The Improving Quality of Concentrate Diet with Fibrolytic Enzyme and Its Effect on Rumen Metabolism and Blood Parameter of Fattening Holstein Male

(Perbaikan Mutu Pakan Konsentrat dengan Enzim Fibrolitik dan Pengaruhnya terhadap Metabolism Rumen dan Parameter Darah pada Penggemukan Sapi Jantan Holstein)

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Abstrak

Suatu penelitian telah dilakukan untuk mengevaluasi peningkatan kualitas pakan konsentrat pada penggemukan sapi jantan Holstein dengan enzim selulase komplek terhadap metabolisme rumen dan parameter darah. Dua belas ekor sapi jantan Holstein dengan umur 12 bulan dibagi dalam tiga kelompok berdasarkan bobot awal dalam Rancangan Acak Kelompok digunakan dalam penelitian ini. Sebagai perlakuan adalah pakan A, B, C dan D. Pakan A adalah kontrol yang tersusun atas rumput gajah, dedak padi, bungkil biji kapas, dedak sorgum, ampas tahu, onggok dan mineral mix dengan komposisi (%BK) berturut-turut adalah 30; 18; 17,5; 23; 4,5; 6 and 1. Pakan B, C dan D mempunyai bahan dan komposisi seperti pakan A, tetapi dedak padi pada B, bungkil biji kapas pada C dan baik dedak maupun bungkil biji kapas pada D disuplementasi dengan enzim celulase 1,5 g kg⁻¹ BK 24 jam sebelum pemberian pakan. Proporsi propionat (C_3) cenderung (P>0.05) meningkat bila dedak padi disuplementasi dengan enzim (B) dan sebaliknya pada (C). Oleh karena itu, efisiensi energi juga cenderung meningkat pada sapi yang diberi pakan B. Tidak terjadi perberdaan yang nyata dari penambahan enzim selulase terhadap N-NH₃ rumen dan cenderung tinggi pada C dan D. Penambahan enzim pada bungkil biji kapas cenderung menekan plasma urea walaupun kandungan protein pada pakan ini tinggi. Glukosa darah berturut-turut untuk pakan A, B, C dan D adalah 83,15, 88,25, 82,30 and 88,60 mg/L. Penambahan enzim pada bungkil biji kapas cenderung menurunkan glukosa darah pada pakan C. Disimpulkan bahwa penambahan enzim selulase pada dedak padi tidak mengganggu metabolisme rumen dan cenderung untuk memperbaiki energi efisiensi.

Kata Kunci: Enzim, Selulase, VFA, Glukosa, Energi.

Introduction

High crude fibre contents such rice bran and cottonseed meal in the concentrate diet of fattening ruminants limits utilisation of them because fermentation in the rumen produces a predominance of acetate production. Efficiency of utilisation of metabolisable energy (ME) from acetate is lower compared to propionate or butyrate (Armstrong and Blaxter, 1957). Utilisation of acetate for fat synthesis requires an adequate supply of NADPH (Armstrong, 1965). NADPH will be more readily available if ample supplies of absorbed amino acids or glucose are present (Annison and Bryden, 1999). Therefore, one alternative of improving feed quality especially of concentrates with high crude fibre may be done by increasing of glucose and amino acids though supplementation with specific enzyme which hydrolyse the fibre to glucose and release amino acids.

Enzyme treatments such as Cellulase complex have been extensively used in diets for monogastrics but positive effects of adding those enzymes to ruminant diets have also recently been reported. Addition of fibrolytic enzyme mixtures to forage diet has lead to improved live weight gain in cattle by as much as 35 per cent and feed conversion ratio by up to 10 per cent (Beauchemin *et al.*, 1995). While addition of fibrolytic enzymes to concentrates in lactating dairy cows has led to increased nutrient digestibility and milk production (Rode *et al.*, 1999). Enzyme mixtures may also be beneficial in high concentrate diets and may help to overcome the depression in fiber digestion that occurs

when using high concentrate diets (Boyles *et al.*, 1992; Hunt *et al.*, 1996; Beauchemin *et al.*, 1997; Krause *et al.*, 1998). Therefore, evaluation of utilization of fibrolytic enzyme to improve quality of rice bran and cottonseed meal in ruminant diet was needed.

