

Aus dem Institut für Tierernährung und Stoffwechselphysiologie  
der Christian-Albrechts-Universität zu Kiel

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# **The lysine requirement for maintenance and the efficiency of energy utilization for growth in pigs**

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**MEINER FAMILIE**



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## TABLE OF CONTENTS

### GENERAL INTRODUCTION

.....1

### CHAPTER ONE

#### **Literature overview**

.....3

### CHAPTER TWO

#### **The effect of lysine intake on energy partitioning and utilization in growing pigs**

.....17

### CHAPTER THREE

#### **Lysine requirement for maintenance in growing pigs**

.....31

### GENERAL SUMMARY

.....45

### ZUSAMMENFASSUNG

.....49



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**List of tables**

- Table I. Interspecies comparison of lysine requirements
- Table II. Composition (g/kg; as-fed basis) and chemical analysis (g/kg DM) of the basal diet
- Table III. Concentration of essential amino acids in protein
- Table IV. Body weight (BW) (kg), body weight gain (BW gain) (g/d), protein (RP) and fat accretion (RF) (g/d), intake of metabolizable energy (IME), energy retained in protein (RPE) and fat (RFE) (kJ/kg BW<sup>0.75</sup>) and RPE/RE (%) at two different levels of lysine intake (mean  $\pm$  SD; n = 6)
- Table V. Energy retention (RE) and heat production (H) (kJ/kg BW<sup>0.75</sup>) as affected by lysine intake and body weight; corrected values after adjustment to the same energy intake
- Table VI. Composition (g/kg; as-fed basis) and chemical analysis (g/kg DM) of the basal diet
- Table VII. Concentration of essential amino acids in protein
- Table VIII. The effects of lysine intake and body weight on nitrogen metabolism (mean  $\pm$  SD)
- Table IX. Lysine requirement estimates for maintenance in pigs according to different authors

**List of figures**

Figure 1. Energy partition of food energy in the growing animal

(Wenk et al., 2001; modified)

Figure 2. Energy retention in relation to the intake of metabolizable energy

(Whittemore, 1993; modified)

Figure 3. Metabolic fate of amino acid-nitrogen

Figure 4. Energy retention ( $\text{kJ/kg BW}^{0.75}$ ) at different levels of lysine intake ( $\pm$  SEM)

Figure 5. Heat production ( $\text{kJ/kg BW}^{0.75}$ ) at different levels of lysine intake ( $\pm$  SEM)

Figure 6. The relationship between retained protein and metabolic body weight



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**Abbreviations**

AA	amino acid(s)
BP	body protein
BW	body weight(s)
BW <sup>0.75</sup>	metabolic body weight
CP	crude protein
DE	digestible energy
DM	dry matter
D <sub>2</sub> O	deuterium oxide
EBW	empty body water
FFS	fat free substance
FHP	fasting heat production
FN	fecal nitrogen
GE	gross energy
H	heat production
IME	intake of metabolizable energy
IN	intake of nitrogen
I.U.	international unit
k <sub>f</sub>	efficiency of fat accretion
k <sub>m</sub>	efficiency of energy utilization below maintenance
k <sub>p</sub>	efficiency of protein accretion
k <sub>pf</sub>	efficiency of energy retention
Lysine-HCl	lysine-hydrogen chloride
ME	metabolizable energy
ME <sub>m</sub>	energy requirement for maintenance
ME <sub>g</sub>	energy requirement for growth
N	nitrogen
n	number of observations
P	probability (level of significance)
Pd <sub>max</sub>	maximum capacity for protein accretion
ppm	parts per million

$r^2$	coefficient of correlation
RE	energy retention (retained energy)
RF	fat accretion (retained fat)
RFE	energy retained in fat
RN	retained nitrogen
RP	protein accretion (retained protein)
RPE	energy retained in protein
SD	standard deviation
SEM	standard error of means
TBW	total body water
UN	urinary nitrogen
VDLUFA	Verband deutscher landwirtschaftlicher Untersuchungs- und Forschungsanstalten

## GENERAL INTRODUCTION

The performance-related feeding of livestock requires distinct knowledge of the requirements for energy and nutrients. With regard to the metabolic rate it is essential to be able to predict performance at a given level of energy and nutrient intake or -vice versa- to derive energy and nutrient requirements from the respective animal products in terms of e.g. growth or milk production. Precise knowledge of the amino acid (AA) maintenance requirements, defined as the inevitable nutrient input to maintain nitrogen (N)-equilibrium, is furthermore regarded as a matter of great importance. Both determination of the latter and of the partition and utilization of metabolizable energy is afflicted with substantial uncertainties concerning growing pigs in particular. Therefore, the intention of this thesis is to improve the knowledge in this field of study.

Chapter One provides a short insight into the main factors affecting the energy requirement for maintenance, furthermore illustrating the partitioning of energy retention (RE) between protein and fat accretion with special emphasis on the efficiency of energy utilization for growth and the process of body protein (BP) turnover. Moreover, the lysine requirement for maintenance is described in the context of its associated irreversible and diet-dependent losses.

In Chapter Two the efficiency of energy utilization for growth is examined at two different levels of lysine intake to ascertain whether the alteration of protein quality is influencing RE and heat production (H). The efficiency of energy utilization for growth has been intensively reviewed by several authors for a fairly long time (Kleiber, 1965; Kielanowski, 1972) and was recently found to be independent from the composition of body weight (BW) gain, age, breed, gender and protein or AA supply, stating a constant value (0.70) for the efficiency of energy retention ( $k_{pf}$ ). This observation seems to be in contrast to the fact that the efficiency of protein accretion ( $k_p$ ) is lower than the respective efficiency of fat accretion ( $k_f$ ).

Due to methodological problems involved in the determination of the lysine requirement for maintenance, a new approach serves as basis for the investigations presented in Chapter Three. The problems of transferring results from experiments with a feeding strategy close to the expected values for maintenance to an effectively growing animal and the inaccuracy of a vast extrapolation are circumvented by assuming a constant marginal

efficiency of lysine utilization for RP. Prerequisites for the latter are the following hypotheses of independence: 1) linearity of RP-lysine intake; 2) independence of lysine utilization from animal size; 3) independence of lysine utilization from the animals capacity for growth; 4) independence of lysine utilization from the level of protein supply; 5) a constant marginal efficiency for lysine utilization, independent from energy intake and 6) independence of lysine utilization from lysine intake level. In this chapter various reference points other than the conventionally used term of metabolic BW ( $BW^{0.75}$ ) (body protein (BP) or fat free substance (FFS)) for the lysine requirement for maintenance are furthermore considered.

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CHAPTER ONE

**Literature overview**

## The energy requirements for maintenance and growth

### General remarks

The particular processes linked with the energy requirements of growing pigs are of considerable complexity. In the majority of energy systems conventionally used in pig nutrition, a distinction is made between the metabolizable energy (ME), defined as the energy supplied to an animal from dietary nutrients after accounting for fecal, gaseous and urinary losses, which is utilized for maintenance purposes and the equivalent ME for growth (see Figure 1). Expecting additivity of the two processes, maintenance is assumed to have first priority (van Milgen and Noblet, 1999) including several significant body processes such as those associated with fasting heat production (FHP), moderate physical activity, activity due to ingestion of feed and the maintenance of body temperature (Black, 1995). The energy requirement for maintenance ( $ME_m$ ) is usually expressed as a function of BW raised to the power of 0.75 (ARC, 1981), nevertheless several authors proposed other exponents to be more appropriate for intra-species comparisons (Brown and Mount, 1982; Noblet et al., 1999). The latter requirement can be determined from various types of experiments: 1) measurements of FHP, using values for the efficiency of energy utilization below maintenance ( $k_m$ ) to calculate  $ME_m$ ; 2) from linear regression equations relating RE to intake of metabolizable energy (IME) calculating  $ME_m$  at the point where RE equals zero and 3) from the relationship between IME and the rates of protein and fat accretion, identifying  $ME_m$  as the intercept of the multiple regression analysis. Estimated maintenance requirements of pigs range from 385 kJ ME/kg  $BW^{0.75}$  to 670 kJ ME/kg  $BW^{0.75}$  per day. On the basis of a considerable amount of experiments 444 kJ ME/kg  $BW^{0.75}$  per day form the mean estimate (NRC, 1998). The utilization of IME above maintenance depends on the partitioning of energy between protein and fat accretion and the corresponding efficiencies. Consequently the energy requirement for growth ( $ME_g$ ) is composed of the energy retained in protein (RPE) and fat (RFE) plus the energy associated with the respective synthesis.

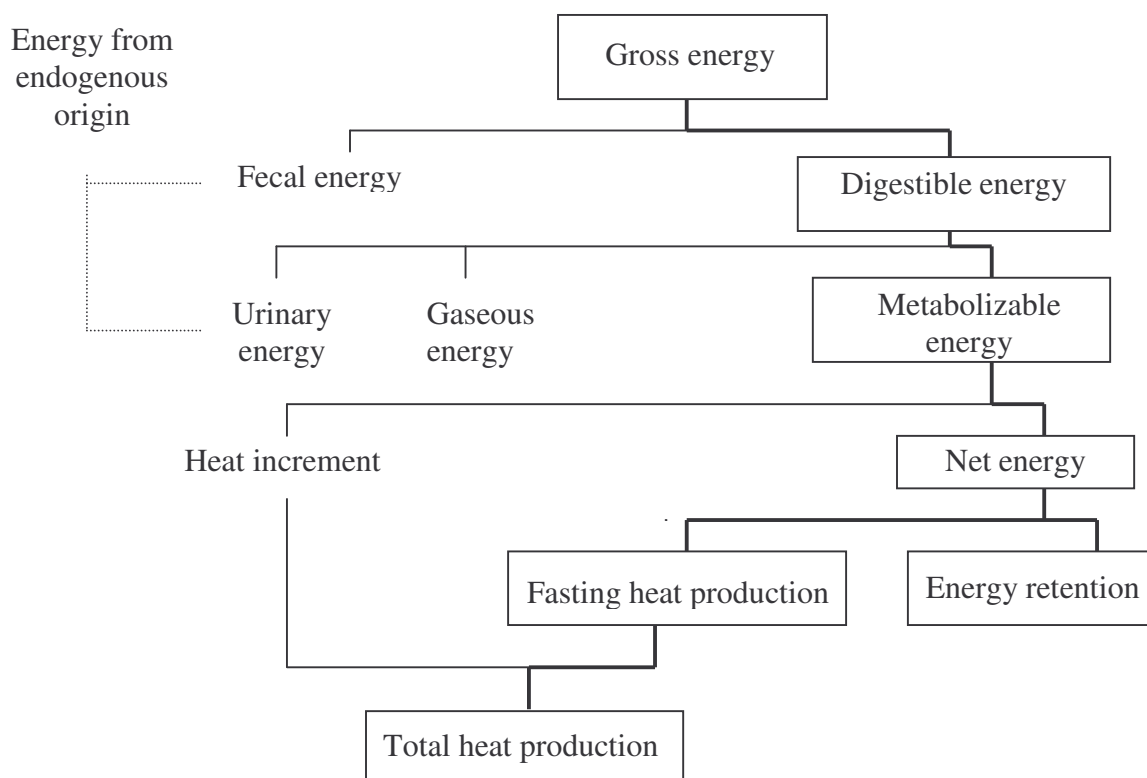


Figure 1. Energy partition of food energy in the growing animal  
(Wenk et al., 2001; modified)

