Diet components as internal indicators in the determination of the apparent digestibility coefficients for Nile tilapia

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Abstract – The objective of this work was to evaluate the diet components – crude fiber, neutral detergent fiber, acid detergent fiber, hemicellulose, cellulose, and lignin – as internal indicators in the determination of the apparent digestibility coefficients of dry matter, gross energy, and of the nutrients – crude protein, phosphorus, and amino acids – in Nile tilapia (*Oreochromis niloticus*). Groups of ten juveniles (80.3±1 g) were randomly distributed in six tanks of 250 L and fed two practical diets, either of a plant-origin diet or of a plant- and animal-origin diet. Both diets were supplemented with 0.1% chromium (III) oxide (Cr_2O_3). Faeces were collected by the modified Guelph system. The apparent digestibility coefficients were determined by the content difference of the internal indicators, present in the diets and faeces, and compared by Dunnett's test to those obtained by the use of Cr_2O_3. Cellulose was effective in the determination of the apparent digestibility of dry matter, energy, and nutrients of both experimental diets; and the acid detergent fiber was effective only for the diet composed exclusively of plant-origin ingredients. The use of crude fiber, neutral detergent fiber, hemicellulose, and lignin as digestibility indicators was inefficient for the analyzed nutrients of both diets. Therefore, cellulose is the most suitable indicator for digestibility evaluation in Nile tilapia.

Index terms: *Oreochromis niloticus*, cellulose, fiber, nutrients.

Componentes da dieta como indicadores internos na determinação dos coeficientes de digestibilidade aparente em tilápia-do-nilo

Resumo – O objetivo deste trabalho foi avaliar os componentes da dieta – fibra bruta, fibra em detergente neutro, fibra em detergente ácido, hemicelulose, celulose e lignina – como indicadores internos na determinação dos coeficientes de digestibilidade aparente da matéria seca, da energia bruta e dos nutrientes – proteína bruta, fósforo e aminoácidos – em tilápia-do-nilo (*Oreochromis niloticus*). Grupos de dez juvenis (80,3±1 g) foram aleatoriamente distribuídos em seis tanques de 250 L e alimentados com duas dietas práticas, ou com ingredientes de origem vegetal ou de origem vegetal e animal. Ambas as dietas foram suplementadas com 0,1% de óxido de cromo-III (Cr_2O_3). As fezes foram coletadas pelo sistema Guelph modificado. Os coeficientes de digestibilidade aparente foram determinados pela diferença de teor dos indicadores internos, presentes nas dietas e nas fezes, e comparados, pelo teste de Dunnett, aos obtidos pelo uso de Cr_2O_3. A celulose foi efetiva na determinação dos coeficientes de digestibilidade aparente da matéria seca, da energia e dos nutrientes de ambas as dietas; e a fibra em detergente ácido foi efetiva apenas para a dieta que continha exclusivamente ingredientes de origem vegetal. A utilização de fibra bruta, fibra em detergente neutro, hemicelulose e lignina, como indicadores de digestibilidade, mostrou-se ineficiente quanto aos nutrientes analisados de ambas as dietas. Assim, a celulose é o indicador mais apropriado para a avaliação de digestibilidade em tilápia-do-nilo.

Termos para indexação: *Oreochromis niloticus*, celulose, fibra, nutrientes.

Introduction

In intensive animal farming, feed is the main or exclusive source of nutrients for fish, accounting for 50–70% of production costs (Guimarães et al., 2008a). However, the use of feeds that are not properly balanced reduces the absorption of nutrients by fish, which results in excess organic

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matter in the production systems. Under tropical conditions, this excess is rapidly mineralized and becomes readily available for phytoplankton growth, with a consequent decrease of water transparency and change of water quality. As a result, a decrease occurs in the concentration of dissolved oxygen, especially at night, leading to respiratory and biochemical stresses that induce to serious fish health risks, and to possible production losses (Cyrino et al., 2010).

Nile tilapia is the sixth most cultivated fish species in the world (Michelato et al., 2013; Tacon & Metian, 2013) and the most cultivated one in Brazil. In the country, the animal feed industry certifies the feed quality by the performance responses obtained mainly by weight gain and apparent feed conversion. Unfortunately, the apparent digestibility coefficient of the nutrients is not determinant in the choice of feeds, whether due to the absence of these values as a rule, or to the difficulty of obtaining them, without reprocessing the feeds, for the inclusion of an external indicator of digestibility.

