Aus dem Institut für Tierernährung

Sven Dänicke

Estimation of endogenous N-losses from the digestive tract of broilers by N-balance technique and linear regression analysis : Effect of xylanase addition to a rye-based diet

Manuskript, zu finden in www.fal.de

Published in: Landbauforschung Völkenrode 51(2001)1/2, pp. 33-40

Braunschweig
Bundesforschungsanstalt für Landwirtschaft (FAL)
2001

Estimation of endogenous N-losses from the digestive tract of broilers by N-balance technique and linear regression analysis: Effect of xylanase addition to a rye-based diet

Sven Dänicke

Summary

An N- balance experiment was carried out with broilers in order to examine the effects of supplementation of a tallow containing rye-based diet with a xylanase-containing enzyme preparation on several measures of protein quality. The experiment was designed according to a linear regression approach yielding an estimate for basal endogenous faecal N-losses and for N-digestibility corrected for these losses which were at 91 $mg \cdot d^{-1} \cdot kg^{-0.67}$. The so-calculated N-digestibility which is termed "true N-digestibility" was estimated at 86.9 % and 94.2 % for unsupplemented and xylanase-treated groups, respectively.

In combination with a previously reported experiment (Dänicke et al., 2000a), the results of the present study made it possible to ascribe the differences in true N-digestibility to the elevated specific endogenous faecal N-losses in broilers fed the unsupplemented diet. By correction for these specific endogenous losses it was also possible to determine the so-called real true N-digestibility, which was only slightly different for both diets (92.0 % vs. 93.9 %).

Key words: Boiler, xylanase, N-balance, endogenous N-loses, N-digestibility

Zusammenfassung

Schätzung der endogenen Darm-Stickstoffverluste von Broilern mittels N-Bilanztechnik und linearer Regressionsanalyse: Einfluss einer Xylanase-Zulage zu einer Roggen-basierenden Futtermischung

Es wurde ein N-Bilanzversuch mit Broilern durchgeführt, um den Einfluss der Zulage eines Xylanase-enthaltenden Enzympräparates zu einer Talg- und Roggen-basierenden Futtermischung auf verschiedene Parameter der Proteinqualität zu untersuchen. Der Versuch war nach entsprechend einem linearen Regressionsansatz angelegt und ermöglichte die Schätzung der basalen faecalen N-Verluste, die bei 91 $mg \cdot d^{-1} \cdot kg^{-0.67}$ lagen. Die damit kalkulierte N-Verdaulichkeit, die als wahre N-Verdaulichkeit bezeichnet wird, betrug 86.9 % und 94.2 % für die unsupplementierte bzw. Xylanase-behandelte Gruppe.

Durch Kombination der Ergebnisse eines früheren Versuches (Dänicke et al., 2000a) mit den Daten des vorliegenden Experimentes war es möglich, die Unterschiede in der wahren N-Verdaulichkeit den erhöhten spezifischen endogenen faecalen N-Verlusten der Broiler, denen die unsupplementierte Diät gefüttert wurde, zuzuschreiben und die sogenannte reale wahre N-Verdaulichkeit zu berechnen, welche sich zwischen beiden Versuchsgruppen nur noch geringfügig unterschied (92,0 % vs. 93,9 %).

Schlüsselwörter: Broiler, Xylanase, N-Bilanz, endogene N-Verluste, N-Verdaulichkeit

[1] Dänicke, Sven; Institute of Animal Nutrition of the Federal Agricultural Research Centre (FAL), Bundesallee 50, 38116 Braunschweig

1 Introduction

Apparent digestibility of exogenous or dietary protein is greatly influenced by endogenous N-losses from the digestive tract since true protein digestibility or protein absorbability is assumed to reach nearly 100 % for the majority of dietary protein sources (Reeds et al., 1999). Endogenous N-losses from the digestive tract are a composite of basal N-losses which would occur under conditions of feeding an N-free diet and of specific or protein type-related N-losses. Thus, the former are assumed to be independent of dietary protein quality whereas the latter are consequently protein quality-dependent.

It was demonstrated by Dänicke et al. (2000a) that feeding of a rye-based diet in combination with beef tallow increased the endogenous N-losses from the broiler's digestive tract dramatically when compared with the xylanase-supplemented group. The so-estimated endogenous N-losses were basically mixed losses resulting from the applied experimental design and from the [^{15}N]isotope-dilution-technique used. Therefore, the specific endogenous N-losses, i.e., the N-losses caused by dietary treatments remained to be determined.

