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Effect of dietary crude protein and energy content on nitrogen utilisation, water intake and urinary output in growing pigs

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A digestibility and balance trial was carried out with four intact castrated male pigs (live weight 33–82 kg) to study the effects of dietary crude protein and energy content on nutrient digestibility, nitrogen metabolism, water intake and urinary output. In a 4 x 4 Latin square design, four barley-oats-soya bean meal based diets were arranged 2 x 2 factorially. The corresponding factors were dietary crude protein (CP) content: high (180 g/kg CP) or low protein diet (140 g/kg CP) supplemented with free lysine, methionine and threonine; and dietary net energy content: high (1.05 feed units (FU)/kg) (feed unit=9.3 MJ net energy) or low net energy content (0.95 FU/kg).

Lowering dietary CP content (mean values of 189 to 152 g/kg dry matter, respectively) by supplementation of free amino acids decreased urinary nitrogen (N) excretion by 6.9 g/day (32%) ($P<0.01$) with no effect on faecal N excretion. Dietary protein reduction also increased the proportion of N retained per unit intake ($P<0.10$) and absorption ($P<0.05$). Daily N retention of pigs tended to decline with reduced dietary CP content ($P<0.10$). Dietary energy intake had no effect on N metabolism. Water intake and urinary output of pigs was unaffected by variations in protein and energy content which may be due to limitations in change-over experimental designs. The present experiment clearly demonstrated benefical effects of lowering dietary protein supply by free amino acid supplementation on N excretion.

Key words: amino acids, digestibility, lysine, methionine, nitrogen excretion, protein, threonine

Introduction

In pig production environmental problems related to nitrogen (N) excretion in animal manure have altered feeding strategies. Formerly, feeding strategies aimed at maximising production performance without special concern for protein and amino acid supply. Environmental constraints have directed pig feeding towards low-

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est possible levels of nitrogen output by adjustment of protein and amino acid supply relative to requirements (Henry and Dourmad 1993). Availability of free amino acids, lysine, methionine, threonine and tryptophan, for practical feed formulation has given new opportunities to match amino acid and protein supply closer to requirements based on the ideal protein concept.

Feeding of pigs with free amino acid supplements to low protein diets based on barley (Näsi 1985, Gatel and Grosjean 1992) or corn (Pierce et al. 1994, Kerr and Easter 1995) have clearly decreased N excretion in urine. In many experiments, however, daily N retention of pigs has simultaneusly been depressed indicating a deficiency of certain amino acids or total protein supply (Näsi 1985, Lee et al. 1993, Pierce et al. 1994, Kerr and Easter 1995). Lowering of protein supply of pigs has also decreased water intake and urine production (Wahlstrom et al. 1970, Close et al. 1983) and can therereby affect slurry output (Kay and Lee 1997). N retention is related to energy intake if an adequate amount of protein is available (Campbell et al. 1985, Dunkin et al. 1986) and can threrefore influence N excretion of pigs. However, quantitative effects of varying both energy and protein supply on N excretion and water intake of pigs have not been assessed in balance studies.

The aim of this study was to investigate the effects of dietary crude protein (CP) and energy content on nutrient digestibility and N metabolism of growing pigs. In addition, the effects of dietary treatments on water intake and urinary output were studied. Dietary CP was reduced by supplementation of free lysine, threonine and methionine in order to maintain an equal dietary content of ileal digestible amino acids.

Material and methods

The study was conducted on four castrated male pigs (Landrace × Finnish Yorkshire) between an average weight range of 33.5 (SE 1.03) and 82.0 (SE 0.91) kg. Experimental design was a 4 x 4 latin square, where four experimental diets were arranged 2 x 2 factorially. The corresponding factors were dietary CP content: high protein diet (180 g/kg CP) without amino acid supplementation or low protein diet (140 g/kg CP) supplemented with free lysine, methionine and threonine; and dietary net energy content: high (1.05 feed units (FU)/kg) (feed unit = 9.3 MJ net energy) or low net energy content (0.95 FU/kg). Animals were kept individually in pens of 1.43 m x 1.23 m with a slatted plastic floor throughout the study.

