Comparison of *in vitro* and *in situ* techniques for estimating protein degradability of selected feedstuffs

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Abstract The objective of this study was to estimate the protein degradability parameters of 6 common ruminant feedstuff using the nylon bags (*in situ*) and the innovative *in vitro* method-the Daisy^{II} incubator. Significant differences were observed between the *in situ* and *in vitro* methods in estimation of the rapidly soluble (a) fraction (P<0.01), except for soybean meal. The estimation method significantly affected the potentially degradable protein (b) fraction of barley grain, corn grain, alfalfa hay, and corn silage (P<0.05). The fractional rate constant (parameter c) for the disappearance of fraction b (/h) was not affected by the degradability determination method. The estimated b fraction, parameter c, potential of degradability (PD) and effective degradability (ED) in the *in vitro* method were highly correlated with the estimated values by the *in situ* method (P<0.01). In conclusion, results showed that the *in vitro* Daisy^{II} method can be used for estimating some protein degradability parameters such as parameter c, PD and ED but not for others (a, b and lag time). In addition, the ability of Daisy^{II} method to estimate some parameters was better for some feeds (soybean meal, canola meal and corn grain) than for others (barley grain, alfalfa hay and corn silage).

Keywords: ruminants, nutritive evaluation, nylon bag method, Daisy apparatus

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Introduction

Feed protein is, to a large extent, degraded in the rumen and ruminal protein degradability is one of the most important qualitative factors determining the protein value of a feed (Hvelplund and Weisbjerg, 2000). The *in situ* technique is one of the most practical methods for evaluation of ruminant feed proteins. This technique allows the estimation of feed protein degradability in the rumen and involves suspending the bags containing feeds in the rumen and measuring nutrient disappearance at various time intervals (Stern et al., 1997). Besides protein degradability, the method has been used to determine rumen degradability of dry matter (DM), organic matter, neutral detergent fiber (NDF) (Stensig et al., 1997) and starch (Tamminga et al., 1990).

Current feeding systems recognize the need to select feeds based on the supply of ruminal degradable and undegradable protein (AFRC, 1993; NRC, 2001; Van Amburghet al., 2015) and most systems have used *in situ* data to develop feed tables. Determination of protein degradation in the rumen using this technique is expensive, labor intensive and time-consuming for industry and field application (Calsamiglia et al., 2000). This technique involves the use of ruminally fistulated animals that, in addition to the costs of surgical operation, it entails removing the animals from the production cycle and thus greater costs. Therefore, any attempts at developing an alternative method for the *in situ* technique that eliminates the use of fistulated animals will be valuable.

It has been shown that there is substantial variation among and within laboratories on estimates of protein degradation obtained with *in situ* technique (Madsen and Hvelplund, 1994; Wilkerson et al., 1995) and the lack of standardization of the *in situ* protocol is the major source of variation (Vanzant et al., 1998). To take full practical advantage of the new feeding systems, it is necessary to develop techniques that allow fast, affordable, reliable and accurate measurements of ruminal degradation and intestinal digestion of proteins. These techniques need to be validated with true estimates of protein degradability and digestibility, but these measurements are difficult to obtain in vivo (Stern et al., 1997). Therefore, the use of values obtained with the *in situ* technique provides the most valuable data to validate the *in vitro* degradability data (Calsamiglia et al., 2000; Belanche et al., 2014).

Many efforts have been made for estimating degradability parameters by alternative methods instead of *in*

situ nylon bag technique (Graham and Aman, 1984; Aufrère et al., 1991; Susmel et al., 1993; Varel and Kreikemeier, 1995). These studies were mostly based on the use of pure commercial enzymes especially protease enzymes of *Streptomyces griseus* (Aufrère et al., 1991 and Susmel et al., 1993), buffers such as phosphate buffer (Susmel et al., 1993), buffered ruminal fluid by the method of Tilly and Terry (Varel and Kreikemeier, 1995), using whole or centrifuged rumen fluid (Susmel et al., 1993) and by *in vitro* gas production technique (Karlsson et al., 2009; Krizsan et al., 2013).

