

Fermentation profiles and degradability measurements in extrusa diet samples collected from brome-suppressed and undisturbed pastures and their relationship to weight gain of steers¹

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Blümmel, M., Grings, E. E. and Haferkamp, M. R. 2004. Fermentation profiles and degradability measurements in extrusa diet samples collected from brome-suppressed and undisturbed pastures and their relationship to weight gain of steers. *Can. J. Anim. Sci.* **84**: 105–111. The effects of suppression of annual bromes (*Bromus japonicus* Thunb. and *Bromus tectorum* L.) by atrazine application on the nutritive quality of extrusa diet samples (EDS) collected from the esophagus were investigated, and EDS quality estimates were compared with weight gain of grazing steers. Analysis on EDS included crude protein (CP), in vitro organic matter degradability (IVOMD), and gas production profiles in N supplemented and unsupplemented incubation media. Brome-suppression tended ($P = 0.07$) to increase CP content but effects on gas production kinetics and IVOMD were dependent on incubation medium N-level. In N-unsupplemented incubations, asymptotic gas production was less and rates of gas production were greater in EDS from brome-suppressed compared to undisturbed pasture. No such differences were found for N-supplemented incubations. Weight gains of steers grazing brome-suppressed pastures were 16% greater ($P = 0.007$) than from control pastures. The R^2 for the comparison of predicted and measured gains were 0.90 ($P < 0.0001$), 0.96 ($P < 0.0001$), and 0.90 ($P < 0.0001$) using CP, IVOMD (N-low), and IVOMD (N-rich) as the predicting variable, respectively. Best predictions using in vitro gas production measurements were obtained from 24 h gas volume recording ($R^2 = 0.93$, $P < 0.0001$). Best-fit model (sigmoidal vs. exponential) depended on grazing period and N-level, and the sigmoidal Gompertz model best described most gas production profiles.

Key words: Forage quality, gas production, weight gain, beef steers

Blümmel, M., Grings, E. E. et Haferkamp, M. R. 2004. Profil de la fermentation et mesure de la dégradabilité des échantillons venant de pâturages naturels et de pâturages débarrassés du brome prélevés *in extrusa*, et relations avec le gain de poids des bouvillons. *Can. J. Anim. Sci.* **84**: 105–111. Les auteurs ont étudié les conséquences de la suppression des espèces annuelles de brome (*Bromus japonicus* Thunb. et *Bromus tectorum* L.) par l'application d'atrazine sur la valeur nutritive des échantillons d'aliments obtenus de l'œsophage par extrusion (EAE) puis ont comparé la qualité estimative des EAE au poids gagné par les bouvillons mis à l'herbe. L'examen des EAE comprenait le dosage des protéines brutes (PB), la détermination de la dégradation de la matière organique *in vitro* (DMOIV) et l'analyse des gaz libérés par un milieu d'incubation enrichi d'azote (N) ou pas. L'élimination du brome a tendance ($P = 0,07$) à augmenter la teneur en PB, mais la cinétique des gaz produits et la DMOIV dépendent de la concentration de N dans le milieu d'incubation. Quand celui-ci n'est pas enrichi, la production asymptotique de gaz est plus faible et les EAE venant des prés sans brome libèrent plus de gaz que ceux issus des pâturages naturels. On n'a pas observé cette variation lors de l'incubation dans les milieux enrichis de N. Les bouvillons qui paissent dans les prés sans brome ont gagné 16 % plus de poids ($P = 0,007$) que ceux mis à l'herbe dans les pâturages témoins. Le gain prévu et le gain réel ont une valeur R^2 de 0,90 ($P < 0,0001$), de 0,96 ($P < 0,0001$) et de 0,90 ($P < 0,0001$), respectivement, quand on sert des PB, de la DMOIV (faible concentration de N) et de la DMOIV (concentration élevée de N) comme variable de prévi-

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Abbreviations: CP, crude protein; CPS, clipped forage samples; EDS, extrusa diet samples; IVOMD, in vitro organic matter digestibility

sion. Les meilleures prévisions résultant de la production de gaz *in vitro* émanent de la mesure du volume libéré en 24 h ($R^2 = 0,93$; $P < 0,0001$). Le modèle le mieux ajusté (sigmoïdal c. exponentiel) dépend de la période de paissance et de la concentration de N. Le modèle sigmoïdal de Gompertz décrit le mieux la plupart des modes de production des gaz.

