

**EFFECTS OF DIETARY PROTEIN AND FIBRE ON NITROGEN EXCRETION  
PATTERNS IN SWINE**

A Thesis Submitted to the College of  
Graduate Studies and Research  
in Partial Fulfillment of the Requirements  
for the Degree of Master of Science  
in the Department of Animal and Poultry Science  
University of Saskatchewan  
Saskatoon, Saskatchewan

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Spring, 2001

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## ABSTRACT

Successful management of nitrogen (N) excretion is important for sustainable pork production. Two experiments were conducted and their objectives were to study the effect of dietary protein and fibre on N excretion patterns and to relate plasma urea (PU) to urinary N excretion.

In the first experiment, three dietary protein contents (high, 197; medium, 169; low, 138 g kg<sup>-1</sup>) and two levels of fibre (low and high) were tested. Diets (wheat, barley, soybean meal; oat-hulls as the fibre source) were formulated to an equal digestible energy (DE) content (3.25 Mcal DE kg<sup>-1</sup>) and at least 2.18 g digestible lysine per Mcal DE, and were supplemented with Lys, Met, Trp, Thr, Ile, or Val. Pigs (32 ± 3.4 kg; n = 42) were housed in confinement-type metabolism crates for 19 d. On d 10 or 11, catheters were installed by cranial vena cava venipuncture. Daily feeding rates were adjusted to three times maintenance and daily rations were halved to two equal meals. Faeces and urine were collected from d 15 to 19. Five blood samples were collected in two-h intervals on d 16 and 19. Faecal N, urinary N, and total N excretion were reduced linearly with a reduction of dietary protein content ( $P < 0.001$ ). Reduction was greater for urinary (48%) and total N excretion (40%) than for faecal N (23%) excretion. The ratio of urinary N to faecal N was reduced linearly with a reduction of dietary protein content ( $P < 0.001$ ). Retention of N (g d<sup>-1</sup>) was reduced linearly but N retention as percentage of intake was increased linearly with a reduction of dietary protein content ( $P < 0.001$ ). Addition of oat hulls did not affect N excretion patterns ( $P > 0.10$ ). Dietary treatments did not affect average daily gain (ADG) or feed efficiency ( $P > 0.10$ ). Plasma urea increased after feeding, peaked at 4 h and then decreased toward pre-feeding concentrations. A linear relationship of urinary N to PU concentration ( $R^2 = 0.66$ ) was observed.

In the second experiment, effects of dietary protein content (high, 185; and low, 157 g kg<sup>-1</sup>) and fibre sources on N excretion patterns were studied in a 2x3 factorial arrangement. The three fibre sources were control, soybean hulls (SH; 15%), and sugar beet pulp (SBP; 20%). Diets were formulated to 3.3 Mcal DE kg<sup>-1</sup> and 2.4 g digestible Lys per Mcal DE, and were supplemented with Lys, Met, Trp, Thr, Ile, or Val. Pigs (30.5 ± 3 kg; n = 36) were housed in confinement-type metabolism crates, with

restricted access to feed (3 x maintenance DE) from d 1 to 18, and free access to feed from d 19 to 26. Faeces and urine were collected from d 15 to 18 and d 23 to 26, and blood samples on d 17 and 25. With restricted access to feed, faecal N (as % of N intake) was increased 3 percentage units for low compared to high protein content, and increased 4 percentage units for SH and 6.5 percentage units for SBP compared to control ( $P < 0.05$ ). Urinary N was reduced 5 percentage units for low compared to high protein content, and reduced 9 percentage units for SBP compared to control ( $P < 0.05$ ). Retention of N ( $\text{g d}^{-1}$ ) was reduced 12% for low compared to high protein content (17.9 compared to 20.4  $\text{g d}^{-1}$ ;  $P < 0.05$ ), and was similar among fibre treatments ( $P > 0.10$ ). With free access to feed, faecal N (as % of N intake) was increased 2.5 percentage units for low compared to high protein content, and increased 5 percentage units for SH and 9 percentage units for SBP compared to control ( $P < 0.05$ ). Urinary N was reduced 5 percentage units for low compared to high protein content, and reduced 9 percentage units for SH and 10 percentage units for SBP compared to control ( $P < 0.05$ ). Retention of N ( $\text{g d}^{-1}$ ) was similar for dietary protein content (30.5  $\text{g d}^{-1}$ ;  $P > 0.10$ ), and reduced for SH (27  $\text{g d}^{-1}$ ;  $P < 0.05$ ) compared to control (31  $\text{g d}^{-1}$ ), while was intermediate for SBP (29  $\text{g d}^{-1}$ ). A linear relationship of urinary N to PU concentration was observed with both restricted ( $R^2 = 0.66$ ) and free access to feed ( $R^2 = 0.71$ ).

Reduction of dietary protein content is an efficient way to reduce total and urinary N excretion, which may reduce ammonia emission. Inclusion of fibre sources high in fermentable fibre shifted N excretion from urine N to faeces N while fibre sources resistant to fermentation did not have any effects on N excretion patterns. Combined effects of dietary protein content and fibre reduced urinary N excretion further than single effects. Level of feed intake is an important consideration when effectiveness of a nutrient management strategy is studied. Diets with a low protein content and containing synthetic AA should be studied using pigs with free access to feed to verify that N retention is maintained. A linear relationship of urinary N to PU concentration was observed under both restricted and free access to feed indicating that urinary N excretion could be predicted from PU concentration.

**Key Words:** Nitrogen excretion, Plasma urea, Protein, Fibre, Grower pigs

## ACKNOWLEDGMENTS

I sincerely thank my supervisor Dr. Ruurd Zijlstra for his guidance, support, and friendship throughout the entire process of my thesis. His guidance and insight were invaluable.

I would like to thank Dr. David Christensen, Dr. Gordon Zello, and Dr. Bernard Laarveld for serving as my advisory committee and providing expertise in their individual areas. I would also like to thank Dr. Phyllis Paterson for agreeing to be my external examiner.

I would like to acknowledge the State Scholarship Foundation of Greece for the scholarship granted to me.

I would like to acknowledge the financial support for the project by the AAFC/NSERC Research Partnership Program and AARI. Pork producers from Saskatchewan, Manitoba, and Alberta, together with Saskatchewan Agriculture & Food provided program funding. I would like to acknowledge Degussa AG for amino acid analysis.

I would like to thank Brian Andries and the barn staff for their assistance and the office staff at the Prairie Swine Centre for their encouragement and friendship.

I would like to thank Dr. Robert Baker for all of the statistical support and Dr. Ronald Chaplin for teaching me the catheterization procedure.

I want to thank my family and friends for their love and support. Special thanks to Dr. Tala Awada for her help, support and friendship. And finally to Emmy Yiannaka, my fiance, for her love, support and patience all these years.

## **DEDICATION**

Dedicated to my father Spiridon and my mother Lambrini. I owe them more than I can say. Their love and support helped me to overcome any difficulty.

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## LIST OF ABBREVIATIONS

AA	Amino Acid
ADF	Acid Detergent Fibre
ADG	Average Daily Gain
BW	Body Weight
CF	Crude Fibre
CP	Crude Protein
D	Day
DE	Digestible Energy
DF	Dietary Fibre
DM	Dry Matter
EE	Ether Extract
FE	Feed Efficiency
H	Hour
N	Nitrogen
NDF	Neutral Detergent Fibre
NE	Net Energy
NSP	Non-Starch Polysaccharides
P	Phosphorus
PU	Plasma Urea
SBP	Sugar Beet Pulp
SH	Soybean Hulls
TSAA	Total Sulfur Amino Acids
VFA	Volatile Fatty Acids
WK	Week

## **1. LITERATURE REVIEW**

### **1.1 Introduction**

#### **1.1.1 Pig production and the environment**

During the last decades, swine production has undergone great expansion and intensification, feasible by advances in housing, genetics, and nutrition. Intensification of pig production was dictated in part by economics because cost minimisation was required for survival in the global economy. Beside the economic advantages, modern production systems have raised environmental concerns (Backus et al. 1998). Increased density of pig production has resulted in local production of vast quantities of manure, which exceeded nutrient requirements of locally-grown crops (Williams 1995). Application of manure in soil as fertiliser, a common practise of manure disposal, might then not be efficient because only part of the applied manure will be utilised by the crops (Williams 1995). Thus, manure might become a serious threat to the local environment in areas with a dense pig population.

Major concerns associated with pig production are manure disposal and odour control, specifically quality of soil, air, and surface and ground water (Paik 2000). Excessive application of manure has resulted in accumulation of heavy metals in soil, with negative consequences for plant growth and potential risks for human and animal health and soil life (Jongbloed and Lenis 1998). Phosphorus (P) may accumulate in soil and subsequent run-off to surface water can cause eutrophication resulting in a major deterioration of water quality and increased fish mortality (Correll 1999).

North America and especially Western Canada have great opportunities to expand pig production because of the low density of pigs and ample supply of locally-produced ingredients that can be used as feed and to which manure can be applied as fertiliser. However, the threat of environmental pollution has restricted expansion of pig production in some areas and has created friction between neighbours. While the

prospects for pig production in North America remain strong, environmental issues should be clearly addressed to ensure long-term sustainability of pig production.

Various legislative measures with degrees of restriction toward expansion and manure storage and application have been introduced to reduce negative effects of swine production (Jongbloed and Lenis 1998). Procedures for manure application were provided to maximize benefits to soil and crops and to eliminate run-off or leaching into water resources (Williams 1995; Jongbloed and Lenis 1998). In some countries, restrictions on animal density were imposed together with requirements for improved utilisation of nitrogen (N) from manure (Backus et al. 1998; Jongbloed and Lenis 1998). In Denmark, current law permits application of pig manure from 1.7 animal units per hectare of arable land (Fernandez et al. 1999). One animal unit is equivalent to 100 kg of manure-N and corresponds to manure production from 30 grower-finisher pigs or from 3 sows (including piglets up to 25 kg). In France, the amount of N from animal manure should not exceed 170 kg per hectare (Dourmad et al. 1999). In the Netherlands, application of manure is legislated by controlling P application (Lenis 1989; Van der Peet-Schwering et al. 1999b).

### **1.1.2 Nitrogen excretion**

Nitrogen in pig manure originates from faeces, urine, and wasted feed. Faecal N originates from undigested dietary protein, endogenous components secreted into the gastrointestinal tract, and bacterial protein. Urinary N originates from excess absorbed amino acids and catabolized protein, and is mainly in the form of urea. Digestibility of N in swine diets is generally high (>80%), but utilisation of dietary nitrogen is variable, and within a range of 30 to 55 % (NRC 1998). Thus, 45 to 70% of dietary N will be excreted in manure. Grower-finisher pigs excrete 65 to 70% of the manure N produced at farrow-to-finish swine production units (Table 1.1; Van der Peet-Schwering et al. 1999b; Dourmad et al. 1999; Fernandez et al. 1999) and are therefore the focus of research to reduce N excretion.

Nitrogen excretion is of major concern because improper management might have a great impact on environment. Two nitrogenous compounds of concern are ammonia and nitrate. Ammonia emission from pig housing is an environmental

**Table 1.1** Nitrogen excretion from swine production

	Days	Feed Intake	Intake	Retained	Feces	Urine	Total	Relative
Denmark <sup>1</sup>								
Piglet (0-7.5) <sup>2</sup>	27	-	1.42	0.25	0.28	0.89	1.17	22
Weaner (7.5-30)	53	0.74	1.26	0.59	0.19	0.49	0.67	13
Growers (30-100)	94	2.04	5.35	1.97	1.07	2.31	3.38	65
Total	308	-	8.03	2.81	1.54	3.69	5.22	100
Relative	-		100	35	19	46	65	
France <sup>3</sup>								
Piglet (0-8) <sup>2</sup>	27	-	1.44	0.38	0.24	0.81	1.05	19
Weaner (8-28)	42	0.83	1.07	0.48	0.16	0.43	0.59	10
Growers (28-108)	110	2.11	6.13	2.01	1.04	3.08	4.12	71
Total	179	-	8.64	2.87	1.44	4.32	5.76	100
Relative	-		100	33	16	51	67	
Netherlands <sup>4</sup>								
Piglet (1.5-7.5) <sup>2</sup>	27	-	1.17	0.28	0.24	0.65	0.89	16
Weaner (7.5-26)	48	0.64	0.87	0.44	0.12	0.26	0.38	7
Growers (26-113)	119	2.02	6.4	2.14	1.09	3.17	4.26	77
Total	179	-	8.44	2.86	1.45	4.08	5.53	100
Relative	-		100	34	17	48	66	

<sup>1</sup>Adapted from Fernandez et al. 1999<sup>2</sup>The intake, retention and the losses related to the sow are also included<sup>3</sup>Adapted from Dourmad et al. 1999<sup>4</sup>Adapted from Van der Peet-Schwering et al. 1999

problem, because ammonia is a noxious gas for humans and animals, and contributes to odour emission and acidification of the environment. Ammonia emission from manure originates mainly from urea in urine (Canh et al. 1997). Urea is easily hydrolysed to ammonium and carbonate in a reaction catalysed by the microbial urease present in faeces but not in urine (Elzing and Swiestra 1993). In water, ammonium is in equilibrium with ammonia, which is easily volatilised. Faecal N is less volatile than urinary N, because faecal N is bound organically within proteins or other compounds, which are broken down by anaerobic digestion in a much slower process and thus is of lesser importance than that of urea for producing ammonia (Aarnink 1993). Approximately 50% of ammonia emission from swine production is emitted from housing and manure storage while the other 50% is emitted during surface application of manure to the soil (Van der Peet-Schwering et al. 1999a). Ammonia may be converted to nitrate by nitrification or to gaseous N by denitrification during storage or after its application as a fertiliser. As a result of both processes, oxides such as dinitrogen oxide, nitrogen oxide, and nitrogen dioxide are produced and might volatilise into the air. Nitrogen that remains trapped in manure used as soil fertiliser might eventually leach as nitrate into surface and underground water sources.

## **1.2 Amino Acid Requirements**

### **1.2.1 Ideal protein**

The primary objective of the swine industry is to maximize net income, which can be achieved in part by maximizing protein deposition. However, protein (or N retention) was maximized while N excretion was ignored. Diets are presently formulated using least-cost diet formulation, which does not incorporate a cost for excess N excretion. For long-term sustainability of the industry, the future objective should be to combine maximizing N retention with minimising N excretion. The objective will mean meeting AA requirements of pigs while avoiding excessive supply, which requires a good knowledge of AA digestibility of feedstuffs, and AA requirements during the grower-finisher phase. Use of modelling techniques for predicting requirements combined with a better knowledge of variations in ileal AA digestibility of feedstuffs are tools to

increase precision in swine nutrition and thus minimise nutrient excretion. The factorial approach has allowed prediction of energy and AA requirements for maintenance and deposition of protein and lipid. The first step of a feeding strategy is to determine daily amounts of nutrients required for a specific group of pigs according to production objectives.

Pigs do not have a requirement for protein but for AA, specifically essential AA. The role of dietary protein is to provide correct amounts and proportions of essential AA as well as enough N for synthesis of non-essential AA. The most important single factor affecting efficiency of protein utilisation is the dietary balance of AA. The excess AA have to be catabolized and the N is excreted mainly as urea in urine. This process of deamination and excretion has an energy cost, and thus may reduce performance. Overformulation of the diet may increase diet cost, reduce utilisation of protein, and raise environmental concerns.

Based on the idea that the balance of AA determines the nutritional value of protein, the concept of "ideal protein" was introduced. Ideal protein is the dietary protein balance in which each essential AA and the sum of the nonessential AA are equally limiting (Wang and Fuller 1990). In the ideal protein concept the essential AA are related to one reference AA. Lysine is usually this AA because lysine is the first limiting amino acid in regular swine diets (cereal-soybean meal), and requirements for lysine have thus been studied intensively. The ideal protein concept assures that if the lysine requirement for a given group of pigs is altered, requirements for other AA will be adjusted according to ideal protein ratios. Finally, the balance of essential and non-essential AA must be considered, and a ratio of essential to non-essential AA of approximately 50:50% has been suggested for optimal N retention and utilisation (Lenis et al. 1999).

For grower-finisher pigs, the total AA requirements consist of a requirement for maintenance and a requirement for lean tissue growth. The maintenance requirement includes losses due to protein turnover, production of enzymes and gut secretions, and replacement of intestinal epithelial cells and skin. The dietary AA requirement for maintenance will increase linearly with an increase in metabolic body weight. The requirement for lean tissue growth is curvilinear; there is an increase during the grower



phase until the maximum potential growth rate is reached, followed by a decline towards slaughter weight (Fuller 1994). The AA requirement for maintenance and growth differ and their relative contribution to the total requirement changes during the grower-finisher phase. Consequently, the ideal protein will include a larger maintenance component with an increase in body weight. Thus, for AA with a higher ideal ratio for maintenance than for protein accretion (threonine, tryptophan, sulphur AA), total ratios will increase slightly as body weight increases. For lysine, the relative amount required for maintenance is lower than that for growth. Consequently, the total requirement for lysine will decrease faster with an increase in BW than for AA with a large requirement for lean growth. The AA requirements of grower-finisher pigs can be expressed in  $\text{g d}^{-1}$  as total, apparent ileal digestible, true or standardised ileal digestible; after division by voluntary feed intake, AA requirements can then be expressed on a dietary concentration basis.

### **1.2.2 Factors that affect amino acid requirements of grower pigs**

The AA requirements should be determined using the factorial approach, including requirements for maintenance and protein deposition. The maintenance requirement is considered a function of body weight (lysine requirement for maintenance is  $36 \text{ mg kg}^{-1} \text{ BW}^{0.75} \text{ d}^{-1}$ ; Fuller et al. 1989). Requirement for protein deposition is more dynamic and is affected by several factors including energy intake, gender, live weight, genotype, health, temperature, stocking density, and stress (NRC 1998).

Protein deposition increases during early growth, reaches a peak between 50 to 80 kg, and then decreases towards slaughter weight (Yen et al. 1986a, b). The decline in protein deposition as pigs approach maturity is accompanied by an increase in lipid deposition (Whittemore 1998). The AA concentration per kilogram feed is decreased as the BW of the pig increases from 25 to 110 kg because voluntary feed intake increases. Whittemore and Fawcett (1976) proposed that potential protein deposition is limited by voluntary feed intake in young pigs, but is maximized below voluntary feed intake in older pigs.

Gender of the animal has a strong effect on maximum protein deposition potential. Boars have the highest rate, barrows the lowest, and gilts are intermediate

(Yen et al. 1986b), and differences in lysine requirement between barrows and gilts have thus been observed (Cromwell et al. 1993). Gilts require a diet with a higher concentration of AA than barrows due to their higher protein deposition rate and reduced voluntary feed intake compared to barrows.

Genotype is well known to affect body composition and growth. Genotypes with a high potential for protein deposition (or lean growth) have a higher dietary requirement for AA and energy than genotypes with a low potential. Pigs with fast rates of lean growth utilise feed more efficiently and produce carcasses with more muscle and less fat. Consequently, a higher concentration of dietary AA is required to achieve their genetic potential for lean growth (Campbell et al. 1985).

The criterion of response that is used for determination of requirements affects the final estimation. Thus, the AA requirements by using weight gain as criterion may be different than by using feed efficiency. For example, maximum carcass leanness requires a greater intake of AA than does the maximization of weight gain (NRC 1988).

### **1.2.3 Energy-amino acid interaction**

Energy and AA requirements are closely related. To achieve maximum performance, sufficient dietary AA and energy are needed. If energy intake is less than required for maintenance and growth, then maximum protein deposition will not be achieved even though AA intake might be adequate. Until energy intake is sufficient to maximize lean tissue growth, the pig will deposit a minimum of fat during growth (Whittemore 1998). Once energy requirement to maximize lean deposition have been reached, fat deposition rate will increase rapidly with further increases in energy intake, because energy intake in excess of requirements for maintenance and growth is stored as body fat (Whittemore 1998).

Voluntary feed intake is determined by the energy content of the diet, because pigs try to satisfy their energy requirements. Thus, to achieve the maximum lean gain and minimise lipid gain, dietary energy content should be related to lysine content (Chiba et al., 1991a, b).

### 1.3 Effects of Dietary Factors and Feeding on Plasma Urea Concentration

Plasma urea concentration is a valuable variable in understanding N metabolism and has been used as an indicator of AA status in animals (Brown and Cline 1974; Yen et al. 1986a, b; Coma and Zimmerman 1993). Plasma urea concentration depends on quality and quantity of dietary protein (Eggum 1970). Urea is the main nitrogenous end product arising from AA catabolism in mammals. Consumption of excess protein increases urea synthesis in the liver, which is reflected by an increase in plasma urea concentration (Eggum 1970). With an improvement in dietary AA balance, protein synthesis is increased and the excess AA is reduced. When the requirement of the first-limiting AA in the diet is met, urea synthesis is minimised and plasma urea concentrations tend to reach a plateau (Brown and Cline 1974).

