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Animal Feed Science and Technology
119 (2005) 107–115
ANIMAL FEED SCIENCE AND TECHNOLOGY
www.elsevier.com/locate/anifeedsci

Apparent and standardized ileal digestibility of amino acids in gelatin-based diets by growing pigs☆

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Received 8 August 2004; received in revised form 15 November 2004; accepted 16 November 2004

Abstract

A study was conducted with the objectives of measuring the coefficients of ileal apparent digestibility (CIAD) and coefficients of ileal standardized digestibility (CISD) of amino acids (AA) in two gelatin-based diets and in soybean meal by growing pigs. Four individually housed pigs (initial BW: 44.1 ± 1.45 kg) were equipped with T-cannulas in the distal ileum. Two diets based on gelatin of either pork or beef origin were formulated using mainly corn starch, sucrose, and gelatin. These diets were fortified with L-histidine, L-isoleucine, DL-methionine, and L-tryptophan. Two additional diets were formulated: one included soybean meal as the only AA-containing ingredient, and the other was a nitrogen-free (N-free) diet. The N-free diet was used to estimate the basal endogenous losses of crude protein (CP) and AA by the pigs. Chromic oxide (4 g/kg) was included in all diets as an indigestible marker. Each of the four diets was fed to each of the four pigs during a 7-day period with digesta being collected in 10 h periods on day-6 and day-7. The CIAD and CISD of CP and AA in the two gelatin-based diets were compared to the CIAD and CISD in soybean meal. Results of the experiment showed that there was no difference ($P > 0.05$) in CIAD and CISD for any of the indispensable AA between the pork gelatin-based diet and soybean meal. The beef gelatin-based diet had a lower CIAD for His and Thr, and a lower CISD for Thr compared to soybean meal ($P < 0.05$), but no differences ($P > 0.05$) for the remaining indispensable AA were observed. It is concluded that the AA in both pork and beef gelatin are highly digestible and both these feed ingredients may

☆ Publication No. 3427 from the South Dakota Agric. Experiment Station Journal Series.
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doi:10.1016/j.anifeedsci.2004.11.006

be used as a protein source in diets for growing pigs provided that crystalline His, Ile, Met and Trp are added to balance the indispensable AA.

Keywords: Amino acids; Digestibility; Gelatin; Pigs

1. Introduction

Gelatin is a protein source that is commonly included in human food and also in feed for companion animals. Recently, gelatin has also been used as a protein source in phosphorus-free diets used to estimate the endogenous losses of phosphorus by pigs. Because gelatin is virtually void of Trp and contains only limited quantities of some of the other indispensable amino acids (AA), it is necessary to fortify gelatin-containing diets with crystalline AA (i.e. His, Ile, Met, and Trp) to meet the needs for indispensable AA by growing pigs. However, the digestibility by pigs of the AA present in gelatin has not been reported and there are no reports on the digestibility of AA from different sources of gelatin. To successfully incorporate gelatin in diets for pigs, it is necessary that such information be generated. The current experiment was conducted with the objective of measuring apparent and standardized ileal digestibility coefficients of AA in two gelatin-based diets and in soybean meal (SBM) by growing pigs.

2. Materials and methods

2.1. Animals and housing

Four growing castrates (initial BW: 44.1 ± 1.45 kg) were equipped with T-cannulas in the distal ileum using procedures adapted from Stein et al. (1998). Following the surgery, pigs were individually housed in metabolism cages for the duration of the experiment. The room was environmentally controlled and room temperature was maintained at 22 °C. The cages were equipped with a feeder and a nipple drinker and had expanded metal slatted floors. The experiment was approved by the Institutional Animal Care and Use Committee at South Dakota State University (# 03-A012).

2.2. Diets and feeding

Two sources of gelatin were obtained from Gelita Gelatine USA Inc., Sioux City, IA (Table 1). One of the sources was produced from porcine raw materials while the other source was based on bovine raw materials. Both gelatin sources had a bloom of 100. The bloom indicates the gelling capabilities of a gelatin source and a value of 100 is low indicating that the two gelatin sources used in this experiment had relatively low gelling capabilities. Four experimental diets were prepared (Tables 2 and 3). Two of the diets contained pork gelatin and beef gelatin, respectively. To compensate for the low concentrations of His,

Table 1
Analyzed nutrient composition of the gelatin used in the gelatin-based diets (as-is basis)

