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Effects of Brewery Meal-Based Fermented Feedstuff Supplemented with *Aspergillus Oryzae* or *Saccharomyces Cerevisiae* on Ruminal Microorganism

Byung Ki Park*, Chang Six Ra, Byong Wan Kim, Jin Seok Oh and Jong Suh Shin

Aspergillus Oryzae 혹은 *Saccharomyces Cerevisiae*를 첨가하여 제조한 맥주박 위주 발효사료가 반추위 미생물에 미치는 영향

박병기* · 라창식 · 김병완 · 오진석 · 신종서

요 약

본 연구는 *Aspergillus oryzae*(AO) 혹은 *Saccharomyces cerevisiae*(SC)를 첨가하여 제조한 맥주박 위주 발효사료의 급여가 한우의 반추위내 미생물에 미치는 영향을 조사하기 위해 실시하였다. 공시동물은 반추위 cannula가 장착된 한우 암소 2두를 이용하였다. 시험은 시판 배합사료 71.5% 및 corn silage 28.5% 급여구(대조구), 시판 배합사료 45.0%, AO 첨가 발효사료 26.5% 및 corn silage 28.5% 급여구(TAO)와 시판 배합사료 45.0%, SC 첨가 발효사료 26.5% 및 corn silage 28.5% 급여구(TSC)의 3처리구로 구분하여 수행하였다. Total viable bacteria($p<0.05$), 혐기성 곰팡이 및 protozoa($p<0.05$)의 수는 대조구에 비해 TAO 및 TSC에서 높았다. 단백질 분해 박테리아($p<0.05$), 섬유소 분해 박테리아 및 xylan 이용 박테리아 수는 대조구에 비해 TAO 및 TSC구에서 높은 경향이었다. Protozoa의 건물회수율은 대조구에 비해 TAO 및 TSC구에서 높았다($p<0.05$). 총미생물 및 protozoa의 조단백질 함량은 대조구 및 TAO구에 비해 TSC구에서 높았다($p<0.05$). 박테리아의 조단백질 함량은 대조구에 비해 TAO 및 TSC구에서 높았다($p<0.05$). 총미생물의 조지방 함량은 대조구 및 TSC구에 비해 TAO구에서 높았으며($p<0.05$), protozoa 및 박테리아의 조지방 함량은 대조구 및 TAO구에 비해 TSC구에서 높았다($p<0.05$). 총미생물의 필수아미노산 비율은 TAO 및 TSC구에 비해 대조구에서 높았다. 박테리아의 methionin 및 alanine 비율은 대조구에 비해 TAO 및 TSC구에서 높았다($p<0.05$). 이상의 결과에서 AO 혹은 SC를 첨가하여 제조한 발효사료의 급여는 반추위내 미생물 수 및 균체 조성 변화에 영향을 미치는 것으로 판단된다.

(**Key words** : *Aspergillus oryzae, Saccharomyces cerevisiae*, Bacteria, Protozoa, Anaerobic fungi)

Ⅰ. INTRODUCTION

There has been much interest recently in the use of biological feed additives to improve productivity in beef and dairy industries. The principal concerns from these additives have been concentrated specially in microbial feed additives such as *Aspergillus oryzae*(AO) and *Saccharomyces cerevisiae*(SC)(Beharka and Nagaraja, 1998; Garcia et al., 2000; Soder and Holden,

강원대학교 동물자원학부(Division of Animal Resources Science, Kangwon National University, Chuncheon, 200 701, Korea)
*농촌진흥청 축산연구소(National Livestock Research Institute, R.D.A., Pyeongchang, 232-952, Korea)
Corresponding author : J. S. Shin, Division of Animal Resource Science, Kangwon National University, Chuncheon, 200-701, Korea.
Tel : 033-250-8628, Fax : 033-244-2532, E-mail : jsshin@kangwon.ac.kr.

1999; Shon et al., 2005 ; Sullivan and Martin, 1999; Wang et al., 2001).

