Impact of dietary cation anion difference in fish and pigs: a comparative study

Yueming Dersjant-Li

CENTRALE LANDBOUWCATALOGUS
0000 0795 8602

Promotoren: Dr. ir. M. W. A. Verstegen

Hoogleraar in Veevoeding,

i.h.b. de voeding van éénmagigen

Dr. E. A. Huisman

Hoogleraar in de Visteelt en Visserij

Co-promotoren: Dr. J. A. J. Verreth

Universitair hoofddocent, leerstoelgroep Visteelt en Visserij

Dr. ir. J. W. Schrama

Universitair docent, leerstoelgroep dierhouderij

NIOB201, 2237

Impact of dietary cation anion difference in fish and pigs: a comparative study

Yueming Dersjant-Li

Proefschrift

ter verkrijging van de graad van doctor

op gezag van de rector magnificus

van de Wageningen Universiteit,

Dr. Ir. L. Speelman,

in het openbaar te verdedigen

op maandag 18 september 2000

des namiddags te half twee in de Aula.

ISBN 90-5808-252-

Cover design: Pablo Almazan Rueda
Printing: Ponsen & Looijen, Wageningen

CIP-DATA KONINLIJKE BIBLIOTHEEK, DEN HAAG

Dersjant-Li, Yueming

Impact of dietary cation anion difference in fish and pigs: a comparative study / Yueming Dersjant-Li. [S.I.:s.n.]. – III Thesis Wageningen University. – With ref. – With summary in English, Chinese and Dutch.
ISBN: 90-5808-297-0

Dersjant-Li, Y. 2000. Impact of dietary cation anion difference in fish and pigs: a comparative study

Dietary cation anion difference (CAD, Na + K – Cl, mEq kg^{-1}) determines the pH and acid base status of a diet, consequently affecting the acid base balance in the body compartments of animals. After feeding, a low dietary CAD will contribute more acids to the animals than a high dietary CAD. An optimal dietary CAD will increase the acid buffer capacity of a diet and this will help animals to compensate for metabolic acidosis. It is hypothesized that with an optimal dietary CAD, less energy will be needed for acid base regulation, indirectly improving feed intake and growth of animals. In the present study the effect of dietary CAD on growth performance, energy metabolism, acid base balance in the blood and in the digestive system were investigated in African catfish and pigs. The study consisted of 3 parts. Part 1 dealt with the growth response to dietary CAD and dietary Na/K ratio. Part 2 dealt with the energetic response to dietary CAD. Parts 3 dealt with the acid base balance in the blood and in the digestive system in response to dietary CAD. A negative dietary CAD (-100 mEq kg^{-1}) resulted in a low feed intake and growth in both African catfish and young pigs. In African catfish, increasing dietary CAD from –100 to 700 mEq kg^{-1} led to a linear increase in growth. In pigs, the optimal dietary CAD was observed to be between 200 and 500 mEq kg^{-1}. The optimal dietary Na/K ratio in formulating dietary CAD was 1.5 to 2.5 (mol/mol) for African catfish. The lowest maintenance cost was observed at a dietary CAD level of 700 mEq kg^{-1} for African catfish. In pigs, dietary CAD of 200 mEq kg^{-1} tended to increase energy requirement for maintenance compared with dietary CAD of –100 mEq kg^{-1}, at restricted feeding. In pigs, a –100 mEq kg^{-1} CAD diet resulted in low blood pH, oxygen and HCO_3^- content (mmol L^{-1}) compared to a 200 mEq kg^{-1} CAD diet. During the postprandial period, however, pigs maintained a relative constant pH level in both portal and arterial blood within each CAD group. African catfish fed 700 mEq kg^{-1} CAD diet showed higher stomach digesta pH than fish fed –100 mEq kg^{-1} CAD diet both 0.5 and 3 h after feeding. However, no difference in pH of small intestine digesta was observed. In pigs, dietary CAD levels of –100 and 200 mEq kg^{-1} did not affect either stomach or small intestine digesta pH 2.5 h after feeding. The possible mechanisms of dietary CAD effect on feed intake and growth were discussed.
PhD thesis, Fish Culture and Fisheries Group, Animal Nutrition Group and Adaptation Physiology Group, Wageningen Institute of Animal Science (WIAS), Wageningen University, Marijkweg 40, 6709 PG Wageningen, The Netherlands.

This study was financially supported by Finn Feeds Int., Provimi BV. and Wageningen University.

BIBLIOTHEEK
LANDBOUWUNIVERSITEIT
WAGENINGEN

NN08201, 2287

Stellingen belonging to the thesis

"Impact of dietary cation anion difference in fish and pigs: a comparative study"

Yueming Dersjant-Li

Wageningen, September 18, 2000

Table of contents

For my family

GENERAL INTRODUCTION

Characteristics of dietary cation anion difference

Definition of dietary cation anion difference

In animal nutrition, for decades the main concern was to meet dietary protein (amino acids) and fat (lipids) requirements. However, the metabolic consequences of diets should also be taken into consideration when formulating diets. In this regard, also the dietary cation anion difference (CAD) became an issue of interest as it directly influences the acid base homeostasis of animals. The CAD in a diet determines the dietary pH and the acid base buffering capacity (the amount of acid or base needed to change one unit pH) of the diet. A low dietary CAD gives a low dietary pH while an increase in dietary CAD results in a more alkaline diet. Therefore ingesting diets with different CAD levels will contribute acid or base to animals and consequently affect acid base homeostasis in the body compartments of animals.

Dietary CAD refers to the sum of dietary mineral cations minus the sum of dietary mineral anions, expressed as mEq kg^{-1} diet. The dietary mineral cations include sodium, potassium, magnesium and calcium. The dietary mineral anions comprise of chloride, phosphorus, and inorganic sulfur. In general three methods are used to calculate dietary CAD:

$$CAD = (Na + K - Cl),\ mEq\ kg^{-1} \quad (1)$$

$$CAD = (Na + K - Cl - 2S),\ mEq\ kg^{-1} \quad (2)$$

$$\text{or } CAD = (Na + K + 2Mg + 2Ca - Cl - 2S - 2P),\ mEq\ kg^{-1} \quad (3)$$

Equation (1) is also defined as *dietary electrolyte balance* and equation (3) is also expressed as the *dietary undetermined anion.*

The prevailing method of formulating CAD in a diet is by adjustment of the Na and Cl content through addition of salts such as $CaCl_2$ and $NaHCO_3$ while maintaining all other ions constant (Table 1).

Sodium, potassium and chloride are the most important ions in determining the acid base status of a diet. These ions, especially Na and Cl, are also the major electrolytes in the blood of animals and human (Table 2). Literature has shown that formulation of the dietary CAD by adjustment of the Na and Cl content had a more pronounced effect on the animal's performance than by using other cations or anions. Waterman *et al.* (1991) compared the efficacy of using Mg or of using the sum of Na and K, to increase dietary CAD in ruminants. They found that Na + K supplementation increased feed intake significantly but Mg supplementation had no effect on the performance of animals. Johnson and Karunajeewa (1985) studied the effect of the total cation anion balance (Ca + Na + K + Mg) - (Cl + H_2PO_4

+ HPO_4) and of the electrolyte balance (Na + K –Cl) on the performance of chicks and found that the latter described the growth of chicks better than total cation anion balance. Patience and Wolynetz (1990) used equation 3 to calculate dietary undetermined anion. The different levels of dietary undetermined anion were formulated by adjusting the Na and/or K and Cl content while the other ions (Ca, Mg, S and P) were kept constant. In this circumstance, dietary CAD should be expressed as Na + K - Cl.

Table 1. Additives used in formulation of dietary CAD in different studies

Authors	Year	Animals	Additives used
Patience *et al.*	1987a	Pig	$CaCl_2$ and $NaHCO_3$
Wilson *et al.*	1985	Fish	$CaCl_2$ and $NaHCO_3$
Chiu *et al.*	1988	Fish	$CaCl_2$, K_2CO_3 and $NaHCO_3$
Ross *et al.*	1994	Steers	NH_4Cl and $NaHCO_3$
Patience and Wolynetz	1990	Pig	$CaCl_2$, $NaHCO_3$ and $KHCO_3$
Fauchon *et al.*	1995	Lambs	NH_4Cl, $CaCl_2$, and $NaHCO_3$
Haydon and West	1990	Pig	$CaCl_2$ and $NaHCO_3$
West *et al.*	1991	Dairy cows	$CaCl_2$ and $KHCO_3$
Johnson and Karunajeewa	1985	Chick	NH_4Cl, $NaHCO_3$ and K_2CO_3

Table 2. Plasma electrolyte concentrations (mEq L^{-1}) of young pigs (25kg)[1], fish [2] and human[3]

Electrolyte	Pigs	Fish		Human
		Myxine	*Cyprinus carpio*	
Na	148	558	130	142
K	4.5	9.6	6.3	4.3
Cl	112	576	127	104
CAD	40.5	-8.4	9.3	42.3
Ca	1.5	6.3	1.5	2.5*
P	2.2⊗			2.0**
Mg	0.4	19.4	0.7	1.1*

[1] from Scott & McIntosh (1975). [2] from Albers (1970). [3] from Rose (1989) * the values for Ca^{2+} and Mg^{2+} include only the ionized (unbound) form of these ions. ** in the form of $H_2PO_4^-$, HPO_4^{2-}. ⊗ in mmol L^{-1}.

Furthermore, the Na:K ratio should also be taken into account when formulating different CAD diets. Johnson and Karunajeewa (1985) reported that using excess K (1.2 –

2%) to increase dietary CAD decreased the performance of chicks compared to the excessive use of Na. They stated that the Na:K ratio should be between 0.5 to 1.8.

In this thesis, dietary CAD refers to the calculation of Na + K - Cl, expressed as mEq kg^{-1} diet.

CAD in some feed ingredients

In animal feeds, cereal products are commonly used ingredients. In these feed ingredients, large variations exist in electrolyte concentrations and the acid buffer capacity (the amount of HCl needed for stomach digestion (pH = 3.0) of 1 kg diet) (Table 3).

Table 3. Sodium, potassium, chloride contents and dietary CAD (Na + K – Cl) of several feed ingredients[1]

Ingredients	Na	K ($g\ kg^{-1}$)	Cl	CAD ($mEq\ kg^{-1}$)	Acid buffer capacity[2] ($mEq\ kg^{-1}$)
Maize meal	0.1	3.7	5.0	-42	211
Maize starch					44
Maize gluten feed (cp > 20%)	1.2	11.8	2.1	295	
Barley	0.1	4.6	1.0	94	
Wheat	0.2	4.4	0.4	110	
Wheat gluten meal					353
Sorghum (cf < 3%)	0.1	3.7	0.7	79	
Tapioca	0.1	7.6	0.5	213	
Rice bran	0.1	10.4	0.3	258	
Wheat bran	0.4	13.4	1.3	324	
Soybean meal-44 solv.extr.	0.2	21.5	0.3	551	1000
Soya flour					986
Soya concentrate					1149
Soya isolate					926
Sunflower meal solv.extr.	0.1	16.4	1.1	393	
Skim milk powder	6	15.0	10.9	338	
Fish meal (cp > 68%)	8.9	12.9	17.9	213	
Whey powder	7.2	22.9	20.3	327	
Meat and bone meal	7.6	4.0	3.7	329	

[1]From Kemme-Kroonsberg (1993). [2] The amount of HCl needed for stomach digestion (pH = 3.0) of 1 kg diet (ingredients), measured in the laboratory of the Animal Nutrition Group.

For example, maize meal has a CAD level of –42 mEq kg^{-1} and a low acid buffer capacity of 211 mEq kg^{-1}. While soybean meal shows a high CAD of 551 mEq kg^{-1} and a high buffer capacity of 1000 mEq kg^{-1}. Therefore, when an animal feed is formulated with different feed ingredients, the dietary CAD and acid buffer capacity will change accordingly.

The role of CAD on acid base regulation

Animals try to maintain a stable pH and electrolyte balance in the body fluids. Homeostasis of the internal environment in the body is of vital importance for proper physiological function and survival (Kemme-Kroonsberg, 1993). However, the dietary electrolyte balance may be different from the one in body fluids and in cells. This means that, to maintain a stable acid base balance and electrolyte balance in their body fluids, animals have to correct for inappropriate CAD's of the diet.

Different dietary CAD levels give rise to different dietary pH and acid base buffer capacity of the diets. After ingestion of diets with different CAD levels, the acid base balance in the stomach and in the small intestine of animals will be directly influenced. This happens shortly after feeding and is considered a short-term effect. Normally animals need to maintain a low pH (approximately 3) in the stomach and a high pH (about 6-7) in the small intestine. Therefore, animals that received a high CAD diet need to secrete more HCl to maintain a proper pH in the stomach. On the other hand, with a low dietary CAD, animals need to secrete more $NaHCO_3$ from the pancreas to compensate for the acidity of the diet and to maintain optimal pH required for digestion. It is generally accepted that animals have a high ability to maintain the acid base balance in the gastro-intestinal tract after ingesting different levels of dietary CAD.

The absorption of dietary CAD will further influence the acid base balance in the cells and in the blood. This effect is a gradual process and is considered a long-term effect. The absorption of monovalent ions in the gut lumen is through the Na^+/H^+ and Cl^-/HCO_3^- ion exchange system. In the small intestine, Cl^- is absorbed in exchange for HCO_3^- to maintain electrical neutrality. If insufficient Na^+ is present to allow the absorption of (neutral) NaCl, an excessive drain of blood HCO_3^- can lead to acidosis (Block, 1994). On the other hand, Na^+ is absorbed by exchange of H^+. If Na^+ absorption is in excess of Cl^-, this would lead to a metabolic alkalosis.

The pH levels in the blood and cells are maintained by acid base buffer systems, namely the Pco_2 (HCO_3^-) buffer system, the hemoglobin buffer system and the ion exchange system. Ion exchange plays an important role in regulating intracellular and extracellular pH. This system consists of a Na^+/H^+ exchanger, a Cl^-/HCO_3^- exchanger and the Na^+/HCO_3^- cotransporter. Figure 1 shows the ionic pathways for intracellular pH regulation in the mammalian liver.

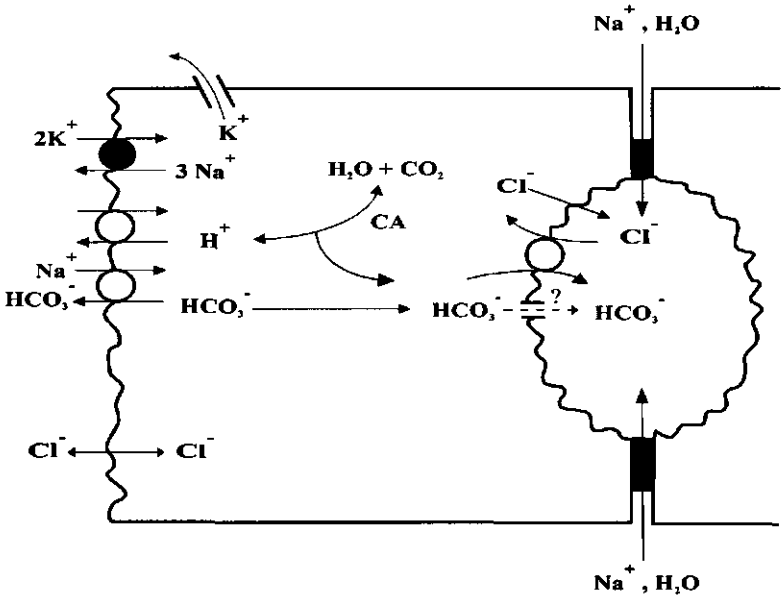

Figure 1. Ionic pathways for intracellular pH regulation in the mammalian liver, adapted from Walsh and Mommsen (1992).

When acidosis occurs, H^+ will be excreted and replaced by Na^+, thereby increasing the pH. In alkaline disturbed conditions, HCO_3^- or OH^- will be exchanged by Cl^- and the pH will decrease. K^+ is mainly present in the intracellular fluid. The balance between dietary Na, K and Cl plays thus a major role in determining the acid base balance in biological fluids (Block, 1994).

In the kidney, normally H^+ and ammonia are secreted and $NaHCO_3$ is reabsorbed. If Cl^- is in excess in the kidney, it may exchange with HCO_3^-, resulting in NaCl re-absorption and in a reduced HCO_3^- re-absorption. This will further reduce the acid buffer in the blood.

Therefore, dietary CAD can challenge acid base homeostasis in the body compartments of animals.

Mechanism of acid base and ion regulation in fish

The mechanism for maintaining the acid base balance differs between fish and higher vertebrates. In fish, buffer values (defined as the amount of H^+ ions required to change the pH by one unit) of the blood and of the intracellular compartments are generally much smaller than in higher vertebrates. The buffer value of the bicarbonate-CO_2 buffer system, for instance, is smaller in fish by a factor of three to eight (Heisler, 1984, 1986), due to a low exchange rate of Pco_2 in the water. The pH in fish is mainly maintained by the ion exchange system. This contrasts with terrestrial animals, where regulation of the pH is mainly through the ventilatory control of Pco_2. The overall implication is that fish are more sensitive to dietary CAD changes and that dietary CAD changes exert a greater influence on the acid base system in fish compared to air breathing animals. Furthermore, the high ion exchange ability with the direct environment that fish possess might indicate that fish could cope with high dietary ion's concentration.

In addition, acid base balance is closely related to osmo-regulation in fish. In freshwater fish, the osmotic concentration of blood lies in the range 260-330 mOsm kg^{-1}, which is much higher than the osmotic concentration in freshwater (Jobling, 1995). Hence, fish will tend to gain water by diffusion through the body surface (gills) and loose salt by diffusion from the body to the water. Freshwater fish have to maintain salt balance by dietary salt intake and by active uptake of ions across the gills (Eddy, 1982). The uptake of ions by the gills is against the ion's gradients between fish body and water, and therefore, this process requires energy. Absorption of the ions from the intestine may spare the energy required for ion's uptake from gills. Thus an optimal dietary CAD may aid freshwater fish to maintain both acid base balance and osmo-regulation.

The effect of dietary CAD on feed intake and growth

In many animal species, it has been reported that dietary CAD can influence feed intake and growth. In lambs, a linear increase in feed intake and growth was found with increasing dietary CAD from 100 to 700 mEq kg^{-1} (Fauchon *et al.*, 1995). In growing steers,

feed intake increased linearly and weight gain increased quadratically with increasing dietary CAD from 0 to 450 mEq kg^{-1} (Ross *et al.*, 1994). In cows, dry matter intake and milk yield increased when dietary CAD was increased from –100 to 200 mEq kg^{-1} (Tucker *et al.*, 1988). The optimal dietary CAD for dry matter intake and milk production was found at a dietary CAD between 300 and 500 mEq kg^{-1} (Sanchez and Beede, 1994). In weanling pigs, feed consumption and feed efficiency improved with dietary CAD increasing from 50 to 350 mEq kg^{-1} (Park *et al.*, 1994). In poultry, a CAD level between 250-300 mEq kg^{-1} caused better weight gain compared to lower or higher CAD levels (Mongin and Sauveur, 1977; Johnson and Karunajeewa, 1985). However, Chui *et al.* (1984) and Wilson *et al.* (1985) found that dietary CAD did not significantly influence feed intake and growth of rainbow trout.

Problem formulation

By choosing both the African catfish (*Clarias gariepinus*) and the pig as model animals, the proposed study focuses on both a water breathing animal and an air breathing animal. Such a comparative study would allow conclusions to be drawn on dietary CAD effect for more animals in general.

