PROTEIN FRACTIONS AND *In Vitro* FERMENTATION OF PROTEIN FEEDS FOR RUMINANTS

[FRACCIONES DE PROTEÍNA Y FERMENTACIÓN *In Vitro* DE INGREDIENTES PROTEÍNICOS PARA RUMIANTES]

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SUMMARY

The objective of this study was to evaluate 20 protein feeds grouped in forages, vegetal by- products and animal by-products used for ruminant diets. Protein fractions (PF): A, non-protein nitrogen (NPN); B_1, buffer-soluble protein; B_2, buffer-insoluble, NDF-soluble protein; B_3, NDF-insoluble, ADF-soluble protein; and C, ADF-insoluble protein, were determined for each ingredient. Protein composition was correlated with total gas production in vitro (GP), gas production rate (S), lag time (L), DM disappearance (DMDIV) and residual protein (RPIV). The completely randomised designed was analysed using mixed proc. and Tukey contrasts. Forages contained 18.29, 7.86, 66.00, 2.96, 4.89% of fractions A, B_1, B_2, B_3 and C, respectively. Vegetable by-products contained 22.55, 4.55, 59.51, 8.84, 4.55% of each fraction, in the same order. Animal by-products contained 19.13, 4.52, 70.24, 3.74, 2.37% of each fraction, in the same order. Vetch, wheat bran and poultry litter had the greatest Vmax in each group. Vmax was correlated ($P \leq 0.01$) with total protein ($r = -0.45$), ADF ($r = 0.27$) and DMDIV ($r = 0.61$). In conclusion, there were differences in protein composition and kinetics of in vitro gas production among ingredients.

Key words: protein ingredients; protein fractions; in vitro gas production; ruminants.

RESUMEN

El objetivo de este estudio fue evaluar 20 ingredientes proteínicos agrupados en forrajes, subproductos vegetales e ingredientes de origen animal para rumiantes. Se determinaron las fracciones de proteína (PF): A (nitrógeno no proteínico (NPN)), B_1 (proteína soluble en amortiguador), B_2 (proteína insoluble en amortiguador pero soluble en detergente neutro), B_3 (proteína insoluble en detergente neutro pero soluble en detergente ácido) y C (proteína insoluble en detergente ácido) en cada ingrediente; esos valores se correlacionaron con variables de producción de gas in vitro (GP) (volumen máximo de gas (Vmax;mL g^{-1}), tasa de producción de gas (S;h^{-1}) y tiempo de retardo (L;h)), desaparición de MS in vitro (DMDIV) y proteína total residual in vitro (RPIV). El diseño fue completamente al azar con un modelo mixto y comparación de medias con la prueba de Tukey ($P \leq 0.05$). Los resultados para forrajes, subproductos de origen vegetal y animal, y fracciones de proteína fueron; A, B1, B2, B3 y C 18.29, 7.86, 66.00, 2.96, 4.89 %; 22.55, 4.55, 59.51, 8.84, 4.55%, 19.13, 4.52, 70.24, 3.74, 2.37%. Para Vmax , S y DMDIV: la veza, salvado de trigo y pollinaza presentaron el valor mayor en cada grupo. Hubo correlaciones significativas ($P \leq 0.01$) entre Vmax; y proteína total ($r = -0.45$), con FDA ($r = 0.27$) y con DMDIV ($r = 0.61$). En conclusión, los ingredientes proteínicos analizados presentaron diferentes proporciones de FP; además, hubo diferencias en las variables cinéticas de producción de gas In vitro entre ingredientes.

Palabras clave: ingredientes proteínicos; fracciones de proteína; producción de gas in vitro; rumiantes.

INTRODUCTION

The biological value of proteins is essential for feeding ruminants. Rumen degradable protein (RDP) provides nitrogen to the microorganisms for microbial protein synthesis (VanSoest, 1994), whereas in rumen undegraded protein and endogenous secretions provide nitrogen compounds and amino acids to the animal (Broderick *et al*., 1991, NRC 2001). The Cornell Net Carbohydrate and Protein System (CNCPS)) described by Sniffen *et al*. (1992), indicates the dynamics of protein degradation and it is divided into five fractions: A, B1, B2, B3, and C. Fraction A corresponds to non-protein nitrogen (NNP x 6.25), fractions B_1, B_2 and B_3 are soluble in different solvents, and fraction C is considered unavailable. Gas production *in vitro* technique describes the fermentation kinetics of the substrate incubated with rumen fluid; their regulation occurs with a buffer control and minerals supplemented, optimising the microbial activity with anaerobiosis and temperature maintained at 39 ºC (Beuvink and Spoelstra, 1992; Getachew *et al*., 2004; Makkar *et al*., 2005); this process is causing gas production, which is an indicator of fermentation kinetics (Theodorou *et al*., 1994; Mould *et al*., 2005).

