Kansas Agricultural Experiment Station Research Reports

Volume 0
Issue 10 *Swine Day (1968-2014)*

Article 256

1983

Prediction by a rapid enzymatic procedure of energy values and nitrogen digestibility of diets and feed ingredients for swine

A Hsu

G L. Allee

Recommended Citation

Hsu, A and Allee, G L. (1983) "Prediction by a rapid enzymatic procedure of energy values and nitrogen digestibility of diets and feed ingredients for swine," *Kansas Agricultural Experiment Station Research Reports*: Vol. 0: Iss. 10. https://doi.org/10.4148/2378-5977.6096

PREDICTION BY A RAPID ENZYMATIC PROCEDURE OF ENERGY VALUES AND NITROGEN DIGESTIBILITY OF DIETS AND FEED INGREDIENTS FOR SWINE

Ali Hsu and Gary L. Allee

Summary

A rapid in vitro method has been developed to predict digestibilities of nitrogen, dry matter (DM) and energy in feed ingredients and mixed diets for swine. A total of 21 samples, including nine feed ingredients and 12 mixed diets in which in vivo dry matter digestibility (DMD), nitrogen digestibility (ND), digestible engergy (DE) and metabolizable energy (ME) had been measured previously by conventional pig digestion trials were used in the in vitro assays. The in vitro procedure involved digestion of the sample with pepsin followed with amylase and pancreatin. The digestibilities of DM and protein were measured and compared to the values determined in vivo. A high correlation was found between the in vitro and in vivo results for the digestibilities of DM, nitrogen and energy. The relationships between in vivo (y) and in vitro (x) values for mixed diets were: DMD_y, % = 1.155 (DMD_x) - 3.43, (r = .92, P < .0001); ND_y, % = 2.346 (ND_x) - 134.10, (r= .85, P < .001); $(DE/GE)_y$,% = 1.151 (DMD_x) - 3.290, (r = .91, P < .0001); $(ME/GE)_y$, % = 1.161 (DMD_x) - 5.85, (r = .90, P < .0001). For feed ingredients, the relationships between in vivo (y), and in vitro (x) were: DMD_y,% = .960 (DMD_x) + 10.91, (r = .97, P < .0001); $(DE/GE)_y$, % = .897 (DMD_x) + 16.05, (r = .97, P < .0001); DE, kcal/gDM = .0416 (DMD_x) + 7.67, (r = .95, P < .0001); $(ME/GE)_y$, % = .905 (DMD_x)+ 12.81, (r = .96, P < .0001). The prediction of protein digestibility for feed ingredients, excluding alfalfa meal and meat and bone meal, was ND_y, % = 1.317 (ND_x) - 38.96, (r = .84, P < .05). These results suggest that the digestible and metabolizable energy and nitrogen digestibility of feed ingredients and diets for swine can be predicted from enzymatic procedures.

Introduction

Conventional digestion and balance trials have contributed considerable knowledge of the nutritional values of feeds, but these approaches are very time consuming and expensive. A simple, reproducible, inexpensive and rapid method to predict the nutritive value for a wide variety of feedstuffs is needed. There has been considerable interest in the development of in vitro techniques with rumen fluid , semipurified enzymes, or both for predicting dry matter digestibility (DMD) of forages for ruminants. A two-stage in vitro method has been developed using pepsin and intestinal fluid obtained from a fistulated pig to estimate the digestibilities of dry matter and protein in diets for pigs. An inherent disadvantage of this technique is the dependence on a supply of intestinal fluid, requiring access to pigs fitted with a cannula and fed under controlled conditions to minimize variation in the inoculum composition.

The objectives of the present study were to develop a rapid in vitro procedure using pepsin, pancreatin, and amylase for predicting the digestibilities of nitrogen, dry matter and energy in a range of feed ingredients and feeds in which in vivo digestibilities had been measured previously in pigs. In addition, the

relationships between in vivo and in vitro digestion of nitrogen and dry matter and the relationship between in vivo DE and in vitro DMD also were studied.