The four 10-day experimental periods comprised of preliminary feeding for 5 days followed by 5 days of total faeces and urine collection. Faeces was collected using plastic bags attached around the anus with glued adhesive tape and snap-fasteners (Van Kleef et al. 1994). Bags were replaced after the pigs had defecated. Collected faeces was weighed and stored at –20°C until the end of experimental period. Urine was quantitatively collected via collection trays installed under the pens into 40 ml of 10 N H_2SO_4, subsampled and frozen at –20°C.

Four experimental diets consisted of barley, oats, soya bean meal and wheat syrup (Table 1). Diets were supplemented with minerals and vitamins to meet the requirements of growing pigs (Tuori et al. 1995). Ileal digestible amino acid balance (lysine, threonine and sulphur containing methionine and cystine) of experimental diets was calculated according to suggested ideal protein balance of Lenis (1996). Mean calculated dietary contents of ileal digestible lysine, threonine and methionine and cystine were 8.1, 5.1 and 4.8 g/kg, respectively. CP content of diets 1 and 3 was reduced by substitution of soya bean meal with oats, while dietary content of ileal digestible lysine, threonine and sulphur-containing amino acids was maintained by supplements of L-lysine-HCl, DL-methionine and L-threonine-premix. Net energy content of diets 3 and 4 was increased by the inclusion of animal fat. All diets were expanded and pelleted. Net energy content and feed unit values of experimental diets were calculated with the Finnish energy

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Table 1. Dietary ingredients (g/kg) and chemical composition (g/kg DM).

	Diet			
	1	2	3	4
Dietary ingredients				
Barley	657.4	631.2	630.3	603.2
Oats	170.0	74.0	144.0	50.0
Soya bean meal	110.0	242.0	123.0	254.0
Animal fat	–	–	40.0	40.0
Wheat syrup	20.0	20.0	20.0	20.0
Limestone	12.0	12.5	12.0	12.5
Monocalcium phosphate	12.5	10.0	13.0	10.0
Natrium cloride	4.0	4.0	4.0	4.0
Vitamins and trace elements[1, 2]	6.3	6.3	6.3	6.3
L-lysine-HCl	3.9	–	3.6	–
DL-Methionine	1.1	–	1.1	–
L-Threonine-premix (50% L-threonine)	2.8	–	2.7	–
Chemical composition				
Feed units (calculated) FU/kg	0.95	0.95	1.05	1.05
Dry matter, g/kg	898.2	895.7	902.2	905.1
Organic matter	948.1	952.1	952.6	951.8
Ash	51.9	47.9	47.4	48.2
Crude protein	151.7	186.7	153.2	190.7
Ether extract	39.2	36.7	74.7	73.8
Crude fibre	53.1	49.3	54.9	47.5
N-free extracts	704.1	679.4	669.8	639.8
Amino acids				
Lysine	8.9	9.6	9.1	9.4
Threonine	6.1	6.9	6.2	7.1
Methionine	2.9	2.6	2.8	2.8
Cystine	3.0	3.4	3.0	3.4
Alanine	6.3	7.7	6.3	7.8
Arginine	8.8	11.1	8.5	11.2
Aspargic acid	12.4	16.7	12.4	17.3
Glutamic acid	30.8	37.6	30.3	37.4
Glycine	6.1	7.4	6.1	7.5
Histidine	3.4	4.2	3.5	5.2
Isoleucine	5.3	6.8	5.2	7.0
Leucine	10.3	12.4	10.0	12.8
Phenylalanine	7.1	8.3	6.8	8.7
Serine	6.8	8.5	6.7	8.8
Tyrosine	3.9	4.6	3.5	4.9
Valine	6.6	7.9	6.3	8.0

[1] Vitamins and trace elements provided (per kg diet): vitamin A, 5000 IU; vitamin D, 1000 IU; vitamin E, 47 mg; vitamin K, 0.3 mg; vitamin B_1, 1 mg; vitamin B_2, 3.5 mg; vitamin B_6, 3.5 mg; vitamin B_{12}, 0.018 mg; biotin, 0.12 mg; calcium pantothenate, 12 mg; folic acid, 1.2 mg; nicotinic acid, 18 mg; choline cloride, 125 mg; Mn, 40 mg; Fe, 90 mg; Zn, 160 mg; Cu, 105 mg; Co, 2.4 mg; J, 0.6 mg; Se, 0.16 mg.