By regressing N-intake on faecal N-excretion it becomes possible to estimate both non-specific faecal N-excretion (intercept on ordinate) and true digestibility of protein (regression slope). Consequently, specific endogenous N-losses could be estimated by combining both methods. Hence, the aim of the present study was to estimate non-specific N-losses with a N-balance technique and to relate these results to those reported by Dänicke et al. (2000a) in order to get more information on the effects of xylanase supplementation to rye-based broiler diets on specific endogenous N-losses from the digestive tract and on further parameters of protein quality.

2 Material and methods

2.1 Experimental design

A rye based diet was formulated to contain beef tallow and was used as a basal diet (Table 1). Supplementation of this basal diet with vitamins and minerals gave a "normal" broiler fattening diet with respect to energy and nutrient concentration (Group 3, Table 2) which meets the requirements of broilers under practical feeding conditions. The decrease in N-intake as a precondition for regression was achieved by stepwise dilution of the basal diet with starch (Groups 1 and 2, corresponding to a low and medium protein supply, respectively, Table 2). Vitamins and minerals were supplemented at the same level in all experimental diets. In combination with pair-feeding conditions, it was ensured that all broilers consumed the same quantity of dry matter, a critical factor in the estimation and comparability of endogenous N-losses from the digestive tract. Consequently, this experimental design yielded a total of 6 diets to be tested.

Table 1.
Composition of basal diet (g·kg^{-1})

Rye[1]	644.1
Soy protein isolate	189.9
DL-methionine	4.4
L-lysine HCl	1.5
L-threonine	2.6
Beef tallow	104.9
Maize starch	52.5

[1] Variety "Marder", water extract viscosity, 2.48 mP·s (dilution 1:10, Brookfield viscometer, model DV-II+LV); insoluble pentosans, 66.2 g/kg dry matter, soluble pentosans, 35.5 g/kg dry matter

2.2 Balance experiment

Each of the 6 diets was tested on 5 male broilers of the LOHMANN-MEAT strain. The experiment was carried out according to the total collection method as described by Schiemann (1981). Briefly, broilers were raised up to 7 days of age on a commercial broiler starter diet (220 g crude protein and 13.1 MJ AME_N per kg of diet) before they were transferred to single metabolic cages enabling quantitative recording of feed consumption and of excreta voided by the birds. The mean live weight was 163 g per chick at this age. Experimental diets were introduced over the next 3 days. The subsequent 5-day pre-collection period was used to adjust all broilers to a similar daily feed intake. During the following collection period, from day 15 to 20 of age, excreta were collected quantitatively twice daily and kept frozen between each collection. Each day 50g of feed were given to each broiler. Feed was consumed completely by all broilers.

At the end of the experiment, excreta were freeze dried, ground to pass through a 1 mm screen and subjected to analysis.

2.3 Analysis

Freeze dried excreta and diets were analysed for dry matter and total nitrogen according to Naumann and Bassler (1993) and for α-amino-N according to Pahle et al. (1983). The latter analysis is required for calculation of apparent protein digestibility and faecal N-excretion.

2.4 Calculations and statistics

Feed intake, N- and α-amino-N concentration in diets and excreta were used to calculate several parameters from the N-balance experiment. Apparent protein digestibility was calculated from the α-amino-N-intake and excretion assuming that the ninhydrine positive nitrogen in excreta originates exclusively from faeces (Pahle et al., 1983) according to Equation 1. Based on this method,

Table 2.
Composition of experimental diets (g·kg^{-1})

	Protein supply					
	Low		Medium		Normal	
Group:	1(-)	1(+)	2(-)	2(+)	3(-)	3(+)
Components:						
Basal diet	571.7	570.7	762.3	761.3	952.9	951.9
Maize starch	381.2	381.2	190.6	190.6	-	-
Limestone	5.4	5.4	5.4	5.4	5.4	5.4
Dicalcium phosphate	28.7	28.7	28.7	28.7	28.7	28.7
Sodium chloride	3	3	3	3	3	3
Premix[1]	10	10	10	10	10	10
Enzyme preparation [2]	-	1	-	1		- 1
Calculated composition:						
AME_N (MJ/kg)	13.8	13.8	13.6	13.6	13.3	13.3
Crude protein	126.0	126.0	168.0	168.0	210.0	210.0
Lysine	7.9	7.9	10.6	10.6	13.2	13.2
Methionine	4.2	4.2	5.6	5.6	7.0	7.0
Methionine + cystine	6.0	6.0	8.0	8.0	10.0	10.0
Ca	9	9	9	9	9	9
Total P	7	7	7	7	7	7
Na	1.2	1.2	1.2	1.2	1.2	1.2

