Response to lysine in a wheat gluten diet in adult minipigs after short- and long-term dietary adaptation as assessed with an indicator amino acid oxidation and balance technique\textsuperscript{1,2}

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ABSTRACT: An experiment was conducted to examine the response to wheat gluten (WG)-based diets at two lysine levels in adult minipigs (23 kg BW) using the indicator AA oxidation (IAAO) approach and N balance. Twenty minipigs (n = five per group), fitted with reentrant ileoileal cannulas allowing collection of ileal effluents, were fed restrictively two WG-based diets (WG and WG + Lys; 2.7 and 6.6 g of lysine/kg, respectively) for adaptation periods of 10 and 100 d. On d 7 and 9, for pigs fed the diets for 10 d, and on d 97 and 99, for pigs fed the diets for 100 d, primed i.v. fasted/fed tracer protocols with \([13C]\)bicarbonate, and \([13C]\)leucine were performed. With the WG diet, \([13C]\)bicarbonate recoveries (%) were lower irrespective of the adaptation period, and higher during the fed period (fasted: WG + Lys = 82.5, and WG = 69.1; fed: WG + Lys = 90.6, and WG = 85.9; \(P < 0.05\)). Leucine oxidation rate was higher with the lower lysine intake (WG = 194.6 vs. 109.5 mg/[kg BW/d]; \(P < 0.05\)). Wheat gluten feeding resulted in a negative leucine balance independent of the adaptation period (WG = \(-29.1\), and WG + Lys = 48.2 mg/[kg BW/d]; \(P < 0.05\)). In contrast with the IAAO method, N balance did not differ between the two lysine intakes, possibly because of an underestimation of N losses. The finding of a lower \([13C]\) bicarbonate recovery with the lower dietary lysine intake suggests that caution should be taken in using a single recovery factor for all AA oxidation studies.

Key Words: \([13C]\)Carbon Bicarbonate Recovery, Indicator Amino Acid Oxidation, Leucine Tracer Balance, Minipigs, Nitrogen Balance, Wheat Gluten

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Introduction

Nitrogen balance (e.g., Dourmad and Étienne, 2002) has been routinely used to determine AA adequacy in pigs. Other methods such as growth assays (Parr et al., 2003), carcass traits evaluation (Owen et al., 1994; Hahn et al., 1995), or plasma urea N assays (Coma et al., 1995; Parr et al., 2003) also have been used. A method applied in human subjects (e.g., Zello et al., 1995) is the indicator AA oxidation (IAAO) method, which relies on stable or radioactive isotope tracer balance. Moehn et al. (2004) reported that results of the IAAO are more accurate than results obtained by N balance, especially under maintenance conditions. These authors also showed that a further advantage of the IAAO method in contrast with the N balance method was the short adaptation time.

Minipigs are increasingly used as model animals in nutrition and medical research (Johansen et al., 2001; Haberer et al., 2003). Miniature pig breeds are different from conventional pigs in terms of leanness, appetite, and apparent N digestibility (Hennig et al., 2004). For example, because minipigs gain large amounts of body fat if they are allowed to eat ad libitum, it is necessary to limit their energy intake.

Our objective was to comparatively assess the response to a wheat gluten (WG)-based diet at two levels of lysine in adult minipigs using the IAAO method, with \([13C]\)leucine as tracer and N balance. Wheat gluten has a low lysine concentration. Further, in an attempt to
consider a potential effect of the period of adaptation, we compared experimental dietary periods of 10 and 100 d, respectively.