Experimental Procedures

A total of 21 samples, including nine feed ingredients and 12 mixed diets of known in vivo dry matter digestibility (DMD), nitrogen digestibility (ND), digestible energy (DE) and metabolizable energy (ME), were used to evaluate the value of an in vitro enzymatic digestion procedure. Feed ingredients evaluated were corn, sorghum grain and wheat (trial 1), alfalfa meal, wheat bran and rice bran (trial 2), soybean meal, meat and bone meal and sunflower meal (trial 3). Feed ingredients were substituted for the basal diets at 50, 30 and 20% in trial 1, 2 and 3, respectively. The DMD, ND, DE and ME were determined for the test feed ingredients (table 1) and then were measured by difference between the basal diet and the basal diet plus the test feed ingredient (table 2).

The in vitro procedure involved digestion of the samples with pepsin followed with pancreatin and amylase. All samples for in vitro analysis were done in duplicate. A sample, 1.1 g of air-dried material, ground to pass through a 1 mm screen or .5 mm screen, was weighed into a 125 ml Erlenmeyer flask, dried 24 hr at 65 C, cooled and weighed. Twenty ml of .2% pepsin[1] (activity; 1200-2000 units per g from porcine stomach mucosa) in .075 N hydrochloric acid was added to the flask and shaken at 80 oscillations/min for 2 hr in a 37 C precision shaking water bath. After neutralization with 7.1 ml of .2 N sodium hyroxide, 80 mg pancreatin[2] (grade III from porcine) and 40 mg amylase[3] (type VI from porcine pancrease) were added in 20 ml of .1M phosphate buffer at pH 7.6. The digestion mixtures were incubated and shaken at 100 oscillations/min for an additional 6 hr at 37 C in a water bath. At the end of incubation, the contents of the flask were transferred with 20 ml distilled water to a 250 ml centrifuge bottle and centrifuged immediately for 15 min at 1880 g in Beckman (Model J) centrifuge. The supernatant was discarded and the insoluble residues were washed with water (3 x 75 ml), centrifuged and the supernatant removed by careful aspiration after each washing. The residue was dried at 65 C for 24 hr, cooled, weighed and the indigestible residue calculated. The percent residue remaining was called in vitro dry matter digestibility (DMD). The residue was quantitatively transferred to a Kjeldahl flask for nitrogen determination. The calculation is as follows: nitrogen digestibility (%) = (N in feeds - N in indigested fragment)/ (N in feeds) x 100.

The relationships between in vivo and in vitro digestion of nitrogen and dry matter and energy were computed with linear regression equations.

---

*[1]Sigma Chemical Company, Saint Louis, MO

Results and Discussion

From the conventional digestion and balance trials, a high correlation (r = .97) was found between dry matter digestibility (DMD) and energy digestibility (DE/GE) or metabolizable energy expressed as percentage of gross energy (ME/GE) in the diets (table 3). A high correlation was found between in vitro and the conventional in vivo results for the digestibilities of dry matter, nitrogen and energy in the diets (table 4). The correlation coefficient between in vivo and in vitro DMD was .92 with a regression line ($DMD_{vivo} = 1.155\ DMD_{vivo} - 3.432$) (figure 1). In vitro DMD also was closely correlated to in vivo energy digestibility (DG/GE, %) (figure 2) and metabolizable energy expressed as percentage of gross energy (ME/GE, %) (figure 3). The correlation coefficient between in vitro DMD and in vivo energy value was higher when energy value was expressed as the percentage of gross energy instead of kcal/g DM in the diets. The correlation coefficient was .85 for ND in diets with .5 mm particle size (table 4).

