Evaluation of in vitro digestibility and pH of Acacia seed pods (Acacia sp.)

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Abstract. This study aimed to determine the rumen fluid’s in-vitro dry matter digestibility (IVDMD), in-vitro organic matter digestibility (IVOMD), and pH of different species Acacia seed pods as a single feed ingredient. The Acacia species in this study were Acacia mangium, Acacia auriculiformis, and Acacia crassicarpa. The method used was in vitro two-stages with the first 48 hours of incubation (the rumen) and the second 48 hours of incubation (post-rumen), and analyses of pH. The research design used was a completely randomized design (CRD) with 3 treatments and 5 replications based on the Acacia species. The data were analysed with one-way analysis of variance (ANOVA) followed by Duncan’s multiple range test (DMRT). The results showed that IVDMD stage one was 34.59-37.59%; IVOMD stage one was 38.66-43.06%. Stage two of IVDMD was 58.02-59.23%; stage two of IVOMD was 51.67-55.01% and rumen fluid pH were 6.58-7.02. Different Acacia seed pods had significant differences in IVDMD and IVOMD stage one in the rumen and stage two in the post-rumen (P<0.05), but not in the pH value (P>0.05). We concluded that different acacia seed pods had different digestibility values in the rumen and post-rumen. However, it does not affect the rumen acidity.

1. Introduction
Livestock productivity is determined by the quality and quantity of feed consumed and the content of anti-nutritional substances such as tannins, lignins, saponins, and tagitinin [1]. Acacia (Acacia sp.) is a leguminous plant that can be used as an alternative source of protein for livestock. The use of Acacia leaf flour can be a cheap and easily available feed ingredient by farmers and can be a source of protein, minerals, and energy [2,3].

Acacia seed pods are identified as having various functions. The fruit can be used to fight hypertension, diarrhea, tightness, platelet aggregation activity, diabetes, and other fertility problems [4]. Phytochemically tannins 25%-60%, mucus 20%-30%, flavonoids, resins, saponins & alkaloids have been isolated from various parts of Acacia sp.. The acacia leaves contain 8% digestible protein (12.4% crude protein) and seedless pods contain 7% of total phenol, and 15.30% of total tannins throughout the pod [5,6]. The acacia seed pods contain higher amounts of flavonoids, minerals, and tannins. In addition, several types of polyphenols contained in the seed pods of acacia plants and identified were gallic acid, m-digalic acid, (+) catechins, robidandiol, chlorogenic acid, m-digalic acid, flavan-3–4-diol galloylated [6–8]. The acacia seed pods can be used as an energy source in mixed concentrates for ruminants and increase the efficiency of energy utilization in livestock. Acacia seed pods also contain essential amino acids which are almost comparable to egg protein [9].
In vitro methods were developed to estimate the digestibility and degradation rate of feed in the rumen, as well as to study various responses to changes in rumen conditions. This method is commonly used for the evaluation of feed, researching the mechanism of microbial fermentation, and to study the action on antinutrient factors, additives, and feed supplements [10,11]. The results of testing Acacia saligna leaves in different seasons in vitro Telly and Terry using several different rumen fluids (sheep, cattle, and buffalo) showed different results also on the level of degradation and fermentability of rumen fluid [12,13]. Based on this description, it is necessary to conduct a study on evaluating the digestibility value of acacia seed pods in vitro so that it can be used as an alternative feed for ruminants.

2. Materials and methods

2.1. Sample preparation
The acacia seed pods used in this study were *Acacia mangium*, *Acacia auriculiformis*, and *Acacia crassicarpa*. Samples were taken from 5 different trees of each species in the Karanganyar, Central Java, and then made a composite of 500 g of each tree in fresh condition. Selected seed pods that are still green and not too old or young. Acacia seed pods were cut into ± 1 cm long. The acacia seed pods that have been cut into pieces are then dried in the sun at 55 °C for 3-4 days until they reach a constant weight. After drying, it was ground using a hammer mill with a screen of ± 2 mm for in vitro incubation. Furthermore, the samples were analyzed for nutrient content and in vitro two-stage digestibility.

2.2. Chemical analysis
The chemical composition of Acacia seed pods analysis [14] was conducted on oven drying samples at 50 °C (moisture, ash, ether extract, crude fiber, crude protein). Organic matter was obtained by difference that is ash = 100% - ash %.

2.3. pH measurement
Rumen fluid pH was measured at the beginning and end of in vitro using a digital pH meter LUTRON PH-201.

