

Rumen Protozoa Diversity of Indonesian Indigenous Cattle

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ABSTRACT: Information on the dominant protozoa in local cattle, especially on Ongole Crossbred (OCB), Bali cattle (BC), and Madura cattle (MC), are still little disclosed in the article. This study aimed to provide information and characteristics of the dominant protozoa in the rumen of local cattle. The experiment used digesta and rumen fluid which were collected from Pegirian Abattoir at Surabaya city. This study used observation under an electron microscope (10 x 10) using a Sedgewick Rafter counting chamber S52. The results showed that the basal feed given in the form of forage was indicated by the NDF and ADF digesta values and closely related to the presence of the dominant genus of protozoa. The dominant genus of OCB and BC is *Eudiplodinium*, while MC dominant genus of *Entodinium*.

Keywords: Indonesian indigenous cattle; Protozoa; Rumen

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INTRODUCTION

The ruminant livestock business is dominated by smallholder farmers (Huda *et al.*, 2020). The characteristics of smallholder farmers are conventional rearing management, ownership of 2-3 cows, and depending on the available feed around them (Huda *et al.*, 2021). Feed is one factor that determines the microbial ecosystem in the rumen. Microbes in the rumen have their function in digesting the feed consumed.

The feed provided by farmers is generally high in fiber content, as evidenced by the *Neutral Detergent Fiber* (NDF) and (*Acid Detergent Fiber*) ADF content in the feed. NDF is a nutrient that is insoluble in neutral detergent and contains hemicellulose, cellulose, and lignin (Jaimes *et al.*, 2019); however, ADF residues contain cellulose and lignin and variable proportions of other cell wall polysaccharides (Harris and Ferguson., 2014). The dominant feed is in the form of forage and a small amount of concentrate, with forages commonly given in corn stalks, elephant grass, and rice straw (Huda *et al.*, 2021). High fiber content will increase the presence of fiber-digesting microbes. Bacteria and Protozoa in the rumen have a role in digesting fiber (Vasta *et al.*, 2010). Information about bacteria in fiber digesters has been widely discussed in several previous reports, but the identification of protozoa is still very little to be discussed. Thus, this study was conducted to provide information about the predominant presence of protozoa in the rumen of Indonesian indigenous cattle; Bali (BC), Madura (MC), and Ongole Crossbred (OCB).

MATERIALS AND METHODS

This research uses rumen digesta from OCB, Madura, and Bali cattle. Rumen digesta collected from Pegirian Abattoir at Surabaya city. Cattle selection was carried

out to determine the characteristics of each cattle and then continued with permanent incisive (PI) observation to cattle age estimation. Rumen temperature and pH were also observed in this research. Rumen fluid sample was prepared with formal saline (1:1).

Protozoa population was observed in Epidemiology Laboratory, Animal Science Faculty, and Life Science Laboratory Center, Universitas Brawijaya. The protozoa population was counted with a Nikon monocular microscope and Sedgewick Rafter counting chamber S52 with 1 ml volume. The Chamber size used was 50 mm x 20 mm x 1mm, and the inside grid was 100 x 1 mm. This counting chamber was also equipped with a 60 mm x 30 mm x 1 mm cover that works to keep the volume precisely 1 ml. Observations use microscope light with 10 x 10 magnification, and documentation of dominant protozoa performed with application NIS elements D.440.0064-bit and Genus's similarity determination is carried out according to Dehority (1993).

RESULT AND DISCUSSION

Characteristics of Local Cattles Rumen Fluid

Observations of permanent incisive (PI) were made to estimate the age of cattle. The result of the estimation showed in Table 1. Based on data that showed in Table 1, the age of cattle collected was between 2-4 years old.

The differences in cattle age are one factor that affected degradation processes in the rumen due to differences in the dominant microbes. Rey *et al.* (2013) reported that the age of cattle would rapidly affect changes in the dominant microbial phylum. Guo *et al.* (2020) also reported that the microbial group of bacteria and protozoa had changes that were more sensitive to changes in the age of cattle.

