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Effect of Different Silages for TMR on *In vitro* Rumen Simulative Fermentation

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ABSTRACT

In this study, the *in vitro* fermentation parameters of whole crop barley (WCBS-TMR) and Italian ryegrass (IRGS-TMR) silage total mixed rations were compared. A rice straw based diet (RSBD), which was a mixture of rice straw and concentrate (60:40), was used as the control. The feeds were incubated in buffered rumen fluid for 3, 6, 9, 12, 24, 48 and 72 hours at 39°C. At the end of each incubation period the following parameters were determined, total gas, pH, ammonia nitrogen (NH_3-N), volatile fatty acids (VFA) and then the acetate to propionate ratio (A/P) was calculated. The dietary treatments did not affect ($p>0.05$) the overall production of NH_3-N, gas, total VFA and all the individual VFA, with the exception of n-butyrate ($p<0.001$). The treatment diets significantly affected the A/P ratio ($p<0.01$). The control diet resulted in the lowest A/P ratios, followed by WCBS-TMR and lastly IRGS-TMR had the highest ratios. Gas production was not different between treatments, suggesting a probable similar level of digestibility when treatments are fed to animals. It can therefore be concluded from the present study that WCBS and IRGS are of almost an equivalent nutritional value when incubated in a TMR form. WCBS-TMR however resulted in lower A/P ratios than IRGS-TMR, which is indicative of a more energy efficient diet.

(**Key words** : *In vitro* rumen fermentation, Italian ryegrass, Roughage source, Total mixed rations, Whole crop barley)

Ⅰ. INTRODUCTION

Feed costs in an intensive beef production account for a critical percentage of the production costs. Provision of productively efficient feed is therefore of greatest importance in sustaining a viable beef production entity. Key to achieving this is the choice of forage source. Forages are the basis for good feed formulation (Van Saun, 2007). Rice is the staple crop of Korea and consequently, rice straw has been widely used as a forage source for ruminants. However, in an effort to improve feed quality whole crop barley (WCB) and Italian ryegrass (IRG) have gained popularity as forage sources among beef producers. These roughage sources are either provided alone or as constituents of total mixed rations (TMR). They are used as straws or silages. Ensiling is one way of preserving forages whilst maintaining their high nutritional value. Silages also make more palatable straws (Gao et al., 2008). In our previous study (Mbiriri et al., 2012), we investigated the *in vitro* fermentation patterns of the forages being investigated in the present study when incubated alone. From the results we concluded that there were no significant differences in total and individual VFA production between forages. However, WCBS was reported a better forage source on the basis of a

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significantly higher NH_3-N output than the other treatment diets. There is need, however, to investigate on the fermentation parameters when these forages are incorporated into TMRs. The objective of this research was to compare *in vitro* fermentation patterns of WCBS-TMR and IRGS-TMR.

Ⅱ. MATERIALS AND METHODS

1. Diets

Three diets, WCBS-TMR, IRGS-TMR and RSBD (the control diet) were used as substrates. The control diet was a mixture of rice straw and a concentrate in a 60:40 ratio. Whole crop barley silage TMR and IRGS-TMR were freeze-dried, ground and passed through 1 mm screen. The nutritional information of the diets was shown in Table 1.

2. Rumen fluid

Rumen fluid was collected before morning feeding from a rumen cannulated Hanwoo steer weighing about 350 kg. The steer was being fed a commercial TMR (forage:concentrate was 50:50) individually in a metabolic pen twice a day, at 0900 and 1700 h. The fluid was strained through 4 layers of cheese cloth into a flask previously filled with N_2 gas. The flask was then transported to the laboratory in less than 1 hour. The rumen fluid was further strained through another 4 layers of cheese cloth on arrival at the laboratory. The strained rumen fluid was then mixed with McDougall's buffer prepared according to Troelsen and Donna (1966), at a rate of 1:4 (rumen fluid:buffer). The buffer (pH 6.5) contained 9.8 g of $NaHCO_3$, 4.62 g of $Na_2HPO4 \cdot 2H_2O$, 0.57 g KCl, 0.47 g NaCl, 0.12 g $MgSO_4 \cdot 7H_2O$ and 4 g $CaCl_2$ per 1 L of distilled water.

