METABOLISM AND NUTRITION

Effect of Chemical Composition of Sunflower Seed Meal on its True Metabolizable Energy and Amino Acid Digestibility[1]

M. J. VILLAMIDE[2] and L. D. SAN JUAN

Departamento de Producción Animal, E.T.S.I. Agrónomos, Universidad Politécnica, 28040 Madrid, Spain

ABSTRACT The effect of chemical composition of sunflower seed meal (SFSM) on TME_n and true amino acid digestibility (TAAD) was studied. In Experiment 1, the excretion pattern of three SFSM samples force-fed (30 g) to 10 adult cockerels (Hy-Line) each was followed for 84 h to determine the time interval for complete excretion of SFSM. Type of SFSM did not affect the excretion pattern of DM and energy ($P = 0.438$, and $P = 0.189$, respectively). Dry matter and energy excreted every 12 h decreased linear and quadratically ($P < 0.001$) with collection time. No differences were found from 48 h collection time on. So, an excreta collection period of 48 h was considered adequate for determining the TME_n of SFSM. In Experiment 2, 135 adult cockerels were force-fed to determine the TME_n of 11 samples of SFSM. Type of SFSM affected TME_n ($P < 0.001$), which ranged from 1,558 to 2,023 kcal/kg DM for SFSM of 31 to 42% CP, respectively. The TME_n was highly correlated ($P < 0.001$) to hemicellulose (r= –0.90), acid detergent lignin (r= –0.84), neutral detergent fiber (r= –0.82), and CP (r= 0.77). Four prediction equations are proposed, the most practical being: TME_n (kcal/kg DM) = 2,816.8 – 109.5 hemicellulose (%DM), RSD = 70.2. Three out of the 11 samples of SFSM were selected for determining TAAD and the effect of endogenous amino acid correction. The methodology used was that of the TME_n assay, but one more estimation of amino acid endogenous excretion was made using a N-free diet with 85% cornstarch and 15% cellulose. Endogenous amino acid excretion was greater for roosters fed the N-free diet than those deprived of feed, resulting in a higher digestibility (from 0.7 to 2.7%, $P < 0.05$) only for six amino acids: threonine, valine, alanine, proline, and aspartic and glutamic acids. No interaction was detected ($P = 0.94$) between type of SFSM and method of estimation of endogenous amino acid excretion. The TAAD of SFSM increased significantly ($P < 0.001$) with the CP content, total TAAD being 86, 88, and 89% for SFSM of 32, 35, and 37% CP, respectively. Attention should be paid when including high fiber-low protein SFSM in poultry diets to balance its lesser digestible amino acids contribution, mainly in lysine (from 0.77 to 1.06% for SFSM of 32 and 37% CP, respectively).

(*Key words*: sunflower seed meal, nitrogen-corrected true metabolizable energy, true amino acid digestibility)

1998 Poultry Science 77:1884–1892

INTRODUCTION

Sunflower is an oilseed cultivated worldwide for oil extraction, due to its great capability of adaptation to different climatic and soil conditions (Ravindran and Blair, 1992). The by-product rendered by the oil industry, sunflower seed meal (SFSM), is used as an alternative source of protein in animal nutrition. Its CP content depends on dehulling and oil extraction process, ranging from 29 to 45% (Spanish cultivars, FEDNA, 1994), in inverse relation to fiber content (from 32 to 14% of crude fiber, CF). This high fiber content and its deficiency of lysine are responsible for the limited use of SFSM in poultry diets. Nevertheless, Zatari and Sell (1989) and Vieira *et al.* (1992) reported successful results in broiler chickens and laying hens using high levels of SFSM (20%) in diets formulated with adequate levels of lysine and ME.

Additional problems in the use of SFSM are: 1) the scarce information about its nutritive value, and 2) its variability, which supports the need for the use of prediction equations. A fast, simple, and precise method for assessing both energy value and amino acid digestibility of single feedstuffs is the precision-fed cockerel assay developed by Sibbald (1976) and modified 10 yr later (Sibbald, 1986; excreta collection period of 48 h). However, when this method has been applied to high fiber feedstuffs, a great variability in TME_n

Received for publication October 14, 1997.
Accepted for publication July 21, 1998.
[1]Financial support provided by Universidad Politécnica de Madrid. Project Number A 9000 0 200 168.
[2]To whom correspondence should be addressed: mvillamide@pan.etsia.upm.es

Abbreviation Key: ADF = acid detergent fiber; ADL = acid detergent lignin; CF = crude fiber; NDF = neutral detergent fiber; SFSM = sunflower seed meal; TAAD = true amino acid digestibility.

