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In situ and *in vitro* degradation kinetics and prediction of the digestible neutral detergent fiber of agricultural and agro-industrial byproducts

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ABSTRACT - The objective of this study was to evaluate the *in situ* and *in vitro* degradation kinetics and to predict the digestible neutral detergent fiber (dNDF) from the incubation times; *in situ* and *in vitro* degradation kinetic parameters; and equations fitted for agricultural and agro-industrial byproducts. Byproducts from pineapple, cocoa, palm kernel, corn gluten meal, common bean, sunflower, guava, cassava bark, cassava stems, cassava foliage, papaya, mango, passion fruit and turnips were evaluated. There were differences between the byproducts as for the potentially neutral detergent fiber (NDF) fraction and the *in situ* NDF degradation rate in the final volume of the gases generated by fibrous carbohydrates (FC), for the lag time and for the *in vitro* fractional degradation rate of the FC. There was equivalence between the dNDF values predicted *in situ* and those observed *in vivo*; however, there was low precision of estimates. The degradability in the *in vitro* incubation times of 30 and 48 hours presented equivalence with the values observed, but also did not present precision in the estimates. The equations fitted without lignin were not precise and accurate to estimate the dNDF of agricultural and agro-industrial byproducts. The equation with lignin and with the digestion rate obtained by the *in vitro* method presented more precise estimates. Byproducts from common bean, cassava bark and papaya presented greater NDF availability, whereas those of guava had the lowest NDF availability. The digestible NDF fraction was best predicted with the *in situ* incubation time of 72 hours. The equation fitted utilizing *in vitro* or *in situ* digestion rates enables the prediction of the NDF availability of agricultural and agro-industrial byproducts.

Key Words: fibrous carbohydrates, gas production, residues, rumen degradability

Introduction

Before utilizing alternative feedstuffs in diets for livestock, it is important to know about their chemical composition, the availability of their nutrients, the behavior in the digestive tract, and to evaluate these feedstuffs in diets for these animals.

Among the different components of the feeds for ruminants, the fibrous fraction is of utmost importance in tropical production systems, for it supplies significant amount of energy at a low cost (Detmann et al., 2004). For Kendall et al. (2009), maximizing the ingestion of digestible carbohydrates is important, once the energy necessary for maintenance and production often exceeds the ingestion consumption capacity of high-production animals.

The neutral detergent fiber (NDF) is the main component of the feedstuff that affects the dry matter intake (DMI) of animals of high production (Waldo, 1986). For Oliveira et al. (2011), the following are expected in diets with a high concentration of NDF: reduction of the potentially digestible fraction of the dry matter (DM); increase in the selective ruminal retention as a natural mechanism of compensation and increase in the probability of NDF digestion.

However, the NDF is not a homogeneous component. Therefore, feedstuffs with high NDF degradation rate are positively correlated with DMI (Van Soest, 1994). Nevertheless, this does not apply to the NDF content, i.e., feedstuffs with concentration similar to the NDF may have different DMI levels, which is limited by the amount of rumen-undegraded NDF (Andriguetto et al., 1993).

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The digestibility of NDF is dependent on the time it remains inside the digestive tract for hydrolysis and, consequently, its use is affected both by digestion and passage (retention time) rates. The NDF rumen digestibility of the feedstuffs can vary from 0.25 to 0.75 kg/kg for different types of forage (NRC, 2001).

The summative equations to predict the digestible fractions of the feedstuffs were developed considering the relations between cause and effect of chemical components and their digestibility in the rumen tract (Van Soest, 1967). However, the biological methods are usually utilized to better characterize the digestible fibrous fraction of the feedstuff (Weiss, 1998).

There are doubts as for the exactness of the digestible neutral detergent fiber (dNDF) estimated from the dNDF *in vitro* (Weiss & Wyatt, 2002). According to Kendall et al. (2009), differences of 5.5 and 8.5% have been observed between the values obtained *in vivo* and those estimated *in vitro* during 48 h of incubation for diets with 280 and 320 g/kg NDF, respectively. Likewise, Oba & Allen (2000) observed differences of 9.4 percentage units between the dNDF obtained *in vitro* during 30 hours of incubation and those observed *in vivo* with cattle receiving diets based on corn silage.

Magalhães (2007) and Silva et al. (2007) observed that the incubation time of 72 hours was the best time for correlation between dNDF *in vivo* and the degradation of this fraction *in situ* for tropical forages.

Thus, the objective of this study was to evaluate the *in situ* degradation kinetics, the *in vitro* gas production kinetics and to predict the dNDF from the incubation times, parameters of *in situ* and *in vivo* degradation kinetics and equations fitted for agricultural and agro-industrial byproducts.

