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The Effect of Zinc-Proteinate Supplementation on the In Vitro Digestibility and Ruminal Fermentation in Goat

Gilbert Nathaniel, Tiara Annisa, Anis Muktiani*, Dian Wahyu Harjanti and Widiyanto

Faculty of Animal and Agricultural Sciences, Diponegoro University, Semarang, Central Java 50275, Indonesia
***Corresponding author email: anismuktiani@lecturer.undip.ac.id**

Abstract. This study aimed primarily to investigate the effect of Zn-Proteinate (Zn-Prot) supplementation on in vitro rumen digestibility and rumen fermentation. This research used a completely randomized design with four treatments and four replicates. The experimental treatment was the supplementation of various levels of Zn-Prot (0; 12.5 ppm; 25 ppm, and 37.5 ppm) on a dry matter basis. Parameters determined were in vitro dry matter digestibility (IVDMD), in vitro organic matter digestibility (IVOMD), partial volatile fatty acid (VFA) (acetate, propionate, and butyrate), A/P ratio, CH4, and the efficiency of the conversion hexose to VFA. Data were analyzed using ANOVA. The results showed that goats fed with a diet supplemented with 25 ppm Zn-Prot had the highest IVDMD and IVOMD values. There was no significant effect on the VFA and CH4 concentrations, A/P ratio, and the efficiency of the hexose-VFA conversion within treatment groups. In conclusion, supplementing 25 ppm of Zn-Prot into the diet of dairy goat increase the dry matter and organic matter digestibility.

Keywords: zinc-proteinate, digestibility, rumen fermentation, in vitro, goat

Abstrak. Penelitian ini bertujuan untuk melihat pengaruh dari suplementasi Zn-proteinat (Zn-Prot) terhadap kecernaan dan fermentabilitas rumen secara *in vitro*. Rancangan percobaan pada penelitian ini yaitu rancangan acak lengkap (RAL) dengan empat perlakuan dan empat ulangan. Suplementasi Zn-Prot yang di berikan yaitu 0 (tanpa supplementasi), 12,5 ppm, 25 ppm dan 37,5 ppm. Parameter pada penelitian ini meliputi kecernaan bahan kering (KCBK), kecernaan bahan organik (KCBO), *volatile fatty acid* (VFA) parsial (asetat, propionat dan butirat), rasio A/P, CH_4 dan efisiensi konversi heksosa menjadi VFA. Analisis data yang di gunakan yaitu ANOVA. Hasil penelitian ini menunjukan bahwa pemberian suplementasi Zn-Prot dengan level 25 ppm menghasilkan nilai kecernaan bahan kering (KCBK) dan kercernaan bahan organik (KCBO) tertinggi sedangkan suplementasi Zn-Prot tidak berpengaruh terhadap parameter VFA parsial, rasio A/P, CH_4 dan efisiensi konversi heksosa menjadi VFA. Dari hasil penelitian yang di peroleh dapat di simpulkan bahwa suplementasi Zn-Prot dengan taraf 25 ppm pada kambing perah dapat meningkatkan kecernaan bahan kering (KCBK) dan kecernaan bahan organik (KCBO).

Kata kunci: Zinc-proteinat, kecernaan, fermentabilitas rumen, *in vitro*, kambing

Introduction

Feed management is one of the factors that significantly impact animal husbandry, especially ruminants. Feed-in ruminants consist of fibrous feed that provides energy for ruminants (Widodo et al., 2012); therefore, the fiber digestion process is vital for ruminants, especially in Indonesia. Some feed ingredients in Indonesia, especially in smallholder farms, still contain agricultural waste dominated by fiber content. Fiber digestion in ruminants is greatly influenced by feed composition, lignin content, cellulose content, the activity of fibrinolytic, cellulolytic microorganisms, and microbial protein synthesis (Pathak, 2008; Noziére et al., 2010; Ransa et al., 2020; Hambakodu et al., 2020). Another factor that impacts fiber digestion in the rumen is the sufficiency of mineral zinc (Zn) (Wang et al., 2013; Arelovich et al., 2014; Hartati et al., 2020).