### Factors affecting the energy requirement for maintenance

#### *Measurement of fasting heat production*

Fasting heat production is a measure of the pigs basal metabolism and can be used to predict  $ME_m$  if  $k_m$  is known. According to van Es (1972) the following equation relating  $ME_m$  to FHP applies:  $ME_m = FHP/k_m$  with  $k_m$  representing the regression of RE on IME when  $RE < 0$  (Figure 2). However, measurements of FHP vary with the duration of the fasting period (Close and Mount, 1975) and with previous nutritional history (Gray and McCracken, 1980). Determining  $ME_m$  via FHP, an extended low feed intake adaptation period may be counterproductive for young growing pigs bearing in mind that throughout the latter period body composition is subject to change towards mobilization of fat reserves and simultaneous protein accretion, consequently making the transfer to an intensively fed animal rather problematical.

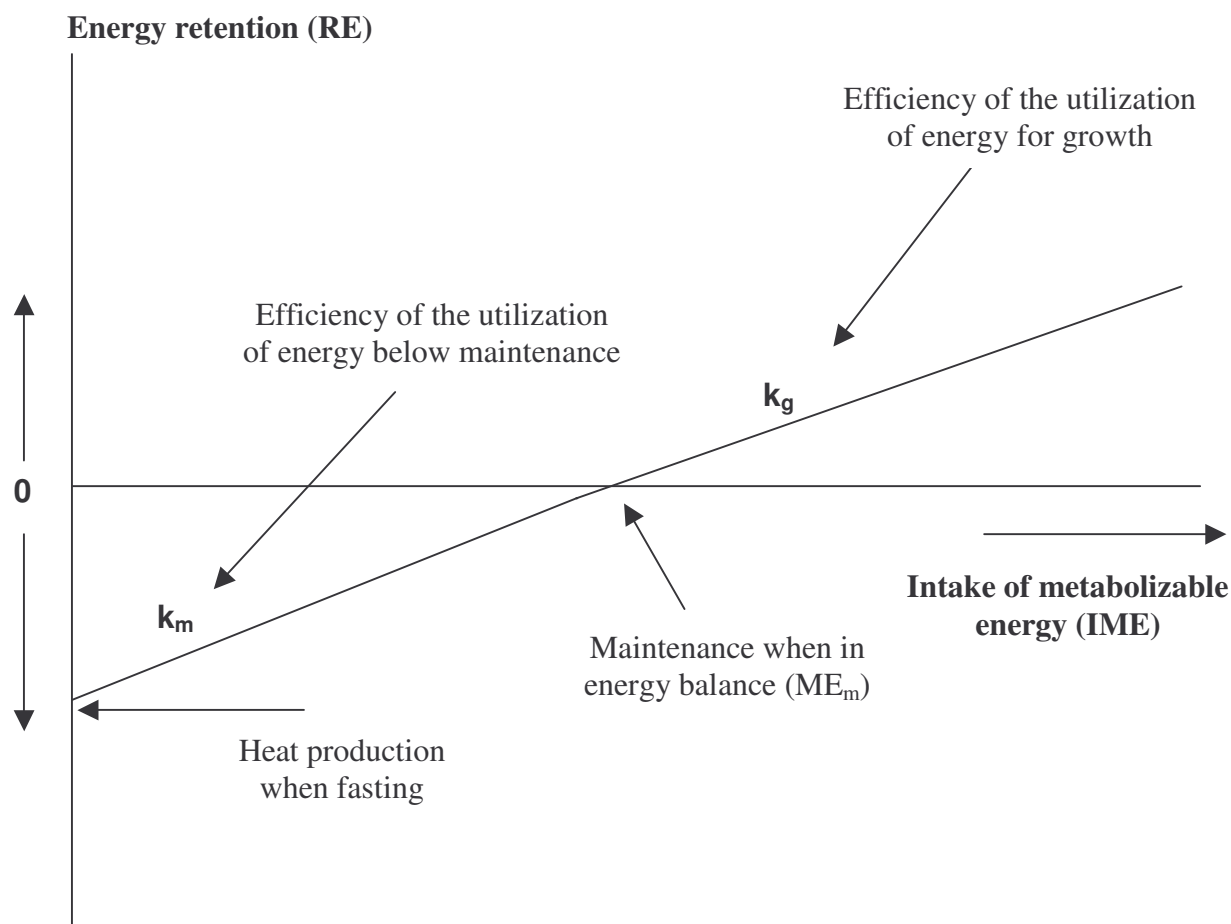


Figure 2. Energy retention in relation to the intake of metabolizable energy (Whittemore, 1993; modified)

### *Physical activity*

Physical activity represents an important source of variation of the ME<sub>m</sub> in growing pigs. In an experiment conducted with growing barrows van Milgen et al. (2001) reported the coefficient of variation for physical activity to equal 2.5 times that of FHP, illustrating the variability between pigs although the latter were housed individually with limited possibility for locomotion. Halter et al. (1980) found out that in group-housed pigs (5-40 kg BW) physical activity accounted for 22% of the ME<sub>m</sub> at ad libitum feed intake, whereas in restrictively-fed pigs the energy requirement for physical activity showed more variation and was on the average about 30% higher than in ad libitum-fed animals. Schrama et al. (1996) furthermore concluded that not only feeding level and housing management affects physical activity, but also the type of carbohydrate supplied to the



animal. Recently, Rijnen et al. (2003) reported that physical activity of group-housed sows tended to decrease with increasing dietary fiber content.

#### *Maintenance of body temperature*

Thermal neutrality is defined as the range of environmental temperature within which the metabolic rate is smallest and constant. In homoiotherms metabolic heat production and the control of heat loss form the means of adjusting the deep-body temperature in a changing thermal environment (Mount, 1980). On the one hand shivering and other thermogenic mechanisms, activated to maintain body temperature, cause an increase in energy expenditure of pigs when the ambient temperature falls below the animals lower critical temperature, while on the other hand energy expenditure also rises as soon as the ambient temperature is above the animals critical temperature due to an activation for increased evaporation.

The energy requirement for growth

#### *Protein and fat accretion*

Depending on several animal characteristics such as age, breed and gender, energy intake has a major impact on protein accretion, among other nutritional factors, namely protein and AA supply. Due to the high water-binding capacity of protein, protein accretion remarkably determines the level of growth.

Fat accretion can be interpreted as the result of the difference between RE and protein accretion. Therefore, fat accretion is predominantly affected by energy intake, the energy maintenance requirement and by factors influencing protein accretion.

#### *Efficiency of energy utilization*

According to the ARC (1981)  $k_p$  and  $k_f$  are enumerated with 0.54 and 0.74, respectively. The determination of the latter is carried out via multiple regression analysis (Kielanowski, 1965):  $IME = b_0 + b_1 RPE + b_2 RFE$  (MJ/kg BW<sup>0.75</sup>) where  $k_p$  and  $k_f$  are derived from the reciprocal of  $b_1$  and  $b_2$ , accordingly, while  $b_0$  represents an estimation of  $ME_m$ .

Predicted and measured values for  $k_p$  differ to a considerable extent and these differences may be related to the high adenosine triphosphate requirement for protein synthesis due to irregular changes in protein turnover.

Therefore the question remains whether increased protein accretion is obtained from an enhancement of protein synthesis, a suppression of proteolysis, or in part from both (Reeds et al., 1998).

### *Protein and fat turnover*

Protein turnover describes the indispensable process to maintain homeostasis where BP is continually being degraded and resynthesized. It is assumed that the major nutritional factors determining protein and AA turnover are feed intake, energy, protein and AA supply.

In adipose tissue a dynamic equilibrium between esterification, hydrolysis and re-esterification exists. The adipose tissue is composed of at least two components, a small component characterized by a fast turnover rate and a large component underlying rather slow modification. While there is sufficient evidence for an influence of age, gender and genotype on lipid metabolism, however, no adequate experimental data for the rate of synthesis or degradation of lipids with respect to different animal traits and nutritional situations can be found.

## **The lysine requirements for maintenance and growth**

### General remarks

Regarding AA requirements, maintenance is defined as N equilibrium, the state in which N intake equals the sum of N losses, however, granting a constant body N content. Maintaining N equilibrium requires an AA supply at the same rate at which AA are lost through metabolism, secretion or excretion from the body. According to Moughan (2003) metabolic processes leading to the loss of AA from the body are subsumed under the following: 1) epithelial AA losses; 2) N losses of AA origin in urine displaying inefficiency in the process of BP turnover; 3) basal gut endogenous AA losses; 4) the irreversible loss of AA in synthesizing essential non-AA nitrogenous metabolites;

5) irreversible chemical alterations of AA and 6) the loss of free AA in the urine. The maintenance requirement for lysine represents a small but significant portion (less than 10%) of growing pigs total lysine requirement. While the AA requirement for maintenance represents a necessity at any stage of life, the remaining part depends upon the productive state of the animal, mostly dominated by the requirement for BP accretion.

Protein and AA requirements are functions of the organisms metabolic demand and the efficiency with which a diet can be utilized to fulfill the latter. Figure 3 meets these concerns and takes into account the metabolic processes leading to the loss of AA from the body.