Table 1. Estimation of cattle age

Cattle Breed	Permanent Incisives (PI)	Age (years)
OCB	4	2-3
Madura	6	3-4
Bali	6	3-4

The population of bacteria and protozoa will be stable when the livestock is eight years old. Based on this statement, it can be concluded that the presence of the dominant microbes that will be obtained will be different for each cattle. Identification of the feedstuff cannot be carried out, so

identifying rumen samples becomes the alternative approach to obtain the value of cattle digesta fiber content. The results of these observations are presented in Table 2 the crude fiber component in the rumen samples can be seen from the content of NDF and ADF.

Table 2. Rumen digesta characteristics

Characteristics	OCB	Madura	Bali
Temperature (°C)	40	39	39
pH	6.9	7.2	7.0
The fiber content of digesta (%)			
NDF	68.95 ± 0.203	72.06 ± 1.498	65.92 ± 0.069
ADF	51.27 ± 0.682	44.91 ± 3.127	41.99 ± 0.950

The result in Table 2 showed that the average value of NDF from local cattle is between 65.92-72.06% and ADF 41.99-51.42 %. These results indicate that the dominant basal feed is given in the form of forage which contains a high fiber content compared to concentrate.

This statement is supported by the experiment results of Ransa *et al.* (2020), which reported the NDF and ADF content of basal feed types such as corn straw, namely 69.81 and 40.20%, elephant grass 73.52 dan 44.49%, while concentrate 27.23 dan 14.39%. Another result also showed that NDF and ADF of corn straw are 66.20 ± 0.47 % and 43.64 ± 0.40 % (Usman *et al.*, 2019).

Based on these statements, overall, it provides basal feed in the form of agricultural by-products. This is supported by the results of pH in the rumen. The pH shown in the rumen of local cattle has ideal conditions, which is in the range of 6.9-7.2, so that there is no acidosis process in the

rumen. The pH of rumen cattle with acidosis is less than 5.0-6.0 (Desnoyes *et al.*, 2008).

Population of protozoa

The group of microbes in the rumen consists of bacteria, fungi, and protozoa. Interaction of this microbial group carries out the digestion of structural carbohydrates to produce Volatile Fatty Acid (VFA). The presence of ciliates in the rumen varies widely between 10⁴ and 10⁶/ ml rumen fluid, and their presence represents 50% of the ruminal biomass (Duarte *et al.*, 2018).

Based on Table 3. showed that OCB, Bali, and Madura cattle have a higher protozoa population. Based on Duarte *et al.* (2018), age difference affected on protozoa population, steers (20-40 months) had the highest population (4.83 x 10⁶) while the lowest protozoa population (1.72 x 10⁴ and 9.50 x 10⁴) were calves and cows (6-8 months and four years old). Through these statements, it can be mentioned that the older cattle will decrease the population of protozoa; this can be seen in Table 3.

Table 3. Population protozoa in the rumen

Cattle Type	Population protozoa (/ml rumen fluid)
Ongole Crossbred	1.0 x 10 ⁴
Madura	1.2 x 10 ³
Bali	1.8 x 10 ³