TABLE 1. Chemical composition of sunflower seed meals used in this study (DM basis)

Sunflower seed meal	Crude protein	Crude fiber	Neutral detergent fiber	Acid detergent fiber	Acid detergent lignin	Ash	Ether extract	Gross energy
	(%)							(kcal/kg)
A	31.46	30.13	45.96	34.35	11.35	7.23	1.97	4,661
B	32.09	27.62	42.52	31.85	10.82	7.24	1.31	4,645
C	33.66	26.95	40.02	31.30	9.61	6.92	2.23	4,699
D	34.42	20.54	32.88	24.75	6.96	9.01	0.73	4,557
E	34.84	23.74	34.97	26.89	7.68	7.27	1.60	4,644
F	35.49	23.23	35.67	26.72	7.55	7.04	3.61	4,710
G	36.41	21.21	34.12	25.24	7.09	7.62	1.68	4,630
H	36.79	20.94	32.99	24.64	7.18	7.36	1.42	4,616
I	37.10	20.70	32.26	24.34	7.12	7.35	2.11	4,643
J	37.19	20.72	32.48	23.64	6.68	7.26	1.39	4,634
K	41.75	19.23	28.41	20.87	5.94	8.74	1.68	4,617

values has been observed (Sibbald, 1979), probably due to incomplete excretion. Thus, Sibbald and Morse (1983), collecting feces every 12 h, obtained a complete excretion of alfalfa hay only 60 h after force-feeding. Likewise, Fisher and McNab (1987) overestimated the energy value of pea hulls with 48 h of collection. Therefore, knowledge of the excretion pattern of SFSM (high fiber content) seems to be necessary before the application of the TME_n assay to obtain reliable values by this method.

The estimation of the endogenous amino acid excretion and the microbial activity of ceca are two important problems in the determination of the digestibility of amino acids. Comparisons between feed-deprived birds and those fed a N-free diet indicate greater amino acid excretion for the latter (Parsons *et al*., 1983). The effect of fiber content on endogenous amino acid excretion from force-fed birds is unclear. Thus, Muztar and Slinger (1980a) and Parsons (1984) reported an increase in amino acid output with fiber content, this effect being attributable in part to an increased abrasion of the intestinal epithelium and a greater microbial synthesis of protein in the ceca. In contrast, Muztar and Slinger (1980b), Sibbald (1980), and Green (1988) found no response to graded levels of fiber on amino acid excretion. On the other hand, removal of the ceca significantly decreased amino acid digestibility in some studies (Parsons, 1985; Johns *et al*., 1986), whereas small or nonsignificant effects have been found in others (Picard *et al*., 1983; Green *et al*., 1987; Green and Kiener, 1989). According to Angkanaporn *et al.* (1997), cecectomy has more importance for determining amino acid digestibility of protein sources of poor quality. However, Engster *et al.* (1985), in a collaborative study to evaluate the precision-fed rooster assay, found good agreement among laboratories in true amino acid digestibility (TAAD), despite considerable methodological and analytical differences.

The objectives of the current work were threefold: 1) to study the excretion pattern of SFSM when force-fed to adult roosters, in order to establish the optimal duration of excreta collection period, 2) to determine the effect of chemical composition of SFSM on their TME_n and to develop prediction equations, and 3) to determine the effect of SFSM and of endogenous correction method on TAAD.

MATERIALS AND METHODS

Feedstuffs

Eleven samples of SFSM with a wide variability in chemical composition were selected for this work. Their chemical composition (on DM basis), sorted by CP content, is shown in Table 1. All the samples came from Spanish oil extraction factories following a similar oil extraction process: conditioning, partial dehulling, press extraction, and solvent extraction.

Birds and Management

One hundred and sixty-five adult Leghorn-type roosters (Hy-Line), 1.5 yr old weighing from 2.3 to 2.8 kg, were used in this study (30 and 135 roosters in Experiment 1 and 2, respectively). Birds were housed in individual metabolism cages, 0.30 m wide, 0.40 m long, and 0.50 m high, 4 d before the beginning of the experiments to allow them to become used to their surroundings. Roosters were held in an environmentally controlled room with a temperature ranging from 20 to 25 C. Throughout the experiments, the animals were handled according to the principles for the care of animals in experimentation established by the Royal Decree 223/88 of Spain (1988).

