

Asian-Aust. J. Anim. Sci. Vol. 21, No. 5 : 701 - 706 May 2008

www.ajas.info

Digestibility of Amino Acids of Maize, Low Tannin Sorghum, Pearl Millet and Finger Millet in Caecectomized Roosters

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ABSTRACT : The aim of the present study was to determine the apparent and true digestibilities of amino acids of maize, low tannin sorghum, pearl millet and finger millet in adult caecectomized cockerels. Adult cockerels (n = 60), 25-weeks old, were used in this study of which 30 birds were caecectomized as per a standard method. The apparent digestibilities of amino acids of sorghum were not affected by caecectomy, but were higher for maize, finger millet and pearl millet in caecectomized cockerels. Caecectomy had no influence on the true digestibilities of amino acids of maize, but higher digestibilities were observed for most of the amino acids of sorghum and finger millet in caecectomized cockerels. Caecectomy lowered the true digestibility of cystine, threonine and serine of pearl millet. The apparent digestibilities of amino acids of sorghum and finger millet in caecectomized cockerels. Caecectomy and finger millet were underestimated in intact cockerels. The true digestibilities of most of the amino acids of sorghum and finger millet were overestimated in intact cockerels. The findings suggest that the amino acid digestibility values of cereal grains determined using caecectomized cockerels might be appropriate and reliable for poultry diet formulations. Moreover, the digestibilities of amino acids of finger millet were inferior to other cereal grains, while those of pearl millet were comparable to maize and sorghum. (**Key Words :** Amino Acid, Digestibility, Caecectomized Cockerels, Cereal Grains)

INTRODUCTION

The dietary protein comprises a major element of the cost of poultry feed. It is universally accepted that the contribution of dietary protein to the animal depends not only on its amino acid composition but also on how effectively the amino acids are utilized (McNab, 1973). Corn is the most common cereal grain used in many parts of the world. It has a high amino acid digestibility coefficient. However, economic reasons have compelled the poultry industry to switch over to an array of alternative cereal grains such as sorghum, pearl millet and finger millet in many countries. Knowledge on the digestibilities of nitrogen and amino acids in cereal proteins aids in improving the utilization of dietary protein and to minimize nitrogen output through excreta (Lemme et al., 2004), thereby enhancing the performance of birds, reducing stress to birds and environmental pollution.

It is well understood that the microbial fauna inhabiting

¹ Central Avian Research Institute, Izatnagar, U.P, India. Received May 25, 2007; Accepted October 16, 2007 the large intestine interfere with amino acid digestibility determinations. Austic (1983) suggested that surgical removal of the caecum, the major site of gut microflora activity, might minimize the influence of microbial effects. A large volume of published data on the amino acid digestibility of corn and sorghum for poultry is available (Green et al., 1987; Ravindran et al., 1996a and 1996b), but there are no reports for millet grains. Hence, the present study was designed to determine the digestibility of amino acids of pearl millet and finger millet, in addition to the native variety of maize and low tannin sorghum, using caecectomized cockerels.

MATERIALS AND METHODS

Experimental procedure

Adult single comb white leghorn cockerels (n = 60), 25 weeks of age and weighing between 1.60 and 1.75 kg, were selected from a flock of same hatch for use in the present study. About 30 cockerels were caecectomized using the methodology described by Green et al. (1987) with slight modifications.

The caecectomized cockerels (30 in total) and the intact birds (total 30) were divided into five replicates of six birds

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Table 1. Amino acid composition of cereal grains (g/100 g)

Attributes	Maize	Finger millet	Pearl millet	Sorghum
Crude protein	9.46	8.80	10.95	11.02
Methionine	0.24	0.28	0.24	0.16
Cystine	0.23	0.19	0.22	0.15
Lysine	0.31	0.24	0.28	0.24
Threonine	0.41	0.39	0.38	0.30
Arginine	0.50	0.38	0.43	0.36
Isoleucine	0.40	0.39	0.41	0.34
Leucine	1.50	0.93	0.98	1.05
Valine	0.52	0.57	0.55	0.44
Histidine	0.31	0.23	0.25	0.19
Phenylalanine	0.61	0.49	0.48	0.42
Glycine	0.40	0.33	0.35	0.32
Serine	0.57	0.48	0.44	0.38
Proline	0.91	0.66	0.67	0.67
Alanine	0.89	0.58	0.75	0.72
Aspartic acid	0.74	0.57	0.76	0.63