Determining the values for apparent digestibility coefficients by dietary components would enable investigations of the nutritional value of commercial feeds, which could help with the development of low-polluting feeds. In this sense, some components have been evaluated and used as indicators of internal digestibility for fish, such as crude fiber (Morales et al., 1999; Vidal Junior et al., 2004; Krontveit et al., 2014), neutral detergent fiber (Furuya et al., 2004), acid detergent fiber (Vidal Junior et al., 2004), hydrolysis-resistant organic matter (Buddington, 1980), acid-insoluble ash (Vidal Junior et al., 2004; Li et al., 2008; Da et al., 2013) and acid-detergent insoluble ash (Vidal Junior et al., 2004). However, the use of these components remains contradictory because the chemical composition of the evaluated diet may affect the obtained coefficients (Morales et al., 1999).

The objective of this work was to evaluate the diet components – crude fiber (CF), neutral detergent fiber (NDF), acid detergent fiber (ADF), hemicellulose, cellulose, and lignin – as internal indicators in the determination of apparent digestibility coefficients (ADC) of dry matter (DM), gross energy, and of the nutrients – crude protein (CP), phosphorus, and amino acids – in Nile tilapia.

Materials and Methods

The experiment was carried out at the laboratory for nutrition of aquatic organisms (AquaNutri) of Faculdade de Medicina Veterinária e Zootecnia, Universidade Estadual Paulista Júlio de Mesquita Filho, in the municipality of Botucatu, in the state of São Paulo, Brazil. The adopted procedures were approved by the institution's animal ethics committee (CEUA), with the protocol No. 132/2014.

To meet the Nile tilapia nutritional requirements (Furuya, 2010), two practical diets were prepared: the first one was made exclusively with plant-origin ingredients, and the second one was made with animal- and plant-origin ingredients (Table 1). Chromium (III) oxide (Cr_2O_3) at 0.1% (m/m) was added to both diets, and used as the standard external indicator, which is widely used in fish digestibility assays (Guimarães et al., 2008b, 2008c; Xavier et al., 2014; Vidal et al., 2015).

For the preparation of the feeds, all ingredients were milled (0.4 mm diameter), weighed, and homogenized.

Table 1. Composition of the experimental diets.

Ingredient (%)	Diet	
	PD[1]	APD[2]
Soybean meal	48.1	43.62
Corn gluten	4.5	–
Meat and bone meal	–	4
Poultry by-product meal	–	6
Corn	33.96	33.96
Wheat middlings	10	10
Soybean oil	0.19	0.11
L-Lysine	0.22	0.02
DL-Methionine	0.31	0.26
L-Threonine	0.33	0.31
Dicalcium phosphate	1.63	0.96
Sodium chloride (NaCl)	0.1	0.1
Premix - vitamin and mineral mix[3]	0.5	0.5
Vitamin C[4]	0.04	0.04
Antioxidant (BHT)[5]	0.02	0.02
Chromium (III) oxide	0.1	0.1

[1]PD, diet containing exclusively plant-origin ingredients. [2]APD, diet containing animal- and plant-origin ingredients. [3]Premix (Tectron, Toledo, PR), vitamin and mineral mix (kg of product): vitamin A, 1,000,000 IU; vitamin D_3, 500,000 IU; vitamin E, 20,000 IU; vitamin K3, 500 mg; vitamin B_1, 1,900 mg; vitamin B2, 2,000 mg; vitamin B_6, 2,400 mg; vitamin B12, 3,500 mcg; vitamin C, 25 g; niacin, 5,000 mg; calcium pantothenate, 4,800 mg; folic acid, 200 mg; biotin, 40 mg; Mn, 7,500 mg; Zn, 25 g; Fe, 12.5 g; Cu, 2,000 mg; I, 200 mg; Se, 70 mg; antioxidant, 300 mg. [4]Vitamin C Rovimix Stay-35 (DMS Nutritional Products, Switzerland). [5]Butylated hydroxytoluene (antioxidant).

