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Associative effects in diets composed of alfalfa and corn-soybean concentrate fed to growing cashmere goats

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Abstract

The associative effects resulting from the proportions of neutral detergent fibre (NDF) and non-fibre carbohydrate (NFC) were explored and assessed by in vitro gas production. Total mixed rations (TMR) composed primarily of alfalfa and corn-soybean concentrate were fed to growing cashmere goats. Treatments were defined by three proportions of NFC and NDF, namely 2.00 (TMR1), 2.35 (TMR2), and 3.00 (TMR3) that were used to grow cashmere goats, and these TRMs were incubated for 48 hours to evaluate their influence on associative effects. The results indicated that the associative influences of these treatments on gas production occurred within the cultures predominantly at 2 - 8 hours, and disappeared gradually as culture time was extended. TMR2 and TMR3 incubation increased gas production compared with that observed in the other groups at all incubation times ($P > 0.05$), and these groups exhibited positive associative effects, particularly during the early hours of incubation ($P < 0.05$). TMR3 displayed the best associative effect.

Keywords: gas production, neutral detergent fibre, non-fibre carbohydrate
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Introduction

Economic, environmental, and societal considerations have increasingly encouraged farmers to feed ruminant animals a TMR that is composed of forage and concentrate. This renewed focus on the use of TMR feed is widespread, particularly in large and medium-sized farms throughout the world. However, on small farms, because of the need for simplicity and various management factors, the feeding method of choice is to grow the individual dietary components. Additionally, the existing cashmere goat feed evaluation system assumes that the nutritional value of the feed ingredients is additive. However, this system fails to consider potential interactions among feed ingredients. Nutritional values can be different when feedstuffs are fed as TMR or are fed separately (Sandoval-Castro *et al*., 2002; Robinson *et al*., 2009). These interactions are known as associative effects. They can modify rumen fermentation in a way that causes the properties of a TMR to differ from those of the individual components (Metzler-Zebeli *et al*., 2012).

Associative effects between forages and concentrate supplements are well documented throughout the literature (Huhtanen, 1991; Doyle *et al*., 2005). The fermentation of high quantities of easily fermentable carbohydrates provided by concentrate feeds results in a decrease in the ruminal pH, and this affects cellulolytic activity and the digestibility of plant cell walls negatively (Mould *et al.*, 1983). However, associative effects can be positive. For example, protein supplementation of straw may improve its digestibility and voluntary intake, thus alleviating nitrogen deficiency and stimulating the growth of rumen microbes (Mawuenyegah *et al.*, 1997). In recent years, there have been many advances in research (i.e., genetics, biotechnology, physiology) on goats (Asroush *et al.*, 2018; Pophiwa *et al.*, 2019; Zubair *et al.*, 2020). However, there is a dearth of information about associative effects of TMR ingredients that are fed to cashmere goats.

The classic methods for evaluating feed mixtures involve the use of in vivo techniques (Zhou *et al.*, 2020). However, these methods are expensive, laborious and time consuming, and require large quantities of feed (Pashaei *et al.*, 2010). Currently, in vitro gas methods are more commonly used (Makkar *et al*., 2005). These methods allow evaluators to maintain experimental conditions more precisely than do the classic in vivo methods. This new type of laboratory approach is based on a gas fermentation system, rumen fluid, and batch culture, combined with mathematical curve fitting. It can aid in differentiating between fast and slow fermentation of carbohydrate pools (B1, B2, and B3) in a single feed or TMR sample (Mahanna, 2010). The fibre carbohydrate and fermentable carbohydrate contents in the diet are the indexes used to describe the dietary structure, whereas the non-fibre carbohydrate (NFC) and the neutral detergent fibre (NDF) contents are used for accurate evaluation. Reasonable proportioning of NFC and NDF in a given TMR is vital for health, rumen fermentation and rumination activity, particularly for cashmere goats fed on large amounts of forage. Thus, the proportion between these components may affect the gas production in vitro, thus affecting the nutrition content of the measured feed. It is speculated that when the ratio of NFC and NDF in a given feed is not the same, the fermentation and the potential association may both be different. Here, the associative effects of three varying proportions of NFC and NDF in feed mixtures and their separate components were assessed in vitro using a gas production method.