Nitrogen metabolism responds rapidly to changes in dietary AA concentrations (Brown and Cline 1974). Plasma urea responded to dietary treatments in less than 24 h and a new equilibrium in PU concentration was reached 3 d after diet changes (Coma et al. 1995). Similarly, the rate of urinary urea excretion reached a new equilibrium within 3 d of adding the limiting AA to the diet (Brown and Cline 1974). In this study, plasma urea concentration responded similarly to dietary treatments, but the reduction was less consistent than urinary urea concentrations. Because renal filtration of urea is directly proportional to plasma urea concentrations, re-equilibration of urinary urea and plasma urea concentrations after dietary changes should require a similar length of time.

Time of feeding and plasma urea concentration are strongly related (Eggum 1970; Cai et al. 1994). Eggum (1970) reported that blood urea concentration increased for the first 4 h after feeding and then reached a plateau during a 5-h study. Malmlof et al. (1989) observed that the peak plasma urea concentration appeared between 4 and 5 h after feeding during an 8-h study. Cai et al. (1994) found that pigs given free access to feed exhibited only an 8% rhythmic fluctuation in PU concentrations. In contrast pigs fed twice daily had a peak PU at 3.6 h after feeding and the peak was 32% higher than pre-feeding concentration.

Pigs fed a diet high in anions had lower PU concentrations and ammonia excretion in urine was increased at the expense of urea excretion while N balance was unaffected (Patience and Chaplin 1997; Sladge and Zimmerman 1979). Furthermore,

feeding pigs excess cation tended to increase PU concentrations and increased urea excretion, although total N excretion remained constant (Cai et al. 1992). The decrease of PU concentration by dietary anions is attributed to their acidifying properties. Acidogenic diets result in a reduced concentration of metabolizable anions in the diet, generating metabolic acidosis (Patience and Chaplin 1997). Metabolic acidosis redirects inter-organ flow of glutamine from hepatic ureagenesis to renal ammoniagenesis in an effort to maintain acid-base balance in the body (Welbourne et al. 1986).

Endogenous urea enters the gastrointestinal tract via digestive secretions. The main site of urea secretion is the small intestine and only traces are found in the large intestine (Bergner et al. 1986; Mosenthin et al. 1992a). Hydrolysis of urea to carbon dioxide and ammonia occurs in the digestive tract of animals and appears to depend on urease of bacterial origin (Wrong 1978). The ammonia produced enters the portal vein and is transported to the liver where it enters a nitrogen pool available for synthesis of AA or urea. The majority of ammonia is converted into urea and a portion may be used for protein synthesis.

Nitrogen balance has been used extensively for determination of AA requirements in pigs (Fuller et al. 1989; Wang and Fuller 1989). In a N balance experiment, the dietary AA concentration with the highest N retention is considered optimum, because the absorbed AA are being used for protein deposition at the highest rate. An increase in AA utilisation decreases urea synthesis and thereby, decreases PU concentrations. Coma et al. (1995) used N retention and PU concentrations as criteria to assess requirements in grower pigs. Maximum N retention and minimum PU concentrations occurred at identical dietary lysine concentrations, indicating that PU concentrations can be used in short-term trials to accurately estimate dietary AA requirements of pigs.

Chen et al. (1995) found an increase in PU concentration over time in pigs receiving the same diet indicating that AA requirements of pigs (as % of diet) dropped with age and body weight. The lysine level that was appropriate for the beginning of the period was in excess later.

## 1.4 Effect of Fibre on Digestion and Absorption in Pigs

### 1.4.1 Fibre digestion and absorption

A major constraint in the study of fibre has been its definition and analysis because fibre is a group of heterogeneous compounds differing widely in chemical composition and physico-chemical characteristics (Graham and Aman 1991). Fibre content of feeds has been described interchangeably as crude fibre (CF), neutral or acid detergent fibre (NDF or ADF), non-starch polysaccharides (NSP), or dietary fibre (DF). The latter is defined either physiologically as the dietary components resistant to hydrolysis by mammalian enzymes or chemically as the sum of NSP and lignin (Graham and Aman 1991). The components of DF are structural polysaccharides (cellulose, hemicellulose, some pectins), nonstructural polysaccharides (pectins, gums) and structural non-polysaccharides such as lignin (Figure 1.1). Furthermore, differentiation of DF in a soluble and insoluble fraction is important as each elicits different effects on the gastrointestinal tract. Soluble fibre is mostly fermentable while the insoluble fibre is fermented to a variable degree depending on its composition.

In recent years, various methods for analysis of dietary fibre have been developed. The methods for measuring total, soluble and insoluble fibre can be divided into two categories based on the approach used; they are the gravimetric method in which the DF is isolated and weighed (Prosky et al. 1988; Lee et al. 1992) and the component analysis method in which the DF is determined by gas chromatography or colorimetry following hydrolysis (Englyst et al. 1988; Theander and Westerlund 1986). Using older analytical methods, fibre is determined as the insoluble residue in a specific solvent, which leads to wide ranges of determined fibre content. For example the CF method that recovers most cellulose and some lignin, results in a lower fibre content than DF. The ADF and NDF detergent methods result in an intermediate fibre content because the former recovers almost all cellulose and lignin and the latter cellulose, lignin, and hemicellulose (Low 1993).

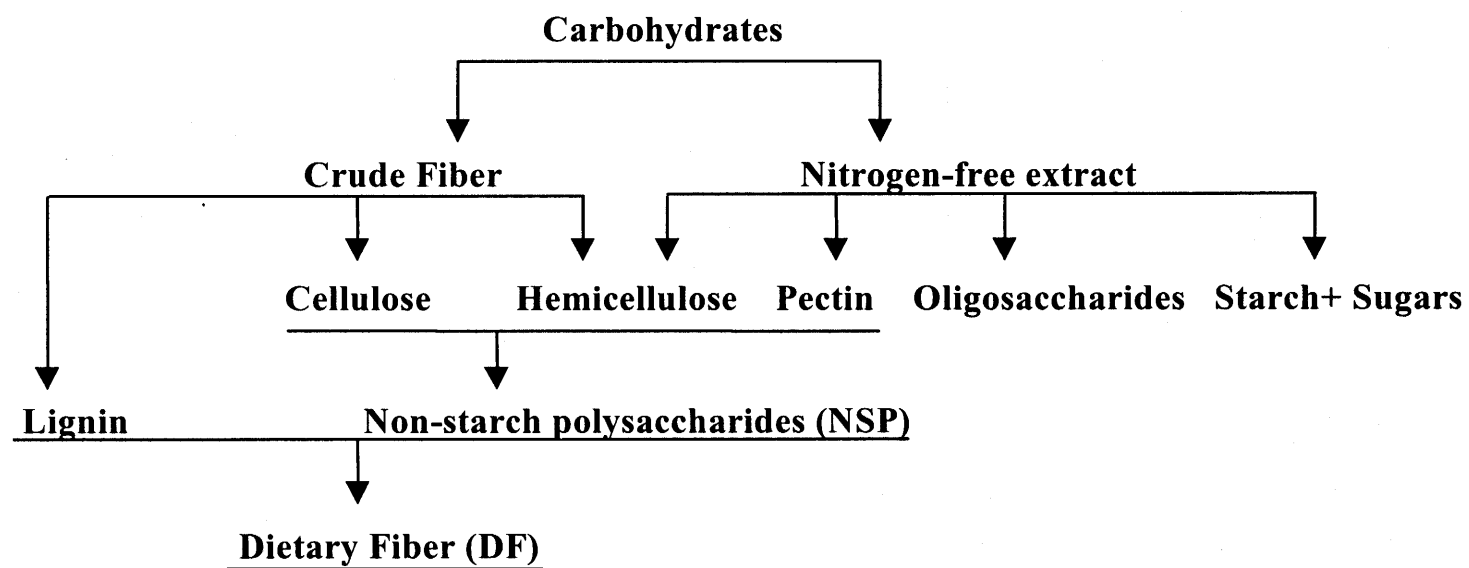


Figure 1.1 Classification of carbohydrates.

Pigs digest simple carbohydrates and starch with endogenous enzymes but lack enzymes to digest DF. Dietary fibre is fermented by intestinal microflora, which produces NSP-degrading enzymes such as cellulases, hemicellulases, and pectinases. Microbial fermentation has important implications for nutrient assimilation (Bach Knudsen et al. 1991). The NSP are broken down to volatile fatty acids (VFA: acetate, propionate, butyrate) and various gasses (carbon dioxide, methane, and hydrogen). The degree of fermentation depends on the source, and amount of DF (Stanogias and Pearce 1985; Graham et al. 1986; Chabeauti et al. 1991) and presence of N, minerals, and vitamins that are essential nutrients for microbial growth (Varel and Yen 1997). Fermentation is carried out by a diverse population of obligate anaerobic bacteria and some aerobic and facultative micro-organisms. The density of the microbial population in the caecum and colon, consisting of more than 500 species, is  $10^{10}$  to  $10^{11}$  viable counts per gram digesta (Moore et al. 1987). The microbial population can adapt to substrates entering the large intestine. For example, dietary cellulose increases the cellulolytic microflora after prolonged feeding of high-fibre diets (Varel et al. 1987).

The physico-chemical properties of soluble and insoluble DF may affect the digestion and absorption in the different segments of the gastrointestinal tract to a variable degree. The DF is mainly fermented in the large intestine after passing through the stomach and small intestine. Digestion of NSP in the stomach is negligible, although some NSP are broken down (Keys and DeBarthe 1974). This degradation of NSP in the stomach is probably due to direct hydrolysis of some carbohydrates under low-pH conditions.

Some breakdown of NSP might occur during passage through the small intestine, but overall pre-cecal fermentation is limited in pigs (Graham et al. 1986; Bach Knudsen and Hansen, 1991). The degree of fermentation varies with the source of DF. For example, 44% of NSP in beet pulp was degraded at the terminal ileum, whereas less than 3% in wheat bran (Graham et al. 1986), which might be related to the proportion of soluble NSP in beet pulp. The degradation of fibre at the end of the ileum was of bacterial origin (Graham et al. 1986). In summary, pre-cecal digestion of cellulose is

extremely low, and higher but limited for hemicellulose and pectic substances (Drohner, 1993).

The digesta is gradually depleted from fermentable sources during passage through the gastrointestinal tract. The digesta in the distal colon is thus comprised of the cell wall materials of the ligno-cellulose type that are most resistant to degradation (Bach Knudsen and Hansen 1991). Digestibility of lignin in the large intestine is negligible and a lignin coating of digestible material will thus diminish bacterial access and thereby limit their degradation (Graham et al. 1986; Chabeauti et al. 1991).

The variation in degradability of NSP is large. The breakdown within the entire gastrointestinal tract is highest for  $\beta$ -glucans, intermediate for arabinoxylan,s and lowest for cellulose (Bach Knudsen et al. 1993a). Cell wall lignification plays an important role for the breakdown of NSP (Bach Knudsen et al. 1993a). Dietary particle size and retention time in the gut are also important factors (Ehle et al. 1982).

The physico-chemical properties of DF can affect the function of the gastrointestinal tract. Potkins et al (1991) observed that soluble DF sources (guar gum and pectin) accelerate gastric emptying whereas insoluble DF sources (bran) did not. On the other hand soluble fibre did not affect overall transit time while bran accelerated it. It appears that overall transit time is primarily determined by the retention time in the hindgut while gastric emptying and transit through the small intestine have minimal effects on retention time. The role of viscosity in gastric emptying appears to be confined to the liquid phase of digesta, because emptying of the solid phase was not reduced (Johansen et al. 1996). Soluble DF sources may absorb other nutrients in a gel matrix and delay also their emptying (Drochner 1993). The physical properties of digesta bulk, volume, and viscosity increase due to fibre, which might slow digestion and absorption. Bulk is increased because fibre is less digestible and hence more matter remains during the passage of digesta through the small intestine. Volume is increased due to increased water-holding capacity of certain fibres.

The DF increases secretion of gastric, biliary, and pancreatic juice (Dierick et al. 1989), mechanical erosion of the mucosal surface leading to increased losses of endogenous materials (Shah et al. 1982), and ileocecal flow of digesta partly due to higher water flow (Jorgensen et al. 1996). The increased ileocecal flow of water,



electrolytes, secreted products from liver, pancreas and mucosa cells is correlated with increasing fibre levels in the diet (Low 1989), resulting in a decreased absorption of nutrients, especially AA and minerals.

The presence and physico-chemical characteristics of fibre are important determinants of faecal output. An increased DF intake will increase faecal bulk, which has been attributed to an increase in bacterial cell mass, undegraded fibre residue, or faecal water (Drohner 1993). Insoluble fibre may increase faecal bulk more than soluble fibre, which is extensively fermented by the microflora (Graham et al. 1986). Fermentation may increase cell mass in faeces but will not increase faecal bulk.

The VFA, end products of microbial fermentation, are rapidly absorbed by colonic mucosa (Binder 1989). The absorbed VFA serve as an energy source for the host. The energy losses in the form of hydrogen gas, methane, and heat produced during fermentation and low efficiency of VFA utilisation by the pig make this route energetically less efficient than utilisation of glucose derived from enzymatic digestion of carbohydrates in the small intestine. The efficiency of energy utilisation from VFA is lower than energy from glucose. The VFA may provide up to 30% of the maintenance energy requirements for growing pigs (Rerat et al. 1987; Yen et al. 1991) and even more for adult swine (Varel 1987). Levels of VFA in the large intestine of pigs range from 150 to 250 mM as opposed to 5 to 40 mM in stomach and small intestine (Low 1993). The specific VFA formed depend on both the source and level of fibre (Kass et al. 1980; Bach Knudsen et al. 1993a, b).

The DF may modify small intestine morphology, by affecting appearance, villus length and number, cell proliferation, mucosal cell division and absorptive function. In weaned pigs, a high fibre diet increased crypt depth and enterocyte depth in the jejunum and ileum compared to low-fibre diet (Jin et al. 1994). The crypts are the principal site of cell proliferation in the intestine, thus DF increased the turnover rate of the intestinal enterocytes. Proliferation of large intestine mucosa is related to VFA (Jin et al. 1994) especially butyrate (Sakata 1987). Dietary fibre likely influences intestinal cell proliferation through several interactive mechanisms some of which include luminal factors. Increased levels of DF from different sources increased weight of different

segments across the gastrointestinal tract, particularly that of the colon (Kass et al. 1980; Anugwa et al. 1989).

#### **1.4.2 Effect of dietary fibre on the digestion and absorption of nutrients**

Dietary fibre has negative effects on apparent ileal and total tract digestibility of other nutrients (Dierick et al. 1989; Graham et al. 1986). Inclusion of fibre resulted in a decrease in apparent ileal digestibility of DM and energy (Sauer et al. 1991; Schulze et al. 1994). Graham et al. (1986) observed a decreased ileal digestibility for DM, ash, fat and protein. Total tract digestibility of energy and N was decreased with increasing levels of dietary fibre (Chabeauti et al. 1991; Stanogias and Pearce 1985). The reduction was affected by the source of fibre (Chabeauti et al. 1991; Stanogias and Pearce 1985). Mroz et al. (2000) observed that the apparent ileal and total tract digestibility of protein was lowest for soybean hulls, medium for sugar beet pulp and highest for tapioca, following their NSP profiles. Dietary fibre can increase the excretion of N into the small intestine of pigs (Potkins et al. 1991; Sauer et al. 1991; Schulze et al. 1995) because of the increase in endogenous secretions into the gastrointestinal tract (Low 1989) and sloughing of the epithelial cells (Shah et al. 1982). Increase of dietary fibre intake resulted in an increase DM and N flow at the terminal ileum (Schulze et al. 1995, 1994). The increase in N flow was composed of 59% endogenous and 41% exogenous N.

The negative effects of NSP on digestibility of other nutrients, especially N, have been attributed to a reduction in absorption of nutrients (Bach Knudsen et al. 1993a; Graham et al. 1986) resulting in a decreased measured true nutrient digestibility and to an increase in endogenous secretions or microbial synthesis of protein (Low 1989) resulting in a reduced apparent nutrient digestibility. The reduction in absorption of nutrients is caused by many factors. First, the enclosure of digestible nutrients by fibre reduces enzymatic breakdown of these nutrients and therefore reduces their digestibility (Drochner 1993). Second, the reduced retention time of the digesta in the gastrointestinal tract caused by NSP, (Ehle et al. 1982; Kass et al. 1980) may reduce nutrient digestion. Finally, the increased viscosity reduces diffusion of substrates and enzymes thus obstructing their effective interaction (Dierick et al. 1989).

Fermentable substrates available for microbial digestion in the large intestine of pigs increase faecal nitrogen output resulting from an increased assimilation of N in bacterial protein. Most of the N in faeces (up to 90%) is of bacterial origin (Sauer et al. 1991; Mosenthin et al. 1992b). Apart from undigested dietary protein and AA, endogenous urea secreted into the small intestine may enter the large intestine and degrade to ammonia (Mosenthin et al. 1992a, b). Provided that a fermentable energy source is available to bacteria in the large intestine, ammonia is used for synthesis of bacterial protein instead of being absorbed, resulting in increased faecal N excretion. However, N retention by pigs is not affected because the increase in faecal N is off set by a reduction in urinary N excretion (Morgan and Whittemore 1988; Misir and Sauer 1982; Canh et al. 1997). Reduced ammonia absorption from the large intestine resulting from increased bacterial assimilation of N will save metabolic energy. Less absorbed ammonia will have to be converted to urea by the liver, a process that requires energy.

### **1.5 Alteration of Nitrogen Excretion Patterns by Nutritional Means**

Total N excretion and ammonia emission need to be reduced to alleviate the negative effects of pig production on the environment. Decreasing manure N content may increase the amount of manure that can be applied per hectare of land. Reducing ammonia emission will result in an improvement of air quality inside and outside the barn.

Total N excretion can be reduced by increasing efficiency of N utilisation. The maximum N retention is set without the application of biotechnological measures to stimulate protein deposition. Thus, the most logical manner to reduce N excretion is to reduce N intake without affecting N retention, resulting in increased N utilisation. Dietary manipulation has been identified as a part of the solution to reduce the environmental impact of swine production. Two feeding strategies that have shown promising results in altering N excretion are reduction of dietary protein content while balancing for AA and inclusion of fermentable fibre in diets.

### 1.5.1 Reduction of dietary protein level

Lowering the dietary protein content may reduce total N excretion, urinary N excretion, and consequently ammonia emission (Gatel and Grosjean 1992; Canh et al. 1998a; Kreuzer et al. 1998) for diets that are properly balanced for AA.

Over-formulation is often employed to maximise pig performance and to account for fluctuations in ingredient quality. In contrast, ensuring an adequate but not excessive supply of nutrients according to the projected growth of the pigs can be an efficient way to reduce N excretion. Accurate diet formulation requires an accurate evaluation of AA requirements and of the digestible AA content in the ingredients used. Reduction of dietary protein content should be handled carefully because any essential AA can potentially become limiting and a slight variation from the calculated AA content can result in a reduced performance.

Reduction of dietary protein content while balancing for AA can be achieved by formulating diets to the same essential AA content using more synthetic AA (Lys, Met, Trp, and Thr) and less intact protein sources. Commercially available AA are the AA that usually are limiting in pig diets. Inclusion of synthetic AA, especially lysine, in pig diets is common because feed costs can be reduced by replacing a portion of the protein source with synthetic AA.

Based on many studies, Kerr and Easter (1995) calculated an average of 8% reduction in N excretion for each percent reduction in dietary protein content. Dourmad et al. (1993) compared three dietary crude protein (CP) levels (17.8, 15.5 and 13.6%) in grower pigs, with increasing levels of synthetic AA supplementation. Faecal, urinary and total N excretion decreased linearly by decreasing the dietary protein level. The improvement of AA profile resulted in a 33% reduction of nitrogen excretion ( $2 \text{ g d}^{-1}$  or 9% reduction for urinary N and  $2.1 \text{ g d}^{-1}$  or 8% reduction in total N for each percent reduction in protein). Latimier and Dourmad (1993) observed a similar relative reduction in N excretion and ammonia emission (7% for each percent reduction in protein) by using low protein diets. Gatel and Grosjean (1992) observed a decrease in urinary N but not in faecal N excretion by reducing dietary protein level from 16.9 and 15.6% to 14.6 and 13.5%, respectively, for grower and finisher pigs ( $1.8$  to  $4 \text{ g/d}$  for each percent protein reduction).

The reduction in N excretion has been related to reduction in ammonia emissions. Aarnick et al. (1993) estimated a 9% reduction of ammonium N content in the manure when dietary protein was reduced by 1%. Canh et al. (1998a) used three dietary protein levels (16.5, 14.5, and 12.5%) with a similar content of net energy (NE) in grower-finisher pigs, and observed for each percentage reduction in protein level a reduction of 11% in urinary N excretion, 9% in total N excretion, 10 to 12.5% in ammonia emission, and 0.25 unit of manure pH. Faecal N excretion was not affected in this study. In grower-finisher pigs, a low protein diet (10% CP) supplemented with synthetic AA reduced ammonium and total N in manure by 41% and 28% compared to 18 and 13% CP diets, respectively (Sutton et al. 1996). Hobbs et al. (1996) showed that reducing the CP content from 21 to 14% CP supplemented with synthetic AA in grower diets and from 19 to 13% CP supplemented with synthetic AA in finisher diets reduced N excretion by 40% and also reduced concentrations for a majority of odorants in the manure. Reduction of the dietary CP level resulted in reduced ammonium concentration and manure pH and consequently in reduction of ammonia emission (Canh et al. 1998a; Sutton et al. 1996). Overall, reduction of urinary N excretion was the main cause for the reduction in total N excretion and ammonia emission when the dietary protein content was decreased.

