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Bacterial protein meal in diets for growing pigs: effects on protein and energy metabolism

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This experiment investigated the effects of increasing the dietary content of bacterial protein meal (BPM) on the protein and energy metabolism of pigs from weaning to a live weight of 80 kg. Four litters with four castrated male pigs in each litter were used. The litters were divided into two blocks according to age. One pig from each litter was fed one of the four experimental diets. Soya-bean meal was replaced with BPM on the basis of digestible protein, and the BPM contents in the four diets were 0% (BP0), 5% (BP5), 10% (BP10) and 15% (BP15), corresponding to 0%, 17%, 35% and 52% of the digestible nitrogen (N), respectively. Four balance periods were performed, at the start of which the pigs weighed 9.5 kg, 20.7 kg, 45.3 kg and 77.2 kg, respectively. Once during each balance period, 22-h respiration experiments were performed using indirect calorimetry. Daily weight gain, feed intake and feed conversion rate were the same for all diets. The apparent digestibility of N was lower on diet BP10 than on BP0 ($P = 0.002$), whereas the apparent digestibility of energy was similar on all diets. The retention of nitrogen did not differ between diets and was 1.50, 1.53, 1.33 and 1.46 g N per kg$^{0.75}$ per day on BP0, BP5, BP10 and BP15, respectively. Neither metabolisable energy intake nor heat production were affected by inclusion level of BPM. Retention of energy was 620 (BP0), 696 (BP5), 613 (BP10) and 664 kJ/kg$^{0.75}$ per day (BP15), the differences among diets being non-significant. The N-free respiratory quotient was similar on all diets. It was concluded that the overall protein and energy metabolism in growing pigs were not affected when up to 50% of dietary N was derived from BPM.

Keywords: bacterial protein meal, energy metabolism, pigs, protein metabolism

Introduction

The global demand for high-quality protein feedstuffs for use in animal nutrition is increasing: fish for the production of fish meal is a limited resource, meat and bone meal and other animal by-products are banned in many countries owing to bovine spongiform encephalitis, and important vegetable protein sources are increasingly genetically modified and thus considered unsuitable for diets of food producing animals in many countries. Therefore, alternative protein sources must continuously be evaluated and, if proved suitable, be included in the diets of farm and companion animals. The prerequisites for a protein feedstuff to be considered of high nutritional quality include good palatability, high biological value, harmlessness and being beneficial to product quality. Evaluation studies conducted with fast-growing animals such as pigs and chickens may form a suitable basis for conclusions on the usefulness of such alternative protein feedstuffs.

Bacterial protein meal (BPM) produced by the continuous aerobic fermentation of natural gas (99% methane) as the energy and carbon source and ammonium as the nitrogen source is a new interesting potential protein source (Skrede *et al*., 1998). The bacterial biomass comprises *Methylococcus capsulatus* (Bath; >90%), *Ralstonia* sp., *Brevibacillus agri* and *Aneurinibacillus* sp. After fermentation the biomass is spray dried and heat-treated to obtain a dry and storage-stable reddish/brown product with a dry matter (DM) content of approximately 96%. The crude protein (CP), fat and ash contents are approximately 70%, 10% and 7%, respectively (Skrede *et al*., 1998). Nitrogen (N) from the purine and pyrimidine bases in RNA and DNA makes up approximately 12% of the N in BPM. This level is low compared with that of many other single-cell proteins of bacterial origin (Braude

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et al., 1977; Tiemeyer *et al*., 1981; Rumsey *et al*., 1991; Kiessling and Askbrandt, 1993) but considerably higher than that of fish meal (Greife, 1984), wheat, barley, maize and soya beans (Herbel and Montag, 1987; Imafidon and Sosulski, 1990; Lassek and Montag, 1990).

In pig diets, BPM may be used to replace soya-bean meal (SBM). Compared with that of SBM, the amino acid composition of BPM mainly differs in having a slightly higher content of S-containing amino acids (with a somewhat lower cystine but a higher methionine content). For other essential amino acids, only minor differences between SBM and BPM have been reported (Øverland *et al*., 2001).

Growth trials suggest that BPM may be a promising alternative protein source, but some varying and contradictory results have been observed (Øverland *et al*., 2001 and 2004; Skrede *et al*., 2003; Storebakken *et al*., 2004). Dietary BPM providing up to one-third of the N intake was found to sustain animal performance and health in slaughter chickens (Skrede *et al*., 2003) and blue foxes (Skrede and Ahlstrøm, 2002). When BPM made up approximately 50% of dietary N, no adverse effects were reported for growing-finishing pigs (Øverland *et al*., 2001) or Atlantic salmon (Storebakken *et al*., 2004). In piglets, however, when 40 to 50% of dietary N originated from BPM, reduced performance during the piglet period was noted in some experiments (Øverland *et al*., 2001 and 2004).

A complete feedstuff evaluation cannot be based solely on performance data (Øverland *et al*., 2001 and 2004) but also needs to consider effects on protein and fat deposition. A high-quality protein source must sustain high N retention and not cause elevated heat production (HE). Effects of including BPM in the diet on protein and energy deposition are still limited to studies in chicken and mink, which showed that retained nitrogen (RN) remained unaffected when BPM made up 20 to 60% of digestible N in the mink diet (Hellwing *et al*., 2005) or 6.5 to 20% in the chicken diet (Hellwing *et al*., 2006). The heat production in mink (Hellwing *et al*., 2005) as well as in chickens (Hellwing *et al*., 2006) was the same on the control diet and the diets with BPM.

