4. General Discussion and Conclusion

In the first experiment it was concluded that the digesta from the last two thirds of the gut between the MD and the ICCJ should be sampled in AA PC digestibility measurements. In the second experiment this procedure was applied to measure AA PPD for TS and MG. The chosen approach used the linear relationship between intake and digested amounts, and 2 supplemented levels of the test protein were investigated to calculate the regression. Because the relationship always was clearly linear it was of interest whether the consideration of only one supplementary level could yield the same accuracy of measurements. In model calculations, therefore only the data from the diets containing zero and the highest inclusion rate of RM, SM, TS or MG in experiments 1, 2 and 6 were included. The slopes in no case differed significantly from the values calculated when all the three inclusion levels were included (Appendices A-4, A-5, B-4, B-5, F-9, F-10). In the face of the very strong linear relationships that were described in these experiments and assuming that such linearity is observed with other protein ingredients as well, the conclusion that 2 instead of 3 levels of inclusion are sufficient for the regression approach appears justified.

In the third, fourth and fifth experiment the possibility of measuring diet UP of AAs and N and energy metabolisability in caecectomised hens and effects of age and using marker on it were studied. In Experiment 4 it was shown that age has a significant effect on AA and N UP and energy metabolisability. It was concluded that the effect of age on AA digestibility should be considered in standard feed protein evaluation. Based on the present data it is suggested to use hens that are not older than 40 weeks.

Linear regression relationship between AAs (except for glycine) intake and unexcreted amounts of them in caecectomised laying hens for TS and MG were confirmed in Experiment 6. In this experiment caecectomised hens were used for measuring AAs and N PTD for TS and MG. In this chapter the results will be compared with the results of Experiment 2 where the AA and N PPD of the same diets and protein ingredients were measured. The comparison between these two methods of measurements (PC and TT) for diets was done based on pooled data for all 5 diets and using t-test of SAS software (V 9.1, SAS Institute

Inc.; Table 4-1). Comparisons for test protein ingredients (TS and MG) were independently done by using simple linear approach of Prism software (V. 4, Graph pad software, Inc. 2003; Tables 4-2 and 4-3, Figure 4-1). These comparisons showed that the diet UP of all AAs (except for glycine) in TT method was significantly higher than diet DC in PC method (Table 4-1). The reason for this may originate from postileal degradation of AAs by gastrointestinal tract micro-organisms (Kadim *et al.,* 2002) or by AA absorption. The other fact is that the caecectomised hens used for the TT method were older than the hens used for the PC method and, therefore, as shown in Experiment 3, they had higher UP of AAs in TT method than DC in younger intact hens in PC method. For glycine it seems that the significantly lower amount in TT method in comparison with PC method originates from urine (Parsons *et al.*, 1983; Jirjis *et al.*, 1997). By author's experiences the problem for detection of glycine during laboratory analyses in high uric acid-containing samples like excreta samples was observed (Appendix F-5). Jirjis *et al.* (1997) reported that uric acid may be converted to glycine upon hydrolysis during laboratory analyses. This problem increased also the standard error (SE) of un-excretion measurements for glycine in TT method. The other reason may be that the GIT micro-organisms produce postileally single amino acid like glycine and nitrogenous compound like ammonia rather than other complex amino acids but this hypothesis needs more investigations. Diet digestibility of N in TT method was lower than diet digestibility in PC method. It is clear that this difference also originates from the excretion of nitrogenous compounds into the urine in TT method.

Further comparisons showed that, although there are significant differences in diet DC and UP of AAs between these two methods, after correction for basal EAA by regression approach no differences between AAs PPD and PTD for protein ingredients remained (except for glycine and N; Tables 4-2 and 4-3). These results suggest that protein ingredients may be investigated for their AA digestibility with caecectomised hens using the regression approach. Regression approach as a standard method for correction of basal EAA can be approved also in caecectomised hens. It seems that by using the regression approach the criticism that arises from age effect on measurement of protein ingredient digestibility has no sense because the digestibility data originated from

regression approach can correct the basal EAA at the same time for measuring protein ingredient digestibility.