A high correlation was found between the in vitro DMD and the conventional in vivo procedures for the digestibilities of DM and energy, suggesting that this in vitro procedure can be used to predict the energy value of feed ingredients. The correlation coefficient for energy digestibility $(DE/GE)_y$ was .97 with a correlation line $(DE/GE)_y$, % = .897 DMD_x + 16.05 (figure 4). The correlation coefficient for dry matter digestibility, digestible energy and metabolizable energy were .97, .95 and .94, respectively (table 6). The correlation coefficient for digestible energy was .95 with a correlation line for DE_y kcal/g DM = .0416 (DMD_x + 7.67 ($P < .0001$). Reducing particle of the sample increased in vitro nitrogen digestibility (table 7). Increasing particle size from .5 mm (table 4 and 6) to 1.0 mm (table 5 and 8), using in vitro digestion, had little effect on correlation coefficient or residual standard deviation but resulted in the different regression equations. Alfalfa meal had the lowest digestibility in dry matter and nitrogen when added to diets (table 9) and among feed ingredients (table 10). For predicting nitrogen digestibility (ND) in feed ingredients, the correlation coefficient between in vitro and in vivo was increased from .62 to .84, respectively, when alfalfa meal and meat and bone meal were excluded. This might be due to the high ash contained in alfalfa meal (10.23%) and meat and bone meal (27.31%). The regression line between nitrogen digestibility by in vivo (ND_y) and in vitro (ND_x) were $ND_y = 1.317\ ND_x - 38.96$ and $PD_y = 1.090\ PD_x - 17.64$ (r^2= .84, $P < .05$) with particle size .5 mm and 1.0 mm, respectively, used in in vitro digestion (table 8).

These results suggest that the digestible and metabolizable energy and nitrogen digestibility of feed ingredients and diets for swine can be predicted from in vitro enzymatic procedures.

Table 1. Nitrogen Digestibility and Energy Value of Feed Ingredients

Feed ingredient	DMD	ND	GE	DE	ME
	%		kcal/g DM		
Corn	88.99	77.66	4.66	4.12	4.05
Sorghum	88.08	70.90	4.63	4.07	4.02
Wheat	88.95	89.54	4.62	4.12	4.04
Alfalfa meal	49.50	55.16	4.44	2.08	1.98
Wheat bran	60.42	72.60	4.60	2.85	2.75
Rice bran	61.12	67.16	5.08	3.30	3.25
Soybean meal	87.05	90.05	4.68	4.03	3.79
Meat & bone meal	54.20	81.95	4.61	2.85	2.68
Sunflower meal	64.26	83.67	4.66	3.06	2.78

Table 2. Nitrogen Digestibility and Energy Value of Diets[a]

Diet			DMD	ND	GE	DE	ME
Number	Feed	Feed ingredient	%		kcal/g DM		
Trial 1.							
A	Basal 1		86.2	88.7	4.45	3.89	3.81
B	50% B 1 +	corn	87.6	85.2	4.62	3.98	3.90
C	50% B 1 +	sorghum	87.1	82.7	4.61	4.04	3.97
D	50% B 1 +	wheat	87.6	88.9	4.55	4.01	3.94
Trial 2.							
E	Basal 2		84.6	82.3	4.40	3.72	3.65
F	70% B 2 +	alfalfa meal	73.6	74.1	4.55	3.33	3.25
G	70% B 2 +	wheat bran	77.2	79.2	4.57	3.54	3.46
H	70% B 2 +	rice bran	77.3	78.2	4.66	3.65	3.57
Trial 3.							
I	Basal 3		86.2	82.6	4.28	3.61	3.54
J	80% B 3 +	soybean meal	86.4	85.7	4.35	3.69	3.59
K	80% B 3 +	meat & bone meal	79.6	82.3	4.38	3.49	3.39
L	80% B 3 +	sunflower meal	81.8	83.0	4.36	3.51	3.40

[a] Each value is the mean for six pigs.

Table 3. Simple Correlation Coefficients Between Dry Matter and Nitrogen Digestibilities and Energy Value of Diets[a]

DMD	ND	DE	ME	DE/GE	MG/GE
%		kcal/g DM		%	
1.00***	.82***	.82**	.81**	.98***	.97***

[a] All data measured by in vivo method.
* P < .01
*** P < .001

Table 4. Prediction of Nitrogen Digestibility and Energy Value of Diets (.5 mm particle size)

In vivo	In vitro	Intercept	r	RSD
DMD_y, %	= 1.155 (DMD_x)	- 3.432	.92****	2.00
ND_y, %	= 2.346 (ND_x)	- 134.10	.85***	2.31
DE_y, kcal/g DM	= .0504 (DMD_x)	- .063	.85***	.126
ME_y, kcal/g DM	= .0508 (DMD_x)	- .180	.83***	.136
$(DE/GE)_y$, %	= 1.151 (DMD_x)	- 3.29	.91****	2.11
$(ME/GE)_y$, %	= 1.161 (DMD_x)	- 5.85	.90****	2.24