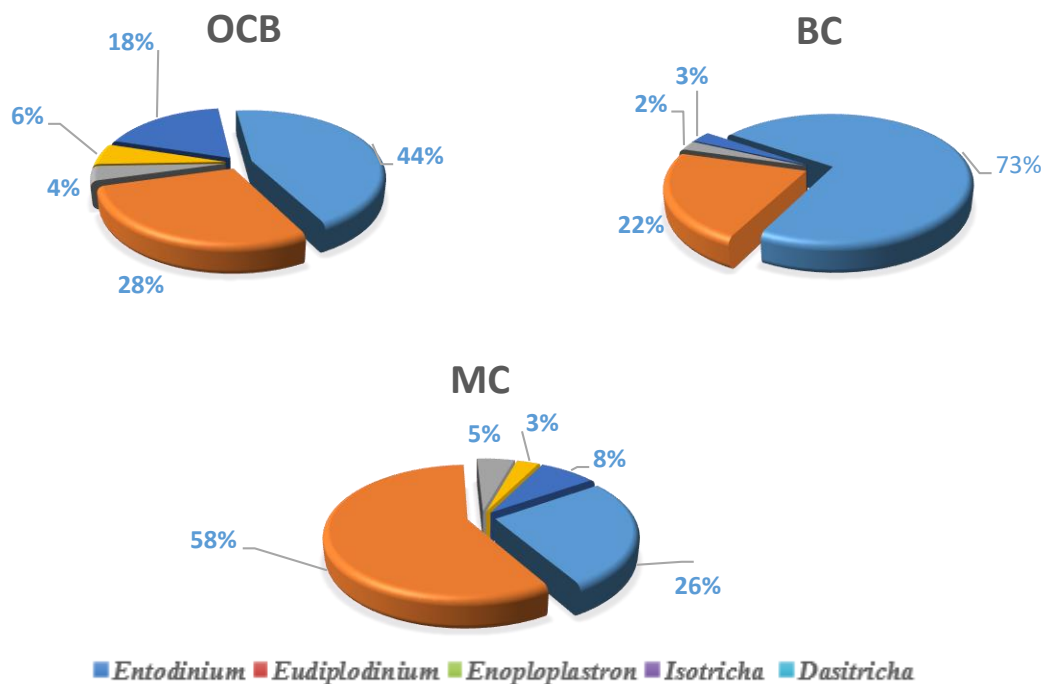
The lowest populations are Madura and Bali's cattle, estimated at 3-4 years old, while the OCB cattle are estimated 2-3 years old, and it has the highest protozoa population. According to Zhang *et al.* (2020), cattle breeds affect the abundance of protozoa in the rumen. So, it can be concluded that the factors that affect the population of protozoa in the rumen are age, feed, and breed of livestock.

Identification of protozoa

The group of protozoa in the rumen consists of *Holotricha* and *Oligotricha*. The *Holotricha* group has a role in digesting carbohydrates, while the *Oligotricha* group digests plant fiber and cellulose (Soetanto,

2019). Observations showed that all local cattle had high protozoa levels in the *Genus Eudiplodinium*, *Entodinium*, and *Polyplastron*, *Dasytricha*, and *Isotricha*. The results of observations of the protozoa genus are presented in Figure 1.

OCB and Bali cattle showed the dominant genus in *Eudiplodinium*, *Entodinium*, and *Dasitricha*, while in Madura cattle, the dominant genus was *Entodinium*, *Eudiplodinium*, and *Polyplastron*. The dominant protozoa have a role in digesting fiber, especially cellulolytic. Cellulolytic protozoa produce cellulolytic enzymes to reduce sugars, ammonia, and VFA (Gonzales *et al.*, 2014).



*Ongole Crossbred (OCB); Bali Cattle (BC), and Madura cattle (MC)

Figure 1. Percentage of dominant protozoa genus in local Indonesian cattle

Eudiplodinium, *Polypaston*, and *Entodinium* are *Oligotricha* groups of protozoa with higher prey activity than the *Holotricha* group (Newbold *et al.*, 2015). The *Oligotricha* genus-group, about 90%,

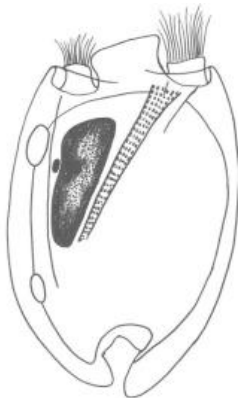
has a role in cellulose's hydrolysis and fermentation process (YanezO-Ruiz *et al.*, 2004). This group *Eudiplodinium* and *Polypaston*, can digest plant crude fiber and cellulose. Other results were also reported

