https://doi.org/10.17221/94/2019-CJAS

Multiple-factor associative effects of peanut shell combined with alfalfa and concentrate determined by *in vitro* gas production method

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Citation: Yuan J., Wan X.J. (2019): Multiple-factor associative effects of peanut shell combined with alfalfa and concentrate determined by *in vitro* gas production method. Czech J. Anim. Sci., 64, 352–360.

Abstract: The associative effects (AE) between concentrate (C), peanut shell (P) and alfalfa (A) were investigated by means of an automated gas production (GP) system. The C, P and A were incubated alone or as 40 : 60 : 0, 40 : 45 : 15, 40 : 30 : 30, 40 : 15 : 45, 40 : 0 : 60 and 30 : 70 : 0, 30 : 55 : 15, 30 : 40 : 30, 30 : 25 : 45, 30 : 10 : 60, 30 : 0 : 70 mixtures where the C : roughage (R) ratios were 40 : 60 and 30 : 70. Samples (0.2000 ± 0.0010 g) of single feeds or mixtures were incubated for 96 h in individual bottles (100 ml) with 30 ml of buffered rumen fluid. GP parameters were analysed using a single exponential equation. After incubation, the residues were used to determine pH, dry matter digestibility (DMD), organic matter digestibility (OMD), volatile fatty acids (VFA) and ammonia nitrogen (NH_3-N) of the incubation fluid, and their single factor AE indices (SFAEI) and multiple-factors AE indices (MFAEI) were determined. The results showed that group of 30 peanut shell had higher SFAEI of $GP_{48\ h}$, DMD, OMD and total volatile fatty acids ($P < 0.05$) and MFAEI ($P < 0.05$) than groups 60, 45 and 0 when C : R was 40 : 60. The group of 10 peanut shell showed higher SFAEI of $GP_{48\ h}$, DMD and OMD ($P < 0.05$) than groups 70, 55 and 40 and MFAEI ($P < 0.01$) when C : R was 30 : 70. It is concluded that optimal SFAEI and MFAEI were obtained when the C : P : A ratios were 40 : 30 : 30 and 30 : 10 : 60.

Keywords: combination effect; peanut hull; clover hay; concentrate supplement; *in vitro* gas production technology

Peanut (*Arachis hypogaea* Linn.*)* is one of the most common economic crops and annual output of peanut is over 5 000 000 t in China. China has the largest production, consumption and export trade of peanut worldwide. Peanut shell is a by-product of peanut and it is abundant and usually discarded or burned directly without further use. Peanut shell can lower blood pressure, reduce inflammation and diuresis. Many holes on the surface of peanut shell are capable of adsorbing intestinal toxins, and thus improving the intestinal health of human and animals. However, peanut shell may be low in digestibility and thus unable to meet the nutritional requirements of animals when fed alone. These limitations can be overcome by the positive associative effects (AE) when a low-quality roughage is combined with other feed. The available energy or digestibility of a mixed feed or diet is not equal to the weighted sum of the values of the available energy or digestibility of the feed included in the diet, meaning that the associative effect is an outcome of the interaction (Dixon and Stockdale 1999; Doyle et al. 2005). These interactions can alter metabolic processes in the ruminant gastrointestinal tract, especially in the rumen. It has been demonstrated that utilization of low-quality roughage can be improved with alfalfa (*Medicago sativa*) supplementation (Mosi and Butterworth

Supported by the Discipline construction fund project of Gansu Agricultural University, China (Project No. GSAU-XKJS-2018-051).

https://doi.org/10.17221/94/2019-CJAS

1985). For forage-based diets, however, in many cases dietary inclusion of concentrated feed is necessary to achieve the desired level of performance, with the amount and composition of the supplement depending on characteristics of the basal forage and the target animal (Goetsch and Gipson 2014). Therefore, it is necessary to take the concentrated feed into consideration.

Tagliapietra et al. (2015) found out that *in vitro* fermentation ability of low-quality forage crops (crown daisy and milk thistle) could be improved by combining these two plant sources with apple pomace or citrus pulp. A prediction method for negative AE in feed using a dietary concentrate level and a hay source was determined for Boer goat wethers; the method demonstrated that metabolisable energy (ME) intake was accurately predicted for use of low levels of concentrate and with moderate levels in growing wethers (Asrat et al. 2017). Haddad and Nasr (2007) stated that a minimum of 20% replacement of dietary dry matter (DM) from barley with corn in high concentrate diets was needed to positively improve both performance and feeding efficiency of growing lambs.