Experiment 1. Energy Excretion of SFSM

Three samples of SFSM (B, C, and E, Table 1) with high content of fiber [from 35 to 43% of neutral detergent fiber (NDF)] were used in this experiment. Thirty roosters were sorted by weight and divided into 10 groups of 3. Each SFSM was randomly assigned to one bird in each group of three. Twenty-four hours prior to the beginning of the experiment, the birds were deprived of feed for clearance

TABLE 2. Amino acid composition (DM basis) of sunflower seed meals used in Experiment 2

Amino acid	Sunflower seed meal		
	32% CP	35% CP	37% CP
	(%)		
Lysine	1.01	1.21	1.27
Methionine	0.67	0.76	0.81
Cystine	0.53	0.56	0.58
Methionine + cystine	1.20	1.32	1.39
Threonine	1.21	1.43	1.50
Isoleucine	1.20	1.36	1.41
Leucine	2.07	2.36	2.44
Valine	1.52	1.71	1.77
Histidine	0.73	0.85	0.89
Arginine	2.43	2.92	3.06
Glycine	1.88	2.11	2.23
Serine	1.29	1.57	1.64
Phenylalanine	1.42	1.65	1.68
Alanine	1.36	1.53	1.60
Tyrosine	0.71	0.86	0.87
Aspartic acid	2.88	3.29	3.39
Glutamic acid	5.99	6.60	7.15
Proline	1.21	1.44	1.47

of digestive tract. Thirty grams of each SFSM sample ground at 2 mm were fed by crop intubation (steel funnel 12 mm diameter, 40 cm high) to 10 roosters. Dry matter contents of SFSM samples were determined at feeding time. Excreta were collected and weighed at 24, 36, 48, 60, 72, and 84 h after force-feeding to determine the time interval for complete excretion of SFSM. Each sample of excreta was stored at –20 C until subsequent drying in a forced-air draft oven at 70 C for 48 h, and ground for determination of gross energy. Nitrogen analysis was not performed because of the insufficient amount remaining in most of the samples.

Experiment 2. TME_n and TAAD

True ME_n of 11 samples of SFSM (Table 1) were determined in this experiment applying the precision-fed cockerels assay described by Sibbald (1986). Three of these (Samples B, F, I) were selected for additional determination of TAAD, (Sibbald, 1987). Their amino acid composition is shown in Table 2. The coefficients of variability of the amino acid analyses of SFSM were from 2% for cystine to 5.2% for proline. The experiment was conducted in 3 consecutive wk. Samples chosen for TAAD were assessed in the same period. Following a feed deprivation period of 24 h, 30 g of each SFSM was fed by crop intubation to nine roosters. Total excreta voided over the following 48 h period was collected twice (at 24 and 48 h), frozen, and then dried and ground for subsequent analyses of gross energy and nitrogen, and amino acids in the corresponding period, as described for Experiment 1. At the same time, nine different cockerels in each week were deprived of feed to estimate the endogenous losses to be applied in each week. During the 3rd wk, another nine cockerels were force-fed 30 g of a nitrogen-free diet composed at 85% cornstarch and 15% cellulose to obtain another estimation of endogenous amino acid excretion. Excreta voided by each group of control birds were homogeneously mixed to perform chemical analyses, and about one half of the excreta samples was freeze-dried[3] for amino acid analysis. Correction to zero nitrogen retention was made using 8.22 kcal/g of retained nitrogen (Hill and Anderson, 1958).

Analyses

Dry matter, ash, CP, CF, and ether extract were analyzed according to AOAC (1984) methods. Neutral detergent fiber analysis was performed as described by Van Soest *et al.* (1991), and sequentially, acid detergent fiber (ADF), and acid detergent lignin (ADL) according to Robertson and Van Soest (1981). Gross energy was measured with an adiabatic bomb calorimeter.[4] Amino acid composition of SFSM and of pooled excreta was performed by HPLC[5] after 24 h hydrolysis with 6 *N* hydrochloric acid at 120 C, following the methodology stated by NFIA (1991). Before analysis of sulfur amino acids, a performic acid oxidation treatment was applied to prevent destruction of cystine (Moore, 1963). Phenol was added (Mason *et al.*, 1979) to prevent a partial destruction of tyrosine, phenylalanine, histidine, and arginine.

Statistical analyses were performed using SAS® (SAS Institute, 1990). For the Experiment 1, an analysis of repeated measurements using General Linear Models (GLM) procedure was applied to study the effect of excreta collection time and type of SFSM on DM and energy excretion. Orthogonal contrasts were performed to evaluate the collection time from which there were no differences in DM and energy excreted. One-way analysis of variance was performed in Experiment 2 for evaluating the effect of SFSM on TME_n. Treatment means were compared using Duncan's multiple range test. Correlation and stepwise regression analyses were applied to determine the chemical components that best predicted the TME_n of SFSM. A two-way analysis of variance was carried out by GLM procedure to study the effect of SFSM, endogenous correction, and their interaction on TAAD in Experiment 2.