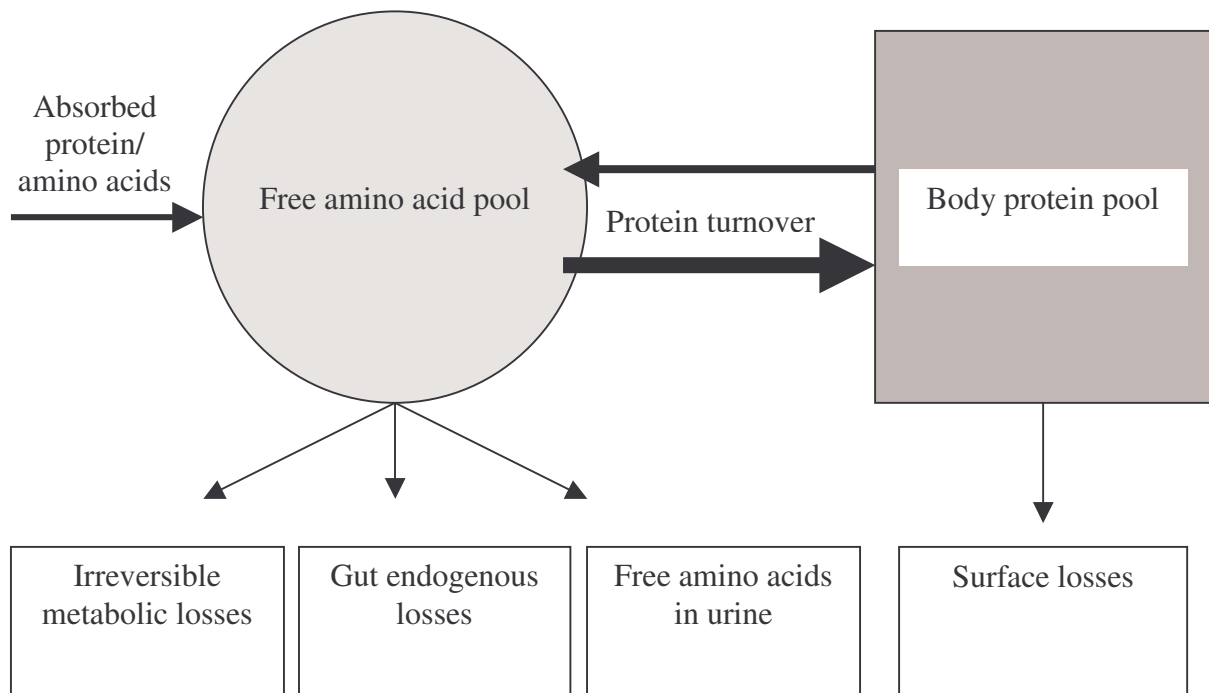


Figure 3. Metabolic fate of amino acid-nitrogen

The lysine requirement for maintenance

A comparison across species for the lysine requirement for maintenance is shown in Table I expressing that the values for pigs are in good agreement with the corresponding values for man.

Table I. Interspecies comparison of lysine requirements

Maintenance requirement	Species				
	Growing pig <sup>1</sup>	Adult pig <sup>2</sup>	Growing rat <sup>3</sup>	Adult rat <sup>4</sup>	Human <sup>5</sup>
Lysine (mg/kg <sup>0.75</sup> )	36	38	23	33	35

<sup>1</sup> Fuller et al. (1989)

<sup>2</sup> Roth et al. (2003)

<sup>3</sup> Benevenga et al. (1994)

<sup>4</sup> Said and Hegstedt (1970)

<sup>5</sup> FAO/WHO (1973)

#### *Losses from epithelia and hair*

Amino acid losses occur through shedding and continuous replacement of skin and hair in terms of proteins. In experiments with growing pigs Fuller and Boyne (1971) quantified daily N losses to account for 250-500 mg N depending on feed intake and environmental temperature. Jahn (2000) were able to confirm these findings (losses of 500 mg N/d) and related the aforementioned to  $BW^{0.75}$  (10 mg N/kg  $BW^{0.75}$ /d). Furthermore setting the N balance to 0 and 10 mg/kg  $BW^{0.75}$  the maintenance requirement for lysine was achieved to be 38 mg/kg  $BW^{0.75}$  and 47 mg/kg  $BW^{0.75}$ , respectively.

#### *Urinary excretion*

Urinary excretion of free AA represents only a small part of the AA maintenance requirements in pigs. In an experiment conducted by Southern and Baker (1984) AA-related N sparsely contributed to the amount of total N excretion in young barrows, with lysine displaying 4.2% of the total AA-N excretion.

### *Basal gut endogenous losses*

The ileal digestibility is the preferred means for describing AA uptake from the gut. In an experiment involving growing barrows and sows Stein and Easter (1997) concluded that apparent ileal AA digestibility was affected by the physiological stage of the animal. Furthermore, as shown by different authors, several diet-dependent factors influence the magnitude of endogenous protein and AA recovered at the distal ileum (Souffrant, 1991; Tamminga et al., 1995; Boisen and Moughan, 1996). In addition to the nutritional factors there is also clear evidence for an effect of total dry matter (DM) intake on endogenous AA losses (Butts et al., 1993a; Jansmann et al., 1999). Consequently, estimating the true ileal digestibility, the aforementioned need to be taken into account while corrections for the non-dietary components such as mucus, bile and desquamated cells must be made. The use of true ileal digestibility, will therewith lead to a more accurate description of the AA uptake from diets for pigs.

Basal gut endogenous AA losses can be determined either traditionally from measurements of AA flow at the terminal ileum of pigs fed a protein-free diet, by feeding a variety of graded levels of protein with subsequent extrapolation or by the use of marker techniques (e.g. homoarginine technique, stable isotopes). To overcome the unphysiological conditions of a protein-free diet Moughan et al. (1990) introduced the enzyme-hydrolyzed protein (peptide alimentation) method measuring gut endogenous AA losses in animals fed a peptide- containing diet. Comparisons between the protein-free and enzyme-hydrolyzed protein methods have shown a significantly higher endogenous ileal AA loss for animals receiving a peptide-containing diet (Moughan et al., 1992; Butts et al., 1993b; Leterme et al., 1996). The enzyme-hydrolyzed protein method, combined with the homoarginine technique, can be regarded as a valuable tool for generating true ileal digestibility coefficients.

### The lysine requirement for growth

In growing animals total AA needs are dominated by the requirement for BP accretion. In a protein-limiting diet, protein and AA intake causes an increase in the rate of protein accretion until the animals accretion potential is reached. The slope of the response is affected by the pattern of available AA relative to the one needed for BP synthesis and the efficiency of utilization of the first limiting AA (Black and de Lange, 1995).

The maximum capacity for protein accretion ( $Pd_{max}$ ) can be regarded as an important limitation on growth as the cell has a finite capacity for protein synthesis and is not able to save AA for later use (Moughan and Fuller, 2003).  $Pd_{max}$  is affected by age, breed, gender and potentially by nutritional history (Moughan, 1995) and has to be experimentally determined for each of these categories.

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CHAPTER TWO

**The effect of lysine intake on energy partitioning and utilization  
in growing pigs**

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**Abstract**

An experiment utilizing twelve castrated male pigs within a body weight range of 23 - 147 kg was conducted to ascertain whether the alteration of protein quality by varying the level of lysine intake is influencing total energy retention, heat production and therewith efficiency of energy utilization for growth. The animals were allotted to two treatments of a constant medium (11.5 g/d) or high lysine intake (13.5 g/d) level on the basis of an isonitrogenous diet at an energy intake level of 1.3 MJ ME/kg BW<sup>0.75</sup>. Representing a tool for determining body composition, at target body weights of 35, 55, 80, 115 and 145 kg measurements of deuterium dilution space were undertaken. Protein and lipid accretion were calculated by difference, assuming accretion to contain 23.8 and 39.0 kJ/g, respectively. The results show a significant effect ( $P < 0.05$ ) between treatment groups for the values of energy retained in protein, thus ensuring the intended alteration by protein quality. Furthermore total energy retention, heat production (difference between ME intake and energy retention) and therewith energy utilization demonstrate independence from the composition of body weight gain. These observations confirm earlier results, however, seem to be in contrast to the supposition of a constant efficiency for protein ( $k_p$ ) and fat ( $k_f$ ) accretion, respectively. This may be attributed to a variable  $k_p$ , in fact to a smaller  $k_p$  at minor values for protein accretion due to an increased whole body protein turnover. Lacking evidence from experimental data for advantages in using constant values for  $k_p$  and  $k_f$  to determine the accurate energy requirement for growth, a uniform value for the efficiency of total energy retention seems to be more adequate.

Keywords: Growing pigs, Lysine, Energy partitioning, Energy utilization

## Introduction

The performance-related supply and the efficient conversion of nutrients play major roles in animal nutrition. It is considered useful to represent overall metabolism in maintenance and growth. Provided that the energetic efficiency of protein ( $k_p$ ) and fat ( $k_f$ ) accretion integrates energy expenditures occurring within the respective accretion processes, the efficiency of energy utilization for tissue accretion in growing pigs is predominantly dependent on  $k_p$  and  $k_f$ . Although it is acknowledged that there is considerable variation in the respective energy efficiencies reported in the literature, typically lower values are achieved for  $k_p$  (ARC, 1981), the latter being partly due to protein accretion as a very dynamic process requiring considerably increased amounts of energy for the repeated arrangement of peptide bonds. Consequently, the efficiency for energy retention ( $k_{pf}$ ) is expected to vary according to differences in the ratio of protein to lipid accretion. Recommendations for an adequate energy supply are based on this assumption in many systems for feeding standards (GfE, 1987; NRC, 1998). Nevertheless several studies indicated that on the contrary  $k_{pf}$  was not affected by variation in the protein-fat ratio in BW gain (Campbell and Dunkin, 1983; Reeds, 1991; Susenbeth and Menke, 1991; Susenbeth, 1996). The aim of this study was to ascertain whether a change of the proportion of protein and fat in BW gain, caused by different levels of lysine supplementation in an isonitrogenous diet, is influencing total energy retention (RE), heat production (H) and therewith efficiency of energy utilization for growth.

## Materials and Methods

*Animals.* Twelve castrated male pigs of the strain PIC Germany were bought from a commercial pig farm at an average BW of 23 kg and utilized in this experiment until they reached 147 kg.

