Influence of caecectomy and source of dietary fibre or starch on excretion of endogenous amino acids by laying hens

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1. Two experiments were conducted to evaluate the effects of caecectomy and source of dietary fibre and starch on excretion of amino acids by laying hens. In Expt 1, caecectomized hens excreted significantly higher levels of several amino acids than intact hens during a 24 h fasting period. These differences were greatest for threonine, proline and leucine.

2. In Expt 2, caecectomized and intact hens were given either a nitrogen-free diet containing 500 g cellulose/kg or one containing 400 g uncooked potato starch/kg and 100 g citrus pectin/kg. The potato starch–pectin (PSP) diet increased excretion of most amino acids by both caecectomized and intact hens compared with the cellulose diet, with this response being larger for intact hens. When compared across diets, caecectomized hens excreted more threonine and serine than intact hens.

3. Excreta voided by hens given PSP contained higher levels of alanine and valine and lower levels of aspartic acid, glutamic acid and methionine than excreta from the cellulose diet. When compared across diets, excreta from caecectomized hens contained more threonine, serine and isoleucine and less aspartic acid and alanine than excreta from intact hens.

4. Multiple regression analyses of excreta amino acid profiles on profiles of endogenous and microbial protein suggested that the intestinal microflora had greater influence on amino acids excreted by caecectomized hens than on those excreted by intact hens.

Several studies have demonstrated that dietary carbohydrate and fibre substantially affect the excretion of nitrogen and amino acids by animals. High levels of dietary fibre result in increased N excretion by rats (Meyer, 1956; Beames & Eggum, 1981) and swine (Whiting & Bezeau, 1957). Both dietary potato and yam (*Dioscorea*) starches increased N excretion by rats (Mason & Palmer, 1973). Also, Rotenburg *et al.* (1982) found that the amino acid excretion of rats was elevated by dietary pectin. These effects of dietary fibre and starch are apparently due to both increased digestive enzyme secretion and sloughing of intestinal mucosal cells (Beames & Eggum, 1981) and increased bacterial synthesis of amino acids in the hind-gut (Mason & Palmer, 1973).

Parsons *et al.* (1983) recently found that elevated dietary fibre also increased amino acid excretion by poultry. A portion of this response was apparently due to increased bacterial synthesis of amino acids in the hind gut. Kessler *et al.* (1981) found that fasted, caecectomized roosters excreted higher levels of amino acids than did fasted, intact roosters, suggesting substantial influence of hind-gut metabolism. The effects of dietary carbohydrate and fibre on excretion of amino acids by poultry are of particular interest because of the recent increase in use of excreta collection assays to determine amino acid digestibility of food ingredients (Likuski & Dorrell, 1978; Sibbald, 1979; Parsons *et al.* 1981). These assays are rapid and usually involve the force-feeding of birds with a known quantity of test ingredients followed by quantitative collection of excreta for 48 h. Morever, fasted birds are used to measure excretion of endogenous amino acids for calculation of true digestibility. Thus, any influence of diet on endogenous amino acid excretion could yield inaccurate digestibility estimates when using this type of procedure.

Since the caeca comprise the major part of the large intestinal area in poultry, the

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caecectomized bird provides a good model for studying the effects of the hind-gut microflora on nutrient excretion. Therefore, the present study evaluated the influence of dietary regimen on endogenous amino acid excretion by caecectomized and intact laying hens. The results showed excretion of endogenous amino acids to be affected by both caecectomy and a diet containing 400 g uncooked potato starch/kg and 100 g citrus pectin/kg.

EXPERIMENTAL

Animals

Two experiments were conducted in the present study. The animals used in both trials were single comb, white Leghorn hens approximately 50 weeks of age. The hens were housed in an environmentally-controlled room and kept in individual cages with raised wire floors. Artificial light was provided for 16 h daily and food and water were supplied *ad lib.* before the start of the experiments.

Caecectomy was performed as described by Parsons *et al.* (1983). Hens were given at least 6 weeks to recover from surgery before being used in the experiments.

Feeding regimen and diets

The effect of caecectomy on excretion of amino acids by fasted hens was evaluated in Expt 1. Twelve caecectomized and twelve intact hens were fasted for 24 h to clear their digestive tracts of food residues. A plastic tray was then placed under each cage and excreta were collected quantitatively for 24 h.