Materials and Methods

Experimental Design and Diets

The study protocol was approved by the Animal Care and Use Committee of the Ministry of Nutrition, Agriculture, Forestry, and Fishery, Schwerin, State Mecklenburg-Vorpommern, Germany (permission No. VI 522a-7221.31-1-031/97). Twenty male, castrated minipigs (strain Minilewe) were obtained from the animal facilities of Humboldt University in Berlin and the Technical University of Dresden, Germany, at a mean age of 8 wk, and a mean BW of 8 kg. Before the start of the experiment, the minipigs were fed a commercial diet based on cereals (barley, wheat, rye, triticale, and soy; Masta Universal, Nordkorn Agrarhandel GmbH, Karstädt, Germany; 13 MJ of ME, 155 g of CP, 61 g of crude fat, 57 g of crude fiber, and 8.8 g of lysine per kg of DM). The experimental diets contained WG as the only protein source, with or without supplementation with crystalline lysine (WG and WG + Lys Table 1) and were fed individually for 10 (short-term adaptation) or 100 d (long-term adaptation). To achieve similar BW among groups when performing the leucine tracer protocol at the end of the study, the pigs assigned to receive the experimental diets differed in BW when they started the experimental feeding. Animals to be adapted short-term had a mean BW of 21.3 kg, and those pigs to be adapted long-term to the diets started the experimental diets at a mean BW of 17.3 kg. At the start of the tracer protocols (see below), the BW of the short-term adapted pigs was 21.5 kg (WG + Lys) and 21.9 kg (WG), and the BW of the long-term adapted pigs was 25.3 kg (WG + Lys) and 24.9 kg (WG).

There are no specific data on the lysine requirement of adult minipigs at a BW of 15 to 25 kg (Ritskes-Hoitinga and Bollen, 1997, 1998). Thus, we assumed that the developmental stage of the minipigs at approximately 25 kg is comparable to young adult conventional pigs. To compare the IAAO response between diets with different AA patterns, we chose WG as the protein source because it is known to contain low concentrations of indispensable AA (e.g., lysine, threonine, and tryptophan) compared with soy or milk proteins (WPSA, 1992; Young and Pellett, 1994; NRC, 1998). In addition to the WG basal diet, a WG-based, lysine-supplemented diet was used (Table 1). Lysine intakes were on either side of a putative mean lysine requirement in conventional pigs (young nonpregnant sows) of 120 to 150 kg BW. This is equivalent to approximately 80 mg of lysine·kg−1·d−1 (Deutsche Landwirtschafts-gesellschaft [1984]; total lysine requirement 268 mg lysine/kg0.75 BW). In this regard, the targeted lysine intake of approximately 110 mg of lysine·kg−1·d−1 in the WG + Lys groups should have been more than adequate, whereas the WG groups received lysine at approximately 40% of this amount (Table 1).

The measured CP content was 131.2 g/kg of DM, and lysine concentrations were 2.73 g/kg in WG and 6.63 g/kg in WG + Lys diets, respectively. The latter being in the range of the estimated lysine requirement of growing pigs (60 to 110 kg BW; NRC, 1998). The diets were fed in amounts of 40 g of DM/kg BW0.75·d) for periods of 10 and 100 d, respectively. The minipigs consumed daily 0.288 MJ of ME/kg BW. The energy level chosen is 18% less than that recommended for 120-kg-BW growing pigs to avoid excessive body fat retention (NRC, 1998). The minipigs were fed two equal meals per day (0800 and 1500), and water was offered ad libitum.

Surgencies

The animals were surgically prepared with an ileoileal reentrant cannula (Hennig et al., 1990) 28 d before the isotope infusion studies to collect ileal efflu-

Table 1. Composition and nutrient content of experimental diets based on wheat gluten (WG) or wheat gluten supplemented with lysine (WG + Lys)

<table>
<thead>
<tr>
<th>Item</th>
<th>WG</th>
<th>WG + Lys</th>
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</thead>
<tbody>
<tr>
<td>Components, g/kg of DM</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Wheat gluten*</td>
<td>150</td>
<td>150</td>
</tr>
<tr>
<td>Wheat starch*</td>
<td>640</td>
<td>635</td>
</tr>
<tr>
<td>Sucrose*</td>
<td>40</td>
<td>40</td>
</tr>
<tr>
<td>Microcellulose*</td>
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<td>40</td>
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<td>Soy oil*</td>
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<td>Vitamin mixture*</td>
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<td>30</td>
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<tr>
<td>Mineral mixture*</td>
<td>60</td>
<td>60</td>
</tr>
<tr>
<td>Lysine-HClg</td>
<td>—</td>
<td>5</td>
</tr>
<tr>
<td>Analyzed content, g/kg of DM</td>
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<td></td>
</tr>
<tr>
<td>ME, MJ/kg</td>
<td>15.8</td>
<td>131.2</td>
</tr>
<tr>
<td>CP</td>
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<tr>
<td>Lys</td>
<td>2.7</td>
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<tr>
<td>Met + Cys</td>
<td>4.9</td>
<td>4.9</td>
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<tr>
<td>Ile</td>
<td>4.8</td>
<td>4.6</td>
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<tr>
<td>Thr</td>
<td>3.3</td>
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</tr>
<tr>
<td>Trp</td>
<td>1.0</td>
<td>1.0</td>
</tr>
</tbody>
</table>