Inclusion of AO in the diets of ruminants has been shown to increase the number of total bacteria(Wiedmeier et al., 1987), cellulolytic bacteria (Yoon and Stern, 1996), pectin fermenters (Beharka et al., 1991), protozoa(Fondevila et al., 1990) and anaerobic fungi(Gomez-Alarcon et al., 1990; Oellermann et al., 1990) in the rumen. Inclusion of SC in the diets of ruminant has been shown to increase the number of total bacteria, total viable bacteria and cellulolytic bacteria(El Hassan et al., 1996; Harrison et al., 1988 ; Kumar et al., 1997; Newbold et al., 1998 ; Yoon and Stern, 1996), protozoa(Edwards, 1991 ; Plata et al., 1994) and anaerobic fungi(Ollermann et al., 1990). However, host animal and ruminal microorganism response to these microbial feed additives have been inconsistent(Caton et al, 1993; Kumar et al., 1994; Newbold et al., 1995; Varel and Kreikemeier, 1994; Williams and Newbold, 1990).

Therefore, various researches have reported that dietary AO or SC causes a range of effects on ruminal microorganism, but, to our knowledge, no data are available on the effect of fermented feedstuff supplemented with AO or SC on ruminal microorganism. Thus, the objective of this study was to evaluate the effects of brewery meal-based fermented feedstuff supplemented with AO or SC on ruminal microorganism and their body composition.

Ⅱ. MATERIALS AND METHODS

1. Experimental animals, experimental diets and treatments

Two Korean native cattle(400 and 450 kg) equipped with ruminal cannula were used as experimental animals. Experimental diets were formulated using the procedure described by Lin et al.(2001). Cracked corn and brewery meal was mixed at a ratio of 50:50(as-fed basis), and it was remixed after adding 5% rice bran, 5% molasses, and 1% micro mineral solution. Experimental diets were fermented anaerobically in a incubator at 30 ℃ during 48 hours after adding 1% AO or 1% SC, respectively. Chemical compositions of experimental diets were shown in Table 1.

Table 1. Chemical composition of experimental diets

Items	Commercial feed	FFAO[1)]	FFSC[2)]	Silage
Dry matter (%)	91.80 ± 0.10	55.74 ± 2.09	56.70 ± 2.68	29.42 ± 0.24
	..... % of dry matter			
Crude protein (%)	15.20 ± 0.35	12.53 ± 0.12	13.07 ± 0.28	8.49 ± 0.18
Ether extract (%)	3.82 ± 0.15	5.93 ± 0.27	6.33 ± 0.17	1.81 ± 0.15
Crude ash (%)	8.06 ± 0.49	2.90 ± 0.17	3.45 ± 0.08	5.99 ± 0.07
Neutral detergent fiber (%)	33.91 ± 0.99	27.36 ± 0.36	30.37 ± 0.89	60.94 ± 0.93
Acid detergent fiber (%)	12.24 ± 0.61	7.49 ± 0.27	8.98 ± 0.08	34.35 ± 0.52
Acid detergent lignin (%)	3.91 ± 0.38	2.86 ± 0.26	2.88 ± 0.29	5.85 ± 0.42

Means ± standard deviation.
[1)] FFAO : fermented feedstuff supplemented with AO.
[2)] FFSC : fermented feedstuff supplemented with SC.

Treatments were composed of three treatment groups: 71.5% of commercial feed and 28.5% of corn silage(control); 45.0% of commercial feed, 26.5% of fermented feedstuff supplemented with AO and 28.5% of corn silage(TAO); 45.0% of commercial feed, 26.5% of fermented feedstuff supplemented with SC and 28.5% of corn silage(TSC). The experiments were accomplished in duplicate. The experimental diets were offered twice a day at 09:00 a.m. and 6:00 p.m. and the total feeding amount of experimental diet was 1.7% of experimental animal weight, and water was offered always a day.

2. Microbiological Measurements

For microbial counting, animals were first adapted for 14 days on experimental diets and the experimental period lasted for 3 days, and 200 ml of ruminal fluid and 200 g of ruminal digesta were collected into 100 ml plastic bottles only at 3 hours(12:00 a.m.) after the morning feeding during the experimental period. These bottles were filled with O_2 free CO_2 gas to minimize exposure to the atmosphere, sealed immediately and stored at 39℃ for transporting to the laboratory within 15 minutes. The ruminal fluid and the ruminal digesta were homogenized anaerobically in the homogenizer(Heidolph DIAX 800, Germany) for 3 minutes(× 3 times) at low speed to separate loosely associated microbes from particulate matter, and the homogenized ruminal fluid and digesta were strained anaerobically through two layers of cheese cloth. The ruminal fluid was serially diluted in dilution media as described by Bryant and Burkey(1953) and the roll tube method(Hungate, 1969) was used for enumeration of ruminal microorganism except protozoa. Total viable, proteolytic, cellulolytic bacteria in diluted ruminal fluid were determined by using the media as described by Dehority (1965), Abou Akkada and Blackburn(1963) and Hungate(1957), respectively. Also, amylolytic bacteria, pectin fermenters, and xylan fermenters in diluted ruminal fluid were determined by using the media as described by Minato et al.(1989). Anaerobic fungi in diluted ruminal fluid was determined by using the media as described by Lowe et al.(1985). and protozoa in diluted ruminal fluid was determined by hemocytometer under a microscope of 400 magnifications using MFS(methylgreen-formaline-saline) solution.

