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# Studies on energy and tryptophan requirements of growing pigs

Dissertation zur Erlangung des Doktorgrades der Agrar- und Ernährungswissenschaftlichen Fakultät der Christian-Albrechts-Universität zu Kiel

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# LIST OF ABBREVIATIONS

AA	Amino acid
BF	Body fat mass
BP	Body protein mass
BW	Body weight
BW <sup>0,75</sup>	Metabolic body weight
BWG	Body weight gain
CA	Crude ash
CP	Crude protein
DM	Dry matter
EBW	Empty body weight
EE	Ether extract
FFM	Fat-free mass
FOM	Fat-O-meater
FI	Feed intake
FGR	Feed to gain ratio
IME	Intake metabolizable energy
k <sub>pf</sub>	Partial efficiency of utilization of ME for energy retention as protein plus fat
LCT	Lower critical temperature
LNAA	Large neutral amino acid
Lys	Lysine
ME	Metabolizable energy
$ME_a$	ME requirement for activity
$ME_{g}$	ME requirement for body weight gain
ME <sub>f</sub>	ME requirement for fat accretion
$ME_{m}$	ME requirement for maintenance
$ME_{p}$	ME requirement for protein accretion
ME <sub>x</sub>	Energy requirement of ME above ME <sub>m</sub> and ME <sub>g</sub>
Ν	Nitrogen
Р	Probability (level of significance)
pcd	Precaecally digestible
RE	Energy retention
SD	Standard deviation
SEM	Standard error of means
Trp	Tryptophan

1. GENERAL INTRODUCTION

## 1.1 Background

Published recommendations for the nutrient and energy supply to growing pigs were commonly derived from animal trials conducted under controlled experimental conditions. The main objective of these trials was to separately determine the animals' requirements for maintenance, body weight gain (BWG), and other (production) purposes as well as the respective nutrient and energy use efficiencies in order to be able to estimate the total nutrient and energy requirements of animals at different production levels. They try to simultaneously optimize several production goals such as animal growth rates, feed to gain ratios, or carcass quality. Nevertheless, these estimates do not fully account for differences in the animals' nutrient and energy requirements due to varying management and feeding practices in commercial production. However, every deprivation in the daily BWG of growing pigs will delay their slaughter age and thus, reduce economical profit. Hence, a more accurate quantification of the energy and nutrient requirements of growing pigs with respect to varying housing and environmental conditions as well as diet compositions are needed.

## 1.2 Energy requirement

Under commercial housing conditions pronounced differences can be observed between the actual BWG of growing pigs and that predicted from published feeding recommendations for the metabolizable energy (ME) supply for maintenance (ME<sub>m</sub>) and growth (ME<sub>g</sub>). These recommendations were commonly derived from studies conducted under well-controlled experimental conditions with pigs kept in individual metabolic cages. However, these estimates cannot fully be applied to commercial production systems, because factors such as climatic and housing conditions as well as social interactions between pigs and hence, their physical activity may largely increase their ME expenditures. Hence, the differences between actual ME<sub>m</sub> requirements of pigs and those considered in published feeding recommendations may partly be responsible for the discrepancy between expected and observed BWGs. Moreover, ME requirements of animals of different genotypes and sexes may vary. For example, selecting for higher lean percentages in pigs in the last years has indirectly selected for greater aggression of pigs (Knap *et al.*, 2002) and thus, increased their energy needs for activity. Furthermore, not all pigs will be affected equally when exposed to the same stress factor, since they not only react differently to their environment, but may also influence each other (Muir and Schinckel, 2002). Hence, in group-housed pigs, dominant pen mates may constantly stress others, while they themselves remain relatively unaffected (Nielsen, 1999). As a consequence, ME intake may be sufficient to satisfy the ME needs of some pigs for maintenance, growth, and their responses to environmental stress factors, while it suppresses performance of others. Hence, the aim of our study was to quantify the ME requirements of growing pigs kept under commercial housing conditions for maintenance and growth as well as for their responses to a combination of mentioned factors. The latter was seen as the difference between the animals' ME intake and their ME<sub>m</sub> and ME<sub>q</sub> expenditures.

# 1.3 Tryptophan

Tryptophan (Trp) is involved in several biological functions. Apart from being an essential precursor for body protein synthesis, Trp intake increases serotonin production (Meunier-Salaün *et al.*, 1991, Sève, 1999) and can thus play a key role in appetite and feed intake regulation (Eder, 2001). Furthermore, Trp has been associated with the control of stress and immune responses (Le Floc'h and Sève, 2007), and pigs' Trp requirements may therefore differ between different husbandry conditions.

Pig diets in Europe are commonly based on wheat and barley, so that Trp is frequently the fourth limiting amino acid (AA) for growth after lysine, threonine and the sulphur containing AAs. Because of the low Trp content and digestibility in corn, it is even the second limiting AA in corn-based diets that are commonly used to feed growing pigs in North America (Jansman *et al.*, 2009). The adequate Trp supply in relation to other AAs may thus increase the animals' BWG and improve nutrient use efficiency, and can thereby reduce the need of an excess feeding of protein. It may thus largely contribute to minimize faecal and urinary nitrogen excretion (Mosenthin and Rademacher, 2003), which has become a major environmental

concern in the last decades. However, this cannot be done unless the availability of dietary AAs as well as the actual AA requirements of pigs are known.

Trp requirements are commonly expressed in relation to dietary Lys, the first limiting AA in most pig diets and several studies have already been published that analyzed the response of pigs to different dietary Trp : Lys ratios. Their aim was to derive recommendations for the critical dietary Trp : Lys ratio that allows for maximum growth and lean deposition of growing pigs from dose-response curves. However, a wide range of critical ratios were determined in these studies which can partly be attributed to differences in the laboratory methods and the mathematical models used for data analysis (Susenbeth, 2006). Furthermore, the animals' Trp requirements and hence, the critical Trp : Lys ratio appear to be affected by diet composition, for example due to differences in the AA digestibility or the contents of other large neutral AAs in different feed stuffs (Jansman *et al.*, 2009).

Hence, the aim of the second study was to determine the critical Trp : Lys ratio required for maximum response in young pigs of 15 - 35 kg body weight (BW) kept under commercial housing conditions and to investigate, whether this ratio depends on the type of cereal (wheat-barley *versus* corn) used in diet formulation and to which extent results may differ, if different mathematical models are used for data analysis.

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2. ENERGY REQUIREMENTS OF GROWING PIGS UNDER COMMERCIAL HOUSING CONDITIONS

# 2.1 Abstract

Scientifically derived recommendations for the energy supply to growing pigs are generally based on estimates of the metabolizable energy (ME) requirements for maintenance ( $ME_m$ ) and protein ( $ME_p$ ) and fat ( $ME_l$ ) retention. It is supposed that animals are kept within the zone of thermoneutrality and that their physical activity is not elevated. These assumptions might not always be true for practical housing conditions, and it is difficult to quantify the additional energy needed for thermoregulation and physical activity. Hence, at a given ME intake, differences can occur between the actual growth rates and those predicted from the recommendations. To quantify such differences three trials were carried out under commercial farming conditions with pigs growing from 25 to 120 kg of body weight (BW). In each trial, 624 castrated males and females pigs were allocated to four different feeding groups that were distributed over 24 double pens. The animals received a liquid diet, which consisted of cereal grains, soybean meal, and a mineral-vitamin premix and varied in dietary lysine concentration. The rations were provided by a sensor-controlled liquid feeder four times daily according to the animals' feed intake capacity. BW of individual animals was recorded every three weeks. Protein, fat, and energy retention (RE) was derived from carcass composition and BW gain. The efficiency of ME utilization for growth ( $k_{of}$ ) and ME<sub>m</sub> requirements were taken from the current German feeding standards. The difference between ME intake and ME<sub>m</sub> plus ME required for growth (ME<sub>a</sub> =  $RE/k_{pf}$ ) was calculated and seen as the ME required for purposes other than maintenance and growth (ME<sub>x</sub>). ME<sub>x</sub> accounted for 2.1, 16.8, and 21.4% of the animals' ME intake in trial 1, 2, and 3, respectively, showing a small variation within trials and being higher in female than in castrated male pigs. Dietary Lys concentration did not affect MEx. It is concluded from this study that total ME requirements of pigs kept under commercial housing conditions can be considerably higher or, vice versa, growth levels lower than those predicted from feeding standards since they ignore ME<sub>x</sub>. ME<sub>x</sub> can be used as an indicator for the guality of livestock systems. However, further studies are needed to identify and to quantify the key factors responsible for ME<sub>x</sub> to allow for more precise recommendations for the energy supply to commercially raised pigs.

# 2.2 Introduction

Since feed prices have largely increased in the last years, feed costs currently account for more than half of total cost in livestock production (Thieme, 2008). It is likely that this proportion will also remain high in future due to the world-wide growing demand for food of animal origin as well as for the alternative use of feedstuffs for bio-fuel production. According to estimates of commercial feed mills (personal communication), the supply of metabolizable energy (ME) to animals accounts for 70 to 80% of the total feed cost. Therefore, a reduction in the amount of ME required per unit produced is a key factor for economical productivity. In growing pigs as well as in other livestock species, recommendations for an adequate energy supply to the animals are generally based on the factorial approach, which relies on estimates of the energy requirements for maintenance and growth (ARC, 1981; NRC, 1998; GfE, 2008). However, these estimates are commonly derived from data of balance studies performed under experimental housing conditions, where animals are kept individually in metabolic cages. At a similar feed intake animal performance in these studies might be higher than under commercial housing conditions due to good climatic and hygienic conditions, the absence of social stress, and/or a lower physical activity. Correspondingly, e.g. Campbell and Taverner (1988) and Black (1999) observed 20 to 30% lower growth rates of pigs raised under commercial conditions than of those in experimental trials.

It is unlikely that variations in the efficiency of energy utilisation for growth ( $k_{pf}$ ) are responsible for those differences, since energy retention (RE) is linearly related to energy intake, which means that  $k_{pf}$  did not change with level of growth rate, and  $k_{pf}$  is independent of composition of gain (Noblet, 1999), age, gender, and genotype (Susenbeth, 1996). Furthermore, partial efficiency of ME utilization for RE was not altered by chronic immune system activations (Williams *et al.*, 1997). Similarly, only minor variations in the efficiency of amino acid (AA) utilisation when being first-limiting were observed (Susenbeth, 1995; Sandberg *et al.*, 2005). Neither body weight (BW) (Dunkin and Black, 1985), genotype or gender (Kyriazakis *et al.*, 1995), nor environmental temperature (Wellock *et al.*, 2003) affected the response of protein retention to energy supply in energy limited diets. Hence, it appears that lower performance of animals under commercial housing conditions are mainly caused by their higher energy demand for physical activity, thermoregulatory heat production, and/or social stress, which reduce the amount of energy available for growth. The aim of this study was therefore to quantify the difference between the ME intake of growing pigs kept under commercial housing conditions and their ME requirements for maintenance and growth. These differences were determined in castrated male and female animals at several growth stages.

## 2.3 Material and methods

### 2.3.1 Animals and housing

Three trials (trial 1, 2, and 3) were performed on a commercial livestock farm in 2004-2005. In each trial, 624 animals were used with a mean initial BW of 25.3, 25.4, and 34.2 kg, respectively (Table 2.1). Pigs were of the genetic line German PIC X Pietrain in trial 1, while Danish crossbreed pigs of Landrace X Yorkshire and Pietrain were used in trials 2 and 3. The animals were randomly distributed among 24 double pens with either 26 castrated male or 26 female pigs each. The double pens were sub-divided by one long feeder into two single pens of  $4.5 \times 2 \text{ m}^2$ , equivalent to 0.7 m<sup>2</sup> per animal. Since 35 cm of feeder space were available to each pig, all animals could eat simultaneously during feeding.

Slurry was stored in a circulating dunging 1.8 m below the pens. The stable was ventilated by a low-pressure system that distributed the air through eight 4 × 10 m<sup>2</sup> permeable reed mattings. A climate logger continuously recorded humidity and air temperatures in- and outside the rooms in order to adjust air conditioning and heating. Mean daily temperature and humidity across all trials was 20.4°C and 56% (minimum 44%, maximum 69%). Air temperature ranged between 17.6 to 26.3°C, 18.1 to 27.8°C, and 16.4 to 21.8°C in trial 1, 2, and 3, respectively. In the respective growing periods, it was never below the minimum ambient temperatures recommended for growing pigs of  $\leq$ 60 kg (19.5°C) and  $\geq$ 60 kg BW (13-15°C; GfE, 2008) and only exceeded the recommended maximum comfortable temperature of 24°C (Mayer and Hauser, 1999) during less than 5% of the total growing period.

Pigs were weighed 7 to 10 days after arrival, were ear-tagged, and weighed again every three weeks until they reached mean BW's of 116.4 (trial 1), 114.2 (trial 2), and 116.6 kg (trial 3) at slaughter. Carcass weights were recorded, and lean and fat percentages were determined using a Fat-O-Meater (FOM; SFK Technology A/S, Herlev, Denmark) according to the formula of the EU regulation (EU-VO No. 2967/85).

### 2.3.2 Diets, feeding, and experimental design

Each trial was divided into five periods of three weeks each (period 1 - 5). Animals received a liquid diet (feed : water ratio about 1 : 2.8) consisting of cereals, soybean meal, and a mineral-vitamin premix (for details see Table 2.2). Dietary ME content was 15.2 MJ/kg dry matter (DM), while mean crude protein contents were 221, 217, 204, 190, and 182 g/kg DM in periods 1 to 5, respectively. Four diets differing in the level of supplemented free Lys were tested. Mean Lys concentration in the unsupplemented diets (Lys 0; basal diet) decreased from 10.2, 9.7, 8.5, and 7.6 to 6.9 g/kg DM in periods 1 to 5 (Table 2.3). In each period three experimental diets were created by supplementing HCI-Lys to the basal diets with 1.1 (Lys 1), 2.3 (Lys 2), and 3.4 g Lys/kg DM (Lys 3). Feed was prepared separately for each meal and diet and offered to pigs by a sensor-controlled liquidfeeder (Bio-Feeder, Hölscher&Leuschner, Germany) at 0700 h, 1130 h, 1600 h, and 2100 h. Feed supply was adjusted to the animals' ingestion capacity. A sensor placed in the troughs recorded the amount of feed left after 10 minutes of eating time at each meal. When any feed was left, the amount of feed offered at the following meal was reduced by 4%. When all feed was consumed, the amount was increased by 4%. In period 5 of trial 1, the amount of feed offered was lower than in the other periods due to an incorrect programming of the feed distribution system. Animals always had free access to water by one drinking nipple per single pen.