Material and Methods

The Animal Care and Use Committee of Inner Mongolia Academy of Agriculture and Animal Sciences (Hohhot, Inner Mongolia, China) approved the use of animals.

The TMR1, TMR2 and TMR3 mixtures were characterised as differing in the ratio of NFC to NDF, and were typical of diets used for growing cashmere goats. TMR1 had an NFC to NDF ratio of 2.00, TMR2 had NFC : NDF of 2.35, and TMR3 had NFC : NDF of 3.00. The chemical compositions of the three TMR are shown in Table 1. Standard methods (Association of Official Analytical Chemists, 2000) were used to determine DM (method 930.15), crude protein (method 976.05), calcium, and phosphorous (method 935.13). Acid detergent fibre (ADF) and NDF contents were determined following the procedure of Van Soest *et al*. (1991) using an Ankom 220 fibre analyser (Ankom Co., USA), and the values were expressed exclusive of residual ash. The buffer solution was produced in accordance with Menke and Steingass (1988). The solution was heated using a water bath set to 39 °C and then purified in a continuous manner using CO_2 for 30 min. Rumen fluid was collected from three ruminally cannulated cashmere goats prior to morning feeding. A total of 500 mg of each TMR and samples of separate ingredients were incubated in 310-mL culture flasks containing 40 mL of buffer solution and 20 mL of rumen inoculum.

Table 1 Composition and nutrient levels of the total mixed rations varying in the ratio of non-fibrous carbohydrate to neutral detergent fibre (DM basis)

Ingredient	TMR1	TMR2	TMR3
Alfalfa	20.15	16.13	15.12
Corn	50.35	55.63	64.90
Soybean meal	13.90	13.91	14.50
Chaff	7.79	7.79	4.00
Rice bran	7.53	6.26	1.20
Soda powder	0.06	0.06	0.06
Premix	0.11	0.11	0.11
Iodized salt	0.11	0.11	0.11
Nutrient level			
Metabolizable energy, MJ/kg DM	10.29	10.51	10.57
Dry matter (DM), %	94.02	93.89	93.60
Crude protein, %DM	15.48	15.45	15.47
Calcium, %DM	0.48	0.42	0.40
Phosphorous, %DM	0.41	0.42	0.39
Neutral detergent fibre, g	229.53	206.86	177.18
Non-fibrous carbohydrates, g	459.10	486.14	530.99
NFC/NDF	2.00	2.35	3.00
Effective rumen degradable protein (ERDP), g	71.78	71.18	70.03
Rumen non-degradable protein (UDP), g	44.21	44.32	45.01
ERDP/UDP	1.62	1.61	1.56

NFC: non-fibre carbohydrate; NDF: neutral detergent fibre

To measure in vitro gas production (GP), the laboratory used the wireless ANKOM Gas Production System (Ankom Technology). During incubation, changes in pressure in the top space of the bottle (measured as a difference from the atmospheric pressure measured at the same time) were transmitted to a PC by radio frequency every 30 minutes. When the pressure inside the units reached 3.4 kPa, gas that accumulated in the headspace of the bottles was released automatically.

The feed samples were incubated for 48 hours, and gas production kinetics were analysed. With PROC NLIN of SAS version 9.0 (SAS Institute Inc., Cary, North Carolina, USA), the cumulative gas production was calculated using the monophasic model described by Groot *et al.* (1996). Specifically

$$G = \frac{A}{1 + \left(\frac{C}{t}\right)^{B}}$$

where: G = the volume of gas produced per gram of TMR or TMR component incubated for time t,
C = time at which one-half of the asymptote had been reached ($t_{1/2}$),
B = a constant indicating the sharpness of the curve, and
A = the asymptotic gas production.