For examination of the changes of ruminal microbial body compositions, animals were first adapted for 14 days on experimental diets and the experimental period lasted for 3 days, and 200 ml of ruminal fluid and 200 g of ruminal digesta were collected into 100 ml plastic bottles at 3, 6, 9 and 12 hours after the morning feeding during the first day, at 1, 4, 7 and 10 hours after the morning feeding during the second day, and 2, 5, 8 and 11 hours after the morning feeding during the third day. This sequence of sampling allowed a sample to be taken every hour during 12 hours. These bottles were filled with O_2 free-CO_2 gas to minimize exposure to the atmosphere, sealed immediately and stored at 39℃ for transporting to the laboratory within 15 minutes. The 12 ruminal fluid samples and the 12 ruminal digesta samples were mixed on an equal volume and weight basis, and frozen at −25℃ before being homogenized. The ruminal fluid and the ruminal digesta were homogenized anaerobically in the homogenizer for 3 minutes(× 3 times) at low speed to separate loosely associated microbes from particulate matter, and the homogenized ruminal fluid and digesta were strained anaerobically through two

layers of cheese cloth. Total microbes, bacteria and protozoa were isolated by using the method as described by Shultz and Shultz(1970), Kalscheur et al.(1997), and Ogimoto and Imai(1981), respectively, and were refrigerated at 4℃ after lyophilization. The microbial samples were analyzed for dry matter recovery(DMR), crude protein(CP), ether extract(EE) and crude ash by the method of AOAC (1995), and were analyzed for amino acids by the HPLC(Waters 510 Pump; Waters™ Automated Gradient Controller; Waters™ 486 Tunable Absorbance Detector; Waters Temperature Control Module, USA) by using the method as described by Mason et al.(1980).

3. Statistical Analysis

Data were analyzed by Duncan's procedure using GLM of SAS(1999). The statistical model was as follow;

$Y_{ij} = \mu + T_i + e_{ij}$

Where, Y_{ij} was response variable, μ was overall mean, T_i was effect of treat, and e_{ij} was residual error.

Ⅲ. RESULTS AND DISCUSSIONS

The numbers of total viable bacteria of control, TAO and TSC were 1.97, 2.40 and 2.23×10^{10} CFU/ml, respectively. And then the number of total viable bacteria was shown similar tendency between TAO and TSC, but the number was significantly higher in TAO than in control ($p<0.05$). The numbers of anaerobic fungi of control, TAO and TSC were 8.62, 9.97, and 8.78×10^4 CFU/ml, respectively. And then the number tended to be higher in TAO and TSC than in control, but was not significant. The numbers of protozoa for control, TAO and TSC were 7.33, 9.33 and 7.50×10^5 count/ml, respectively. And then the number was higher in TAO than in TSC and control ($p<0.05$), and there was no significant difference between TSC and control. (Table. 2)

Like this, the numbers of ruminal total viable bacteria, anaerobic fungi, and protozoa tended to be higher in TAO and TSC than in control, and this result was to agree with reports that the inclusion of AO or SC increased the number of ruminal total bacteria and total viable bacteria(El Hassan et al., 1996; Kumar et al., 1997; Yoon and Stern, 1996). Also, Ollermann et al.(1990) reported that the number of ruminal anaerobic fungi was increased as adding levels of AO and SC were increased. The number of anaerobic fungi was increased in TAO and TSC compared

Table 2. Effects of fermented feedstuff supplemented with AO or SC on the number of total viable bacteria, anaerobic fungi, protozoa and proteolytic bacteria in the rumen of Korean native cattle