3. Incubation procedure

In vitro rumen simulated fermentation was performed in 250 mL serum bottles under the stream of N_2 gas to maintain anaerobic condition. 0.5 g of each diet was mixed with 50 mL of buffered rumen fluid in the serum bottles whilst gassing with N_2 gas. The bottles were then sealed and capped using aluminium sealed

Table 1. Chemical composition of experimental diets

Items[1)]	Experimental diets[3)]			
	Concentrate	Rice straw	WCBS-TMR	IRGS-TMR
Dry matter (%)	95.02	91.63	73.15	76.05
Crude protein (% DM)	12.57	5.21	13.68	13.02
Ether extract (% DM)	2.34	1.42	2.45	2.66
Crude fiber (% DM)	15.23	34.27	22.71	19.42
Crude ash (% DM)	11.26	12.99	9.26	7.68
NDF (% DM)	ND[2)]	76.31	ND	ND
ADF (% DM)	ND	52.35	ND	ND

[1)] NDF: Neutral detergent fiber; ADF: Acid detergent fiber.
[2)] ND: Not determined.
[3)] WCBS-TMR: Whole crop barley silage total mixed ration; IRGS-TMR: Italian ryegrass silage total mixed ration.

rubber stoppers. They were then placed in an incubator at 39°C. All treatments were performed in triplicate. The procedure followed the method outlined by Tilley and Terry (1963). At each sampling time, total gas production was measured by way of displacing a glass syringe. The bottles were then uncapped and the pH measured using a pH meter. A cell and diet particle free supernatant was prepared by centrifugation (10,000 rpm for 15 min at 4°C) and stored at −20°C for further analysis. Ammonia nitrogen was analysed using a spectrophotometer according to the method of Chaney and Marbach (1962). Volatile fatty acids were determined using gas chromatography (Agilent 6890, Agilent Technology Inc., USA) according to Erwin et al. (1961).

4. Statistical analysis

Data on the measured fermentation parameters were subjected to ANOVA using SPSS program (version 18, IBM, New York, USA). The following model was used for all observed patterns

$Y_{ijk} = \mu + T_i + H_j + (T \times H)_{ij} + e_{ijk}$, where;

Y_{ijk} is response variable (pH, gas e.t.c). μ and T_i are overall mean common to all observations and the i^{th} treatment diet, respectively. H_j and $(T \times H)_{ij}$ are the j^{th} incubation time and interaction between the i^{th} treatment diet and the j^{th} time, respectively. e_{ijk} is random residual error. The model used to evaluate overall effect of treatment diets did not include time effects.

Ⅲ. RESULTS AND DISCUSSION

Significantly different fermentation patterns ($p<0.01$) were observed for all the measured fermentation parameters. The observed pH profiles were shown in Table 2. The RSBD

Table 2. Effect of treatment diets on pH patterns

Incubation time, h	Treatments[1)]			SEM	Significance[2)]
	RSBD	WCBS-TMR	IRGS-TMR		
3	6.66[a]	6.89[b]	6.91[b]	0.041	***
6	6.59[a]	6.87[b]	6.88[b]	0.047	***
9	6.54[a]	6.83[b]	6.85[c]	0.050	***
12	6.50[a]	6.79[b]	6.83[c]	0.051	***
24	6.40[a]	6.74[b]	6.76[c]	0.058	***
48	6.34[a]	6.69[b]	6.71[c]	0.060	***
72	6.31[a]	6.68[b]	6.70[b]	0.063	***

Source of variation	*F* value	Significance
Treatment	7639.293	***
Time	953.759	***
(Treatment*Time)	24.681	***

[abc] Means with different superscripts in a row are significantly different ($p<0.05$).
[1)] RSBD: Rise straw based diet; WCBS-TMR, IRGS-TMR: Whole crop barley and Italian ryegrass silage total mixed rations respectively.
[2)] Level of significance code *** means $p<0.001$.

resulted in the lowest pH values throughout the incubation period. The pH values, for all diets, decreased with increasing incubation time, which is an expected trend as VFA accumulate with time. Between 9 and 48 h, IRGS-TMR had higher pH values than RSBD and WCBS-TMR.