[2] Vitamin and trace element mixture contains (g/kg): barley 740.1, iron sulphate 71.4, copper sulphate 69.4, zinc oxide 39.7, potassium iodine 0.1, sodium selenite 0.1 and vitamin products 78.4.

evaluation system for pigs using constant digestibility coefficients for feed ingredients (Tuori et al. 1995).

Pigs were fed twice daily, at 0600 and 1600. Daily feed intake was maintained at 93 g/kg $W^{0.75}$ during the experiment and was adjusted for each period according to the increase in body weight (from 1512 to 2382 g DM/day). Water was added to the diets prior to feeding in a ratio of 2:1. Between meals water was offered *ad libitum* from troughs. Water intake was measured during the collection period.

Feeds and faeces were freeze-dried and ground through a 1-mm mesh before analysis. Proximate analysis of feeds and faeces was performed using standard methods (AOAC 1984). Ether extract was determined after acid hydrolysis with 4 N HCl. N content of urine was analysed by Kjeldahl method and that of freeze-dried feeds and faeces by the method of Sweeney (1989) on a Leco FP 428 nitrogen analyser. Amino acid content of diets was determined with a Beckman 6300 amino acid analyser after a 23 h hydrolysis with 6.0 N HCl at 110°C. Methionine and cystine were oxidated with performic acid to methionine sulphone and cysteic acid prior hydrolysis. All analyses were performed in duplicate.

Apparent total tract digestibility of diets was calculated by total collection method. Data were subjected to analysis of variance by the GLM procedure of SAS (1985) using the following model:

$$y_{ijkl} = \mu + p_i + a_j + d_k + e_{ijkl}$$

where y_{ijkl} is the dependent variable; μ is the overall mean; p_i is period effect; a_j is animal effect; d_k is diet effect; and e_{ijkl} is a normally distributed residual error. Three orthogonal contrasts were formed to test the following effects: high CP vs. low CP diets (diets 1 and 3 vs. diets 2 and 4); high energy vs. low energy diets (diets 1 and 2 vs. diets 3 and 4); and interaction between CP and energy level (diets 1 and 4 vs. diets 2 and 3).

Results and discussion

Chemical composition

Analysed composition of the experimental diets was in close agreement with calculated values. CP contents of the low protein (Diets 1 and 3) and high protein diets (Diets 2 and 4) were very close to each other (Table 1). The mean CP content of high protein diets was 37 g/kg DM higher than that of low protein diets. Similarly, the analysed contents of lysine, threonine, methionine and cystine were maintained at similar levels in all diets. The contents of lysine and threonine were slightly higher for high than low protein diets, because lower ileal amino acid digestibilities were used for dietary ingredients than for free amino acids during diet formulation. Due to animal fat inclusion dietary ether extract content was 36 g/kg DM higher in high than low energy diets.

Digestibility

Pigs completed the experiment successfully with an average daily weight gain of 1121 g/day. Generally, pigs ate their diets readily except minor diet refusals of two pigs during the last two periods, which resulted in slightly higher average feed intake of the pigs on low protein diets ($P<0.01$). Apparent total tract nutrient digestibility was higher in high (1 and 3) than low protein diets (2 and 4) (Table 2) being significant for the digestibility of dry matter ($P<0.05$), organic matter ($P<0.05$), ash ($P<0.10$) and CP ($P<0.05$). Apparent total tract digestibility of ether extract was higher in high than low energy diets ($P<0.001$). These differences in digestibility of nutrients can be explained by variations in diet composition and nutrient intake. Slightly lower dry matter and organic matter digestibility of low protein diets mainly resulted from increased amounts of oat inclusion. In the earlier

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Table 2. Total tract nutrient digestibility, nitrogen metabolism, water intake and urinary output of pigs fed diets containing different amounts of crude protein and feed units.