[1] Vitamin-mineral premix provided per kg of diet:

Fe, 60 mg; Cu, 5 mg; Zn, 51.4; Mn, 60.8; Se, 0.2; I, 0.6; vitamin A, 12000 IU; vitamin D3, 3000 IU; vitamin E, 42 mg; vitamin B1, 2.1 mg; vitamin B2, 6.6 mg; vitamin B6, 4.1 mg; vitamin B12, 20.7 mg; pantothenic acid, 15 mg; nicotinic acid, 36 mg; folic acid, 1 mg; biotin, 102 mg; choline chloride, 700 mg; ethoxyquin, 120 mg; Zn-bacitracin, 50 mg

[2] Avizyme 1300 (Finnfeeds, Marlborough, UK) contained xylanse from Trichoderma longibrachiatum, 3000 IU/g, measured at pH 6.0 on the basis of reducing substance formation; ß-glucanase activity and cellulase activity measured under the same conditions, were 40 and 14 IU/g, respectively

Table 3.
N-intake, faecal N-excretion, N-balance and apparent protein digestibility in response to protein supply and xylanase supplementation (15.-20. day of age)

Protein supply	Enzyme addition[1]	N-intake (mg·d^{-1}·kg$^{-0.67}$)	Faecal N-excretion (mg·d^{-1}·kg$^{-0.67}$)	Apparent protein digestibility (%)	N-balance (mg·d^{-1}·kg$^{-0.67}$)
Low	-	2069	354	82.9	998
Medium	-	2890	487	83.1	1490
Normal	-	3719	569	84.6	2037
Low	+	2113	209	90.1	1274
Medium	+	2955	237	92.0	1925
Normal	+	3679	334	90.8	2355
ANOVA (Probability):					
Enzyme addition		0.739	<0.001	<0.001	0.001
Protein supply		<0.001	0.002	0.604	<0.001
Linear		<0.001	<0.001	0.365	<0.001
Quadratic		0.707	0.903	0.682	0.671
Enzyme addition x protein supply		0.807	0.394	0.588	0.766
PSEM		81	39	1.2	110

[1] "-" = without enzyme addition; "+" = xylanase activity of 3000 IU per kg of diet

the faecal N-excretion is calculated from protein intake and undigested protein (see equation [4]). Faecal N-excretion was used to regress against N-intake (equation [4]) in order to estimate the basal N-excretion and the true N-digestibility from the regression slope which equates the definition of true protein digestibility according to equation [2] . Specific endogenous N-losses were calculated by subtracting the basal N-losses obtained from equation [4] from the mixed basal and specific N-losses as reported by Dänicke et al. (2000a). The real true N-digestibility, i.e., the N-digestibility which can truly be ascribed to the dietary nitrogen, was calculated by consideration of both basal and specific N-losses according to equation [3].

The N-balance, which equates the difference between N-intake and total N-excretion (faeces plus urine), was regressed against N-intake (equation [5]) in order to estimate the total basal N-losses, i.e., the intercept on ordinate, and the net protein utilization which is equivalent to the respective regression slope.

$$\text{Apparent protein digestibility (\%)} = \frac{\alpha\text{-amino-N-intake} - \alpha\text{-amino-N-excretion}}{\alpha\text{-amino-N-intake}} \cdot 100 \quad [1]$$

$$\text{True N-digestibility (\%)} = \frac{\text{N-intake} - \text{faecal N} + \text{basal faecal N}}{\text{N-intake}} \cdot 100 \quad [2]$$

$$\text{Real true N-digestibility (\%)} = \frac{\text{N-intake} - \text{faecal N} + \text{basal faecal N} + \text{specific faecal N}}{\text{N-intake}} \cdot 100 \quad [3]$$