In spite of increasing research on the topic of AE, little is known regarding the AE of peanut shell combined with alfalfa and concentrate on gas production (GP) and rumen fermentation parameters. Given peanut shell is abundant worldwide and easily available, the hypothesis of the present study is that AE can be promoted by the optimum ratio of concentrate, peanut shell and alfalfa. The objective of this study was to evaluate the AE of mixtures of peanut shell, alfalfa and concentrate on $GP_{48\,h}$, dry matter digestibility (DMD), organic matter digestibility (OMD), total volatile fatty acids (TVFA), ammonia nitrogen (NH_3-N) and multiple-factors AE indices (MFAEI) using the *in vitro* GP method. The results of this study are expected to guide the scientific utilization of peanut shell as roughage for ruminants.

MATERIAL AND METHODS

Feedstuffs and chemical analysis. A single incubation (96 h) was performed in which concentrate (C), peanut shell (P), and alfalfa hay (A) were previously milled at 1 mm and then incubated alone or in the following proportions: C : P : A ratios of 40 : 60 : 0, 40 : 45 : 15, 40 : 30 : 30, 40 : 15 : 45, 40 : 0 : 60 and 30 : 70 : 0, 30 : 55 : 15, 30 : 40 : 30, 30 : 25 : 45, 30 : 10 : 60, 30 : 0 : 70 for the concentrate : roughage ratios (C : R) of 40 : 60 and 30 : 70, respectively. The experimental protocol of this study was approved by the Institutional Committee for Animal Use and Ethics of Gansu Agricultural University. The formulation of the commercial concentrate was corn 85.17%, soybean meal 7.22%, cotton seed meal 3.46%, salt 1.65% and premix 2.50%.

In vitro fermentation process. Prior to the grinding, C, P and A were dried. The three substrates and the eleven mixtures were incubated in individual glass bottles with 20 replicates plus two blanks each, for a total of 282 bottles. Each bottle (100 ml) was filled with 0.2000 ± 0.0010 g of feed sample or mixture, 20 ml of buffer and 10 ml of rumen fluid (headspace = 70 ml) and placed into a constant temperature water bath pot at 39 ± 0.5°C (Menke and Steingass 1988). Buffer solution was prepared according to Menke and Steingass (1988). Rumen fluid was collected by an esophageal probe 2 h before morning feeding from four dry Holstein-Friesian cows (approximately 5 years old, body weight 550 ± 7.8 kg, fed hay *ad libitum* plus concentrates (composition and nutrition levels are presented in Table 1) 4 kg/day/head twice a day), stored in two thermal flasks preheated to 39 ± 0.5°C, and transferred to the laboratory. The rumen fluid was filtered through four layers of cheesecloth to eliminate feed particles, and the filtered fluid was mixed with buffer solution (1 : 2 ratio). All of these operations were conducted under anaerobic conditions by continuous flushing with CO_2 and were completed in 30 min or less. Gas collection tubes (häberle LABORTECHNIK GmbH & Co. KG, Germany) were used to measure GP at various incubation times (2, 4, 6, 9, 12, 24, 36, 48, 72, 96 h).

Calculation of GP. The collected cumulative gas production (GP) profiles (Orskov and McDonald 1979) were fitted to a simple exponential equation to evaluate the kinetics of GP:

$$GP\ (ml) = a + b(1 - e^{-ct})$$

where:

a = rapid GP (ml)
b = slow GP (ml)
(a + b) = potential GP (ml)
c = rate constant of slow GP (%/h)
t = time (2, 4, 6, 9, 12, 24, 36, 48, 72, 96 h) since commencement of incubation (h)

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Table 1. Ingredients and nutrient levels of the concentrate of the experimental diet for three Holstein-Friesian cows (%, DM basis)

Ingredients (%)	
Corn	60.50
Soybean	23.00
Wheat bran	11.50
$CaHPO_4$	2.00
NaCl	1.00
Premix[1]	2.00
Total	100.00
Nutrient levels (%)	
DM	83.20
CP	16.70
NE_L (MJ/kg)[2]	7.15
NDF	12.68
ADF	5.23
Ca	0.51
P	0.45

DM = dry matter, CP = crude protein, NE_L = net energy of lactation, NDF = neutral detergent fibre, ADF = acid detergent fibre

[1]1 kg of premix contained: vitamin A 650 000 IU, vitamin D_3 300 000 IU, vitamin E 400 000 IU, Fe 500 mg, Cu 500 mg, Mn 1000 mg, Zn 500 mg, Co 15 mg, Se 40 mg

[2]estimated value: NE_L (Mcal/kg milk) = 0.3512 + 0.0962 × milk fat rate (Yang 2005)

The predicted values of cumulative GP at 48 h were calculated by summation of three single feed results considering the proportions of peanut shell, alfalfa and concentrate. The cumulative GP was corrected for blanks.