Varel and Kreikemeier (1995), using the method of Tilly and Terry, estimated the degradability parameters of alfalfa and bromegrass NDF. Their results indicated that the lag time was 3.5 ± 0.3 h less, rate was 0.03 ± 0.002 /h faster, and the extent of degradation was 6.0 ± 0.5% greater for the *in situ* than for the *in vitro* method. Trujillo et al. (2010) used the Daisy^{II} method to estimate DM and NDF degradation kinetics of some fibrous feedstuffs. To our knowledge, no study has attempted to estimate protein degradability parameters by the Daisy^{II} apparatus. Therefore, the objective of this study was to estimate the degradability of several feeds by using the Daisy^{II} apparatus as an *in vitro* alternative method and compare the estimates with the *in situ* values.

Materials and methods

The experiment was conducted using six common ruminant feeds including soybean meal, canola meal (as representatives of plant protein sources), barley and corn grain (as representatives of grain sources), and alfalfa hay and corn silage (as representatives of forage sources). A sample of each feed was ground through a Wiley mill (1-mm screen) and analyzed for dry matter (DM, method 930.15), ash (method 924.05), crude protein (CP, Kjeldahl N×6.25, method 984.13), and ether extract (EE, method 920.39) according to the procedures of AOAC (1990). Neutral detergent fiber (NDF) and acid detergent fiber (ADF) were analyzed by the procedure of Van Soest et al. (1991). The NDF content was assayed without a heat stable amylase and sodium sulfite. Both NDF and ADF were expressed as inclusive residual ash (Table 1).

In situ experiment

Ruminal degradability of feed protein was measured using three rumen-fistulated Moghani rams (55 ± 4 kg body weight). Rams were fed a total mixed ration with forage to concentrate ratio of 60:40 that was balanced by sheep CNCPS software (version 1.0.21) for 10 percent above maintenance requirements (AFRC, 1992; Vanzant et al., 1998). The ingredients and chemical composition of the diet are presented in Table 1 and 2, respectively. Rams were housed in individual pens (2.0 × 1.2 m) and fed twice daily at 0800 and 1800. For the *in situ* experiment, feed samples were ground through a Willey mill (2-mm screen) and triplicate sub-samples (3 g per bag) were incubated in heat-sealed polyester bags (7 × 10 cm, 52 ± 5 µm pore size, Gol Pooneh Safahan, Isfahan, Iran) for 0, 2, 4, 8, 16, 24 and 48 h in the case of concentrate feeds, and for 0, 8, 16, 24, 48 and 72 h in the case of forage feeds (AFRC, 1992).The bags were simultaneously inserted in the rumen immediately after the morning meal for three consecutive periods and removed sequentially at end of each incubation time. The ratio of sample size to bag surface area was about 21 mg per cm^2. After incubation, the bags were immediately rinsed in cold water and washed in a commercial washing machine for 21 minutes with two gaps (three 7 minutes cycles). The 0 h bags were not incubated in the rumen but followed the same washing procedure. The bags were then dried in a forced-air oven at 65 °C for 48 h to determine DM disappearance and the residues were analyzed for crude protein content (AOAC, 1990; Kjeldahl N×6.25, method 984.13).

In vitro experiment

The *in vitro* degradability was determined by using the ruminal digestion stage (first stage) of *in vitro* DM digestibility determination by Daisy^{II} apparatus (Holden, 1999). For removing any variation from sample preparations, the procedure was the same as that used for the *in situ* experiment. Ruminal fluid was collected from

Table 1. Ingredients and chemical composition of the diet

Ingredients (g/kg DM)	
Alfalfa hay	600
Barley grain	240
Canola meal	98
Wheat bran	47
Salt	3
Calcium carbonate	3
Mineral and Vitamin premix[1]	9
Chemical composition	
Metabolizable energy (Mcal/kg DM)	2.24
Crude protein (g/kg DM)	120
Ether extract (g/kg DM)	26
Neutral detergent fiber (g/kg DM)	334
Acid detergent fiber (g/kg DM)	243
Calcium (g/kg DM)	7
Phosphorous (g/kg DM)	4

[1]Contained 195 g/kg calcium, 80 g/Kg phosphorous, 21 g/Kg magnesium, 1000 mg/Kg cobalt, 300 mg/Kg copper, 120 mg/Kg iodine, 3000 mg/Kg iron, 2200 mg/Kg manganese, 3000 mg/Kg zinc, 1.1 mg/Kg selenium, 600000 IU/Kg vitamin A, 200000 IU/Kg vitamin D, 2000 mg/Kg vitamin E, 2500 mg/Kg antioxidant.