Non-ruminant animal by-products can be used as ruminant protein supplements in Mexico (SAGARPA. Guideline NOM-O60-ZOO-1999). Ruminants can also be fed poultry litter with certain restrictions (SAGARPA. Guideline NOM-O61-ZOO-1999). The objective of the present study was to identify the proteins fractions, *in vitro* gas production kinetics, dry matter and protein disappearance of different protein supplements typically utilised in the central region of Mexico.

MATERIALS AND METHODS

Protein samples was collected, including a) forages (alfalfa (*Medicago sativa*), betch *(Vicia sativa)* and orchard grass (*Dactylis glomerata*)), b) vegetable by-products and seeds (corn gluten meal, cottonseed, canola meal, safflower paste, coconut meal, soybean meal, malt sprouts, corn bran, wheat bran, cottonseeds), and c) animal by-products (meat and bone meal, fishmeal, feather meal, Mexican poultry meal, imported poultry meal, blood-meal and poultry litter). Forages samples were obtained from the Colegio de Postgraduados Research Farm. Mexican vegetables and animal by-products were supplied by Malta Clayton and National Renderers Association (NRA). Imported samples were supplied by NRA. Dry samples were ground through a 1-mm screen and they were stored until analysis.

Chemical composition and partitioning protein

The dry matter, ash and crude protein contents were analyzed according to the procedure of AOAC (2000). Both, acid detergent fiber (ADF) and neutral detergent fiber (NDF) were analyzed according to the procedure of Van Soest *et al*. (1991; without sodium sulphite). The NDF and ADF components were further processed for their acid detergent insoluble N (ADIN) and neutral detergent insoluble N (NDIN) (Licitra *et al*., 1996). ADIP and NDIP were obtained in protein values (ADIP = ADIN x 6.25; NDIP = NDIN x 6.25, respectively). The non-protein nitrogen (NPN) was obtained by precipitation of true protein in the filtrate with tungstic acid (10% sodium tungstate solution) and determined as the difference between total N and the N content of the residue after filtration. Total soluble protein was obtained by incubating the sample with borate-phosphate buffer and filtering through Whatman (541) filter paper (Licitra *et al*., 1996). Protein fractions as percentage of total protein were determined as described Sniffen *et al.* (1992): A, non-protein nitrogen; B_1, buffer-soluble protein; B_2, buffer-insoluble, neutral detergent-soluble protein; B_3, neutral detergent-insoluble, acid detergent-soluble protein, and C, acid detergent-insoluble protein. PNDR was determined from protein fractions and NDF according to NRC (2001). Samples were analysed in duplicate and the difference between determinations was always less than 1%.

Gas production kinetic, dry matter disappearance and *in vitro* residual protein

Ruminal fluid of two 480 kg body weight steers was obtained through the ruminal cannula 4 h after feeding them a diet composed by 70% oats and 30% commercial concentrate (12% CP and 4.2 Mcal ME). Ruminal fluid was strained through four layers of cheese cloth and mixed with buffer 1:9 (v/v) at 39 ºC and under oxygen-free CO_2 (Menke y Steingass, 1998; Krishnamoorthy *et al.*, 2005). *In vitro* incubation was conducted as by Theodorou *et al*. (1994) with the following modifications: 0.5 g DM of each ingredient ground through a 1 mm screen were placed into 120 mL amber serum bottles with 90 mL ruminal fluid:buffer mixture and sealed, and immediately placed into the water bath at 39 °C. Two bottles without substrates were used as blanks to correct for inoculums fermentation.

At determined times of incubation a needle connected to a pressure gauge with a scale 0-1 kg cm-2 was inserted through the stopper, and gas pressure was recorded from the first hour to 48 hours of incubation time, at intervals of every two hours. The units of pressure (kg cm-2) were transformed to volume (V = (P + 0.0273)/0.0186)) and the cumulative gas production was adjusted to the logistical model

proposed by Pitt et al. (1999): $Y = v/(1+exp(2-4 \times S \times (t - L)))$, where: Y= total volume of gas produced, mL g^{-1}DM; v=volume; s=rate of gas production, mL h^{-1}; t=time and L=lag time. DM disappearance (DIVMS) and total residual protein (RPIV) were determined through mass difference between time 0 and 48 h.