Determination of nutritional index. For the eleven mixtures, the "expected GP" was calculated as the weighted average of the GP supplied by substrates when they were incubated alone (this supposes that there is no AE between substrates). The three substrates were analysed in triplicate for DM, crude protein (CP), ether extract (EE) and crude ash (ASH) according to AOAC (2003). The CP content was determined by Kjeldahl method No. 2001.14 using a Kjeldahl nitrogen analyzer (FOSS, Denmark). The content of EE was determined by Soxhlet extraction (method No. 993.20). The ASH content was determined by muffle furnace method and the organic matter was calculated as OM = DM – ASH. The NDF and ADF were determined according to the method by VanSoest et al. (1991) using an ANKOM A2000i automated fiber analyser (ANKOM Technology, USA). The water-soluble carbohydrate (WSC) content was determined by anthrone reagent (TCVN 5285-1990) and non-fibrous carbohydrate (NFC) was calculated based on the formula NFC = OM – (CP + NDF + EE).

Determination of IVDMD and IVOMD. After finishing a 96-hour incubation, the residues were filtered through a filter paper and the *in vitro* dry matter digestibility (IVDMD) was assessed by measuring the difference between the initial and final weight of samples (correcting for blanks). The *in vitro* organic matter digestibility (IVOMD) was estimated by incineration of the dry residues (550°C) and also assessed by measuring the difference between the initial and final weight of samples (correcting for blanks) (Sandoval-Castro et al. 2002).

Determination of pH, ammonia-N and volatile fatty acid. After a 96-hour fermentation, the samples of ruminal fluid were collected for determination of pH, VFA and NH_3-N concentration. The pH was measured with a digital pH meter fitted with a glass electrode, and then 4 ml were placed into a tube with 1 ml of a 25% (w/v) metaphosphoric acid solution and frozen at –20°C for later VFA analysis. Likewise, 3 ml were placed into a tube with 2 ml of 3 M HCl and frozen at –20°C for NH_3-N analysis. Analyses of the VFA and NH_3-N were done by the procedures of Lu et al. (1990) and Broderick and Kang (1980), respectively.

Calculation of associative effects. Single-factor associative effects index (SFAEI) and multiple-factor associative effects index (MFAEI) were determined according to the method of Wang (2011):

SFAEI = (measured value after combination – value of weighted estimate)/value of weighted estimate, where the value of the weighted estimate = measured value of the type of feed × proportion of the feed in the combination + measured value of another type of feed × proportion of the other type of feed in the combination.

MFAEI = the sum of each single-factor associative effect value. In this experiment, MFAEI is the sum of the five SFAEI (AE of GP, DMD, OMD, NH_3-N and TVFA).

Statistical analysis. Data were statistically processed by one-way analysis of variance (ANOVA) using SAS software (Version 7.0, 1996). The significance of differences between means for the treatments was tested using Tukey-Kramer multiple

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comparisons. The differences between treatment means were considered to be significant at $P < 0.05$. The results are presented as the mean and standard error of the mean (SEM).

RESULTS

Chemical composition and GP parameters of single feedstuffs. Table 2 shows that peanut shell had the highest NDF content and the lowest CP content. The a value of peanut shell was higher than that of concentrate and alfalfa. The values b, $GP_{48\,h}$ and c of peanut shell were lower than those of concentrate, but higher than those of alfalfa. The (a + b) of peanut shell and concentrate was higher than that of alfalfa.

