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Digestibility, Blood Metabolites, Faecal Bacterial Population and Performance of Madura Cattle Inoculated with Cellulolytic Bacteria Consortium

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ABSTRACT

This study aimed to evaluate the effect of cellulolytic bacteria consortium inoculation on Madura cattle's digestibility, blood metabolites, fecal microbial population, and performance. This study used 15 Madura cattle with an initial body weight of 150-300 kg with a standard deviation of 38.45 kg. A complete group randomized design with 3 treatments and 5 replications was used in this study. The grouping of the cattle is based on initial body weight. The treatments consisted of T1: concentrate: rice straw with a ratio of 50:50 as the control ratio, T2: T1 + 5 ml 10^6 CFU/ml of cellulolytic bacteria consortium, and T3: T1 + 10 ml 10^6 CFU/ml cellulolytic bacteria consortium. The data obtained were analyzed using analysis of variance (ANOVA), and the differences among treatments were further analyzed using Duncan's multiple range test. The result showed that inoculation of cellulolytic bacteria consortium until 10^6 CFU/ml had no significant effect on performance, blood metabolites, digestibility, and total microbial population during 30 days of treatment. In conclusion, inoculation of cellulolytic bacteria consortium did not improve performance and digestibility in short periods and did not interfere with blood metabolites.

Keywords: Cellulolytic bacteria, performance, digestibility, blood metabolites, Madura cattle

INTRODUCTION

Livestock farming in Indonesia highly depends on feed availability in the form of forage or agricultural waste. However, during the dry season, feed availability becomes limited due to decreased production, accompanied by the low nutritional quality of the available feed. This condition is one of the factors inhibiting the increase in livestock production, especially ruminants. Low feed quality is generally caused by feed ingredients dominated by agricultural waste and forage with low digestibility. Low-quality feed also adversely affects the rumen function of livestock.

In Madurese cattle farms managed by smallholder farmers, the main feed is mostly low-quality forages such as elephant grass and rice straw. These forages have a low level of digestibility, resulting in low body weight gain. Increasing forage digestibility can be done by adding fiber-degrading bacteria, especially cellulolytic bacteria. The rumen of Madura cattle has a high microbial population, including bacteria, protozoa, and fungi (Muslim et al., 2014). However, research shows that adding cellulolytic bacteria to ruminants can provide significant benefits, such as increased feed digestibility and livestock performance. Adding cellulolytic bacterial isolates can help these bacteria develop in the rumen, break down feed fibre, and improve digestibility. In addition, the production of volatile fatty acid (VFA) and digestibility of dry matter and crude fibre also increases. Adding cellulolytic isolates also accelerates the fermentation process in the rumen (Suharti et al., 2023). The cellulolytic bacteria in this study came from herbivorous animals' endemic to Indonesia, such as deer, bull, anoa, and deer. Suharti et al. (2023) reported the presence of Enterococcus faecium, which has cellulolytic properties in the feces of anoa and banteng.

Cellulolytic bacteria produce cellulase enzymes that break down cellulose, thus allowing ruminants to survive despite consuming low-quality forage (Arora, 1992). The effectiveness of the biodegradation process of cellulosic feed is highly dependent on the ability of cellulolytic microbes to produce cellulase enzymes with high activity (Asenjo et al., 1986). Previous studies have successfully isolated cellulolytic bacteria from Indonesian endemic herbivores such as deer, banteng, anoa, and deer. According to Suharti et al. (2023), adding cellulolytic bacterial isolates increased fibre degradation, indicating that the isolates could thrive in the rumen system and break down feed

fibres. However, although the results of in vitro tests have shown this potential, further in vivo studies are needed to analyze the ability of cellulolytic bacterial isolates to degrade fibre in Madura cattle-fed rice straw-based rations.

This study aims to analyze the effect of cellulolytic bacteria consortium inoculation on digestibility, blood metabolites, fecal microbial population, and performance of Madura cattle.

MATERIALS AND METHODS

Time and Location of Research

This research was conducted in May - June 2024. This research was conducted in several laboratories, including Microbial propagation carried out at the Biochemistry Laboratory and Microbiology of Nutrition (BMN); in vivo, tests on cattle were carried out at the Baratajaya Farm field laboratory, a partner of the Faculty of Animal Husbandry, Bogor Agricultural University. Blood analysis was conducted at the Meat and Work Animal Nutrition Laboratory, Faculty of Animal Husbandry, Bogor Agricultural University; Proximate analysis was carried out at the Biological Material Analysis Laboratory, IPB Biotechnology Center.