Material and Methods

Byproducts from pineapple, cocoa, palm kernel, corn gluten meal, common bean, sunflower, guava, cassava bark, cassava stems, cassava foliage, papaya, mango, passion fruit and turnip were evaluated (Table 1).

Byproducts from pineapple (*Ananas comosus*), guava (*Psidium guajava*), papaya (*Carica papaya*), mango (*Mangifera indica*) and passion fruit (*Passiflora ligularis* originated from the processing for production of fruit juice, so they were comprised of peel, seeds, the fibrous part retained in the strainers and fruit unsuitable for processing. The byproduct from cocoa was basically composed of the integument which surrounds the seed (nut) after its industrial processing, but also contained little pieces of seeds. The byproduct cassava hulls was composed of strain, tip, bark and inner bark of its root resulting from the pre-cleaning for fabrication of cassava flour and was dried in industrial dryer at 60 ºC. The cassava stem was composed only of the stalk without leaves and was dried in industrial dryer at 60 ºC. The byproduct corn gluten meal was composed of the part of the external membrane of the corn grain which remains after the extraction of the biggest part of the starch, of the gluten and of the germ by the process employed in the production of starch or syrup via wet process.

All the byproducts collected were subjected to pre-drying at 60 ºC for 72 hours and ground in knife mill with 1mm pore diameter, for subsequent analysis of DM, crude protein (CP), organic matter (OM), ether extract (EE) and acid

Table 1 - Chemical composition of 14 agricultural and agro-industrial byproducts

Item	Dry matter[1]	Organic matter[2]	Crude protein[2]	NDIP[3]	ADIP[3]	Ether extract[2]	NDFap[2]	NFC[2]	ADF[2]	Lignin[2]	iNDF[2]	iADF[2]
Pineapple	139.1	952.7	70.9	504.9	443.8	7.8	602.0	272.0	341.1	37.1	149.4	70.2
Cocoa	893.4	925.8	143.3	551.4	474.6	50.7	369.7	362.2	400.9	185.4	321.3	298.4
Palm kernel	924.4	970.4	161.0	949.1	583.2	107.1	523.0	179.3	359.4	111.8	252.9	162.2
Corn gluten meal	857.6	942.2	218.6	382.4	132.6	28.4	226.8	468.4	124.6	14.4	40.0	10.4
Common bean	872.2	955.4	239.2	358.3	254.4	19.5	294.8	401.9	66.9	01.8	4.9	0.0
Sunflower	927.3	948.7	368.8	118.0	55.7	21.3	186.3	372.2	133.1	52.4	95.0	76.1
Guava	285.6	986.1	86.2	267.7	188.5	76.8	729.6	93.5	597.4	221.0	657.0	508.8
Papaya	100.7	949.3	148.0	868.7	274.1	72.8	293.8	434.8	327.4	77.4	78.7	52.7
Cassava hulls	255.6	966.3	37.3	588.2	74.1	05.9	157.5	765.6	155.6	55.4	141.6	110.7
Cassava stem	899.8	962.6	54.4	471.5	213.4	9.2	651.1	247.9	571.2	200.1	646.2	529.3
Cassava foliage	218.5	918.1	241.7	598.0	559.6	23.8	404.4	248.2	362.6	96.1	339.9	252.7
Mango	345.0	976.1	50.5	791.7	226.4	40.0	325.5	560.1	237.8	72.5	199.7	147.5
Passion fruit	195.3	963.2	99.7	179.8	77.0	122.0	547.7	193.8	427.0	77.9	336.7	261.4
Turnip	916.2	942.9	275.9	199.3	187.3	242.8	227.2	196.9	196.9	75.3	179.1	125.1

NDIP - neutral detergent insoluble protein; ADIP - acid detergent insoluble protein; NDFap - neutral detergent fiber corrected for ash and protein; NFC - non-fibrous carbohydrates; ADF - acid detergent fiber; iNDF - indigestible neutral detergent fiber; iADF - indigestible acid detergent fiber.
[1] g/kg of natural matter.
[2] g/kg of dry matter.
[3] g/kg of crude protein.

detergent fiber (ADF), according to the methods of the AOAC (1990).

In the NDF analyses, samples were treated with thermostable alpha-amylase, without the use of sodium sulfite and corrected for residual ash (Mertens, 1992). The correction of NDF and ADF for the nitrogenous compounds and the estimation of neutral (NDIN) and acid detergent insoluble nitrogen (ADIN) compounds were performed according to Licitra et al. (1996). The lignin contents were obtained by means of solubilization of cellulose by sulfur acid (Van Soest & Robertson, 1985).