GP and fermentation parameters and VFA of feedstuffs mixtures. When the C : R ratio was 40 : 60, the b value of the 30 peanut shell group (group 30, the same as below) was higher than in groups 60 ($P < 0.01$), 45 ($P < 0.01$) and 0 ($P < 0.05$) (Table 3). The $GP_{48\,h}$ value of group 30 was higher than in group 60 ($P < 0.05$). Rumen pH value of

Table 2. Nutrient levels and *in vitro* gas production (GP) parameters of experimental diets

Items	Nutrient levels (%)								*In vitro* gas production parameters (ml)				
	DM	OM	CP	EE	NDF	ADF	NFC	WSC	a	b	c (%/h)	a + b	$GP_{48\,h}$
P	96.3	93.7	6.4	2.3	66.6	58.5	19.4	11.2	2.7	44.3	0.060	47.0	36.5
C	91.5	94.3	19.1	3.9	26.2	nd	nd	nd	–10.5	56.1	0.121	45.5	42.5
A	95.3	90.4	18.3	6.0	31.6	25.4	40.7	6.4	–2.9	29.6	0.056	26.7	15.8

C = concentrate, P = peanut shell, A = alfalfa, nd = not determined, DM = dry matter, OM = organic matter, CP = crude protein, EE = ether extract, NDF = neutral detergent fibre, ADF = acid detergent fibre, NFC = non-fibrous carbohydrate (calculated as OM – (CP + NDF + EE)), WSC = water-soluble carbohydrate, a = rapid GP, b = slow GP, c = rate constant of slow GP, a + b = GP potential, $GP_{48\,h}$ = GP at 48 h

Table 3. Gas production (GP) and fermentation parameters at 48 h when peanut shell was incubated with alfalfa and concentrate *in vitro*

C : P : A	GP parameters (ml)					Fermentation parameters (%)			
	a	b	c (%/h)	a + b	$GP_{48\,h}$	pH	DMD	OMD	NH_3–N (mg/dl)
40 : 60 : 0	–5.6	39.4^{Bc}	0.14	33.9	33.3^{b}	6.72^{b}	38.9^{b}	40.2^{b}	10.6^{b}
40 : 45 : 15	–3.9	45.6^{B}	0.11	41.7	39.5^{ab}	6.68^{b}	44.2^{b}	46.7^{b}	10.8^{b}
40 : 30 : 30	–11.5	63.5^{Aa}	0.16	52.0	51.3^{a}	6.61^{c}	66.4^{a}	69.5^{a}	16.9^{a}
40 : 15 : 45	–7.0	54.8^{ABab}	0.12	47.8	42.8^{ab}	6.71^{b}	65.1^{a}	66.6^{a}	17.2^{a}
40 : 0 : 60	–3.1	49.1^{ABbc}	0.11	46.1	42.3^{ab}	6.84^{a}	66.0^{a}	68.9^{a}	16.0^{a}
P-value	0.357	0.001	0.571	0.137	0.040	0.038	0.003	0.002	0.036
SEM	1.335	1.416	0.009	1.247	1.896	0.064	1.058	1.131	0.14
30 : 70 : 0	-6.36^{b}	37.8	0.06^{b}	40.5	31.2	6.89^{a}	28.0^{B}	30.1^{B}	12.8
30 : 55 : 15	-4.14^{ab}	38.1	0.10^{ab}	34.0	32.5	6.84^{a}	31.4^{B}	32.9^{B}	14.4
30 : 40 : 30	0.41^{ab}	44.6	0.07^{ab}	45.0	35.2	6.90^{a}	49.4^{AB}	51.2^{AB}	15.0
30 : 25 : 45	2.71^{a}	44.3	0.12^{ab}	38.0	35.3	6.66^{b}	53.6^{AB}	55.0^{AB}	16.1
30 : 10 : 60	-3.97^{ab}	51.4	0.13^{a}	47.4	41.3	6.71^{b}	64.7^{A}	67.3^{A}	16.8
30 : 0 : 70	-4.03^{ab}	51.2	0.11^{ab}	47.2	43.3	6.69^{b}	47.1^{AB}	47.9^{AB}	16.3
P-value	0.011	0.163	0.017	0.257	0.083	0.041	0.001	0.002	0.068
SEM	0.978	1.929	0.009	1.825	1.501	0.075	1.078	1.103	0.044

C = concentrate, P = peanut shell, A = alfalfa, a = rapid GP, b = slow GP, c = rate constant of slow GP, a + b = GP potential, $GP_{48\,h}$ = GP at 48 h, DMD = dry matter digestibility, OMD = organic matter digestibility, SEM = standard error of the means
means within a column differ at $^{a–c}(P < 0.05)$, $^{A–C}(P < 0.01)$