Diet	1	2	3	4	SEM	Contrasts		
Crude protein, g/kg	140	180	140	180		High vs. low CP	High vs. low energy	CP/energy interaction
Feed unit, FU/kg	0.95	0.95	1.05	1.05				
n	4	4	4	4				
Dry matter	81.9	84.2	82.5	85.0	0.68	*	ns	ns
Organic matter	83.6	85.9	84.2	86.6	0.66	*	ns	ns
Ash	53.3	54.8	51.6	55.3	1.31	o	ns	ns
Crude protein	83.7	85.8	83.1	85.9	0.75	*	ns	ns
Ether extract	68.4	67.4	78.5	78.6	1.17	ns	***	ns
N intake, g/day	54.0	64.9	54.8	65.6	0.58	***	ns	ns
N in faeces, g/day	8.9	9.2	9.0	9.2	0.54	ns	ns	ns
N in urine, g/day	14.1	21.5	15.7	22.2	1.47	**	ns	ns
Total N excretion, g/day	22.9	30.7	24.7	31.3	1.65	**	ns	ns
N retained, g/day	31.1	34.2	30.0	34.3	1.59	o	ns	ns
% of intake	58.7	52.7	56.4	51.6	2.33	o	ns	ns
% of absorbed	70.0	61.4	67.7	60.1	2.49	*	ns	ns
Feed intake, g DM/day	2000	1946	2016	1946	17.3	*	ns	ns
Daily weight gain, g/day	1067	1065	1247	1106	49.9	ns	o	ns
Water intake, g/day	11700	11350	11600	11951	1026.3	ns	ns	ns
Urinary output, g/day	8171	7554	8174	8532	1016.4	ns	ns	ns

Significance: ns=non significant, o = P<0.10, * = P<0.05, ** = P<0.01 and *** = P<0.001. SEM = standard error of the means.

studies with pigs and rats, from 11 to 12%-units lower apparent total tract organic matter and energy digestibilities have been obtained in oats than in barley (Homb et al. 1988, Eggum et al. 1992). The higher fibre content of oats than of barley is the main reason for the lower digestibility. Enhancement in apparent ether extract digestibility was caused by increased fat intake of pigs on high energy diets which has been well demonstrated in earlier experiments (Jørgensen et al. 1993, Powles et al. 1994, Jørgensen et al. 1996, Valaja and Siljander-Rasi 1997). Similarly higher apparent CP digestibility in high protein diets mainly resulted from the increased intake of dietary protein. This is in agreement with the results of Eggum (1973), who found with rats, that apparent total tract protein digestibility improved with increasing dietary protein content.

Nitrogen metabolism and excretion

Lower N intake of pigs on low protein diets resulted in a simultaneous decrease in N excretion (Table 2). N excretion in urine was decreased by 6.9 g/day and 32% when dietary protein content was lowered by the supplementation of free lysine, methionine and threonine (P<0.01) Faecal N excretion, however, remained the same. Total N excretion decreased by 7.1 g/day per 3.7%-unit reduction in dietary protein content (P<0.01). The decline in N excretion in our experiment was of a similar magnitude observed in earlier studies (Näsi 1985, Gatel and Grosjean 1992, Pierce et al. 1994, Kerr and Easter 1995) where decreases in total N excretion have ranged from 3.3 to 9.0 g/day (from 9 to 37%). Reduction in N excretion in the above studies has been higher in finishing than growing pigs,