Mots clés: Qualité des fourrages, production de gaz, gain de poids, bouillons de boucherie

A reliable prediction of the effect of the presence of annual bromes on animal performance by *in vitro* techniques could assist forage and range scientists in preliminary investigations of various research hypotheses within a smaller, less costly and more closely controlled experimental framework than direct animal experimentation. Forage is largely utilized ruminally, and routine *in vitro* procedures that estimate the extent of ruminal feed degradation exist (Tilley and Terry 1963; Goering and Van Soest 1970). However, these measurements alone may not be sensitive enough for prediction of animal response, and additional information such as rate of forage degradation [National Research Council (NRC) 1996] may contribute to a more comprehensive understanding of forage quality and possibly to a better prediction of animal performance.

Free-ranging animals graze selectively, and diet quality is often assessed by extrusa samples (EDS) collected from esophageally fistulated animals (Scales et al. 1974; Cohen 1979; Sankhyan et al. 1999). While EDS closely reflect the botanical composition of the forages consumed, mastication can impact the actual nutritive nature of the diet (Scales et al. 1974; Cohen 1979; Sankhyan et al. 1999). Rumen-hepatic N recycling may increase N content of EDS relative to forage N through saliva, and *in vitro* procedures might need to take this into account by using different N-levels in the incubation medium than those recommended for feed or clipped forage samples. It was the aim of this work to investigate the effect of annual bromegrass reduction in natural pasture on various *in vitro* ruminal fermentation characteristics of EDS collected monthly and to compare this *in vitro* information with the seasonal weight gain of yearling steers grazing these pastures. *In vitro* fermentation characteristics of EDS were assessed using the manual *in vitro* gas production system of Menke et al. (1979) as modified for kinetic measurements by Blümmel and Becker (1997).

MATERIALS AND METHODS

Experimental Design, Pasture Intervention, and Grazing Experiments

Experimental design, treatment for suppression of bromes (*Bromus japonicus* Thunb. and *Bromus tectorum* L.), vegetational responses, and animal performance have been reported in detail by Haferkamp et al. (2001a,b). Briefly, six 12-ha pastures were randomly allocated to three replications of a treatment consisting of brome suppression by atrazine application to dormant vegetation or to three replications of undisturbed pasture. Each 12-ha pasture was stocked with eight British-type predominantly Angus-sired crossbred yearling steers and grazed from mid-May until mid-September 1995. Initially, steers were weighed (mean weight 272 kg, ± 14 kg), stratified by weight and allocated

to pasture by random-stratified procedures. Thereafter weights were obtained every 30 d from non-fasted (non-shrunk) animals. Diet quality was assessed by EDS collected on each pasture every 30 d using at least three esophageally fistulated yearling heifers per pasture. Heifers had experience in pastures of similar vegetation types. Before EDS collection, heifers were penned at 1600 and, with access to water, fasted overnight. Collections began the following morning at 0700, lasting approximately 30 min. Individual EDS were thoroughly mixed by hand, freeze dried, and subsampled for chemical and *in vitro* analysis. All cattle were handled in a manner meeting the guidelines of the Canadian Council on Animal Care.

In Vitro Analysis

In vitro gas production of EDS samples was measured essentially as described by Blümmel and Becker (1997) using 100-mL calibrated glass syringes as incubation vessels (Menke et al. 1979) with the modification that replicates of 250 mg of substrate were incubated in 20 mL of rumen suspension. Ruminal fluid and particulate matter (approximately 60:40) were collected from two rumen-fistulated crossbred cows at 0700 into a pre-warmed CO₂-filled thermos bottle. The cows were offered *ad libitum* access to sudan-grass hay and received 1.5 kg of alfalfa pellets every other day, with supplementation occurring on the opposite day from ruminal fluid collections. Ruminal contents were homogenized in a laboratory blender, filtered first through nylon cloth and afterwards through glass wool. All handling of rumen contents was done under continuous flushing with CO₂. The filtrate was added to reduced distilled water, buffer and mineral solution. Twenty milliliter of incubation medium consisted of 5 mL of rumen liquor, 5 mL of hydrogen carbonate buffer, 2.5 mL of macro- and micromineral solution, and 7.5 mL of distilled water. The N level in the incubation medium was varied by replacing ammonium hydrogen carbonate (NH₄HCO₃) in the buffer with sodium hydrogen carbonate (NaHCO₃) on carbonate equivalents (Blümmel and Lebzién 2000). Twenty milliliters of N-supplemented incubation medium contained 175 mg of NaHCO₃ and 20 mg NH₄HCO₃ while 20 mL of N-unsupplemented incubation medium contained 196.3 mg of NaHCO₃ for hydrogen carbonate buffer. *In vitro* gas volumes were recorded after 2, 4, 6, 8, 10, 12, 14, 16, 24, 30, 36, 48, 54, 60, 72, and 96 h of incubation. *In vitro* organic matter digestibility of EDS was determined after 48 h of incubation by the two-stage Tilley and Terry (1963) procedure using pepsin HCl, but as modified by White et al. (1981) in N-low and N-rich incubation media where N was supplemented in the form of urea. Mean IVOMD values for a grazing period were computed by averaging the IVOMD value from the beginning