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Table 4. Volatile fatty acids at 48 h when peanut shell was incubated with alfalfa and concentrate *in vitro* (mmol/l)

C : P : A	Acetic acid	Propionic acid	Isobutyric acid	Butyric acid	Isovaleric acid	Valerianic acid	A/P	TVFA
40 : 60 : 0	71.83^{ab}	21.78^{ab}	1.21	6.52	2.51	1.33	3.30	105.18^{ab}
40 : 45 : 15	71.76^{ab}	21.90^{ab}	1.21	6.49	3.20	1.54	3.28	106.10^{ab}
40 : 30 : 30	84.72^{a}	23.77^{a}	1.35	7.11	2.93	1.55	3.56	121.43^{a}
40 : 15 : 45	65.44^{b}	20.26^{b}	1.06	5.74	2.18	1.21	3.23	95.89^{b}
40 : 0 : 60	66.40^{b}	21.86^{ab}	1.13	5.94	2.40	1.26	3.04	98.99^{b}
P-value	0.010	0.037	0.066	0.067	0.401	0.298	0.241	0.041
SEM	0.723	0.301	0.036	0.177	0.177	0.064	0.013	0.384
30 : 70 : 0	68.60^{b}	22.40	1.19	6.12	2.55	1.41	3.06	102.27^{b}
30 : 55 : 15	68.03^{b}	23.00	1.22	6.08	2.62	1.26	2.96	102.21^{b}
30 : 40 : 30	67.67^{b}	22.50	1.18	6.17	2.48	1.37	3.01	101.37^{b}
30 : 25 : 45	67.98^{b}	22.10	1.15	6.15	2.37	1.28	3.08	101.03^{b}
30 : 10 : 60	78.27^{a}	22.00	1.17	6.23	2.45	1.29	3.56	111.41^{a}
30 : 0 : 70	78.40^{a}	23.00	1.12	6.04	2.27	1.27	3.41	112.10^{a}
P-value	0.038	0.308	0.835	0.995	0.656	0.241	0.085	0.041
SEM	0.497	0.412	0.020	0.090	0.058	0.021	0.105	0.545

C = concentrate, P = peanut shell, A = alfalfa, A/P = acetic acid to propionic acid ratio, TVFA = total volatile fatty acids, SEM = standard error of the means

^{a,b}means within a column with different superscripts differ ($P < 0.05$)

group 30 was lower than that of other groups ($P < 0.05$). DMD, OMD and NH_3-N of groups 30, 15 and 0 were all higher than in groups 60 and 45 ($P < 0.05$). Acetic acid and TVFA of group 30 were higher than in groups 15 and 0 ($P < 0.05$), propionic acid of group 30 was higher than in group 15 ($P < 0.05$) (Table 4).

When the C : R ratio was 30 : 70, the a value of group 25 was higher than in group 70 ($P < 0.01$) (Table 3). The c value of group 10 was higher than in group 70 ($P < 0.05$). Rumen pH in groups 0, 10 and 25 was lower than in the other groups ($P < 0.05$). DMD and OMD of group 10 were higher than in groups 70 and 55 ($P < 0.01$). Acetic acid and TVFA of group 10 were higher than in the other groups ($P < 0.05$) (Table 4).

AE of feedstuffs mixtures. When the C : R ratio was 40 : 60, the AE of $GP_{48\,h}$ of group 30 was higher than in groups 60 and 45 ($P < 0.05$) and that of groups 15 and 0 was higher than in group 60 ($P < 0.05$) (Table 5). The AE of DMD of group 30 was higher than in groups 60, 45 and 0 ($P < 0.01$). The AE of OMD and TVFA of group 30 was higher than in other groups ($P < 0.05$). The MFAEI of group 30 were higher than in other groups ($P < 0.05$) and those of groups 0 and 15 were higher than in groups 60 and 45 ($P < 0.05$).

When the C : R ratio was 30 : 70, the AE for $GP_{48\,h}$ of group 10 was higher than in groups 70, 55, 40 and 25 ($P < 0.01$) and that of group 0 was higher than in groups 70 ($P < 0.01$), 55 ($P < 0.05$) and 40 ($P < 0.05$) (Table 5). The AE of DMD and OMD of group 10 was higher than in groups 70, 55, 40 and 25 ($P < 0.05$) and AE of DMD of groups 40, 25 and 0 was higher than in groups 70 and 55 ($P < 0.05$). The MFAEI of group 10 were higher than in other groups ($P < 0.01$) and those of group 0 were higher than in groups 25, 40, 55 and 70 ($P < 0.01$), and those of groups 25 and 40 were higher than in groups 55 and 70 ($P < 0.01$).