The influence of source of dietary fibre and starch on excretion of amino acids by caecectomized and intact hens was investigated in Expt 2. Ten caecectomized and ten intact hens were fasted for 24 h. Five hens of each type were then force-fed with 40 g of an N-free diet containing either 500 g cellulose/kg (Solka Floc; Brown Co., Chicago, Illinois) or one containing 400 g uncooked potato starch/kg and 100 g citrus pectin/kg (US Biochemical Co., Cleveland, Ohio; PSP diet). The remainder of the diets were composed of 250 g each of maize starch and glucose monohydrate/kg. Thus, the treatments were arranged in a 2×2 factorial design. Excreta from each hen were collected quantitatively for 36 h post-feeding as described for Expt 1. The 36 h collection period was chosen on the basis of results obtained by Parsons *et al.* (1983) with a high-fibre diet.

Chemical analyses

Excreta from both experiments were dried to constant weight in a forced-draft oven at 60° and ground to pass through a 60-mesh screen. Amino acid content of at least two replicate samples of excreta from each bird was determined by ion-exchange chromatography after hydrolysis in 6 M-hydrochloric acid for 24 h at 110°. Amino acid concentrations were measured with a Beckman 119C automated amino acid analyser. Values for aspartate, threonine, serine, glutamate, proline, alanine, valine, methionine, isoleucine, leucine, tyrosine, phenylalanine, histidine, lysine and arginine were determined. Amino acid compositions are expressed as mmol of each individual amino acid in 1 mol of the total.

Statistical analyses

Results were assessed by analysis of variance procedures. Student's t test and the least significant difference calculated with the pooled standard error from analysis of variance were used to assess treatment differences where appropriate. The influence of endogenous *v.* microbial protein on excreta amino acid profiles was evaluated using multiple regression: $Y = b_0 + b_1 X_1 + b_2 X_2$, where Y is the amino acid composition (mmol/mol) of excreta, X_1 is the amino acid composition of endogenous protein collected from the terminal ileum of surgically-modified roosters (Parsons *et al.* 1983) and X_2 is the amino acid composition of

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Table 1. *Expt 1. Amino acid excretion (mg) and amino acid composition (mmol/mol) of excreta voided by fasted caecectomized and intact laying hens*

(Values are means with their standard errors for nine hens each)

	Amino acid excreted				Amino acid composition			
Amino acid	Caecectomized (A)	Intact (B)	SEM	A/B	Caecectomized (C)	Intact (D)	SEM	C/D
Aspartic acid	40·6	29·8*	3·5	1·36	109·7	109·9	1·8	1·00
Threonine	25·1	15·2*	2·3	1·65	75·1	63·1*	3·5	1·19
Serine	25·3	20·0	2·6	1·26	86·0	92·2	3·1	0·93
Glutamic acid	50·6	41·0	4·3	1·23	124·6	137·5*	2·6	0·91
Proline	23·9	14·6*	2·7	1·66	72·8	63·5	5·1	1·15
Alanine	24·6	18·5	2·1	1·33	100·2	102·5	3·4	0·98
Valine	22·8	16·7*	2·0	1·37	70·7	70·1	1·9	1·01
Methionine	5·9	5·2	0·8	1·13	14·3	17·1	1·3	0·84
Isoleucine	18·4	13·2*	1·6	1·39	50·5	49·4	1·5	1·02
Leucine	37·8	23·1*	3·4	1·64	103·0	86·4*	3·8	1·19
Tyrosine	14·0	11·4	1·4	1·23	28·0	31·1	1·2	0·90
Phenylalanine	15·1	12·3	1·6	1·23	33·1	36·5	1·5	0·91
Histidine	14·6	11·9	1·7	1·23	33·1	37·9	2·4	0·87
Lysine	24·1	17·7*	1·9	1·36	60·0	60·4	2·7	0·99
Arginine	18·6	15·1	1·6	1·23	38·9	42·6	2·2	0·91
Total	361·4	265·7*	31·3	1·36				

* Within rows, differences between means were significant ($P < 0{\cdot}05$) according to Student's t test.

microbial cells harvested from poultry excreta (Parsons *et al.* 1981). The regression procedure has been described by Mendes-Pereira *et al.* (1977) and Parsons *et al.* (1982). Methionine and histidine were not included in these regressions due to variation in amino acid analysis methods among the studies. Regression coefficients were compared statistically by the methods described by Steel & Torrie (1980).