Carcass characteristics and composition of grower-finisher pigs fed low protein diets vary in studies reported in the literature. Pigs fed with diets reduced by 2 to 3 percent in CP had a similar performance to control pigs (Noblet et al. 1987; Tuitoek et al. 1997; Canh et al. 1998a) without any effect on back fat thickness (Lopez et al. 1994; Hahn et al. 1995; Knowles et al. 1998). Canh et al. (1998a) and Dourmad et al. (1993) used low and high protein diets with a similar NE content and observed a similar performance and carcass characteristics in grower-finisher pigs. Overall, pigs fed low protein diets had a performance and carcass composition similar to those of pigs fed diets in which all AA were provided from an intact protein source.

In some studies, the reduction in protein content was associated with an increase in fat content in the carcass (Noblet et al. 1987; Kerr et al. 1995; Kerr and Easter 1995; Tuitoek et al. 1997). The reason for the increased fatness might be due to an increased NE content of low CP diets. Degradation of excess AA resulted in an increased energy

loss in urine and an elevated heat loss (Noblet et al. 1987). The energy spared due to reduced dietary protein content resulted in increased fat deposition which was not observed when the diets were adjusted to the same net energy content (Dourmad et al. 1993; Canh et al. 1998a). Diets containing dietary fibre have reduced backfat in pigs (Pond et al. 1988, 1989). Reduced backfat may not be a direct effect of fibre but a result of dietary energy dilution since fibre reduces the dietary NE content (Baird et al. 1970). In contrast, Knowles et al. (1998) reported that the inclusion of a source of dietary fibre (3 to 4%) did not reduce fatness of pigs; this was attributed to the low inclusion rate of dietary fibre that could not elicit the energy-diluting effect of fibre.

Plasma urea concentration is also affected by dietary CP and AA levels, similar to changes that occur for N balance. Addition of limiting AA to diets with imbalanced AA patterns results in decrease in blood urea concentrations (Kerr and Easter 1995). Pigs fed low protein, AA-supplemented diets, have reduced plasma urea concentration (Lopez et al. 1994; Kerr and Easter 1995; Ward and Southern 1995), compared with pigs fed diets in which AA were provided entirely from intact protein. A reduced plasma urea content is indicative of a lesser need for deamination of excess AA.

### **1.5.2 Inclusion of the fibre in diets**

Ammonia emission from manure is affected mainly by urea concentration and pH. Nutritional strategies that reduce excretion of urinary urea and manure pH will thus result in a decreased ammonia emission.

In properly formulated diets, inclusion of ingredients high in fermentable fibre does not affect performance or N retention of grower-finisher pigs (Canh 1998b,c). Inclusion of fermentable NSP in the diet shifts N excretion from urea in urine to bacterial protein in the faeces (Morgan and Whittemore 1988; Mroz et al. 1993; Canh et al. 1997). The shift in N excretion pattern occurs because fermentable carbohydrates serve as an energy source for intestinal microflora. The microflora also requires N for protein synthesis, provided by dietary protein that escapes digestion, endogenous protein and diffusion of blood urea into gastrointestinal tract (Mason 1984). Blood urea is the largest and most-readily available source of N for bacterial protein synthesis in the caecum (Levrat et al. 1993). Urea in the large intestine is broken down by bacterial

urease to ammonia, which is subsequently used for microbial protein synthesis. Synthesis of microbial protein causes less ammonia to be reabsorbed from the colon resulting in a reduced urea and ammonia content in the portal plasma (Younes et al. 1993). Addition of starch in the caecum to stimulate bacterial growth increased faecal N excretion of bacterial N and reduced ammonia absorption in the colon, thereby reducing urinary N excretion (Moshenthin et al. 1992b). In summary, at unchanged body N retention, N excretion is shifted from urea in urine to bacterial protein in faeces by dietary inclusion of fermentable NSP in the diets.

An increased intake of fermentable fibre increased faecal N excretion and reduced urinary N excretion while N retention was increased (Morgan and Whittemore 1988). Pigs fed high NSP diets (by-products and SBP) excreted less N in urine and more in faeces than pigs fed with low NSP content diets but total N excretion and N retention were not affected by the diets (Canh et al. 1997). The effect of the high NSP was attributed to cellulose of the diets although SBP is high in pectin (Bach Knudsen 1997). Mroz et al. (1993) observed that cellulose had the highest effect on shifting N excretion from urine to faeces, pectins had the lowest effects, whereas hemicellulose gave intermediate results.

Urinary N is easily converted into ammonia, which is subsequently emitted into the air. Fecal N is less easily degraded into ammonia. Thus, shifting N excretion patterns from urea in urine to less degradable bacterial protein in faeces, by inclusion of fibrous ingredients in diets, results in a reduction of ammonia emission. Kreuzer and Machmuller (1993) showed that an increase of fermentable NSP in pig diets from 10 to 22% reduced urinary N excretion from 39 to 35% and increased faecal N excretion from 20 to 28%. Gaseous N losses were reduced linearly by 0.6% for each additional percent of dietary NSP. Mroz et al. (1993) showed that cellulose reduced ammonia emissions from manure compared to manure from pigs fed cornstarch, hemicellulose, or pectin.

Fermentable NSP also decrease manure pH by VFA formation in faeces and manure (Canh et al. 1997; 1998b, c) and consequently reduce the ammonia emission from manure (Canh et al. 1998b, c). The VFA are mainly formed by bacterial fermentation of dietary fibre in the hindgut of the pigs and by anaerobic digestion of the manure during storage (Canh et al. 1998b, c). Rates of VFA production depend on the

inclusion rate and characteristics of fermentable fibre. Total amount of VFA in the manure was positively related to cellulose and hemicellulose whereas lignin content had a negative effect (Canh et al. 1997; 1998c).

Canh et al. (1998c) found that 30% SBP in the diet reduced manure pH by 0.8 units (from 8.9 to 8.1) and ammonia emission by 52% compared to the control diet. Total N, ammonia and pH were reduced in fresh manure from pigs fed with low-protein AA supplemented diets, and the addition of 5% cellulose further reduced ammonia N and total N excretion (Sutton et al. 1997). Canh et al. (1998b) found a linear relationship between intake of dietary NSP and ammonia emission by using coconut expeller, soybean hulls, and sugar beet pulp. For each 100 g increase in intake of dietary NSP, manure pH decreased by 0.12 units and ammonia emission from manure was decreased by 5.4%. Soybean hulls had a greater effect on decreasing pH and ammonia emission than sugar beet pulp and coconut expeller. Recently, inclusion of soybean hull and beet pulp in diets did not affect partitioning of N excretion but reduced ammonia emission, with soybean hulls being the most efficient (Mroz et al. 2000). The reduction of ammonia emission was attributed to a reduced pH of the manure.

In conclusion, dietary fermentable NSP reduces urinary urea excretion and decreases manure pH by VFA formation in faeces and manure resulting in a reduction of ammonia emission from manure (Canh et al. 1997; 1998b, c).



## 2. EFFECTS OF DIETARY PROTEIN AND OAT HULLS ON NITROGEN EXCRETION PATTERNS AND PLASMA UREA PROFILES

### 2.1 Abstract

The objectives of the experiment were to study the effect of dietary protein content and oat hulls on nitrogen (N) excretion patterns and to relate plasma urea (PU) to urinary N excretion. Three dietary protein contents (high, 197; medium, 169; low, 138 g kg<sup>-1</sup>) and two levels of fibre (high and low) were tested. Diets (wheat, barley, soybean meal; oat-hulls as a fibre source) were formulated to 3250 kcal DE kg<sup>-1</sup> and at least 2.18 g digestible lysine per Mcal DE, supplemented with Lys, Met, Trp, Thr, Ile, or Val to ensure meeting an ideal AA profile. Pigs (32 ± 3.4 kg; n = 42) were housed in confinement-type metabolism crates for 19 d. On d 10 or 11, catheters were installed by cranial vena cava venipuncture. Daily feeding rates were adjusted to three times maintenance (3 x 110 kcal DE kg<sup>-1</sup> BW<sup>0.75</sup>), and daily rations were halved to two equal meals. Faeces and urine were collected from d 15 to 19. Five blood samples were collected in two-h intervals on d 16 and 19. Faecal N, urinary N, and total N excretion were reduced linearly with a reduction of dietary protein content (P < 0.001) with the reduction being greater for urinary (48%) and total N excretion (40%) than faecal N (23%) excretion. The ratio of urinary N to faecal N was reduced linearly with a reduction of dietary protein content (P < 0.001). Retention of N (g d<sup>-1</sup>) was reduced linearly but N retention as percentage of intake was increased linearly with a reduction of dietary protein content (P < 0.001). Addition of oat hulls did not affect N excretion patterns and PU concentrations (P > 0.10). Dietary treatments did not affect ADG or feed efficiency (P > 0.10). Plasma urea increased after feeding, peaked at 4 h after feeding and then decreased toward pre-feeding concentrations. A linear relationship of urinary N to PU concentration (R<sup>2</sup> = 0.66) was observed. Reduction of dietary protein

content is an efficient way to reduce total and urinary N excretion, which may result in substantial reduction of ammonia emission.

## **2.2 Introduction**

The intensification and expansion of the pig production in the last decades has raised environmental concerns because pig manure can cause pollution, if not managed properly. Nitrogen (N) excretion is of great concern because of its impact on inside and outside barn environment. Both total N excretion and ammonia emission should be reduced to alleviate the environmental impact of N. Reduction in total N excretion will result in higher quantities of manure per hectare of arable land. Ammonia emission, which is a noxious gas for humans and animals and contributes to bad odour and the acidification of the environment, should be minimised to improve the air quality inside the pig barn. The main component of ammonia emission originates from urea in urine. Urinary N is easily emitted into the air as ammonia. Faecal nitrogen is less volatile than urinary nitrogen, because faecal nitrogen is bound chemically within proteins or other compounds. Thus reduction in urinary N excretion will reduce ammonia emission (Canh et al. 1997).

Dietary manipulation might become a powerful tool to reduce the negative effects of swine production on the environment. Reduction of dietary protein is a direct manner to reduce N excretion and ammonia emission (Dourmad et al. 1993; Canh et al. 1998a). Nitrogen excretion is shifted from urea in urine to bacterial protein in faeces when fermentable carbohydrates are included in the diet resulting in a reduction of ammonia emission (Morgan and Whittemore 1998; Canh et al. 1998b). The combined effects of these two nutritional strategies to alter N excretion patterns has not been investigated.

Blood urea concentration and urinary urea are related (Brown and Cline 1974). An increase in the plasma urea coincides with an increase in urinary urea excretion; however, models to predict the relationship have not been established.

The objectives of the experiment were to study the effect of dietary protein and fibre on nitrogen (N) excretion patterns and to relate plasma urea (PU) to urinary N excretion.

## 2.3 Materials and Methods

### 2.3.1 Experimental protocol

The animal protocol was approved by the University of Saskatchewan-Committee on Animal Care and Supply, and followed principles established by the Canadian Council on Animal Care (1993). Three dietary protein contents (high, 197; medium, 169; and low, 138 g CP kg<sup>-1</sup>) and two levels of dietary fibre (low and high) were tested in a 3 x 2 factorial arrangement for a total of 6 treatments. The main ingredients of the diets were barley, wheat, soybean meal, corn starch, canola oil, and synthetic AA; chromic oxide was included as an indigestible marker (Table 2.1). Oat hulls were added as a fibre source for the high fibre diets. Experimental diets were formulated to 3.25 Mcal DE kg<sup>-1</sup> and at least 2.18 g digestible lysine per Mcal DE (Table 2.2). Diets were supplemented with synthetic AA to balance to an ideal AA ratio (Table 2.2). Brill<sup>®</sup> feed formulation software (Version 7, Brill Corporation) was used for the diet formulation to ensure that diets met nutrients requirements. A total of 42 crossbred barrows (Camborough-15 x Canabrid, Pig Improvement Canada, Acme, AB, initial BW 32 ± 3.4 kg) were selected in three groups. In the first and second group, 12 pigs were assigned to the treatments and 18 pigs in the third group, for a total of seven observations per treatment.

The 19-d experimental period consisted of a 14-d adaptation to diets and metabolism crates and a 5-d collection of faeces and urine. Pigs were placed individually in steel confinement-type metabolic crates (0.71 x 1.83 m), which allowed separate collection of urine and faeces. Throughout the experiment, diets were fed in wet-mash form, in a 1:1 water to mash ratio. Daily feed allowance was adjusted to 3 x maintenance (3 x 110 kcal DE kg<sup>-1</sup> BW<sup>0.75</sup>), which was fed in two equal meals at 0800 and 1600. Water was supplied ad libitum through a nipple drinker. Pigs were housed in an environmentally controlled room with an average temperature of 21°C. Lights were turned on at 0700 and off at 1900.

During the 5-d collection, representative samples were collected for each diet. Faeces were collected twice daily, pooled and stored at -20°C. Urine was collected twice daily, weighed, and a 5% aliquot was stored at -20°C. Twenty mL of hydrochloric

**Table 2.1** Composition of experimental diets (as-fed basis).

Protein content Ingredient, %	Diets					
	Low Fibre			High Fibre		
	Low	Medium	High	Low	Medium	High
Barley	49.4	40.5	40.0	50.1	49.9	43.5
Wheat	21.8	28.3	25.7	20.0	21.3	20.4
Soybean meal	10.0	17.2	24.8	10.0	16.7	25.1
Corn starch	13.8	9.9	5.6	7.4	-	-
Oat hulls	-	-	-	5.0	5.0	5.0
Canola oil	0.5	-	-	3.0	3.3	2.5
Mineral premix <sup>1</sup>	0.5	0.5	0.5	0.5	0.5	0.5
Vitamin premix <sup>2</sup>	0.5	0.5	0.5	0.5	0.5	0.5
Salt	0.4	0.4	0.4	0.4	0.4	0.4
Chromic oxide	0.4	0.4	0.4	0.4	0.4	0.4
Limestone	1.130	1.400	1.400	1.113	1.150	1.150
Dicalcium phosphate	0.897	0.740	0.650	0.878	0.673	0.556
HCl-Lysine	0.363	0.143	-	0.361	0.147	-
L-Threonine	0.158	0.055	-	0.157	0.054	-
DL-Methionine	0.094	0.012	-	0.096	0.017	-
L-Isoleucine	0.020	-	-	0.022	-	-
L-Valine	0.035	-	-	0.029	-	-
L-Tryptophan	0.020	-	-	0.021	-	-

<sup>1</sup>Provided per kg of premix: zinc, 200 mg; iron, 160 mg; manganese, 50 mg; copper, 100 mg; iodine 100 mg; selenium, 20 mg.

<sup>2</sup>Provided per kg of premix: vitamin A, 650,000 IU; vitamin D<sub>3</sub>, 165,000 IU; vitamin E, 8,000 IU; menadione, 800 mg; thiamine, 200 mg; riboflavin, 1,000 mg; d-pantothenic acid, 3,000 mg; niacin 7,000 mg; B<sub>12</sub>, 5 mg; d-biotin, 40 mg; folic acid, 400 mg.

**Table 2.2** Chemical characteristics of experimental diets (as-fed basis).

Protein content	Diets					
	Low Fibre			High Fibre		
	Low	Medium	High	Low	Medium	High
Calculated nutrient level						
DE, Mcal kg <sup>-1</sup>	3.25	3.25	3.25	3.25	3.25	3.25
CP, %	13.60	16.50	19.50	13.60	16.50	19.50
EE, %	1.87	1.48	1.53	4.42	4.77	3.93
CF, %	3.50	3.50	3.70	5.00	5.30	5.28
ADF, %	5.00	5.13	5.57	7.19	7.71	7.85
NDF, %	12.10	11.86	12.20	16.00	16.74	16.30
Dlysine <sup>1</sup> , g Mcal <sup>-1</sup> DE	2.18	2.18	2.39	2.18	2.18	2.40
Total lysine, %	0.84	0.86	0.96	0.84	0.87	0.97
Calcium, %	0.65	0.75	0.75	0.65	0.65	0.65
Phosphorus						
Total, %	0.50	0.50	0.52	0.50	0.50	0.50
Available, %	0.28	0.27	0.27	0.28	0.26	0.25
Apparent Ileal Digestible AA <sup>2</sup>						
Lysine, %	0.71	0.71	0.78	0.71	0.71	0.78
Threonine, %	0.47	0.47	0.52	0.47	0.47	0.52
Methionine, %	0.26	0.22	0.25	0.26	0.23	0.25
Tryptophan, %	0.13	0.14	0.17	0.13	0.14	0.17
Isoleucine, %	0.41	0.50	0.62	0.41	0.51	0.62
Leucine, %	0.72	0.93	1.13	0.71	0.93	1.14
Valine, %	0.48	0.57	0.69	0.47	0.56	0.68
Analysed nutrient level %						
DE, Mcal kg <sup>-1</sup>	3.23	3.17	3.22	3.11	3.17	3.14
CP, %	13.6	16.8	19.1	14.2	17.0	20.2
EE, %	1.9	1.5	1.4	4.3	4.7	3.8
CF, %	3.4	3.7	3.8	5.0	4.9	5.1
ADF, %	4.0	3.8	4.3	5.6	5.3	5.4
NDF, %	11.5	12.1	12.8	15.9	15.7	15.8

<sup>1</sup>Digestible lysine

<sup>2</sup>Ideal pattern of apparent digestible AA compared to lysine (%): lysine 100; threonine 61; methionine 28; tryptophan 17; leucine 99; isoleucine 55; valine 66; phenylalanine 59 (PSCI 2000).

acid were added to the collection container at the start of each collection to prevent volatilization of urinary nitrogen. On d 16 and 19, five blood samples were collected from pigs with catheters in 2-h intervals starting immediately before the morning feeding, for a total of 10 blood samples per pig. For pigs without the catheters, blood samples were collected before morning feeding (0800) and 4 h (1200) after feeding on d 19 via jugular vein venipuncture. Blood samples were centrifuged, and plasma was frozen at -20°C until analyses. After the collection, faecal samples were thawed, homogenised, sub-sampled, and freeze-dried. Urine was thawed, homogenised, and sub-sampled.

### 2.3.2 Catheterization procedure

On d 10 or 11, pigs were catheterized in the vena cava using clear vinyl tubing (Dural Plastics and Engineering, Auburn, NSW, Australia, O.D 1.5 mm, I.D. 1.0 mm), 60 cm length according to procedures described by Kingsbury and Rawlings (1993) with modifications. The procedure lasted up to 20 min, and general anaesthesia was not required.

Catheters were inserted under diazepam (Valium, Hoffmann-LaRoche Ltd, Etobicoke, Ontario, Canada; 0.5 mg kg<sup>-1</sup>) and ketamine (M.T.C. Pharmaceuticals, Cambridge, Ontario, Canada; 5 mg kg<sup>-1</sup>) anaesthesia, which were administered through the ear vein. With the pig in dorsal recumbency, the catheter was inserted through a 14-gauge thin-walled needle, directed toward the midline at an angle of 30° to both the median and frontal planes approximately 2.5 cm anterior to and 2.5 cm lateral to the manubrium sterni. Once the catheter was in the vein, the 14-gauge needle was removed and an adapter and plug was fitted to the end of the tubing. The catheter was withdrawn until approximately 15 cm remained inside the pig. The catheter was secured and protected in place with Elastoplast tape (Smith and Nephew, Lachine, Quebec, Canada) and was accessible from the side of the neck. Catheters were maintained potent by flushing heparinized saline (10 U ml<sup>-1</sup>) once daily.

Pigs recovered quickly from the catheterization procedure, and were fed their allowance in the afternoon. Out of 36 attempts, 25 catheters were installed successfully. Twenty-one catheters remained functional throughout the entire collection.

### 2.3.3 Chemical analyses

Feed and freeze-dried faecal samples were ground through a 1-mm screen on a Retch mill. Chemical analyses were conducted in duplicate. Dry matter content of feed and faeces was determined by drying at 135°C in an airflow type oven for 2 h (method 930.15; AOAC 1990). Feed, faecal, and urinary samples were analysed for nitrogen by combustion method (method 968.06; AOAC 1990) using a Leco protein/nitrogen determinator (model FP-528, Leco Corporation, St Joseph, MI). Chromic oxide content was analysed in feed and faeces according to the Fenton and Fenton (1979) procedure with a Pharmacia LKB-Ultrospec III spectrophotometer (model 80-2097-62), at 440 nm after ashing at 450°C overnight.

Gross energy in feed and faeces was measured in a Parr adiabatic bomb calorimeter (model 1281, Parr Instrument Co. Moline, IL). Benzoic acid (3415, Parr Instrument Co.) was utilised as a caloric standard for calibration. Feed was analysed for crude fibre (method 978.10; AOAC 1990) and EE content (method 920.39; AOAC 1990). Acid detergent fibre (ADF) and neutral detergent fibre (NDF) content were determined using Ankom<sup>200</sup> fibre analyser (Ankom Technology Co., Fairport, MI) according to the procedures described by the Ankom 220/200 Fiber Analyzer Operator's Manual (Ankom 1998). Plasma samples were analysed for urea using the Abbott Spectrum Urea Nitrogen Test (Abbott Spectrum Series II, Abbot Laboratories, Dallas, TX). Following analyses, nitrogen retention, digestibility of nitrogen and energy, and digestible energy were calculated using chromium oxide concentration in diets and faeces. Degussa AG (Allandale, NJ) conducted AA analyses of feed samples (method 994.12; AOAC 1995). Methionine and cystine were determined as methionine sulfone and cysteic acid after oxidation with performic acid. Tryptophan content was determined after alkaline hydrolysis with lithium hydroxide by means of reversed phase high performance liquid chromatography.