Table 1. Weight gain of steers grazing control (CO) and treated rangeland (TR) from May to September and crude protein (CP, %) and 48-h in vitro organic matter digestibility (IVOMD, %)

Periods	May–June		June–July		July–August		August–September	
	CO	TR	CO	TR	CO	TR	CO	TR
Weight gain (kg d ⁻¹)	1.72	1.88*	1.02	1.31*	0.72	0.71	0.17	0.42
CP	19.3	20.2	11.4	12.6*	7.3	7.4	5.2	6.0
IVOMD N-low	65.8a	67.5a	52.3a	56.8a*	43.6a	45.2a	35.4a	38.7a
IVOMD N-rich	76.0b	74.6b	66.1b	66.2b	58.4b	58.9b	54.9b	55.8b

*Denote differences ($P < 0.05$) between CO and TR in a given period.

a, b Different superscripts denote differences ($P < 0.05$) between N-low and N-rich incubations.

and end of a grazing period. Total N (organic matter basis) of EDS was analyzed using Technicon Auto Analyzer (Technicon Industrial Systems 1977) and CP content was calculated by multiplying N by 6.25. Mean CP values for a period were computed analogously to IVOMD values.

Curve Fitting and Statistical Analysis

Gas volume recordings from 2 to 96 h were fit to exponential and sigmoidal models using the curve fitting features of GraphPad Prism (1994). Hourly gas volume recordings from EDS were averaged for periods as described for IVOMD and CP, and these averaged values were used for curve fitting. Exponential models used were the modified Ørskov and McDonald (1979).

$$y = B(1 - \exp(-ct))$$

and

$$y = B(1 - \exp(-c(t-\text{lag})))$$

where B represents total potential gas production, c , the fractional rate at which B is produced per hour; lag, a delay in the onset of gas production, and where y equals gas production at time t . Sigmoidal models were the modified Boltzmann model

$$y = \text{Top} / (1 + \exp((v50 - t)/\text{slope})),$$

and the modified Gompertz model

$$y = AS(\exp(-\exp((2.718(MR/AS))(\text{lag} - t) + 1)))$$

where Top and AS represent total potential gas production; v50 the time when half of the total gas was produced, slope, the shape of the curve of gas production, MR, the maximum rate of gas production, lag, a delay in the onset of gas production, and where y equals gas production at time t . The best-fit model was identified using F -test features of GraphPad Prism (1994).

Statistical mean differences of treatments were calculated by paired t -tests. Simple correlations and stepwise multiple regressions between in vitro variables and weight gain of steers were calculated using SAS Institute, Inc. (1997) software. Prediction of weight gains by N and in vitro variables were based on cross-validation procedures (SAS Institute, Inc. 1997) where the respective variable of a predicted

observation was not part of the regression equation developed for the prediction.

RESULTS

Animal Performance and Chemical and In Vitro Analysis

Weight gains of steers, extrusa CP concentrations and IVOMD for the four monthly periods are presented in Table 1. Brome suppression had a beneficial effect on weight gain. Over the entire grazing period mean weight gain was about 16% ($P = 0.007$) greater on treated relative to undisturbed pasture. Mean CP content of EDS tended ($P = 0.07$) to be greater in treated than undisturbed pastures when compared over the whole grazing period. In N-unsupplemented incubations, mean IVOMD of EDS from brome-suppressed pastures was 52.1%, which is 5.4% greater ($P = 0.005$) than the mean IVOMD of EDS collected from untreated pastures (49.4%). However, significant differences in IVOMD between undisturbed and treated pasture within a given period was observed only for the June to July period. In N-supplemented incubations, IVOMD of EDS of treated and untreated pastures were identical (63.7%) and substantially higher than IVOMD in the N-unsupplemented medium.