	: Body w ferent pe error of n	eight (BW) riods of th <del>t</del> neans (SEI	Table 2.1: Body weight (BW) at the beginning and pigs in different periods of the three trials as well a standard error of means (SEM), minimum (Min) an	ning and a as well as (Min) and	at slaughter (LS-means; st s the differences between i id maximum (Max) values).	(LS-means; ces betwee (Max) value	the	viation (± ured and ces betw	dard deviation (±)) of castrated male and female ir measured and estimated BW gain (LS-means; Differences between measured and estimated BW	ated male I BW gair ured and	eand fem (LS-mea estimated	ale ns; d BW
			BW (Kg)	(kg)					gain (kg/d)	g/d)		
	Ц	Trial 1	Trial 2	al 2	Trial 3	13	Tria	Trial 1*	Trial 2	2	Trial 3	e
Casti male	Castrated male	Female	Castrated male	Female	Castrated male	Female	Mean (SEM)	Min Max	Mean (SEM)	Min Max	Mean (SEM)	Min Max
			Initial BW	BW								
ч <del>П</del>	25.7 (±3.6)	24.9 (±2.1)	25.9 (±1.1)	25.2 (±1.1)	33.9 (±2.9)	34.4 (±2.6)	0.019 (0.002)	0.007 0.057	0.043 (0.002)	0.003 0.063	0.047 (0.001)	0.039 0.058
ъ Ĥ	54.9 (±3.3)	51.5 (±2.9)	53.1 (±1.5)	50.3 (±1.3)	57.9 (±3.4)	58.9 (±3.6)	-0.023 (0.002)	-0.033 0.015	0.009 (0.002)	-0.035 0.019	0.031 (0.001)	0.016 0.046
► Ħ	74.8 (±2.8)	70.8 (±3.6)	69.5 (±2.0)	65.4 (±1.9)	74.6 (±4.3)	75.7 (±4.7)	-0.054 (0.002)	-0.060 -0.016	-0.011 (0.002)	-0.063 -0.002	0.008 (0.002)	-0.011 0.017
တ ၂	95.0 (±3.5)	88.6 (±3.4)	86.8 (±2.9)	80.7 (±2.4)	94.2 (±4.4)	93.2 (±4.2)	-0.070 (0.002)	-0.097 -0.039	-0.032 (0.002)	-0.067 0.025	-0.017 (0.001)	-0.033 -0.010
← Ţ	111.2 (±2.5)	104.8 (±4.5)	102.8 (±2.4)	95.8 (±2.2)	110.2 (±4.4)	108.6 (±4.8)	0.060 (0.002)	0.021 0.104	-0.046 (0.002)	-0.092 -0.037	-0.039 (0.002)	-0.057 -0.023
			BW at slaughter	aughter					Overall mean	nean		
	116.4 (±2.6)	116.3 (±4.3)	116.2 (±2.9)	112.6 (±2.9)	115.9 (±2.8)	117.1 (±2.2)	-0.014 (0.001)	-0.097 0.104	-0.007 (0.003)	-0.092 0.063	0.006 (0.003)	-0.057 0.058

\* for the reason for higher variation in trial 1 see material and methods.

Diet ingredient	Period 1	Period 2	Period 3	Period 4	Period 5
Wheat	387	363	404	528	500
Rye	150	200	200	135	200
Wheat gluten	120	120	120	120	120
Soybean meal	127	100	60	18	0
Soybean meal HP*	100	100	100	100	77
Barley	70	70	70	70	70
Molasses	10	10	10	0	0
Soybean oil	12.5	13.5	12.3	5.0	8.2
Calcium carbonate	11.8	11.8	11.3	11.5	11.5
Dicalcium-phosphate	2.0	2.0	2.8	2.8	3.5
Sodium chloride	2.6	2.5	2.6	2.6	2.7
Mineral-vitamin-premix**	7.1	7.1	7.1	7.1	7.1

Table 2.2: Composition (g/kg as fed) of the basal diets in the five periods of the three trials (means of the trial 1, 2, and 3)

\* HP = with high protein content

\*\* Mineral-vitamin-premix contributed to the basal diet (per kg feed with 88% dry matter): Magnesium 0.4 g; Ferrous sulphate 160 mg; Copper sulphate 40 mg; Manganese 80 mg; Zinc sulphate 160 mg; Iodine 2.0 mg; Sodium selenite 0.6 mg; Cobalt 0.8 mg; Vitamin A 7410 IU; Vitamin D 890 IU; Vitamin E 59 mg; Vitamin K 1.5 mg; Vitamin B1 0.7 mg; Vitamin B2 3 mg; Vitamin B6 1.5 mg; Vitamin B12 0.02 mg; Niacin 7.4 mg; Pantothenic acid 3 mg.

			Trial 1					Trial 2					Trial 3		
			Period					Period					Period		
Ingredient	~	7	ო	4	5	~	7	ო	4	5	~	7	с	4	Ð
Crude ash	58	55	52	50	52	56	55	52	51	51	64	56	53	53	52
Crude fiber	44	41	37	34	34	39	38	36	33	33	46	44	39	37	37
Crude protein	219	220	201	195	183	214	220	203	184	175	229	213	207	191	188
Ether extract	44	35	34	26	30	39	25	28	21	27	43	34	34	25	25
Starch	375	427	449	472	486	400	441	432	425	467	370	407	421	424	430
Lysine	10.2	10.0	8.5	7.7	7.1	10.1	9.6	8.1	7.6	6.1	10.4	9.5	8.9	7.6	6.9
Pcd lysine <sup>1</sup>	8.1	7.3	6.4	5.8	4.8	8.1	7.3	6.4	5.8	4.8	8.1	7.3	6.4	5.8	4.8
$ME^2$	15.2	15.3	15.5	15.5	15.5	15.2	15.3	15.5	15.3	15.3	15.1	15.2	15.4	15.3	15.3

Table 2.3: Chemical composition of the basal diets and metabolizable energy (ME) content offered in the five periods of the three

the feed table of AminoDat (2005). <sup>2</sup> ME contents were calculated according to the equation of Noblet and Perez (1993): ME = 4.168 – 12.3 × crude ash + 1.4 × crude protein + 4.1× ether extract - 6.1 × crude fiber

### 2.3.3 Chemical analyses

Feed samples were taken twice daily immediately after feed preparation by a magnetic valve placed in the feed distribution system. Samples were stored at 6°C, pooled by dietary treatment at the end of each period, and analysed in duplicates for crude ash, crude protein (N × 6.25), crude lipid, and crude fibre concentrations according to Naumann and Bassler (2001). DM content was determined by freeze-drying and subsequent oven-drying at 105°C over night. Starch content was determined by enzyme hydrolysis of starch to glucose using the heat-stable alpha-amylase Termamyl type 120 L (Novo Industrials, Bagsvard, Denmark) according to Brandt *et al.* (1987). AA concentrations were analyzed according to the Commission Directives (1998 and 2000). Precaecal digestible (pcd) Lys content was calculated from the analysed Lys content and the preacecal digestibility values given in the feed table of AminoDat (2005). ME concentrations of the diets were calculated according to the equation of Noblet and Perez (1993): ME (kcal/kg DM) = 4.168 - 12.3 × crude ash + 1.4 × crude protein + 4.1 × ether extract - 6.1 × crude fiber (in g/kg DM), Table 2.3.

### 2.3.4 Calculation of protein and fat accretion and animals' ME requirements

Protein and fat accretions of the pigs were calculated according to a growth model described in detail by Susenbeth (2008). The calculation procedure was as follows: individual protein accretions across the whole trial periods were calculated from protein mass at slaughter minus protein mass at the beginning of the experiment. Protein mass at slaughter was derived from lean percentages of the carcasses determined by FOM assuming a protein content of fat-free lean of 22% and that 56% of the total body protein are located in lean. Protein mass at beginning was estimated from BW according to the equation of GfE (2008) describing the relationship between the empty body weight (EBW, kg) and the body protein mass (BP, kg):

where EBW was assumed to be 0.96 BW (Susenbeth, 1984; Quiniou and Noblet, 1995). Since protein accretion is not constant during growth and the mean protein accretion across the whole trial therefore deviates from that within periods, protein

accretions during periods 1-5 were corrected as follows: The first derivative of equation 1 was calculated to determine the decreasing protein content of BWG with increasing BW. Then the ratio between the calculated protein content of BWG within BW ranges corresponding to the growth periods in the trials and the protein content of BWG across whole period were taken to adjust (by multiplication) experimentally determined whole trial protein accretion values to period specific values.

Similarly, fat accretions across the whole trial periods were calculated as the difference between body fat mass at slaughter and fat mass at the beginning. While a proportion of body fat of 20% of EBW was assumed for the latter (GfE, 2008), fat mass at slaughter was calculated as the difference between EBW and body fat-free matter (FFM). FFM was derived from protein mass (as described above) assuming a protein content of 22% in FFM. Since fat accretion progressively increases with age, fat accretions within the five periods were derived from fat accretion across the whole trial periods analogous to the correction of protein accretions. For this purpose the equation of GfE (2008) describing the relationship between EBW (kg) and body fat mass (BF, kg; GfE (2008)) was used:

To evaluate the accuracy of our protein and fat accretions estimates, we calculated the animals' BWG for the respective growth periods as the sum of fat and FFM accretion (= EBW gain) divided by 0.96. The differences between calculated and measured BWG are presented in Table 2.1 and evaluated in section Discussion.

ME requirements for growth (ME<sub>g</sub>) were calculated from protein and fat accretions using the energy content of protein and fat of 23.8 kJ/g and 39.7 kJ/g, respectively, and the efficiency of ME utilisation for RE of 0.70 (GfE, 2008). Values for ME requirements for maintenance (ME<sub>m</sub>) were taken from GfE (2008) as well. The difference between ME intake and the sum of ME<sub>m</sub> and ME<sub>g</sub> was defined as ME<sub>x</sub> and seen as that proportion of the energy budget used for purposes other than growth and maintenance. We hypothesised that ME<sub>x</sub> is close to zero under experimental and elevated under commercial housing conditions.

## 2.3.5 Statistical analyses and calculations

Mean values of all 26 animals per pen were considered as one observation. According to the completely randomised experimental design, BWG and FI data were analysed using the mixed model procedure (ProcMixed) of SAS (1996). The following model was used:

 $Y_{ijkl} = \mu + T_i + P_j + TxP_{ij} + L_k + S_l + TxPxS_{ijl} + TxPxSxL_{ijkl} + TxPxL_{ijk} + e_{ijkl},$ 

where  $Y_{ijkl}$  is the observed response,  $\mu$  the overall mean,  $T_i$  the effect of trial number *i*,  $P_j$  the effect of period *j*,  $L_k$  the effect of Lys supplementation *k*,  $S_l$  the effect of gender *l*,  $TxP_{ij}$  the effect of period within each trial,  $TxPxS_{ijl}$  the effect of gender within each period and trial,  $TxPxL_{ijk}$  the effect of Lys within each period and trial,  $TxPxSxL_{ijkl}$  the effect of Lys within each gender, period and trial, and  $e_{ijkl}$ the residual error. Adjusted means were compared pair-wise using the t-test with a Tukey correction for multiple comparisons.

Sick animals were removed and weighed, and the feed amount offered was immediately adjusted to the number of the remaining animals in the pen. In total, data of 575, 595, and 574 animals were recorded in trial 1, 2, and 3, respectively. Since variables were measured in two sexes, in four different diets during five periods with three replications each, and in three trials 360 observations were obtained.

# 2.4 Results

Mean BWGs were 0.795, 0.737, and 0.797 kg/d in trial 1, 2, and 3, respectively. Except for trial 2, BWG increased from start to 90 kg BW (Table 2.4). BWG differed between sexes (P<0.05) with 0.04 kg/d in period 2, 0.10 kg/d in period 3 (P<0.001), and 0.05 kg/d across the whole period (P<0.05). However, no differences were observed in periods 1, 4, and 5 (P>0.05). Carcass weights were similar between trials and gender. The few significant differences are biologically not meaningful. Mean lean percentages in carcass were 55.6, 55.1, and 55.3% in trial 1, 2, and 3, respectively. They differed between trial 1 and 2 (P<0.05) and were higher in female than in castrated male pigs (P<0.001; Table 2.4). Mean daily DM intake differed between trials (P<0.05) and was 1.96, 2.06, and 2.30 kg in trial 1, 2, and 3, respectively, and increased from 1.48, 1.41, and 1.62

kg in period 1 to 2.15, 2.40, and 2.73 kg in period 4 of the respective trials, when

animals reached a BW of 80 to 95 kg (Table 2.5). Average daily DM intake was 0.2 kg higher in castrated male than in female pigs (P<0.001). Mean ME intake was 30.2, 31.7, and 35.9 MJ/d in trial 1, 2, and 3, respectively (P<0.001), and was 3 MJ/d higher in castrated male than in female pigs across all trials (P<0.001; Table 2.6). ME intake related to metabolic BW (BW<sup>0.75</sup>) was 1.19, 1.29, and 1.32 MJ in trial 1, 2, and 3, respectively (P<0.05; Table 2.7) and was 0.10 MJ higher in castrated male than in female pigs (P<0.05). ME<sub>x</sub> amounted to 0.6, 5.4, and 7.7 MJ/d on average in trial 1, 2, and 3, respectively (P<0.001). It continuously increased with BW from period 1 to period 5 with 3.8 and 7.4 MJ/d in trial 2 and 4.2 (period 1) and with 11.0 MJ/d in trial 3. In trial 1 and 2, mean ME<sub>x</sub> was almost 1 MJ/d higher in female than in castrated male pigs (P<0.001), while it was similar in animals of both sexes in trial 3 (P>0.05). ME<sub>x</sub> related to BW<sup>0.75</sup> did not differ between trial periods (P>0.05), but was different between trial 1, 2, and 3 at 0.07, 0.22, and 0.30 MJ/d, respectively (P<0.001; Table 2.7). MEx per kg  $BW^{0.75}$  was higher in female than in castrated male pigs in trial 1 and 2 (P<0.001), while no effect of gender was observed in trial 3 (P>0.05). This means that 4.5, 19.5, and 21.3% of ME intake in females and 0.0, 14.5, and 21.5% in castrated males were not used for maintenance and growth in the trials 1, 2, and 3, respectively. When related to BW<sup>0.75</sup>, ME<sub>x</sub> was 7.8, 20.0, and 22.8% of ME intake of female and 1.6, 14.3, and 21.9% of castrated male pigs. No effect of Lys supply on ME<sub>x</sub> was observed (P>0.05).