The objectives of this study were to evaluate the effect of Cellupract AS 100® enzyme treatment of RB and CSM on efficiency rumen, blood and growth parameters of Holstein weaned bulls in Indonesia.

Research Methods

Animals and Housing

Twelve months old male weaner Holstein bulls averaging 160.54 ± 14.04 kg (mean ± S.D.) initial body weight were used in this experiment. All animals were born in February 2000 at the Centre of Dairy Breeding and Forages (BBPTU) Baturraden, Purwokerto, Central Java Province, Indonesia. They were given a prophylactic dose of piperazine at the beginning of the experiment. The animals were housed in individual stalls of 2 m length, 0.75 width and 2.5 height. The stalls had separate feed and drinking troughs.

Diets

Four dietary treatments were compounded. The diet consisted of Napier Grass and concentrate with 30 : 70 ratio of dry matter (DM). The concentrate ingredients and composition were rice bran (RB), cottonseed meal (CSM), pollard bran, cassava waste, soybean cake waste and mineral mix for 18; 17.5; 23; 4.5; 6 and 1 (%DM), respectively. Mineral mix percentage composition were 50.00 $CaCO_3$; 25.00 P; 0.35 Mn; 0.20 I; 0.10 K; 0.15 Cu; 22.00 Na; 0.8 Fe; 0.20 Zn; 0.15 Mg; 1.05 Cl Nutrient compositions of the diets are presented in Table 1. The first diet consisted of RB, CSM, polar bran, cassava waste, and soybean cake waste and mineral mix and had 15 per cent crude protein (CP) content. This diet was the control (diet A). In diet B, the RB was treated with fibrolytic enzyme. In diet C, CSM was treated with fibrolytic enzyme and in diet D both RB and CSM were treated. Treatment was effected by thoroughly mixing 1.5 g fibrolytic enzyme kg^{-1} dry matter (DM) of feed 24 hours before feeding. The fibrolytic enzyme used was Cellupract AS100® and its activity is 96±4, 2800±8, 4400±3, and 5600±4 U g^{-1} ± SD for Hemicellulase, Carboxymethylcellulase, ß-glucanase and Xylanase, respectively. The enzyme was dissolved in distilled water at 100 g/L according to Krause *et al.* (1998) before mixing it with RB or CSM. These diets constituted the concentrate portions of the diets. Napier (*Pennisetum purpureum*) grass constituted the forage component.

Table 1. Nutrient composition of the diets (% DM)

Nutrients	Diet			
	A	B	C	D
ME, MJ/kg DM*	8.13	8.25	8.28	8.40
Crude protein	4.03	14.49	15.27	15.73
Crude fibre	21.25	21.83	20.77	21.35
Ethyl extract	13.20	13.21	13.45	13.46
Ash	11.90	11.05	11.18	11.51
NFE	39.62	39.42	39.33	37.95

*Calculation from gas test

Experimental Design

The twelve animals were blocked by initial weight into three blocks of four animals per block and assigned within block, randomly to one of four dietary treatments. Therefore, the randomised block design used in this experiment with weight used as the blocking factor. The animals were kept in individual pens.

Feeding

The animals were offered Napier grass and concentrate at 3.4 per cent of body weight on DM basis. The concentrate to Napier grass DM ratio was maintained at 70:30, respectively. Diets were offered to the animals four times a day at 0700; 0900; 1400 and 1700 hours. Concentrates were offered at 0700 and 1400 hours while Napier grass was offered 0900 and 1700 hours. Drinking water was available freely. The trial lasted for 15 weeks including two weeks for adaptation. Data was recorded for 13 weeks. Feed offered and refused was weighed and recorded daily to calculate nutrient intake. Experimental diets were sampled weekly, immediately dried at 55°C for 48 hours and then milled with a blender (Super Grinder HL 1641, Philips Pvt. Ltd.) to pass through a 1 mm screen before chemical analysis.

