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Determination of amino acid digestibility with different methods in birds

A comperative study

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ABSTACT

A comparative study was conducted with layer hens either intact, or cecectomized or cannulated in the ileum or colon to determine the amino acid digestibility of the same diet. The studies used Shaver Star Cross 288 layer hybrids, with 6 birds per each test method, in 2 replicates (n=12). Birds were placed in metabolic cages during the studies. Nutrient content of the trial diets followed the NRC (1994) recommendations. Amino acid content of the diet, excreta, faeces and digesta samples was determined according to Bech et al (1990). Trial data were subjected to variance analysis (ANOVA) (SAS 2001). The trial data led to the conclusion, that no statistically verifiable differences (Pł0.05) could be found between the amino acid digestibilities of intact or cecectomized birds. Neither was a statistically verifiable difference (Pł0.05) found between the amino acid digestibilities measured at the terminal ileum or terminal colon. The digestibility values found with intact and cecectomized birds, however, were significantly lower (PŁ0.05) than the digestibility measured at the terminal ileum or terminal colon. Also these trial data lead to the conclusion - in accordance with the relevant data in literature - that diet formulations should be based on the digestible amino acid content in poultry nutrition as well. In contrast to what was found for pigs, however, there is no difference in whether we use faecal or ileal digestibility in the formulation of poultry diets.

Keywords: layer, amino acid, digestibility, measuring techniques

INTRODUCTION

The methods used for determining the digestibility of amino acids in poultry are by no means as uniform as in the case of pig, where during the last decades it has become obvious that feed formulas should be calculated on the basis of ileal digestible amino acid contents. Several methods are known for determining the digestibility of amino acids in poultry as well (McNab, 1989; Babinszky et al, 1999). The oldest and at the same time the simplest procedure is the determination of the dropping digestibility (based on the collection of excreta). Another procedure is based on the cecectomization of the birds, which differs from the dropping digestibility study in that prior to the tests the double ceca of the trial birds is removed. The latest study methods however are based on cannulation techniques. Subject to which section of the intestinal tract the cannula is implanted we speak of ileal or faecal digestibility determination. When these methods are applied it is also possible to separately collect urine, digesta and faeces.

Post mortem studies should also be mentioned. These studies essentially consist of collecting the intestinal contents of the euthanized birds and the amino acid digestibility of the diets is determined based on the composition of the digesta collected. This trial method, however, allows for many failures and in consequence is used less and less.

No systematic comparative study of the trial methods listed above (except the post mortem studies) has been performed so far, hence the differences resulting from these methods are not known either. It is therefore indispensable to perform such studies for selecting the appropriate method.

The objective of these studies is therefore to determine the similarities and discrepancies between data resulting from various digestibility studies (based on the collection of excreta: intact birds, cecectomized birds; ileal cannulated birds and colon cannulated birds) when a commercial layer diet is fed.

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MATERIALS AND METHODS

Animals and housing

The studies were conducted with adult Shaver Star Cross 288 layer hybrids weighing 1.6 ±0.15 kg, using 6 birds per treatment (study method) in two replicates (n=12). The birds were placed in metabolic cages during the study. The temperature, relative humidity, length and intensity of lighting in the trial room corresponded to the values recommended for layer hens.

Table 1: Composition, nutrient and amino acid content of the diet (g/kg)

Ingredients	Amount
Corn	540.9
Wheat	178.7
Soya (full-fat)	24.7
Soya (extr.)	107.0
Gluten	59.0
Na-bicarbonate	1.4
NaCl	1.3
Limestone	72.5
Monocalcium-phosphat	5.8
Premixa	5.0
Lysine-HCl	0.6
DL-methionine	2.6
L-threonine	0.5
Total	**1000.00**
Nutrients	
Dry matter	892.0
AMEn(MJ/kg)b	11.6
Crude protein	155.0
Crude fat	28.0
Crude fiber	29.0
Crude ash	103.0
N-free extract	577.0
Calcium	34.8
Phosphorous	5.1
Amino acids	
Lysine	8.8
Methionine	5.3
Cystine	1.7
Threonine	6.4
Arginine	9.7
Isoleucine	6.7