Fish

According to available literature, most work in fish focused on the influence of dietary CAD on amino acids metabolism (Chiu *et al.*, 1984, 1987, 1988). In these studies, the response of growth and feed efficiency to dietary CAD was tested. However, different results were found among various experiments. Wilson *et al.* (1985) reported that a dietary CAD level of 155 mEq kg^{-1} resulted in a numerically higher growth rate and feed efficiency in rainbow trout than other (higher or lower) dietary CAD levels. Based on this information, it is uncertain whether dietary CAD will have a significant effect on performance of fish, as the source of protein (Chiu *et al.*, 1988) and the use of purified diets may influence the response of fish to dietary CAD. Evidence of the effect of dietary CAD as a result of different formulations of commercial feeds (e.g. different ingredient composition) is lacking and will therefore have to be studied.

Pigs

Some observations on the effect of dietary CAD levels on food intake and growth in pigs have been reported in literature. However, results are not consistent and different studies reported different optimal dietary CAD levels. It was found that both growth and food intake was maximal at a dietary CAD level ranging from 0 to 340 mEq kg^{-1}, but decreased at a dietary CAD level of -85 mEq kg^{-1} (Patience *et al.*, 1987a). Park *et al.* (1994) observed that weight gain improved at dietary CAD from 50 to 150 mEq kg^{-1} while maximum food consumption occurred at dietary CAD of 350 mEq kg^{-1}. Austic *et al.* (1983) suggested that the dietary CAD levels between 100 and 300 mEq kg^{-1} resulted in optimal weight gain. Haydon *et al.* (1990), however, reported a linear increase in weight gain and food intake with dietary CAD increasing from 25 to 400 mEq kg^{-1}. Therefore, determining an optimal dietary CAD for pigs from available information is a difficult procedure. On the other hand, interactions between dietary CAD and feed composition may be expected. Moreover, the addition of feed enzymes may influence the digestibility of minerals, and this may interact with dietary CAD.

Hypothesis and the objective of this thesis

Acid is generated in considerable quantity as a result of normal metabolic activity (Patience *et al.*, 1987b). These acids consist of volatile (or CO_2) and fixed (such as sulfuric, lactic) acids. CO_2 can be readily removed by the lungs of an animal (for fish, by gas exchange through gills). The fixed acids will rapidly decrease the blood pH and the intracellular pH (pHi). Ketelaars and Tolkamp (1991) suggested that intracellular pH may be an important parameter used by animals to adjust feed intake.

The diet can play a crucial role in increasing or decreasing the total acid or alkaline load, resulting from acids generated by metabolic activities. Diet containing excess of sulfur amino acids can contribute to the acid load through oxidation of these amino acids. The absorption of cations and anions by the gastrointestinal tract plays also an important role in the acid load. For example, when more anions than cations are absorbed, the acid load will increase compared to equal absorption of both anion and cation (Kemme-Kroonsberg, 1993).

Since most enzyme systems require an optimum pH for catalysing metabolic reactions, changes in pH are expected to result in changed enzyme activity, and this results in further fluctuations in metabolic performance (Heisler, 1984). To maintain an optimal pH, an

acid load produced by metabolic activities has to be compensated by the acid base regulation system.

In addition, in an animal's active transport system, the Na-K pump mechanism maintains a high level of Na^+ concentration in the extracellular fluid and a high level of K^+ concentration in the intracellular fluid. By maintaining this gradient, nutrients (e.g., glucose) are taken up by the cells. The gradient of K^+ and Na^+ is established by action of Na-K ATPase and therefore requires energy (Lehninger, 1971; Goss *et al.*, 1992). Obviously, excess of one cation to the other can change the efficiency of the Na-K pump. Therefore, dietary Na/K ratio may indirectly affect the efficiency of the Na-K pump and the associated energy expenditures.

The working hypotheses of this study are, therefore, as follows. Firstly, feed intake is closely related to the acid base balance of animals. With a low CAD diet, animals may have to eat less to reduce accumulations of acid, while maintaining the acid base balance in the body. An optimal dietary CAD will improve the dietary acid buffering capacity and reduce acidity of a diet. This in turn will improve the acid buffering capacity in the animal's body compartments and help to prevent acidosis resulting from metabolic activity. Maintenance costs for homeostasis will thereby be reduced and feed intake and growth will be improved. Secondly, feed intake is (among others) regulated through a mechanism that optimizes net energy intake, e.g. an animal balances costs/benefits from extra feed and will regulate it's intake level accordingly. With a non-optimal dietary CAD, extra energy will be required for acid base balance regulations and maintenance of homeostasis. To optimize energy utilization efficiency, feed intake may be reduced. Thirdly, dietary CAD can have both a short-term effect on the acid base balance in the digestive system and a long-term effect on the acid base balance in the other body compartments (blood and cells) of animals.

Therefore, the general objective of this thesis is to investigate the physiological and the energetic responses of fish and pigs to changing dietary CAD levels.

The specific objectives are:

1. Dietary CAD and performance: to ascertain an optimal dietary CAD for fish and pigs; to investigate the interaction between dietary CAD and other feed components, such as feed enzyme addition; to determine optimal dietary Na/K ratio for formulating an optimal CAD diet.

2. Dietary CAD and maintenance costs: to investigate the effect of dietary CAD on nitrogen balance and energy metabolism; to estimate the energy requirements for maintenance costs in fish and pigs.
3. Dietary CAD and acid base balance: to investigate to what extent animals can maintain the acid base balance in the blood during the postprandial period; to test to what extent animals can regulate the acid base balance in the digestive system after ingesting different CAD diets.

Outline of this thesis

This study investigated physiological and energetic responses of fish and pigs fed different CAD diets. The thesis consists of 3 parts. Part 1 deals with the growth performance in response to dietary CAD and dietary Na/K ratio. Part 2 deals with the energetic response to dietary CAD. Parts 3 deals with acid base balances in the blood and in the digestive system in response to dietary CAD.

Part 1. The first step of this study is to determine an optimal dietary CAD for African catfish (chapter 1) and young pigs (chapter 2) at a given dietary composition. In chapter 2 the interaction of dietary CAD with feed enzyme was studied also. Chapter 3 determined an optimal dietary Na/K ratio for formulation of CAD's in fish diets.

Part 2. To test the hypothesis that dietary CAD is related to maintenance costs for homeostasis, two experiments were conducted in fish (chapter 4) and pigs (chapter 5) respectively. In these experiments nitrogen and energy balances were determined and the energy requirements for maintenance at different dietary CAD levels were estimated.

Part 3. Changing dietary CAD may influence arterial and portal blood oxygen content and affect acid base balances in the blood during the postprandial period. In chapter 6, changes in arterial and portal blood oxygen content, postprandial blood pH, Pco_2, HCO_3^-, base excess, Na^+, K^+ and Cl^- were investigated in pigs receiving different levels of dietary CAD.

A high dietary CAD results in high dietary pH and a low dietary CAD gives a low dietary pH. Therefore, ingesting different CAD diets may interfere with the acid base balance in the digestive system. To answer whether animals can maintain acid base

balances in the GI tract, the pH in the chyme was measured in fish (chapter 7) and pigs (chapter 8) respectively.

References

Albers, C. 1970. Acid-base balance. In: Hoar W. S. and Randall, D. J. (Ed), Fish Physiology, Vol. IV, The Nervous System, Circulation, and Respiration. Academic Press, New York, pp 173-208.

Austic, R. E., Boyd, R. D., Klasing, K. C. and Riley, W. W. 1983. Effect of dietary electrolyte balance on growth performance in swine. J. Anim. Sci. (Suppl. 1): 236 (Abstr.).

Block, E. 1994. Manipulation of dietary cation-anion difference on nutritionally related production diseases, productivity, and metabolic responses of dairy cows. J. Dairy Sci. 77:1437-1450.

Chiu, Y. U., Austic, R. E. and Rumsey, G. L. 1984. Effect of dietary electrolytes and histidine on histidine metabolism and acid-base balance in rainbow trout (*Salmo Gairdneri*). Comp. Biochem. Physiol. 78B: 777-783.

Chiu, Y. U., Austic, R. E. and Rumsey, G. L. 1987. Interactions among minerals, arginine and lysine in rainbow trout (*Salmo Gairdneri*). Fish Physiol. Biochem. 4: 45-55.

Chiu, Y. U., Austic, R. E. and Rumsey, G. L. 1988. Effect of feeding level and dietary electrolytes on the arginine requirement of rainbow trout (*Salmo Gairdneri*). Aquaculture 69: 79-91.

Eddy, F. B. 1982. Osmotic and ionic regulation in captive fish with particular reference to salmonids. Comp. Biochem. Physiol. 73B: 125-141.

Fauchon, C., Seoane, J. R. and Bernier, J. F. 1995. Effects of dietary cation-anion concentrations on performance and acid-base balance in growing lambs. Can. J. Anim. Sci. 75: 145-151.

Goss, G. G., Perry, S. F., Wood, C. M. and Laurent, P. 1992. Mechanisms of ion and acid-base regulation at the gills of freshwater fish. J. Exp. Zool. 263: 143-159.

Haydon, K. D. and West, J. W. 1990. Effect of dietary electrolyte balance on nutrient digestibility determined at the end of the small intestine and over the total digestive tract in growing pigs. J. Anim. Sci. 68: 3687-3693.

Haydon, K. D., West, J. W. and McCarter, M. N. 1990. Effect of dietary electrolyte balance on performance and blood parameters of growing-finishing swine fed in high ambient temperature. J. Anim. Sci. 68: 2400-2406.

Heisler, N. 1984. Acid-base regulation in fishes. In: Hoar W.S., Randall, D.J. and Brett, J.R. (Ed), Fish Physiology, Vol. XA, Anatomy, Gas Transfer, and Acid-Base Regulation. Academic Press, New York, pp 315-401.

Heisler, N. 1986. Mechanisms and limitations of fish acid-base regulation. In: Nilsson S. and Holmgren S. (Ed), Fish Physiology: Recent Advance. Dover, New Hampshire. pp 24-49.

Jobling, M. 1995. Osmotic and ionic regulation – water and salt balance. In: Environmental Biology of Fishes. Chapman & Hall, London, pp211-249.

Johnson, R. J. and Karunajeewa, H. 1985. The effects of dietary minerals and electrolytes on the growth and physiology of the young chick. J. Nutr. 115:1680-1690.

Kemme-Kroonsberg, C. 1993. Nutrition and acid-base balance of pigs: a review. Research Institute for Livestock Feeding and Nutrition (IVVO-DLO), Lelystad, Netherlands. No. 243. P69.

Ketelaars, J. J. M. H. and Tolkamp, B. J. 1991. Toward a new theory of feed intake regulation in ruminants. PhD thesis, Wageningen University. Wageningen, the Netherlands. P251.

Lehninger, A. L. 1971. Bioenergetics. The molecular basis of biological energy transformations. Second edition. W.A.Benjamin, INC. Menlo Park, California. P246.

Mongin, P. and Sauveur, B. 1977. Interrelationships between mineral nutrition, acid-base balance, growth and cartilage abnormalities. In: Boorman, K. N. and Wilson, B. J. (Ed), Growth and Poultry Meat Production, British Poultry Science ltd., London, U.K. pp. 235-247.

Park, J. H., Han, I. K. and Kim, I. B. 1994. Effects of electrolyte balance (EB: Na+K-Cl) on the growth, blood parameters, urine parameters and nutrient availabilities of weaning pigs. Korean J. of Anim. Nutr. and Feed Stuffs 18: 147-154.

Patience, J. F., Austic, R. E. and Boyd, R. D. 1987a. Effect of dietary electrolyte balance on growth and acid-base status in swine. J. Anim. Sci. 64: 457-466.

Patience, J. F., Austic, R. E. and Boyd, R. D. 1987b. Acid-base homeostasis in swine: Nutritional perspective. Feedstuffs, July 6: 13-19.

Patience, J. F. and Wolynetz., M. S. 1990. Influence of dietary undetermined anion on acid-base status and performance in pigs. J. Nutr. 120: 579-587.

Rose, B. D., 1989. Clinical physiology of acid-base and electrolyte disorders. 3rd edition. McGraw-Hill International editions. Medical series. New York, p853.

Ross, J. G., Spears, J. W. and Garlich, J. D. 1994. Dietary electrolyte balance effects on performance and metabolic characteristics in growing steers. J. Anim. Sci. 72:1842-1848.

Sanchez, W. K. and Beede, D. K. 1994. Interactions of sodium, potassium, and chloride on lactation, acid-base status, and mineral concentrations. J. Dairy Sci. 77: 1661-1675.

Scott, D. and Mclntosh, G. H. 1975. Changes in blood composition and urinary mineral excretion in the pig in response to acute acid-base disturbance. Q. J. Exp. Physiol. 60: 131-140.

Tucker, W. B., Harrison, G. A. and Hemken, R. W. 1988. Influence of dietary cation-anion balance on milk, blood, urine, and rumen fluid in lactating dairy cattle. J. Dairy Sci. 71: 346.

Walsh, P. J. and Mommsen, T. P. 1992. Intracellular pH regulation and metabolic interactions in hepatic tissues. J. Exp. Zool. 263: 176-185.

Waterman, D. F., Swenson, T. S., Tucker, W. B. and Hemken, R. W. 1991. Role of magnesium in the dietary cation-anion balance equation for ruminants. J. Dairy Sci. 74: 1866-1873.

West, J. W., Mullinix, B. G. and Sandifer, T. G. 1991. Changing dietary electrolyte balance for dairy cows in cool and hot environments. J. Dairy Sci. 74: 1662-1674.

Wilson, R. P., Cowey, C. B. and Adron, J. W. 1985. Effect of dietary electrolyte balance on growth and acid-base balance in rainbow trout (*Salmo gairdneri*). Comp. Biochem. Physiol. 82A: 257-260.

PART 1

Effect of dietary cation anion difference on performance of fish and pigs

CHAPTER 1

The influence of dietary cation anion difference on acid base balance, food intake, growth and nutrient utilization of juvenile African catfish *Clarias gariepinus* (Burchell).

Y. Dersjant-Li[1,3], J. A. J. Verreth[1], F. Evers[2], P. A. T. Tijssen[1], R. Booms[1] and M. W. A. Verstegen[3]

[1] *Fish Culture and Fisheries Group,* [3] *Animal Nutrition Group, Wageningen Institute of Animal Sciences, Wageningen Agricultural University, P.O.Box 338, 6700 AH Wageningen, The Netherlands;* [2] *Provimi BV, P.O.Box 5063, 3008 AB Rotterdam, The Netherlands*

Published in: Fish Physiology and Biochemistry (1999), 20: 305-311

Abstract: A study was conducted to examine the effect of dietary cation anion difference (CAD, Na + K - Cl, mEq kg^{-1}) on acid base balance, food consumption and growth of juvenile African catfish *Clarias gariepinus*. Four dietary CAD levels (-100, 100, 500, 700 mEq kg^{-1}) were established by altering levels of $NaHCO_3$, $CaCl_2$ and NH_4Cl in the diets. The group fed the diet of excess anions (CAD: -100 mEq kg^{-1}) had low food consumption and growth rate. Food consumption and growth increased linearly with an increase in dietary CAD within the range -100 to 700 mEq kg^{-1}. Blood pH was not significantly influenced by dietary CAD. A quadratic relationship between Na^+, Cl^- and CAD level in plasma and dietary CAD was found. Body composition of fish and nitrogen/energy utilization efficiency differed between dietary CAD treatments. The results suggest that dietary CAD influence acid base regulation and thus the energy costs for maintaining homeostasis. The increased food intake and growth performance of *Clarias gariepinus* at higher CAD levels may be related to a reduced maintenance costs for homeostasis.

Keywords: blood pH, chloride, electrolyte balance, minerals, potassium, sodium

Introduction

In higher vertebrates, dietary cation anion difference (CAD), i.e. the sum of Na and K minus Cl, in mEq kg^{-1} diet, has an influence on blood pH, feed intake and growth (Johnson and Karunajeewa, 1985; Patience *et al.*, 1987; Tucker *et al.*, 1988; West *et al.*, 1991; Ross *et al.*, 1994; Sanchez and Beede, 1994; Fauchon *et al.*, 1995). In fish Chiu *et al.* (1984, 1987, 1988) investigated the relation between dietary CAD and amino acid metabolism. Apart from CAD also other factors differed between experiments (Chiu *et al.*, 1988). Moreover Wilson *et al.* (1985) found that in rainbow trout a dietary CAD level of 155 mEq kg^{-1} gave numerically better growth and feed efficiency than higher or lower CAD levels. Thus there exists only limited data on the impact of dietary CAD on feed consumption and growth in fish.

The objective of the present study was to determine the effect of dietary CAD on blood acid base balance, food intake, growth and nutrient utilization in African catfish *Clarias gariepinus* (Burchell).

Materials and methods

Fish and facilities

The study was conducted at Wageningen University, Netherlands. Juvenile African catfish (*Clarias gariepinus*) were size-sorted, weighed and 55 fish of 36.4 ± 1.6g (mean ± sd) were distributed at random to each of 12 aquaria (70L). Four dietary CAD treatments with 3 replications each were tested over a five week period. The fast growth rate reported earlier for this species (Hogendoorn, 1983: 20g $kg^{-0.8}$; Torreele *et al.*, 1993: 30-35g $kg^{-0.8}$) ensured that during the given period size would sufficiently increase to enable the assessment of dietary effects in the body constituents. Fish were reared at a temperature of 27 ± 0.5 °C in a recirculation system with aeration in each aquarium, under a 12L:12D light regime. The O_2 concentration was maintained above 65% saturation. To avoid bacterial infections, the water salinity was maintained between 2.1-2.5 g L^{-1} by adding sea salt or fresh water into the system storage tanks.

Diets

The experimental diets with the dietary CAD levels of -100, 100, 500 and 700 mEq kg^{-1} were obtained by mixing alternate levels of 3 different salts to the ingredients of a commercial diet (provided by PROVIMI BV, Rotterdam, the Netherlands). NH_4Cl and $CaCl_2$

were added to the commercial diet ingredients to elevate Cl levels thereby producing a diet with excess anions. For the formulation of the cation excess diets, $NaHCO_3$ was added increasing the Na concentration, while maintaining the K concentration. K concentration was kept relatively constant due to the reported negative effect of K on growth rate (Johnson and Karunajeewa, 1985). Calcium was maintained at about 2.5% in all diets by addition of $CaCO_3$. Diets were pelleted and following pellet manufacture dietary CAD levels were measured (Table 1).

Feeding

Feed was supplied twice daily at 08.00 and 16.00h, over periods of about 1 h. Meals were supplied in small portions at a time thus ensuring that fish were fed to satiation and that feed was not wasted. The amount of feed given per meal and per day was recorded. Fish were fed 2 mm pellets during the first three weeks and 2.5 mm pellets during last two weeks of the experimental period.

Sampling and measurements

At the start of the experiment and before fish were assigned to the rearing aquaria, a sample of 20 fish was taken for dry weight and body composition. At the end of the experimental period a sample of 10 fish was taken from each aquarium allowing data to be gathered per dietary treatment. Samples were kept at -20 °C before analysis. Blood (1-2 ml) was sampled from groups of anaesthetized (200 mg TMS l^{-1}) fish (15 at the beginning and 6 per aquaria at the end of the experiment), by inserting a syringe into the caudal vessels. EDTA-LiOH solution (pH =7.36) was used as anticoagulant for the samples from the beginning of the experiment. The volume of the samples was corrected for the amount of anticoagulant solution in the syringe. Anticoagulant solution was not used for the samples at the end of the experiment because of blood pH was measured immediately (ORION pH meter, model 920A). The blood sample was centrifuged (1599g for 15 min.) to obtain plasma. Samples were stored at -20 °C until analysis of mineral content. Fish carcass samples were pooled per aquarium and were ground subsequently. Fresh samples were taken for dry matter and nitrogen content analysis. Subsamples were freeze dried for analysis of dry matter, ash and energy content. Dry matter and ash were measured according to the procedure described by Henken *et al.* (1986). Nitrogen was analyzed by the Kjeldahl method and energy was determined by bomb-calorimetry (IKA Calorimeter C 7000).