Microbial counts	Control[1)]	TAO[2)]	TSC[3)]
Total viable bacteri($\times 10^{10}$, CFU[4)]/ ml rumen content)	1.97 ± 0.21^b	2.40 ± 0.10^a	2.23 ± 0.15^{ab}
Fungi($\times 10^4$, CFU/ ml rumen content)	8.62 ± 0.49	9.97 ± 0.56	8.78 ± 0.48
Protozoa($\times 10^5$, count/ ml rumen content)	7.33 ± 1.26^b	9.33 ± 0.29^a	7.50 ± 0.50^b
Proteolytic bacteria($\times 10^9$, CFU/ ml rumen content)	1.43 ± 0.06^c	3.20 ± 0.20^a	2.77 ± 0.15^b

Means ± standard deviation.
[1)] Control : commercial feed + corn silage ; [2)] TAO : commercial feed + FFAO + corn silage ;
[3)] TSC : commercial feed + FFSC + corn silage; [4)] CFU: cell formation unit.
[a,b,c] Means in same row with different superscripts differ ($p<0.05$).

with control in this experiment, and this result was similar with the result of Oellermann et al.(1990). The range of ruminal protozoa number was $10^5 \sim 10^6$ ml^{-1} (Hungate, 1966). It has been known generally that the number of ruminal protozoa is increased as the soluble carbohydrate content of feed is elevated. And, Lee and Lee(1982) found that the number of protozoa was increased as the number of bacteria was increased. Fondevila et al.(1990). reported that the number of protozoa by addition of AO was increased by 59%, and the number of protozoa was also increased in other research results(Edwards, 1991; Plata et al., 1994). The number of protozoa was significantly increased in TAO compared with control but the number did not have significant difference in TSC compared with control in this experiment. It may be verified that the result to agree generally with research results stated above.

The numbers of proteolytic bacteria in control, TAO and TSC were 1.43, 3.20 and 2.77 CFU/ml, respectively. The numbers were higher in TAO and TSC than in control($p<0.05$) and the number was higher in TAO than in TSC ($p<0.05$). The number of amylolytic bacteria in control, TAO and TSC were 8.00, 5.00 and 3.00×10^9/ml, respectively, and the numbers of cellulolytic bacteria of control, TAO and TSC were 0.28, 0.90 and 0.60×10^9/ml, and then the numbers of cellulolytic bacteria tended to be higher in TAO and TSC than in control. (Table. 3). The numbers of pectin fermenters did not show significant difference between treatments, but the numbers of xylan fermenters tended to be higher in TAO and TSC than in control. Yoon and Stern(1996) found that the number of cellulolytic and proteolytic bacteria was increased by inclusion of AO, and Beharka et al. (1991) also reported that the numbers of cellulolytic, hemicellulolytic and pectinolytic bacteria were increased by inclusion of AO; therefore, the result of this experiment agreed with their results. Kumar et al.(1997). found that the number of cellulolytic bacteria was increased by 57% through inclusion of AO, and the number of cellulolytic bacteria was also increased by inclusion of AO in many research results. Therefore, these results were supporting the results of this experiment.

The number of cellulolytic bacteria and xylan fermenters that these bacteria can degrade cell wall material(hemicellulose, cellulose and xylan) tended to be higher in TAO and TSC than in control, and the number of proteolytic bacteria was also increased. The number of amylolytic bacteria was higher in control which a feeding amount of commercial feed is relatively high compared with TAO and TSC. Therefore, it was estimated that increased number of the cellulolytic bacteria by the feeding of fermented feedstuff supplemented with AO or SC was the reason in the promotion activity of AO or SC to be including in these fermented feedstuffs.

Table 3. Effects of fermented feedstuff supplemented with AO or SC on microbial numbers in the rumen of Korean native cattle

Microbial counts ($\times 10^9$, MPN[4]/ml rumen content)	Control[1]	TAO[2]	TSC[3]
Amylolytic bacteria	8.00	5.00	3.00
Cellulolytic bacteria	0.28	0.90	0.60
Pectin fermenters	2.10	2.60	2.20
Xylan fermenters	2.20	3.50	3.40

[1] Control : commercial feed + corn silage;
[2] TAO : commercial feed + FFAO + corn silage ;
[3] TSC : commercial feed + FFSC + corn silage ;
[4] MPN : most probable number.

DMR for total microbes of control, TAO and TSC were 4.18, 4.36 and 4.25 g/dl, respectively, and then DMR of control, TAO and TSC were similar. DMR for protozoa of control, TAO and TSC were 2.11, 2.30 and 2.50 g/dl, and then the DMR was higher in TAO and TSC than in control($p<0.05$). DMR of bacteria tended to be higher in TAO and TSC than in control, but was not significant. Therefore, DMR for total microbes, protozoa and bacteria tended to be higher in TAO and TSC than in control. Especially, DMR for protozoa was increased by adding of AO or SC. (Table. 4).

