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GROWTH RESPONSE OF SHEEP FED CONCENTRATE WITH *ASPERGILLUS ORYZAE* FERMENTATION CULTURE

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Abstract

Probiotics have recently been of interest of nutritionists and to be used to modify rumen fermentation pattern and to improve digestion process to enhance ruminant production. *Aspergillus oryzae* fermentation culture (AOFC) was used in this experiment to increase sheep production performance. Fifteen growing male 'Garut' sheep were fed with chopped King grass and commercial concentrate added with 5 and 10% AOFC during 16 weeks. A randomised block design was applied to put dietary treatments allocation. The sheep was weighed early in the morning once a week, feed refusals were also weighed every morning during 14 weeks data collection period. Faecal samples were taken in the last week of the experiment. Both feed and faecal samples were analysed for nutrient contents. Duncan's MRT was used for treatment means comparisons. The AOFC gave a good result in sheep production, with average daily gain reaching 122.08 g/d for sheep that consumed concentrate with 5% AOFC (CF_1) and 140.52 g/d for sheep fed with concentrate with 10% AOFC (CF_2), compared to 94.81 g/d for control sheep (CF_0). No significant differences determined in feed efficiency, dry matter and organic matter digestibilities. Crude protein digestibility was significantly improved in sheep having treatment CF_2, while NDF digestibility increased significantly by feeding AOFC in both treatments (CF_1 and CF_2).

Key words: Sheep, *A. oryzae*, Daily gain, Digestibility

Introduction

Various attempts have been made to improve ruminal microbial fermentation through the use of methane inhibitors, propionate enhancers or ionofors (monensin, rumensin, avoparcin, lasalocid), microbial growth promoters, and dietary modifications. The uses of probiotics, including bacteria and fungi, have also been studied recently in order to decrease protein degradation and energy loss as gasses in the rumen. *Aspergillus oryzae* is one of fungi species that has been recently used as a probiotic. Experimental results varied according mainly to the diets offered and physiological status of the animals, and have been clearly reviewed by Yoon and Stern (1995).

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Most studies dealing with *A. oryzae* were done related to digestive physiology of dairy cows and milk production. It was reported that feeding *A. oryzae* fermentation extract to lactating dairy cows increased ration consumption and milk production (Huber *et al.*, 1986; Kellems *et al.*, 1990), associated with the increased (Harris *et al.*, 1983) or decreased (Van Horn *et al.*, 1984) milk fat content. Beharka *et al.* (1991) found that feeding *A. oryzae* fermentation extract on calves and steers could increase feed consumption and daily gain. Furthermore, it has also been concluded that the positive effects of *A. oryzae* supplementation on daily gain was more realized in high concentrate feeding (Huber *et al.*, 1985). Although *A. oryzae* is known as a producer of starch-degrading enzymes (amylase and amyloglucosidase) and in much lesser amount of cellulase, its fermentation culture can increase digestibility of dry matter, NDF, ADF, and crude protein (Ayala *et al.*, 1992; Campos *et al.*, 1990; Gomez-Alarcon *et al.*, 1990). Some of these enhanced digestive activities may be related to fungal stimulation on microbial growth and activity. Several experiment results indicated that certain substances (thiamin, niacin, and branched-chain volatile fatty acids) contained in *A. oryzae* fermentation extract could promote the growth of cellulolytic and lactate utilizing bacteria in the rumen (Yoon and Stern, 1995). This study is aimed to improve weight gain through altering rumen fermentation with addition of *A. oryzae* fermentation culture.

Materials and Method

The *Aspergillus oryzae* (AO) used in this experiment was derived from the result of previous study concerning the isolation of AO from several fermented products that has been cultured in potato dextrose agar. *Onggok* (tapioca processing waste) was used as a media for production scale of AO after being enriched with some minerals (sodium hypophosphate, potassium chloride, calcium chloride, ferro sulphate, magnesium sulphate, and urea). This media was processed and placed in sterilized plastic trays, cultured with AO and incubated at room temperature (26 – 30°C) for 5 days, then the whole media with the AO culture was oven-dried at 40°C and ground to be used as a feed additive, which is called as *Aspergillus oryzae* fermentation culture (AOFC).