Experimental design

Chemical composition, protein fractions and PNDR data were analyzed in a complete randomized with three replicates per ingredient in each group classified. Fermentation *in vitro* was performed twice and each assay container three replicates per substrate test and their respective blanks. Data were analyzed as a completely randomized block design using the incubation as blocking criteria (repeated twice).
Statistical analyzes were performed using the Mixed Model procedure of SAS (1999). Means were compared with Tukey contrasts (Steel and Torrie, 1992) with significance declared at $P \leq 0.05$. Pearson's correlation coefficients between chemical composition and kinetics of gas production were obtained using CORR (SAS, 1999). Correlations were considered significant at $P \leq 0.05$.

RESULTS

Table 1 shows the calculations of the chemical constituents of the three groups of ingredients classified as forages and by-product of vegetable and animal. NDF, SolP, NPN, NDPI and ADIP were similar in all groups ($p>0.05$). Numerically, coconut meal had the highest NDIP (62.19) than other ingredients; this value was reflected in high concentration of B3 and C fractions (Table 2). Protein fractions and rumen-undegradable protein (PNDR) were similar between groups. B2 fractions of all groups were the highest concentration than other fractions, and insoluble fraction (C) had the lowest concentration (Table 2).

V max, S, L, DMDIV and RPIV are shown in Table 3. Average Vmax of wheat bran, corn bran and coconut meal were 29% greater ($P \leq 0.01$) than the average of the rest vegetable by-products. Numerically, poultry litter had the greatest V max compared with all animal by-products, but it was similar ($P>0.01$) with Mexican poultry meal. There were no differences ($p>0.01$) in Vmax between wheat bran, corn bran and coconut meal, and these three supplements were on average 29% greater ($P \leq 0.01$) than the average of the rest plant by-products. Numerically, poultry litter had the greatest Vmax, but this was similar ($P>0.01$) to Mexican poultry meal, and 72% greater than the average of the rest of the animal by-products. Gas production rate was similar ($P>0.01$) for feather meal and blood meal, and their average was 22% lower than the average of the rest animal by-products. Lag time was similar ($P>0.01$) for blood meal, poultry litter and imported poultry meal, and their average was 3-fold greater ($P \leq 0.05$) than the average of the rest of the animal by-products. Betch, wheat bran and poultry litter had different ($P \leq 0.01$) DMDIV within their groups. Soybean meal and blood meal had different ($P \leq 0.01$) RPIV than the other ingredients in their respective groups.

Gas production *in vitro* showed significant difference ($P \leq 0.01$) in each incubation, while the Vmax was lower in forages high in protein. There were correlations between Vmax and DMDIV ($r = 0.61$) and Vmax and efficiency per gram of DM disappeared ($r = 0.57$). In this study there was a low correlation between Vmax and ADF ($r=0.27$). But, Vmax and total protein ($r = -0.45$) had better correlation (Table 4).

DISCUSSION

The content of protein fractions and the amount in the PNDR were in the range of values reported by other authors, with minimal differences (NRC, 2001; Sniffen *et al.*, 1992; Vanzant *et al.*, 1996, Elizalde *et al*., 1999; Shannak *et al*., 2000). In the literature reviewed there were no information about chicken meal, corn bran and malt sprouts. Other authors (Coblentz *et al.*, 1998; Faria-Marmol *et al.*, 2002) have reported more NDIN of pastures (without affecting ADIN) as compared drying feeds. However, in this study the data variation between the types of ingredients is high, but this did not occur between groups. The differences should be attributed to the technique used in the nitrogen fraction (Licitra *et al*., 1996) and modifications in chemical structure, caused by nitrogen compounds of different molecular weight (Shannak *et al*., 2000; Schwab *et al*., 2003).

The values of forages evaluated, are attributed to the characteristics of the species and maturity, changing the fibre content as mentioned by Van Soest, (1994). Additionally, cell wall glycoproteins, tannins and products formed by the Maillard reaction, causing a protein ligation, limit the degradation of nitrogen compounds (Krishnamoorthy *et al.*, 1982; Licitra *et al.*, 1996 , Elizalde *et al*., 1999). In sequence, the amount of soluble protein can be modified.