$$y = a + b \cdot x \quad [4]$$

where

y = faecal N-excretion ($mg \cdot d^{-1} \cdot kg^{-0.67}$)
= protein intake (mg)·(1—apparent protein digestibility·100^{-1})·6.25^{-1}·$kg^{-0.67}$
x = N-intake ($mg \cdot d^{-1} \cdot kg^{-0.67}$)
a = basal endogenous faecal N-loss ($mg \cdot d^{-1} \cdot kg^{-0.67}$)
$(1-b) \cdot 100$ = True protein digestibility (%)

$$y = a + b \cdot x \quad [5]$$

where

y = N-balance ($mg \cdot d^{-1} \cdot kg^{-0.67}$)
x = N-intake ($mg \cdot d^{-1} \cdot kg^{-0.67}$)
a = basal endogenous faecal plus urinary N-loss ($mg \cdot d^{-1} \cdot kg^{-0.67}$)
$b \cdot 100$ = Net protein utilization (%)

The slopes of the regressions were compared according to procedure 3/62/5251 of the Verfahrensbibliothek (Rasch et al., 1978) in order to evaluate differences between dietary treatments (enzyme supplementation).

Moreover, data of the balance experiment were analysed according to a complete two-factorial design of analysis of variance (ANOVA):

$$y_{ijk} = \mu + a_i + b_j + a_i x b_j + e_{ijk}$$

where y_{ijk} = k^{th} observation for protein supply i and enzyme supplementation j ; a_i = protein supply (low, medium, normal), b_j = enzyme supplementation ('-' - without and '+' - with xylanase supplementation);, $a_i x b_j$ = interactions between protein supply and enzyme supplementation, e_{ijk} = error term.

All statistics were carried out using the Statistica for the Windows^{TM} operating system (StatSoft Inc., 1994).

3 Results

3.1 Balance study

N-intake showed a strong increase, clearly linear to protein supply because broilers of all groups consumed a similar quantity of the respective diets (Table 3). Faecal N-excretion also increased linearly with protein supply, but at a significantly lower level in groups fed the xylanase-supplemented diets. Consequently, apparent protein digestibility was markedly influenced by the addition of enzymes but appeared unaffected by protein supply. N-balance as the difference between N-intake and total N-excretion increased linearly with dietary protein concentration, but again, at a higher level for the enzyme treated groups.

3.2 Regression analyses

N-intake was regressed on faecal N-excretion according to equation [4]. The regression analysis was modified in such a way that regression slopes could be estimated separately for unsupplemented and xylanase-treated groups at the same time, while both regressions were forced through a common intercept on ordinate (Table 4, Fig. 1). Birds fed the enzyme-supplemented diets excreted significantly less nitrogen with the faeces per unit N-intake than their unsupplemented counterparts. The term (1-b)×100 yields a protein digestibility measure which can be interpreted as true protein or N-digestibility according to equation [2]. Consequently, the so-calculated true N-digestibility was significantly higher due to xylanase addition. Moreover,

Table 4.
Parameters estimated by regression of N-intake on faecal N-excretion according to equation [4](parameters are given together with standard errors and probabilities)

Enzyme addition [1]	a[2]	b	RSD	r²
- +	91± 45 (0.236)	0.131[a] ± 0.025 (<0.001) 0.058[b] ± 0.026 (0.035)	64	0.809

[1] "-" = without enzyme addition; "+" = xylanase activity of 3000 IU per kg of diet
[2] regressions were forced to a common intercept on ordinate
ab = regression slopes are significantly different (p<0.05)

Table 5.
Parameters estimated by regression of N-intake on N-balance according to equation [5](parameters are given together with standard errors and probabilities)

Enzyme addition [1]	a[2]	b	RSD	r²
- +	-291± 75 (<0.042)	0.623[a] ± 0.05 (<0.001) 0.736[b] ± 0.05 (<0.001)	169	0.889

[1] "-" = without enzyme addition; "+" = xylanase activity of 3000 IU per kg of diet
[2] regressions were forced to a common intercept on ordinate
ab = regression slopes are significantly different (p<0.05)

the common intercept on ordinate can be taken as an indicator for basal endogenous N-loss from the digestive tract. Fitting of N-intake and N-balance data to model [5] (Figure 1) yielded the parameters given in Table 5. The regression slope indicates an increase of N-balance per unit of N-intake as the net protein utilization, and it was estimated to be significantly higher in enzyme-treated broilers. The commonly estimated intercept on ordinate can be interpreted as nitrogen maintenance requirement and includes both endogenous urinary and faecal N-losses.