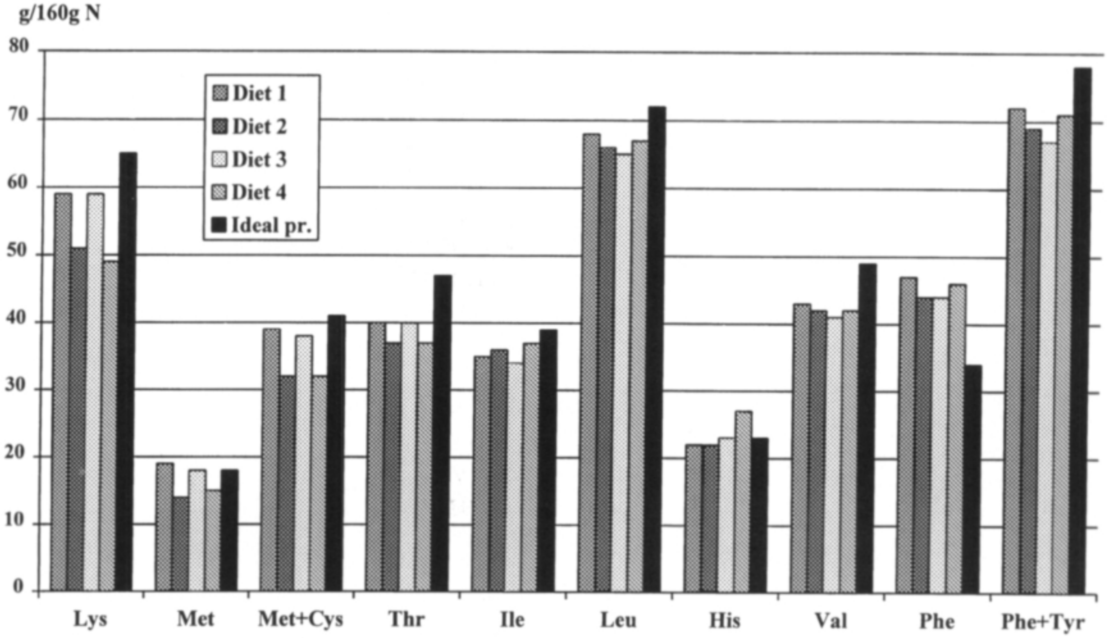

Fig. 1. Essential amino acid profiles of experimental diets and ideal protein (g/160g N) (Wang and Fuller 1989).

because of higher daily feed and protein intakes during finishing. The greatest response on N excretion can be achieved when dietary crude protein content is adjusted during the whole growing-finishing period (Gatel and Grosjean 1992). Lower N excretion of low protein diets was accounted for entirely by reduced urinary N excretion in our experiment which may also affect emission of ammonia from slurry. Kay and Lee (1997) observed that ammonia emission rates from pig slurry were reduced by 53% from high to low protein diets.

Dietary protein reduction tended to decrease daily N retention of the pigs ($P<0.10$). In the majority of earlier studies, N retention has significantly declined with the reduction dietary protein indicating an imbalance or deficiency in amino acid or total protein supply (Näsi 1985, Lee et al. 1993, Pierce et al. 1994, Kerr and Easter 1995). In comparison to ideal protein composition (Wang and Fuller 1989), the largest difference was found in the content of lysine, methionine and threonine in high protein diets (Figure 1). After protein reduction with supplementation of these amino acids, valine, isoleucine and threonine seemed to be the most limiting amino acids. For practical feed formulation, lysine, threonine, methionine and tryptophan are the only amino acids currently commercially available which limits the potential to reduce N excretion. In spite of lowered N retention, daily weight gain of the pigs was the same on low and high protein diets and indicated a shift in growth composition from protein to fat deposition, which is in agreement with the results of Kerr and Easter (1995). Increased fatness in pigs receiving low protein amino acid supplemented diets compared to those fed high protein diets has also been observed in growth experiments (Noblet et al. 1987, Valaja et al. 1993, Kerr et al. 1995), which suggests higher heat production and lower utilisation of energy on high protein diets. Excess protein is a poor source of energy, with net energy only accounting for 50% of gross energy (Just 1982).

Protein quality of the experimental diets

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could be calculated from the relationship between total amino acid composition of the feeds and ideal protein (Boisen 1997). The proportion of ideal protein is equal to the relationship between the most limiting amino acid in the feed and ideal protein. In our experiment, lowering of dietary protein content with supplements of free lysine, methionine and threonine increased the calculated amount of dietary ideal protein from 77% to 86% (Figure 1). These estimates are in close agreement with the increase in N retained per unit absorption ($P<0.05$). Total N utilisation was also improved as the amount of retained N per intake was increased from 51.1% to 57.5% ($P<0.10$). In agreement with our results, Boisen (1997) indicated that ideal protein-N in typical pig diets cannot exceed 90% of absorbed N due to the limited number of free amino acids commercially available for practical feed formulation.

Dietary energy content had no effect on N retention of the pigs, but daily weight gain tended to be higher for pigs on high energy diets ($P<0.10$). A linear-plateau type relationship has usually been established between N retention or protein deposition and energy intake in pigs above 40 kg live weight if protein supply is at an adequate level (Campbell et al. 1985, Dunkin et al. 1986, Quiniou et al. 1996). In our experiment, protein retention of the pigs had likely reached plateau levels since no response was found in N retention with increasing energy intake. Slight improvements in daily gain with increased energy intake indicated that extra energy was deposited as fat. Our experimental design is likely to be inadequate to determine relationships between energy intake and protein deposition because only two energy levels over a narrow range were used.