*** P < .001
**** P < .0001

Table 5. Prediction of Nitrogen Digestibility and Energy Value From In Vitro Nitrogen and Dry Matter Digestibilities of Diets (1 mm particle size)

In vivo[a]	In vitro	Intercept	r	RSD
DMD_y, %	= 1.158 (DMD_x)	- .78	.88****	2.40
ND_y, %	= 1.620 (ND_x)	- 65.39	.72**	3.06
DE_y, kcal/g DM	= .0559 (DMD_x)	- .339	.90****	.103
ME_y, kcal/g DM	= .0567 (DMD_x)	- .474	.89****	.113
$(DE/GE)_y$, %	= 1.199 (DMD_x)	- 3.82	.91****	2.16
$(ME/GE)_y$, %	= 1.216 (DMD_x)	- 6.89	.90****	2.23

[a] Abbreviation as in table 4.
** P < .01
**** P < .0001

Table 6. Prediction of Nitrogen Digestibility and Energy Value From In Vitro Nitrogen and Dry Matter Digestibilities of Feed Ingredients[a] (.5 mm particle size)

In vivo[b]	In vitro	Intercept	r	RSD
ND_y, %	= 1.317 (ND_x)	- 38.96	.84*	5.47
DMD_y, %	= .960 (DMD_x)	+ 10.91	.97****	3.91
DE, kcal/g DM	= .0416 (DMD_x)	+ 7.67	.95****	.25
ME, kcal/g DM	= .0419 (DMD_x)	+ .620	.94***	.28
$(DE/GE)_y$, %	= .897 (DMD_x)	+ 16.05	.97****	4.23
$(ME/GE)_y$, %	= .905 (DMD_x)	+ 12.81	.96****	4.89

[a] Nine feed ingredients with the exception of ND, which excludes alfalfa meal and meat and bone meal.
[b] Abbreviation as in table 4.
* P < .05
*** P < .001
**** P < .0001

Table 7. Effect of Particle Size on In Vitro Digestion

Feeds	Particle size .5 mm	Particle size 1 mm	SE	CV[c]
Diet				
Dry matter digestibility, %[a]	74.79	72.28	1.41	1.90
Nitrogen digestibility, %[a]	92.39	91.50	.88	.96
Feed ingredient				
Dry matter digestibilitiy, %[b]	63.23	63.04	1.13	1.79
Nitrogen digestibility, %[a]	87.53	86.38	1.12	1.27

[a] $P < .0001$
[b] $P > .95$
[c] CV = coefficient of variation = standard error/least-square mean.

Table 8. Prediction of Nitrogen Digestibility and Energy Value From In Vitro Nitrogen and Dry Matter Digestibilities of Feed Ingredients[a] (1 mm particle size)

In vivo[b]	In vitro	Intercept	r	RSD
ND_y, %	= 1.090 (ND_x)	- 17.64	.84*	5.50
DMD_y, %	= 1.027 (DMD_x)	+ 6.46	.97****	4.03
DE_y, (kcal/g DM)	= .0441 (DMD_x)	+ .600	.94***	.276
ME_y, (kcal/g DM)	= .0442 (DMD_x)	+ .467	.92***	.315
$(DE/GE)_y$, %	= .952 (DMD_x)	+ 12.36	.96****	4.77
$(ME/GE)_y$, %	= .956 (DMD_x)	+ 9.43	.94****	5.65

[a] Nine feed ingredients with the exception of ND which excludes alfalfa meal and meat and bone meal.
[b] Abbreviation as in table 4.
* $P < .05$
*** $P < .001$
**** $P < .0001$