### 2.3.4 Statistical analyses

Individual pig was considered the experimental unit. Variables were analysed using the General Linear Models (GLM) procedure of SAS (1996). The statistical model included effects for dietary treatment (dietary protein content, fibre level, and dietary protein by fibre interaction) and period. Orthogonal contrasts for linear and quadratic effect of dietary protein content were examined. Means comparisons were performed using the probability of difference (Pdiff). Pearson's correlation coefficients were calculated to determine the degree of association between urinary N and PU. Regression analysis was used to predict urinary N as a function of PU. Repeated measures analysis was used to evaluate the time effect on PU concentration. Values are reported as least square means.

## 2.4 Results

### 2.4.1 Nitrogen balance

Health problems did not occur during the experiment, and feed refusals were not observed. Amino acid analysis showed that the actual AA concentration was very close to the calculated values and that diets were balanced according to ideal protein ratio (Table 2.3). Lysine content increased with increasing the dietary protein content and it was higher for the high fibre compared to low fibre diets. As expected, N intake was different among treatments (Table 2.4;  $P < 0.001$ ) following dietary protein patterns. Nitrogen intake decreased linearly and quadratically with decreasing dietary protein content ( $P < 0.001$ ) with a dietary protein and fibre interaction ( $P < 0.05$ ). The quadratic response was due to unequal spacing among analysed dietary protein contents within low fibre levels and equal spacing within high fibre diets, which also explains the interaction observed between dietary protein and fibre.

Faecal, urinary, and total N excretion ( $\text{g d}^{-1}$ ) decreased linearly with decreasing dietary protein content (Table 2.4;  $P < 0.01$ ). For low compared to high protein diets, faecal, urinary and total N excretion were reduced by 23%, 48%, and 40%, respectively.



**Table 2.3** Total amino acid content of experimental diets (as-fed basis).

Protein content	Diets					
	Low Fibre			High Fibre		
	Low	Medium	High	Low	Medium	High
Amino acid, %						
Calculated						
Lysine	0.84	0.86	0.96	0.84	0.87	0.97
Threonine	0.60	0.63	0.71	0.60	0.63	0.71
Methionine	0.29	0.26	0.29	0.30	0.27	0.30
Tryptophan	0.17	0.19	0.23	0.17	0.19	0.23
Analysed						
Lysine	0.83	0.92	0.99	0.86	0.88	1.01
Threonine	0.64	0.67	0.74	0.65	0.68	0.76
Methionine	0.30	0.27	0.30	0.31	0.29	0.31
Tryptophan	0.20	0.25	ND <sup>2</sup>	0.19	0.23	ND
Cysteine	0.28	0.32	0.36	0.28	0.33	0.37
TSAA <sup>1</sup>	0.58	0.59	0.66	0.59	0.62	0.68
Arginine	0.78	1.02	1.28	0.82	1.02	1.30
Isoleucine	0.55	0.70	0.84	0.57	0.68	0.86
Leucine	0.93	1.19	0.14	0.96	1.18	1.45
Valine	0.69	0.81	0.96	0.70	0.81	0.98
Histidine	0.32	0.41	0.49	0.32	0.40	0.49
Phenylalanine	0.65	0.81	0.97	0.67	0.81	0.99

<sup>1</sup>TSAA = Total sulfur amino acids

<sup>2</sup>ND = not determined

**Table 2.4** Effects of dietary protein content and fibre level on N balance, energy and N digestibility, DE content, PU concentration and performance in grower pigs.

Protein content Variable	Diets						<i>Pooled</i> <i>SEM</i>	Effects <sup>1</sup>		Contrast <sup>2</sup>	
	Low Fibre			High Fibre				Fibre	CP x Fibre	Linear	Quadratic
	Low	Medium	High	Low	Medium	High					
Number of pigs	7	7	7	7	7	7					
N intake, g d <sup>-1</sup>	34.1	42.1	47.9	35.6	42.6	50.6	0.02	.0001	.0001	.0001	.0001
Faecal N, g d <sup>-1</sup>	6.1	6.8	8.0	6.7	7.5	8.6	0.4	.0584	NS <sup>3</sup>	.0001	NS
Urinary N, g d <sup>-1</sup>	9.5	14.4	17.6	8.8	13.8	17.6	0.8	NS	NS	.0001	NS
N excretion, g d <sup>-1</sup>	15.6	21.2	25.6	15.5	21.2	26.2	0.7	NS	NS	.0001	NS
N retention, g d <sup>-1</sup>	18.5	20.9	22.3	20.0	21.4	24.4	0.7	.0287	NS	.0001	NS
Urinary/faecal N	1.6	2.2	2.3	1.3	1.9	2.1	0.2	.0514	NS	.0004	NS
Faecal N, % of intake	17.8	16.1	16.7	18.8	17.5	17.0	1.0	NS	NS	NS	NS
Urinary N, % of intake	27.9	34.3	36.7	24.8	32.3	34.6	1.8	NS	NS	.0001	NS
N excretion, % of intake	45.7	50.3	53.4	43.7	49.8	51.8	1.8	NS	NS	.0001	NS
N retention, % of intake	54.3	49.7	46.6	56.3	50.2	48.2	1.8	NS	NS	.0001	NS
N digestibility, %	82.9	83.9	83.6	81.2	82.5	82.9	0.9	NS	NS	NS	NS
Plasma urea <sup>4</sup> , mmol L <sup>-1</sup>											
Before feeding	2.9	4.0	5.5	3.3	3.7	4.8	0.4	NS	NS	.0001	NS
4 h after feeding	3.4	4.9	6.7	3.5	5.0	6.3	0.3	NS	NS	.0001	NS
Energy											
Digestibility, %	86.1	84.2	84.1	80.5	80.6	80.3	0.5	.0001	.0811	.0404	NS
DE, Mcal kg <sup>-1</sup>	3.473	3.249	3.274	3.109	3.211	3.239	0.020	.0001	.0001	.0821	.0143
ADG <sup>5</sup> , kg d <sup>-1</sup>	0.550	0.543	0.581	0.537	0.558	0.579	0.026	NS	NS	NS	NS
Feed efficiency <sup>6</sup>	0.368	0.370	0.396	0.366	0.380	0.395	0.018	NS	NS	NS	NS

<sup>1</sup>Fibre = Main effect of fibre; CP x Fibre = protein level by fibre interaction.

<sup>2</sup>Linear and quadratic effects of protein

<sup>3</sup>NS = not significant (P > 0.10)

<sup>4</sup>Plasma urea concentration on d19

<sup>5</sup>Calculated for a period of 8 days.

<sup>6</sup>Feed efficiency = kg weight gain per kg of feed intake

The ratio of urinary to faecal N decreased linearly with decreasing dietary protein content ( $P < 0.001$ ), resulting in a 32% reduced ratio for low compared to high protein diets. Nitrogen retention was reduced linearly with decreasing dietary protein content ( $P < 0.001$ ) resulting in a 17% reduction for low compared to high protein diets.

Expressed as percentage of intake, urinary and total N excretion decreased linearly, whereas N retention was increased linearly with decreasing dietary protein content (Table 2.4;  $P < 0.01$ ). Faecal N excretion was not affected ( $P > 0.10$ ). Urinary N was reduced by 9 percentage units for the low compared to high protein diet. Total N excretion was decreased by 8 percentage units for the low compared to high fibre diet whereas N retention was increased to the same extent.

Nitrogen intake was higher for high fibre than low fibre diets (Table 2.4;  $P < 0.01$ ). The differences observed for N excretion patterns and N retention, expressed as  $\text{g d}^{-1}$ , may be due to differences in N intake. Therefore, effects of dietary fibre in N excretion patterns will be discussed as a % of N intake. Dietary fibre did not affect faecal, urinary, total N excretion and N retention ( $P > 0.10$ ).

#### **2.4.2 Energy and nitrogen digestibility and animal performance**

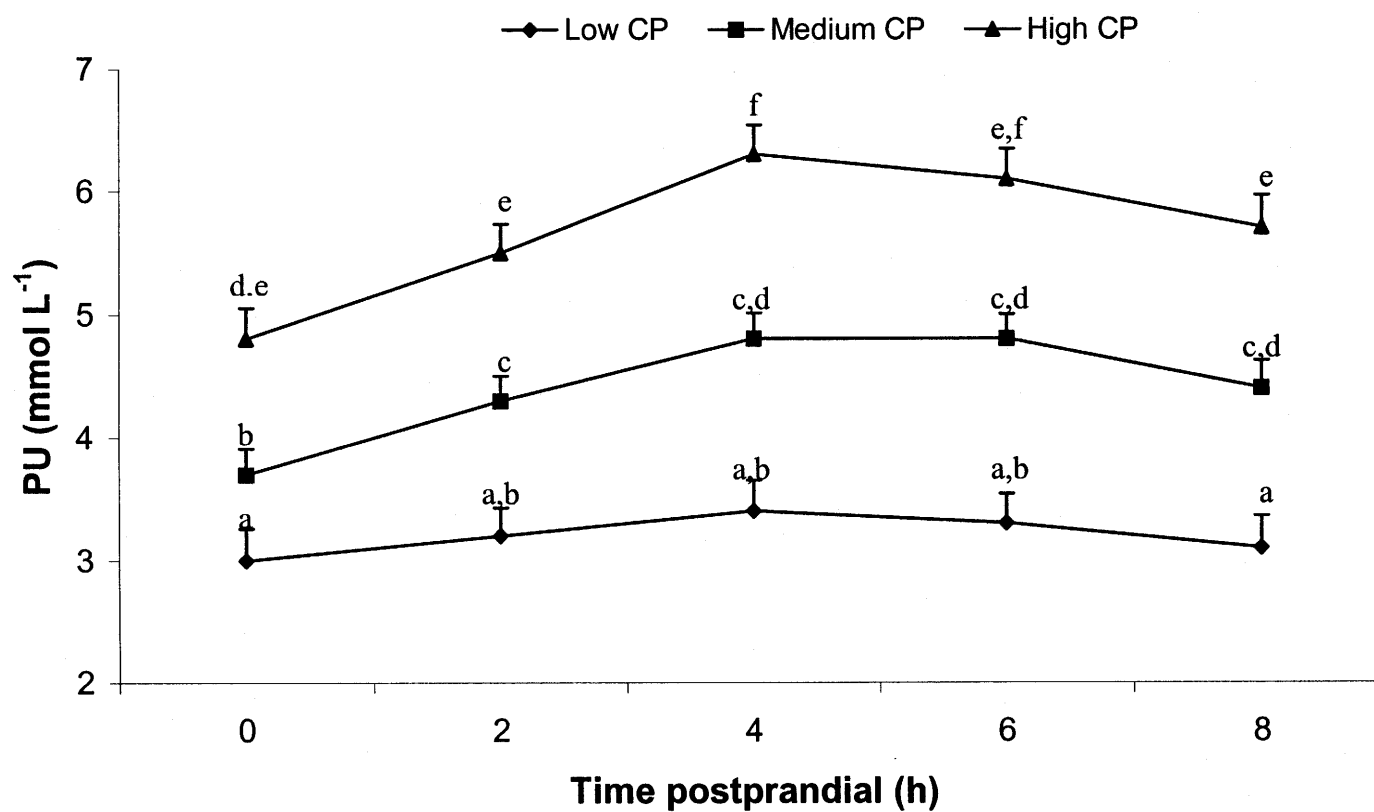
Although diets were formulated to an equal DE content ( $3.25 \text{ Mcal kg}^{-1}$ ), differences among diets were measured (Table 2.4). Digestible energy content was 4.3% lower for the low protein-high fibre diet and 6.8% higher for the low protein-low fibre diet, compared to calculated values. Medium and high protein diets for both fibre levels were closer to calculated values. A quadratic response ( $P < 0.05$ ) of DE to dietary protein content was observed. Digestible energy content was affected by dietary fibre content ( $P < 0.001$ ) with a dietary protein by fibre interaction ( $P < 0.001$ ). Overall, DE content was 4.4% lower for oat hulls compared to control diets ( $P < 0.001$ ). Specifically, for the high fibre diets, DE content was reduced 10.5% for low and 1.1% for high protein diets compared to low fibre diets ( $P < 0.05$ ). For medium protein diets, DE content was similar for low and high fibre diets ( $P > 0.10$ ).

Energy digestibility increased linearly (Table 2.4;  $P < 0.05$ ) with decreasing dietary protein content, resulting in 1.2% increase in energy digestibility for the low compared to high protein diets. Energy digestibility was affected by dietary fibre ( $P < 0.001$ ), resulting in a reduction of digestibility by 4.3 percentage units for the high compared to low fibre diet. The interaction of dietary protein with fibre tended to be significant ( $P < 0.10$ ). Energy digestibility was similar among high fibre diets, but 2 percentage units higher for low compared to medium and high protein diets within the low fibre diets. Nitrogen digestibility, although it ranged from 81.2 to 83.9% among treatments, was not affected by dietary protein or fibre ( $P > 0.10$ ). Dietary treatments did not affect ADG or feed efficiency ( $P > 0.10$ ).

### 2.4.3 Plasma urea

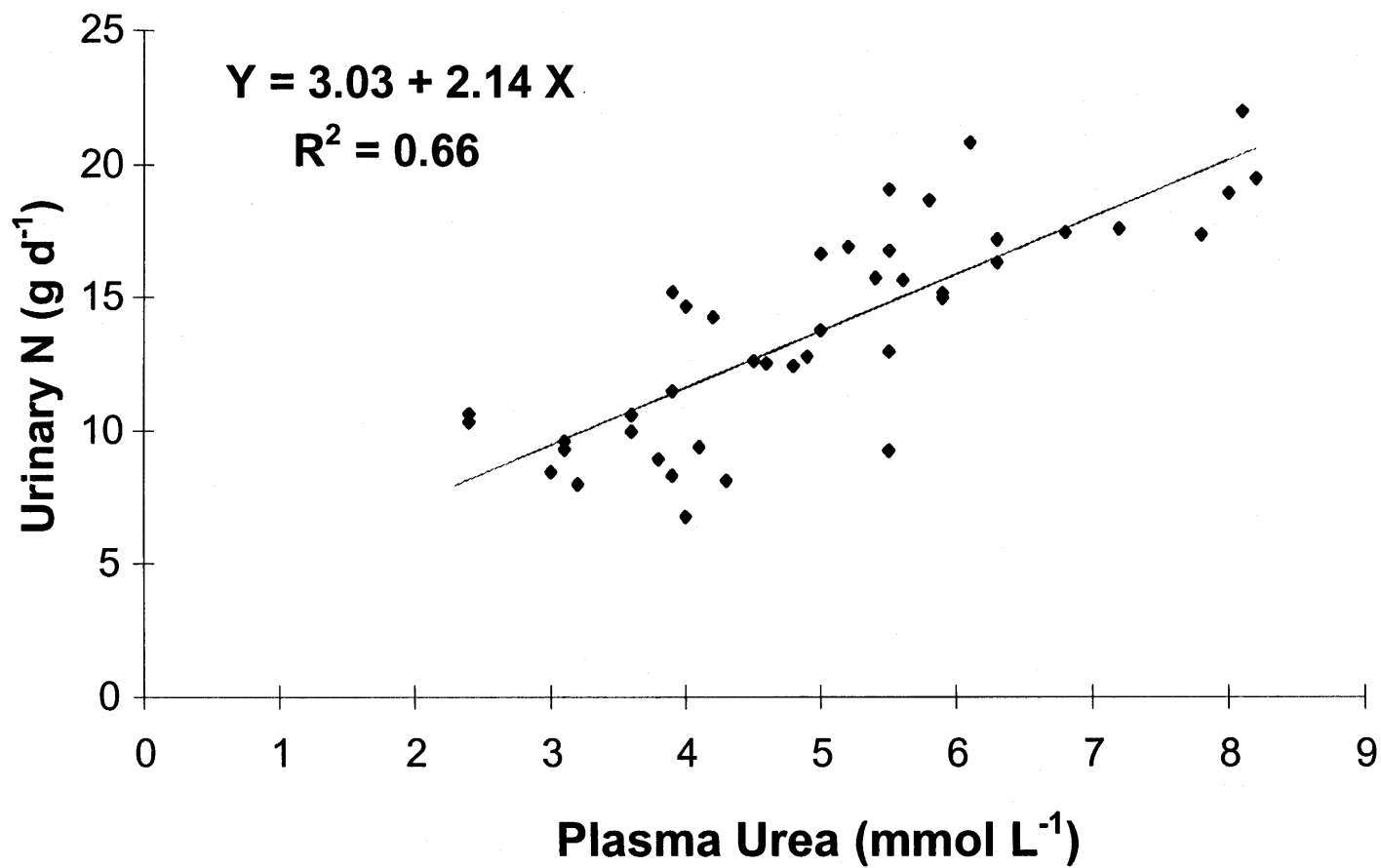
Plasma urea (PU) concentration was measured for 42 pigs (observations) before feeding and 4 h after feeding on d 19. A reduction of dietary protein reduced PU linearly (Table 2.4;  $P < 0.001$ ) at both sampling times. Before feeding, PU concentration was reduced 40% for low ( $3.1 \text{ mmol L}^{-1}$ ) compared to high ( $5.2 \text{ mmol L}^{-1}$ ) protein diets. Four h after feeding, PU was reduced 48% for low protein ( $3.4 \text{ mmol L}^{-1}$ ) compared to high ( $7 \text{ mmol L}^{-1}$ ) protein diets. Dietary fibre did not affect PU concentration ( $P > 0.10$ ).

The PU data from catheterized pigs indicated the dietary protein, time, and dietary protein by time interaction exhibited a significant effect on PU concentration (Figure 2.1;  $P < 0.001$ ). Plasma urea increased after feeding for high and medium protein diets, and peaked approximately 4 h after feeding, before declining towards pre-feeding concentrations by 10 h after feeding (Figure 2.1;  $P < 0.05$ ). Differences for the low protein diet among the different sampling times were not detected ( $P > 0.05$ ). Plasma urea values at 4 h after feeding had the highest correlation to urinary N ( $r = 0.81$  for d 16 and 19) compared to the other sampling times, coinciding with the sampling time with maximum PU concentrations. Models to predict urinary N as a function of PU were developed using regression analyses. Among sampling times, the best model was obtained from PU concentration 4 h after feeding ( $R^2 = 0.66$ , Figure 2.3).



**Figure 2.1** Effects of dietary protein and time of sampling on plasma urea (PU) concentration (average from d 16 and 19). Sampling time interacted with dietary protein content ( $P < 0.001$ ).

<sup>a-f</sup> Different letters denote significant differences ( $P < 0.05$ ).



**Figure 2.2** Relationship of urinary N to plasma urea concentration at 4 h after feeding on d 19 ( $R^2 = 0.66$ ;  $n = 42$ ).

## 2.5 Discussion

In the present study, reduction of dietary protein content while balancing for digestible AA reduced faecal, urinary, and total N excretion. The reduction in urinary N excretion coincided with a reduction in PU concentration. The increase in dietary fibre by using oat hulls in the diet did not affect N excretion patterns or PU concentration.

Reduction of dietary protein content decreased faecal, urinary, and total N excretion similar to Dourmad et al. (1993). In contrast, Gatel and Grosjean (1992) and Canh et al. (1998a) observed a reduced urinary N and total N excretion by reducing dietary protein content without a reduction of faecal N excretion. Faecal N excretion was not reduced due to a decrease in N digestibility, which compensated for N intake differences. In the present study, digestibility of N was not different among dietary protein content, indicating that differences in faecal N excretion were due to changes in N intake. The effect of dietary protein content was greater for urinary and total N excretion than for faecal N excretion. For each percent reduction in dietary protein content, urinary N excretion was reduced 8% or ( $1.4 \text{ g d}^{-1}$ ) and total N excretion was reduced by 7% (or  $1.7 \text{ g d}^{-1}$ ), whereas faecal N excretion was reduced only by 4% (or  $0.3 \text{ g d}^{-1}$ ). Ideally, each percent reduction in dietary protein content will result in  $1.6 \text{ g d}^{-1}$  reduction in urinary N and total N excretion for each kg of feed consumed, if N retention and fecal N excretion are not affected. For each percent reduction of dietary protein content, the reduction in N excretion ranged from 8 to 20% (or  $1.9$  to  $4 \text{ g d}^{-1}$ ) in urine and 8 to 19% (or  $2$  to  $5 \text{ g d}^{-1}$ ) for total (Dourmad et al. 1993; Gatel and Grosjean 1992; Canh et al. 1998a). Values observed in the present study are closer to the lower end of the range reported. Results from this study support the concept that low protein diets that are supplemented with AA supplemented can reduce total N excretion, and especially urinary N excretion.