RESULTS AND DISCUSSION

Experiment 1

Type of SFSM did not affect the excretion pattern of DM and energy ($P = 0.44$, and $P = 0.19$, Figure 1 and Table 3, respectively), the three samples following the same tendency. Dry matter excreted decreased linearly and quadratically ($P < 0.001$) with collection time, as shown in Figure 1. Most of DM excreted was obtained during the

[3]Cryodos, –45C, 10^{-1} mbar, Telstar, Terrassa, Spain.
[4]IKA-4000, Janke and Kunkel GmbH and Co., Staufen, Germany.
[5]Beckman 6300, Beckman Instruments Corp., Palo Alto, CA 93402.

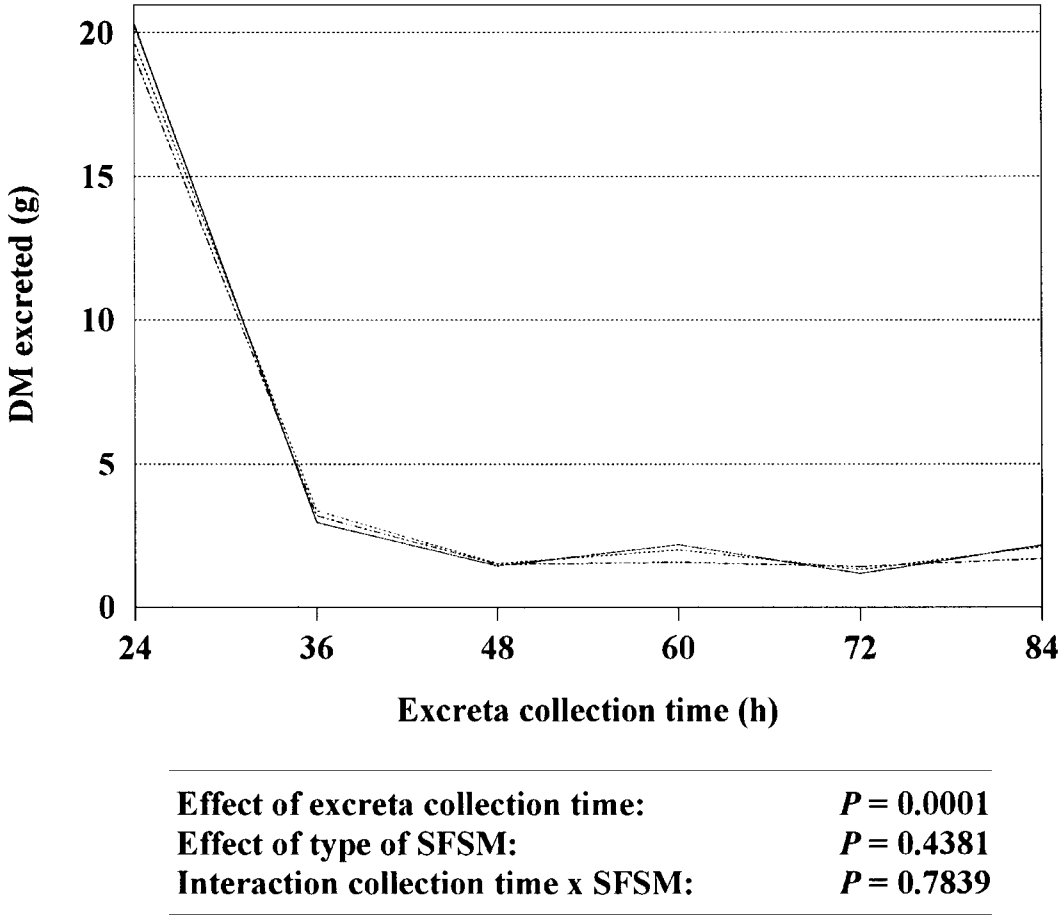

FIGURE 1. Dry matter excreted (grams) at each collection time [hours after force feeding sunflower seed meals (SFSM)], Experiment 1 (——— 43% NDF; – – – 40% NDF; ···· 35% NDF SFSM).

first 24 h (70%), and about 10% more was obtained from 24 to 36 h. No differences were found for DM excreted every 12 h from 48 to 84 h collection times. Thus, any DM excreted after 48 h was considered to be endogenous DM.