2.4. Rumen fluid preparation
Rumen fluid was obtained from the three sheep that were slaughtered at the abattoir in the morning before being fed, rinsed with CO₂, filtered using 3 layers of nylon cloth so that the particles of the rumen contents were not mixed and mixed (1:2, v/v) with the solution. anaerobic mineral buffer using McDougall's solution.

2.4.1. Preparation of 1000 ml of McDougall solution. McDougall's solution was used as an artificial salivary solution/buffer. The first step was accurately weighed: 9.8 grams of NaHCO₃, 10 grams of Na2HPO4.12H2O, 0.57 grams of KCl, 0.47 grams of NaCl, 0.12 grams of MgSO4 7H2O. Then it was dissolved with 500 ml of distilled water in a glass beaker (1000 ml) (Solution 1). The dissolution was carried out at 39oC and using a magnetic stirrer to speed up the process. Furthermore, 5.3 grams of CaCl₂ were weighed accurately and then put into a measuring cup and dissolved with 100 ml of distilled water (Solution 2). Then 1 ml of Solution 2 was added to Solution 1. Then stir until homogeneous (Solution 3). Furthermore, distilled water is added to Solution 3 until the volume becomes 1000 ml, then 1 liter of McDougall's solution is formed. To neutralize the pH, 0.1 m HCl was added to the solution. 0.1 m HCl solution was prepared by diluting 455.75 ml of concentrated HCl (normality 11.3) with 44.25 ml of distilled water.

2.4.2. Preparation of HCl-pepsin solution. The pepsin solution was made by dissolving 0.2 grams of pepsin with 0.1 N HCl and then adding aquadest to make it 50 ml.

2.5. In vitro dry matter degradability (IVDMD) and in vitro organic matter digestibility (IVOMD)
In vitro testing of treatment rations based on the in vitro two-stage refers to the [15] method which has been modified by [16], where without washing the residue in the rumen phase procedure to the post-rumen phase, so that HCl and pepsin are added immediately. The first stage (in the rumen) lasts for 48 hours. A total of 0.5 g of treatment samples were fermented in a 50 mL in vitro fermenter tube filled with rumen fluid: artificial saliva (1:4 v/v). Mc Dougall solution/artificial saliva which functions as an artificial rumen was then given CO$_2$ to create anaerobic conditions and incubated in a water bath at ±39°C. Every 2 hours the fermenter bottles were shaken manually to get the conditions of movement like in the rumen. After 48 hours, ±1-2 drops of saturated HgCl$_2$ were added to each fermenter tube to stop rumen microbial activity.

The second stage of the abomasum condition (post rumen) was made by adding 20% HCl into each test tube as much as 3 mL (by giving gradually 0.5, 0.5, 1, and 1 mL) and after that 5% pepsin was added as much as 1 mL. Digestion in the abomasum lasts for 48 hours. Each incubation point of the incubated treatment, blank, and standard was replicated 5 times. Blanks are tubes filled without treatment samples, whose function is as a correction factor. Standard is a tube filled with pangola grass. Shaking is done manually every 8 hours.

2.6. Data analysis
This research used one-way randomized design. The data were analyzed using analysis of variance (ANOVA) and differences between treatment means were further analyzed using Duncan’s New Multiple Range Test (DMRT) with significance level of p<0.05.

3. Results and discussions

3.1. Chemical composition of different acacia species
The nutrient content of three species of acacia seed pods showed different results between species (Table 1).

| Species              | DM     | OM     | CP     | Ash    | NDF   | ADF    | Hemicellulose |
|----------------------|--------|--------|--------|--------|-------|--------|---------------|
| Acacia mangium       | 29.44a | 94.56  | 8.03a  | 5.44a  | 54.50a| 29.81a | 24.69a        |
| Acacia auriculiformis| 36.47b | 96.03  | 6.15b  | 3.97b  | 44.35b| 28.07a | 16.28b        |
| Acacia crassicarpa.  | 35.60b | 95.02  | 5.98a  | 4.98a  | 43.00b| 21.86b | 21.14a        |
| P values             | 0.038  | 0.072  | 0.011  | 0.065  | 0.034 | 0.042  | 0.022         |

DM= dry matter; OM = organic matter, CP = crude protein, NDF = neutral detergent fibre, ADF = acid detergent fibre
Means with different letters in the same raw differ (P < 0.05).

Based on the results showed that the content of DM, CP, NDF, ADF, and hemicellulose of the three acacia species ranged from 29.44-36.47% respectively; 5.98-8.03%; 43.00-54.50%; 21.86-29.81%, and 16.28-24.69%(P<0.05). While the OM and ash content of several acacia species showed results between 94.56-96.03% and 3.97-5.44% (p>0.05). This can be caused by differences in species, plant age, drying process, and seasons that can affect the nutrient content of acacia. According to [13], the age of maturity and season can affect the level of digestibility in vitro gas tests. Therefore, in the dry season acacia plants can be used as forage for ruminants. Although there are limiting factors such as secondary metabolic compounds in its administration. Leguminous plants can meet the nutritional needs of microbes in the rumen [17].