Table 9. In Vitro Nitrogen and Dry Matter Digestibilities of Diets

Diet Number	Feed	Feed ingredient	Digestibility, % Dry matter[a]	Nitrogen[b]
A	Basal 1		73.39	92.61
B	50% B1 + corn		76.61	92.55
C	50% B1 + sorghum		78.25	92.38
D	50% B1 + wheat		78.60	93.82
E	Basal 2		74.76	92.76
F	70% B2 + alfalfa meal		66.18	89.11
G	70% B2 + wheat bran		69.92	89.63
H	70% B2 + rice bran		71.56	91.18
I	Basal 3		73.67	91.71
J	80% B3 + soybean meal		77.19	94.26
K	80% B3 + meat & bone meal		69.90	90.67
L	80% B3 + sunflower meal		72.39	92.54

[a]Least square mean with standard error = .58

[b]Least square mean with standard error = .36

Table 10. In Vitro Nitrogen and Dry Matter Digestibilities of Feed Ingredients

Feed ingredient	Digestibility, % Dry matter[a]	Nitrogen[b]
Corn	78.15	90.87
Sorghum	77.90	85.75
Wheat	85.18	93.68
Alfalfa meal	42.79	82.39
Wheat bran	46.01	78.23
Rice bran	53.12	83.85
Soybean meal	76.18	95.44
Meat & bone meal	47.93	78.72
Sunflower	60.93	93.65

[a]Least square mean with standard error = .51.

[b]Least square mean with standard error = .50.

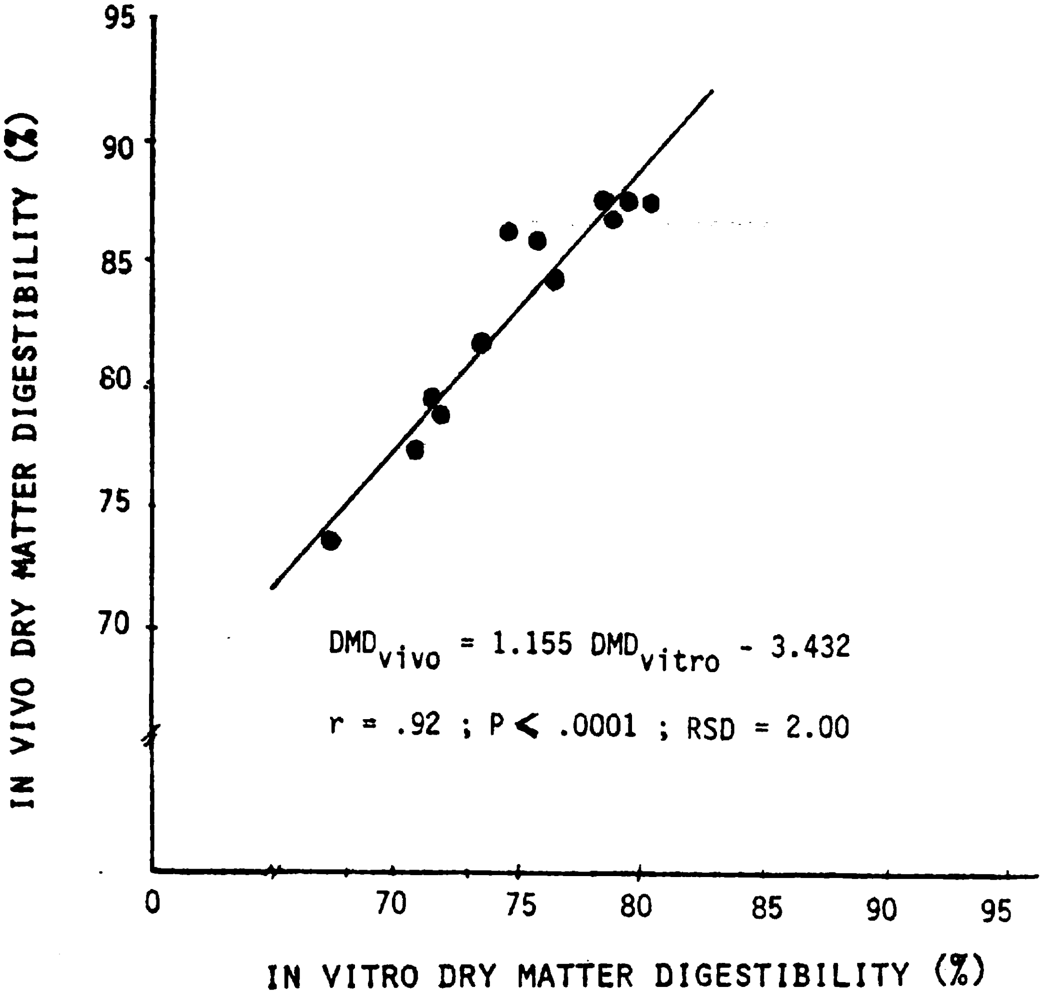

Figure 1. Relationship between in vivo and in vitro dry matter digestibility (DMD) in diets.

