Effect of Sex on Rumen Fermentation Characteristics and Enzyme Activities of Garut Sheep

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ABSTRACT

This study aims to determine effect of sex on ruminal fermentation characteristics and enzyme activities in Garut sheep. Twelve Garut sheep (6 females and 6 males) were used. The sheep were about twelve months old with an average initial body weight of 25±2 kg, and were divided into two groups according to sex. The sheep were housed individually, and were allowed for free access to fresh clean water. Animals were fed separate feeding of concentrate (bran pollard) and roughage (king grass) with ratio of feed 40:60 dry matter basis. The feed contained CP 12.10% and TDN 64.31%. At the end of the study, rumen contents were sampled to measured pH, microbial protein content, VFA, ammonia concentration, protozoa, and enzyme activities. There was no significant difference ($P>0.05$) among sheep in ruminal pH, microbial protein, ammonia, protozoa, and propionic acid, but there were significant differences in acetic and butyric acid, total FVA, CMC-ase, and protease activities in rumen fluid ($P<0.05$) between females and males. The females had lower acetic, butyric acid and total FVA ($P<0.05$), as well as amylase activities ($P<0.01$) than the males. It could be concluded that males Garut sheep had more efficient ruminal fermentation of feed than the females.

Keywords: *Sex, Rumen fermentation, Enzyme activities, Garut sheep*

1. INTRODUCTION

Sheep is one of animal commodities which may fulfill the need of national food requirement. One of indigenous sheep from Indonesia is Garut sheep, the local sheep from Garut district, West Jawa Provence. Garut sheep is known as meat and fighting type sheep [1]. Some of the advantages of Garut sheep compared to other sheep include has a fairly good productivity and resistant to disease, has advantages comparative especially in terms of performance and strength and has a good body weight can compete with imported sheep in terms of quality and productivity [2]. The availability of feed is the common problem facing in sheep production in tropical countries.

Metabolic ability of ruminant depends on species, physiological characteristics, age, sex and breed [3]. Microbial activities in the rumen and their relation with host functions may be conducted by measure the amount and kind of material disappearing during passage of food, and the turnover rates of the materials in the rumen. Rumen microbial activities are evalued using the continuous fermentation model [4]. Rumen microbial fermentation converts feed to volatile fatty acids (VFAs), which are the main energy supply in ruminants. There is a significant relationship between ruminal pH and absorption of VFAs [5]. Ruminants have rumen where microorganisms consisted of bacteria, protozoa, and fungi are able to digest nutrients of feed in anaerobiccondition. The main products of rumen microbial fermentation are volatile fatty acids (acetic, propionic, and butyric acids) and microbial biomass, which are utilized as energy and protein source by the host ruminant. Rumen microorganisms produce the degrading enzymes to degrade the feed nutrients. Ruminal microorganisms produce the enzymes important for fermentation processes to convert forages into the energy [6]. Therefore, ruminants such as goat, sheep, cattle, and buffalo may utilize fibrous feed efficiently.

There is limited information on feeding system in sheep, and its effects on rumen fermentation characteristics. We here in report effect of sex on fermentation characteristics and enzyme activities in rumen of Garut sheep.

2. MATERIALS AND METHOD

2.1. Digestion Trial

Twelve Garut sheep (6 females and 6 males) were used. The sheep were about twelve months old with an average body weight of 25±2 kg, and were grouped according to sex so that there were 6 females and 6 males in each treatment. The sheep were housed individually, and were allowed free access to fresh clean water. Animals were fed separate feeding of concentrate (bran pollard) and roughage (king grass) with ratio of feed 40:60 dry matter basis. The feed contained CP 12.10% and TDN 64.31%. Animals were fed with each feed at the level of 3% body weights (DM basis) at 7:00 AM and 3:00 PM during the study.

