3. Own Work

3.1. Objectives of own studies

The general objective of these studies was to standardise AA digestibility measurement in laying hens. Precaecal and total tract measurements were compared. Then in addition the effect of protein ingredients inclusion rates, marker recovery and age on AA digestibility were investigated. Specific questions were:

1. Is the digestion of crude protein and AA completed at Meckel's diverticulum in laying hens?
2. How do protein ingredients compare to each other in AA digestibility based on regression method and what are the minimum inclusion levels for protein ingredients?
3. Is it possible to use total tract excreta instead of precaecal digesta to estimate partial digestibility of feedstuffs by using caecectomised hens in balance trials?
4. What is the effect of age on AA excretion in laying hens?

There are different terminologies and assay methods for estimation of AA digestibility. In this thesis for the sake of clarity, the term ''digestibility'' for a protein ingredient is used only when the potential or capacity of a protein source is meant. Then in PC and TT measurements they were called partial (ingredient) PC digestibility (PPD) and partial TT digestibility (PTD). This capacity will be measured by slope of linear regression between intake and digested or unexcreted amount of AAs in corresponding diets. The more general term ''net disappearance (ND)'' describes the proportion of intake that is not recovered in any part of intestine.

3.2. Experiment 1: Effect of ileum segment and protein source on net disappearance of crude protein and amino acids from the ileum of laying hens

3.2.1. Introduction

Modern rapeseed *(Brassica napus ssp. oleifera or Brassica rapa ssp. oleifera)* varieties containing moderate or low amounts of goitrogenic glucosinolates have been found to be suitable protein sources, partially replacing soybean products in diets for broiler chickens in numerous experiments. In most of the published papers, 150 to 200 g/kg feed mixture has been found to be the maximum content of rapeseed meal not leading to problems associated with metabolic disorders due to goitrogenic glucosinolates. The modern moist pressure processing further reduces some anti-nutritional factors of rapeseed as well as soybean, but high processing temperatures may negatively influence protein digestibility (Palander *et al.,* 2004b).

However, new data in rapeseed AA digestibility for laying hens are scarce. On the other hand, one of the problems associated with rapeseed products has been a high fibre content leading to low digestibility and energy values. One could assume that this problem might be relieved if the ability of laying hens to digest fibre improves with age. Rapeseed meal, especially high glucosinolates varieties, showed a lower digestibility for lysine, cystine and threonine. For the other oilseed meals, lysine is the most variable AA in terms of digestibility (Dalibard and Paillard, 1995).

Using PC digestibility as a measure of protein quality implies a description of feedstuff potential. In this method, digestibility is measured at the end of the ileum and before the ICCJ. Meckel's diverticulum in the intestine is commonly used as the starting point of the gut section to be isolated, but the sub-section considered for digesta sampling was different in past studies, and studies were not done with laying hens. While, for instance, Kadim and Moughan (1997a) used the 15 cm anterior to the ICCJ, others used the entire section beginning at MD (Short *et al.,* 1999; Wiseman *et al.*, 2003) or the last two sub-sections between MD and ICCJ in broilers (Kluth

et al., 2005b). In some studies, the last few centimetres prior to the ICCJ were not sampled in order to avoid contamination from content of the postileal part of the gut (Rodehutscord *et al.*, 2004). These differences in the sampled sub-section may be irrelevant if the net disappearance of AA does not further change posterior to MD. For feed protein evaluation, it is of crucial importance, however, that measurements from different studies are comparable and methods are standardised (Rodehutscord and Mosenthin, 2005; Kluth *et al.,* 2005b).

The role of EAAs also must be considered. A method where basal losses are automatically corrected in the digestibility determination is the 'regression method', which involves feeding diets containing increasing levels of the test protein. In the regression of AA disappearance (mg/day) in relation to AA intake (mg/day), the slope of the regression line corresponds to a digestibility corrected for basal EAA (Rodehutscord *et al.,* 2004). Furthermore, regression method is the least stressful one for poultry in comparison with others methods that imply feeding the birds with unphysiological diets like N-free ones (Ishibashi and Yonemochi, 2003).

By our knowledge, there has not yet been conducted an experiment to evaluate AA PC digestibility and sampling places in laying hens to allow specification of a standard procedure. The aim of the present study was to investigate whether, in laying hens, the ND of CP and AAs is different in sub-sections of the ileum and whether such differences may be relevant for AA digestibility studies. Furthermore, in regards to CP and AA ND, solvent extracted meals from either soybean (SM) or rapeseed meal (RM) were compared in laying hens.

3.2.2. Materials and methods

Dietary treatments

Five diets were used in this experiment. One basal diet (BD) was based mainly on maize, wheat gluten and maize starch. A crude protein level of about 15 % was chosen for the basal diet. This was intended as a compromise to avoid a severely reduced intake of the BD as well as to

provide a wide range for supplementing the test protein. In the four other diets RM or SM was included, each at 14 and 28 % (Table 3-1). Such it was achieved a range in CP concentration in DM from 15.6 % in the BD to 24.4 % in RM containing diets and 26.7 % in SM containing diets. Highly digestible wheat gluten was the dominant protein source in the BD. RM and SM replaced maize starch in equal proportions so that the change in the AA concentrations of the experimental diets resulted from RM and SM only. Titanium dioxide (TiO_2) was included as an indigestible dietary marker.

Table 3-1. *Composition (g/kg) of experimental diets (BD = basal diet, RM = rapeseed meal, SM = soybean meal)*

		Diets with inclusion of			
	BD	14 % RM	28 % RM	14 % SM	28 % SM
Maize	425	425	425	425	425
Wheat gluten	113	113	113	113	113
Maize starch	282	141	0	141	0
Solvent extracted soybean meal	0	0	0	141	282
Solvent extracted rapeseed meal	0	141	282	0	0
Soybean oil	42	42	42	42	42
TiO_2	5	5	5	5	5
Di-calcium phosphate	38	38	38	38	38
Salt	3	3	3	3	3
Limestone	73	73	73	73	73
Premix (vitamins and minerals)*	9	9	9	9	9
L-Lysine.HCl	6	6	6	6	6
DL-Methionine	3	3	3	3	3
L-Threonine	1	1	1	1	1

***** *Supplied by Hohburg Mineralfutter GmbH, Hohburg, Germany. Contained* (*per kg): 233 g Ca; 410 mg retinol acetate; 0.8 mg cholecalciferol; 4.200 mg alpha tocopherol acetate; 200 mg vit. K_3; 200 mg vit. B_1; 664 mg vit. B_2; 500 mg vit. B_6, 2 mg vit. B_{12}; 100 mg folic acid; 15 mg biotin; 1.500 mg Ca-Di-panthothenate; 70 g choline chloride, 12 g antioxidant; 500 mg Cu; 5.135 mg Zn; 6.000 mg Fe; 7.100 mg Mg; 62 mg I; 20 mg Se.*

All the dietary ingredients, with the exception of the RM, SM and maize starch, were mixed in one lot. This mix was subsequently divided in 5 equal parts and each part was mixed with the respective amounts of RM, SM and maize starch. Similar diets have been already used for broiler, turkey and duck AA digestibility trials (Kluth and Rodehutscord, 2006).
The difference in this trial was that limestone was additionally included to achieve the required calcium level in the diet for laying hens. Diets were pelleted without steam through a 3 mm die. Results of the proximate nutrients and AA analyses for the diets are summarised in Table 3-2.

Table 3-2. *Analysed concentrations of proximate nutrients and amino acids (g/kg in DM) for the experimental diets (BD = basal diet, RM = Rapeseed meal, SM = Soybean meal)*

	Pure		BD	Diets with inclusion of			
	RM	SM		RM		SM	
				14 %	28 %	14 %	28 %
Dry matter (g/kg)	912	897	909	910	911	913	910
Crude Protein	372	448	156	197	244	219	267
Crude fat	47	30	73	78	83	71	74
Crude fibre	170	108	18	31	60	34	49
Alanine	18.2	18.4	6.2	8.4	10.1	9.0	11.1
Arginine	21.3	30.3	5.7	8.6	11.4	10.1	13.7
Aspartic acid	28.4	50.4	6.94	10.5	13.9	14.1	20.2
Cystine	8.6	7.0	3.8	4.2	5.4	4.0	4.9
Glutamic acid	63.9	82.5	48.2	54.2	63.1	60.9	68.5
Glycine	17.7	18.5	5.1	7.3	9.6	8.0	10.2
Isoleucine	14.2	20.3	5.5	6.9	8.9	8.7	10.4
Leucine	25.5	33.2	13.5	16.6	20.1	18.6	22.2
Lysine	19.7	27.4	7.8	10.7	13.1	11.4	14.8
Methionine	7.2	6.4	5.2	5.8	6.9	6.0	6.4
Phenylalanine	17.0	22.4	7.7	9.6	11.1	10.8	12.9
Serine	15.8	21.7	7.1	9.4	11.5	10.5	13.3
Threonine	16.1	17.2	5.2	7.4	9.5	7.8	9.5
Tryptophan	5.6	7.5	1.3	1.9	2.5	2.1	2.7
Valine	18.2	21.0	6.7	8.7	10.9	9.5	11.3
Calcium	6.9	3.1	45.0	45.3	47.3	45.7	45.0
Total Phosphorus	11.9	7.0	8.6	10.0	12.0	10.0	10.0
TiO_2	n. d.	n. d.	4.9	4.8	4.9	4.7	4.9

n. d. = Not detected

Animals, housing and feeding

The experiment was conducted at the Research Centre for Animal Sciences, Merbiz, of the Martin-Luther-Universität Halle-Wittenberg and was approved by authorities in accordance with the animal welfare legislation. Two hundred and fifty five, 20 week old pullets (Lohmann Brown) were obtained from Gefügelzuchtbetriebe Gudendorf (Ankum, Germany) and were housed in individual crates in a temperature and illumination controlled room. Each six neighbouring crates were an experimental unit, forming a group set. In this Experiment 7 rows of crates were considered as 7 blocks in the room. Each row was allocated the 5 random experimental diets for each unit. All experimental performance data were recorded for individual hens but analysed on a unit basis. There were 7 replicate units per diet. Feed was supplied from individual feeders and drinking water from nipple drinkers *ad libitum* throughout. Until week 26 (90-95% total egg production) all birds received the same commercial layer diet.

In week 25, individual egg production (EP) was recorded. Then the birds were weighed and 210 birds were selected based on individual best EP and minimum variation in BW of each experimental unit. In week 26 the individual feeders were installed and individual hen performance (EP and FI) was recorded still using the commercial diet. In week 27 the experimental diets were offered *ad libitum* for 7 days.

Sampling

In the last day of feeding the experimental diets, the birds were killed by asphyxiation with carbon dioxide and weighed again. After slaughtering the birds, body cavity was immediately opened and the section between MD and 2 cm anterior to the ICCJ was isolated. This part was cut into three equal sub-sections, proximal, central and terminal. The contents of each sub-section were gently flushed out with distilled water, pooled between the six birds of one unit, immediately frozen and subsequently freeze-dried to await analyses.

Analyses and calculations

Dietary concentrations of proximate nutrients were analysed according to the VDLUFA official methods. CP was calculated as N × 6.25. Amino acid analysis followed standard procedures (Naumann and Bassler, 1976) and was described in details by Rodehutscord *et al.* (2004). In brief, 250 mg of sample was weighed (equivalent to 10 mg N) and oxidised in an ice bath for 24 hours after addition of 5 mL freshly prepared performic acid reagent [mix of 0.5 mL hydrogen peroxide, 4.5 mL 88 % phenol formic acid solution (889 g formic acid, 111 g water, 4.73 g phenol) and 25 mg phenol]. Performic acid was decomposed thereafter with sodium metabisulphite (~ 0.9 g). Samples were then hydrolysed for 24 h at 110 ºC after the addition of 50 mL hydrochloric acid solution (6 M, containing 1 g phenol / l). After cooling to room temperature, citrate buffer (0.2 M, pH 2.20) was added and pH of samples was adjusted with hydrochloric acid and sodium hydroxide solution to 2.20. After mixing, samples were filtered through sintered glass membrane filters (0.20 µm). The pH was controlled again and adjusted with hydrochloric acid and sodium hydroxide solution to pH 2.20 if necessary. Norleucine was used as the external standard. After this sample treatment, the determination of histidine, tryptophan and tyrosine is not possible. AAs were separated and detected using an AA Analyser (Eppendorf LC3000), using different buffer solutions, and ninhydrin. Extinction was determined at 570 nm, with the exception of proline, which was measured at 440 nm.

Tryptophan analysis followed standard procedures and was described by Fatufe *et al.* (2005). Two hundred and fifty mg of sample was weighed (equivalent to 10 mg N) in a bottle. Then 8.4 g of barium hydroxide and 12 mL distilled water were added over an electric shaker to the samples. The bottles were placed in an autoclave at 110º C for 2 hours (the caps must be over the bottles in open position). After autoclaving (opening the autoclave door at below 90º C), approximately 30 mL distilled water and 2 mL internal tryptophan standard were added to the samples. The samples were then cooled in ice water over an electric shaker. Five mL phosphoric acid (0.5 M) and 7.5 mL hydrochloric acid (6 M) were added for hydrolysis.

The pH of the sample was adjusted to 3.0 by using hydrochloric acid (1 M). The bottles were filled to 100 mL by using distilled water. The solutions were then filtered through filter paper. 0.5 mL of solution and 2 mL methanol of 30 % concentration were filtered through sintered glass membrane filters (0.22 µm). Separation and detection of tryptophan was conducted with HPLC (High Performance Liquid Chromatography) apparatus, using different solutions. Standard solutions of AAs were obtained from Sigma Aldrich Chemie (Taufkichen, Germany).
The concentrations of TiO_2 in diets and digesta were determined spectophotometrically according to the method described by Brandt and Allam (1987).
The net disappearance (ND) of the AAs and CP for each diet was calculated, on a unit basis, according to the following equation:

$$ND_{AA\ Diet} = 1 - [(TiO_{2\ Diet} \times AA_{\ Digesta}) / (TiO_{2\ Digesta} \times AA_{\ Diet})]$$

With

$TiO_{2\ Diet}$ and $TiO_{2\ Digesta}$: concentrations of TiO_2 in the diet and digesta samples (g/kg)

$AA_{\ Diet}$ and $AA_{\ Digesta}$: concentrations of the AAs (or CP) in the diet and digesta samples (g/kg)

The quantitative daily intakes of each AA and CP were calculated as FI (g/day) multiplied by the analysed AA (or CP) concentration in the diet. The quantity of AA (or CP) that disappeared up to the terminal ileum was calculated as AA (or CP) intake (g/d) multiplied by ND. The partial ND of each AA from the supplemented RM and SM was obtained by calculating the multiple linear regressions between the quantitative AA intake and the amount of AA that disappeared in each sub-section as described by Kluth *et al.* (2005a).
The following model was applied to simultaneously determine the partial ND of AAs originating from the two solvent extracted meals in each sub-section (modification of method described by Kluth *et al.* 2005a):

$Y = \alpha + \beta_b \times X_b + \beta_i \times X\ (s_i)$

With
Y: daily amount of disappeared AA (or CP) in each sub-section
α: intercept
β_b: partial PC ND of AA (or CP) originating from BD
X_b: daily intake of AA (or CP) originating from BD
β_i: partial PC ND of AA (or CP) originating from protein ingredient (SM or RM)
$X\ (s_i)$: daily intake of AA (or CP) originating from protein ingredient (SM or RM) in each sub-section.

The model was fitted for each of the three sub-sections of the gut. The resulting data were analysed using the statistical software package SAS (V 9.1, SAS Institute Inc.). Differences in ND of CP and AA between RM and SM containing diets and partial ND in each sub-section of each AA and CP from the supplementary RM and SM were tested for significance using the GLM and MIX procedures and ESTIMATE statement.