Previous production experiments with BPM in diets for monogastric species (Øverland *et al*., 2001 and 2004; Skrede and Ahlstrøm, 2002; Skrede *et al*., 2003; Storebakken *et al*., 2004), and N and energy metabolism studies in mink and chickens (Hellwing *et al*., 2005 and 2006), have given somewhat varying and contradictory results. Therefore, the objective of the present study was, by means of balance and respiration experiments, to reveal effects of replacement of SBM by BPM on N and energy metabolism in balanced diets for growing-finishing pigs. Our hypothesis was that protein and energy retention would be negatively affected by an increasing proportion of dietary N from RNA and DNA in the diets with increasing dietary supply of BPM.

Material and methods

Animals and experimental design

The experiment was conducted as a block experiment with four dietary treatments and the same sixteen pigs were measured four times from weaning until 80 kg. Two blocks (A and B) were used and the blocks were measured with one-week in-between. In each block eight recently weaned barrows [(Landrace × Yorkshire) × (Hampshire × Duroc)] from two litters were used. One pig from each litter was allocated to one of the four dietary treatments. At arrival the barrows in block A weighed 8.2 ± 0.6 kg (mean ± s.d.) and the barrows in block B 9.8 ± 0.9 kg. Four spare pigs having the same age as the barrows in block A were fed the control diet.

The experimental diets were fed from day one after arrival. At 9 days after arrival the pigs were placed in metabolism cages and the first 4-day balance period started at 12 days after arrival. Another three balance periods were conducted at 40, 68 and 96 days after arrival. Once during each balance period the barrows were subjected to a 22-h respiration experiment by means of indirect calorimetry in an open-air circulation system (Chwalibog *et al*., 2004).

Diets

The batch of BPM (Bioprotein®, Norferm AS, Stavanger, Norway) used in this experiment was pelleted with inclusion of approximately 1% soya oil after spray-drying in order to minimise dusting. All ingredients were then mixed and the diets pelleted. In order to use diets that were in agreement with the actual nutritional requirement of the pigs, diets designed for weaned piglets were used in periods 1 and 2, and diets designed for growing-finishing pigs were used in periods 3 and 4. The BPM content of the starter and growing finishing diets remained the same, but the N and energy contents were somewhat reduced in the growing-finishing diet in order to comply with the animals' decreasing protein requirements. The starter diet was used from arrival until the end of balance period 2, and the growing-finishing diet was used during the rest of the experiment. One of the experimental diets served as the control diet and contained no BPM (BP0), while the remaining diets contained 5% BPM (BP5), 10% BPM (BP10) and 15% BPM (BP15) (Table 1). The BPM replaced SBM on a digestible protein basis, providing 0/0, 17/17, 33/35 and 49/52% of N in the starter and growing-finishing diets, respectively. The contents of digestible protein were calculated by using digestibility values indicated by Skrede *et al.* (1998) for BPM, and table values for the other feed ingredients (Norwegian Feed Table, 2002). The diets were formulated to meet or exceed the requirements for essential amino acids and all other nutrients established by the National Research Council (NRC, 1998). The diets were produced by the Centre for Feed Technology (Ås, Norway) and pelleted using a 3-mm die on a Münch pellet press (Münch-Edelstahl GmbH, Hilden, Germany). For a more detailed description of the diet formulation, see Øverland *et al.* (2004). All diets were

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Table 1 *Composition and chemical content of the diets used in balance and respiration experiments with pigs*

	Starter diet				Growing–finishing diet			
	BP0	BP5	BP10	BP15	BP0	BP5	BP10	BP15
Barley (g/kg)	226	246	250	270	487	518	541	563
Wheat (g/kg)	480	480	500	497	250	250	250	250
Bacterial protein meal (BPM) (g/kg)	0	52	101	153	0	50	100	150
Soya-bean meal (45% crude protein) (g/kg)	210	140	68	0	224	146	72	0
Soya oil (g/kg)	40	40	40	40	6.0	3.6	5.0	5.0
Limestone (g/kg)	15.8	16.2	16.6	16.9	14.2	14.2	14.2	14.2
Monocalcium phosphate (g/kg)	15.4	14.8	14.4	13.9	11.7	11.7	11.8	11.8
Sodium chloride (g/kg)	4.7	4.6	4.5	4.40	3.8	3.7	3.6	3.5
Iron fumarate (g/kg)	0.33	0.33	0.33	0.33				
Zinc oxide (g/kg)	0.10	0.10	0.10	0.10				
Pre-mix starter diet[†] (g/kg)	3.03	3.03	3.03	3.03				
Pre-mix growing–finishing diet[‡] (g/kg)					1.8	1.8	1.8	1.8
L-lysine HCl (98%) (g/kg)	3.4	2.5	1.8	0.7	0.65	0.64	0.51	0.34
DL-methionine (g/kg)	0.30	0.0	0.0	0.0	0.54	0.32	0.06	0.0
L-threonine (g/kg)	1.0	0.63	0.4	0.0	0.60	0.37	0.09	0.0
L-tryptophan (g/kg)	0.15	0.21	0.3	0.35				
Chemical composition								
Dry matter (g/kg)	919	917	918	916	881	886	892	890
Ash (g/kg)	67.4	59.3	59.6	59.0	55.8	54.9	50.5	54.6
Crude protein (N × 6.25) (g/kg)	198	198	200	206	191	197	189	190
Fat (g/kg)	36.9	40.3	52.1	73.5	27	32	40	43
Gross energy (MJ/kg)	17.5	17.4	17.5	17.8	16.0	16.3	16.7	16.6
Lysine (g/kg)	10.7	10.2	9.8	8.6	9.9	10.0	9.1	8.2
Methionine (g/kg)	2.8	2.9	3.4	3.8	3.0	3.6	3.5	3.7
Cystine (g/kg)	3.1	3.0	2.8	2.5	3.2	2.9	2.6	2.4
Threonine (g/kg)	7.0	6.9	7.1	6.8	7.1	7.4	6.8	6.6
Tryptophan (g/kg)	2.5	2.7	2.9	3.2	2.6	2.9	3.0	3.2
Purine bases								
Adenine (mg/kg)	392	899	1272	1905	446	1027	1345	1743
Guanine (mg/kg)	347	770	1148	1617	370	876	1155	1518
Pyrimidine bases								
Cytosine (mg/kg)	506	939	1328	1794	522	1083	1428	1783
Uracil (mg/kg)	722	1031	1177	1541	790	1267	1508	1732
Thymine (mg/kg)	139	208	265	347	126	240	252	284
N from purine and pyrimidine bases (% of all nitrogen)	2.5	5.0	7.1	9.6	2.7	5.6	7.7	9.7