Table 1. Chemical analysis of the test diets[1]

	CAD (mEq kg^{-1})			
	-100	100	500	700
2 mm pellets				
Dry matter (%)	88.8	88.7	87.9	87.8
Crude protein (%)	46.1	46.4	46.6	46.9
Crude fat (%)	11.4	11.5	11.4	11.6
Crude fiber (%)	1.5	1.6	1.5	1.5
Ash (%)	11.2	11.3	12.1	12.2
Ca (g kg^{-1})	25.7	26.9	25.1	21.5
P (g kg^{-1})	13.6	13.7	13.8	13.7
Na (g kg^{-1})	6.0	6.1	12.3	16.1
K (g kg^{-1})	7.8	7.7	7.9	8.1
Cl (g kg^{-1})	18.5	9.0	9.0	9.0
Gross energy (kJ g^{-1})	19.59	19.68	19.40	19.41
pH[2]	5.75	5.84	7.10	7.50
Measured CAD (mEq kg^{-1} diet)	-64	205	480	654
2.5 mm pellets				
Dry matter (%)	92.9	93.4	93.0	93.2
Crude protein (%)	46.6	46.7	46.9	47.2
Crude fat (%)	11.4	11.5	11.4	11.6
Crude fiber (%)	1.6	1.5	1.3	1.6
Ash (%)	11.4	11.2	12.1	12.4
Ca (g kg^{-1})	26.9	27.2	25.2	22.1
P (g kg^{-1})	13.6	13.6	13.7	12.8
Na (g kg^{-1})	6.0	5.8	13.2	17.3
K (g kg^{-1})	6.2	6.0	7.3	8.7
Cl (g kg^{-1})	20.0	10.3	9.3	9.3
Gross energy (kJ g^{-1})	19.52	19.60	19.42	19.33
pH	5.76	5.95	7.06	7.42
Measured CAD (mEq kg^{-1} diet)	-146	116	497	713

[1] Chemical analyses of the test diets were performed by Provimi BV, Rotterdam, the Netherlands. Same dietary mix was used for making the 2 and 2.5 mm pellets. [2] Dietary pH was measured in the lab. of Animal Nutrition group.

All chemical analyses were done in three replicates. Cl^- content of the plasma was measured using a chloride meter (Jenway PCLM 3) and Na^+ and K^+ were measured using a flame photometer (Jenway PFP 7). Mineral content in body samples was determined after solubilization of the ash samples in demineralized water (Cl^-) or in 1N HCl solution (Na^+, K^+) as described by Fauchon *et al.* (1995). Dietary pH was measured as described by Tacon and De Silva (1983).

Data analysis

Growth and feed consumption were calculated per kg metabolic weight using 0.8 as the weight exponent (Heinsbroek, 1990). Feed conversion (FC) was defined as:

$$FC = FI/WG$$

with FI is feed consumed (wet weight, g) and WG is weight gain (wet weight, g). Apparent net nitrogen utilization efficiency (NUE) was calculated as:

$$NUE\% = RN/FN \times 100\%$$

$$RN = Wt \times Nt - Wo \times No$$

With RN is retained N and FN is N fed. Wt and Wo are the mean weights of fish at the end and the beginning of the experiment respectively (in g). Nt and No represent the nitrogen content of the fish body (per g of body weight) at the end and the beginning of the experiment respectively. Energy conversion efficiency (ECE) was estimated as:

$$ECE\% = RE/FE \times 100\%$$

$$RE = Wt \times Et - Wo \times Eo$$

With RE is retained energy in the body of fish and FE is energy fed to the fish, Et, Eo are the energy concentration of fish body (kJ per g) at the end and the beginning of the experiment respectively.

The proportion of retained energy as protein was calculated by converting the retained protein (retained nitrogen × 6.25) to energy by using an average energy content of 23.6 kJ g^{-1} protein (Brafield, 1985).

Statistical analysis

Data were tested by a one way ANOVA by use of the GLM procedure of SAS (SAS, 1990), according to the model:

$$Y_{ij} = \mu + CAD_i + e_{ij}$$

Where Y_{ij} is dependent variable (growth, intake, feed conversion etc.) and CAD_i is dietary CAD level (i = 1..4) with μ and e_{ij} representing the mean and error term respectively.

The Bonferroni t -test was used for multiple comparison of treatment means. The significance level was set at $\alpha = 0.05$. Curves were fitted using the nonlinear regression algorithm procedures from the NONLIN package (Method Levenberg-Marquadt, (Dennis *et al.*, 1981), convergence criterion 10^{-10}). For curve fitting treatment means were used.

Results

Blood pH, mineral contents in plasma and body

Dietary CAD treatment had no significant effect on blood pH (Table 2). During the course of the experiment CAD decreased in plasma, but tended to increase in the whole body (Table 2). After 5 weeks plasma Cl^- concentration was significantly higher than the initial value, whereas K^+ concentration decreased ($P < 0.0002$). Plasma Na^+ concentration was not significantly influenced by dietary CAD (Table 2).

Na^+ concentration increased significantly ($P < 0.01$) in the whole body samples at the end of the experimental period in comparison to initial values (Table 2). But no significant differences were found for whole body Cl^- and K^+ concentration between beginning and end of the experiment.

Plasma Cl^- concentration increased quadratically with increasing dietary CAD (Figure 1), while plasma Na^+ concentration and CAD levels showed a tendency to quadratically decrease. The relationship can be described as: $Cl^-=103.65-0.018X+0.111X^2$ ($R^2=0.9999$, $P<0.01$); $Na^+=143.88-3.5X+0.466X^2$ ($R^2=0.99$, $P<0.105$); $CAD=42.21-3.86X+0.466X^2$ ($R^2=0.995$, $P<0.07$); with X representing 100 mEq kg^{-1} dietary CAD.

Food consumption and growth

Both food intake and growth increased linearly with increasing dietary CAD (Figure 2). The linear relationship between dietary CAD and feed intake / growth can be described as: feed intake (g $kg^{-0.8}$ d^{-1}) = 30.93+0.9X ($R^2=0.91$, $P<0.05$, $n=4$); growth (g $kg^{-0.8}$ d^{-1}) = 39.63+0.67X ($R^2=0.97$, $P<0.02$, $n=4$) respectively, where X represents 100 mEq kg^{-1} dietary CAD. Feed conversion ranged from 0.77 to 0.86 among dietary groups and was not significantly influenced by dietary CAD (Table 3).

Table 2. Blood pH and mineral concentration in the plasma and whole body of *Clarias gariepinus* at the beginning (n=15) and the end (n=3, each n represents mean of 6 individuals) of the experiment

		Dietary CAD (mEq kg^{-1})				
		-100	100	500	700	Effect[1]
	Initial	End of the exp.				
Blood pH, plasma mineral contents (mmol L^{-1})						
pH	7.21 ± 0.02	7.29 ± 0.02	7.26 ± 0.01	7.23 ± 0.03	7.23 ± 0.04	NS
Cl^-	97.5 ± 0.69	103.7 ± 1.56	104.1 ± 0.66	106.1 ± 1.03	108.3 ± 2.09	Q^{++}
Na^+	142.0 ± 1.72	145.0 ± 1.56	138.3 ± 1.84	138.4 ± 3.70	140.8 ± 1.65	Q^+
K^+	10.4 ± 1.46	2.0 ± 0.44	2.3 ± 0.11	1.8 ± 0.07	3.7 ± 0.70	NS
CAD (mEq L^{-1})	54.8	44.8	36.5	33.5	36.2	Q^+
Mineral contents in the whole body (g kg^{-1} DM)						
Cl^-	2.6 ± 0.16	2.6 ± 0.10	2.6 ± 0.06	2.4 ± 0.07	2.8 ± 0.14	NS
Na^+	2.5 ± 0.09	3.4 ± 0.17	3.3 ± 0.08	3.1 ± 0.04	3.2 ± 0.09	NS
K^+	7.1 ± 0.09	6.1 ± 0.42	6.7 ± 0.29	7.2 ± 0.35	7.0 ± 0.59	NS
CAD (mEq kg^{-1} dm)	218	231	240	249	238	NS

[1] Statistical analysis of the data from the end of the experiment: Q^{++} -- Quadratic effect significant at $p<0.05$, Q^+ -- Quadratic effect significant at $p< 0.10$, NS -- no significant ($p>0.10$) difference. Data shown as mean ± SEM.

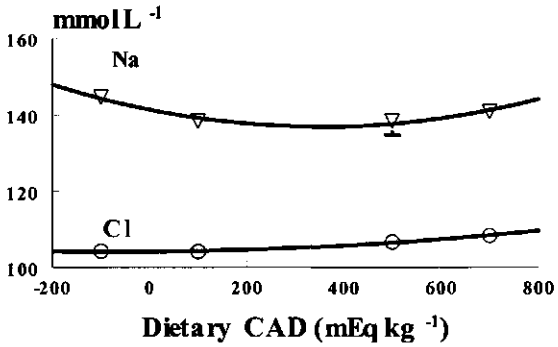

Figure 1. Plasma Na^+ and Cl^- concentration in juvenile African catfish *Clarias gariepinus* to dietary CAD levels (error bars indicate SEM of three replicates).

Nitrogen and energy utilization

The energy concentration in body dry matter was significantly higher in fish fed a dietary CAD of 500 mEq kg^{-1} compared to the other treatment groups (Table 3).

Table 3. Body composition, nitrogen and energy utilization

		Dietary CAD (mEq kg^{-1})				
		-100	100	500	700	Effect[1]
	Initial	End of the exp.				
Body composition						
Dry matter %	23.4 ± 0.08	25.8 ± 0 07	26.7 ± 0.11	26.2 ± 0.21	26.0 ± 0.12	NS
Ash % in dm	11.5 ± 0.26	10.3 ± 0.17	10.3 ± 0.08	9.6 ± 0.09	10.2 ± 0.17	NS
Nitrogen (g kg^{-1} dm)	101.0 ± 0.09	89.3 ± 0.18	90.0 ± 1.18	89.9 ± 0.92	90.6 ± 0.67	NS
Energy (kJ g^{-1} dm)	24.4 ± 0.06	25.5^{b} ± 0.06	25.8^{b} ± 0.06	26.3^{a} ± 0.03	25.6^{b} ± 0.35	*
Feed conversion, nitrogen and energy utilization						
Feed conversion		0.79 ± 0.004	0.77 ± 0.02	0.86 ± 0.01	0.82 ± 0.01	NS
ECE (% RE/GE)[2]		44.5^{ab} ± 0.95	47.7^{a} ± 0.55	43.2^{b} ± 0.65	42.8^{b} ± 0.30	*
NUE (%RN/GN)[3]		40.8^{ab} ± 0.72	43.5^{a} ± 0.70	38.0^{b} ± 0.74	39.3^{ab} ± 0.65	*
Retained energy as protein (%)		50.4	50.2	49.2	51.0	

[1] Statistical analysis: NS-- no significant difference, * different letters in rows means there is significant difference between treatment means ($p<0.05$). [2] NUE--apparent net nitrogen utilization (% RN/GN). [3] ECE--energy conversion efficiency (% RE/GE). Data shown as mean ± SEM.

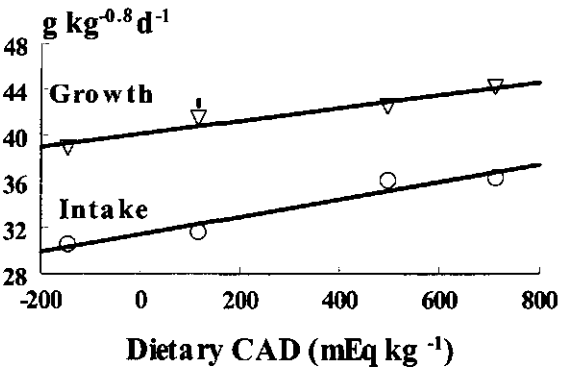

Figure 2. Food intake and growth at different dietary CAD levels in juvenile African catfish *Clarias gariepinus* (error bars indicate SEM of three replicates).

Apparent nitrogen utilization efficiency (NUE) and energy conversion efficiency (ECE) reached their maxima when dietary CAD was 100 mEq kg^{-1} (Table 3). The lowest retained protein energy as percentage of total retained energy was found at a dietary CAD level of 500 mEq kg^{-1}. This dietary group had also the highest energy concentration in body dry matter (Table 3).

Discussion

Blood pH and mineral contents

At the end of the experiment blood pH was similar in all treatment groups, indicating that African catfish is able to maintain blood pH at different CAD. Chiu *et al.* (1984) reported that blood pH of rainbow trout was not affected by changing dietary CAD from –200 to 0 mEq kg^{-1}. Wilson *et al.* (1985) also reported that blood pH of rainbow trout was not influenced by dietary CAD ranging from –90 to 322 mEq kg^{-1}, however, blood pH significantly decreased at a dietary CAD level of 638 mEq kg^{-1} compared to dietary CAD levels of –90 and 155 mEq kg^{-1}. In contrast to studies conducted in fish, most studies with terrestrial animals showed an increase in blood pH with increasing dietary CAD levels (Haydon and West, 1990; Ross *et al.*, 1994; Fauchon *et al.*, 1995).

A linear increase in plasma CAD with increasing dietary CAD levels was observed in cows (Tucker *et al.*, 1988; West *et al.*, 1991), whereas a quadratic relationship was reported for steers (Ross *et al.*, 1994). In the present study with catfish, CAD levels in both plasma and whole body changed quadratically with increasing dietary CAD. In plasma, CAD reached a minimum at a dietary CAD level of 500 mEq kg^{-1}, while whole body CAD level was at a maximum at this dietary CAD level. The results show that African catfish maintains its acid-base balance in body and plasma, this is probably also done by adjusting the Na^+, K^+ and Cl^- levels in the various body compartments.

With increasing dietary CAD (i.e. a lower concentration of dietary Cl^-), plasma Cl^- concentration increased quadratically. This contrasts with the observations of Tucker *et al.* (1988) and Ross *et al.* (1994) in mammals. Similar to the results found in steers (Ross *et al.*, 1994), an increase in dietary CAD, i.e. an increase in Na content in the diet, was associated with decreased plasma Na^+ concentration. No clear effect of dietary CAD on $[K^+]$ in plasma was observed, but whole body $[K^+]$ increased with increasing dietary CAD. This might be expected because K^+ is mainly present in intracellular fluid (Walsh and Mommsen, 1992).

Compared to mammals fish respond differently to dietary CAD because of the high ion-exchange rate with the environment (Heisler, 1984). The results of the present study indicate that the studied species can maintain its acid-base buffering capacity by both retaining more chloride in plasma after ingestion an alkaline diet and also by retaining the Na^+ concentration in plasma after ingestion of acidic diets. It is very well possible that these plasma mineral levels are maintained by absorption from the water when dietary supply is decreased. The present study did not include measurements to test this hypothesis.

Food intake and growth

Based on the equations provided by Eding and van Weerd (1999), maximum growth of 150g African catfish (*Clarias gariepinus*) can be predicted at about 20g $kg^{-0.8}$. Torreele *et al.* (1993) obtained maximum growth rates of 30-35g $kg^{-0.8}$. In the present study, the observed growth rate (39-44g $kg^{-0.8}$) was substantially higher than the predicted and observed values from the previous studies, indicating that the nutritional value of the experimental diets was high.

Increasing dietary CAD often leads to increased feed consumption: e.g. in dairy cows (West *et al.*, 1991), growing steers (Ross *et al.*, 1994), young dairy calves (Jackson *et al.*, 1992), lambs (Fauchon *et al.*, 1995) and in pigs (Patience *et al.*, 1987). In catfish feed consumption was highest at the highest dietary CAD tested (700 mEq kg^{-1}). This CAD level is higher than the results obtained in terrestrial animals (277 mEq kg^{-1} in pigs, Patience *et al.*, 1987; 370 mEq kg^{-1} in calves, Jackson *et al.*, 1992; 500 mEq kg^{-1} in lambs, Fauchon *et al.*, 1995). The results indicate that fish can tolerate higher dietary CAD levels than terrestrial animals.

The observed growth response of the studied fish species to dietary CAD is similar to the results obtained by Fauchon *et al.* (1995) with lambs and Ross *et al.* (1994) with steers. They found that daily gain increased linearly with increasing dietary CAD. Studies with swine and chicks showed a lower optimal dietary CAD level (175 mEq kg^{-1} in swine, Patience *et al.*, 1987; 200-350 mEq kg^{-1} in chicks, Johnson and Karunajeewa, 1985). In contrast to all the previous studies, Wilson *et al.* (1985) found no significant growth effects in rainbow trout for dietary CAD ranging from -90 to 638 mEq kg^{-1}. The latter result may be attributed to the feeding levels (2 - 2.5 % of body weight) used in their study.

The results of the present study with African catfish indicate that the improved growth rate with increasing dietary CAD is achieved by higher food consumption. In the present

study we observed no apparent taste effect of diets with high levels of Cl^- (as measured by the time to ingest 20g diet). In accordance to West *et al.* (1991), we suggest that a negative dietary CAD (-100 mEq kg^{-1}) is associated with a negative electrolyte balance, resulting in a low dietary pH and inducing metabolic acidosis. According to Ketelaars and Tolkamp (1991) feed intake of an animal may be related to maintenance costs for homeostasis. At the dietary CAD of -100 mEq kg^{-1}, fish require more energy to maintain an acid-base balance and therefore less energy will be available for feeding activity. This may explain the low intake and growth in this treatment group. Increasing the dietary CAD helped fish to compensate for metabolic acidosis requiring less energy for maintenance of acid base balance, resulting in high feed intake and growth. Hence the increase in food intake with increasing dietary CAD may be related to reduced maintenance costs for homeostasis.

Acknowledgements

The first author was financially supported by a grant from the feed company PROVIMI BV, Rotterdam, the Netherlands. The authors are grateful to E. Eding, S. Leenstra, A. Hutten, F. Dersjant and G. Rutjes for their help in system management, sampling and providing experimental materials. Mr. P.Roeleveld and his colleagues from ILOB-TNO helped in processing the pellets. Sincerely thanks to Dr. Th. van de Poel and ing. T. Zandstra for oil coating of the pellets.

References

Brafield, A.E. 1985. Laboratory studies of energy budgets. In: Tytler, P. and Calow, P. (Ed), Fish Energetics: New Perspectives. Croom Helm, Beckenham, pp. 257-281.

Chiu, Y.U., Austic, R.E. and Rumsey, G.L. 1984. Effect of dietary electrolytes and histidine on histidine metabolism and acid-base balance in rainbow trout (*Salmo gairdneri*). Comp. Biochem. Physiol. 78B: 777-783.

Chiu, Y.U., Austic, R.E. and Rumsey, G.L. 1987. Interactions among minerals, arginine and lysine in rainbow trout (*Salmo gairdneri*). Fish Physiol. Biochem. 4: 45-55.

Chiu, Y.U., Austic, R.E. and Rumsey, G.L. 1988. Effect of feeding level and dietary electrolytes on the arginine requirement of rainbow trout (*Salmo gairdneri*). Aquaculture 69: 79-91.

Dennis, J.E., Gay, D.M. and Welsch, R.E. 1981. An Adaptive Nonlinear Least - Squares Algorithm. ACM. Transaction on Mathematical Software 7,3.

Eding, E. and van Weerd, H. 1999. Grundlagen, Aufbau und Management von Kreislaufanlage. In: Bohl, M. (Ed), Zucht und Produktion von Süsswasserfischen. DIG-Verlag, Frankfurt. pp 436-491.

Fauchon, C., Seoane, J.R. and Bernier, J.F. 1995. Effects of dietary cation-anion concentrations on performance

and acid-base balance in growing lambs. Can. J. Anim. Sci. 75: 145-151.

Haydon, K.D. and West, J.W. 1990. Effect of dietary electrolyte balance on nutrient digestibility determined at the end of the small intestine and over the total digestive tract in growing pigs. J. Anim. Sci. 68: 3687-3693.