*Diets and Feeding.* All animals received 1.3 kg/d of a basal pelleted diet, consisting of wheat, barley, wheat gluten, soybean meal, soybean oil and a mineral-vitamin premix (see Table II). Throughout the whole growing period they were allotted to two treatments of a constant medium (11.5 g/d) or high lysine intake (13.5 g/d) level, the latter was reached by supplementation of lysine-HCl (2.6 g/d). Concentration of essential amino acids in protein (Table III) ranged slightly above the level recommended by Fuller et al. (1989) ensuring that lysine remained first-limiting. Two equal meals were provided at 0730 and 1730. Pigs

were weighed weekly while feed allowances per group were adjusted according to the mean anticipated BW for the following week. Therefore appropriate amounts of wheat starch, offered as powder mixed with water to enable intake, were added to meet the increasing demand for energy, to maintain an isonitrogenous level and to assure an energy intake level of 1.3 MJ ME/kg BW<sup>0.75</sup>.

Table II. Composition (g/kg; as-fed basis) and chemical analysis (g/kg DM) of the basal diet

Ingredients (g/kg)	Value
Barley	365
Wheat	365
Wheat gluten	120
Soybean meal	100
Soybean oil	20
Vitamin mineral premix <sup>a</sup>	30
Components, as analyzed (g/kg DM)	
Dry matter	917
Crude protein (N x 6.25)	235
Crude fiber	41
Crude fat	6
Crude ash	36

<sup>a</sup>Provided the following per kg DM of the diet: vitamin A, 7410 I.U.; vitamin D, 890 I.U.; vitamin E, 59 mg; vitamin K, 1.5 mg; vitamin B<sub>1</sub>, 0.7 mg; vitamin B<sub>2</sub>, 3 mg; vitamin B<sub>6</sub>, 1.5 mg; vitamin B<sub>12</sub>, 0.02 mg; niacin, 7.4 mg; pantothenic acid, 3 mg; Co 0.5 mg; Cu 15 mg; Fe 93 mg; I 1.1 mg; Mn 26 mg; Se 0.2 mg; Zn 52 mg.

Table III. Concentration of essential amino acids in protein

		g/16g N	relative to lysine (%)	
Level of lysine supplementation	+	5.00	100	
	0	4.26	100	
Methionine		1.57	31	37
Cystine		1.71	34	40
Threonine		3.01	60	71
Tryptophan		1.16	23	27
Isoleucine		3.80	76	89
Leucine		6.94	139	163
Valine		4.12	82	97
Tyrosine		3.06	61	72
Phenylalanine		5.46	109	128
Histidine		2.73	55	64
Arginine		5.14	103	121

*Housing and Management.* The barrows were housed in pens with concrete floors in an insulated building at ambient temperature of approximately 20-22°C. At each target BW of 35, 55, 80, 115 and 145 kg measurements of deuterium dilution space were undertaken. After animals were being fasted for 14 h, 0.25 g deuterium oxide (D<sub>2</sub>O) per kg BW was administered with a small amount of feed (about 20% of the daily allowance). The basal level of D<sub>2</sub>O was determined via blood sampling from the *vena jugularis* prior D<sub>2</sub>O application. Blood samples were taken 4.5 hours after application (Schloerb et al., 1950; Sutcliffe, 1996) and stored at -20°C until further processing.

*Chemical Analysis.* Dry matter content of the diet samples was determined by oven-drying for 4 h at 103°C while the proximate analysis was utilized to determine crude fiber, fat and ash. Both amino acid as well as sample analyses were carried out according to the methods of the VDLUFA (Naumann et al., 1997). For extraction of D<sub>2</sub>O and H<sub>2</sub>O from blood, samples were firstly vacuum sublimated and the blood water was afterwards detected quantitatively in cold finger condensers (Tissier et al., 1978).

The received substance, consisting of H<sub>2</sub>O-D<sub>2</sub>O, was analyzed with an infrared spectral photometer (Spectran 677, Bodenseewerk Geosystem GmbH, Germany) at a wave length of 3960 nm against water for determination of the D<sub>2</sub>O content.

*Calculations.* Based on the applied amount of D<sub>2</sub>O and its concentration in blood water, total body water (TBW) content was calculated as follows (Susenbeth, 1984):

$$\text{TBW (\%)} = \frac{\text{D}_2\text{O}_{\text{appl. (g)}}}{\text{D}_2\text{O}_{\text{blood (ppm)}}} \times \frac{100000}{\text{BW (kg)}} \quad [1]$$

Since 2% - 4% of the hydrogen tracers exchange with nonaqueous hydrogen (Ellis, 2000), TBW was corrected by multiplication with 0.97. Losses to expired air and urine are negligible because of the long half-life of isotopic hydrogens in the body (Culebras and Moore, 1977). The empty body is defined as the difference between BW and gut content (including urine content of the bladder). Empty body water (EBW) was calculated assuming 155 g gut content/kg BW<sup>0.75</sup> (Susenbeth and Keitel, 1988) and taking into account the water fraction of the last-mentioned, estimated as 82% (Houseman and McDonald, 1976). According to Susenbeth (1984), fat free substance (FFS) was calculated from EBW and the water concentration in FFS as a function of BW:

$$\text{FFS (\%)} = \frac{\text{EBW (\%)}}{82.43 \text{ BW}^{-0.0219}} \quad [2]$$

while body protein (BP) content was estimated from FFS and the protein concentration in FFS as a function of BW:

$$\text{BP (\%)} = \text{FFS (\%)} \times 0.1402 \text{ BW}^{0.0940}. \quad [3]$$

Standard deviations for the two latter equations were 1.7% and 1.0% of the empty body mass, respectively. With respect to the two treatment groups, protein and fat accretion were calculated by difference while protein and fat were assumed to contain 23.8 (ARC, 1981) and 39.0 kJ/g (Menke and Huss, 1987), respectively. Regarding the diet, ME concentration was determined by measuring energy digestibility prior D<sub>2</sub>O application in metabolism cages within a 7-day period. Feed and feces were analyzed by performing calorimetry while the energy content of urine was assumed to be 40 kJ/g N (Hoffmann et al., 1971) assigning methane losses to be negligible.



Statistical analyses were performed using the PROC MIXED procedure of SAS (1996) for repeated measures data. The following model applied:  $y_{ijk} = \mu + BW_i + L_j + BW_i * L_j + A_k$ , where  $y$  is the observed value,  $\mu$  the total mean,  $BW$  the effect of body weight ( $i = 5$ ), while  $L$  represents the effect of lysine intake ( $j = 2$ ),  $BW_i * L_j$  the interaction between body weight and lysine intake and  $A$  is the animal effect ( $k = 12$ ).

## Results

The effects of lysine intake and average BW on BW gain, protein (RP) and fat accretion (RF) are presented in Table IV. Furthermore values for the intake of metabolizable energy (IME), energy retained in protein (RPE) and fat (RFE) as well as for the RPE/RE (%) ratio at the two different levels of lysine intake are displayed. Forming a prerequisite, intended BW was reached consistently for both treatment groups. By reason of improving protein quality, BW gain increased with ascending BW ( $P < 0.001$ ), further showing a significant effect ( $P < 0.001$ ) regarding the level of lysine. As a result of complete ingestion of the provided diets, IME corresponded well between treatment groups. An exception formed the last period, in fact the group on a medium level of lysine, where intake varied due to partial wheat starch refusal of two individuals; the latter were not excluded from statistical analysis. When related to  $BW^{0.75}$ , RPE diminished with increasing BW ( $P < 0.001$ ), displaying a significant effect ( $P < 0.05$ ) between treatment groups, while RFE rose ( $P < 0.001$ ), thus leading to a distinct decrease in the RPE/RE ratio ( $P < 0.001$ ). The aforementioned was positively affected by lysine level within each BW category. However, RE and H remained uninfluenced (Figure 4 and Figure 5); this independence is emphasized when RE and H were adjusted to the same IME (Table V).

Table IV. Body weight (BW) (kg), body weight gain (BW gain) (g/d), protein (RP) and fat accretion (RF) (g/d), intake of metabolizable energy (IME), energy retained in protein (RPE) and fat (RFE) (kJ/kg BW<sup>0.75</sup>) and RPE/RE (%) at two different levels of lysine intake (mean  $\pm$  SD; n = 6)

Lysine intake	BW	BW gain <sup>ab</sup>	RP <sup>ab</sup>	RF <sup>a</sup>	IME	RPE <sup>ac</sup>	RFE <sup>a</sup>	RPE/RE <sup>a</sup>
medium	46.4 $\pm$ 1.4	636 $\pm$ 50	108 $\pm$ 7	62 $\pm$ 26	1266 $\pm$ 30	145 $\pm$ 10	136 $\pm$ 58	53.6 $\pm$ 10.9
high	47.8 $\pm$ 1.4	708 $\pm$ 26	114 $\pm$ 28	55 $\pm$ 17	1241 $\pm$ 34	149 $\pm$ 36	119 $\pm$ 38	55.7 $\pm$ 9.2
medium	69.0 $\pm$ 1.4	785 $\pm$ 36	135 $\pm$ 5	107 $\pm$ 27	1255 $\pm$ 22	134 $\pm$ 5	174 $\pm$ 45	44.4 $\pm$ 7.0
high	69.7 $\pm$ 1.1	851 $\pm$ 52	150 $\pm$ 6	100 $\pm$ 28	1251 $\pm$ 19	147 $\pm$ 7	163 $\pm$ 46	48.6 $\pm$ 9.1
medium	97.5 $\pm$ 1.7	841 $\pm$ 16	139 $\pm$ 4	164 $\pm$ 20	1235 $\pm$ 19	107 $\pm$ 3	206 $\pm$ 25	34.3 $\pm$ 3.7
high	97.4 $\pm$ 1.2	902 $\pm$ 27	156 $\pm$ 9	142 $\pm$ 22	1213 $\pm$ 17	122 $\pm$ 13	179 $\pm$ 28	40.9 $\pm$ 5.9
medium	126.6 $\pm$ 2.4	824 $\pm$ 74	129 $\pm$ 11	210 $\pm$ 36	1188 $\pm$ 60	82 $\pm$ 7	217 $\pm$ 35	27.6 $\pm$ 3.6
high	129.9 $\pm$ 1.1	958 $\pm$ 56	156 $\pm$ 12	212 $\pm$ 26	1211 $\pm$ 9	96 $\pm$ 7	215 $\pm$ 26	31.1 $\pm$ 3.6

<sup>a</sup> Body weight effect, P < 0.001.