DISCUSSION

GP parameters of single feedstuffs. In this study, peanut shell (a = 2.7) had no lag time (LT) of GP and alfalfa (a = –2.9) had shorter LT of GP than concentrate (a = –10.5) (Table 2). Corn had higher LT than barley for the *in vitro* GP method (Aye Sandar et al. 2012). In the present study, concentrate was composed of 85.17% corn. Therefore, the result that concentrate had longer LT was consistent with results of previous studies. Peanut shell had no LT due to a higher WSC content than

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Table 5. SFAEI and MFAEI after fermentation for 48 h when peanut shell was incubated with alfalfa and concentrate *in vitro* (%)

C : P : A	SFAEI					MFAEI
	AE of $GP_{48\ h}$	AE of DMD	AE of OMD	AE of TVFA	AE of NH_3-N	
40 : 60 : 0	-14.5^{c}	-1.82^{B}	0.89^{b}	-0.15^{b}	−19.69	-35.27^{c}
40 : 45 : 15	2.7^{bc}	1.14^{B}	1.23^{b}	-14.54^{b}	−21.93	-31.40^{c}
40 : 30 : 30	56.9^{a}	6.96^{A}	5.04^{a}	17.90^{a}	−11.26	75.54^{a}
40 : 15 : 45	44.6^{ab}	4.11^{AB}	1.03^{b}	-8.07^{b}	−8.59	33.08^{b}
40 : 0 : 60	45.0^{ab}	0.97^{B}	1.91^{b}	-2.51^{b}	−17.67	27.70^{b}
P-value	0.006	0.002	0.008	0.039	0.913	0.007
SEM	2.163	1.045	1.351	1.5572	2.3722	2.442
30 : 70 : 0	-18.6^{CD}	-3.22^{c}	-2.54^{b}	−4.02	−25.94	-54.32^{D}
30 : 55 : 15	-7.6^{BCb}	-2.07^{c}	-2.69^{b}	−2.09	−17.77	-32.22^{D}
30 : 40 : 30	9.6^{BCb}	1.96^{b}	-0.43^{b}	0.81	−17.97	-6.03^{C}
30 : 25 : 45	22.0^{BC}	5.34^{ab}	1.79^{b}	−1.67	−24.86	2.60^{C}
30 : 10 : 60	82.3^{A}	8.71^{a}	6.78^{a}	3.48	−8.80	92.47^{A}
30 : 0 : 70	59.6^{ABa}	2.11^{b}	3.05^{ab}	0.43	−31.83	33.36^{B}
P-value	< 0.001	0.009	0.010	0.342	0.363	0.002
SEM	1.833	2.013	1.892	0.9739	2.2213	2.156

C = concentrate, P = peanut shell, A = alfalfa, AE = associative effects, $GP_{48\ h}$ = GP at 48 h, DMD = dry matter digestibility, OMD = organic matter digestibility, TVFA = total volatile fatty acids, SFAEI = single-factor associative effects index, MFAEI = multiple-factor associative effects index, SEM = standard error of the means

means within a column differ at $^{a–c}(P < 0.05)$, $^{A–D}(P < 0.01)$

alfalfa. The WSC fraction was almost instantly and completely degraded in the rumen (Boudon et al. 2002). The values of a, b, c, (a + b) and $GP_{48\ h}$ for peanut shell were higher than those for alfalfa (Table 2). This indicated that GP performance of peanut shell was higher than that of alfalfa.

GP of feedstuffs mixtures. GP is one of the most important indicators for predicting digestibility of feedstuff in the rumen of ruminants. Mixture and simplex designs are useful tools to identify and study AE of feed mixtures using an *in vitro* GP technique. For example, a positive AE on GP was observed when forage tree leaves were incubated with concentrate (Sandoval-Castro et al. 2002). There was a positive AE on GP parameters when wheat straw was mixed with alfalfa (Tang et al. 2008). The benefits associated with the use of legume forage result from the integration of many factors, demonstrating that positive AE can help develop the utilization of straws (Haddad 2000). Two sources of slowly fermentable fibre (milk thistle and pure cellulose) and three rapidly fermentable fibre sources (tomato peels without seeds, citrus pulp, and pectin) were incubated as 75 : 25 or 25 : 75 mixtures *in vitro*, and each enhanced the GP provided by milk thistle and pure cellulose (Maccarana et al. 2013). AEs between forage and concentrate are well documented in the literature. In this study, GP and fermentation parameters were higher when the C : P : A ratios were 40 : 30 : 30 and 30 : 10 : 60 (Table 3). These above-mentioned reports are consistent with the present study.