3.2.3. Results

During the 7 days of treatment, the FI of the hens decreased from a pre-experimental average of 123 g/d to 63 g/d, 62 g/d, 86 g/d, 90 g/d and 86 g/d, the BW from a pre-experimental average of 2008 g to 1832 g, 1863 g, 1945 g, 1972 g and 1956 g, and the EP from a pre-experimental average of 88 % to 65 %, 64 %, 72 %, 76 % and 77 % for BD, 14 % RM, 28 % RM, 14 % SM and 28 % SM respectively. The difference between treatments in EP and ileum length (IL) was not significant but there was a significant difference ($P<0.05$) in BW between the BD and 14 % and 28 % SM and in egg weight (EW) between the BD and 28 % SM, respectively (Table 3-3; Appendix A-1).
Net disappearance of CP and AAs was calculated for all diets (Table 3-4; Appendix A-2). Diet ND of CP and all studied AAs was significantly lower ($P<0.05$) in the proximal sub-section than in the central or terminal sub-

sections. The average disappearance of AA from the proximal sub-section was 10 percentage units lower than in the other two sub-sections, without significant differences between the central and the terminal sub-section. No significant interactions between diets and ileum sub-sections were detected. The amounts of CP and AAs that disappeared in the ileum depended linearly on the intake of CP and AAs. Examples are shown in the Figure 3-1. Partial PC ND of AAs and CP in RM and SM were calculated and compared separately in the different sub-sections (Table 3-5). RM had significantly ($P<0.05$) lower partial ND for CP and all studied AAs in the proximal sub-section than in the central or terminal sub-sections but SM had significantly lower ($P<0.05$) partial ND only for arginine, aspartic acid, glutamic acid and phenylalanine in the proximal sub-section than in the central or terminal sub-sections. Partial ND of CP and all studied AAs were not significantly different between central and terminal sub-sections in both protein ingredients (Table 3-5).

SM had significantly higher ($P < 0.05$) CP and AAs (except cystine and methionine) partial ND than RM in the proximal sub-section, but these differences were not significant in the central and terminal sub-sections (Table 3-5). In the next stage partial ND of SM and RM was calculated only for the pooled data in the last two sub-sections (central and terminal) and was named partial PC digestibility (PPD; Table 3-6). The differences between RM and SM PC digestibility were sometimes as high as 0.13 (aspartic acid) but never reached a significant level. Partial PC digestibility ranged from 0.63 (threonine) to 0.80 (arginine and glutamic acid) in RM and from 0.58 (cystine) to 0.83 (arginine) in SM. The chosen multiple linear regression model explained 0.94 to 0.99 of the observed variance (Table 3-6).

Table 3-3. *Hen performance data (BD = basal diet, RM = rapeseed meal, SM = soybean meal, FI = feed intake, EP = egg production, BW = body weight)*

	BD		14 % RM		28 % RM		14 % SM		28 % SM	
	Mean	SE	Mean	SE	Mean	SE	Mean	SE	Mean	SE
Pre-experimental FI (g/d)	122	± *2.4*	121	± *1.6*	124	± *1.8*	124	± *1.6*	123	± *1.9*
FI during the experiment (g/d)	62.6 ^{b}	± *7.2*	62.5 ^{b}	± *9.4*	85.8 ^{ab}	± *10.3*	90.3 ^{a}	± *9.7*	85.9 ^{ab}	± *9.6*
Pre-experimental EP (%)	89.9 ^{ab}	± *1.5*	85.7 ^{b}	± *1.8*	91.4 ^{a}	± *1.5*	89.3 ^{ab}	± *1.7*	87.5 ^{ab}	± *1.8*
EP during the experiment (%)	64.6	± *4.2*	63.9	± *4.7*	72.1	± *5.0*	76.5	± *5.3*	77.2	± *5.6*
Pre-experimental BW (g)	2008	± *23.5*	2000	± *21.3*	2015	± *22.6*	2012	± *22.7*	2006	± *21.6*
BW during the experiment (g)	1832 ^{b}	± *39.0*	1863 ^{ab}	± *46.7*	1945 ^{ab}	± *47.1*	1972 ^{a}	± *48.4*	1956 ^{ab}	± *44.3*
Ileum length[1] (cm)	59.1	± *1.6*	59.0	± *1.8*	62.7	± *1.8*	63.7	± *1.7*	63.7	± *1.8*
Egg weight during the experiment (g)	57.1 ^{b}	± *0.4*	59.0 ^{ab}	± *0.5*	59.9 ^{ab}	± *1.0*	60.2 ^{ab}	± *0.7*	62.4 ^{a}	± *0.5*

$^{a, b}$ *Parameters in one row not sharing a common superscript are significantly different between diets ($P < 0.05$)*

[1]*Section between Meckel's diverticulum and ileo-caeca-colonic junction*

Table 3-4. *Net disappearance of crude protein and amino acids determined in the proximal (p), central (c), and terminal (t) sub-sections of sampled gut of laying hens for the basal diet (BD) and the other diets with different inclusion rates of soybean meal (SM) and rapeseed meal (RM)*

Diets	BD			14 % RM			28 % RM			14 % SM			28 % SM			Pooled	P (ANOVA)		
Sub-sections	p	C	T	p	c	t	P	c	t	p	c	t	p	c	t	SE	Diet	Section	Diet × Section
Crude protein	0.67	0.76	0.76	0.68	0.73	0.69	0.61	0.70	0.74	0.70	0.75	0.77	0.70	0.76	0.74	0.01	0.19	<0.01	0.39
Alanine	0.56	0.69	0.71	0.60	0.67	0.62	0.51	0.65	0.71	0.59	0.67	0.70	0.61	0.68	0.69	0.01	0.79	<0.01	0.23
Arginine	0.59	0.74	0.74	0.67	0.75	0.73	0.60	0.73	0.78	0.69	0.75	0.76	0.69	0.78	0.79	0.01	0.47	<0.01	0.45
Aspartic acid	0.35	0.56	0.56	0.50	0.58	0.51	0.39	0.56	0.62	0.56	0.64	0.66	0.59	0.69	0.70	0.01	0.00	<0.01	0.15
Cystine	0.67	0.77	0.75	0.65	0.69	0.68	0.57	0.69	0.71	0.62	0.69	0.72	0.61	0.68	0.68	0.01	0.09	<0.01	0.63
Glutamic acid	0.85	0.90	0.90	0.84	0.87	0.85	0.79	0.86	0.88	0.83	0.87	0.88	0.82	0.86	0.87	0.00	0.04	<0.01	0.42
Glycine	0.55	0.69	0.69	0.60	0.68	0.64	0.50	0.65	0.70	0.61	0.68	0.70	0.61	0.68	0.69	0.01	0.61	<0.01	0.24
Isoleucine	0.58	0.72	0.74	0.62	0.70	0.66	0.54	0.68	0.73	0.67	0.73	0.75	0.65	0.71	0.73	0.01	0.21	<0.01	0.15
Leucine	0.68	0.80	0.81	0.68	0.75	0.72	0.61	0.73	0.78	0.68	0.75	0.78	0.68	0.75	0.77	0.01	0.37	<0.01	0.33
Lysine	0.72	0.79	0.79	0.72	0.76	0.72	0.60	0.72	0.75	0.71	0.76	0.75	0.71	0.77	0.77	0.01	0.10	<0.01	0.18
Methionine	0.82	0.89	0.89	0.82	0.85	0.82	0.74	0.82	0.86	0.81	0.85	0.84	0.77	0.82	0.83	0.01	0.04	<0.01	0.16
Phenylalanine	0.70	0.82	0.81	0.71	0.77	0.77	0.62	0.75	0.79	0.72	0.78	0.80	0.70	0.77	0.79	0.01	0.19	<0.01	0.33
Serine	0.59	0.73	0.73	0.65	0.72	0.68	0.54	0.67	0.72	0.66	0.72	0.74	0.66	0.73	0.74	0.01	0.28	<0.01	0.20
Threonine	0.46	0.62	0.63	0.57	0.63	0.57	0.45	0.59	0.64	0.58	0.63	0.64	0.57	0.65	0.65	0.01	0.43	<0.01	0.24
Tryptophan	0.47	0.64	0.70	0.56	0.69	0.65	0.51	0.69	0.72	0.58	0.65	0.70	0.61	0.70	0.71	0.01	0.49	<0.01	0.43
Valine	0.63	0.73	0.75	0.62	0.70	0.67	0.54	0.66	0.72	0.64	0.71	0.74	0.62	0.68	0.71	0.01	0.30	<0.01	0.42

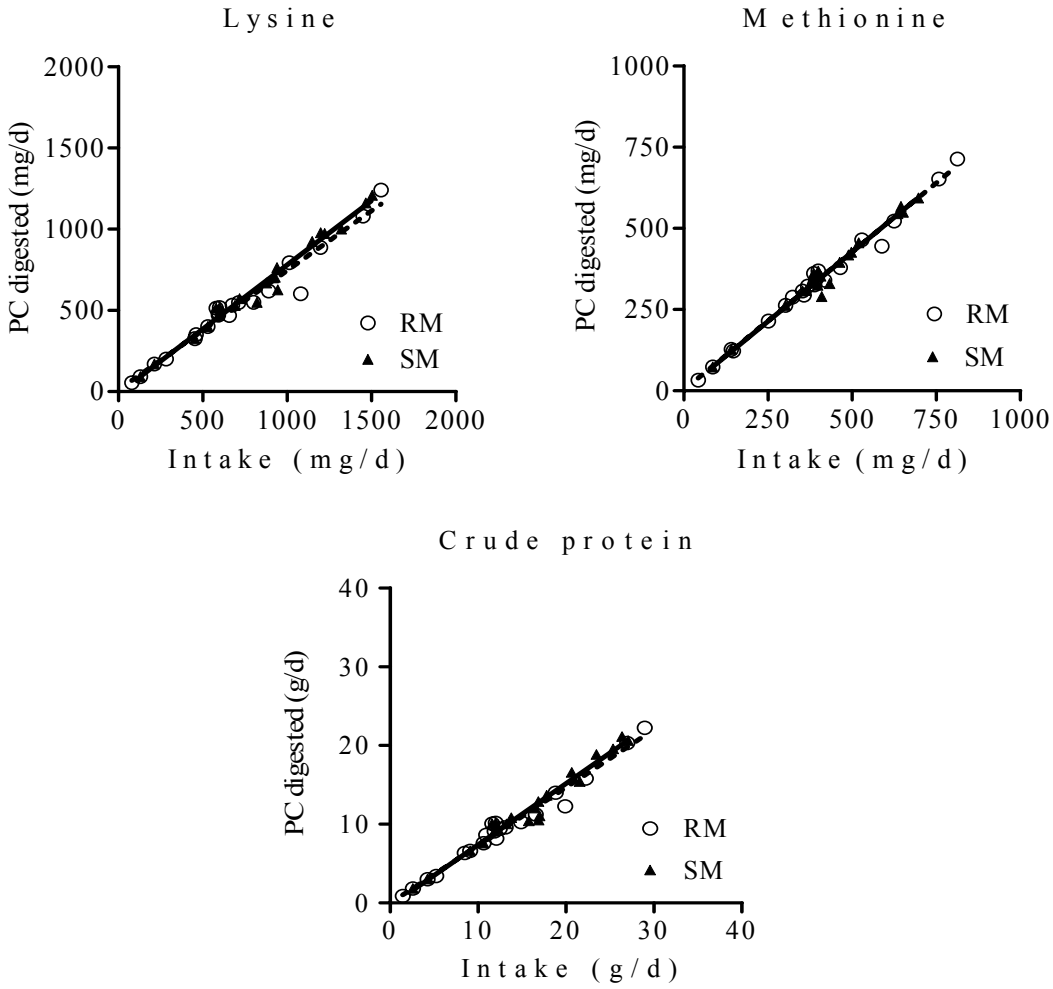

Figure 3-1. *Relationship between intake and digested (mean of central and terminal sub-sections) amount of Lysine, methionine and crude protein up to the terminal ileum in laying hens fed different dietary concentration of rapeseed meal (RM) and soybean meal (SM)*

Table 3-5. *Partial precaecal net disappearance of amino acids and crude protein for soybean meal (SM) and rapeseed meal (RM) in three sub-sections determined by multiple linear regression analysis (estimate and SE of estimate for the regression coefficient)*

	Proximal sub-section		Central sub-section		Terminal sub-section	
	SM	RM	SM	RM	SM	RM
Crude protein	$0.62^{A} \pm 0.07$	$0.34^{Bb} \pm 0.09$	0.71 ± 0.08	$0.62^{a} \pm 0.09$	0.72 ± 0.07	$0.64^{a} \pm 0.09$
Alanine	$0.57^{A} \pm 0.10$	$0.36^{Bb} \pm 0.12$	0.66 ± 0.10	$0.64^{a} \pm 0.12$	0.74 ± 0.10	$0.71^{a} \pm 0.12$
Arginine	$0.69^{Ab} \pm 0.06$	$0.53^{Bb} \pm 0.08$	$0.80^{a} \pm 0.04$	$0.76^{a} \pm 0.05$	$0.84^{a} \pm 0.04$	$0.82^{a} \pm 0.06$
Aspartic acid	$0.64^{Ab} \pm 0.06$	$0.31^{Bb} \pm 0.10$	$0.74^{a} \pm 0.06$	$0.62^{a} \pm 0.10$	$0.80^{a} \pm 0.06$	$0.67^{a} \pm 0.10$
Cystine	0.31 ± 0.15	$0.30^{b} \pm 0.10$	0.48 ± 0.15	$0.62^{a} \pm 0.10$	0.58 ± 0.15	$0.68^{a} \pm 0.10$
Glutamic acid	$0.67^{Ab} \pm .06$	$0.48^{Bb} \pm 0.08$	$0.76^{a} \pm 0.06$	$0.73^{a} \pm 0.08$	$0.84^{a} \pm 0.06$	$0.82^{a} \pm 0.08$
Glycine	$0.56^{A} \pm 0.07$	$0.35^{Bb} \pm 0.08$	0.66 ± 0.08	$0.64^{a} \pm 0.08$	0.75 ± 0.07	$0.71^{a} \pm 0.08$
Isoleucine	$0.62^{A} \pm 0.07$	$0.30^{Bb} \pm 0.10$	0.68 ± 0.07	$0.62^{a} \pm 0.10$	0.77 ± 0.07	$0.69^{a} \pm 0.10$
Leucine	$0.58^{A} \pm 0.08$	$0.37^{Bb} \pm 0.10$	0.67 ± 0.08	$0.64^{a} \pm 0.10$	0.77 ± 0.08	$0.75^{a} \pm 0.10$
Lysine	$0.64^{A} \pm 0.06$	$0.39^{Bb} \pm 0.08$	0.74 ± 0.07	$0.68^{a} \pm 0.08$	0.80 ± 0.06	$0.72^{a} \pm 0.08$
Methionine	0.40 ± 0.12	$0.35^{b} \pm 0.09$	0.56 ± 0.13	$0.66^{a} \pm 0.09$	0.70 ± 0.12	$0.80^{a} \pm 0.09$
Phenylalanine	$0.58^{Ab} \pm 0.07$	$0.31^{Bb} \pm 0.10$	$0.68^{a} \pm 0.07$	$0.61^{a} \pm 0.10$	$0.77^{a} \pm 0.07$	$0.70^{a} \pm 0.10$
Serine	$0.63^{A} \pm 0.07$	$0.36^{Bb} \pm 0.10$	0.71 ± 0.07	$0.62^{a} \pm 0.10$	0.78 ± 0.07	$0.68^{a} \pm 0.10$
Threonine	$0.54^{A} \pm 0.10$	$0.32^{Bb} \pm 0.10$	0.63 ± 0.10	$0.59^{a} \pm 0.10$	0.72 ± 0.10	$0.64^{a} \pm 0.10$
Tryptophan	$0.62^{A} \pm 0.07$	$0.35^{Bb} \pm 0.08$	0.71 ± 0.07	$0.70^{a} \pm 0.08$	0.72 ± 0.07	$0.68^{a} \pm 0.08$
Valine	$0.50^{A} \pm 0.10$	$0.28^{Bb} \pm 0.10$	0.61 ± 0.10	$0.57^{a} \pm 0.10$	0.73 ± 0.09	$0.69^{a} \pm 0.10$

$^{A, B; a, b}$ *Amino acids not sharing a common superscript are significantly different between the two sources within sub-sections (upper case) and between sub-sections within protein source (lower case)($P < 0.05$)*

Table 3-6. *Partial precaecal digestibilities of amino acids and crude protein (pooled data from central and terminal sub-sections) for soybean meal (SM) and rapeseed meal (RM) determined by multiple linear regression analysis (estimate and SE of estimate for the regression coefficient)*

	R^2	SM		RM		Difference		P value
		Estimate	SE	Estimate	SE	Estimate	SE	
Crude protein	0.97	0.70	*0.06*	0.63	*0.08*	0.07	*0.06*	0.18
Alanine	0.94	0.73	*0.10*	0.69	*0.11*	0.04	*0.08*	0.57
Arginine	0.99	0.83	*0.03*	0.80	*0.04*	0.03	*0.03*	0.30
Aspartic acid	0.95	0.80	*0.05*	0.67	*0.09*	0.13	*0.07*	0.07
Cystine	0.96	0.58	*0.12*	0.66	*0.08*	- 0.08	*0.11*	0.44
Glutamic acid	0.99	0.83	*0.05*	0.80	*0.07*	0.03	*0.05*	0.53
Glycine	0.96	0.74	*0.06*	0.69	*0.07*	0.05	*0.05*	0.34
Isoleucine	0.97	0.76	*0.06*	0.67	*0.09*	0.09	*0.07*	0.20
Leucine	0.97	0.76	*0.07*	0.72	*0.09*	0.04	*0.07*	0.61
Lysine	0.97	0.80	*0.06*	0.71	*0.07*	0.09	*0.06*	0.10
Methionine	0.98	0.70	*0.11*	0.76	*0.09*	- 0.06	*0.08*	0.47
Phenylalanine	0.98	0.75	*0.06*	0.67	*0.09*	0.08	*0.06*	0.21
Serine	0.97	0.78	*0.06*	0.67	*0.08*	0.11	*0.06*	0.07
Threonine	0.94	0.72	*0.08*	0.63	*0.08*	0.09	*0.07*	0.19
Tryptophan	0.97	0.72	*0.05*	0.69	*0.06*	0.03	*0.05*	0.55
Valine	0.95	0.71	*0.09*	0.65	*0.09*	0.06	*0.07*	0.55

3.2.4. Discussion

During this experiment, average BW and EW decreased significantly ($P < 0.05$) in comparison with the pre-treatment period. This may have been because of FI decreasing based on pellet diet usage, experimental feed

ingredients or some other stress during the experimental period in comparison with pre-treatment mash diet.

