

Metabolizable Energy and Amino Acids Relationships with the Soluble Fractions of Protein and Fiber of Vegetable Feed Ingredients

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ABSTRACT - To estimate nutrient and energy digestion with animal from *in vitro* variables it is necessary to control factors that may interfere on digestion such as: source and concentration of digester substance, as well as the origin and processing of test ingredients. In this study there were performed five laboratory assays, two with poultry and finally regression equations were established based on variables generated with twelve vegetable ingredients. The results showed that there is greater solubility of proteins with NaOH than with KOH. On average, both alkali produced better discrimination of soluble protein with concentration of 0.02% (range of 68.61%) in relation to a concentration of 0.2% (range of 37.97%). Considering interaction between alkali and concentration, the best discrimination on protein solubility was reached with NaOH at 0.02%. The methods of protein or fiber solubilization together with the chemical analysis showed significant results variation among ingredients and when they were used to estimate *in vivo* variables they produced good estimates. It was concluded that reliable digestion estimates for energy or amino acids from vegetable feed ingredients could be obtained from *in vitro* variables, when considering solubilities of protein and fiber with chemical analysis.

Key Words: prediction equations, energy, total dietary fiber, nitrogen solubility, fiber solubility, alkali digestion, pepsin digestion.

Energia Metabolizável e Relações de Aminoácidos com as Frações Solúveis de Proteína e Fibra de Ingredientes Vegetais

RESUMO - Para estimar a digestão de nutrientes e de energia dos animais a partir de variáveis *in vitro*, é necessário controlar os fatores que interferem na digestão – origem e concentração da substância digestora – e ter conhecimento da fonte e do processo que envolve o ingrediente em teste. Neste trabalho, foram conduzidos cinco ensaios laboratoriais, dois com animais e, por fim, estabelecidas equações de regressão a partir das variáveis geradas com a utilização de 12 ingredientes vegetais. Os resultados mostraram que há maior solubilidade das proteínas com o NaOH que com o KOH e que, na média de ambos os álcalis, a concentração de 0,02% permite maior discriminação na solubilidade das proteínas (diferença de 68,61%) em relação a 0,2% (37,97%). Considerando a interação entre álcalis e concentrações, a melhor discriminação foi com NaOH a 0,02%. Os métodos de solubilização da proteína ou fibra, juntos com as análises bromatológicas, mostraram variações significativas e, quando usados para relacionar as variáveis *in vivo*, produziram bons resultados estimadores. Concluiu-se que podem ser obtidas estimativas confiáveis da digestão animal de ingredientes vegetais com a determinação de variáveis *in vitro*, desde que considerados fatores relacionados à solubilidade da proteína e fibra e variáveis químico-bromatológicas.

Palavras-chave: equações de predição, energia, fibra dietética total, solubilidade do nitrogênio, solubilidade da fibra, digestão em alcali, digestão em pepsina

Introduction

Many times the difficulty in estimating the *in vitro* nutrients and energy digestion is associated with the misunderstanding in knowing or controlling the factors which interfere with the digestion, such as the protein source (Johnson, 1992), the enzymatic concentration (Johnston & Coon, 1979; Parsons et al., 1997), the enzyme and the pH (Cone, 1993), as well as the

processing to what the ingredients were submitted (Bellaver et al., 2001). The *in vivo* variables and the relationship with those *in vitro* has been studied over one decade as it is described by Bellaver et al. (1991), Boisen & Fernandez (1995), Bellaver et al. (1997) and Boisen & Fernandez (1997). The *in vitro* protein solubility paradigm for vegetable origin ingredients is the nitrogen solubility in KOH 0.2%, being this alkali and the concentration recommended by Pomeranz

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and Lindner (1960) and AOAC (1995). Based on the information obtained by Parsons et al. (1997) and Bellaver et al. (2000) it is known that the digester substance concentration must be smaller than the originally recommended by AOAC (1995) for animal origin meals and also that the enzymatic method could be used to vegetable ingredients. Soybean meal has been analyzed more frequently than the rest of vegetable ingredients for the digestion in KOH. However, other methodology proposed by Prosky et al. (1992) is more useful in the establishment of relationships among *in vivo* and *in vitro* variables. This method uses three enzymes in the routine, having been found that the total dietary fiber (TDF) introduces high repeatability and that the used vegetable species show significant differences regarding TDF contents. Thus, the goal of this paper was to obtain chemical data, protein and fiber solubility of the vegetable ingredients and to relate them with the obtained *in vivo* values for the same ingredients.