4 Discussion

With linear regression of N-intake on faecal N-excretion it becomes possible to estimate the true protein digestibility according to equation [2] by considering basal endoge-

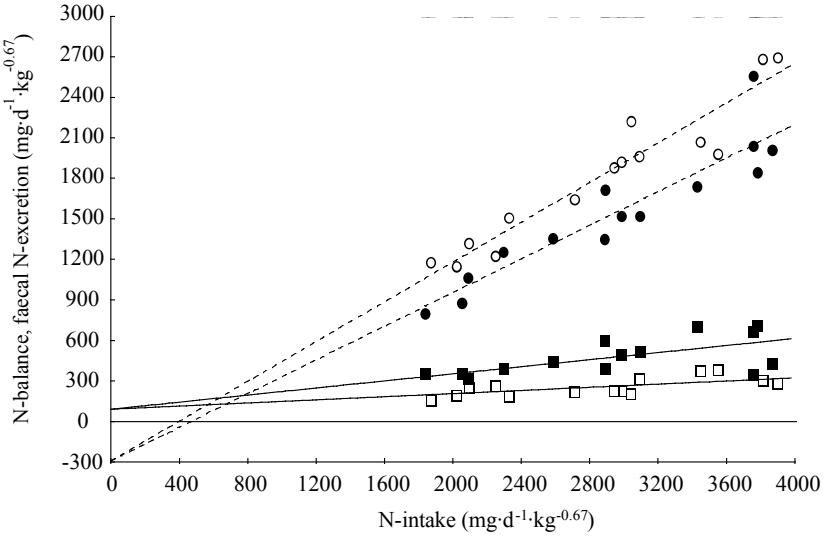

Figure 1.
N-balance and faecal N-excretion in response to N-intake of male broilers (15 to 20 days of age)
(Faecal N-excretion, without xylanase, —■— , y = 91 + 0.131·x;
Faecal N-excretion, with xylanase, ——— ; y = 91 + 0.058·x;
N-balance, without xylanase, ·····●····· ; y = -291 + 0.623·x;
N-balance, with xylanase, ····· ; y = -291 + 0.736·x;
regressions for each parameter sets were forced through a common intercept on ordinate)

Table 6.
Endogenous N-losses from the digestive tract and protein digestibility measures as influenced by xylanase supplementation according to the linear regression approach or to a [^{15}N]isotope dilution method

	[^{15}N]isotope dilution technique		Linear regression	
Enzyme addition [1]:	-	+	-	+
N-intake (mg·d^{-1}·kg$^{-0.67}$)	3000	3000	3000	3000
Faecal N-excretion (mg·d^{-1}·kg$^{-0.67}$)	540	360	484	265
Relative to total faecal N-excretion (%)	*100*	*100*	*100*	*100*
Real indigestible faecal N-excretion (mg·d^{-1}·kg$^{-0.67}$)	296	279	240	184
Relative to total faecal N-excretion (%)	*55*	*78*	*49*	*69*
Mixed endogenous faecal N-loss (mg·d^{-1}·kg$^{-0.67}$)	244	81		
Basal endogenous faecal N-loss (mg·d^{-1}·kg-$^{-0.67}$)	91[2]	91[2]	91	91
Relative to total faecal N-excretion (%)	*17*	*25*	*19*	*34*
Specific endogenous faecal N-loss (mg·d^{-1}·kg$^{-0.67}$)	153	-10	153[3]	-10[3]
Relative to total faecal N-excretion (%)	*28*	*-3*	*32*	*-3*
Apparent N-digestibility (%)	82.0	88.0	83.9	91.2
True N-digestibility (%)	85.0	91.0	86.9	94.2
Real true N-digestibility (%)	90.1	90.7	92.0	93.9

[1] "-" = without enzyme addition; "+" = xylanase activity of 3000 IU per kg of diet
[2] Similar basal endogenous N-loss assumed as derived from regression
[3] Similar specific endogenous faecal N-loss assumed as derived from isotope dilution experiment

nous N-losses. On theoretical grounds it is reasonable to assume that these basal N-losses are not influenced by protein quality since extrapolation to zero-N-intake gives that faecal N-excretion which should be measurable by feeding an N-free diet and which gives an estimate on basal faecal N-losses. The so-estimated basal faecal N-losses are in accordance with those given by Dänicke et al. (1998) which ranged between 86-116 mg×d^{-1}·kg$^{-0.67}$.