The maximum gas production rate (Rmax, in mL h^{-1}) and the time at which this maximum occurs (Tmax, in hours) were calculated using the formulas:

$$T_{max} = C\left(\frac{B-1}{B+1}\right)^{1/B}, \text{ and}$$

$$R_{max} = \left(\frac{(A\ x\ C^{B})\left(B\ x\ T_{max}^{-(B-1)}\right)}{(1 + C^{B})\ x\ T_{max}^{-B}}\right)^{2}$$

To achieve an assessment of the association of substrates to gas generation, the percentage difference between the calculated value of the separated components and the measured value of the TMR were calculated as:

$$Associative\ effect\ (\%) = 100\frac{observed\ value - calculated\ value}{calculated\ value}$$

The model that was used to assess cumulative gas production was:

$$V_t = \frac{V_{f1}}{1 + \exp(2 - 4k_1(t - L)} + \frac{V_{f2}}{1 + \exp(2 - 4k_2(t - L)}$$

where: V_t = cumulative gas production (ml) in time t,
V_{f1}= final volume of gases derived from the degradation of the rapid digestion soluble fraction,
V_{f2}= final volume of gases derived from the degradation of the slow digestion insoluble fraction,
k_1= specific rate of gas production because of soluble fraction reduction (%/h),
k_2= specific rate of gas production because of insoluble fraction reduction (%/h),
t = incubation time in hours, and
L = colonization time in hours.

Analysis of variance and Duncan's multiple range tests were used to evaluate differences in the parameter estimates for these models using SAS software (SAS Institute Inc., Cary, North Carolina, USA).

Results and Discussion

A logistic dual-pool model was used to divide the cumulative gas production of three TMR diets during the incubation period. No differences in the gas production profiles were observed during the initial hours. However, differences were observed in fast pool gas production (Figure 1). The final fast pool values of the TMR1, TMR2, and TMR3 were 23.03, 43.03, and 25.32 mL, respectively (Table 2). TMR2 exhibited the highest relative fast pool contribution.

The amounts and relative proportions of the amount (ml) of gas produced by each pool (fast and slow) could help to characterize the fermentation (Johnston & Tricarico, 2007). Gas is generated from the buffering of acids produced by rumen bacteria and from fibre fermentation (e.g., methane). Field experience involving hundreds of herds undergoing production challenges indicated that the vast majority of problem herds were fed a TMR that possessed an excessively large amount of starch and soluble fibre and a relatively small amount of insoluble available fibre or NDF (Mahanna, 2010). But these pools were not homogeneous, as there could be slow and fast pools within each carbohydrate fraction. For example, the slow pool might contain some slowly digested starch. This analysis did approximate the nature of ruminal fermentation and provided a practical means to evaluate rations, predict the productive response, and make sound nutrition decisions that affect animal productivity and, ultimately, economic profitability (Johnston & Tricarico, 2007). In the current results, TMR2 had a fast pool that yielded a gas volume that exceeded 40 ml, thus suggesting that higher gas production may arise from the rapidly digested soluble fibre that produces large amounts of methane and carbon dioxide gas rather than from an overabundance of available starch.

