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Associative Effects of Supplementing Rice Straw with Rapeseed Meal on Performance, Digestion and Ruminal Microbes for Growing Lambs

[1,2]M.R. Ma, [2]W.J. Chen, [2]X.D. Zhang and [2]J.X. Liu
[1]Jinhua Polytechnic, 321007 Jinhua, Zhejiang, China
[2]College of Animal Sciences, Zhejiang University,
310058 Hangzhou, Zhejiang, China

Abstract: Thirty six male Huzhou lambs consuming a rice straw-based diet were used in a 60 days trial to study the associate effects of Rapeseed Meal (RSM) supplementation on digestion, ruminal fermentation parameters, blood biochemical indices and growth performance. All animals were fed rice straw *ad libitum* together with 120 g day^{-1} of cornstarch and supplemented with RSM at levels of 0 (control), 80, 160 or 240 g day^{-1}, respectively. Supplemental RSM had no significant effects on feed intake of rice straw ($p>0.05$) but enhanced the apparent digestibility of organic matter, crude proteins and neutral detergent fiber significantly ($p<0.05$) with the maximum value at 160 g RSM per day. Daily weight gain of lambs increased linearly with the increasing RSM, reaching 108 g day^{-1} from 26 for the control. Supplementation of rapeseed with 160 or 240 g day^{-1} increased significantly serum concentrations of total protein and urea nitrogen ($p<0.05$) but with no significant effects on triglyceride ($p>0.05$). The blood glucose concentration rose as the level of supplementation increased. Rumen concentrations of volatile fatty acids, microbial protein and carboxymethyl cellulase activity increased as the proportion of rapeseed increased. When the quantity of supplemented rapeseed reached to 240 g day^{-1}, the increasing rate was mitigated.

Key words: Growth performance, rapeseed meal, rice straw, rumen fermentation, Hu sheep

INTRODUCTION

Main constraints of crop by products such as rice straw as the feed for ruminal animals included high fiber content and low content of nitrogen and available energy and then low nutritional value. In order to make effective use of these resources, the stalks should be processed before feeding to animals or supplemented with available energy and protein source. A large quantity of studies indicated the negative associate effects when a high level of fermentable carbohydrate is supplemented to a forage-based diet while low level of supplementary feeding can improve the intake and digestibility of forage (Huhtanen, 1991; Lardy *et al*., 2004; Zhang *et al*., 2010). The supplemented protein source can improve the utilization of low-quality roughage by ruminal animals and therefore enhance the productivity (Mould, 1988; Jaakkola and Huhtanen, 1990; Bodine and Purvis, 2003). Weixian *et al.* (1994) found that when being fed with only low quality roughage, sheep had a low growth performance. With a certain quantity of supplemented oil seed meal, the daily gain showed a dramatic increase. The roughage and oil seed meal had positive associate effects (Liu *et al*., 1998; Xu and Liu, 2005). Due to the influence from multiple factors, the associate effects have a complicated manifestation. Concentrate to forage ratio, protein level and proportion of energy and available nitrogen all can affect the associate effects.

Recently, the rumen cellulolytic bacteria have been studied extensively and have been shown to be the primary degraders of fibre. *R. albus*, *R. flavefaciens* and *F. succinogenes* are considered as the representative cellulolytic species in the rumen and *B. fibrisolvens* is not only cellulolytic but also proteolytic species. They require ammonia N for their optimum growth when host was fed fibre basal diet (Ranilla *et al*., 2001). Therefore, quantification of population sizes of cellulolytic bacteria would be appropriate way to reveal the effect of protein supplementation on microbial growth and fiber digestion.

China is abundant in Rapeseed Meal (RSM) used mainly for swine and poultry. Information is limited on the use of RSM as a protein supplement to ruminants fed on straw based diet. Many of the reported research on oilseed-straw combination diets have dealt with the associative effects themselves rather than the mechanism causing such effects. The objectives of this experiment were to investigate the positive associative effects of supplementing RS-based diets with different levels of RSM on animal performance, intake and digestibility and to explore possible mechanisms in terms of rumen fermentation and microbial populations.