Three types of concentrate feeds were prepared for feeding trial, i.e. commercial concentrate (CC) which is called as CF_0, CC + 5% (w/w) AOFC (as CF_1), and CC + 10% AOFC (as CF_2). As much as 15 growing male 'Garut' sheep were fed with the experimental diets, where each kind of diet was allotted to 5 sheep (replications) based on a randomised block design. The amount of the concentrate feed offered was based on the capacity of the sheep to consume during two weeks preliminary period, which was come up to 500 g/d. Chopped King grass was fed as a basal diet at 3 kg/d and drinking water was available continuously every day.

Table 1. Nutrient content (in dry matter) of King grass and concentrate with or without addition of *A. oryzae* fermentation culture

Nutrient	King grass	CF_0*	CF_1*	CF_2*
Dry matter, %	28.40	83.41	88.02	87.39
Gross energy, Mcal/kg	3.131	3.744	4.004	4.287
Crude protein, %	7.97	14.27	14.99	15.16
Total fibre (NDF), %	82.52	40.35	39.40	36.81
Ash, %	11.54	8.87	8.02	8.50

*Indicating concentrate with 0, 5, and 10% (w/w) of *A. oryzae* fermentation culture, respectively

All sheep were weighed once a week in the morning before they were being fed. Feed refusals were also weighed every day at early morning during 14 weeks data collection period. Faecal samples were collected and weighed in the last 10 days of the experiment. All feed and faecal samples were analysed for nutrient contents (dry matter, crude protein, total fibre (NDF), and ash). Analysis of variance was done for the data collected and Duncan's MRT was applied for treatment means comparison by using the GLM procedure according to statistical analysis system (SAS, 1996).

Results and Discussion

Total dry matter consumption (grass and concentrate) varied from 593 g/d to 971 g/d and statistically different between treatment means (Table 2), in which sheep that receive treatment CF_1 and CF_2 have much higher intake than that of CF_0. Same pattern also occur for organic matter intake. Such increment was caused by significant increase in the intake of concentrate with addition of *A. oryzae* fermentation culture (AOFC), while grass consumption has no significant differences among treatments. Dry matter consumption ratio of grass and concentrate in this experiment was 49:51. Such increased concentrate consumption was probably because the concentrate added with AOFC was more palatable than the commercial concentrate without AOFC, and more important, the sheep that consume AOFC might have better digestion/fermentation process in their rumen and increase the flow of digesta to the lower gut. The same circumstances have also been reported in lactating dairy cows fed with *A.oryzae* fermentation extract (Van Horn *et al.*, 1984; Huber *et al.*, 1986; Gomez-Alarcon *et al.*, 1991; Denigan *et al.*, 1992; Sievert and Shaver, 1993).

Table 2. Dry matter and organic matter consumption (g/d) according to dietary treatments

Nutrient	Dietary Treatment*			Significance Level (P =)
	CF_0	CF_1	CF_2	
Dry Matter:				
Grass	$355.8^a \pm 110.9$	$457.8^a \pm 70.0$	$452.6^a \pm 57.8$	0.1746
Concentrate	$414.0^a \pm 8.9$	$447.4^b \pm 4.3$	$442.0^b \pm 8.5$	0.0002
Total ration	$769.8^a \pm 106.8$	$905.2^b \pm 74.1$	$894.6^b \pm 58.4$	0.0579
Organic Matter:				
Grass	$314.7^a \pm 97.2$	$404.9^a \pm 61.9$	$400.4^a \pm 51.1$	0.1746
Concentrate	$377.3^a \pm 8.1$	$411.5^b \pm 4.0$	$404.4^b \pm 7.7$	0.0001
Total ration	$692.0^a \pm 94.4$	$816.5^b \pm 65.7$	$804.8^b \pm 51.7$	0.0599

* Indicating concentrate with 0, 5, and 10% (w/w) of *A. oryzae* fermentation culture, respectively. Numbers with different superscripts in the same row indicate significantly difference.