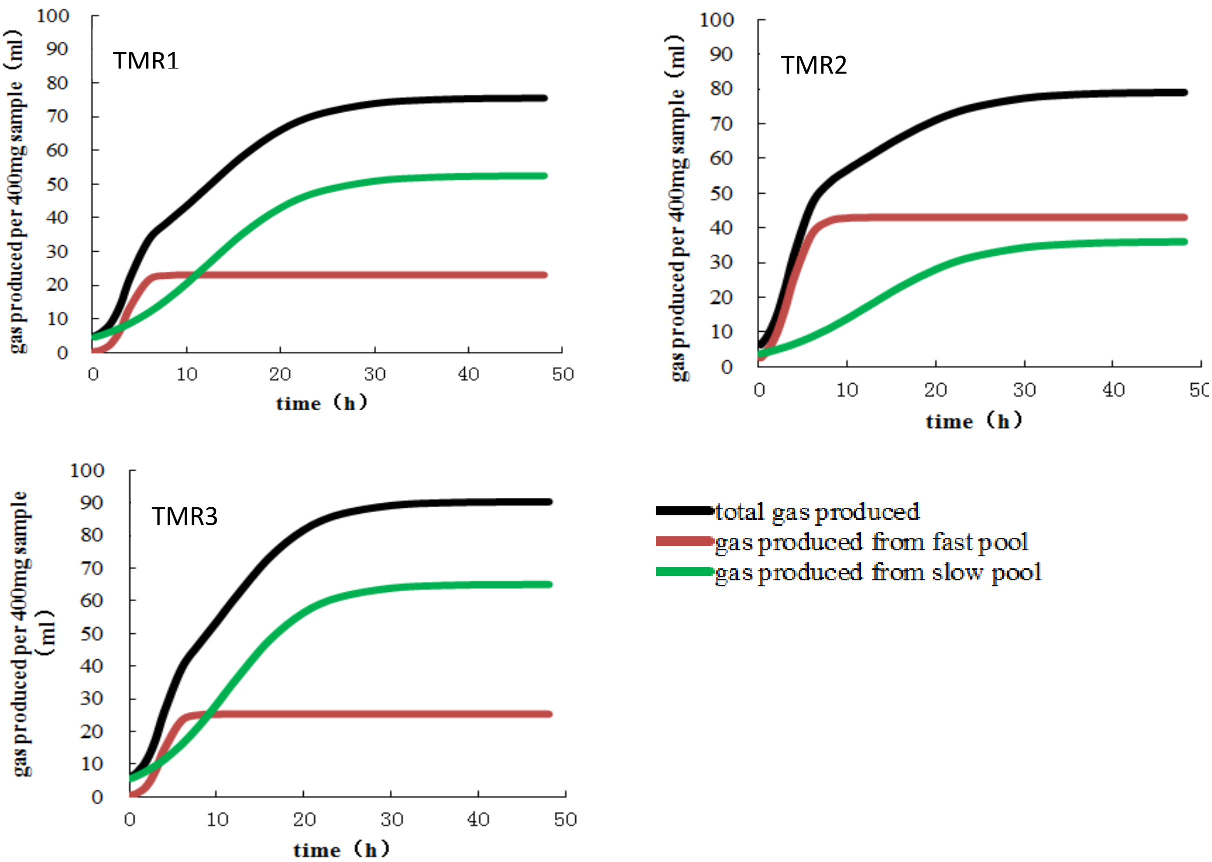

Figure 1 Plots detailing the results from a logistic dual-pool model that was used to partition cumulative gas production from three total mixed rations (TMR) during in vitro incubation

Table 2 Quantities and rates of gas produced in the fast and slow pools from in vitro digestion of three total mixed rations

	TMR1			TMR2			TMR3		
	ml gas	% total	Max rate	ml gas	% total	Max rate	ml gas	% total	Max rate
Fast pool	23.0	30.5	3.25	43.0	54.4	2.62	25.3	28.0	3.19
Slow pool	52.5	69.5	6.39	36.0	45.6	5.26	65.0	72.0	5.98
Total	75.5			79.0			90.4		

TMR1: ration with NFC : NDF = 2.00, TMR2: ration with NFC : NDF = 2.35, TMR3: ration with NFC : NDF = 3.00

The total gas production values recorded after 48 hours of incubation were similar among the treatments (Table 3). Fermentation of TMR3 resulted in a slightly larger amount of gas production throughout the incubation process compared with that produced during the incubation of TMR1 and TMR2. The associative influence on gas production occurred primarily in the culture at 2–8 hours, and this influence weakened as culture time was extended. Incubation of TMR2 and TMR3 increased gas production compared to that observed with TMR1 at all early incubation times, and these treatments exhibited positive associative effects ($P < 0.05$), particularly during the early hours of incubation.