Using caecectomised hens in comparison to measurements with the PC method have some advantages. By using caecectomised hens fewer animals are needed, repeated measurements with each hen are possible and because of less SE of observations, the existing differences between different protein ingredients are easier to detect (Figure 4-2). Less SE of observations in PTD measurements with caecectomised hens may be because of no necessity for pooling the samples within each experimental unit. In this method quantitative excreta collection always provides a sample size that is big enough for chemical analyses. This helps also to avoid slaughtering a large number of birds in the process of sample collection.

It is concluded that regression between intake and unexcretd amount of amino acid in caecectomised laying hens can be used as a standard method in AAD measurements for protein ingredients only with two inclusion levels of protein source. If the PC method is used, the digesta of last two subsections of the intestine between MD and 2 cm anterior to ICCJ for AAD measurements are advisable.

Table 4-1. *Comparison between precaecal digestibility coefficient (DC) and total tract unexcreted proportion (UP) calculated based on pooled data in all 5 diets and using marker*

	DC		UP		T test
	Mean	SE	Mean	SE	
Nitrogen	0.87 [a]	± *0.01*	0.34 [b]	± *0.01*	<0.01
Alanine	0.84 [b]	± *0.01*	0.87 [a]	± *0.01*	0.04
Arginine	0.85 [b]	± *0.01*	0.92 [a]	± *0.00*	<0.01
Aspartic acid	0.78 [b]	± *0.01*	0.84 [a]	± *0.01*	<0.01
Cystine	0.81 [b]	± *0.01*	0.84 [a]	± *0.00*	<0.01
Glutamic acid	0.94 [b]	± *0.00*	0.96 [a]	± *0.00*	<0.01
Glycine	0.81 [a]	± *0.01*	0.48 [b]	± *0.02*	<0.01
Isoleucine	0.87 [b]	± *0.01*	0.91 [a]	± *0.00*	<0.01
Leucine	0.89 [b]	± *0.01*	0.94 [a]	± *0.00*	<0.01
Lysine	0.84 [b]	± *0.01*	0.86 [a]	± *0.01*	0.05
Methionine	0.88 [b]	± *0.01*	0.93 [a]	± *0.00*	<0.01
Phenylalanine	0.90 [b]	± *0.01*	0.94 [a]	± *0.00*	<0.01
Proline	0.92 [b]	± *0.00*	0.95 [a]	± *0.00*	<0.01
Serine	0.85 [b]	± *0.01*	0.91 [a]	± *0.00*	<0.01
Threonine	0.75 [b]	± *0.01*	0.82 [a]	± *0.00*	<0.01
Tryptophan	0.78 [b]	± *0.01*	0.85 [a]	± *0.00*	<0.01
Valine	0.85 [b]	± *0.01*	0.91 [a]	± *0.00*	<0.01

[a, b] *Parameters in one row not sharing a common superscript are significantly different between methods (P < 0.05; pooled data from all diet in Experiment 2 and Experiment 6)*

Table 4-2. *Comparison between partial precaecal digestibility (PPD) and partial total tract digestibility (PTD) of amino acids and nitrogen for toasted soybeans, calculated based on marker and determined by simple linear regression analysis*

	PPD			PTD			Slope	Intercept
	Slope	*SE*	R^2	Slope	*SE*	R^2	P value	P value
Nitrogen	0.92[a]	*0.03*	0.98	0.30[b]	*0.05*	0.65	<0.01	<0.01
Alanine	0.91	*0.04*	0.97	0.88	*0.01*	0.99	0.49	0.06
Arginine	0.95	*0.02*	0.99	0.96	*0.00*	1.00	0.77	<0.01
Aspartic acid	0.90	*0.02*	0.99	0.89	*0.01*	1.00	0.71	<0.01
Cystine	0.85	*0.04*	0.97	0.81	*0.02*	0.99	0.34	0.01
Glutamic acid	0.95	*0.02*	0.99	0.96	*0.00*	1.00	0.70	<0.01
Glycine	0.88[a]	*0.03*	0.98	0.67[b]	*0.06*	0.86	0.01	<0.01
Isoleucine	0.92	*0.03*	0.98	0.92	*0.01*	1.00	0.94	<0.01
Leucine	0.92	*0.03*	0.98	0.93	*0.01*	1.00	0.78	<0.01
Lysine	0.91	*0.03*	0.99	0.92	*0.01*	1.00	0.82	0.05
Methionine	0.94	*0.03*	0.98	0.95	*0.01*	1.00	0.81	<0.01
Phenylalanine	0.94	*0.02*	0.99	0.95	*0.01*	1.00	0.72	<0.01
Proline	0.93	*0.04*	0.97	0.94	*0.01*	1.00	0.70	<0.01
Serine	0.92	*0.03*	0.99	0.91	*0.01*	1.00	0.80	<0.01
Threonine	0.87	*0.04*	0.97	0.85	*0.01*	0.99	0.57	<0.01
Tryptophan	0.86	*0.03*	0.97	0.87	*0.01*	1.00	0.64	<0.01
Valine	0.90	*0.03*	0.98	0.92	*0.01*	1.00	0.44	<0.01