by Fondevila and Dehority (2001) in an *in vitro* study showing that protozoa of the genera *Polyplastron* and *Eudiplodinium* can digest crystalline cellulose and xylan. The review results conducted by Gonzales *et al.* (2014) stated that protozoa that produce cellulolytic enzymes are *Eudiplodinium maggii*, *Enoploplastron triloricaum* with fermentation products in the form of reducing sugars. *Entodinium* is a harmful protozoa because its presence can degrade bacterial protein cells (turnover) and reduce protein utilization by cattle (animal hosts) (Newbold *et al.*, 2015). Ivan *et al.* (2000) reported that the presence of *Entodinium* in the rumen can reduce up to 18% of bypass protein supplementation, so it is recommended to do specific defaunation in this genus.

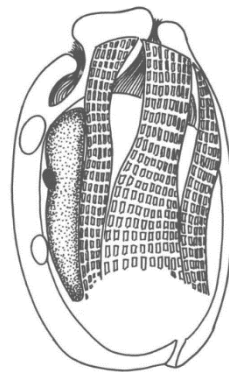
Isotricha and *Dasytricha* are the *Holotricha* group. The genus *Isotricha* is found in almost all livestock and has the highest percentage of OCB with a percentage of 6%; this is very good even though in the OCB, the dominant genus is *Entodinium* which can reduce protein bypass, the presence of *Isotricha* can suppress the degradation. This is because the role of *Isotricha* can reduce protein degradation in the rumen to increase bypass supplementation into the abomasum (Ivan *et al.*, 2000), while the presence of the genus *Dasytricha* is a protozoa that does not have crude fiber degradation activity. However, *Dasytricha* has glucosidase and cellobiosidase enzyme. However, its presence is neglected (Takenaka *et al.*, 2004).

Dehoriry (1993)

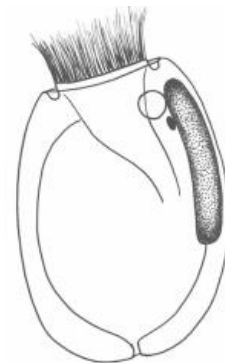
(a)



(b)



(c)



Result



(10 x 10)

Note: *Eudiplodinium* (a); *Enoploplastron* (b); *Entodinium* (c)

Figure 2. Protozoa Genus of *indigenous* cattle

The body size of the dominant genus shown in Figure 2 has different sizes depending on the species (Dehority, 1993). The results showed that the average genus *Eudiplodinium* identified was average Length $122.72 \pm 11.657 \mu\text{m}$ with width $76.61 \pm 4.380 \mu\text{m}$ and included in species *Eudiplodinium maggi*, which has an average length of $115 \mu\text{m}$ with a range of $115\text{-}212 \mu\text{m}$ and a width of $100 \mu\text{m}$ with a range of $73\text{-}143 \mu\text{m}$. Genus of *Enoploplastron* identified's average a length $97.03 \pm 3.648 \mu\text{m}$ with a width of $60.04 \pm 1.919 \mu\text{m}$ and included in species *Enoploplastron triloricastrum* which has an average length of $100 \mu\text{m}$ with a range of $60\text{-}112$ and a width of $61 \mu\text{m}$ with a range of $37\text{-}70 \mu\text{m}$. Genus *Entodinium* identified having an average length of $64.82 \pm 19.183 \mu\text{m}$ with a width of $40.86 \pm 10.547 \mu\text{m}$ and included in species *Entodinium longinucleatum* has an average length of $54 \mu\text{m}$ with a range of $44\text{-}110 \mu\text{m}$ and a width of $37 \mu\text{m}$ with a range of $27\text{-}87 \mu\text{m}$; (Dehority, 1993).

CONCLUSIONS

There is a relationship between feed and the presence of the protozoa population to the dominant genus in the rumen. The dominant genus of OCB and Bali Cattle is *Eudiplodinium*, while in Madura cattle, it is the *Entodinium* genus group. *Eudiplodinium* is closely related to fiber degradation, while *Entodinium* tends to increase the degradation of bacterial protein cells in the rumen.

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