Gas production trends differed significantly ($p<0.001$) between treatments (Table 3). Gas output increased with time although after 72 h it either decreased or tended to show smaller increment than at the 48 h sampling time. The observation suggests an almost complete degradation of the substrate. Since there was no significant difference ($p>0.05$) in the overall gas output, it indicates that the diets would most likely to be of the same *in vivo* digestibility when fed to animals (Menke et al., 1979).

Ammonia nitrogen values for all treatments started-off higher than at 6 to 12 h incubation (Table 4). This observation is a result of the breaking down of the rapidly degraded protein in the concentrate portion of the diets. Mbiriri et al. (2012) who incubated 4 forage sources that included WCBS, IRGS and rice straw, without a concentrate, did not observe such a trend. However, they noted a steeper rise in NH_3-N output after 12 h incubation of the forages. The same pattern is apparent in this study, although in this case the overall NH_3-N for the diets did not differ significantly ($p>0.05$). At the 72 h mark, WCBS-TMR registered a lower value than at the 48 h mark, as is shown in Table 4. This suggests an almost complete degradation of the substrate. Contrary to NH_3-N values out of the optimal range (15~20 mg/100 mL) (Perdok and Leng, 1989) observed when the same forages were incubated alone, when incubated in a TMR form, the NH_3-N reached the optimal values after 48 h of incubation.

There was no significant difference between

Table 3. Effect of treatment diets on gas production (mL) patterns

Incubation time, h	Treatments[1)]			SEM	Significance[2)]
	RSBD	WCBS-TMR	IRGS-TMR		
3	19.67[b]	14.00[a]	10.33[a]	1.563	*
6	23.33[a]	30.33[b]	25.00[a]	1.256	*
9	35.67[a]	45.00[c]	38.67[b]	1.412	***
12	45.00[a]	55.00[b]	44.00[a]	1.795	***
24	63.33[a]	72.33[b]	63.00[a]	1.631	**
48	81.00[a]	85.67[b]	80.00[a]	0.940	**
72	79.67[a]	85.67[b]	82.00[ab]	1.144	*

Source of variation	*F* value	Significance
Treatment	37.10	***
Time	2195.54	***
(Treatment*Time)	73.32	***

[abc] Means with different superscripts in a row are significantly different ($p<0.05$).

[1)] RSBD: Rise straw based diet; WCBS-TMR, IRGS-TMR: Whole crop barley and Italian rye grass silage total mixed rations respectively.

[2)] Level of significance codes ***, **, * mean $p<0.001$, $p<0.01$ and $p<0.05$ respectively.

Table 4. Effect of treatment diets on ammonia nitrogen (mg/100 mL) patterns

Incubation time, h	Treatments[1)]			SEM	Significance[2)]
	RSBD	WCBS-TMR	IRGS-TMR		
3	2.68[ab]	3.54[b]	2.38[a]	0.218	*
6	1.61[a]	2.46[b]	1.30[a]	0.180	**
9	1.46[b]	2.91[c]	0.72[a]	0.326	***
12	2.33[b]	3.41[c]	1.01[a]	0.364	***
24	7.51[c]	3.42[b]	2.14[a]	0.817	***
48	15.01[a]	15.37[a]	14.00[a]	0.371	NS
72	19.14[b]	14.26[a]	15.25[a]	0.912	*

Source of variation	*F* value	Significance
Treatment	66.657	***
Time	1624.543	***
(Treatment*Time)	6.866	***

[abc] Means with different superscripts in a row are significantly different ($p<0.05$).
[1)] RSBD: Rise straw based diet; WCBS-TMR, IRGS-TMR: Whole crop barley and Italian ryegrass silage total mixed rations respectively.
[2)] Level of significance codes ***, **, *, NS mean $p<0.001$, $p<0.01$, $p<0.05$ and non significant respectively.

