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Lysine efficiency in piglets fed diets with a phytogenic feed additive and conclusion of lysine requirement data

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ABSTRACT

Two experiments were conducted to examine effects of a commercial phytogenic feed additive (PFA), containing inulin, an essential oil mix (carvacrol, thymol), and chest nut meal (polyphenols) on nutrient digestibility, protein utilization, and lysine efficiency in piglets. Each experiment utilized sixteen piglets (male castrated, 8 wk) and four experimental groups. Experiment 1 studied graded levels of the PFA (0, 0.05, 0.1, 0.15%) in lysine limited diets (wheat, barley, soyabean meal, fish meal). Experiment 2 examined 0.1% of the PFA and two dietary levels of lysine. The results indicated that apparent nutrient digestibility, protein utilization, and lysine efficiency were not significantly affected by the PFA (P>0.05). Observed daily lysine requirements (7.3, 9.9 and 13.1 g for 76, 100, 124 g daily protein deposition) in growing barrows (20-30 kg body weight) were in line with recommendations and contribute to the limited database for modelling of lysine requirements in piglets.

KEY WORDS: piglets, protein utilization, feed additives, lysine

INTRODUCTION

Mixtures of herbs, spices and essential oils are currently applied to achieve feed intake, growth and health promoting effects in several animal species. However, mode of action and potential of phytogenic feed additives (PFA) *in vivo*

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is mostly unknown. Currently, contradictory observations do not withstand an objective and critical judgement. Additionally, the diversity of PFA mixtures allows no general conclusion (Jeroch et al., 2008). Earlier studies with a commercial PFA examined zootechnical data, parameters of gut microflora, digestive enzyme activity and unspecific immune response in weaned piglets (Muhl and Liebert, 2007a,b). The current study investigated parameters of digestibility, protein utilization and dietary lysine efficiency. A validated procedure (Liebert, 2008; Liebert and Wecke, 2008; Wecke and Liebert, 2009) for modelling of protein utilization depending on observed dietary amino acid efficiency was applied for conclusion of lysine requirement data for piglets.

MATERIAL AND METHODS

N-balance experiments were conducted making use of a commercial PFA containing the fructopolysaccharide inulin (53%) from Jerusalem artichoke, an essential oil mix from oregano oil (8%), tannins from chest nut meal (3%) and cellulose powder (36%), respectively. An external gas chromatographic analysis of the PFA yielded 6.0% carvacrol and 0.14% thymol.

Animals and diets

Each of the experiments was carried out on sixteen male castrated eight week old piglets [Pietrain × (Large White × German Landrace)]. Piglet mash diets were based on the main ingredients: wheat, soyabean meal, barley and fish meal, respectively.

Experiment 1 examined three graded levels of the PFA under study (Table 1) for investigation of effects of the additive on dietary lysine efficiency. To adjust lysine in the first limiting position, the dietary lysine supply was approximately 80% of the NRC (1998) recommendation (Table 2). Experiment 2 utilized only one level of PFA addition, but supplemented crystalline lysine (Table 1, diets C, D) to approve the limiting position of lysine and to examine the PFA effect at optimal lysine supply.

Balance studies were conducted with individual metabolism cages making use of an adaptation period of five days and two consecutive collecting periods (five days each). For standardization of feed intake during the collecting periods, piglets were restrictive fed twice a day.

Sampling

Faeces were quantitatively collected twice a day during morning and afternoon feeding and stored at -18°C for further analyses. In order to minimize ammonia losses, the urine was acidified (pH below 2) with 60 g of sulphuric acid (30%). Individual aliquots of the daily urine sampling were stored at 4°C, carefully mixed and further analysed.

Table 1. Composition of the basal diet in experiment 1 and 2, g/kg

Ingredient	g/kg	Experiment 1	Experiment 2, treatment	
			A/B	C/D
Wheat	530			
Soyabean meal	230			
Barley	140			
Fish meal	25			
Soyabean oil	20			
Wheat starch		18.9	19.0	14.5
Premix*	15			
$CaCO_3$	11.7			
Sodium chloride	4.1			
Dicalcium phosphate	1.0			
L-tryptophan	0.4			
L-threonine	2.1			
DL-methionine		1.8	1.7	1.7
L-lysine		0	0	4.5
PFA2		A: 0 B: 0.5 C: 1 D: 1.5	A: 0 B: 1	C: 0 D: 1