The contents of non-fibrous carbohydrates (NFC) of the byproducts, expressed in g/kg DM, were calculated according to Hall (2000) as 100- (g/kgNDF + g/kgCP + g/kgEE + g/kgMM) and total digestible nutrients (TDN) were calculated as: TDN = g/kg digestible CP + g/kg digestible NDF + g/kg digestible NFC + 2.25*g/kg digestible EE.

For *in situ* incubations, samples were dried in oven at 60 ºC for 72 hours, processed in knife mill with 2 mm sieve and homogenized, forming a composite sample for each residue for further incubation. For the evaluation of indigestible NDF (iNDF) and indigestible ADF (iADF) contents, three replicates of each byproduct were conditioned in non-woven fabric bags of 100 g/m^2 measuring 4 × 5 cm and following the ratio of 20 mg DM/cm^2 surface suggested by Nocek (1988) and incubation time of 264 hours, proposed by Casali et al. (2008). For incubations, three crossbred (Holstein × Zebu) rumen-cannulated castrated cattle of average weight of 260 kg were utilized. Animals received a diet containing 700 g/kg roughage and 300 g/kg concentrate.

Byproducts were incubated (2 g DM in each bag) in duplicate, in non-woven textile bags of 100 g/m^2, following the ratio of 20 gm DM/cm^2 suggested by Nocek (1988), in the following incubation times, in descending order: 144, 120, 96, 72, 48, 24, 12, 6 and 3 h. After removed from the rumen, along with time zero, they were taken to running water until total clearing and then transferred to forced-ventilation oven (60 ºC), where they were kept for 72 hours. They were then sequentially dried in non-ventilated oven (105 ºC for 45 minutes), conditioned in dissector and weighed for the obtainment of the non-digested DM.

After, bags were treated with neutral detergent fiber (Mertens, 2002) for 60 minutes, in autoclave (Pell & Schofield, 1993) at 105 ºC, washed in hot water, acetone, weighed and dried, according to the aforementioned procedure, for quantification of non-digested NDF.

The *in situ* degradation data were obtained by the difference of weight, found for each component, between the weighings done before and after ruminal incubation and expressed in percentage. For the estimation of the potentially degradable fraction, the exponential decay model was utilized, corrected for the lag period (L), described by Mertens (1976), according to the formula:

$$\hat{Y} = B^* \exp^{(-kd^*(t - L))} + I,$$

in which $\hat{Y}$ accounts for the non-digested DM or NDF residue in time t (%); B is the potentially degradable fraction of the fiber (%); kd is the rumen degradation dynamics of fraction B (h^{-1}); t is the incubation time, in hours; L is the lag time (h); and I is the undegradable fraction (%), which represents the iNDF contents.

The NDF effectively degraded fraction (NDFEDF) was estimated by the equation (Mertens & Loften, 1980):

$$NDFEDF = B^*kd^*\exp(-kp^*L)/(kd + kp),$$

in which kd accounts for the passage rate of the digesta through the rumen, assuming kp value equal to 0.02 h^{-1}.

For the *in vitro* incubations, calibrated syringes were utilized, according to procedure described by Getchew et al. (2004).

Syringes with capacity of 100 mL were previously washed with distilled water, dried in oven and subsequently lubricated with Vaseline, in which approximately 200 mg of the byproduct studied were placed.

The macro and mineral buffer solution, described by Menke & Steingass (1988), was prepared prior to incubation and kept heated at 39 ºC, under continuous gasification by CO_2, on a stirrer.

The animals which donated the inoculate were the same of the *in situ* experiment. The ruminal fluid was taken manually in the morning, before the supply of diet, from several parts of the rumen, and stored in previously heated (39 ºC) thermos bottles and immediately taken to laboratory. In an acclimatized room of the lab, (39 ºC), the rumen fluid was filtered, passing through two layers of cotton gauze. After, the rumen fluid was added to the buffer solution at a 2:1 ratio (v/v), under continuous CO_2 injection.

Three syringes were utilized per feedstuff, in which 30 mL of the buffered ruminal fluid were added to the substrate. After closure of the tip of the syringe with a clip attached to a silicone rubber to impede sample leaking, the piston was introduced until there was total removal of gases, and then the syringe was gently agitated.