Heinsbroek, L.T.N., van Thoor, R.M.H. and Elizondo, L.J. 1990. The effect of feeding level on the apparent digestibilities of nutrients and energy of a reference diet for the European eel, *Anguilla anguilla* L. and the African catfish, *Clarias gariepinus* (Burchell). In: Takeda, M. and Watanabe, T. (Ed), The Current Status of Fish Nutrition in Aquaculture. Toba, Japan. pp 175-188.

Heisler, N. 1984. Acid-base regulation in fishes. In: Hoar W.S., Randall, D.J. and Brett, J.R. (Ed), Fish Physiology, Vol. XA, Anatomy, Gas Transfer, and Acid-Base Regulation. Academic Press, New York, pp 315-401.

Henken, A.M., Lucas, H., Tijssen, P.A.T. and Machiels M.A.M. 1986. A comparison between methods used to determine the energy content of feed, fish and faeces samples. Aquaculture 58: 195-201.

Hogendoorn, H. 1983. Growth and production of the African catfish, *Clarias lazera* (C. & V.) III. Bioenergetic relations of body weight and feeding level. Aquaculture, 35: 1-17.

Jackson, J.A., Hopkins, D.M., Xin, Z. and Hemken, R.W. 1992. Influence of cation-anion balance on feed intake, body weight gain, and humoral response of dairy calves. J. Dairy Sci. 75: 1281-1286.

Johnson, R.J. and Karunajeewa, H. 1985. The effects of dietary minerals and electrolytes on the growth and physiology of the young chick. J. Nutr. 115: 1680-1690.

Ketelaars, J. J. M. H. and Tolkamp, B. J. 1991. Toward a new theory of feed intake regulation in ruminants. PhD thesis, Wageningen University. Wageningen, the Netherlands. P251.

Patience, J.F., Austic, R.E. and Boyd, R.D. 1987. Effect of dietary electrolyte balance on growth and acid-base status in swine. J. Anim. Sci. 64: 457-466.

Ross, J.G., Spears, J.W. and Garlich, J.D. 1994. Dietary electrolyte balance effects on performance and metabolic characteristics in growing steers. J. Anim. Sci. 72: 1842-1848.

Sanchez, W.K. and Beede, D.K. 1994. Interactions of sodium, potassium, and chloride on actation, acid-base status, and mineral concentrations. J. Dairy Sci. 77: 1661-1675.

SAS. 1990. SAS User's Guide: Statistics (Version 6 Ed.). SAS Inst. Cary, NC

Tacon, A.G.J. and De Silva, S.S. 1983. Mineral composition of some commercial fish feeds available in Europe. Aquaculture, 31: 11-20.

Torreele, E., van der Sluiszen, A. and Verreth, J. 1993. The effect of dietary L-carnitine on the growth performance in fingerlings of the African catfish (*Clarias gariepinus*) in relation to dietary lipid. British J. of Nutrition, 69: 289-299.

Tucker, W.B., Harrison, G.A. and Hemken, R.W. 1988. Influence of dietary cation-anion balance on milk, blood, urine, and rumen fluid in lactating dairy cattle. J. Dairy Sci. 71: 346.

Walsh, P.J. and Mommsen, T.P. 1992. Intracellular pH regulation and metabolic interactions in hepatic tissues. J. Exp. Zool. 263: 176-185.

West, J.W., Mullinix, B.G. and Sandifer, T.G. 1991. Changing dietary electrolyte balance for dairy cows in cool and hot environments. J. Dairy Sci. 74: 1662-1674.

Wilson, R.P., Cowey, C.B. and Adron, J.W. 1985. Effect of dietary electrolyte balance on growth and acid-base balance in rainbow trout (*Salmo gairdneri*). Comp. Biochem. Physiol. 82A: 257-260.

CHAPTER 2

Feed intake, growth, digestibility of dry matter and nitrogen in young pigs as affected by dietary cation anion difference and supplementation of xylanase

Y. Dersjant-Li [1,2], H. Schulze [4*], J. W. Schrama[3], J. A. Verreth[2] and M. W. A. Verstegen[1]

*[1]Animal Nutrition Group, [2] Fish Culture and Fisheries Group, [3] Animal Husbandry Group, Wageningen Institute of Animal Sciences, Wageningen Agricultural University, Wageningen, The Netherlands. [4] Finnfeeds International Ltd., Marlborough, United Kingdom, * Present address: Provimi B.V., Rotterdam, The Netherlands*

Submitted to: J. Anim. Physiol. Anim. Nutrition

Abstract: An experiment was conducted to test the effect of dietary cation anion difference (CAD, Na + K - Cl, mEq kg^{-1} diet) and xylanase addition on plasma electrolyte balance, feed consumption, growth and digestibility of nutrients in young pigs. A 2 × 3 factorial arrangement with 3 dietary CAD levels (-100, 200, and 500 mEq kg^{-1}) and two levels xylanase supplementation (0 and 0.1% xylanase derived from *Trichoderma longibrachiatum*) was used. Thirty-six individually housed, castrated pigs (5 wks of age) with an initial body weight of 9.34 ± 0.28 kg (mean ± SE) were randomly assigned to the six treatments. Diets were provided to pigs as cold pellets. Pigs had *ad libitum* access to feed and water. Venous plasma Cl^- concentration was higher ($p < 0.0001$) in dietary CAD of –100 mEq kg^{-1} group compared with the other two CAD groups. Dietary CAD did not affect Na^+ and K^+ concentrations in the venous plasma. Growth rates were higher ($p < 0.05$) in pigs receiving dietary CAD of 200 mEq kg^{-1} (657 g pig^{-1} d^{-1}) and dietary CAD of 500 mEq kg^{-1} (603 g pig^{-1} d^{-1}) than in pigs receiving dietary CAD of -100 mEq kg^{-1} (484 g pig^{-1} d^{-1}). Fecal dry matter and nitrogen decreased with increasing dietary CAD. Fecal apparent digestibility of dry matter and nitrogen was higher ($p < 0.05$) in the dietary CAD of 500 mEq kg^{-1} compared to the two lower level CAD groups. Supplementation of xylanase did not effect on the performance of pigs. Xylanase addition in the diet significantly increased apparent fecal digestibility of dry matter and tended to increase apparent digestibility of nitrogen. No interaction between dietary CAD and xylanase was found.

In conclusion, dietary CAD influenced the performance and digestibility of nutrients of pigs. Xylanase supplementation improved digestibility of dry matter.

Keywords: cation anion difference, feed intake, growth, nutrient utilization, piglets, xylanase

Introduction

In pigs, feed intake and growth are related to dietary cation anion difference (CAD, Na + K - Cl, mEq kg^{-1} diet) (Patience *et al.*, 1987; Haydon *et al.*, 1990). However, different optimal dietary CAD was observed in different studies (Austic *et al.*, 1983; Patience *et al.*, 1987, Patience and Chaplin, 1997; Park *et al.*, 1994). Haydon *et al.* (1990) observed a linear increase in feed intake and growth with dietary CAD ranging from 25 to 400 mEq kg^{-1}. While Patience *et al.* (1987) observed that feed intake and growth were maximal for dietary CAD between 0 to 340 mEq kg^{-1}. Austic *et al.* (1983) suggested that dietary CAD could influence lysine utilization in pigs. The inconsistent optimal dietary CAD levels may be caused by different diet composition which were used in the mentioned studies.

Wheat and barley based pig diet containing high proportion of dietary fiber. Pigs are not capable of digesting dietary fiber by means of their own digestive enzymes (Trowell *et al.* as cited by Inborr, 1994). Adding fiber-degrading enzymes to diets, which contained high concentration of dietary fiber, has improved pig performance (Newman *et al.*, 1983) and nutrients digestibility (Graham *et al.*, 1989; Bedford *et al.*, 1992; Inborr *et al.*, 1993). Minerals digestibility may also influenced by supplementation of fiber-degrading enzymes (van der Klis, 1993). Therefore inclusion of feed enzyme may interact with dietary CAD.

The objectives of this study are 1) to determine an optimal dietary CAD on a wheat/barley based diet in young pigs; 2) to test the effect of exogenous feed enzyme (xylanase) on digestibility of nutrients; 3) to test if there is an interaction between feed enzymes addition and dietary CAD.

Materials and methods

Pigs and experimental design

A 2×3 factorial arrangement with three levels of dietary CAD and two levels of enzyme supplementation was used in this experiment. The experiment consisted of a 1-wk adaptation period followed by a 5-wk test period. A total of 36 castrated pigs (F1 of female [Dutch Landrace × Finnish Landrace] × male [Cofok breed × Great York]) of 5-wk old and with initial body weight of 9.34 ± 0.28 kg (mean ± SE) were randomly assigned to the 6 treatment groups. The pigs were individually housed in pens (1.08 × 2.75 m, 1/3 slatted concrete floor, feeding trough made of baked clay). Pigs were allowed to adapt to the new environment for 1-wk with a commercial diet before the experiment. Temperature of the room was maintained between 22 (night) and 29 °C (day) throughout the experimental period.

Animal welfare committee of Wageningen University approved the protocol of this experiment.

Diets and feeding

Six treatment diets were formulated in a 3×2 factorial arrangement: included 3 dietary CAD levels (-100, 200, and 500 mEq kg^{-1}), and 2 enzyme levels (with and without enzyme supplementation, 0.1% xylanase derived from *Trichoderma longibrachiatum*, supplied by Finnfeeds Ltd). The basal diet consisted of wheat, barley, rye, soybean isolate and premix (Table 1), with a calculated CAD level of 172 mEq kg^{-1}. By addition of $CaCl_2$ or $NaHCO_3$, respectively, the designed dietary CAD levels were achieved. Calcium levels were maintained constant in each diet by addition of $CaCO_3$ or $CaCl_2$. Diamol (diatomaceous shell powder, Biakon N.V., Grobbendonk, Belgium) was added to the diet to keep ash content constant. Enzyme was added by replacement of diamol. All test diets were formulated to meet or exceed the nutrient requirements (NRC, 1988) for young pigs (Table 2).

Table 1. Basal diet composition

Ingredients (%)	
Barley	41.6
Wheat	20
Rye	20
Soybean isolate	13
Tallow	2
Limestone	0.8
Monocalcium phosphate	0.8
Salt (KCl)	0.3
L-lysine HCl	0.3
DL-methionine	0.1
Premix[1]	1.0
L-threonine	0.1

[1] The vitamin and mineral premix supplied per kg of the basal diet 9000 IU of vitamin A, 1800 IU of vitamin D_3, 40 mg of vitamin E, 5 mg of riboflavin, 30 mg of niacin, 12 mg of d-pantothenic acid, 350 mg of choline chloride, 40 µg of vitamin B_{12}, 3 mg of vitamin K, 50 mg of vitamin C, 1 mg of folic acid, 0.1 mg of biotin, 0.52 mg of Co from $CoSO_4.7H_2O$, 0.06 mg of Se from $Na_2SeO_3.5H_2O$, 0.12 mg of I from KI, 80 mg of Fe from $FeSO_4.7H_2O$, 170 mg of Cu from $CuSO_4.5H_2O$, 44 mg of Mn from MnO_2, 73 mg of Zn from $ZnSO_4.H_2O$.

The feed was provided *ad libitum*, as cold pellet, 23 hours daily. In the middle and the end of the experiment before blood sampling, pigs were starved for one night and fed to satiation in the next morning. Water was freely available throughout the experimental period.

Table 2. The composition of test diets as fed

Dietary CAD (mEq kg^{-1})	-100		200		500	
Xylanase	0	0.1%	0	0.1%	0	0.1%
Ingredients %						
Basal diet	95		95		95	
$CaCl_2.2H_2O$	1.93					
$NaHCO_3$			0.31		2.83	
$CaCO_3$			1.36		1.36	
Diamol[1]	3.07		3.33		0.81	
Analyzed composition (%)						
Dry matter	88.7	89.4	89.5	89.8	89.3	89.3
Crude protein	19.6	19.8	19.9	20.0	19.9	19.9
Crude fiber	2.83	2.86	2.86	2.77	2.91	2.92
Ash	7.31	7.37	7.84	7.95	7.15	7.15
P	0.53	0.53	0.53	0.53	0.54	0.53
Ca	1.0	1.0	0.97	0.98	0.99	1.0
Na	0.19	0.17	0.30	0.31	1.07	1.07
K	0.60	0.57	0.52	0.53	0.54	0.55
Cl	1.1	1.2	0.28	0.31	0.33	0.31
Dietary CAD (mEq kg^{-1})[2]	-84	-115	182	185	510	517
Acid binding capacity (mEq kg^{-1})[3]	499	509	800	817	1193	1168

[1] Diamol is diatomaceous shell powder (Biakon N.V., Grobbendonk, Belgium), [2] Calculated as Na + K – Cl, [3] Quantity of 1.0N HCl required for the gastric digestion pH (3.0) of 1 kg feed.

Sampling and measurements

Feed intake was recorded daily. Weight gain was measured weekly. Fecal apparent digestibility of nitrogen and dry matter was measured using acid insoluble ash (AIA) as an indicator (McCarthy *et al.*, 1974; Wunsche *et al.*, 1991). Diet samples were taken every day during the experiment for feed composition analysis. Fecal samples (grab samples) were collected during experimental wk 1, 3, and 5. In each of these weeks the fecal samples were taken on three successive days. These samples were stored in a freezer (-20°C) until the end of the experiment. Before analysis the samples from all three days per week 1, 3 and 5 were

pooled, then sub-samples were taken for dry matter and nitrogen analysis. The remained samples were freeze-dried. Dry matter and acid insoluble ash were measured from freeze-dried samples. Nitrogen content of feed and feces was analyzed by the Kjeldahl method (ISO 5983, 1979). Dry matter content of feed and feces, ash content of feed and acid insoluble ash content of feed and feces were measured according to ISO procedures [ISO 6496 (1983); ISO 5984 (1978) and ISO 5985 (1978) respectively]. Dietary Ca content was measured using atomic absorption spectrometry (ISO 6869, 1997) and dietary P content was measured using spectrophotometry (ISO 6491, 1995).

Blood samples were taken by puncture of the vena cava. At the start of the experiment (day 0), blood samples were taken from randomly selected 12 pigs (2 from each treatment) for initial values. At the middle (day 16 to 18) and the end (day 34 to 36) of the experiment the blood samples were taken from all 36 pigs on three successive days with 12 piglets (2 from each treatment) on each day. Before blood sampling the pigs were given *ad libitum* access to feed for 30 min. at 0800, after an overnight fast. The blood samples were taken 2.5 hours after feeding, when an important part of the nutrients in the ingested feed would have been absorbed in the small intestine. The blood samples were centrifuged at 1600 × *g* for 15 min to obtain the blood plasma fraction. The plasma samples were then stored at -20 °C until analyses of Cl^-, $Na^+$and K^+ contents.

Plasma Cl^- content was measured by using a chloride meter (PCLM digital chloride meter, Jenway Ltd. Dunmow, England), Plasma Na^+ and K^+ were measured by using a flame photometer (PFP 7 flame photometer, Jenway Ltd. Dunmow, England). Chloride, Na and K in the diets were determined as described by Fauchon *et al.* (1995) with some modifications. One gram feed sample was dissolved in 10 ml demineralized water, incubated in 37°C oven for an hour and Cl^- concentration in the solution was measured using the same instrument as for plasma Cl^-. The diet sample was used instead of ash sample for measuring Cl^- in the feed because using ash samples resulted in unexplained low values. For measuring Na^+ and K^+, 0.5 g ash samples were dissolved in 1M HCl solution and the Na^+ and K^+ concentration in the solution were measured using the same instrument as for plasma. Acid binding capacity of the feed was measured by the method as described by Prohaszka and Baron (1980). Two grams of feed was incubated in 20ml 0.1M HCl solution at 37°C for one hour. Afterwards the suspension was titrated with 0.1M NaOH till pH = 3.0. Acid binding capacity was calculated as the quantity of 1.0M HCl required for the gastric digestion at pH = 3.0 of 1kg feed (mEq kg^{-1}).

Data analysis

The average value of 5 weeks were calculated for weight gain, feed intake and feed conversion. Feed conversion (FC) was defined as kg feed consumed per kg weight gain. Digestibility was calculated according to the following equation:

Di% = 100-100×[(% AIA in feed × % Nutrient in feces)/(% AIA in feces × % Nutrient in feed)]

Where Di% is the digestibility of nutrients in percentage and AIA is the acid insoluble ash. Mean plasma Na^+, K^+ and Cl^- concentrations were calculated as the average of the values measured from middle and the end of the experiment. The measuring values from week 1, 3 and 5 were averaged for fecal dry matter, nitrogen and their digestibility. For all measured parameters the mean values were used for statistical analysis.

Statistical analysis

Data were analyzed according to the General Linear Model procedure of SAS (SAS 1990). The effects of dietary CAD, enzyme and their interaction were tested by two-way ANOVA. LSM procedure was used to compare treatment means.

Results

Plasma minerals

Venous plasma Cl^- concentration was significantly lower ($p < 0.001$) in dietary CAD of –100 mEq kg^{-1} group compared to dietary CAD of 200 and 500 mEq kg^{-1} groups. Plasma Cl^- concentration and the growth (in g $kg^{-0.75}$ d^{-1}) of the pigs were negatively correlated ($r = -0.71$) (Figure 1). However, neither dietary CAD nor feed enzyme had an effect ($p > 0.10$) on venous plasma Na^+ and K^+ concentrations (Table 3).

Feed consumption, growth and feed utilization

Dietary CAD levels significantly affected the growth of pigs. At a dietary CAD level of -100 mEq kg^{-1}, growth was lower ($p < 0.01$) than at other two dietary CAD levels (Table 4). Dietary CAD tended to influence feed intake and feed conversion ($p < 0.10$). Supplementation of xylanase had no effect on feed intake, growth and feed conversion of pigs. No dietary CAD and xylanase interaction was found for feed intake and growth.

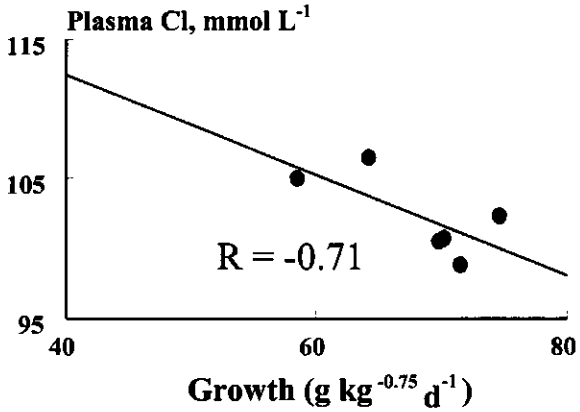

Figure 1. Correlation between plasma Cl^- concentration (mmol L^{-1}) and the growth (g $kg^{-0.75}$ d^{-1}) of pigs.

Table 3. Plasma Na^+, K^+ and Cl^- concentrations (mmol L^{-1}) of pigs, the average values of the middle and the end of the experiment[1]

Dietary CAD (mEq kg^{-1})[2]	-100		200		500		SEM	P values[3]	
Xylanase	0	0.1%	0	0.1%	0	0.1%		CAD	Enzyme
Plasma Cl^-[4]	105.0	106.5	102.3	100.7	98.8	100.5	1.24	0.0001	0.6
Plasma Na^+	146.6	143.3	143.2	141.4	140.2	146.2	4.45	0.7	0.9
Plasma K^+	5.82	5.94	5.80	6.02	6.10	6.24	0.297	0.6	0.5

[1] The start values for plasma Cl^-, Na^+ and K^+ were 101.5, 125.6 and 4.88 mmol L^{-1}, respectively, [2] CAD is defined as Na + K - Cl (mEq kg^{-1}), [3] No CAD and feed enzyme interaction was found ($p > 0.3$). , [4] CAD group of –100 mEq kg^{-1} significantly differ from CAD groups of 200 and 500 mEq kg^{-1} ($p < 0.05$).