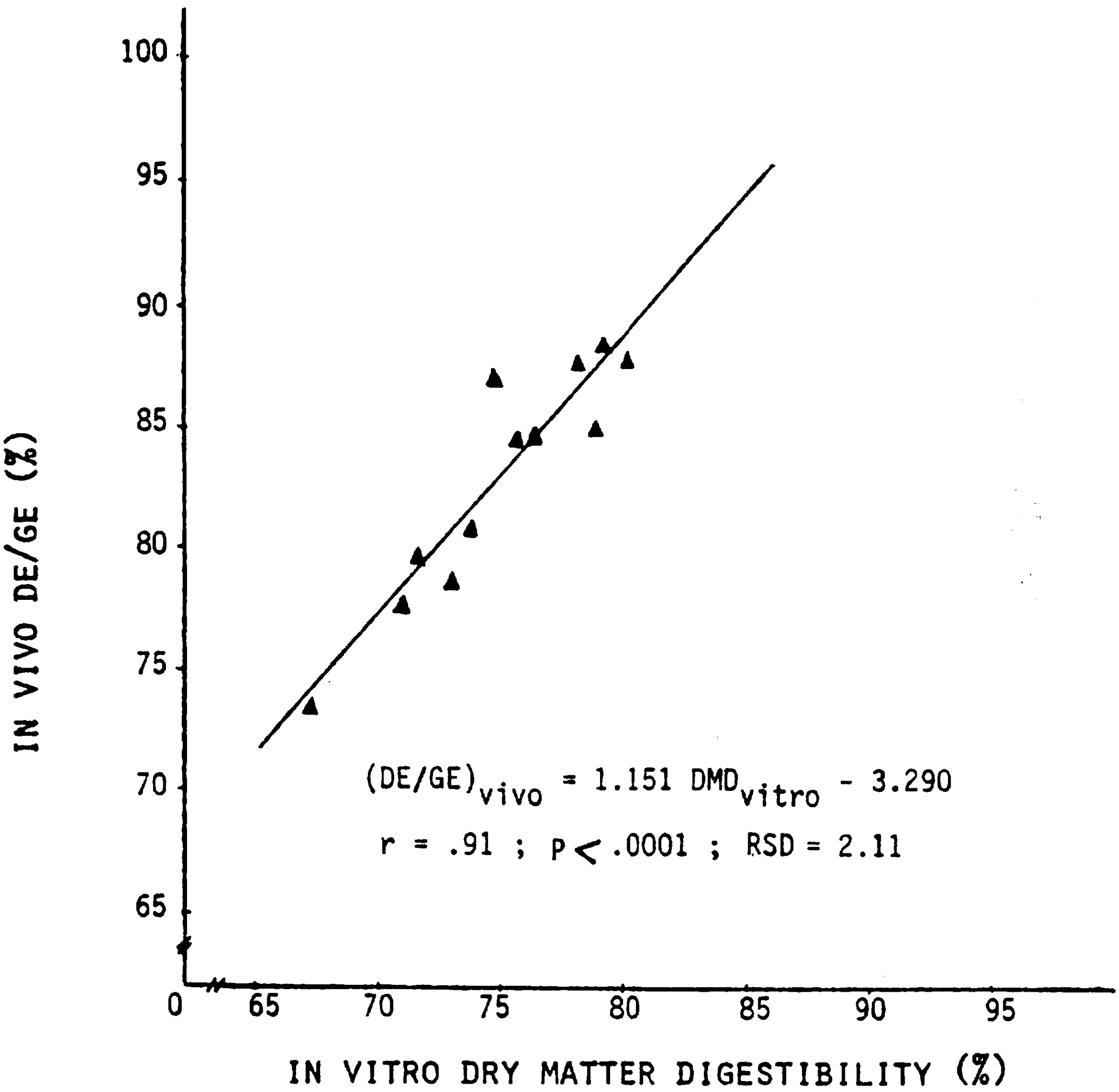

Figure 2. Relationship between in vivo energy digestibility (DE/GE) and in vitro dry matter digestibility (DMD) in diets.