In this experiment ND of CP and all studied AAs in all diets were significantly lower ($P < 0.05$) in the proximal sub-section than in the central and terminal sub-sections. Central and terminal sub-sections were not significantly different in ND. The results of the present study therefore suggest that AAs disappear from the ileum of hens still posterior to the MD. This should be accounted for in protocols for AA digestibility studies by limiting the sampled ileum to the last two thirds. A similar result also was found for broilers by Kluth *et al.* (2005b) which only the terminal and medial sub-sections between MD and ICCJ should be sampled, which correspond to a length of 25 – 41 cm in broilers of that body size.

Kadim and Moughan (1997a) stated that there was no significant effect on the apparent ileal digestibility of dietary N with varying sampling places. They considered the terminal 15 cm of ileum a preferred site for sampling ileal digesta from broiler chickens. They studied the ND of N with diets that contained CP from soybean meal, blood meal, or wheat bran. They used sections of different lengths anterior to the ICCJ from 28-day old broilers (10, 15, 20, and 25 cm) and found differences in N disappearance between diets, but not between sections of different lengths. These authors discuss the differences that exist in ileum length between individual animals depending on age or body size, and suggest making the sampled sub-section as short as possible. As a compromise regarding the need for a sufficient sample size, they suggest using the last 15 cm anterior ICCJ for digestibility studies. According to the present study only the central and terminal sub-sections between MD and ICCJ should be sampled, which corresponded to a length of 20 – 58 cm (Table 3-3, Appendix A-1) in laying hens of this body size. This length will be more practical because it provides more digesta for analyses and needs fewer animals in each replicate unit.

Endogenous AA is contained in the digesta and they contribute to different extents to the calculated digestibility. Different techniques have been shown to lead to great differences in the estimate of endogenous losses (Donkoh and Moughan, 1999; Jansman *et al.*, 2002; Lemme *et al.*, 2004;

Rodehutscord *et al.,* 2004; Rodehutscord and Mosenthin, 2005) and all techniques are subject to certain limitation and criticisms (Sauer *et al.,* 2000). Thus, approaches like the regression method that do not depend on a separate determination of endogenous losses appear advantageous for the purpose of feed evaluation.

By using multiple regression analysis, the PPD of the AAs from SM or RM is separated from the digestibility of the entire diet, where the RM or SM contributed only part of the total protein. In this condition the basal endogenous loss is contained in the intercept and hence does not need to be further accounted for. Separation into unabsorbed AAs and specific EAAs secretion is not possible by regression analysis (Rodehutscord *et al.,* 2004). The high R^2 (Table 3-6) in the chosen model indicates the high relationship between AAs (or CP) intake and disappeared amounts, which is consistent with previous reports. Net disappearance determined by regression analysis following the above restrictions is a suitable measure for AA digestibility because it does not need any correction for basal EAA losses (Fan and Sauer, 1997; Short *et al.,* 1999; Rodehutscord *et al.*, 2004; Kluth and Rodehutscord, 2006; Kluth *et al.,* 2005a).

Soybean meal had significantly higher AAs (except cystine and methionine) and CP partial ND than RM in the proximal sub-section, but these differences were not significant in the central and terminal sub-sections (Table 3-5). This may be the consequence of interaction between GIT enzymes activity and concentrations of fibre or ANFs in RM. It seems that protein content in RM needs more time and enzymes than SM to hydrolyse into absorbable AAs and small peptides.

For RM the partial ND of all AAs was lower in the proximal sub-section than in central and terminal sub-sections and for SM it was so only for four AA (arginine, aspartic acid, glutamic acid and phenylalanine). The reasons for this difference in absorptive place may be described by difference in pH and absorptive surface conditions like the higher microvillus intense in central and terminal sub-sections rather than proximal sub-section, but it needs more investigations.

Variation exists in PPD of AA between RM and SM and within one protein source for hens. The ranking of individual AAs regarding their digestibility

is different between SM and RM (Appendix A-3). Digestibility values determined in one poultry species cannot be applied to another species (Huang *et al.*, 2000; Kluth and Rodehutscord, 2006). Partial PC digestibility of CP and all AAs for RM and SM in this experiment was compared with broilers results in Kluth and Rodehutscord (2006) experiment (Appendix A-6). These results showed lower PPD of CP and nearly all AAs in laying hens than in broilers for RM and SM. The exceptions were aspartic acid and glycine PPD for SM that was higher in laying hens than in broilers. Therefore these calculated SM and RM PC digestibilities will be very useful for practical feed formulation in laying hens.

3.2.5. Conclusion

Crude protein and AAs disappear from the ileum of hens still posterior to MD. This should be accounted for in protocols for AA digestibility studies by limiting the sampled ileum section to the last two thirds. ND determined by regression analysis following above restrictions is a suitable measure for AA digestibility because it does not need any correction for basal EAA losses. Variation exists in AA PC digestibility between RM and SM and within one protein source for hens. The ranking of individual AAs regarding their digestibility is different between SM and RM.

3.3. Experiment 2: Partial precaecal digestibility of amino acids for toasted soybeans and maize gluten

3.3.1. Introduction

For a number of years un-extracted or full-fat soybeans have been used in poultry diets. They provide an excellent source of energy and protein because of their high oil (180 to 220 g/kg) and protein contents (370 to 420 g/kg) with an acceptable AA profile (Perez-Maldonado *et al.* 2003). Unfortunately, raw soybean seeds contain various anti-nutritional factors (ANF) like antitrypsin and antichymotrypsin that have principally anti-proteases activity in poultry. Processing is necessary to destroy ANF. There are a number of full-fat soybean products available, which differ in the way of processing. Full-fat soybeans digestibility can be influenced by the processing, as shown by the differences between toasted and extruded soybeans (Dalibard and Paillard, 1995).

The use of heat processing for toasting the soybean seeds to reduce ANF activity thus allows higher inclusion of soybeans in the diets but at the same time over-heating may negatively affect AA digestibility of protein ingredients. In this experiment for controlling the quality of toasted soybeans (TS), partial PC AA digestibility of it will be compared with maize gluten (MG) as a presumably highly digestible feed ingredient in laying hens.

As concluded from the first experiment and also by Kluth *et al.* (2005b) in broilers, ileal digesta from the last two thirds of the intestine between MD and 2 cm anterior to the ICCJ are to be sampled after killing the birds for protein ingredients PC AA digestibility studies. In this method the digesta of the birds in each replication are pooled in order to obtain a more reliable sample closer to the physiological condition of the feed digestion during the transit time in the GIT. Also it is necessary to use markers in the feed in order to be able to calculate digestibility.

3.3.2. Materials and methods

Dietary treatment

Five diets were prepared, a basal diet (BD) mainly based on maize, wheat gluten and maize starch that met the requirements recommended by NRC (1994) and four diets including increased levels of TS or MG each at 15 % and 30 % (Table 3-7), such that a range in CP concentration in DM from 18.0 % in the BD to 28.6 % in TS and 35.7 % in MG containing diets was achieved. Highly digestible wheat gluten was the dominant protein source in the BD. TS and MG replaced maize starch in equal proportions so that the changes in the AA concentrations of experimental diets resulted from TS and MG alone. Titanium dioxide (TiO_2) was included as an indigestible dietary marker. All the dietary ingredients, with the exception of TS, MG and maize starch, were mixed in one lot. This mix was subsequently divided in 5 equal parts and each part was mixed with the respective amounts of TS, MG and maize starch. Diets were pelleted without steam through a 3 mm die, but were crumbled in order to increase FI of birds. Results of the proximate nutrients and AA analyses for diets are summarised in Table 3-8.

Table 3-7. *Composition (g/kg) of the experimental diets (TS = toasted soybeans, MG = maize gluten)*

	Basal diet	Diets with inclusion of			
		15 % TS	30 % TS	15 % MG	30 % MG
Maize	418	418	418	418	418
Wheat gluten	155	155	155	155	155
Maize starch	300	150	0	150	0
Toasted soybeans	0	150	300	0	0
Maize gluten	0	0	0	150	300
TiO_2	5	5	5	5	5
Di-calcium phosphate	16	16	16	16	16
Salt	3	3	3	3	3
Limestone	89.5	89.5	89.5	89.5	89.5
Premix (vitamins and minerals)	10	10	10	10	10
L-Lysine.HCl	3.3	3.3	3.3	3.3	3.3
DL-Methionine	0.2	0.2	0.2	0.2	0.2
AME_N (MJ/kg) (calculated)	13.8	13.6	13.4	13.5	13.2
Crude Protein (calculated)	185	241	297	289	394

Table 3-8. *Analysed concentrations of proximate nutrients and amino acids (g/kg in DM) in the experimental diets and in pure toasted soybeans (TS) and maize gluten (MG)*

	Pure		Basal	Diets with inclusion of			
	TS	MG	diet	15 % TS	30 % TS	15 % MG	30 % MG
Dry matter (g/kg)	915	918	927	927	930	933	932
Crude Protein	395	571	180	258	286	270	357
Crude ash	62	192	130	137	151	134	136
Crude fat	212	49	9	45	77	34	41
Crude fibre	109	9	29	39	56	34	33
Alanine	17.0	51.2	6.0	8.7	12.0	14.9	21.9
Arginine	26.6	19.7	4.3	8.2	13.1	8.3	10.5
Aspartic acid	46.9	39.3	6.6	14.1	21.2	13.6	19.0
Cystine	6.7	11.0	4.4	5.7	6.6	6.0	7.1
Glutamic acid	75.4	138.2	58.0	73.3	88.0	87.9	103.2
Glycine	17.0	17.0	5.8	8.9	11.5	9.1	11.4
Isoleucine	17.5	24.4	6.0	8.4	11.8	10.5	13.1
Leucine	31.4	101.2	14.0	19.3	24.9	31.1	44.7
Lysine	24.9	12.1	5.4	9.6	13.1	8.0	9.1
Methionine	6.1	13.2	2.9	3.5	5.1	5.3	6.9
Phenylalanine	20.2	37.8	8.3	11.7	15.6	15.1	20.1
Proline	19.0	53.2	20.6	24.0	27.2	30.5	38.3
Serine	20.8	32.0	7.8	11.7	15.0	13.9	18.1
Threonine	14.9	20.8	4.3	7.0	9.4	8.2	10.8
Tryptophan	5.4	3.3	1.4	2.3	2.9	1.9	2.4
Valine	17.3	29.2	6.9	9.9	12.5	11.9	15.1
Calcium	2.5	2.8	43.7	44.8	45.5	44.1	46.6
Total Phosphorus	7.2	6.4	5.4	6.4	7.4	6.4	7.3
TiO_2	n. d.	n. d.	5.0	5.2	4.9	5.2	4.5

n. d. = Not detected

Animals, housing and feeding

The experiment was conducted at the Research Centre for Animal Sciences, Merbiz, of the Martin-Luther-Universität Halle-Wittenberg and was approved by authorities in accordance with the animal welfare legislation. Two hundred and ten, 18 weeks old pullets (Tetra Brown) were obtained from Robert's Bio-Geflügel GmbH & Co. KG (Schöneck,

Germany) and were housed in individual crates in a temperature and illumination controlled room. Each seven neighbouring crates were an experimental unit. All experimental performance data were recorded for individual hens but analysed on a unit basis. There were 6 replicate units per diet. Feed was supplied from individual feeders and drinking water from nipple drinkers *ad libitum* throughout.

Until week 26, when hens had achieved EP of 90 % to 95 % all birds received the same commercial layer diet. In week 26 the individual feeders were installed and individual hen performance (EP and FI) and also the BW in the last day were recorded. Then the birds were distributed within units to minimise variation in BW. Birds with very low EP and BW were excluded. In week 27 experimental diets were offered *ad libitum* for 7 days before the birds were weighed again and slaughtered by asphyxiation by carbon dioxide. One day before asphyxiation FI of hens was measured and the number of hens in each group was reduced to six based on best FI.

Sampling

In week 27 after slaughtering of birds by carbon dioxide, body cavity of each bird was immediately opened and the section between MD and 2 cm anterior to the ICCJ was isolated. This section was cut into three equal sub-sections (proximal, central and terminal). The digesta of the last two sub-sections (central and terminal) anterior to the ICCJ were gently flushed out with distilled water, pooled between the contents obtained from the other five birds from the same unit, immediately frozen and subsequently freeze-dried to await analyses.

Analyses and calculations

Dietary concentrations of proximate nutrients were analysed according to the VDLUFA official methods (Naumann and Bassler, 1976) as described in detail for Experiment 1. The concentrations of TiO_2 in the diets and digesta were determined spectrophotometrically according to the method described by Brandt and Allam (1987).

The PC digestibility coefficient (DC) of the AAs and N for each diet was calculated, on a unit basis, according to the following equation:

$$DC_{Diet} = 1 - [(TiO_{2\ Diet} \times AA_{Digesta}) / (TiO_{2\ Digesta} \times AA_{Diet})]$$

With
$TiO_{2\ Diet}$ and $TiO_{2\ Digesta}$: concentrations of TiO_2 in the diet and digesta samples (g/kg),
AA_{Diet} and $AA_{Digesta}$: concentrations of the AAs or N in the diet and digesta samples (g/kg).

The quantitative daily intakes of each AA and N were calculated as FI (g/day) multiplied by the analysed AAs or N concentration in the diet. The quantity of AAs or N digested precaecally was calculated as AA or N intake (g/d) multiplied by DC. The partial PC digestibility (PPD) of each AA or N from the supplemented TS and MG was obtained by calculating the multiple linear regressions between the quantitative AAs or N intake and the PC digested amount of AAs or N.
The following model was applied to simultaneously determine the PPD of AAs or N originating from the two protein ingredients of feed:

$$Y = \alpha + \beta_b \times X_b + \beta_i \times X_i$$

With
Y: daily amount of digested AA or N (g)
α: intercept
β_b: partial PC digestibility of AA or N originating from BD
X_b: daily intake of AA or N originating from BD (g)
β_i: partial PC digestibility of AA or N originating from protein ingredient (TS or MG)
X_i: daily intake of AA or N originating from protein ingredient (TS or MG) (g)

The resulting data were analysed using the GLM procedures of the statistical software package SAS (V 9.1, SAS Institute Inc.). Differences between N and AA DC of TS and MG containing diets and amino acids and N PC digestibility of supplemented TS and MG were tested for significance using GLM procedure and the ESTIMATE statement.

3.3.3. Results

During the 7 days of treatment, the daily FI of hens changed from a pre-experimental average of 107 g to 93 g, 103 g, 99 g, 88 g and 86 g, the BW of hens changed from a pre-experimental overall average of 1919 g to 1958 g, 2006 g, 2017 g, 1952 g and 1919 g and the EP from a pre-experimental average of 97 % to 96 %, 97 %, 99 %, 96 % and 96 % for the BD, 15 % TS, 30 % TS, 15 % MG and 30 % MG containing diets, respectively. The difference between treatments in EP was not significant but there was a significant difference ($P < 0.05$) in BW after feeding by experimental diets between 30 % MG with 15 % and 30 % TS containing diet and in FI between the BD and TS and MG containing diets, respectively. Hens fed the BD had significantly higher FI than those fed MG containing diets but significantly lower FI than those fed TS containing diets (Table 3-9; Appendix B-1).