[†]Vitamins and trace elements included to provide the following per kg of feed: 140 mg Zn; 201 mg Fe; 80 mg Mn; 20 mg Cu; 10 mg I, 0.4 mg Se; 3 300 μg retinol; 34.4 μg cholecalciferol; 137.5 mg D-α-tocopheryl acetate; 6.9 mg riboflavin; 22.9 mg D-pantothenic acid; 27.5 μg cyanocobalamin.
[‡]Vitamins and trace elements included to provide the following amounts per kg of feed: 105 mg Zn; 75 mg Fe; 60 mg Mn; 15 mg Cu; 7.44 mg I; 0.3 mg Se; 2 520 μg retinol, 17.5 μg cholecalciferol; 115.9 mg D-α-tocopheryl acetate; 5 mg riboflavin; 15 mg D-panthothenic acid; 20 mg cyanocobalamin.

analysed for DM, ash, N, fat, energy, amino acids, purine and pyrimidine bases (Table 1).

Housing and feeding

The pigs were housed individually for the duration of the experiment. For the first 9 days after arrival and between balance periods they were housed in pens with concrete floors covered with wood shavings. During the balance periods the pigs were housed in metabolic cages of stainless steel, (1.65 m × 0.75 m with a slatted floor of round stainless steel bars). When kept in the balance cages and the pens, but not in the respiration chambers, the pigs had visual contact. The light schedule was 12 h light and 12 h dark. Between balance periods the pigs were fed twice daily (0830 and 1530 h) but during balance periods they were fed once daily between 1130 and 1200 h. The feed supply was regulated on a daily basis to a level where pigs had a minimum of feed residues, i.e. were fed as close to *ad libitum* as possible. Between balance periods, water was provided *ad libitum* from drinking nipples. During the balance periods, water (twice the weight of the feed) was mixed into the diets. In addition, pigs were given drinking water in a trough. The temperature was kept at 20 to 22 °C throughout the experimental period. All pigs were provided with a rubber mat in the front of the metabolism cages during the first and second balance periods.

Experimental techniques

Pigs were weighed at the start and end of each 4-days balance period. The collection procedures were performed

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between 0800 and 1200 h every day. Feed residues, faeces and urine were quantitatively collected from the individual pigs, weighed and frozen at −18°C. Urine was collected in 30 ml of 5% sulphuric acid in periods 1 and 2 and in 50 ml of 5% sulphuric acid in periods 3 and 4. For 2 days in periods 2 and 4 when protein turn-over was estimated by means of the ^{15}N-glycine end-point technique the addition of acid was omitted. The results from the protein turn-over experiment will be reported elsewhere (Hellwing *et al*., 2007).

The inside surfaces of the metabolic cage, the mat and collection plate were washed with citric acid daily after completion of the collection procedures. After each balance period feed residues, faeces and urine were thawed and mixed to homogeneity. Samples for chemical analyses were taken and frozen at −18°C for later analyses. Furthermore, samples of faeces were freeze-dried for analyses of ash, fat and energy. The citric acid rinse was thawed and centrifuged at 3000 × **g** for 10 min to separate the solid particles (assumed to be faeces residues) from the fluid (assumed mainly to contain N residues derived from urine). The sediment was weighed and freeze-dried. A sample of the supernatant was stored at −18°C.

The day when the pigs were subjected to the respiration experiment they were brought to the respiration unit between 0930 and 1030 h. Each respiration experiment lasted 22 h, starting at 1100 h and ending at 0900 h the following morning. The volume of each respiration chamber was 3500 l and the chambers were constructed for animals with live weights of 5 to 200 kg. For a detailed description of the calibration and measurement procedures, see Chwalibog *et al.* (2004).

Analyses

Samples of diets and freeze-dried faeces were ground and homogenised before analyses. Feed residues were analysed for DM, assuming that the composition of the DM was the same as in the DM of the feed. Wet faeces were analysed for DM and N and freeze-dried faeces for ash, fat and energy. Urine was analysed for N. The supernatant of the citric acid rinse was analysed for N, and the DM content of the sediment was determined by freeze-drying.

DM was determined by evaporation at 105°C to constant weight. Ash was determined by incineration at 525°C. N was determined by the micro-Kjeldahl technique using the Tecator–Kjeltec system 1030 (Tecator AB, Höganäs, Sweden). CP was calculated as N × 6.25. Fat was determined by petroleum ether extraction in a Soxtec system after HCl hydrolysis. Gross energy was determined using an adiabatic bomb calorimeter (IKA Calorimeter system, IKA Gmbh and Co. KG, Staufen, Germany). The amino acids, except tryptophan, in the diets were determined according to the European Community (1998). Tryptophan was analysed according to the Bech-Andersen (1991) procedure. Adenine, guanine, thymine, uracil, and cytosine in the diets were determined by a HPLC method. A diet sample of 0.100 g was mixed with 2 ml of $HClO_4$ and heated for 1 h at 95°C. The sample was chilled and mixed with 5 ml borate buffer and 3 ml 12.4 mmol/l KOH. A sample of 1 ml was taken and centrifuged at 15 000 r.p.m. for 10 min. To the centrifuged sample 4 ml borate buffer was added and micro-filtrated (0.45 μm). The sample was injected into the column and eluents were detected by UV-absorbance at 254 nm. Data were analysed against external standards using the Hewlett-Packard HP ChemStation Software (Møller-Hansen, personal communication).

