THE INTERACTION EFFECTS OF SARDINES OIL AND ZINC ON CONCENTRATIONS OF PROSTAGLANDIN-E_2, SERUM ZINC LEVELS, ZINC ABSORPTION AND ALKALINE PHOSPHATASE ACTIVITY, OF RAM FED WITH BASAL RATION OF SUGAR CANE PULP SILAGE

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Abstract

The purpose of this study were to find out the interaction between the sardines oil with zinc on concentrations of prostaglandin-E_2, serum zinc levels, zinc absorption, alkaline phosphatase activity of ram fed by basal ration of sugar cane pulp that have been fermented and urea added. (2) To what extent the effect of the interaction between sardines oil and zinc to concentrations of prostaglandin-E_2, serum zinc levels, zinc absorption, alkaline phosphatase activiy. (3.) What level of zinc and sardines oil gave the best to concentrations of prostaglandin-E_2, serum zinc levels, zinc absorption and alkaline phosphatase activity. Experiments conducted in the cage experiment Test Farm, Laboratory of Physiology and Biochemistry and at Livestock Research Center (Balitnak) Ciawi Bogor. The experiments were carried out in two stages, namely 1) an experiment in vitro to explore the level of use of urea. Fermented sugar cane pulp using rumen fluid plus buffer fluid compared to fermentation with the bacteria Cytophaga sp. Performed with completely randomized design (CRD) with four levels of Urea (0, 0.5 1.0 and 1.5%) as a treatment and five replications. 2) experiment in vivo to explore the level sardines oil supplementation and zinc. Analysis Statistic was conducted by randomized block design (RBD). A factor is a supplement of zinc ($ZnSO_4$) with stage 0, 50 and 100 mg.kg^{-1} DM rations, factor B is the sardines oil level with 0, 1.5 and 3% kg^{-1} DM rations. Sheep were divided into 3 groups based on body weight.. Experiments in vivo performed using rams age 5-7 months weighing between 17-20 kg. The basal ration used was sugar cane pulp silage of urea which has the highest fermentability (experimental results 1) of the rumen fluid plus buffer with urea levels of 0.5% in comparison DM and concentrates 25%: 75% are prepared with CP 13.61 % and 63.71% TDN. There is interaction between the sardines oil with zinc on concentrations of prostaglandin-E2, serum zinc levels, zinc absorption, alkaline phosphatase activity, of ram fed by basal ration of sugar cane pulp that have been fermented and added urea. Interaction shown in, serum concentrations of PGE_2 ($P < 0.01$) and absorption of zinc ($P < 0.01$). At the level of sardines oil 1.5% and zinc 50 mg provides the best concentrations of prostaglandin-E2, serum zinc levels, zinc absorption, alkaline phosphatase activity from the rams were given the rations of sugar cane pulp that have been fermented and urea added.

Key words: sardines oil, zinc, prostaglandin-E_2, alkaline-phosphatase actvity, sugar cane pulp

INTRODUCTION

Fat in feed is a source of essential fatty acids for livestock. Fatty acid deficiency can lead to hyperkeratosis that interfere with absorption and subsequently will affect livestock production, this is one reason to be suspected of why productivity is low, although there is no empirical data that reveals the prevalence of hyperkeratosis incidence of sheep in Indonesia. The addition of fish oil sardine (*Sardinella sardine*) suspected to be a solution to the shortage of essential fatty acids. Sardine fish oil as a source of unsaturated fatty acid compound (Poly-unsaturated Fatty Acid = PUFA), such as chain C20: 5 n-3 (eicosapentaenoic acid = EPA) and C22: 6 n-3 (docosahexaenoic acid = DHA). Fatty acids are not easily hydrogenated in the rumen compared with unsaturated fatty acids C18

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chain. In Ruminant ingesta extensive experience biohidrogenation PUFA by rumen microbes chemical Treatment with formaldehyde to reduce biohidrogenation-linolenic acid (C18: 3n-3), while fish oil has a natural protectionα[1]. It is expected that these PUFA than useful as a source of energy, vitamin D and careers defaunation agents can also reduce methane gas emissions and serve as precursors of prostaglandins, each of which increases the benefits of energy use and absorption of zinc.