Corresponding Author: Jianxin Liu, College of Animal Sciences, Zhejiang University, 310058 Hangzhou, China

MATERIALS AND METHODS

Animals, diets and experimental designs: Thirty six male Hu lambs with an average live weight of 21±0.9 kg were divided into four equal groups of nine each according to body weight. Lambs in each group were kept in 3 pens (three lambs each) and given free access to RS and water during 15 days of adaptation and 45 days of measurements. The RS used was from the late season rice, cultivated in Zhejiang Province. Following the harvest, RS was air-dried and manually chopped to 5-10 cm lengths. The RSM was supplemented at levels of 0 (control), 80, 160 and 240 g day^{-1} dry matter, respectively. All lambs were also offered 120 g corns per day which was chosen based on previous research (Xu and Liu, 2005). Supplements were given twice daily at 8:00 and 16:00. Chemical compositions of feeds are shown in Table 1.

Intake, growth performance and digestion: The amounts of RS offered and refused by the animals were recorded daily and feed intake was measured at intervals of 5 days. Each lamb was weighed for two consecutive days on arrival before the morning feeding at the beginning and end of the feeding trial and at the intervals of 15 days over the trial.

Digestion trial was conducted on the 20th day of the measurement period and consisted of 3 days adjustment to fecal bags and a 6 days total fecal collection. At the completion of the digestion trial, subsamples of feed offered and refused and dried fecal samples were ground in a mill to pass a 1 mm screen and stored at room temperature in sealed containers until they were analyzed. Averages of daily feed offered and refused and feces excreted during the 6 days total collection period were used for digestibility calculation.

Slaughtering trial: Hot carcass weight was taken on the floor within 10 min after slaughter including kidneys and kidney fat. Dressing percentage was the ratio of hot carcass weight to the unshorn weight just before slaughter, multiplied by 100. The GR value (the depth of muscle and fat tissue from the surface of the carcass to the lateral surface of the twelfth rib 110 mm from the midline) was directly measured using a GR knife. Ribeye area was measured by tracing an outline of the cross-section of the longissimus muscles from both the right and left sides of the carcass between the 12th and 13th thoracic vertebrae on a piece of acetate. The area was then measured in square centimeters by a polar planimeter (Model WDY-500, Harbin Optical Gaging Products Co., Ltd. China) and the two sides were averaged. The muscle and bone of each right side of the carcasses were separated for calculation of bone to muscle ratio. All the necessary cutting of the carcasses was made by experienced technicians.

Table 1: Chemical composition of rice, rapeseed meal and corn

Compositions	Rice straw	Rapeseed meal	Corn
Dry matter (%)	88.0	87.6	84.9
Organic matter (DM %)	85.5	90.5	97.5
Crude protein (DM %)	5.7	42.8	8.1
Neutral detergent fiber (DM %)	68.6	23.1	7.9

Ruminal fluid measurements: Rumen fluid was sampled immediately after the animal was slaughtered and strained through two layers of gauze into tubes for analysis of pH, ammonia N, Microbial Crude Protein (MCP) and Volatile Fatty Acid (VFA) using the methods as described by Hu *et al.* (2005). In brief, the pH of rumen fluid was determined immediately after removal using a pH meter (Model PB-20, Sartorius). Concentration of ammonia N was determined by a spectrometer (Model 721) using colorimetry (Searle, 1984) with ammonium chloride solution as a standard. The MCP was determined by the method of Zinn and Owens (1986) based on purine and estimated from the ratio of purines to N of isolated microbes. Yeast RNA was used as a standard. The VFA was determined with a gas chromatograph (GC-2010, Shimadzu) equipped with a flame ionization detector and a capillary column (HP-INNOWAX, 1909N-133). Enzyme extraction from the solid-bound microbes was performed according to the methods by Manyuchi *et al.* (1992). Carboxymethyl Cellulase (CMCase) activity was determined by measuring reducing sugar formation from sodium carboxymethyl cellulose (Manyuchi *et al.*, 1992).