3.2. In vitro dry matter degradability (IVDMD) and in vitro organic matter digestibility (IVOMD)
Based on the results of the in vitro two-stage test, dry matter digestibility and organic matter digestibility in the rumen and post-rumen as well as pH can be seen in table 2.
Table 2. In vitro dry matter degradability (IVDMD) and in vitro organic matter digestibility (IVOMD) of acacia seed pods

| Spesies of acacia seed pods  | Acacia mangium | Acacia auriculiformis | Acacia crassicarpa | p-values |
|-----------------------------|----------------|----------------------|-------------------|---------|
| IVDMD stage 1               | 34.59±5.51b    | 36.59±0.54a          | 37.59±0.38a       | 0.035   |
| IVDMD stage 2ns             | 58.02±1.29     | 59.23±0.15           | 58.23±0.57        | 0.065   |
| IVOMD stage 1               | 38.66±0.47a    | 40.79±0.61ab         | 43.06±0.91b       | 0.012   |
| IVOMD stage 2               | 55.01±0.01b    | 51.67±0.68a          | 53.37±0.95c       | 0.023   |
| pHns                        | 7.03±0.09      | 7.02±0.58            | 6.58±0.41         | 0.091   |

a, b, c, means in the same column with different superscript differ significantly (P<0.05)

The results showed that IVDMD stage one was 34.59-37.59% (P<0.05); IVOMD stage one was 38.66-43.06% (P<0.05). Stage two of IVDMD was 58.02-59.23% (P<0.05); stage two of IVOMD was 51.67-55.01% (P<0.05); and rumen fluid pH was 6.58-7.02 (P>0.05) (Table 2). The difference in these figures is due to differences in plant species and the nutrient content in them. Dry matter digestibility is one indicator to determine the quality of the ration. The higher the dry matter digestibility, the higher the opportunity for nutrients that can be utilized by livestock for growth. This is in accordance with the opinion of [5,18] which states that feed digestibility is influenced by the treatment of feed (processing, storage, and administration) type, amount, and composition of feed given to livestock. The value of the digestibility of organic matter is in line with the digestibility of dry matter, this is because the content of organic matter is present in dry matter

The low IVDMD and IVOMD values of acacia seed pods were caused by the content of secondary metabolic compounds (tannins) in acacia seed pods. In addition, the high content of fiber fractions such as NDF and ADF has an impact on the digestibility value in the rumen (Table 2.). The phytochemical content of Acacia seed pods extract revealed the presence of phytochemicals such as tannins, proteins, flavonoids, carbohydrates, and phenolic compounds [19]. Acacia leaf tannin compounds can reduce the in vitro digestibility of dry matter, organic matter, and crude protein. Degraded products of tannins from Acacia seed pods in goat rumen fluid are phloroglucinol, gallic acid, resorcinol, and catechins [9]. Rumen microbes digest more soluble carbohydrates so that the percentage of propionic acid is higher than acetic acid. The concentration of propionic acid is high due to the higher availability of easily degraded carbohydrates in the rumen which is thought to come from concentrates [20]. The lower of fiber fraction component, the less energy needed by microbes to digest cellulose, hemicellulose, and lignin, so that it can increase digestibility. Lignin content in the feed is one of the factors that affect digestibility [5,9].

The pH value obtained was still in the normal range of rumen pH between 6.58-7.02 (P>0.05) so that it could maintain the feed fermentation process in the rumen running optimally. According to earlier study [21], the ideal fermentation in the rumen requires a pH in the range of 5-7.5. This is supported by [22] that pH values that are outside the range of 5.7-7.8 will have a negative impact on the rumen so that the pH in this study was still in a normal state which did not interfere with rumen function. When livestock consumes feed that contains a lot of fiber or structural carbohydrates, the pH tends towards 7.5, but if the feed contains more soluble carbohydrates, the pH tends towards 5 [23]. The changes in feed can result in a shift in the population of cellulyotic and amyolytic microbes in the rumen. The number of cellulyotic microbes decreases when starch fermentation occurs in the rumen, which in turn affects the pH conditions in the rumen [24].

4. Conclusion
We concluded that different acacia seed pods had different digestibility values in the rumen and post-rumen. However, it does not affect the rumen acidity.
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