<sup>b</sup> Lysine effect, P < 0.001.

<sup>c</sup> Lysine effect, P < 0.05.

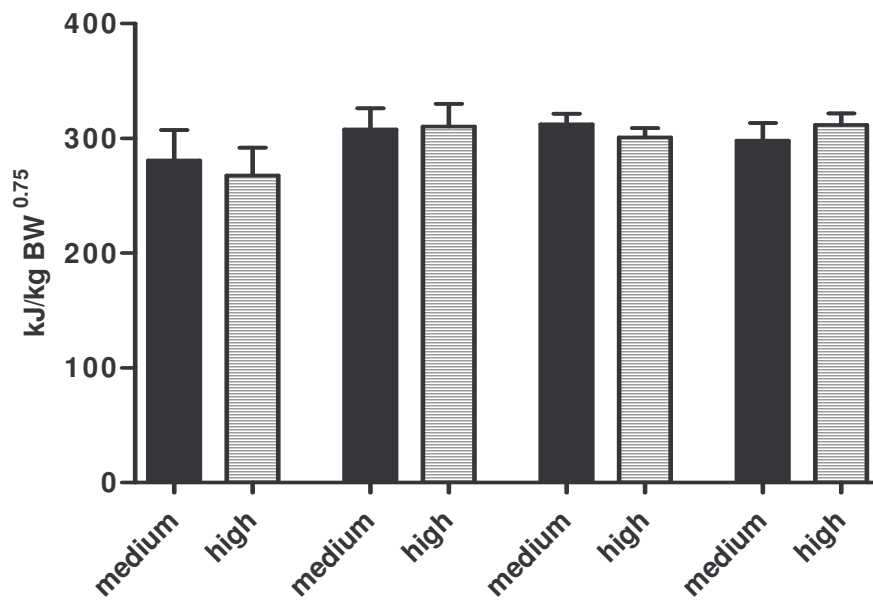


Figure 4. Energy retention (kJ/kg BW<sup>0.75</sup>) at different levels of lysine intake ( $\pm$  SEM)

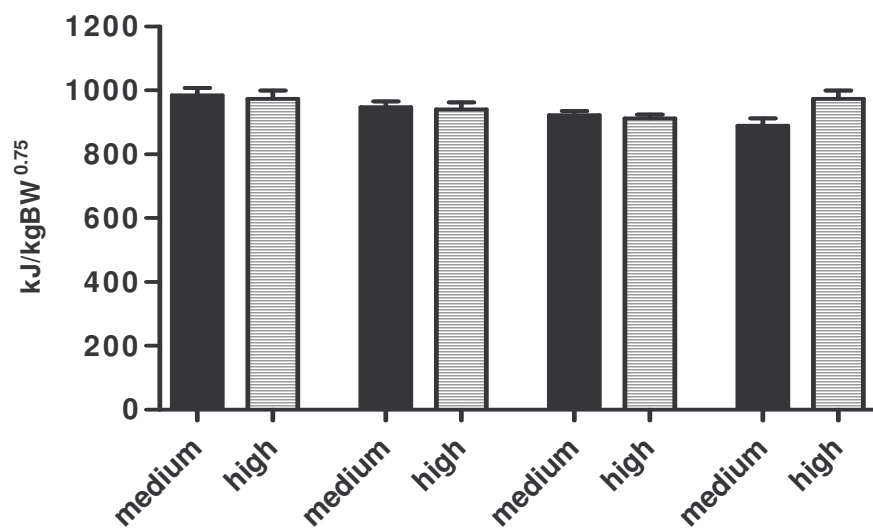


Figure 5. Heat production (kJ/kg BW<sup>0.75</sup>) at different levels of lysine intake ( $\pm$  SEM)

Table V. Energy retention (RE) and heat production (H) (kJ/kg BW<sup>0.75</sup>) as affected by lysine intake and body weight; corrected values after adjustment to the same energy intake (mean  $\pm$  SD; n = 6)

Lysine intake	RE	RE <sup>a</sup>	H	H <sup>a</sup>
medium (46.4 kg)	281 $\pm$ 65		985 $\pm$ 55	
high (47.8 kg)	268 $\pm$ 60	286	973 $\pm$ 62	980
medium (69.0 kg)	308 $\pm$ 45		947 $\pm$ 44	
high (69.7 kg)	310 $\pm$ 48	313	941 $\pm$ 52	942
medium (97.5 kg)	313 $\pm$ 22		922 $\pm$ 32	
high (97.4 kg)	301 $\pm$ 20	316	912 $\pm$ 29	919
medium (126.6 kg)	299 $\pm$ 37		889 $\pm$ 57	
high (129.9 kg)	312 $\pm$ 25	295	899 $\pm$ 25	893

<sup>a</sup> adjusted to the same intake of metabolizable energy of the respective BW group on the medium level of lysine, assuming  $k_{pf} = 0.70$ .

## Discussion

Throughout the study animals remained healthy, consumed their daily feed allowance readily, therefore reaching the target BW consistently. Measurements of deuterium dilution space provided valuable information on body composition. However, compared to results of other studies, FFS seems to be overestimated, consequently displacing the observed values in a systematic manner without influencing the objective of the study.

Following from a shift in the proportion of protein in energy retention, caused by different levels of lysine supplementation, RE, H and therewith energy utilization demonstrate independence from the composition of BW gain. This observation seems to be in contrast to the fact that  $k_p < k_f$  and may presumably be attributed to a variable  $k_p$ , actually to a smaller  $k_p$  at minor values for protein accretion due to a possibly increased whole body protein turnover. In the literature, several experiments with growing pigs have been conducted to investigate protein quality as a potentially determining factor of whole body protein turnover (Fuller et al., 1987; Salter et al., 1990; Roy et al., 1997) by altering the level of lysine supplementation in isoenergetic diets. Fuller et al. (1987) studied whether increases in protein accretion, caused by improving protein quality, were achieved by changes in the rates of protein synthesis and degradation. The findings indicate that the supplementation of lysine led to a decrease in protein breakdown without influencing protein synthesis. On the contrary, experiments

carried out by Salter et al. (1990) showed that whole body protein synthesis and degradation increased significantly at “adequate” (7.8 g/kg DM) levels of lysine supplementation in comparison to lysine “deficient” (3.5 g/kg DM) diets. Roy et al. (1997) confirmed the latter by investigating the effect of lysine supplementation, covering the range from “adequate” (7.6 g/kg DM) to “slight deficiency” (3.6 g/kg DM). However, studies with growing chickens confirmed the findings of Fuller et al. (1987): Providing a reduced supply of lysine (50% of the optimal amount for young growing chicks) Maruyama et al. (1978) found a considerable increase in the protein breakdown rate in breast muscles while protein synthesis remained unchanged. Concordant information was obtained from a more recent study by Tesseraud et al. (1996), also showing that lysine deficiency increased the protein breakdown in the muscle while maintaining the same level of protein synthesis. These observations show that whole body protein turnover can be affected by the quality of dietary protein, supporting the hypothesis of a variable and probably even improved  $k_p$  with a decreasing protein turnover. Furthermore, high values for  $k_p$ , found in young growing pigs (Gädeken et al., 1985) may also give indication for a more efficient use of energy for protein retention at lower rates of protein turnover, the aforementioned being assumed for young animals.

The findings of the present study affirmed that RE, H and therewith energy utilization show independence from the composition of BW gain. Protein quality, as reviewed in the literature, acts as a determining factor for whole body protein turnover and may in part explain a lower  $k_p$  at minor values for protein accretion. Therefore it cannot be concluded from experimental data that the use of constant values for  $k_p$  and  $k_f$  leads to either a more precise prediction of energy retention from energy intake or to a more accurate calculation of the ME requirement for growth from protein and fat accretion. Consequently a uniform value for  $k_{pf}$  seems to be more accurate.

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## CHAPTER THREE

**Lysine requirement for maintenance in growing pigs**

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**Abstract**

A series of five N balance experiments utilizing growing pigs was conducted to test whether the decrease in protein accretion with ascending BW reflects the increasing requirement of lysine for maintenance when offering lysine as the first-limiting AA. The well-known lysine concentration in body protein and the efficiency with which absorbed lysine is incorporated into body protein were used to estimate the lysine requirement for maintenance in growing pigs. In search of a reference point other than  $BW^{0.75}$ , body composition was determined via  $D_2O$  dilution technique. For this reason twelve castrated male pigs (23 to 147 kg BW) were fed a basal diet at an energy intake level of 1.3 MJ ME/kg  $BW^{0.75}$  while being allotted to two treatments of a constant medium or high lysine intake level. Furthermore appropriate amounts of wheat starch were offered to reach the intended energy intake level. The results indicate that retained protein tends to show higher values for the treatment group kept on a high level of lysine, thus ensuring an undersupply of lysine for the other group. Based on this undersupply, the following estimates for the lysine requirement for maintenance were obtained: 18 mg/kg BW, 71 mg/kg  $BW^{0.75}$ , 29 mg/kg fat free substance and 121 mg/kg body protein. Retrieving a similar coefficient of correlation for protein retention and the examined reference points, the conventionally used reference point of  $BW^{0.75}$  is appropriate.

Keywords: Growing pigs, Lysine, Maintenance requirement

## Introduction

Due to methodological problems profound knowledge about pigs AA requirement for maintenance is rather scarce. Roth et al. (2003) investigated the requirement of essential AA for adult sows to maintain N equilibrium whereas it was estimated as the x-intercept of the linear regression while relating N accretion to AA intake. This approach requires a feeding strategy close to the expected values for maintenance consequently implying a strong reduction of nutrient intake in growing pigs while raising the question of transferability. The approach used in experiments undertaken by Fuller et al. (1989) for growing pigs was to measure the response of protein accretion at different AA intake levels. The regression coefficients reflected the dietary AA requirement for the accretion of 1 g body protein whereas the daily amount of the respective AA needed to maintain N equilibrium was estimated by extrapolating the linear regression to the x-intercept. Although estimates of the latter approach are derived from young animals in a physiologically normal nutritional situation, the extrapolation to N equilibrium strongly reduces accuracy. Based on the assumption of a constant marginal efficiency of lysine utilization for protein retention (Susenbeth, 1995), the present study used a third approach to determine the lysine requirement for maintenance. Provided that lysine intake is below the required level to meet protein retention capacity and that the ratios of other essential AA to lysine do not fall below the critical values of the ideal protein (Wang and Fuller, 1989), the same amount of lysine ingested by animals of different BW implies that the decrease in protein retention with ascending BW reflects the increasing requirement of lysine for maintenance. A further aim of the study was to identify whether body protein (BP) or fat free substance (FFS) may be more closely related to the maintenance requirement for lysine than  $BW^{0.75}$ .