a: 1 kg premix contains: Zn:21600 mg, Cu:3600 mg, Fe:11654 mg, Mn:17280 mg, I:288 mg, Se:43 mg, Co: 86 mg, Vit.A:1640000 IE, Vit.D3: 388000 IE, Vit.E:3880 mg, Vit.K3:312 mg, Vit.B1:312 mg, Vit.B2:1160 mg, Vit.B3:8001 mg, Vit.B5:2400 mg, Vit.B6:520 mg, Vit. B12:2.56 mg, Cholin:34355 mg, Folic acid:128 mg, Biotin: 25.8 mg.
b: calculated value

Treatment and diets, birds feeding system

The corn-soy based diet was formulated according to the NRC (1994) nutrient recommendations. The birds received in their diet 1.5 times their maintenance energy requirement. Drinking water was freely available from self-drinkers. The composition, nutrient and amino acid contents of the diet is shown in Table 1.

Preparation of the trial birds

Three procedures from the methods we investigated, i.e. cecectomization, ileal-digesta collection (ileal-cannula) and faeces collection (colon-cannula) necessitate operated birds. Surgical operations were conducted according to the recommendations of van Leeuwen et al (2000).

Cecectomization

During the surgery the double caecum of the birds are removed. The ceca were separated from the digestive tract so that the length of the remnants should not exceed 15 mm. The importance of the shortest possible cecal remnant is to eliminate the possibility of remaining bacterial activity in the stump. Collection of the excreta (faeces and urine) was enabled by a polythene bag attached to the cloaca opening during the studies. The schematic diagram of the surgery is shown in Figure 1.

Implantation of the ileal cannula

During the preparatory operation the ileum was separated from the postileal section of the digestive tract. Next a simple T-cannula was implanted in the terminal section of the ileum. The cannula enabled quantitative collection of the ileal digesta during the studies. The digesta was collected in polythene bags attached to the T-cannula. The schematic diagram of the surgery is shown in Figure 2.

Implantation of the colon cannula

During the operation after the abdominal cavity was opened the colon was separated from the cloaca about 15 mms before the colon sphincter, and then a simple T-cannula was implanted in the terminal section of the colon. The cannula enabled quantitative collection of the faeces during the studies. Faeces were collected in a polythene bag attached to the T-cannula. The schematic diagram of the surgery is shown in Figure 3.

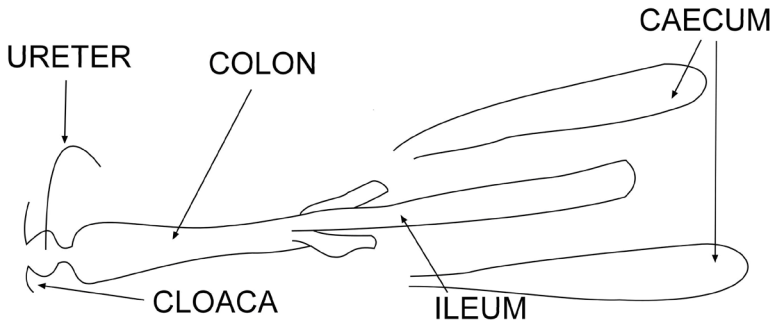

Fig. 1. Schematic diagram of removing the double ceca (cecectomization)