* Wheat gluten and wheat starch; Ferdinand Kreutzer Sabamühle GmbH, Nürnberg, Germany.
* Sucrose from beet sugar; Nordzucker GmbH & Co. KG, Uelzen, Germany.
* Microcellulose; Arboce B600, J. Rettenmaier u. Söhne, Rosenberg, Germany.
* Soybean oil; Sedina, Oelmühle Hamburg, Aktiengesellschaft, Germany.
* Provided per kilogram of diet as fed basis; composition after Anke and Groppel, 1989: vitamin A, 6,000 IU; vitamin D, 600 IU; vitamin E, 37 mg; vitamin K, 3 mg; thiamine, 7 mg; riboflavin, 7 mg; pyridoxine, 5 mg; cobalamin, 15 μg; niacin, 19 mg; calcium pantothenate, 19 mg; folic acid, 3.7 mg; biotin, 0.2 mg; choline chloride, 750 mg; and ascorbic acid, 37 mg.
* Provided per kilogram of diet as-fed basis; composition of mixture after Anke and Groppel, 1989: Ca, 6.8 g (dicalcium phosphate, and carbonate); Mg, 1.6 g (magnesium oxide); Na, 2.3 g (sodium chloride); K, 9.8 g (potassium sulfate); Fe, 150 mg (ferrous sulfate); Mn, 72 mg (manganese carbonate); Zn, 150 mg (zinc sulfate); Cu, 30 mg (copper sulfate); I, 1.1 mg (potassium iodate); P, 3 mg (sodium fluoride); and Se, 0.5 mg (sodium selenite).
*g-Lysine-monohydrochloride, feed-grade, 98.5% pure; Ajinomoto Co., Inc., Tokyo, Japan.
ents and determine ileal loss of intestinal microbial lysine (Backes et al., 2002). Briefly, following a feed-deprivation period (18 h), pigs were fitted with two curved flexible interconnected cannulas at the distal ileum approximately 10 cm proximal to the ileocecal valve. The ileum was transected and the two tubes were sewed antimesenterically into both intestinal stumps. The two cannulas were exteriorized through incisions in the abdominal wall and the barrel ends were inter-connected. The cannulas were secured in place with a retainer plate. Systemic postoperative antibiotic therapy (Pen Strep, Vetoquinol, Goch, Germany) administered i.m. at 5 mL per animal was used to prevent infection and to eliminate the need for antibiotic therapy during the surgical recovery period. After 10 d of recovery, minipigs were transferred to metabolic cages (0.8 × 1.3 m), where they were individually housed in a temperature- and light-controlled room (18 to 20°C, light from 0600 to 1800) until the end of the experiment.

Ten days before the tracer protocols, we implanted jugular vein and carotid artery catheters (customized using sterile silastic tubing 602-285, Aromando Medi-zintechnik GmbH, Düsseldorf, Germany) for tracer infusions and blood sampling, respectively. The jugular catheter was inserted 12 cm through the external jugular vein into the cranial vena cava; and the carotid catheter was placed 10 cm into the external carotid artery (U. Hennig, personal communication). The minipigs resumed full feed intake 2 d after catheter implantation. Before and between tracer infusion studies, catheters were kept open for a maximum period of 9 d by a constant infusion (0.7 mL/h) of physiological saline containing heparin (10,000 IU/L; heparin-sodium, B. Braun Melsungen AG, Melsungen, Germany) and sulfonamide (2 mL/L; sulfadimidin as sodium salt, Serumwerk Bernburg AG, Bernburg, Germany).

**Tracer Infusion Protocol**

After an overnight fast, primed 10-h tracer-infusion protocols with [13C]bicarbonate, and [13C]leucine were conducted according to a standard design on dietary d 7 (short-term adaptation) or d 97 (long-term adaptation), and d 9 or 99, respectively. At 0800 on d 7 and 97, a continuous i.v. infusion (3.5 mg/kg BW−1 h−1) of sodium [13C]bicarbonate (NaH13CO3, 99 atom%; Cambridge Isotope Laboratory Inc., Andover, MA), with a priming dose of 5 mg/kg of BW was started using a screw-driven syringe pump (model 22, Harvard Apparatus, South Natick, MA). At 0800 on d 7 and 99, a continuous i.v. infusion (3.5 mg/kg BW-h) of L-[1,13C]leucine; MassTrace, Woburn, MA) 99 atom%, with a priming dose of 5.25 mg/kg BW [13C]leucine and 1 mg/kg BW NaH13CO3 was started using the same pump. During the first 4 h of the infusions, no feed was given, whereas between 1200 to 1800, minipigs were fed small meals of their respective diet at consecutive 30-min intervals.