Microbial Propagation Procedure

The total bacterial population was calculated using the roller tube method according to Ogimoto and Imai (1981). This study used faeces from Timor deer, the anoa, muntjak, and bison as a source of cellulolytic bacteria. Brain Heart Infusion (BHI) medium was used to culture all bacterial isolates. The composition of BHI media includes 3.7 grams of BHI powder, 0.5 ml haemin, 0.05 ml resazurin, and 10 ml Carboxy Methyl Cellulose (CMC) 1% dissolved in 100 ml distilled water. This mixture was heated, CO_2 was added to maintain anaerobic conditions, and 0.1 gram of HCl-cysteine was added. A total of 9 ml of BHI broth was put into a test tube and then sterilized in an autoclave at 121 ℃ for 15 minutes. 1 ml of culture from each isolate was inoculated into the medium and then incubated at 39℃. The incubation lasted 8 hours for bacterial isolates from Timor deer, muntjak, and anoa and 12 hours for bacterial isolates from bison.

Research Rations

The research ration consisted of rice straw and concentrate with a ratio of 50:50. The nutrient composition of rice straw, concentrate, and total ration is presented in Table 1.

Table 1. Nutrient composition of rice straw, concentrate and total research ration

Nutrient Content	Rice Straw	Concentrate	Total Rations
Dry Matter (%)	95.63	87.55	91.59
Crude Protein (%)	10.04	17.55	15.86
Crude Fat (%)	2.15	3.98	3.53
Crude Fiber (%)	28.10	14.66	24.01
Ash (%)	19.37	5.36	13.74
NFE (%)	40.34	58.45	56.59

Source: Biological Material Analysis Laboratory, IPB Biotechnology Center 2024, Note: NFE = Nitrogen-Free Extract

Cattle Maintenance

Fifteen adult Madurese cattle with a weight range of 150-300 kg with a standard deviation of 38.45 kg were kept for 30 days in a pen. Cattle were randomized based on body weight into 3 treatments. In vivo tests will be given a control ratio. Feed and drinking water were provided ad libitum. The remaining feed is weighed every morning on the following day before feeding. The treatments in this study are as follows:

T1: Control ration

T2: T1 + 5 ml 10^6 CFU/ml cellulolytic bacteria consortium

T3: T1+ 10 ml 10^6 CFU/ml cellulolytic bacteria consortium

Probiotic feeding was conducted for 21 days. Microbes were given to cows orally in amounts of as much as 5 ml and 10 ml 10^6 CFU/ml using a syringe.

Research Parameters

Research parameters included nutrient digestibility (crude fibre), blood metabolites (glucose, triglycerides, and BUN), bacterial

population in faeces, and performance (feed consumption, body weight gain, and feed efficiency).

Measurement of Crude Fiber Digestibility

Feces collection was carried out for five consecutive days in the last week of rearing to determine the nutrient content of the feces. Feces were taken after 24 hours of collection, then weighed and stirred (homogenised) to be taken as a sample of 500 g of feces. The sample was placed in an oven at 60℃ and weighed until constant weight. After air drying, the samples were homogenized per treatment and mashed with a mortar. Samples were separated into plastic for proximate analysis, namely crude fiber.

$$Crude\ Fiber\ Digestibility = \frac{Crude\ fiber\ consumption - Fecal\ crude\ fiber}{Crude\ fiber\ consumption}\ x\ 100\%$$

Blood Sample Collection

Blood sampling was done in the morning. Blood sampling for metabolite analysis was done at the end of the study. In blood metabolite testing, blood samples were taken from the jugular vein; 3 ml of blood was taken with a syringe and then put into an EDTA tube.

Blood Metabolite Analysis

Glucose, triglyceride, and BUN levels were measured by centrifuging blood samples for 15 minutes at 3000 rpm to obtain blood plasma. The obtained plasma is analyzed for glucose, triglyceride, and BUN levels using a Microlab 300 tool based on enzymatic reactions with the KIT method (DyaSis brand).