Material and Methods

The methodology used in this work consisted of five *in vitro* assays, two *in vivo* and a regression analysis involving the *in vitro* and *in vivo* variables of a set of vegetable origin ingredients. Statistical analyses were carried out using the procedures described in the SAS program (2001). The assays are described as follow.

Assay 1 - Determination of the ingredients chemical composition

The twelve analyzed ingredients were: 1) corn gluten meal - 21% crude protein (21 CP), 2) soybean meal - 46% crude protein (46 CP), 3) corn gluten meal - 11% crude protein (11 CP), 4) citric pulp, 5) corn gluten meal - 60% crude protein (60 CP), 6) cotton seed meal - 28% crude protein (28 CP), 7) wheat bran, 8) soybean meal - 48% crude protein (48 CP), 9) corn gluten meal - 10% crude protein (10 CP), 10) soybean hulls, 11) corn and 12) canola meal. These ingredients were submitted to lab analyses determination for dry matter (DM), gross energy (GE), crude protein (CP), ether extract (EE), mineral matter (MM), crude fiber (CF), acid detergent fiber (ADF), neutral detergent fiber (NDF), calcium (CA), phosphorus (P), copper (Cu), iron (Fe), manganese (Mn), zinc (Zn), potassium (K) and amino acid (AA). The methods used for the accomplishment of these determinations have been described by AOAC (1995).

Assay 2 – Test of protein solubilization in alkalis with different concentration

These tests were used to determine the *in vitro* protein solubility using two alkalis (NaOH and KOH), two alkali concentrations (0.2 and 0.02%) and four ingredients (two soybean meals, micronized and protein isolated soya meals). The experimental design was the completely randomized, with 16 treatments, in factorial structure 2 x 2 x 4 (two alkalis, two concentrations and four ingredients), with six replications of each treatment. The grinding and treatment of the ingredients were accomplished according to the procedures established by Araba & Dale (1990). The procedure used to solubilize the protein was established according to the Brazilian Compendium of Animal Feeding (1998) considering alkali and respective concentration variations.

Assay 3 - Protein vegetable ingredient solubility determination with alkalis

This assay was carried out with twelve vegetable ingredients and two alkalis. The studied ingredients were the same of the assay 1. The analysis using protein solubility method was the same described by the Brazilian Compendium of Animal Feeding (1998), having the digesting substances KOH in the concentration of 0.2% and NaOH in the concentration of 0.02%. KOH at 0.2% was used as a standard procedure (Pomeranz & Lindner, 1960) and NaOH at 0.02%, for being an alkali and at a concentration that allow a larger solubilization of the proteins, as indicated in the assay 2. The experimental design was the completely randomized, with factorial structure 12 x 2 (twelve ingredients x two alkalis), with four replications.

Assay 4 – Protein vegetable ingredient solubility determination with pepsin

This assay was completed with the same assay ingredients described on assay 1. The methodology adopted for solubilization of the protein in pepsin was that employed by Bellaver et al. (2000), and the used pepsin concentrations equivalent to 0.02%, 0.002% and 0.0002%. The experimental design was the completely randomized with factorial structure 12 x 3 (twelve ingredients x three pepsin concentrations) with four replicates.

Assay 5 – Total dietary fiber determination (TDF) of vegetable ingredients

This assay was conducted with the same twelve ingredients studied on assay 1. The method used to determine TDF was enzyme/weighing, according to

Prosky et al. (1992). The ingredient samples were previously dried at 105°C, grounded to obtain smaller particles than 0.5 mm and degreased. This method is based in gelatinization of the sample with Thermamyl (stable alpha-amylase to the warmth), followed by enzymatic digestion with protease and amiloglucosidase to remove proteins and starch. The experimental design was the completely randomized with twelve ingredients and six replications.

