

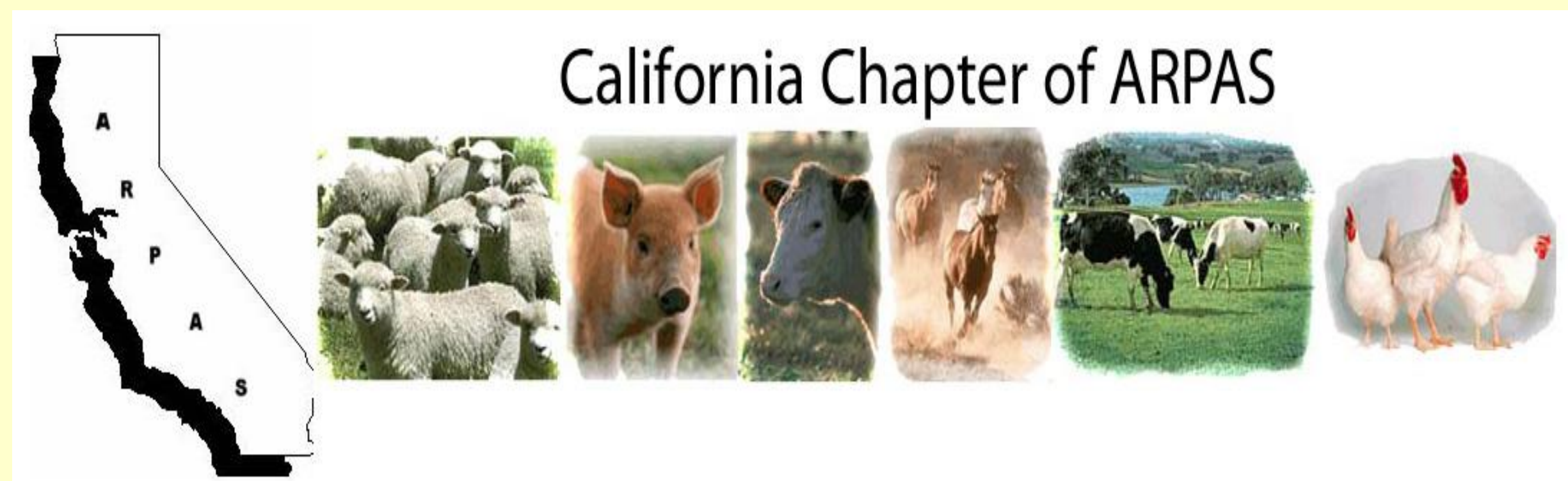
Abstract: M448

A Comparison of Models Used to Estimate Kinetics of In-vitro

Degradation of Components in Alfalfa Hay

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Introduction

- Current systems used to predict alfalfa hay quality are fiber based.
- These systems do not adequately predict alfalfa quality nor animal performance.
- California ARPAS proposed system uses a property of both feed and animal to describe quality and performance.
- Rate and extent of degradation for chemical and proximate entities are estimated.
- Use of NIR allows for rapid and quantitative results.

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Materials and Methods

Samples of each alfalfa hay sample (In-vivo 11 plus the remainder of the collection) were dried (62°C, to a constant weight) and ground (Wiley Mill fitted with a 6mm screen, Thomas Scientific, Swedesboro, NJ). For each in-vitro (IV) evaluation, aliquots of a composite sample of rumen contents from 4 donor Jersey steers were distributed among 3 L vessels pre-filled with 1920 mL incubation medium (Bossen et al., 2008) and equilibrated to 20% CO₂ and to 39°C. Also prior to the introduction of the composited rumen fluid, the pH of the incubation medium was adjusted to the pH of the collected rumen fluid (generally 6.4 to 6.7 pH) using citric acid solution. When these pre-steps were completed, four IV bags (10x20 cm with 40 micron pores, ANKOM, Macedon, NY) containing 10 to 15 g of dry, ground alfalfa hay were introduced. Replication was achieved by incubating 4 IV bags per sample per constituent per ruminal incubation time (4 to 120 hours). Also, after 16 hours of ruminal incubation, a set of 4 IV bags per sample were incubated by an in-vitro procedure that mimics gastric and ileal post-ruminal digestion (Babinszky et al., 2006).