[a, b] Parameters in one row not sharing a common superscript are significantly different between methods ($P < 0.05$; data from Experiment 2 and Experiment 6)

Table 4-3. *Comparison between partial precaecal digestibility (PPD) and partial total tract digestibility of amino acids and nitrogen for maize gluten, calculated based on marker and determined by simple linear regression analysis*

	PPD			PTD			Slope	Intercept
	Slope	*SE*	R^2	Slope	*SE*	R^2	P value	P value
Nitrogen	0.91[a]	*0.04*	0.97	0.23[b]	*0.03*	0.74	<0.01	<0.01
Alanine	0.94	*0.03*	0.99	0.95	*0.01*	1.00	0.66	0.01
Arginine	0.93	*0.04*	0.98	0.95	*0.01*	1.00	0.62	<0.01
Aspartic acid	0.89	*0.04*	0.97	0.90	*0.01*	1.00	0.98	0.01
Cystine	0.87	*0.05*	0.94	0.80	*0.02*	0.99	0.19	<0.01
Glutamic acid	0.93	*0.03*	0.98	0.96	*0.00*	1.00	0.26	<0.01
Glycine	0.88[a]	*0.04*	0.97	0.49[b]	*0.06*	0.80	<0.01	<0.01
Isoleucine	0.92	*0.04*	0.98	0.92	*0.01*	1.00	0.86	<0.01
Leucine	0.94	*0.02*	0.99	0.97	*0.00*	1.00	0.22	<0.01
Lysine	0.85	*0.08*	0.88	0.86	*0.02*	0.99	0.89	0.10
Methionine	0.93	*0.03*	0.98	0.95	*0.01*	1.00	0.40	<0.01
Phenylalanine	0.94	*0.03*	0.99	0.96	*0.00*	1.00	0.36	<0.01
Proline	0.95	*0.03*	0.99	0.96	*0.01*	1.00	0.58	<0.01
Serine	0.92	*0.03*	0.98	0.93	*0.01*	1.00	0.74	<0.01
Threonine	0.89	*0.05*	0.96	0.89	*0.01*	1.00	0.91	<0.01
Tryptophan	0.83	*0.08*	0.87	0.84	*0.02*	0.99	0.80	<0.01
Valine	0.92	*0.04*	0.97	0.94	*0.01*	1.00	0.50	<0.01

[a, b] *Parameters in one row not sharing a common superscript are significantly different between methods ($P < 0.05$; data from Experiment 2 and Experiment 6)*