each. All the birds were starved for 48 h. Each replicate of operated birds and intact birds was fed with one test cereal grain. Maize, sorghum and pearl millet were fed according to Farrell's (1978) rapid ME method. Finger millet (50 g) was force fed by Sibbald's (1976) crop intubation method. Drinking water was provided ad libitum. The last group of birds of both treatments were force fed with 50 g of nitrogen-free diet, which was previously cold pelleted. The composition (g/kg) of the protein-free diet was starch 400.00, cellulose 76.00, glucose 487.15, and vitamin and mineral mixture 36.85. The vitamin and mineral mixture contained calcium phosphate, 7 g; calcium carbonate 8 g; dicalcium phosphate 15 g; iodized salt 4 g; trace mineral mixture 0.5 g; vitamin premix 0.2 g; B complex 0.15 g; and choline chloride (50%) 2.0 g. The vitamin premix contained (per g) vitamin A 82,500 IU; B2 50 mg; D3 62,000 IU and K 10 mg. The trace mineral premix contained (per 100 g) FeSO4 8 g; ZnSO4 10 g; MnSO4 10 g; CuSO4 1 g; KI 30 mg and starch 70.97 g.

The total excreta were collected for the following 48 h at 8 h intervals (three times daily). The excreta of each bird were oven dried individually at 80°C for 24 h, finely ground and stored separately in a sealed airtight polythene bag at 24°C (deep freezer) until further analysis. The dry matter and nitrogen were estimated by the AOAC (1995) methods.

Amino acid analysis

The amino acid concentrations of test ingredients and excreta samples were analyzed by ion-exchange chromatography (Llames and Fontaine, 1994) following hydrolysis of samples in 6N HCl under N for 24 h at 110°C. Methionine and cystine were analyzed after performic acid oxidation (Moore, 1963). Apparent amino acid digestibility values were calculated using the following equation:

Apparent amino acid digestibility (%)

$$=\frac{(AAI_{f} - AAE_{f})}{AAI_{f}} \times 100$$

Where, AAI_f is the amount of each amino acid consumed by the fed cockerel and AAE_f is the amount of each amino acid excreted by the fed cockerel during the 48 h period post-feeding.

True amino acid digestibility (%)
=
$$\frac{AAI_{f} - (AAE_{f} - EAAE_{pf})}{AAI_{f}} \times 100$$

Where, AAI_f is the amount of each amino acid consumed by the fed rooster, AAE_f is the amount of each amino acid excreted by the fed rooster and $EAAE_{pf}$ is the endogenous amino acid excreted by the rooster fed the protein-free diet.

All the experiments were approved by the Animal Ethics Committee of the University and Indian Council of Agricultural Research. The data obtained from the above experiments were subjected to statistical analysis (independent 't' test, and ANOVA) by standard procedures (Snedecor and Cochran, 1980).

RESULTS

The amino acid concentrations of cereal grains are presented in Table 1. The amino acid contents of the different cereal grains studied were similar.

The apparent amino acid digestibilities of maize and tannin sorghum (white variety) estimated in low caecectomized and intact cockerels are presented in Table 2. The apparent digestibilities of methionine, lysine, isoleucine, leucine, valine, phenylalanine, proline, alanine, aspartic and glutamic acids in maize were significantly higher (p<0.05 to 0.001) in caecectomized cockerels than in the intact birds. The apparent digestibility of methionine in white sorghum was markedly higher (p<0.05) in caecectomized cockerels as compared to intact birds (85.22 vs. 75.46%). The apparent digestibilities of amino acids in finger millet are summarized in Table 3. Almost all the amino acids of finger millet (except threonine) were more (p<0.001) digestible in caecectomized birds than in intact ones. In comparison to maize, the apparent digestibilities of most of the essential amino acids of finger millet with caecectomized cockerels determined (except methionine, valine and phenylalanine) were markedly lower by about 7-15 per cent. On the contrary, the digestibilities were similar to sorghum. Significant differences (p<0.05 to 0.01) in the apparent amino acid digestibilities of methionine, lysine, arginine, isoleucine, leucine, valine, histidine, alanine and glutamic acid were evident for pearl