Measuring Bacterial Population (Ogimoto and Imai, 1981)

The total population of fecal bacteria was calculated according to Ogimoto and Imai (1981) using the roller tube method. The media used in this study was brain heart infusion (BHI). The 100 ml BHI media solution uses the formula: 3.7 g BHI powder; 0.05 g glucose and starch; 10 ml CMC 1%; 0.5 ml Hemin 0.5%; 0.05 ml resazurin and 100 ml distilled water. All ingredients were heated until they turned golden yellow. The solution was cooled while CO_2 gas flowed, and after cooling, 0.1 g of l-cysteine was added. Bacto agar was put as much as 0.9 g into a Hungate tube, added to the BHI solution, and then heated until homogeneous while flowing CO_2. The media was sterilized in an autoclave at 121℃ for 15 minutes at a 1.2 Kg f cm^{-3} pressure. In this study, feces that had been dissolved using glycerol in a ratio of 1:3 (glycerol: feces) were used. Dilution was carried out until the 7th dilution, then 1 gram of feces was weighed and homogenized with a dilution of 10 ml. Then the fecal suspension was taken using a 0.5 ml syringe into the previously prepared BHI media, after which the tube was rotated (so that it covered the hungate tube) and put into a roller tube. The samples were incubated for 48 hours at 39℃. The bacterial population was calculated using the following formula:

$$Total\ bacterial\ population = \frac{Bacterial\ population}{ml\ dilution\ x\ 10_n x\ ml\ of\ BHI\ media}$$

Livestock Performance Measurement

Livestock performance observations were made daily during maintenance to measure feed consumption. Body weight weighing was conducted before and after the research period. Livestock performance measured included consumption of Dry matter, Crude Protein, Crude Fiber, Crude Fat, Nitrogen-Free Extract, Daily Weight Gain, and Feed Efficiency.

Experimental Design and Data Analysis

The experimental design was a group-randomized design consisting of three treatments and 5 replicates. The data obtained were analyzed using ANOVA analysis of variance and t-test using SPSS software version 25. Test results that show significant differences ($P < 0.05$) will be tested with Duncan's further test.

RESULTS AND DISCUSSION

Feed Nutrient Consumption

Effect of inoculation of cellulolytic bacteria in Madura cattle for 30 days at doses of 5 and 10 ml 10^6 CFU/ml on feed nutrient consumption of Madura cattle (Table 2).

Table 2. Effect of Cellulolytic Bacteria Inoculation on feed Nutrient Consumption

Variables		Treatment			P-*value*
		Control (C)	C+5 ml isolate (10^6 CFU)	C+10 ml isolate (10^6 CFU)	
Consumption Dry Matter	(kg tail^{-1} day^{-1})				
	Concentrate	4.98 ± 0.26	5.05 ± 0.11	5.03 ± 0.09	0.79
	Forage	4.41 ± 0.19	4.42 ± 0.22	4.33 ± 0.27	0.66
	Total	9.39 ± 0.38	9.47 ± 0.32	9.36 ± 0.28	0.58
Crude Protein	(g tail^{-1} day^{-1})	1316.98 ± 56.4	1329.46 ± 39.2	1235.68 ± 28.4	0.02
	%	14.02	14.04	13.20	
Crude Fat	(g tail^{-1} day^{-1})	293.07 ± 12.62	295.89 ± 8.61	293.28 ± 6.52	0.66
	%	3.12	3.13	3.13	
Crude Fiber	(g tail^{-1} day^{-1})	1969.07 ± 77.32	1981.70 ± 75.64	1953.52 ± 76.09	0.58
	%	20.97	20.93	20.87	
Nitrogen-Free Extract	(g tail^{-1} day^{-1})	4690.42 ± 196.55	4732.76 ± 145.13	4686.39 ± 116.24	0.63
	%	49.95	50.00	50.08	

The results showed that inoculation of cellulolytic bacterial consortium did not significantly affect feed consumption in Madura cattle. The absence of significant differences in feed consumption between treatments indicates that isolate inoculation does not affect palatability or the level of acceptance of livestock to feed. Church and Pond (1988) explained that feed consumption is influenced by palatability, which is related to the odor, taste, texture, and temperature characteristics of the feed provided.

Crude Fiber Digestibility

Inoculation of cellulolytic bacteria in Madura cattle for 30 days did not affect nutrient digestibility and bacterial population (Table 3).