Microbial populations: Total Deoxyribonucleic Acid (DNA) from the rumen fluid was extracted as described by Chen *et al.* (2007b). To minimize animal-to-animal variations, each sample of one group (4 samples) was mixed with a same concentration of DNA (final concentration of 3 ng mL^{-1}) before the real-time PCR operation.

The primer pairs of total bacteria, *F. succinogenes*, *B. fibrisolvens*, *R. albus* and *R. flavefaciens* as described by Denman and McSweeney (2006), Koike and Kobayashi (2001) and Arakaki were listed in Table 2. Species-specific real-time quantitative PCR was performed using Bio-Rad iCycler iQ real-time PCR detection System (Bio-Rad laboratories, INC) with fluorescence detection of SYBR Green dye. Amplification consisted of an initial hold for 3 min followed by 40 cycles of 95°C for 30 sec and 60°C for 60 sec. Detection of the fluorescent product was

Table 2: Primers for qPCR assay

Target species	Forward/Reverse	Primers sequence	Amplicon (base pairs)
Total bacteria[1]	F	CGGCAACGAGCGCAACCC	130
	R	CCATTGTAGCACGTGTGTAGCC	
R. albus[2]	F	CGGCAACGAGCGCAACCC	176
	R	CCATTGTAGCACGTGTGTAGCC	
R. flavefaciens[1]	F	CGAACGGAGATAATTTGAGTTTACTTAGG	132
	R	CGGTCTCTGTATGTTATGAGGTATTACC	
F. succinogenes[1]	F	GTTCGGAATTACTGGGCGTAAA	121
	R	CGCCTGCCCCTGAACTATC	
B. fibrisolvens[3]	F	GAGGAAGTAAAAGTCGTAACAAGGTTTC	160
	R	CAAATTCACAAAGGGTAGGATGATT	

[1]Cited from Denman and McSweeney (2006); [2]Cited from Koike and Kobayashi (2001); [3]Cited from Arakaki

set at the last step of each cycle. To determine the specificity of amplification, analysis of product melting was conducted after each amplification. The melting curve was obtained by slow heating with a 0.1°C sec^{-1} increment from 65-95°C with fluorescence collection at 0.1°C intervals. Amplification efficiencies for each primer pairs were investigated by examining dilution series of total rumen microbial DNA template on the same plate in triplicate.

Statistical analyses: Quantification for *F. succinogenes*, *B. fibrisolvens*, *R. albus* and *R. flavefacien* were expressed as a proportion of total rumen bacterial 16S rDNA according to the equation: Relative Quantification = $2^{-(ct\ target-ct\ total\ bacteria)}$, where Ct represents threshold cycle. Data were analysed using the General Linear Model (GLM) procedure of SAS (2000, SAS Inst., Inc., Cary, NC). All multiple comparisons among means were performed using Duncan's new multiple range test. Linear and quadratic effects of increasing levels of RSM supplementation were determined using polynomial contrasts (Steel and Torrie, 1984).

RESULTS

Feed intake and digestibility: Chemical compositions of the diet ingredient are shown in Table 1. The supplementary RSM had no significant effects on straw intake ($p>0.05$, Table 2). The intake of Dry Matter (DM), Organic Matter (OM), Crude Protein (CP) and Neutral Detergent Fiber (NDF) increased linearly with the increasing RSM level. Supplementary RSM significantly increased the apparent digestibility of OM, CP and NDF ($p<0.05$). Thus, intake of the digestible nutrients also increased with the level of RSM and reached to a peak at 160 g day^{-1} of RSM. Ratio of Digestible Protein (DIP) to Digestible Organic Matter (DOM) was 4.7, 11.1, 18.0 and 21.3 for 0, 120, 160 and 240 g RSM day^{-1}, respectively.