After the addition of water at 55°C (20% of the total mass), the mixtures were extruded (Exteec, Ribeirão Preto, SP, Brazil). Subsequently, the mixtures were dried in an oven at 55°C, for 24 hours, and stored in labelled plastic containers in a cold chamber (5°C) until use. The composition of the diets are shown in the Tables 1 and 2.

For fish feeding, six circular fiberglass tanks (250 L), connected to a physical and biological filter with a continuous water-recirculation system, were used. For the collection of faeces, six cylindrical fiberglass tanks of 300 L, each with a conical bottom, were used, with a physical-biological filter and individual water recirculation system. The physicochemical parameters of water, such as pH (6.66±0.04) and dissolved oxygen (6.23±0.22 mg L^{-1}) were measured weekly using a YSI-556 multiparameter probe (YSI Environmental, Yellow Spring, OH, USA). Total ammonia content (0.002±0.000 ppm) was measured weekly using a Labcon commercial kit (Alcon, Camboriú, SC, Brazil). The temperature was measured daily (25.3±0.5°C). The photoperiod was of 12 hours, and it was controlled with fluorescent lamps.

Table 2. Composition of the experimental diets on a dry-matter basis.

Composition	Diet	
	PD[(1)] (%)	APD[(2)] (%)
Dry matter	94.36	93.98
Gross energy (kcal kg^{-1})	4,390	4,338
Crude protein	31.76	32.18
Ether extract	3.29	4.18
Ash	5.60	6.91
Phosphorus	0.80	1.10
Essential amino acids		
Arginine	2.26	2.43
Histidine	0.74	0.97
Isoleucine	0.95	0.85
Leucine	2.84	2.50
Lysine	1.99	1.97
Phenylalanine	1.47	1.39
Methionine	0.52	0.61
Threonine	1.13	1.31
Tryptophan	0.28	0.23
Valine	0.93	0.96
Nonessential amino acids		
Alanine	1.67	1.76
Aspartic acid	3.14	3.18
Cystine	0.67	0.45
Glycine	1.77	2.49
Glutamic acid	6.09	5.84
Proline	2.79	2.88
Serine	1.66	1.62
Tyrosine	0.74	0.74
Indicator		
Chromium (III) oxide	0.10	0.10
Crude fiber	4.33	4.46
Neutral detergent fiber	19.19	21.56
Acid detergent fiber	6.78	8.61
Hemicellulose	12.41	12.95
Cellulose	5.00	6.25
Lignin	0.87	0.80

[(1)]PD, diet containing exclusively plant-origin ingredients. [(2)]PAD, diet containing animal- and plant-origin ingredients.

Masculinized juveniles of Nile tilapia (80.3±1 g) were stocked (ten fish per cage) in six cylindrical cages of 120 L made of a plastic screen (1.5 cm mesh). The cages, placed in the feeding tanks, were used to house fish and to facilitate handling between the feeding and fecal collection tanks. For seven days, fish remained in the feeding tanks for adaptation to the handling and to the feeds, which were given four times a day – at 8:00, 11:00, 14:00, and 15:00 h until apparent satiation.

Subsequently, the feed was supplied hourly (from 8:00 to 5:00 h); at the end of the day (6:00 h), fish were transferred to the fecal collection tanks, where they remained overnight. In the morning of the following day (8:00 h), fish were returned to their respective feeding tanks, and faeces were collected with sampling vials (200 mL) connected to the bottom of the collection tanks. This procedure was carried out for 14 days, which allowed of the obtention of a representative fecal volume for the chemical-nutritional analysis (±6 g per tank, in the DM basis). The collected faeces were dehydrated in an oven at 55°C, for 48 hours, ground, and stored at -20°C.

The determinations of DM, CP, ether extract, and mineral matter of the feeds and faeces were performed according to the methodologies described by AOAC (Silva & Queiroz, 2006). Gross energy was estimated by the combustion of samples in a bomb calorimeter C200 (IKA, Staufen, BW, Germany). Phosphorus was quantified by the vanadomolybdophosphoric acid method (Moraes et al., 2009). Amino acid analysis was performed by high-performance liquid chromatography (HPLC) at the laboratory CBO Análises Laboratoriais (Campinas, SP, Brazil).