Mineral Zn is known as a multi-functional micro mineral, such as the co-factor of 300 enzymes, protein and DNA synthesis, and metabolism of essential fatty acid (Elamin et al., 2013; Aliarabi et al., 2015). Zn minerals in rumen microbial growth are due to their function as a metalloenzyme that plays volatile roles in carboxypeptidase A and B enzymes and alkaline phosphatase, which significantly contribute to protein digestion and protein synthesis, including microbial protein synthesis (Hartati et al., 2020). Increased microbial protein synthesis will impact the increase of total rumen microbes, especially cellulolytic bacteria, thus increasing the efficiency of digestion of fibrous feed as well as the final product (acetate, propionate, and butyrate) (Noziére et al., 2010; Suhendra et al., 2015; Ransa et al., 2020). Increased digestibility of fibrous feed will improve energy availability for body metabolism, and hence, increase livestock productivity. The common problem in Indonesia is the very low mineral content in the feed. Suhada et al. (2012) stated that the mineral content of Zn in elephant grass, bagasse, and sugar cane shoot ranges between 4 and 8 ppm. also, the mineral content of feed ingredients is strongly influenced by season (Fariani, 2008).

According to NRC (1981), the requirement of Zn for dairy goats is estimated at 10 ppm/day relative to the type, breed, and physiological status. Supplementing 20 ppm Zn peptide to Zandi lambs increased in vitro dry matter digestibility (IVDMD) and neutral detergent fiber (NDF) digestibility (Mallaki et al., 2015), while 30 ppm Zn methionine supplemented to Muzaffarnagri lambs have increased acids detergent fiber (ADF) digestibility (Grag et al., 2008). It shows differences in the requirement of Zn mineral in each breed of livestock. In addition to the level of supplementation, the form of Zn contributes significantly to the level of effectiveness. Zinc mineral in organic form (Zn-Prot) is more effective than inorganic form ($ZnSO_4$) (Muktiani and Prastiwi, 2014). Mineral in the organic form is the process of chelating dissolved metal slats with hydrolyzed amino acids or proteins, so they further assist the activity of enzymes in the rumen (Supriyati et al., 2000). The purpose of this research is to investigate the effect of Zn mineral (Zn-Prot) supplementation at various levels on rumen digestibility as well as rumen fermentation.

Materials and Methods

This research has conducted within June – August 2020 at the Laboratory of Animal Nutrition, Faculty of Animal and Agricultural Sciences, Diponegoro University, Semarang.

The research was conducted using a completely randomized design with four treatments and four replications. The experimental treatment was the supplementation of basal diets with various levels of Zn-Prot (0 ppm, 12.5 ppm, 25 ppm, and 37.5 ppm) on a dry matter basis. The materials used in this research are goat rumen fluid, McDougall's solution, CO_2, centrifuge, shaker bath, fermentor tube, ventilated rubber cap, ash-free filter paper (Whatman no. 41), exicator, vacuum pump, porcelain cup, oven, electric furnace, and Zn-Prot. The basal diet consisted of concentrate, soybean husk, dried kale, odot grass (Pennisetum purpureum cv. Mott), calliandra (Calliandra calothyrsus), and Indigofera (Indigofera tinctoria). The composition and nutrient content of the concentrate and basal diet in this research are presented in Table 1 and Table 2.