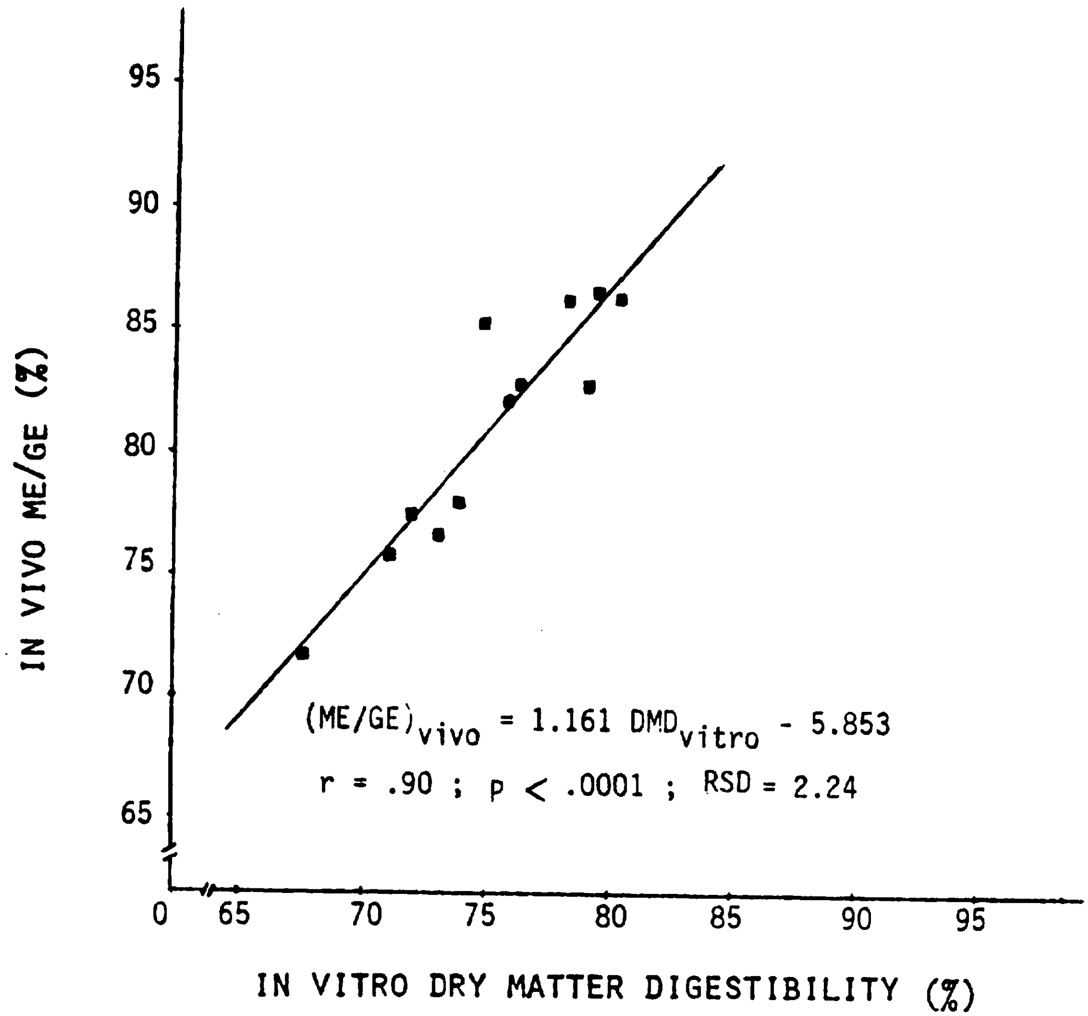

Figure 3. Relationship between in vivo metabolizable energy as percentage of gross energy (ME/GE) and in vitro day matter digestibility in diets.