Component (g/kg)	Gelatin source	
	Pork gelatin	Beef gelatin
Dry matter	846.2	890.0
Nitrogen	149.5	151.0
Indispensable AA		
Arginine	82.1	79.7
Histidine	8.8	5.9
Isoleucine	13.2	14.2
Leucine	29.3	30.3
Lysine	43.0	37.4
Methionine	10.1	9.0
Phenylalanine	20.5	19.6
Threonine	17.3	18.4
Tryptophan	<0.4	<0.4
Valine	24.2	24.3
Dispensable AA		
Alanine	86.1	93.4
Aspartic acid	57.9	55.4
Cysteine	1.6	0.6
Glutamic acid	101.8	107.4
Glycine	213.0	212.7
Proline	127.4	124.2
Serine	26.9	27.3
Tyrosine	7.1	2.8

Ile, Met, and Trp in gelatin, both diets were supplemented with crystalline sources of these AA. Diet 3 contained SBM as the only AA-containing ingredient and diet 4 was a nitrogen-free (N-free) diet. Dextrose and soybean oil were included in all diets at 150 and 40 g/kg, respectively. Chromic oxide (4 g/kg) was included in all diets as an inert marker; vitamins and minerals were included at concentrations that met or exceeded the estimated requirements for growing pigs (NRC, 1998).

Feed was supplied in a daily amount equal to 2.5 times the energy requirement for maintenance (i.e., 444 kJ ME/kg$^{0.75}$; NRC, 1998). The daily feed allowance was divided into two equal meals that were fed at 800 and 1700 h. Water was provided for ad libitum consumption.

2.3. *Experimental design and digesta collection*

Pigs were arranged in a 4 × 4 Latin square design with four periods and four animals representing the rows and the columns, respectively. Each experimental period lasted 7 days. The initial 5 days of each period were considered an adaptation period while the remaining 2 days were used for digesta collections in 10 h periods as described by Stein et al. (1999a). Briefly, a plastic bag was attached to the cannula barrel and digesta flowing into the bag were collected. Bags were removed whenever they were filled with digesta – or at least

Table 2
Ingredient composition of experimental diets (as-is basis)

Ingredient (g/kg)	Diet			
	Pork gelatin	Beef gelatin	Soybean meal	N-free
Gelatin, pork	300.0	–	–	–
Gelatin, beef	–	300.0	–	–
Soybean meal, dehulled	–	–	400.0	–
Solka floc[a]	40.0	40.0	–	40.0
Dextrose	150.0	150.0	150.0	150.0
Soybean oil	40.0	40.0	40.0	40.0
Chromic oxide	4.0	4.0	4.0	4.0
Dicalcium phosphate	27.0	27.0	13.0	27.0
Limestone	1.5	1.5	6.0	1.5
DL-Methionine	3.4	3.4	–	–
L-Tryptophan	1.4	1.4	–	–
L-Isoleucine	1.2	1.2	–	–
L-Histidine	0.5	0.5	–	–
Salt	4.0	4.0	4.0	4.0
Vitamins[b]	1.0	1.0	1.0	1.0
Micro minerals[c]	1.0	1.0	1.0	1.0
Maize starch	425.0	425.0	381.0	731.5

[a] Fiber Sales and Development Corporation, Urbana, OH.

[b] Vitamin premix provided the following quantities of vitamins per kg of diet: 10,032 IU of Vitamin A acetate, 992 IU of Vitamin D_3 as D-activated animal sterol, 88 IU of Vitamin E as DL-alpha-tocopheryl acetate, 1.5 mg of Vitamin K as menadione dimethylpyrimidinol bisulfate, 0.4 mg of biotin, 60 mg of niacin, 25 mg of pantothenic acid, 10 mg of riboflavin, and 0.05 mg of Vitamin B_{12}.

[c] Trace mineral premix provided the following quantities of minerals per kg of diet: Cu, 23 mg as copper sulfate; Fe, 110 mg as iron sulfate; I, 0.275 mg as potassium iodate; Mn, 23 mg as manganese sulfate; Se, 0.275 mg as sodium selenite; Zn, 114 mg as zinc oxide.

every 30 min – and immediately frozen at −20 °C to prevent bacterial degradation of the digesta proteins.