With the syringe tip closed, the initial volume was recorded. At every two hours, reading was performed through records of gases volume, and the syringe was gently agitated. The incubation times utilized were 0, 2, 4, 6, 8, 10, 12, 24, 26, 28, 30, 32, 36, 48, 52, 54, 56, 60 and 72 hours. The results were corrected for blank (syringe containing buffered ruminal fluid, without sample) and for the standard (tifton 85 grass hay), at 24 h of incubation.

The kinetic variables of fibrous carbohydrates (FC) and NFC were estimated by the technique of *in vitro* gas production. Bicompartimental model was used, fitted to the curves of cumulative gas production (Schofield et al., 1994):

$$V = VF_{NFC} / (1 + \exp(2 - 4*kd_{NFC}*(T - L))) + VF_{FC} / (1 + \exp(2 - 4*kd_{FC}*(T - L))),$$

in which: VF_{NFC} is equivalent to the maximum volume of gases from the NFC fraction; kd_{NFC} is the degradation rate (h^{-1}) of this fraction (NFC); VF_{FC} is the maximum volume of the gases from the FC fraction; kd_{FC} is the degradation rate (h^{-1}) of FC; and T and L are the incubation (hours) and lag (hours) times, respectively.

After estimation of the kinetic variables of production of gases from carbohydrates, the degradation curves of FC in function of incubation time were constructed, for the data obtained by the method of gas production.

For incubations, glass flasks were utilized according to procedure described by Schofield et al. (1994). Flaks with capacity of 50 mL were previously washed with distilled water, dried in oven and had approximately 200 mg of the byproduct studied inserted in them.

Macro and micromineral buffer solution, in addition to the ruminal fluid, was the same as described in the experiment for kinetics of *in vitro* gas production.

Three flasks were utilized per byproduct; every one of them had 30 mL of the buffered ruminal liquid added to the substrates and were immediately sealed with rubber corks and aluminum rings aiming to ensure complete maintenance of the gases inside them.

After 30 and 48 hours of incubation, flasks were removed from the acclimatized room and taken to refrigerator for 4 ºC, for ceasing the fermentative process. After, 30 mL of neutral detergent solution were added to each flask (Mertens, 2002) and then taken to be autoclaved for 60 minutes, at 105 ºC, according to technique proposed by Pell & Schofield (1993). Next, the contents of each glass flasks were filtered in filter crucible of zero-porosity, washed with hot distilled water and acetone and dried in oven at 105 ºC for 16 hours.

For the prediction of the NDF digestible fiber, the equation proposed by Conrad et al. (1984), adapted by Tedeschi et al. (2009) and a modification of the equation adapted by Tedeschi et al. (2009) were evaluated, resulting in the following equations:

- Equation proposed by Conrad et al. (1984) and adapted by Tedeschi et al. (2009):

$$\hat{dNDF} \text{ (g/kg DM)} = [(\frac{Kd_{FC}}{Kd_{FC} + Kp}) + 0.2] x (NDF - NDIN)$$

- Modified equation:

$$\hat{dNDF} \text{ (g/kg DM)} = (\frac{Kd_{FC}}{Kd_{FC} + Kp}) x (NDF_{cp} - LIG)$$

In this evaluation, passage rate (Kp) of 0.02 h^{-1} and two digestion rates (Kd) were considered (obtained *in vitro* by the technique of gas production and *in situ*).

The results obtained by Azevêdo (2009) were utilized for the *in vivo* dNDF information of agricultural and agro-industrial byproducts (Table 2).

For the procedures of validation of the digestible fractions observed and predicted by the incubation times, model parameters of the fitted equations were based on the fitting of the simple linear regression models, and estimates of the regression parameters tested by the joint null hypothesis according to Mayer et al. (1994): Ho: $\beta_0 = 0$ and $\beta_1 = 1$ X Ha: non-Ho. In the case of non-rejection of the null hypothesis, it is concluded that there is equivalence between the observed and predicted values.

The mean bias (MB) was calculated (Cochran & Cox, 1957) according to the following equation:

$$MB = \frac{1}{n}\sum_{i=1}^{n}(xi - yi);$$

in which: x = observed values; y = predicted values.

The concordance correlation coefficient (CCC), also known as reproducibility index, which considers exactness and precision simultaneously, was calculated according to Lin (1989).

The comparative evaluation of the prediction efficiency was performed by the evaluation of the mean square prediction error (MSPE), as described by Bibby & Toutenburg (1977), following the equation below:

$$MSPE = \frac{1}{n}\sum_{i=1}^{n}(xi - yi)^2,$$

in which: x = observed values; y = predicted values. It is necessary to stress that for all the variance calculations, the total observations (n) was utilized as divisor.