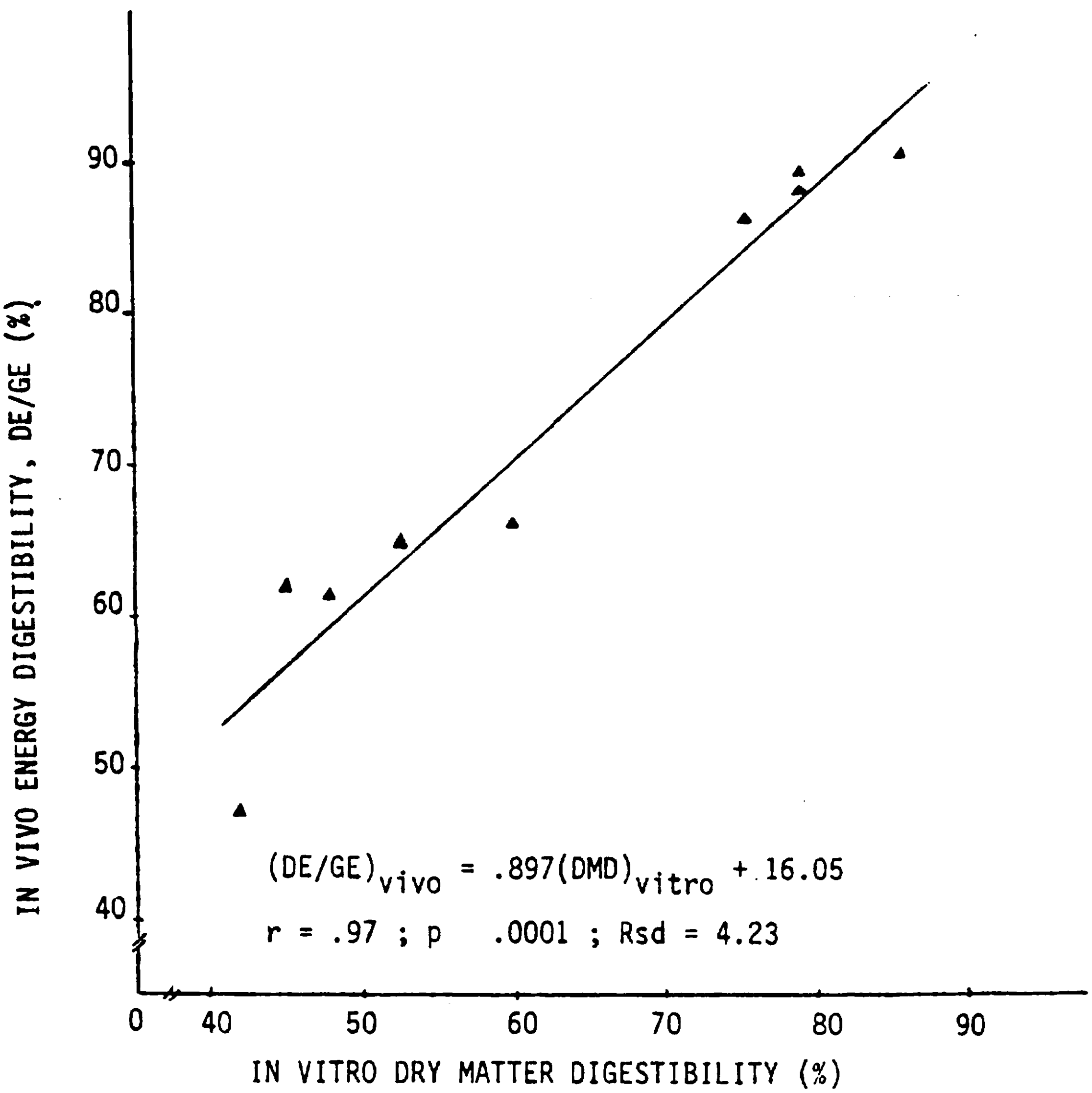

Figure 4. Relationship between in vivo energy digestibility (DE/GE) and in vitro dry matter digestibility in feed ingredients.