Assay 6 - Amino acid digestibility coefficient determination

This experiment was performed in the metabolism room of Embrapa Swine and Poultry National Center, Brazil, where the roosters were allocated in individual cages and maintained in an environment with natural and/or artificial light for periods of 18 hours/day. The temperature was maintained in the thermal neutrality zone. The ingredients of the amino acid metabolizable determination were the same of the assay 1 and the forced feeding method of Sibbald (1979) was used, with the adaptation of Parsons (1985b) for cecotomized adult roosters (2,3±0,2 kg). The formula used to calculations was that given by Parsons (1985a). The experimental design was the completely randomized with twelve ingredients plus the control treatment (starvation), with four replications of one rooster per experimental unit. At the beginning of the assay, the roosters were maintained in fasting for a period of 36 hours, in order to empty the digestive content. Then they were forced to ingest 30 g of the ingredient in test, all at once, through a funnel introduced inside the crop, placed way intra-esophagus. After the roosters' feeding, excretas were collected twice a day from the plastic trays continuously placed for a period of 48 hours under the cages. At the same time four roosters were maintained with no feed in order to collect and calculate the endogenous and metabolic losses. The material, after collected, was weighed, identified, placed in plastic bags, and frozen until the final collection. Thus, the material was defrosted, homogenized and placed in the freeze drier for dehydration and posterior determination of DM, N and amino acids according with the methodologies described by AOAC (1995).

Assay 7 - Determination of apparent metabolizable energy N corrected

This experiment was carried out with broiler chicks, using excretas total collection method, as proposed by Hill & Anderson (1958), described by

Albino (1992). Broilers, 780, were distributed in blocks at random, with 13 treatments (the same twelve previous assays ingredients and one more reference diet), with six replications of ten birds each (five males and five females). Broilers were in experimental period with 15 days of age and maintained during 9 days, being four days for adaptation and five days for the collection of total excreta. Test ingredients were included at 40% in a reference diet. General care with the birds' hygiene and health were adopted, keeping the environment on the thermal comfort zone.

Regression analyses – Analyses between apparent metabolizable energy N corrected (AMEn) and the amino acid digestibility coefficients, with the in vitro variables.

Among all the *in vitro* variables for the same twelve ingredients, 13 were selected and correlated with values obtained by the *in vivo* assays. The *in vitro* variables involved in the estimates were: mineral matter (MM), ether extract (EE), crude protein (CP), crude fiber (CF), acid detergent fiber (ADF), neutral detergent fiber (NDF), gross energy (GE), total dietary fiber (TDF), solubility in KOH 0.2% (KOH), solubility in NaOH 0.02% (NaOH), digestibility/solubility in pepsin 0.02% (DPP02), digestibility/solubility in pepsin 0.002% (DPP002), digestibility/solubility in pepsin 0.0002% (DPP0002). These variables were combined up to six parameters (intercept + 5 *in vitro* variables) in the equation, once this number was the parameters limit in function of observations number (twelve ingredients) used in the study. For each answer variable (metabolizable energy and digestibility coefficients of ten amino acids) 2.379 equations were calculated for each variable. The selection of the more important equations was based on the larger correlation (R^2 best fitting to the model parameters), and it was done in combination with the ones that had introduced the minimal error on the estimation.

Results and Discussion

The results of chemical composition and total amino acid analyses of the studied ingredients (assay 1) are shown in Tables 1 to 3. The protein solubility values in alkali are displayed in Table 4 and the solubility statistics in Table 5. These results showed higher protein solubility with KOH 0.2% or NaOH 0.2% as used by Pomeranz & Lindner (1960).

However, the variance analysis revealed that there were differences in the solubility due to protein sources ($P < 0.001$), alkalis ($P = 0.025$), concentration of alkalis ($P < 0.001$) and also due to the interaction between the last factors ($P = 0.054$), which is presented in Table 5. The solubility averages were calculated with their deviations and amplitude, assuming that the biggest difference of solubility can better characterize the differences between samples, when related with *in vivo* values, like protein solubility in pepsin (Bellaver et al., 2000). In this assay it was observed that the better protein solubility was obtained with NaOH (57.56%) than with KOH (54.02%) and that in the average from both alkalis the concentration of 0.02% allows larger discrimination in the proteins solubility (difference of 68.61%) in respect to 0.2% (37.97%). Considering the existing interaction between alkalis

and concentrations, the best discrimination was obtained with NaOH to 0.02%.