The exponential model with lag fitted the gas volume recordings consistently better ($P < 0.05$) than the exponential model without lag. For the sigmoidal models, Gompertz consistently provided a better fit ($P < 0.05$) than Boltzmann. Therefore, only data from the Gompertz and the exponential with lag model are presented. Gas production profiles of EDS were influenced by grazing period, pasture treatment, and N-supplementation of the incubation medium. Figure 1 presents mean gas production profiles and kinetic models of EDS collected from untreated pasture from May to June and from August to September and incubated in N-unsupplemented and N-supplemented incubation media.

Neither the exponential with lag nor the sigmoidal Gompertz model fitted all grazing periods and N-levels equally well. Gas production profiles of EDS collected in the late grazing period were better characterized by the sigmoidal than by the exponential with lag model. In general, the reverse applied to the early grazing period but these gas production profiles were also affected by N-supplementation (Fig. 1). Table 2 presents the kinetic parameters of in vitro gas production averaged for treatment and monthly periods as calculated by the exponential with lag and by the Gompertz model. Except for the May to June period, the

Table 2. Potential gas production (ml 250 mg⁻¹ organic matter), rates of gas production, lag values and sums of squares (SS) for observed and fitted gas volumes as obtained by exponential and sigmoidal models for EDS collected from control (CO) and treated (TR) rangeland^a

Model component	Incubation medium	Periods							
		May – June		June – July		July – August		August – September	
		CO	TR	CO	TR	CO	TR	CO	TR
		$y = B(1 - \exp(-c(t - \text{lag})))$							
B	N-low	83.6	79.6	87.8a	83.8*a	89.3a	88.1a	93.5a	91.4
B	N-rich	83.3	80.8	83.7b	79.5b	81.2b	78.8b	79.6b	76.2
c	N-low	0.0670a	0.0797*a	0.0426a	0.0471*a	0.0307a	0.0313	0.0255a	0.0251a
c	N-rich	0.0873b	0.0935b	0.0626b	0.0615b	0.0514b	0.0500	0.0489b	0.0512b
lag	N-low	1.09	1.03	1.61a	1.26*a	1.51a	1.42a	1.22a	1.14
lag	N-rich	1.27	1.14	1.79b	1.52*b	2.23b	2.18b	2.52b	2.06
SS	N-low	30.0	15.7	115.0a	52.0*	105.9	100.3a	112.7a	108.7a
SS	N-rich	20.7	21.0	56.3b	36.7*	117.3	133.3*b	148.3b	140.0b
		$y = AS(\exp(-\exp((2.718(MR/AS))(\text{Lag} - t) + 1)))$							
AS	N-low	81.2	77.8	82.4a	79.5	80.6a	79.9a	80.7a	79.9
AS	N-rich	81.5	79.2	80.4b	76.5	76.7b	74.3b	74.6b	71.1
MR	N-low	3.44a	3.81a	2.53a	2.55	1.92a	1.92a	1.58a	1.64a
MR	N-rich	4.39b	4.42b	3.48b	3.15	2.96b	2.82b	2.85b	2.93b
Lag	N-low	-0.24	-0.26	-0.85	-0.01*w	0.79a	0.64a	0.76a	0.55a
Lag	N-rich	-0.13	-0.17	1.13	0.54*w	2.13b	2.12b	2.78b	2.49b
SS	N-low	22.3a	27.3a	9.3a	13.3a	5.9a	5.0a	13.0	10.7a
SS	N-rich	75.1b	99.6b	41.3b	35.7b	25.7b	30.0b	29.0	38.0b

^aB = total gas production, c = fractional rate of gas production, t = time, MR = maximum rate of gas production, AS = total potential gas production, lag and Lag = delay in onset of gas production.

*Denotes differences (P < 0.05) between CO and TR in a given period.

a, b Different letters denote differences (P < 0.05) between N-low and N-rich incubations.

Table 3. Correlation (r) between equation parameters of exponential with lag and Gompertz sigmoidal model and weight gain of steers (N = 24)^a

Equation parameter	B	c	lag	AS	MR	Lag
N-low	-0.85***	0.95***	-0.35	-0.20	0.96***	-0.74***
N-rich	0.36	0.90***	-0.90***	0.60*	0.83***	-0.96***

^aB = total gas production, c = fractional rate of gas production, lag and Lag = delay in onset of gas production, AS = total potential gas production, MR = maximum rate of gas production.