RESULTS

Expt 1

Three hens had to be eliminated from each treatment because of excreta contamination by broken eggs in the collection trays. Fasted, caecectomized hens excreted significantly ($P < 0{\cdot}05$) higher levels of several amino acids than fasted, intact hens during the 24 h collection period (Table 1). These differences were greatest for threonine, proline and leucine. Total amino acid excretion was also significantly different ($P < 0{\cdot}05$), with caecectomized hens excreting a 36% higher level of amino acids than intact hens.

In general, amino acid composition of excreta voided by fasted, caecectomized and intact hens was similar (Table 1). However, excreta from caecectomized hens contained higher levels ($P < 0{\cdot}05$) of threonine and leucine but a lower level of glutamic acid than excreta from intact hens.

Expt 2

Both caecectomized and intact hens given the high-cellulose diet excreted more dry matter (DM) than those given the PSP diet (Table 2). Bird type had no significant effect on DM excretion from the two diets.

In contrast to DM, the PSP diet increased excretion of several amino acids by both

Table 2. *Expt. 2. Dry matter (g) and amino acid (mg) excretion by caecectomized and intact hens given a high-cellulose or a high-potato starch–pectin (PSP) diet*

(Values are means for five hens each)

Bird type...	Caecectomized		Intact		Pooled	Statistical significance of factors†	
Diet...	Cellulose	PSP	Cellulose	PSP	SEM	Bird type	Diet
Dry matter	16·6	14·5	19·7	14·8	1·19	NS	*
Amino acids							
Aspartic acid	53·0	58·5	49·5	62·4	4·77	NS	NS
Threonine	35·1	43·6	22·6	33·7	4·13	*	*
Serine	30·7	41·6	24·0	33·2	3·14	*	**
Glutamic acid	67·5	68·9	59·0	70·5	5·07	NS	NS
Proline	35·5	40·0	21·8	37·5	4·54	NS	*
Alanine	33·3	48·2	32·5	53·7	4·62	NS	**
Valine	27·2	39·3	23·7	35·9	3·48	NS	**
Methionine	5·5	3·3	5·8	4·1	0·81	NS	*
Isoleucine	25·9	31·8	16·3	29·2	3·05	NS	**
Leucine	34·3	46·2	30·3	45·8	3·43	NS	**
Tyrosine	13·4	16·0	11·2	17·6	1·95	NS	*
Phenylalanine	18·8	24·5	16·6	24·0	2·39	NS	*
Histidine	18·5	17·2	15·8	19·2	2·78	NS	NS
Lysine	30·3	38·0	25·5	33·4	3·40	NS	*
Arginine	19·8	24·6	17·1	21·3	1·54	NS	**
Total	448·7	541·8	371·8	521·8	40·26	NS	**

NS, not significant ($P > 0·05$).
* $P < 0·05$, ** $P < 0·01$.
† No significant ($P > 0·1$) interactions between bird type and diet.

caecectomized and intact hens compared with the cellulose diet (Table 2). The response to the PSP diet was greater for intact than caecectomized hens, with total amino acid excretion being increased by 40% ($P < 0·05$) for intact hens and 20% ($P > 0·10$) for caecectomized hens.

On comparing hens given the cellulose diet, it was found that caecectomized hens excreted larger amounts of amino acids than intact hens, with differences being significant ($P < 0·05$) for threonine, proline and isoleucine. On the other hand, amino acid excretion by the two types of hen was similar when the PSP diet was given.

Type of diet also had a substantial influence on amino acid composition of excreta (Table 3). Excreta from both types of hen given the PSP diet contained significantly ($P < 0·05$) more alanine and valine but less aspartic acid, glutamic acid and methionine than did excreta from hens given the cellulose diet. Additional responses of bird type to the PSP diet included increased serine and valine in excreta of caecectomized hens, and increased isoleucine but decreased aspartic acid and arginine in excreta of intact hens.

On comparing hens given the same diets, it was found that excreta from caecectomized hens contained more ($P < 0·05$) threonine, serine and isoleucine but less aspartic acid and alanine than did excreta from intact hens. In addition, the cellulose diet increased excreta proline for caecectomized hens compared with intact hens.

A significant ($P < 0·07$) bird type × diet interaction was observed for serine and arginine. The PSP diet increased excreta serine and arginine levels of caecectomized hens but decreased these amino acids in excreta of intact hens.