Table 3. Effect of cellulolytic bacteria inoculation on nutrient digestibility and bacterial population

Variables	Treatment			P-*value*
	Control (C)	C+5 ml isolate (10^6 CFU)	C+10 ml isolate (10^6 CFU)	
Crude Fiber Digestibility (%)	65.17 ± 4.15	63.96 ± 4.85	64.96 ± 2.28	0.91
Fecal bacteria population (Log10 CFU/g)	6.88 ± 0.49	6.67 ± 0.79	6.16 ± 0.80	0.40

The study showed that inoculation of cellulolytic bacterial consortium in Madura cattle-fed rice straw and concentrate rations did not significantly affect crude fibre digestibility (P>0,05). The value of oil fibre digestibility in the control group and treatment with isolate inoculation showed almost the same range. This indicates that isolate inoculation does not provide additional benefits in increasing crude fibre digestibility in rice straw-based rations. As a comparison, Suharti et al. (2023) reported *vitro* crude fibre digestibility values of 24.30-28.98% using the same cellulolytic bacteria in a ratio consisting of elephant grass, palm oil, and concentrate with a ratio of 30:30:40. This difference indicates that the type of ration used plays a vital role in determining the level of crude fibre digestibility.

The low effectiveness of cellulolytic bacteria inoculation in rice and the characteristics of the rice straw itself can explain straw-based rations. Rice straw has a high crude fibre content, especially hemicellulose and lignin, which makes its cell wall thicker and more difficult to degrade. According to Anggorodi (1979), the higher the crude fibre content in the feed, the denser the cell wall of the material, so fibre-digesting microbes have difficulty breaking down the crude fibre structure into nutrients that the livestock's body can absorb.

In addition, rice straw also contains high levels of lignin. Lignin is insoluble and highly resistant to degradation by microbes. Although cellulolytic bacteria can break down cellulose, lignin inhibits the access of enzymes produced by bacteria to the cellulose substrate, thus

reducing the efficiency of the crude fibre degradation process.

Blood Metabolites

Inoculation of cellulolytic bacteria in Madura cattle for 30 days did not affect blood metabolites, including glucose, triglycerides, and blood urea nitrogen (BUN) (Table 4).

Table 4. Effect of blood metabolites from the use of cellulolytic bacteria consortium isolates

Variables	Treatment			Normal	P-*value*
	Control (C)	C+5 ml isolate (10^6 CFU)	C+10 ml isolate (10^6 CFU)		
Glucose (mg/dl)	81.12 ± 11.48	78.51 ± 8.14	85.45 ± 5.86	43-100*	0.53
Triglycerides (mg/dl)	24.94 ± 5.83	25.10 ± 11.70	17.90 ± 9.16	10-19**	0.36
BUN (mg/dl)	8.57 ± 1.83	8.53 ± 2.27	8.05 ± 1.24	6-27***	0.88

Note: BUN= Blood Urea Nitrogen
*Mitruka et al. (1977)
** Cornell University Clinical Pathology Laboratory
***Mitruka et al. (1981)

The results showed that the blood glucose levels produced were still within the normal range, according to the opinion of Mitruka et al. (1977), which states that blood glucose levels in regular cattle range from 43-100 mg/dl. Normal blood glucose levels are influenced by energy derived from feed and the regulation of insulin and glucagon hormones. These two hormones maintain blood glucose balance through glycolysis, glycogenesis, and gluconeogenesis, which help maintain blood glucose levels in ruminants. Blood glucose levels are also influenced by carbohydrates in feed, including crude fibre and extractable material without nitrogen, which contribute to increased blood glucose levels (Maynard et al. 1979). Rumen microbes ferment crude fibre and BETN into volatile fatty acids (VFA) and simple sugars, which are then converted into blood glucose in the liver (Tillman et al. 1991). Propionic acid supplies about 30% of the body's glucose needs (Parakkasi, 1999). This indicates that feeding the consortium of cellulolytic bacterial isolates did not affect blood glucose levels, as shown in Table 3.

Based on the data in Table 3, the average triglyceride concentration in this study was high, at 17.90-25.10 mg/dl. According to Meyer and Harvey (2004), the normal range of triglycerides in cattle is between 0-14 mg/dl, while the Cornell University Clinical Pathology Laboratory reports the expected value is 10-19 mg/dl. Increased triglyceride levels in cattle occur because when cattle consume feed containing carbohydrates, the digested carbohydrates are converted into glucose, which is then used as an energy source. However, if glucose levels increase, the excess glucose will be converted into fatty acids through lipogenesis. These fatty acids then combine with glycerol to form triglycerides stored in fat cells as energy reserves. Increased triglyceride levels can also be triggered by feeding high-fat feed. Damron (2003) explains that triglyceride levels in the blood are influenced by the amount of fat digested from feed or fat entering from outside the body. In addition, Katan et al. (1997) and Voisinet et al. (1997) mentioned that high carbohydrate content in feed can also increase blood triglyceride levels.