Calculations

Urinary nitrogen (UN) was calculated as the sum of the N in the urine and the citric acid rinse. Faecal nitrogen (FN) was calculated as the N content of the faeces plus the N content of the sediment from citric acid rinse. It was assumed that the N content of the sediment was the same as that of the faeces. Energy in urine (UE) was calculated as 53.5 kJ/g × UN (Chwalibog *et al*., 2004). RN was calculated as ingested nitrogen (IN) minus UN and FN. Metabolisable energy (ME) was calculated by subtracting FE and UE from gross energy intake. Heat production (HE) was calculated according to Brouwer (1965) as HE, kJ= $16.18\times O_2,L+5.02\times CO_2,L-5.99\times UN,g-2.17\times CH_4,$ L. The production of CH_4 was low and therefore omitted from the equation. Retained energy (RE) was calculated as ME minus HE. The non-protein respiratory quotient (RQ_{np}) was calculated as $RQ_{np}=(CO_2,L-[UN,g\times 6.25\times 0.774])/(O_2,L-[UN,g\times 6.25\times 0.957])$. Furthermore, the maximum protein retention was calculated according to a second order equation as described, and under the same assumptions, as given by Chwalibog *et al.* (1996) and Tauson *et al.* (1998).

Statistical analyses

Statistical analyses of data from the balance and respiration experiments were carried out by means of the MIXED Procedure in Statistical Analysis Systems Institute (SAS) (Littell *et al*., 1996) using the following model:

$$Y_{ijkl} = \mu + \alpha_i + \beta_j + (\alpha\beta)_{ij} + \gamma_k + \varepsilon_{ijkl}$$

where Y_{ijkl} is the Y_{ijkl}th observation, μ is the general mean, α_i is the fixed effect of diet (BP0, BP5, BP10 and BP15), β_j is the fixed effect of balance period (1 to 4), $\alpha\beta_{ij}$ is the interaction between diet and balance period, γ_k is the fixed effect of block (A and B) and ε_{ijkl} is the residual error. Data were analysed as repeated measurements and the heterogeneous autoregressive order 1 (ARH(1)) covariance structure was fitted (Littell *et al*., 1996). Results are presented as least squares means (LSmeans) and the square root of residuals (RR) is given for each variable. Pair-wise comparisons of LSmeans were made using the PDIFF option, and effects were considered significant if $P < 0.05$. One observation from period 2 was omitted because of an injury not related to the dietary treatment. Another three observations (regarding one pig in period 2 and two pigs in period 3) were omitted from the analysis of the respiration data

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because of technical problems. A linear regression analysis of the percent of N derived from BPM and apparent digestibility of N was performed using PROC REG in SAS (1990).

Results

From period 2 and onwards all pigs performed well. After arrival, piglets in block A suffered from diarrhoea and pneumonia. The sick piglets were treated with antibiotics and no health problems were observed during the first balance period. During the interval between the first and the second periods, one pig in block A and one spare pig died and the post mortem examination showed that death was caused by oedema disease. Another pig with clinical signs of the disease was treated with antibiotics and the remaining pigs were given a prophylactic vaccination (Danish Institute for Food and Veterinary Research, Copenhagen, Denmark). The dead pig from block A was replaced with a spare pig. All results regarding intake of nutrients, amino acids, protein and energy metabolism are presented in relation to metabolic body size ($kg^{0.75}$).

Intake of nutrients, digestibility and performance

Effect of diet. Intake of DM did not differ between diets ($P = 0.38$) and the intake was measured to 93 g/$kg^{0.75}$ on diets BP0 and BP10 and 97 g/$kg^{0.75}$ on BP5 and BP15. The intake of fat increased significantly ($P < 0.001$) with increasing dietary BPM (Table 2), because the content of fat in both the starter and growing-finishing diets increased with increasing BPM content (Table 1). The intake of lysine was significantly higher ($P < 0.001$) on diets BP0 and BP5 than on BP10 and BP15. The intake of methionine plus cysteine ($P = 0.07$) was the same on all diets.

The apparent digestibility of N was lower ($P < 0.01$) on diets BP10 and BP15 than on BP0. The apparent digestibility of fat increased significantly ($P < 0.001$) with increasing dietary BPM, whereas the apparent digestibility of energy ($P = 0.11$) was unaffected by diet (Table 2).

The daily gain during the balance periods was similar among groups, however numerically lowest on BP10 (688 g) and highest on BP0 (852 g). The feed conversion rate ($P = 0.12$) did not differ between diets but there was a slight decrease with increasing dietary BPM (Table 2).

Effect of period. Intake of DM, CP, fat, carbohydrate and gross energy (all $P < 0.001$) increased from period 1 to period 3, but in period 4 the intake of nutrients was intermediate to the levels in periods 1 and 2 (Table 2).

The apparent digestibility of N was the same in periods 1 through 3, whereas it was significantly higher in period 4. The apparent digestibility of fat was highest in period 2 and lowest in period 1. The apparent digestibility of energy was lowest in period 3.