Variables Measured and Chemical Analysis

Ruminal fluid was taken 3 hours after feeding at day 5 of collection periods. After collection, pH of the fresh rumen fluid was measured using an electric pH meter (Hanna Instrument, HI 9321). Production of $N-NH_3$ in the rumen was analysed by using Micro-Diffuse Conway Technique. Rumen fluid was separated for ammonia analysis by centrifuging at 5.000 g for 10 minutes. The supernatant fraction was decanted and kept frozen at –20°C until analysed for ammonia. Adding 1 ml of 1% sodium azide preserved nine milliliters of filtrate, and then the

samples were frozen. Ruminal VFA was separated by gas chromatography according to Krause *et al.* (1998). Efficiency of conversion of hexose energy to VFA was calculated according to Ørskov and Ryle (1990).

Samples of blood were taken from the jugular vein 3 hours after feeding. Heparin was used as anticoagulant. Centrifuging of whole blood at 5.000 g for 10 minutes separated blood plasma. Samples were kept frozen at -20° C until analysed. Blood urea and glucose were detected by using a Biochemical analyser (WAKO® Model 20R).

Napier grass was core-sampled and concentrates were grab-sampled and composited for each treatment for analysis of chemical composition (Lewis *et al.,* 1996). Napier grass and concentrates samples were analysed for dry matter, crude protein, ether extract, crude fiber, ash (AOAC, 1990)

Statistical Analysis

The data collected were analysed using the general linear models procedure (SAS, 1989). Analysis of variance (ANOVA) was undertaken an all other data. Duncan's multiple range tests was used to test for differences between treatment means.

Results and Discussion

The results of blood and rumen parameters are presented in Table 2. The proportion of propionate (C_3) tended (P>0.05) to be decreased when the cattle were fed a diet where the cottonseed meal was treated with enzyme. On the other hand, there was a positive effect to increase the proportion of C_3 when rice bran in the diet was treated with enzyme, therefore the energy efficiency from this group also tended to increase compared with control and other treatments (Table 2). High-energy efficiency in the rumen fermentation is shown by a pattern of

fermentation producing low methane. This result is consistent with performance of the cattle that received diet B in which rice bran was treated with fibrolytic enzymes (Bata, 2004). Orskov and Ryle (1990) suggested that the amount of fermentation energy converted into energy that can be utilised by the host can vary considerably and this variation will be indicated by differences in methane production, not by differences in the heat of fermentation. Generally, methane production in the rumen have a positive correlation with acetate production, high acetate levels will produce high methane levels. Yang et al. (1999) reported that the proportion of propionate in the total VFA's was numerically higher for cows fed diets containing enzymes than for cows fed the control. Consequently, the ratio of acetate to propionate was numerically lower for cow fed diets containing enzymes than for cows' fed the control diet. The same result was also reported by Iwaasa et al. (1998) whose enzyme supplement to alfalfa forage resulted in higher acetic, propionic acid and total VFAs production and in acetate: propionate ratios which were lower compared with control for all levels of treatments. A high propionate acid production is very important for the body metabolism because propionate is a glucogenic compound that can be changed to glucose. There have been many attempts to manipulate the rumen fermentation with the intention to increase the production of propionate and reduce the production of methane (Fernandez, 1980). There are advantages of both directions, since the production of methane represents a direct loss of energy while propionic acid is an important precursor for gluconeogenesis. It can thus have a protein sparing role since amino acids are the other main source of glucose precursors (Leng, 1970). This is the one reason why the cattle, which received diet B had a better performance compared with the

control group and other treatment groups. However, Feng et al. (1996) reported no effect of enzyme supplementation on molar proportion of individual VFAs or total VFA concentration. The proportion of butyrate (C_4) showed the same pattern as the C_2 proportion and the cattle fed diet B tended (P>0.05) to have the highest C_4 proportion in the rumen of weaned Holstein bulls.

There were only small changes of the isobutyrate (iC_4) and isovalerate (iC_5) proportions in this experiment. Data in Table 2 show that only iC_5 tended (P>0.05) to be increased when the cattle were fed diet B, C and D. This may be caused by the increasing of protein content of diet where protein is main source of long fatty acids such as Valerat (C_5).