The order of CP content appeared in the sequence of TSC > TAO > control(23.74, 23.30 and 23.15%, respectively) for total microbes, and the order appeared in the sequence of TSC > TAO > control(43.30, 43.26 and 42.79%, respectively) for bacteria; thereby, CP content of ruminal microorganism was increased by adding of SC.

Regardless of treatments, ranges of CP content for total microbes, protozoa and bacteria were 23.15~23.74%, 25.45~28.02% and 42.79~43.30%, respectively; Therefore, CP content tended to be higher in bacteria than in total microbes and protozoa. In addition, the order of EE appeared in the sequence of TAO>TSC> control (14.35, 14.28 and 13.33%, respectively) for total microbes, and then EE content was higher in TAO and TSC than in control($p<0.05$), and EE content of protozoa and bacteria was also higher in TSC than in control and TAO ($p<0.05$); therefore, addition of SC increased EE content of ruminal microorganism. Crude ash content of total microbes and bacteria was higher in TAO than in control and TSC ($p<0.05$), crude ash content of protozoa was higher in control than in TAO and TSC($p<0.05$).

Regardless of treatments, ranges of EE content for total microbes, protozoa and bacteria were 13.33~14.35%, 9.09~10.68% and 10.49~11.75%, respectively. In addition, ranges of crude ash for total microbes, protozoa and bacteria were 37.27~38.73%, 13.67~15.53% and 19.27~23.12%,

Table 4. Effects of fermented feedstuff supplemented with AO or SC on microbial body compositions in the rumen of Korean native cattle

Items	DMR[4](g/dl)	Crude protein(%)	Ether extract(%)	Crude Ash(%)
Total microbes				
Control[1]	4.18 ± 0.05	23.15 ± 0.07^{b}	13.33 ± 0.13^{c}	38.26 ± 0.16^{b}
TAO[2]	4.36 ± 0.12	23.30 ± 0.06^{b}	14.35 ± 0.84^{a}	38.73 ± 0.25^{a}
TSC[3]	4.25 ± 0.06	23.74 ± 0.11^{a}	14.28 ± 0.08^{b}	37.27 ± 0.20^{c}
Protozoa				
Control	2.11 ± 0.12^{c}	25.45 ± 0.05^{c}	9.79 ± 0.04^{b}	15.53 ± 0.16^{a}
TAO	2.30 ± 0.03^{b}	26.91 ± 0.03^{b}	9.09 ± 0.25^{b}	13.67 ± 0.24^{c}
TSC	2.50 ± 0.06^{a}	28.02 ± 0.13^{a}	10.68 ± 0.13^{a}	14.87 ± 0.16^{b}
Bacteria				
Control	0.76 ± 0.16	42.79 ± 0.21^{b}	10.25 ± 0.09^{b}	19.27 ± 0.13^{c}
TAO	0.84 ± 0.05	43.26 ± 0.15^{a}	10.49 ± 0.19^{b}	23.12 ± 0.08^{a}
TSC	0.83 ± 0.02	43.30 ± 0.04^{a}	11.75 ± 0.06^{a}	21.38 ± 0.01^{b}

Means ± standard deviation.
[1] Control : commercial feed + corn silage; [2] TAO : commercial feed + FFAO + corn silage;
[3] TSC : commercial feed + FFSC + corn silage; [4] DMR : dry matter recovery.
[a,b,c] Means in same column with different superscripts differ($p<0.05$).

respectively. The crude ash content tended to be higher in bacteria than in protozoa.

Generally, it has been reported that the range of CP of ruminal total microbes was from 29%(Smith, 1975) to 55%(Baldwin, 1970), CP content of total microbes tended to be low in this experiment, this was presumed by the difference in feed CP content, feeding method, collection time interval of ruminal fluid and isolation method of microbes. Also, the range of EE of ruminal total microbes was reported by 2 ~25% in the research results of some researchers (Czerkawski, 1986; Henderickz et al., 1972; Baldwin, 1970), and the result of this experiment was similar to previous results. Furthermore, EE content of protozoa and bacteria were significantly increased by adding of SC, although we can not clarify clear conclusion in this experiment. However, it was presumed by the existence of an unknown factor which activate EE synthesis of ruminal microorganism within SC, and we think as the thing by needing more research about this phenomenon.