Zinc content of ruminant feed in Indonesia ranges from 20-38 mg^{-1} [2]. The amount is lower than that provided adequate levels of between 40-50 mg kg^{-1} ration dry matter[3]. Zinc requirement of micro-organisms in the rumen ranged from 130-220 mg kg^{-1} [4]. Zinc deficiency can cause intestinal tissue parakeratosis same result with fatty acid deficiency, in addition it can also interfere with the role of zinc in the metabolism of rumen microorganisms [4], [5] , alkaline phosphatase activity of enzymes that play a role in energy metabolism [6] and carboxy peptidase enzymes that play a role in the process of digestion and absorption of protein[7].

Was one of sugar cane pulp waste agricultural industry with huge potential as an energy source, because a lot of production throughout the year. When compared with other components derived from sugarcane, sugar cane pulp is the largest component. According [8] sugar cane pulp content ranges from 24 to 36%, while according to [9] ranged from 30 to 35%. If the production of milled sugar cane production for the whole region in Indonesia is 23,947,174.90 tons [10] the sugar cane pulp produced is 5,747,321.8 tons.

One way is processing with the help of microorganisms found in rumen fluid or the use of digestive bacteria have been isolated such as *Cytophaga sp* and then supplemented urea for the easily fermented feed and also to supply the ammonia that is necessary for microbial protein synthesis.

MATERIAL AND METHOD

Experiment Stage 1: Testing in vitro addition of urea on Fermentation Method Cane Sugar cane pulps between rumen fluid with bacteria *Cytophaga sp.*

This is a pretrial experiment. The purpose of this experiment was to determine the appropriate level of use of urea in the fermentation process sugar cane pulp using rumen fluid plus buffer and bacteria *Cytophaga sp.* making it easier and simultaneously fermented to supply the ammonia (NH_3) for microbial protein synthesis. From this experiment only one level of urea taken the best of all Treatments both rumen fluid and bacteria Cythophaga sp. for further experiments.

Variables measured in this experiment is the Dry Matter Digestibility (DMD) and Organic Matter Digestibility (OMD) in vitro by using the method of [11].

Experimental Design and Data Analysis

The experimental design used was completely randomized design (CRD) with four levels of Treatment doses of urea were 0, 0.5, 1.0, and 1.5% per kg of dry sugar cane pulp and five replications. Parameters measured were dry matter digestibility and organic matter sugar cane pulp.

Data were analyzed using ANOVA followed by Duncan's Multiple Range Test to determine the average difference between Treatments. The best response is the most high OMD and DMD or her to be used as the basis of urea in rations containing sugar cane pulp silage in experiment 2.

Experiment 2 : Effect of oil supplementation levels sardine and zinc ($Zn SO_4$) in the diet stimulated the growth of research in sheep through measurement variables of concentrations of prostaglandin-E_2, serum zinc levels, zinc absorption, and alkaline phosphatase activity.

The main purpose of this experiment is to trace levels sardine fish oil supplementation and zinc which can optimize the absorption of zinc and activity of enzymes that play a role in utilizing nutrients diet containing sugar cane pulp silage to spur growth in sheep.

Materials and Equipment

Experiment using 5-6 months old ram as much as 27 animals had a weight of about 17-20 kg. Obtained from livestock and animal market around sub-district Tanjungsari.

The cage used was wood piled onstage cage, walled board, with size: length 120 cm,

width 60 cm, height 100cm, and 120 cm high basic cage. Place feed in form of pyramid made of boards with a length of 60 cm, base width 40 cm, width of 60 cm and 40 cm high.

Basal diet used consisted of 25% sugar cane pulp silage and 75% concentrate. Sugar cane pulp silage used was the result of fermentation with rumen fluid, buffer and addition of urea at 0.5% (results of experiment 1 that is generating the highest digestibility). Concentrates prepared from cassava dregs (*onggok*), polar, bran, coconut meal and soybean meal, so that the ration contained 63.7% TDN and 13.65% PK, nutrient content of rations in accordance with the[3]. The diet done twice a day. Concentrate and fermentation of sugar cane dregs provided as required are mixed evenly to reduce the chances of picking out the feed. Drinking water is provided throughout the day.