Table 2 *Intake of nutrients and amino acids, digestibility of nutrients and performance in pigs fed increasing levels of bacterial protein meal (BPM) from weaning to a weight of approximately 80 kg*

	Diet				Period					Significance			
No. of pigs	BP0 15	BP5 16	BP10 16	BP15 16	1 16	2 15	3 16	4 16	RR†	Diet (D)	Period (P)	D × P	Block
Intake of nutrients													
Dry matter (g/$kg^{0.75}$ per day)	93	97	93	97	67^{D}	107^{B}	118^{A}	88^{C}	0.95		***		***
Protein (N × 6.25) (g/$kg^{0.75}$ per day)	20	21	20	21	15^{D}	24^{B}	25^{A}	19^{C}	0.87		***	‡	***
Fat (g/$kg^{0.75}$ per day)	3.2^{d}	3.9^{c}	4.7^{b}	6.1^{a}	3.7^{C}	6.0^{B}	4.7^{A}	3.5^{C}	0.15	***	***	***	***
Carbohydrate (g/$kg^{0.75}$ per day)	63	66	62	64	44^{D}	71^{B}	80^{A}	60^{C}	0.95		***		***
Gross energy (MJ/$kg^{0.75}$ per day)	1.72	1.81	1.75	1.84	1.28^{D}	2.06^{B}	2.17^{A}	1.62^{C}	0.07		***		***
Lysine (g/$kg^{0.75}$ per day)	1.1^{a}	1.1^{a}	1.0^{b}	0.9^{b}	0.7^{C}	1.2^{A}	1.2^{A}	0.9^{B}	0.04	***	***	‡	***
Methionine plus cysteine (g/$kg^{0.75}$ per day)	0.6	0.7	0.6	0.7	0.5^{D}	0.7^{B}	0.8^{A}	0.6^{C}	0.04	‡	***	‡	***
Threonine (g/$kg^{0.75}$ per day)	0.7	0.8	0.7	0.7	0.5^{D}	0.8^{B}	0.9^{A}	0.7^{C}	0.02	‡	***	*	***
Tryptophan (g/$kg^{0.75}$ per day)	0.27^{c}	0.31^{b}	0.31^{b}	0.35^{a}	0.21^{D}	0.35^{B}	0.39^{A}	0.29^{C}	0.01	***	***	‡	***
Apparent digestibility													
Nitrogen	0.78^{a}	0.77^{ab}	0.75^{c}	0.76^{bc}	0.76^{B}	0.75^{B}	0.75^{B}	0.79^{A}	0.02	**	***		
Fat	0.66^{c}	0.74^{b}	0.76^{b}	0.81^{a}	0.71^{C}	0.77^{A}	0.74^{BC}	0.75^{B}	0.001	***	***	‡	
Energy	0.82	0.82	0.81	0.81	0.82^{A}	0.82^{A}	0.80^{B}	0.82^{A}	0.01		***		
Animal performance													
Live weight (kg)	38.1	40.2	40.4	39.7	10.1^{D}	21.7^{C}	47.5^{B}	79.1^{A}	0.43		***		***
Daily gain (g/day)	852	806	688	772	322^{C}	596^{B}	$1\,150^{A}$	$1\,050^{A}$	1.00		***		**
Feed conversion rate (kg feed/kg gain)	1.8	1.9	1.9	2.1	1.4^{B}	2.0^{A}	2.0^{A}	2.1^{A}	0.25		***	*	

[a,b,c] Values with different superscripts differ significantly, effect of diet ($P < 0.05$).
[A,B,C,D] Values with different superscripts differ significantly, effect of period ($P < 0.05$).
† Residual error.
‡ Approaching significance ($P < 0.10$).

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The pigs weighed approximately 10, 22, 48 and 79 kg at the midpoint of each balance period. The body weight gain was lowest in the first period and then increased significantly until balance period 3 ($P < 0.001$), after which the level remained the same in period 4. The feed conversion was most efficient ($P < 0.001$) in the first period and then declined to a constant level for the rest of the experiment (Table 2).

Interaction between diet and period. There was a significant interaction effect between diet and period for the intake of fat ($P < 0.001$). For all diets the intake of fat increased with increasing dietary content of BPM but feed intake only increased from period 1 to 2 and then declined (Figure 1a).

Nitrogen metabolism

Effect of diet. Because of the lower DM intake, IN was slightly lower (3.2 g/kg$^{0.75}$) on diets BP0 and BP10 than on BP5 and BP15 (3.4 g/kg$^{0.75}$) ($P = 0.15$). The intake of nucleic acid nitrogen (NAN) increased with increasing dietary BPM. Due to the lower IN and apparent digestibility of N, the amount of digested nitrogen (DN) was significantly lower on BP10 than on BP5. The faecal excretion of N increased slightly ($P = 0.12$) with increasing dietary BPM. The excretion of N in urine ranged from 0.99 (BP0) to 1.06 g/kg$^{0.75}$ (BP5 and BP15) ($P = 0.44$). There was a tendency ($P = 0.08$) for diet effect on RN, which was lowest on BP10 (1.33 g/kg$^{0.75}$) and highest on BP5 (1.53 g/kg$^{0.75}$). The utilisation of digested N for retention (RN/DN) ranged from 60% and 55% ($P = 0.15$). The partitioning of excreted N between faeces and urine was similar on all diets ($P = 0.90$) (Table 3).

Effect of period. Both IN and DN increased significantly until period 3, after which they decreased significantly in period 4. UN excretion was higher ($P < 0.001$) in periods 3 and 4 than in periods 1 and 2. N retention was higher ($P < 0.001$) in periods 2 and 3 than in periods 1 and 4. Utilisation of DN for retention (RN/DN) was most efficient in period 2 (64%), after which it declined significantly to below 50% in period 4 ($P < 0.001$). This decline was reflected in the partitioning of N excretion between faeces and urine ($P < 0.001$), the latter increasing continuously from period 2 to period 4 (Table 3).