In the present study, N retention was reduced for the low and medium protein diet compared to the high protein similar to Kerr and Easter (1995) and Lenis et al. (1999). In contrast, Canh et al. (1998a) and Dourmad et al. (1993) did not observe any effect of protein level on the N retention although N retention was reduced numerically following dietary protein pattern. A reduction in N retention observed in the present study might be attributed to AA imbalances (Kerr and Easter 1995) or to the higher

levels of intake for most of the AA for the medium and high protein diets. Diets were formulated with six amino acids being equally limiting. Thus, deviations of actual ileal digestible AA content from estimated values might have affected the AA balance. Total AA analysis of diets indicated that AA content of the diets was balanced according to ideal protein ratio. Because pigs were under restricted feed intake the maximum potential for lean tissue was not achieved. Thus, based on total AA analysis, higher level of AA intake resulted in the differences in N retention. The profile of AA indicated that lysine was the first limiting AA responsible for the linear reduction in N retention with the reduction of dietary protein content. High fibre diets, which also had slightly higher lysine content than the lower fibre diets, responded with an increased N retention, supporting the hypothesis that lysine was the limiting factor for N retention. According to another theory, N retention of low protein diets might have been reduced due to a lower utilisation of synthetic AA when pigs are fed infrequently instead of having free access to feed (Partridge et al. 1985; Batterham et al. 1984). Expressed as percentage of intake, N retention increased linearly with decreasing dietary protein content indicating that efficiency of N utilisation was increased.

Reduction of dietary protein level did not affect ADG and feed efficiency. Similar results have been reported by Dourmad et al. (1993), Canh et al. (1998a), and Latimier and Dourmad (1993). Tuitoek et al. (1997) reduced dietary protein content from 16.6 to 13% without negative effect on growth rate, feed intake, feed efficiency and carcass characteristics. In the present study, although ADG and feed efficiency were not affected by dietary protein content, N retention was reduced for low and medium compared to high protein diet. It would be expected that ADG and N retention would follow the same pattern. The discrepancy between ADG and N retention can be attributed to a higher fat deposition for pigs fed low and medium protein diets because of energy sparing due to low protein content (Noblet et al. 1987). In addition, numerically ADG and feed efficiency were in agreement with the N retention. Considering that ADG and feed efficiency were measured over a short period of time, differences might have been detected if performance was measured for a longer period or if more observations were made.



Oat hulls are a by-product of oats during milling of oats for human and animal consumption (oat groats) and represent 25% of the total grain. Oat hulls have a high concentration of dietary fibre primarily in cellulose and lignin (Bach Knudsen 1997). Inclusion of the hulls in diets did not affect N retention, digestibility, and excretion patterns (% of intake) or PU. The small differences observed in faecal N excretion and N retention (in  $\text{g d}^{-1}$ ), for high compared to low fibre diets, are due to slightly higher N intake.

Oat hulls might not have affected N excretion in urine for three reasons. First, 5% of oat hulls were included in the high fibre diet, which might be an inclusion level that was too low to cause any effect on N excretion patterns. Second, because of the high cellulose content (Bach Knudsen 1997), a longer adaptation period than two weeks might be required to stimulate hindgut fermentation (Gargallo and Zimmerman 1981; Longland et al. 1993). Finisher pigs might have responded better to ingredients high in cellulose such as oat hulls (Kennely and Aherne 1980a). Third, oat hulls have a high degree of lignification (Bach Knudsen 1997) that might result in resistance to bacterial fermentation in the large intestine (Stanogias and Pearce 1985; Moore et al. 1986). Oat hulls have very low digestibility, indicating that they are not very fermentable (Moore et al. 1986).

Dietary protein content, time of sampling, and dietary protein by time interaction exhibit a significant effect on affected PU concentration. Plasma urea concentration before the morning feeding and 4 h after feeding was linearly decreased with a decrease of dietary protein content (Lopez et al. 1994; Lenis et al. 1999). The PU concentration increased after feeding and peaked at 4 h after feeding similar to Eggum (1970) and Malmlof et al. (1989) who observed a peak in plasma urea concentration at 4 and 5 h after feeding. In addition, Cai et al. (1994) found that pigs fed twice daily had a peak PUN at 3.6 h after feeding and the peak was 32% higher than pre-feeding concentration. The effect of time was significant for the high and medium protein diets while time effect was not detected for the low protein diet, confirmed by the interaction of time by dietary protein content on PU in the present study. A low PU concentration reflects a high quality of dietary protein (Eggum 1970); thus, the low PU for low protein diet indicates that these diets were better balanced for AA. In addition, PU did not increase

significantly over time, indicating that the excess of AA not used for protein synthesis was minimal. For the medium protein diet the excess of AA were lower compared to the high protein diet, which showed the higher PU concentrations. Plasma urea concentrations were not affected by dietary fibre content, in agreement with N balance data that were also not affected by dietary fibre.

The PU concentrations were in agreement with urinary N excretion validating findings of Brown and Cline (1974) who found a strong relationship between PU concentration and urea in urine. Because urea is the major nitrogenous compound in urine, the relationship could be extrapolated to the total N in urine. The relationship of PU and urinary N excretion was investigated using regression analysis. A high relationship was found ( $R^2 = 0.65$ ), raising the possibility that urinary N excretion could be predicted to a sufficient degree from the PU concentration. Because time of sampling affected PU concentration, choice of sampling time is crucial to establish an accurate prediction. In the present study, the best prediction of urinary N excretion was obtained from PU concentration at 4h after feeding.

A linear relationship of dietary protein and ammonia emission from manure was observed (Canh et al. 1998a). For each percent reduction in dietary protein content, ammonia emission was reduced by about 10%. Because most of urinary N is in the form of urea, which is easily converted to ammonia by the action of bacterial urease in feces, the reduction in urinary N resulted in reduced ammonia content of the manure and consequently reduced ammonia emission from manure (Canh et al. 1998a). Thus, the reduction in urinary N observed in the present study will result in a reduction of ammonia emission.

Inclusion of oat hulls in the diets reduced energy digestibility but did not affect N digestibility similar to Stanogias and Pearce (1985), Moore et al. (1986), and Kennely and Aherne (1980b) who observed that oat hulls had a small effect on N digestibility while they had a large effect on energy digestibility. For energy digestibility, the interaction of dietary protein with the fibre tended to be significant. The decrease of energy digestibility for the high compared to low fibre was higher for low than for medium and high protein diets. A possible explanation for this interaction is that the

higher level of cornstarch and canola oil in the low protein-high fibre resulted in a higher digestibility of energy compared to medium and high protein diets.

Reduction of dietary protein content while balancing for AA reduced both faecal and urinary N excretion and consequently total N excretion. The reductions were higher for urinary N because AA that are in excess of requirements are excreted as urea in the urine. The reduction of dietary protein content thus increased the efficiency of N utilisation resulting in reduced N excretion. The reduction in N excretion will reduce ammonia emission from manure. Although ADG and FE were not affected by reducing dietary protein content, further research is required to maintain nitrogen retention. Plasma urea was correlated to urinary N excretion suggesting that urinary N excretion could be predicted from plasma urea concentrations. Development of a model to predict urinary N excretion has several benefits. Blood sampling from pigs in a production setting is easier than the collection of urine. Thus, the developed model might be functional to assess N status under commercial farm conditions.

### 3. EFFECTS OF DIETARY PROTEIN AND FIBRE SOURCE ON NITROGEN EXCRETION PATTERNS

#### 3.1 Abstract

Successful management of nitrogen (N) excretion is important for sustainable pork production. Effects of dietary protein content (high 185; and low, 157 g kg<sup>-1</sup>) and fibre sources on N excretion patterns and PU concentrations were studied in a 2x3 factorial arrangement. The three fibre sources were control, soybean hulls (SH; 15%), and sugar beet pulp (SBP; 20%). Diets were formulated to 3.3 Mcal DE kg<sup>-1</sup> and 2.4 g digestible Lys per Mcal DE, supplemented with Lys, Met, Trp, Thr, Ile, or Val to ensure meeting an ideal AA profile. Pigs (30.5 ± 3 kg; n = 36) were housed in confinement-type metabolism crates, with restricted access to feed (3 x maintenance DE) from d 1 to 18, and free access to feed from d 19 to 26. Faeces and urine were collected from d 15 to 18 and d 23 to 26, and blood samples on d 17 and 25. Under restricted access to feed, faecal N (as % of N intake) was increased 3 percentage units for low compared to high protein content, and increased 4 percentage units for SH and 6.5 percentage units for SBP compared to control (P < 0.01); urinary N was reduced 5 percentage units for low compared to high protein content, and reduced 9 percentage units for SBP compared to control (P < 0.05). Retention of N (g d<sup>-1</sup>) was reduced 12% for low compared to high protein content (17.9 compared to 20.4 g d<sup>-1</sup>; P < 0.05), and was similar among fibre treatments (P > 0.10). With free access to feed, faecal N (as % of N intake) was increased 2.5 percentage units for low compared to high protein content, and increased 5 percentage units for SH and 9 percentage units for SBP compared to control (P < 0.05); urinary N was reduced 5 percentage units for low compared to high protein content, and reduced 9 percentage units for SH and 10 percentage units for SBP compared to control (P < 0.05). Retention of N (g d<sup>-1</sup>) was similar for dietary protein content (30.5 g d<sup>-1</sup>; P > 0.10), and reduced for SH (27 g d<sup>-1</sup>; P < 0.05) compared to control (31 g d<sup>-1</sup>), and

intermediate for SBP (29 g d<sup>-1</sup>). A linear relationship of urinary N to PU concentration was observed under both restricted ( $R^2 = 0.65$ ) and free access to feed ( $R^2 = 0.71$ ) raising the possibility that urinary N excretion could be predicted to a sufficient degree from PU concentration. Reduction of dietary protein content reduced urine N, and fibre sources high in fermentable fibre shifted N excretion from urine N to faeces N. Combined effects of dietary protein content and fibre resulted in a larger reduction of urinary N excretion. Level of feed intake is an important consideration when effectiveness of a nutrient management strategy is studied. Diets with a low protein content and containing synthetic AA should be studied using pigs with free access to feed to verify that nitrogen retention is maintained.

### 3.2 Introduction

Despite great advantages of modern intensified swine production, negative environmental effects of swine production have drawn public attention. Nitrogen (N) excretion is of major concern, and total N excretion and ammonia emission should be reduced to alleviate negative effects of N. Reduction of total N excretion might allow a higher manure application rate per hectare of land in a sustainable manner. Ammonia is a noxious gas for humans and animals and contributes to bad odour and acidification of the environment. Ammonia volatilisation from pig manure should be minimised to improve air quality inside the pig barn and to prevent high emissions into the environment. Urea in urine, by the action of urease, is converted to ammonia, which is easily emitted into the air. Degradation of faecal N to ammonia is a much slower process compared to urinary N because faecal N is bound chemically within proteins or other compounds.

Research has identified dietary manipulation as a powerful tool to reduce negative environmental effects of swine production. Reduction of dietary protein is a direct manner to reduce urinary and total N excretion (Chapter 2; Dourmad et al. 1993) and ammonia emission (Canh et al. 1998a). Nitrogen excretion is shifted from urea in urine to bacterial protein in faeces by using dietary fermentable carbohydrates resulting in a reduction of ammonia emission (Canh 1997, 1998b) whereas addition of insoluble

fibre did not affect N excretion patterns (Chapter 2). The effect of combining these two feeding strategies on N excretion patterns has not been fully investigated.

Blood urea concentration and urinary N are related (Brown and Cline 1974; Chapter 2), and a model to predict this relation with restricted feed intake has been established (Chapter 2). However, models to predict this relationship with free access to feed have not been established. A model might be functional to assess nitrogen status under commercial farm conditions.

The objectives of the experiment were to study the effect of dietary protein content and fibre source on nitrogen (N) excretion patterns and to relate plasma urea (PU) to urinary N excretion both with restricted and free access to feed.

### **3.3 Materials and Methods**

#### **3.3.1 Experimental protocol**

The animal protocol was approved by the University of Saskatchewan-Committee on Animal Care and Supply, and followed principles established by the Canadian Council on Animal Care (1993). Two levels of dietary protein content (high 185 g kg<sup>-1</sup>; and low, 157 g kg<sup>-1</sup>) and three sources of fibre (control, sugar beet pulp and soybean hulls) were compared in a 2 x 3 factorial arrangement for a total of 6 dietary treatments. The main ingredients of the diets were barley, wheat, soybean meal, corn starch, canola oil, sugar beet pulp, and soybean hulls; chromic oxide was included as an indigestible marker (Table 3.1). Experimental diets were formulated to 3.3 Mcal DE kg<sup>-1</sup> and 2.4 g digestible lysine per Mcal DE (Table 3.2). Diets were supplemented with synthetic AA to balance to an ideal AA ratio (Table 3.2). Brill<sup>®</sup> feed formulation software (Version 7, Brill Corporation) was used for the diet formulation to ensure that diets met nutrients requirements. A total of 36 crossbred growing barrows (Camborough-15 x Canabrid, Pig Improvement Canada, Acme, AB; initial BW 23.7 ± 1.3 kg) were selected in two periods of 18 pigs each. Per period, three pigs were allotted to each treatment, for a total of six observations per treatment.

**Table 3.1** Composition of experimental diets (as-fed basis).

Protein content Fibre source Ingredient, %	Diets					
	High			Low		
	Control	SH <sup>1</sup>	SBP <sup>2</sup>	Control	SH	SBP
Wheat	64.8	41.2	42.0	60.6	42.7	40.0
Barley	15.0	15.0	16.2	18.1	15.0	15.0
Soybean meal	14.3	17.6	16.4	7.5	9.8	9.2
Corn starch	0.8	3.4	-	8.0	9.0	9.0
Canola oil	0.5	4.0	1.6	0.5	4.0	1.6
Soybean hulls	-	15.0	-	-	15.0	-
Sugar beet pulp	-	-	20.0	-	-	20.0
Mineral premix <sup>3</sup>	0.5	0.5	0.5	0.5	0.5	0.5
Vitamin premix <sup>4</sup>	0.5	0.5	0.5	0.5	0.5	0.5
Salt	0.4	0.4	0.4	0.4	0.4	0.4
Chromic oxide	0.4	0.4	0.4	0.4	0.4	0.4
Dicalcium phosphate	1.080	0.831	0.858	1.328	1.020	1.120
Limestone	1.297	0.953	0.759	1.250	0.927	0.982
Lysine-HCL	0.293	0.169	0.265	0.512	0.409	0.500
L-Threonine	0.079	0.039	0.087	0.184	0.151	0.202
DL-Methionine	0.001	0.008	0.031	0.063	0.082	0.125
L-Valine	-	-	-	0.066	0.065	0.141
L-Leucine	-	-	-	0.022	0.021	0.137
L-Isoleucine	-	-	-	-	-	0.069
L-Tryptophan	-	-	-	0.025	0.025	0.034

<sup>1</sup>SH = Soybean hulls<sup>2</sup>SBP = Sugar beet pulp<sup>3</sup>Provided per kg of premix: zinc, 200 mg; iron, 160 mg; manganese, 50 mg; copper, 100 mg; iodine 100 mg; selenium, 20 mg.<sup>4</sup>Provided per kg of premix: vitamin A, 650,000IU; vitamin D<sub>3</sub>, 165,000 IU; vitamin E, 8,000 IU; menadione, 800 mg; thiamine, 200 mg; riboflavin, 1,000 mg; d-pantothenic acid, 3,000 mg; niacin 7,000 mg; B<sub>12</sub>, 5 mg; d-biotin, 40 mg; folic acid, 400 mg.

**Table 3.2** Chemical characteristics of experimental diets (as-fed basis).

Protein content Fibre source	Diets <sup>1</sup>					
	High			Low		
	Control	SH <sup>1</sup>	SBP <sup>2</sup>	Control	SH	SBP
Calculated nutrient level						
DE (Mcal kg <sup>-1</sup> )	3.30	3.30	3.30	3.30	3.30	3.30
CP, %	17.50	17.50	17.50	14.50	14.50	14.50
EE, %	2.13	5.52	3.05	2.02	5.44	2.85
CF, %	3.07	7.78	6.20	2.85	7.52	5.80
ADF, %	4.39	10.56	8.69	3.96	10.03	8.01
NDF, %	11.00	17.80	17.40	10.50	17.22	16.33
Dlysine <sup>3</sup> , g Mcal <sup>-1</sup> DE	2.40	2.40	2.40	2.40	2.40	2.40
Lysine, %	0.94	0.97	0.97	0.91	0.94	0.94
Ca, %	0.75	0.65	0.65	0.75	0.66	0.75
Phosphorus, %						
Total	0.59	0.50	0.50	0.60	0.50	0.50
Available	0.37	0.29	0.29	0.40	0.31	0.32
Apparent Ileal Digestible AA <sup>4</sup>						
Lysine, %	0.79	0.79	0.79	0.79	0.79	0.79
Threonine, %	0.48	0.48	0.48	0.48	0.48	0.48
Methionine, %	0.22	0.22	0.23	0.24	0.25	0.28
Tryptophan, %	0.14	0.15	0.14	0.14	0.14	0.14
Isoleucine, %	0.56	0.57	0.50	0.44	0.44	0.44
Leucine, %	0.99	0.99	0.89	0.78	0.78	0.78
Valine, %	0.59	0.60	0.53	0.53	0.53	0.53
Analysed nutrient level %						
CP	19.1	18.0	18.4	16.1	15.6	15.5
ADF	3.9	10.0	7.8	3.6	9.2	7.4
NDF	11.7	17.9	18.3	11.5	17.5	16.9

<sup>1</sup>SH = Soybean hulls

<sup>2</sup>SBP = Sugar beet pulp

<sup>3</sup>Digestible lysine

<sup>4</sup>Ideal pattern of apparent digestible AA compared to lysine (%): lysine 100; threonine 61; methionine 28; tryptophan 17; leucine 99; isoleucine 55; valine 66; phenylalanine 59 (PSCI 2000).



Prior to the experiment, pigs were housed in groups and fed for two wk an acclimation diet, containing 10% soybean hulls and 5% sugar beet pulp to enhance adaptation to high fibre diets.

The 26-d experimental period consisted of a 14-d adaptation to experimental diets and metabolism crates under restricted feed intake, followed by a 4-d collection of faeces and urine and a 5-d adaptation to free access to feed followed by a 3-d collection of faeces and urine. Blood samples were collected before the morning feeding (or 0800) and 4 h after the morning feeding (or 1200 for the free access phase) on d 17 and d 25. Pigs were housed in steel confinement-type metabolic crates.

From d 0 to 17, pigs were fed the diet as a wet-mash, in 1:1 water to mash ratio. Daily feed allowance was adjusted to three times maintenance ( $3 \times 110 \text{ kcal DE kg}^{-1} \text{ BW}^{0.75}$ ), which was fed in two equal meals (0800 and 1600). Water was supplied ad libitum through a nipple drinker. During d 18 to 26, pigs had free access to feed. Pigs were housed in an environmentally-controlled room with an average temperature of 21°C. The lights turned on at 0700 and off at 1900.

The procedures for collection of the samples and preparation of samples for analysis were followed as described for the first experiment (Chapter 2).

### 3.3.2 Diet preparation

Diets were each mixed in two batches of 200 kg. For two diets (low protein-soybean hulls and high protein-soybean hulls) the analysed protein content was low for one of the two batches. In combination with the low levels of chromic oxide, we suspect that part of the soybean meal that should have been included in the diet was not. For this reason, observations from these two batches were not included in the statistical analysis for the N balance study. Plasma urea and urinary N values from these pigs were used in regression analysis to investigate the relationship between plasma urea and urinary N excretion.

### 3.3.3 Chemical analyses

Feed and freeze-dried faecal samples were ground through a 1-mm screen on a Retch mill. Chemical analyses were conducted in duplicate. Dry matter content of feed and faeces was determined by drying at 135°C in an airflow type oven for 2 h (method 930.15; AOAC 1990). Feed, faecal, and urinary samples were analysed for nitrogen by combustion method (method 968.06; AOAC 1990) using a Leco protein/nitrogen determinator (model FP-528, Leco Co., St Joseph, MI). Chromic oxide content was analysed in feed and faeces according to the Fenton and Fenton (1979) procedure with a Pharmacia LKB-Ultrospec III spectrophotometer (model 80-2097-62), at 440 nm after ashing at 450°C overnight.

Gross energy in feed and faeces was measured in a Parr adiabatic bomb calorimeter (model 1281, Parr Instrument Co., Moline, IL). Benzoic acid (3415, Parr Instrument Co.) was utilised as a caloric standard for calibration. Acid detergent fibre (ADF) and neutral detergent fibre (NDF) content were determined using Ankom<sup>200</sup> fibre analyser (Ankom Technology Co., Fairport, NY) according to the procedures described by the Ankom 220/200 Fiber Analyzer Operator's Manual (Ankom 1998). Plasma samples were analysed for urea using the Abbott Spectrum Urea Nitrogen Test (Abbott Spectrum Series II, Abbot Laboratories, Dallas, TX). Degussa AG (Allendale, NJ) conducted AA analysis of feed samples (method 994.12; AOAC 1995). Methionine and cystine were determined as methionine sulfone and cysteic acid after oxidation with performic acid. Tryptophan content was determined after alkaline hydrolysis with lithium hydroxide by means of reversed phase high performance liquid chromatography. Following analyses, nitrogen retention, digestibility of nitrogen and energy, and digestible energy were calculated using chromium oxide concentration in diets and faeces.