The results of protein solubility under these conditions for the twelve vegetable ingredients are displayed in Table 6. Also the table shows the pepsin digestibility values for three enzyme concentrations (0.2, 0.02 and 0.002%). The results of total dietary fiber determination (TDF) of the twelve ingredients are exhibited in Table 7.

As it is observed by the analyzes of the ingredients composition, the acid detergent fiber (ADF) oscillated between 5.26 and 50.69%, showing substantial variation in the chemical composition of these ingredients like shown in Tables 1 to 3. The total dietary fiber variation (TDF) gave even larger difference between tested ingredients (6.06 - 77.65%). The *in vitro* assay in alkali (NaOH 0.02% and KOH

Table 1 - Chemical composition¹ and gross energy values for the tested ingredients

Ingredients	DM (%)	CP (%)	EE (%)	CF (%)	MM (%)	ADF (%)	NDF (%)	GE (kcal/kg)
Corn gluten meal (21 CP)	88.26	21.64	2.45	6.63	6.40	9.82	44.40	4182
Soybean meal (46 CP)	89.88	46.08	1.85	5.43	6.09	9.56	25.75	3944
Corn gluten meal (11 CP)	89.40	11.05	2.97	5.07	2.65	9.20	48.27	3980
Citric pulp	90.41	5.93	1.25	10.45	6.97	25.78	28.00	3615
Corn gluten meal (60 CP)	91.15	61.03	1.65	0.03	1.12	9.85	19.32	5141
Cotton seed meal (28 CP)	90.45	27.00	0.89	25.38	4.14	32.88	57.00	4112
Wheat bran	88.58	13.42	3.24	9.14	4.42	12.30	40.35	4020
Soybean meal (48 CP)	88.69	48.45	1.52	3.53	6.06	7.56	27.05	4144
Corn gluten meal (10 CP)	89.20	10.35	1.35	5.16	5.75	9.09	47.70	3661
Soybean hulls	89.78	9.43	1.12	40.67	3.91	50.69	66.39	3713
Corn	87.55	7.20	3.05	1.68	0.97	5.26	14.42	3850
Canola meal	88.80	37.49	2.08	11.31	5.36	19.27	34.18	4249

¹ Dry matter (DM), crude protein (CP), ether extract (EE), crude fiber (CF), mineral matter (MM), acid detergent fiber (ADF), neutral detergent fiber (NDF) and gross energy (GE).

Table 2 - Chemical composition of the ingredients (macro and microelements)

Ingredients	Ca (%)	P (%)	Cu (mg/kg)	Fe (mg/kg)	Mn (mg/kg)	Zn (mg/kg)	K (%)
Corn gluten meal (21 CP)	0.01	0.84	7.05	142.84	26.41	79.11	1.98
Soybean meal (46 CP)	0.32	0.53	16.39	219.45	36.39	59.57	2.42
Corn gluten meal (11 CP)	0.01	0.49	4.99	186.84	19.75	50.22	0.70
Citric pulp	1.57	0.11	5.57	739.29	23.58	14.88	1.03
Corn gluten meal (60 CP)	0.01	0.39	13.08	150.77	5.99	28.85	0.14
Cotton seed meal (28 CP)	0.11	0.56	7.22	98.79	15.09	38.02	1.29
Wheat bran	0.07	0.92	12.22	123.24	114.41	78.47	1.05
Soybean meal (48 CP)	0.32	0.57	19.92	208.02	332.49	60.98	2.26
Corn gluten meal (10 CP)	1.03	0.52	5.13	169.21	27.26	53.44	0.68
Soybean hulls	0.58	0.09	6.77	878.73	30.16	55.68	1.12
Corn	0.01	0.24	2.11	75.98	5.81	22.80	0.28
Canola meal	0.66	0.754	9.71	324.07	69.21	57.97	1.13