The indicators were determined as below described. Crude fiber was quantified by the Weende method (Silva & Queiroz, 2006), whereas the NDF, ADF,

cellulose, and lignin were measured according to the sequential methodology described by Van Soest et al. (1991). The hemicellulose content was obtained by the equation (NDF - ADF). The content of chromium (III) oxide was determined according to the methodology of Bremer Neto et al. (2005).

The calculations of the ADCs of the DM, energy, and nutrients (CP, phosphorus, and amino acids) of the experimental diets were carried out using the external Cr_2O_3 standard indicator, and the calculations of the remaining internal indicators were performed using the equations described by Maynard & Loosli (1969) and Bureau & Hua (2006), respectively, as follows: $ADC_{DM} = 1 - (I_d / I_f)$, in which ADC_{DM} is the apparent DM digestibility coefficient, I_d is the indicator percentage in the diet, and I_f is the indicator percentage in the faeces. Furthermore, $ADCN = 1 - [(I_d / I_f) \times (N_f / N_d)]$, in which ADC_N is the ADC of the nutrient, I_d is the indicator percentage in the diet, I_f is the indicator percentage in the faeces, N_f is the nutrient percentage (or kcal kg^{-1} gross energy) in the faeces, and N_d is the nutrient percentage (or kcal kg^{-1} gross energy) in the diet.

The experiment was carried out in a completely randomized design in a split-plot arrangement, with repeated measures, and three replicates for each diet. The plots consisted of the two diets, and the subplots consisted of the seven digestibility indicators.

Data were previously analyzed for normality by the Shapiro-Wilk's test, and based on this premise, they were subjected to the analysis of variance for the one-factor model, at 5% probability. The Dunnett's test was used to compare the ADC means, obtained when using the standard external indicator Cr_2O_3, to those means provided by the evaluated internal indicators. The analyses were performed by the Minitab statistical software, version 16 (Minitab, Inc., State College, PA, USA).

Results and Discussion

The diet with animal- and plant-origin ingredients showed a higher percentage of CF, NDF, ADF, hemicellulose, and cellulose than the diet containing exclusively plant-origin ingredients (Table 2). This result may be associated with the ingredients meat-and-bone meal and poultry by-product meal, whose inclusion, although at lower levels than the maximum recommended one for the species (Hernández et al., 2010; Abimorad et al., 2014), may have affected the determination of the obtained values due to the chemical composition of these ingredients, which comprised a high content of ether extract and mineral matter (Van Soest et al., 1991).

The ADF compared with the values obtained with the Cr_2O_3 standard indicator provided an adequate determination of the ADCs of the DM, energy, and nutrients of the diet that exclusively contained ingredients of plant origin (Table 3). However, there is a result reported on a study on tambaqui (*Colossoma macropomum*) whose ADF provided values which differed from those obtained with Cr_2O_3, when the digestibility coefficients of DM and CP of finely ground corn and soybean meal were evaluated (Vidal Junior et al., 2004). Like ADF, the use of cellulose as an indicator of digestibility provided similar ADCs for the DM, energy, and nutrients in the diet with plant-origin ingredients only (Table 3), in comparison with the values obtained by using the external standard indicator Cr_2O_3.

In the determination of ADC and cellulose of the diet with animal- and plant-origin ingredients, it was observed that ADF provided values lower than those obtained using Cr_2O_3 for the DM, energy, and nutrients ($p<0.05$), while the cellulose provided lower values only for CP and for the essential amino acids phenylalanine and valine ($p<0.05$) (Table 4). The ADF consists mostly of cellulose and lignin, and it is basically used as a pre-extracted material for the determination of lignin, by the methods of acid detergent lignin or potassium permanganate lignin (Silva & Queiroz, 2006). Although Saha et al. (2006) reported the isolation of bacteria strains with cellulase activity, in the intestine of Mozambique tilapia (*O. mossambicus*), the coefficients obtained with the ADF and cellulose in the present study for the diet with exclusively plant-origin ingredients indicate that no degradation occurred in these components by Nile tilapia. The lower ADCs provided by using ADF and cellulose (for CP and the amino acids phenylalanine and valine) as indicators of diet digestibility, with animal- and plant-origin ingredients, may be attributed to the influence of the meat-and-bone meal and poultry by-product meal on the values (Van Soest et al., 1991).