Table 2. Chemical composition of test feedstuffs (g/kg DM)

	Soybean meal	Canola meal	Barley grain	Corn grain	Alfalfa hay	Corn silage
DM	918	905	878	859	895	308
CP	485	366	112	89	137	82
EE	19	17	21	36	23	31
NDF	175	270	195	103	552	600
Ash	69	78	43	35	105	85

three rams immediately after slaughtering (slaughterhouse of Ardebil city, Iran), mixed and approximately 2 liters of pooled ruminal fluid and 300 g of ruminal particulate matter per Daisy^{II} digestion bottle were transported to the laboratory in a pre-warmed container. Preparation of the inocula included blending the ruminal fluid and particulate matter for 2 min in a blender, followed by filtering through four-layer cheesecloth with constant purging with CO_2. Buffered ruminal fluid was prepared as described by Holden (1999). The buffer solution was made just prior to each digestion run by warming solutions A and B to 39 °C and adding 20 mL of solution B to 1 L of solution A. The pH of the buffer solution was adjusted to 6.8 (if needed) by adding small additional amounts (1 to 2 mL) of solution B. The reagents used were: solution A (10 g of KH_2PO_4, 0.5 g of $MgSO_4.7H_2O$, 0.5 g of NaCl, 0.1 g of $CaCl_2 \cdot H_2O$ and 0.5 g of urea in 1 L of deionized water) and solution B (15 g of Na_2CO_3 and 1 g of $Na_2S.9H_2O$ in 100 ml of deionized water) (Holden, 1999). Overall, each digestion bottle was filled with 1440 mL buffer solution and 360 mL rumen fluid (1800 mL in total).

For each incubation time, 5 polyester bags (4 × 6 cm; 52 ± 5 µm pore size, Gol Pooneh Safahan, Isfahan, Iran) were filled with 1 g of each of the 6 feed samples, heat sealed, placed in 2 L digestion bottles that were filled with buffered ruminal fluid and incubated in the Daisy^{II} apparatus at 39°C for incubation times similar to the *in situ* method. A thread was used for attaching bags to each other at approximately 5-cm distance between them. Thereafter, the bags were inserted and hanged in the digestion bottles with the end of each thread remaining out of the bottles. For simulating ruminal condition, the feeds samples were placed in each digestion bottle because Holden (1999) showed that there were no significant differences in using the Daisy^{II} method either with the same feeds or with different feeds in a digestion bottle. The number of bags in each digestion bottle was not more than 20 bags per bottle. At each incubation time, the door of the digestion bottle was opened, the threaded bags of that incubation time were taken out and the door of the digestion bottle was closed again. These procedures were conducted under constant purging with CO_2. The bags of three incubation times were inserted in each bottle, and the opening and reclosing cycles for each bottle were not more than two occasions. Overall digestion process of *in vitro* method for the test feeds was performed in two runs by two Daisy^{II} apparatuses at the same time. The 0 h bags were not incubated in the bottles, but the same washing procedure similar to the *in situ* experiment was used. After incubation, the bags were immediately rinsed, dried, weighed and analyzed for CP content.

Statistical analysis

The kinetic parameters of protein disappearance were estimated by the Fitcurve software for both methods (Chen, 1995) by fitting the modified model of Orskov and McDonald (1979) by McDonald (1981): $P = a + b\,(1-e^{-c\,(t-L)})$, if $t \geq L$, Where P is the proportion of protein that disappears at time t, *a* is the fraction of rapidly solubilized protein, *b* is the fraction of potentially degradable protein , *c* is the fractional rate constant for the disappearance of fraction *b* (/h), t is the time of incubation (h) and L is the lag time.

Data were analyzed by the GLM procedure of SAS (2003) according to the completely randomized complete design using the following model: $y_{ij} = \mu + T_i + e$, where y was the dependent variable, µ was overall mean, T_i was the estimation method effect and e was random error. Comparison between methods for each parameter was done by LSMEANS and the significant differences were declared at P<0.05. Also, the Pearson correlation coefficients between *in situ* and *in vitro* methods for estimating the degradability parameters were calculated by the CORR procedure of SAS (2003) and P<0.05 was considered as the significance level.