2.4. Chemical analysis

At the conclusion of the experiment, samples were thawed, mixed within animal and diet, and a sub-sample was taken for chemical analysis. All digesta samples were lyophilized and finely ground prior to chemical analysis. Dry matter (DM) and crude protein (CP) were analyzed in digesta samples, diets, and the gelatin sources according to AOAC procedures (Procedure 4.1.06 and 4.2.09, respectively; AOAC, 1998). Amino acids were also analyzed in these samples on a Chrom-tech HPLC AA analyzer, using ninhydrine for post-column derivatization and nor-leucine as the internal standard. Prior to analysis, samples were hydrolyzed with 6 NHCl for 24 h at 110 °C (Procedure 4.1.11 alternative 3; AOAC, 1998). Methionine and Cys were determined as Met sulfone and cysteic acid after cold performic acid oxidation overnight prior to hydrolysis (Procedure 4.1.11. alternative 1; AOAC, 1998). Tryptophan was determined after NaOH hydrolysis for 22 h at 110 °C (Procedure 988.15; AOAC, 1995). The chromium content of diets and digesta samples were determined using spectrophotometry as described by Fenton and Fenton (1979).

Table 3
Analyzed nutrient composition of diets (as-is basis)

Item	Diet			
	Pork gelatin	Beef gelatin	Soybean meal	N-free
Energy (MJ ME/kg)	13.46	13.46	15.36	15.81
Dry matter (g/kg)	913.1	917.7	922.2	929.8
Crude protein (g/kg)	321.1	298.7	187.1	2.4
Calcium (g/kg)	6.2	6.2	6.2	6.2
Phosphorus (g/kg)	5.0	5.0	5.0	5.0
Neutral detergent fiber (g/kg)	40	40	35.6	40
Indispensable AA				
Arginine (g/kg)	25.4	24.9	13.6	0.2
Histidine (g/kg)	4.1	3.1	5.0	0.0
Isoleucine (g/kg)	9.7	9.9	8.9	0.1
Leucine (g/kg)	9.5	9.8	14.9	0.2
Lysine (g/kg)	12.1	11.7	12.2	0.1
Methionine (g/kg)	11.7	10.7	3.4	0.1
Phenylalanine (g/kg)	6.4	6.4	9.8	0.1
Threonine (g/kg)	12.2	10.9	11.3	0.1
Tryptophan (g/kg)	1.7	1.8	3.1	0.0
Valine (g/kg)	7.7	7.6	9.4	0.1
Dispensable AA				
Alanine (g/kg)	28.7	31.6	8.5	0.2
Aspartic acid (g/kg)	19.8	19.1	22.8	0.3
Cysteine (g/kg)	0.2	0.1	2.3	0.0
Glutamic acid (g/kg)	33.0	32.8	35.8	0.4
Glycine (g/kg)	80.6	80.7	8.3	0.1
Proline (g/kg)	40.9	40.8	9.4	0.0
Serine (g/kg)	4.9	5.2	6.8	0.1
Tyrosine (g/kg)	2.3	0.9	7.0	0.1

Values for ME, Ca, P and NDF were calculated (NRC, 1998) while all other values were analyzed.

2.5. Calculations and statistical analysis

The coefficients of ileal apparent digestibility (CIAD) for AA in the two gelatin-based diets and in SBM were calculated using the following equation (Stein et al., 1999a):

$$\mathrm{CIAD} = \left(1 - \left[\left(\frac{\mathrm{AA_d}}{\mathrm{AA_f}}\right)\left(\frac{\mathrm{Cr_f}}{\mathrm{Cr_d}}\right)\right]\right) \quad (1)$$

where CIAD is the coefficient of ileal apparent digestibility of an AA, AA_d the AA concentration in the ileal digesta DM, AA_f the AA concentration in the feed DM, Cr_f the chromium concentration in the feed DM, and Cr_d the chromium concentration in the ileal digesta DM. The CIAD of CP was calculated using the same equation.

The basal endogenous loss at the distal ileum of each AA (IAA_{end}) was determined based on the flow obtained after feeding the N-free diet using the following equation (Stein et al., 1999b):

$$IAA_{end} = \left[AA_d \left(\frac{Cr_f}{Cr_d} \right) \right] \quad (2)$$

where IAA_{end} is the basal endogenous loss of an AA (mg/kg dry matter intake).

By correcting the CIAD for the IAA_{end} of each AA, coefficients of ileal standardized AA digestibility (CISD) were calculated using the following equation (Stein et al., 2001):

$$CISD = \left[CIAD + \frac{IAA_{end}}{AA_d} \right] \quad (3)$$

where CISD is the coefficients of ileal standardized digestibility.

The CIAD and CISD for CP and AA in each of the three protein containing ingredients were compared statistically using the Proc Mixed procedure of SAS (Littell et al., 1996). An analysis of variance was conducted with pig, period, and diet as the main effects. Pig was the experimental unit. Treatment means were separated using the LSMeans statement and the DIFF option of Proc Mixed, and an alpha level of 0.05 was used to assess significance between means.