Experimental Design and Data Analysis

The experimental design used was randomized block design (RAK) 3 x 3 factorial pattern. Factor A is the supplementation of zinc ($ZnSO_4$) at 0, 50, and 100 mg per kg of ration dry matter and factor B is sardine oil supplementation of 0, 1.5, and 3% of ration dry matter.

RESULTS AND DISCUSSIONS

Digestibility Dry Matter and Organic Matter In vitro Silage Waste of Fermented Cane with Rumen Fluid

Measurement of dry matter digestibility and organic matter carried out a preliminary experiment to determine the appropriate level of use of urea, so that sugar cane pulp silage to be used in experiments in vivo more fermentable and also to supply ammonia for microbial protein synthesis. Value averaging effect of adding urea on sugar cane pulp silage on dry matter digestibility and organic matter are presented in Table 1.

Statistical analysis showed that the level of the addition of urea greatly affect dry matter digestibility (DMD) and organic matter digestibility (OMD) sugar cane pulp silage. From the data above shows that the digestibility of dry matter and organic matter reached the highest respectively on the addition of urea 0.5%, significantly different than without the addition of urea, urea addition of 1% and 1.5%. As a comparative material also analyzed sugar cane pulp without Treatment and the results are much lower compared with the processing or manufacture of silage.

Based on these results, the addition of urea 0.5% will be benchmark in the processing of sugar cane pulp to be used for further experiments in vivo. Value of 0.5% is a low number when compared with the addition of non-protein nitrogen (NPN) on rumen fermentation in ruminant livestock, it is logical because the fermentation process outside the body of cattle is different than the process of fermentation in the rumen of cattle with the installation homeostation both in body and in his own cattle rumen ecology that support good life rument microbiology.

Table 1 Effect of Urea Addition on Digestibility Dry Matter and Organic Matter Silage Waste Fermented Sugar Cane in vitro with rumen fluid of

Variables	Addition of Urea (%)				Controls (Without treatment)	P
	0	0.5	1.0	1.5		
DMD (%)	17,97b	24,47a	17,71b	14,69b	9.61c	0,01
OMD (%)	19.65b	27.09a	20.22b	17.22b	7,95c	0,01

***Note**: DMD (Dry Matter Digestibility)*
OMD(Organic Matter Digestibility)
The same letter within a column indicate no significant difference (P <0.01)

Digestibility Dry Matter and Organic Matter in vitro of Fermented Sugar cane Silage Waste withBacteria*Cytophaga sp*

This research is a comparison in the processing of sugar cane sugar cane pulp. The average dry matter digestibility and

digestibility of organic matter are presented in Table 2. In contrast to the fermentation of sugar cane pulp which is done by using rumen fluid and OMD and DMD where the highest value at 0.5% of urea is 24.47 and 27.09%, sugar cane pulp fermentation with the bacterium *Cytophaga sp* DMD and OMD highest value on providing urea be produced 1% that is equal to 15.47 and 19.44%, and this result was still lower compared with rumen fluid. Based on these results then used in further research is sugar cane pulp fermented with rumen fluid and buffer with the addition of 0.5% urea.

Table 2 Effect of Urea Addition on Digestibility Dry Matter and Organic Matter Silage Waste in vitro Sugar Cane Silage Waste Bacteria *Cytophaga sp.*

Variables	Addition of Urea (%)				**Controls** (Without treatment)	P
	0	0.5	1.0	1.5		
DMD (%)	12,34ab	11.15b	15.47a	13.50a	9.61b	0.01
OMD (%)	15.11b	14.17b	19.44a	15.67b	7,95c	0.01

***Note**: DMD (Dry Matter Digestibility)*
OMD(Organic Matter Digestibility)
The same letter within a column indicate no significant difference (P <0.01)

The concentration of prostaglandin-E2 and Zinc, Serum Alkaline Phosphatase Activity and Zin Absorption.

Sardine oil supplementation and zinc simultaneously significant effect (P <0.01) in the concentration of PGE2, zinc absorption and activity of alkaline phosphatase in serum as well as the absorption of zinc (Table 3) and partially sardine oil supplementation tended to increase the activity of alkaline phosphatase and serum zinc concentrations. Then the adequacy of zinc in the diet, the fatty acids are used as precursors and arachidonic acid gamalinolenat dehomo to sintesis PGE2 which will further increase the concentration of PGE2. Should look at the diet without supplementation of PGE2 concentration much lower than that of oil sardine. This is due to the low concentration of linolenic acid were used as precursors dehomo gamma-linolenic and arachidonic to form PGE2.