Growth performance and carcass quality: Results for growth performance and carcass quality of Hu sheep are shown in Table 3. Supplementary RSM significantly improved growth performance of lambs compared to the control ($p<0.05$). When the level of supplemented RSM was at 80, 160 and 240 g day^{-1}, supplementation efficiency, expressed as increased weight gain relative to the control per unit of added supplement was 0.40, 0.45 and 0.34, respectively.

Table 3: Effects of supplementary Rapeseed Meal (RSM) on digestibility and digestible nutrients intake

	Supplemental RSM (g day^{-1})					Effect[1]	
Compositions	0	80	160	240	SEM	L	Q
Dry matter intake (g day^{-1})							
Rice straw	375.8	387.6	370.2	369.8	12.93	NS	NS
Total	495.8^{d}	587.6^{c}	650.2^{b}	729.8^{a}	12.93	**	NS
Nutrient intake (g day^{-1})							
Organic matter	429.4^{d}	517.5^{c}	580.6^{b}	658.3^{a}	11.05	**	NS
Crude protein	31.4^{d}	66.2^{c}	99.5^{b}	133.6^{a}	0.73	**	NS
Neutral detergent fiber	267.2^{b}	293.7^{ab}	300.2^{a}	318.4^{a}	8.87	**	NS
Apparent nutrient digestibility (%)							
Organic matter	52.2^{b}	54.6^{ab}	56.7^{a}	56.1^{a}	1.00	*	NS
Crude protein	33.7^{c}	47.3^{b}	59.2^{a}	59.0^{a}	2.10	**	*
Neutral detergent fiber	46.3^{b}	47.9^{ab}	50.7^{a}	49.4^{ab}	1.05	*	NS
Digestible nutrient intake (g day^{-1})							
Organic matter	224.2^{d}	282.8^{c}	329.1^{b}	369.5^{a}	5.97	**	NS
Crude protein	10.6^{d}	31.3^{c}	58.9^{b}	78.8^{a}	0.34	**	NS
Neutral detergent fiber	123.9^{c}	140.6^{b}	152.2^{ab}	157.4^{a}	4.24	**	NS
DIP:DOM[2]	4.7^{d}	11.1^{c}	18.0^{b}	21.3^{a}	0.15	**	**

[a-d]Within rows, means with different letters are different ($p<0.05$); [1]L = Linear; Q = Quadratic; **$p<0.01$, *$p<0.05$; NS = Not Significant; [2]Ratios of Degradable Intake Protein (DIP) nitrogen to Digestible Organic Matter (DOM)

The carcass weight, slaughter rate, eye muscle area and GR value were all significantly higher in the RSM-supplemented groups than those for the control ($p<0.05$) with the maximum value at 160 g day^{-1} of supplemental RSM.

Blood biochemical parameters: Results of the blood biochemical indices are shown in Table 4. Compared with the control, serum concentrations of total protein and glucose urea nitrogen concentration were increased significantly ($p<0.05$). The supplementary RSM had no effects on triglyceride levels ($p>0.05$).

Ruminal fluid measurement: The pH values were not significantly affected by RSM supplementation with all values within the normal range (Table 5). Rumen ammonia

Table 4: Growth performance and carcass characteristics in Huzhou sheep influenced by Rapeseed Meal (RSM) supplementation