2.2. Rumen Fermentation Characteristics and Enzyme Activities

Rumen fluid was sampled at the end of the study, the rumen fluid was taken using a vacuum pump via the esophagus. Ruminal fluid samples were taken after 3 h of the morning feeding, and homogenized with centrifuge 4,000 x *g* for 15 min to separate feed particles from sample, and then passed to 10,000 x *g* for 15 min to separate microbial cells and rumen fluid supernatant. Microbial cells was used to analysis of microbial protein content according method of Lowry [7]. Rumen fluid supernatant was analyzed pH, profile of VFA, ammonia, protozoa, as well as enzyme activities.

Ruminal pH was evalued by pH meter. Profile of VFA was analyzed by gas chromatography [8]. Ammonia content was measured using the indophenol method [9]. Protozoa measurement were prepared by pipetting 1 ml of bottle content and be added to 0.8 ml of formaldehyde saline solution (1ml of 37%formaldehyde + 9 ml 0.9% NaCl), then 0.02 ml of sample was transferred to haemocytometer (width 16 mm^2, depth 0.2 mm) for direct calculation under microscope with magnification 40x [10]. Activity of celullase (CMCase), amylase [11], and protease [12] were measured.

2.3. Statistical Analysis

Treatment means were analysed using a t-test [13], and the differences between means were considered significant when $P < 0.05$ or 0.01.

3. RESULTS AND DISCUSSION

3.1. Rumen Fermentation Characteristics

Data of rumen fermentation characteristics are presented in Table 1. There was no significant difference ($P > 0.05$) among sheep in ruminal pH, microbial protein, ammonia, protozoa, and propionic acid, but there were significant differences in acetic and butyric acid, as well as total FVA in rumen fluid ($P < 0.05$) between females and males. The females had lower ($P < 0.05$) acetic, butyric acid and total FVA than the males. Statistically, also ruminal propionic acid of females lower ($P < 0.1$) than the males.

Under normal fermentation conditions, the rumen environment is weakly acidic and populated by rumen microbes that are adapted to pH values between 5.5 and 6.5 [14]. Ruminal pH indicated normal condition where the pH value ranges from 6.40 to 6.49, there was no significant different. The pH value of rumen fluid is influenced by ration composition, stage of lactation, time of day, milk yield, and age. The ruminal pH decreased with increasing percentage of concentrates in the ration [15]. In this study, sheep were fed the same ration, such as there were either difference in rumen pH value nor ammonia concentration. Forage species or the feeding level affected pH and ammonia concentration in rumen of sheep, rumen fluid ammonia concentrations were lower when sheep were fed chicory (lower potein), compared with perennial ryegrass (higher protein) [16]. Ammonia concentrations in rumen fluid are measured as an indicator of rumen nitrogen metabolism with particular reference to ruminal protein degradation. In rumen, protein is degraded by proteolytic bacteria to peptides and amino acids, and then is deaminated to release ammonia. Rumen microbes use ammonia as source of nitrogen to synthesis microbial cells.

Total protozoa in Garut sheep had normal concentration. The rumen microorganisms are consisted of bacteria, protozoa, and fungi, at concentrations of 10^{10}, 10^6, and 10^4 cells/ml, respectively [6]. Protozoa constitute 40-80% of the microbial biomass, approximately 90% of total protozoa are *Entodiniomorphida*, which are able to hydrolyze and ferment cellulose [17]. While other protozoa has amylolytic activity that produce two isoforms of α-amylase and maltase, it hydrolize amylum into maltose, maltotriose, and glucose [18]. Protozoa also has proteolytic activity [6]. Males had higher total VFA production than females. This is because males have higher energy and protein requirements for growth [19,20]. As we know that volatile fatty acids are the main energy supply in ruminants. Nutrients were better digested by ram lambs than by ewe lambs of the three rations offered, based on crop residues [21].