Fecal composition and digestibility

Fecal dry matter (DM) ($p < 0.001$) and fecal nitrogen ($p < 0.05$) content decreased with increasing of dietary CAD levels. Dietary CAD of –100 mEq kg^{-1} showed higher fecal dry matter and nitrogen compared to dietary CAD of 200 and 500 mEq kg^{-1}. Enzyme supplementation ($p < 0.052$) increased fecal DM content but did not influence fecal nitrogen content. No dietary CAD and feed enzyme interaction was found (Table 5).

Dietary CAD of 500 mEq kg^{-1} resulted in significantly higher fecal apparent

digestibility of DM and nitrogen than other two dietary CAD levels (Table 5). Fecal apparent digestibility of DM increased ($p < 0.04$) and apparent digestibility of nitrogen tended to increase when xylanase was added to the diets ($p < 0.07$).

Table 4. Feed intake, growth and feed conversion, average value of 5 weeks

Dietary CAD (mEq kg^{-1})[1]	-100		200		500		SEM	P values[2]	
Xylanase	0	0.1%	0	0.1%	0	0.1%		CAD	Enzyme
Feed intake (g pig^{-1} d^{-1})	787	839	980	858	938	1006	62.8	0.08	0.8
Growth (g pig^{-1} d^{-1})[3]	484	532	657	593	603	651	34.1	0.002	0.5
Feed conversion	1.78	1.60	1.50	1.47	1.59	1.61	0.085	0.07	0.4

[1] CAD is defined as $Na^+ + K^+ - Cl^-$ (mEq kg^{-1}), [2] No CAD and feed enzyme interaction was found ($p > 0.10$), [3] CAD group of –100 mEq kg^{-1} significantly differ from CAD groups of 200 and 500 mEq kg^{-1} ($p < 0.05$).

Table 5. Fecal dry matter and nitrogen content and their apparent digestibility, average value of week 1, 3 and 5

Dietary CAD (mEq kg^{-1})[1]	-100		200		500		SEM	P values[2]	
Xylanase	0	0.1%	0	0.1%	0	0.1%		CAD	Enzyme
Fecal composition (g kg^{-1})									
Dry matter[3]	380	398	341	362	294	323	14.3	0.0001	0.052
Nitrogen[4]	12.3	13.1	11.5	12.0	11.9	11.4	0.45	0.05	0.5
Digestibility (%)									
Dry matter[5]	81.8	82.7	80.6	80.7	88.7	89.7	0.424	0.0001	0.036
Nitrogen[6]	83.2	83.7	81.4	81.9	87.6	89.6	0.865	0.0001	0.07

[1] CAD is defined as $Na^+ + K^+ - Cl^-$ (mEq kg^{-1}), [2] No CAD and feed enzyme interaction was found ($p > 0.10$), [3] In the order of significant: –100 mEq kg^{-1} CAD > 200 mEq kg^{-1} CAD > 500 mEq kg^{-1} CAD ($p < 0.05$), [4] – 100 mEq kg^{-1} CAD significantly higher than 200 and 500 mEq kg^{-1} CAD ($p < 0.05$), [5] In the order of significant: 500 mEq kg^{-1} CAD > -100 mEq kg^{-1} CAD > 200 mEq kg^{-1} CAD ($p < 0.05$), [6] 500 mEq kg^{-1} CAD significantly higher than -100 and 200 mEq kg^{-1} CAD ($p < 0.05$).

Discussion

Plasma minerals

The high plasma Cl^- concentration in low dietary CAD group as found in the present study agrees with the observations in literature (Patience and Wolynetz, 1990; Patience and Chaplin, 1997). The high plasma Cl^- concentration may be associated with high Cl content in the –100 mEq kg^{-1} CAD diet. Yen *et al.* (1981) also found an increase in plasma Cl^- concentration in pigs fed a diet containing high Cl (4% of $CaCl_2.2H_2O$). Patience and Chaplin

(1997) compared a diet with –20 mEq kg^{-1} CAD level to a diet with 104 mEq kg^{-1} CAD level on minerals balance. The two diets had similar chloride content. But the 104mEq kg^{-1} CAD diet had additional Na and K. They found that Cl^- concentration in the serum was significantly lower in –20 mEq kg^{-1} CAD group than the other dietary CAD group. This result indicates that plasma Cl^- is influenced by dietary CAD component other than dietary Cl. Furthermore the high Cl^- in the serum was associated with low HCO_3^- concentration in the serum or vise versa. Increasing dietary CAD can increase HCO_3^- concentration in the blood (Patience *et al.*, 1987). Kemme-Kroonsberg (1993) suggested that when HCO_3^- is increased in the plasma, consequently an equivalent amount of Cl^- has to be excreted in order to maintain electroneutrality in the extracellular fluid. This might explain why plasma Cl^- concentration decreased with increase of dietary CAD or vise versa. In the present study, although Na content in the diet increased with increasing dietary CAD, plasma Na^+ concentration was not changed. This result is in agreement with the study of Patience and Chaplin (1997), who also found that Na^+ and K^+ concentration in the serum were not influenced by dietary CAD in pigs. Haydon *et al.* (1990) as well as Patience and Wolynetz (1990), however, observed a linear increase in blood Na^+ concentration with increasing dietary CAD levels. In our previous study (unpublished data), we also found that blood Na^+ concentration was higher at 200 mEq kg^{-1} CAD level compared to –100 mEq kg^{-1} CAD level. In the present study, dietary K was kept constant among treatment groups, this may explain the constant K^+ concentration in the plasma. Some other studies also support this result (Haydon *et al.*, 1990; Patience and Wolynetz, 1990).

Feed consumption, growth and feed conversion

Growth rate was depressed in pigs after feeding the –100 mEq kg^{-1} CAD diet. Increasing dietary CAD to 200 mEq kg^{-1} significantly improved the performance of pigs. Further increase of dietary CAD from 200 to 500 mEq kg^{-1} slightly decreased intake and weight gain in the group without enzyme addition. This suggested that an optimal dietary CAD could be between 200 and 500 mEq kg^{-1}.

In general a negative dietary CAD results in poor performance. Increasing dietary CAD improves feed intake and growth in many animal species. In this study increasing dietary CAD from -100 to 200 mEq kg^{-1} resulted in an increase of 24% in feed intake and 36% in weight gain. Patience *et al.* (1987) found that optimal dietary CAD for young pigs was around 175 mEq kg^{-1}. The results of the study of Austic *et al.* (1983) suggested that dietary

CAD ranging between 100 to 300 mEq kg^{-1} permitted optimal performance of pigs. On the other hand, Park *et al.* (1994) found an optimal dietary CAD of 50 to 150 mEq kg^{-1} for weaning pigs.

Many studies showed that a diet with excess anion (Cl^-) resulted in high Cl^- content in the plasma, reduced blood pH, bicarbonate and base excess (Yen *et al.*, 1981; Patience *et al.*, 1987; Patience and Chaplin, 1997). This may depress feed intake and growth. Yen *et al.* (1981) found that inclusion of 4% $CaCl_2.2H_2O$ in pig feed resulted in metabolic acidosis and reduced feed intake and growth.

Dietary CAD did not significantly influence feed intake, this was associated with large within group variations. However the data showed a clear trend of a low feed intake at low dietary CAD level, especially for non-enzyme supplementation groups. The effect of dietary CAD on feed intake has been observed in many other studies. Patience *et al.* (1987) observed a linear and quadratic increase in feed intake with increasing dietary CAD from –85 to 341 mEq kg^{-1} in pigs. Haydon *et al.* (1990) showed a linear increase in daily feed intake with increasing dietary CAD from 25 to 400 mEq kg^{-1}. In the present study, feed conversion tended to decrease with increasing dietary CAD levels. Patience and Wolynetz (1990) reported a linear increase in feed efficiency with increasing dietary CAD from –176 to 248 mEq kg^{-1}. In contrast Patience *et al.* (1987) and Haydon *et al.* (1990) found that dietary CAD affected feed intake and growth, but feed efficiency was not changed.

Overall, xylanase addition did not influence on performance of pigs. Dietary CAD and xylanase did not interact with regard to performance. However, comparing performance responses at individual dietary CAD levels, xylanase addition numerically increased feed intake and growth at dietary CAD levels of –100 and 500 mEq kg^{-1}. But it numerically decreased feed intake and growth at a dietary CAD level of 200 mEq kg^{-1}.

Fecal composition and digestibility

A high dietary CAD resulted in low dry matter content in feces. This may be a consequence of high water intake in the high dietary CAD group. In a previous experiment we observed a higher water intake in dietary CAD of 200 mEq kg^{-1} group compared to –200 mEq kg^{-1} group (unpublished data). Patience and Chaplin (1997) observed an increase in water intake and water to feed ratio in pigs fed a diet with CAD level of 104 mEq kg^{-1} compared to a diet with a CAD level of –20 mEq kg^{-1}. However dietary CAD did not affect the water retention.

The digestibility of DM and nitrogen was higher in the dietary CAD of 500 mEq kg^{-1} group compared to the other two lower CAD groups. This was associated with low fecal DM and nitrogen content at a CAD of 500 mEq kg^{-1}. This result is in contrast to the observations by Haydon and West (1990), they found that dietary CAD levels did not effect fecal nitrogen, although it numerically decreased with increasing dietary CAD.

The inclusion of xylanase significantly increased fecal digestibility of dry matter and tended to improve fecal digestibility of nitrogen. This is in agreement with literature (Bedford *et al.*, 1992; Inborr *et al.*, 1993). Xylanase inclusion increased fecal apparent digestibility of dry matter at dietary CAD levels of –100 and 500 mEq kg^{-1} compared to dietary CAD level of 200 mEq kg^{-1}. This may partly explain the numerical increased growth by addition of feed enzyme at these two dietary CAD levels. Addition of feed enzyme did not interact with dietary CAD. This may imply that enzyme did not influence minerals digestibility or the enzyme effect on nutrient digestibility was not strong enough to obtain this interaction.

In conclusion, this study showed that a dietary CAD level of –100 mEq kg^{-1} depressed growth of young pigs. While dietary CAD between 200 and 500 mEq kg^{-1} improved the performance of young pigs. When a pig feed is formulated, an optimal dietary CAD should be considered. Impaired feed intake and growth may be the result of sub-optimal dietary CAD level. Enzyme addition improved digestibility of dry matter. No dietary CAD and feed enzyme interaction was found in this study.

Acknowledgement

This study was supported by Finnfeeds International Ltd., Marlborough, UK. The experiment was conducted in the facility of 'de Haar' in the Department of Animal Science, Wageningen University. The authors would like to express their thanks to all the people in the 'de Haar' for their help during the experiment. The assistant of all the people in the lab. of Animal Nutrition Group on samples' chemical analysis are appreciated. The special thank is given to Tamme Zandstra for all the arrangement of experiment.

References

Austic, R. E., Boyd, R. D., Klasing, K. C. and Riley, W. W. 1983. Effect of dietary electrolyte balance on growth performance in swine. J. Anim. Sci. 57 (Suppl. 1): 236 (Abstr.).

Bedford, M. R. and Classen, H. L. 1992. Reduction of intestinal viscosity through manipulation of dietary rye and pentosanase concentration is effected through changes in the carbohydrate composition of the intestinal aqueous phase and results in improved growth rate and food conversion efficiency of broiler chicks. J. Nutr. 122: 560-569.

Fauchon, C., Seoane, J. R. and Bernier, J. F. 1995. Effects of dietary cation-anion concentrations on performance and acid-base balance in growing lambs. Can. J. Anim. Sci. 75: 145-151.

Graham, H., Fadel, J. G., Newman, C. W. and Newman, R. K. 1989. Effect of pelleting and β-glucanase supplementation on the ileal and whole – tract digestibility of a barley-based diet in the pig. J. Anim. Sci. 67: 1293-1298.

Haydon, K. D. and West, J. W. 1990. Effect of dietary electrolyte balance on nutrient digestibility determined at the end of the small intestine and over the total digestive tract in growing pigs. J. Anim. Sci. 68: 3687-3693.

Haydon, K. D., West J. W. and McCarter, M. N. 1990. Effect of dietary electrolyte balance on performance and blood parameters of growing-finishing swine fed in high ambient temperature. J. Anim. Sci. 68: 2400-2406

Inborr, J., Schmitz, M. and Ahrens, F. 1993. Effect of adding fibre and starch degrading enzymes to a barley / wheat based diet on performance and nutrient digestibility in different segments of the small intestine of early weaned pigs. Anim. Feed Sci. Technol. 44: 113-127.

Inborr, J. 1994. Supplementation of pig starter diets with carbohydrate-degrading enzymes-stability, activity and mode of action. Agri. Sci. in Finland. Vol. 3, supplement no. 2.

ISO 5984. 1978. International Organization for Standardization. Animal feeding stuffs – Determination of crude ash. Genève, Switzerland.

ISO 5985. 1978. International Organization for Standardization. Animal feeding stuffs – Determination of ash insoluble in hydrochloride acid content. Genève, Switzerland.

ISO 5983. 1979. International Organization for Standardization. Animal feeding stuffs – Determination of *nitrogen content* and calculation of *crude protein content*. Genève, Switzerland.

ISO 6496. 1983. International Organization for Standardization. Animal feeding stuffs – Determination of moisture content. Genève, Switzerland.

ISO 6491. 1995. International Organization for Standardization. Animal feeding stuffs – Determination of *phosphorus content- Spectrometric method*. Genève, Switzerland..

ISO 6869. 1997. International Organization for Standardization. Animal feeding stuffs – Determination of the calcium, copper, iron, magnesium, manganese, potassium, sodium and zinc contents – Atomic absorption spectrometric method. Genève, Switzerland.

Kemme-Kroonsberg, C. 1993. Nutrition and acid-base balance of pigs: a review. Research Institute for Livestock Feeding and Nutrition (IVVO-DLO), Lelystad, Netherlands. No. 243. P 69.

Van der Klis, J.D., Verstegen, M.W.A. and Van Voorst, A. 1993. Effect of a soluble polysaccharide (carboxy methyl cellulose) on the absorption of minerals from the gastrointestinal tract of broilers. Bri. Poul. Sci. 34: 377-389.

McCarthy, J. F., Aherne, F. X. and Okai, D. B. 1974. Use of HCl insoluble ash as an index material for determining apparent digestibility with pigs. Can. J. Anim. Sci. 54: 107-109

Newman, C. W., Eslick R. F. and E$_L$Negoumy, A. M. 1983. Bacterial diastase effect on the feed value of two hulless barleys for pigs. Nutr. Report. Int. 28: 139-146.

NRC. 1988. Nutrient Requirements of Swine (9th Ed.). National Academy Press, Washington, DC.

Park, J. H., Han, I. K. and Kim, I. B. 1994. Effects of electrolyte balance (EB: Na+K-Cl) on the growth, blood parameters, urine parameters and nutrient availabilities of weaning pigs. Korean J. Anim. Nutr. and Feedstuffs 18: 147-154.

Patience, J. F., Austic, R. E. and Boyd, R. D. 1987. Effect of dietary electrolyte balance on growth and acid-base status in swine. J. Anim. Sci. 64: 457-466.

Patience, J. F. and Wolynetz, M. S. 1990. Influence of dietary undetermined anion on acid-base status and performance in pigs. J. Nutr. 120: 579-587.

Patience, J. F. and Chaplin, R. K. 1997. The relationship among dietary undetermined anion, acid-base balance, and nutrient metabolism in swine. J. Anim. Sci. 75: 2445-2452.

Prohaszka, L. and Baron, F. 1980. The predisposing role of high dietary protein supplies in Eneteropathogenic E. Coli infections of weaned pigs. Zbl. Veterinary Medicine B 27: 222-232.

SAS. 1990. SAS User's Guide: Statistics (Version 6 Ed.). SAS Inst. Inc., Cary, NC.

Wunsche, J., Borgmann, E., Souffrant, S., Hennig, U. and Souffrant, W.B. 1991. Nutrient digestibility and energetic feed value of finisher diets under practical conditions based on HCl insoluble ash as marker. Archiv-fur-Tierenahrung. 34: 561-567.

Yen, J. T., Pond, W. G. and Prior, R. L. 1981. Calcium chloride as a regulator of feed intake and weight gain in pigs. J. Anim. Sci. 52: 778-782.

CHAPTER 3

The impact of changing dietary Na/K ratios on growth and nutrient utilization in juvenile African catfish, *Clarias gariepinus*

Yueming Dersjant-Li[1, 2], Sheng Wu[1], Martin W. A. Verstegen[2], Johan W. Schrama[3] and Johan A. J. Verreth[1]

[1] *Fish Culture and Fisheries Group,* [2] *Animal Nutrition Group,* [3] *Adaptation Physiology Group, Wageningen Institute of Animal Sciences, Wageningen University, Wageningen, The Netherlands*

Submitted to Aquaculture

Abstract: The effects of dietary Na/K ratios on feed intake, growth, nutrient utilization, plasma and body mineral concentration in juvenile African catfish, *Clarias gariepinus* were investigated. Four Na/K ratios, 0.2, 0.7, 1.5, 2.5 (mmol/mmol), were tested in a randomized experimental design with three replications in each treatment. Juvenile African catfish (n = 360) with a mean initial body weight of 42.9g were used. The experiment was conducted in 12 (70 L) aquaria with biofilter and recirculating water supply system. Dietary Na/K ratios were obtained by altering levels of Na_2CO_3 and K_2CO_3 concentrations. Feed was provided manually twice a day to satiation. Feed consumption was not influenced by the mineral composition of the diets. Growth, feed conversion, nitrogen and energy utilization efficiency as well as body dry matter, fat, nitrogen and energy content increased linearly with increasing dietary Na/K ratio. Potassium, chloride concentrations and the ratios between Na/K, Na/Cl and K/Cl in the body remained constant among treatment groups at the end of a 4 weeks feeding period. In conclusion, an excess K in the diet depresses growth and nutrient utilization efficiency, reduces body fat and protein deposition. The optimal dietary Na/K ratio can be considered between 1.5 to 2.5 (mol/mol) for the growth of African catfish.

Keywords: African catfish, dietary Na/K ratio, energy utilization, growth

Introduction

In African catfish, feed intake and growth increase linearly with dietary cation anion difference from –100 to 700 mEq kg^{-1} (Dersjant-Li *et al.*, 1999). Dietary cation anion difference (CAD) is defined as Na + K - Cl, expressed as mEq kg^{-1}. High dietary CAD can be formulated by addition of $NaHCO_3$ and/or $KHCO_3$, low dietary CAD can be made by addition of $CaCl_2$ in a diet. By doing so, a fixed dietary CAD level can be formulated with different Na/K ratios. When excess potassium is used to increase dietary CAD, growth of chickens is depressed (Johnson and Karunajeewa, 1985). For juvenile chinook salmon, insufficient K in the diet causes mortality and depresses growth, while an adequate K concentration (0.6 – 1.2%) improves growth (Shearer, 1988). In vertebrates, the Na^+ concentration in the extracellular fluid surpasses that in the cytosol whereas K^+ is higher in the intracellular fluid compared to the plasma. The gradient of K^+ and Na^+ over the cell's plasma membrane is maintained by Na-K ATPase activity (Lehninger, 1971). To maintain the Na^+ and K^+ gradients between intra- and extracellular fluids, energy is needed. Obviously, excess of one cation to the other can change the efficiency of the Na-K pump. As a working hypothesis of this study, it was supposed that an optimal dietary Na/K ratio might reduce the energy requirement to maintain this gradient. As plasma Na^+, K^+, and Cl^- levels contribute significantly to the osmotic balance and influence the acid base equilibrium, the Na/K ratio in the diet may have also an indirect influence on feed intake and growth of animals. We therefore investigated the effect of dietary Na/K ratios on feed intake, growth and nutrient utilization of fish at a dietary CAD level of 700 mEq kg^{-1}, which was considered as optimal for African catfish (Dersjant-Li *et al.*, 1999, 2000).

