energy among all treatments. Our experiment using baker's yeast at level 5 % fermented AM was higher than Nabila et al. [45] Supplementation. Therefore, the bird-fed dietary yeast fermented caused an increase in the apparent metabolizable energy in this study.

4. Conclusions

The study shows that the fermentation AM with yeast (*Saccharomyces cerevisiae*) improved nutrient composition, enhanced nutrient digestibility, protein utilization, and apparent metabolizable energy of *Acacia mangium* leaf meal in Japanese quail. The apparent metabolizable energy of AM and YFAM was basic information for calculating feed formulation in Japanese quail.

5. Acknowledgements

The author would like to thank Asst. Prof. Dr. Seksom Attamangkune and Assoc. Prof. Dr. Neramit Sookmanee for help and support throughout this research. Moreover, I would like to thank The Agricultural Research Development Agency (ARDA) and King Mongkut's Institute of Technology Ladkrabang Prince of Chumphon Campus for funding and research equipment.

Author Contributions: Conceptualization, S.L. and W.N.; performed the experiments, S.L., D.P. and W.N.; formal analysis, S.L.; data collection, S. L., D. P. and W. N.; writing—original draft preparation, S.L.; writing—review and editing, S.L.; supervision, S.L.; project administration, S.L.; funding acquisition, S.L., and W.N.. All authors have read and agreed to the published version of the manuscript.

Funding: This research was funded by Agricultural Research Development Agency (ARDA), PRP6005011290.

Conflicts of Interest: The authors declare that there is no conflict of interest regarding the publication of this article.

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