Energy followed the same excretion pattern, as shown in Table 3. There were no differences for energy excreted every 12 h after the 48-h collection time. The excreta collection period suggested by Sibbald (1986) seems to be appropriate for SFSM, not expecting overestimation of their TME_n values. On the contrary, 60 h were necessary for an accurate determination of TME_n of SFSM in a study carried out by Gous and Denninson (1983) using higher

TABLE 3. Energy excreted at each collection time, Experiment 1

		Collection time[1]						
Sunflower seed meal (SFSM)	n[2]	24 h	36 h	48 h	60 h	72 h	84 h	SEM
		(kcal)						
B	6	80.46	10.98	4.35	7.21	3.21	6.61	1.58
C	7	74.25	11.92	4.71	4.91	4.04	5.21	1.78
E	10	75.50	12.61	4.45	6.45	3.75	6.61	1.60
Mean		76.21	11.97	4.51	6.18	3.70	6.18	

Source of variation	Probability	Orthogonal contrast	Probability
Collection Time (CT)	0.0001	24 vs 36, 38, 60, 72, 84 h	0.0001
		36 vs 48, 60, 72, 84 h	0.0001
		48 vs 60, 72, 84 h	0.4252
		60 vs 72, 84 h	0.2725
		72 vs 84 h	0.0573
SFSM	0.1894		
CT × SFSM	0.5905		

[1]Hours after force feeding SFSM.

[2]Number of replicates.

TABLE 4. Nitrogen-corrected true metabolizable energy of sunflower seed meals, Experiment 2

Sunflower seed meal	n[1]	TME_n	SE
		(kcal/kg DM)	
A	9	1,558[c]	76.1
B	9	1,558[c]	62.9
C	6	1,933[ab]	134.0
D	8	1,863[ab]	87.5
E	8	1,893[ab]	74.1
F	8	1,924[ab]	71.2
G	8	1,876[ab]	38.1
H	8	1,922[ab]	109.4
I	8	1,837[b]	48.0
J	6	1,902[ab]	34.6
K	8	2,023[a]	28.0

[a,b]Means in the same column with no common superscript differ significantly ($P < 0.05$).

[1]Number of replicates.

levels of intake (50 g). Crop compactation, and subsequently delayed excretion, could happen with these low density feedstuffs. In fact, 13% of the roosters (four) in Experiment 1 and 8% of the roosters (eight) used in Experiment 2 showed this problem and were eliminated from the experiments.

A slight difference in energy excreted (3.7 vs 6.2 kcal, $P = 0.06$) was observed (Table 3) between the 72 h (morning) and 84 h (evening) collection times. Similar results can be observed in Figure 1 for collections of 48 and 72 h (morning) against 60 and 84 h (evening collections). The lesser endogenous excretion in morning hours could be a consequence of the lower activity of roosters during the night. So, periods of complete days (48, 72 h) seem to be more appropriate for the application of force-feeding method.

Experiment 2

The chemical composition of the samples chosen for this experiment (Table 1) agreed in distribution and mean value with composition data of more than 400 samples of the Spanish market of SFSM (FEDNA, 1994). However, this data set did not quite meet some other recommendations of design, such as having a regular distribution of samples throughout the entire range of reported values (Wiseman *et al*., 1991). Most of them had a CP ranging from 34 to 38% (DM basis), two samples were hulled (about 32% CP) and only one had a CP content above 40%. Despite this, the actual distribution reflects the real variability of this feedstuff.

Type of SFSM significantly affected the TME_n ($P < 0.001$, Table 4), which varied from 1,558 to 2,023 kcal/kg DM, the mean value being 1,834. However, most of the samples, 8 out of 11, showed energy values between 1,838 and 1,933 kcal TME_n/kg DM. This low TME_n content of SFSM (about 40% of their GE content) contrasts with the high TAAD (87.8%, see below) obtained in the current work and also reported in the literature (Green *et al*., 1987; Green and Kiener, 1989). This fact could indicate a poor utilization of carbohydrates, and perhaps an interaction of fiber with the utilization of other nutrients. The fiber content of our samples ranged from 29 to 46% NDF, and they presented a high degree of lignification (from 21 to 25% ADL/NDF). Such lignification impairs accessibility to the enzymatic degradation as stated by Düsterhöft (1993), who determined that cellulose, hemicellulose, and pectic compounds are the major constituents of nonstarch polysaccharides of SFSM. Pectic compounds, presumably those derived from parenchyma tissues of seed, have relatively high degradability to enzyme attack, whereas the less solubilized constituents (cellulose and hemicellulose) are derived from the hulls. Unfortunately, common analyses of cell walls (i.e., NDF) solubilize pectic compounds, and hemicellulose contains a heterogeneous mix of polysaccharides, which are associated with lignin (Van Soest, 1994).