Table 3 - Chemical composition of ingredients total amino acids

Ingredients	Amino Acids ¹ (%)								
	Lys	His	Arg	Asp	Thr	Ser	Glu	Pro	Gly
Corn gluten meal (21 CP)	0.41	0.59	0.61	1.19	0.79	0.84	4.30	1.87	1.13
Soybean meal (46 CP)	2.81	1.11	3.06	9.01	1.75	2.49	10.93	2.11	2.17
Corn gluten meal (11 CP)	0.54	0.33	0.83	0.87	0.58	0.55	1.66	0.61	0.52
Citric pulp	0.19	0.09	0.24	0.52	0.23	0.22	0.46	0.49	0.21
Corn gluten meal (60 CP)	0.84	1.12	2.75	3.95	0.25	3.09	12.72	4.50	1.49
Cotton seed meal (28 CP)	1.08	0.66	3.44	2.20	1.04	1.12	4.83	0.98	0.95
Wheat bran	0.58	0.38	0.85	1.27	0.47	0.65	3.58	0.85	0.99
Soybean meal (48 CP)	3.45	1.35	5.09	6.63	1.38	1.00	3.95	2.05	2.20
Corn gluten meal (10 CP)	0.53	0.30	0.81	0.86	0.57	0.53	1.62	0.59	0.49
Soybean hulls	0.69	0.22	0.30	0.85	0.42	0.58	0.95	0.38	0.83
Corn	0.21	0.21	0.30	0.51	0.26	0.37	1.70	0.69	0.32
Canola meal	2.40	1.03	2.52	2.76	2.28	1.75	7.08	2.32	1.86

Ingredients	Amino Acids ² (%)								
	Ala	Cys	Val	Met	Ile	Leu	Tyr	Phe	Try
Corn gluten meal (21 CP)	1.59	0.64	0.98	0.31	0.56	2.07	0.38	0.72	0.09
Soybean meal (46 CP)	1.65	0.78	1.77	0.58	1.91	3.67	1.46	2.19	0.64
Corn gluten meal (11 CP)	0.68	0.21	0.58	0.16	0.35	0.98	0.40	0.46	0.12
Citric pulp	0.21	0.08	0.19	0.06	0.15	0.29	0.14	0.19	0.03
Corn gluten meal (60 CP)	5.04	1.29	2.84	1.28	2.20	9.45	3.81	3.54	0.46
Cotton seed meal (28 CP)	0.91	0.69	0.92	0.49	0.69	1.35	0.79	1.26	0.33
Wheat bran	0.61	0.39	0.51	0.22	0.41	0.89	0.30	0.53	0.19
Soybean meal (48 CP)	2.23	0.90	3.33	0.61	2.15	3.97	2.03	1.28	0.54
Corn gluten meal (10 CP)	0.62	0.26	0.55	0.19	0.30	0.90	0.35	0.42	0.06
Soybean hulls	0.33	0.17	0.35	0.67	0.28	0.50	0.45	0.31	0.05
Corn	0.50	0.20	0.22	0.35	0.22	0.98	0.18	0.31	0.03
Canola meal	1.67	1.13	2.07	0.69	1.38	2.67	1.36	0.73	0.46

¹ Lys = lysine, His = histidine, Arg = arginine, Asp = aspartic acid, Thr = threonine, Ser = serine, Glu = glutamic acid, Pro = proline, Gly = glycine.

² Ala = alanine, Cys = cystine, Val = valine, Met = methionine, Ile = isoleucine, Leu = leucine, Tyr = tyrosine, Phe = phenylalanine, Try = tryptophan.

Table 4 - Averages of protein solubility as function of alkali and concentration

Ingredient	Protein solubility (%)			
	KOH 0.02 %	KOH 0.2 %	NaOH 0.02 %	NaOH 0.2 %
Soybean meal 48 CP	7.93	66.82	10.33	69.06
Soybean meal 45 CP	13.94	77.40	20.92	77.68
Micronized soybean	19.73	80.09	27.66	79.48
Soya isolated protein	67.77	98.46	76.75	98.58

Table 5 - Statistics on the protein solubilities by alkali and concentration

Alkali	Concentration	N	Average ¹	SD	Minimal values	Maximal values	Difference
KOH	0.02	24	27.34 c	24.31	7.64	71.91	64.27
KOH	0.2	24	80.69 a	11.72	64.14	99.83	35.69
NaOH	0.02	24	33.92 b	26.13	9.44	82.38	72.94
NaOH	0.2	24	81.20 a	11.28	66.54	106.78	40.24

¹ Non-similar letters indicates significant differences (P<0.01); interaction between concentration and alkali (P=0.054).