The differences in values between vegetable and animal by-products were due to the characteristics of each ingredient and chemical processes carried out in the by-products, modifying the content of nitrogen compounds (Calsamiglia and Stern 1995). Thermal processing in animal-meals denatures proteins, specifically fraction B2 becomes insoluble, and increase the fraction B3 and C. This process causes the Maillard reaction, producing compounds with lower solubility (Licitra *et al*., 1996; Calsamiglia and Stern

1995). Fractions B3 and C represent a small amount, they do not possess nitrogen compounds associated with fiber (Sniffen *et al.*, 1992, Krishnamoorthy *et al.*, 1982) and is preferable to maintain low amounts by the unavailability of this fraction (Licitra *et al.*, 1996).

As already mentioned, gas production *in vitro* showed significant difference ($P \leq 0.01$) in each incubation time, while the Vmax was lower in forages high in protein. Forages by-products contain more NDF structure, compared with animal by-products. Nsahlai *et al*. (1995) found a relationship between gas production with the disappearance of NDF. However, in this study there was a low correlation between Vmax and ADF (r=0.27). But, Vmax and total protein (r = -0.45) had better correlation; this result was similar with that reported by Getachew *et al*. (2004) and theoretically by Wolin, (1960). Protein fermentation produces less gas compared with carbohydrates (Cone and Van Gelder 1999), but in this study there was no significant correlation ($P > 0.01$) between the two variables, which may be due to protein diet is used mainly for protein synthesis and is catabolized as an energy source only if the organisms increase their energy requirements and nitrogen compounds (Bach *et al*., 2005).

Table 1. Chemical composition of three groups of ingredients classified as forages, vegetables and animal by-products

Ingredients	NDF (% DM)	ADF (% DM)	N * 6.25 (% DM)	SOLP (% TP)	NPN* 6.25 (% SOL P)	NDIP (% CP)	ADIP (% CP)
Forages							
Alfalfa *(Medicago sativa)*	46.42	36.22	17.28	36.20	83.21	6.08	2.03
Orchardgrass*(Dactylis glomerata)*	49.94	26.12	14.02	8.77	1.23	8.74	6.24
Betch *(Vicia sativa)*	41.02	32.76	24.05	33.49	73.87	8.73	6.40
Average	45.79[a]	31.7 [a]	18.45[b]	26.15 [a]	52.77[a]	7.85 [a]	4.89 [a]
Vegetable by-products							
corn gluten meal	12.10	5.06	61.39	23.71	87.02	7.70	1.71
Cottonseed meal	22.22	11.00	45.04	16.47	83.48	9.32	4.66
Canola meal	38.44	17.98	38.39	69.92	96.09	22.40	10.93
Safflower paste	37.4	19.00	31.06	37.40	84.93	5.64	3.16
Coconut meal	58.36	30.18	22.21	14.56	78.35	62.19	14.17
Soybean meal	13.02	10.84	49.16	12.97	80.50	5.70	2.85
Malt sprouts	35.92	31.90	25.58	25.82	86.75	3.42	1.37
Corn bran	44.22	32.12	17.12	59.11	90.32	5.72	2.04
Wheat bran	56.06	40.04	14.82	8.30	30.98	8.26	2.36
Cottonseed	51.06	38.80	18.35	22.75	66.45	7.63	2.29
Average	36.88 [a]	23.69 [ab]	32.31 [b]	29.10 [a]	78.49 [a]	13.80 [a]	4.55 [a]
Animal by-products							
Meat and bones meal	34.92	6.02	45.63	14.40	20.07	11.50	3.83
Fishmeal	34.00	5.32	63.61	8.53	80.64	7.70	2.42
Feathermeal	39.60	27.86	80.57	6.80	42.47	3.91	2.61
Mexican poultry meal	34.12	9.68	60.88	15.72	84.44	7.53	1.72
Imported poultry meal	40.72	28.8	54.64	4.17	78.03	4.16	1.99
Blood meal	15.20	2.16	81.43	57.63	93.29	3.87	1.85
Poultry litter	36.80	15.5	25.59	58.32	92.96	4.10	2.19
Average	33.62 [a]	13.62 [b]	58.91 [a]	23.65 [a]	70.27 [a]	6.11 [a]	2.37 [a]
SEM	7.62	6.51	9.4	12.2	15.2	7.54	4.89

[ab] Average value in each group within column with different superscript differ ($P \leq 0.05$).

NDF: neutral detergent fibre; ADF: acid detergent fibre; NPN (% CP): percentage of crude protein of the j^{th} feedstuff that is non-protein nitrogen x 6.25; SOLP (% CP): percentage of the crude protein of the j^{th} feedstuff that is soluble protein; NDIP (%DM)= percentage of the j^{th} feedstuff that is neutral detergent insoluble protein; ADIP (%DM)= percentage of the j^{th} feedstuff that is acid detergent insoluble protein. Average of duplicate determinations.