	Supplemental RSM (g day^{-1})					Effect	
Compositions	0	80	160	240	SEM	L	Q
Growth performance							
Initial weight (kg)	19.90	20.70	20.80	20.80	0.560	NS	NS
Final weight (kg)	21.00^{c}	23.30^{b}	25.30^{a}	25.70^{a}	0.620	**	NS
Average daily gain (g day^{-1})	26.40^{c}	58.10^{b}	98.50^{a}	108.00^{a}	4.300	**	*
Supplementation efficiency[2]	-	0.40^{ab}	0.45^{a}	0.34^{b}	0.020	**	**
Carcass characteristics							
Slaughter weight (kg)	21.30^{c}	23.70^{b}	25.70^{a}	25.90^{a}	0.650	**	NS
Carcass weight (kg)	7.80^{c}	9.30^{b}	10.50^{a}	10.30^{ab}	0.370	**	*
Dressing percent (%)	36.20^{b}	39.40^{a}	40.70^{a}	39.60^{a}	0.890	*	*
Bone:Muscle ratio	35.40^{a}	31.30^{ab}	28.30^{b}	28.30^{b}	1.330	**	*
Ribeye area (cm)[2]	6.50^{b}	9.40^{a}	10.50^{a}	10.10^{a}	0.680	**	*
GR[3] (cm)	0.75^{b}	0.88^{ab}	1.00^{a}	1.00^{a}	0.073	*	NS

$^{a-c}$Within rows, means with different letters are different ($p<0.05$); [1]L = Linear; Q = Quadratic; **$p<0.01$, *$p<0.05$; NS = Not Significant; [2]Expressed as increased weight gain relative to the control per unit of added supplement; [3]The depth of muscle and fat tissue from the surface of the carcass to the lateral surface of the twelfth rib 110 mm from the midline

Table 5: Effects of rapeseed meal supplementation on rumen fermentation parameters and population of the ruminal cellulolytic bacteria

	Supplemental RSM (g day^{-1})					Effect[1]	
Compositions	0	80	160	240	SEM	L	Q
Fermentation parameters							
pH	7.20	7.00	7.10	7.10	0.070	NS	NS
Ammonia-N (mg dL^{-1})	5.30^{c}	11.10^{b}	13.00^{ab}	14.20^{a}	0.930	**	*
Microbial protein (mg mL^{-1})	0.69^{c}	1.06^{b}	1.42^{a}	1.47^{a}	0.096	**	NS
Total VFA[2] (mmol L^{-1})	34.40^{b}	39.30^{b}	48.50^{a}	49.80^{a}	2.510	**	NS
Molar proportions (%)							
Acetate	75.10	76.00	76.70	76.50	0.870	NS	NS
Propionate	22.70	21.30	21.00	21.20	0.910	NS	NS
Butyrate	2.10	2.70	2.30	2.30	0.210	NS	NS
CMCase[3] (IU gmin^{-1})	7.10^{c}	11.90^{b}	16.80^{a}	14.90^{ab}	1.030	**	*
Cellulolytic microbes (percentage of total bacterial 16S rDNA)							
B. fibrisolvens	0.45^{c}	0.77^{b}	1.39^{a}	0.32^{d}	0.019	*	**
F. succinogenes	0.75^{c}	1.63^{a}	1.60^{a}	1.12^{b}	0.051	**	**
R.albus (×10^{-2})	4.34^{b}	3.60^{c}	5.97^{a}	3.15^{c}	0.170	NS	**
R.flavefaciens (×10^{-2})	0.49^{b}	1.17^{a}	1.00^{a}	1.15^{a}	0.076	**	*

$^{a-c}$Within rows, means with different letters are different ($p<0.05$); [1]L = Linear; Q = Quadratic; **$p<0.01$, *$p<0.05$; NS = Not Significant; [2]VFA = Volatile Fatty Acids; [3]CMCase = Carboxymethyl Cellulose

N concentration increased ($p<0.05$) with RSM supplementation to straw being highest ($p<0.05$) with the highest level of RSM. Concentration of MCP increased with the increasing levels of RSM but the incremental response was diminished when supplemental RSM reached 240 g day^{-1}. The concentrations of VFAs showed the similar tendency to MCP concentration. Molar proportions of VFA were not affected by the supplementation. CMCase activity increased with the increasing levels of RSM and slight decline was observed when supplemental RSM reached 240 g day^{-1}.