Digestibility coefficient (DC) of AA and N was calculated for all diets (Table 3-10; Appendix B-2). Diet DC of all studied AAs and N, was higher in most cases for the diets with higher concentration of AAs and N than in the diets with lower concentration of AAs and N but this difference was only significant ($P < 0.05$) for alanine, arginine, aspartic acid, glycine, leucine, serine and threonine. The amounts of PC digested AAs and N was regressed linearly on the intake of AAs and N. Examples are shown in Figure 3-2. Partial PC digestibility (PPD) of AAs and N for MG and TS was calculated and compared (Table 3-11). The differences between TS and MG PPD of AAs and N were sometimes as high as 0.06 (lysine) but never reached the level of significance. Partial PC digestibility ranged from 0.84 (cystine) to 0.96 (arginine) in TS and from 0.82 (tryptophan) to 0.95

(proline) in MG. The chosen multiple linear regression model explained 0.94 to 0.99 of the observed variance (Table 3-11).

Table 3-9. *Hen performance data (BD = basal diet, TS = toasted soybeans, MG = maize gluten, FI = feed intake, EP = egg production, BW = body weight)*

	BD		15 % TS		30 % TS		15 % MG		30 % MG	
	Mean	SE	Mean	SE	Mean	SE	Mean	SE	Mean	SE
Pre-experimental FI (g/d)	107	± *2.2*	108	± *1.8*	109	± *1.4*	106	± *24.2*	103	± *22.0*
FI during the experiment (g/d)	93.3 *b*	± *2.0*	102.8 *a*	± *1.5*	99.2 *a*	± *1.8*	88.0 *c*	± *2.0*	86.4 *c*	± *2.2*
Pre-experimental EP (%)	96.0	± *1.2*	96.8	± *1.2*	97.2	± *1.4*	97.2	± *1.7*	99.2	± *1.1*
EP during the experiment (%)	96.4	± *1.2*	96.8	± *1.2*	98.8	± *0.7*	96.4	± *1.2*	96.0	± *1.5*
Pre-experimental BW (g)	1949	± *22.9*	1902	± *25.9*	1936	± *24.2*	1927	± *24.2*	1880	± *22.0*
BW during the experiment (g)	1958 *ab*	± *26.2*	2006 *a*	± *25.6*	2017 *a*	± *24.1*	1952 *ab*	± *19.7*	1919 *b*	± *22.4*
Ileum length[1] (cm)	58.1 *c*	± *1.3*	61.8 *b*	± *0.9*	65.1 *a*	± *0.8*	60.9 *b*	± *1.1*	64.6 *a*	± *0.9*

[a, b] Parameters in one row not sharing a common superscript are significantly different between diets (P < 0.05)

[1]Section between Meckel's diverticulum and ileo-caeca-colonic junction

Table 3-10. *Digestibility coefficient of nitrogen and amino acids for the basal diet (BD) and the other diets with different inclusion rates of toasted soybeans (TS) and maize gluten (MG)*

	BD		15 % TS		30 % TS		15 % MG		30 % MG		Pooled P value
	Mean	SE	Mean	SE	Mean	SE	Mean	SE	Mean	SE	
Nitrogen	0.86	± *0.02*	0.88	± *0.01*	0.88	± *0.01*	0.87	± *0.02*	0.88	± *0.01*	0.71
Alanine	0.79 [b]	± *0.04*	0.82 [b]	± *0.02*	0.85 [a]	± *0.02*	0.87 [a]	± *0.02*	0.89 [a]	± *0.02*	0.02
Arginine	0.77 [b]	± *0.03*	0.86 [a]	± *0.02*	0.90 [a]	± *0.01*	0.85 [a]	± *0.02*	0.86 [a]	± *0.02*	<0.01
Aspartic acid	0.67 [b]	± *0.05*	0.79 [a]	± *0.01*	0.84 [a]	± *0.01*	0.77 [a]	± *0.03*	0.81 [a]	± *0.02*	<0.01
Cystine	0.80	± *0.02*	0.82	± *0.01*	0.82	± *0.01*	0.81	± *0.01*	0.81	± *0.02*	0.90
Glutamic acid	0.94	± *0.01*	0.94	± *0.00*	0.95	± *0.01*	0.94	± *0.01*	0.93	± *0.01*	0.94
Glycine	0.78 [b]	± *0.02*	0.82 [b]	± *0.01*	0.84 [a]	± *0.01*	0.81 [b]	± *0.02*	0.82 [b]	± *0.02*	0.19
Isoleucine	0.85	± *0.02*	0.86	± *0.01*	0.89	± *0.01*	0.88	± *0.02*	0.88	± *0.02*	0.45
Leucine	0.86 [b]	± *0.02*	0.87 [b]	± *0.01*	0.89 [b]	± *0.01*	0.91 [a]	± *0.02*	0.91 [a]	± *0.02*	0.10
Lysine	0.82	± *0.03*	0.86	± *0.01*	0.88	± *0.01*	0.82	± *0.03*	0.82	± *0.02*	0.20
Methionine	0.86	± *0.02*	0.86	± *0.01*	0.90	± *0.01*	0.89	± *0.02*	0.90	± *0.02*	0.35
Phenylalanine	0.88	± *0.02*	0.89	± *0.01*	0.91	± *0.01*	0.91	± *0.01*	0.91	± *0.01*	0.38
Proline	0.91	± *0.01*	0.92	± *0.01*	0.92	± *0.01*	0.93	± *0.01*	0.92	± *0.01*	0.79
Serine	0.81 [b]	± *0.02*	0.85 [b]	± *0.01*	0.87 [a]	± *0.01*	0.86 [a]	± *0.02*	0.87 [a]	± *0.02*	0.06
Threonine	0.66 [b]	± *0.04*	0.74 [b]	± *0.01*	0.79 [a]	± *0.02*	0.76 [a]	± *0.02*	0.79 [a]	± *0.02*	0.02
Tryptophan	0.76	± *0.03*	0.79	± *0.01*	0.82	± *0.02*	0.76	± *0.02*	0.77	± *0.02*	0.37
Valine	0.81	± *0.02*	0.84	± *0.01*	0.86	± *0.01*	0.86	± *0.02*	0.86	± *0.02*	0.28

[a, b] *Parameters in one row not sharing a common superscript are significantly different between diets ($P < 0.05$)*

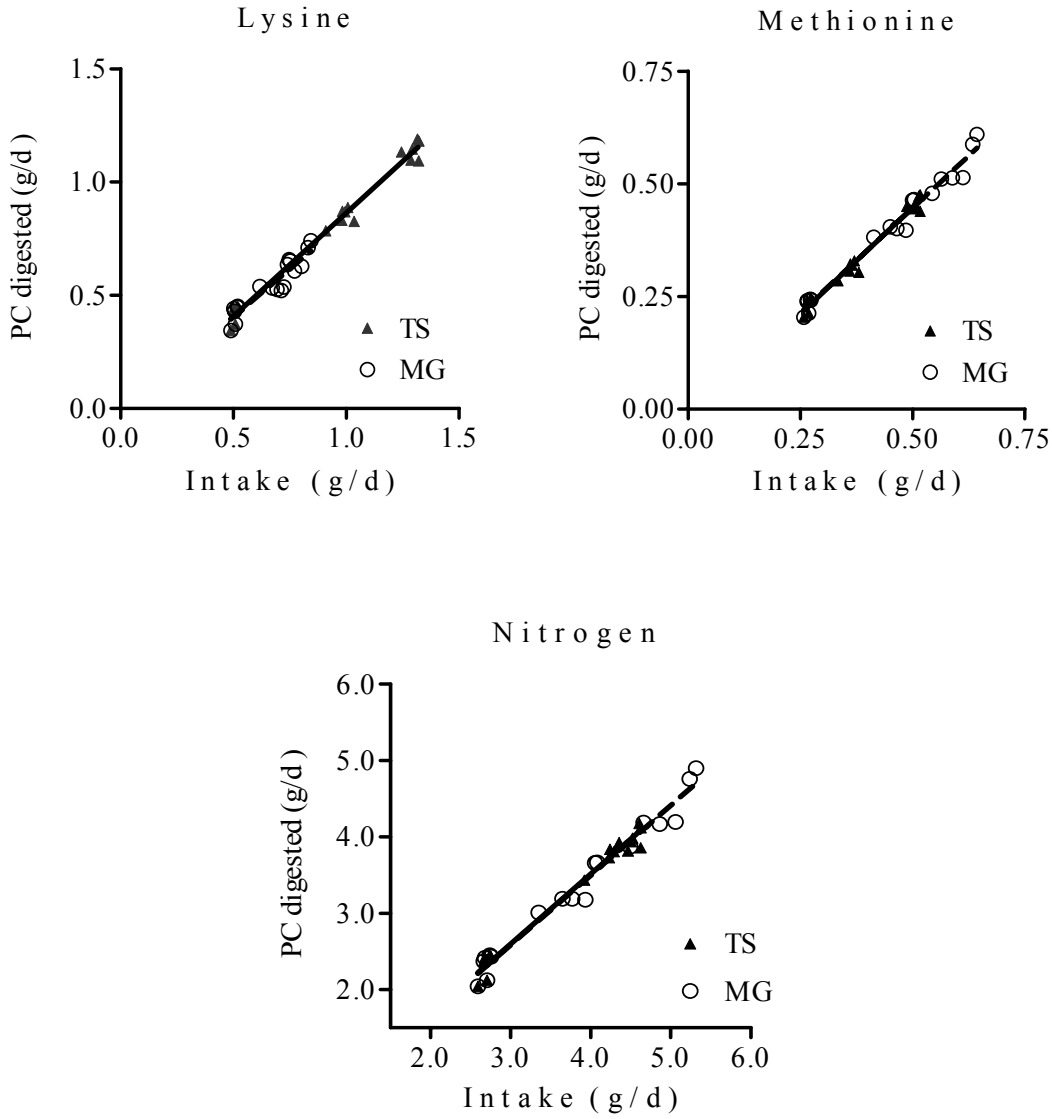

Figure 3-2. *Relationship between intake and precaecal digested amount of lysine, methionine and nitrogen up to the terminal ileum in laying hens fed on different dietary concentration of toasted soybeans (TS) and maize gluten (MG)*

Table 3-11. *Partial precaecal digestibility of nitrogen and amino acids for toasted soybeans (TS) and maize gluten (MG) determined by multiple linear regression analysis (estimate and SE of estimate for the regression coefficient)*

		TS		MG		Difference		P value
	R^2	Estimate	SE	Estimate	SE	Estimate	SE	
Nitrogen	0.97	0.91	*0.05*	0.92	*0.03*	- 0.01	*0.06*	0.90
Alanine	0.98	0.92	*0.06*	0.94	*0.03*	- 0.02	*0.06*	0.74
Arginine	0.99	0.96	*0.02*	0.93	*0.04*	0.03	*0.04*	0.50
Aspartic acid	0.98	0.91	*0.03*	0.89	*0.04*	0.02	*0.05*	0.80
Cystine	0.95	0.84	*0.05*	0.86	*0.05*	- 0.02	*0.06*	0.77
Glutamic acid	0.98	0.95	*0.04*	0.94	*0.03*	0.01	*0.04*	0.78
Glycine	0.97	0.88	*0.04*	0.88	*0.04*	0.00	*0.05*	0.85
Isoleucine	0.98	0.92	*0.04*	0.92	*0.04*	0.00	*0.04*	0.90
Leucine	0.99	0.92	*0.06*	0.94	*0.02*	- 0.02	*0.05*	0.71
Lysine	0.98	0.91	*0.03*	0.85	*0.08*	0.06	*0.08*	0.41
Methionine	0.98	0.95	*0.05*	0.93	*0.03*	0.02	*0.05*	0.70
Phenylalanine	0.98	0.94	*0.04*	0.94	*0.03*	0.00	*0.04*	1.00
Proline	0.98	0.92	*0.07*	0.95	*0.03*	- 0.03	*0.06*	0.62
Serine	0.98	0.92	*0.04*	0.93	*0.03*	- 0.01	*0.04*	0.87
Threonine	0.94	0.87	*0.05*	0.89	*0.05*	- 0.02	*0.06*	0.75
Tryptophan	0.96	0.87	*0.04*	0.82	*0.08*	0.05	*0.08*	0.56
Valine	0.97	0.90	*0.05*	0.91	*0.04*	- 0.01	*0.05*	0.83

3.3.4. Discussion

Significant differences in BW between 30 % MG with 15 % and 30 % TS containing diet can be an effect of lower FI in TS containing diets as compared with MG containing diets (Table 3-7). Diet DC of all studied AAs and N was mostly higher in the diets with higher concentrations of AAs and N than in the diets with lower concentrations of AAs and N. This

fact is in agreement with the studies of Sauer *et al.* (2000) that an increase in concentration of N and AAs in diet will increase DC of it.

In this study R^2 of all regression lines was high and this fact confirms the close relationship between intake of AAs and the digested amounts. The PC digested amounts of AAs and N depended linearly on the intake of N and AAs for all the studied AAs. These results agree with earlier reports on this subject (Mitchell and Bert, 1954; Short *et al.,* 1999; Ishibashi and Yonemochi, 2003; Lemme *et al.,* 2004; Rodehutscord *et al.*, 2004; Rodehutscord and Mosenthin, 2005).

After completing statistical analyses by multiple linear regression method for all studied AAs and N, no any significant difference in PPD between the two protein sources were found. These results may demonstrate the high quality of toasted soybeans after heat processing because it shows a high digestibility for this seed like maize gluten. The differences between TS and MG were sometimes as high as 6 percent (lysine) but because of high standard error (between 2 to 8 percent) never reached the level of significance. In comparison between SM in Experiment 1 and TS in Experiment 2 it was revealed that the content of all AAs and CP of SM was higher (between 7.1 % in glutamic acid to 0.3 % in methionine) than TS (Tables 3-2 and 3-8), but in contrast the PPD of all AAs and CP in TS was higher (between 26 % in cystine to 11 % in aspartic acid and lysine)) than SM (Tables 3-6 and 3-11). The hen's performance (FI, EP and BW gain) was better with TS diets than RM diets (Tables 3-3 and 3-9). It seems that these different AA digestibilities originate from different feed intake (Zuprizal *et al.,* 1991; Furuya and Kaji, 1992; Butts *et al.*, 1993; Kadim and Moughan, 1997b; Hess and Seve, 1999; Stein *et al.,* 1999; Albin *et al.,* 2001; Stein *et al.,* 2001; Moter and Stein, 2004), feed ANFs (King *et al.,* 2000; Wiseman *et al.,* 2003), process method (Zuprizal *et al.,* 1991; Amornthewaphat *et al.*, 2005), other nutrients of these protein ingredients like higher crude fat concentration in TS than SM (Li and Sauer, 1994; Dänicke *et al.,* 2000) or smaller feed particle size in pelleted-crumbled TS containing diets than pelleted SM containing diets (Svihus and Hetland, 2001; Fastinger and Mahan, 2003). These results demonstrate that higher AA or CP in one feed ingredients does not correspond always to better

quality for poultry. Finding the exact reasons for this hypothesises needs several experiments, further more the effect of ingredients additivity on digestibility measurements is not clear until now. Using PC methods depends on slaughtering the hens and this makes the experiments expensive. Approving the caecectomised hens for digestibility as a constant material for partial amino acid digestibility studies could save the time and money for doing more experiments and finding the exact reasons for these differences. By knowledge of the author there is not any other literature for comparison of digestibility with regard to the details of the feed processing in laying hens.

The results of this study again confirm regression approaches as a method for protein ingredient AA digestibility determination without the need to measure endogenous losses. Variation exists in AA digestibility between TS and MG and within one protein source for hens. The ranking of individual AAs regarding their digestibility is different between TS and MG (Appendix B-3). Apart from N, alanine, cystine, leucine, proline, serine, threonine and valine regression coefficients were higher (but not significantly) in TS than in MG. Hence these calculated TS and MG PPD can be very useful for practical feed formulation in laying hens.

3.3.5. Conclusion

Variation exists in PC AA digestibility between TS and MG and within one protein source for hens. The ranking of individual AAs regarding their digestibility is different between TS and MG. These results also may demonstrate the high quality of toasted soybeans after heat processing because it shows a high digestibility for such feed similar as for maize gluten.