Table 6 - Protein solubility coefficients as function of alkali or pepsin on different concentrations¹

Ingredients	KOH 0.2 %	NaOH 0.02 %	DPP 0.2 %	DPP 0.02 %	DPP 0.002 %
Corn gluten meal (21 CP)	78.43	71.62	70.49	47.43	33.35
Soybean meal (46 CP)	82.79	25.07	90.15	82.99	62.06
Corn gluten meal (11 CP)	40.03	25.47	80.59	58.13	32.50
Citric pulp	52.62	47.60	87.01	54.08	33.97
Corn gluten meal (60 CP)	34.07	6.67	89.20	65.28	17.87
Cotton seed meal (28 CP)	52.91	21.92	66.35	58.42	49.19
Wheat bran	66.73	66.34	76.14	59.46	50.11
Soybean meal (48 CP)	82.17	22.58	86.74	81.14	61.02
Corn gluten meal (10 CP)	32.91	15.52	76.96	57.98	33.37
Soybean hulls	34.65	29.97	51.20	33.08	22.05
Corn	55.93	28.93	88.85	63.29	41.29
Canola meal	57.21	23.37	79.78	68.24	42.73
Standard error of means	18.51	19.71	11.55	13.49	13.87

¹ Solubilities in KOH 0.2% or NaOH 0.02%, solubility in pepsin (DPP) at 0.2, 0.02 and 0.002% concentration.

0.2%) and the solubility of the N through the digestibility in pepsin with different concentrations also presented a considerable variation among the ingredients (Table 6).

The results of amino acid digestibility coefficients for each ingredient were calculated and shown in Table 8. The apparent metabolizable energy corrected for nitrogen (AMEn) is shown in Table 9. These values are similar to the conventional digestibility values shown in known animal nutrition tables.

The obtained *in vitro* variables were correlated with AMEn values and with the *in vivo* amino acid digestibility coefficients and are displayed in Table 10. These results are consistent and facilitated the prediction equations adjustment shown in Table 10. Eliminating R² fittings for values lower than 80%, R² averages values for all the equations were close to 88% and the value estimated average error prediction is situated at 2,95% which encourages additional work on this line. This demonstrates that all the equations except the ones with R² values lower than 80% represent good to excellent estimates.

In the table of equations the AMEn can be better estimated by an equation that considers the variables TDF, ADF, MM and NaOH. An interesting and important fact is that TDF enters in the formulation of all the equations for AMEn. In general CF or TDF also entered in the digestibility prediction equations of AA. This means that these *in vitro* variables are essential for AMEn estimates and for the digestibility coefficients of the AA. The lysine digestibility coefficient can be estimated with CF, ADF, CP and DPP02 with a R² fitting of 95.70% and average error

in the estimate close to 2.75%. Threonine presented a very poor fitting and is not reliable in the estimation by any of the studied equations, being unknown the reason for the failure.

These data allow the improvement of the tables for the amino acid ingredients composition and digestibility. The literature support other predicting methods with alkali to estimate soya quality but actually the methods studied in this paper, as well as, TDF and DPP also are very useful and should be better known for vegetable ingredients.

Due to the promising results found in this work, a continuous research line is expected in this area because it means quickness in the estimates for amino acid and metabolizable energy digestibility, with lower cost and acceptable reliability. With that it

Table 7 - Average values of total dietary fiber (TDF) and their coefficient of variation (CV)