Table 2. Protein fractions in total protein and rumen-undegradable protein of three groups of ingredients classified as forages, vegetables and animal by-products*

	A	B1	B2	B3	C	PNDR
Forages						
Alfalfa *(Medicago sativa)*	30.12	6.08	57.73	4.05	2.03	29.31
Orchardgrass*(Dactylis glomerata)*	0.00	8.77	82.49	2.50	6.24	38.39
Betch *(Vicia sativa)*	24.76	8.73	57.78	2.33	6.40	32.39
Average	18.29[a]	7.86[a]	66.00 [a]	2.96 [a]	4.89 [a]	33.36 [a]
Vegetable by-products						
Corn gluten meal	0.00	3.71	92.59	1.99	1.71	76.84
Cottonseed meal	13.75	2.72	74.21	4.66	4.66	45.08
Canola meal	67.18	2.73	7.68	11.47	10.93	25.44
Safflower paste	31.77	5.64	56.96	2.48	3.16	26.37
Coconut meal	11.41	3.15	23.26	48.02	14.17	70.53
Soybean meal	10.48	2.49	81.33	2.85	2.85	44.82
Malt sprouts	22.40	3.42	70.76	2.05	1.37	28.20
Corn bran	53.39	5.72	35.16	3.68	2.04	20.11
Wheat bran	0.00	8.30	83.44	5.90	2.36	45.26
Cottonseeds	15.12	7.63	69.62	5.34	2.29	37.47
Average	22.55[a]	4.55[a]	59.51[a]	8.84 [a]	4.55 [a]	42.01 [a]
Animal by-products						
Meat and bone meal	2.89	11.51	74.10	7.67	3.83	56.44
Fishmeal	6.88	1.65	83.77	5.29	2.42	57.76
Feathermeal	2.89	3.91	89.29	1.30	2.61	67.72
Mexican poultry meal	13.28	2.44	76.75	5.80	1.72	57.45
Imported poultry meal	9.09	4.17	82.58	2.18	1.99	63.85
Blood meal	53.76	3.87	38.50	2.02	1.85	34.67
Poultry litter	54.22	4.10	37.57	1.91	2.19	26.79
Average	20.43[a]	4.52[a]	68.93[a]	3.74 [a]	2.37 [a]	52.09 [a]
EEM	12.67	7.86	14.28	5.96	1.91	9.61

[a] Average value in each group within column with similar superscript no differ (P>0.05).

* Protein fraction content calculated as*:*

A (% CP)= NPN (% SOLP)*0.01*SOLP (% CP)

B1 (% CP)= SOLP (% CP) – Fraction A (%CP)

B2 (% CP) =100-Fraction A (%CP)-B1 (%CP)-B3 (%CP) C (%CP)

B3 (% CP) =NDIP (% CP) - ADIP (%CP)

C (% CP) = ADIP (%CP)

A (%CP)= percentage of crude protein in the j^{th} feedstuff that is non-protein nitrogen; B1 (%CP)= percentage of crude protein in the j^{th} feedstuff that is rapidly degraded protein; B2 (%CP)= percentage of crude protein in the j^{th} feedstuff that is intermediately degraded protein; B3 (%CP)= percentage of crude protein in the j^{th} feedstuff that is slowly degraded protein, and C (%CP)= percentage of crude protein in the j^{th} feedstuff that is bound protein. Rumen-undegradable protein (PNDR) calculated according to Sniffen *et al.* (1992) and NRC (2001) PNDR = fraction B [kp/(kd + kp)] + fraction C. Degradation rate according to Sniffen *et al.* (1992). Passage rate calculated according to NRC (2001) for 4% bodymass DM intake, 50% forage, and own data on NDF.

The differences in gas production rates are attributed to changes in the structure and bonding of the fiber components of forages and by-products of plant origin (Van Soest, 1994). Fibrolytic microorganisms are predominant with high forage (France *et al*. 2005), in the present study, there was better response in the lag phase, in the case of ingredients of animal by-products were more dependent on the proportions of soluble particles insoluble, degradable and non degradable (Getachew *et al*., 1998). The little variation in lag phase should be attributed to the microbial population of rumen fluid from the donor who consumed diets with 70:30 forage concentrate ration and, therefore, the microbial population were probably predominant as fibrolytic bacteria that have affinity to protein substrates to release ammonia (Weimer, 1996). In addition, the values of the lag phase is related to the IVDMD and determined by the difference in protein composition in each ingredient. However, although some have the same origin differences were found because their components may resist degradation, which made the difference in gas production and amount of substrate degraded (Groot *et al*., 1996).