**Sample Collection and Analyses**

On the day of the leucine tracer infusion, appropriate baseline blood samples were drawn from the carotid catheter into Li-heparinized tubes (Monovette, Sarstedt AG and Co., Nürnberg, Germany). During the last 2 h of the fasted (120 to 240 min) and fed (480 to 600 min) periods, respectively, blood was drawn every 30 min. Plasma was separated by centrifugation (10 min, 4°C, 900 × g) and stored frozen at −80°C until analysis. Before (−15, −5 min) and during the tracer leucine and bicarbonate infusions, breath samples were collected at timed intervals in gas-tight bags (Tecobag, Tesseraux Container GmbH, Burstadt, Germany) using a customized face mask (Heiland Vet GmbH and Co. KG, Hamburg, Germany), and then transferred into evacuated tubes (10-mL Exetainer, Labco Ltd., Buckinghamshire, U.K.) for storage and eventual measurement. The pigs were trained to tolerate the facemask 2 wk before the tracer protocols.

Twenty-four hour CO2 production rate was measured by indirect calorimetry in an open-circuit respiration chamber (4 m3, 24°C environmental temperature, 50% relative humidity; Junghans et al., 1997) after recovery from the cannulation procedure, following the feeding regimen and the respective experimental diets during the standard tracer protocol mentioned above. For this purpose, animals housed in metabolic cages were moved into the respiration chamber.

Twenty-four–hour collections of urine, ileal contents, and feces were made in chilled containers on d 8, 9, and 10, and on d 98, 99, and 100 as described elsewhere (Backes et al., 2002) to estimate apparent N balance. To collect ileal effluents, the two interconnected cannulas were detached, and the proximal cannula was attached to a plastic bag placed on ice (0°C). Immediately after ileal effluent appeared in the bag, it was weighed, and divided into aliquots of 15 and 85% (by weight). The 15% samples were pooled for each collection day, stored at −20°C, and then freeze-dried. Feces and urine were collected whenever present with a sieve in the floor of the metabolic cages. Urine from each pig was collected in containers prepared with 100 mL of 5% 6 N HCl. The pH values in these containers were regularly checked, and additional HCl was added if needed to ensure pH 2. Samples were pooled over 24 h, and stored frozen as daily aliquots. Feces was weighed, pooled for 24 h in containers on ice, and stored frozen at −20°C in plastic bags until freeze-dried. Fecal mass was very small because ileal effluents were collected simultaneously.

The isotopic abundance of plasma α-[13C]ketoisocaproic acid ([13C]KIC) was considered to represent the enrichment of the intracellular leucine pool (Matthews et al., 1982). The KIC enrichment was measured in quinoxalino-tert-butyl-dimethyl-silyl derivatives by electron-impact gas chromatography-mass spectrometry (SSQ710, ThermoFinnigan, Bremen, Germany). Plasma was precipitated with methanol and the super-


13CO2 Recovery from [13C]Bicarbonate Administration

Because CO2 from tracer oxidation is not quantitatively recovered in breath (Leijssen and Elia, 1996), 13CO2 production must be corrected for incomplete recovery of 13CO2 in breath. Individual correction factors for the specific experimental conditions were determined by means of infusion of sodium [13C]bicarbonate.

The recovery of 13CO2 (%) was calculated as the ratio of the 13CO2 output to the amount of [13C]bicarbonate infused. A mean value for each animal derived for fasted (120 to 240 min) and fed (540 to 600 min) states was used to correct the 13CO2 production measured during [13C]leucine infusions.

\[
\text{Recovery, \%} = \frac{\text{VCO}_2, \text{ mmol/(kg-h)} \times 13\text{CO}_2 \text{breath enrichment, APE/100}}{\text{[13C]bicarbonate infused, \mu mol/(kg-h)}}
\]

where VCO2 is the volume of CO2 produced.

