

# The Study of Relationship Between the Number of Protozoa and Inoculum pH on the *in vitro* Technique Incubating Treated *Colleus amboinicus*

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## ABSTRACT

This experiment aimed to reveal the relationship between the number of protozoa and the pH of inoculum in incubating different treated *Colleus amboinicus in vitro* technique. A sample of dry *Colleus amboinicus* was powdered and then treated with four treatments. There were four treatments including *Colleus amboinicus* without treatment as control, *Colleus amboinicus* put into a capsule, *Colleus amboinicus* treated with tannin, and *Colleus amboinicus* treated with saponin for T0, T1, T2, and T3 respectively. Treated *Colleus amboinicus* was incubated following *in vitro* gas technique. At the end of the incubation period, inoculum pH was measured and around 3 ml of samples was used for calculating protozoa number. Data were statistically analyzed for regression and correlation. The result of this experiment shows that there was a low negative correlation between protozoa number and inoculum pH ( $r^2 = -0.1999$ ) but the effect of protozoa number on the inoculum pH was also negative on the inoculum pH.

**Keywords:** Protozoa, pH, *Colleus Amboinicus*, Inoculum, Relationship.

## 1. INTRODUCTION

*Colleus amboinicus* (CAL) is a tropical local herb which potentially increases the *in vitro* digestibility of ration [1]. The addition of CAL into ration could be possible. However, the protection of CAL nutrients is very crucial to maximize the utilization of its nutrient content especially protein in rumen. Some studies regarding protein protection using tannin and saponin have been done [2],[3]. Apart from this, the use of tannin and saponin within ration will influence the rumen microbial diversity. Saponin is a natural detergent as defaunation agent on protozoa [4]. Defaunation could increase 18 % of protein into post rumen digestive tract rumen [5]. Defaunation could also influence the methanogen metabolism and the rumen environment like rumen pH, ammonia concentration. Yanuartono et al [6] stated that protozoa represent almost 50% of total microbial biomass in rumen. The existence of protozoa within rumen could influence the amount of bacteria, kind of bacteria, proportion and concentration of volatile fatty acid, rumen pH, and ammonia concentration [6].

The effect of protozoa on the rumen pH is still

debatable as the rumen ecosystem is very complex. Protozoa could have a reciprocal effect on rumen pH. An increasing amount of protozoa in rumen would reduce the bacteria number and then also reduce the fatty acid production which maintains the rumen pH to be neutral. However, protozoa are very sensitive to acid, and when the pH is low the number of protozoa will decrease [7]. Martin et al [8] reported that the decrease of protozoa population in rumen could reduce methane production and increase the efficiency of use of energy and the use of microbial protein for ruminant, therefore it could hopefully increase animal production as a whole. Some studies have been done concerning the ration modification to reduce the protozoa population or defaunation in the rumen [9][10]. Wahyuni et al [2] try to use some dosages of tannin and saponin for defaunation and increase the fermentability of feed-in ongle cross-breed cow. In this case, the amount of protozoa population could influence the rumen pH. This study aimed to reveal the relationship between the total amount of protozoa population and the inoculum pH.

## 2. MATERIAL AND METHOD

### 2.1. Sample preparation

A sample of dry *Colleus amboinicus* (DCA) was imported from Bogor through Tokopedia online. The dry sample was re-oven-dried at 60 °C overnight and then powdered into a 1 mm sieve. The powder sample was kept until further treatment with saponin and tannin. Extract Saponin and tannin were prepared following procedure [11] with slight modification. For the extraction of saponin, 600 g of fresh *Hibiscus rosinansia* leaf was sliced and put into a food blender (Philips Ltd). Furthermore, 900 ml of water was also added into a blender and blended until becoming juice for 10 minutes. The juice was filtered through a two-layer cotton sheet to get an extract of saponin. The extract was then used to waddled DCA homogeneously. The waddled DCA was oven-dried for 60 °C overnight until dry and kept for further use. For the extraction of tannin, 400 g of the fresh banana stem was sliced and put into a food blender (Philips Ltd). Furthermore, 400 ml of water was also added into a blender and blended until becoming juice for 10 minutes. The next step was the same with saponin extract procedure.