Table 3. Gas production kinetic, dry matter disappearance and residual protein *in vitro*.

	Vmax (mL g^{-1}DM)		S (ln mL h^{-1})		L (h)		DMDIV (%DM)		RPIV (%DM)	
Forages										
Alfalfa *(Medicago sativa)*	352.75	bcde	0.0313	cdef	0.382	b	42.00	fgh	15.10	hij
Orchardgrass*(Dactylis glomerata)*	345.00	bcdef	0.0309	def	0.970	b	49.15	def	18.78	hg
Betch *(Vicia sativa)*	380.08	bcd	0.0362	bcd	1.464	b	63.85	ab	19.95	hg
Vegetables by-products										
Corn gluten meal	338.90	cdef	0.0367	abc	1.762	ab	46.15	efg	70.11	b
Cottonseed meal	307.43	defg	0.0343	bcde	1.042	b	37.85	hij	47.48	d
Canola meal	395.38	bc	0.0341	bcdef	0.413	b	55.70	cd	39.71	e
Safflower paste	365.53	bcde	0.0327	bcdef	1.757	ab	42.90	efgh	11.01	j
Coconut meal	428.50	ab	0.0329	bcdef	0.547	b	50.35	de	33.23	f
Soybean meal	398.18	bc	0.0335	bcdef	0.388	b	64.20	ab	78.05	a
Malt sprouts	355.70	bcde	0.0339	bcdef	0.278	b	57.00	bcd	16.92	hi
Corn bran	419.40	abc	0.0374	ab	1.696	ab	59.20	bc	22.73	g
Wheat bran	492.33	a	0.0421	a	1.172	b	71.35	a	12.62	ij
Cottonseeds	263.53	fghi	0.0296	efg	0.282	b	27.10	kl	15.89	hij
Animal by-products										
Meat and bone meal	225.98	ghi	0.0312	cdef	0.949	b	39.75	ghi	36.50	ef
Fishmeal	219.30	hi	0.0335	bcdef	1.150	b	23.95	l	55.57	c
Feathermeal	189.38	i	0.0286	g	0.260	b	31.45	jkl	70.90	b
Mexican poultry meal	282.58	defg	0.0348	bcde	1.178	b	31.50	jkl	49.77	d
Imported poultry meal	261.05	fghi	0.0336	bcdef	2.069	ab	40.90	hg	45.79	d
Bloodmeal	221.68	hi	0.0245	g	3.589	a	32.35	ijk	83.15	a
Poultry litter	384.23	bcd	0.0367	abc	2.195	ab	69.10	a	18.38	hg
SEM	14.216		0.001		0.162		1.64		2.63	

[ab] Means within column with different superscript differ ($p \leq 0.01$).Vmax: gas volume at 48 h incubation; S: gas production rate; L: lag period; DMDIV: DM disappearance *in vitro*; RPIV: residual protein *in vitro* SEM: standard error of the mean.

Table 4. Pearson correlation coefficients between chemical components and gas production volume at 48 h

	Vmax	EFG	N*6.25	NDF	ADF	ISP	SOLP	DMDIV	NPN	NDIP	ADIP
Vmax	1.00	0.57	-0.45	0.20	0.27	-0.12	0.15	0.61	-0.15	0.21	0.23
		(<.0001)	(<.0001)	(0.074)	(0.016)	(0.294)	(0.174)	(<.0001)	(0.196)	(0.067)	(0.043)
EFGR		1.00	-0.08	0.12	0.03	0.11	-0.02	-0.07	0.02	0.13	0.07
			(0.454)	(0.278)	(0.769)	(0.313)	(0.828)	(0.554)	(0.833)	(0.247)	(0.547)
N*6.25			1.00	-0.66	-0.67	0.11	-0.24	0.37	0.16	-0.23	-0.28
				(<.0001)	(<.0001)	(0.330)	(0.034)	(0.001)	(0.157)	(0.038)	(0.011)
NDF				1.00	0.80	0.03	-0.02	-0.39	0.05	0.42	0.36
					(<.0001)	(0.809)	(0.840)	(0.0003)	(0.654)	(<.0001)	(0.001)
ADF					1.00	0.00	-0.02	-0.28	-0.08	0.14	0.11
						(0.970)	(0.836)	(0.013)	(0.478)	(0.227)	(0.326)
ISP						1.00	-0.80	0.40	-0.37	0.00	-0.10
							(<.0001)	(0.0002)	(0.001)	(0.980)	(0.360)
SOLP							1.00	-0.46	0.36	-0.01	0.15
								(<.0001)	(0.001)	(0.899)	(0.176)
DMDIV								1.00	-0.86	-0.18	-0.22
									(<.0001)	(0.119)	(0.050)
NPN									1.00	0.06	0.08
										(0.616)	(0.499)
NDIP										1.00	0.88
											(<.0001)
ADIP											1.00