### 3.3.4 Statistical analyses

Individual pig was considered the experimental unit. Variables were analysed using the General Linear Models (GLM) procedure of SAS (1996). The statistical model included effects for dietary treatment (dietary protein content, fibre level, and dietary protein by

fibre interaction) and period; initial BW was included as covariate. Means comparisons were performed using the probability of difference (Pdiff). Pearson's correlation coefficients were calculated to determine the degree of association between urinary N and PU. Regression analysis was used to predict urinary N as a function of PU. Values are reported as least square means.

### **3.4 Results: Restricted Access to Feed**

#### **3.4.1 Nitrogen balance**

Health problems and feed refusals were not observed during the experiment. Dietary protein content was higher the calculated due to higher CP content of barley and wheat. Analysis showed that the actual dietary AA concentrations were slightly higher compared to the calculated values and that diets were balanced according to an ideal AA ratio (Table 3.3). Within the same level of protein, lysine content was highest for the SBP, intermediate for the control, and lowest for the SH diets. Lysine content was higher for high protein compared to low protein diets. As expected, N intake was different among treatments following dietary patterns, with a dietary protein by fibre source interaction (Table 3.4,  $P < 0.05$ ). Overall, N intake was 15% lower for low protein compared to high protein diets ( $P < 0.001$ ). For the low protein compared to high protein diets, N intake was reduced 15.4% for control, 13.3% for SH, and 15.7% for SBP diets.

Faecal N excretion was affected by fibre source (Table 3.4;  $P < 0.001$ ), with a dietary protein content by fibre source interaction ( $P < 0.05$ ). Overall, faecal N excretion was highest for SBP, intermediate for SH, and lowest for control diets. For low protein compared to high protein diets, faecal N was decreased by 13% for SBP ( $P < 0.05$ ) and was similar for control and SH diets ( $P > 0.10$ ).

Dietary protein content affected urinary N excretion, total N excretion and N retention (Table 3.4;  $P < 0.01$ ). Urinary N and total N excretion were reduced 28% and 18%, respectively, for low protein compared to high protein diets ( $P < 0.001$ ). Nitrogen retention was 12% lower for low protein compared to high protein diets ( $P < 0.001$ ).

**Table 3.3** Total amino acid content of experimental diets (as-fed basis).

Protein content Fibre source	Diets					
	High			Low		
	Control	SH <sup>1</sup>	SBP <sup>2</sup>	Control	SH	SBP
Amino acid, %						
Calculated						
Lysine	0.94	0.97	0.97	0.90	0.94	0.94
Threonine	0.64	0.65	0.67	0.61	0.62	0.64
Methionine	0.26	0.27	0.28	0.27	0.29	0.32
Tryptophan	0.20	0.20	0.19	0.18	0.18	0.18
Analysed						
Lysine	1.01	0.99	1.07	0.94	0.97	1.00
Threonine	0.70	0.69	0.76	0.67	0.67	0.70
Methionine	0.29	0.28	0.31	0.29	0.30	0.32
Tryptophan	0.24	0.24	0.24	0.22	0.23	0.21
Cysteine	0.36	0.34	0.34	0.32	0.30	0.28
TSAA <sup>3</sup>	0.65	0.62	0.65	0.61	0.60	0.60
Arginine	1.04	1.08	1.07	0.83	0.86	0.83
Isoleucine	0.73	0.74	0.74	0.59	0.60	0.64
Leucine	1.25	1.25	1.25	1.06	1.05	1.10
Valine	0.88	0.84	0.87	0.75	0.77	0.83
Histidine	0.44	0.45	0.45	0.36	0.37	0.36
Phenylalanine	0.87	0.84	0.85	0.73	0.71	0.69

<sup>1</sup>SH = Soybean hulls<sup>2</sup>SBP = Sugar beet pulp<sup>3</sup>TSAA = Total sulfur amino acids

**Table 3.4** Effect of dietary protein content and fibre source on N balance in grower pigs with restricted access to feed.

Protein content Fibre source Variable	Dietary Treatments						Effects <sup>1</sup>		
	High			Low			CP	Fibre	CP x Fibre
	Control	SH <sup>2</sup>	SBP <sup>3</sup>	Control	SH	SBP			
Number of pigs	6	3	6	6	3	6			
N intake, g d <sup>-1</sup>	39.6	37.5	38.2	33.5	32.5	32.2	.0001	.0001	.0044
SEM	0.04	0.07	0.04	0.04	0.07	0.04			
Fecal N, g d <sup>-1</sup>	5.1	5.7	7.7	5.1	6.9	6.8	NS <sup>4</sup>	.0001	.0495
SEM	0.3	0.5	0.3	0.3	0.5	0.3			
Urinary N, g d <sup>-1</sup>	14.1	10.8	10.8	10.3	8.8	6.7	.0003	.0008	NS
SEM	0.8	1.2	0.8	0.8	1.2	0.8			
N excretion, g d <sup>-1</sup>	19.2	16.5	18.5	15.4	15.7	13.5	.0009	NS	NS
SEM	0.9	1.3	0.9	0.9	1.3	0.9			
N retention, g d <sup>-1</sup>	20.4	21.0	19.7	18.1	16.8	18.8	.0081	NS	NS
SEM	0.9	1.3	0.9	0.9	1.3	0.9			
Urinary N:fecal N	2.7	1.9	1.4	2.0	1.3	1.0	.0004	.0001	NS
SEM	0.1	0.2	0.1	0.1	0.2	0.1			
Fecal N, % of intake	13.0	15.1	20.3	15.4	21.3	21.1	.0034	.0001	NS
SEM	1.0	1.4	1.0	1.0	1.4	1.0			
Urinary N, % of intake	35.5	28.7	28.2	30.6	27.0	20.7	.0386	.0022	NS
SEM	2.1	3.2	2.1	2.1	3.2	2.1			
N excretion, % of N intake	48.5	43.8	48.5	46.0	48.3	41.8	NS	NS	NS
SEM	2.4	3.6	2.4	2.4	3.6	2.4			
N retention, % of N intake	51.5	56.2	51.5	54.0	51.7	58.2	NS	NS	NS
SEM	2.4	3.6	2.4	2.4	3.6	2.4			

<sup>1</sup>CP = Main effect of protein level; Fibre = Main effect of fibre; CP x Fibre = protein level by fibre interaction.

<sup>2</sup>SH = Soybean hulls

<sup>3</sup>SBP = Sugar beet pulp

<sup>4</sup>NS = not significant (P > 0.10)

Expressed as percentages of intake, faecal N and urinary N were affected by dietary protein content (Table 3.4;  $P < 0.05$ ) whereas total N excretion and retention were not affected ( $P > 0.10$ ). Faecal N excretion was increased 3 percentage units and urinary N was reduced 5 percentage units for low compared to high protein diets ( $P < 0.05$ ).

Nitrogen intake was 4% lower for SH and SBP compared to control diets (Table 3.4;  $P < 0.01$ ). The differences observed for N excretion patterns and N retention, expressed as  $\text{g d}^{-1}$ , may be due to differences in N intake. Therefore, effects of dietary fibre in N excretion patterns will be discussed on a percentage of N intake basis. Dietary fibre source affected faecal and urinary N excretion ( $P < 0.01$ ) but did not affect total N excretion and N retention ( $P > 0.10$ ). Faecal N excretion was increased 4 percentage units for SH and 6.5 percentage units for SBP compared to control diets ( $P < 0.01$ ). Urinary N excretion was 9 percentage units lower for the SBP compared to control diets ( $P < 0.05$ ), while SH diet did not differ from either the control or the SBP diets.

The ratio of urinary N to faecal N excretion was affected by protein level and fibre source ( $P < 0.001$ ). For low protein diets, the ratio was reduced by 30% compared to high protein diets ( $P < 0.001$ ). Regarding the fibre sources, the ratio was reduced 33% and 50% for SH and SBP diets, respectively, compared to control diets ( $P < 0.001$ ) and was 33% higher for SH compared to SBP diets ( $P < 0.05$ ).

### 3.4.2 Energy and nitrogen digestibility and animal performance

Although diets were formulated to an equal DE content ( $3.3 \text{ Mcal kg}^{-1}$ ), measured DE content was lower for most diets except for the high protein-SH diet ( $3.365 \text{ Mcal kg}^{-1}$ ). The DE content was affected by fibre source (Table 3.5;  $P < 0.001$ ), with a protein by fibre source interaction ( $P < 0.05$ ). Overall, DE content was higher for SH diets ( $3.321 \text{ Mcal kg}^{-1}$ ;  $P < 0.001$ ) and lower for SBP diets ( $3.187 \text{ Mcal kg}^{-1}$ ;  $P > 0.10$ ) compared to control diet ( $3.216 \text{ Mcal kg}^{-1}$ ). Specifically, for the low protein compared to high protein, DE content was decreased by 2.3% for SH ( $P < 0.05$ ) and was similar for control diets and SBP diets ( $P > 0.05$ ). Energy digestibility was affected by fibre source (Table 3.5;  $P < 0.001$ ) but not by protein level ( $P > 0.10$ ). Digestibility of energy was 2 percentage units lower for SH and SBP diets compared to control diets ( $P$

< 0.05). Protein and fibre source affected N digestibility ( $P < 0.001$ ). Digestibility of N was 3 percentage units lower for low protein compared to high protein diets ( $P < 0.001$ ). Nitrogen digestibility was reduced 4 percentage units and 6.5 percentage units for SH and SBP diets, respectively, compared to control diets ( $P < 0.01$ ). For the low compared to high protein diets N digestibility was reduced 8 percentage units for the SH ( $P < 0.001$ ).

Average daily gain and feed efficiency were not affected by treatments ( $P > 0.10$ )

### 3.4.3 Plasma urea

Plasma urea concentration was affected by dietary protein level and fibre source (Table 3.5;  $P < 0.05$ ). The PU concentration before morning feeding was 29% lower for the low compared to high protein diets (2.4 vs 3.4 mmol L<sup>-1</sup>;  $P < 0.01$ ). Furthermore, PU concentration was 17% lower for the SH (3.0 mmol L<sup>-1</sup>) and 39% lower for SBP (2.2 mmol L<sup>-1</sup>) compared to control diet (3.6 mmol L<sup>-1</sup>;  $P < 0.05$ ).

At four h after feeding, PU concentration was 32% lower for low compared to high protein diets (3.2 vs 4.7 mmol L<sup>-1</sup>;  $P < 0.001$ ). Also, PU concentration was 15% lower for SH (3.9 mmol L<sup>-1</sup>;  $P = 0.0974$ ) and 26% lower for SBP diets (3.4 mmol L<sup>-1</sup>;  $P < 0.01$ ) compared to control diets (4.6 mmol L<sup>-1</sup>). Plasma urea concentration at 4 h after feeding increased 38% for high protein and 33% for low protein diets compared to PU concentration before feeding. For the fibre sources, PU increased by 28%, 30% and 54 % for SH, SBP, and control diets, respectively, at 4 h after feeding compared to PU values before feeding.

Models to predict urinary N as a function of PU were developed using regression analyses. The models obtained are shown in Figures 3.1 and 3.2.

**Table 3.5** Effect of dietary protein content and fibre source on energy and N digestibility, DE content, PU concentration, and performance in grower pigs with restricted access to feed.

Protein content Fibre source	Dietary Treatments						Effects <sup>1</sup>		
	High			Low			CP	Fibre	CP x Fibre
Number of pigs	6	3	6	6	3	6			
Variable									
Plasma urea, mmol L <sup>-1</sup>									
Before feeding	4.1	3.4	2.8	3.2	2.5	1.7	.0020	.0003	NS <sup>4</sup>
SEM	0.3	0.4	0.3	0.3	0.4	0.3			
4 H after feeding	5.3	4.6	4.3	3.9	3.3	2.6	.0001	.0041	NS
SEM	0.3	0.5	0.3	0.3	0.5	0.3			
N digestibility, %	87.0	84.9	79.7	84.7	78.7	79.0	.0034	.0001	NS
SEM	1.0	1.4	1.0	1.0	1.4	1.0			
Energy digestibility, %	85.4	84.5	82.3	85.2	82.8	83.5	NS	.0001	.0541
SEM	0.4	0.7	0.4	0.4	0.7	0.4			
DE, Mcal kg <sup>-1</sup>	3.238	3.365	3.162	3.194	3.279	3.211	NS	.0001	.0103
SEM	0.018	0.027	0.018	0.018	0.027	0.018			
ADG <sup>5</sup> , g d <sup>-1</sup>	0.569	0.600	0.592	0.544	0.591	0.616	NS	.0795	NS
SEM	0.021	0.031	0.021	0.021	0.031	0.021			
Feed efficiency <sup>6</sup>	0.473	0.499	0.492	0.452	0.492	0.512	NS	.0793	NS
SEM	0.017	0.026	0.017	0.017	0.026	0.017			

<sup>1</sup>CP = Main effect of protein level; Fibre = Main effect of fibre source; CP x Fibre = protein level by fibre interaction.

<sup>2</sup>SH = Soybean hulls

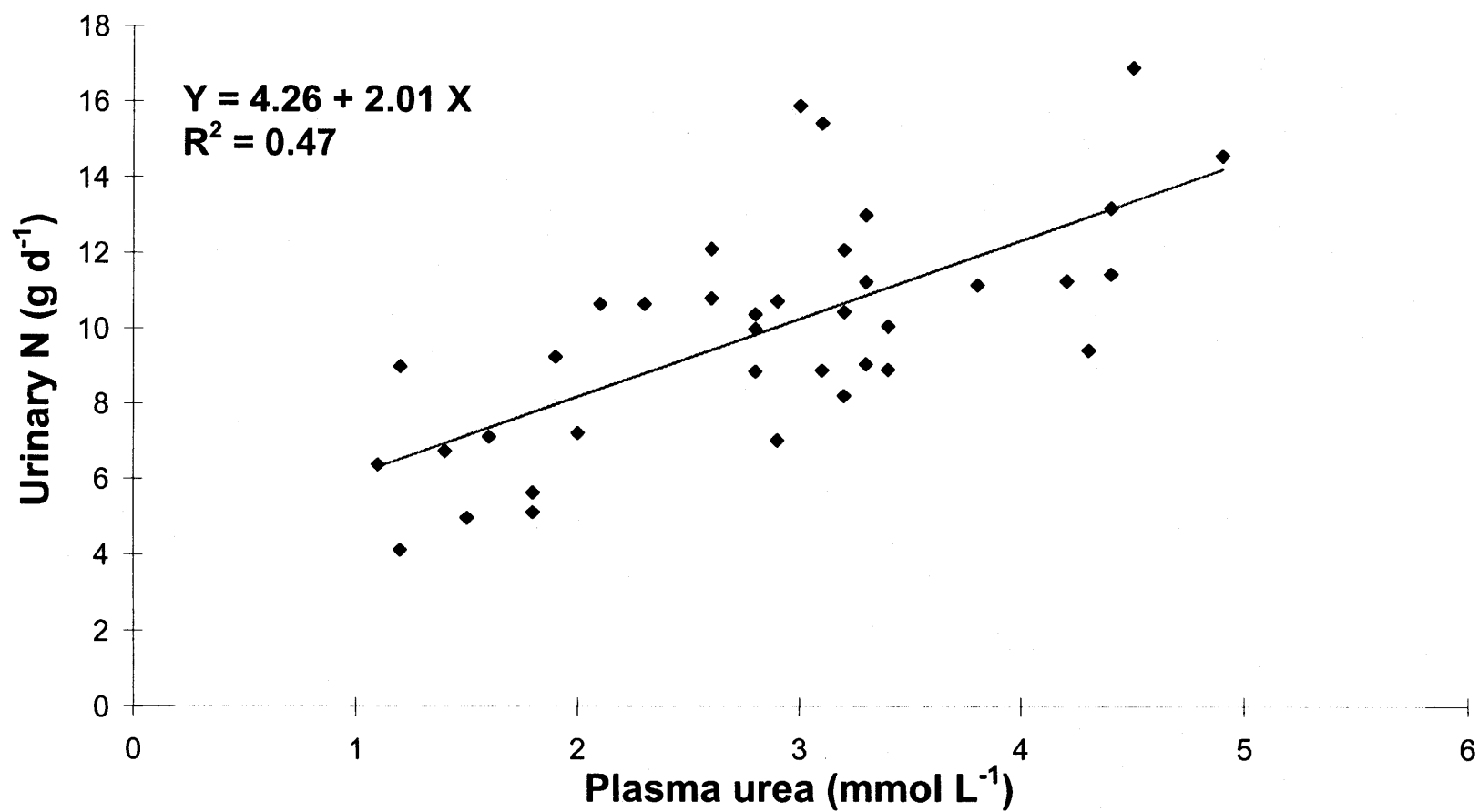
<sup>3</sup>SBP = Sugar beet pulp

<sup>4</sup>NS = not significant (P > 0.10)

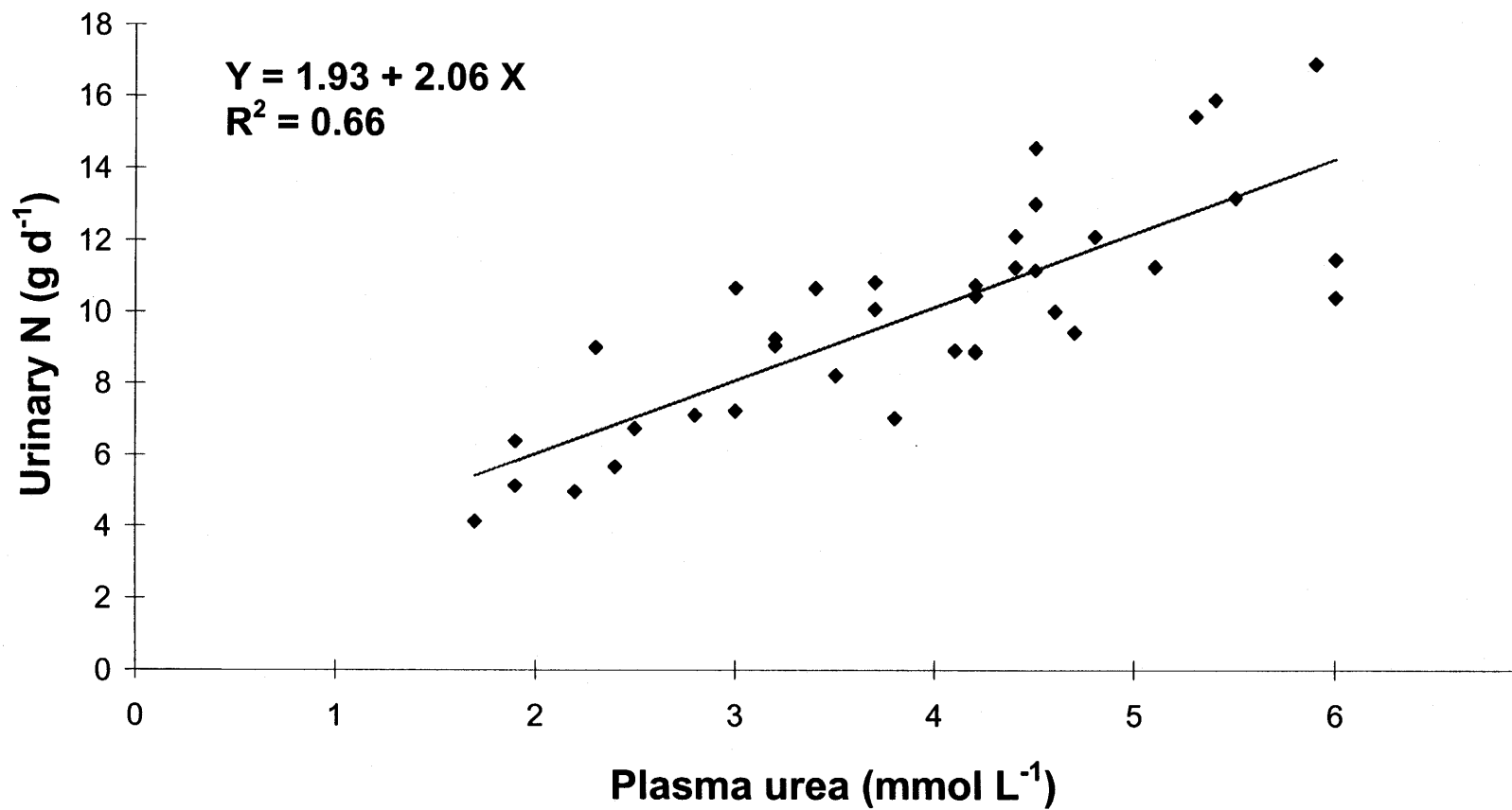
<sup>5</sup>Calculated for a period of 18 days

<sup>6</sup>Feed efficiency = kg of weight gain per kg of feed





**Figure 3.1** Relationship of urinary N to plasma urea concentration before feeding with restricted access to feed ( $R^2 = 0.47$ ;  $n = 36$ ).



**Figure 3.2** Relationship of urinary N to plasma urea concentration at 4 hours after feeding with restricted access to feed ( $R^2 = 0.66$ ;  $n = 36$ ).