Adding enzyme did not affect rumen pH (Table 2). This result agree with the results of Krause et al. (1998) and Beauchemin et al. (1999), who reported no differences in ruminal pH or VFA concentration by enzyme supplementation of diets based on barley fed to growing steers despite large increases in total fibre digestibility. Arambel and Wiedmeier (1986), who reported no influence of fungal additions (A. oryzae) on ruminal pH of dairy heifers, also reported similar results.

Ruminal $N-NH_3$ concentration was not different among treatments. Cattle fed enzyme treatment tended to have a greater ruminal $N-NH_3$ production than control. Similar result was reported by Lewis et al. (1996), who found that steers fed enzyme treatments tended to increase the $N-NH_3$ production, whereas it was lower in steers receiving barley treated with enzymes at feeding than forage treated with enzyme 24 h before or at feeding. The difference of this is due to that the availability of energy between them where availability of energy from barley treated with enzyme is high than forage, therefore, nitrogen sources like $N-NH_3$ needed by microbes.

Table 2. Rumen and blood parameters of weaned Holstein bulls offered diets treated with fibrolytic enzymes

Parameters	Diets				SEM
	A	B	C	D	
Rumen					
N-NH$_3$ (mM)	9.42	9.90	10.56	10.68	0.337
pH	7.12	7.37	7.40	7.12	0.191
Acetate (%)	60.27	64.27	64.19	62.82	0.318
Propionate (%)	21.30	22.61	19.75	20.26	1.978
Butyrate (%)	8.54	10.65	9.78	9.48	0.875
Isobutyrate (%)	1.15	1.15	1.20	1.13	0.144
Isovalerate (%)	1.15	1.54	2.14	1.37	0.427
Efficiency Energy (%)*	82.19	83.35	81.81	82.05	1.418
Blood					
Glucose (mg/L)	83.15	88.25	82.30	88.60	4.598
Urea plasma mg/dL	33.13	33.37	29.43	32.43	2.064

SEM = Standard error of mean

*Calculated according to Ørskov and Ryle (1990)

The concentration of ammonia in the rumen varies widely ranging from 2 – 40 mM (Mackie and Morrison, 1995). Satter and Slyter (1974) found that when the concentration of N-NH$_3$ is lower than 3.5 mM, microbial growth decreased significantly. Wohlt et al. (1976) found that levels of ammonia in dairy cow range from 7 – 13.5 mM. Under adequate feeding regimes, prevailing ammonia concentration should always be adequate for optimal growth of rumen bacteria (Mackie and Morrison, 1995). Ruminal N-NH$_3$ concentrations from this research were in the range from 9.42 mM for control treatment to 10.68 mM in cattle receiving diet D where both rice bran and cottonseed meal were treated with enzymes. Many factors affect ruminal N-NH$_3$ production such as protein level in the diets, time after feeding, rate of absorption by rumen wall, level and rate of protein degradation and uptake by micro-organisms. Lewis et al. (1996) found in his study that low level of N-NH$_3$ (below 3.5 mM) might be attributed to a greater N-NH$_3$ uptake by ruminal micro-organisms because the dietary CP concentrations (11.5%) would seemingly provide abundant degradable protein. This level of CP is lower than CP contained in the diet of all treatment in this experiment (14.03 – 15.73), therefore, the high concentrations of N-NH$_3$ measured in this research may be caused by the high CP levels of the diets. In high-concentrate diets, N-NH$_3$ concentration is not limiting microbial growth. The decrease in ruminal N-NH3 concentration is caused by the greater use of N-NH$_3$ by microorganisms (Devant et al., 2000) due to that the availability of energy in the rumen is high.

Theoretically, optimal ruminal N-NH$_3$ concentration required for maximal microbial growth are in the range of 2 – 3 mM; concentrations below 1 mM are limiting it (Mackie and Morrison, 1995). However, this assumes that there is no other limiting nutrient and is an oversimplification since many factors need to be considered. Atasoglu et al. (1999) concluded that different individual amino acids are synthesised de novo to different extents by mixed rumen micro-organisms when pre-formed amino acids are present. Also, the source of N used for synthesis of cell-N and amino acids depends on the respective concentrations of the different N sources available; however,

supplementing only with amino acids whose synthesis is lowest when pre-formed amino acids are present does not stimulate fermentation or microbial growth. Additionally, N-NH$_3$ concentrations that facilitate maximal levels of fibre degradation may be lower than those that maximise microbial protein synthesis and feed intake (Mackie and Morrison, 1995). When the low-degradable protein source was supplemented and (or) CP concentration was low, ruminal NH$_3$N concentrations fell below 5 mg/100 mL (Devant et al., 2000).