Interaction between diet and period. Interaction effects were observed for the utilisation of DN for retention (RN/DN), excretion of UN and the total excretion of N ($P < 0.001$) (Figure 1b to d). The utilisation of DN for retention was lower on BP10 and BP15 than on BP0 and BP5 in periods and 1 and 2 whereas it was the same or better in periods 3 and 4. The excretion of UN increased

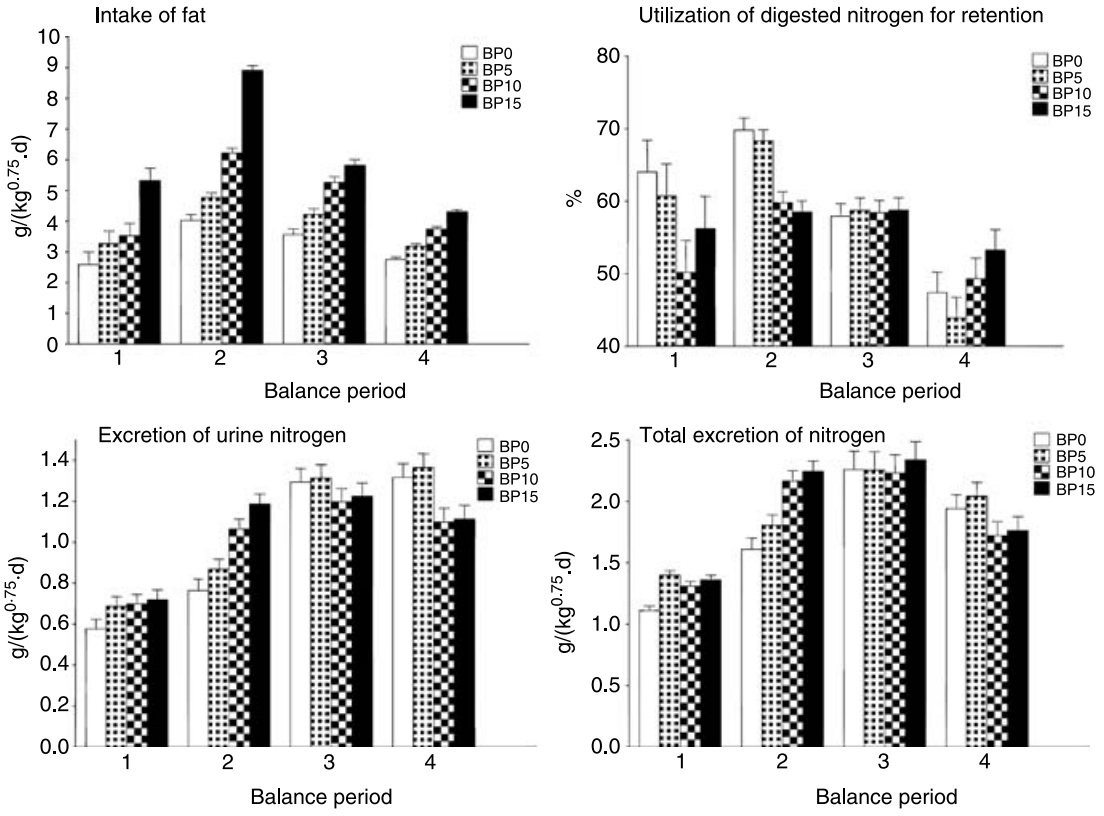

Figure 1 (a) Intake of fat, (b) utilisation of digested nitrogen for retention, (c) excretion of urinary nitrogen, and (d) total excretion of nitrogen for each balance period and diet for pigs fed increasing levels of dietary bacterial protein meal. The pigs weighed about 10.1, 21.7, 47.5, and 79.1 kg in the first, second, third, and fourth balance periods, respectively.

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Table 3 *Protein metabolism in pigs fed increasing levels of bacterial protein meal (BPM) from weaning to a weight of approximately 80 kg*[†]

	Diet				Period					Significance			
	BP0	BP5	BP10	BP15	1	2	3	4					
No. of pigs	15	16	16	16	16	15	16	16	RR	Diet (D)	Period (P)	D × P	Block
IN (g/kg$^{0.75}$ per day)	3.21	3.40	3.18	3.38	2.33^{D}	3.76^{B}	4.07^{A}	3.03^{C}	0.14		***	‡	***
IN−NAN (g/kg$^{0.75}$ per day)	3.13	3.22	2.95	3.06	2.19^{D}	3.52^{B}	3.80^{A}	2.84^{C}	0.13	‡	***	‡	***
DN (g/kg$^{0.75}$ per day)	2.51^{ab}	2.62^{a}	2.37^{b}	2.55^{ab}	1.78^{D}	2.82^{B}	3.07^{A}	2.39^{C}	0.13	*	***		***
FN (g/kg$^{0.75}$ per day)	0.73	0.82	0.84	0.86	0.62^{B}	0.98^{A}	1.01^{A}	0.64^{B}	0.002		***		
UN (g/kg$^{0.75}$ per day)	0.99	1.06	1.01	1.06	0.67^{C}	0.97^{B}	1.26^{A}	1.22^{A}	0.09		***	***	
RN (g/kg$^{0.75}$ per day)	1.50	1.53	1.33	1.46	1.04^{B}	1.81^{A}	1.80^{A}	1.16^{B}	0.13	‡	***		***
RN/DN (%)	59.8	58.0	54.4	56.7	57.8^{B}	64.1^{A}	58.5^{B}	48.5^{C}	0.94		***	***	**
N excretion	1.73	1.87	1.86	1.92	1.29^{C}	1.95^{B}	2.27^{A}	1.87^{B}	0.08		***	***	***
– in faeces (%)	43	44	45	45	48^{AB}	50^{A}	44^{B}	35^{C}	0.87		***	‡	**
– in urine (%)	57	56	55	55	52^{BC}	50^{C}	56^{B}	65^{A}	0.87		***	‡	**

