

Determination of PDCAAS and DIAAS values for raw and roasted American pistachio nuts

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MATERIALS AND METHODS

The protocol for this experiment was reviewed and approved by the Institutional Animal Care and Use Committee at the University of Illinois.

Diets, Animals, and Feeding

Two proteins, raw pistachio nuts and roasted pistachio nuts, were used in the experiment (Table 1). Three diets were prepared (Tables 2 and 3) with the 2 pistachio proteins included in one diet each as the only amino acid (AA) containing ingredient. A nitrogen-free diet was also formulated and used to measure basal endogenous losses of AA and crude protein (CP). All diets were formulated to 13% CP, and vitamins and minerals were included to meet or exceed current nutrient requirement estimates for growing pigs (National Research Council; NRC, 2012). All diets contained 0.4% titanium dioxide as an indigestible marker, and all diets were provided in meal form. A sample of each pistachio protein and of all diets were collected at the time of diet mixing and used for chemical and compositional analysis.

Twelve growing barrows (initial body weight: 60.9 ± 3.2 kg) were equipped with a T-cannula in the distal ileum (Stein et al., 1998). Following surgery, pigs were randomly allotted to a 2-period switch-back design with 3 diets and 4 replicate pigs per period. Pigs were housed in individual pens (1.2×1.5 m) in an environmentally controlled room. Each pen had smooth sides and fully slatted tribar floors. A feeder and a nipple drinker were installed in each pen.

All pigs were fed their assigned diets in a daily amount equivalent to 4% of the body weight for each pig. Two equal meals were provided daily at 0800 and 1600 h, and water was available at all times. Pig weights were recorded at the beginning of each period and at the conclusion of the experiment. The amount of feed supplied each day was also recorded.

Sample Collection

Experimental periods were 9 d with the initial 5 d being the adaptation period to the diet. Fecal samples were collected on d 6 and 7 and stored at -20 °C, and ileal digesta were collected for 9 hours September 12th 2019

(from 0800 to 1700 h) on d 8 and 9 following standard procedures. In short, a plastic bag was attached to the cannula barrel and digesta flowing into the bag were collected. Bags were removed when filled with ileal digesta, or at least once every 30 minutes, and immediately frozen at – 20 °C to prevent bacterial degradation of AA in the digesta (Appendix 1). On the completion of one experimental period, animals were deprived of feed overnight and the following morning, the new experimental diet was offered.

Chemical Analysis

At the end of the test, fecal samples were dried and ground through a 1-mm screen in a Wiley Mill (model 4; Thomas Scientific) prior to chemical analysis. Ileal digesta samples were thawed, mixed within animal and diet, and a sub-sample was collected for chemical analysis. Ileal digesta samples were lyophilized and finely ground prior to chemical analysis. Samples of all ingredients, diets, fecal samples, and ileal digesta were analyzed for dry matter (**DM**; Method 927.05; AOAC International, 2007) and for CP by combustion (Method 990.03; AOAC International, 2007) using a LECO FP628 analyzer (LECO Corp., Saint Joseph, MI) and a nitrogen-to-protein conversion factor of 6.25 (FAO, 1991; FAO, 2013). All diets, fecal samples, and ileal digesta samples were also analyzed for titanium (Method 990.08; Myers et al., 2004), and all diets, ingredients, and ileal digesta samples were analyzed for AA [Method 982.30 E (a, b, c); AOAC International, 2007].

Calculations

Apparent ileal digestibility (**AID**) values for CP and AA in each diet were calculated using equation [1] (Stein et al., 2007):

$$\text{AID (\%)} = [1 - [(AA_d/AA_f) \times (Ti_f/Ti_d)] \times 100 \quad [1]$$

where AID is the apparent ileal digestibility value of an AA (%), AA_d is the concentration of that AA in the ileal digesta DM, AA_f is the AA concentration of that AA in the feed DM, Ti_f is the titanium concentration in the feed DM, and Ti_d is the titanium concentration in the ileal digesta DM. The AID for CP was also calculated using this equation.