Table 2.4: Body weight gain (kg/d), carcass weight (kg), and lean percentage in carcass (%) of castrated male and female pigs in the five periods of the three trials (LS-means, standard deviation ( $\pm$ ), standard error of means (SEM)).

Period         Castrated male           1         ±0.06           1         ±0.06           2         ±0.06           0.964         0.958 <sup>a</sup> 3         ±0.05           0.021)         0.958 <sup>a</sup> 0.958 <sup>a</sup> 0.021	ed Female 0.766 <sup>b</sup> ±0.08 ±0.07 0.919 ±0.07 0.859 <sup>b</sup> ±0.06 0.748 ±0.05	Castrated male $0.781^{a}$ $\pm 0.05$ (0.024) 0.776 $\pm 0.04$ (0.024) $0.840^{a}$ $\pm 0.09$	Female 0.727 <sup>b</sup> ±0.05 (0.021) 0.725 ±0.05	Castrated male	Female	Ţ	2	S	Castrated	
		$0.781^{a}$ $\pm 0.05$ (0.024) 0.776 $\pm 0.04$ (0.024) $0.840^{a}$ $\pm 0.09$	$0.727^{b}$ $\pm 0.05$ (0.021) 0.725 $\pm 0.05$ 0.05			-		)	male	remare
		$\pm 0.05$ (0.024) 0.776 $\pm 0.04$ (0.024) 0.840 <sup>a</sup> $\pm 0.09$	±0.05 (0.021) 0.725 ±0.05	0.707	0.723	$0.795^{a}$	0.754 <sup>5</sup>	0.716 <sup>c,b</sup>	0.769 <sup>a</sup>	0.739 <sup>a</sup>
		(0.024) 0.776 $\pm 0.04$ (0.024) $0.840^{a}$ $\pm 0.09$	(0.021) 0.725 ±0.05	±0.12	±0.14	±0.08	±0.05	±0.06	±0.08	±0.07
		0.776 ±0.04 (0.024) 0.840 <sup>a</sup> ±0.09	0.725 ±0.05	(0.021)	(0.021)	(0.016)	(0.017)	(0.016)	(0.017)	(0.016)
		±0.04 (0.024) 0.840 <sup>a</sup> ±0.09	±0.05	0.834	0.811	0.941 <sup>a</sup>	0.751 <sup>b</sup>	0.822 <sup>c</sup>	0.861 <sup>a</sup>	0.818 <sup>5</sup>
		(0.024) 0.840ª ±0.09		±0.07	±0.07	±0.07	±0.05	±0.07	±0.10	±0.10
	_	0.840ª ±0.09	(0.021)	(0.021)	(0.021)	(0.016)	(0.017)	(0.016)	(0.017)	(0.016)
		±0.09	0.735 <sup>b</sup>	$0.946^{a}$	0.838 <sup>b</sup>	0.908 <sup>a</sup>	0.787 <sup>b</sup>	0.892 <sup>a</sup>	0.914 <sup>a</sup>	0.811 <sup>b</sup>
(0.021) 0 770			±0.07	±0.05	±0.06	±0.07	±0.09	±0.08	±0.08	±0.08
0 770	0.748 ±0.05	(0.024)	(0.021)	(0.021)	(0.021)	(0.016)	(0.017)	(0.016)	(0.017)	(0.016)
0 0	±0.05	0.774 <sup>a</sup>	0.709 <sup>b</sup>	0.792	0.743	0.759 <sup>a</sup>	0.741 <sup>b</sup>	0.767 <sup>a</sup>	0.776 <sup>a</sup>	$0.734^{a}$
4 ±0.04	7 F - F - F	±0.11	±0.06	±0.06	±0.08	±0.15	±0.09	±0.07	±0.10	±0.11
(0.021)	(0.021)	(0.024)	(0.021)	(0.021)	(0.021)	(0.016)	(0.017)	(0.016)	(0.017)	(0.016)
0.560	0.574	0.650	0.647	0.802	0.773	0.567 <sup>a</sup>	0.648 <sup>b</sup>	0.787 <sup>c</sup>	0.670 <sup>a</sup>	$0.665^{a}$
5 ±0.11	±0.10	±0.06	±0.07	±0.03	±0.04	±0.10	±0.07	±0.04	±0.13	±0.11
(0.021)	(0.021)	(0.024)	(0.021)	(0.021)	(0.021)	(0.016)	(0.017)	(0.016)	(0.017)	(0.016)
0.816 <sup>a</sup>	0.773 <sup>b</sup>	0.764 <sup>a</sup>	0.708 <sup>b</sup>	$0.816^{a}$	0.778 <sup>b</sup>	$0.795^{a}$	0.737 <sup>b</sup>	0.797 <sup>a</sup>	0.798 <sup>a</sup>	0.753 <sup>5</sup>
Overall ±0.17	±0.15	±0.09	±0.07	±0.09	±0.07	±0.16	±0.08	±0.09	±0.13	±0.11
(0.009) (0.009)	(0.009)	(0.010)	(0000)	(600.0)	(0.010)	(0.015)	(0.016)	(0.015)	(0.006)	(0.006)
92.0	91.9	91.7 <sup>a</sup>	89.0 <sup>5</sup>	91.6	92.5	91.9 <sup>a</sup>	90.3 <sup>b</sup>	92.0 <sup>a</sup>	91.7 <sup>a</sup>	91.1 <sup>b</sup>
Carcass ±2.06	±3.41	±2.06	±2.26	±2.18	±1.77	±2.75	±2.63	±1.95	±2.03	±2.98
weigili (0.58)	(0.58)	(0.61)	(0.53)	(0.61)	(0.53)	(0.41)	(0.40)	(0.40)	(0.35)	(0.31)
Lean 53.9 <sup>a</sup>	57.4 <sup>b</sup>	$53.3^{a}$	56.8 <sup>b</sup>	54.4 <sup>a</sup>	56.3 <sup>b</sup>	55.6 <sup>a</sup>	55.1 <sup>b</sup>	55.3 <sup>a,b</sup>	53.9 <sup>a</sup>	56.9 <sup>b</sup>
percentage ±0.33	±0.78	±1.14	±1.16	±0.76	±1.50	±1.91	±2.04	±1.47	±0.93	±1.30
(0.27)	(0.27)	(0.28)	(0.25)	(0.29)	(0.25)	(0.19)	(0.18)	(0.18)	(0.16)	(0.14)

castrated males and females, respectively (P<0.05).

	Trial 1	_	Trial 2	7	Trial 3	e		Trial		Gender	er
Period	Castrated male	Female	Castrated male	Female	Castrated male	Female	٢	~	ო	Castrated male	Female
<del>.  </del>	1.52	1.43	1.47	1.36	1.63	1.60	1.48 <sup>a</sup>	1.41 <sup>a</sup>	1.62 <sup>b</sup>	1.55 <sup>a</sup>	1.46 <sup>a</sup>
	±0.13	±0.17	±0.06	±0.09	±0.12	±0.14	±0.16	±0.09	±0.13	±0.13	±0.17
7	2.13ª	1.89 <sup>b</sup>	1.98ª	1.76 <sup>b</sup>	2.20	2.10	2.01 <sup>a</sup>	1.86 <sup>b</sup>	2.15°	2.11ª	1.92 <sup>b</sup>
	±0.11	±0.16	±0.08	±0.15	±0.16	±0.14	±0.18	±0.16	±0.15	±0.15	±0.20
ო	2.44ª	2.19 <sup>b</sup>	2.34ª	2.03 <sup>b</sup>	2.70 <sup>a</sup>	2.46 <sup>b</sup>	2.32 <sup>a</sup>	2.17 <sup>b</sup>	2.58 <sup>c</sup>	2.50 <sup>a</sup>	2.23 <sup>b</sup>
	±0.14	±0.16	±0.12	±0.14	±0.10	±0.10	±0.19	±0.20	±0.16	±0.20	±0.23
4	2.28ª	2.03 <sup>b</sup>	2.56ª	2.28 <sup>b</sup>	2.88 <sup>a</sup>	2.58 <sup>b</sup>	2.15 <sup>a</sup>	2.40 <sup>b</sup>	2.73 <sup>c</sup>	2.57ª	2.29 <sup>b</sup>
	±0.18	±0.28	±0.14	±0.12	±0.05	±0.12	±0.26	±0.19	±0.17	±0.29	±0.29
5	1.84	1.78	2.56	2.34	2.59	2.41	1.81 <sup>a</sup>	2.45 <sup>b</sup>	2.50 <sup>b</sup>	2.26	2.16
	±0.21	±0.14	±0.17	±0.11	±0.02	±0.11	±0.18	±0.17	±0.12	±0.42	±0.31
Overall mean	2.04 <sup>a</sup>	1.87 <sup>b</sup>	2.18 <sup>a</sup>	1.95 <sup>b</sup>	2.36 <sup>a</sup>	2.19 <sup>b</sup>	1.96 <sup>a</sup>	2.06 <sup>b</sup>	2.30 <sup>c</sup>	2.20 <sup>a</sup>	2.01 <sup>b</sup>
SEM	0.05	0.05	0.05	0.05	0.05	0.05	0.018	0.019	0.021	0.016	0.015
Values w between	ith different s castrated me	superscripts ales and fem	Values with different superscripts within rows significantly differ between castrated male and female within trials, between trials, and between castrated male and female within trials, between trials, and	gnificantly di /ely (P<0.05	ffer between o	astrated ma	ale and fe	male wit	hin trials, l	between trials	, and

Table 2.5: Dry matter intake (kg/d) of castrated male and female pigs in the five periods of the three trials (LS-means, standard deviation (±), standard error of mean (SEM)).

Table 2.6: Metabolizable energy intake (ME) of castrated male and female pigs in the five periods of the three trials and differences between ME intake and their estimated ME requirements for maintenance and growth ( $ME_x$ ; MJ/d;

			Trial	1*	Trial	2	Trial	3
Dariad			Castrated		Castrated		Castrated	
Period			male	Female	male	Female	male	Female
		Mean	23.2 <sup>a,c</sup>	21.8 <sup>a</sup>	22.6 <sup>a,c</sup>	20.9 <sup>b,a</sup>	24.4 <sup>c</sup>	23.9 <sup>c</sup>
1	ME	SD	±2.1	±2.6	±0.9	±1.4	±1.8	±2.1
		SEM	(0.59)	(0.67)	(0.27)	(0.40)	(0.51)	(0.61)
	ME <sub>x</sub>	Mean	1.7 <sup>a</sup>	4.4 <sup>b</sup>	2.6 <sup>a</sup>	4.8 <sup>b</sup>	3.9 <sup>b</sup>	4.4 <sup>b</sup>
		SD	±2.3	±2.7	±1.3	±1.1	±0.8	±1.3
		SEM	(0.66)	(0.27)	(0.42)	(0.31)	(0.23)	(0.38)
_		Mean	32.7 <sup>a</sup>	29.0 <sup>b</sup>	30.2 <sup>b</sup>	26.9 <sup>b</sup>	33.6 <sup>a</sup>	31.9 <sup>a</sup>
2	ME	SD	±1.7	±2.4	±1.3	±2.2	±2.4	±2.0
		SEM	(0.50)	(0.69)	(0.39)	(0.64)	(0.68)	(0.57)
	ME <sub>x</sub>	Mean	0.5 <sup>a</sup>	1.6 <sup>b</sup>	3.5 <sup>°</sup>	4.5 <sup>c</sup>	7.3 <sup>d</sup>	7.4 <sup>d</sup>
		SD	±2.0	±2.2	±1.9	±1.4	±1.6	±1.3
		SEM	(0.66)	(0.63)	(0.63)	(0.04)	(0.46)	(0.38)
		Mean	37.9 <sup>a</sup>	34.1 <sup>b</sup>	36.2 <sup>a</sup>	31.3 <sup>⊳</sup>	41.9 <sup>a</sup>	38.1 <sup>a</sup>
3	ME	SD	±2.1	±2.4	±1.9	±2.2	±1.6	±1.5
		SEM	(0.59)	(0.71)	(0.59)	0.64	(0.45)	(0.44)
		Mean	1.3 <sup>a</sup>	3.6 <sup>b</sup>	4.7 <sup>c,b</sup>	5.6 <sup>c</sup>	9.9 <sup>d</sup>	9.7 <sup>d</sup>
	$ME_{x}$	SD	±2.8	±2.1	±2.0	±1.6	±1.7	±1.6
		SEM	(0.80)	(0.61)	(0.64)	(0.45)	(0.47)	(0.48)
		Mean	35.6 <sup>ª</sup>	31.6 <sup>b</sup>	39.7 <sup>c</sup>	35.2 <sup>a</sup>	44.3 <sup>c</sup>	39.7 <sup>c</sup>
4	ME	SD	±2.8	±4.3	±2.2	±1.8	±0.8	±1.8
		SEM	(0.79)	(0.91)	(0.70)	(0.51)	(0.22)	(0.51)
		Mean	0.1 <sup>a</sup>	0.1 <sup>a</sup>	6.4 <sup>b</sup>	7.1 <sup>b</sup>	11.9 <sup>c</sup>	10.1 <sup>c</sup>
	$ME_x$	SD	±3.5	±3.3	±3.2	±2.1	±1.5	±1.5
		SEM	(1.10)	(0.92)	(1.01)	(0.59)	(0.43)	(0.44)
		Mean	28.5 <sup>ª</sup>	27.8 <sup>a</sup>	39.1 <sup>b</sup>	36.6 <sup>b</sup>	42.5 <sup>b</sup>	39.5 <sup>b</sup>
5	ME	SD	±3.3	±2.3	±2.7	±1.7	±0.3	±1.8
		SEM	(0.94)	(0.65)	(0.83)	(0.48)	(0.08)	(0.52)
		Mean	-3.8 <sup>a</sup>	-2.9 <sup>a</sup>	7.3 <sup>b</sup>	7.4 <sup>b</sup>	7.1 <sup>b</sup>	5.2 <sup>c</sup>
	$ME_{x}$	SD	±3.9	±3.3	±3.6	±1.3	±2.0	±2.8
		SEM	(1.10)	(0.91)	(1.22)	(0.38)	(0.56)	(0.80)
		Mean	31.6 <sup>a,c</sup>	28.8 <sup>b</sup>	33.7 <sup>c</sup>	30.2 <sup>a,b</sup>	37.3 <sup>d</sup>	34.6 <sup>c</sup>
Over-	ME	SD	±5.8	±3.2	±6.9	±6.1	±7.6	±6.4
all		SEM	(0.74)	(0.41)	(0.97)	(0.78)	(0.91)	(0.82)
mean		Mean	-0.04 <sup>a</sup>	1.29 <sup>b</sup>	4.89 <sup>c</sup>	5.88 <sup>d</sup>	8.02 <sup>e</sup>	7.37 <sup>e</sup>
	$ME_{x}$	SD	±3.5	±3.7	±3.0	±1.9	±3.1	±2.9
		SEM	(0.45)	(0.48)	(0.42)	(0.24)	(0.40)	(0.37)
· · · · ·								

LS-means, standard deviation (±SD), standard error of means (SEM)).