Table 3. *Expt 2. Amino acid composition (mmol/mol) of excreta voided by hens given a high-cellulose or a high-potato starch–pectin (PSP) diet*

(Values are means for five hens each)

Bird type...	Caecectomized		Intact		Pooled	Statistical significance of factors†	
Diet...	Cellulose	PSP	Cellulose	PSP	SEM	Bird type	Diet
Amino acid							
Aspartic acid	115·8	103·5	129·5	113·3	4·17	**	**
Threonine	82·6	86·7	65·8	68·8	4·49	**	NS
Serine	83·4	93·6	79·2	77·4	3·09	**	NS
Glutamic acid	133·5	110·1	139·4	117·8	5·57	NS	**
Proline	86·6	82·0	66·5	80·3	6·53	NS	NS
Alanine	106·8	125·7	126·9	146·3	6·13	**	**
Valine	66·8	78·1	70·1	74·4	2·98	NS	*
Methionine	11·1	5·5	13·3	6·6	1·52	NS	**
Isoleucine	57·3	56·3	43·2	53·4	3·30	*	NS
Leucine	75·9	82·7	80·0	85·6	2·98	NS	NS
Tyrosine	22·4	21·2	21·0	24·4	3·42	NS	NS
Phenylalanine	33·3	34·2	34·7	35·4	1·88	NS	NS
Histidine	32·4	26·2	35·5	30·8	2·71	NS	NS
Lysine	58·9	60·8	61·0	55·6	2·71	NS	NS
Arginine	33·1	33·4	33·9	29·8	1·09	NS	NS

NS, not significant ($P > 0·05$).
* $P < 0·05$, ** $P < 0·01$.
† Significant bird type × diet interaction ($P < 0·07$) for serine and arginine. No other interactions were significant ($P > 0·1$).

Table 4. *Regression coefficients obtained from multiple regression analysis of amino acid composition (mmol/mol) of excreta (Y) on that of endogenous protein (X_1) and microbial protein (X_2)*

(Mean values with their standard errors)

Expt no.	Excreta source	Independent variable: Endogenous protein* Mean	SE	Microbial protein† Mean	SE	r^2
1	Caecectomized, fasted	0·60	0·13	0·34	0·12	0·91
	Intact, fasted	0·49	0·15	0·45	0·13	0·89
2	Caecectomized, cellulose	0·64	0·15	0·36	0·13	0·91
	Intact, cellulose	0·41	0·12	0·73	0·11	0·95
	Caecectomized, PSP	0·66	0·13	0·30	0·12	0·92
	Intact, PSP	0·47	0·16	0·58	0·14	0·90

All the regression coefficients were significant ($P < 0·05$) but no intercept estimates were significant ($P > 0·10$).
Cellulose, high-cellulose diet.
PSP, high-potato starch–pectin diet.
* Endogenous protein collected at terminal ileum of surgically-modified roosters given a nitrogen-free diet (Parsons *et al.* 1983).
† Microbial cells harvested from poultry excreta (Parsons *et al.* 1981).

Regression analysis of amino acid profiles

The results of the multiple regression analyses used to evaluate the influence of endogenous *v.* microbial protein on excreta amino acid composition are presented in Table 4. No significant ($P > 0.10$) intercepts were obtained in any of the regressions. Significant regression coefficients ($P < 0.05$) were obtained for both endogenous and microbial protein in Expts 1 and 2. No significant ($P > 0.05$) differences were observed between regression coefficients for any of the analyses. However, the coefficients for endogenous protein were consistently larger than those for microbial protein with caecectomized hens in both experiments, whereas the reverse was observed for fasted, intact hens.

DISCUSSION

Effect of caecectomy: fasted hens. The results of the present study showed that the caeca had a substantial influence on amino acid excretion by fasted hens. Fasted, caecectomized hens excreted greater amounts of most analysed amino acids than fasted, intact hens. This observation is in agreement with the results of similar work by Kessler *et al.* (1981) with adult roosters. The values obtained in the present study for fasted, intact laying hens are also in agreement with those obtained for fasted, intact adult roosters by Sibbald (1980) and Kessler *et al.* (1981).