3.4. Experiment 3: Comparison of unexcreted proportion of amino acids and nitrogen and energy metabolisability for diet between intact and caecectomised laying hens

3.4.1. Introduction

The use of excreta as a basis for digestibility measurements are criticised because of the possibly spurious influence of bacteria in the hindgut (Wallis and Balnave, 1984; Ten Doeschate *et al.,* 1991; Ten Doeschate *et al.,* 1993; Ravindran *et al.,* 1999; Kadim *et al.,* 2002; Ogle *et al.,* 2002). It has been reported that hindgut micro-flora may be able to synthesise AAs or utilise undigested AAs without having any benefit to the birds. Excreta in intact (non-caecectomised) laying hens were affected by micro-flora of the hindgut, especially in the caecum. Caecum micro-flora changes the profile of AA during flow of digesta through this part of the GIT. Because the caeca are the main sites of bacterial activity in the hindgut, caecectomised or surgically caeca removed poultry have been proposed for reducing bacterial influence on digestibility measurement of AAs for many years (Parsons, 1984; Johns *et al.,* 1986b; Parsons, 1986; Green *et al.,* 1987; Green, 1988; Angkanaporn *et al.,* 1997a; Parsons *et al.,* 1997; Ragland *et al.,* 1999; Son *et al.,* 2000).

With the aim of excluding the post ileal micro-organisms effects on AA digestibility, nowadays researchers use precaecal digesta after slaughtering the birds for AA digestibility calculations (Johns *et al.,* 1986a; Ravindran *et al.,* 1999; Kadim *et al.,* 2002), but by using the excreta of caecectomised laying hens it would be possible to repeat the experiment with the same hens, thus reducing the number of animals in the trials considerably.

The objective of this experiment was to use caecectomised laying hens to study AA digestibility with a minimum usage of hens, without the need for markers and without using different hens at different ages. In a preliminary experiment some birds were caecectomised and compared with intact laying hens in order to establish this method and knowing the extent of changes in AAs and N unexcreted proportion (UP) and energy metabolisability (EM) by caecectomy.

3.4.2. Materials and methods

Animals involved

This experiment was conducted in the Institute of Nutritional Sciences of the Martin-Luther-Universität Halle-Wittenberg and was approved by authorities in accordance with the animal welfare legislation. Fifteen pullets (Lohmann Brown) 15 weeks old were obtained from Deubener Geflügelhof GmbH (Altenbach, Germany) and kept individually in balance crates for quantitative measure of FI and excretion (faeces plus urine) in a temperature and illumination controlled room. The room temperature was kept at 20° C and provided with fourteen hours of light (from 7 am to 9 pm) automatically.
Six birds were caecectomised between the ages of 20 to 21 weeks and also six intact birds (selected based on FI, broken egg) grown on a commercial diet until reaching peak production (90-95 % EP) were used. In the 27th week of age caecectomised hens were compared with 6 intact hens for production performance, UP of AAs and N and EM. Feed was supplied from individual feeders and drinking water from nipple drinkers *ad libitum* throughout. FI and EP were recorded individually five days before and five days during the excreta collection period.

Caecectomy surgery

The surgery was done following the descriptions by Angkanaporn *et al.* (1997b) and Green *et al.* (1987) when the hens were 20 to 21 weeks old (Figure 3-3). The hens were deprived from feed 12 hours before surgery. 0.1 mL Diazepam Ratiopharm® 10 injection liquid (Ratiopharm GmbH) was injected intramuscularly into the breast muscle before surgery. After some minutes each hen was placed on the surgery table in a position of dorsal recumbence. Each hen was anaesthetised by using a VMC Anaesthesia machine (Motrx Company). This machine uses Isofluran (Isoba®, Essex Tierarznei) as an anaesthetising substance. A mask was

placed over the beak and nostrils of the hens and anaesthesia was induced by a mixture of oxygen and isofluran.

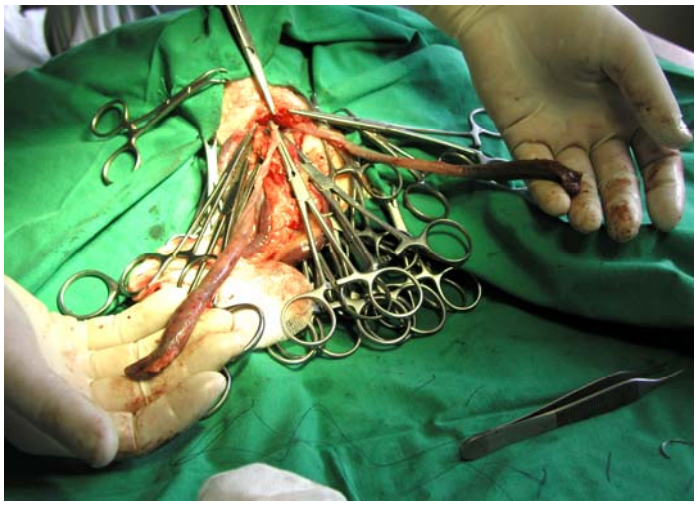

Figure 3-3. *Photograph showing caecectomy surgery*

At first oxygen flow was adjusted to 1000 mL/min and Isofluran to 5 vol.-%. When hens became completely unconscious, the anaesthesia was maintained by adjusting the oxygen flow to approximately 300 mL/min and the flow of liquid to 2 vol.-%. Feathers between the sternum and rectum were removed. This region was disinfected with an iodine spray. Then 5 cm transversal cutting was made about 4 cm below the sternum. The body cavity was opened and the abdominal layers were separately cut with operating scissors very carefully. Attachment layers and vessels were cut by scissors and blocked by suturing. This procedure was continued until reaching the ICCJ for both caeca. Caeca were blocked with forceps as near as possible to the ICCJ and bound with a sterile absorbable cut cot string. Caeca were cut next to the binding with scissors. The free ends were disinfected with alcohol-iodine solution, sutured and rubbed with an antibiotic cream. Now the abdominal layers were sutured with absorbable cut cot string and the skin with a polyester string. The surgery area was then disinfected. When the hens regained consciousness, 0.5 mL antibiotic (Ursocyclin® 10 % per injection, Serumwerk Bernburg GmbH) and 0.15 mL anodyne (Rimadyl®, injection solution, Pfizer GmbH) were injected subcutaneously. These injections were repeated the day following the surgery. Polyester sutures were removed from the skin after one week.

Surgery of one hen required at least 1.5 hours. Hens were completely healthy after only one day. Within one week after surgery hens returned to their previous levels of FI and laying performance.

Dietary treatment

Only one experimental diet was used throughout this experiment (Table 3-12). One week before excreta collection, at 26 weeks of age, hens were fed the experimental diet *ad libitum*. At 27 weeks of age, six caecectomised laying hens and also six intact laying hens were offered individually 120 g in two equal meals per day (8 am and 2 pm). These amounts were offered for five days of adjustment and the five days of excreta collection period. Feed wastes in the feeder were collected in separate and weighed buckets and frozen (–20 °C) daily before offering the first meal in the morning. Results of nutrients and AA analyses for the experimental diet are summarised in Table 3-13. FI and EP were recorded five days before and during the five days of excreta collection. Hens were weighed before and at the end of the excreta collection period.

Table 3-12. *Experimental diet composition*

Composition	**g/kg**
Wheat	408
Maize	115
Soybean meal (solvent extracted, 48 % CP)	165
Peas	117
Sunflower meal (solvent extracted, 30 % CP)	47
Soybean oil	40
Limestone	91
Mono-di-calcium phosphate	6.5
Premix (vitamins and minerals)	5
Alimet (Feed supplement)	1.5
Salt	3
Sodium carbonate	1

Table 3-13. *Chemical analyses of the experimental diet*

Analysed	**g/kg**
Dry matter	960
AME, calculated (MJ/kg)	11
Crude protein	203
Crude fibre	49
Crude lipid	45
Crude ash	131
Alanine	7.8
Arginine	11.7
Aspartic acid	16.5
Cystine	3.6
Glutamic acid	38.5
Glycine	7.8
Isoleucine	7.3
Leucine	13.8
Lysine	8.7
Methionine	2.8
Phenylalanine	8.9
Proline	11.8
Serine	8.3
Threonine	4.1
Valine	8.5

Sampling

Excreta and daily feed wastes in drinkers and on trays were collected from the trays three times per day (8 am, 2 pm and 8 pm) in separate and weighed buckets, to minimise volatilisation of nitrogenous compounds and were frozen (–20°C) immediately. Excreta of each hen were pooled for the five days of the collection period. The crates and net under each bird were cleaned at each excreta collection. Feathers were removed from excreta before each collection.

Chemical analyses

At the end of the experiment buckets were weighed again and their contents analysed for DM, N, AAs and energy. DM of feed and feed wastes was determined after oven drying (3 hours at 105°C). Frozen excreta were defrosted and homogenised. DM of these excreta also was measured in the oven by using sand (24 hours at 105 °C). About 200 g of excreta per hen was freeze dried. Freeze dried excreta and also feed and feed wastes were ground (0.5 mm screen) and DM of them measured before nutrient analyses. Dietary concentrations of proximate nutrients were analysed according to the VDLUFA official methods (Naumann and Bassler, 1976). AA analyses also followed standard procedures (Naumann and Bassler, 1976) with laboratory details as described in Experiment 1. Energy content of samples was measured by a bomb calorimeter (IKA-Calorimeter C7000 isoperibolic, Janke & Kunkel IKA Analysentechnik, Staufen, Germany).

Calculations and statistical evaluation

The UP of the AAs and N and energy metabolisability (EM) were calculated for each hen, according to the following equation:

UP or EM = (DI – DE) / DI

Where:
DI: daily intake of DM, AA, N or E (g or MJ)
DE: daily excretion of DM, AA, N or E (g or MJ)
All parameters were compared statistically by using software package SAS (9.1, SAS Institute Inc.).

3.4.3. Results

Mean BW in intact and caecectomised hens was 1.81 and 1.73 kg, and laying performance 97 and 100 %. The mean FI for both was 101 g/d, and DM excretion 35 and 38 g/d. No significant differences in body weight, EP,

FI and excreted DM due to the caecectomy operation were recorded but disappeared DM was significantly higher ($P < 0.05$) in intact hens (66 g/d) than caecectomised hens (64 g/d) (Table 3-14; Appendix C-1).
The range in UP for all the 15 studied AAs was from 0.69 (glycine) to 0.89 (arginine, glutamic acid and proline) for intact laying hens and from 0.63(glycine) to 0.89 (arginine, glutamic acid) for caecectomised laying hens (Table 3-15; Appendix C).

Table 3-14. *Comparison of production performance between caecectomised and intact laying hens (EP = egg production, BW = body weight)*

Treatment	Intact		Caecectomised	
	Mean	*SE*	Mean	*SE*
EP (%)	97.2	± *2.8*	100.0	± *0.0*
DM intake (g/d)	101	± *1.8*	101	± *1.1*
Excreted DM (g/d)	35.0	± *1.2*	37.7	± *0.8*
Disappeared DM (g/d)	66.1 [a]	± *0.8*	63.6 [b]	± *0.7*
Initial BW (g)	1876	± *67.1*	1873	± *38.6*
Final BW (g)	1809	± *55.2*	1734	± *28.6*

[a, b] *Parameters in one row not sharing a common superscript are significantly different between hens ($P < 0.05$)*

The mean UP of all AAs was 0.82 and 0.80 in intact and caecectomised laying hens. UP for DM and 6 AAs (aspartic acid, cystine, glycine, proline, serine and threonine) and also EM was significantly higher ($P < 0.05$) in intact than caecectomised laying hens. The maximum difference in UP of AAs between intact and caecectomised laying hens was 5 % for glycine. For most of the other AAs and N, UP was higher in intact laying hens than caecectomised hens but not significantly different.

Table 3-15. *Comparison of unexcreted proportion of dry matter, nitrogen and amino acids and energy metabolisability between caecectomised and intact hens*

Treatment	Intact		Caecectomised		
	Mean	*SE*	Mean	*SE*	Diff
Dry matter	0.65 [a]	± *0.007*	0.63 [b]	± *0.004*	0.03
Nitrogen	0.40	± *0.010*	0.39	± *0.014*	0.01
Alanine	0.74	± *0.008*	0.75	± *0.013*	-0.02
Arginine	0.89	± *0.004*	0.89	± *0.006*	0.01
Aspartic acid	0.82 [a]	± *0.005*	0.80 [b]	± *0.004*	0.02
Cystine	0.80 [a]	± *0.006*	0.76 [b]	± *0.009*	0.04
Glutamic acid	0.89	± *0.003*	0.89	± *0.003*	0.00
Glycine	0.69 [a]	± *0.012*	0.63 [b]	± *0.008*	0.05
Isoleucine	0.83	± *0.009*	0.84	± *0.005*	-0.01
Leucine	0.84	± *0.005*	0.83	± *0.006*	0.01
Lysine	0.83	± *0.004*	0.82	± *0.005*	0.01
Methionine	0.83	± *0.011*	0.83	± *0.013*	0.00
Phenylalanine	0.85	± *0.007*	0.84	± *0.007*	0.01
Proline	0.89 [a]	± *0.003*	0.86 [b]	± *0.009*	0.03
Serine	0.83 [a]	± *0.004*	0.80 [b]	± *0.004*	0.03
Threonine	0.76 [a]	± *0.007*	0.73 [b]	± *0.007*	0.04
Valine	0.81	± *0.005*	0.80	± *0.007*	0.01
Energy	0.73 [a]	± *0.004*	0.70 [b]	± *0.003*	0.04

[a, b] *Parameters in one row not sharing a common superscript are significantly different between hens (P < 0.05)*

3.4.4. Discussion

Caecectomy had no effect on hen performance such as FI, EP and BW. These results confirm those by Son *et al.* (2000). In this experiment the results showed that UP of more than one third of all studied AAs and DM and EM were significantly lower ($P < 0.05$) in caecectomised rather than intact laying hens. Published studies using caecectomised poultry for AA digestibility studies are abundant and most of them reported the same results as obtained here. They mentioned that caecectomised poultry should be used in AA digestibility studies to prevent overestimation of digestibility of AAs in feedstuffs that are caused by further breakdown of

AAs and also change the profile of AA in excreta by micro-organism in caecum (Parsons, 1984; Parsons, 1986; Parsons *et al.,* 1997; Angkanaporn *et al.,* 1997a; Ragland *et al.,* 1999).

Although the UP of some AAs and EM were significantly higher in intact than caecectomised hens such differences were not seen in higher hen performance. These results may be the consequence of using a highly digestible experimental diet and being the hens in excess of requirement. In other conditions like using less digestible diets, these differences in AA UP perhaps had resulted in different hen performance between intact and caecectomised laying hens.

It is now clear that caecectomised laying hens are different from intact laying hens in AAs excretion. No significant differences in hen performance between caecectomised and intact laying hens together with a reduced effect of micro-organisms on feed digestibility may confirm caecectomised hens as models for protein ingredients AA digestibility measurements. Literatures show that there is no significant absorption of AAs in caeca (Webb, 1990), but this is stated in other papers controversially (Obst and Diamond, 1989; Whittow, 2000). It is also worth noting that faeces after voiding can be ingested again by the hens and AAs or micro-organisms of them can be absorbed. This mechanism may declare the usefulness of cooperation between poultry and microbes in nature, but is not so important for experimental birds.

3.4.5. Conclusion

Caecectomised laying hens had similar production performance like intact hens, but UP of more than one third of studied AAs and EM of them was significantly lower ($P < 0.05$) than in intact hens. This experiment confirms that caecectomy can reduce the hindgut micro-organism effect on nutrient degradation. Designing an experiment for comparison of excreta of caecectomised hens with PC digesta from intact hens after correction for EAA losses may prove caecectomised hens as a model for protein ingredients AA digestibility measurements. Using caecectomised hens has some advantages in AA digestibility measurements like being a constant

animal material and collecting samples quantitatively for several feed ingredients and finding the factors that affect it like the effect of age of hens. This will be the objective of further experiments with caecectomised hens described in the next chapters.

3.5. Experiment 4: Amino acid excretion in caecectomised laying hens of different ages

3.5.1. *Introduction*

Some experiments exist that measured the effect of age on AA digestibility with excreta of intact poultry (Batal and Parsons, 2002a; Batal and Parsons, 2002b; Palander *et al.,* 2004a). But as mentioned before, these results are not easy to interpret because of effects of micro-flora especially in caeca on AA digestibility (Wallis and Balnave, 1984; Ten Doeschate *et al.,* 1991; Ten Doeschate *et al.,* 1993; Ravindran *et al.,* 1999; Kadim *et al.,* 2002; Ogle *et al.,* 2002).