\* for the reason for the higher variation in trial 1 see *material and methods*. Values with different superscripts within rows significantly differ (P<0.05).

Table 2.7: Metabolizable energy intake (ME) related to metabolic body weight (BW) of castrated male and female pigs in the five periods of the three trials and the difference between ME intake and their ME requirements for maintenance and growth (ME<sub>x</sub>; MJ/kg BW<sup>0.75</sup>; LS-means standard deviation ( $\pm$ SD), standard error of means (SEM)).

			Trial 1	*	Trial	2	Trial	3
Period			Castrated	Femal	Castrated		Castrated	
Tenou			male	е	male	Female	male	Female
4		Mean	1.47 <sup>a</sup>	1.43 <sup>a</sup>	1.45 <sup>a</sup>	1.39 <sup>b</sup>	1.29 <sup>c</sup>	1.26 <sup>c</sup>
1	ME	SD SEM	±0.26	±0.30	±0.09	±0.12	±0.07	±0.09
			(0.07)	(0.08)	(0.02)	(0.03)	(0.02)	(0.03)
	$ME_{x}$	Mean SD	0.11 <sup>a</sup>	0.29 <sup>b</sup>	0.17 <sup>a,c</sup>	0.32 <sup>b</sup>	0.21 <sup>a,c</sup>	0.23 <sup>c</sup>
		SEM	±0.20 (0.05)	±0.26 (0.07)	±0.11 (0.04)	±0.09 (0.03)	±0.05 (0.01)	±0.08 (0.02)
								. ,
2	ME	Mean SD	1.43 <sup>a</sup>	1.33 <sup>b</sup>	1.38 <sup>a,b</sup>	1.29 <sup>b</sup>	1.45 <sup>a</sup>	1.36 <sup>b</sup>
2	IVIC	SEM	$\pm 0.09$	±0.12	$\pm 0.06$	$\pm 0.11$	±0.08	$\pm 0.07$
			(0.02)	(0.03)	(0.02)	(0.03)	(0.02)	(0.02)
	$ME_{x}$	Mean SD	0.02 <sup>a</sup>	0.07 <sup>a</sup>	0.16 <sup>b</sup>	0.22 <sup>b</sup> ±0.07	0.32 <sup>c</sup>	0.32 <sup>c</sup>
		SEM	±0.10 (0.02)	±0.11 (0.03)	±0.10 (0.03)	±0.07 (0.02)	±0.07 (0.02)	±0.06 (0.02)
3	ME	Mean SD	1.35 <sup>a</sup>	1.28 <sup>b</sup>	1.38 <sup>a</sup>	1.25 <sup>b</sup>	1.50 <sup>c</sup>	1.36 <sup>a</sup>
Ū		SEM	±0.08 (0.02)	±0.10 (0.03)	±0.07 (0.02)	±0.08 (0.02)	±0.07 (0.02)	±0.05 (0.01)
		Mean	(0.02) 0.05 <sup>a</sup>	(0.03) 0.14 <sup>b</sup>	(0.02) 0.18 <sup>b,c</sup>	(0.02) 0.22 <sup>c</sup>	(0.02) 0.36 <sup>d</sup>	(0.01) 0.35 <sup>d</sup>
	$ME_{x}$	SD	0.05° ±0.10	$\frac{0.14}{\pm 0.08}$	0.18 <sup>m</sup> ±0.08	0.22* ±0.06	0.36° ±0.07	$0.35^{\circ}$ ±0.06
		SEM	(0.03)	(0.25)	(0.03)	(0.02)	(0.02)	(0.02)
		Mean	1.07 <sup>a</sup>	0.98 <sup>a</sup>	1.31 <sup>b</sup>	1.22 <sup>b</sup>	1.38 <sup>c</sup>	1.24 <sup>b</sup>
4	ME	SD	±0.10	0.98 ±0.14	±0.06	±0.06	1.38 ±0.06	1.24 ±0.06
		SEM	(0.03)	(0.04)	(0.02)	(0.02)	(0.02)	(0.02)
		Mean	0.003 <sup>a</sup>	0.003 <sup>a</sup>	0.21 <sup>b</sup>	0.25 <sup>b</sup>	0.37 <sup>c</sup>	0.32 <sup>c</sup>
	$ME_{x}$	SD	±0.11	±0.11	±0.10	±0.07	±0.06	±0.05
		SEM	(0.03)	(0.03)	(0.03)	(0.02)	(0.02)	(0.01)
		Mean	0.79 <sup>a</sup>	0.78 <sup>a</sup>	1.14 <sup>b</sup>	1.09 <sup>b</sup>	1.21 <sup>c</sup>	1.13 <sup>b</sup>
5	ME	SD	±0.09	±0.07	±0.07	±0.06	±0.04	±0.06
		SEM	(0.03)	(0.02)	(0.02)	(0.02)	(0.01)	(0.02)
	ME <sub>x</sub>	Mean	-0.11 <sup>a</sup>	-0.08 <sup>a</sup>	0.21 <sup>b</sup>	0.22 <sup>b</sup>	0.24 <sup>b</sup>	0.23 <sup>b</sup>
	ΝΠ <sub>X</sub>	SD	±0.11	±0.09	±0.11	±0.04	±0.06	±0.08
		SEM	(0.03)	(0.02)	(0.04)	(0.01)	(0.02)	(0.02)
		Mean	1.22 <sup>a</sup>	1.16 <sup>ª</sup>	1.33 <sup>a,b</sup>	1.25 <sup>a</sup>	1.37 <sup>b</sup>	1.27 <sup>a</sup>
Over-	ME	SD	1.22 ±0.44	$\pm 0.43$	±0.25	1.25 ±0.25	±0.15	±0.13
		SEM	(0.05)	(0.06)	(0.04)	(0.03)	(0.02)	(0.02)
all		Mean	0.02 <sup>a</sup>	0.09 <sup>b</sup>	0.19 <sup>c</sup>	0.25 <sup>d</sup>	0.30 <sup>e</sup>	0.29 <sup>e</sup>
mean	$ME_{x}$	SD	±0.15	±0.21	±0.11	±0.10	±0.10	±0.11
		SEM	(0.02)	(0.03)	(0.01)	(0.01)	(0.01)	(0.01)

\* for the reason for the higher variation in trial 1 see *material and methods*. Values with different superscripts within rows significantly differ (P<0.05).

#### 2.5 Discussion

All three trials were conducted without any problems. The comprehensive data set on FI, BWG, and lean percentages of carcasses of growing pigs raised under commercial housing conditions allowed for a reliable estimate of their ME needed for purposes other than maintenance and growth. ME<sub>g</sub> requirements of pigs in our study were estimated from the animals' protein and fat accretions, which were derived from their lean and fat mass at slaughter. As mentioned above, we evaluated the accuracy of our accretion estimates by calculating BWG from these accretion values and by comparing the calculated with the measured BWGs in the respective growth periods. The mean difference between measured and estimated BWGs was small at -0.014, -0.007, and 0.006 kg/d in trial 1, 2, and 3, respectively (Table 2.1). However at -0.097 to 0.104 kg/d, it was greatest in trial 1, since the animals were fed restrictively in the fifth period and their BWG was therefore overestimated in the last and underestimated in the preceding periods. Nevertheless, the relatively small differences underline the validity of our calculated protein and fat accretion values. Since the efficiency of ME utilization for growth  $k_{pf}$  is very constant, the estimates of ME<sub>g</sub> requirements can be seen as valid as well.

However, both, experimental methodology and variability between pigs, result in variations in ME<sub>m</sub> requirement values (Noblet *et al.*, 1999; Birkett and de Lange, 2001). These range between 413 to 482 kJ/kg BW<sup>0.75</sup> (values were recalculated by the authors from 936 to 1122 kJ/kg BW<sup>0.60</sup>) depending on factors such as age, gender, and environmental influences related to different housing conditions. In our study ME<sub>m</sub> requirement values were taken from GfE (2008). These decrease from 550 kJ at 30 kg BW to 440 kJ/kg BW<sup>0.75</sup> at 100 kg BW and are similar to those given by ARC (1981) and NRC (1998). Consequently, ME<sub>x</sub> would be similar, if ME<sub>m</sub> and ME<sub>g</sub> values were taken from ARC (1981) or NRC (1998). In the following, possible factors that might be responsible for ME<sub>x</sub> such as the animals' physical activity, genotype and gender, their immune responses as well as ambient temperatures are discussed.

### 2.5.1 Physical activity

Animals' physical activities are influenced by group size, space allowance, feeding frequency, and animal mixing (Black et al., 1986). However, the activities under commercial housing conditions and the resulting energy expenditures are difficult to quantify. Energy requirements for different activities (ME<sub>a</sub>) have been determined under controlled experimental conditions with individual or small groups of animals. Van Milgen and Noblet (2000) observed a large variation in heat production between individual animals for the same activities which confirmed results of earlier studies of Noblet et al. (1993) where mean daily heat production of pigs associated with physical activity was 0.15 (62 kJ/kg BW<sup>0.75</sup>) of their total heat production ranging between 0.08 to 0.21. Recommendations for the energy supply to growing pigs usually consider ME<sub>a</sub> as part of ME<sub>m</sub>. GfE (2008) supposes a daily ME<sub>a</sub> requirement of 55 kJ/kg BW<sup>0.75</sup> as part of in their ME<sub>m</sub> recommendations to account for activities such as standing, walking, sitting, and eating as well as getting up and lying down that last in total for approximately 10 to 15% of the day. ARC (1981) and Noblet et al. (1993) assumed ME<sub>a</sub> to be 0.20 and 0.15 of ME<sub>m</sub>, respectively. However, Van Milgen et al. (1998) found ME<sub>a</sub> to be less than 0.10 of the fasting heat production in pigs kept individually in metabolic cages and a quiet environment. Heat production of pigs during standing is increased by approximately 60% of the resting heat production and considerably higher than in any other domestic species (Noblet et al., 1993). Van Milgen and Noblet (2000) showed that 0.08 to 0.13 of the ME intake (91 and 155 kJ /kg BW<sup>0.75</sup>) was used for physical activity in growing pigs housed individually or in groups and concluded that ME<sub>a</sub> amounts to 0.26 to 0.34 of fasting heat production. This effect is confirmed by observations of Gonyou et al. (1992) that pigs kept in groups spent 20% more time standing than those penned individually.

Even if the time periods of the different activities were known, the use of experimentally determined requirement values might not lead to appropriate  $ME_a$  estimates: e. g. using a standard value of 18.5 kJ ME/kg BW<sup>0.75</sup>/h for activity (GfE, 2008), a pig of 60 kg BW would have had to be active for 15 h/d to fully explain  $ME_x$  determined in our study (5 MJ ME/d). Hence, it appears that either factors other than physical activity must also have contributed to  $ME_x$  or - which seems to be more realistic (see discussion below) - higher values for  $ME_a$  per unit of time for

the same activity under commercial housing conditions are true due to a more intensive movement, an aggressive behaviour, more frequent changes in position, and social stress.

#### 2.5.2 Genotype and gender

Since energy costs for maintaining visceral mass are 3.1 times higher than for muscle mass, the ratio between muscle and visceral tissue affects the animals'  $ME_m$  requirements (Noblet *et al.*, 1999). This not only explains why  $ME_m$  per kg  $BW^{0.75}$  is higher in young animals and decreases as the ratio between muscle to visceral mass increases with advancing maturity (Noblet *et al.*, 1999), but may also result in differences between the  $ME_m$  requirements of animals of different genotype. Whittemore (1993) found that animals with higher proportions of lean tissue reach maturity at a higher BW. As a consequence, animals of different genotypes can differ in age and their physiological development despite a similar BW. Hence, Noblet *et al.* (1999) determined significantly higher  $ME_m$  requirements in Large White pigs (482 kJ/kg BW<sup>0.75</sup>) than in Meishan X Large White (471 kJ/kg BW<sup>0.75</sup>) and pure Meishan pigs (449 kJ/kg BW<sup>0.75</sup>). However, it seems unlikely that similar differences existed between the genotypes used in our study and those, from which the recommendations were derived.

Furthermore,  $ME_m$  requirements vary between pigs of different breeds due to different behaviour and activity levels (van Milgen *et al.*, 1998). These authors found a significant breed effect on the frequency of activity and therefore, on the animals'  $ME_a$  expenditures with 16.7 kJ in Large White, 22.0 kJ in Piétrain, and 11.2 kJ/kg  $BW^{0.75}$ /h in Meishan male pigs; 1 h of sitting or standing increased heat production by 69% in Large White and Meishan, and by 105% in Piétrain pigs. Similarly, McPhee *et al.* (2001) observed that restrictively fed sows selected for high lean-growth had prolonged lying periods and spent less time standing, sitting, or changing postures than sows selected for low lean-growth. Therefore, it can be speculated that the different genotypes used in trial 1 compared to trial 2 and 3 might be responsible for the differences in  $ME_x$  observed between trials. However, Susenbeth and Menke (1991) showed that differences in physical activity of pigs of different genotypes only exist at low feeding level. Since animals were fed for *ad libitum* intake in our study, we assume that it is rather unlikely that activities differ.