Leucine Oxidation Rates

A steady state of plasma [13C]KIC enrichment was achieved in the fasted and fed periods 2 and 8 h (i.e., 4 h after start of feeding small meals) after the start of the [13C]leucine continuous infusion, respectively (Figure 1). Leucine oxidation was computed for consecutive 30-min intervals during the fasted (120 to 240 min) and fed (540 to 600 min) states as follows:

\[
\text{Leucine oxidation rate, \mu mol/(kg-30 min) =} \frac{13\text{CO}_2 \text{production, \mu mol/(kg-30 min)}}{\text{Plasma [13C]KIC enrichment, MPE/100}}
\]

where [13C]KIC enrichment (mole % excess, MPE) is the average of the two enrichments determining the specific 30 min interval.

\[
\text{13CO}_2 \text{ production, \mu mol/(kg-30 min) =} \frac{\text{VCO}_2, \text{ \mu mol/(kg-30 min)} \times 13\text{CO}_2 \text{ breath enrichment, APE-1/R}}{}
\]

where R is the recovery factor determined in each individual animal adapted to either WG or WG + Lys diet, and 13CO2 breath enrichment during the [13C]leucine infusion, respectively.

Mean fasted and fed leucine oxidation rates were calculated from individual values obtained during the time periods given above. Twenty-four-hour leucine oxidation rates were extrapolated from the measured mean fasted and fed oxidation rates, assuming that the animals were in the fasted and fed state for 12 h each.

Leucine and N balances

According to the concept of the IAAO method, the criterion for the adequacy of test AA intake is zero balance of the indicator AA (i.e., leucine [difference between measured input and output is zero]). Because
Leucine balance in minipigs

Figure 1. Mean plasma \([^{13}\text{C}]\)ketoisocaproic acid (KIC) enrichment during i.v. infusion of \([^{13}\text{C}]\)leucine in minipigs fed wheat gluten-based diets with or without lysine supplementation (WG and WG + Lys) after short-term and long-term adaptation (five pigs per mean). For clarity, SE bars were omitted.

Total ileal effluents were collected during the day of isotope infusions, we considered ileal N losses in the calculation of 24-h N balances. Ileal AA losses are thought to be of no further value to the body because AA are not absorbed from the large intestine. Thus, we did not consider ileal leucine losses in the leucine balance.

\[
\text{Leucine balance} = (\text{dietary leucine intake} + \text{tracer leucine intake}) - (\text{leucine oxidation})
\]

\[
\text{N balance} = (\text{dietary N intake}) - (\text{N losses [urine, fecal, and ileal]})
\]

Statistics
Data were analyzed by a mixed model, with two fixed effects (lysine level and adaptation period), a repeated factor metabolic state (fasted, fed), a random factor (animal), and corresponding interactions using the Mixed procedure of SAS (v. 8.2; SAS Inst., Inc., Cary, NC). Two-way fixed effects (lysine level and adaptation period) and one interaction ANOVA were performed with the GLM procedure of SAS when measurements occurred only once (e.g., 24-h leucine oxidation rate). When significant interactions were detected, post-hoc tests of subclasses with a Tukey-Cramer correction (to ensure a multiple test risk of first kind < 0.05) were applied. An alpha level of 0.05 was used for determination of statistical significance.

Results
Mean BW on the day before the tracer infusions was 23.3 ± 0.5 kg and did not differ between diets. Weight gain by the pigs adapted long-term to the diets was 59 ± 49 (WG + Lys) and 65 ± 56 (WG) g/d. Weight gain by the pigs adapted short-term to the diets was 75 ± 71 (WG + Lys) and 61 ± 50 (WG) g/d; BW gain did not differ between groups. Measured total daily lysine intakes with the WG + Lys and WG diets were 126 ± 9 and 57 ± 3 mg/(kg BW·d), respectively. Total daily leucine intake was 162 ± 11 mg/kg BW in all groups.

CO₂ Production Rate and \([^{13}\text{CO}_2]\) Bicarbonate Recovery
The CO₂ production was only affected by metabolic state (fasted = 886.8; fed = 1,557.7 mL/(kg⁰.⁷⁵ BW·h); \(P < 0.001\)), and did not differ between diets. The \([^{13}\text{CO}_2]\) bicarbonate recovery factors (%) differed according to the lysine concentration in the diet (Table 2). The \([^{13}\text{CO}_2]\) recoveries were lower with the WG diets (\(P = 0.041\)) and higher in the fed vs. the fasted state, regardless of the adaptation period (Table 2; \(P < 0.001\)).