Attributes	Maize			Sorghum		
	Caecectomized	Intact	Pooled SEM	Caecectomized	Intact	Pooled SEM
Methionine	91.92 ^a	88.20 ^b	0.69***	85.22 ^a	75.46 ^b	1.44*
Cystine	83.68	81.83	0.76	64.19	62.44	1.42
Lysine	81.14 ^a	70.63 ^b	1.85***	67.16	62.63	1.75
Threonine	78.24	76.14	0.99	67.96	60.35	1.62
Arginine	88.72	86.48	0.61	79.52	71.18	1.90
Isoleucine	88.44^{a}	83.95 ^b	0.87**	81.61	74.68	1.56
Leucine	94.66 ^a	93.12 ^b	0.34*	89.88	86.44	0.38
Valine	87.23 ^a	83.84 ^b	0.78*	79.66	74.50	1.67
Histidine	87.91	86.54	0.56	74.64	68.09	1.34
Phenylalanine	92.02 ^a	90.21 ^b	0.45*	84.62	79.96	1.07
Serine	86.16	85.46	0.60	70.74	68.76	0.62
Proline	90.53 ^a	88.23 ^b	0.55*	81.59	79.46	0.31
Alanine	90.79 ^a	88.14 ^b	0.58*	84.95	81.05	0.99
Aspartic acid	84.33 ^a	80.68^{b}	0.90*	74.92	68.76	1.28
Glutamic acid	93.30 ^a	91.53 ^b	0.41*	87.22	83.48	0.71

Table 2. Apparent amino acid digestibility (%) of maize and sorghum in caecectomized and intact cockerels

The values are means of six cockerels.

^{a, b} Means in a row bearing different superscripts are significantly different (p<0.05).

* p<0.05; ** p<0.01; *** p<0.001.

Table 3. Apparent amino acid digestibility (%) of finger millet and pearl millet in caecectomized and intact cockerels

Attributes	Finger millet			Pearl millet		
	Caecectomized	Intact	Pooled SEM	Caecectomized	Intact	Pooled SEM
Methionine	88.18 ^a	78.87 ^b	1.56*	92.57 ^a	88.76 ^b	0.73**
Cystine	71.37 ^a	60.45 ^b	2.06**	82.08	79.94	0.94
Lysine	65.23 ^a	41.69 ^b	4.01***	80.70^{a}	71.69 ^b	1.79**
Threonine	68.81	63.99	1.37	76.78	76.90	1.05
Arginine	75.77 ^a	59.98 ^b	2.70***	87.30 ^a	81.85 ^b	1.11**
Isoleucine	81.59 ^a	68.93 ^b	2.11***	89.36 ^a	85.38 ^b	0.85*
Leucine	86.64 ^a	78.33 ^b	1.43***	91.92 ^a	89.24 ^b	0.60*
Valine	81.92 ^a	72.15 ^b	1.72***	88.18 ^a	84.88^{b}	0.81*
Histidine	74.79^{a}	60.38 ^b	2.51***	86.46 ^a	81.03 ^b	1.13**
Phenylalanine	85.04^{a}	75.43 ^b	1.65***	89.86	87.47	0.64
Serine	77.24 ^a	69.70 ^b	1.48**	82.90	81.39	0.85
Proline	80.52^{a}	71.44 ^b	1.64***	86.77	85.54	0.66
Alanine	76.14 ^a	68.93 ^b	1.46**	89.68 ^a	86.53 ^b	0.74*
Aspartic acid	69.75 ^a	56.30 ^b	2.45***	85.18	82.12	0.89
Glutamic acid	87.11 ^a	79.72 ^b	1.29***	92.16 ^a	90.08 ^b	0.52*

The values are means of six cockerels.

^{a, b} Means in a row bearing different superscripts are significantly different (p<0.05).

* p<0.05; ** p<0.01; *** p<0.001.

millet in caecectomized cockerels. The apparent digestibilities of almost all the essential amino acids of pearl millet determined using caecectomized cockerels were similar to maize, but higher than sorghum and finger millet.

The amino acids of metabolic origin excreted by cockerels fed the protein-free diet are presented in Table 4. The endogenous output of almost all the amino acids, with the exception of basic amino acids (His, Arg and Lys), was significantly higher (p<0.01) in caecectomized cockerels.

The true digestibilities of amino acids in maize and sorghum (low tannin) determined using caecectomized and intact cockerels are listed in Table 5. Lysine and histidine of maize showed a significant difference (p<0.05) in true digestibility. The true digestibility of lysine was

significantly higher in caecectomized cockerels (88.90 vs. 83.30%), while that of histidine showed a higher digestibility in intact cockerels (96.26 vs. 93.08%). The true digestibilities of most of the amino acids in sorghum were higher (p<0.05 to 0.001) in caecectomized cockerels. Similarly, the true digestibilities of methionine, lysine, arginine, isoleucine, leucine, valine, phenylalanine, proline and glutamic acid in finger millet (Table 6) were higher (p<0.05 to 0.01) in caecectomized cockerels as compared to intact birds. The true digestibilities of basic amino acids (His, Arg and Lys) and non-essential amino acids viz., alanine and aspartate of finger millet determined using caecectomized cockerels were markedly lower when compared to that of maize, low tannin sorghum and pearl