In trial 1 and 2, significant differences in  $ME_x$  were observed between female and castrated male animals, whereas in trial 3, no difference occurred. Similar to trial 3, van Milgen and Noblet (1999) and van Milgen *et al.* (1998) did not find any difference in  $ME_m$  (as the total requirement minus  $ME_g$ ) between male, female, and castrated male pigs.

#### 2.5.3 Ambient temperature

Since thermoregulation can require considerable amounts of energy (Knap, 1998), ARC (1981), NRC (1998), and GfE (2008) take into account energy needs for thermoregulation in their recommendations. Many studies have analysed the effect of ambient temperature on the amount of energy, which pigs require for thermoregulation with respect to their BW, performance, and feeding level as well as to different production systems (Black et al., 1999). According to GfE (2008), the lower critical temperature (LCT) ranges between 15 to 19°C, 13 to 15°C, and 12 to 15°C for growing pigs of 20, 60, and 100 kg BW, respectively. However, these values are only valid at free convection, dry conditions, a relative air humidity of 50%, and an insulated floor. These LCT values are below the air temperature of 17.6 - 26.3°C, 18.1 - 27.8°C, and 16.4 - 21.8°C in trial 1, 2, and 3, respectively. Nevertheless, it cannot be excluded that LCT of animals in our study was sometimes higher, since the slatted floor was made out of concrete without any insulation, and energy losses due to conduction are thus likely. Hence, ME<sub>x</sub> might have been caused to a certain extent by energy expenditures for thermoregulation in our study. Assuming an energy requirement for thermoregulation of 16 kJ/kg BW<sup>0.75</sup> per 1°C below the LCT (GfE, 2008), the ambient air temperatures in trial 1, 2, and 3 would have had to be 4°C, 15°C, and 21°C below the LCT, if ME<sub>x</sub> was exclusively caused by thermoregulation, which shows that thermoregulation cannot be seen as the main factor responsible for  $ME_x$  in our study.

#### 2.5.4 Immune response

Animals' physiological and immunological responses to diseases increase their energy needs and may thus contribute to the discrepancy between expected and actual animal performances (Black *et al.*, 1999). The increased energy needs may be due to changes in animal behaviour, digestion of nutrients, or impaired anabolic

processes in skeletal muscles, bones, and other tissues (Klasing, 2006). Moreover, increased energy requirements for immune responses, repair of damaged tissues, replacement of lost body fluids, and an increased body temperature can affect animal performance (Sandberg et al., 2007) and therefore would have an impact on ME<sub>x</sub>. Pigs vaccinated against porcine respiratory and reproductive syndrome or bacterial endotoxin infections (Escherichia coli) showed a decline of 15% in feed intake and of 21% in BWG compared to unvaccinated animals during the challenge period (Spurlock et al., 1997). Germ-free chicks had greater rates of protein and RE, lower  $ME_m$  requirements, and higher BWGs (5 to 30%) despite lower ME intakes than conventionally reared chicks (Lochmiller and Deerenberg, 2000). In immune system-activated, un-medicated pigs kept in a pathogenic environment, Williams et al. (1997) determined ME<sub>m</sub> requirements that were 54 kJ/kg BW<sup>0.75</sup> higher than those of early-weaned medicated pigs kept in a non-pathogenic environment. However, immune activation did not influence the partial efficiency of energy utilization for protein and lipid accretion in this study. Based on their literature review, Lochmiller and Deerenberg (2000) summarized that a mild up-regulation of the immune system can result in 15-30% increases of the basal metabolic rate in the vertebrate host. This would correspond to 5-10% of the ME-intake in a fast growing animal at a feeding level of three times maintenance. In trial 1, 2, and 3 of our study, 3.5, 3.4, and 3.7% of the animals got obviously ill, were medicated and separated from the group. Other than that, there were no animal health issues that required medical care. Nevertheless, it cannot be excluded that reactions to sub-clinical infections might have had increased the animals' ME needs and partly explain ME<sub>x</sub>. However, based on the estimate of 5-10% of ME needed for an immune challenge and assuming that half of the animals were challenged during 1/3 of the time (which reduces this estimate to 1-2% of the ME intake) we would conclude that energy expenditures for immune responses only marginally contributed to  $ME_x$  in our study.

### 2.6 Conclusions

With this study it has been shown that fattening pigs kept under commercial housing conditions need a considerable amount of energy for other purposes than maintenance and growth ( $ME_x$ ).  $ME_x$  can be used as a sensitive indicator for the

efficiency and quality of production systems. Enhanced physical activity is likely the main responsible factor for  $ME_x$  in our study, while environmental temperature, genotype, and immune responses might have contributed to a smaller extent only. However, further studies are needed to identify and to quantify the factors responsible for  $ME_x$  to provide more precise recommendations for the energy supply to growing pigs.

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3. TRYPTOPHAN REQUIREMENTS OF GROWING PIGS IN CONSIDERATION OF TWO DIFFERENT DIETS

#### 3.1 Abstract

There is considerable variation in the recommendations for the optimum tryptophan (Trp):lysine (Lys) ratio in diets of growing pigs which may partly be due to differences in the composition of experimental diets, the experimental approach, and the mathematical model used for data analysis. The objective of this study was therefore to determine the Trp:Lys ratio required for maximum response of pigs (15 - 35 kg body weight) offered a wheat-barley-soybean meal (diet type A) or a corn-soybean meal based diet (diet type B). Three mathematical models were applied to test to which extent the choice of model will affect the estimate of the optimum Trp:Lys ratio. A 4-week dose-response growth study was conducted with 880 crossbred castrated male and female pigs. The pigs were randomly allotted to eight dietary treatments per diet type. A Trp-deficient diet (T1) was formulated for each diet type that met the animals' requirements for all amino acids other than Trp (1.4 g precaecal digestible (pcd) Trp/kg diet) and Lys (10.5 g pcd Lys/kg diet). L-Trp was added to T1 diets to create six additional diets per diet type (T2 - T7)with pcd Trp concentrations that increased from 13.1 to 19.7% in type A diets and from 14.3 to 20.9% in type B diets. To test whether Lys was the second-limiting amino acid, a Lys adequate diet was formulated (T8) for each diet type by adding free Lys to the respective T7 diet. Pigs had free access to feed and water. Their body weight was recorded every week, while feed intake was measured every day. For both diet types body weight gain and feed to gain ratio increased with increasing Trp:Lys ratio. In the Trp-deficient diets feed intake was reduced by 60g and 100g in diet A and B, respectively, compared to diets 2-8. According to the broken-line model, the curvilinear increase-plateau model, and an exponential model, body weight gain reached the plateau phase Trp:Lys ratios of 15.9, 18.6, and >19.7% for diet type A and 17.8, >20.9, and >20.9% for diet type B, respectively. The respective ratios for the feed to gain ratio were 17.0, 18.9, and >19.7% and 19.7, 19.9, and >20.9% for diet type A and B, respectively.

The results confirm published ratios determined in barley-wheat based diets and indicate a higher optimum ratio in corn based diets. However, the mathematical model used for data analysis, the chosen response parameter, and the performance level aimed for strongly affect estimates of the optimum dietary Trp:Lys ratio. The results confirm recommended ratios in barley-wheat based diets, however clearly show a higher optimum ratio in maize based diets.

### 3.2 Introduction

Tryptophan (Trp) is usually considered the fourth limiting amino acid (AA) in cereal-based diets of pigs and it is required for protein synthesis as well as for various metabolic functions such as the synthesis of serotonin which plays an important role in feed intake (FI) regulation (Cortamira et al., 1991; Henry et al., 1992; Eder, 2001). Supplementation of free Trp to commercial diets becomes necessary to reach optimum AA-balance, when dietary protein concentration is reduced and free lysine (Lys) is supplemented, or higher proportions of feedstuffs with low Trp concentrations are used. Recommendations for optimum Trp supply are generally expressed in relation to dietary Lys concentration. In literature a relatively wide range of optimum Trp:Lys ratios in diets of growing pigs are reported (Susenbeth, 2006). GfE (2008) recommends a ratio of 17 to 19% on the basis of dietary precaecal digestible (pcd) AA concentrations. Based on total AA NRC (1998) and ARC (1988) suggest Trp requirements of 18.0 - 18.7% and 15%, respectively. Other, more recent studies determined higher optimum pcd Trp:Lys ratios of 19 to 22% (Roth et al., 2005, Fernándes and Strathe, 2009). Jansman and van Diepen (2007) recommended optimum pcd Trp:Lys ratios of even 21.4 -23.1%. Environmental factors (L'Floc, 2007) or diet composition (Jansman and van Diepen, 2009) may increase pigs' Trp requirements, so that Trp:Lys ratios above the general recommendations may by the German recommendations, (GfE 2008) may improve growth performance of pigs (Fernández and Strathe, 2009). The aim of the present study was therefore to determine the Trp:Lys ratio required for maximum growth response in young pigs of 15 - 35 kg body weight (BW) kept under commercial housing conditions and to investigate, whether this ratio depends on the type of cereal (wheat-barley versus corn) used in the diets.

# 3.3 Material and methods

# 3.3.1 Animals and housing

A total of 880 crossbred pigs (Pietrain X dbNaima/Porcus, 50% female and 50% castrated male) with an initial age of 21 - 28 days and a mean initial BW of 8.5 kg (6.3 - 10.6 kg) were kept in flat deck compartments at the experimental farm of the Agricultural Chamber Schleswig-Holstein. The compartments were heated over a twin-pipe system to keep ambient temperatures at 22 °C and air humidity at 65 - 70%. Each compartment comprised eight pens with slatted plastic floors. Fresh air was supplied from underneath the slab. In each compartment, 88 animals were evenly distributed among the eight pens. Prior to the experimental period, piglets were allowed to adapt to housing and feeding conditions for 19 days (pre-experimental period). The experimental period lasted from day 20 to 48.

# 3.3.2 Diets, feeding, and experimental design

Two experimental diet types were formulated (Table 3.1) using analyzed AA concentrations of diet ingredients and their precaecal digestibilities given in feed tables of AminoDat<sup>®</sup> 3.0 (2005; Table 3.2). While diet type A consisted of wheat, barley, corn gluten feed, corn gluten meal, and soybean meal, diet type B was composed corn, corn gluten feed, and soybean meal (Table 3.1). For both diet types a basal diet (T1) was formulated that met the animals' requirements for all AAs other than Trp (1.4 g pcd Trp/kg diet) and Lys (10.5 g pcd Lys/kg diet). A dietary Lys concentration that was marginally limiting (according to GfE, 2008) was chosen to enable to express Trp requirement in relation to Lys. By supplementing free Trp to T1 diets of each diet type the high Trp diets (T7) were formulated (Table 2). To create diets T2, T3, T4, T5, and T6 with increasing pcd Trp:Lys ratios diets T1 were blended with diets T7 with following proportionens: 167, 333, 500, 667, and 833 g T7/kg diet. To test if Lys was the second limiting AA in diets T1 -T7, two high-Lys diets (T8: 11.5 g pcd Lys/kg feed) were created by supplementing free Lys to the T7 diets of both diet types (positive control). Analysed total dietary Trp concentrations were 1.6, 1.7, 1.9, 2.0, 2.1, 2.2, 2.4, and 2.4 g/kg feed in T1 to T8 diets of diet type A and 1.9, 2.0, 2.1, 2.3, 2.4, 2.5, 2.7, and 2.7 g/kg feed in T1 to T8 diets of diet type B. Respective pcd Trp:Lys ratios were 13.1, 14.2, 15.3, 16.4, 17.5, 18.6, 19.7, and 18.3% (diet type A) and 14.3, 15.4, 16.5, 17.6, 18.7, 19.8, 20.9, and 19.3% (diet type B).

A commercial standard diet containing 190 g crude protein (CP), 14.5 g Lys, and 14.6 MJ ME/kg feed was fed to all pigs during the eight days (pre-experimental period). Starting at day 9, pigs were stepwise adapted to the experimental diets types by feeding the respective T8 diets until day 19 (adaptation period). Subsequently, they received the specific experimental diets from day 20 to day 48 (experimental period). Each pen received one of the eight diets of the two diet types. Dietary treatments were tested in five replicates each. The animals were fed for *ad libitum* intake in groups by a spot-mix system (Schauer, Germany). A feeding-break during night lasted 8 hours.

	Diet ty	/pe A	Diet t	уре В
Ingredients	T1	T7	T1	T7
Wheat	574.0	573.0	-	-
Barley	200.0	200.0	-	-
Corn	-	-	657.0	656.0
Soybean meal (48% CP)	47.2	47.2	195.0	195.0
Corn gluten feed	61.7	61.7	88.8	88.8
Corn gluten meal	53.5	53.5	-	-
Limestone	12.3	12.3	9.7	9.7
Dicalcium phosphate	8.3	8.3	12.4	12.4
Soybean oil	12.2	12.2	11.0	11.0
L-Lysine-HCl	8.3	8.3	5.4	5.4
DL-Methionin	2.3	2.3	2.8	2.8
L-Threonin	3.4	3.4	2.7	2.7
L-Tryptophan	-	0.95	-	0.95
L-Isoleucin	1.9	1.9	1.2	1.2
L-Valin	2.0	2.0	1.5	1.5
L-Histidin	0.5	0.5	-	-
Mineral-vitamin premix <sup>1</sup>	12.5	12.5	12.2	12.2

**Table 3.1:** Composition (g/kg feed) of the wheat-barley-soybean meal (diet type A) and a corn-soybean meal based diets (diet type B) with low (T1) or high (T7) tryptophan concentrations.