Leucine Oxidation Rate, and Ileal N and Leucine Losses
Minipigs fed the WG diets showed a higher leucine oxidation rate than those fed the WG + Lys diets (\(P < 0.013\)). Although no interaction between diet and adaptation period was detected, the means of all four groups are presented (Table 2). With the WG diet, the fed leucine oxidation rates were approximately three times higher than those for fasting pigs (\(P < 0.001\)), whereas with WG + Lys, fed leucine oxidation rates were approximately twice the fasted rate (\(P = 0.031\); Table 2). Ileal N and leucine losses were affected neither by adaptation period nor by lysine level in the diet (Table 3).
Table 2. Fasted and fed \(^{13}\)C bicarbonate recoveries and leucine oxidation rates in minipigs fed wheat gluten-based diets with or without lysine supplementation (WG and WG + Lys, respectively) after short-term and long-term adaptation\(^a\)

<table>
<thead>
<tr>
<th>Responses</th>
<th>Diet:</th>
<th>Diet:</th>
<th>P-value(^b)</th>
</tr>
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<tbody>
<tr>
<td></td>
<td>WG</td>
<td>WG + Lys</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Adaptiation period:</td>
<td>Short-term</td>
<td>Long-term</td>
</tr>
<tr>
<td></td>
<td></td>
<td>0.041</td>
<td>0.001</td>
</tr>
<tr>
<td>(^{13})C bicarbonate recovery, % Fasted period</td>
<td>73.1</td>
<td>65.2</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Fed period</td>
<td>90.7</td>
<td>81.1</td>
</tr>
<tr>
<td>Leucine oxidation rate, (\mu)mol/(kg(^{-1})30 min) Fasted period</td>
<td>14.8</td>
<td>14.6</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Fed period</td>
<td>38.1</td>
<td>56.1</td>
</tr>
</tbody>
</table>

\(^a\)Five pigs per mean.

\(^b\)Mixed model evaluating effects of dietary lysine level (Lys), adaptation period (Adapt), and metabolic state (MS; fasted or fed). There were no significant main effects of Adapt, or Lys \(\times\) Adapt, Adapt \(\times\) MS, and Lys \(\times\) Adapt \(\times\) MS interactions (\(P = 0.10\) to 0.59).

Leucine and N Balances

Daily leucine balance was affected by the dietary lysine level (\(P = 0.046\)) but was independent of the adaptation period (\(P = 0.780\); Table 3). The WG + Lys feeding resulted in a positive leucine balance. With the WG diet, leucine balance was negative but not different from zero. Apparent N balance was positive with both diets and was not affected by diet and adaptation period (WG + Lys = 129.7, and WG = 87.1 mg/[kg BW\(^{-1}\)d]).

Discussion

This investigation compared two balance criteria (tracer leucine and N balance) in the same individuals to assess responses to two WG-based diets with or without lysine supplementation in minipigs. The N balance method as the classical method for determination of AA requirement has limitations, mainly because of systematic underestimation of N losses and thereby overestimation of balance (Wallace, 1959; Fuller and Garlick, 1994; Rasch and Benevenga, 2004).

One recent method increasingly applied in human subjects (e.g., Zello et al., 1995) is the IAAO method, which relies on stable or radioactive isotope tracer balance. Moehn et al. (2004) claim that, especially under maintenance condition, the IAAO yields more accurate results than the N balance. To date, the IAAO method has been used in pigs mainly by a group at the University of Toronto, Canada, and particularly in piglets, for the determination of indispensable AA requirements (e.g., Lin et al., 1986; Elango et al., 2002; Shoveller et al., 2003). This technique relies on the fact that the animal requires a balanced supply of AA, whereas an imbalance caused by the deficiency of an indispensable AA results in the oxidation of any surplus of the other indispensable AA. To our knowledge, studies on pigs have been largely performed using phenylalanine and lysine tracers. In humans, however, labeled leucine also has been used (Kurpad et al., 2002).