Water intake and urinary output

Water intake and urinary output of the pigs was the same for all diets (Table 2). In agreement with our results, Mroz et al. (1995a) obtained similar water intakes and urinary output of reproductive sows when dietary crude protein was decreased from 15.8 to 12.8%. Likewise, Hobbs et al. (1996) found no difference in slurry output of pigs between high and low protein level feeding strategies. In contrast, Wahlstrom et al. (1970) and Close et al. (1983) found that water intake of growing pigs was reduced as dietary protein supply decreased. Likewise, Pfeiffer and Henkel (1991) concluded from two balance experiments with growing pigs that both water intake and urine production were decreased with lower protein supply. According to Mroz et al. (1995b) both dietary protein quantity and quality affect both water intake and manure production of pigs. Absence of water intake and urinary output responses in this study may result from current experimental methods using a change-over design with short adaptation periods. Most of the studies reporting a positive effect on water intake and urine production were observed in continous experimental designs (Wahlstrom et al. 1970, Close et al. 1983). In a few studies where cross over design have been used, the adaptation period was longer than that used in this study (Pfeiffer and Henkel 1991). Use of adaptation period of five days, on the other hand, is suitable for nitrogen balance measurements as only three days are needed for N metabolism adaptation to changes in dietary protein content (Fuller et al. 1979).

Mean daily water intake of pigs was 11 650 g/day which is clearly higher than that reported in earlier studies of Wahlstrom et al. (1970), Close et al. (1983) and Pfeiffer and Henkel (1991). Mroz et al. (1995a), however, reported higher water intakes for non-pregnant sows. Spillage of water during this experiment may have influenced intake measurements. Variation in water intake between individual animals was high (from 8691 to 14 465 g/day) in our experiment. Likewise to Pfeiffer and Henkel (1991), we observed that some of the pigs maintained their water intake after changing from the high to low protein diet indicating that other factors affect the regulation of water intake.

Conclusions

The present study showed that N excretion of the pigs can be substantially reduced by lowering dietary protein content of barley-soya bean meal based diets supplemented with the free amino acids lysine, methionine and threonine. The reduction was almost accounted for decreased urinary N excretion which may also affect volatilisation of ammonia. Balancing of protein supply with free amino acid supplementation improved the utilisation of dietary protein since the amount of ideal protein in the diets was increased.

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SELOSTUS

Rehun valkuais- ja energiapitoisuuden vaikutus sikojen typen hyväksikäyttöön, veden kulutukseen ja virtsan eritykseen

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Tutkimuksessa selvitettiin rehun valkuaispitoisuuden ja rehuyksikköarvon vaikutuksia ravintoaineiden sulavuuteen ja typen hyväksikäyttöön sekä veden kulutukseen ja virtsan eritykseen lihasikojen ruokinnassa. Koe-eläiminä oli neljä leikkosikaa (elopaino 33–82 kg). Koerehuina oli neljä ohra-kaura-soijarouhepohjaista rakeistettua seosta, joissa rehun raakavalkuaispitoisuus: korkea (180 g/kg raakavalkuaista) tai matala raakavalkuaispitoisuus (140 g/kg raakavalkuaista) ja rehuyksikköarvo: matala (0,95 ry/kg) tai korkea ry-arvo (1,05 ry/kg) vaihtelivat. Matalavalkuaisrehuja täydennettiin puhtailla aminohapoilla, lysiinillä, metioniinilla ja treoniinilla, jotta tärkeimpien välttämättömien aminohappojen määrät pysyivät samoina kuin korkeavalkuaisrehuissa.

Korkeavalkuaisrehujen raakavalkuaisen sulavuus oli parempi kuin matalavalkuaisrehujen. Valkuaispitoisuuden alentaminen pienensi selvästi virtsan mukana erittyvän typen määrää (6,9 g/pv) ja tehosti rehutypen hyväksikäyttöä. Matalavalkuaisrehuilla sikojen päivittäinen typen pidättyminen jäi kuitenkin hieman alemmaksi kuin korkeavalkuaisrehuilla. Valkuaispitoisuuden alentaminen ei vaikuttanut sikojen veden kulutukseen tai virtsan eritykseen. Rehuyksikköarvon nosto ei vaikuttanut rehutypen hyväksikäyttöön, veden juontiin tai virtsan eritykseen. Tulokset osoittivat, että sikojen typen eritystä voidaan selvästi vähentää, kun rehuvalkuaisen määrää pienennetään ja aminohappokoostumusta tasapainotetaan puhtaalla lysiinillä, metioniinilla ja treoniinilla lähemmäksi tarvetta vastaavaa ihannevalkuaista.