Although the fibrolytic enzymes treatment increased CP digestibility (Bata, 2004), plasma urea was relative similar among treatments. This is in agreement with findings of Hristov et al. (1998) that fibrolytic enzymes treatment to diets based on rolled barley grain and corn silage or infused directly to abomasum had no effect on the plasma urea although the CP content of the diet after treatment with enzyme increased from 13.7 to 14.6% of DM. Table 2 shows that adding fibrolytic enzymes to cottonseed meal (diet C) tended to depress plasma urea compared with control and diet B although diet C had a higher CP content than diet B. Ferrel et al. (1999) reported that high CP content in the diet could increase plasma urea of sheep fed low-quality forage supplemented with energy, nitrogen and protein. With a CP content of 3.3%; 33.6%; 29.9% and 34.4% the plasma urea levels measured were (mM) 1.81; 1.88; 4.34; and 3.85, respectively. Nitrogen arising from deamination of pre-formed protein in the rumen, from intake or recycled N (in the form of ammonia) is largely absorbed by the liver, and, to a great extent, converted to urea (Reynolds, 1995). Alterations in energy, rather than N intake, organic matter digestibility, microbial protein synthesis, and tissue metabolism are detected at the level of the liver, whereby the liver manipulates absorption and release of ammonia (Reynolds, 1995). Therefore, it is the liver that integrates N metabolism and determines the fate of N in the ruminant body (Dicostanso et al., 2002).

In ruminants, the main energy source is VFAs but glucose is essential, since it is absolutely required for cellular metabolism and also since adequate precursors and control mechanisms must exist for its synthesis (Bergman, 1983). The main part (90% or more) of the carbohydrate, which is available for metabolism by ruminant is in the form of VFAs. Thus, very little glucose is available for absorption and the micro-organisms ferment carbohydrates that potentially would be available to the ruminant directly (starches) as well as or rather than structural carbohydrates that are unavailable directly (Russel and Gahr, 2000). Ruminants, therefore, are very dependent on gluconeogenesis for maintaining an adequate blood glucose concentration. In this present study, the cattle fed the diet containing rice bran treated with fibrolytic enzymes (diet B) tended to increase plasma glucose compared with those fed cottonseed meal treated with fibrolytic enzymes (diet C) or control (88.25 mg/L VS 83.15 or VS 82.30 mg/L). This concentration is still in the range of normal blood glucose concentrations (2 to 6 mM or 35-110 mg/100 ml) in all species of mammals. Adult ruminants have the lowest concentration, usually 2 to 3.5 mM as compared with simple-stomached animals (Bergman, 1983). At the same treatment, propionate also tended to increase. Only four groups of compounds serve as significant precursors for gluconeogenesis: (a) propionate, (b) glycerol, (c) amino acids, and (d) lactate and pyruvate (Bergman, 1983). In ruminants, of the VFAs, only propionate is a major source of carbon for gluconeogenesis (Russel and Gahr, 2000). Therefore, most of glucose in this present study may come from propionate. Of the glucose present in ruminants 40 - 60% originates from propionic acid and the remainder from branched VFA,

lactic acid and glycerol (McDonald *et al.*, 1995). In fattening regimes, like in this experiment where cattle received high concentrate rations treated with fibrolytic enzymes, glucose can also be absorbed directly from the small intestine because high amounts of concentrate will pass to the small intestine and the fibrolytic enzymes is still remain active in the small intestine. Therefore, it can hydrolyse polymers of carbohydrates to glucose which is then absorbed by the small intestine.

Conclusions

It can be concluded that Cellupract As 100® fibrolytic enzyme treatment of rice bran do not disturb rumen metabolism and tended to improve energi efficiency of glucose conversion to VFA.

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