Table 2 - Digestible neutral detergent fiber (dNDF) in different agricultural and agro-industrial byproducts[1]

Agricultural and agro-industrial byproducts	dNDF g/kg of DM
Pineapple	461.1
Cocoa	64.9
Palm kernel	333.1
Corn gluten meal	128.5
Common bean	209.8
Sunflower	120.2
Guava	145.6
Papaya	201.3
Cassava hulls	55.6
Cassava stem	434.2
Cassava foliage	350.7
Mango	221.2
Passion fruit	258.6
Turnip	105.2

[1] Information obtained *in vivo* by Azevêdo (2009).

The data on the NDF degradability parameters and production of gases from NFC and FC obtained in the different methods (*in situ* and *in vitro*) and incubation times were fitted by non-linear regression through the Gauss-Newton method, according to the respective models previously informed. Variance analyses were conducted, by applying the F test. For the variables whose F test was significant, the means were compared utilizing the Scott Knott criterion.

For all statistical procedures, the critical level for probability of type I error was fixed at 0.05. All statistical procedures were performed with software SAS (Statistical Analysis System, version 9.0) and MES (Model Evaluation System, version 3.0.11).

Results and Discussion

The values estimated for the *in situ* NDF degradability indicate that the byproducts from pineapple, palm kernel, corn gluten meal, common bean, sunflower, papaya and mango showed greater (P<0.05) potentially degradable fraction (B) and lower (P<0.05) undegradable fraction (I), indicating that the NDF components serve as a source of energy to the animal and that these byproducts do not have limitations in their cell wall for use by the microbial rumen population (Table 3).

For the NDF degradation rate (kd), the byproduct cassava bark differed (P<0.05) from most of the byproducts studied for presenting the highest value (0.250 h^{-1}), and was also that with the lowest NDFap concentration (157.5 g/kg in the DM) of the byproducts studied. This indicates that the NDF of the cassava bark ferments quickly and can be considered an additional source of energy to ruminal microorganisms, since it has a high concentration of NFC (765.6 g/kg in the DM).

Usually, the degradation of FC tends to be affected by variation in the rate of passage through the rumen-reticulum, once they present lower digestion rate when compared with the NFC (Mertens, 1993). However, although the cassava bark did not differ (P>0.05) from byproducts cocoa, cassava stem, cassava foliage, guava, passion fruit and turnip for the adjusted parameters of the potentially degradable and undegradable NDF fractions, it behaved differently from the other byproducts as for the NDF degradation, for presenting greater (P<0.05) degradation rate of this fraction. In addition to the estimates indicating NDF promptly available to the cassava bark, the estimates of the degraded fractions were of approximately 54% in the incubation time of 144 hours, whereas for the other byproducts, they were inferior to 38%.

The average estimates of the parameters of *in vitro* kinetic degradations of FC and NFC (Table 4) indicate differences (P<0.05) between the agricultural and agro-industrial byproducts. Considering linear relation between NDF disappearance and VF_{CF} (Pell et al., 1994), one can infer that the cassava barks would present greater (P<0.05) NDF disappearance in relation to the other byproducts. Byproducts corn gluten meal, common beans and papaya

Table 3 - Estimate of *in situ* degradation kinetic parameters of the neutral detergent fiber from agricultural and agro-industrial byproducts

Byproducts	B	kd	L	I
Pineapple	0.8522a	0.012b	4.50	0.0778b
Cocoa	0.3618b	0.026b	4.73	0.6297a
Palm kernel	0.7175a	0.013b	6.53	0.2481b
Corn gluten meal	0.8068a	0.012b	12.10	0.0001b
Common bean	0.8218a	0.024b	7.91	0.0001b
Sunflower	0.5525a	0.044b	1.35	0.3854b
Guava	0.0908b	0.060b	4.95	0.8885a
Papaya	0.7653a	0.022b	8.55	0.1296b
Cassava hulls	0.2885b	0.250a	4.50	0.4392a
Cassava stem	0.1162b	0.063b	7.88	0.8257a
Cassava foliage	0.3208b	0.032b	4.95	0.6303a
Mango	0.6937a	0.010b	5.20	0.2874b
Passion fruit	0.3460b	0.039b	4.50	0.6239a
Turnip	0.3204b	0.037b	3.83	0.6276a
P value	0.0024	<0.0001	0.3214	0.0002
CV (%)	33.58	53.56	55.79	35.42

Means in columns with the same letter do not differ by the Scott Knott test (P<0.05).
B - potentially degradable fraction of the fiber (kg/kg); kd - rumen degradation dynamics of fraction B (h^{-1}); L - lag time (h); I - undegradable fraction of the neutral detergent fiber (kg/kg).