<sup>1</sup>:Mineral-vitamin-premix contributed to the basal diet (per 880.0 g/kg feed): NaCl 4.5 g; Ferrous sulphate monohydrate 180 mg; Copper sulphate 155 mg; Manganous sulphate 50 mg; Zinc sulphate 50 mg; Calcium iodate 2.0 mg; Seodium selenite 0.36 mg; Cobalt 0.4 mg; vitamin A 15000 I.U; vitamin D3 1500 IU; vitamin E 105 mg; vitamin K3 4.0 mg; vitamin B1 2.5 mg; vitamin B2 6 mg; vitamin B6 4.0 mg; vitamin B12 40.0 mg; pantothenic acid 20 mg; folic acid 2.0 mg; vitamin K3 4.0 mg; biotin 200.0 mg; betaine 500 mg. Feed supply for each pen was adjusted via sensors which recorded the feed amount in the troughs every 25 minutes. Animals had free access to water by one drinking nipple per pen. Daily FI per pen was recorded every day. BW was determined weekly during the experimental period per pen group and also individually every two weeks. Mean BWG and FI per pen were used to calculate FGR.

#### 3.3.3 Chemical analyses

From each diet, three feed samples were taken across the experimental period. They were analysed in duplicates for crude ash, CP (N x 6.25), crude lipid, and crude fibre concentrations according to Naumann and Bassler (2001). Dry matter (DM) concentrations were determined by oven-drying at 105°C over night. Starch contents were determined by enzyme hydrolysis of starch to glucose (Brandt *et al.*, 1987), using the heat-stable alpha-amylase Termamyl type 120 L (Novo Industrials, Bagsvard, Denmark). AA analyses of the diets were performed according to the Commission Directives (1998 and 2000), Table 3.2.

# 3.3.4 Statistical analyses

Statistical analyses were carried out using the ANOVA procedure (GLM) of SAS (1999). The following model was used:  $Yij = \mu + Di + Pj + eij$ , where Yij is the observed value,  $\mu$  the overall mean, Di the effect of diet type (diets A and B), Pj the effect of diet (T1 - T8), and *eij* the residual error. Least squares means and standard errors of the means for BW, BWG, FI, and feed to gain ratio (FGR) were calculated. Adjusted means were compared pair-wise using the Tukey-Kramer-Test. To describe the dose-response-relationship the following models were applied (Vedenov and Pesti, 2008): (1) the broken-line model, (2) the curvilinear increase–plateau model, and (3) an exponential model according to Robbins *et al.* (1979). The critical Trp:Lys ratio (commonly taken as the optimum; see discussion below) for the response parameter in model 1 is given at its breakpoint, in model (2) at the maximum of the quadratic function which coincidences with the beginning of the plateau phase, and in model (3) at that ratio at which 95% of the asymptote value is reached (Table 4).

	Diet ty	vpe A	Diet ty	уре В
Ingredient	T 1	Т 7	T1	Τ7
Crude protein	165.6	168.2	194.6	196.2
Lysine	11.2	11.8	12.2	12.8
Lysine, supp <sup>1</sup>	6.6	7.1	4.9	5.1
pcd Lysine <sup>2</sup>	10.3	10.9	11.1	11.6
Tryptophan	1.6	2.4	1.9	2.7
Tryptophan, supp <sup>1</sup>	<0.1	0.7	<0.1	0.9
pcd Tryptophan <sup>2</sup>	1.36	2.15	1.61	2.42
Threonin	8.1	8.2	9.3	9.6
Threonin, supp <sup>1</sup>	3.3	3.5	2.7	2.8
Methionin	4.6	4.8	6.0	6.0
Methionin, supp <sup>1</sup>	2.2	2.4	2.7	2.7
Methionin + Cystein	7.6	7.8	9.2	9.3
Isoleucin	7.2	7.4	8.8	8.9
Isoleucin, supp <sup>1</sup>	1.7	1.9	1.2	1.3
Valin	8.6	8.9	10.3	10.5
Valin, supp <sup>1</sup>	1.9	2.1	1.5	1.6
Leucin	13.2	13.7	20.4	20.6
Phenylalanin	7.4	7.6	9.8	9.9
Histidin	3.9	4.0	4.9	4.9
Arginin	7.2	7.3	10.2	10.5
Crude fiber	16.6	19.1	13.8	15.1
Ether extract	30.5	34.2	38.5	38.5
Ash	45.0	44.2	44.5	43.7
ME <sup>3</sup>	14.3	14.4	14.4	14.4

**Table 3.2:** Analysed composition (g/kg DM) and metabolizable energy (ME) content (MJ/kg DM) of the wheat-barley-soybean meal and the based diet with low (T1) and high (T7) tryptophan concentrations.

<sup>1</sup>Supp = supplemented

<sup>2</sup> pcd content were calculated according feed table of Evonik degussa (AminoDat.3,2005)

<sup>3</sup>ME contents were calculated according to the equation of Noblet and Perez (1993):

ME (MJ/kg DM) =  $4.168 - 12.3 \cdot Ash + 1.4 \cdot Crude protein + 4.1 \cdot Ether extract$ 

- 6.1 • Crude fiber (all in g/kg DM)

#### 3.4 Results

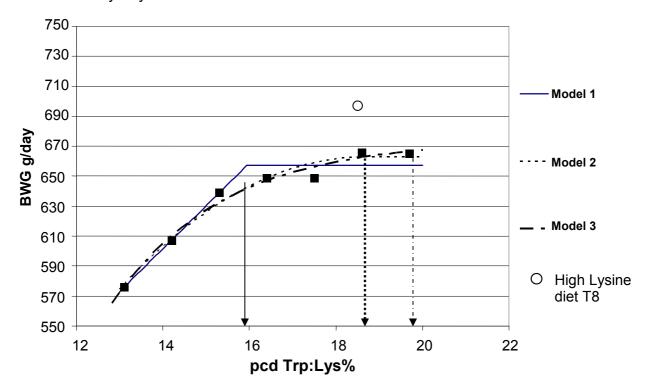
The experiment was carried out without any technical problems. Ten pigs got ill, were separated, and weighed. Pigs' FI, BW gain (BWG), and FGR are presented in Table 3.3. At the beginning of the experimental period, mean BW was similar between animals offered diet type A (14.1 kg; SD 2.2) and B (14.6 kg; SD 2.2). However, BW was lower in diet type A (32.3 kg; SD 5.6) than diet type B pigs (34.5 kg; SD 4.6; P=0.01) at the end of the experiment. Daily FI did not differ between diet type A and B at 1.17 kg (SD 0.13) and 1.19 kg (SD 0.07), respectively (P>0.05). Within diet types FI was lowest in pigs receiving diets T1 at 1.08 kg (SD 0.19; P=0.008) for diet type A and 1.14 kg (SD 0.09; P=0.09) for diet type B. Daily BWG was 700 g/d in pigs offered type B diets and higher than of those offered type A diets (640 g; P<0.001). Daily BWG increased with increasing pcd Trp:Lys ratio in both diet types. BWG tended to be highest at the high Lys diets T8 at 693 g/d (SD 0.06) for diet type A and at 750 g/d (SD 0.04) for diet type B, compared to the mean BWG in T1 - T7 the difference to T8 was significant (P=0.01) in both diet types. FGR was higher in type A (1.85; SD 0.17) than in type B diets (1.75; SD 0.25; P=0.03). FGR in diets T8 were lower compared to the mean FGR across diets T1 - T7 (diet type A: P=0.003, diet type B: P=0.014). However, it was only numerically lower when compared to diets T7 (diet type A: P=0.14; diet type B: P=0.42).

The effects of increasing pcd Trp:Lys ratios on BWG and FGR according to the three models are illustrated in Figure 1 and 2. The respective regression equations are given in Table 3.4. The critical pcd Trp:Lys ratios differed between models and response parameters. According to model 1 and 2 the respective critical ratios are 15.9% and 18.6% for pigs offered type A and 17.8% and >20.9% for those offered type B diets when BWG is taken as response parameter. When FGR is used, model 1 and 3 yield critical ratios of 17.0% and 18.9% (diet type A) as well as 19.7% and 19.9% (diet type B), respectively. Optimum ratios could not be determined by model 3, since ratios at which 95% of the asymptote value were reached exceeded the maximum ratios tested in diet type A (19.7%) and B (20.9%).

					Diet						P-values	
	11	Т2	Т3	Т4	T5	T6	17	Т8	SEM	Trp effect within T1 - T7	Lys effect T1 – T7 vs. T8	Lys effect T7 vs. T8
Wheat-barley-soybean meal based diet	ybean m	eal based	l diet									
Total Trp:Lys ratio,	14.3	15.3	16.3	17.3	18.3	19.3	20.3	19.7				
Pcd * Trp:Lys ratio,	13.1	14.2	15.3	16.4	17.5	18.6	19.7	18.3				
Trp intake, g/d	1.73	1.97	2.26	2.30	2.46	2.75	2.83	2.80				
Initial BW, kg	14.1	14.2	14.1	14.3	14.0	14.7	13.6	13.9	0.293			
Final BW, kg	30.3	31.2	32.3	32.5	32.4	33.8	32.2	32.8	0.817	0.017	0.463	0.639
FI, kg dry matter/d	1.08	1.16	1.19	1.15	1.17	1.23	1.18	1.17	0.029	0.008	0.787	0.854
BWG, g/d	576	607	639	649	649	666	665	693	0.021	0.002	0.014	0.341
FGR	1.91	1.91	1.86	1.80	1.77	1.83	1.78	1.69	0.046	0.017	0.003	0.137
Corn-soybean meal based diet	eal base	diet										
Total Trp:Lys ratio,	15.6	16.5	17.4	18.3	19.3	20.2	21.1	20.4				
Pcd <sup>*</sup> Trp:Lvs ratio.	14.3	15.4	16.5	17.6	18.7	19.8	20.9	19.3				
Trp intake, g/d	2.17	2.40	2.52	2.71	2.98	2.98	3.27	3.24				
Initial BW, kg	14.6	15.1	15.1	14.4	14.5	14.5	14.7	14.4	0.240			
Final BW, kg	32.6	34.6	34.4	34.3	34.0	34.0	35.2	35.3	0.692	0.102	0.131	0.922
FI, kg dry matter/d	1.14	1.20	1.19	1.18	1.24	1.19	1.21	1.20	0.024	0.097	0.847	0.829
BWG, g/d	644	674	069	209	695	716	735	750	0.019	0.001	0.008	0.556
FGR	1.77	1.77	1.73	1.67	1.68	1.64	1.64	1.59	0.039	0,002	0.014	0.418

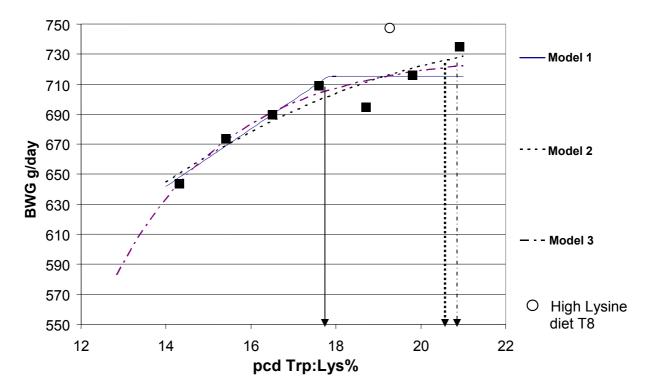
Table 3.3: Total and precaecal digestible (pcd) tryptophan (Trp) to lysine (Lys) ratio (%) in eight dietary treatments (T1 - T8) of a wheat-

**Figure 3.1:** The critical ratio of precaecal digestible (pcd) Tryptophan (Trp) to lysine (Lys)(arrows) using body weight gain (BWG) in g/day as response variable for the wheat-barley-soybean meal (Diet A) and corn-soybean meal (Diet B) based diet and as determined by three different mathematical models for growing pigs (for details see text).

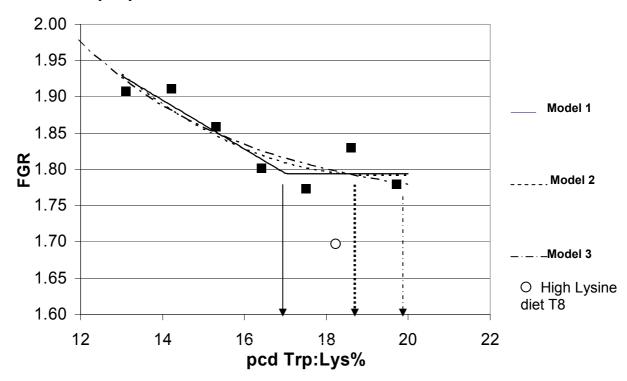


Wheat-barley-soybean meal based diet

Corn-soybean meal based diet

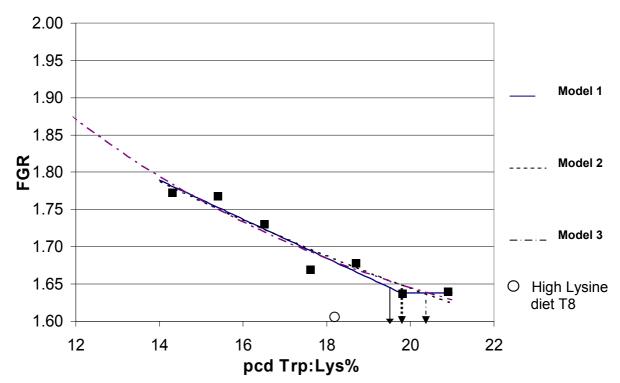


**Figure 3. 2:** The critical ratio of precaecal digestible (pcd) Tryptophan (Trp) to lysine (Lys) (arrows) using feed to gain ratio (FGR) as response variable for the wheat-barley-soybean meal (Diet A) and the corn-soybean meal (Diet B) based diet and as determined by three different mathematical models in growing pigs (for details see text.).