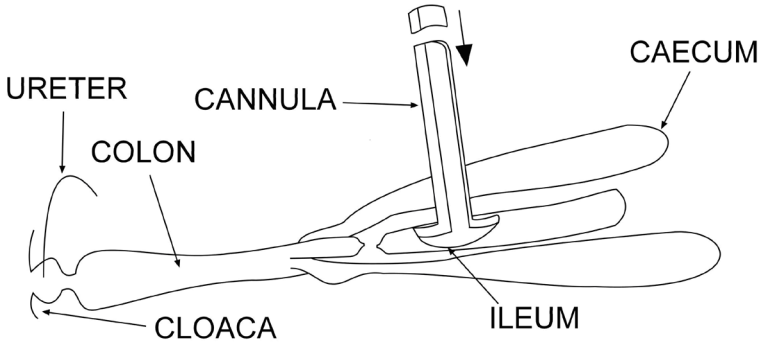

Fig. 2. Schematic diagram of implanting the ileal cannula

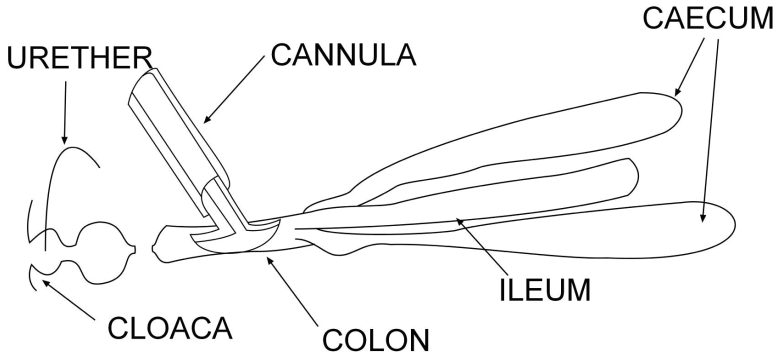

Fig. 3. Schematic diagram of implanting the colon cannula

Experimental procedure

The metabolic studies consisted of a 5 days adaptation and a 5 days collection period in all study methods investigated (van Leeuwen et al, 2000, Babinszky et al, 2003). During the trial the feed intake of the birds was weighed daily with gram precision. The discharged digesta, faeces and excreta were continuously collected in polythene bags attached to the cloaca and to the cannula, and these were weighed with gram precision also, and then stored for further processing at a temperature of -18 oC. At the end of the trial phase the samples were prepared for laboratory analysis.

Laboratory analyses

The nutrient content of the diets were determined in accordance with AOAC (1989). The amino acid contents of the diet, the excreta, digesta and faeces samples were determined in accordance with Bech et al (1990).

Statistical analysis of the trial data

The trial data were subjected to variance analysis (SAS, 2001). In case of a significant treatment effect the statistical reliability of differences between treatments (study methods) was verified by Tukey's test (SAS, 2001).

RESULTS AND DISCUSSION

The more important findings of the trial are summarized in Table 2. According to our data amino acid digestibilities measured with the method based on collection of faeces (dropping digestibility) and on cecectomy were - with the exception of methionine - identical (Pł0.05). The higher amino acid digestibility (Pł0.05) measured in the case of cecectomized birds indicates the likelihood of a limited amino acid synthesis in the ceca of the intact birds. In consequence their amino acid excretion was higher resulting in the lower digestibility value. The difference between the digestibilities determined by the two methods did not exceed 1 % on average - with the exception of methionine - which suggests a limited level of amino acid synthesis occurring in the ceca of intact birds, resulting in the higher amino acid excretion and lower amino acid digestibility levels. Cecal amino acid synthesis is also supported by the earlier studies of Green et al (1987), who found in their studies, that in addition to the deamination taking place in the ceca of poultry a low level of amino acid synthesis occurs as well. Bearing in mind that in our studies the additional amino acid excretion probably attributable to cecal amino acid synthesis had - with the exception of methionine - no significant effect on the digestibility of amino acids (Pł0.05) in case of any of the amino acids studied, the finding of Picard (1983) and Green et al (1987) also appears to be acceptable, namely that the modifying impact on amino acid digestibility of the amino acid synthesis occurring in the ceca - due to its low volume - is negligible, and thus need not be taken into account under practical circumstances.