Table 1. Rumen fermentation characteristics of females and males of Garut sheep (average±SEM)

Rumen fermentation characteristics	Females	Males	SEM	Significance
pH	6.49	6.40	0.18	NS
Microbial protein (mg/100ml)	44.35	50.11	4.25	NS
Ammonia (mg/100ml)	7.56	7.17	0.30	NS
Protozoa (10^3/ml)	12.72	14.46	2.74	NS
Acetic acid (mM)	28.99	51.91	5.68	*
Propionc acid (mM)	37.78	68.58	9.36	NS
Butyric acid (mM)	16.31	37.25	4.68	*
Total FVA	83.08	157.75	18.02	*
Ratio Acetic:Propionic:Butyric acid	39.25: 43.10: 17.65	47.27: 36.05: 16.68		

Table 2. Rumen enzyme activities of females and males of Garut sheep (average±SEM)

Enzyme activities (U/g)	Females	Males	SEM	Significance
CMC-ase	14.78	14.57	3.56	NS
Amylase	7.43	20.01	2.03	**
Protease	46.01	42.42	6.70	NS

3.2 Rumen Enzyme Activities

Table 2. presented data rumen enzyme activities, it showed there was either significant differences in CMC-ase nor protease activities in rumen fluid, but male sheep had higher ($P<0.01$) amylase activities than the females.

The ruminant has multiple digestive system consisted of reticulum, rumen, omasum, and abomasum, while the major digestion of feed are held in rumen. There are enzymes produced by rumen microorganisms, and used to digest and ferment food eaten by ruminants. Therefore, ruminants can utilize fiber of feed by converting into energy source. The fibrous degrading-enzymes produced by ruminal microorganisms important for fermentation processes, so ruminant obtain the energy from forages efficiently[6]. Rumen microorganisms provide superior cellulase activity which degrade cellulose into simple saccharides then fermented them into volatile fatty acids and gases [14]. In this study showed cellulase, amylase, and protease activities in rumen fluid. Interestingly, high VFA concentration in male (as shown in Table 1) was linier with higher amylase activities in male than female; even though cellulase activities in female and male sheep were similar. Several Rumen bacterias, including *Bacteroides ruminicola*, *Ruminobacter amylophilus*, *Selenomonas ruminantium* and *Succinomonas amylolytica* provide starch-degrading enzyme may produce formate, acetate, propionate, and succinate. Ruminal microorganisms degrade forage proteins and structural polysaccharides by 50-70%, ruminal proteolysis acts in protein hydrolysis processes, degradation of peptides, and deamination of amino acid [6].

4. CONCLUSION

Males Garut sheep had more efficient ruminal fermentation of feed than the females.

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REFERENCES

[1] A. Gunawan, R.R. Noor, Pendugaan nilai heritabilitas bobot lahir dan bobot sapih domba Garut tipe laga, Media Peternak. 29 (2006).

[2] A. Gunawan, R.H. Mulyono, C. Sumantri, Identification of Body Size and Body Shape of Garut Sheep Fighting Type and Meat Type and Garut Cross Based on Principal Component Analysis, Anim. Prod. 11 (2009) 8–14.

[3] E.S. Pereira, A.C.N. Campos, E.L. Heinzen, J.A.D. Barbosa Filho, M.S.S. Carneiro, D.R. Fernandes, L.R. Bezerra, R.L. Oliveira, Effect of dietary reduction and sex class on nutrient digestibility, nitrogen balance, excreted purine derivatives and infrared thermography of hair lambs, J. Agric. Sci. 156 (2018) 1028–1038. DOI:https://doi.org/10.1017/S0021859618001016.

[4] R.E. Hungate, J. Reichl, R. Prins, Parameters of rumen fermentation in a continuously fed sheep: evidence of a microbial rumination pool, Appl.

Microbiol. 22 (1971) 1104–1113. DOI: https://doi.org/10.1128/am.22.6.1104-1113.1971.

[5] K. Liu, Y. Li, G. Luo, H. Xin, Y. Zhang, G. Li, Relations of ruminal fermentation parameters and microbial matters to odd-and branched-chain fatty acids in rumen fluid of dairy cows at different milk stages, Animals. 9 (2019) 1019. DOI: https://doi.org/10.3390/ani9121019.

[6] A.R. Castillo-González, M.E. Burrola-Barraza, J. Domínguez-Viveros, A. Chávez-Martínez, Rumen microorganisms and fermentation, Arch. Med. Vet. 46 (2014) 349–361.