[1] provided per kg of feed, IU: vit. A 9.000, vit. D_3 1.050; mg: vit. E 30, thiamin 1.5, riboflavin 3.0, vit. B_6 3, vit. K_3 3.0, nicotinic acid 18.8, Ca-pantothenate 11.3, folic acid 0.6, Fe 150, Cu 30, Mn 37.5, Zn 150, I 0.23, Se 0.23, Co 0.75; g: Ca 1.8, P 2.1, Na 0.08, Mg 0.1; μg: biotin 225, vit. B_{12}, 30; [2] PFA composition, per kg; g: inulin 530, essential oil mix (mainly thymol and carvacrol) 80, tannins 30, cellulose powder 360

Table 2. Nutrient composition of the basal diets, g/kg DM

Ingredient	Experiment	
	1	2
Crude protein	225.8	229.0
Ether extract	50.1	51.9
Crude fibre	38.3	36.5
Crude ash	65.4	66.1
N-free extractives	620.4	616.5
Lysine	11.5	11.5 (Diets A + B) - 15.4 (Diets C + D)
Threonine	10.0	10.0
Methionine + cystine	9.3	9.3
Metabolizable energy[1]	15.9 MJ	16.0 MJ

[1] calculated according to Kirchgessner and Roth (1983)

All experimental protocols were approved by the Animal Welfare Committee of the Agricultural Faculty of Goettingen University.

Chemical analysis

Diets were analysed for dry matter (DM), crude protein (CP), crude fibre (CF), ether extract (EE), ash, starch and sugar. Nitrogen content of feed and faeces was estimated by Dumas-method (LECO® FP-2000), nitrogen content of urine with the Micro-Kjeldahl-Apparatus (BUECHI 315). CP was calculated from nitrogen content by factor 6.25. Crude fat analyses were conducted following HCl-hydrolysis. Analyses of ingredients, mixed diets and excreta were according to the German VDLUFA standards. Amino acid analyses run by ion-exchange chromatography (LC 3000, Biotronik) using acid hydrolysis with and without oxidation step for quantification of sulphur containing amino acids.

Data analysis

Apparent faecal digestibility of crude nutrients was established. Analysis of N-balance data and modelling procedure was conducted according to Liebert and Wecke (2008). Additional current applications of the N-utilization model are reported elsewhere (Thong and Liebert, 2004a,b,c; Liebert, 2008; Wecke and Liebert, 2009).

The basic function of the model is given in equation (1):

$$NR = NR_{max}T\ (1\text{-}e^{-b \cdot NI}) \qquad (1)$$

where: NR - daily N-retention ($mg/BW_{kg}^{0.67}$) = Daily N-deposition + NMR; NMR - daily N-maintenance requirement ($mg/BW_{kg}^{0.67}$); $NR_{max}T$ - theoretical maximum for daily N-retention ($mg/BW_{kg}^{0.67}$); b - model parameter for the slope of the exponential function, depending on the dietary protein quality; NI - daily N-intake ($mg/BW_{kg}^{0.67}$); e - basic number of natural logarithm (ln).

Model parameters NMR and $NR_{max}T$ were taken from earlier studies with similar genotype barrows (Wecke and Liebert, 2009). Assessment of dietary protein quality (b) utilized equation (2):

$$b = \ln\ [NR_{max}\ T - \ln\ (NR_{max}\ T - NR)] : NI \qquad (2)$$

Net protein utilization (NPU) data were standardized for equal daily N-intake (3650 $mg/BW_{kg}^{0.67}$) according to Thong and Liebert (2004a). The applied procedure for modelling lysine requirements was according to earlier reports (Thong and

Liebert, 2004b,c).

Equation (3) summarises the derived requirement dependent on NR and observed dietary amino acid efficiency (bc^{-1}):

$$LAAI = [\ln NR_{max}T - \ln (NR_{max}T - NR)] : 16bc^{-1} \quad (3)$$

where: LAAI - required daily intake of the limiting amino acid for a given NR, $mg/BW_{kg}^{0.67}$; bc^{-1} - model parameter for the dietary amino acid efficiency.

Statistical analysis

Statistical data analysis utilized ANOVA ($P<0.05$ or higher) within the programme SPSS 12.0 for Windows. Data were subjected to a verification of variance homogeneity according to Levene test, following LSD in case of homogeneity of variance. Following in-homogeneity of variance, the Games-Howell post-hoc test was applied.

RESULTS

Apparent nutrient digestibility. Digestibility of crude nutrients (Table 3) did not significantly respond to the added PFA. The dietary lysine supply was not a significant factor for the observed results of the digestibility study.