Table 3 Mean concentrations of prostaglandin E_2 and Zinc, Serum Alkaline Phosphatase Activity and Zinc Absorption at Various Levels and Zinc Supplementation Oil Sardine

Treatment		Concentration of Serum PGE_2 (pg. ml^{-1})	Zinc Absorption ($mg.h^{-1}$)	Serum Alkaline Phosphatase Activity ($I\mu.L^{-1}$)	Concentration of Serum Zinc (ppm)
SO (%)	Zn(mg)				
0	0	97.80	38.40	77.07	0.76
1.5	0	254.40	41.83	81.53	0.82
3.0	0	96.00	45.63	92.77	1.13
0	50	193.20	69.03	111.63	0.79
1.5	50	342.90	79.17	119.50	0.92
3.0	50	403.83	88.87	126.17	1.20
0	100	153.93	212.00	108.57	0.84
1.5	100	284.10	237.70	118.93	0.92
3.0	100	259.20	250.50	128.30	1.27
Statistical Analysis					
Treatment					
Zn		*	*	*	*
SO		*	*	*	*
SO x Zn		*	*	ns	ns

***Note**: * significant (P,0.01)*
ns= non significant
SO= Sardine oil

[12] to the contrary, that if animals deficient in essential fatty acids continuously, although not supplemented with zinc will increase drastically weight gain. The results of this study support the statement in which up to 100 mg of zinc supplementation level. Kg-1 dry matter ration and without sardine oil supplements, weight gain is not as good as sheep in sheep that received rations with sardine oil supplementation (Table 3). The situation is caused by the absorption of zinc was lower in sheep that received rations without sardine oil supplements.

The rate of absorption of zinc in this study is within the range reported zinc absorption rate [13] that is equal to 20-80%. The amount of zinc absorption rate is highly influenced by age, the balance of other minerals and zinc content in the ration. The results showed that supplementation sardine oil tends to further increase the concentration of PGE2 and PGE2 was also shown to affect the absorption of zinc. Trend of increasing concentrations of PGE2 were allegedly related to arachidonic acid content is quite high 79.86 mg / g (7.99%) on sardine oil that can be used as precursors for the synthesis of PGE2. Besides directly used for the synthesis of PGE2, arachidonic acid joined in part by phospholipid fatty acid and if necessary will be released by membrane phospholipids to synthesis of PGE2. The prostaglandin-E2 by [14] is necessary for muscle contraction-like longitudinal smooth muscle in the gut, in his capacity as the absorption of zinc and other nutrient substances necessary for basic living and production.This research results also confirms the results of in vitro studies proposed by [15] that the addition of PGE2 can increase the absorption of zinc up to 54%.

The enzyme alkaline phosphatase is an enzyme whose activity is one of the very influenced by the fat content in the diet in addition to zinc status. One indicator of whether livestock zinc deficiency or excess can seen from zinc levels in serum. Blood alkaline phosphatase activity are used as an indication of zinc status in cattle. [16] and [17] reported plasma alkaline phosphatase activity was higher in mice with sufficient Zn than Zn deficient. [18] reported that increased plasma alkaline phosphatase activity was higher in lambs fed hay ration of 100% whole grain wheat or 67% hay and 33% alfalfa than lambs fed 33% hay and 67% wheat or alfalfa ration containing 100% alfalfa. These results reflect that the concentration of Zn from wheat and alfalfa hay. In studies[19] plasma alkaline phosphatase activity did not differ between ewes supplemented and control. In pigs, plasma alkaline phosphatase activity was not influenced by sources other than Zn sulfate or zinc as Zn chelated[20].

CONCLUSIONS

1. There is interaction between the sardine oil with zinc on physiological conditions rams fed by the basal ration sugar cane pulp which is already fermented and urea added.
2. Interaction shown in serum concentrations of PGE_2 ($P < 0.01$) and zinc absorption ($P < 0.01$).
3. At the level of 1.5% sardine oil and 50 mg of zinc gives the best physiological conditions in rams fed by the sugar cane pulp ration that has been fermented and urea added.

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