Nowadays researchers use PC digesta for AA digestibility calculations after slaughtering the birds (Donkoh and Moughan, 1994). By using the excreta of caecectomised laying hens it will be possible to perform repeated measures with the same hens and without the need for using indigestible markers (Parsons, 1984; Johns *et al.,* 1986b; Parsons, 1986; Green *et al.,* 1987; Green, 1988; Parsons *et al.,* 1997; Ragland *et al.,* 1999; Son *et al.,* 2000). However, by the knowledge of the author the effect of age on AA digestibility in caecectomised hens has not yet been studied. In this experiment, AAs unexcreted proportion (UP) and energy metabolisability (EM) of a diet were compared at 27, 40 and 57 weeks of age in the same caecectomised laying hens.

3.5.2. Materials and methods

Animals involved

The same six caecectomised birds as in Experiment 3 were used. These birds had been caecectomised between the 20th and 21st week of age and grown on a commercial diet until peak production (90-95 % EP). Housing and handling of them were as described for Experiment 3. All experimental data given for the 27th week of age are the same as in Experiment 3. The trial was repeated in week 40 and 57 of age. All parameters as in the

previous experiment were recorded. Feed was supplied from individual feeders, at the rate of 120 g per day, and drinking water from nipple drinkers *ad libitum* throughout. Feed intake and EP were recorded five days before and five days during each excreta collection period. Hens were weighed before and at the end of each excreta collection period.

Dietary treatment

Only one experimental diet (Table 3-12) was used. Feed was offered for five days of adjustment and the five days of excreta collection. One week before excreta collection in the 40^{th} and 57^{th} week of age, hens were fed the experimental diet *ad libitum.* Hens were offered 120 g in two equal meals per day (8 am and 2 pm) during the excreta collection period. Feed residues in the feeder were collected in separate and pre-weighed buckets daily and frozen (–20°C) before offering the first meal in the morning. Results of nutrients and AA analyses for the experimental diet are summarised in Table 3-13. FI was recorded as in Experiment 3.

Sampling

All sampling procedures were as described for Experiment 3. Voided excreta and daily feed wastes (in feeders, drinkers and on trays) were collected three times per day (8 am, 2 pm and 8 pm) and were frozen (–20^{o} C) immediately. Excreta of each hen were pooled between the five days of the collection period. At the end of the experiment, buckets were weighed again and their content analysed for DM, N, AAs and gross energy. The crates and net under each bird were cleaned at each excreta collection. Feathers were removed from excreta before each collection period.

Chemical analyses and calculations

All laboratorial analyses and calculations were applied as in Experiment 3. At each age, the experimental diet was analysed again completely. All

parameters were compared statistically by using the GLM procedures of the statistical software package SAS (V 9.1, SAS Institute Inc.).

3.5.3. Results

In order to make the comparison of the 3 periods, earlier results of Experiment 3 are given her again. Mean BW in the 3 phases was 1.73 kg, 1.89 kg and 2.00 kg, and laying performance 100 %, 97 % and 93 %. The mean FI was 101 g/d, 104 g/d and 103 g/d. Egg production, DM intake, excreted DM and initial BW were not significantly different in the three age periods, but disappeared DM and final BW in week 57 was significantly higher ($P < 0.05$) than in week 27 (Table 3-16; Appendix D-1).

Table 3-16. *Production performance of caecectomised hens at different ages (EP = egg production, FI = feed intake, BW = body weight)*

	27th week			40th week			57th week		
	Mean		*SE*	Mean		*SE*	Mean		*SE*
EP (%)	100	±	*0.0*	97	±	*3.3*	93	±	*3.2*
DM intake (g/d)	101	±	*1.1*	104	±	*0.8*	103	±	*0.5*
Excreted DM (g/d)	38	±	*0.8*	38	±	*1.2*	36	±	*0.5*
Disappeared DM (g/d)	64 ^{b}	±	*0.7*	66 ^{ab}	±	*1.3*	67 ^{a}	±	*0.6*
Initial BW (g)	1873	±	*38.6*	1889	±	*55.5*	2043	±	*71.8*
Final BW (g)	1734 ^{b}	±	*28.6*	1893 ^{ab}	±	*54.4*	2009 ^{a}	±	*73.6*

$^{a, b}$ *Parameters in one row not sharing a common superscript are significantly different between ages ($P < 0.05$)*

The UP of DM, N, alanine, arginine, cystine, glutamic acid, methionine, phenylalanine, proline, serine, valine and also EM was significantly affected ($P < 0.05$) by age. Hens had higher UP for DM, N, alanine, arginine, cystine, glutamic acid, methionine, phenylalanine, serine and valine and also energy metabolisability in week 57 than in weeks 27 and

40. Significant differences ($P < 0.05$) for arginine and proline UP were detected between week 27 and 40. The range in UP for all the 15 AAs studied across all weeks was from 0.64 (glycine) to 0.89 (glutamic acid) and for the essential AAs from 0.73 (threonine) to 0.88 (arginine). The mean UP of all AAs was 0.80, 0.80, and 0.82 in week 27, 40 and 57, respectively (Table 3-17; Appendix D-2).

Table 3-17. *Comparison of unexcreted proportion of dry matter, nitrogen and amino acids and energy metabolisability between different age periods*

	27th week				40th week				57th week			
	Mean			*SE*	Mean			*SE*	Mean			*SE*
Dry matter	0.63	*b*	±	*0.004*	0.64	*ab*	±	*0.012*	0.65	*a*	±	*0.005*
Nitrogen	0.39	*b*	±	*0.014*	0.42	*b*	±	*0.016*	0.48	*a*	±	*0.011*
Alanine	0.75	*b*	±	*0.013*	0.75	*b*	±	*0.009*	0.79	*a*	±	*0.008*
Arginine	0.89	*a*	±	*0.006*	0.86	*b*	±	*0.007*	0.88	*a*	±	*0.007*
Aspartic acid	0.79	*a*	±	*0.004*	0.80	*a*	±	*0.007*	0.80	*a*	±	*0.005*
Cystine	0.76	*ab*	±	*0.009*	0.72	*b*	±	*0.014*	0.77	*a*	±	*0.007*
Glutamic acid	0.89	*b*	±	*0.003*	0.89	*ab*	±	*0.004*	0.90	*a*	±	*0.003*
Glycine	0.63	*a*	±	*0.008*	0.63	*a*	±	*0.018*	0.66	*a*	±	*0.019*
Isoleucine	0.84	*a*	±	*0.005*	0.83	*a*	±	*0.006*	0.83	*a*	±	*0.006*
Leucine	0.83	*a*	±	*0.006*	0.85	*a*	±	*0.005*	0.85	*a*	±	*0.004*
Lysine	0.82	*a*	±	*0.005*	0.81	*a*	±	*0.011*	0.82	*a*	±	*0.006*
Methionine	0.83	*b*	±	*0.013*	0.84	*b*	±	*0.007*	0.87	*a*	±	*0.007*
Phenylalanine	0.84	*b*	±	*0.007*	0.83	*b*	±	*0.007*	0.87	*a*	±	*0.004*
Proline	0.86	*b*	±	*0.009*	0.90	*a*	±	*0.009*	0.87	*ab*	±	*0.005*
Serine	0.80	*b*	±	*0.004*	0.81	*ab*	±	*0.007*	0.82	*a*	±	*0.005*
Threonine	0.73	*a*	±	*0.007*	0.73	*a*	±	*0.011*	0.74	*a*	±	*0.008*
Valine	0.80	*ab*	±	*0.007*	0.79	*b*	±	*0.007*	0.82	*a*	±	*0.011*
Energy	0.70	*b*	±	*0.003*	0.70	*b*	±	*0.010*	0.72	*a*	±	*0.003*

[a, b] *Parameters in one row not sharing a common superscript are significantly different between weeks ($P < 0.05$)*

3.5.4. Discussion

Results of this experiment demonstrated that for 8 of 15 AAs under study the UP was significantly higher in week 57 than in week 27 and 40. Significant differences between 27th and 40th week were detected only for 2 AA. The UP of DM and N and also the EM were affected positively by increase in age. It showed that laying hens digested nutrients better as age increased. This fact was also found in other poultry species using intact birds and by other approaches but not in caecectomised hens (Batal and Parsons, 2002a, Batal and Parsons, 2002b; Palander *et al.,* 2004a; Ravindran and Hendriks, 2004b; Wu *et al.,* 2004; Huang *et al.,* 2005; Thomas and Ravindran, 2005).

Huang *et al.* (2005) found that the age of broilers between 11 and 42 days post-hatching significantly influenced the ileal AAAD. The effects, however, varied among AAs and feed ingredients. Analysis of the combined results for the 8 feed ingredients showed that, in general, the digestibility coefficients of AAs increased with advancing age of broiler chickens. Batal and Parson (2002b) concluded that nutrient digestibility increases with increasing age between 0 to 21 day post-hatching for chicks and found that the utilisation rate of energy-yielding feedstuffs is age-dependent. They concluded that the increased ME_N of a maize-soybean meal-based diet with age was due to a combination of increased utilisation of starch in the maize, fat in the maize and added soybean oil, the protein in both the maize and soybean meal, and possibly other carbohydrates in the maize and soybean meal.

Ravindran and Hendriks (2004b) measured recovery and composition of endogenous protein at the terminal ileum of broiler chickens 14 and 42 days post-hatching using the peptide alimentation method. The ileal endogenous flows of N and AAs, expressed in mg/kg DM intake, differed significantly ($P < 0.05–0.01$) between the two age groups, with flows increasing with age, except for lysine, histidine and glycine. The flows of lysine and histidine were unaffected ($P > 0.05$) by age, whereas a tendency ($P = 0.07$) for increased loss with age was observed for glycine. These findings suggest that, when determining true digestibility, corrections using

EAA flows determined with broilers of a particular age to AAAD values determined with birds of a different age would result in less accurate estimates. Palander *et al.* (2004a) showed that UP of protein in growing turkeys decreased from 4 to 8 weeks of age for soybean meal and rapeseed meal but increased for soybean cake and rapeseed cake. From 8 to 12 weeks of age UP of protein decreased for all the products tested. In above mentioned experiments as well as in the present experiment the reasons for the differences in UP or digestibility may be seen in the amount of secretion of endogenous digestive enzymes (Ravindran and Hendriks, 2004b) and also growing and developing the absorption surface by age increase.

3.5.5. Conclusion

It is concluded that AA UP as an estimate for digestibility in caecectomised hens may increase with age. Nitrogen and DM UP and energy metabolisability increase also with age. This effect of age on AA digestibility should be considered in standard measurements of feedstuffs AAs digestibility approaches**.** Based on the present data it is suggested to use hens that are not older than 40 weeks. Furthermore this standard method should measure the ingredient AA digestibility independently from measuring EAAs that they generally obtain by the birds of a different age. This kind of standard method will be discussed in detail in the general discussion and conclusion chapter.

3.6. Experiment 5: Marker transit time in the gastrointestinal tract of caecectomised laying hens

3.6.1. Introduction

The time that feed components are retained in successive segments of the GIT determines the time available for digestion and absorption of nutrients. Time between oral intake of a marker and its first appearance in the faeces (transit time) is often used as a parameter for the feed transit time in the GIT. The transit time, however, is determined by the rate of passage of the chyme fraction, which is transported at the highest rate through the GIT. Whether it gives any information about the average time available for digestion and absorption is doubtful (Van Der Klis and Van Voorst, 1993).
The rate of passage of material through the digestive tract has been measured in many ways. Since digesta consists of both solid and liquid components, different types of markers have been used. Insoluble markers such as chromium-mordanted rice, cerium-mordanted rice, Cr_2O_3 or radiopaque plastic pellets have been used as indicators of solid transit time whereas a soluble marker such as Cr-EDTA or phenol red has been used to measure liquid transit time. In general, it was found that larger particles are retained longer in the digestive tract. In chickens, insoluble markers first appear in the excreta 1.6 to 2.6 hours after ingestion. However, mean retention time is a better indicator of transit time than the time of initial appearance of the marker in the excreta. Mean retention time for insoluble markers can vary form 5 to 9 hours depending on the nature of the ingesta and its size. Transit time of digesta is influenced by genetics. When comparing broiler and Leghorn-type chickens, the overall mean retention time is not different, but the time food spends in various parts of the digestive tract is different. The rate of food passage is affected by many factors. Feed transit time through the small and large intestine increases with age. This may account for increases in metabolisable energy values of feedstuffs noted in older birds. Adding lipid or protein to the diet can increase passage time. Increase in environmental temperature slows transit time (Whittow, 2000).

Feed passage rate, together with digesta volume, will be the bird-related factors setting the limits for maximum daily FI. Feed passage rate is therefore an important factor which may affect performance, nutrient digestibility and health (Svihus *et al.,* 2002).
It is clear that all previous diet from GIT must be voided before measuring digestibility of each new diet. It is possible to measure passage rate of diets from GIT by using different markers. This experiment was conducted to determine the approximate time for adjustment to a new diet before starting the excreta collection period in caecectomised laying hens.

3.6.2. Materials and methods

Animals involved

The experiment was conducted in the Institute of Nutritional Sciences of the Martin-Luther-Universität Halle-Wittenberg and was approved by authorities in accordance with the animal welfare legislation. Five pullets at 15 weeks of age (Lohmann Brown) were obtained from Deubener Geflügelhof GmbH (Altenbach, Germany) and were housed in individual metabolism crates in a temperature and illumination controlled room. 14 hours lighting period (from 7 am to 9 pm) and 20° C constant temperature were controlled automatically in the experimental house.
For this experiment five birds aged between 29 and 30 weeks were caecectomised as described in Experiment 3. These hens were grown on a commercial diet until starting this experiment, at 37 weeks of age. Feed was supplied from individual feeders and drinking water from nipple drinkers *ad libitum* throughout.

Dietary treatment

Only one experimental diet (Table 3-12) was used. Feed was offered for five days of adjustment and the five days of excreta collection at the rate of 120 g for each hen. TiO_2 was included in the diet during the first 24 hours of excreta collection.

Sampling and analyses

Excreta of the hens were collected each day separately during the 24 hours of feeding and 4 subsequent days three times daily, as described in Experiment 3. The excreta of each day were pooled into one sample. TiO_2 concentration in excreta was measured spectrophotometrically in excreta samples according to the method described by Brandt and Allam (1987).

3.6.3. Results

TiO_2 concentration in excreta was 22.5 g/kg DM during the first 24 hours, 5 g/kg DM in the first day, 0.2 g/kg DM in the second day and below 0.1 g/kg DM in the third and fourth days after TiO_2 withdrawal from the diet (Figure 3-4, Appendix E-1).

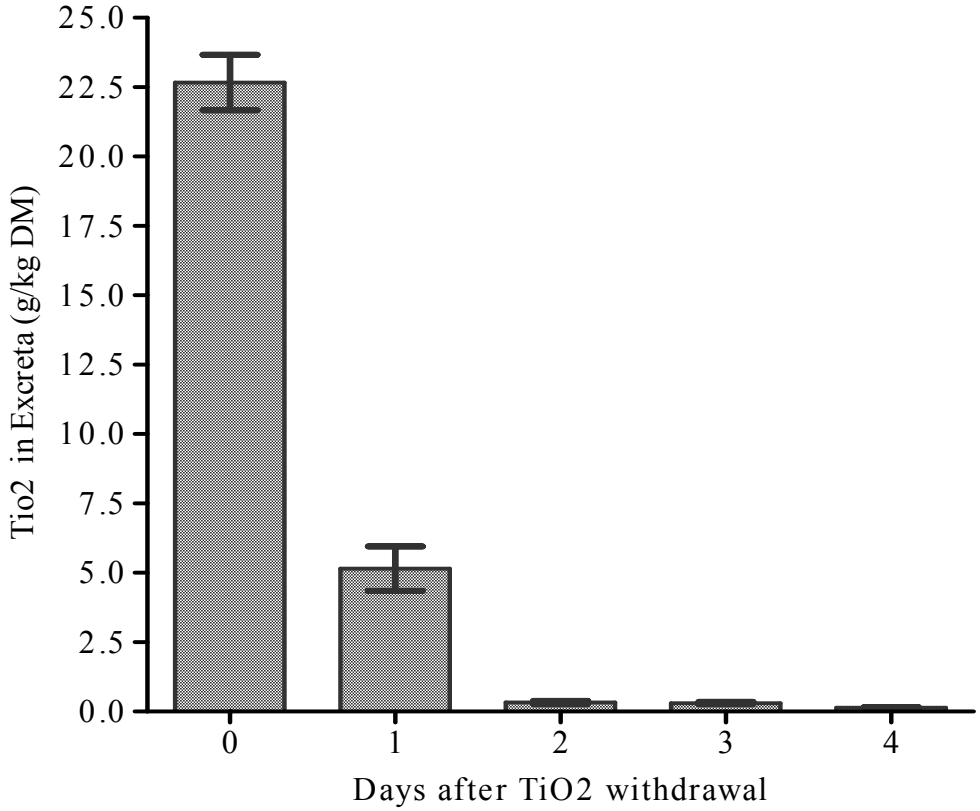

Figure 3-4. *TiO_2 concentration in excreta (g/kg in DM) following TiO_2 withdrawal from the diet (Mean and SE)*

3.6.4. Discussion

The TiO_2 concentration in excreta came close to zero level after only two days of TiO_2 withdrawal from the diet. It can be concluded that using a 5 day period will be a suitable time for adjustment to a new diet in order to measure digestibility in the following 5 days. However, it needs to be presumed that the marker behaves in the same way as the feed ingredients. Jagger *et al.* (1992) compared Cr_2O_3, TiO_2 and acid insoluble lignin as inert markers for determination of digestibility in pigs. They found the smallest difference between the faecal digestibility of N and AAs determined by total faecal collection and by the use of markers for TiO_2 with a recovery rate of 97 %. They concluded that the most appropriate marker to use in digestibility studies was TiO_2. Based on the present study it is speculated that during 5 days, the pre-experimental diet is voided from GIT and substituted by the new experimental diet. It is proposed that collection of excreta for 5 days will give a representative sample to make sure that all feed components pass through GIT and collect for digestibility measurements.