Table 3 Gas production (mL) and associative effects (%) from in vitro digestion of three total mixed rations

Group	Incubation time (h)	TMR	Alfalfa	Concentrate	Calculated value	Associative effect, %
TMR1	2	27.33	22.89	30.95	29.33	-6.80
	4	54.51	34.15	59.60	54.47	0.07
	8	96.46	49.59	113.95	100.98	-4.48
	12	124.92	58.13	146.10	128.38	-2.69
	24	178.14	72.98	211.11	183.28	-2.81
	30	185.58	77.12	215.93	187.96	-1.26
	48	191.18	89.05	221.99	195.20	-2.06
TMR2	2	43.40	22.89	39.68	36.97	17.39
	4	79.60	34.15	74.23	67.76	17.47
	8	132.31	49.59	136.18	122.22	8.26
	12	153.14	58.13	164.41	147.27	3.98
	24	184.45	72.98	210.41	188.24	-2.02
	30	191.86	77.12	222.63	199.16	-3.66
	48	199.74	89.05	230.21	207.44	-3.72
TMR3	2	33.36	22.89	31.06	29.83	11.85
	4	64.31	34.15	59.60	55.75	15.35
	8	116.21	49.59	117.41	107.16	8.44
	12	153.56	58.13	163.32	147.42	4.17
	24	219.17	72.98	232.67	208.52	5.11
	30	222.14	77.12	241.32	216.49	2.61
	48	228.25	89.05	246.80	222.95	2.38
SE		26.09	9.03	30.60	26.93	2.35
P-value		0.78	1.00	0.95	0.91	0.02

TMR1: ration with ratio of NFC to NDF = 2.00, TMR2: ration with ratio of NFC to NDF = 2.35, TMR3: ration with ratio of NFC to NDF = 3.00

Although this concept was often discussed, the amount of research literature that examined feed association effects was not extensive. During digestion, different feed components can influence each other, and associative effects typically occur (Niderkorn *et al*., 2009). Owing to differences in microbial fermentation rates caused by the divergence between individual and combination feeds, the risk of the occurrence of rumen disease in cashmere goats may be misjudged. Rapid ruminal fermentation of the diet is known to be a risk factor for sub-acute rumen acidosis in ruminants, particularly in dairy cattle, owing to their high intake levels of concentrate to meet their energy demands (Allen, 1997).

Numerous studies have reported that mixtures of feed leaves, concentrate diets, straw, and tree leaves exert positive effects in gas production (Liu *et al.*, 2002; Sandoval-Castro *et al.*, 2002). Jiang and Diao (2006) reported that different proportions of concentrate and roughage may produce different associative effects. When the non-structural carbohydrates (NSC) in the diet were too high, the fermentation of sugar and starch produced a large amount of volatile fatty acids under the action of rumen bacteria, and this led to a sharp decrease in pH within the rumen. In response, the activity of cellulolytic bacteria was inhibited, and the degradation of roughage by rumen microorganisms was also inhibited, thus reducing the degradation and flow rate of roughage, which led to a change in rumen capacity and a decrease in forage intake. This resulted in a negative associative effect. The current study revealed that associative effects were more significant early in culture and decreased over time, and this was consistent with the results of Liu *et al*. (2002), who used in vitro systems to support microbial growth with sufficient nitrogen. The current results demonstrated a reduction in associative influences over incubation time, where TMR3 exhibited the best associative effect that reached approximately 8 - 11% during the early hours of incubation. Similar to previous studies (Kiran & Krishnamoorthy, 2007; Robinson *et al.*, 2009), the associative effects were more pronounced during the early hours of incubation (i.e., 2 and 4 hours) and declined as the incubation time progressed. Given that the association effect can serve as an index of immediate fermentability of the rations, a positive association effect should be evaluated carefully, as the high fermentation capacity of TMR may increase the content of short-chain fatty acids significantly. These results suggest that the TMR3 group possessed a significant potential for more efficient rumen fermentation and better feed efficiency. Additionally, the differences in gas production patterns between TMR and the individual TMR components in the current experiment were probably caused by different microbial profiles, which could be related to the substrate preferences of the protozoal, bacterial, and archaeal species in the rumen and to differences in the energy-to-protein ratio (Berthiaume *et al.*, 2010; Williams *et al.*, 2010).