Rumen pH, DMD and OMD of feedstuffs mixtures. Rumen pH is an important indicator of rumen fermentation and of changes in rumen environment. The normal range of pH is between 6 and 7; values too high or too low affect normal rumen fermentation. It has been reported that VFA lowers ruminal pH (Stritzler et al. 1998). The pH is lowered when VFA is produced faster than it is absorbed from the rumen. The decrease in pH tends to be linearly related to the intake levels of readily fermentable carbohydrates (Abdelhadi et al. 2005). In this study, groups 30 and 10 had higher TVFA and lower pH, in agreement with the reports cited above.

With the increase in GP, the fermentation activities of microorganisms in the rumen become

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more intense, and the digestibility of feed increases (Menke and Steingass 1988). The DMD and OMD indicate the availability of OM in ruminants and are also important indices of the nutritional value of feed. The DMD and OMD were higher when C : P : A ratios were 40 : 30 : 30, 40 : 15 : 45, 40 : 0 : 60, 30 : 10 : 60 (Table 3). The main reason is that peanut shell also has better GP performance and effective degradation rate, despite alfalfa itself has a high effective degradation rate, and the C/N ratio of alfalfa is more conducive to growth and reproduction of microbes (Gunun et al. 2013). Another reason may be that the contents of non-structural carbohydrates and degradable nutrients increased in these dietary combinations (40 : 30 : 30, 40 : 15 : 45, 40 : 0 : 60 and 30 : 10 : 60). Associative effects values for supplementation of alfalfa on digestibility, intake and utilization of poor-quality feeds are often reported (Haddad 2000). Table 3 showed that DMD and OMD at C : P : A ratios 30 : 0 : 70 (47.1, 47.9%) were lower than at 30 : 10 : 60 ratios (64.7, 67.3%) and the main reason was that AE occurred between low proportion (10, 25, 40) of peanut shell and high proportion (60, 45, 30) of alfalfa and the optimal AE group was 30 : 10 : 60. The DMD and OMD values were lower in groups with the concentrate proportion of 30 than in groups with the concentrate proportion of 40, and the reason was that WSC of concentrate is higher and it is easier to degrade than that of roughage, therefore the optimal AE group was 40 : 30 : 30. Wang et al. (2008) found that the optimal range to achieve beneficial AE was alfalfa hay of 150–300 g per day for cornstalk-based diets for sheep. Increasing amounts of crushed wheat fed with Persian clover herbage reduced ruminal pH and dietary fibre digestibility in lactating dairy cows (Leddin et al. 2010). Associative effects between red clover and kikuyu grass silage involved proteolysis reduction and synergy during IVOMD (Guzatti et al. 2017). The results of the present study are consistent with the reports mentioned above.

Positive AEs on the rates of GP, DMD and organic matter effective degradability were observed when spring pasture was incubated with corn and when autumn pasture was incubated with either corn or barley (Aye Sandar et al. 2012). Palmgren et al. (2000) studied AE on total tract digestibility in horses fed different ratios of grass hay and whole oats and found that the digestibility of DM and OM as well as the amount of energy increased in a curvilinear pattern with increasing inclusion levels of oats. An investigation of AE of date palm leaves mixed with *Aristida pungens* and *Astragalus gombiformis* on the aptitudes of ruminal microbiota in small ruminants revealed that the IVOMD decreased linearly with increasing amounts of date palm leaves (Djamila and Rabah 2016). Zhang et al. (2010) studied AE of a rice straw-based diet supplemented with cornstarch and found that high levels of cornstarch decreased cellulase activity, populations of cellulolytic bacteria, and the digestibility of forage in Huzhou lambs; proper amounts of starch supplementation had little adverse effect on forage utilization but could effectively improve growth performance. All of these reports demonstrate that utilization of low-quality roughage is improved with alfalfa supplementation (Mosi and Butterworth 1985). The results with peanut shell, alfalfa and concentrate in this study are basically consistent with the above studies.