Ruminal IV incubation followed the procedure described by Goering and Van Soest (1970) with some modifications of both preparation of ruminal inocula and incubation media as described by Bossen et al. and developed by SALLC. Extracted rumen fluid was not strained but used in the condition it was collected. Rumen fluid used was a composite of approximately 3 l withdrawn from each of 4 Jersey steers. This procedure enabled rumen fluid to be delivered to the incubation vessels within 20 minutes of extraction from each of the donor animals in order to limit the negative effects on ruminal protozoa. A portion of the natural fiber mat was included in the extracted ruminal inocula to help maintain the integrity of the natural population of fiber-degrading micro-organisms.

Post-ruminal incubations followed a SALLC modification of the gastric/ileal procedure described by Babinszky et al. (2006). The first modification was to cleanse the IV bags of adhering micro-organisms using a wash by neutral detergent solution. The gastric phase was performed in a medium of acid, pepsin. The in-vitro ileal phase was performed at pH 8 with a mixture of pancreatin, lipase and amylase.

After completion of each digestion time course, all bags were recovered, thoroughly washed in 11°C water, dried (62°C, to a constant weight) and sent for compositional analysis. The data were plotted and calculations performed that estimated lag time, rate and extent of DM, CP and NDF digestion. Kinetics of degradation were estimated using three models; a heterogeneous stochastic model (HS) assuming a gamma distribution of rate constants, a single exponential model (SE) or a biexponential model (BE). These models are similar to those described by Ellis et al. (1984)

Ruminal and post-ruminal availabilities of selected amino acids, asparagine (asp), glutamine (glu), lysine (lys), cysteine (cys), methionine(met) and tryptophan (trp) were determined for the 11 alfalfa hays used in the metabolism study. Ruminal availability was determined using a combination of the SALLC ruminal and post-ruminal IV procedures.

Results and Discussion

Goodness of fit, as estimated by residual sums of squares (RSS), was best for model HS (RSS = .012). Kinetics of dry matter degradation were poorly described by model SE (RSS = 4.29) and adequately described by model BE (RSS = .160). Model SE assumes, that for all particles, there exists one, and only one, degradation rate k. For model BE the assumption is that two degradation rates, k₁ and k₂, exist. Data sets lack precision to describe models for i>2 so these were not examined. Given the heterogeneity in particle sizes and degradation rates it is expected that model HS would provide for the best fit. Models assuming arbitrary pool separation times were not investigated; it is well understood that such a practice produces biased estimators.

In addition to dry matter degradation, we also investigated kinetics of crude protein and neutral detergent fiber degradation. As was the case with dry matter, model HS had lower RSS than did either model SE or BE.

Data for ruminal amino acid disappearance are found in Table 2. Column means, standard deviations (Std Dev) and 95 percent confidence intervals (CI) are shown. The variability in ruminal disappearance of

individual amino acids is evident. For an individual amino acid, it is more likely that ruminal disappearance is different from the average than it is to be not different. Total amino acid disappearance was similar to ruminal disappearance for glu, lys, cys and trp. Ruminal disappearance of asp and met were different from the total ruminal amino acid disappearance.

Apparent intestinal disappearances of ruminally undegraded amino acids are shown in Table 3. Similar to what was seen with ruminal disappearance, intestinal disappearance was variable. The coefficient of variation for total amino acid disappearance was 19 percent. As was the case for ruminal disappearance it was more likely that, for an individual amino acid, apparent intestinal disappearance was more likely to be different from the mean disappearance than it was similar. Apparent intestinal disappearance of glu, cys and met differed from total intestinal amino acid disappearance. These data indicate that current assumptions regarding amino acid availability, ruminal and intestinal, such as those found in the current Nutrient Requirements of Dairy Cattle, may not be correct for alfalfa hay.

Table 3. Apparent intestinal disappearance of selected amino acids in the In-vivo 11.