[7] P. Mu, D.T. Plummer, Introduction to practical biochemistry, Tata McGraw-Hill Education, 2001.

[8] J. Filípek, R. Dvořák, Determination of the volatile fatty acid content in the rumen liquid: comparison of gas chromatography and capillary isotachophoresis, Acta Vet. Brno. 78 (2009)627–633.DOI: https://doi.org/10.2754/avb200978040627.

[9] M.W. Weatherburn, Phenol-hypochlorite reaction for determination of ammonia, Anal. Chem. 39 (1967) 971–974. DOI: https://doi.org/10.1021/ac60252a045.

[10] A. Diaz, M. Avendano, A. Escobar, Evaluation of Sapindus saponaria as a defaunating agent and its effects on different ruminal digestion parameters, Livest. Res. Rural Dev. 5 (1993) 1–6.

[11] G. Halliwell, The action of cellulolytic enzymes from Myrothecium verrucaria, Biochem. J. 79 (1961) 185.

[12] H.U. Bergmeyer, J. Bergmeyer, M. Grass, Methods of Enzymatic Analysis Weinheim, Verlag Chemie, (1974).

[13] R.G.D. Steel, J.H. Torrie, Principles and procedures of statistics., Princ. Proced. Stat. (1960).

[14] L.U. Zhang, J. Chung, Q. Jiang, R. Sun, J. Zhang, Y. Zhong, N. Ren, Characteristics of rumen microorganisms involved in anaerobic degradation of cellulose at various pH values, Rsc Adv. 7 (2017) 40303–40310. DOI: https://doi.org/10.1039/C7RA06588D.

[15] T. Geishauser, N. Linhart, A. Neidl, A. Reimann, Factors associated with ruminal pH at herd level, J. Dairy Sci. 95 (2012) 4556–4567. DOI: https://doi.org/10.3168/jds.2012-5380.

[16] X. Sun, A. Chen, D. Pacheco, S.O. Hoskin, D. Luo, Sheep rumen fermentation characteristics affected by feeding frequency and feeding level when fed fresh forage, Animals. 10 (2020) 7. DOI: https://doi.org/10.3390/ani10010007.

[17] D.R. Yanez Ruiz, A. Moumen, A.I. Martin Garcia, E. Molina Alcaide, Ruminal fermentation and degradation patterns, protozoa population, and urinary purine derivatives excretion in goats and wethers fed diets based on two-stage olive cake: Effect of PEG supply, J. Anim. Sci. 82 (2004) 2023–2032. DOI: https://doi.org/10.2527/2004.8272023x.

[18] K. Wereszka, T. Michałowski, The ability of the rumen ciliate protozoan Diploplastron affine to digest and ferment starch, Folia Microbiol. (Praha). 57 (2012) 375–377. DOI: https://doi.org/10.1007/s12223-012-0146-1.

[19] M. Costa, E.S. Pereira, A.M.A. Silva, P.V.R. Paulino, I.Y. Mizubuti, P.G. Pimentel, A.P. Pinto, J.N.R. Junior, Body composition and net energy and protein requirements of Morada Nova lambs, Small Rumin. Res. 114 (2013) 206–213. DOI:https://doi.org/10.1016/j.smallrumres.2013.06.014.

[20] E.S. Pereira, M.W.F. Pereira, M.I. Marcondes, A.N. de Medeiros, R.L. de Oliveira, L.P. da Silva, I.Y. Mizubuti, A.C.N. Campos, E.L. Heinzen, A.S.C. Veras, Maintenance and growth requirements in male and female hair lambs, Small Rumin. Res. 159 (2018) 75–83. DOI:https://doi.org/10.1016/j.smallrumres.2017.11.003.

[21] E.M. Aregheore, Effect of sex on feed intake, growth and nutrients digestibility in Blackhead sheep fed complete mash rations of crop residues, J. Anim. Feed Sci. 6 (1997) 71–79. DOI: https://doi.org/10.22358/jafs/69505/1997.