## Materials and Methods

*Animals.* Twelve castrated male pigs of the strain PIC Germany were bought from a commercial pig farm at a BW of 23 kg and utilized in this experiment until they reached 147 kg.

*Diets and Feeding.* All animals received 1.3 kg/d of a basal pelleted diet, consisting of wheat, barley, wheat gluten, soybean meal, oil and a mineral-vitamin-premix at an energy intake level of 1.3 MJ ME/kg  $BW^{0.75}$  (Table VI). They were allotted to two treatments of a

constant medium (11.5 g/d) or high lysine intake (13.5 g/d) level throughout the whole growing period. Concentrations of essential AA in protein are given in Table VII. Two equal meals were provided at 0730 and 1730. Pigs were weighed weekly while feed allowances per group were adjusted according to BW and predicted weight gain for the following week. Therefore appropriate amounts of wheat starch were added to meet the increasing demand for energy, to maintain an isonitrogenous level and to assure the energy intake level of 1.3 MJ ME/kg BW<sup>0.75</sup>. Throughout the N balances trials feed allowances were kept at a constant rate.

Table VI. Composition (g/kg; as-fed basis) and chemical analysis (g/kg DM) of the basal diet

Ingredients (g/kg)	Value
Barley	365
Wheat	365
Wheat gluten	120
Soybean meal	100
Soybean oil	20
Vitamin mineral premix <sup>a</sup>	30
Components, as analyzed (g/kg DM)	
Dry matter	917
Crude protein (N x 6.25)	235
Crude fiber	41
Crude fat	6
Crude ash	36

<sup>a</sup>Provided the following per kg DM of the diet: vitamin A, 7410 I.U.; vitamin D, 890 I.U.; vitamin E, 59 mg; vitamin K, 1.5 mg; vitamin B<sub>1</sub>, 0.7 mg; vitamin B<sub>2</sub>, 3 mg; vitamin B<sub>6</sub>, 1.5 mg; vitamin B<sub>12</sub>, 0.02 mg; niacin, 7.4 mg; pantothenic acid, 3 mg; Co 0.5 mg; Cu 15 mg; Fe 93 mg; I 1.1 mg; Mn 26 mg; Se 0.2 mg; Zn 52 mg.

Table VII. Concentration of essential amino acids in protein

		g/16g N	relative to lysine (%)	
Level of lysine supplementation	+	5.00	100	
	0	4.26	100	
Methionine		1.57	31	37
Cystine		1.71	34	40
Threonine		3.01	60	71
Tryptophan		1.16	23	27
Isoleucine		3.80	76	89
Leucine		6.94	139	163
Valine		4.12	82	97
Tyrosine		3.06	61	72
Phenylalanine		5.46	109	128
Histidine		2.73	55	64
Arginine		5.14	103	121

*Housing and Management.* The barrows were housed in pens with concrete floors in an insulated building at ambient temperature of approximately 20-22°C. Throughout N balance trials the animals were kept individually in metabolism cages. Collection of feces as well as urine was carried out twice daily and the urine was detected in tared bottles with sufficient sulfuric acid to maintain pH < 3. At the end of each balance trial, daily compilations were pooled per pig and sampled for subsequent analysis. At each target BW of 35, 55, 80, 115 and 145 kg measurements of deuterium dilution space were undertaken. While animals were being fasted for 14 h, 0.25 g deuterium oxide (D<sub>2</sub>O) per kg BW was administered with a small amount of feed (about 20% of the daily allowance). The basal level of D<sub>2</sub>O concentration in body water was determined via blood sampling from the *vena jugularis* prior D<sub>2</sub>O application. Blood samples were taken 4.5 hours after application and stored at -20°C until D<sub>2</sub>O concentration was analyzed.

*Chemical Analysis.* Nitrogen concentration in feces and urine was determined in the original substance according to the Kjeldahl procedure; furthermore feces were freeze-dried to perform calorimetry and further analyses. Dry matter content of the diet samples

was determined by oven-drying for 4 h at 103°C while DM content of feces was firstly determined by freeze-drying, followed by oven-drying for 4 h at 103°C. The Weende method was utilized to determine crude fiber, fat and ash. Amino acid and sample analyses were carried out according to the methods of the VDLUFA (Verband deutscher landwirtschaftlicher Untersuchungs- und Forschungsanstalten) (Naumann et al., 1997). Gross energy contents of the diet and feces were determined after complete incineration in an adiabatic bomb calorimeter whereas the energy content of urine was assumed to be 40 kJ/ g N (Hoffmann et al., 1971). For extraction of D<sub>2</sub>O and H<sub>2</sub>O from blood, samples were firstly vacuum sublimated and the blood water was afterwards detected quantitatively in cold finger condensers (Tissier et al., 1978). For determination of the D<sub>2</sub>O content, the substance consisting of H<sub>2</sub>O-D<sub>2</sub>O was analyzed with an infrared spectral photometer (Spectran 677, Bodenseewerk Geosystem GmbH, Germany) at a wave length of 3960 nm against water.

*Calculations.* Based on the applied amount of D<sub>2</sub>O and its concentration in blood water, total body water content (TBW) was calculated as follows:

$$\text{TBW (\%)} = \frac{\text{D}_2\text{O}_{\text{appl. (g)}}}{\text{D}_2\text{O}_{\text{blood (ppm)}}} \times \frac{100000}{\text{BW (kg)}} \quad [1]$$

Since 2% - 4% of the hydrogen tracers exchange with nonaqueous hydrogen (Ellis, 2000), TBW was corrected by multiplication with 0.97. Losses to expired air and urine are negligible because of the long half-life of isotopic hydrogens in the body (Culebras and Moore, 1977). The empty body is defined as the difference between BW and gut content (including urine content of the bladder). Assuming 155 g gut content/kg BW<sup>0.75</sup> (Susenbeth and Keitel, 1988) and taking into account the water content of the gut content, estimated as 82% (Houseman and Mc Donald, 1976), regression on TBW led to empty body water (EBW). The concentration of FFS was calculated from EBW:

$$\text{FFS (\%)} = \frac{\text{EBW (\%)}}{82.43 \text{ BW}^{-0.0219}} \quad [2]$$

while BP content was expressed by the following equation:

$$\text{BP (\%)} = \text{FFS (\%)} \times 0.1402 \text{ BW}^{0.0940} \quad (\text{Susenbeth, 1984}). \quad [3]$$

Statistical analyses were performed using the PROC MIXED procedure of SAS (1996) for repeated measures data. The following model applied:  $y_{ijk} = \mu + BW_i + L_j + BW_i * L_j + A_k$ , where  $y$  is the observed value,  $\mu$  the total mean,  $BW$  the effect of body weight ( $i = 5$ ), while  $L$  represents the effect of lysine intake ( $j = 2$ ),  $BW_i * L_j$  the interaction between body weight and lysine intake and  $A$  is the animal effect ( $k = 12$ ). Adjusting for the animal effect, a regression analysis was conducted to estimate the relationship between retained protein (RP) and  $BW$ ,  $BW^{0.75}$ , FFS and BP as reference points. Additional information on  $BW$ , FFS and BP was obtained by defining  $BW^{0.75}$  as point of origin.

## Results

The effects of lysine intake and  $BW$  on N metabolism are presented in Table VIII. Daily  $BW$  gain was merely determined for the length of each balance period (7 d), consequently serving as additional information only. As a result of complete ingestion of the provided feedstuff, actual energy intake corresponds well with the previously intended one (1.32 - 1.23 MJ ME kg/ $BW^{0.75}$ ), an exception forms the last balance period, namely the group on a medium level of lysine, where intake varied due to partial wheat starch refusal of two individuals; the latter were not excluded from statistical analysis. For the five balance periods intake of nitrogen (IN) remained similar between treatment groups. Retained nitrogen (RN) and therefore RP ( $RN \times 6.25$ ) tend to show greater values for the treatment group kept on a high level of lysine. Consequently the latter caused an increase while the group on a medium level of lysine displayed an undersupply. Regarding the response measures RN, RP, fecal nitrogen (FN) and urinary nitrogen (UN), significant effects ( $P < 0.05$ ) can only be detected for  $BW$ .

Referring to the treatment group on a medium lysine level for further calculations, the following linear regression equations between RP (g/d) and  $BW$ ,  $BW^{0.75}$  as well as the body components FFS or BP, respectively, were achieved:

- 1)  $RP = 135.5 - 0.1735 BW$  ( $r^2 = 0.29$  ;  $n = 30$ )
- 2)  $RP = 139.6 - 0.6848 BW^{0.75}$  ( $r^2 = 0.28$  ;  $n = 30$ )
- 3)  $RP = 138.3 - 0.2785 FFS$  ( $r^2 = 0.27$  ;  $n = 30$ )
- 4)  $RP = 136.7 - 1.1792 BP$  ( $r^2 = 0.28$  ;  $n = 30$ )

Provided that the marginal efficiency of lysine utilization is not affected by BW, the negative effect of body size on protein retention is an indicator for the increasing demand of lysine for maintenance. As a consequence of overestimating protein retention through the N-balance method, a correction of 11% (Susenbeth et al., 1999) was applied. For further calculations, dietary lysine was assumed to be utilized for retention with a gross efficiency of 0.63 (Susenbeth, 1995, Bikker, 1994), additionally 7.2% lysine in retained body protein (Susenbeth, 1995) were utilized. Therefore the respective regression coefficients of equations 1 - 4 were divided by 1.11, multiplied by 0.072 and divided by 0.63, resulting in the following values for the lysine requirement for maintenance: 18 mg/kg BW, 71 mg/kg  $BW^{0.75}$ , 29 mg/kg FFS and 121 mg/kg BP. Obtaining a similar coefficient of correlation for each of the examined reference points and lacking further evidence from statistical analyses, the conventionally used reference point of  $BW^{0.75}$  was equally applicable in this study (Figure 6).