It has been reported that *A. oryzae* fermentation extract causing increased lactate utilization by a species of rumen bacteria, *Selenomonas ruminantium*, hence the rumen pH corrected to normal and digestion/fermentation process become better (Nisbet and Martin, 1990; Martin and Streeter, 1994). Daily gain of the sheep was generally good, but better gain was observed in sheep fed with ration CF_2 (concentrate with 10% AOFC). Average daily gain of the sheep that having treatment CF_1 and CF_2 was increase consecutively by 29% and 48% as compared to the control sheep. However, such relatively large increment in sheep with treatment CF_1 is not different statistically with CF_0 (control), only sheep that consumed treatment CF_2 have significant higher weight gain ($P = 0.0341$). This fact might be due to a relatively high standard deviation of the means, especially in control group (Table 3).

Table 3. Average daily gain (g/d), total weight gain (kg), and feed efficiency of sheep fed concentrate with or without AOFC

Parameters	Ration Treatment*			Significance Level (P =)
	CF_0	CF_1	CF_2	
Daily gain	$94.81^a \pm 30.42$	$122.08^{ab} \pm 18.62$	$140.52^b \pm 18.03$	0.0341
Total gain	$7.30^a \pm 2.34$	$9.49^{ab} \pm 1.43$	$10.82^b \pm 1.39$	0.0347
Feed efficiency	$0.12^a \pm 0.04$	$0.14^a \pm 0.03$	$0.16^a \pm 0.01$	0.0894

*Numbers with different superscripts in the same row indicate significant difference.

The occurrence of increased body weight gain with feeding *A. oryzae* culture has also been observed in steers by Rush *et al.* in 1990 (in Yoon and Stern, 1995), calves

(Beharka *et al.*, 1991), and lambs (Herring *et al.*, 1989). The fastest growth rate occurred in the first 4-week of data collection period, which might be as a compensatory effect of feeding better feed during the experiment. Then, the weight curve has a lesser slope in between week-4 and week-11, but increase again rapidly up to week-14, primarily in sheep fed with AOFC at 5 and 10% level (Figure 1).

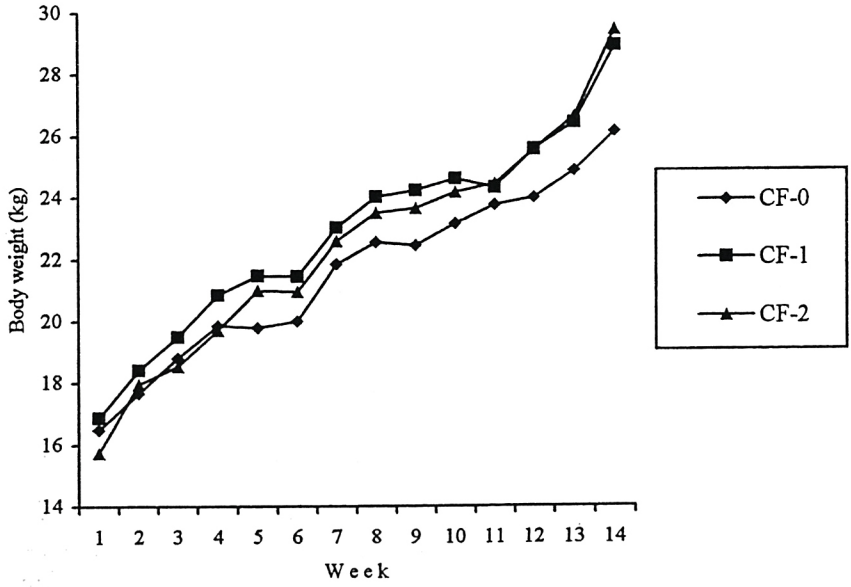

Figure 1. Growth rate of sheep fed concentrate diets with or without supplementation of AOFC