3. Results

The pigs stayed healthy throughout the experiment and readily consumed their diets. There were no significant effects of pig or period for any of the digestibility coefficients.

For CP and the majority of the indispensable AA, no differences in CIAD between the two gelatin-based diets and the SBM diet were observed (Table 4). However, the CIAD for His was lower ($P < 0.05$) for the diet based on beef gelatin than for the other two diets and the CIAD for Thr was lower ($P < 0.05$) for the beef gelatin diet compared to SBM, but not different from the pork gelatin diet. In contrast, for Trp and Ile, a lower ($P < 0.05$) CIAD was observed for SBM compared to the other two diets.

For Cys and Asp, lower CIAD were observed in both gelatin-based diets compared to SBM ($P < 0.05$). For Glu and Tyr, the CIAD for the diet based on beef gelatin was lower than in the other two diets ($P < 0.05$). For Gly, the CIAD for both gelatin-based diets was higher ($P < 0.05$) than in SBM, but for the remaining dispensable AA and for the mean of the dispensable AA, no differences between the three feed ingredients were observed.

For CP, the mean of the indispensable AA, and all indispensable AA except Ile, Phe, Thr, and Trp, no differences in the CISD among diets were observed (Table 5). The CISD for Ile and Trp was lower ($P < 0.05$) in SBM than in the two gelatin-based diets. For Phe, the CISD for the diet based on pork gelatin was higher ($P < 0.05$) than for the SBM diet, while the diet based on beef gelatin had a CISD that was not different from any of the other diets. In contrast, the CISD for Thr was lower ($P < 0.05$) in beef gelatin than in SBM, but pork gelatin was not different from the other two diets.

For Asp, Cys, Glu, and Tyr, a lower CISD was observed for beef gelatin than for SBM ($P < 0.05$), but for pork gelatin, only Asp had a lower ($P < 0.05$) CISD than SBM. For the remaining dispensable AA and for the mean of the dispensable AA, no differences among the three diets were observed.

Table 4
Coefficients of ileal apparent digestibility (CIAD) of CP and AA in experimental diets by growing pigs

Item	Diet			S.E.M.
	Pork gelatin	Beef gelatin	Soybean meal	
CP	0.83	0.81	0.84	0.017
Indispensable AA				
Arginine	0.94	0.94	0.93	0.012
Histidine	0.90 x	0.86 y	0.90 x	0.010
Isoleucine	0.93 x	0.93 x	0.87 y	0.008
Leucine	0.86	0.84	0.87	0.015
Lysine	0.89	0.90	0.89	0.012
Methionine	0.91	0.90	0.90	0.010
Phenylalanine	0.91	0.90	0.88	0.010
Threonine	0.79 xy	0.77 y	0.84 x	0.019
Tryptophan	0.92 x	0.93 x	0.87 y	0.011
Valine	0.86	0.86	0.85	0.011
Mean, indispensable AA	0.85 xy	0.83 y	0.88 x	0.015
Dispensable AA				
Alanine	0.87 x	0.86 xy	0.82 y	0.014
Aspartic acid	0.71 y	0.55 z	0.85 x	0.028
Cysteine	0.22 y	−1.62 y	0.81 x	0.187
Glutamic acid	0.82 xy	0.78 y	0.88 x	0.019
Glycine	0.82 x	0.81 x	0.73 y	0.020
Proline	0.80	0.78	0.75	0.043
Serine	0.79	0.79	0.81	0.021
Tyrosine	0.81 x	0.43 y	0.87 x	0.035
Mean, dispensable AA	0.83	0.81	0.84	0.016
Mean, total AA	0.84	0.81	0.86	0.015

(100 − [(CP or AA in digesta/CP or AA in feed) (Cr in feed/Cr in digesta)]). Data represent least square means of four observations. Means within a row lacking a common letter differ ($P < 0.05$).

4. Discussion

The gelatin sources used in the current experiment were devoid of Trp and contained relatively low quantities of His, Ile, Met and Cys. This observation is in agreement with previous work and the AA composition of the gelatin used in this experiment is close to the concentration previously reported (Boomgaardt and Baker, 1972, 1973; Parsons et al., 1982).

For CP and most AA, no differences in CIAD and CISD among the three feed ingredients were observed. This indicates that most of the AA in the gelatin-based diets are as digestible as are AA in SBM. The CISD for most AA obtained in this study with growing pigs are slightly lower than the values reported for CISD in gelatin measured in poultry (Parsons et al., 1982).