3.6.5. Conclusion

Considering five days as the pre-collection (adaptation) and five subsequent days as collection time seems an appropriate time in digestibility measurements. This may be considered as a representative passage time for all feed components from GIT in caecectomised laying hens.

3.7. Experiment 6: Total tract digestibility of amino acids for toasted soybeans and maize gluten in caecectomised laying hens

3.7.1. Introduction

In Experiment 2, partial PC AA digestibility of toasted soybeans (TS) and maize gluten (MG) were compared. These two protein ingredients had similar partial PC digestibility of AAs. In Experiment 1 also no significant differences in partial PC digestibility of AAs between soybean meal (SM) and rapeseed meal (RM) were found.

As concluded in Experiment 1 for laying hens and by Kluth *et al.* (2005b) for broilers, in PC AA digestibility studies, ileal digesta from the last two thirds of the gut between the MD and 2 cm anterior to the ICCJ should be sampled after asphyxiation of the birds. In this method, the digesta of the birds in each replication are pooled in order to gather a more reliable sample near the physiological condition of feed digestion in the GIT. This method requires the use of markers to calculate digestibility, and this contributes to a higher standard error in measurement. Furthermore birds must be slaughtered for digesta collection and new birds must be used in each new experiment. These disadvantages of PC digestibility may be reduced by using caecectomised birds.

This experiment was conducted in order to do further study with caecectomised laying hens as an experimental model for measuring partial digestibility of AAs by regression approach without the need for slaughtering hens and with the possibility to collect excreta quantitatively. For this purpose, partial total tract (TT) digestibility (PTD) of AAs for TS and MG will be compared in caecectomised laying hens. Furthermore AAs unexcreted proportion (UP) of diets and PTD will be compared between total excreta collection and marker calculations in order to justify using of TiO_2 as indigestible markers in digestibility measurement. In the next chapter the results of PTD of AAs for TS and MG in caecectomised laying hens will be compared with the results of partial PC digestibility (PPD) of AAs from Experiment 2 for the same protein ingredients.

3.7.2. Materials and methods

Animals involved

This experiment was conducted in the Institute of Nutritional Sciences of the Martin-Luther-Universität Halle-Wittenberg and was approved by authorities in accordance with the animal welfare legislation. Fifteen pullets at 17 weeks of age (Lohmann Brown) were obtained from Deubener Geflügelhof GmbH (Altenbach, Germany) and were housed in individual balance crates in a temperature and illumination controlled room. Light was from 7 am to 9 pm and temperature was constant at 20° C on average. Feed was supplied from individual feeders and drinking water from nipple drinkers. Fourteen of these hens had been caecectomised when they were between 20 and 30 weeks old. These hens were reared in individual experimental balance crates with commercial laying hen's diets until 46 weeks of age and then the experimental diets were offered in three subsequent periods (Table 3-18). Parameters like hen body weight (before and after each experimental period) and individual daily hen performance (EP and FI) were measured as described for the previous experiments.

Dietary treatment

The same five diets as in Experiment 2 were used. In brief, these diets comprised a BD and diets with increasing amounts of TS or MG at 15 % and 30 % inclusion rate. TS and MG replaced maize starch in equal proportions so that the change in the AA concentrations of the experimental diets resulted from TS and MG only. Titanium dioxide (TiO_2) was included as an indigestible marker. Diets were pelleted without steam through a 3 mm die, but were crumbled in order to increase FI of birds. Compositions and results of the proximate and AAs analyses for the TS and MG diets are summarised in Tables 3-7 and 3-8. In 46^{th} until 50^{th} weeks of age, the experimental diets were offered at 120 g per day in three periods (Table 3-18) and the daily feed residuals were collected and weighed daily like in previous experiments.

Sampling

Each period consisted of 10 days, 5 days for adjustment to the new diet and 5 days for excreta collection. Between two periods the hens were fed a commercial diet for 4 days. In each period hens were selected based on the best FI, so that for each diet a total of 7 replicates achieved in the three periods. Voided excreta and daily feed wastes (in drinkers and on trays) were collected 3 times per day (8 am, 2 pm and 8 pm) in buckets, maintained in a freezer, weighed and analysed for DM, N and AAs at the end of the experiment. The crates and tray under each bird were cleaned at each time of excreta collection. Feathers were removed from excreta before collection.

Table 3-18. *Experimental diet (I = Basal diet, II = 15 % Toasted soybeans, III = 30 % Toasted soybeans, IV = 15 % Maize gluten, V = 30 % Maize gluten) distribution during three excreta collection periods*

First Period (from 03. Aug until 13. Aug 2005)

Diet No.	I*	I	I	II	II*	II	III	III	III	IV	IV*	V	V	V*
Hen No.	1	2	3	4	5	6	7	8	9	10	11	12	13	14

Second Period (from 17. Aug until 27. Aug 2005)

Diet No.	IV	IV	IV	V	V	V	I	I	II	II	II	III	III	III*
Hen No.	1	2	3	4	5	6	7	8	9	10	11	12	13	14

Third Period (from 31. Aug until 10. Sep 2005)

Diet No.	II	III	III	IV	IV	IV	V	V	I*	I	I	I	II	II*
Hen No.	1	2	3	4	5	6	7	8	9	10	11	12	13	14

Not included in the calculations because of too low feed intake

Analyses and calculations

All chemical analyses and calculations were done as in Experiment 2. The UP of the AAs and N for each diet was calculated according to the following equations. Both marker-based and quantitative measurements were calculated in order to compare them to each other for detecting the accuracy of marker calculations.

By using marker:

$$UP_{AA\ or\ N} = 1 - [(TiO_{2\ Diet} \times AA\ or\ N_{\ Excreta}) / (TiO_{2\ Excreta} \times AA\ or\ N_{\ Diet})]$$

Where:
$TiO_{2\ Diet}$ and $TiO_{2\ Excreta}$: concentrations of TiO_2 in the diet and excreta samples (g/kg).
AA or $N_{\ Diet}$ and AA or $N_{\ Excreta}$: concentrations of the AAs or N in the diet and excreta samples (g/kg).

By using total excreta collection:

$$UP_{AA\ or\ N} = (DI - DE) / DI$$

Where:
DI: daily intake of AAs or N (g/d)
DE: daily excretion of AAs or N (g/d)

The quantitative daily intakes of each AA or N were calculated as FI (g/day) multiplied by the analysed AA or N concentration in the diet. The amount of unexcreted AAs and N was calculated as the amount of AAs and N intake (g/d) multiplied by the UP of them.
Partial total tract digestibility (PTD) of AAs or N from the supplemented TS and MG were obtained by calculating the multiple linear regressions between the quantitative intake and unexcreted amount of AAs or N. Data were pooled across the three periods. The following model was applied to

simultaneously determine the PTD of AAs and N originating from two protein ingredients.

$$Y = \alpha + \beta_b \times X_b + \beta_i \times X_i$$

Where:
Y: daily amount of unexcreted AA or N
α: intercept
β_b: PTD of AA or N originating from the BD
X_b: daily intake of AA or N originating from the BD
β_i: PTD of AA or N originating from protein ingredient (TS or MG)
X_i: daily intake of AA or N originating from protein ingredient (TS or MG)

The resulting data were analysed using the statistical software package SAS (V 9.1, SAS Institute Inc.). Differences between N and AA UP of TS and MG containing diets and amino acids and N PTD of supplemented TS and MG were tested for significance using GLM procedure and the ESTIMATE statement.

3.7.3. Results

During the feeding with the experimental diets, FI of hens decreased from a pre-experimental average of 121 g/d to 86 g/d, 100 g/d, 103 g/d, 89 g/d and 88 g/d, the BW of hens changed from a pre-experimental average of 1971 g to 1877 g, 2099 g, 1979 g, 1955 g and 1937 g and the EP from a pre-experimental average of 94 % to 83 %, 83 %, 93 %, 94 % and 81 % for the BD, 15 % TS, 30 % TS, 15 % MG and 30 % MG containing diets, respectively. The differences between treatments in FI, BW and EP were not significant before and during the feeding with experimental diets (Table 3-19; Appendix F-2).
Unexcreted proportion (UP) of AAs and N was calculated for all diets based on marker measurements (Table 3-20, Appendix F-3). Interaction between diet and experimental period was not significant. The effect of experimental periods on UP of AA and N was not significant, with the

exception of cystine. Unexcreted proportion of N, alanine, arginine, aspartic acid, leucine, lysine, methionine, proline, serine, threonine and valine was significantly different ($P < 0.05$) between diets. UP of all studied AAs and N was statistically the same for the two levels of MG containing diets. UP of all AAs except methionine, serine and thereonine and also N was statistically the same for the two TS levels. The mentioned AAs had higher UP in the diet with the higher TS level than in the diet with the lower TS level, but for N this trend was opposite. Mean UP of all AAs for MG containing diets was 0.88. It was at a minimum for glycine (0.44) and at a maximum for glutamic acid (0.96). Mean UP of all AAs for TS containing diet was 0.87. It was at a minimum for glycine (0.54) and at a maximum for glutamic acid (0.96).

Unexcreted proportion of all AAs (except alanine, glutamic acid, leucine and lysine) and N for pooled data in all 5 diets was significantly higher when calculated based on total excreta than on marker (Table 3-21). Titanium dioxide (TiO_2) recovery was calculated in this experiment based on total excreta collection procedure. For pooled data in all diets the TiO_2 intake of each hen was 0.46 g/d and the excreted amount was 0.42 g/d. This means that 91 % of TiO_2 was recovered in excreta **(**Appendix F-1).

The amounts of unexcreted AAs and N were linearly dependent on the intake of AAs and N for all studied AAs. Examples are shown in Figure 3-5. The chosen multiple linear regression model explained 72 % (N), 85 % (glycine), 99 % (cystine) and 100 % (all other AAs) of the observed variance based on marker calculation. R^2 for all AAs and N except for glycine and N was more than 99 %. This parameter confirms the high relationship between intake and unexcreted amount of AAs (Tables 3-22). For alanine, glutamic acid, glycine, leucine, lysine, proline, serine, threonine and tryptophan a significant difference ($P < 0.05$) in PTD calculated based on marker between the two protein sources existed. Partial total tract digestibilities of AA in marker method calculation ranged from 0.61 (glycine) to 0.97 (arginine) for TS and from 0.45 (glycine) to 0.97 (leucine, methionine and phenylalanine) for MG (Table 3-22).

The chosen multiple linear regression model based on total excreta calculation explained 74 % (N), 87 % (glycine), 99 % (cystine) and 100 %

(all other AAs) of the observed variance. Values for the PTD of AA for TS and MG differed between total excreta method and marker method calculation. It ranged in total excreta method calculation from 0.56 (glycine) to 0.96 (arginine, methionine) for TS and from 0.36 (glycine) to 0.97 (leucine) for MG (Table 3-23).

Table 3-19. *Hen performance data by different diets (BD = basal diet, TS = toasted soybeans, MG = maize gluten, FI = feed intake. EP = egg production, BW = body weight)*

	BD		15 % TS		30 % TS		15 % MG		30 % MG		P value
	Mean	SE	Mean	SE	Mean	SE	Mean	SE	Mean	SE	
Pre-experimental FI (g/d)	121	± *6.3*	120	± *8.0*	122	± *6.5*	126.1	± *5.8*	117.0	± *6.3*	0.86
FI during the experiment (g/d)	86.3	± *7.0*	100.2	± *7.6*	103.0	± *5.8*	89.0	± *3.5*	88.1	± *6.2*	0.33
Pre-experimental EP (%)	89.4	± *12.2*	101.0	± *4.1*	92.2	± *5.8*	91.4	± *6.0*	94.3	± *5.7*	0.91
EP during the experiment (%)	83.0	± *5.8*	83.3	± *10.1*	92.9	± *6.6*	93.9	± *2.9*	81.1	± *7.8*	0.22
Pre-experimental BW (g)	1964	± *77.7*	2036	± *80.6*	1907	± *80.0*	1993	± *71.9*	1954	± *77.0*	0.82
Post-experimental BW (g)	1877	± *86.8*	2099	± *73.6*	1979	± *84.8*	1955	± *78.3*	1937	± *86.1*	0.43

Table 3-20. *Unexcreted proportions of nitrogen and amino acids for the basal diet (BD) and diets with different inclusion rates of toasted soybeans (TS) and maize gluten (MG)*
Data based on marker calculation

	BD		15 % MG		30 % MG		15 % TS		30 % TS		P (ANOVA)		
	Mean	*SE*	Mean	*SE*	Mean	*SE*	Mean	*SE*	Mean	*SE*	Diet	Period	Diet×Period
Nitrogen	0.42 [a]	± *0.01*	0.28 [b]	± *0.01*	0.31 [b]	± *0.01*	0.39 [a]	± *0.02*	0.30 [b]	± *0.02*	<0.01	0.61	0.84
Alanine	0.84 [b]	± *0.01*	0.90 [a]	± *0.00*	0.92 [a]	± *0.00*	0.84 [b]	± *0.01*	0.86 [b]	± *0.01*	<0.01	0.40	0.62
Arginine	0.89 [b]	± *0.00*	0.92 [a]	± *0.00*	0.93 [a]	± *0.00*	0.92 [a]	± *0.00*	0.94 [a]	± *0.00*	<0.01	0.48	0.39
Aspartic acid	0.80 [b]	± *0.01*	0.84 [ab]	± *0.00*	0.86 [ab]	± *0.01*	0.84 [ab]	± *0.01*	0.87 [a]	± *0.01*	<0.01	0.29	0.31
Cystine	0.86	± *0.01*	0.83	± *0.01*	0.83	± *0.01*	0.83	± *0.01*	0.83	± *0.01*	0.04	0.01	0.38
Glutamic acid	0.96	± *0.00*	0.96	± *0.00*	0.96	± *0.00*	0.96	± *0.00*	0.96	± *0.00*	0.29	0.07	0.95
Glycine	0.46	± *0.03*	0.42	± *0.04*	0.46	± *0.02*	0.54	± *0.05*	0.54	± *0.04*	0.81	0.62	0.24
Isoleucine	0.91	± *0.00*	0.92	± *0.01*	0.92	± *0.01*	0.90	± *0.01*	0.91	± *0.01*	0.12	0.14	0.76
Leucine	0.93 [b]	± *0.00*	0.95 [a]	± *0.00*	0.96 [a]	± *0.00*	0.92 [b]	± *0.01*	0.93 [b]	± *0.01*	<0.01	0.66	0.86
Lysine	0.84 [b]	± *0.01*	0.84 [b]	± *0.01*	0.85 [b]	± *0.01*	0.88 [ab]	± *0.01*	0.89 [a]	± *0.01*	0.01	0.45	0.83
Methionine	0.93 [ab]	± *0.00*	0.94 [a]	± *0.00*	0.94 [a]	± *0.00*	0.91 [b]	± *0.00*	0.94 [a]	± *0.01*	<0.01	0.11	0.85
Phenylalanine	0.94	± *0.00*	0.95	± *0.00*	0.95	± *0.00*	0.93	± *0.00*	0.94	± *0.00*	0.04	0.56	0.94
Proline	0.95 [ab]	± *0.00*	0.96 [a]	± *0.00*	0.95 [ab]	± *0.00*	0.94 [b]	± *0.00*	0.95 [ab]	± *0.00*	0.02	0.18	0.81
Serine	0.90 [b]	± *0.01*	0.92 [a]	± *0.00*	0.92 [a]	± *0.00*	0.90 [b]	± *0.00*	0.91 [a]	± *0.01*	0.02	0.07	0.21
Threonine	0.80 [b]	± *0.01*	0.83 [ab]	± *0.00*	0.85 [a]	± *0.01*	0.80 [b]	± *0.01*	0.83 [a]	± *0.01*	<0.01	0.16	0.25
Tryptophan	0.86 [ab]	± *0.00*	0.84 [ab]	± *0.01*	0.84 [b]	± *0.01*	0.85 [ab]	± *0.00*	0.87 [a]	± *0.01*	0.07	0.53	0.60
Valine	0.90 [ab]	± *0.00*	0.92 [a]	± *0.01*	0.92 [ab]	± *0.01*	0.90 [b]	± *0.01*	0.91 [ab]	± *0.01*	0.02	0.42	0.17