Sample ID	Total AA	Asp	Glu	Lys	Cys	Met
1	69.3	72.3	75.0	69.2	58.0	72.4*
2	70.9	72.8	75.9	69.5	60.5	73.7*
3	70.1	72.1	74.8	68.1	61.2	75.0
4	74.5	76.3*	78.6	72.3	65.6*	79.6
5	83.5*	88.0*	90.3*	87.8*	83.4*	93.7*
6	81.2*	82.3*	84.7*	81.9*	73.9*	85.2*
7	36.2*	39.7*	42.0*	37.0*	18.9*	42.8*
8	71.6	73.0	76.1	71.9	58.4*	77.3
9	64.3*	66.2*	70.5*	62.3*	53.1*	72.0*
10	79.5*	79.9*	82.7*	78.7*	68.7*	85.6*
11	83.1*	82.1*	86.2*	82.2*	75.3*	89.9*
Mean	71.7	71.3	76.1	71.0	61.5	77.0
Std Dev	13.70	12.70	12.70	13.50	16.70	13.50
Lower 95% CI	68.7	68.3	73.1	68.0	58.5	74.0
Upper 95% CI	74.7	74.3	79.1	74.0	64.5	80.0

(*) Means within a column differ (P<.05)

Table 1. Compositional values (DM basis) for alfalfa hay.

		Dry Matter	CP	ADF	NDF	TDN	PheMI ¹
US 3000	Average	87.0	19.7	31.9	40.5	58.4	8.54
in vivo 11		90.3	17.4	34.3	43.9	56.5	9.63
US 3000	Maximum	81.1	24.7	39.6	50.1	64.2	10.5
in vivo 11		86.8	23.1	51.0	65.4	66.6	14.4
US 3000	Minimum	93.0	14.7	24.2	31.0	52.6	6.82
in vivo 11		92.8	12.6	21.0	24.0	44.1	3.00
US 3000	Range	11.9	9.92	15.4	19.1	11.6	3.64
in vivo 11		6.00	10.5	30.0	41.4	22.5	11.4

¹PheMI is percent presumed-hemicellulose calculated as NDF-ADF

Table 2. Apparent ruminal disappearance of selected amino acids in the In- vivo 11.

Sample ID	Total AA	Asp	Glu	Lys	Cys	Met	Trp
1	64.4	70.3	63.6	64.1	66.0	58.6	63.2
2	62.0	74.6	61.4	59.2*	63.0*	54.7	65.4
3	64.3	74.3	64.9	63.8	64.5	55.9	56.5*
4	65.8	71.3	65.5	65.4	69.6*	60.9*	70.5*
5	58.1*	67.3*	56.9*	59.9	62.8*	49.7*	59.7*
6	54.2*	65.0*	52.9*	52.2*	59.8*	44.8*	54.6*
7	72.4*	79.5*	70.9*	72.1*	74.1*	67.7*	75.0*
8	66.4	73.0	66.0*	65.4*	70.8*	58.1	70.0*
9	58.6*	68.6*	58.2*	57.5	63.5	49.1*	62.2
10	67.9*	75.3*	67.9*	68.0*	73.1*	60.7*	67.8*
11	63.1	74.8	60.1	60.7	63.5	57.2	59.0*
Mean	63.4	72.2	62.6	62.6	66.4	56.1	64.0
Std Dev	5.06	4.17	5.26	5.44	4.72	6.4	6.38
Lower CI	60.4	69.2	59.6	59.6	63.4	53.1	61.0
Upper CI	66.4	75.2	65.6	65.6	69.4	59.1	67.0

(*) Means within a column differ (P<.05)

The California ARPAS Alfalfa Study

Current forage quality and marketing systems in California are fiber based, a practice dating from the mid-nineteenth century. Alfalfa hay quality is based upon a single laboratory measurement of the content of acid detergent fiber (ADF). ADF is used in a regression equation to calculate total digestible nutrients (TDN = 82.326 - 0.7506*ADF). Variation in acid detergent fiber explains approximately 70 percent of the variation in TDN and 75 percent of that in digestible energy. When the basis for the current system was developed in the 1950s, TDN was, at best, an anachronism. By the latter part of the nineteenth century most countries, other than the USA, based their ruminant feed evaluation systems on some proxy for net energy, either starch or barley equivalents.

The use of both ADF and NDF in prediction equations has been suggested. Acid detergent fiber is a subset of NDF; if the pairwise correlation is significant (.70 or greater) multicollinearities may exist. For multiple predictor variable equations such as:

$$Y = b_0 + a_1 ADF + a_2 NDF$$

it is possible that solutions are unique to the data from which they were developed. For our data, the pairwise correlation between ADF and NDF is greater than .90, strongly suggesting that parameter estimates in multiple predictor variable equations may be biased.