Generally, Ørskov(1982) reported that the amino acids profile of mixed ruminal bacteria was regular and did not take the influence by the

Table 5. Effects of fermented feedstuff supplemented with AO or SC on the amino acid compositions of total microbes in rumen of Korean native cattle

Items	Control[1)]	TAO[2)]	TSC[3)]
Essential amino acids			
Arginine(%)	6.06 ± 0.03^{a}	5.95 ± 0.01^{b}	5.93 ± 0.01^{b}
Histidine(%)	2.40 ± 0.01^{b}	2.34 ± 0.01^{c}	2.54 ± 0.02^{a}
Isoleucine(%)	4.27 ± 0.00^{a}	4.17 ± 0.01^{b}	3.92 ± 0.00^{c}
Leucine(%)	8.34 ± 0.01	8.30 ± 0.02	8.32 ± 0.03
Lysine(%)	7.51 ± 0.10^{a}	6.82 ± 0.52^{b}	6.59 ± 0.20^{b}
Methionine(%)	2.04 ± 0.01^{a}	1.84 ± 0.02^{c}	1.94 ± 0.00^{b}
Phenylalanine(%)	5.66 ± 0.05^{a}	5.38 ± 0.10^{b}	5.38 ± 0.04^{b}
Threonine(%)	4.54 ± 0.02^{a}	4.46 ± 0.01^{b}	4.44 ± 0.01^{b}
Valine(%)	4.81 ± 0.02^{b}	4.74 ± 0.03^{c}	4.89 ± 0.01^{a}
Subtotal(%)	45.64 ± 0.08^{a}	44.00 ± 0.43^{b}	43.95 ± 0.14^{b}
Nonessential amino acids			
Alanine(%)	7.23 ± 0.02^{b}	7.14 ± 0.07^{b}	7.57 ± 0.05^{a}
Aspartic acid(%)	10.36 ± 0.01^{b}	11.42 ± 0.10^{a}	10.47 ± 0.04^{b}
Cystein(%)	0.49 ± 0.01^{b}	0.49 ± 0.00^{b}	0.52 ± 0.00^{a}
Glutamic acid(%)	16.34 ± 0.04	17.96 ± 1.37	18.21 ± 1.34
Glycine(%)	5.49 ± 0.02^{c}	5.58 ± 0.03^{b}	5.66 ± 0.02^{a}
Proline(%)	4.29 ± 0.01^{c}	4.46 ± 0.01^{b}	4.55 ± 0.01^{a}
Serine(%)	6.34 ± 0.02	5.45 ± 0.89	5.55 ± 0.95
Tyrosine(%)	3.82 ± 0.01^{a}	3.48 ± 0.00^{c}	3.52 ± 0.01^{b}
Subtotal(%)	54.36 ± 0.05^{b}	56.00 ± 0.50^{a}	56.05 ± 0.27^{a}
Total(%)	100.00	100.00	100.00

Means ± standard deviation.

[1)] Control : commercial feed + corn silage; [2)] TAO : commercial feed + FFAO + corn silage ;

[3)] TSC : commercial feed + FFSC + corn silage.

[a,b,c] Means in same row with different superscripts differ($p<0.05$).

feed, but Purser and Buechler(1966) found that the range of amino acids content of mixed ruminal bacteria was wide. Namely, the range of each amino acids content(g of each amino acid /100 g of total amino acids) for *Selenomonas ruminantium, Butyrivibrio fibrisolvens, Bacteroides amylophilus,* and *Bacteroides ruminicola* to be most abundant four kinds of bacteria in the rumen was 4.7~5.7(Thr), 6.4~11.4(Val), 2.2~3.3(Met), 6.3~7.4(Ile), 7.7~8.6(Leu), 8.3~14.9 (Lys), and 4.7~5.6(Phe), respectively, and they concluded that the content of the amino acids takes the influence greatly by the feed.