*, **, *** Denote significance at P < 0.05, P < 0.001 and P < 0.0001, respectively.

sigmoidal Gompertz model fitted gas production profiles better (P < 0.05) than the exponential model with lag. Eighteen out of a total of 24 gas production profiles were better described by the sigmoidal Gompertz model.

When averaged across all grazing periods, kinetic gas production parameters of both models differed between treated and untreated pastures. In the exponential with lag model, mean potential gas production and lag phase were lower by 3 (B; P < 0.05) and by 9% (lag; P < 0.05), while rate of gas production was greater by 12% (P < 0.05) in EDS from treated relative to untreated pastures. In the sigmoidal Gompertz model, potential gas production and lag values were 2 (AS; P < 0.05) and 6% less (Lag; P < 0.05) in EDS from treated relative to untreated pastures while rate of gas production (MR) was 5% greater (P < 0.05) in EDS from treated pastures. Nitrogen-supplementation of the incubation medium eliminated (P > 0.05) all these differences.

Prediction of Animal Performance by Chemical and in Vitro Analysis

Crude protein content of EDS accounted for 91% of the variation in weight gain of steers, whereas 95 and 91% of the variation were accounted for by IVOMD measurements

obtained from N-unsupplemented and N-supplemented incubation media, respectively. The relationship between gas production equation parameters of the exponential with lag and the sigmoidal Gompertz model and weight gain of steers is presented in Table 3.

The gas production rates of these models (c and MR) as obtained in N-unsupplemented incubations were highly positively related to weight gain of steers explaining approximately 90 (c) and 92% (MR) of the variation in gain. The relationship between potential gas volumes (B and AS) and weight gain was strongly affected by N-level in the incubation medium. Negative associations between these variables and gain were found for N-unsupplemented incubations, while the reverse was true for N-supplemented incubations. Lag values (lag and LAG) were inversely related to gain, and these relationships were stronger in N-supplemented than in N-unsupplemented medium (Table 3). Combination of gas production equation parameters in stepwise multiple regressions did not result in higher correlations with gain than reported in Table 3 when the entry level of a variable into a model was set to a probability value of 0.05.

With both N levels, gas volumes measured during the first quarter of the incubation period were more closely related to

Table 4. Relationships between measured (x) and cross validations blind predicted (y) weight gain of steers (kg d^{-1}) presented in form of linear regression analysis²

Predicting variable	Intercept	Slope	Sy,x	R ²	P value
Crude protein	0.093 (\pm 0.076)	0.91 (\pm 0.066)	0.181	0.90	0.0001
IVOMD (N-unsuppl.)	0.096 (\pm 0.049)	0.92 (\pm 0.042)	0.116	0.96	0.0001
IVOMD (N-suppl.)	0.105 (\pm 0.073)	0.92 (\pm 0.064)	0.176	0.90	0.0001
MR (N-unsuppl.)	0.091 (\pm 0.075)	0.91 (\pm 0.065)	0.179	0.90	0.0001
c (N-unsuppl.)	0.094 (\pm 0.087)	0.92 (\pm 0.076)	0.209	0.87	0.0001
LAG (N-suppl.)	0.116 (\pm 0.070)	0.91 (\pm 0.061)	0.168	0.91	0.0001
Gas 24 h (N-unsuppl.)	0.069 (\pm 0.063)	0.93 (\pm 0.055)	0.151	0.93	0.0001

²MR = maximum rate of gas production, c = fractional rate of gas production, LAG = delay in onset of gas production.