Microbial populations: The results of relative quantification of *F. succinogenes*, *B. fibrisolvens*, *R. albus* and *R. flavefacien* are showed in Table 5. Relative population of *F. succinogenes* and *B. fibrisolvens* was predominant compared with other microbes. Numbers of *F. succinogenes* and *B. fibrisolvens* relative to total bacterial 16S rDNA were increased by a small quantity of RSM supplemented and decline tendency observed when supplemental RSM reached 240 g day^{-1}. Compared to the control, quantity of *R. flavefaciens* relative to total bacterial 16S rDNA was increased with the RSM supplementation ($p<0.05$), however, no further improvement was observed at the higher levels of RSM.

DISCUSSION

Intake and digestibility: The RSM supplementation did not affect the RS intake. This is consistent with the findings by Fattet *et al.* (1984) that there was not a significant difference in straw intake between unsupplemented and protein supplemented diets. However, Scott and Hibberd (1990) found that forages intake increased in response to increasing quantities of protein supplements to ruminants fed low quality forages. In the present study, no positive associative effects were observed for RS intake when supplemented with RSM. A positive effect of RSM supplementation to RS-based diet

was observed on total feed intake. Total nutrients intakes were increased with the increasing of supplemental RSM. These findings are consistent with (Liu *et al*., 1998) who fed sheep with straw-basal diets supplemented with 0, 100, 200, 300 g day^{-1} RSM.

The apparent digestibility of OM, CP and NDF were increased with the supplementation of 160 g per day RSM, however, no further improvement was noted at the higher levels of RSM. This indicates that the digestibility of diets is greatly improved when supplement level is low.

Performance and carcass characteristics: Supplemental RSM significantly increased average daily gain with 120, 273 and 309% higher in 80, 160 and 240 g day^{-1} of RSM treatments than in control, respectively. A similar result has been reported by Creek *et al.* (1984) who used concentrate mixtures as supplements to cattle offered RS. These results suggested that protein supply is critical for growing animal fed low quality forages. Assuming that the efficiency of utilization of the basal diets did not alter, the supplement conversion ratio may be estimated to be 2.6, 2.2 and 3.0 kg kg^{-1} for 80, 160 and 240 g day^{-1} RSM supplemented groups, respectively. These values are similar to those suggested in a review study by McCollum and Horn (1990) for either protein or energy-supplemented grazing livestock. Supplement conversions of <3:1 could be attributed to positive associative effects (McCollum and Horn, 1990). Bodine and Purvis (2003) found that the supplement conversion was 1.5 kg of added supplement into a kilogram of added gain when the DIP/DOM was 15.0 with corn and soybean meal as supplements to beef steers offered tallgrass. Chen *et al.* (2007a) also found that the balance of N and energy may have a profound effect on associative effects of feedstuffs *in vitro*. In the present study, positive associative effects that were observed in performance might be partly due to the improved balance of the N and energy in the diets resulting from RSM supplementation. And the results in carcass characteristics also showed that 160 g day^{-1} RSM was appropriate level for efficacious protein deposition.

Ruminal fluid measurement: Enhanced ruminal ammonia N concentrations observed in thes study with increasing protein supplementation agree with other research (Lee *et al*., 1987; Stokes *et al*., 1988) and reflect the provision of a readily available N source. The MCP in the rumen provides the majority of protein supplied to the small intestine of ruminants, accounting for 50-80% of total absorbable protein (Storm and Orskov, 1983). Generally, the efficiency of microbial protein synthesis is regulated by the balance of N and energy. In the present study, the small quantity of RSM supplementation improved the protein and energy balance. The balance of N and energy may ensure optimal MCP synthesis, improve the utilization of protein and energy and result in positive associative effects. When the large number of RSM was supplemented, the available energy might be limited and certain amount of N was lost as ammonia but not incorporated into MCP causing the decreased efficiency of MCP synthesis.