[a,b] Values with different superscripts differ significantly, effect of diet ($P < 0.05$).
[A,B,C,D] Values with different superscripts differ significantly, effect of period ($P < 0.05$).
[†]Abbreviations are: IN = ingested nitrogen, IN−NAN = ingested nitrogen minus nucleic acid nitrogen, DN = apparently digested nitrogen, UN = urinary nitrogen, FN = faecal nitrogen, RN = retained nitrogen, RR = residual error.
[‡]Approaching significance ($P < 0.10$).

with increasing dietary level of BPM in periods 1 and 2 whereas it was lower on BP10 and BP15 than on BP0 and BP5 in periods 3 and 4. The ranking of the different diets for the excretion of total N differed in the different periods and no clear pattern could be discerned.

Energy metabolism

Effect of diet. Intake of ME ($P = 0.22$), HE ($P = 0.29$) and RE ($P = 0.25$) were not affected by diet. Heat production was highest on BP15 but did not differ significantly from the levels on the other diets. RE was not significantly affected by diet. Partitioning of RE ($P = 0.07$) between protein and fat ($P = 0.33$) was not affected by diet. The non-protein respiration quotient was not affected by diet ($P = 0.16$).

Effect of period. The intake of ME was higher ($P < 0.001$) in periods 2 and 3 than in periods 1 and 4. This pattern was partly reflected in HE, although the lowest HE values were recorded in period 4. Consequently, RE was low in period 1, increased by almost 0.5 MJ/kg$^{0.75}$ in period 2 and remained at this level in period 3. HE and RE values in the two last periods were probably somewhat affected by the decline in ME intake observed on the days of the respiration experiments (4 g DM per kg$^{0.75}$ in period 3 and 27 g DM per kg$^{0.75}$ in period 4), resulting in RE values in period 4 being lower than expected from the measured body weight gain. The non-protein respiratory coefficient was lower in period 4 than in period 3 (Table 4).

Discussion

When performing an experiment from shortly after weaning until slaughter weight of the pigs, the problem of changing nutrient requirements over the experimental period complicates the diet formulation: if the same diet is used throughout the experiment pigs are inevitably either underfed or overfed during some part of the experiment. On the other hand, if two diets are used, pigs are fed closer to their requirements, but there might be some confounding between diets and periods. We chose the latter solution, because we wanted to feed the pigs as close to their requirements as possible and furthermore, this experiment was run in parallel to a production trial in which pigs were followed from weaning until a live weight of 105 to 110 kg (Øverland *et al*., 2004).

Intake of nutrients, performance and digestibility

The diets were generally readily consumed and the animals performed well even on the diet with highest inclusion level, where BPM supplied approximately 50% of dietary N. Although our performance data, because of the small number of animals per treatment, only have indicative value, our results concur with those of Øverland *et al.* (2004): using the same diets as in the present study, they found no differences in feed intake but a small reduction in average daily gain on the diet containing the most BPM.

The amino acid composition differed somewhat between diets, the control diet (BP0) having an almost ideal pattern of essential amino acids (NRC, 1998), whereas increasing BPM inclusion resulted in gradually lower lysine content and higher methionine and tryptophan contents in the diet. The lysine contents of all starter diets, declining from 10.7 g/kg feed on BP0 to 8.6 g/kg feed on BP15, were lower than recommended by NRC (1998) for pigs weighing 10 to 20 kg. Thus the differences in lysine content may have caused low RN in the two first periods, the level decreasing with increasing dietary content of BPM. All the growing-finishing diets contained sufficient amounts of lysine, according to the NRC (1998) recommendations.

The apparent digestibility of N in BPM at the terminal ileum and of the total digestive tract of pigs has been estimated to be 0.78 and 0.85, respectively, using BPM as the sole source of protein (Skrede *et al*., 1998). Regression

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Table 4 *Energy metabolism in pigs fed increasing levels of bacterial protein meal (BPM) from weaning to a weight of approximately 80 kg*[†]

	Diet				Period					Significance			
	BP0	BP5	BP10	BP15	1	2	3	4					
No. of pigs	14	16	15	15	16	14	14	16	RR	Diet (D)	Period (P)	D × P	Block
ME (kJ/kg$^{0.75}$ per day)	1 362	1 426	1 360	1 442	1 021^{C}	1 637^{B}	1 677^{B}	1 255^{C}	0.89		***		***
HE (kJ/kg$^{0.75}$ per day)	741	730	749	777	678^{C}	821^{B}	869^{A}	629^{D}	0.98		***		***
RE (kJ/kg$^{0.75}$ per day)	620	696	613	664	340^{C}	819^{A}	808^{A}	625^{B}	0.85		***		**
RPE (kJ/kg$^{0.75}$ per day)	220	226	195	217	154^{B}	265^{A}	267^{A}	172^{B}	0.97	‡	***		***
RFE (kJ/kg$^{0.75}$ per day)	400	470	419	446	185^{C}	554^{A}	543^{A}	454^{B}	0.90		***		
RQ_{NP}	1.07	1.06	1.04	1.05	0.97^{C}	1.08^{B}	1.12^{A}	1.05^{B}	0.04		***		

^{A,B,C,D} Values with different superscripts differ significantly, effect of period ($P < 0.05$).
[†]Abbreviations are: ME = metabolizable energy, HE = heat production, RE = retained energy, RPE = energy retained in protein, RFE = energy retained in fat, RQ_{np} = N-free respiratory quotient, RR = residual error.
[‡]Approaching significance ($P < 0.10$).

analysis of our data indicated a lower digestibility, although the equation had a low R^2. However, the apparent digestibility of N of the control diet based on SBM was also low, and the effects of replacing SBM with BPM were minor.