Table 5. Effect of treatment diets on overall mean volatile fatty acid production

Parameter, mM	Treatments[1)]			SEM	Significance[2)]
	RSBD	WCBS-TMR	IRGS-TMR		
Acetate	31.01	32.58	33.51	1.141	NS
Propionate	10.76	10.50	9.88	0.412	NS
Iso-butyrate	0.55	0.54	0.57	0.035	NS
n-butyrate	4.77[b]	6.76[a]	6.27[a]	0.228	***
Iso-valerate	0.92	1.02	1.10	0.067	NS
n-valerate	0.65	0.74	0.70	0.033	NS
Total VFA	48.66	51.65	52.52	1.882	NS
A/P ratio	2.88[c]	3.17[b]	3.45[a]	0.042	***

[abc] Means with different superscripts in a row are significantly different ($p<0.05$).
[1)] RSBD: Rise straw based diet; WCBS-TMR, IRGS-TMR: Whole crop barley and Italian ryegrass silage total mixed rations respectively.
[2)] Level of significance codes *** and NS mean $p<0.001$ and non significant respectively.

Table 6. Volatile fatty acid production patterns of the dietary treatments

Item[1)]	Incubation time, h						
	3	6	9	12	24	48	72
				..... Acetate, mM			
RSBD	16.60[a]	20.90[a]	24.67[a]	26.93[a]	35.83	42.30[a]	45.07[ab]
WCBS-TMR	20.89[b]	25.76[b]	28.72[b]	32.30[b]	35.64	43.70[ab]	40.86[a]
IRGS-TMR	20.16[b]	26.14[b]	28.35[b]	30.87[b]	38.52	45.08[b]	45.46[b]
SEM	0.63	0.895	0.691	0.97	0.91	0.485	0.958
Significance[2)]	***	**	***	***	NS	*	NS
				..... Propionate, mM			
RSBD	5.48[ab]	7.62	8.79	9.28	12.08[b]	14.66[b]	15.65[b]
WCBS-TMR	5.61[b]	7.87	9.3	10.39	11.35[ab]	15.11[c]	13.86[a]
IRGS-TMR	5.15[a]	7.17	8.86	9.18	10.63[a]	13.92[a]	14.22[ab]
SEM	0.09	0.147	0.109	0.295	0.249	0.184	0.351
Significance[2)]	NS	NS	NS	NS	*	***	NS
				..... iso-Butyrate, mM			
RSBD	0.30[b]	0.32	0.34[a]	0.38	0.61[b]	0.90[a]	0.93[ab]
WCBS-TMR	0.24[a]	0.33	0.39[b]	0.45	0.52[a]	0.97[ab]	0.86[a]
IRGS-TMR	0.23[a]	0.34	0.39[b]	0.37	0.59[b]	1.00[b]	1.04[b]
SEM	0.011	0.007	0.009	0.018	0.015	0.022	0.032
Significance[2)]	**	NS	**	NS	*	NS	*
				..... n-Butyrate, mM			
RSBD	2.63[a]	3.16[a]	3.83[a]	4.35[a]	5.16[a]	6.40[a]	6.66[a]
WCBS-TMR	3.99[b]	5.26[b]	5.66[b]	6.10[b]	7.32[b]	8.34[b]	7.20[a]
IRGS-TMR	3.90[b]	5.29[b]	5.88[b]	6.14[b]	8.79[c]	8.55[b]	8.79[b]
SEM	0.211	0.358	0.328	0.325	0.466	0.35	0.345
Significance[2)]	**	***	***	**	***	***	**
				..... iso-Valerate, mM			
RSBD	0.45	0.47[a]	0.54[a]	0.63	0.99[a]	1.54[a]	1.66[a]
WCBS-TMR	0.49	0.66[b]	0.70[b]	0.78	1.06[a]	1.87[b]	1.57[a]
IRGS-TMR	0.47	0.64[b]	0.71[b]	0.7	1.22[b]	1.94[b]	1.99[b]
SEM	0.01	0.032	0.029	0.03	0.041	0.066	0.073
Significance[2)]	NS	**	***	NS	*	**	*
				..... n-Valerate			
RSBD	0.41[b]	0.40[a]	0.48[a]	0.54[a]	0.74[a]	0.94[a]	0.99[ab]
WCBS-TMR	0.35[a]	0.54[b]	0.62[c]	0.71[b]	0.86[b]	1.16[c]	0.97[a]
IRGS-TMR	0.33[a]	0.48[b]	0.56[b]	0.57[a]	0.80[ab]	1.07[b]	1.16[b]
SEM	0.012	0.023	0.021	0.032	0.023	0.034	0.03
Significance[2)]	*	**	***	*	NS	**	NS
				..... Total VFA, mM			
RSBD	25.86[a]	32.89[a]	38.66[a]	42.09[a]	55.84	66.75[a]	70.92
WCBS-TMR	31.57[b]	40.42[b]	45.58[b]	50.73[b]	56.75	71.15[b]	65.33
IRGS-TMR	30.26[b]	40.06[b]	44.76[b]	47.83[ab]	60.55	71.56[b]	72.61
SEM	0.829	1.32	1.132	1.584	1.119	0.836	1.507
Significance[2)]	***	**	***	*	NS	**	NS
				..... A/P ratio[3)]			
RSBD	3.04[a]	2.74[a]	2.81[a]	2.90[a]	2.97[a]	2.89[a]	2.88[a]
WCBS-TMR	3.73[b]	3.28[b]	3.11[b]	3.11[b]	3.13[a]	2.89[a]	2.95[a]
IRGS-TMR	3.91[b]	3.64[c]	3.20[b]	3.36[c]	3.62[b]	3.24[b]	3.20[b]
SEM	0.13	0.132	0.06	0.069	0.107	0.061	0.05
Significance[2)]	**	***	***	***	**	***	***