It is generally known that changes in metabolic rate due to feeding, hormonal changes, and physical activity affect \(^{13}\)CO\(_2\) bicarbonate recovery, which varies approximately from 50 to 100% (Van Hall, 1999). These changes demonstrate the need to determine bicarbonate recovery in farm animals during oxidation studies to derive appropriate correction factors. The \(^{13}\)CO\(_2\) recovery values we observed in the minipigs were well within the range reported for human subjects and animals in resting conditions (Benevenga et al., 1992; Leijssen and Elia, 1996; Tabiri et al., 2002). A rather unexpected result was that the \(^{13}\)C bicarbonate recovery was

Table 3. Ileal leucine and nitrogen losses, nitrogen balance, and leucine oxidation and balance of minipigs fed wheat gluten-based diets with or without lysine supplementation (WG and WG + Lys) after short-term and long-term adaptation\(^a\)

<table>
<thead>
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<th>Responses</th>
<th>Diet:</th>
<th>Diet:</th>
<th>P-value(^b)</th>
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<tr>
<td></td>
<td>WG</td>
<td>WG + Lys</td>
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<tr>
<td></td>
<td>Adaptiation period:</td>
<td>Short-term</td>
<td>Long-term</td>
</tr>
<tr>
<td>Ileal leucine loss, mg/[kg BW(^{-1})d]</td>
<td>34.8</td>
<td>37.6</td>
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<tr>
<td>Ileal N loss, mg/[kg BW(^{-1})d]</td>
<td>47.5</td>
<td>45.7</td>
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<td>N balance, mg/[kg BW(^{-1})d]</td>
<td>86.2</td>
<td>87.9</td>
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<tr>
<td>Leucine oxidation, mg/[kg BW(^{-1})d]</td>
<td>194.6</td>
<td>109.5</td>
<td></td>
</tr>
<tr>
<td>Leucine balance, mg/[kg BW(^{-1})d](^c)</td>
<td>(-29.1)</td>
<td>48.2(^d)</td>
<td></td>
</tr>
</tbody>
</table>

\(^a\)Five pigs per mean.

\(^b\)Mixed model evaluating effects of dietary lysine level (Lys), and adaptation period (Adapt). Adapt \(\times\) Lys interactions were not significant (\(P = 0.45\) to 0.78).

\(^c\)Leucine balance was calculated without consideration of ileal leucine loss.

\(^d\)Designates difference from zero balance.
lower with the lower dietary lysine intake (Table 2). This finding implies that more carbon is fixed in the body with a marginal dietary supply of indispensable AA and suggests that caution should be taken using a single recovery factor for all oxidation studies. In this fixation process, carbon could be sequestered via anaplerotic reactions to replenish citric acid cycle intermediates, which in turn serve as precursors for dispensable AA such as glutamate, glutamine, proline or arginine, and glucose. One additional possibility is that carbon derived from the bicarbonate tracer is used for urea synthesis. Plasma urea concentrations from these same WG-fed pigs were increased (Backes et al., 2002) compared with WG + Lys, suggesting a higher urea synthesis rate, which might partly explain lower bicarbonate recovery rates with the WG diet or generally, with imbalanced dietary AA patterns. Others have found in sheep that NaH$^{13}$CO$_3$ infusion led to urinary enrichments approaching enrichment levels of blood bicarbonate (Ram et al., 1999). To our knowledge, a decreased $^{13}$CO$_2$ bicarbonate recovery in individuals consuming diets with low lysine content, or more generally, a suboptimal AA supply, has not been reported before and deserves further investigation.

It is assumed that the leucine oxidation rates determined by i.v. leucine tracer infusion reflect true whole-body leucine oxidation, and thus, this method is feasible to predict AA adequacy (Kurpad et al., 2002). Whole-body leucine oxidation rate seems to be independent of the route of tracer administration, at least in human subjects and dogs (Hoerr et al., 1993; Yu et al., 1995). It was shown that oral and intravenous tracer protocols of the IAAO method using $^{13}$C phenylalanine as tracer provide the same estimate of lysine requirement in healthy men (Kriengsinyos et al., 2002). Recently, requirement values of dietary lysine for humans were derived by the IAAO method from measurements of leucine balance (Kurpad et al., 2002).