Materials and methods

Fish and facilities

The experiment consisted of a one week of adaptation to the new environment followed by one week of adaptation to the treatment diets and a 4-wks period of genuine exposure to the test diets. Upon arrival at the experimental facility, juvenile African catfish (*Clarias gariepinus*) were allowed to adapt to the new environment with a commercial diet for one week. At the end of this adaptation period, the fish were size-graded, weighed and 45 fish (mean weight of 35.0 ± 0.64g, sd) were distributed at random to each aquarium, with 12 aquaria (70L) in total. Each treatment diet was randomly allocated to 3 of the 12 aquaria. Fish

were allowed to adapt to the treatment diets for one week. During this adaptation period some fish showed bacterial infection possibly because of handling. Therefore, at the start of 4-wks feeding experiment, these infected fish were removed from the tanks. The remaining fish were kept at a density of 30 fish per aquarium with a mean initial body weight of 42.9±1.0 g (mean ± sd). To avoid further bacterial infections, the salinity in the system water was raised by addition of agriculture salt (99.8% NaCl) to the storage tank of the recirculation system. The salinity was maintained at 3.5g L^{-1} (about 10% of the salinity of seawater) for the first three-days of the 4-wks experimental period. Afterwards the water salinity was reduced to 2.5g L^{-1} and maintained at this level until the end of the experiment. All the fish in different treatment groups experienced the same changes in water salinity because they were in the same recirculation system. Fish were reared at a temperature of 27 ± 0.5°C, with aeration in each aquarium, under a 12L:12D light regime. O_2 concentration in each aquarium was maintained above 6.0 mg L^{-1}.

Experimental design, diets and feeding

Four dietary Na/K ratios (0.2, 0.7, 1.5, 2.5, mol/mol) were tested using a randomized experimental design, with three replications in each treatment. Dietary Na/K ratios were formulated by supplementation of a basal diet with designed amounts of Na_2CO_3 and/or K_2CO_3 (Table 1). The addition of salt was done by replacement of wheat in the basal diet. The lowest Na/K ratio (0.2 mol/mol) was formulated by only adding K_2CO_3 to the basal diet, while the highest Na/K ratio was achieved by only adding Na_2CO_3 to the basal diet. The basal diet consisted of wheat, wheat gluten, soybean meal, meat meal, fishmeal, vitamin and mineral premix (Table 1). The sum of Na+K was constant in the diets, the ratio of Na/K varied. The measured dietary CAD levels were all between 500-700 mEq kg^{-1}, which was considered as optimal dietary CAD range for performance of African catfish (Dersjant-Li *et al.*, 1999). Diets were pelleted and coated (4%) with herring oil. During the 1-wk adaptation and the 4–wks feeding period, fish were fed to satiation twice daily, at 9:00am and 5:00pm respectively, for a period of about 1 hour. Meals were supplied by hand in small portions at a time, to ensure that all feed was eaten, until fish do not react to feed supply (satiated).

Table 1. Composition of the treatment diets

	Dietary Na/K ratio (mol/mol)			
	0.2	0.7	1.5	2.5
Basal ingredients (g kg^{-1})				
Fixed ingredients[1]	777	777	777	777
Wheat	189	192.1	194.6	196.5
Additives (g kg^{-1})				
K_2CO_3	33.2	18.7	8.3	
Na_2CO_3	0.8	12.2	20.1	26.5
Analyzed composition (g kg^{-1})				
Dry matter	891	896	894	897
Crude protein	451	457	458	456
Crude fat	114	110	109	108
Ash	144	142	137	135
Gross energy (kJ g^{-1})	18.5	18.5	18.6	18.5
Na	2.4	7.3	9.8	12.7
K	22.3	17.1	12.5	8.9
Cl	4.3	4.3	4.3	4.3
pH	8.8	8.7	8.7	8.4
Buffer capacity (mEq kg^{-1})[3]	1927	1919	1911	1938
Measured CAD[4]	554	633	624	657

[1] Fixed ingredients consist of 360 g kg^{-1} fish meal, 100 g kg^{-1} wheat gluten, 75 g kg^{-1} soybean meal, 30 g kg^{-1} meat meal, 75 g kg^{-1} feather meal, 60 g kg^{-1} herring oil, 35 g kg^{-1} $CaCO_3$, 25 g kg^{-1} binder, 7 g kg^{-1} monocalcium phosphate, 10 g kg^{-1} of vitamin and mineral premix. The premix was provided by Coppens International BV, Helmond, The Netherlands. [2] Diamol (washed sand) is diatomaceous shell powder (Biakon N.V., Grobbendonk, Belgium). [3] Quantity of 1.0N HCl required for the gastric digestion pH (3.0) of 1 kg feed. [4] Calculated from Na, K and Cl content.

Sampling and measurements

Feed intake was recorded daily. Fish body weight was measured, respectively, at the start of the 1-wk adaptation to the experimental diets, at the beginning and at the end of the 4-wks feeding period. Before the fish received the experimental diets, one random sample of 20 fish was taken for analysis of the initial body composition. At the end of the experiment a sample of 10 fish was taken from each aquarium for final body composition analysis. Sampled fish were euthanized by an overdose of tricaine methane sulphonate (TMS 0.6 g L^{-1}) for 15 min. and were then stored at –20°C until later analysis.

Blood (<1ml) was sampled from groups of lightly anaesthetized (0.3g TMS L^{-1}) fish (20 individuals at the start of the study, before fish were receiving experimental diets and 6

fish per aquaria at the end of the experiment), by puncturing vessels in the caudal peduncle. The blood samples were centrifuged (1599g for 15 min.) to obtain plasma. Plasma samples were stored at –20°C until analysis of mineral content (Na^+, K^+, Cl^-). After blood sampling, the same fish were killed and kidney samples were taken for Na-K ATPase activity measurement. The fixation, storage of kidney samples and the measurement of Na-K ATPase activities were done according to the method of Zaugg (1982) with some modifications.

Fish carcass samples were ground before analysis. Each sample from each aquarium was used as one experimental unit (n = 12). Dry matter and nitrogen content was measured from fresh material. Ash, fat and energy content were measured from freeze-dried material. Nitrogen content was analyzed by the Kjeldahl method (ISO 5983, 1979). Dry matter and ash content was measured according to ISO procedures (ISO 6496, 1983 and ISO 5984, 1978, respectively). Energy content was determined by bomb-calorimetry (IKA Calorimeter C 7000). Fat was measured according to ISO/DIS 6492 (1996).

All chemical analyses were carried out in triplicate. Mineral contents in carcass samples were determined after solubilization of ashed samples in demineralized water (Cl^-) or in 3N HCl solution (Na^+, K^+) as described by Fauchon *et al.* (1995) with some modifications. Chloride was measured by using a chloride meter (PCLM digital chloride meter, Jenway Ltd. Dunmow, England). Sodium and potassium were measured by using an atomic absorption spectrometry (SpectrAA 300, Varian Australia Pty Ltd, Mulgrave Victoria, Australia). Dietary pH was measured as described by Tacon and De Silva (1983). Buffer capacity was defined as quantity of 1.0N HCl required for the gastric digestion (pH=3) of 1 kg diet (mEq kg^{-1}). The buffer capacity was measured according to the following procedure: 2 g of diet was incubated in 20ml 0.1N HCl solution in 37°C water bath for one hour. Afterwards the suspension was titrated to pH=3 with 0.1 N NaOH solution.

Data analysis

Feed intake and growth were expressed in g per kg metabolic weight (g $kg^{-0.8}$ BW) (Heinsbroek *et al.*, 1990), and calculated for the 4-wks of feeding period only (excluding the adaptation period). Feed conversion was calculated as feed consumed divided by weight gain. Retained nitrogen (RN), retained fat (RF) and retained energy (RE) were derived from the body composition data (Dersjant-Li *et al.*, 1999). The initial body composition was analyzed from the samples taken before fish received the treatment diets; the final body composition was obtained at the end of the experiment. Thus the RN, RF and RE were calculated for a 5-

wks period, including the 1-wk period of adaptation to the treatment diets. Accordingly gross nitrogen (GN) and gross energy (GE) intake were also calculated based on feed intake data in the same period. Apparent net nitrogen utilization efficiency (NUE) was calculated as the percentage of retained nitrogen over gross nitrogen intake. Energy conversion efficiency (ECE) was estimated as the percentage of retained energy over gross energy intake. The proportion of retained energy as protein (RE_p) was calculated by converting the retained protein (retained nitrogen × 6.25) to energy by using an average energy content of 23.6 kJ g^{-1} protein (Brafield, 1985). Retained energy as fat (RE_f) was calculated by using an average energy content of 39.5 kJ g^{-1} fat (Brafield, 1985). The metabolizable energy (ME) content of diet (kJ g^{-1}) was calculated according to the dietary protein, fat and carbohydrate content. The following equation was derived based on the results of previous experiments as carried out in our laboratory (Heinsbroek, unpublished):

$$ME = 0.173 \times protein\% + 0.356 \times fat\% + 0.125 \times carbo's\%$$

Where ME is metabolizable energy content of diet. Based on the data of RE_p and RE_f, metabolizable energy requirement for maintenance (MEm) was estimated using the following equation:

$$MEm = ME - (RE_p / K_p + RE_f / K_f)$$

Where MEm is metabolizable energy requirement for maintenance, RE_p is retained energy as protein, RE_f is retained energy as fat, K_p and K_f are the efficiencies of metabolizable energy (ME) utilization for protein and fat retention. The sum of $((RE_p / K_p) + (RE_f / K_f))$ is the metabolizable energy requirement for production. Rodehutscord and Pfeffer (1999) used a K_f value of 0.9 and estimated a K_p value of 0.54 for rainbow trout. These values were assumed to be applicable to African catfish also.

Statistical analysis

Data were tested by one-way ANOVA. Linear and quadratic effects were tested using the GLM procedure of the SAS program (SAS, 1990). $P < 0.05$ was considered as significant. The treatment means were compared by the Bonferroni (Dunn) t tests.

Results

Feed consumption, growth, nitrogen/energy retention and nutrient utilization

No significant differences were observed in feed intake among treatments. Growth, nitrogen and energy retention, energy conversion efficiency (ECE) and apparent nitrogen utilization efficiency (NUE) increased linearly and quadratically with increasing dietary Na/K ratio (Table 2). Feed conversion and retained energy as protein decreased in a linear and quadratic manner with increasing dietary Na/K ratio (Table 2).

Table 2. Feed intake, growth, feed conversion, nitrogen and fat retention

	Dietary Na/K ratio (mol/mol)					
	0.2	0.7	1.5	2.5	SEM	Effect[1]
Feed intake (g $kg^{-0.8}$ d^{-1})	23.5	23.1	23.7	25.6	0.71	NS
Growth (g $kg^{-0.8}$ d^{-1})	18.6	23.0	26.8	28.8	1.01	L***, Q*
Feed conversion	1.27	1.0	0.88	0.89	0.02	L***, Q***
Protein retention (kJ $kg^{-0.8}$ d^{-1})	111.3	143.9	181.7	191.5	5.02	L***
Fat retention (kJ $kg^{-0.8}$ d^{-1})	42.3	63.4	95.6	104.8	4.51	L***
ECE (% RE/GE)	23	31	40	41	1.7	L***, Q**
NUE (% RN/GN)	25	31	37	37	1.0	L***, Q***
RE as protein (%)	62.4	58.4	53.6	52.1	0.99	L***, Q*

[1] L-linear effect, Q- quadratic effect, * = $P < 0.05$, ** = $P < 0.01$, *** = $P < 0.001$.

Mineral contents in plasma and fish body

Plasma potassium increased quadratically with increasing Na/K ratio, which showed a maximum plasma K^+ concentration of 5.30 mmol L^{-1}, at dietary Na/K ratio between 1.5-2.5. No significant differences were observed in plasma chloride, sodium and CAD levels among treatments (Table 3).

Sodium content in total body composition decreased linearly with increasing dietary Na/K ratio. However K^+, Cl^-, CAD, Na/K, Na/Cl and K/Cl ratios in the fish body were more or less constant among treatment groups (Table 3).

Table 3. Minerals in plasma and body[1]

	Dietary Na/K ratio (mol/mol)					
	0.2	0.7	1.5	2.5	SEM	Effect[2]
Minerals in plasma (mmol L^{-1})						
Na	120.1	119.6	121.3	128.8	6.47	NS
K[3]	4.70[b]	5.30[a]	5.30[a]	4.87[ab]	0.12	Q**
Cl	106.1	107.8	101.6	104.3	2.48	NS
CAD(mEq L^{-1})	18.7	17.1	25.0	29.3	7.31	NS
Minerals in fish body (g kg^{-1} body wet weight)						
Na	1.03	1.02	0.98	0.98	0.01	L**
K	2.78	2.79	2.76	2.77	0.07	NS
Cl	0.87	0.78	0.80	0.81	0.03	NS
CAD (mEq kg^{-1})	91.5	93.8	91.0	90.8	1.70	NS
Na/K (mol/mol)	0.63	0.62	0.60	0.60	0.01	NS
Na/Cl (mol/mol)	1.83	2.01	1.90	1.88	0.08	NS

[1] The initial values for plasma minerals were 118, 5.1 and 97.2 mmol L^{-1} for Na^+, K^+ and Cl^- respectively and 26.0 mEq L^{-1} for CAD. The initial values of body minerals were 0.96, 2.81 and 0.54 g kg^{-1} wet body weight for Na^+, K^+ and Cl^- respectively and 98.6 mEq kg^{-1} wet weight for CAD. [2] NS- Non significant, L- linear effect, Q- quadratic effect, * = $P < 0.05$, ** = $P < 0.01$, *** = $P < 0.001$. [3] Different superscript in a raw indicates significant differences.

Body composition

Dry matter, fat, nitrogen and energy content in the body increased linearly (and quadratically) with increasing dietary Na/K ratio. Ash content tended to decrease linearly with increasing dietary Na/K ratio (Table 4).

Na-K ATPase activity in the kidney

At the end of the experiment, Na-K ATPase activity in the kidney was not affected by dietary Na/K ratios. The Na-K ATPase activities in the kidney were 4.20±1.75, 2.99±1.61, 3.39±1.32 and 3.28±1.59 (mean ± sd) (μmolPi h^{-1} mg^{-1}) in dietary Na/K ratio of 0.2, 0.7. 1.5 and 2.5 groups respectively. At the start of the experiment, the initial value of Na-K ATPase activity in the kidney was 5.62 (μmolPi h^{-1} mg^{-1}), which was significantly higher than the values of the end of the experiment.

Table 4. Body composition (% wet weight) in different treatment groups[1]

	Dietary Na/K ratio (mol/mol)					
	0.2	0.7	1.5	2.5	SEM	Effect[2]
DM	24.4	25.2	25.9	26.5	0.25	L***
Ash	3.06	3.07	2.98	2.90	0.07	L†
Nitrogen	2.41	2.43	2.44	2.46	0.02	L*
Fat	5.79	6.45	7.50	7.83	0.16	L***, Q*
Energy (kJ g^{-1} wet weight)	5.82	6.12	6.57	6.79	0.07	L***, Q*

[1] The initial values were 25.5, 3.10, 2.46, 6.06% of wet weight for DM, ash, N and fat respectively and 6.17 kJ g^{-1} wet weight for energy. [2] L-linear effect, Q- quadratic effect, † = tendency, $P < 0.10$, * = $P < 0.05$, ** = $P < 0.01$, *** = $P < 0.001$.

Mortality

The highest mortality was found in the dietary Na/K ratio of 0.2 group (29%). While the group with dietary Na/K ratio of 0.7 had a lower mortality (11%). The mortality was lowest in the groups with dietary Na/K ratios of 1.5 (1%) and 2.5 (2%).

Discussion

The results of the present study indicated that a Na/K ratio of between 1.5 and 2.5 (mol/mol) improved growth of African catfish. Fish can perform well with a wide range of Na levels in the diet. With changing of dietary Na/K ratio, the fish maintained a constant K^+, Cl^- and CAD concentration in the body. Although Na^+ linearly decreased ($p < 0.01$) in the body the change was very small among treatments. These data indicated that fish maintained these electrolyte concentrations in the body compartments after ingesting diets with different Na and K concentrations, including a high excess K (Na/K ratio of 0.2) diet. These results are comparable to the observations by Smith *et al.* (1995). They found that in rainbow trout body Na^+ concentration is not significantly altered when fed either a 2.1% NaCl diet or a 12% NaCl diet in comparison to unfed fish. Sodium concentration in the body was numerically higher with fed fish than in unfed fish. It was also numerically higher with fish fed 2.1% NaCl diet than fish fed 12% NaCl. In the present study, the Na^+, K^+ and Cl^- concentrations in the body (58.3, 95.3 and 30.0 mmol kg^{-1} body H_2O respectively) compared well with the data reported by Eddy and Bath (1979). They measured a value of 52.2, 107.4 and 40.4 mmol kg^{-1} body H_2O for Na^+, K^+ and Cl^- concentrations respectively in rainbow trout adapted to fresh

water. For the rainbow trout adapted to 1/3 of seawater, these values were 59.1, 112.3 and 49.7 mmol kg^{-1} body H_2O for Na^+, K^+ and Cl^- concentrations respectively. In the present study, the Cl^- concentration in the body is lower than the value reported by Eddy and Bath (1979), this may be due to the fish species (African catfish) used in the present study.

The constant Na/K, Na/Cl and K/Cl ratios in the fish body imply that fish show strict homeostasis of these minerals. Apparently, fish receiving less optimal dietary mineral balances can adjust the uptake and excretion balance. This process however, may be energetically expensive. With increasing dietary Na/K ratio feed efficiency improved and therefore more fat was deposited. This indicates that in low Na/K ratio groups more energy will be required to maintain mineral balance and homeostasis. This can explain the low growth, the low nutrient utilization efficiency, and the low fat and energy deposition in the low Na/K ratio groups.

In the present experiment, decreasing Na/K ratio in the diet resulted in excess K, which consequently depresses growth. This result is in agreement with some other studies. Johnson and Karunajeewa (1985) reported that excess K in the diet had a negative effect on growth of young chicken. Golz and Crenshaw (1984) showed that growth rate in young pigs was depressed when K levels were increased without a concomitant increase in Cl levels. The optimal dietary K concentration for growth appears to be between 0.9 to 1.3 % in the present study, this agrees with Shearer (1988), who suggested an optimal dietary K level of 0.6-1.2% for the growth of juvenile chinook salmon.

In the present study, the water salinity (by addition of agriculture salt) was kept higher (2.5 to 3.5 g L^{-1}) than the salinity of freshwater. It is generally accepted that freshwater fish can adapt to this salinity (Eddy, 1982). The water salinity was maintained the same for all the treatment groups. Therefore, it will not have interfered with the comparison between treatments. However it may influence Na^+ and K^+ exchange with water. When unbalanced Na/K ratio diets were fed to fish, the excess K^+ (or Na^+) would be excreted and might have exchanged for Na^+ (or K^+) from the water. Therefore the high salinity in the water might serve as a salt resource and help fish to maintain mineral balance in the body. This might also explain why fish can maintain body Na^+ in a narrow range and maintain K^+, Cl^- concentrations and the ratios of Na/K, Na/Cl and K/Cl constant in the body after ingesting diets with different Na/K ratios.

The growth rate obtained in this study is in a range of 19-29 g $kg^{-0.8}$ d^{-1}. This is low compared to other studies in African catfish *Clarias gariepinus*. Torreele *et al.* (1993)

obtained maximum growth rates of 30-35 g $kg^{-0.8}$ d^{-1}. Dersjant-Li *et al.* (1999) observed growth rates of 39-44 g $kg^{-0.8}$ d^{-1}. The low growth rate in the present study may be a consequence of the bacterial infection during the adaptation period. The high mortality in the group fed excess K diet (Na/K ratio of 0.2) might indicate that fish in this group had a low resistance to disease. Another possible explanation is that the dead fish was not able to maintain mineral balance with excess K in the diet. The sum of these two aspects might be the cause of the high mortality in low Na/K ratio group.