Table 4 - Estimates of *in vitro* degradation kinetic parameters of the neutral detergent fiber from agricultural and agro-industrial byproducts

Byproducts	VF_{NFC}	kd_{NFC}	L	VF_{FC}	kd_{FC}
Pineapple	1.157a	0.132	1.75c	0.409c	0.038a
Cocoa	0.291e	0.137	0.88d	0.632c	0.029b
Palm kernel	0.826b	0.076	4.80a	0.321e	0.025b
Corn gluten meal	0.457d	0.144	2.20b	1.263b	0.031b
Common bean	0.729c	0.166	1.67c	1.276b	0.039a
Sunflower	0.484d	0.154	1.25d	0.664c	0.039a
Guava	0.822b	0.222	1.35d	0.072e	0.048a
Papaya	0.690c	0.180	1.77c	1.144b	0.042a
Cassava hulls	0.518d	0.168	2.46b	2.304a	0.036a
Cassava stem	0.565d	0.121	1.07d	0.166e	0.028b
Cassava foliage	0.638c	0.131	4.53a	0.458d	0.032b
Mango	0.536d	0.153	1.12d	0.643c	0.036a
Passion fruit	1.0624a	0.131	1.51c	0.281e	0.040a
Turnip	1.194a	0.130	0.55d	0.615c	0.032b
P value	<0.0001	0.0121	<0.0001	<0.0001	0.0019
CV (%)	13.65	23.83	16.30	16.57	15.80

Means in columns with the same letter do not differ by the Scott Knott test (P<0.05).
VF_{NFC} - maximum volume of gases from the NFC fraction (mL/%NFC); kd_{NFC} - degradation rate (h^{-1}) of this fraction (NFC); VF_{FC} - maximum volume of the gases from the FC fraction mL/ % NDFap; kd_{FC} - degradation rate (h^{-1}) of FC; T and L - incubation (hours) and lag times (hours).

presented higher (P<0.05) FC availability in relation to the other byproducts (except for cassava bark), since they produced higher VF_{CF}.

Among the byproducts, palm kernel, guava, cassava stem and passion fruit promoted lower (P<0.05) FC disappearance. The stratification of the differences between byproducts was greater in the technique of gas production because of the higher precision, for they presented coefficient of variation below 24%, whereas for the *in situ* technique, this value was superior to 33%.

There were differences (P<0.05) between byproducts for the lag period, which is a period in which no degradation of the substrate is verified. Byproducts from palm kernel and cassava foliage were those which promoted greater estimates of the lag period: 4.80 and 4.53 hours, respectively.

For the fractional degradation rate of FC, there was influence of byproduct; pineapple, common beans, sunflower, guava, papaya, cassava bark, mango and passion fruit were superior (P<0.05) to the majority.

There was big dispersion of the values predicted in *in situ* incubation times of 24, 48 and 72 hours and also by the NDF effectively degraded fraction, between the line of equality (Y = X) (Figure 1).

The iNDF is considered unavailable both in the rumen and intestines (Sniffen et al., 1992), as a consequence of the high lignin concentration. The relation between the iNDF obtained after 264 hours of rumen incubation and the estimate of the NDF undegradable fraction obtained by the model of Mertens & Loften (1980) (Figure 2) was linear (r^2 = 0.954). This fact indicates that the byproducts from cocoa, cassava bark, cassava stem, cassava foliage, guava,

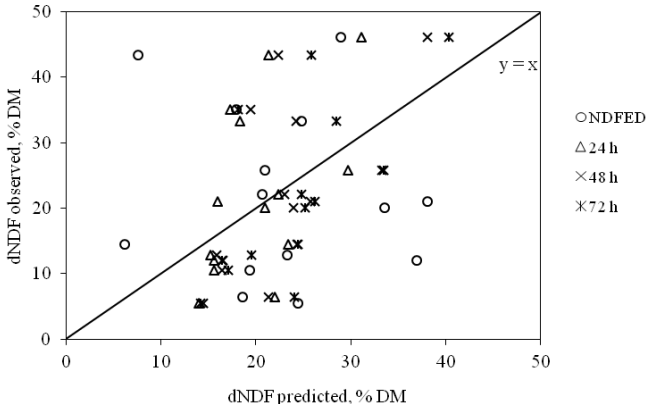

Figure 1 - Relation between digestible neutral detergent fiber (dNDF) values observed and predicted in *in situ* incubation times of 24, 48 and 72 hours and by the neutral detergent fiber effective degradability (NDFED).