Goat rumen fluid was collected from Ettawah Crossbreed goat through slaughtering and incubated at 38˚ – 39˚ C. The in vitro process was carried out by mixing basal diet (0,56 g), Zn-Prot, McDougall solution (40 ml), and rumen fluid (10 ml) into a fermentor tube and added with CO_2 for 10 – 20 seconds, then sealed with a ventilated rubber cap. In the next step, the fermentor tube was incubated in a water bath at 39˚ – 40˚ C. For the partial VFA parameter, the incubation was carried out for 4 hours after then the fermentation process was halted with ice cubes, and then the samples were centrifuged for 10 minutes at 10.000 RPM to take the liquid (supernatant). Partial VFA measurements based on AOAC (1975) using Shimadzu series GC-2010 plus gas chromatography made in Japan.

Table 1. Nutritional composition of concentrate

Feed ingredients	Composition	CP	Ash	CF	EE	NFE	TDN
				%			
Soybean meal	17.61	6.66	3.12	1.16	0.51	6.16	12.86
DDGS	14.79	4.33	0.91	1.58	1.18	6.79	12.31
Copra	8.71	1.71	0.62	3.77	1.00	1.62	4.72
Rice bran	13.90	1.35	2.60	4.98	0.61	4.37	7.27
Soybean husk	14.79	2.40	0.96	6.38	0.90	4.16	7.35
Wheat bran	30.19	5.42	1.19	1.81	2.00	19.77	26.14
Total	100	21.86	9.40	19.68	6.19	42.87	70.65

CP = crude protein, CF = crude fiber, EE = ether extract, NFE = nitrogen free extract dan TDN = *total digestible nutrient*, DDGS = distiller's dried grain with soluble, ND = not detected.

Table 2. Nutritional composition of experimental diet (100% DM)

Feed Ingredients	Composition	CP	Ash	CF	EE	NFE	TDN	NDF	ADF	Zn
					%					Ppm
P. purpureum	7.61	1.27	1.11	2.89	0.31	2.03	3.93	4.55	2.92	1.12
Indigofera tinctoria	1.56	0.39	0.17	0.15	0.03	0.81	1.19	0.29	0.24	ND
Calliandra	2.52	0.49	0.14	0.27	0.01	1.61	1.89	0.59	0.50	0.18
Soybean husk	31.20	5.07	2.02	13.46	1.90	8.76	15.49	18.96	13.12	1.58
Dried Kale	29.64	2.46	3.47	6,44	0.57	16.70	18.70	12.45	11.17	ND
Concentrate	27.47	6.01	2.58	5.41	1.70	11.78	19.41	10.78	5.06	9.44
Total	100	15.69	9.49	28.62	4.52	41.69	60.61	46.23	33.01	12.32

Notes: *P. purpureum: Pennisetum purpureum cv. Mott*; Calliandra: Calliandra calothyrsus CP = crude protein, CF = crude fiber, EE = ether extract, NFE = nitrogen free extract dan TDN = *total digestible nutrient*, DDGS = distiller's dried grain with soluble, ND = not detected.

The measurement procedure was performed by injecting 1 µl of supernatant sample into the gas chromatography, then the column reduction will be captured by a computer recorder for producing a graph and is calculated by the formula. Parameters such as CH4 and the efficiency of the conversion hexose energy to VFA were measured using the estimated individual VFA calculation based on Ørskov and Ryle's (1990) formula.

In vitro dry matter digestibility (IVDMD) and in vitro organic matter digestibility (IVOMD) incubation was carried out for 96 h. The first 48-h incubation constituted fermentative digestion, and the next 48 h was enzymatic digestion. During the incubation, the fermentor tube was shaken every 6 hours. After the fermentative digestion process, the samples were centrifuged for 10 minutes at 10.000 RPM to separate solid and liquid (supernatant). Then the solid was returned to the fermentor tube, added with 50 ml pepsin HCl, and incubated for 48 hours in the water bath at a temperature of 39˚ – 40˚ C. After the enzymatic digestion process was completed, the sample was filtered using an ash-free filter paper then oven-dried for 24 h at 105˚ C. After being removed from the oven, the sample was incorporated into the exicator for 30 minutes before weighing. The last process was placing the sample into the furnace for 6 hours at 400˚ C and weighing. In vitro dry matter digestibility (IVDMD) and in vitro organic matter digestibility (IVOMD) value was calculated based on Tilley and Terry (1963) equation:

$$\text{IVDMD (\%)} = \frac{\text{Initial dry matter input} - (\text{residue} - \text{blanko})}{\text{Initial dry matter input}} \times 100\%$$

$$\text{IVOMD (\%)} = \frac{\text{Initial organic matter input} - (\text{residue} - \text{blanko})}{\text{Initial organic matter input}} \times 100\%$$