Table 4. Endogenous amino acid excretion (mg/bird/d) incaecectomized and intact cockerels fed protein free diet

Attributes	Caecectomized	Intact	Pooled SEM				
Methionine	5.62 ^a	5.08 ^b	0.13**				
Cystine	13.39 ^a	10.90 ^b	0.32***				
Lysine	16.14	15.01	0.40				
Threonine	29.94 ^a	18.22 ^b	1.16***				
Arginine	17.86	17.59	0.35				
Isoleucine	14.52^{a}	12.30 ^b	0.34***				
Leucine	25.42 ^a	20.89 ^b	0.61***				
Valine	22.23 ^a	17.37 ^b	0.59***				
Histidine	11.53	12.04	0.43				
Phenylalanine	16.76 ^a	12.93 ^b	0.46***				
Serine	27.62 ^a	19.92 ^b	0.83***				
Proline	28.29^{a}	21.03 ^b	0.76***				
Alanine	23.68 ^a	19.12 ^b	0.62***				
Aspartic acid	36.84 ^a	28.53 ^b	1.04***				
Glutamic acid	47.51 ^a	39.89 ^b	1.17***				

The values are means of six cockerels.

^{a, b} Means in a row bearing different superscripts are significantly different (p<0.01).

** p<0.01; *** p<0.001.

millet. The true digestibility of cystine, threonine and serine were significantly (p<0.05 to 0.01) higher for pearl millet in intact cockerels than in their caecectomized counterparts. The true digestibilities of all the 15 amino acids of pearl millet determined with caecectomized cockerels were comparable to those of maize and sorghum.

DISCUSSION

The amino acid levels in maize were higher than reported values (Fernandez et al., 1995; Huang et al., 2006). These differences were due to the lower crude protein contents of corn analyzed by earlier workers. The amino acid composition of sorghum observed in this study was comparable to the values reported by NRC (1994) and Mandal et al. (2004). However, the levels of indispensable amino acids (except methionine) in pearl millet were lower than the values reported by NRC (1994) and Mandal et al. (2004).

The apparent digestibilities of most of the amino acids of maize were significantly higher in caecectomized cockerels and the digestibility was remarkably higher than the apparent digestibility values reported by Green et al. (1987) in maize. The lower values obtained in their study might be attributed to the relatively lower intake of amino acids (50 g maize) as compared to the higher intake in the present study (>80 g maize). Similar relationships between intake and digestibility of amino acids were reported earlier (Payne et al., 1971; Sibbald, 1979). In contrast, caecectomy had no influence on the apparent digestibilities of most of the amino acids (except methionine) in sorghum. These observations suggested that the caecal microorganisms of intact birds had only a marginal influence on the amino acid excretion. It may be attributed to the higher solubility of dietary protein that results in minimum quantity of dietary (undigested) amino acids reaching the hindgut.

The apparent digestibilities of most of the amino acids of finger millet and pearl millet were higher in caecectomized cockerels. Moreover, the amino acids of finger millet were less digestible than those in the other three cereal grains. This difference in digestibility might be due to higher fibre content (5.90%) as compared to other cereals (2 to 3%). The higher fibre might lead to higher amounts of undigested dietary protein reaching the hindgut for further degradation by caecal microflora. The profound influence of hind gut microorganisms on the apparent digestibilities of amino acids in millet grains revealed that caecectomized birds were to be preferred for such studies

 Table 5. True amino acid digestibility (%) of maize and sorghum in caecectomized and intact cockerels

Attributes	Maize			Sorghum		
	Caecectomized	Intact	Pooled SEM	Caecectomized	Intact	Pooled SEM
Methionine	95.24	94.17	0.46	97.67 ^a	92.83 ^b	0.81***
Cystine	91.97	94.37	0.87	96.04	96.87	0.60
Lysine	88.90^{a}	83.30 ^b	1.33*	92.06 ^a	84.23 ^b	1.38***
Threonine	89.40	89.13	1.04	96.78	95.23	0.67
Arginine	94.08	94.52	0.56	97.91 ^a	92.60 ^b	0.95***
Isoleucine	93.92	92.10	0.66	97.59 ^a	93.11 ^b	0.79***
Leucine	97.24	96.75	0.28	97.12 ^a	96.53 ^b	0.41***
Valine	93.68	92.77	0.66	93.66 ^a	94.91 ^b	0.69*
Histidine	93.08 ^b	96.26 ^a	0.75*	95.15	96.01	0.46
Phenylalanine	96.23	95.91	0.40	96.94	96.10	0.46
Serine	93.32	95.04	0.71	95.82	96.91	0.50
Proline	95.12	93.65	0.52	97.10 ^a	93.71 ^b	0.61***
Alanine	94.60	93.84	0.47	96.71 ^a	94.75 ^b	0.44*
Aspartic acid	91.99	91.74	0.78	94.51 ^a	94.10 ^b	0.80**
Glutamic acid	96.61	96.55	0.34	96.67 ^a	97.00^{b}	0.41*

The values are means of six cockerels.