[a, b] *Parameters in one row not sharing a common superscript are significantly different between diets ($P < 0.05$)*

Table 3-21. *Comparison of unexcreted proportion of amino acids and nitrogen between calculations based on marker and total excreta collection*

(Pooled data for all 5 diets)

	Total excreta		Marker		T test
	Mean	SE	Mean	SE	
Nitrogen	0.40 [a]	± *0.02*	0.34 [b]	± *0.01*	<0.01
Alanine	0.88	± *0.01*	0.87	± *0.01*	0.15
Arginine	0.93 [a]	± *0.00*	0.92 [b]	± *0.00*	0.05
Aspartic acid	0.86 [a]	± *0.00*	0.84 [b]	± *0.01*	0.02
Cystine	0.85 [a]	± *0.00*	0.84 [b]	± *0.00*	0.04
Glutamic acid	0.96	± *0.00*	0.96	± *0.00*	0.11
Glycine	0.53 [a]	± *0.02*	0.48 [b]	± *0.02*	0.04
Isoleucine	0.92 [a]	± *0.00*	0.91 [b]	± *0.00*	0.03
Leucine	0.94	± *0.00*	0.94	± *0.00*	0.13
Lysine	0.87	± *0.00*	0.86	± *0.01*	0.07
Methionine	0.94 [a]	± *0.00*	0.93 [b]	± *0.00*	0.03
Phenylalanine	0.95 [a]	± *0.00*	0.94 [b]	± *0.00*	0.03
Proline	0.96 [a]	± *0.00*	0.95 [b]	± *0.00*	0.02
Serine	0.92 [a]	± *0.00*	0.91 [b]	± *0.00*	0.01
Threonine	0.84 [a]	± *0.00*	0.82 [b]	± *0.00*	0.01
Tryptophan	0.86 [a]	± *0.00*	0.85 [b]	± *0.00*	0.01
Valine	0.92 [a]	± *0.00*	0.91 [b]	± *0.00*	0.03

[a, b] *Parameters in one row not sharing a common superscript are significantly different between calculation methods ($P < 0.05$)*

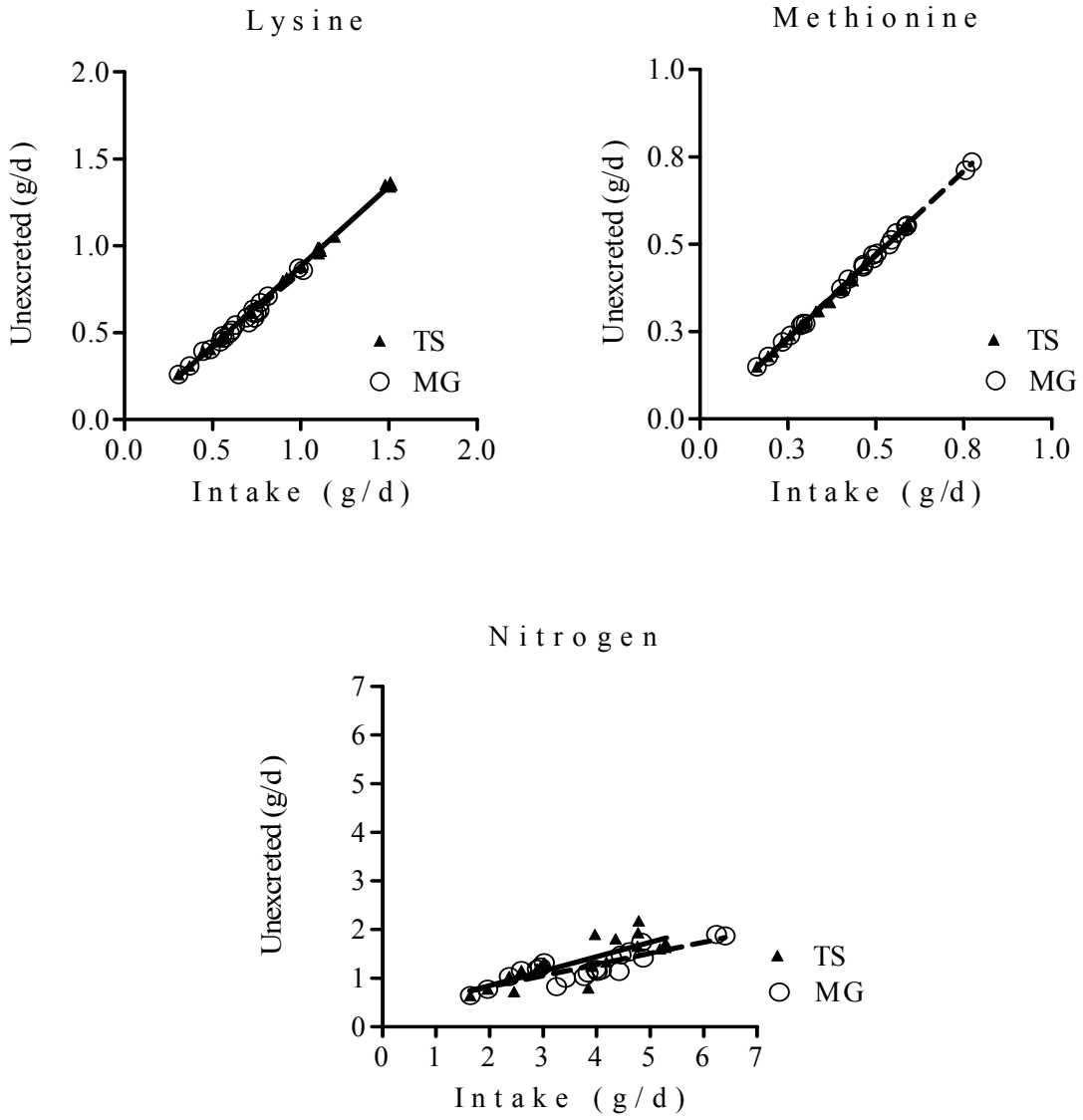

Figure 3-5. R*elationship between intake and unexcreted lysine, methionine and nitrogen in laying hens fed on different dietary concentrations of toasted soybeans (TS) and maize gluten (MG)*

Table 3-22. *Partial total tract digestibility of amino acids and nitrogen for toasted soybeans (TS) and maize gluten (MG) calculated based on marker and determined by multiple linear regression analysis*

	R^2	TS		MG		Difference	P
		Estimate	*SE*	Estimate	*SE*		value
Nitrogen	0.72	0.19	± *0.07*	0.17	± *0.05*	0.02 ± *0.07*	0.75
Alanine	1.00	0.88 [b]	± *0.02*	0.95 [a]	± *0.01*	- 0.07 ± *0.02*	<0.01
Arginine	1.00	0.97	± *0.01*	0.96	± *0.01*	0.01 ± *0.01*	0.12
Aspartic acid	1.00	0.90	± *0.01*	0.90	± *0.01*	0.00 ± *0.01*	0.86
Cystine	0.99	0.77	± *0.04*	0.77	± *0.03*	0.00 ± *0.03*	0.92
Glutamic acid	1.00	0.94 [b]	± *0.01*	0.96 [a]	± *0.00*	- 0.02 ± *0.01*	0.04
Glycine	0.85	0.61 [a]	± *0.08*	0.45 [b]	± *0.08*	0.16 ± *0.07*	0.03
Isoleucine	1.00	0.92	± *0.01*	0.93	± *0.01*	- 0.01 ± *0.01*	0.40
Leucine	1.00	0.92 [b]	± *0.01*	0.97 [a]	± *0.00*	- 0.05 ± *0.01*	<0.01
Lysine	1.00	0.93 [a]	± *0.01*	0.86 [b]	± *0.03*	0.07 ± *0.02*	<0.01
Methionine	1.00	0.96	± *0.01*	0.97	± *0.01*	0.01 ± *0.01*	0.93
Phenylalanine	1.00	0.95	± *0.01*	0.97	± *0.01*	- 0.02 ± *0.01*	0.06
Proline	1.00	0.92 [b]	± *0.02*	0.96 [a]	± *0.01*	- 0.04 ± *0.02*	0.02
Serine	1.00	0.92 [b]	± *0.01*	0.94 [a]	± *0.01*	- 0.02 ± *0.01*	0.01
Threonine	1.00	0.87 [b]	± *0.02*	0.90 [a]	± *0.01*	- 0.03 ± *0.02*	0.03
Tryptophan	1.00	0.88 [a]	± *0.01*	0.83 [b]	± *0.02*	0.05 ± *0.02*	0.01
Valine	1.00	0.92	± *0.02*	0.94	± *0.01*	- 0.02 ± *0.01*	0.16

[a, b] *Parameters in one row not sharing a common superscript are significantly different between protein sources (P < 0.05)*

Table 3-23. *Partial total tract digestibility of amino acids and nitrogen for toasted soybeans (TS) and maize gluten (MG) calculated based on total excreta and determined by multiple linear regression analysis*

	R^2	TS		MG		Difference	P value
		Estimate	*SE*	Estimate	*SE*		
Nitrogen	0.74	0.11	± *0.07*	0.07	± *0.05*	0.04 ± *0.06*	0.57
Alanine	1.00	0.87 [b]	± *0.02*	0.94 [a]	± *0.01*	- 0.07 ± *0.02*	<0.01
Arginine	1.00	0.96 [a]	± *0.01*	0.95 [b]	± *0.01*	0.01 ± *0.01*	0.02
Aspartic acid	1.00	0.90	± *0.01*	0.89	± *0.01*	0.01 ± *0.01*	0.44
Cystine	0.99	0.75	± *0.04*	0.74	± *0.03*	0.01 ± *0.03*	0.63
Glutamic acid	1.00	0.94 [b]	± *0.01*	0.95 [a]	± *0.01*	- 0.01 ± *0.01*	0.05
Glycine	0.87	0.56 [a]	± *0.07*	0.36 [b]	± *0.08*	0.20 ± *0.07*	<0.01
Isoleucine	1.00	0.91	± *0.01*	0.92	± *0.01*	- 0.01 ± *0.01*	0.55
Leucine	1.00	0.91 [b]	± *0.01*	0.97 [a]	± *0.00*	- 0.06 ± *0.01*	<0.01
Lysine	1.00	0.92 [a]	± *0.01*	0.83 [b]	± *0.02*	0.09 ± *0.02*	<0.01
Methionine	1.00	0.96	± *0.01*	0.96	± *0.01*	0.00 ± *0.01*	0.77
Phenylalanine	1.00	0.94	± *0.01*	0.96	± *0.01*	- 0.02 ± *0.01*	0.06
Proline	1.00	0.91 [b]	± *0.02*	0.95 [a]	± *0.01*	- 0.04 ± *0.02*	0.01
Serine	1.00	0.91 [b]	± *0.01*	0.93 [a]	± *0.01*	- 0.02 ± *0.01*	0.02
Threonine	1.00	0.85 [b]	± *0.02*	0.88 [a]	± *0.01*	- 0.03 ± *0.01*	0.05
Tryptophan	1.00	0.87 [a]	± *0.01*	0.80 [b]	± *0.02*	0.07 ± *0.02*	<0.01
Valine	1.00	0.91	± *0.02*	0.93	± *0.01*	- 0.02 ± *0.01*	0.20

[a, b] *Parameters in one row not sharing a common superscript are significantly different between protein sources (P < 0.05)*

3.7.4. Discussion

For most studied AAs, the UP was higher in the diets with higher concentrations of AAs and N than in the diets with lower concentrations. Likewise the reason is that the proportion of endogenous AA by increasing AA intake becomes lower and their role becomes quantitatively less relevant (Sauer *et al.,* 2000; Lemme *et al.,* 2004). However little is known about excretion of protein and free amino acids in urine. Jirjis *et al.* (1997) reported that increasing the protein content of diets fed to turkeys from 228 to 330 g per kg did not influence the urinary excretion of amino acids significantly.

For N the UP was not higher in the diets with higher concentrations of N than with lower concentrations. This is probably the consequence of nitrogenous

compounds excreted with urine. Higher concentrations of protein in the diet may exceed the requirements of hens and then nitrogenous compounds are excreted in urine. Fernández-Fígares *et al.* (1996) showed that the excretion of total N, uric acid-N, ammonia-N and urea-N significantly ($P < 0.05$) increased with increase in protein intake and significantly ($P < 0.05$) decreased with improvement in dietary protein quality by free AA supplementation.

In the present study R^2 of all regression lines except N and glycine was high (99 to 100 %) and this confirms the high relationship between intake of AAs and their unexcreted amount. It also means that the amounts of unexcreted AAs depend linearly on the intake of AAs for all the studied AAs (except glycine; Figure 3-5). These results were reported precaecally also by other researches (Short *et al.,* 1999; Ishibashi and Yonemochi, 2003; Rodehutscord *et al.,* 2004). The lower R^2 for N and glycine may be the effect of nitrogenous compounds that originate from urine. By the author's experiences the problem for detection of glycine during laboratory analysis in high uric acid-containing samples like excreta (Appendix F-5) may be the other reason for the lower R^2 of glycine found in comparison with the other AAs. It may be because uric acid is converted to glycine upon hydrolysis during laboratory analyses (Jirjis *et al.*, 1997). This problem also increased the SE of UP of glycine for the diets.

Multiple linear regression analysis demonstrated significant differences ($P <0.05$) in PTD between the two protein sources for some AAs and N. These results calculated based on marker and total excreta collection showed higher PTD of glycine, lysine and tryptophan for TS than MG, equal PTD of aspartic acid, cystine, isoleucine, methionine, phenylalanine and valine for TS and MG, and in the other AAs except arginine the PTD for TS was less than MG. In calculation based on total excreta collection PTD of arginine was significantly higher for TS than MG, but in marker calculation it was similar between TS and MG. These differences between TS and MG were sometimes as high as 7 percent (alanine). The standard error of digestibility estimates in this experiment except N and glycine was low (between 1 to 3 percent). These results confirm regression approaches as a method for protein ingredient AA digestibility determination by using the excreta of caecectomised hens without the need for measuring endogenous AA losses.

Significant variation existed in PTD of AAs between TS and MG and within one protein source for hens. It seems that the main reason for the detected differences between these two protein ingredients is the low SE of measurements in this method except for nitrogen and glycine. The ranking of individual AAs regarding their PTD is different between TS and MG (Appendix F-6). In this experiment it was revealed that the recovery rate of markers in excreta was 91 % (Appendix F-1). This may bring some criticisms against the usefulness of titanium dioxide as an indigestible marker for digestibility studies. But the differences of diet UP of N and AA between calculations by marker and total excreta collection were low and when PTD of AAs and N calculated based on marker and total excreta collection were compared, it was revealed that there are no significant differences between these two methods of calculation (Appendices F-7 and F-8). In the next chapter PTD of AA values measured in this experiment will be compared with PPD values from Experiment 2.

3.7.5. Conclusion

It was concluded that significant variation exists in PTD of AAs between TS and MG and within one protein source in caecectomised laying hens. The ranking of individual AAs regarding their PTD is different between TS and MG. The main reason for detected differences between these two protein ingredients is the low SE of measurements in this method except for nitrogen and glycine. This fact will be discussed in detail in the general discussion and conclusion chapter.