The use of CF, in comparison with the data obtained with Cr_2O_3 resulted in higher ADCs for the DM, energy, and nutrients for both diets ($p<0.05$) (Tables 3 and 4).

Crude fiber does not represent a homogeneous group of substances, as it is composed mainly of nonstarch polysaccharides (NSP) (Goñi et al., 2009). The NSP fraction can be classified as insoluble (cellulose and hemicellulose) and soluble (pectins, gums, and mucilages), according to its solubility in water. The soluble NSP is digestible during the passage through the digestive tract (Amirkolaie et al., 2005; Krogdahl et al., 2005). In the present study, the ADC of the NSP fraction was not determined. However, according to the coefficients provided by the CF as an indicator of digestibility, it is possible that NSP fraction has not undergone degradation. Determination of the CF by the Weende's method may have affected the obtained coefficients because, in the Weende's method, parts of the hemicellulose and the lignin are solubilized and become part of the nitrogen-free extract (Silva & Queiroz, 2006).

The use of NDF and hemicellulose as indicators of digestibility provided lower ADCs for the DM, energy, and nutrients, in both diets ($p<0.05$), in comparison with the standard indicator Cr_2O_3 (Tables 3 and 4), which may be associated with the solubilization of pectin by the neutral detergent solution (Van Soest et al., 1991). Pectin constitutes the cell wall of plants, together with the structural carbohydrates (cellulose and hemicellulose) and lignin (Van Soest et al., 1991), and it is described by Brito et al. (2008) as nondigestible by monogastric animals. As pectin constitutes a relevant percentage of the cell wall of soybean meal (Queiroz et al., 2010), and this ingredient corresponds to the protein base of both diets, its solubilization may have affected the determination of the coefficients, when the NDF was used as an indicator. In turn, the hemicellulose, obtained by subtraction of NDF by ADF, may also have been changed by this solubilization.

Table 3. Apparent digestibility coefficients[1] (ADC, %) of dry matter, gross energy, and nutrients estimated using chromium (III) oxide (Cr_2O_3), crude fiber (CF), neutral detergent fiber (NDF), acid detergent fiber (ADF), hemicellulose, cellulose, and lignin as indicators in Nile tilapia fed plant-origin diet[2].