Results

Significant differences were observed between the *in situ* and *in vitro* methods in the estimation of the rapidly soluble (*a*) fraction for all feeds (P<0.01), except for soybean meal (Tables3, 4 and 5). The estimation method significantly affected the potentially degradable protein (*b*) fraction of barley grain, corn grain, alfalfa hay (P<0.01) and corn silage (P<0.05), with the *in situ* method resulting in smaller values. But, the *b* fraction of soybean meal and canola meal was not significantly

Table 3. Estimated degradability parameters of plant protein sources by *in situ* and *in vitro* methods

	in situ	*in vitro*	SEM	P-value[1]
Soybean meal				
a[2]	0.21	0.20	0.02	ns
b[3]	0.77	0.80	0.02	ns
c[4] (/h)	0.059	0.063	0.001	ns
Lag time (h)	2.20	2.27	0.39	ns
PD[5]	0.99	1.0	0.01	ns
ED[6] $_{(0.05)}$	0.64	0.65	0.01	ns
RSD[7]	3.39	7.34	0.01	**
Canola meal				
a	0.28	0.22	0.01	**
b	0.61	0.66	0.02	ns
c (/h)	0.058	0.060	0.002	ns
LT (h)	0.00	1.33	0.002	*
PD	0.89	0.88	0.01	ns
ED $_{(0.05)}$	0.60	0.58	0.01	*
RSD	2.44	4.85	0.51	*

[1]ns: not significant, *P≤0.05, **P≤0.01.
[2] the fraction of rapidly solubilized protein.
[3] the fraction of potentially degradable protein.
[4] the fractional rate constant for the disappearance of fraction b (/h) with the time, t.
[5] potential of degradability.
[6] effective degradability at the passage rate 0.05 (/h), respectively.
[7]residual standard deviation.

Table 4. Estimated degradability parameters of grain concentrates by *in situ* and *in vitro* methods

	in situ	*in vitro*	SEM	P-value[1]
Barley grain				
a[2]	0.55	0.22	0.01	**
b[3]	0.36	0.70	0.01	**
c[4] (/h)	0.16	0.21	0.02	ns
Lag time (h)	0.00	0.60	0.04	**
PD[5]	0.94	0.92	0.003	**
ED[6] $_{(0.05)}$	0.84	0.78	0.01	*
RSD[7]	3.88	3.67	0.90	ns
Corn grain				
a	0.26	0.23	0.01	**
b	0.63	0.68	0.01	**
c (/h)	0.066	0.073	0.003	ns
LT (h)	0.00	0.60	0.11	*
PD	0.89	0.90	0.003	0.08
ED $_{(0.05)}$	0.62	0.63	0.01	ns
RSD	3.43	3.72	0.24	ns

[1] ns: not significant, *P≤0.05, **P≤0.01.
[2] the fraction of rapidly solubilized protein.
[3] the fraction of potentially degradable protein.
[4] the fractional rate constant for the disappearance of fraction b (/h) with the time, t.
[5] potential of degradability.
[6] effective degradability at the passage rate 0.05 (/h), respectively.
[7] residual standard deviation.

Table 5. Estimated degradability parameters of forages by *in situ* and *in vitro* methods

	in situ	*in vitro*	SEM	P-value[1]
Alfalfa hay				
a[2]	0.38	0.20	0.01	**
b[3]	0.44	0.61	0.01	**
c[4] (/h)	0.077	0.075	0.003	ns
Lag time (h)	0.00	3.77	0.08	**
PD[5]	0.82	0.81	0.001	**
ED[6]$_{(0.05)}$	0.65	0.58	0.001	**
RSD[7]	1.41	1.67	0.30	ns
Corn silage				
a	0.40	0.27	0.02	**
b	0.30	0.41	0.02	*
c (/h)	0.048	0.057	0.003	ns
LT (h)	0.00	3.60	0.18	**
PD	0.69	0.69	0.01	ns
ED $_{(0.05)}$	0.54	0.50	0.01	*
RSD	1.43	1.73	0.80	ns

[1] ns: not significant, *P≤0.05, **P≤0.01.
[2] the fraction of rapidly solubilized protein.
[3] the fraction of potentially degradable protein.
[4] the fractional rate constant for the disappearance of fraction b (/h) with the time, t.
[5] potential of degradability.
[6] effective degradability at the passage rate 0.05 (/h), respectively.
[7] residual standard deviation

affected by the method. For all feeds, the fractional rate constant (parameter c) for the disappearance of fraction b (/h) was not affected by the degradability determination method.

Excepting soybean meal, the incubation of feeds by *in vitro* method resulted in longer lag time than the *in situ* method (P<0.01). The method of degradability determination had significant effect on the estimation of degradability potential (PD) of barley grain and alfalfa hay (P<0.01), but not for other feeds. The *in situ* method gave significantly higher values for effective degradability (ED) at the passage rate of 0.05 h^{-1} than *in vitro* method for canola meal, barley grain, corn silage (P<0.05) and alfalfa hay (P<0.01).