The accuracy of TME_n determination was relatively low and with high variation among samples of SFSM (SE from 28 to 134 kcal/kg DM, Table 4). Part of this variation could be reduced by taking into account the individual variability in endogenous excretion. Thus, although the average value of endogenous energy (not corrected to N equilibrium) determined in Experiment 1 (energy excreted from 48 to 84 h) and Experiment 2 (feed deprived cockerels) were similar (20.57 and 18.14 kcal/48 h, respectively, data not shown), high variability among animals (CV = 25%, with extreme values from 13.0 to 32.7 kcal/48 h) were detected in Experiment 1. The application of its own endogenous correction to each individual in Experiment 1 led to much lower standard errors in TME determinations than in Experiment 2 (47.3 vs 94.7).

An attempt was made to relate chemical analyses and TME_n. Thus, correlation analyses (Table 5) showed the large inverse relationship between fiber and TME_n. By contrast, CP was positively related to TME_n, although the correlation coefficient was lower than that of some fiber measurements (r = 0.77 vs –0.90, –0.84, and –0.82 for hemicellulose, ADL, and NDF, respectively). As expected, CP and fiber measurements also had high correlations (from –0.76 to –0.92), the reason for this relationship being the degree of dehulling. Ether extract had no significant correlation with any of the variables analyzed, except GE (r = 0.85). This lack of correlation is a reflection of the low ether extract content (1.79 on average) and variability (SD = 0.73) of SFSM.

To obtain equations to predict the TME_n of SFSM, stepwise regression analyses were performed using different chemical analyses. The best single predictor of TME_n was hemicellulose concentration, which explained 80% of TME_n variation:

$$TME_n \text{ (kcal/kg DM)} = 2{,}816.82 - 109.5 \text{ Hem (\% DM)} \quad [1]$$

$$r^2 = 0.80, \text{ RSD} = 70.2, P < 0.001$$

TABLE 5. Correlation coefficients among chemical analyses and TME_n of SFSM

	Crude protein	Crude fiber	Neutral detergent fiber	Acid detergent fiber	Acid detergent lignin	Hemicellulose[1]	Cellulose[2]	Ash	Ether extract	Gross energy
TME_n	0.77	–0.76	–0.82	–0.77	–0.84	–0.90	–0.70	0.28	0.15	–0.08
Crude protein		–0.85	–0.90	–0.91	–0.86	–0.76	–0.92	0.45	–0.01	–0.27
Crude fiber			0.98	0.99	0.98	0.85	0.97	–0.55	0.20	0.54
Neutral detergent fiber				0.99	0.99	0.91	0.97	–0.53	0.15	0.47
Acid detergent fiber					0.98	0.85	0.99	–0.55	0.17	0.50
Acid detergent lignin						0.88	0.94	–0.49	0.09	0.43
Hemicellulose							0.80	–0.42	0.08	0.32
Cellulose								–0.57	0.22	0.53
Ash									–0.53	–0.80
Ether extract										0.85

[1]Calculated as NDF – ADF.

[2]Calculated as ADF – ADL.

followed by ADL (r^2 = 0.71, RSD = 89.2, $P < 0.001$). Both single predictors need at least two sequential laboratory analyses, which implies high cost and labor and low accuracy. However, when a single predictor from single analysis was selected (NDF), the precision of the estimation decreased:

$$TME_n \text{ (kcal/kg DM)} = 2{,}697.99 - 23.93 \text{ NDF (\% DM)} \quad [2]$$

$$r^2 = 0.68, \text{ RSD} = 89.2, P < 0.001$$

The inclusion of a second variable (ADF with positive sign) resulted in a similar equation as [1].

On the other hand, it is well known that the most common analysis performed to control SFSM quality is CP, so this parameter could be interesting to predict TME_n:

$$TME_n \text{ (kcal/kg DM)} = 397.28 + 40.69 \text{ CP (\% DM)} \quad [3]$$

$$r^2 = 0.59, \text{ RSD} = 100.3, P = 0.006$$

This equation shows lower accuracy than previous ones, and no other terms were selected in stepwise procedure.