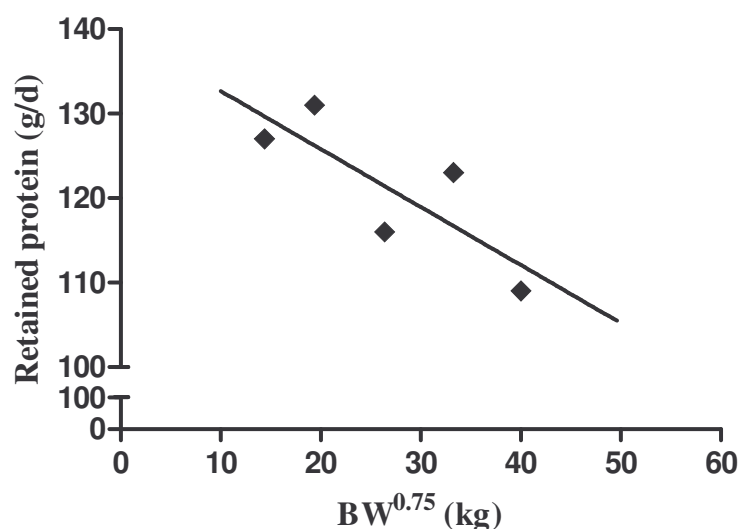


Figure 6. The relationship between retained protein and metabolic body weight  
( $RP = 139.6 - 0.6848 BW^{0.75}$ ) ( $r^2 = 0.28$ ;  $n = 30$ )



Table VIII. The effects of lysine intake and body weight on nitrogen metabolism (mean<sup>a</sup> ± SD)

N-balance	1	1	2	2	3	3	4	4	5	5
Level of lysine	medium	high	medium	high	medium	high	medium	high	medium	high
BW (kg) <sup>b</sup>	34.8 ± 1.3	35.0 ± 1.8	52.3 ± 1.6	54.5 ± 1.2	78.5 ± 1.5	77.0 ± 1.5	107.3 ± 1.8	108.3 ± 1.4	136.5 ± 3.0	141.6 ± 1.4
BW gain (kg/d) <sup>c</sup>	0.60 ± 0.15	0.64 ± 0.21	0.66 ± 0.12	0.72 ± 0.10	0.94 ± 0.09	1.02 ± 0.17	1.08 ± 0.13	1.09 ± 0.08	1.00 ± 0.14	1.14 ± 0.20
Basal intake (g DM/d)	1159	1159	1158	1158	1154	1152	1160	1148	1137	1143
Starch (g DM/d) <sup>d</sup>	0	0	103	103	830	848	1343	1325	1525 ± 456 <sup>e</sup>	2205
Lysine-HCl(g DM/d)	0	5.2 <sup>f</sup>	0	2.6	0	2.6	0	2.6	0	2.6
GE (MJ/d)	22.1	22.2	28.9	29.0	36.5	36.9	45.6	45.1	48.4 ± 8.0	54.9
DE (MJ/d) <sup>g</sup>	19.7 ± 0.1	19.6 ± 0.3	26.4 ± 0.2	26.5 ± 0.1	34.0 ± 0.3	34.0 ± 0.2	43.0 ± 0.3	42.0 ± 0.2	45.7 ± 8.1	51.8 ± 0.5
ME (MJ/d)	18.9 ± 0.1	18.9 ± 0.4	25.6 ± 0.1	25.7 ± 0.2	33.2 ± 0.3	33.2 ± 0.2	42.2 ± 0.3	41.3 ± 0.3	44.8 ± 8.1	51.1 ± 0.5
ME (MJ/kg BW <sup>0.75</sup> )	1.32 ± 0.04	1.31 ± 0.08	1.31 ± 0.03	1.28 ± 0.02	1.26 ± 0.02	1.28 ± 0.03	1.27 ± 0.02	1.23 ± 0.02	1.12 ± 0.20	1.25 ± 0.01
Intake of nitrogen (g/d)	45.7	45.7	45.0	45.0	44.1	44.1	43.8	43.3	42.0	42.2
Fecal nitrogen (g/d) <sup>hi</sup>	4.0 ± 0.5	4.2 ± 0.6	3.8 ± 0.3	3.5 ± 0.3	3.4 ± 0.4	3.6 ± 0.2	3.4 ± 0.3	3.9 ± 0.2	3.4 ± 0.4	3.8 ± 0.5
Urinary nitrogen (g/d) <sup>h</sup>	21.4 ± 1.7	19.1 ± 2.9	20.2 ± 1.7	19.2 ± 1.5	22.1 ± 0.7	20.5 ± 1.8	20.7 ± 0.6	19.7 ± 1.9	21.2 ± 2.2	19.2 ± 6.6
Retained nitrogen (g/d) <sup>h</sup>	20.3 ± 1.6	22.5 ± 3.3	21.0 ± 1.5	22.2 ± 1.6	18.6 ± 1.0	19.9 ± 1.9	19.7 ± 0.8	19.8 ± 2.1	17.4 ± 2.4	19.3 ± 6.6
Retained protein (g/d) <sup>h</sup>	127 ± 10	140 ± 21	131 ± 9	139 ± 10	116 ± 6	124 ± 12	123 ± 5	123 ± 13	109 ± 15	120 ± 41

<sup>a</sup> Six observations per treatment group.

<sup>b</sup> Mean BW during balance period.

<sup>c</sup> Merely determined for the length of each balance period (7 d).

<sup>d</sup> Wheat starch (DM = 864 g/kg; GE = 17.5 kJ/g DM) ingested in addition to the constant amount of 1.3 kg/d (basal diet).

<sup>e</sup> Different ingestion of wheat starch, consequently affecting energy parameters.

<sup>f</sup> Amount of supplemented lysine twice as high for balance period 1.

<sup>g</sup> Performing calorimetry, samples of feces were not analyzed individually, but pooled within each group instead.

<sup>h</sup> Body weight (N-balance periods 1 - 5) effect, P < 0.05.

<sup>i</sup> Body weight (N-balance periods 1 - 5) and lysine (medium or high) interaction effect, P < 0.05.

## Discussion

Several hypotheses of independence were serving as the basis for the present study. Forming the first prerequisite, the linearity of protein retention-lysine intake was revealed by Susenbeth (1995) in an extensive literature study. Furthermore evidence can be found for postulating independence of lysine utilization from animal size. According to literature, the efficiency of digestible lysine for growth is close to 70%, derived from experiments with growing pigs between 20 and 55 kg BW (Noblet et al., 1987; Batterham et al., 1990; Bikker et al., 1994). Dourmad et al. (1996) were able to confirm these findings for growing pigs within a body weight range of 50 to 110 kg (marginal efficiency of true digestible lysine for growth between 65-70%). Verifying the hypothesis that lysine utilization is independent from the animals capacity for growth, Batterham et al. (1990) could not detect any differences regarding protein retention between gilts and boars up to an intake level of 12 g lysine/d; additionally Susenbeth et al. (1999) reaffirmed the latter for an intake level of 13 g lysine/d considering different genetics (German Landrace and Pietrain). The assumption that lysine utilization for protein accretion shows independence from the level of protein supply is validated by Susenbeth et al. (1994) as well as Langer and Fuller (1996). The latter found no differences in lysine utilization for protein accretion even at extremely varying levels of protein supply (100, 200 and 300 g/kg). Campbell et al. (1984, 1985) reported a constant marginal efficiency of lysine utilization, independent of energy intake. Bikker (1994) and Möhn et al. (2000) approved the last-mentioned and further showed independence of lysine utilization from lysine intake level.

In the present study values of 18 mg/kg BW, 71 mg/kg BW<sup>0.75</sup>, 29 mg/kg FFS and 121 mg/kg BP were achieved. With respect to BW<sup>0.75</sup> lysine requirement estimates for maintenance in pigs, according to different authors, are displayed in Table IX. Although the determination of the lysine requirement for maintenance holds uncertainties, values found by Fuller et al. (1989), Heger et al. (2002), Roth et al. (2003) and Dourmad and Etienne (2002) correspond despite the fact that the weight under consideration varied to a large extent. Dealing with growing pigs from 35 to 140 kg, greater values could be observed in the present study, being partly in concordance with findings of Susenbeth et al. (1999).

Table IX. Lysine requirement estimates for maintenance in pigs according to different authors

	Body weight (kg)	Lysine (mg/kg BW <sup>0.75</sup> )	Energy intake level (MJ ME/kg BW <sup>0.75</sup> )
Fuller et al.(1989)	40 - 45	36	0.83 <sup>b</sup>
Susenbeth et al. (1999)	44/77	69	1.20
Heger et al. (2002)	45	39	1.24 <sup>b</sup>
Roth et al.(2003)	170 - 250	38	0.45
Dourmad and Etienne (2002)	245	35 <sup>a</sup>	0.61 <sup>b</sup>
Present study	35 - 140	70	1.30

<sup>a</sup> converted from ileal digestible to total lysine.

<sup>b</sup> ME/DE = 0.96 (NRC,1998).

In a recent literature overview Sandberg et al. (2005) studied the marginal response in protein retention on energy-limiting foods and demonstrated that there was no effect of live weight, genotype including gender and temperature. Furthermore it should be noted that the aforementioned calculations used for the determination of the lysine requirement for maintenance were related to total dietary lysine intake while ileal digestibility or bioavailability of protein and lysine were not defined. Higher values achieved in the present study may be caused by the correspondingly high feeding level of 1.3 MJ ME/kg BW<sup>0.75</sup> compared to other studies (see Table IX). This is supported by Jansmann et al. (2002), who related endogenous losses to DM intake. For further observations it is therefore equally important to study endogenous CP and AA losses at high feeding levels to ensure application under practical conditions.

Based on a constant marginal efficiency of lysine, the results of the present study validate the hypothesis that the same amount of lysine ingested by pigs of ascending BW leads to decreasing values for retained protein, the latter implying a varying lysine requirement for maintenance. In comparison to other studies explicitly higher estimates were achieved. Lacking further evidence for a reference point other than BW<sup>0.75</sup>, the commonly used term of BW<sup>0.75</sup> is equally applicable.