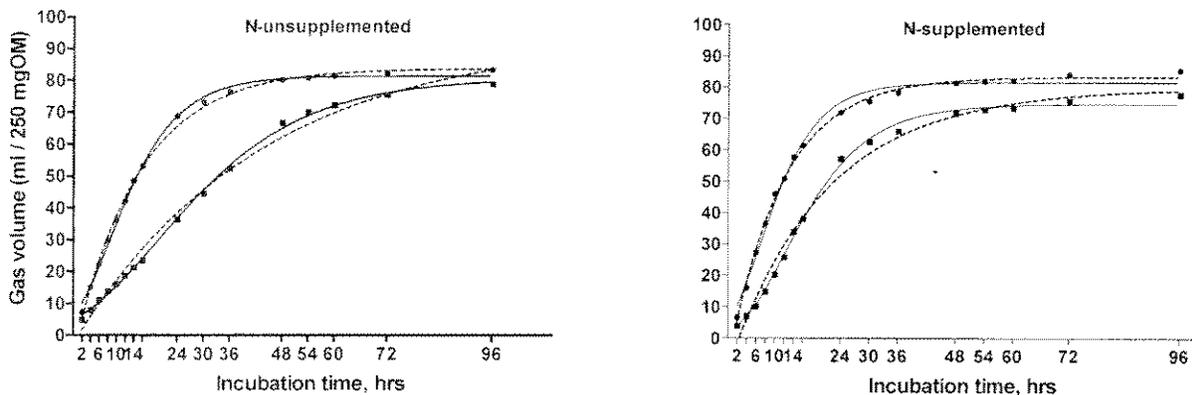


Fig. 1. Gas production curves for EDS collected early (Period I) or late (Period IV) in the grazing season and incubated in N-supplemented or N-unsupplemented media (circles and square symbols denote Periods I and IV, respectively, and dotted and solid lines denote exponential with a lag and Gompertz models, respectively)

gain than later volume recordings (Fig. 2). Gas volumes measured between 14 and 24 h in the N-unsupplemented incubation medium accounted for 93 to 94% (24 h) of the variation in gain. Less of the variation in gain was accounted for by gas volume recordings in the N-supplemented medium where maximum R^2 was 0.88 (6- and 8-h gas volumes).

Cross-validation blind-predicted weight gains and actual weight gains are compared in Table 4. Among the predicting variables employed, IVOMD obtained from N-unsupplemented incubations resulted in the most accurate prediction of weight gain. The slopes of the regression equations reported in Table 4 suggest a rather constant underestimation of predicted weight gains of about 10% relative to observed weight gains.

DISCUSSION

Brome Suppression, Grazing Periods, Animal Performance, and their Relationship with Chemical and in Vitro Measurements

Major characteristics of annual bromegrasses affecting livestock management decisions include erratic fluctuations in annual forage production (Gartner et al. 1986), reduction in perennial plant production (Rummell 1946; Haferkamp et al. 1997), and early plant maturity (Vallentine and Stevens 1994). These negative effects on livestock production were confirmed in the current work where brome suppression

increased total weight gain of steers during the 4-mo grazing period by about 16% over gain of steers grazing untreated pastures (Table 1). The overall pattern in seasonal weight gain of steers observed agrees well with studies conducted by Heitschmidt et al. (1993) and Grings et al. (1994) within similar rangeland environments and grazing periods in the Northern Great Plains. Decreasing weight gains with progressing grazing season suggest the need for strategic supplementation in the second half of the grazing season. In the present work, the main effect of brome suppression was observed during the first half of the grazing season indicating that a lack of annual bromes in the environment will not significantly reduce the need for later-season supplementation.

While weight gain on brome-suppressed pastures was significantly higher than on untreated pasture in May to June and June to July, this treatment effect was only partly (June to July) related to higher CP concentrations of EDS and IVOMD of EDS conducted in N-unsupplemented incubations (Table 2). In comparison, brome suppression in May to June was related to significantly higher rates of gas production (exponential model with lag) of EDS fermented in N-unsupplemented incubations (Table 2). These findings could suggest that rate measurements of fermentation are potentially more sensitive in detecting changes in forage quality than nitrogen or 48-h IVOMD Tilley and Terry (1963) measurements. However, interpretation of these rate

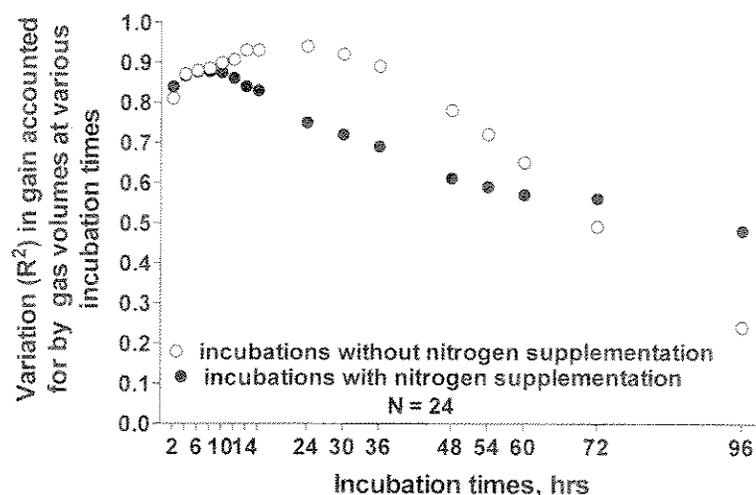


Fig. 2. Variation in weight gain of steers accounted for by gas volumes at various incubation times for EDS incubated in medium with and without supplemental N (circles and square symbols denote Periods I and IV, respectively, and dotted and solid lines denote exponential with a lag and Gompertz models, respectively).