The introduction of a second independent variable to the equations of hemicellulose and ADL (GE and CF, respectively), significantly improved the accuracy of TME_n prediction (R^2 =0.85 and R^2 = 0.81, respectively). The former was:

$$TME_n \text{ (kcal/kg DM)} = -1{,}126.6 - 118.9 \text{ Hem (\%DM)} + 0.87 \text{ GE (kcal/kg DM)} \quad [4]$$

$$R^2 = 0.85, \text{ RSD} = 64.2, P < 0.001$$

TABLE 6. Effect of sunflower seed meal and endogenous correction on true amino acid digestibility, Experiment 2

	Sunflower seed meal					Endogenous correction		
Amino acid	32% CP[1]	35% CP[2]	37% CP[3]	SEM	Probability	N-Free	Feed-deprived	Probability
	(%)					(%)		
Lysine	76.39[c]	81.75[b]	83.69[a]	0.53	0.001	80.76	80.12	0.298
Methionine	89.61[c]	91.69[b]	93.00[a]	0.24	0.001	91.64	91.08	0.053
Cystine	80.72[b]	82.94[a]	83.75[a]	0.54	0.001	82.44	82.36	0.899
Met + Cys	85.61[c]	88.00[b]	89.13[a]	0.37	0.001	87.72	87.28	0.310
Threonine	82.72[b]	83.44[b]	85.94[a]	0.43	0.001	84.72	83.24[b]	0.005
Isoleucine	87.72[c]	89.69[b]	90.88[a]	0.30	0.001	89.68	89.04	0.071
Leucine	87.94[c]	89.88[b]	91.38[a]	0.31	0.000	89.92	89.40	0.149
Valine	86.78[c]	88.06[b]	90.00[a]	0.35	0.001	89.56	86.88[b]	0.001
Histidine	86.72[b]	87.50[ab]	88.44[a]	0.36	0.006	87.88	87.16	0.091
Arginine	90.22[b]	91.94[a]	92.00[a]	0.23	0.001	91.44	91.24	0.446
Serine	84.33[b]	85.00[b]	86.94[a]	0.42	0.001	85.56	85.20	0.465
Phenylalanine	89.50[c]	91.38[b]	92.69[a]	0.21	0.001	91.57	90.80	0.075
Alanine	80.94[c]	84.31[b]	86.38[a]	0.42	0.001	84.40	83.12[b]	0.011
Tyrosine	84.33[b]	82.96[c]	87.38[a]	0.15	0.001	84.89	84.89	1.000
Aspartic acid	86.00[b]	87.81[a]	88.25[a]	0.34	0.001	87.84	86.76[b]	0.009
Glutamic acid	91.72[b]	92.75[b]	94.00[a]	0.21	0.001	93.20	92.36[b]	0.002
Proline	86.83[b]	88.06[ab]	89.04[a]	0.33	0.001	88.35	87.60[b]	0.031
Total amino acids	85.78[c]	88.00[b]	89.44[a]	0.35	0.001	88.16	87.16[b]	0.028

[a,b]Means in the same row with no common superscript differ significantly ($P < 0.05$).

[1]n = 9.

[2]n = 8.

[3]n = 8.

However, this equation makes little biological sense because of the negative independent term, and has low applicability because several and noncomplementary analysis are required. Besides, GE is an analysis seldom performed in feed plants. Then, the three first equations should be more useful for practical purposes. Among them, the one based on hemicellulose is preferred because of its larger coefficient of determination and its smaller residual standard error.

The comparison of these prediction equations with those of the literature is a difficult task because of 1) differences in ME unit (AME vs AME_n vs TME_n): applying the equations from literature to the chemical composition of our SFSM, the average values (DM basis) are: 1,311 and 1,618 kcal AME [Janssen and Carré (1985) and Lessire and Conan (1990); quoted by Carré and Rozo (1990), respectively], and 1,595 kcal AME_n (Janssen and Van Schagen, 1987), lower than 1,844 kcal TME_n obtained from Equation [1] of the current study, because of endogenous correction; 2) differences in sunflower seed products used for prediction, and 3) differences in the independent variables analyzed. Thus, the two first equations quoted above were obtained not only for SFSM but also for partially defatted SFS, and included ether extract (with coefficients of 60 and 63, respectively) because of its importance in energy value. The equation proposed by Janssen and Carré (1985) includes CF with a negative coefficient, whereas some equations proposed by Carré and Rozo (1990) include insoluble fiber (similar values to NDF in sunflower products).