In other parts of the country relative feed value (RFV) is used to estimate alfalfa quality. Neutral detergent fiber (NDF) is the predictor variable for dry matter intake (DMI) and ADF is the predictor variable for dry matter digestibility (DDM), RFV is calculated as f(DMI*DDM). Calculations of this sort may prevent some of the problems associated with multicollinearity. However, Sanson and Kercher (1996) reported that variation in ADF content accounted for only 20 percent of the variation in DDM and that the slopes of the lines for DMI=f(NDF) and RFV_{DMI}= f(RFV_{DDM}) were not different from zero. Hackman et al. (2008) also commented on the poor predictability of RFV from the RFV equation. As a tool to estimate alfalfa quality, RFV appears to be lacking. *Si non e vero e molto ben trovato*. Detergent residues are related to the extraction medium and reaction conditions, as well as plant maturity and growing conditions; therefore, prediction equations based on detergent residues may not accurately reflect the utilization of alfalfa hay in the ruminant digestive system. Graham made this comment in 1966 "... a simple but demonstrably misleading system is no substitute for a reliable though complex one. The real choice is not between unmanageable truth and facile fiction, but between science and rule of thumb." It is as true today as it was 44 years ago.

The California Chapter of the American Registry of Professional Animal Scientists (California ARPAS) has undertaken a study whose goal is to replace current fiber based methods of alfalfa hay evaluation with an alternative method of measuring the quality of alfalfa hay that will be based on a property of both feed and animal, metabolizable energy. The proposed energy-based system will include the calculation:

$$DE = \sum_{i=1}^n e_i c_i d_i$$

where:

DE = digestible energy (Mcal/kg)

e_i = ΔH_c of ith entity (Mcal/kg)

c_i = digestibility of ith entity (%)

d_i = concentration of ith entity (%)

It will have the advantage of describing feed inputs and animal requirements in the same terms and will allow for better estimates of alfalfa value. As the plane of nutrition increases, the proportion of gross energy lost as fecal energy increases. However, numerous investigations have shown that energy losses in methane and urine decrease as plane of nutrition increases. The net result is that increased losses in fecal energy are offset by decreased urine and methane energy losses; percent metabolizability (Q) is unchanged for Q of approximately .60 (Blaxter, 1967). The lack of uniqueness of solution for DE, ME or NE, in commonly used equations, based on either ADF or NDF, is apparent and indicates that some or all of the equations are incorrectly specified. Our proposed system does not have that shortcoming as items of interest are directly measured. Accurate determinations of ME or NE_g can be used to aid in pricing alfalfa hay based on estimated milk production per unit of feed consumed.

California ARPAS began this study to determine if near infrared spectrophotometry (NIRS) could be used to predict rate, site and extent of degradation of selected chemical and proximate entities in pure-stand alfalfa hay along with the content of each entity. The system must be an improvement on existing methodologies used to estimate alfalfa hay quality. More than 150 samples of alfalfa hay were collected in California during the 2008 growing season. Growing regions and stacks of hay were selected in an attempt to represent the maximum possible variability in composition. Sites included, but were not limited to, the Imperial Valley, San Joaquin Valley, the San Joaquin River-Sacramento River Delta, Sacramento Valley and the intermountain growing areas of northeastern California. Samples were analyzed on site using a portable near infrared spectrophotometer (AgrinIR, *Dinamica Generale* srl, Poggio Rusco (MN) Italy). Eleven lots of hay, two tons each, representing the expected range in ADF and crude protein contents were purchased. Subsequently, these 11 lots of hay (In-vivo 11) were used in a lamb metabolism trial conducted at the USDA Dairy Forage Research Center (USDA DFRC, Madison, WI).

The diversity in composition can be seen from the ranges shown in Table One. The USA 3000 are compositional data for 3,000 samples analyzed by Dairyland Laboratories Inc. (Arcadia, WI) during 2007. Of major interest is the apparent larger numerical range for each of the components in the In-vivo 11. These samples are more diverse, in ADF content, than those used to develop the currently used California TDN equation.

California- it ain't all LA!