However, the nature of faecal N-losses above the basal N-losses remains obscure in the linear regression approach. No differentiation is made in this approach between non-absorbed faecal nitrogen and that faecal endogenous N-excretion which might have been caused by dietary (anti-nutritive) components (Figure 2). The importance of these so-called specific endogenous faecal N-losses and the associated problems in evaluation of dietary protein quality were discussed in detail, for example, by Krawielitzki and Bock (1975), Krawielitzki and Smulikowska (1977) and Sève and Hess (2000).

Using an isotope dilution technique and similar diet types as in the presented in this experiment, Dänicke et al. (2000a) succeeded in demonstrating that endogenous faecal N-proportions and excretion were markedly increased when an unsupplemented tallow-containing rye-based diet was fed to broilers compared to the xylanase supplemented group. However, with this isotope dilution technique it was not possible to differentiate between basal and specific endogenous faecal N-losses. On the other hand, calcu-

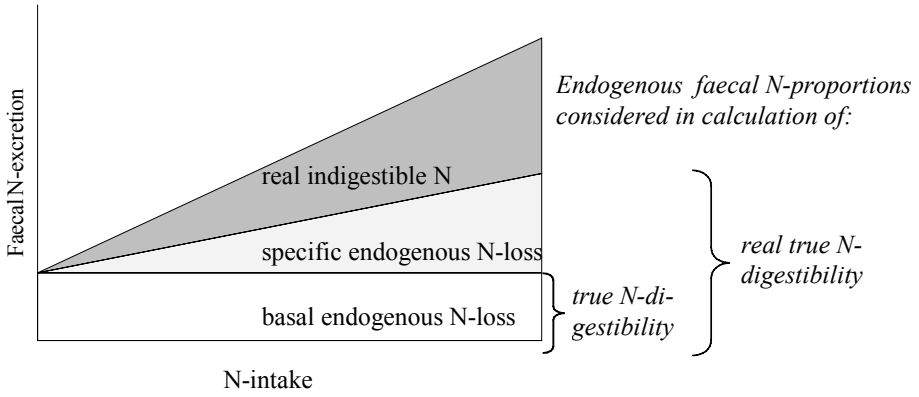

Figure 2.
Schematic illustration of the relationships between N-intake and faecal N-excretion from different sources (after Krawielitzki and Bock, 1975, modified)

lation of N-digestibility by consideration of these N-losses can be interpreted as real true N-digestibility according to Equation 3.

With the results of the present experiment taken together with those reported by Dänicke et al. (2000a), it should be possible to estimate the specific endogenous faecal N-losses by subtruction. The regression approach provides the basal endogenous faecal N-losses, whereas the isotope dilution technique gives an estimate of the sum of specific and basal endogenous faecal N-losses. Such an approach is justified by the fact that similar diet types were used in both experiments. By further assumption of a similar N-intake, the specific N-losses were estimated (Table 6). Moreover, it became possible to calculate both the true N-digestibility for the isotope dilution experiment and the real true N-digestibility for the regression approach with the information available.

It can be concluded from these calculations that specific endogenous faecal N-excretion amounted to approximately 30 % of total faecal N-excretion in broilers fed the unsupplemented diet, whereas practically no specific N-losses occurred in the xylanase-supplemented group. Therefore, the differences in apparent protein digestibility according to equation [1] between both diets were caused by the differences in specific endogenous faecal N-losses. Similarly, the diet differences remained by evaluation as true N-digestibility calculated by equation [2] because only the constant basal endogenous faecal N-losses are considered in this N-digestibility measure. Consequently, these differences disappeared completely in calculation of the real true N-digestibility by using equation [3]. From the latter measure it can be deduced that approximately 90 % to 94 % of the dietary protein were indeed absorbed.