The ratio of essential amino acids(EAA) of ruminal total microbes and protozoa was lower in TAO and TSC than in control($p<0.05$), the ratio of EAA of total microbes did not differ between TAO and TSC but the ratio of EAA of protozoa was higher in TAO than in TSC ($p<0.05$). The ratio of nonessential amino acids(NEAA) of total microbes and protozoa was higher in TSC than in control and TAO ($p<0.05$), and the ratio of NEAA was also higher in TAO than in control($p<0.05$). Also, the ratio of EAA and NEAA of bacteria did not differ among the treatments. (Tables 5, 6, 7)

Table 6. Effects of fermented feedstuff supplemented with AO or SC on the amino acid compositions of protozoa in the rumen of Korean native cattle

Items	Control[1)]	TAO[2)]	TSC[3)]
Essential amino acids			
Arginine(%)	6.64 ± 0.01^{b}	6.74 ± 0.07^{a}	6.74 ± 0.05^{a}
Histidine(%)	3.03 ± 0.01^{b}	2.84 ± 0.03^{c}	3.16 ± 0.00^{a}
Isoleucine(%)	4.89 ± 0.04^{a}	4.80 ± 0.02^{a}	4.19 ± 0.27^{b}
Leucine(%)	9.75 ± 0.04^{a}	9.49 ± 0.04^{b}	9.56 ± 0.05^{b}
Lysine(%)	6.73 ± 0.04^{b}	7.18 ± 0.30^{a}	5.75 ± 0.10^{c}
Methionine(%)	3.33 ± 0.01^{a}	2.94 ± 0.14^{b}	3.06 ± 0.01^{b}
Phenylalanine(%)	6.72 ± 0.02^{a}	6.66 ± 0.02^{b}	6.20 ± 0.04^{c}
Threonine(%)	4.98 ± 0.01	5.05 ± 0.05	5.05 ± 0.03
Valine(%)	5.53 ± 0.03^{a}	5.44 ± 0.05^{b}	5.56 ± 0.04^{a}
Subtotal(%)	51.59 ± 0.04^{a}	51.13 ± 0.12^{b}	49.27 ± 0.14^{c}
Nonessential amino acids			
Alanine(%)	8.52 ± 0.00^{a}	7.71 ± 0.02^{c}	8.17 ± 0.06^{b}
Aspartic acid(%)	5.02 ± 0.00^{c}	5.32 ± 0.05^{b}	5.48 ± 0.01^{a}
Cystein(%)	0.48 ± 0.00^{b}	0.63 ± 0.11^{a}	0.61 ± 0.00^{ab}
Glutamic acid(%)	11.80 ± 0.04^{c}	12.76 ± 0.05^{b}	13.71 ± 0.06^{a}
Glycine(%)	5.70 ± 0.02^{a}	5.55 ± 0.03^{b}	5.64 ± 0.05^{a}
Proline(%)	5.35 ± 0.01^{c}	5.59 ± 0.02^{b}	5.87 ± 0.02^{a}
Serine(%)	6.97 ± 0.00^{a}	6.83 ± 0.03^{c}	6.89 ± 0.03^{b}
Tyrosine(%)	4.58 ± 0.01^{a}	4.49 ± 0.01^{b}	4.37 ± 0.01^{c}
Subtotal(%)	48.41 ± 0.03^{c}	48.87 ± 0.29^{b}	50.73 ± 0.23^{a}
Total (%)	100.00	100.00	100.00

Means ± standard deviation.

[1)] Control : commercial feed + corn silage ; [2)] TAO : commercial feed + FFAO + corn silage;

[3)] TSC : commercial feed + FFSC + corn silage.

[a,b,c] Means in same row with different superscripts differ($p<0.05$).

Table 7. Effects of fermented feedstuff supplemented with AO or SC on the amino acid compositions of bacteria in rumen of Korean native cattle