### 2.2. Experimental procedure

Method of first step Tilley and Terry [12] was applied. Twenty samples of CAL treated with four different treatments including control of dry CAL, capsuled CAL, saponin treated CAL and tannin treated CAL were incubated following technique Afdal et al [13]. At the end of the incubation period, pH was measured and around 3 ml of inoculum liquid was taken for counting protozoa population according to procedure Ogimoto and Imai [14]. Both data of protozoa count and inoculum pH were statistically analyzed for regression and correlation between both parameters [15].

## 3. RESULT AND DISCUSSION

A picture of the relationship between the protozoa number and the inoculum pH can be seen in Figure 1. The correlation coefficient (R) between protozoa number and inoculum pH was 0.8385, with the determination coefficient (R<sup>2</sup>) of -70.3%. It shows a strong negative linear relationship between the two parameters. There is no information from a previous study concerning the relationship between two parameters. Protozoa is theoretically a predator of bacteria. Therefore it consumes bacteria that will influence the fermentation process in rumen and other side effect like rumen methanogenesis, acid production, and Ph.

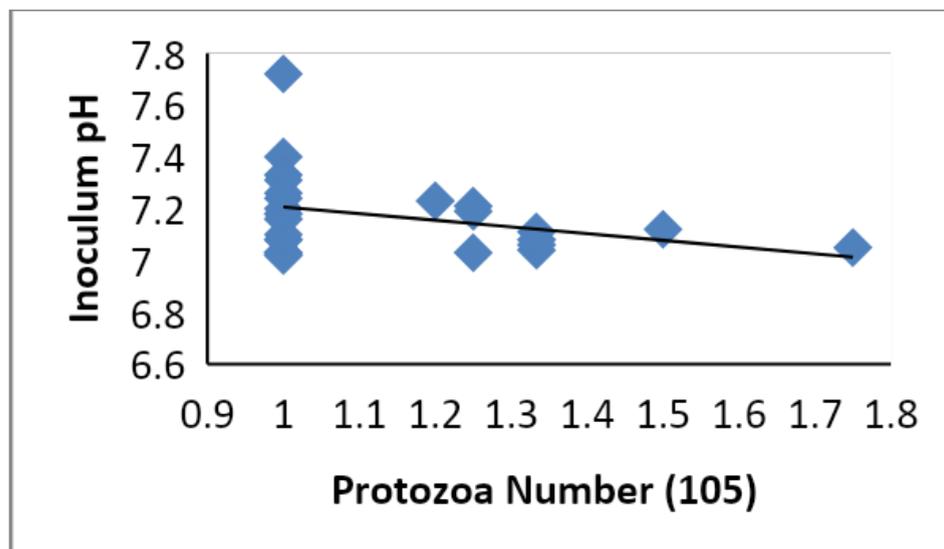


Figure 1. The relationship between the protozoa number and inoculum pH

In this study, CAL was protected with tannin and saponin to prevent the degradation of the protein. Sugoro and Yuniarto [16] reported that the number of protozoa decreases when buffalo was fed with

supplementation of tannin in ration. They added that there were a tolerant and intolerant type or species of protozoain rumen. This might depend on the ration eaten by the ruminant. Some studies have been done

about the effect of feed on the protozoa number [2][3][16].

The protection of CAL with tannin and saponin could provide some effects on the fermentation in the rumen. Tannin and saponin can reduce protein degradation and synthesize microbial protein [3]. This can provide more bypass protein into the hindgut. This could improve nitrogen utilization, animal performance such as milk yields, and milk quality. Apart from this, the effect of tannin and saponin within ration will decrease ruminal ammonia concentration N excretion from the rumen, ammonia emission from manure [17]. Additionally, Avila et al [18] stated that Condensed tannins did not affect intake and ruminal protozoa population, but reduced protein digestibility and decreased ruminal pH and acetate proportion

#### 4. CONCLUSION

It could be concluded there is a negative correlation between protozoa number and the inoculum pH on the incubation of treated CAL.

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