When data for all feeds were pooled, there were significant differences between *in situ* and *in vitro* methods in estimated *a* fraction, lag time (P<0.01) and the b fraction (P<0.05). The methods did not affect other parameters (Table 6).

The estimated *b* fraction, parameter *c*, PD and ED by the *in vitro* method were highly correlated with the estimated values by the *in situ* method (P<0.01). But, the correlation coefficient between the two methods for the estimation of fraction *a*, lag time and RSD was low and non-significant (Table 7).

Discussion

In the present study, ruminal fluid for *in vitro* method was taken from slaughterhouse materials to estimate degradation parameters but not from fistulated animals. In most feeds, the *in vitro* method resulted in a lower *a* fraction and longer lag time than the *in situ* method. These results indicated slow start of degradation process in the *in vitro* method that was predictable because of lower concentration of microorganisms in the *in vitro* inocula as reported earlier by others (Varel and Kreikemeier, 1995; Trujillo et al., 2010). In the present study, the ruminal fluid was mixed with buffer solution at a ratio of 1 to 4 (Holden, 1999) that resulted in lower concentration of microorganisms in the digestive bottles of Daisy^{II} at the initiation of degradation process. Other factors that may explain the higher *a* fraction obtained by the *in situ* are larger particle loss due to physical pressure exerted on bags by rumen contractions and faster rate of rumen liquor flow through the bags (Trujillo et al., 2010). In addition, lower microbial degradation in the bottles of Daisy^{II} method may be partly attributed to ruminal microorganisms exposed to improper temperature and aerobic condition, even for short time, during the inoculum preparation that may influence microbial degradation ability, particularly at early incubation times (Varel and Kreikemeier, 1995, Trujillo et al., 2010). This effect may be more important for feeds with faster degradation rate because when the data on barley grain were excluded and the correlation of two methods for estimation of the *a* value recalculated, the correlation

Table 6. Pooled estimates of the degradability parameters of feedstuffs by *in situ* and *in vitro* methods

	in situ	*in vitro*	SEM	P-value[1]
a[2]	0.35	0.21	0.02	**
b[3]	0.52	0.64	0.03	*
c[4] (/h)	0.078	0.090	0.02	ns
Lag time (h)	0.37	2.03	0.27	**
PD[5]	0.87	0.86	0.02	ns
$ED^{6}_{(0.05)}$	0.65	0.62	0.02	ns
RSD[7]	2.66	3.85	0.42	0.06

[1] ns: not significant, *P≤0.05, **P≤0.01.
[2] the fraction of rapidly solubilized protein.
[3] the fraction of potentially degradable protein.
[4] the fractional rate constant for the disappearance of fraction b (/h) with the time, t.
[5] potential of degradability.
[6] effective degradability at the passage rate 0.05 (/h), respectively.
[7] residual standard deviation.

Table 7. Correlation coefficients between estimated degradability parameters by *in situ* and *in vitro* methods

a[1]	b[2]	c[3]	Lag time	PD[4]	$ED^{5}_{(0.05)}$	RSD[6]
0.22	0.78**	0.89**	0.10	0.98**	0.94**	0.29

[1] the fraction of rapidly solubilized protein.
[2] the fraction of potentially degradable protein.
[3] the fractional rate constant for the disappearance of fraction b (/h) with the time, t.
[4] potential of degradability.
[5] effective degradability at the passage rate 0.05 (/h), respectively.
[6] residual standard deviation.
*P≤0.05, **P≤0.01.

coefficient was improved (r=0.47) and tended to be significant (P=0.07). Among the test feeds, close estimations of the *a* value by *in vitro* method compared to *in situ* results were observed for soybean meal, canola meal and corn grain. Based on the present results, it seems that the *in vitro* Daisy[II] method was not a suitable method for estimating the *a* value in feeds with very fast (barley grain) or very low (forages) degradability in the rumen. Terramoccia et al. (1992) estimated the protein degradability parameters of 8 feeds using the protease enzyme of *Streptomyces griseus* and reported that the estimates obtained from the *in vitro* method, except for the 2 full fat samples (full fat soybean and maize germ), were close to the *in situ* data.