Table 3. Apparent digestibility of crude nutrients, %

Experiment	Treatment	Crude protein %	Crude fibre %	Ether extract %	N-free extractives %
1	A	$89.5^a \pm 2.31$	$46.7^a \pm 6.55$	$74.4^a \pm 4.31$	$93.7^a \pm 0.67$
	B	$89.1^a \pm 1.98$	$48.2^a \pm 4.43$	$74.8^a \pm 5.17$	$93.7^a \pm 0.50$
	C	$89.8^a \pm 1.02$	$49.4^a \pm 4.43$	$76.0^a \pm 2.56$	$93.6^a \pm 0.58$
	D	$89.8^a \pm 1.12$	$49.1^a \pm 5.13$	$76.0^a \pm 3.12$	$93.9^a \pm 0.33$
2	A	$90.6^a \pm 0.90$	$50.7^a \pm 6.29$	$77.2^a \pm 1.23$	$93.7^a \pm 0.57$
	B	$90.8^a \pm 2.13$	$49.7^a \pm 7.36$	$77.8^a \pm 2.22$	$93.9^a \pm 0.49$
	C	$89.8^a \pm 2.10$	$49.4^a \pm 6.84$	$79.1^a \pm 1.91$	$93.9^a \pm 0.56$
	D	$89.6^a \pm 0.61$	$49.5^a \pm 3.38$	$78.3^a \pm 2.10$	$93.8^a \pm 0.45$

means with different superscript letters are significantly different ($P<0.05$)

Nitrogen balance studies. Daily N-balance, N-utilization and dietary lysine efficiency were not significantly affected by the added PFA (Table 4). Supplementation of crystalline lysine in diets C and D (Experiment 2) improved N-utilization significantly. Consequently, lysine was confirmed to be in first limiting position in all of the diets not supplemented with crystalline lysine.

Table 4. Results of the N-balance study (n=8; mean BW: experiment I = 23.2 kg, experiment II = 25.21 kg)

Experiment	Treat-ment	N-intake $mg/BW_{kg}^{0.67}/d$	N-balance g/d	N-digestibility %	N-utilization* %	NPU** %	Lysine efficiency bc^{-1}
1	A	3567^{a}	15.25^{a}	89.54^{a}	50.33^{a}	61.80^{a}	37^{a}
	B	3570^{a}	14.62^{a}	89.10^{a}	49.66^{a}	69.90^{a}	35^{a}
	C	3710^{a}	15.18^{a}	89.80^{a}	51.70^{a}	62.00^{a}	37^{a}
	D	3693^{a}	15.41^{a}	89.76^{a}	50.99^{a}	62.54^{a}	37^{a}
2	A	3598^{a}	15.67^{a}	90.56^{a}	51.95^{a}	63.14^{a}	38^{a}
	B	3405^{a}	14.63^{a}	90.75^{a}	51.81^{a}	59.89^{a}	35^{a}
	C	3459^{a}	19.73^{b}	89.84^{a}	63.34^{b}	73.93^{b}	37^{a}
	D	3431^{a}	19.67^{b}	89.55^{a}	63.47^{b}	73.80^{b}	37^{a}

means with different superscript letters are significantly different ($P<0.05$); * N-balance: N-intake, %; ** net protein utilization standardized for 3650 $mg/BW_{kg}^{0.67}/d$ N-intake; N-retention:N-intake

Modelling of lysine requirement data. Conclusion of lysine requirement data (Table 5) was not in first focus of the conducted experiments. However, approved limiting position of lysine provided an additional database to improve information about quantitative lysine requirements for piglets. Requirements were derived for 40, 50 and 60% of the growth potential ($NR_{max}T$), according to graded daily protein deposition (76, 100 and 124 g daily protein gain at 24.2 kg average BW). Assuming 17.0% crude protein in daily gain (GRRS 2006. 2008), these protein deposition data were equal with 447, 588 and 729 g daily gain of BW. Additionally, according to the applied modelling procedure the requirements were also derived for graded dietary efficiency of lysine (Table 6).

Table 5. Model calculation of lysine requirement for piglets (20 to 30 kg) depending on daily CP-deposition, observed efficiency of dietary lysine utilization and predicted feed intake

CP-deposition, g/d	76[1]	100[2]	124[3]
Lys-efficiency, bc^{-1}		37	
Lys-requirement, $mg/BW_{kg}^{0.67}/d$	863	1171	1548
g/d[4]	7.3	9.9	13.1
Optimal dietary lysine concentration (%) depending on feed intake			
predicted feed intake, g/d			
900	0.81	1.10	1.46
1000	0.73	0.99	1.31
1100	0.66	0.90	1.19

[1] 40% of NR_{max} T at BW = 24.2 kg; [2] 50% of NR_{max} T at BW = 24:2 kg; [3] 60% of NR_{max} T at BW = 24.2 kg; [4] BW = 24.2 kg

Based on predictions for the daily feed intake of piglets which were in line with feed intake pattern observed in the current experiments, recommendations for the optimal dietary lysine supply (as % of diet) were concluded.