The concentrations of VFAs in RSM treatments were higher than in control, consistent with the increase in ammonia N and MCP concentration. Koster *et al.* (1996) reported that total ruminal VFA increased in response to supplemental DIP fed to beef cows that were consuming forage. Burroughs *et al.* (1975) suggested that limiting growth of ruminal microbes by restricting dietary protein intake would reduce fermentative activity. Supplementing RS with RSM would increase N supply and stimulate the growth of ruminal microbes in the rumen, hence improve the digestion of fibre and get higher fermentability. In addition, the increase in total diet intake would also contribute to a higher total VFA concentration when greater amounts of protein were supplemented.

CMCase produced by major cellulolytic ruminal bacteria are thought to initiate cellulolysis. Using hay and concentrate diets (Silva *et al*., 1987) found that there was high correlation between CMCase and fibre degradation. The ammonia N concentrations were quite low in the study when no RSM was supplemented and probably limited ruminal microbial growth and fiber digestion. Therefore, it seems likely that the increases in CMCase activity observed with the initial increment of supplemental RSM were due to provision of N as well as other nutrients (e.g., branched-chain VFA) to the fiber-digesting microbes. Because increased concentrate intake and passage rate result in a shorter retention time of OM in the rumen (Staples *et al*., 1984) less time is available for cellulolytic microorganisms to digest fiber. Hence, the slight decline was observed in CMCase activity when higher levels of RSM were supplemented.

Microbial populations: Ruminal cellulolytic microbes such as *F. succinogenes*, *B. fibrisolvens*, *R. albus* and *R. flavefacien* play an important role in fibre digestion. The relative population size of the *F. succinogenes* was much greater than that of *R. flavefaciens* (almost 100 fold). In agreement with the results (Koike and Kobayashi, 2001) reported a higher populations of *F. succinogenes* (10^6-10^7 mL^{-1} of rumen fluid) compared to *R. flavefaciens* (10^5 mL^{-1}) in the rumen of sheep fed different proportions of alfalfa hay. Michalet-Doreau *et al.* (2001) found that the relative population size of *F. succinogenes* was always higher than that of ruminococcus in the rumen of sheep fed

alfalfa hay diet. *R. flavefaciens*, *F. succinogenes* and *B. fibrisolvens* were increased by the improvement of protein-energy balance. Chen *et al.* (2007b) reported that addition of N resource increased the populations of *F. succinogenes*, *B. fibrisolvens in vitro*. Koike *et al.* (2003) found that high N content of hay could stimulate growth of *B. fibrisolvens*. Bacterial growth is largely dependent on the amount of ammonia and fermentable organic matter present in the rumen (Bryant and Robinson, 1962). There is more available N supplied by adequate RSM supplementation to meet requirement of microbial growth. However, the large amount of RSM supplemented, the higher passage rate can be obtained. As a result, less time is available for cellulolytic microorganisms to adhere to fiber. Hence, the decline of cellulolytic microbial relative population is observed. And it might be a reasonable explanation for the slight decline in CMCase activity and NDF digestibility in high level of RSM supplementation. Silva *et al.* (1987) reported that the rate of fibre degradation depends on the extent to which the rumen environment allows an adherent cellulolytic microbial population to develop. The present study provides additional support to this observation.

CONCLUSION

The results indicated that an appropriate supplementation of rapeseed has a positive associate effect on utilization of the rice-straw-based diet in growing lambs, indicating the improved digestibility and growth performance, enhanced microbial protein synthesis and promoted activity of fiber-degrading enzyme.

IMPLICATIONS

Positive associative effects in growth performance were observed for rice straw-based diets when supplemented with 160 g day^{-1} RSM. The more quantities of RSM supplementation can be less effective when adequate DIP is included. The balance between degradable N and dietary available energy has great effects on associative effects. The increased digestibility, MCP synthesis, cellulolytic microbial populations and cellulolytic bacteria activity may be responsible for the positive associative effects.

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