measurements appears to be complicated by the fact that no single kinetic model fitted the EDS fermentation characteristics in all grazing periods (see also Fig. 1 and Table 2). Where the sigmoidal Gompertz model provided, with the exception of May to June, a better fit than the exponential with lag model for the gas production profiles of EDS, it failed to reflect the difference between gas production profiles of treated and untreated pastures in the May to June period (Table 2). These findings underline the importance of choosing the appropriate kinetic model and more than one model appears to be required for pasture evaluation. Level 2 of the NRC (1996) manual for nutrient requirements of beef cattle uses fractional rate constants of protein and carbohydrate degradation to ultimately improve the prediction of ruminal microbial protein production. Pitt et al. (1999) discussed the use of *in vitro* tests for estimating these fermentation rates, particularly of carbohydrates. Clearly, the extended applications of gas production rates for providing rate input variables for carbohydrate fermentation of pasture forages into the NRC (1996) level 2 matrix will be complicated by the possible need for different kinetic models.

Prediction of Animal Performance by Nitrogen and *In Vitro* Measurements

Measurements of fermentation kinetics did not result in more accurate predictions of weight gain of steers than were obtained by some individual gas volume recordings (Table 4, see also Fig. 2). While previously discussed problems may have contributed to the fact that simple individual gas volume recordings could account for more of the variation in gain than any kinetic model parameter, regardless of the model used (compare Fig. 2 and Table 3), more explanations appear warranted to account for these observations. In this context it is interesting to note that weight gain was

most accurately predicted by the 48-h IVOMD measurement (Table 4) while gas volume measurements at this incubation time exhibited a weaker relationship with gain (Fig. 2). This emphasizes that these two measurements reflect different fermentative events. It was pointed out in a recent review (Blümmel 2000) that *in vitro* gas production tests give only limited information about substrate degradability in that they reflect only microbial feed conversion into SCFA and ignore the production of microbial protein. Furthermore the interpretation of gas production at later incubation times is complicated by the secondary fermentation of lysed microbial biomass into SCFA and thus gases (Blümmel 2000). Both reservations raised against *in vitro* gas tests (Blümmel 2000) appear to apply in the current work considering the better prediction of gain obtained by the IVOMD than by gas volume measurement, together with the weakening relationships between gas volumes and gain with increasing incubation time (Fig. 2).

Regardless of the kind of *in vitro* measurement, variables obtained in N-unsupplemented incubations predicted gain more accurately than measurements obtained in N-supplemented incubations (Table 4). These findings are in contrast with work by Blümmel and Grings (2000) who reported significantly better prediction of weight gain of steers from *in vitro* gas measurements when clipped pasture samples (CPS) were incubated in the N-supplemented rather than in the N-unsupplemented incubation medium. These authors argued that the incubation of CPS in N-unsupplemented incubation medium might result in an underestimation of ruminal fermentability relative to actual utilization in the animal where additional N will be made available by way of rumino-hepatic N recycling. Nitrogen contamination is one of the problems frequently associated with EDS (Scales et al. 1974; Cohen 1979; Sankhyan et al. 1999), but this N originated

from N recycling and N content of EDS samples may, therefore, reflect a truer picture of N supply to the fermentation than the N content in CPS. Incubating EDS in N-supplemented medium might result in an overestimation of fermentation resulting in the less-accurate relationship between *in vitro* and *in vivo* measurements evident in Table 4.

The need for supplementing pasture in the Northern Great Plains in the second half of the grazing season was pointed out by several authors (e.g., Heitschmidt et al. 1993; Grings et al. 1994). The results reported in Table 1 show that *in vitro* N supplementation to EDS increased IVOMD in the second grazing period by about 10 to 20 percentage units. These findings suggest that N-supplementation in this period may have a potentially beneficial effect on animal performance under these grazing conditions.

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