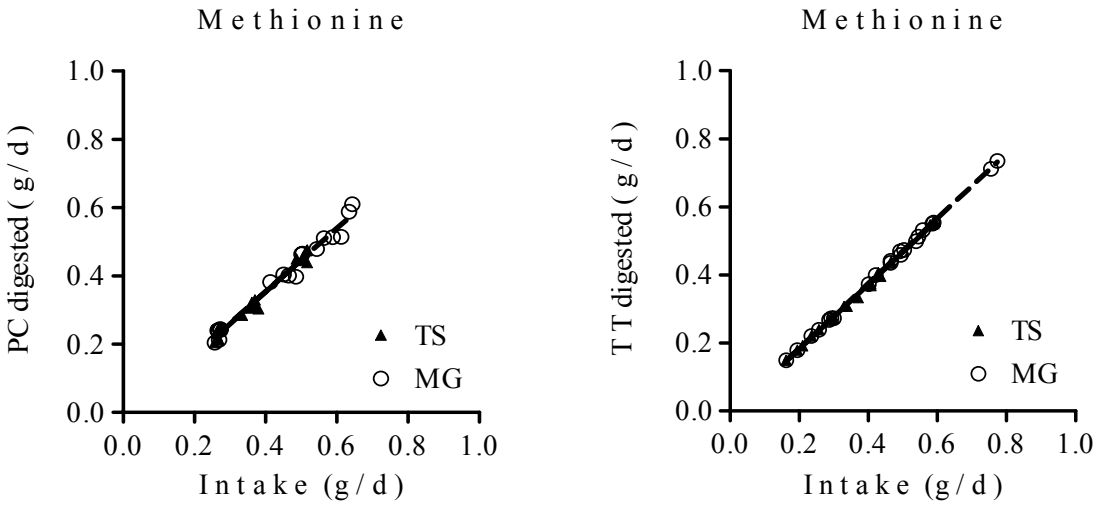

Figure 4-1: *Relationship between intake and digested amounts of methionine from toasted soybeans (TS) and maize gluten (MG), determined precaecally (PC) or based on total tract (TT) method*

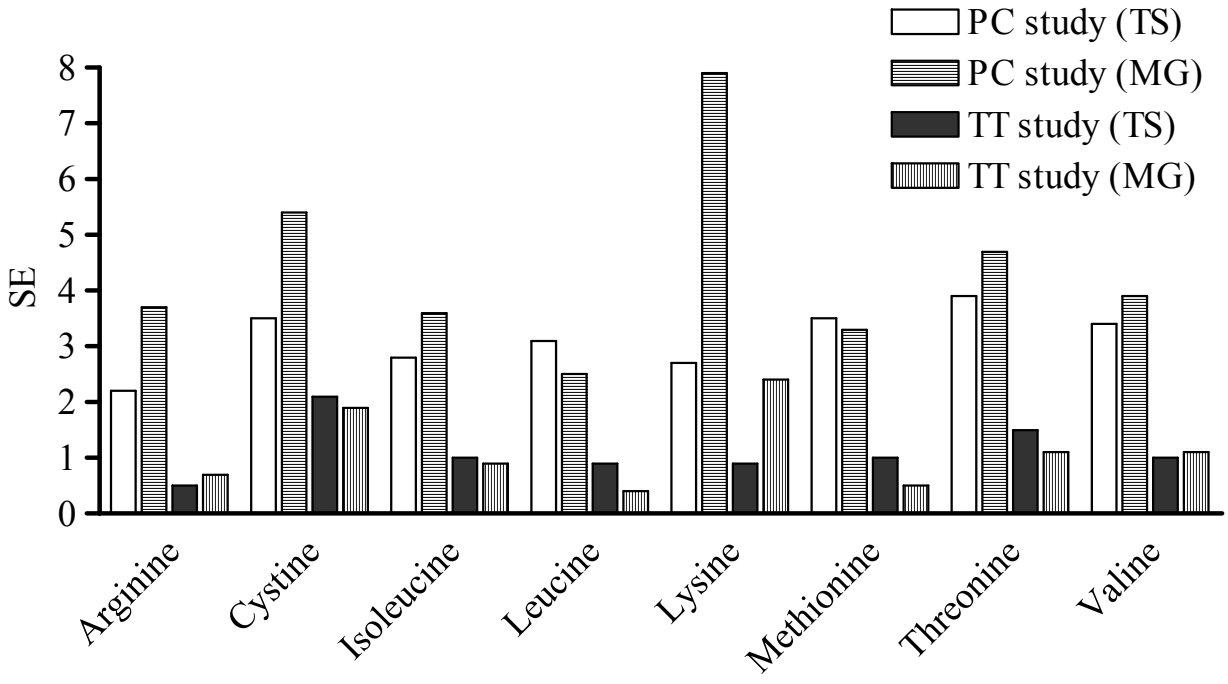

Figure 4-2. *Standard error (SE) of amino acid digestibility measurements in precaecal (PC) and total tract (TT) method (%) for toasted soybeans (TS) and maize gluten (MG)*