The reason why the CIAD and CISD for Trp in the two gelatin-based diets are higher than in SBM is that the Trp in the gelatin-based diets was furnished by crystalline Trp. That explains why the CISD for Trp in those diets are close to 100%. The low CIAD and

Table 5
Coefficients of ileal standardized digestibility (CISD) of CP and AA in experimental diets by growing pigs

Item	Diet			S.E.M.
	Pork gelatin	Beef gelatin	Soybean meal	
CP	0.85	0.84	0.88	0.018
Indispensable AA				
Arginine	0.95	0.95	0.95	0.012
Histidine	0.92	0.89	0.91	0.009
Isoleucine	0.96 x	0.95 x	0.89 y	0.009
Leucine	0.89	0.87	0.89	0.015
Lysine	0.91	0.92	0.90	0.013
Methionine	0.92	0.91	0.92	0.010
Phenylalanine	0.94 x	0.92 xy	0.90 y	0.011
Threonine	0.82 xy	0.80 y	0.88 x	0.018
Tryptophan	0.97 x	0.99 x	0.90 y	0.010
Valine	0.90	0.90	0.88	0.016
Mean, indispensable AA	0.88	0.86	0.91	0.018
Dispensable AA				
Alanine	0.88	0.87	0.86	0.015
Aspartic acid	0.74 y	0.58 z	0.87 x	0.028
Cysteine	0.56 xy	−0.03 y	0.86 x	0.230
Glutamic acid	0.83 xy	0.80 y	0.89 x	0.019
Glycine	0.83	0.82	0.83	0.020
Proline	0.84	0.82	0.92	0.040
Serine	0.85	0.85	0.86	0.020
Tyrosine	0.89 x	0.62 y	0.90 x	0.040
Mean, dispensable AA	0.85	0.82	0.87	0.016
Mean, total AA	0.86 xy	0.84 y	0.89 x	0.016

Apparent ileal digestibility of the diet + (endogenous loss/intake). Endogenous losses (g/kg DMI) of CP and AA were calculated as the following quantities; CP, 8.67; Arg, 0.27; His, 0.10; Ile, 0.25; Leu, 0.37; Lys, 0.25; Met, 0.07; Phe, 0.22; Thr, 0.38; Trp, 0.10; Val, 0.29; Ala, 0.37; Asp, 0.57; Cys, 0.09; Glu, 0.63; Gly, 0.91; Pro, 1.77; Ser, 0.31; Tyr, 0.20. Data represent least square means of four observations. Means within a row lacking a common letter differ ($P < 0.05$).

CISD that were calculated for Thr in the diet based on beef gelatin indicate that there is a real difference in the digestibility of this AA between the diets because all diets contained approximately the same quantity of Thr and no crystalline Thr was added to any of the diets. For Ile, His, and Met, the CIAD and CISD in both gelatin-based diets represent the digestibility of the mixture of AA from gelatin and crystalline sources of these AA. This is the reason why the digestibility of Ile in both gelatin-based diets was higher than in SBM. The reason why the CIAD, but not the CISD, was lower for His in beef gelatin compared to the other diets is that the diet based on beef gelatin had a lower concentration of His compared to the diets based on pork gelatin and SBM. As a result, the endogenous His contributed more on a percentage basis to the total ileal output of His in the pigs fed the beef gelatin-based diet compared to the pigs fed the other two diets, which in turn resulted in a lower CIAD. However, when calculating CISD, the effect of endogenous His is eliminated; that is the reason why no differences in CISD for His between the three feed ingredients were observed. For Cys, very low CIAD and CISD were found in both gelatin-based diets. This is undoubtedly a result of the low concentrations of Cys in the gelatin-based diets because the endogenous Cys makes up a large proportion of the total ileal output of Cys, resulting in low digestibility coefficients. However, the very low CISD for Cys in beef gelatin indicate that the endogenous loss of this AA may have been underestimated. Theoretically, all values for CISD should be positive. Nevertheless, pigs fed diets based on gelatin have to satisfy their Cys requirement by synthesizing Cys from Met. As a consequence, such diets need to contain enough Met to satisfy the requirements for both Met and Cys. For this reason, relatively large quantities of crystalline Met were included in the two gelatin-based diets used in the current experiment. Both the CIAD and the CISD for Met that were measured in both these diets were relatively high, as would be expected for crystalline Met.

5. Conclusions

Most AA in gelatin sourced from pork or beef raw materials have CIAD and CISD that are similar to soybean meal. Therefore, both gelatin sources may be used as a protein source in diets for swine provided that crystalline His, Ile, Met, and Trp are added to balance the indispensable AA.

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