From a physiological point of view, the increased specific endogenous faecal N-losses in broilers fed the unsupplemented diets are a reflection of an elevated endogenous N-secretion into the gut, combined with a decreased rate of re-absorption of these excessively secreted nitrogenous compounds up to the end of the ileum. Using similar diet types, it was shown by Dänicke et al. (2000b) that protein synthesis rates of the pancreas and of the small intestine were significantly enhanced in the broilers for which the increased endogenous faecal N-losses were measured in the present experiment and in the isotope dilution experiment (Dänicke et al., 2000a). The elevated protein synthesis of tissues involved in endogenous N-secretion was closely related to the dramatically increased intestinal viscosity in broilers fed the unsupplemented diet which, in turn, was caused by dietary soluble arabinoxylans from rye. This viscosity increase was avoided by the xylanase-caused partial hydrolysis of these anti-nutritive compounds. Therefore, the highly soluble arabinoxylans apparently induced the measured excessive protein synthesis of tissues contributing to endogenous secretions and the increased specific endogenous faecal N-losses via the increased intestinal viscosity.

In contrast to faecal N-excretion which was calculated on the basis of the analysis of α-amino-N in diets and excreta, N-balance data (Table 5, Figure 1) are based on analysis of total nitrogen of both specimens but yielded similar conclusions. N-balance per unit N-intake was significantly lower in broilers fed the unsupplemented diet, which indicates an increased N-excretion with urine and faeces at the same time. Therefore, it is reasonable to assume that these elevated total N-excretions were also due to the higher specific endogenous faecal N-losses, at least in part.

From a methodological standpoint, both the linear regression approach and isotope dilution technique are sensitive indicators for detection of dietary related differences in protein quality and endogenous faecal N-losses. By combining both methods it became possible to obtain information about specific endogenous faecal N-losses. For future experiments, the isotope dilution technique should be the preferred method because it allows assumption of a constant basal endogenous faecal N-loss related to metabolic body size. Once determined, this value should be applicable in calculation of specific endogenous faecal N-losses from the mixed endogenous faecal N-losses obtained by isotope dilution experiments investigating a variety of conceivable nutritional influences.

References

Dänicke S, Böttcher W, Simon O, Jeroch H, Thielebein J (2000b) Replacement of soybean oil with tallow in rye-based diets without xylanase increases protein synthesis in small intestine of broilers. J Nutr 130:827-834

Dänicke S, Simon O, Jeroch H (2000a) Estimation of endogenous N-losses in broilers by a [^{15}N] isotope dilution technique : effect of dietary fat type and xylanase addition. Arch Anim Nutr 53:75-97

Dänicke S, Jeroch H, Kracht W (1998) Untersuchungen zum Einfluß eines thermischen Behandlungsverfahrens von Rapssaat auf die Proteinqualität für Broiler. Arch Geflügelkde 62:163-168

Krawielitzki K, Bock H-D (1976) Zur Problematik des Stickstoff-Stoffwechsels monogastrischer Tierarten. Arch Anim Nutr 26:83-98

Krawielitzki K, Smulikowska S (1977) Versuche zur Bestimmung des endogenen und exogenen fäkalen N-Anteiles monogastrischer Tierarten. Arch Tierernährung 27:39-47

Naumann C, Bassler R (1993) Die chemische Untersuchung von Futtermitteln. VDLUFA, Darmstadt, Germany

Pahle T, Köhler R, Halle I, Jeroch H, Gebhardt G (1983) Methodische Untersuchungen zur Bestimmung der Verdaulichkeit des Rohproteins beim Hühnergeflügel. Arch Anim Nutr 33:363-370

Rasch D, Herrendörfer G, Bock J, Busch K (1978) Verfahrensbibliothek Versuchsplanung und -Auswertung. Deutscher Landwirtschaftsverlag, Berlin (1978, 1978, 1981)

Reeds PJ, Burrin DG, Stoll B, van Goudoever JB (1999) Consequences and regulation of gut metabolism. In: Lobley GE, White A, MacRae JC (eds) Proc of the VIIIth International Symposium on Protein Metabolism and Nutrition. Aberdeen, UK, 1-4 September 1999. pp 127-153

Schiemann R (1981) Methodische Richtlinien zur Durchführung von Verdauungsversuchen für die Futterwertschätzung. Arch Anim Nutr 31:13-18

Sève B, Hess V (2000) Amino acid digestibility in formulation of diets for pigs : present interest and limitations, future prospects. In: Gransworthy PC, Wiseman J (eds) Recent advances in animal nutrition 2000. Nottingham University Press, UK

StatSoft Inc (1994) Statistica for the Windows™ Operating System. Tulsa OK, USA