Statistical analysis indicates no significant differences in feed efficiency between treatment means although the values tend to increase along with the increasing of amount of AOFC addition. Previous experiment done by other researchers also showed no significant improvement in feed efficiency by feeding *A. oryzae* fermentation extract to fine-wool lambs (Herring *et al.*, 1989), young calves (Beharka *et al.*, 1991), and lactating cows (Gomez-Alarcon *et al.*, 1991). Feed efficiency in this case was calculated based on rough calculation concerning feed intake and body weight gain, but there might be a significant increase metabolically which was not observed/measured in this experiment. Frumholtz *et al.* (1989) reported that methane production in the rumen (*in vitro*) was reduced by the presence of *A. oryzae* fermentation extract. Reduced methane production reflecting (in part) a decrease energy loss in the rumen, indicating an increase in fermentation efficiency. Even the declining in methane production is small, such change is important in confirming changes in fermentation stoichiometry (Williams and

Newbold, 1990). Another fact was reported by Caton *et al.* (1993), who found that both duodenal essential and non-essential amino acid flows increased by supplementation of *A. oryzae* fermentation extract to steers grazing cool-season pasture.

Table 4. Nutrient digestibility (%) in sheep fed concentrate with/without AOFC supplementation

Nutrient	Ration Treatment*			Significance Level (P =)
	CF_0	CF_1	CF_2	
Dry matter	$63.6^{a} \pm 4.8$	$65.7^{a} \pm 1.5$	$66.0^{a} \pm 1.2$	0.4700
Organic matter	$67.1^{a} \pm 4.5$	$68.8^{a} \pm 1.8$	$69.3^{a} \pm 1.5$	0.5560
Crude protein	$59.6^{a} \pm 4.9$	$61.7^{ab} \pm 3.1$	$65.5^{b} \pm 1.3$	0.0472
Total fibre (NDF)	$52.7^{a} \pm 2.3$	$57.6^{b} \pm 7.7$	$56.5^{b} \pm 2.3$	0.0412

*Numbers with different superscripts in the same row indicate significant difference

Supplementing AOFC to concentrate feed in this study had no significant effect on both dry matter and organic matter digestibility (Table 4). A significant increase, however, occurred on crude protein digestibility of the sheep that received treatment CF_2, while total fibre (NDF) digestibility was significantly higher in sheep fed diet CF_1 and CF_2. An increase in crude protein digestibility with supplementation of *A. oryzae* culture to dairy cows diet was also found by Gomez-Alarcon *et al.* (1990).

As shown in Table 4, NDF digestibility in this experiment increased by 7 to 9% magnification with supplementation of AOFC and this is in agreement with what was observed by previous researchers. The *A. oryzae* fermentation culture, 'Amaferm', has been reported could increase digestibility of NDF, ADF, cellulose, and hemicellulose in the rumen (Campos *et al.*, 1990; Miranda *et al.*, 1996) and total digestive tract (Gomez-Alarcon *et al.*, 1990). It has been proved that *A. oryzae* culture can increase rumen bacterial count and stimulate the growth rate of cellulolytic bacteria. The fibrolytic bacteria population increased *in vitro* (Newbold *et al.*, 1991) as well as *in vivo* (Wiedmeier *et al.*, 1987; Newbold *et al.*, 1992). However, Beharka and Nagaraja in 1991 (in Yoon and Stern, 1995) found that not all species of cellulolytic bacteria is stimulated to grow by *A. oryzae* fermentation extract, only positive to *Ruminococcus albus* and *Fibrobacter succinogenes*. Furthermore, Nisbet and Martin (1991) demonstrated that extracts prepared from yeast and *A. oryzae* cultures increased lactate uptake by the rumen bacterium *Selenomonas ruminantium*. Lower lactic acid concentration in rumen fluid usually associated with higher rumen pH moderately and such environment is more suitable for fibre digestion by rumen bacteria. Additionally, the presence of dicarboxylic acid (L-malate) in *A. oryzae* fermentation extract may be partially responsible for the increased lactic acid utilization by *Selenomonas ruminantium* (Nisbet and Martin, 1990; Martin and Streeter, 1994).

Conclusions

Feeding *A. oryzae* fermentation culture to growing male sheep can increase daily gain up to 48%. Crude protein and total fibre digestibility increased significantly, but feed efficiency is not affected. Technical parameters related to digestion processes in the rumen and lower digestive tracts were not completely determined in this experiment. Deeper studies still need to be done, especially in ruminal and post ruminal metabolism that relevance with digestion efficiency.

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