NH_3-N and VFA of feedstuffs mixtures. NH_3-N is an important index of nitrogen metabolism in the rumen, microbial protein synthesis, and protein degradation in feed. Maintaining appropriate NH_3-N is a prerequisite to ensuring microbial protein synthesis in the rumen. Calsamiglia et al (2002) reported that suitable NH_3-N range was 6.3–27.5 mg/dl. In this study, NH_3-N concentrations were all in the normal range, and values in groups 30, 15 and 0 were higher than in other groups (Table 3). The treatments employed in groups 30, 15 and 0 may promote the simultaneous release of energy and ammonia and the synthesis of microbial proteins in the rumen (Zhou et al. 2015). The end products of rumen fermentation are VFA, NH_3-N, and gases such as methane and carbon dioxide. VFA is the main source of energy for the maintenance and growth of rumen microorganisms. In this experiment, acetic acid, propionic acid and TVFA of groups 30, 10 and 0 were higher than in other groups (Table 4). This is in line with the change in $GP_{48\,h}$. $GP_{48\,h}$ of groups 30, 10 and 0 were also higher than in other groups (Table 3). Fermentation of digestible carbohydrates in feed produces VFA, and there is a positive correlation between TVFA and $GP_{48\,h}$. $GP_{48\,h}$ increased with the increase of acetic acid production. A/P reflects the type of rumen fermentation, and A/P values in the present experiment were all greater than 3 (Table 4). Therefore, the rumen fermentation belonged to the acetic acid fermentation type under different proportions of peanut shell, alfalfa and concentrate; this type of fermentation is beneficial to increasing the milk fat rate of ruminants. Taken

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together, the results indicate that groups 30 and 10 were the most optimal for rumen fermentation to produce VFA.

Fieser and Vanzant (2004) studied the interactions between supplemental energy sources and tall fescue hay maturity in forage utilization by beef steers and found that compared with soybean hulls, corn supplementation resulted in greater negative AE on OMD and lower NH_3-N, and that the supplementation did not affect ruminal pH, TVFA or A/P. In the present study, acetic acid, propionic acid and TVFA of groups 30 and 10 were increased (Table 4), and the pH in these groups was lower (Table 3), in agreement with the results of the above research. In this study, the concentration of acetic acid in different feed combinations was higher than the concentration of propionic acid (Table 4), and the reason may be that the rate of absorption of VFA by ruminants is in the order of butyric acid, propionic acid and acetic acid, and the concentration of VFA in the rumen was not affected by the differences between diet types.

AE of feedstuffs mixtures. The indicators of AE include nutrient digestibility, utilization rate, animal growth performance and feed intake. Among these, energy or digestibility is the most commonly used index to measure AE. Gas production *in vitro* is highly correlated with OMD. However it may be inaccurate to measure the nutritional value of feed and evaluate the AE by means of GP alone or several indices because of the complexity of the AE mechanism. *In vitro* GP of feed is highly correlated with carbohydrate digestion. The higher the feed protein, the lower the GP. If the nutritive value of feed is measured by GP alone, it may be inaccurate, and the feed or combination with low GP and high protein production may be eliminated.

The evaluation of feed nutritive value should adopt a comprehensive index or a mathematical model for accurate assessment. In this experiment, the AE of peanut shell, alfalfa and concentrate was evaluated by combining the $GP_{48\ h}$, DMD, OMD, VFA and NH_3-N as a comprehensive index using multiple factors. It was found that MFAEI of groups 30 and 10 were optimal (Table 5). This may be due to the mutual supplementing of nutrients in groups 30 and 10. Increasing the fermentation rate of substrate promotes digestibility of feed. Groups 30 and 10 had improved GP characteristics, rumen fermentation level and feed utilization after the 48-hour fermentation *in vitro* (Table 5).

CONCLUSION

Peanut shell can be used as feed for ruminants. Optimal SFAEI of $GP_{48\ h}$, DMD, OMD, NH_3-N and TVFA and MFAEI were obtained when the concentrate : peanut shell : alfalfa ratios were 40 : 30 : 30 and 30 : 10 : 60.

Ackowledgement. We thank LetPub (www.letpub.com) for language assistance services.

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Received: 2019–04–19
Accepted: 2019–07–17