Table 3 shows that the average concentration of BUN (Blood Urea Nitrogen) in this study is typical for cows in the finishing period, with a range of 6-27 mg/dL (Mitruka et al. 1981). Blood urea levels are influenced by feed, as most of the urea comes from the breakdown of protein consumed. In livestock with high protein intake, most of the protein will undergo fermentation in the rumen, which can increase blood urea levels to exceed typical values. According to Unzaronah et al. (2010), uniformity of protein content in feed will result in relatively stable blood urea levels. This indicates that rumen microbes utilize the feed given to livestock to form ammonia, and protein metabolism runs in balance with the amount of energy available. Most of the ammonia is used by the microbes to synthesize their body proteins, which are then digested by the cattle for production needs rather than absorbed into the bloodstream (Aleena et al. 2016). Blood urea concentrations usually reflect the nitrogen balance in the rumen, which is related to the needs of rumen microbes and their hosts. This balance depends on the extent to which the

amount and composition of amino acids in the feed meet the needs of the livestock (Tahuk et al. 2017).

Fecal Bacterial Population

The results showed that inoculation of cellulolytic bacterial consortium in Madura cattle fed rice straw and concentrate rations did not have a significant effect (P>0.05) on the fecal bacterial population (Table 3). The total fecal bacteria population can reflect the bacteria population in the rumen. However, the bacteria that come out through feces mainly do not contribute directly to rumen fermentation. Inoculation of cellulolytic bacteria could potentially increase the population of these specific bacteria in the rumen, affecting the population of bacteria excreted through the feces. However, the fecal bacterial population is usually lower than the rumen. Microbes in the rumen consist of bacteria (10^{10}-10^{11} cells g-1 rumen contents), protozoa (10^5-10^6 cells ml-1 rumen fluid), and small amounts of fungi (Chruch, 1988), while total fecal bacteria in adult cattle, according to Mantrawan et al. (2018), was 831 x 10^9 CFU/g. The low number of faecal bacteria in this study may be related to the low digestibility of high-fibre feed, such as rice straw, which affects the substrate availability for these bacteria.

Body Weight Gain and Feed Efficiency

The inoculation of cellulolytic bacteria in Madura cattle for 30 days did not affect consumption, final body weight, DWG, or feed efficiency (Table 5).

Table 5. Effect of cellulolytic bacteria consortium inoculation on cow performance

Variables	Treatment			P-*value*
	Control (C)	C+5 ml isolate (10^6 CFU)	C+10 ml isolate (10^6CFU)	
Initial BW (kg tail^{-1})	240.20 ± 46.68	247.20 ± 47.21	244.40 ± 27.10	0.81
Final BW (kg tail^{-1})	253.00 ± 49.60	264.20 ± 46.60	263.60 ± 27.37	0.62
DWG (kg tail^{-1} day^{-1})	0.43 ± 0.19	0.57 ± 0.16	0.64 ± 0.12	0.18
Feed Efficiency (%)	4.54 ± 2.01	6.01 ± 1.78	6.85 ± 1.32	0.17

Note: BW=Body Weight, DWG=Daily Weight Gain

Body weight gain is an essential indicator of livestock growth and feeding efficiency (Safwan et al., 2020). Body weight gain reflects the ability of livestock to convert feed nutrients into meat (Yanuarianto et al. 2021). Nutritional adequacy in cattle plays a vital role in accelerating the increase in body weight (Suryani et al., 2020). Although no significant differences were found in body weight gain in this study, there were still differences in body weight in each treatment.

The results showed that inoculation of cellulolytic bacterial consortium in Madura cattle did not significantly affect feed efficiency. The feed utilization efficiency reflects how feed can be converted into livestock products, such as meat, reflected in body weight gain. This efficiency can be used as an indicator to assess the quality of feed given to livestock by measuring body weight gain and the amount of feed consumed in a certain period. High feed utilization efficiency can occur when the nutritional content of the feed is better so that livestock can consume less feed but produce optimal body weight gain. Increased feed utilization efficiency is often influenced by high protein content in feed, which can increase its usefulness. Some affect animals, livestock factors that influence feed efficiency for meat production include the type of livestock, digestibility of feed ingredients, nutritional adequacy, type and quality of feed, livestock age, and livestock body weight (Wati and Yusuf 2020).

CONCLUSION

Inoculation of the cellulolytic bacterial consortium at 5 and 10 ml 106 CFU/ml doses did not increase crude fibre digestibility, bacterial population, and animal performance, such as consumption, body weight gain, and feed efficiency. It did not interfere with blood metabolites within 30 days.

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