### 3.5 Results: Free Access to Feed

#### 3.5.1 Nitrogen balance

One pig was removed from the experiment during the acclimation period to free access to feed because of rectal prolapse. Nitrogen intake was reduced 11% for low protein compared to high protein diets (Table 3.6;  $P < 0.01$ ). Dietary protein content affected urinary N excretion ( $P < 0.05$ ), N retention and total N excretion ( $P < 0.10$ ) but did not affect faecal N ( $P > 0.10$ ). Urinary N excretion was reduced 27% for low protein compared to high protein diets ( $P < 0.05$ ). Excretion of N was decreased by 16% and N retention was decreased by 7% for low protein diets compared to high protein diets ( $P < 0.10$ ).

Expressed as percentage of intake, faecal N and urinary N were affected by dietary protein content ( $P < 0.05$ ) whereas total N excretion and retention were not affected ( $P > 0.10$ ). Faecal N excretion was increased 2.5 percentage units and urinary N excretion was decreased 5 percentage units for low protein diets compared to high protein diets ( $P < 0.05$ ).

Nitrogen intake was 14% lower for SH compared to control diets ( $P < 0.05$ ) while SBP was not different than the control or the SH diets ( $P > 0.10$ ). Dietary fibre source affected faecal and urinary N excretion ( $P < 0.05$ ) but did not affect total N excretion and N retention ( $P > 0.10$ ). Faecal N excretion was 44% and 26% higher for SBP diets, respectively, compared to control and SH diets ( $P < 0.05$ ). Faecal N excretion was increased 15% for the SH diets compared to control diets ( $P < 0.10$ ). Urinary N excretion was decreased 36% for SH and SBP compared to control diets ( $P < 0.001$ ), while SH and SBP were not different ( $P > 0.10$ ).

Expressed as percentage of intake, faecal N and urinary N were affected by dietary fibre source ( $P < 0.05$ ) whereas total N excretion and retention were not affected ( $P > 0.10$ ). Faecal N was increased 5 percentage units for SH and 9 percentage units for SBP diets compared to control diets ( $P < 0.001$ ). Urinary N was decreased 9 percentage units for SH and 10 percentage units for SBP diets compared to control diets ( $P < 0.05$ ).

**Table 3.6** Effect of dietary protein content and fibre source on N balance in grower pigs with free access to feed.

Protein content Fibre source	Dietary Treatments						Effects <sup>1</sup>		
	High			Low			CP	Fibre	CP x Fibre
	Control	SH <sup>2</sup>	SBP <sup>3</sup>	Control	SH	SBP			
Number of pigs	6	2	6	5	3	6			
Variable									
N intake, g d <sup>-1</sup>	68.8	58.5	63.8	59.9	52.6	56.6	.0072	.0298	NS <sup>4</sup>
SEM	2.4	5.3	2.3	2.5	3.6	2.4			
Fecal N, g d <sup>-1</sup>	10.7	11.9	16.3	11.0	12.9	14.9	NS	.0001	NS
SEM	0.6	1.1	0.6	0.7	1.0	0.6			
Urinary N, g d <sup>-1</sup>	25.5	15.3	16.7	18.4	12.2	11.4	.0288	.0032	NS
SEM	2.1	3.8	2.1	2.3	3.2	2.1			
N excretion, g d <sup>-1</sup>	36.2	27.2	33.0	29.4	25.1	26.3	.0587	NS	NS
SEM	2.5	4.5	2.4	2.7	3.8	2.5			
N retention, g d <sup>-1</sup>	32.6	31.3	30.8	30.6	27.5	30.3	.0837	NS	NS
SEM	1.1	2.0	1.1	1.2	1.7	1.1			
Urinary N:fecal N	2.4	1.3	1.0	1.6	1.0	0.8	.0018	.0001	NS
SEM	0.1	0.2	0.1	0.1	0.2	0.1			
Fecal N, % of N intake	15.5	20.4	25.6	18.5	24.5	26.3	.0055	.0001	NS
SEM	0.8	1.4	0.8	0.8	1.2	0.8			
Urinary N, % of N intake	36.9	26.4	26.1	30.1	22.8	20.3	.0324	.0008	NS
SEM	2.3	4.1	2.1	2.4	3.5	2.3			
N excretion, % of N intake	52.4	46.8	51.7	48.6	47.1	46.6	NS	NS	NS
SEM	2.3	4.1	2.2	2.4	3.5	2.3			
N retention, % of N intake	47.6	53.2	48.3	51.4	52.9	53.4	NS	NS	NS
SEM	2.3	4.1	2.2	2.4	3.5	2.3			

<sup>1</sup>CP = Main effect of protein level; Fibre = Main effect of fibre source; CP x Fibre = protein level by fibre interaction.

<sup>2</sup>SH = Soybean hulls

<sup>3</sup>SBP = Sugar beet pulp

<sup>4</sup>NS = not significant (P > 0.10)

The ratio of urinary N to faecal N excretion was affected by protein level and fibre source ( $P < 0.01$ ). For low protein diets, the ratio was reduced by 31% compared to high protein diets ( $P < 0.01$ ). The urinary N to faecal N ratio was reduced 45% and 55% for SH and SBP diets, respectively, compared to control diets ( $P < 0.001$ ).

### **3.5.2 Energy and nitrogen digestibility and animal performance**

The DE content was affected by fibre source ( $P < 0.001$ ), with a protein by fibre source interaction ( $P < 0.05$ ). Overall, DE content was higher for SH and lower for SBP diets compared to control diet. Specifically, DE for the low protein compared to high protein diets was increased by 2.2% for SBP diets (Table 3.7;  $P < 0.05$ ) while was similar for SH and for control treatments ( $P > 0.10$ ).

Energy digestibility was affected by the fibre source (Table 3.7;  $P < 0.001$ ). For the SH and SBP diets, energy digestibility was reduced by 3% compared to control diet. Dietary protein and fibre source affected ( $P < 0.001$ ) N digestibility. Digestibility of N was 2.5% lower for low protein diets compared to high protein diets (77 vs 79.5%;  $P < 0.01$ ). Also it was decreased 5% for SH (77.7%) and 9% lower for SBP diets (74.1%) compared to control diets (83 %;  $P < 0.01$ ) and 4% lower for SH compared to SBP diets ( $P < 0.001$ ).

Feed intake and FE efficiency were not affected by the treatments (Table 3.7;  $P > 0.10$ ). Average daily gain was affected by fibre source ( $P < 0.05$ ) resulting in 11% and 14% reduction in ADG for SH compared to control and SBP diets, respectively.

### **3.5.3 Plasma urea**

Plasma urea concentration at 0800 was affected by the protein level (Table 3.7;  $P < 0.05$ ) and fibre source ( $P < 0.10$ ). Plasma urea concentration was 26% higher for the high protein diets compared to low protein diets (3.9 vs 5.3 mmol L<sup>-1</sup>;  $P < 0.05$ ). Regarding fibre sources, PU was decreased 29% for SH (3.9 mmol L<sup>-1</sup>) and 22% for SBP diets (4.3 mmol L<sup>-1</sup>) compared to the control diets (5.5 mmol L<sup>-1</sup>;  $P < 0.05$ ).

Plasma urea concentration at 1200 was affected by protein level, resulting in a 22% increase in PU for the high protein diets compared to low protein diets (4.2 vs 5.4

mmol L<sup>-1</sup>;  $P < 0.10$ ). Fibre source affected PU concentration, resulting in a reduction by 32% for the SH (3.9 mmol L<sup>-1</sup>;  $P < 0.05$ ) and by 18% and for SBP diets (4.7 mmol L<sup>-1</sup>;  $P < 0.10$ ) compared to the control diets (5.7 mmol L<sup>-1</sup>). Also initial weight was significant effect on PUN values at 1200h ( $P < 0.05$ ).

Regression analysis was used to build a model to predict urinary N from PUN. The models developed are shown in figures 3.3 and 3.4.

### 3.6 Discussion

Reduction of dietary protein content reduced urinary N and total N excretion whereas inclusion of fermentable carbohydrates shifted N excretion from urinary N to faecal N without affecting total N excretion or N retention. The ratio of urinary N to faecal N excretion was reduced by reducing the protein content or by including fermentable carbohydrates in the diet. Plasma urea concentration was reduced when dietary protein content was reduced or when fermentable carbohydrates were included in the diet.

#### 3.6.1 Low protein diets

Results of the present study support the hypothesis that reduction of dietary protein content while balancing for AA reduces urinary and total N excretion (Canh et al. 1998a; Dourmad et al. 1993; Gatel and Grosjean 1992). In agreement with findings of Canh et al. (1998), faecal N excretion was not affected because reduction of N intake was compensated by a reduction in N digestibility for low compared to high protein diets, resulting in similar faecal N excretion. Under restricted access to feed, for each percent reduction in dietary protein content, urinary N excretion was reduced 10% (or 1.2 g d<sup>-1</sup>) and total N excretion 4% (or 1.2 g d<sup>-1</sup>), which are less compared to lowest values for urinary N (8% or 1.9 g d<sup>-1</sup>) and total N excretion (8% or 2 g d<sup>-1</sup>) reported in other studies (Dourmad et al. 1993; Gatel and Grosjean 1992; and Canh et al. 1998a). Under free access to feed, for each percent reduction in dietary protein content urinary N excretion was reduced 10% (or 1.8 g d<sup>-1</sup>) and total N excretion 6% (or 1.8 g d<sup>-1</sup>). Also, urinary N

**Table 3.7** Effect of dietary protein content and fibre source on energy and N digestibility, DE content, PU concentration, and performance in grower pigs with free access to feed.

Protein content Fibre source	Dietary Treatments						Effects <sup>1</sup>		
	High			Low			CP	Fibre	CP x Fibre
	Control	SH <sup>2</sup>	SBP <sup>3</sup>	Control	SH	SBP			
Number of pigs	6	2	6	5	3	6			
Variable									
Plasma urea, mmol L <sup>-1</sup>									
0800 h	6.4	4.5	4.9	4.6	3.4	3.8	.0284	.0510	NS <sup>4</sup>
SEM	0.5	0.9	0.5	0.5	0.8	0.5			
1200 h	6.8	3.9	5.3	4.6	3.8	4.0	.0573	.0411	NS
SEM	0.5	1.2	0.5	0.5	0.8	0.5			
N digestibility, %	84.5	79.6	74.4	81.5	75.7	73.7	.0055	.0001	NS
SEM	0.8	1.4	0.8	0.8	1.2	0.8			
Energy digestibility, %	84.1	81.2	78.9	83.6	80.7	80.5	NS	.0001	NS
SEM	0.5	0.9	0.5	0.5	0.8	0.5			
DE, Kcal kg <sup>-1</sup>	3.186	3.236	3.033	3.138	3.193	3.101	NS	.0001	.0078
SEM	0.020	0.035	0.019	0.021	0.30	0.020			
Feed intake, kg d <sup>-1</sup>	2.253	2.043	2.169	2.259	2.099	2.288	NS	NS	NS
SEM	0.073	0.131	0.071	0.078	0.111	0.073			
ADG <sup>5</sup> , kg d <sup>-1</sup>	1.275	1.103	1.260	1.196	1.087	1.295	NS	.0321	NS
SEM	0.050	0.089	0.048	0.053	0.076	0.050			
Feed efficiency <sup>6</sup>	0.569	0.536	0.580	0.532	0.520	0.565	NS	NS	NS
SEM	0.022	0.039	0.021	0.023	0.034	0.022			

<sup>1</sup>CP = Main effect of protein level; Fibre = Main effect of fibre; CP x Fibre = protein level by fibre interaction.

<sup>2</sup>SH = Soybean hulls

<sup>3</sup>SBP = Sugar beet pulp

<sup>4</sup>NS = not significant (P > 0.10)

<sup>5</sup>Calculated for a period of 18 days

<sup>6</sup>Feed efficiency = kg of weight gain per kg of feed

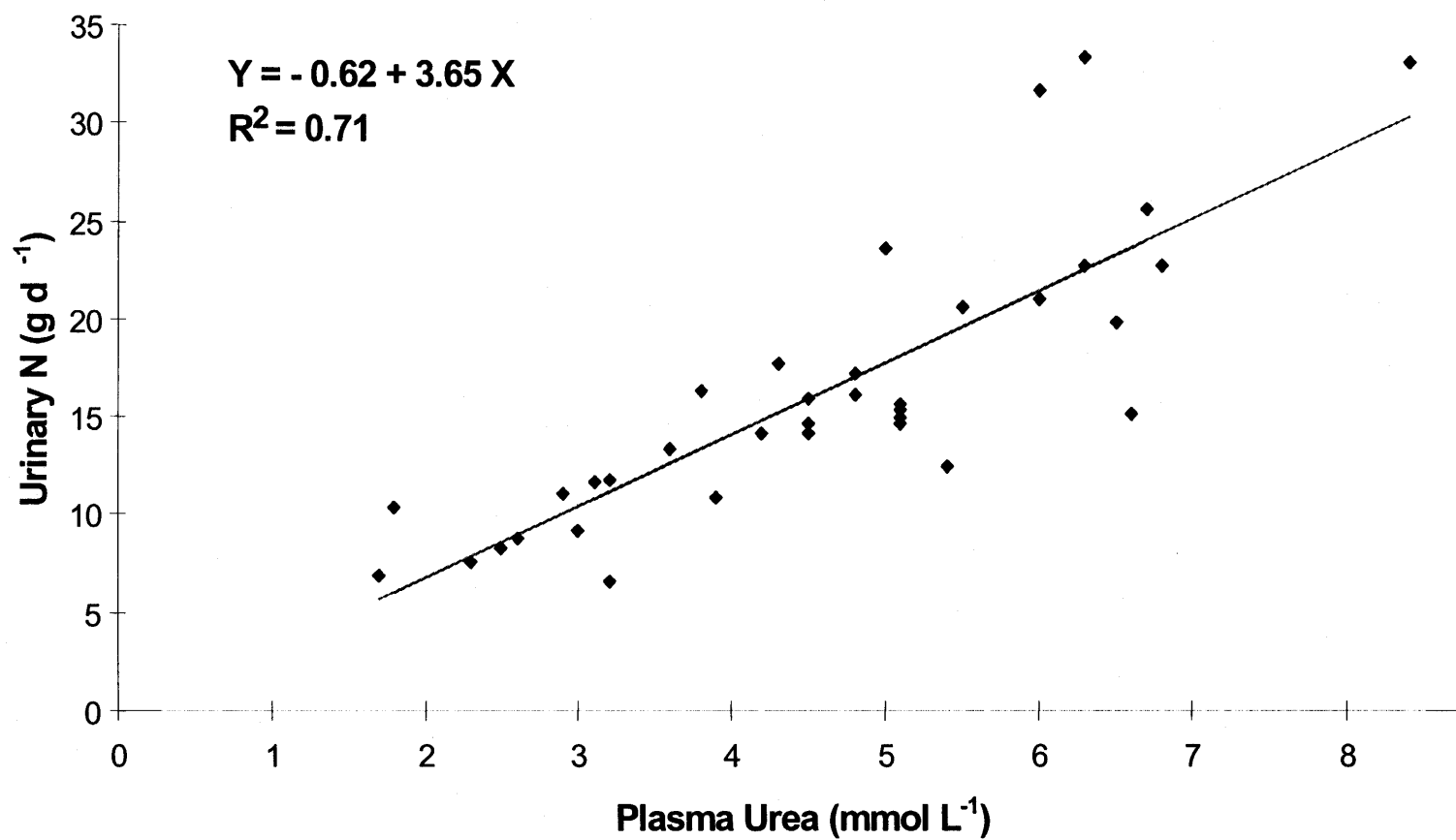


Figure 3.3 Relationship of urinary N to plasma urea concentration at 0800 with free access to feed ( $R^2 = 0.71$ ;  $n = 34$ ).



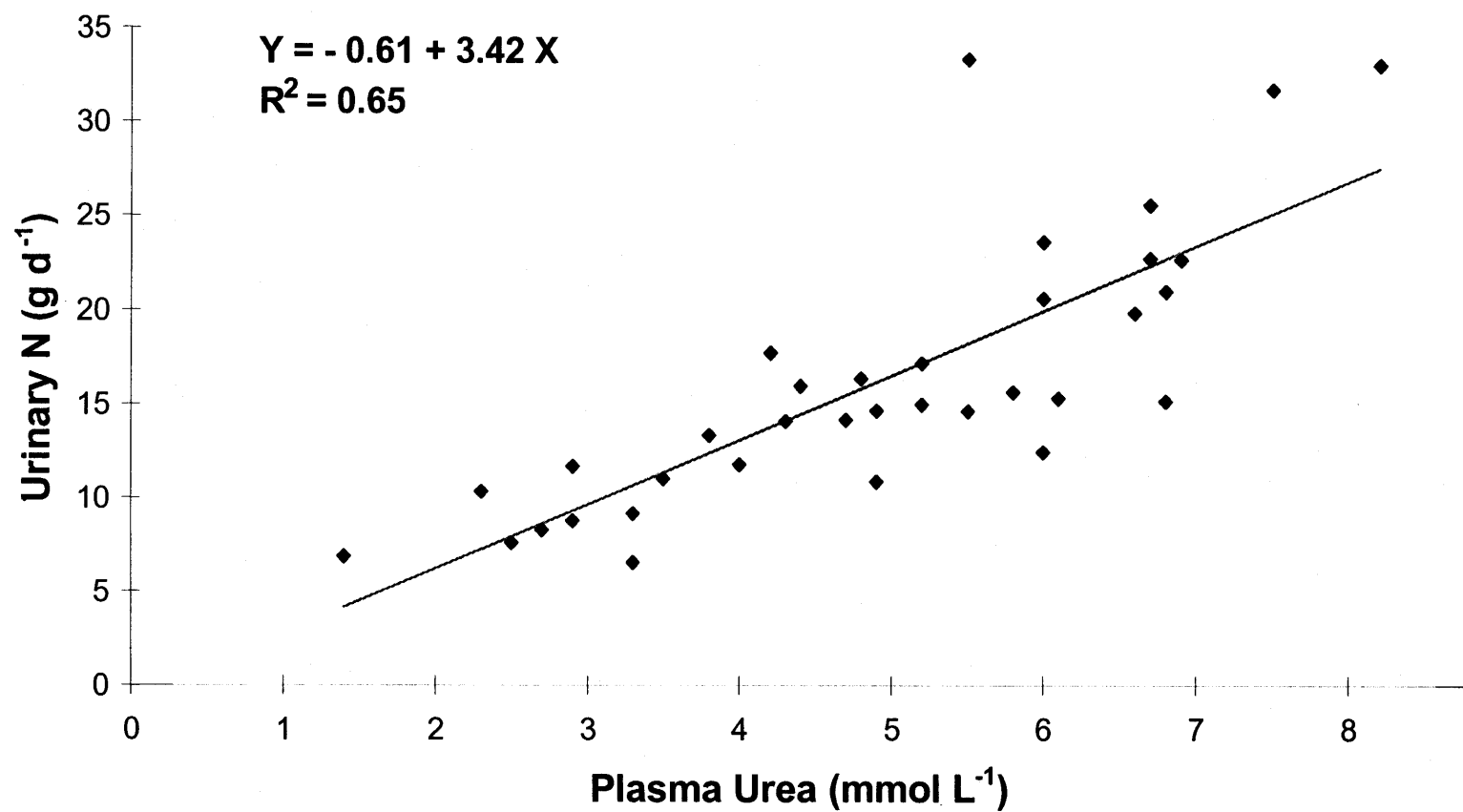


Figure 3.4 Relationship of urinary N to plasma urea concentration at 1200 with free access to feed ( $R^2 = 0.65$ ;  $n = 33$ ).

to faecal N excretion ratio was reduced by reducing dietary protein content. Because faecal N was not affected by protein content this reduction in urinary to faecal N ratio is a result of the reduction in urinary N excretion.

Under restricted access to feed, N retention was reduced by 4% (or  $0.9 \text{ g d}^{-1}$ ) for each percent reduction in dietary protein content. As discussed previously (Chapter 2), the reduction in N retention might be attributed partly to differences in intake of essential AA, especially lysine. Nitrogen retention might have been reduced due to a lower utilisation of synthetic AA when pigs are fed infrequently than when they have free access to feed (Partridge et al. 1985; Batterham et al. 1984). When pigs had free access to feed, N retention was not affected by dietary protein content indicating that intake of AA was the main reason for reduced N retention and not dietary AA balance. In addition, only for the SH diet was N retention reduced for low compared to high protein diets, whereas differences among other treatments were not detected. The reduced N retention for the low protein-SH diet, under restricted and free access to feed is probably an indication that one AA was limiting, most likely lysine.

Expressing the N variables as percentage of N intake, the reduction of protein content reduced faecal N and urinary N excretion while N excretion and retention were not affected, both under restricted and free access to feed.

Plasma urea concentration was reduced by reducing dietary protein content similar to Lopez et al. (1994) and Lenis et al (1999), reflecting differences in protein quality. Low protein diets induced a lower plasma urea concentration at both restricted and free access to feed, at both sampling times. Urinary N excretion was in close agreement with PU data showing a close relationship exists between urinary N excretion and plasma urea concentration.