The data obtained were analyzed using ANOVA (analysis of variance) in a Completely Randomized Design with a significant level of 5% and continued with the Duncan's Multiple Range test (Steel and Torrie, 1994).

Results and Discussion

The results of Zn-Prot supplementation on feed digestibility in Figure 1 show a significant difference of IVDMD and IVOMD value across (P<0,05) between treatments. The highest IVDMD and IVOMD values in this research are in 25 ppm treatment with 68,99 % and 84,51 % (Figure 1). The high digestibility of dry matter and organic matter value represents the higher the nutritional value opportunity to be absorbed. The increase of dry matter and organic matter digestibility value is due to the function of mineral Zn, such as the synthesis of protein, DNA, and nucleic acids, as well as the of >300 enzymes and microbial growth factor (Krisnan et al., 2009; Elamin et al., 2013; Aliarabi et al., 2015). The influence of the mineral Zn on protein, DNA, and nucleic acid synthesis is inseparable from their influence on DNA and RNA polymerase activity (Putra., 2006). In addition, the mineral Zn also acts as metalloenzymes which play a broad role in the carboxylation enzymes of peptidase A&B and alkaline phosphate (Hartati et al., 2020). As a result, enzyme activity in rumen bacteria increases, more microbial proteins are synthesized, eventually improving the growth rate of rumen bacteria (Putra., 2006; Krisnan et al., 2009; Hilal et al., 2016). An increase in the microbial growth rate will directly impact the efficiency of feed digestibility, particularly fibrous feed, thus an increased absorbability (Noziére et al., 2010; Ransa et al., 2020).

The results of rumen fermentability in this research show no significant differences between treatments (Table 4). The different result between digestibility and product fermentability was indicative of zero effect of Zn supplementation at 12.5 – 37.5 ppm on the proportion and number of rumen microbe. The value of individual VFA value is significantly influenced by the proportion and number of rumen microbe, particularly especially cellulolytic, proteolytic, and amylolytic bacteria (Yurleni et al.,2013). Hungate (1966) stated that the requirement of Zn for rumen microbes is within 130 – 220 ppm. The same result was obtained by Hosseini-Vardanjani et al. (2020), where there was no influence Zn supplementation at 30 ppm against individual VFA values although dry matter digestibility increase. This result was indicated due to changes in the rumen fermentation process in the capture of feed energy as VFA (Bateman et al., 2004; Hosseini-Vardanjani et al.,2020). Meanwhile, the proportion of acetate in this research is higher than the standard value (73 – 74%). Butyrate proportion in this study (6%) was lower than the normal range, as reported by Bregman et al. (1965), i.e., 64 – 70%, 17 – 21%, and 12 – 15%.