Vmax: Gas production volume at 48 h; EFG: Efficiency per gram DM; N*6.25: Crude protein; NDF: Neutral detergent fibre; ADF: Acid detergent fibre; ISP: Insoluble crude protein; SOLP: Soluble crude protein; DMDIV: Dry matter disappearance *in vitro*, NPN: Non-protein nitrogen; NDIP: neutral detergent-insoluble crude protein; ADIP: acid detergent-insoluble crude protein. Significance is indicated in parenthesis.

CONCLUSION

In the present study were differences in the protein fractions analyzed, due to the chemical structure of each ingredient and processing. In the gas production kinetic in vitro, the maximum volume was positively correlated with in vitro disappearance of DM and ADF content, but negatively with crude protein content. RPIV differences was relate to the structure of soluble and insoluble particles of the substrate. The concentration of dietary protein and degradation are factors that influence organic matter disappearance and gas production. In general, these data provide important information to have better balancing rations and supplements for ruminants.

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REFERENCES

AOAC. 2000. Association of Official Analytical Chemist. Official Methods of Analysis. 17th ed. Washington, DC. USA.

Bach, A., Calsamiglia, S., Stern, M.D. 2005. Nitrogen metabolism in the rumen. Journal of Dairy Science. 88 (E. Suppl.) E9-E21.

Beuvink, J. M. W., Spoelstra, S. F. 1992. Interactions between substrate, fermentation end-product, buffering systems and gas production upon fermentation of different carbohydrates by mixed rumen organisms *in vitro*. Applied Microbiology and Biotechnology. 37:505-509.

Broderick, G. A., Wallace, R.J., Ørskov, R.E. 1991. Control of rate and extent of protein degradation. *In*: Physiological aspects of digestion and metabolism in ruminants. Tsuda T, Y. Sasaki, and R. Kawashima (eds). Academic Press, Boston, MA. pp:541–592.

Calsamiglia, S., Stern, M.D. 1995. A three-step in vitro procedure for estimating intestinal digestion of protein in ruminants. Journal of Animal Science. 73:1459-1465.

Coblentz, W.K., Fritz, J.O., Fick, W.H., Cochran, R.C. Shirley, J.E. 1998. In situ dry matter, nitrogen and fiber degradation of alfalfa red clover and eastern gamagrass at four maturities. Journal of Dairy Science. 81: 150-161.

Cone, J. W., Van Gelder, A.H. 1999. Influence of protein fermentation on gas production profiles. Animal Feed Science and Technology 76:251-264.

Elizalde, J. C., Merchen, N. R., Faulkner, B.D. 1999. Fractionization of fiber and crude protein in fresh forages during the spring growth. Journal of Animal Science 77:467-484.

Faria-Marmol, J., Gonzalez, J., Rodríguez, C.A., Alvir, M.R. 2002. Effect of diet forage to concentrate ration of rumen degradability and post-ruminal availability of protein from fresh and dried lucerne. Animal Science 74:337-345.

France, J., Lopez, S., Kebreab, E., Bannink, A., Dhanoa, M.S., Dijkstra, J. 2005. A general compartmental model for interpreting gas production profiles. Animal Feed Science and Technology 123-124: 473-485.

Getachew, G., Blümmel, M., Makkar, H.P.S., Becker, K. 1998. *In vitro* gas measuring techniques for assessment of nutritional quality of feeds: A review. Animal Feed Science and Technology 72: 261-281.

Getachew, G., Robinson, P.H., De Peters, E.J., Taylor, S.J. 2004. Relationships between chemical composition dry matter degradation and *in vitro* gas production of several ruminant feeds. Animal Feed Science and Technology 111:57-71.

Groot, C. J. J., Cone, W.J.; Williams, A.B., Debersaques, M.A., Lantinga, A.E.1996. Multiphasic analysis of gas production kinetics for *in vitro* fermentation of ruminant feeds. Animal Feed Science and Technology 64:77-89.