Feed efficiency and ADG were not affected by dietary protein content either under restricted or free access to feed, despite reductions in N retention with restricted feed intake. This can be attributed probably to differences in energy deposition.

Digestibility of N was reduced with a reduction of dietary protein content. The reduction might be attributed to a reduction in inclusion rate of soybean meal, which has a higher N digestibility than wheat and barley. Energy digestibility was not affected by dietary protein content.

### 3.6.2 Fibre effects

Shifting of N excretion from urine to faeces was the primary effect of soybean hulls and sugar beet pulp in the present study. The main reason for this shift in N excretion patterns is that nitrogenous compounds that escape digestion and absorption enter the large intestine and are converted into ammonia. Microflora in the large intestine uses ammonia as N source for synthesis of microbial protein. The incorporation of ammonia into bacterial protein results in a decrease of ammonia available for absorption into blood, subsequent transformation to urea in the liver, and finally excretion in urine. Bacterial protein will be excreted in faeces, resulting in a shift of N excretion pattern from urinary N to faecal N (Mroz et al. 1993; Morgan and Whittemore 1988; Canh et al. 1997). From environmental point of view, faecal N excretion is preferable than urinary N excretion because faecal N is decomposed more slowly to volatile ammonia, reducing the amount of ammonia emitted into air during indoor and outdoor manure storage (Canh et al. 1998b, c).

Inclusion of sugar beet pulp and soybean hulls in the diet reduced urinary N excretion and increased faecal N excretion compared to control diets while N retention and total N excretion were not affected (either in  $\text{g d}^{-1}$  or expressed as % of intake), similar to Canh et al. (1997). Inclusion of 20% SBP reduced urinary N excretion 3.5 and 6  $\text{g d}^{-1}$  under restricted and free access to feed, respectively. Inclusion of 15% SH reduced urinary N excretion 2.5 and 6  $\text{g d}^{-1}$  under restricted and free access to feed, respectively. Canh et al. (1997) reported a reduction in urinary N of 13  $\text{g d}^{-1}$  for SBP and 8.5  $\text{g d}^{-1}$  for by-products. In contrast, Mroz et al. (2000) found that faecal N was increased when SBP and SH were included in the diet, but without a reduction in urinary N excretion. The lack of effects was not explained, but in the case of SBP increase in faecal N was accompanied with a reduction in N retention, indicating an AA imbalance. In the present study, urinary N to faecal nitrogen excretion was lowest for SBP, intermediate for SH, and highest for the control diets.

Sugar beet pulp clearly affected N excretion patterns. The effects of soybean hulls on N excretion patterns were less profound and differences occurred between protein contents. For high protein content, faecal N was not affected while urinary N was reduced compared to control. For low protein content, urinary N was not affected

while faecal N was increased compared to control. The reason that urinary N was not reduced might be an AA imbalance, which was validated by the lower N retention observed for this treatment under free access to feed. Based on AA analysis of the diets and the reduced N digestibility, lysine might have been the limiting AA for the low protein-SH diet. For the soybean hulls diets, faecal N excretion was higher for the low compared to the high protein diet, causing the interaction between dietary protein and fibre with restricted feed intake. For the soybean hulls diets, N digestibility was higher for high compared to low protein diet. Between the two diets, the major difference was that soybean meal in high protein diets was replaced by corn starch; thus, the high N digestibility was hard to explain. An interaction of corn starch with soybean hulls probably reduced the N digestibility. Although a similar replacement was made for SBP diets, differences in N digestibility were not observed.

Inclusion of SH and SBP reduced plasma urea concentration at both sampling times compared to control diets reflecting the reduced urinary N excretion. In rats, fermentable fibre reduces plasma urea concentrations and urinary N excretion as well (Younes et al. 1993).

Digestibility of N and energy was reduced also with the inclusion of SBP and SH in the diets, similar to Chabeauti et al. (1991). Mroz et al. (2000) observed that fibre source affected total tract and ileal digestibility of nutrients. Soybean hulls and sugar beet pulp reduced total tract digestibility of protein by 7.2 and 4.8 percentage units, respectively, compared to tapioca diets whereas total tract digestibility of energy was reduced only for SH. In addition, Mroz et al. (2000) concluded that the large intestine fermentation contributed substantially to digestion of SBP and SH based on the differences of ileal and total tract digestibility. The SBP and SH appear to be ideal sources for microbial fermentation, because of the chemical composition and physicochemical properties. Both sources of fibre have high amounts of soluble fibre in the form of pectin (Chabeauti et al. 1991) and thus are easily fermented in the large intestine (Stanogias and Pearce 1985; Chabeauti et al. 1991). Fermentation of SH and SBP resulted in an increased N assimilation in bacterial protein and excretion in the faeces, reducing N digestibility.

### **3.6.3 Low protein diets and inclusion of fibre source**

Dietary protein and fibre source did not interact with the exception of faecal N excretion under restricted feed intake; thus, the effects of the two factors are additive. Combining the two nutritional strategies will thus reduce urinary and total N excretion further than a single strategy without affecting animal performance. For example, with free access to feed, the low protein-SBP diet resulted in a 55% ( $14 \text{ g d}^{-1}$ ) reduction in urinary N excretion and a 27% (or  $10 \text{ g d}^{-1}$ ) reduction in total N excretion compared to the high protein-control diet without affecting performance. The additive effects are in agreement with Kreuzer et al. (1998) who found that effects of fermentable NSP on reduction of ammonia emission were enhanced by reducing dietary protein content resulting in a combined reduction of ammonia emission by 38%. For total and urinary N excretion, the additive effects were observed for the low protein-SH diet but also resulted in a reduced animal performance (ADG and N retention) for unknown reasons. Feed intake for the SH diets was numerically less compared to SBP and control diets and probably contributed to the reduced performance.

Generally, plasma urea concentration and urinary N excretion were in agreement for each dietary treatment and further confirmed the additive results.

### **3.6.4 Restricted versus free access to feed**

Overall, similar effects of dietary treatments were observed under restricted and free access to feed; however, N retention was higher with free access to feed. For low protein diets, N retention was reduced with restricted access to feed, but not with free access, indicating that effectiveness of low protein diets might be discounted if assessed in pigs with restricted feed intake. Furthermore, the ultimate possibilities to reduce N excretion are not shown under restricted feed intake.

Performance measured as ADG and feed efficiency was not affected by fibre source under restricted feed intake. With free access to feed, feed efficiency and feed intake were maintained but ADG was reduced. Average daily gain was reduced for the soybean hulls compared to control and SBP diets.

Digestibility of energy and N was reduced as feed intake was increased. This was expected because a higher feed intake results in higher passage rate of the digesta through the gastrointestinal tract, reducing digestibility of nutrients (Roth and Kirchgessner 1985).

For all diets DE content was reduced with free access to feed compared to restricted access to feed; however, the hierarchy observed with restricted access to feed was similar except for the low protein-control and -SBP diets.

### **3.6.5 Plasma urea concentration and urinary nitrogen**

Models to predict urinary N as a function of PU under restricted and free access to feed were developed using regression analyses. Under restricted feed intake, the best prediction of urinary N excretion was obtained from PU concentration at 4 h after feeding ( $R^2 = 0.66$ ). Under free access to feed, a good relationship between urinary N and PU was obtained for both sampling times ( $R^2 = 0.71$  at 0800 and  $R^2 = 0.65$  at 1200).

### **3.6.6 Implications**

Canh et al. (1998) found that ammonia emission was reduced 10% for each percent reduction in dietary protein. Because most of urinary N is in the form of urea, the reduction in urinary urea leads to a reduced ammonium content of manure and subsequently, a reduced ammonia emission from manure. Thus, the reduction in urinary N and total N excretion observed in the present study will result in a reduction of ammonia emission. In addition, fermentable NSP not only affects partitioning of N excretion but also decreases the pH of the manure by VFA formation in faeces and manure (Canh et al. 1997; 1998b, c) and consequently reduces the ammonia emission from manure (Canh et al. 1998b, c). A combination of reduction of dietary protein with inclusion of fermentable fibre may be an efficient feeding strategy to further reduce ammonia emission from pig production systems.

#### 4. GENERAL DISCUSSION

Reduction of dietary protein content reduced urinary and total N excretion whereas inclusion of fermentable carbohydrates shifted N excretion from urinary to faecal N without affecting total N excretion or N retention. The ratio of urinary N to faecal N excretion was reduced by reducing protein content or by including fermentable carbohydrates in the diet. Plasma urea concentration was reduced when dietary protein content was reduced or when fermentable carbohydrates were included in the diet.

Results of the present thesis support the hypothesis that reduction of dietary protein content while balancing for AA reduces urinary and total N excretion (Chapter 2 and 3). Under restricted access to feed, for each percent reduction in dietary protein content, urinary N excretion was reduced 8 to 10% (or 1.2 to 1.4 g d<sup>-1</sup>) and total N excretion 4 to 7% (or 1.2 to 1.7 g d<sup>-1</sup>). Under free access to feed, for each percent reduction in dietary protein content urinary N excretion was reduced 27% (or 1.8 g d<sup>-1</sup>) and total N excretion 16% (or 1.8 g d<sup>-1</sup>). Also, urinary N to faecal N excretion ratio was reduced by reducing dietary protein content mainly because of the reduction in urinary N excretion. Under restricted access to feed, N retention was slightly reduced, whereas when pigs had free access to feed, N retention was not affected by dietary protein content. The reason for the reduction in N retention is not clear from the present studies and further studies are required to maintain N retention while feeding low protein diets.

Shifting N excretion patterns from urea in urine to faeces was the primary effect of fermentable fibre sources (soybean hulls and sugar beet pulp) in the present study (Chapter 3). The main reason for this alteration in N excretion pattern is that nitrogenous compounds that have escaped digestion and absorption will enter the large intestine and are converted into ammonia. Microflora in the large intestine use ammonia as N source for synthesis of microbial protein. The incorporation of ammonia into bacterial protein results in a decrease of ammonia available for absorption into portal blood, subsequent transformation to urea in the liver, and finally excretion in urine. Bacterial protein will be excreted in faeces, resulting in a shift of N excretion patterns

from urinary N to faecal N. Inclusion of fibre sources, resistant to microbial fermentation, such as oat hulls does not affect N excretion patterns (Chapter 2). From an environmental point of view, faecal N excretion is preferable to urinary N excretion because faecal N is decomposed more slowly to volatile ammonia, reducing the amount of ammonia emitted into air during indoor and outdoor manure storage.

Dietary protein and fibre source did not interact; thus, the effects of the two factors are additive. The addition of fermentable fibre enhanced the effects of the low protein diet on urinary N excretion by causing an additional reduction in urinary N excretion. Thus, combining the two nutritional strategies will reduce urinary and total N excretion without affecting animal performance. Under free access to feed, the low protein-SBP diet resulted in a 55% ( $14 \text{ g d}^{-1}$ ) reduction in urinary N excretion and 27% (or  $10 \text{ g d}^{-1}$ ) reduction in total N excretion compared to high protein-control diet, without affecting performance. Further studies are required to investigate the effects of SH on animal performance and N retention.

Dietary protein content, time of sampling, and a dietary protein by time interaction affected PU concentration (Chapter 2). Plasma urea concentration was linearly decreased with a decrease of dietary protein content. The PU concentration increased after feeding and peaked at 4 h after feeding. The effect of time was significant for the high and medium protein diets while time effect was not detected for the low protein. A low PU concentration reflects a high quality of dietary protein, indicating a better balance for AA. Plasma urea concentrations were not affected by addition of insoluble fibre in the diets (Chapter 2) but were reduced by addition of fermentable fibre (Chapter 2).

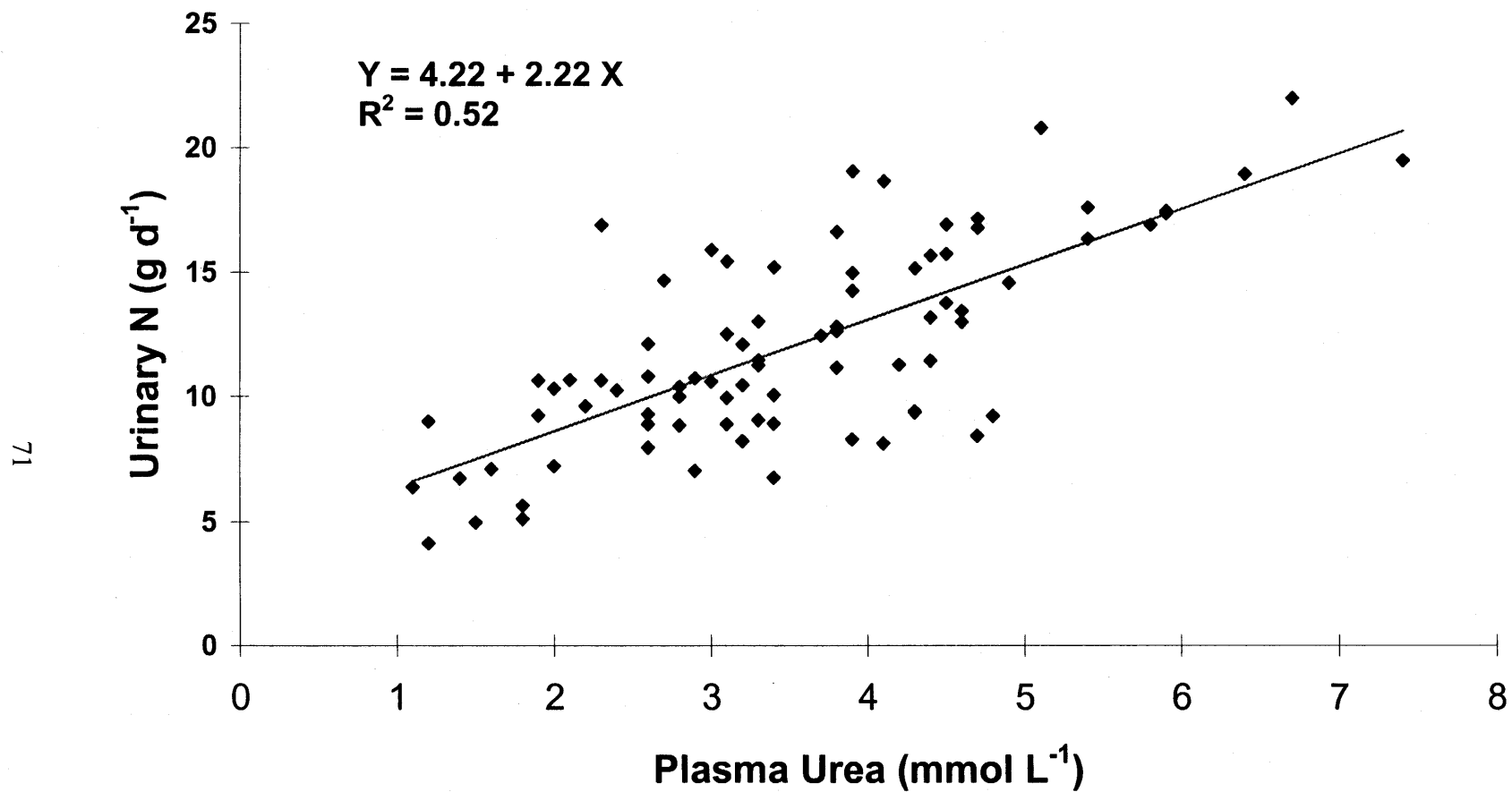
Plasma urea was correlated to urinary N excretion suggesting that urinary N excretion could be predicted from plasma urea concentrations (Chapter 2 and 3). The relationship of PU and urinary N excretion was investigated using regression analysis. Under restricted feed intake, a high relationship was found ( $R^2 = 0.66$ ), raising the possibility that urinary N excretion could be predicted to a sufficient degree from the PU concentration. Because time of sampling affected PU concentration, choice of sampling time is very crucial to prediction. Both experiments showed that the best prediction of urinary N excretion was obtained from PU concentration at 4h after feeding. Data from



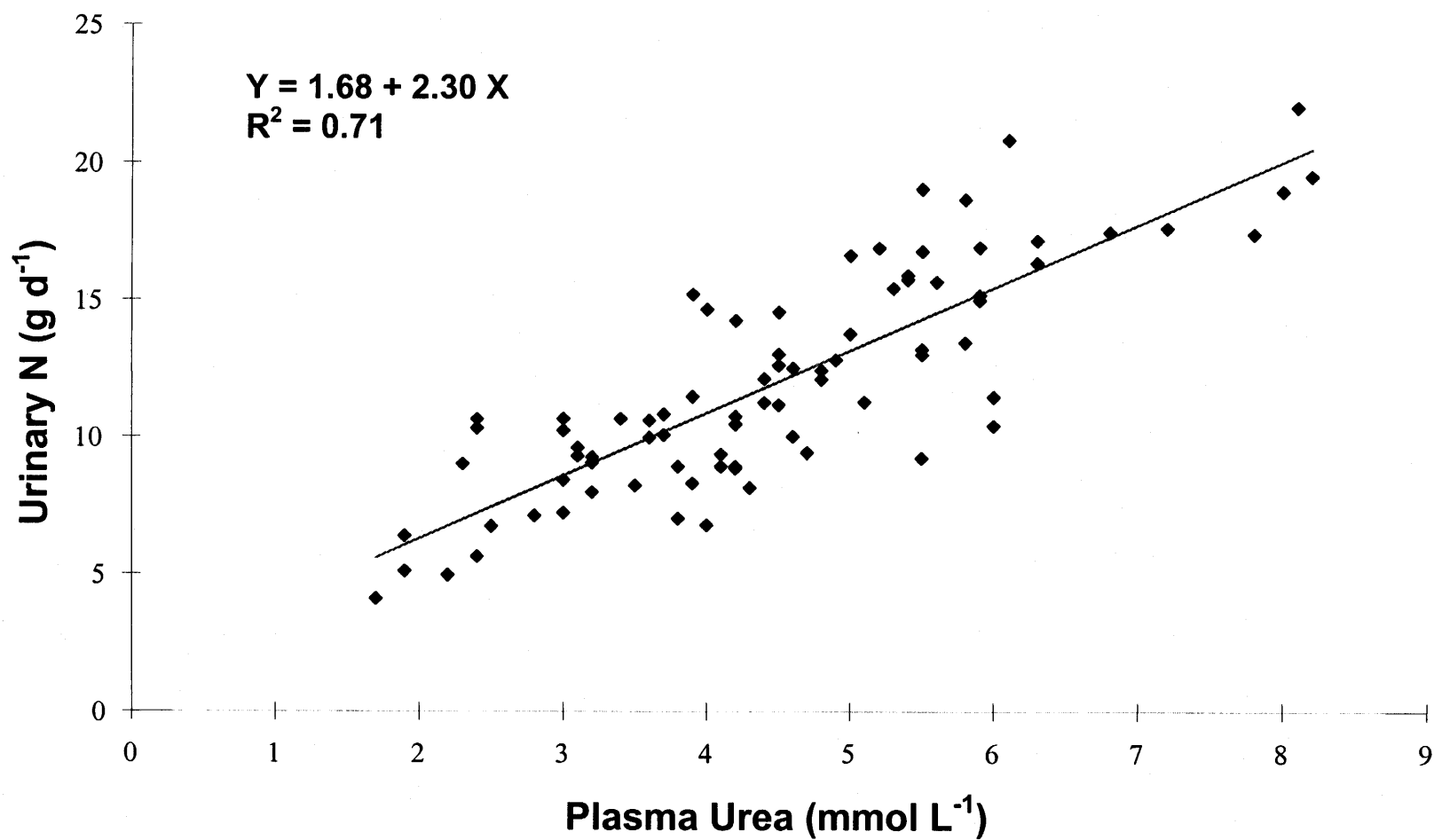
both experiments, under restricted feed intake, were pooled and new models to predict urinary N excretion as a function of PU were developed using regression analyses (Figures 4.1 and 4.2). Under free access to feed, a high relationship between urinary N and PU was obtained for both sampling times ( $R^2 = 0.71$  at 0800 and  $R^2 = 0.65$  at 1200). Development of a model to predict urinary N excretion has several benefits. Blood sampling from pigs in a production setting is easier than the collection of urine. Thus, a model might be functional to assess N status under commercial farm conditions.

Reduction of dietary protein content reduced both faecal and urinary N excretion and consequently total N excretion. The effects were higher for the urinary N because the AA that are in excess are excreted as urea in the urine. Results suggest that reduction of dietary protein content increases the efficiency of N utilisation resulting in reduction of N excretion. Although ADG and FE were not affected by reducing dietary protein content, further research is required to maintain nitrogen retention.

The reduction in urinary N and total N excretion by reducing dietary protein observed in the present study will result in a reduction of ammonia emission. In addition, fermentable NSP affect partitioning of N excretion and also decrease the pH of manure by VFA formation in faeces and manure and consequently reduce ammonia emission from pig manure. This approach may be an efficient nutritional strategy to reduce ammonia emission from pig production systems.



**Figure 4.1** Relationship of urinary N to plasma urea concentration before feeding combined for both experiments ( $R^2 = 0.52$ ;  $n = 78$ ).



**Figure 4.2** Relationship of urinary N to plasma urea nitrogen at 4 hours after feeding combined for both experiments ( $R^2 = 0.71$ ;  $n = 78$ ).

## 5. LITERATURE CITED

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