Feed was supplied in small quantities in a duration of one hour, assuring that fish ate till satiation and no feed was wasted. The recorded feed intake remained similar in different treatment groups, however growth rate increased by 55% in dietary Na/K ratio of 2.5 group compared to dietary Na/K ratio of 0.2 group. Energy utilization efficiency (percentage of retained energy over gross energy, %RE/GE) increased by 85% and nitrogen utilization efficiency (percentage of retained nitrogen over gross nitrogen, %RN/GN) increased by 52%. The increased nitrogen and energy utilization efficiency may indicate a decrease in maintenance cost. Based on the assumption that metabolizable energy of the diets is not affected by dietary Na/K ratios, the metabolizable energy requirement for maintenance (MEm) was calculated (Table 5).

Table 5. Estimation of metabolizable energy requirement for maintenance (MEm) base on an assumption that metabolizable energy intake is not influenced by dietary treatment

	Dietary Na / K ratio (mol/mol)			
	0.2	0.7	1.5	2.5
Gross energy intake (GE) (kJ $kg^{-0.8}$ d^{-1})	422	424	453	480
ME for production (kJ $kg^{-0.8}$ d^{-1})[1]	154	207	277	296
ME for maintenance (kJ $kg^{-0.8}$ d^{-1})[2]	170	117	66	69

[1] calculated as $RE_p/k_p + RE_f/k_f$, as described in M&M. [2] Assuming metabolizable energy is not influenced by dietary treatment, ME for maintenance = ME intake – ME for production, as described in M&M.

The metabolizable energy of the diet was estimated from the composition of protein, fat and carbohydrate. This method may over estimate the ME intake, therefore these data should be interpreted with caution. However this method provides a mean of comparison of possible differences in MEm among different treatment groups. As shown in Table 5, the estimated metabolizable energy requirement for maintenance is about two fold in Na/K ratio

of 0.2 group compared to the one in Na/K ratio of 2.5 group. Because the only difference in the diets is Na/K ratios, therefore the increased energy requirement for maintenance in low Na/K ratio group is related to maintenance of mineral balance and homeostasis. The large difference in growth and nutrient utilization in different treatment groups is probably associated with differences in maintenance costs. The linear increase in nitrogen, fat and energy levels in the body with increasing dietary Na/K ratio can be regarded as the result of an increased scope for growth.

The result of the present study showed that Na-K ATPase activity in the kidney was not influenced by dietary Na/K ratios. In fish, the ion exchange is mainly via gills (Cameron, 1980; Eddy, 1982), this may explain the results of Na-K ATPase activity in the kidney. African catfish maintained Na^+ concentration in the plasma with the changes in sodium concentration in the diets. Also Chiu *et al.* (1984) reported that in rainbow trout, increasing sodium and potassium levels in the diet increased potassium concentration but did not affect sodium concentration in the blood. Smith *et al.* (1995) reported that blood plasma Na^+ concentration was not altered in rainbow trout that absorbed NaCl of 0.5-1 mmol kg^{-1} compared to unfed fish. However, when the fish absorbed more than 18mmol NaCl kg^{-1}, significantly higher plasma Na^+ concentration was found (Smith *et al.*, 1995). In the present study the Na^+ absorption was between 2-13 mmol kg^{-1} fish. Wilson *et al.* (1985) showed that in rainbow trout, plasma chloride was not changed with changes of dietary Na^+ and Cl^- levels. But plasma Na^+ concentration was high and plasma K^+ concentration was low in a high Na /low Cl diet compared to a high Cl/low Na diet.

The high plasma K^+ concentration in the dietary Na/K ratio of 0.7 and 1.5 groups confers with the results of Shearer (1988), who observed that plasma K^+ concentration increased in juvenile chinook salmon, after 10 weeks of feeding high K diets (0.6%-1.2%). In the present study, the fish receiving a dietary Na/K ratio of 0.2 showed a significant low plasma K^+ concentration. This is associated with the low dry matter content of the body. When the body composition contains more water, the mineral's concentration will consequently be diluted. It can be argued that the dilution is a mechanism for maintaining mineral's homeostasis in fish.

The result of this study implies that in African catfish, excess Na should be used for elevating dietary CAD levels instead of using excess K.

Conclusion

Growth, nutrient utilization efficiency, body dry matter, fat, nitrogen and energy content of African catfish linearly increased with increasing dietary Na/K ratios between 0.2 to 2.5 (mol/mol). Excess K in the diet reduced feed efficiency and could be detrimental. Fish, however, were able to maintain concentrations and the ratios of electrolytes relatively constant in the body after ingesting diets with different Na and K levels. The optimal dietary Na/K ratios can be considered between 1.5-2.5 mol/mol for African catfish.

Acknowledgements

The authors are grateful to R. Booms, T. Leffering and N. Ruane for their assistance during samples analyses, to M. ter Veld, S. Leenstra and A. Hutten for their help in fish husbandry, to R. Ozorio for providing the basal diet composition. Sincerely thanks are given to Dr. Gert Flik (University of Nijmegen, The Netherlands) for his comments on an earlier version of this manuscript.

References

Cameron, J. N. 1980. Body fluid pools, kidney function, and acid-base regulation in the freshwater catfish *Ictalurus punctatus*. J. Exp. Biol. 86: 171-185.

Brafield, A. E. 1985. Laboratory studies of energy budgets. In: Tytler, P. and Calow, P. (Ed), Fish Energetics: New Perspectives. Croom Helm, Beckenham, UK. pp. 257-281.

Chiu, Y. U., Austic, R. E. and Rumsey, G. L. 1984. Effect of dietary electrolytes and histidine on histidine metabolism and acid-base balance in rainbow trout (*Salmo gairdneri*). Comp. Biochem. Physiol. 78B: 777-783.

Dersjant-Li, Y., Verreth, J. A. J., Evers, F., Tijssen, P. A. T., Booms, R. and Verstegen, M. W. A. 1999. The influence of dietary cation-anion differences on acid-base balance, food intake, growth and nutrient utilisation of Juvenile African catfish *Clarias gariepinus* (Burchell). Fish Physiol. Biochem. 20: 305-311.

Dersjant-Li, Y., Verreth, J. A. J., Tijssen, P. A. T., Booms, R., Verstegen, M. W. A. and Huisman, E. A. 2000. Metabolic costs of changing the cation-anion difference in the diet of juvenile African catfish *Clarias gariepinus* (Burchell). Aquacult. Nutr. 6: 39-45.

Eddy, F. B. 1982. Osmotic and ionic regulation in captive fish with particular reference to salmonids. Comp. Biochem. Physiol. 73B: 125-141.

Eddy, F. B. and Bath, R. N. 1979. Ionic regulation in rainbow trout (*Salmo gairdneri)* adapted to fresh water and diluted sea water. J. Exp. Biol. 83: 181-192.

Fauchon, C., Seoane, J. R. and Bernier, J. F. 1995. Effects of dietary cation-anoin concentrations on performance and acid-base balance in growing lambs. Can. J. Anim. Sci.75: 145-151.

Golz, D. I. and Crenshaw, T. D. 1984. Interrelationships of dietary sodium, potassium and chloride in 5-10 kg pigs. J. Anim. Sci. 59 (suppl. 1): 268.

Heinsbroek, L. T. N., van Thoor, R. M. H. and Elizondo, L. J. 1990. The effect of feeding level on the apparent digestibilities of nutrients and energy of a reference diet for the European eel, *Anguilla anguilla* L. and the African catfish, *Clarias gariepinus* (Burchell). In: Takeda, M. and Watanabe, T. (Ed), The current Status of Fish Nutrition in Aquaculture. Toba, Japan, pp. 175-188.

ISO 5984. 1978. International Organization for Standardization. Animal feeding stuffs – Determination of crude ash. Genève, Switzerland.

ISO 5983. 1979. International Organization for Standardization. Animal feeding stuffs – Determination of nitrogen content and calculation of crude protein content. Genève, Switzerland.

ISO 6496. 1983. International Organization for Standardization. Animal feeding stuffs – Determination of moisture content. Genève, Switzerland.

ISO/DIS 6492. 1996. International Organization for Standardization. Animal feeding stuffs – Determination of fat content. Genève, Switzerland.

Johnson, R. J. and Karunajeewa, H. 1985. The effects of dietary minerals and electrolytes on the growth and physiology of the young chick. J. Nutr. 115: 1680-1690.

Lehninger, A. L. 1971. Bioenergetics. The molecular basis of biological energy transformations. Second edition. W.A. Benjamin, INC. Menlo Park, California. P246.

Rodehutscord, M. and Pfeffer, E. 1999. Maintenance requirement for digestible energy and efficiency of utilisation of digestible energy for retention in rainbow trout, *Oncorhynchus mykiss*. Aquaculture 179: 95-107.

SAS. 1990. SAS User's Guide: Statistics (Version 6 Ed.). SAS Inst. Cary, NC.

Shearer, K. D. 1988. Dietary potassium requirement of juvenile chinook salmon. Aquaculture. 73: 119-129.

Smith, N. F., Eddy, F. B. and Talbot, C. 1995. Effect of dietary salt load on transepithelial Na^+ exchange in freshwater rainbow trout (*Oncorhynchus mykiss)*. J. Exp. Biol. 198: 2359-2364.

Tacon, A. G. J. and De Silva, S. S. 1983. Mineral composition of some commercial fish feeds available in Europe. Aquaculture 31: 11-20.

Torreele, E., van der Sluiszen, A. and Verreth, J. 1993. The effect of dietary L-carnitine on the growth performance in fingerlings of the African catfish (*Clarias gariepinus*) in relation to dietary lipid. Br.J. Nutr. 69: 289-299.

Wilson, R. P., Cowey, C. B., Adron, J. W. 1985. Effect of dietary electrolyte balance on growth and acid-base balance in rainbow trout (*Salmo gairdnerii*). Comp. Biochem. Physiol. 82A: 257-260.

Zaugg, W. S. 1982. A simplified preparation for adenosine tripolyphosphate determination in gill tissue. Can. J. Fish. Aquat. Sci. 39: 215-217.

PART 2

The effect of dietary cation anion difference on energy metabolism in fish and pigs

CHAPTER 4

Metabolic costs of changing the cation-anion difference in the diet of juvenile African catfish *Clarias gariepinus* (Burchell).

Y. Dersjant-Li[1,2], J. A. J. Verreth[1], P. A. T. Tijssen[1], R. Booms[1], M. W. A. Verstegen[2] and E.A. Huisman[1]

[1] *Fish Culture and Fisheries Group,* [2] *Animal Nutrition Group, Department of Animal Sciences, Wageningen Agricultural University, P.O.Box 338, 6700 AH Wageningen, The Netherlands*

Published in: Aquaculture Nutrition (2000), 6: 39-45

Abstract: The influence of dietary cation anion difference (CAD, Na + K - Cl, mEq kg^{-1}) on energy metabolism and nitrogen losses in juvenile African catfish *Clarias gariepinus* (Burchell) was examined in fish exposed to different dietary CAD levels (-146, 116, 497, 713 and 828 mEq kg^{-1} diet). The experiment was conducted in open circuit balance respiration chambers over a 3-week period. Five 24-h monitoring periods were carried out at 3-day intervals during the experimental period with O_2 consumption, ammonia and nitrate + nitrite (NO_x) and CO_2 production being measured at 5-min intervals for each chamber. The negative dietary CAD (-146 mEq kg^{-1}) resulted in the highest energy expenditures (83 kJ $kg^{-0.8}$ d^{-1}). With increasing dietary CAD levels, heat loss gradually decreased to minimum values of 56 kJ $kg^{-0.8}$ d^{-1} at a dietary CAD level of 713 mEq kg^{-1}. Consequently metabolizable energy utilization efficiency (MEU, percentage of retained energy over metabolizable energy) quadratically ($p < 0.05$) increased and reached a maximum at a dietary CAD of 713 mEq kg^{-1}.

Keywords: African catfish, electrolyte balance, heat production, maintenance costs, metabolic expenditures, O_2 consumption

Introduction

Dersjant-Li *et al.* (1999) examined the growth response of the African catfish, *Clarias gariepinus* to dietary cation anion difference (CAD, Na + K - Cl, mEq kg^{-1}) varying from -100 to 700 mEq kg^{-1}. At high dietary CAD levels growth rate increased, mainly due to higher feed intake.

Dietary CAD is important for both pH and acid-base buffering capacity of a diet. Low dietary CAD gives feeds an acidic property while a high dietary CAD gives an alkaline one. The literature reports that increasing dietary CAD increased blood pH, HCO_3^- and base excess in many animal species (Chiu *et al.*, 1984, in fish; Ross *et al.*, 1994, in steers; Fauchon *et al.*, 1995, in lambs; Petience and Chaplin, 1997, in pigs). Changes in acid base balance may also have an effect on energy metabolism of an animal. Sub optimal dietary CAD may require more energy to correct pH in the stomach and small intestine and to maintain the acid base balance in other body compartments after feed intake (Ketelaars and Tolkamp, 1991). More substrates have to be oxidized to meet this higher energy requirement, resulting in a higher heat production.

To test this hypothesis an experiment was conducted to determine the effect of dietary CAD levels on heat production and nitrogen loss of African catfish, *Clarias gariepinus*.

Materials and methods

Fish and facilities

The experiment was conducted at the Wageningen University, the Netherlands, using cultivated juvenile African catfish *Clarias gariepinus*. The fish were adapted to the experimental conditions for two weeks prior to the experiments. During the first week the fish were fed a commercial diet (9115, PROVIMI BV, Rotterdam, The Netherlands, with 52% crude protein and 13% crude fat) and during the second week they were adapted to the experimental diets.

The experiment was conducted in open circuit respiration chambers as described by Hogendoorn *et al.* (1981) and Heinsbroek *et al.* (1991), and lasted 3 weeks. The facilities allow the measurements of O_2 consumption, ammonia and nitrate + nitrite (NO_x) and CO_2 production. At the start of the experiment the fish were size sorted and distributed at random among five 140L respirometer chambers. Forty-five fish with an initial body weight of 42.1 ±

1.3 gram (mean ± sd) were allocated to each chamber. Five dietary treatments were randomly assigned to five chambers, using repeated measurement as replicates. Water temperature was maintained at about 27°C, light - dark cycle was 12 h:12 h. Dissolved oxygen was above 7-8 mg L^{-1} in inlet water and remained above 3-4 mg L^{-1} in outlet water.

Diet and feeding

The ingredients for the basal diet were provided by PROVIMI BV, in the form of a pre-made mix (9031). Upon arrival at the Feed Processing Central of TNO-Nutrition and Food Research Institute, a mixture of minerals (NH_4Cl, $CaCl_2$ and $NaHCO_3$ alternately) was added to the ingredients mix to formulate the five diets with different CADs (-100, 100, 500, 700 and 900 mEq kg^{-1}) levels. To avoid biased results because of varying Ca levels, the relative concentration of Ca in each diet was set at 2% by adding appropriate amounts of $CaCO_3$ to the respective ingredient/mineral mixes. Subsequently, the diets were pelleted at the Feed Processing Central of TNO. After pelleting, the diets were oil coated (3%) at the Wageningen Feed Processing Centre, Wageningen University. In the course of the experiment, feed samples were taken at regular time intervals and pooled per diet. From each pooled diet sample the proximate composition was analyzed. CAD levels were calculated according to the analyzed Na, K and Cl concentrations in the diets (Table 1).

Daily feed rations were calculated by estimating changes in body size assuming a feed conversion of 0.9, as obtained during the pre-experimental period. Feed was provided at a restricted level of 27 g $kg^{-0.8}$ (body weight, BW) per day to assure equal gross energy provision. This level was equivalent to about 75-90% of voluntary intake levels of the same diets determined in a previous experiment (Dersjant-Li *et al.*, 1999). Feed was given continuously for 24 h by an automatic belt feeder. When uneaten feed pellets appeared on the fecal collector (checked twice a day), they were collected and their estimated dry weight was subtracted from dry matter feed intake.

Sampling and measurement

Five 24-h monitoring periods were carried out at 3-day intervals with O_2 consumption, ammonia and nitrate + nitrite (NO_x) and CO_2 production being measured at 5-min intervals for each chamber using an autoanalyser (Alpkem RFA/2 TM, Alpkem Corporation, Clackamas, OR, USA) as described by Heinsbroek *et al.* (1991).

Table 1. Chemical analysis of the test diets as fed[1]

	CAD (mEq kg^{-1})				
	-100	100	500	700	900
Dry matter (g kg^{-1})	929	934	930	932	925
Crude protein (g kg^{-1})	466	467	469	472	466
Crude fat (g kg^{-1})	114	115	114	116	136
Crude fiber (g kg^{-1})	16	15	13	16	13
Ash (g kg^{-1})	114	112	121	124	132
Ca (g kg^{-1})	26.9	27.2	25.2	22.1	21.4
P (g kg^{-1})	13.6	13.6	13.7	12.8	13.1
Na (g kg^{-1})	6.0	5.8	13.2	17.3	19.5
K (g kg^{-1})	6.2	6.0	7.3	8.7	9.0
Cl (g kg^{-1})	20.0	10.3	9.3	9.3	8.8
Gross energy (kJ g^{-1})	19.52	19.60	19.42	19.33	19.43
Measured CAD (mEq kg^{-1})[2]	-146	116	497	713	828

[1] Formulation and chemical analysis of the test diets were done by Provimi BV., Rotterdam, the Netherlands. [2] CAD was calculated based on Na, K and Cl concentrations.

At the beginning of the experiment and the beginning of each week 10 fish were sampled from each chamber for body analysis. Feces were collected twice daily. Fecal samples were pooled per chamber per week, freeze dried and analyzed for dry matter, ash, energy and nitrogen content. Dry matter and ash were measured according to the procedure as described by Henken *et al.* (1986). Nitrogen content of feed, fish body and feces were analyzed by the Kjeldahl method and energy concentration was determined by bomb-calorimetry (IKA Calorimeter C 7000, Fa. IKA-Analysentechnik, Weitersheim, Germany).

Feed samples were analyzed in the laboratory of PROVIMI BV. Dry matter was done by drying the samples for 4 h at 103 °C (ISO 6496/Netherlands Normalization Institute (NEN) 3332). Crude protein was measured by the Dumas combustion method (ISO/CD 15670). Crude fat was done by Soxhlet extraction (EEG 18.1.84 No 15/29-30). Crude fiber was measured by Gravimetry (ISO 5498/NEN 5415). Ash was done by ashing the samples for 4 h at 550 °C (ISO 5484/NEN 3329/NEN 6327). Ca was measured using atomic absorption spectrometry (ISO 6490-2), P was measured using spectrophotometry (ISO 6491). Na and K

were measured using atomic emission spectrometry (Prop. NEN 3349). Cl^- was measured by titrimetry (ISO 6495).

Data analysis

Growth and intake were calculated on a per week basis with results expressed as g per kg metabolic weight (g $kg^{-0.8}$ BW), using 0.8 as metabolic coefficient (Winberg, 1956), which is also valid for African catfish (Heinsbroek, 1987). The results of three weeks were averaged and only the means were presented.

O_2 consumption was calculated as:

$$O_{2con} = (C_{in}-C_{out})\ FL/N \qquad (1)$$

With O_{2con} is oxygen consumption (g $fish^{-1}$ d^{-1}), C_{in} is the oxygen concentration of inlet water (g L^{-1}) and C_{out} is the oxygen concentration (g L^{-1}) of outlet water, FL is water flow rate (L d^{-1}) and N is number of fish per chamber. CO_2 production and ammonia and nitrate + nitrite (NOx) excretion were also calculated in the same way. *Clarias spp.*, being air breathers, may also consume oxygen from air. Oxygen consumption from air above the water phase was calculated from the data on oxygen concentration of air inlet and outlet samples and air flow rate, in a manner similar to calculation of water oxygen consumption. The calculations were corrected for concentration differences in the chamber at the beginning and the end of the monitoring period (24 h).