[abc] Means with different superscripts in a column are significantly different ($p<0.05$).

[1)] RSBD: Rise straw based diet; WCBS-TMR, IRGS-TMR: Whole crop barley and Italian ryegrass silage total mixed rations, respectively.

[2)] Level of significance codes ***, **, *, NS mean $p<0.001$, $p<0.01$, $p<0.05$ and non significant respectively.

[3)] A/P ratio: acetate to propionate ratio.

treatments in overall VFA production except for n-butyrate ($p<0.001$) (Table 5). Volatile fatty acid production trends over time (Table 6) were however significantly different between treatments diets used in this experiment. The RSBD consistently produced significantly lower ($p<0.05$) total VFA for all incubation periods with the exception of 24 and 72 h incubations where total VFA did not significantly differ ($p>0.05$) between treatments. This is an almost similar result as in the previous incubations where the forages were incubated alone (Mbiriri et al., 2012). Italian ryegrass silage and WCBS-TMRs exhibited a similar production trend although WCBS-TMR tended to tapper-off after 72 h incubation. The RSBD showed a steady rise in acetate. Although it resulted in lower production from 3 to 12 h incubation, it matched the other 2 treatments after 24 h incubation. This is indicative of a higher content of slowly degradable fiber than IRGS and WCBS-TMRs. A similar pattern was observed in propionate production, but in this case the control diet actually tended to result in more propionate than either WCBS-TMR or IRGS-TMR. The significantly different ($p<0.001$) A/P ratios reflect the different acetate and propionate production patterns. RSBD had the lowest A/P ratios followed by WCBS-TMR and lastly IRGS-TMR had the highest values. This suggests that the control diet could prove the most energy efficient to animal production and IRGS-TMR will most likely be the least energy efficient diet to the animal (Wolin, 1960).

Ⅳ. CONCLUSION

In the present *in vitro* study WCBS and IRGS TMRs, resulted in different fermentation patterns. The fermentation of WCBS-TMR resulted in lower overall A/P ratios than IRGS-TMR, making it a more likely energy efficient diet to the animal. However, the overall pH, gas, NH_3-N, total VFA and individual VFA, with the exception of n-butyrate were not affected significantly by the dietary treatments.

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