Krishnamoorthy, U., Muscato, Sniffen, C.J., Van Soest, P.J. 1982. Nitrogen fractions in selected feedstuffs. Journal of Animal Science 65:217-225.

Krishnamoorthy, U., Rymer, C., Robinson, P.H. 2005. The *in vitro* gas production technique: limitations and opportunities: Animal Feed Science and Technology 123-124:1–7.

Licitra, G., Hernandez, T.M., Van Soest, P.J. 1996. Standardization of procedures for nitrogen fractionation of ruminant feeds. Animal Feed Science and Technology 57:347–358.

Makkar, H.P.S. 2005. *In vitro* gas methods for evaluation of feeds containing phytochemicals. Animal Feed Science and Technology 123-124:291-302.

Menke, K. H., Steingass, H. 1998. Estimation of the energetic feed value obtained from chemical analysis and *in vitro* gas production using rumen fluid. Animal Research Development. 28:7-55.

Mould, F. L.; Kliem, K.E.; Morgan, R., Mauricio, R.M. 2005. *In vitro* microbial inoculum: A review of its function and properties. Animal Feed Science and Technology 123-124:31–50.

Nsahlai, I. V., Umunna, N.N., Negassa, D. 1995. The effect of multi-purpose tree digesta on *in vitro* gas production from napier grass or neutral-detergent fiber. Journal of Science Food and Agriculture 69:519-528.

NRC. Nutrient Requirements of Dairy Cattle. 2001. 7th rev. ed. National Academy Press, Washington, DC. pp 43-104.

Pitt, R. E., Cross, T.L., Pell, A.N., Shofield, P., Doane, P.H. 1999. Use of *in vitro* gas production models in ruminal kinetics. Mathematical Biosciences. 159: 145-163.

SAGARPA. Norma Oficial Mexicana. NOM-060-ZOO. 1999. Especificaciones zoosanitarias para la transformación de despojos animales y su empleo en la alimentación animal. México (DF) 1999.

SAGARPA. Norma Oficial Mexicana. NOM-061-ZOO. 1999. Especificaciones zoosanitarias de los productos alimenticios para consumo animal. México (DF).

SAS. 1999. User' s Guide: Statistics, version 8.0. Ed. SAS Institute, Inc., [CD-ROM] Cary N.C.

Schwab, C. G., Tylutki, T.P., Ordway, R.S., Sheaffer, C., Stern, M.D. 2003. Characterization of Proteins in Feeds. Journal of Dairy Science 86:(E. Suppl.):E88–E103 21:353-371.

Shannak, S., Südekum, K.H., Susenbeth, A. 2000. Estimating ruminal crude protein degradation with *in situ* and chemical fractionation

procedures: Animal Feed Science and Technology 85 195–214.

Sniffen, C. J., O'Connor, J.D., Van Soest, P.J., Fox, D.G., Russell, J.B. 1992. A net carbohydrate and protein system for evaluating cattle diets. II. Carbohydrate and protein availability. Journal of Animal Science 70:3562-3577.

Steel, R. G D., Torrie, J.H. 1992. Bioestadística, principios y procedimientos. 2da. Edición. México Ed McGraw-Hill Book Co. New York .622 p.

Theodorou, M. K., Williams, B.A., Dhanoa, M.S., McAllan, A.B., France, J. 1994. A simple gas production method using a pressure transducer to determine the fermentation kinetics of ruminant feeds. Feed Science and Technology 48: 185-197.

VanSoest, P.J., Robertson, J.B., Lewis, B.A. 1991. Methods for dietary fiber, neutral detergent fiber, and nonstarchpolysaccharides in relation to animal nutrition. Journal of Dairy Science. 74: 3583-3597.

Van Soest, P. J. 1994. Nutritional ecology of the ruminant. 2nd Ed, Cornell University Press, Ithaca, NY. pp 436.

Vanzant, E. S., Cochran, C., Titgemeyer, E.C., Stafford K.; Olson, C., Johnson, D.E., Jean J. 1996. *In Vivo* and *in situ* measurements of forage protein degradation in beef cattle. Journal of Animal Science 74:2773–2784.

Wolin, M. J. 1960. A theoretical rumen fermentation balance. Journal of Dairy Science 43:1452-1459.

Weimer, P. J. 1996. Why don't ruminal bacteria digest cellulose faster? Journal of Dairy Science 79:1496-1502.

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