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## GENERAL SUMMARY

Recommendations for an adequate energy supply are based on the assumption that the efficiency of energy retention ( $k_{pf}$ ) is expected to vary according to differences in the ratio of protein to fat accretion in many systems for feeding standards (GfE, 1987; NRC, 1998).

Based on more recent observations, which question this assumption, the objective of the first study was to determine whether a change in the proportion of protein and fat in body weight gain has an impact on the efficiency of energy utilization for growth, namely on energy retention (RE) and heat production (H). The investigation was conducted on two different levels of lysine intake (medium: 11.5 g/d and high: 13.5 g/d) to reach the intended alteration of protein quality and protein accretion; the basal diet for the twelve growing barrows remained isonitrogenous at an energy intake level of 1.3 MJ metabolizable energy/kg metabolic body weight ( $BW^{0.75}$ ). Furthermore measurements of deuterium dilution space were undertaken at target body weights (BW) of 35, 55, 80, 115 and 145 kg to provide information on body composition while protein and fat accretion were calculated by difference. The results show a significant effect (probability ( $P$ ) < 0.05) between treatment groups for the values of energy retained in protein, moreover clearly indicating that RE, H and therewith energy utilization demonstrate independence from the composition of BW gain. Since it is a widely accepted fact that the efficiency of protein accretion ( $k_p$ ) (0.54) is smaller than the efficiency of fat accretion ( $k_f$ ) (0.74) (ARC, 1981), this observation seems to be contradictory unless being attributed to a smaller  $k_p$  at minor values for protein accretion. Relating the latter to a possibly increased body protein turnover, evidence to support this hypothesis can be derived from studies with growing pigs (Fuller et al., 1987) and chickens (Maruyama et al., 1978; Tesseraud et al., 1996). Observations from these studies show that whole body protein turnover can be affected by the quality of dietary protein, supporting the hypothesis of a variable and probably even improved  $k_p$  with a decreasing protein turnover. Therefore, lacking evidence from experimental data for advantages in using constant values for  $k_p$  and  $k_f$ , a higher accuracy for the calculation of energy utilization cannot be expected when separate constant values for  $k_p$  and  $k_f$  are adopted. Consequently, a uniform value for  $k_{pf}$  seems to be more adequate.

The aim of the second study was to test whether the decreasing protein accretion obtained throughout a growing period from 23 – 147 kg, in which the animals received a constant amount of lysine per day, reflects the increasing requirement of lysine for maintenance, provided that lysine represents the first-limiting nutrient. The well-known lysine concentration in body protein (Susenbeth, 1995) and the constant efficiency with which absorbed lysine is incorporated into the latter (Susenbeth, 1995; Bikker, 1994) were used to estimate the lysine requirement for maintenance in growing pigs. A further aim of the study was to identify whether body protein (BP) or fat free substance (FFS), for whose estimation the deuterium oxide dilution technique served as a valuable tool, may be more closely related to the maintenance requirement for lysine than  $BW^{0.75}$ . Assuming a constant marginal efficiency of lysine utilization for protein accretion, the following estimates for the lysine requirement for maintenance were obtained: 18 mg/kg BW, 71 mg/kg  $BW^{0.75}$ , 29 mg/kg FFS and 121 mg/kg BP. Retrieving a similar coefficient of correlation for protein accretion and the examined reference points, the conventionally used reference point of  $BW^{0.75}$  is appropriate. Although the determination of the lysine requirement for maintenance holds uncertainties, values found by Fuller et al. (1989), Heger et al. (2002), Roth et al. (2003) and Dourmad and Etienne (2002) correspond (36 mg/kg  $BW^{0.75}$ ; 39 mg/kg  $BW^{0.75}$ ; 38 mg/kg  $BW^{0.75}$ ; 35 mg/kg  $BW^{0.75}$ ) despite the fact that the weight under consideration varied to a large extent. Nevertheless, higher values for the lysine requirement for maintenance were achieved in the present study, possibly caused by the higher feeding level compared to other studies, the latter being reported to increase fecal endogenous protein losses (Butts et al., 1993).



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## ZUSAMMENFASSUNG

Empfehlungen für eine adäquate Energieversorgung gründen in vielen Systemen für Fütterungsstandards auf der Annahme, dass die Energieverwertung für die Energieretention ( $k_{pf}$ ) variiert, welche Unterschieden im Protein-/Fettansatzverhältnis zugerechnet wird (GfE, 1987; NRC, 1998).

Ausgehend von neueren Untersuchungen, die diese Annahme in Frage stellen, war es Ziel der ersten Studie festzustellen, ob sich eine Veränderung des Protein-/Fettanteils im Körpergewichtszuwachs auf die Energieretention (RE), Wärmeproduktion (H) und somit auf die Energieverwertung für das Wachstum auswirkt. Um die gewünschte Änderung der Proteinqualität und des Proteinansatzes zu erreichen, wurde der Versuch mit je sechs wachsenden Börgen auf zwei unterschiedlichen Lysinaufnahmeniveaus (mittel: 11.5 g/d und hoch: 13.5 g/d) durchgeführt. Bei einer Energieaufnahme von 1.3 MJ umsetzbarer Energie/kg metabolisches Körpergewicht ( $BW^{0.75}$ ) wies die Basalration keine Unterschiede hinsichtlich des Stickstoffgehaltes auf. Mittels der Deuteriumoxid ( $D_2O$ )-Verdünnungstechnik wurden bei Zielgewichten von 35, 55, 80, 115 und 145 kg Messungen zur Bestimmung der Körperzusammensetzung durchgeführt, wobei Protein- und Fettansatz durch Differenz berechnet wurden. Ein signifikanter Effekt (Wahrscheinlichkeit ( $P$ ) < 0.05) zwischen den beiden Gruppen konnte für die Werte der im Protein retinierten Energie festgestellt werden, was verdeutlicht, dass RE, H und damit die Energieverwertung für das Wachstum unabhängig von der Zusammensetzung des Körpergewichtszuwachses sind. Da allgemein angenommen wird, dass die Energieverwertung für den Proteinansatz ( $k_p$ ) (0.54) kleiner ist als die Energieverwertung für den Fettansatz ( $k_f$ ) (0.74) (ARC, 1981) ist diese Aussage zunächst widersprüchlich, es sei denn, sie wird einem niedrigeren  $k_p$ -Wert bei geringerem Proteinansatz zugeordnet. Wird die letztgenannte Aussage zu einem möglichen erhöhten Proteinturnover in Beziehung gesetzt, lassen sich in der Literatur Studien mit wachsenden Schweinen (Fuller et al., 1987) und Hühnern (Maruyama et al., 1978; Tesseraud et al., 1996) finden, die diese Hypothese unterstützen. Hierbei zeigt sich, dass der Proteinturnover von der Qualität des Futterproteins beeinflusst wird. Dies unterstützt die Annahme eines variablen und möglicherweise verbesserten  $k_p$ -Wertes bei abnehmendem Proteinturnover. Aufgrund fehlender experimenteller Daten zum Einsatz konstanter Werte für  $k_p$  und  $k_f$  kann bei deren Verwendung nicht von einer höheren Genauigkeit für die Berechnung der

Energieverwertung ausgegangen werden. Folglich scheint es sinnvoll einen einheitlichen Wert für  $k_{pf}$  anzunehmen.

Das Ziel der zweiten Studie bestand darin zu ermitteln, ob der in einem Wachstumsbereich von 23 – 147 kg abnehmende Proteinansatz den erhöhten Lysinerhaltungsbedarf widerspiegelt; Voraussetzung hierfür war die Aufnahme einer konstanten täglichen Menge der erstlimitierenden Aminosäure Lysin. Zur Schätzung des Lysinerhaltungsbedarfs für wachsende Schweine wurden die Lysinkonzentration im Körperprotein (Susenbeth, 1995) und die konstante Verwertung, mit welcher Lysin in Körperprotein eingebaut wird (Susenbeth, 1995; Bikker, 1994), unterstellt. Desweiteren galt es zu ermitteln, ob eines der Kompartimente Körperprotein (BP) oder fettfreie Körpersubstanz (FFS), für deren Bestimmung die  $D_2O$ -Methode herangezogen wurde, möglicherweise in engerer Beziehung zum Lysinerhaltungsbedarf stehen als der in der Literatur eingesetzte Bezugspunkt  $BW^{0.75}$ . Unter Annahme einer konstanten Lysinverwertung für den Körperproteinansatz konnten die folgenden Werte erzielt werden: 18 mg/kg BW, 71 mg/kg  $BW^{0.75}$ , 29 mg/kg FFS and 121 mg/kg BP. Da zwischen Proteinansatz und den oben genannten Bezugspunkten ähnliche Korrelationskoeffizienten erhalten wurden, ist der konventionelle Bezug auf  $BW^{0.75}$  angemessen. Obwohl die Bestimmung des Lysinerhaltungsbedarfs mit erheblichen Unsicherheiten behaftet ist, stimmen die Werte aus Studien von Fuller et al. (1989), Heger et al. (2002), Roth et al. (2003) und Dourmad and Etienne (2002) trotz verschiedener untersuchter Gewichtsbereiche gut überein (36 mg/kg  $BW^{0.75}$ ; 39 mg/kg  $BW^{0.75}$ ; 38 mg/kg  $BW^{0.75}$ ; 35 mg/kg  $BW^{0.75}$ ). Die höheren Werte aus der vorliegenden Studie können möglicherweise dem erhöhten Fütterungsniveau im Vergleich zu anderen Studien zugerechnet werden, da eine Erhöhung mit einem Anstieg der endogenen Proteinausscheidung einhergeht (Butts et al., 1993).

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## EIDESSTÄTLICHE ERKLÄRUNGEN

Dipl.-Agr. Biol. Judith Ringel

Eidesstattliche Erklärung:

Hiermit erkläre ich an Eides statt, dass ich die vorgelegte Dissertation mit dem Titel “The lysine requirement for maintenance and the efficiency of energy utilization for growth in pigs“ selbständig und ohne unerlaubte Hilfe angefertigt habe und dass ich die Arbeit noch keinem anderen Fachbereich bzw. noch keiner anderen Fakultät vorgelegt habe.

**Kiel, Januar 2006**

Hiermit erkläre ich, dass gegen mich kein strafrechtliches Ermittlungsverfahren schwebt.

**Kiel, Januar 2006**