The basal endogenous flow to the distal ileum of each amino acid was determined based on the flow obtained after feeding the N-free diet using equation [2] (Stein et al., 2007):

$$IAA_{\text{end}} = [AA_d \times (Ti_f / Ti_d)] \quad [2]$$

where IAA_{end} is the basal endogenous loss of an AA (mg per kg DM intake). The basal endogenous loss of CP was determined using the same equation.

By correcting the AID for the IAA_{end} of each AA, standardized ileal digestibility (**SID**) values for AA were calculated using equation [3] (Stein et al., 2007):

$$SID (\%) = AID + [(IAA_{\text{end}} / AA_f) \times 100] \quad [3]$$

where SID is the standardized ileal digestibility value of an AA (%). The SID of CP and STTD of CP were also calculated using this equation.

Values for STTD of CP were used to calculate PDCAAS values using the following equation [4] (FAO, 1991):

$$PDCAAS (\%) = \text{mg of limiting AA in 1g of test protein} / \text{mg of the same AA in 1g of reference protein} \times \text{fecal true digestibility} (\%) \times 100. \quad [4]$$

The calculation of PDCAAS values was accomplished using the reference protein pattern for preschool children from 2 to 5 yr of age (FAO, 1991).

Values for SID of AA were used to calculate DIAAS reference ratios for each AA using the following equation [5] (Cervantes-Pahm et al., 2014):

$$\text{Digestible indispensable AA reference ratio} = \text{digestible indispensable AA content in 1g protein of food (mg)} / \text{mg of the same dietary indispensable AA in 1g of the reference protein.} \quad [5]$$

The DIAAS was calculated for children older than 3 yr, adolescents, and adults as recommended by FAO (2013) using the following equation [6] (FAO, 2013):

$$DIAAS (\%) = 100 \times \text{lowest value of the digestible indispensable AA reference ratio.} \quad [6]$$

Statistical Analysis

At the conclusion of the experiment, data were analyzed by ANOVA using the MIXED procedure in SAS (SAS Institute Inc. Cary, NC). The pig was the experimental unit for all analyses. Diet was the fixed effect and pig and period were random effects. Treatment means were calculated using the LS MEANS statement in SAS, and if significant, means were separated using the PDIFF option in the MIXED procedure. An alpha value of 0.05 was used to assess significance among means.

PDCAAS RESULTS
CONVERSION FACTOR OF 6.25

Table 1. Protein digestibility corrected amino acid score (PDCAAS) for the 2 pistachios^{1,2}.

Item	Raw pistachios	Roasted pistachios
PDCAA reference ratio		
His	0.95	1.04
Ile	1.34	1.48
Leu	0.88	0.96
Lys	0.76	0.81
SAA ³	1.13	1.24
AAA ⁴	1.11	1.21
Thr	0.73	0.81
Trp	1.05	0.90
Val	1.40	1.56
PDCAAS ⁵ , %	73 ^b (Thr)	81 ^a (Thr)

¹First-limiting amino acid (AA) is in parentheses.

²Values for PDCAAS were calculated from the standardized total tract digestibility (STTD) of crude protein in pigs: raw pistachio nuts, 92.1%; roasted pistachio nuts, 91.6%.

³SAA = sulfur AA.

⁴AAA = aromatic AA.

⁵PDCAAS values were calculated using the recommended AA scoring pattern for preschool children (2 to 5 yr). The indispensable AA reference patterns are expressed as mg AA/g protein: His, 19; Ile, 28; Leu, 66; Lys, 58; Sulphur AA, 25; Aromatic AA, 63; Thr, 34; Trp, 11; Val, 35 (FAO, 1991).

Table 2. Digestible indispensable amino acid score (DIAAS) for the 2 pistachios¹.

Reference pattern: older children, adolescents, and adults		
Item	Raw pistachios	Roasted pistachios
DIAA reference ratio		
His	1.09	1.07
Ile	1.18	1.18
Leu	0.91	0.90
Lys	0.86	0.83
SAA ²	1.17	1.18
AAA ³	1.62	1.58
Thr	0.95	0.92
Trp	1.75	1.38
Val	1.17	1.16
DIAAS ⁴ , %	86 ^a (Lys)	83 ^a (Lys)

¹First-limiting amino acid (AA) is in parentheses.

²SAA = sulfur AA.