Wheat-barley-soybean meal based diet

Corn-soybean meal based diet



**Table 3.4** Critical precaecal digestible pcd tryptophan (Trp) to lysine (Lys) ratio (%) in a wheat-barley-soybean meal and a corn-soybean meal based diets as determined by three mathematical models using body weight gain (BWG) and feed to gain ratio (FGR) of growing

laboM	Broken-Line model (1)	l (1)	Curvilinear increase-plateau model (2)	del (2)	Exponential model(3)	
			Wheat-barley-soybean meal based diet	ased diet		
Variable	Equation	Critical Trp:Lys	Equation	Critical Trp:Lys	Equation	Critical Trp:Lys <sup>1</sup>
BWG, g/d	y= 657.2 - 28.6 • (15.9 - x) R² = 0.96	15.9%	y= 662.9 - 2.75 • (18.6 - x) <sup>2</sup> R <sup>2</sup> = 0.97	18.6%	y= 582 + 102.5 • (1 - e <sup>-0.337 (x - 13.1)</sup> ) R <sup>2</sup> = 0.96	> 19.7%
FGR	y= 1.79 + 0.034 • (17.0 - x) R <sup>2</sup> = 0.84	17.0%	y= 1.80 + 0.004 • (18.9 - x) <sup>2</sup> R <sup>2</sup> = 0.85	18.9%	y= 1.93 - 0.168 • (1 - $e^{-0.270}$ (x - <sup>13.1</sup> ) R <sup>2</sup> = 0.82	> 19.7%
			Corn-soybean meal based diet	, t		
Variable	Equation	Critical Trp:Lys	Equation	Critical Trp:Lys	Equation	Critical Trp:Lys <sup>1</sup>
BWG, g/d	y= 715.3 - 19.18 • (17.8-x) R <sup>2</sup> = 0.84	17.8%	y= 738 - 0.915 • (24.7 - x) <sup>2</sup> R <sup>2</sup> = 0.93	> 20.9% <sup>2</sup>	y= 639 + 135 • (1- $e^{-0.162}$ (x - 14.3) R <sup>2</sup> = 0.93	> 20.9%
FGR	y= 1.634 + 0.026 • (19.7-x) R <sup>2</sup> = 0.94	19.7%	y= 1.63 + 0.025 • (19.9 - x) <sup>2</sup> R <sup>2</sup> = 0.96	19.9%	y= $1.77 + 21.4 \cdot (1 - e^{-0.001 (x - 14.3)})$ R <sup>2</sup> = 0.94	> 20.9%
<sup>1</sup> Critical as	Critical assumed to be reached at 95% of asymptotic value,	of asymptotic	value, when this ratio is above	the highest r	when this ratio is above the highest ratio measured, this latter is given.	

<sup>2</sup> Critical ratio is higher than at diet T7, therefore the value at T7 is given.

#### 3.5 Discussion

Many experiments are reported in the literature where the effect of different Trp:Lys ratios on FI and BWG of pigs were studied in order to determine their Trp requirement. However, while Eder *et al.* (2003) and Guzik *et al.* (2005) tested five and Pulske and Mullan (2000) as well as Jansman and de Jong (2000) even less than five ratios, seven Trp levels were tested in two diet types in this study. Together with the large number of animals used (n=874), our results in respect of an adequate Trp supply to growing pigs (14 to 35 kg BW) are based upon a comprehensive data set. In order to be enabled to express Trp requirement in relation to Lys, it is essential that Lys is the second limiting AA and provided at or below the animals' requirement (Boisen, 2003; Barea *et al.*, 2009). Pcd Lys concentrations were 10.5 g /kg feed in diets T1 – T7 in our study which is about 90% of the concentration recommended by GfE (2008) for diets of pigs of similar BW growing at 700 g/d. In both diet types additional Lys supply (T8 *versus* T7) non-significantly increased BWG, sustaining the supposition that Lys was the second limiting AA in diets T1 – T7.

Depending on the chosen response criteria (BWG or FGR) and the mathematical model used for data analysis, critical pcd Trp:Lys ratios varied between 15.5 to >20.9% across both diet types, showing that both, the model as well as the response variables strongly affect the conclusions drawn from measured values. The breakpoint of the broken-line model underestimates the animals' Trp requirements and does not account for gradually diminishing returns before reaching the asymptote value (Robbins et al., 2006). Hence, this model might be appropriate to evaluate data of an individual animal, but does not adequately describe the mean response of a group of animals (Fuller, 1994). The exponential model better describes non-linear relationships (Robbins, 2006) but there is no scientific basis for the definition of performance level (i.e. 95% of the asymptote value in our study) which, however, strongly influences the value for the critical ratio. Although we agree with Simongiovanni et al. (2010) that the curvilinear increase-plateau model (model 2) yields the best estimate of the critical pcd Trp:Lys ratio, none of the mathematical models can be taken as the exclusive approach for estimating the AA requirements of animals from dose-response data (Vedenov, 2008). Thev rather give an indication of the required

range or, *vice versa*, of the performance that can be expected when feeding a certain amount of AAs.

Responses and critical pcd Trp:Lys ratios differed between diet types. BWG of pigs fed diet type A increased up to a pcd Trp:Lys ratio of 18.6%, while it further increased above this ratio in animals offered diet type B. However, no critical pcd Trp:Lys ratio could be identified for this diet type, since neither BWG nor FGR reached the plateau phase. Nevertheless, according to all, optimum pcd Trp:Lys ratios determined from the models or estimated by extrapolation are higher in diet type B than A, indicating that Trp requirements of pigs receiving corn-soybean meal based diets are higher than of those fed wheat-barley-soybean meal based diets. Similarly, Jansman *et al.* (2009) determined higher optimum pcd Trp:Lys ratios in corn-soybean meal-corn gluten meal based diets (23.1%) than in wheat-barley-soybean meal-pea-whey powder based diets (21.4%). However, the latter value is higher than the critical ratio determined in our study for a similar diet.

Similarly, Eder *et al.* (2003) found maximum FI, BWG, and N retention in female pigs (25 - 50 kg BW) offered a corn-barley-pea-based diet at higher critical Trp:Lys ratios (22.6 - 23.0%). Likewise, Schutte *et al.* (1989) determined highest BWG in pigs offered a barley-corn-tapioca-soybean meal based diet at a Trp:Lys ratio of 23.5%. In a recent meta-analysis of literature data on Trp requirements of piglets using the curvilinear increase-plateau model, Simongiovanni *et al.* (2010) determined optimum Trp:Lys ratios of 21.6, 22.2, and 19.7 for BWG, FI, and BWG:FI ratio, respectively. In contrast thereto, Susenbeth and Lucanus (2005) did not find any effect on BWG of pigs (15 - 25 kg BW) when Trp:Lys ratios in a wheat-barley-soybean meal-corn gluten-corn starch based diet were increased above 17%, neither at restricted nor at unrestricted feeding.

Factors such as dietary CP content or age of the animal may also affect the critical Trp:Lys ratio and thus explain the high variation in recommended ratios found in literature (Eder *et al.*, 2003; Jansman *et al.*, 2009). Hence, Jansman and de Jong (2000) who compared two basal diets containing 17 or 20% CP determined similar

BWGs (560 g/d) of pigs (9 - 27 kg LW) at Trp:Lys ratios of 19 and 22% in the high protein diet and 18% in the low protein diet. Feeding the low protein diet, the animals' growth was further improved by increasing the Trp:Lys ratio to 21%. Based on chemical analyses, in our study CP contents were slightly higher in the corn-soybean meal based diets than in the wheat-barley-soybean meal based diet which may thus partly explain the higher Trp requirements of pigs receiving this diet type.

Furthermore, there is evidence that AA other than Trp and Lys affect the critical Trp:Lys ratio which might explain the difference between both diet types. Large neutral AA's (LNAA: valine, isoleucine, leucine, phenylalanine, and tyrosine) compete with Trp for the same transporters during intestinal absorption and passage of the blood-brain barrier. In diets with high LNAA concentrations the quantity of Trp available for serotonin synthesis is reduced (Henry *et al.*, 1996; Sève, 1999). As a consequence, FI of pigs in a study by Henry *et al.* (1996) decreased by approximately 20% when dietary Trp:LNAA ratios were reduced from 2.50 to 1.78%. However, Trp:LNAA ratios were similar in diets of type B (4.4 - 6.4%) and A (3.9 - 5.4%) and FI did not differ between the two diet types. Hence, it appears that differences of LNAA contents might not be the reason for the differences in critical Trp:Lys ratios observed in our study.

Precaecal digestibility of Trp in corn (76%) is lower than in wheat (88%; AminoDat<sup>®</sup> 3.0. 2005). We accounted for this difference in the formulation of our experimental diets and in the calculation of the Trp:Lys ratios as the independent variables. In growing-finishing pigs Urbaityte *et al.* (2009) determined precaecal digestibilities of 68 and 63% of Lys and Trp in corn gluten. Although these values are similar to those given by NRC (1998), GfE (2008), and Rademacher *et al.* (2009), they are 1-12 percent units lower than those of the feed tables of Amipig (2000) and CVB (2003). Differences in the precaecal AA digestibility between piglets and growing pigs were observed in soybean meal and between various studies in wheat gluten (Urbaityte *et al.*, 2009). Despite using animals of similar BW the authors reported 6 - 38 percent units lower AA digestibilities for soybean meal than Rademacher *et al.* (2009). These differences may be due to the methodology used, or related to properties of feeds which can vary according to

their origin and processing treatment. Hence, the different responses of pigs offered diet type A and B to increasing Trp:Lys ratios might to a certain extent be caused by using standard precaecal digestibility values taken from feed tables. Therefore, underestimation of pcd Trp of corn could explain the higher BWG observed in diet type B than in A at similar Trp intake. However, reasons responsible for the higher critical Trp:Lys ratio in the corn-soybean meal than in the wheat-barley-soybean meal based diets cannot be conclusively identified on the basis of our results, whereas the generally higher BWG in diets of type B than on type A is related to the higher Lys and Trp concentration.

Finally, the critical ratio at which maximum response is reached is commonly taken as the optimum. However, the increment per unit intake strongly diminishes before reaching the plateau level. Supposing that 99% of maximum response might be appropriate for commercial production, required Trp:Lys ratio decreases from 18.6% at maximum BWG to 17.5% at 99% of maximum BWG for diet type A and from >20,9% to 19.8% for diet type B, respectively, according to the curvilinear increase-plateau model. When aiming at only 98% of maximum BWG, required Trp:Lys ratio further decreases to 16.7% and 19.0% for diet A and B. Similar changes can be derived from the results of the meta-analysis of Simongiovanni et al. (2010): The optimum Trp:Lys ratio of 21.6% (i.e. ratio at maximum BWG) would be lowered to 19.8%, if 99% of maximum BWG is taken as an adequate response. Defining the optimum as the ratio necessary to reach maximum response includes a considerable safety margin. Thus, recommendations should not be given as fixed values, rather they should provide information about the expected biological response to enable farmers to find the farm-specific optimum depending among others on the actual and local set of pizes (Morris, 1983).

# 3.6 Conclusions

(i) Estimates of the optimum Trp:Lys ratio differ between the mathematical model used to analyze experimental data, on the chosen response parameter as well as on the intended performance level. A more critical discussion about suitable models to describe dose-response relationships, about the performance level to be aimed for in the commercial production, and about the parameters to be chosen is necessary to avoid conflicting recommendations for optimum AA supply which lead to an uncertainty of farmers and the feeding industry. (ii) Trp requirement is influenced by feed ingredients when using standard values for precaecal digestibility of Trp. Further studies are needed to identify the responsible factors for this effect to allow for more diet specific recommendations for the adequate Trp supply to growing pigs.

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# 4. GENERAL DISCUSSION

## 4.1 General discussion

Feeding costs represent a large proportion of total costs in pig production (Thieme, 2008) and public as well as official interest in the reduction of environmental pollution from livestock husbandry has largely increased. From an economic and environmental point of view, it is thus a major challenge in animal feeding to maximize nutrient and energy use efficiencies by adjusting their supply to the animals' requirements. However, detailed and accurate information on the nutrients and energy requirements of animals at different physiological states are necessary in order to be able to optimise nutrient supply to the animals' without risking a decline in their production.

North American and European feeding recommendations (ARC, 1988; NRC, 1998; GfE, 2008) are based upon a separate estimation of the animals' energy requirements for maintenance ( $ME_m$ ) as well as protein and fat deposition ( $ME_g$ ) which were determined in numerous, multi-faceted studies. While  $ME_g$  is derived from the energy value of gain according to its protein and fat content and the efficiency of ME utilization for gain, and  $ME_m$  form dose-response-studies or from trials where animals are fed near maintenance level mainly under controlled experimental conditions, where pigs were kept individually in metabolic cages or respiration chambers.

ME<sub>m</sub> recommendations already consider a certain fraction of the energy requirements of group-housed pigs for physical activities (i.e. eating, standing, walking, lying down, and getting up). Hence, GfE (2008) included 55 kJ/kg BW<sup>0.75</sup> in their ME<sub>m</sub> recommendations. However, on-farm studies showed that there are nevertheless large discrepancies between measured BW gains and those predicted from the animals' ME intake using official feeding recommendations. Campbell and Taverner (1988) and Black *et al.* (2001) reported 20 to 30% lower growth rates of pigs raised on commercial farms than of those used in controlled experiments despite similar energy intakes. Moughan (2003) stressed that farm animals may not achieve the maximum potential performance level determined at breeding stations due to inferior housing and social conditions.

General Discussion

At least part of the ME ingested by the animals but not used for maintenance and gain (ME<sub>x</sub>) can be attributed to environmental factors or housing conditions such as ambient temperatures, humidity, air quality, hygiene, space allowance, group size, or feeding management (Wellock *et al.*, 2003) which increase the animals' activity and/or impose stress on their metabolism and immune system. Increases in ME requirements in response to individual of these factors were partly estimated and considered in the feeding recommendations (GfE, 2008). However, ME<sub>x</sub> as a result of a combination of environmental and housing related factors cannot be estimated under controlled experimental conditions.

In our study, we quantified  $ME_x$  in commercially raised pigs using a large data set from three on-farm trials with in total 1872 individual animals of both sexes (castrated males and females). We estimated protein and fat accretions from lean meat percentages at slaughter adjusting for changes in protein and fat concentrations in measured body weight gain of the pigs according to GfE (2008). A considerable amount of  $ME_x$  was determined in all trials ranging from 2.1, 16.9 to 21.4% of the animals' average ME intake in trial 1,2 and 3.  $ME_x$  differed between trials, but was consistent for animals used within one trial. Physical activity of the animals was likely the main reason for ME<sub>x</sub>. Not only the duration, but also the kind of activity as well as the permanent muscle strain in response to adverse group constellations or environmental conditions may thus largely determine ME<sub>x</sub>. In conclusion, the amount of required ME<sub>x</sub> might be specific for different farming systems and may thus be a useful indicator for evaluating their efficiency. However, further studies that include measurements of the animals' behaviour are needed to identify all factors responsible for ME<sub>x</sub> and to determine whether various production systems with different group sizes or housing conditions affect this value.