The higher *b* parameter estimated by the *in vitro* method can be explained by compensatory microbial digestion. So, the *in vitro* method had lower disappearance values in short incubation times and higher disappearance values in long incubation times compared to *in situ*. This may be supported by the closeness of the estimated values for PD by two methods and similar disappearances at final incubation times between methods (unpublished data). Similar estimations of PD by the *in vitro* method compared to the *in situ* could indicate the digestion ability of the ruminal microorganisms under *in vitro* conditions. Trujillo et al. (2010) used the Daisy[II] apparatus for determining DM and NDF degradation of some fibrous feedstuffs compared to *in situ* method and reported that the *in vitro* Daisy[II] method underestimated DM and NDF disappearance; the largest differences between procedures were observed at early incubation times. They concluded that the *in vitro* Daisy[II] procedure may be a useful tool to compare degradation potential of feedstuffs. In present study, high and significant correlation coefficient was observed between *in vitro* Daisy[II] and *in situ* methods for estimating potential of degradation (r = 0.98) that was in agreement with the results of Trujillo et al. (2010). Susmel et al. (1993) assessed the centrifuged and whole rumen liquid in comparison to the *in situ* method for estimating protein degradability parameters and reported that the *in situ* method gave higher b values than rumen preparations *in vitro*. These results are in contrast to our results. In the present study, the whole ruminal fluid was mixed with buffer solution that insures complete microbial activity including cellulolytic and amylolytic activities. In addition, the buffer solution neutralizes the inhibitory effect of the produced fermentation acids in the degradation process.

Conclusion

Based on the present results, close estimations with high correlation coefficients between *in situ* and *in vitro* methods were observed in the estimation of parameters of c, PD and ED. The Daisy^{II} method underestimated the rapidly degradable fraction of protein for all test feeds. In addition, the ability of the method in estimating some parameters was better for some raw materials (soybean meal, canola meal and corn grain) than for others (barley grain, alfalfa hay and corn silage). The results suggested that if one is interested in estimating the protein degradability parameters by an *in vitro* method, the *in vitro* Daisy^{II} procedure can be a useful method for estimating fractional degradation rate, degradation potential and effective degradability of protein of feedstuffs.

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مقایسه روشهای کیسه نایلونی و آزمایشگاهی در برآورد فراسنجه‌های تجزیه‌پذیری پروتئین برخی مواد خوراکی

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چکیده هدف از این پژوهش برآورد فراسنجه های تجزیه پذیری پروتئین ۶ ماده خوراکی رایج نشخوارکنندگان با استفاده از روش کیسه‌های نایلونی (*in situ*) و روش آزمایشگاهی ابداعی با استفاده از دستگاه Daisy[II] بود. بجز کنجاله سویا، تفاوت های معنی داری بین دو روش *in situ* و *in vitro* در برآورد بخش تجزیه پذیر سریع (فراسنجه a) مشاهده شد ($P < ۰/۰۱$). روش برآورد به طور معنی داری بخش تجزیه پذیر کند (فراسنجه b) دانه جو، دانه ذرت، یونجه خشک و علوفه ذرت سیلو شده را تحت تاثیر قرار داد ($P < ۰/۰۵$). نرخ تجزیه‌پذیری (فراسنجه c) برای ناپدید شدن بخش b (درصد در ساعت) به وسیله روش تعیین تجزیه پذیری تحت تاثیر قرار نگرفت. مقادیر برآورد شده بخش b، فراسنجه c، پتانسیل تجزیه پذیری (PD) و تجزیه پذیری موثر (ED) در روش *in vitro* به میزان زیادی با مقادیر برآورد شده توسط روش *in situ* همبستگی داشت. پتانسیل تجزیه‌پذیری و تجزیه‌پذیری موثر برآورد شده توسط روش *in vitro* به میزان زیادی با برآوردهای روش *in situ* همبستگی داشت ($P < ۰/۰۱$). نتایج نشان داد که روش آزمایشگاهی با استفاده از دستگاه Daisy[II] برای برآورد برخی فراسنجه‌های تجزیه پذیری مانند فراسنجه c، PD و ED می تواند مورد استفاده قرار بگیرد. توانایی روش Daisy[II] برخی فراسنجه ها در مورد برخی مواد خوراکی (کنجاله کانولا، کنجاله سویا و دانه ذرت) بهتر از برخی دیگر (دانه جو، یونجه خشک شده و علوفه ذرت سیلو شده) بود.