Ingredients	TDF (% ± s)	CV (%)
Corn gluten meal (21 CP)	37.78 ± 3.43	9.09
Soybean meal (46 CP)	23.48 ± 2.54	10.81
Corn gluten meal (11 CP)	26.05 ± 1.36	5.22
Citric pulp	47.97 ± 2.97	6.20
Corn gluten meal (60 CP)	6.06 ± 3.01	49.75
Cotton seed meal (28 CP)	59.49 ± 3.29	5.53
Wheat bran	41.59 ± 1.24	2.99
Soybean meal (48 CP)	20.75 ± 5.37	25.87
Corn gluten meal (10 CP)	27.55 ± 2.20	7.98
Soybean hulls	77.65 ± 1.73	2.23
Corn	10.13 ± 1.71	16.92
Canola meal	29.31 ± 5.57	19.00

Table 8 - Digestibility coefficients (%) of amino acids on the natural matter

Ingredients	Amino Acids									
	Lys	His	Arg	Thr	Val	Met	Ile	Leu	Phe	Try
Corn gluten meal (21 CP)	98.76	89.94	97.26	99.68	96.77	97.03	95.31	82.36	95.58	100.00
Soybean meal (46 CP)	87.54	89.00	89.62	95.55	90.08	90.30	88.00	83.07	88.58	90.20
Corn gluten meal (11 CP)	100.00	100.00	100.00	100.00	100.00	100.00	100.00	87.10	100.00	100.00
Citric pulp	100.00	100.00	100.00	100.00	100.00	100.00	100.00	79.05	100.00	100.00
Corn gluten meal (60 CP)	99.37	92.87	91.35	100.00	95.66	93.24	94.20	90.11	93.07	97.29
Cotton seed meal (28 CP)	75.35	88.79	89.99	98.65	90.79	83.56	85.30	71.93	88.06	87.94
Wheat bran	87.42	89.52	91.35	93.31	94.08	77.60	92.17	69.18	91.31	89.74
Soybean meal (48 CP)	84.41	86.63	87.30	79.10	84.39	83.81	81.61	77.28	77.80	85.33
Corn gluten meal (10 CP)	95.72	95.58	94.12	100.00	100.00	100.00	100.00	80.41	98.21	100.00
Soybean hulls	42.06	76.05	80.56	77.86	47.10	56.12	54.25	69.98	96.94	100.13
Corn	100.00	97.95	100.00	100.00	100.00	100.00	94.01	100.00	85.73	100.00
Canola meal	85.41	89.09	89.18	93.12	88.00	79.63	89.07	82.73	92.42	91.83

¹ Lys = lysine, His = histidine, Arg = arginine, Thr = threonine, Val = valine, Met = methionine, Ile = isoleucine, Leu = leucine, Phe = phenylalanine, Try = tryptophan.

Table 9 - Average values for the apparent metabolizable energy corrected for nitrogen (AMEn) and respective coefficients of variation (CV)

Ingredients	AMEn (kcal/kg)	CV (%)
Corn gluten meal (21 CP)	1632 ± 45	2.73
Soybean meal (46 CP)	2387 ± 50	2.07
Corn gluten meal (11 CP)	2693 ± 131	4.86
Citric pulp	1101 ± 270	24.49
Corn gluten meal (60 CP)	3503 ± 137	3.92
Cotton seed meal (28 CP)	1364 ± 116	8.48
Wheat bran	2065 ± 75	3.62
Soybean meal (48 CP)	2229 ± 185	8.29
Corn gluten meal (10 CP)	2419 ± 51	2.10
Soybean hulls	754 ± 206	27.36
Corn	3290 ± 78	2.36
Canola meal	1989 ± 128	6.42

is confirmed the hypothesis that it is possible to estimate the metabolizable energy and amino acid digestibility of vegetables and this result has a great value in the animal feed production systems.

Conclusions

The highest protein solubility was obtained with NaOH and solubility best discrimination occurred in the concentration of 0.02%.

Protein solubility in pepsin and the total dietary fiber introduced considerable and necessary variation among the vegetable samples to use in prediction equations.

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Metabolizable energy N corrected can be estimated by one equation that considers the variables total dietary fiber, acid detergent fiber, mineral matter and the solubility in NaOH.

Total dietary fiber enters in the formulation of all the equations for the metabolizable energy estimation.

In general, the crude fiber or the total dietary fiber enter in the amino acid digestibility prediction estimates.

The amino acid and energy digestibilities estimates produced high determination coefficients, except for threonine and leucine.