Most research evidence indicates that the primary functions of the caeca are water reabsorption and fibre digestion via microbial fermentation (Thornburn & Willcox, 1965; Annison *et al.* 1968). Barnes (1972) has shown that the caeca contain a very high concentration of micro-organisms. Nitsan & Alumot (1963) and Nesheim & Carpenter (1967) demonstrated that substantial proteolysis occurs in the caeca of growing chicks. The results of the present study suggest that microbial metabolism in the caeca significantly affects excretion of amino acids by poultry. In the case of the fasted bird, proteolysis and deamination of amino acids would probably be the primary modes of microbial action due to a paucity of fermentable carbohydrate reaching the lower gut (Rérat, 1978). This hypothesis was supported by regression analyses of excreta amino acid profiles which suggested that microbial protein had a larger effect on excreta from intact hens than on that from caecectomized hens. Moreover, in both the present study and that of Kessler *et al.* (1981), the largest differences observed between fasted, caecectomized and intact hens were for threonine and proline. These amino acids are very abundant in intestinal mucins, pancreatic juices and other endogenous protein (Rérat, 1978). Thus, microbial proteolysis and deamination in the lower gut could explain the reduced excretion of amino acids by fasted, intact hens compared with fasted, caecectomized hens.

Effect of source of dietary fibre and starch. The results of Expt 2 showed that source of fibre and starch affect endogenous amino acid excretion by poultry. When compared with the 500 g cellulose/kg diet, the PSP diet increased excretion of amino acids by both caecectomized and intact hens, although DM excretion from the cellulose diet was greater. Mason & Palmer (1973) also observed that faecal N excretion by rats was not related well to the amount of DM passing through the lower gut.

The response to the PSP diet probably resulted from a combination of factors. The abrasive action of this diet on the intestinal wall may have increased endogenous amino acid excretion (Hallsworth & Coates, 1962). The absorptive and gel-forming properties of the PSP diet could also have contributed (Kay & Strasberg, 1978). Indeed, these characteristics probably accounted for most of the increase in endogenous amino acid excretion observed from the PSP diet with caecectomized hens. This hypothesis is supported by the regression analyses of excreta-amino-acid profiles which suggested that most amino acids excreted by caecectomized hens were of endogenous origin.

Another explanation for differences observed between diets and bird type concerns the influence of the intestinal microflora. The PSP and cellulose diets differed markedly in carbohydrate composition. The PSP diet contained much more potentially-fermentable carbohydrate than did the cellulose diet. Both uncooked potato starch (Mason & Palmer, 1973; Demigne & Remesy, 1982) and pectin (Kay & Strasberg, 1978) provide good sources of fermentable carbohydrate for intestinal micro-organisms. These ingredients also increase N and amino acid excretion due to increased bacterial synthesis of amino acids in the lower gut (Mason & Palmer, 1973; Rotenburg *et al.* 1982). In contrast, the cellulose was primarily wood cellulose, which is not fermentable, although this ingredient does contain a small amount (50–80 g/kg) of potentially-fermentable hemicellulose.

The variation in response between hen types to the experimental diets strongly suggests that the intestinal microflora had substantial influence on amino acid excretion by intact hens. The response to the PSP diet was greater for intact hens than for caecectomized hens. Moreover, regression analyses of excreta-amino-acid profiles from the PSP diet predicted greater microbial influence for intact than for caecectomized hens. The results suggest substantial bacterial synthesis of amino acids in the lower gut of intact hens given the PSP diet.

The regression analyses of amino acid profiles also indicated that microbial influence on excreta composition was substantial for intact hens given either the cellulose or the PSP diet. The much higher levels of aspartic acid and alanine and lower levels of threonine and serine in excreta of intact *v.* caecectomized hens particularly indicated large microbial effects. Aspartic acid and alanine are the most abundant amino acids in microbial cells harvested from poultry excreta (Parsons *et al.* 1981), whereas threonine and serine are abundant in endogenous protein as described previously.

The present study shows that both caecectomy and a dietary mixture of uncooked potato starch and pectin influence excretion of endogenous amino acids by poultry. These effects appear to be due to both the physical characteristics of the fibre and starch in these ingredients and the availability of carbohydrate for microbial fermentation in the hind gut. Such responses could significantly affect amino acid digestibility estimates obtained with excreta collection assays. When carbohydrate is limiting, such as with fasted birds or birds given highly-digestible diets, amino acid excretion will probably be reduced due to bacterial deamination. The latter situation is predominant in most amino acid digestibility trials. Thus, bacterial deamination may partially explain why amino acid digestibility values from excreta collection assays are often higher than availability estimates obtained by other methods (Nesheim & Carpenter, 1967; Parsons *et al.* 1982).

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