True digestibility of amino acids of the three SFSM chosen for this study and the effect of endogenous amino acid correction are shown in Table 6. Type of SFSM significantly affected ($P < 0.001$) the TAAD, digestibility of total amino acids being 89.4, 88.0, and 85.8% for SFSM of 37, 35, and 32% of CP, respectively. Most of the amino acids (16 out of 17) showed a significant decrease ($P < 0.05$) in their digestibility with decreasing CP content of SFSM. For eight of them, this decrease was consistent throughout the range of CP assayed, differences between extreme CP contents ranging from 7.3% for lysine to 3.2% for isoleucine. For the others, this effect was slight (differences between extreme CP contents from 3.0% for cystine to 1.8 for arginine), the intermediate only differing from one of the extremes. Only tyrosine failed to show such tendencies; the SFSM of intermediate CP had the lowest TAAD. Values of glycine were not presented because of the formation of glycine from uric acid by acid hydrolysis (Parsons *et al*., 1983), which produced erratic values of glycine balance. Therefore, true digestible amino acid content of SFSM was clearly affected by their chemical composition.

Green and Kiener (1989) compared two SFSM of the same origin, hulled or partially dehulled (31 and 36% CP, respectively), which were not significantly different in their TAAD, the average value for total amino acids being 93%. They concluded that the only benefit of dehulling was an increase in amino acid concentration. Nevertheless, both Rad and Keshavarz (1976) and Zhang and Parsons (1994) reported a decrease in amino acid digestibility (from 87 to 74%), when the temperature or time of processing for the same SFSM sample increased. According to Ravindran and Blair (1992), high temperatures associated with mechanical pressing damage the protein, destroy amino acids and decrease their availability. Protein-reducing sugars and protein-fat interactions seem to be responsible for the latter effect. As dehulling favors oil extraction, high fiber SFSM has to be subjected to higher temperatures of processing than high protein SFSM samples (Zatari, 1989), which could explain the smaller amino acid content and decreased digestibility of high fiber-low protein SFSM observed in this work.

Despite the high total TAAD (mean 87.8%), there were important differences among amino acids. Lysine was the most poorly digested amino acid (80.4% as average), whereas methionine was, after glutamic acid, the most well digested (91.4% as average). Total sulfur amino acids had the same digestibility as total amino acids, because cystine was less well digested (82.4%). Threonine also had low digestibility (84.0%), and is therefore, after lysine, the second limiting amino acid of SFSM (Cuca *et al*., 1973). Green *et al*. (1987), using intact and cecectomized roosters, reported similar values of TAAD (90.4% for total amino acids) for a 35.7% CP SFSM, observing only an effect of cecectomization for three amino acids. Unexpectedly, our results were nearer to those reported by these authors for cecectomized than for intact birds (i.e. lysine digestibility 83.4 and 61.1%, respectively). Green *et al*. (1987) suggest an important bacterial synthesis of lysine attributable to great quantities of suitable nutrients arriving at the hind gut after feeding SFSM. However, in further work, Green and Kiener (1989) did not find any differences between intact and cecectomized roosters either in lysine or in total TAAD.

Endogenous excretion of amino acids was greater for roosters fed the N-free diet than for those deprived of feed, resulting in a higher digestibility (from 0.7 to 2.68%, $P < 0.05$) only for six amino acids: threonine, valine, alanine, proline, aspartic acid, and glutamic acid (Table 6). Total amino acid digestibility was also affected (88.16 vs 87.16, $P = 0.028$) because of the quantitative importance of the two latter amino acids. Tyrosine was not detected in endogenous excreta, so there was no effect of endogenous correction. No interaction was found between type of SFSM and method of estimation of endogenous excretion for any amino acid ($P = 0.94$ for total TAAD), which seems to indicate the lack of effect of level of fiber on the ratio among amino acid concentrations in the endogenous excretion, as obtained Muztar and Slinger (1980b) and Green (1988).

As a conclusion, the true digestible amino acid contents of SFSM should be accurately calculated in practice. These values could be quite different among SFSM, because of the influence of chemical composition on TAAD observed in this study. Thus, for SFSM of 32, 35, and 37% CP, true digestible lysine content was 0.77, 0.99, and 1.06%, true

digestible methionine content was 0.60, 0.70, and 0.75%, and true digestible threonine content was 1.00, 1.19, and 1.28%, respectively.

This work has some practical implications. An excreta collection period of 48 h is suitable for determining the TME_n of SFSM. Prediction equations of TME_n based on hemicellulose, NDF, or CP (r^2 from 0.8 to 0.6) are proposed. Finally, as TAAD is directly related to CP content, attention should be paid to the balance of lesser digestible amino acids when including high fiber-low protein SFSM in poultry rations.

ACKNOWLEDGMENTS

The authors wish to thank Trouw Ibérica S.A. for amino acid analyses and especially to thank Angel Gago for valuable discussions.

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