Items	Control[1)]	TAO[2)]	TSC[3)]
Essential amino acids			
Arginine(%)	6.52 ± 0.03	6.64 ± 0.03	6.53 ± 0.15
Histidine(%)	2.27 ± 0.01^{c}	2.35 ± 0.00^{a}	2.32 ± 0.00^{b}
Isoleucine(%)	4.62 ± 0.02^{a}	4.52 ± 0.00^{b}	4.57 ± 0.07^{ab}
Leucine(%)	7.44 ± 0.03^{ab}	7.57 ± 0.01^{a}	7.32 ± 0.17^{b}
Lysine(%)	7.18 ± 0.15^{a}	6.52 ± 0.02^{b}	6.55 ± 0.38^{b}
Methionine(%)	2.56 ± 0.00^{b}	2.65 ± 0.00^{a}	2.65 ± 0.02^{a}
Phenylalanine(%)	5.08 ± 0.06^{b}	5.33 ± 0.01^{a}	4.95 ± 0.19^{b}
Threonine(%)	4.89 ± 0.02	4.98 ± 0.02	4.89 ± 0.11
Valine(%)	4.85 ± 0.00^{c}	5.04 ± 0.01^{b}	5.14 ± 0.03^{a}
Subtotal(%)	45.41 ± 0.14	45.61 ± 0.04	44.92 ± 0.17
Nonessential amino acids			
Alanine(%)	7.60 ± 0.09^{b}	8.09 ± 0.01^{a}	8.06 ± 0.09^{a}
Aspartic acid(%)	11.72 ± 0.01^{a}	10.64 ± 0.03^{b}	11.69 ± 0.09^{a}
Cystein(%)	0.75 ± 0.00^{a}	0.74 ± 0.01^{b}	0.66 ± 0.01^{c}
Glutamic acid(%)	14.48 ± 0.07	14.49 ± 0.01	14.34 ± 0.20
Glycine(%)	5.55 ± 0.05^{b}	5.76 ± 0.03^{a}	5.86 ± 0.11^{a}
Proline(%)	4.08 ± 0.02^{a}	3.83 ± 0.02^{b}	4.07 ± 0.03^{a}
Serine(%)	6.20 ± 0.03^{b}	6.49 ± 0.02^{a}	6.23 ± 0.11^{b}
Tyrosine(%)	4.22 ± 0.00^{b}	4.35 ± 0.01^{a}	4.17 ± 0.03^{c}
Subtotal(%)	54.59 ± 0.22	54.39 ± 0.16	55.08 ± 0.59
Total(%)	100.00	100.00	100.00

Means ± standard deviation.
[1)] Control : commercial feed + corn silage ; [2)] TAO : commercial feed + FFAO + corn silage ;
[3)] TSC : commercial feed + FFSC + corn silage.
[a,b,c] Means in same row with different superscripts differ($p<0.05$).

The ratios of lysine of total microbes and bacteria were lower in TAO and TSC than in control($p<0.05$), and the ratio of lysine of protozoa was lower in TSC than in control ($p<0.05$). The ratios of glutamic acid were higher in TAO and TSC than in control for total microbes and protozoa($p<0.05$), and the ratio was especially higher in TAO than in TSC($p<0.05$). Like this, the ratios of lysine of ruminal microorganism were reduced chiefly in bacteria, and the ratios of glutamic acid of ruminal microorganism were increased chiefly in protozoa. Consequently, the addition of AO or SC had an influence on amino acids compositions of ruminal microorganism; thereby, the ratio of lysine was reduced and the ratio of glutamic acid was increased.

Ⅳ. ABSTRACT

This study was conducted to examine effects of brewery meal-based fermented feedstuff supplemented with *Aspergillus oryzae*(AO) or *Saccharomyces cerevisiae*(SC) on ruminal microorganism of Korean native cattle. Two cows equipped with ruminal cannulas were used as

experimental animals. Experiment was done with three treatment groups: 71.5% of commercial feed and 28.5% of corn silage(control); 45.0% of commercial feed, 26.5% of fermented feedstuff supplemented with AO and 28.5% of corn silage(TAO); 45.0% of commercial feed, 26.5% of fermented feedstuff supplemented with SC and 28.5% of corn silage(TSC). The number of total viable bacteria ($p<0.05$), anaerobic fungi and protozoa($p<0.05$) was higher in TAO and TSC than in control. The number of proteolytic bacteria($p<0.05$), cellulolytic bacteria and xylan fermenters tended to be higher in TAO and TSC than in control. The dry matter recovery (DMR) of protozoa was higher in TAO and TSC than in control($p<0.05$). The crude protein (CP) content of total microbes and protozoa was higher in TSC than in control and TAO ($p<0.05$). The CP content of bacteria was higher in TAO and TSC than in control($p<0.05$). The ether extract(EE) content of the total microbes was higher in TAO than in control and TSC($p<0.05$), and the EE of protozoa and bacteria were higher in TSC than in control and TAO($p<0.05$). The ratio of essential amino acids of total microbe was higher in control than in TAO and TSC($p<0.05$). The ratio of methionine and alanine of bacteria was higher in TAO and TSC than in control($p<0.05$). The results suggested that the feeding of fermented feedstuff supplemented with AO or SC had an influence on the numbers of ruminal microorganism and the changes of microbial body composition.

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