Similarly, accurate estimates of the animals' amino acid (AA) requirements of pigs for growth are needed to be able to increase nutrient use efficiency through an optimized diet formulation. In view of the importance of cereal grains in pig nutrition, protein accretion is among AA mainly determined by the quantity of lysine (Lys) available for growth. Hence, recommendations for the adequate supply of AAs other than Lys are often expressed in relation to dietary Lys

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concentration. However, a considerable variation exists in the estimates derived from scientific experiments of the AA requirements of growing pigs (Moughan, 2003, Susenbeth, 2006). Recent studies determined critical ratios between precaecal digestible (pcd) Trp and Lys of 19 to 22% (Roth *et al.*, 2005, Fernándes and Strathe, 2009), while German feeding standards recommend pcd Trp:Lys ratios of 17 to 19% for the adequate Trp supply to growing pigs (GfE, 2008). The Agricultural Research Council (ARC,1988) proposes a pcd AA ratio of 15% which is confirmed by data compiled by the National Research Council (NRC, 1998) who suggests a Trp supply of 18.0 to 18.7% of total Lys concentration.

Trp requirements and hence, recommendations for the adequate Trp:Lys ratio in pig diets may vary due to differences in diet composition and the feed used for their formulation (Jansman and von Diepen, 2009). Moreover, different methods of laboratory and statistical analyses may affect estimates of the critical Trp:Lys ratio. Hence, depending on the analytical procedure variations in the Trp concentrations of feed samples of up to 15% are possible (Landry and Delhaye, 1994). In some feedstuffs due to protein-bound Trp, there is a correction value for losses during This will lead to significant overestimations of actual Trp hydrolysis. concentrations. As a consequence, a standard EU methodology was established for the determination of total AA concentrations. Moreover, estimates of the AA recovery in ileal digesta used to determine precaecal AA digestibility exhibit a large variation between laboratories, even if the same methodological approach was used (AmiPig, 2000). Finally, certain AAs such as Lys and Trp are susceptible to heat treatment. They may form complexes which are unavailable for protein metabolism. In this case, intake of pcd AAs usually overestimates the actual amount of AAs available for protein synthesis (Batterham, 1992). Hence, standard analytical methods for the determination of the content and digestibility of AAs are needed in order to be able to derive reliable estimates of the critical AA supply that account for differences between individual diet components as well as the native variation within one feedstuff.

Dose-response studies are commonly used to derive recommendations for the AA supply to animals. The dietary supply levels tested must include moderately deficient diets as well as those that do not promote any further increment in the

animals' responses (Baker, 1986, Berndtson, 1991). Moreover, the choice of the response parameter as well as of the model and hence, the response curve fitted to the data sets strongly affect the recommendations derived from them. Brokenline regression analyses of dose-response data sets assume a linear increase in the animals' responses to nutrient intake below and no increment above their required intake level (Robbins *et al.*, 1979). The latter is defined as the break point of the model. Aiming at one single value of nutrient input that yields maximum output has thus favoured the use of the broken-line model. However, it implies that nutrients are utilized at constant efficiency until maximum response is achieved. This does not always apply, since the rate of change may also decrease with increasing nutrient intake (Rodehutscord *et al.*, 1999). Hence, there is no general agreement that the broken-line model adequately describes the animals' actual responses to increasing nutrient intake (Robbins 2006, Rodehutscord *et al.*, 1995 and 1999, Vedenov and Pesti, 2008).

Furthermore, a mathematical model must account for differences in doseresponse relationships of individual animals and hence, the differences in their response value at a specific nutrient intake (Pomar *et al.*, 2003), leading to rather curvilinear dose-response relationships. Hence, while the broken-line model may well describe the response of an individual animal, it might not be appropriate for the mean response of a group of animals (Fuller, 1994) and alternative models that include a non-linear component are required instead (Robbins *et al.*, 2006). Exponential models are frequently recommended (Vedenov and Pesti, 2008), since they assume diminishing returns towards the maximum response.

The aim of our study was to derive reliable estimates of the critical pcd Trp:Lys ratio for growing pigs. Unlike to the major published studies, seven diets of two different diet types, a wheat-barley-soybean meal and a corn-soybean meal based diet, were tested with Trp:Lys ratios ranging from 13.1 to 20.9%. In all diets, Lys was the second limiting AA which was confirmed by results obtained for one diet of each diet type that met the animals' Lys requirements (positive control). Data of a total of 880 pigs raised at commercial farming conditions were collected and analysed by three different mathematical models.

Fitting the broken-line model to our experimental data, critical pcd Trp:Lys ratio ranged between 15.9 to 19.7%. When applying the curvilinear increase-plateau model or the exponential regression equations, critical ratios of 18.6 to 20.9% and ≥19.7% were obtained. Critical pcd Trp:Lys ratios also varied according to the chosen response parameter (body weight gain vs. feed to gain ratio) and was higher for the corn-soybean meal than for the wheat-barley-soybean meal based diet. Our estimates of the critical pcd Trp:Lys ratios for wheat-barley-soybean meal based diets (16 to 19%) were within the range of the recommendations presented above. However, LWG and feed to gain ratio of pigs offered corn-soybean meal based diet type appears to further increase with dietary Trp:Lys ratios higher than those tested in our study. Hence, although they exceeded earlier published ratios, no final conclusion on the critical pcd Trp:Lys ratio could be drawn for this diet type. These differences observed in our trial might be related to animal and dietary factors as well as the methods used for data collection and analyses. However, further studies are needed to identify these factors and their causes as well as to quantify to which extent they contribute to increases in Trp requirements of growing pigs in order to be able to consider them in feeding recommendations.

From an economic and ecological point of view, the main aim in commercial pig production is to formulate diets that meet the animals' nutrient and energy requirements as much as possible. However, our studies showed that there are considerable differences between the ME requirements of pigs kept under controlled experimental or commercial husbandry conditions. A number of factors and their combinations appear to be responsible for this discrepancy as well as for the variation in the critical nutrient and energy supply for maximum growth and feed use efficiency. Hence, besides precise estimates of the animals' growth potential and of the performance level that allows for maximum profit with respect to specific socio-economic frame conditions, further studies are needed to quantify AA and energy requirements of commercially raised pigs and to identify the factors that are responsible for the discrepancy between their expected and observed growth performance and the variation in the animals' responses to increasing nutrient and energy intake.

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5. SUMMARY/ZUSAMMENFASSUNG

#### 5.1 Summary

## Studies on the energy and tryptophan requirements of growing pigs

In commercial pig production, pronounced discrepancies can be observed between the actual growth rates and those expected from current feeding recommendations which are based upon measurements of the metabolizable energy (ME) requirements of individually-housed pigs for maintenance as well as protein and fat retention under controlled housing conditions. The aim of the first study was to quantify this amount of the energy required for purposes other than maintenance and body weight (BW) gain of pigs. In three trials, feed intake, BW gain, and carcass composition was determined in 660 castrated male and female pigs each (25 to 120 kg body weight (BW)) kept under commercial housing conditions. Energy retention was derived from carcass composition and BW gain. ME requirements for maintenance and efficiency of ME utilization for BW gain were taken from current German feeding standards.

Differences between the ME intake and the ME requirements for maintenance and growth accounted for 2.1, 16.8, and 21.4% of the animals' ME intake. Although values differed considerably between trials, they were consistent for all animal groups within each trial. Hence, results indicate that under commercial housing conditions factors such as environmental and the animals' physical activity increase their ME requirements. We assume that the activity plays a special role, not only depending on the type of activity and its temporal duration, but also the permanent muscle strain in consequence of the group constellation and environmental conditions. This additional amount of ME required by commercially grown pigs may serve as an useful indicator for the evaluation of the efficiency of different housing systems.

Similarly, published recommendations for the critical supply with the amino acids tryptophan (Trp) and lysine (Lys) strongly vary. Hence, the aim of the second study was to determine the critical Trp:Lys ratio for young pigs (15 to 35 kg BW) under practical farming conditions, and whether this ratio is affected by diet composition. Thus, a four-week dose-response study was conducted with 880 crossbred castrated males and female pigs. Two diet types were formulated: a

wheat-barley-soybean meal (diet type A) and a corn-soybean meal based diet (diet type B). Seven diets with precaecal digestible (pcd) Trp:Lys ratios increasing from 13.1 to 19.7% and 14.3 to 20.9% were formulated for diet type A and B, respectively, by supplementing L-Trp. With the exception of one Lys-adequate diet per diet type (positive control), Lys was the second limiting AA in all experimental diets. Pigs were evenly allotted to dietary treatments and were group-fed ad libitum by a sensor-controlled spot-mix system. BW gain and feed to gain ratio (FGR) were measured and used as response parameter to determine critical Trp:Lys ratio by three mathematical models.

Fitting the broken-line model to BW gain data, critical Trp:Lys ratios were 15.9% (diet type A) and 17.8% (diet type B). Using FGR as response variable the respective ratios were 17.0 and 19.7%. When using the broken-line with an ascending quadratic model to analyse BW gain and FGR data, critical ratios were 18.6% (diet type A) and >20.9% (diet type B) as well as 18.9% (diet type A) and 19.9% (diet type B), respectively. Fitting exponential models to the same respective data sets, the critical Trp:Lys ratios were >19.7% (diet type A) and >20.9% (diet type B).

Results showed that critical Trp:Lys ratios derived from the broken-line model were consistently lower than those determined by the exponential model and stress that the choice of the mathematical model used for data analyses strongly affects the estimated critical amino acid ratio. Moreover, estimates of the critical Trp:Lys ratio differ depending on the chosen response variable (BW gain vs. FGR). Nevertheless, irrespective of the chosen mathematical model and response parameter, critical Trp:Lys ratio was consistently higher in diet type B than A, indicating differences in diet composition will alter Trp requirements of growing pigs.

In conclusion, further research is needed to identify the factors that are responsible for higher energy requirements of commercially raised pigs than of those kept under controlled experimental conditions. Similarly, further studies are needed to determine why and to which extent diet composition alters pigs' Trp requirements. Moreover, revisions of current feeding recommendations for the critical supply of energy and Trp appear to be necessary.

#### 5.2 Zusammenfassung

Untersuchungen zum Energie- und Tryptophanbedarf des wachsenden Schweines Unter praxisüblichen Mastbedingungen gibt es Diskrepanzen zwischen den Wachstumsleistungen, wie sie unter Berücksichtigung der aktuellen Versorgungsempfehlungen zu erwarten wären, und den gemessenen Leistungen bei wachsenden Schweinen. Um diese Abweichungen genauer zu quantifizieren, wurden in drei Mastdurchgängen unter praktischen Haltungsbedingungen Untersuchungen an wachsenden Schweinen im Gewichtsbereich von 25 bis 120 kg durchgeführt. Der Energiebedarf für den Protein- und Fettansatz wurde indirekt über Schlachtkörperparameter der einzelnen Tiere unter Anwendung der entsprechenden Kenngrößen und Verwertungskoeffizienten aus den aktuellen Versorgungsempfehlungen (GfE, 2008) ermittelt. Ebenfalls wurde ein Energiebedarf für die Erhaltung gemäß dieser Empfehlungen angenommen. In allen Mastdurchgängen wurde eine höhere Energieaufnahme ermittelt als sie für den Protein- und Fettansatz sowie zur Deckung des Erhaltungsbedarfes nötig gewesen wäre. Diese Differenz entsprach im Mittel 2.1, 16.8 und 21.4% der aufgenommenen Energiemenge in den Mastdurchgängen 1, 2 und 3, war aber innerhalb eines Mastdurchganges relativ konstant. Es ist daher davon auszugehen, dass die Tiere unter praktischen Bedingungen einen zusätzlichen Energiebedarf aufweisen, der von haltungsbedingten Faktoren wie z.B. der Umgebungstemperatur und der Bewegungsaktivität der Tiere hervorgerufen wird. Unterschiede in diesem hauptsächlich durch Haltungsfaktoren bedingten Energiebedarf können daher auch als sensitiver Indikator bei einer Bewertung der Produktionseffektivität von verschiedenen Haltungssystemen dienen.

In einer zweiten praxisorientierten Studie sollte der Bedarf von Schweinen mit Lebendmassen von 14 bis 35 kg an Tryptophan (Trp) in Relation zu Lysin (Lys) anhand des Lebendmassezuwachses und des Futteraufwandes abgeleitet werden, da es die aktuellen Empfehlungen für eine optimale Versorgung wachsender Schweine mit praecaecal verdaulichem Trp in der Literatur sehr stark schwanken. Weiterhin sollte durch den Einsatz von zwei unterschiedlichen Diäten (Weizen-Gerste-Soja- vs. Mais-Soja basierte Diäten) überprüft werden, ob der Trp-Bedarf durch unterschiedliche Futterkomponenten beeinflusst wird. Durch Zulage von L-Trp wurden acht Versorgungsstufen mit steigenden Trp:Lys-Verhältnissen von 13.1 bis 20.9% für jede Diät formuliert. Die Daten wurden mittels drei verschiedenen mathematischen Modellen mit Lebendmassezunahmen und Futteraufwand als abhängigen Variablen ausgewertet. Die so abgeleiteten optimalen Trp:Lys-Verhältnisse waren abhängig von der Auswahl des Modelles und der abhängigen Variablen und lag zwischen 15.9 und 19.7% für die Weizen-Gerste-Soja- und zwischen 17.8 und >20.9% für die Mais-Soja basierte Diät. Das Ergebnis weist darauf hin, dass unabhängig von dem verwendeten Modell deutliche Unterschiede zwischen den beiden Diäten auftraten.

Aus den Ergebnissen der vorliegenden Studien kann abgeleitet werden, dass weitere Untersuchungen erforderlich sind, um die den Energiehaushalt des Tieres beeinflussenden Haltungsfaktoren näher zu identifizieren. Entsprechendes gilt auch für die Versorgung mit Trp, wo zunächst nur nachgewiesen werden konnte, dass dessen Bedarf nicht unabhängig von der Rationszusammensetzung ist. Auch hier sind weitere Untersuchungen erforderlich, um die Ursachen für diesen Unterschied zu identifizieren. Sie zeigen auch, dass eine entsprechende Präzisierung der Versorgungsempfehlungen für die Energie und die Aminosäure Trp erforderlich ist.

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