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Table 10 - Estimated prediction equations with respective parameter errors, values for significance of the model, adjusted R2 and average prediction equation errors for AMEn and the ten amino acid, as function of different candidate variables

Variable	Parameters estimates with errors for predicting variables										Model		R2		Avg. Maximal Error	
	AMEn	LYSc	HISc	ARGc	THRc	VALc	METc	ILEc	LEUc	PHEc	TRYc	Prob. F%	Adjust. %	Error %	Maximal %	
	3893.91±111.46	-25.95±5.7790TDF	-10.613±8.2990ADF***	-159.045±24.5691MM							0.00	97.07	4.79	10.11		
	3767.69±140.41	-36.94±3.6580TDF	6.266±4.4280NDF**	-137.951±22.2491MM							0.00	97.18	4.36	9.23		
	4317.15±272.20	-20.31±6.2130TDF	-22.408±10.2900ADF*	-143.238±85.5851EE**							0.00	97.61	4.17	8.07		
	3959.96±99.12	-15.87±6.8810TDF*	-24.285±9.6150ADF	-159.679±20.6891MM							0.00	97.93	4.13	6.40		
	146.53±19.07	-3.77±0.5650CF	1.553±0.3580ADF	-0.205±0.0601CP							0.00	95.70	2.75	7.69		
	88.28±7.04	0.49±0.1560TDF	-3.002±0.4160CF	0.770±0.3790ADF*							0.00	95.27	2.52	7.19		
	136.70±5.98	-2.19±0.1920CF	1.230±0.1380ADF	0.197±0.0440NDF							0.00	95.71	0.84	1.99		
	96.56±2.76	-1.79±0.2700CF	0.962±0.1960ADF	0.131±0.0660NDF*							0.02	90.71	1.30	2.96		
	100.24±1.84	-1.05±0.2750CF	0.508±0.2240ADF	-0.218±0.0431CP							0.03	81.83	1.93	3.77		
	131.05±11.40	0.35±0.1310TDF	-1.717±0.3230CF	0.617±0.2340ADF							0.12	83.15	1.44	3.90		
	179.85±18.42	-2.12±0.4480CF	1.109±0.2860ADF	-1.383±0.4461MM							0.15	81.61	1.65	3.67		
	97.30±9.78	0.41±0.2480TDF**	-1.052±0.4880CF*	3.281±3.1321EE***							5.17	40.12	4.11	11.52		
	104.65±6.89	0.64±0.3050TDF*	-1.511±0.5350CF	-1.477±1.2881MM***							4.70	41.68	4.19	13.05		
	71.46±10.81	1.31±0.3900TDF	-2.944±0.5990CF	8.862±4.3101EE*							0.05	83.15	4.28	10.11		
	102.37±6.36	0.68±0.2260TDF	-2.196±0.4260CF	-0.280±0.1001KOH							0.04	80.43	4.50	14.10		
	102.12±5.95	0.66±0.2110TDF	-2.174±0.3990CF	-0.228±0.0931KOH							0.02	82.99	3.86	9.65		
	104.20±3.72	-2.14±0.5560CF	0.878±0.4530ADF*	-0.284±0.0871CP							0.02	84.06	3.78	11.51		
	106.12±6.64	0.48±0.2500TDF*	-1.926±0.4370CF	-0.179±0.0991KOH**							0.05	83.97	3.55	9.65		
	153.56±30.40	11.53±2.4341EE	-0.024±0.0091CF	-0.358±0.0881KOH							0.36	71.49	2.86	6.72		
	91.46±4.40	0.22±0.0650TDF	-0.219±0.0810NDF	8.466±1.2661EE							0.05	86.96	1.67	2.81		
	159.84±16.17	-0.07±0.0410TDF**	2.100±1.3451EE**	-0.013±0.0051CF							0.02	90.25	1.11	2.86		
	159.42±16.21	-0.08±0.0440NDF**	2.542±1.2021EE*	-0.013±0.0051CF							0.02	90.20	1.19	2.48		

* (P<0.10) ** (P<0.20) *** (P<0.30) **** (P<0.40); otherwise (P<0.05)

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Recebido em: 04/02/04

Aceito em: 24/08/04