Nutrient/ amino acids	Cr_2O_3	CF	NDF	ADF	Hemicellulose	Cellulose	Lignin
Dry matter	76.77±0.24a	82.3±0.24b	72.46±0.31b	75.93±0.31a	70.09±0.55b	77.17±0.2a	70.33±0.35b
Gross energy	81.31±0.19a	85.75±0.19b	77.83±0.25b	80.63±0.25a	75.93±0.44b	81.63±0.16a	76.12±0.28b
Crude protein	94.79±0.11a	96.03±0.05b	93.83±0.07b	94.61±0.02a	93.3±0.14b	94.88±0.02a	93.35±0.13b
Phosphorus	51.23±0.83a	62.81±1.02b	42.18±0.53b	49.44±1.16a	37.23±0.26b	52.05±0.9a	37.72±0.33b
Essential amino acids							
Arginine	97.39±0.04a	98.01±0.07b	96.91±0.1b	97.29±0.11a	96.64±0.11b	97.43±0.09a	96.67±0.08b
Histidine	97.93±0.02a	98.42±0.02b	97.54±0.03b	97.85±0.03a	97.33±0.05b	97.96±0.02a	97.36±0.03b
Isoleucine	92.35±0.01a	94.17±0.13b	90.93±0.18b	92.07±0.2a	90.15±0.22b	92.48±0.16a	90.23±0.13b
Leucine	95.94±0.02a	96.91±0.08b	95.19±0.11b	95.79±0.12a	94.78±0.12b	96.01±0.09a	94.82±0.08b
Lysine	96.27±0.11a	97.16±0.04b	95.58±0.07b	96.14±0.02a	95.2±0.12b	96.34±0.03a	95.24±0.12b
Phenylalanine	95.14±0.06a	96.29±0.12b	94.23±0.17b	94.95±0.18a	93.73±0.18b	95.21±0.15a	93.79±0.13b
Methionine	99.51±0a	99.62±0.01b	99.42±0.01b	99.49±0.01a	99.37±0.01b	99.52±0.01a	99.37±0.01b
Threonine	94.47±0.15a	95.78±0.2b	93.44±0.28b	94.26±0.29a	92.88±0.3b	94.56±0.25a	92.94±0.24b
Tryptophan	96.74±0.30a	97.53±0.18a	96.15±0.29a	96.64±0.23a	95.82±0.34a	96.81±0.23a	95.84±0.35a
Valine	92.18±0.01a	94.04±0.14b	90.72±0.18b	91.89±0.2a	89.93±0.22b	92.31±0.16a	90.01±0.13b
Nonessential amino acids							
Alanine	94.04±0.04a	95.45±0.12b	92.93±0.17b	93.82±0.18a	92.32±0.19b	94.14±0.15a	92.38±0.12b
Aspartic acid	98.61±0.08a	98.94±0.08a	98.35±0.12a	98.56±0.12a	98.21±0.13a	98.63±0.11a	98.23±0.12a
Cystine	94.63±0.05a	95.91±0.06b	93.63±0.08b	94.44±0.08a	93.09±0.13b	94.72±0.06a	93.14±0.08b
Glycine	92.92±0.36a	94.59±0.38a	91.59±0.56a	92.64±0.54a	90.87±0.58b	93.02±0.48a	90.95±0.51a
Glutamic acid	98.34±0.02a	98.73±0.04b	98.03±0.06b	98.28±0.06a	97.86±0.06b	98.37±0.05a	97.88±0.05b
Proline	95.68±0.07a	96.71±0.12b	94.88±0.17b	95.52±0.17a	94.44±0.18b	95.75±0.15a	94.49±0.13b
Serine	95.62±0.1a	96.66±0.14b	94.8±0.20b	95.45±0.2a	94.35±0.21b	95.69±0.18a	94.4±0.16b
Tyrosine	94.14±0.15a	95.53±0.20b	93.04±0.29b	93.91±0.29a	92.44±0.3b	94.23±0.26a	92.51±0.24b

[1]Mean±standard error of the mean (n=3). [2]Means followed by equal letters, in the rows, do not differ from one another by the Dunnett's test, at 5% probability.

Table 4. Apparent digestibility coefficients[1] (ADC, %) of dry matter, gross energy, and nutrients estimated using the external indicator chromium (III) oxide (Cr_2O_3), and the internal indicators crude fiber (CF), neutral detergent fiber (NDF), acid detergent fiber (ADF), hemicellulose, cellulose, and lignin as indicators in Nile tilapia fed animal- and plant-origin diet[2].