^{a, b} Means in a row bearing different superscripts are significantly different (p<0.05).

* p<0.05; ** p<0.01; *** p<0.001.

Attributes		Finger millet			Pearl millet	
	Caecectomized	Intact	Pooled SEM	Caecectomized	Intact	Pooled SEM
Methionine	92.59 ^a	87.96 ^b	0.91**	95.37	95.31	0.37
Cystine	86.76	87.20	1.14	89.44 ^b	94.41 ^a	1.04**
Lysine	80.98^{a}	71.21 ^b	2.18*	87.77	86.68	0.96
Threonine	87.45	88.90	1.17	87.05 ^b	92.42 ^a	1.19*
Arginine	87.04^{a}	79.40^{b}	1.61*	92.54	92.04	0.61
Isoleucine	90.51 ^a	84.12 ^b	1.30**	93.86	94.06	0.49
Leucine	93.20 ^a	88.92 ^b	0.89*	95.26	95.34	0.37
Valine	91.29 ^a	87.03 ^b	1.02*	93.39	94.25	0.53
Histidine	85.81	84.15	1.11	91.81	94.09	0.72
Phenylalanine	93.37 ^a	88.41 ^b	1.02**	94.42	95.50	0.46
Serine	90.68	90.37	0.89	90.64 ^b	94.84 ^a	0.93*
Proline	90.49^{a}	84.98 ^b	1.18*	92.02	93.59	0.58
Alanine	85.32	84.73	0.93	93.47	93.91	0.47
Aspartic acid	85.46	82.34	1.33	91.44	93.87	0.72
Glutamic acid	92.89 ^a	89.81 ^b	0.73*	95.50	96.68	0.38

Table 6. True amino acid digestibility (%) of finger millet and pearl millet in caecectomized and intact cockerels

The values are means of six cockerels.

^{a, b} Means in a row bearing different superscripts are significantly different (p<0.05).

* p<0.05; ** p<0.01.

involving poorly digestible protein sources. The apparent digestibilities of amino acids of pearl millet were similar to those of maize and better than for sorghum and finger millet.

In the present study caecectomy had a marked effect on the endogenous output of amino acids. The higher amounts of endogenous losses by caecectomized cockerels reflected that the greater bacterial activity in intact birds might have contributed markedly to lessen the endogenous amino acid output. Similar observations were also reported by Kessler et al. (1981) and Parsons (1984) in caecectomized birds after fasting. In contrast, only threonine output was significantly higher in caecectomized birds (Green et al., 1987), while the excretion of other amino acids differed numerically rather than statistically between caecectomized and intact birds.

The true digestibilities of most of the amino acids of maize, sorghum and finger millet were higher in caecectomized cockerels. As a consequence the intact birds tended to underestimate the true digestibility values. On the contrary, the true digestibilities of cystine, threonine and serine of pearl millet and histidine of maize were higher in intact birds. This observation suggested significant degradation of these amino acids by the microflora in the hindgut of intact birds, thereby overestimating the digestibility values of these amino acids. Literature relevant to apparent or true digestibilities of amino acids of millet grains is very scarce. The digestible amino acid contents of maize and finger millet grains observed in this study using caecectomized cockerels were similar to the values reported by degussa laboratories in broilers. However, the digestible leucine content of low tannin sorghum was higher (1.04 vs. 0.92%) by 11.5% as compared to that reported by degussa laboratories (Fontaine, 2006). Due to lack of related literature, it is difficult to compare and interpret the

digestible amino acid contents of pearl millet.

The present study revealed that the apparent digestibility of amino acids of sorghum could be determined using intact birds, while caecectomized birds are preferred for maize, finger millet and pearl millet grains. On the other hand, it would be appropriate to determine the true digestibility of amino acids of all four cereal grains using caecectomized cockerels. Further, the results indicated that the digestibilities of amino acids of finger millet were lower as compared to other cereal grains, while pearl millet was comparable to maize and sorghum.

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