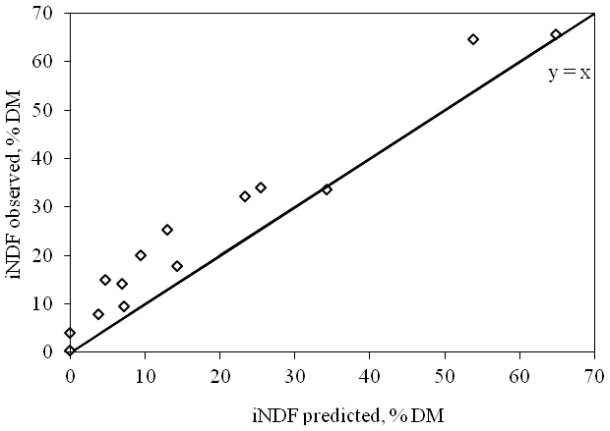

Figure 2 - Relation between indigestible neutral detergent fiber (iNDF) observed *in situ* after 264 hours of incubation and predicted by the model of Mertens & Loften (1980).

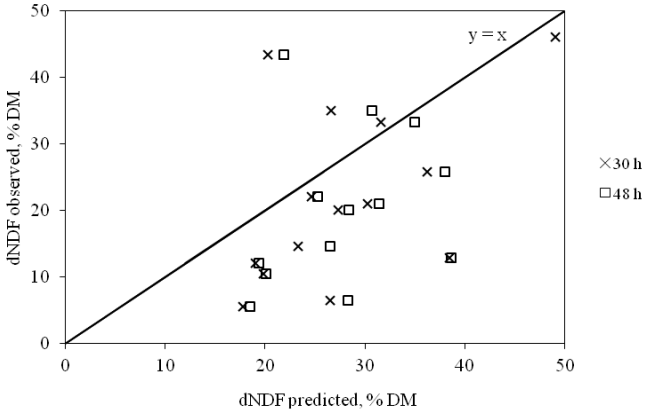

Figure 3 - Relation between digestible neutral detergent fiber (dNDF) values observed and predicted in *in vitro* incubation times of 30 and 48 hours.

passion fruit and turnip, which showed higher (P<0.05) estimates of iNDF, promoted lower energy availability of the NDF fraction to ruminal microorganisms, and the estimates obtained by the model of Mertens & Loften (1980) were coherent with the values observed.

For the results of regressions between the dNDF values observed *in vivo* and the estimates obtained *in vitro*, there was overestimation of the dNDF values and few differences between the values obtained in the two incubation times (Figure 3).

Based on the regression statistics (Table 5) and more particularly for the significance test of the joint null hypothesis, one can state that all parameters evaluated *in situ* presented equivalence (P>0.05) between the predicted and observed dNDF values for agricultural and agro-

Table 5 - Statistics for regression between *in situ* and *in vitro* observed and predicted values for the digestible neutral detergent fiber (dNDF)

Item	dNDF *in situ*				dNDF *in vitro*	
	NDFED	24 hours	48 hours	72 hours	30 h	48 h
Intercept	26.136	-3.575	-6.476	-7.723	2.049	-0.834
Slope	-0.177	1.270	1.253	1.233	0.717	0.777
r^2	0.016	0.262	0.399	0.438	0.227	0.275
P value (Ho: a = 0 and b = 1)	0.036	0.765	0.826	0.643	0.187	0.087
Mean bias, %	-0.886	1.871	-0.715	-2.087	-5.853	-7.369
CCC	-0.121	0.349	0.506	0.541	0.386	0.399
MSPE, $\%^2$	273.421	123.489	99.312	96.800	163.602	174.258

CCC - concordance correlation coefficient; MSPE - mean square prediction error; NDFED - neutral detergent fiber effectively degraded fraction; incubation times *in situ* of 24, 48, 72 hours and *in vitro* of 30 and 48 hours.

industrial byproducts, except for neutral detergent fiber effective degradability. However, after 72 hours of incubation, dNDF was the best predictor of the dNDF among the other values obtained *in situ*, because it presented greater r^2 (0.438), lower MB (-2.087%), higher CCC (0.541) and lower MSPE (96.800%^{2}).

The overestimate of the values observed *in vivo* can also be confirmed by the MB values of -5.853 and -7.396 for *in vitro* incubation times of 30 and 48 hours, respectively. Kendall et al. (2009) also observed that, numerically, the *in vivo* dNDF values were inferior to the *in vitro* dNDF values.