Table 4 presents data detailing the associative influences exerted by the gas production kinetic parameters of the three TMRs. The proportions of NFC and NDF slightly affected asymptotic gas production, B, $t_{1/2}$, T_{max}, and R_{max}. The rate of gas production for TMR (NFC/NDF= 2.35) was faster than that observed using the other treatments. Positive and negative associative effects were observed for all TMR. The associative effects on Rmax were less (P <0.05) pronounced with TMR1 compared with those observed with TMR2 and TMR3, whereas the associative effects were positive in regard to asymptotic gas production with TMR3 compared with the slightly negative associative influences observed with TMR1 and TMR2 (P <0.05).

Table 4 Associative effects on estimates of gas production kinetic parameters for in vitro digestion of three total mixed rations

Group	A(mL/g DM)		B		$C_{(t1/2)}$		Tmax(h)		Rmax(mL/h)	
		Associative effect, %		Associative effect, %		Associative effect, %		Associative effect, %		Associative effect, %
TMR1	219.60	-2.09[b]	1.31	1.63	9.43	-6.66	2.04	-10.88[a]	17.11	-10.97[c]
TMR2	213.40	-7.47[c]	1.30	4.16	5.79	-31.86	1.21	-25.24[b]	24.66	10.76[a]
TMR3	255.00	1.05[a]	1.42	1.57	8.60	-14.44	2.51	-12.90[a]	22.88	2.57[b]
SE	3.05	0.24	0.11	0.12	0.22	1.09	0.16	0.91	1.04	0.43
P-value	0.35	0.03	0.64	0.42	0.56	0.61	0.26	0.49	0.58	0.00

A: asymptotic gas production, B: constant indicating the sharpness of the curve, C: time that one-half of the asymptote was reached ($t_{1/2}$), T_{max}: time that maximum gas production rate occurs, R_{max}: maximum rate of gas production, TMR1: ration with NFC:NDF = 2.00, TMR2: ration with NFC:NDF = 2.35, TMR3: ration with NFC:NDF = 3.00
[a,b,c] Values within a column with a common superscript did not differ at P <0.05

During the early stage of fermentation, the maximum fermentation capacity of TMR was supported by the observed negative effect on T_{max}. These results indicated that the maximum gas yield actually occurred prior to the calculated time point, and the positive effect on R_{max} indicated that the maximum gas yield was typically higher than the calculated value. Taken together, these results indicated that the assessment of rations based on the calculation of values for TMR components may underestimate the fermentation that occurs at the beginning of culture. This is likely to affect the accurate prediction of fermentation products in the rumen and during normal rumen physiology (Zebeli *et al*., 2008). Although these in vitro kinetic data provide valuable indicators that suggest an association with the rumen digestion physiology of cashmere goats, validation of these findings and an assessment of the in vitro data for comparison to the in vivo data must be performed prior to making detailed conclusions. To achieve this, more detailed research must be performed, and additional in vivo studies are required.

Conclusion

The observed associative effects reflect the complexity and multiplicity of nutritional situations that affect intake and the ruminal ecosystem activity in cashmere goats. A more detailed understanding of the associative effects between TMR and individual TMR components could help to improve current feed systems in efficiency of feed use and environmental impact, and this would contribute to the development of more sustainable animal production.

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Authors' Contributions

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Conflict of Interest Declaration

The authors have no conflicts of interest to declare.

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