According to the tracer leucine balance results, a WG-based diet providing 2.7 g of lysine/kg of diet does not support AA homeostasis in minipigs, whereas a dietary lysine concentration of 6.6 g of lysine/kg of diet resulted in a positive balance, indicating that the AA intake provided by the WG diet was insufficient to achieve leucine balance. Thus, we tentatively conclude that AA requirements in adult minipigs are possibly higher than those provided by a WG-based diet. However, because we considered two lysine levels only, and WG provides only marginal quantities of indispensable AA other than lysine, our data are not suitable to draw any conclusions regarding the lysine requirement of minipigs.

It is thought that AA passing beyond the ileocecal valve are of no further value for the body because there is no quantitatively important AA absorption in the large intestine. However, there is some evidence for large intestinal N or AA absorption (Metges, 2000), and the occurrence of peptide transporters in the large intestine has been shown in rabbits and humans (Doring et al., 1998; Ford et al., 2003). Whether these peptide transporters bear functional importance regarding whole body AA utilization, and whether it turns out to be true for the pig, remains to be determined. Thus, for the sake of comparison, assuming 100% whole-body availability of terminal ileal AA, and considering leucine losses in ileal effluents in addition to obligatory oxidative losses, leucine balance would be achieved at 20 and $-46$ mg/(kg BW·d) with the WG + Lys and WG diets, respectively ($P = 0.045$).

By means of the N balance data, it was not possible to distinguish between the two diets. Even with the lower lysine intake in the WG groups, N balance was still positive and not different from the WG + Lys diet. Possibly, we overestimated the N balance by not measuring miscellaneous N losses such as skin (hair) losses (Wallace, 1959), and by a comparably short sampling period of 3 d, although the adaptation period was at least 10 d. A further issue could have been that acidification of the urine collection tray was not performed (Van Kempen et al., 2003). All of these factors can lead to an underestimation of N losses. Our observation that BW gain was very low or close to zero during the experimental period also is in favor of our conclusion that the N balance measured was overestimated for methodological reasons. For example, based on the N balance in the short-term-adapted WG + Lys pigs, protein accretion amounted to 21 g/d, which is equivalent to a lean tissue gain of approximately 90 g/d. The mean total gain in these pigs was 75 g/d. Others have shown that maintenance of N equilibrium in growing pigs has been achieved despite the fact that the diet was devoid of lysine (Mnilk et al., 1996). Wallace (1959) noted that “. . . the numerical value obtained for intake is always larger than actuality, while the numerical value for output is always smaller.” Further, a recent study suggests that there is a N excretory product not detected by standard methods (Rasch and Benevenga, 2004). In conclusion, N balance can lead to an overestimation of N retention even if properly conducted (Fuller and Garlick, 1994; Rasch and Benevenga, 2004). Thus, these results further emphasize the need to take care when conducting N balance trials, as recommended earlier (Van Kempen et al., 2003).

Another question we aimed to answer was whether adaptation to the diets affects leucine balance data. Because lysine is an AA that is particularly well conserved in the body (Waterlow, 1981; Flodin, 1997), we anticipated that changes of leucine oxidation might only occur after long-term adaptation in adult individuals. The leucine tracer balance data suggest that adaptation to the lower lysine level in adult minipigs occurs within 10 d. This finding is confirmed by two reports showing that using the IAAO method of adaptation to a low-lysine diet in human subjects is complete within 7 d, whereas pigs respond to changes in lysine intake within 2 d (Kurpad et al., 2002; Mohr et al., 2004).

**Implications**

According to the tracer leucine balance results, a wheat gluten-based diet providing 2.7 g of lysine/kg of...
diet is insufficient to support amino acid homeostasis in minipigs, whereas a wheat gluten-based diet supplemented by lysine at a dietary concentration of 6.6 g of lysine/kg resulted in a positive leucine balance. With regard to the two wheat gluten-based diets differing in the dietary lysine level, we found that the indicator amino acid oxidation method was more discriminating than the nitrogen balance technique, which might be due to an underestimation of nitrogen losses. The period of dietary adaptation (10 or 100 d) had no effect on any of the measurements. The finding of a lower 13C bicarbonate recovery with the lower lysine intake suggests that caution should be taken using a single recovery factor for all amino acid oxidation studies.

**Literature Cited**


Deutsche Landwirtschaftsgesellschaft (German Agricultural Society). 1984. Pages 15–35 in Feed Tables for Pigs. 6th ed. DLG-Verlag, Frankfurt/Main, Germany. [In German]