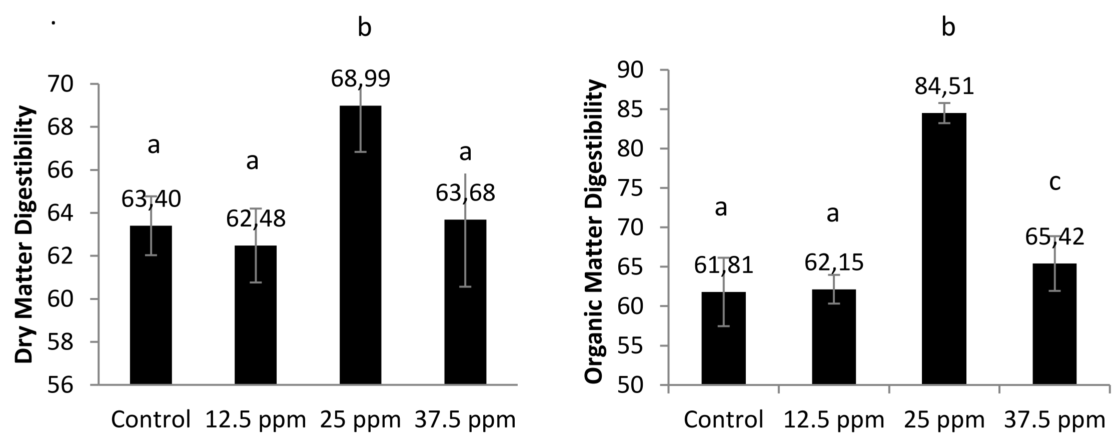

Figure 1. The effect of zn-proteinate supplementation on dry matter and organic matter digestibility (Different superscript in same table shows significant different p<0.05).

Table 4. The Effects of Zn-Proteinate on Rumen Fermentation

Parameter	Zn-Proteinate levels (ppm)				SEM
	0	12.5	25	37.5	
Acetate (mM)	15.14	16.33	19.83	14.50	0.855
Proportion of acetate (%)	74.91	73.34	74.65	75.92	0.22
Propionate (mM)	3.80	4.50	5.05	3.51	0.278
Proportion of propionate (%)	18.62	20.01	19.02	18.05	0.23
Butyrate (mM)	1.31	1.50	1.69	1.17	0.097
Proportion of butyrate (%)	6.47	6.65	6.32	6.03	0.38
A/P Ratio	4.04	3.68	3.93	4.24	0.095
CH_4 (mM)	7.27	7.79	9.50	6.90	0.423
The Efficiency of the Conversion Hexose to VFA (%)	72.34	72.99	72.49	71.95	0.168

SEM (Standard error of the mean).

The value of the A/P ratio in this research was 3.68 – 4.24 or higher than reported by Muktiani et al. (2019). The A/P, which illustrates the efficiency of energy utilization in ruminants (Rahayu et al., 2018), is strongly influenced by fiber and non-structural carbohydrates digestion (Purbowati et al., 2014; Puastuti et al., 2010).

Therefore, the higher the A/P value, the higher the acetate concentration, thus increasing CH4 and decreasing energy efficiency. While the value of CH4 in this research was 6.90 - 9.50 mM, Petriĉ et al., (2021) reported that supplementing 70 ppm Zn mineral in the diet produced 1.74 – 2.80 mM of CH4. According to Ekawati et al. (2015), the value of CH4 in sheep that consume diets containing 25 % crude fiber and 13 % crude protein is 9.11 mM.

The values of CH4 are highly varied, depending on the digestibility of fiber in the diet (Imanda et al., 2016). The higher the fiber digestibility, the higher the acetate and butyrate value, as well as the number of hydrogen gas (H2) (Puniya et al., 2015). Hydrogen gas (H2) is the raw material for methanogenesis, so the increase of CH4 is parallel to H2 (Hapsari et al., 2018; Susilo et al., 2019). The higher A/P ratio and CH4 values will impact decreasing energy efficiency (Muktiani et al., 2019). In this research, the value of efficiency hexose conversion to VFA (71 – 72%) was not affected by Zn supplementation. This result was higher than the 70% by Wahyuni et al. (2014) but lower than the 77% by Muktiani et al. (2019). The high variation in conversion efficiency of hexose to VFA depends on individual VFA values, particularly propionate (Ørskov and Ryle 1990; Faizah et al., 2019).

Conclusions

The supplementation of 25 ppm Zn-Prot into the diet of dairy goat increased the dry matter and organic matter digestibility.

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