The explanation for the slightly lower apparent N digestibility with increasing inclusion of BPM in the diet may be that the cell wall of the bacteria either had a low digestibility, or even was indigestible. However, Soeder (1977) stated that a completely indigestible cell wall would only cause a minor decline in N digestibility. The explanation as to why the present study found that BPM consumption resulted in slightly lower apparent digestibility of N than those resulting from SBM consumption may be that *M. capsulatus*, in addition to the cell walls, contains a complex system of poorly digestible internal membranes. This is in accordance with a higher N digestibility in a membrane-reduced extract of autolysed BPM than the crude autolysed BPM (Schøyen *et al*., 2005).

The increasing apparent digestibility of fat with increasing dietary levels of BPM found in our study was likely due to the increasing fat content of the diets, and hence reduced contribution to faecal fat from endogenous losses (Jørgensen *et al*., 1992 and 1993). An estimate of the true fat digestibility, assuming an endogenous loss of 4.4 g/kg DM (Jørgensen *et al*., 1993), indicated a higher true fat digestibility in BPM than in the fat sources of the control diet.

Protein metabolism

Our diets were designed to be iso-nitrogenous, and protein metabolism traits were generally found to be independent of the inclusion level of BPM in the diet. The exception was the low apparent digestibility of N and DN on diet BP10, which was at least partly caused by a low DM intake. Despite less protein being available for retention and the lysine content in the diets being slightly below the NRC (1998) recommendations, the RN on BP15 was only slightly and non-significantly lower than the highest value, which was found on diet BP5. This result concurs with our findings in mink, where RN was unaffected by dietary BPM level (Hellwing *et al*., 2005). In slaughter chickens, Hellwing *et al.* (2006) also found similar RN levels for all BPM-containing diets.

The utilisation of DN for N retention was numerically higher on diet BP0 than on BP10, but the N derived from nucleic acids increased from 2.5% on BP0 to approximately 10% on BP15 and this fraction cannot be directly used for protein synthesis. The fate of nucleic acid N is important in relation to protein metabolism. Pig experiments have shown that up to 40% of adenine, 15% of guanine and 20% of the pyrimidine bases in the diet were retained in the body (Greife and Molnar, 1984a and b). From studies by Roth and Kirchgessner (1978) and Greife and Molnar (1984a) it has been indicated that NH_3 from the decomposition of purine and pyrimidine bases can be used for synthesis of non-essential amino acids and/or incorporated directly in the body, which might be an explanation for the unchanged RN in BPM diets compared with the control diet. Furthermore, it was shown that the excretion of purine base derivatives in urine in pigs on BP5, BP10 and BP15 were lower than the intake (Hellwing *et al*., 2007). Collectively, our data did not support the hypothesis that increasing dietary supply of BPM would have a negative effect on protein retention.

The total retention of protein (g per pig and day) in different balance periods was in good agreement with results obtained by Whittemore *et al.* (1988), although the highest retention we found was in balance period 3 at an average pig live weight of 48 kg, while Whittemore *et al.* (1988) recorded maximum protein retention at 75 kg live weight. To achieve maximum protein retention the supply of both digestible protein and ME must be sufficient. The criteria for this (DN > 1.9 g/kg$^{0.75}$.per day, ME > 1100 kJ/kg$^{0.75}$.per day; Chwalibog *et al*., 1996; Tauson *et al*., 1998) were fulfilled in this study. The pattern of protein retention can be modelled according to a second order function. Data pertaining to pigs of mixed sexes from 2 to 120 kg (Chwalibog *et al*., 1996) or intact boars (Tauson *et al*., 1998) gave peak values of 180 g/day at 98 kg and 227 g/day at 135 kg, respectively. Using a similar approach with the present material gave a peak value of 210 g/day

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at 62 kg, but the equation had a significant negative intercept, so the result has only indicative value. Considering the effects of sex, our estimated maximum protein retention level seems reasonable, even though it was achieved at a lower than expected live weight.

Energy metabolism

Increasing inclusion of BPM in the diet did not affect HE in growing pigs, which is in agreement with the results of previous studies on mink (Hellwing *et al.*, 2005) and slaughter chickens (Hellwing *et al.*, 2006). This means that even if assuming 100% digestion of the purine and pyrimidine bases, the energy cost for the excretion of their metabolites would be small in relation to other metabolic processes. Therefore, the hypothesis that increasing dietary supply of BPM would have a negative impact on energy retention, was not supported by our results.

Until period 3, HE increased linearly in relation to the metabolic body size of the pigs, as also shown by Thorbek (1975). In period 4, however, the lowest HE values were recorded, and RQ_{np} was found to be lower than in periods 2 and 3, which was caused by a lower feed intake during the respiration experiments. During the last period the pigs were observed to spend most of the time sleeping. The feed intake during the complete balance period did not differ between diets, so comparisons between diets are still valid, whereas comparisons to other balance periods should be made with caution.

Conclusion

The present data suggest that inclusion of BPM up to 50% of dietary N in the diets of growing pigs does not affect protein and energy metabolism. With increasing dietary levels of BPM, the amount of ingested amino acid N decreased, but the animals were still able to maintain protein retention at the same level. This indicated a slightly more efficient utilisation of dietary amino acid N. The hypothesis that increased intake of N from RNA and DNA with increasing dietary levels of BPM would result in decreased retention of protein and energy was therefore rejected.

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