In spite of the big dispersion of the degradability data in the *in vitro* incubation time of 30 and 48 hours, their estimates did not differ (P>0.05) by the test of joint null hypothesis (Mayer et al., 1994), indicating that the values predicted in these incubation times presented equivalence to the values observed for the agricultural and agro-industrial byproducts studied.

By comparing the estimates obtained *in situ* and *in vitro*, one can observe that those with *in situ* incubation time of 72 hours presented greater CCC and lower MSPE than those *in vitro*, suggesting better prediction results.

For the prediction of the values of the dNDF observed by means of the equation which considers the chemical characteristics of the feedstuff and also parameters obtained via biological methods, a regression was carried out (Figure 4). There was better fit for dNDF around the line of equivalence (Y = X) in the equations with lignin and degradation rates obtained by the *in vitro* method.

According to the variables analyzed in the regression statistics (Table 6), one can affirm that the equations fitted without lignin, as proposed by Tedeschi et al. (2009), were

Table 6 - Statistics for regression between values observed and predicted by equations fitted for digestible neutral detergent fiber (dNDF)

Item	With lignin		Without lignin	
	in vitro	*in situ*	*in vitro*	*in situ*
Intercept	3.401	11.108	2.839	11.746
Slope	0.973	0.633	0.553	0.313
r^2	0.516	0.244	0.384	0.168
P value (Ho: a = 0 and b = 1)	0.541	0.212	0.001	0.002
Mean bias, %	2.877	4.754	-12.717	-10.857
CCC	0.666	0.443	0.435	0.316
MSPE, $\%^2$	85.792	156.666	300.452	379.620

CCC - concordance correlation coefficient; MSPE - mean square prediction error.

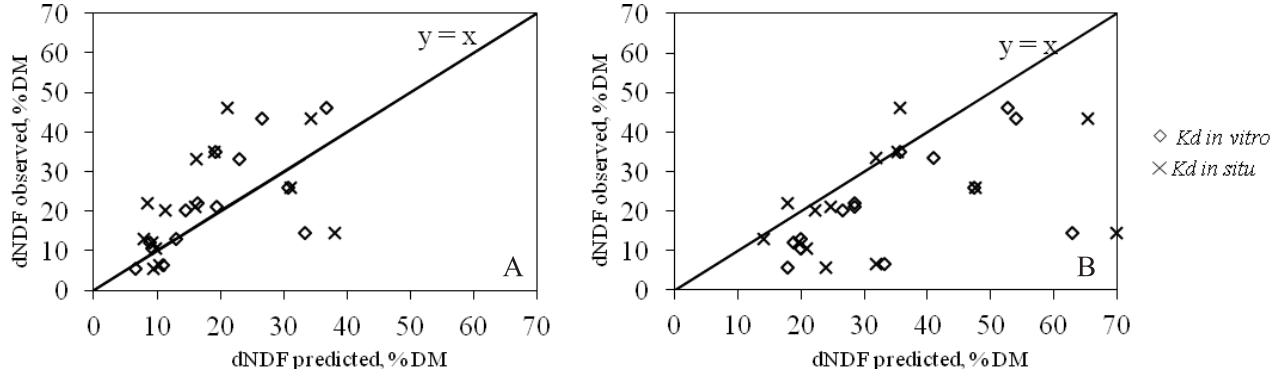

Figure 4 - Relation between digestible neutral detergent fiber (dNDF) observed and predicted by equations: with lignin (A) and without lignin (B).

not precise and accurate to estimate the dNDF of agricultural and agro-industrial byproducts, for they were the equations which presented the highest MSPE.

It was also observed that the equations with lignin and digestion rates obtained by the *in situ* method presented less precise estimates in relation to those obtained by the *in vitro* method, and this can be a consequence of the lower variability of the second method. Tedeschi et al. (2009) recommended the use of digestion rates obtained *in vitro* by the technique of gas production to estimate the dNDF.

Conclusions

Byproducts common bean, cassava bark and papaya presented greater availability of neutral detergent fiber, whereas that from guava is of lower availability of neutral detergent fiber. Incubation times of 30 and 72 hours are recommended for the prediction of the *in vitro* and *in situ* neutral detergent fiber digestible fractions, respectively. The fitted equation: $\hat{dNDF}$ (% of the DM) = $(\frac{Kd_{CC}}{Kd_{FC}+Kp})x(NDF_{ap} - LIG)$, utilizing the *in vitro* or *in situ* digestion rates, enables the prediction of the neutral detergent fiber availability of agricultural and agro-industrial byproducts.

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