Nutrient/ amino acids	Cr_2O_3	CF	NDF	ADF	Hemicellulose	Cellulose	Lignin
Dry matter	74.67±0.6a	80.62±0.84b	68.94±0.37b	66.83±0.75b	70.17±0.64b	71.39±1.03a	61.88±1.27b
Gross energy	79.14±0.49a	84.04±0.69b	74.43±0.31b	72.69±0.61b	75.44±0.52b	76.44±0.85a	68.61±1.04b
Crude protein	92.38±0.08a	94.18±0.17b	90.66±0.14b	90.03±0.08b	91.02±0.26b	91.4±0.2b	88.53±0.47b
Phosphorus	55.13±1.82a	65.66±2b	45.03±1.46b	41.27±2.26b	47.21±1.49a	49.32±2.53a	32.55±2.46b
Essential amino acids							
Arginine	96.55±0.03a	97.36±0.04b	95.76±0.11b	95.48±0.03b	95.93±0.16b	96.1±0.06a	94.79±0.27b
Histidine	97.02±0.02a	97.72±0.04b	96.35±0.09b	96.1±0.02b	96.49±0.14b	96.64±0.05a	95.51±0.23b
Isoleucine	87.74±0.12a	90.63±0.14b	84.96±0.42b	83.95±0.14b	85.54±0.59b	86.17±0.2a	81.52±0.97b
Leucine	92.92±0.04a	94.58±0.12b	91.31±0.18b	90.72±0.03b	91.65±0.29b	92±0.14a	89.32±0.5b
Lysine	94.14±0.04a	95.52±0.08b	92.81±0.18b	92.33±0.05b	93.09±0.27b	93.39±0.1a	91.17±0.44b
Phenylalanine	92.12±0.08a	93.97±0.18b	90.33±0.15b	89.68±0.09b	90.71±0.27b	91.1±0.21b	88.12±0.49b
Methionine	99.09±0.02a	99.31±0.03b	98.89±0.01b	98.81±0.03b	98.93±0.02b	98.98±0.04a	98.64±0.05b
Threonine	93.49±0.05a	95.02±0.13b	92.02±0.15b	91.48±0.04b	92.33±0.24b	92.65±0.15a	90.2±0.43b
Tryptophan	89.3±2.94a	91.74±2.44a	87.01±3.35a	86±3.88a	87.59±3.07a	87.86±3.51a	84.21±3.78a
Valine	88.09±0.21a	90.89±0.33b	85.39±0.17b	84.41±0.25b	85.97±0.34b	86.55±0.4b	82.07±0.65b
Nonessential amino acids							
Alanine	91.32±0.06a	93.36±0.12b	89.35±0.26b	88.63±0.06b	89.76±0.38b	90.2±0.16a	86.91±0.64b
Aspartic acid	97.71±0.08a	98.25±0.10b	97.19±0.05b	97±0.1b	97.30±0.06b	97.41±0.12a	96.56±0.1b
Cystine	87.45±0.95a	90.43±0.56a	84.55±1.45a	83.56±1.26a	85.13±1.55a	85.85±0.97a	80.97±2.15b
Glycine	91.73±0.14a	93.68±0.06b	89.85±0.36b	89.18±0.18b	90.24±0.47a	90.67±0.14a	87.52±0.74b
Glutamic acid	97.44±0.02a	98.04±0.04b	96.86±0.08b	96.65±0.02b	96.98±0.11b	97.11±0.05a	96.14±0.19b
Proline	94±0.05a	95.41±0.12b	92.64±0.13b	92.14±0.04b	92.93±0.22b	93.22±0.14a	90.96±0.39b
Serine	93.72±0.15a	95.21±0.05b	92.29±0.34b	91.78±0.2b	92.59±0.41a	92.92±0.14a	90.52±0.63b
Tyrosine	91.33±0.11a	93.37±0.21b	89.37±0.15b	88.65±0.12b	89.78±0.28b	90.21±0.25b	86.94±0.52b

[1]Mean±standard error of the mean (n=3). [2]Means followed by equal letters, in the rows, do not differ from one another by the Dunnett's test, at 5% probability.

As observed with the use of NDF and hemicellulose, the use of lignin as an indicator of digestibility resulted in lower ADCs for the DM, energy, and nutrients ($p<0.05$), except for the values obtained for the amino acids tryptophan, aspartic acid, and glycine of the diet with exclusively plant-origin ingredients (Tables 3 and 4).

The ADF and the cellulose were effective in determining the ADC of the DM, energy, and nutrients of the diet that contained exclusively plant-origin ingredients. Regarding the diet with animal- and plant-origin ingredients, only cellulose was effective in determining the ADCs. This result indicates the possible interference of the ingredients of animal-origin in the determination of the components evaluated as internal indicators. Furthermore, it indicates the importance of subsequently evaluating diets that contain higher levels of inclusion of animal-origin ingredients, in order to verify the possible influence of these ingredients on determinations of the dietary cellulose and, therefore, the validity of cellulose as an internal indicator of digestibility.

Conclusions

1. Dietary cellulose is effective in determining the apparent digestibility components of dry matter, gross energy, and nutrients of diets that exclusively contain plant-origin ingredients, or animal- and plant-origin ingredients.

2. Acid detergent fiber is effective as an indicator of digestibility only for the diet with plant-origin ingredients.

3. The use of crude fiber, neutral detergent fiber, hemicellulose, and lignin is not effective in the estimation of the apparent digestibility coefficients of dry matter, gross energy, and nutrients, irrespective of the diet composition.

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