³AAA = aromatic AA.

⁴DIAAS values were calculated using the recommended AA scoring pattern for older child, adolescent, and adult. The indispensable AA reference patterns are expressed as mg AA/g protein: His, 16; Ile, 30; Leu, 61; Lys, 48; Sulphur AA, 23; Aromatic AA, 41; Thr, 25; Trp, 6.6; Val, 40 (FAO, 2013).

**CONVERSION FACTOR OF 5.3 FOR DIETS AND INGREDIENTS AND 6.25 FOR FECAL
CRUDE PROTEIN DATA**

Table 3. Protein digestibility corrected amino acid score (PDCAAS) for the 2 pistachios^{1,2}.

Item	Raw pistachios	Roasted pistachios
PDCAA reference ratio		
His	1.10	1.21
Ile	1.55	1.71
Leu	1.02	1.12
Lys	0.88	0.94
SAA ³	1.31	1.44
AAA ⁴	1.29	1.41
Thr	0.85	0.94
Trp	1.22	1.04
Val	1.63	1.81
PDCAAS ⁵ , %	85 ^b (Thr)	94 ^a (Thr)

¹First-limiting amino acid (AA) is in parentheses.

²Values for PDCAAS were calculated from the standardized total tract digestibility (STTD) of crude protein in pigs: raw pistachio nuts, 90.7%; roasted pistachio nuts, 90.2%.

³SAA = sulfur AA.

⁴AAA = aromatic AA.

⁵PDCAAS values were calculated using the recommended AA scoring pattern for preschool children (2 to 5 yr). The indispensable AA reference patterns are expressed as mg AA/g protein: His, 19; Ile, 28; Leu, 66; Lys, 58; Sulphur AA, 25; Aromatic AA, 63; Thr, 34; Trp, 11; Val, 35 (FAO, 1991).

Table 4. Digestible indispensable amino acid score (DIAAS) for the 2 pistachios¹.

Reference pattern: older children, adolescents, and adults		
Item	Raw pistachios	Roasted pistachios
DIAA reference ratio		
His	1.28	1.26
Ile	1.39	1.39
Leu	1.07	1.06
Lys	1.01	0.97
SAA ²	1.38	1.39
AAA ³	1.91	1.86
Thr	1.12	1.09
Trp	2.06	1.63
Val	1.38	1.37
DIAAS ⁴ , %	101 ^a (Lys)	97 ^a (Lys)

¹First-limiting amino acid (AA) is in parentheses.

²SAA = sulfur AA.

³AAA = aromatic AA.

⁴DIAAS values were calculated using the recommended AA scoring pattern for older child, adolescent, and adult. The indispensable AA reference patterns are expressed as mg AA/g protein: His, 16; Ile, 30; Leu, 61; Lys, 48; Sulphur AA, 23; Aromatic AA, 41; Thr, 25; Trp, 6.6; Val, 40 (FAO, 2013).

CONVERSION FACTOR OF 5.3

Table 5. Protein digestibility corrected amino acid score (PDCAAS) for the 2 pistachios^{1,2}.

Item	Raw pistachios	Roasted pistachios
PDCAA reference ratio		
His	1.12	1.23
Ile	1.58	1.74
Leu	1.03	1.14
Lys	0.89	0.96
SAA ³	1.33	1.47
AAA ⁴	1.31	1.43
Thr	0.86	0.95
Trp	1.24	1.06
Val	1.65	1.84
PDCAAS ⁴ , %	86 ^b (Thr)	95 ^a (Thr)

¹First-limiting amino acid (AA) is in parentheses.

² Values for PDCAAS were calculated from the standardized total tract digestibility (STTD) of crude protein in pigs: raw pistachio nuts, 92.1%; roasted pistachio nuts, 91.6%.

³SAA = sulfur AA.

⁴AAA = aromatic AA.

⁵PDCAAS values were calculated using the recommended AA scoring pattern for preschool children (2 to 5 yr). The indispensable AA reference patterns are expressed as mg AA/g protein: His, 19; Ile, 28; Leu, 66; Lys, 58; Sulphur AA, 25; Aromatic AA, 63; Thr, 34; Trp, 11; Val, 35 (FAO, 1991).

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