Our data, however, also call attention to the fact, that of the amino acids investigated it is methionine which is synthesized in the biggest amount in the ceca. In consequence the intact birds excreted significantly more methionine (data are not shown). For this reason the digestibility of methionine in intact birds is 3.9 % lower (PŁ0.05) than the value determined for cecectomized birds. Comparing the results achieved with the two methods it is apparent, that the rate of amino acid excretion will be lower in case of cecectomy, which results in higher digestibility coefficients. Data resulting from the trials conducted with colon-cannulated and ileum-cannulated birds lead to the conclusion, that these two methods resulted no statistically verifiable differences (Pł0.05) for any of the amino acids. It is apparent from the data, however, that although the digestibility levels measured at the terminal colon are about 1.5 % higher - in the average of amino acids studied - than values measured at the terminal ileum, these differences are not significant (Pł0.05).

The slightly higher (Pł0.05) faecal digestibility suggests the possibility of a limited amino acid absorption still occurring in the colon of layer hens. This surplus amino acid, however - similarly to the findings for pigs - is not available for protein synthesis.

It is remarkable though, that dropping digestibility values and digestibility values measured with cecectomized birds were significantly lower (PŁ0.05) in case of most amino acids studied than the digestibility measured at the terminal ileum or colon. This means, that intact and cecectomized layer hens excrete more amino acid. A possible source of amino acid excretion is the amount of amino acids excreted in the urine. The hypothesis of urinary amino acid excretion is contrary to the generally accepted view, that the amount of amino acid excreted in the urine is negligible for poultry. Parsons (1983), however disagrees to this. The trial findings of Parsons (1983) are also confirmed by the data of Babinszky et al (2003) according to which urinary lysine excretion in poultry may reach even 25 % of total (faecal + urinary) lysine excretion, and will exceed 10 % even in the average of all amino acids. This amino acid level, however may result a considerable distortion of "digestibility" values.

Table 2: Digestibility of amino acids (%), determined by different methods

Amino acids	M E T H O D S							
	Dropping digestibility (intakt birds)		Cecectomisation (Cecectomised birds)		Faecal digestibility (birds with colon-cannula)		Ileal digestibility (birds with ileum-cannula)	
	x̄	sd	x̄	sd	x̄	sd	x̄	sd
Lysine	80.0b	5.7	81.1b	4.5	87.1a	2.1	86.5a	3.4
Methionine	88.7b	3.9	92.6a	1.9	93.1a	1.6	92.6a	2.0
Cystine	73.8b	10.6	70.4b	8.7	90.0a	3.6	87.2a	4.4
Threonine	77.4c	4.7	80.1bc	4.8	86.6a	2.9	84.2ab	4.1
Arginine	88.1b	3.5	90.4b	2.7	94.1a	1.3	94.1a	0.9
Leucine	82.7b	4.6	83.9b	4.2	87.7a	1.5	85.8ab	2.2
Isoleucine	79.2b	5.4	80.6ab	4.5	84.8a	1.8	82.4ab	3.3

a - c : Means within the same row without a common superscript letter differ, P≤0,05

To explain, in case of excessively high lysine intakes the activity of L-amino acid oxidase increases in the liver, in consequence of which lysine will be catabolized in the liver and its volume excreted in the urine will also decrease (Babinszky et al, 2003). These implications clearly support, that study methods based on collection of excreta, where the test samples (excreta) contain urine as well are less suitable for determining amino acid digestibility than methods based on cannulation of the birds.

CONCLUSIONS

The following main conclusions can be drawn from the trial - when high quality diets are fed:

1. No significant differences can be found (Pł0.05) between the digestibility values measured with intact and cecectomized birds;
2. there is no significant difference (Pł0.05) between the amino acid digestibility values measured at the terminal ileum and colon, either;
3. digestibility values determined for intact and cecectomized birds were significantly lower (PŁ0.05) than the amino acid digestibility measured at the terminal ileum and colon, which is probably attributable to the distorting influence of the urinary amino acid content;
4. for the purpose of determining amino acid digestibility of poultry diets both the ileal (ileum cannula) and the faecal (colon cannula) test methods can be suggested.

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