Table 6. Model calculation of lysine requirement for piglets (20 to 30 kg) with varying dietary efficiency of lysine utilization

CP-deposition, g/d		100[1]	
Lys-efficiency, bc^{-1}	33[2]	37[3]	41[4]
Lys-requirement, $mg/BW_{kg}^{0.67}/d$	1313	1171	1057
g/d[5]	11.1	9.9	8.9
Optimal dietary lysine concentration (%) depending on feed intake			
predicted feed intake, g/d			
900	1.23	1.10	0.99
1000	1.11	0.99	0.89
1100	1.01	0.90	0.81

[1] 50% of $NR_{max}T$ at BW = 24.2 kg; [2] 10% below the observed efficiency of dietary lysine utilization; [3] observed lysine efficiency; [4] 10% above the observed efficiency of dietary lysine utilization; [5] BW = 24.2 kg

DISCUSSION

Commercial phytogenic feed additives are varying in supply of individual bioactive substances. Consequently, several studies observed improved nutrient digestibility by supplementation of essential oils, but other reports failed to yield significant effects (Möller, 2001). Wald (2002) summarized that positive effects of essential oils are only caused by their antimicrobial action which could contribute to improve nutrient and energy utilization in the host animal. However, according to Wald (2002) our previous studies (Muhl and Liebert, 2007a,b) did not yield significant growth effects. Enhanced activity of digestive enzymes (Platel et al., 2002) was not supported by results of Muhl and Liebert (2007b).

According to Möller (2001), presented N-balance data failed to show significant effects of the PFA under study. According to Branner et al. (2004), no effect on total N-excretion, N-retention and N-excretion *via* urine was attributed to supplementation of inulin. However, due to supplied native prebiotic compounds in pig feeds effects of low quantity of added prebiotics is questionable. Furthermore, masking or dilution by dietary oligosaccharides from cereals is also discussed (Gabert et al., 1995). However, experimental data for inulin effects on availability and retention of nutrients are scarce (Verdonk et al., 2005). In the current study, the very low supply of added inulin from the PFA under study was not sufficient to induce any significant effect. Mosenthin and Zimmermann (2000) concluded that healthy and well-kept animals not crucial exposed to pathogenic bacteria and stress factors will scarcely respond to this type of feed additives.

The insignificant effects of the PFA on observed dietary lysine efficiency provided data for modelling of lysine requirements. In growing barrows (20-30 kg BW) 7.3, 9.9 and 13.1 g lysine per day were required for 76, 100 and 124 g daily protein deposition. These data were in line with NRC (1998) recommendations (10-20 kg BW: 11.5 g/d; 20-50 kg BW: 17.5 g/d). In terms of total lysine, earlier German recommendations vary between 7.2 and 12.3 g lysine per day (20-25 kg BW, 300-600 g daily gain). Actual German recommendations (GRRS, 2006, 2008) advise 10.1 and 12.1 g lysine per day for 500 and 600 g daily gain (25 kg BW) in terms of standardized praecaecal digestible lysine. Assuming 85% as an average of ileal lysine digestibility, these recommendations are in the scope of presented requirement data for 100 g daily protein deposition. Our reported lysine requirement data for piglets are also in line with several other studies (Gatel et al., 1992; Whittemore et al., 2003; Wecke and Liebert, 2009). For feed formulation, Bertolo et al. (2005) recommended 0.91% ileal digestible lysine (24.1 kg BW; 982 g daily feed intake), which are in line with our data (85% predicted ileal lysine digestibility) and several other studies (Martinez and Knabe, 1990; Coma et al., 1995). In contrast, Fu et al. (2004) recommended 1.32% true digestible lysine for late nursery pig diets (29 kg BW). Generally, the applied procedure makes use of benchmarks for modelling amino acid requirements which can be adapted to different needs, like genotype, individual BW and the aimed daily protein deposition as important factors of influence (Martinez and Knabe, 1990; Cromwell et al., 1993; Thong and Liebert, 2004a,b,c).

CONCLUSIONS

According to our previous studies, no significant response was observed due to application of a commercial phytogenic feed additive (PFA) in piglet diets, but more investigations are needed to clarify mode of action and efficacy of PFA mixtures under several environmental circumstances. The applied modelling procedure was confirmed as a reliable tool to improve database for lysine recommendations in piglets.

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