The nitrogen and energy balances were calculated as described by Heinsbroek *et al.* (1990). Retained nitrogen (RN) and retained energy (RE) were calculated as:

$$RN\ (RE) = Wt \times Ct - Wo \times Co \qquad (2)$$

With Wt and Wo being the final and initial body weight of fish respectively while Co and Ct, respectively, represent the nitrogen/energy content of the fish body at the beginning and the end of the experimental period.

Metabolizable energy (ME) was calculated as the sum of the retained energy (RE) and the heat production (H). Metabolizable energy utilization (MEU) was calculated as percentage of RE over ME. Branchial and urinary nitrogen loss (BUN) was derived from ammonia and nitrate + nitrite (NO_x) excretion data. Branchial and urinary energy (BUE) was calculated as BUN multiplied by 24.85 kJ g N^{-1} (Cho and Kaushik, 1985; Heinsbroek, 1987).

Retained protein energy (kJ $kg^{-0.8}$ d^{-1}) was calculated as retained protein (retained N × 6.25) multiplied by 23.6 kJ g $protein^{-1}$ (Brafield, 1985). Retained energy as lipid (kJ $kg^{-0.8}$ d^{-1}) was calculated as total retained energy minus retained energy as protein, assuming total energy equal to protein plus lipid.

Energy costs for maintenance was estimated indirectly. Literature data (Wieser, 1994; Hogendoorn, 1983) gave a value (0.81) for the net growth efficiency (Kg = RE/MEp, in which RE is retained energy and MEp is metabolizable energy for production, see equation 4) for African catfish. This enable separates the metabolizable energy available for production (MEp) and the part available for maintenance (MEm). Subsequently the energy expenditure was divided over the part associated with growth (Hg) and the part used for maintenance (Hm). The procedure followed the following calculations:

$$ME = RE + H \quad (3)$$

$$Kg = RE / MEp \quad \text{or} \quad MEp = RE / Kg \quad (4)$$

$$MEp = RE + Hg \quad \text{or} \quad Hg = MEp - RE \quad (5)$$

$$H = Hm + Hg \quad \text{or} \quad Hm = H - Hg \quad (6)$$

Where ME = metabolizable energy intake (kJ $kg^{-0.8}$ d^{-1}) (calculated), RE = retained energy (kJ $kg^{-0.8}$ d^{-1}) (measured), MEp = ME requirement for production (kJ $kg^{-0.8}$ d^{-1}), H = total metabolic expenditures (kJ $kg^{-0.8}$ d^{-1}) (measured), MEm, Hm = metabolic expenditures for maintenance (kJ $kg^{-0.8}$ d^{-1}), Hg = metabolic expenditures for growth (kJ $kg^{-0.8}$ d^{-1}), and Kg = net growth efficiency (from Wieser, 1994; Hogendoorn, 1983).

The respiratory quotient (RQ) was calculated as CO_2 produced (mmol L^{-1}) over O_2 consumed (mmol L^{-1}). Heat production (H) was calculated by 1), using an oxycaloric equivalent of 13.6 kJ g O_2^{-1} (Heinsbroek *et al.*, 1990) and 2), from the equation of Brafield (1985):

$$H = 11.18\ O_2 + 2.61\ CO_2 - 9.55\ NH_3 \quad (7)$$

Where H is the total heat production (J) and where O_2 consumed and CO_2 and NH_3 produced are expressed in mg. The protein oxidization was estimated as ammonia nitrogen excreted multiplied by 6.25 (Van Waversveld *et al.*, 1988). The quantity of oxidized substrates was calculated according to the equations described by Van Waversveld *et al.* (1988), based on the O_2 consumption, CO_2 and nitrogen excretion data.

Statistical analysis

Curves were fitted using the nonlinear regression algorithm procedures from the NONLIN package (Method Levenberg-Marquadt, (Dennis *et al.*, 1981), convergence criterion 10^{-10}). Treatment means were used for curve fitting.

Results

O_2 consumption and CO_2 production

O_2 consumption tended ($p = 0.10$) to decrease quadratically with increasing dietary CAD. CO_2 production changed in a similar manner. The lowest O_2 consumption was observed (3.84g $kg^{-0.8}$ d^{-1}) at a dietary CAD level of 713 mEq kg^{-1}. The lowest CO_2 production was found (5.89g $kg^{-0.8}$ d^{-1}) at a dietary CAD level of 497 mEq kg^{-1} (Figure 1).

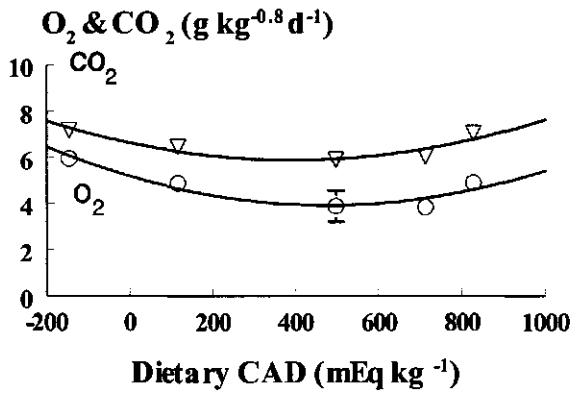

Figure 1. O_2 (o) consumption and CO_2 (∇) production (g $kg^{-0.8}$ d^{-1}) in relation to dietary CAD levels. Error bars indicate the SEM of five repeated measurements during a 3-week period.

Food consumption and growth

Feed intake remained similar and varied around 25g $kg^{-0.8}$ d^{-1} for each treatment (see Table 2). The group receiving a diet with negative CAD (-146 mEq kg^{-1}) showed a lower growth rate than the groups receiving positive dietary CAD's (ranging from 116 to 828 mEq kg^{-1}). Among the latter groups growth rates were comparable (Table 2).

Table 2. Feed consumption and growth (mean ± SEM, n = 3) in each treatment

	Dietary CAD (mEq kg^{-1})				
	-146	116	497	713	828
Growth (g $kg^{-0.8}$ d^{-1})	27.9 ± 2.08	31.5 ± 3.08	29.8 ± 2.35	31.5 ± 0.90	30.7 ± 1.18
Feed intake (g $kg^{-0.8}$ d^{-1})	25.1 ± 1.10	25.2 ± 0.44	25.1 ± 0.81	25.5 ± 0.37	25.2 ± 0.49
Feed conversion[1]	0.91 ± 0.04	0.82 ± 0.08	0.86 ± 0.05	0.81 ± 0.01	0.82 ± 0.02

[1] Feed conversion is defined as wet weight gain: weight of feed administered.

Body and fecal composition

The nitrogen content of the fish body increased curvilinearly with increasing dietary CAD. The highest nitrogen content (96.5 g kg^{-1} dry body weight) was obtained at a dietary CAD of 713 mEq kg^{-1}. Ash and dry matter in the body decreased with increasing dietary CAD, however, these changes were not significant. Fecal nitrogen and energy losses were higher in the group receiving a low CAD diet (-146 mEq kg^{-1}) than in the other groups (Table 3).

Table 3. Body and fecal composition (mean ± SEM, n = 3) in each treatment group

	Dietary CAD (mEq kg^{-1})				
	-146	116	497	713	828
Body composition					
Dry matter (g kg^{-1})	256 ± 3.0	251 ± 5.5	249 ± 3.4	246 ± 5.0	252 ± 5.1
Ash (g kg^{-1} DM)	100 ± 1.0	101 ± 1.8	96 ± 1.4	97 ± 1.3	96 ± 1.4
Nitrogen (g kg^{-1} DM)	94.0 ± 1.07	95.8 ± 1.23	96.1 ± 0.81	96.5 ± 1.20	94.5 ± 1.42
Gross energy (kJ g^{-1} DM)	25.0 ± 0.08	25.1 ± 0.25	25.2 ± 0.08	25.0 ± 0.02	25.5 ± 0.18
Fecal composition					
Ash (g kg^{-1} DM)	244	280	317	240	336
Fecal N (g kg^{-1} DM)	59.3	46.6	47.9	48.7	50.7
Fecal energy (kJ g^{-1} DM)	17.1	15.7	15.5	15.9	14.8

Energy and nitrogen utilization

Compared with the other treatment groups, retained nitrogen (RN) and retained energy (RE) was low at a dietary CAD level of -146 mEq kg^{-1} (Table 4). Metabolizable energy utilization efficiency (MEU, RE/ME × 100%) increased quadratically ($P < 0.05$, $R^2 = 0.95$) and reached a maximum at a dietary CAD level of 713 mEq kg^{-1} (Figure 2). The heat loss was lowest at the latter dietary CAD level. In the group fed at a CAD level of -146 mEq kg^{-1}, heat loss was approximately 1.5 times higher than in the group receiving a diet with a CAD level of 713 mEq kg^{-1}.

Table 4. Nitrogen and energy utilization in relation to dietary CAD (no significant differences were found in different treatment groups)

	Dietary CAD (mEq kg^{-1})				
	-146	116	497	713	828
Nitrogen utilization (g $kg^{-0.8}$ d^{-1})					
GN (gross nitrogen intake)	1.84±0.08	1.85±0.03	1.84±0.06	1.87±0.03	1.83±0.04
RN (retained nitrogen)	0.69±0.06	0.79±0.08	0.70±0.08	0.77±0.05	0.73±0.08
BUN (branchial and urinary N)	0.26	0.29	0.29	0.28	0.30
Energy utilization (kJ $kg^{-0.8}$ d^{-1})					
GE (gross energy intake)	491±21.5	496±8.59	490±15.8	496±7.20	490±9.43
ME (metabolizable energy)[1]	269	293	247	263	286
RE (retained energy)	186±21.9	224±21.3	191±15.8	207±27.7	216±27.8
BUE (branchial and urinary energy)	6.5	7.1	7.2	7.0	7.4
H (heat production)[2]	80.9	66.5	52.9	52.2	66.6
H[3]	82.5	68.5	55.9	55.7	70
Retained energy as protein (REp)	102	117	104	114	108
Retained energy as lipid[4]	84	107	87	93	108

[1] ME is calculated as RE + H[3]. [2] Using an oxycaloric equivalent of 13.6 kJ g O_2^{-1} (Heinsbroek *et al.*, 1990). [3] From the equation of Brafield (1985), i.e. $H = 11.18\ O_2 + 2.61\ CO_2 - 9.55\ NH_3$, where H is the total heat production (in joules) and where O_2 consumed and CO_2 and NH_3 produced are expressed in milligrammes. [4] Estimated as RE – REp.

Heat production responded to changing dietary CAD in a quadratic manner but the relation was not significant. From curve fitting the minimal heat loss and maximal MEU were

estimated at a dietary CAD level of 460 mEq kg^{-1}.

Respiratory quotient (RQ) and substrate oxidization

As shown in Table 5, fish receiving a diet with CAD of -146 mEq kg^{-1} produced a lower respiratory quotient (RQ) than the other groups. Protein oxidization rate (mg g^{-1} d^{-1}) was low and fat oxidization rate was high in this group. The RQ value was highest at a dietary CAD level of 713 mEq kg^{-1}. At dietary CAD levels between 497 to 828 mEq kg^{-1} a negative value of fat oxidization was found, indicating that, in these groups, fat was deposited.

Table 5. Respiratory quotient and quantity of substrates oxidized per fish per day

	Dietary CAD (mEq kg^{-1})				
	-146	116	497	713	828
Respiratory quotient (RQ)	0.88	0.96	1.10	1.14	1.05
Substrates oxidized:					
Protein (mg $fish^{-1} \cdot d^{-1}$)	196.9	227.1	224.1	216.1	233.0
Fat (mg $fish^{-1} \cdot d^{-1}$)	132.1	60.5	-62.4	-50.1	-8.44
Carbohydrates (mg $fish^{-1} \cdot d^{-1}$)	68.2	121.4	287.5	292.4	286.7

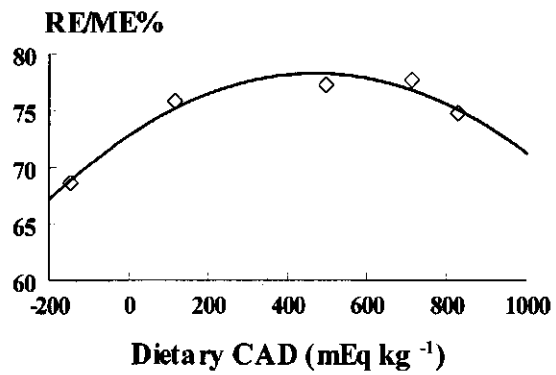

Figure 2. Metabolizable energy utilization efficiency in relation to dietary CAD levels. ME, metabolizable energy; RE, retained energy.

Discussion

Owing to incomplete fecal collection (catfish produce very soluble feces; personal observation), it was not possible to estimate digestibility coefficients.

Feed was provided at a calculated ration of 27g $kg^{-0.8}$ d^{-1}, however the feed intake was around 25g $kg^{-0.8}$ d^{-1}. This is possibly related to the feed conversion of 0.9, which was used to estimate changes in body size for calculating feed ration, while the actual feed conversion was about 0.84.

We expected a quadratic response of growth and feed conversion to dietary CAD levels. At a deviating (too low or too high) dietary CAD, growth should be lower than at optimal dietary CAD. The present study revealed that a dietary CAD level of -146 mEq kg^{-1} resulted in poor growth performance of juvenile African catfish. At this CAD level, growth rate, nitrogen and energy utilization were low while fecal and heat losses were high. When dietary CAD ranged from 116 to 828 mEq kg^{-1}, juvenile African catfish showed higher growth than in animals receiving a negative dietary CAD (-146 mEq kg^{-1}). At dietary CAD level of 828 mEq kg^{-1} growth rate decreased slightly compared with CAD level of 713 mEq kg^{-1}, but this difference is not statistically significant. This fish species apparently can cope with high dietary CAD, although the data also suggest that CAD level above 800 mEq kg^{-1} may become slightly too high.

In the present study, metabolizable energy (ME) was estimated as the sum of retained energy (RE) and heat energy (H). Both (RE) and (H) are measured in each group, thus this is a reliable approach. The other approach by estimating ME from GE – FE - BUE is much less precise because the measurements of the components (especially FE) have more analytical error than the RE and H measurements. RE is easy to measure and based on the proximate analysis of body samples. Previous studies have also shown that the measurements on heat production are precise (a CV of 4% for air oxygen measurements and 2% for water oxygen measurements; Moreau *et al.*, 1991). The approach presented is corroborated by the views of Huisman (1974) and the results of balance studies in *Clarias gariepinus* by Heinsbroek *et al.* (1990).

The results revealed a clear relationship between metabolizable energy utilization efficiency (MEU) and dietary CAD. The highest MEU was found at a dietary CAD level of 713 mEq kg^{-1}. This might be related to low metabolic expenditures in this dietary group.

As shown in Table 4, the estimated H from the two different methods is rather similar.

However the values estimated from the Brafield (1985) equation, based on O_2 consumption, CO_2 production and NH_4 excretion, were higher than the values estimated from O_{ox}. This difference is more pronounced in the higher CAD groups, indicating that the ratio of oxidized substrates (i.e. protein, fat and carbohydrates) changed in different CAD groups. As a consequence using a general value of O_{ox} (as was done in the first method) may lead to (small) errors. The H values calculated according to Brafield (1985) are closer to the experimental results and will be used in further discussion. At dietary CAD levels between 497 and 828 mEq kg^{-1}, the high RQ values indicated that more carbohydrates were oxidized. The O_{ox} value of carbohydrates (14.76 kJ g O_2^{-1}) is higher than of protein (13.36 kJ g O_2^{-1} if NH_4 is produced) and of fat (13.71 kJ g O_2^{-1}) (Brafield, 1985). This might explain why, when using an O_{ox} of 13.6 kJ g O_2^{-1}, low values of H were obtained in the high CAD groups.

In the present study metabolic expenditures of 56 kJ $kg^{-0.8}$ d^{-1} were obtained at dietary CAD levels of 497 and 713 mEq kg^{-1}. This is comparable to the results reported by Heinsbroek *et al.* (1990). They found a metabolic expenditure of 45.9 kJ $kg^{-0.8}$ d^{-1} for juvenile African catfish *Clarias gariepinus* fed at 15 g $kg^{-0.8}$ d^{-1}. However the metabolic expenditures in the dietary CAD of -146 mEq kg^{-1} group (82 kJ $kg^{-0.8}$ d^{-1}) were considerably higher (approximately 1.5 times higher) than these values. This indicated that the fish that received the negative dietary CAD used more energy to maintain homeostasis than the other groups. If this is so, this could explain the poor performance of this group.

Maintenance costs were estimated using equations 4-6. Some of the assumptions associated with this approach can be criticized, e.g., net growth efficiency Kg. However it is believed that the results give a valuable picture of the relative maintenance costs when the different CAD groups are compared one to another. The lowest Hm (about 7 kJ $kg^{-0.8}$ d^{-1}) was found at a CAD level of 713 mEq kg^{-1}, while at a CAD level of –146 mEq kg^{-1}, Hm reached 39 kJ $kg^{-0.8}$ d^{-1}. This means that fish receiving the diet with negative CAD (–146 mEq kg^{-1}) needed 5 times more energy to maintain homeostasis than the fish fed with a diet of 713 mEq kg^{-1} CAD (Table 6).

The changes in nitrogen utilization at different CAD levels demonstrate that dietary CAD may also influence protein metabolism. Chiu *et al.* (1988) observed an increase in arginine requirement in rainbow trout *Salmo gairdneri* with an increase in dietary CAD level.

At a dietary CAD level of -146 mEq kg^{-1}, a low RQ value indicated that more fat was oxidized for energy. The RQ values above 1, as found at dietary CAD levels between 497 to

828 mEq kg^{-1}, suggest that animals synthesized lipid from carbohydrates (Chwalibog *et al.*, 1992) (Table 5). It can be concluded that an optimal dietary CAD may reduce the maintenance costs and thereby improve the performance of fish.

Table 6. Maintenance energy costs (Hm, kJ $kg^{-0.8}$ d^{-1}) at different dietary CAD levels. By subtracting Hm of the group receiving a CAD of 713 mEq kg^{-1}, dHm was obtained. This difference (dHm) gives the extra cost for maintaining homeostasis in all test groups when compared to the reference group receiving a dietary CAD of 713 mEq kg^{-1}

	Dietary CAD (mEq kg^{-1})				
	-146	116	497	713	828
Hm kJ $kg^{-0.8}$ d^{-1}	39	15.97	11.24	7.19	19.30
DHm kJ $kg^{-0.8}$ d^{-1}	31.82	8.78	4.05	0	12.11

Acknowledgements

The first author was financially supported by a fellowship from the feed company PROVIMI BV, Rotterdam, the Netherlands and from Finnfeeds International Ltd. Marlborough, UK. The authors are grateful to E. Eding, S. Leenstra, A. Hutten and F. Dersjant for their help in system management and sampling. Mr. P. Roeleveld and his colleagues from ILOB-TNO helped in processing the pellets. Dr. Th. van de Poel and ing. T. Zandstra are thanked for oil coating of the pellets.

References

Brafield, A. E. 1985. Laboratory studies of energy budgets. In: Tytler, P. and Calow, P. (Ed), Fish Energetics: New Perspectives. Croom Helm, Beckenham, UK. pp. 257-281.

Chiu, Y.U., Austic, R.E. and Rumsey, G.L. 1984. Effect of dietary electrolytes and histidine on histidine metabolism and acid-base balance in rainbow trout (*Salmo gairdneri*). Comp. Biochem. Physiol. 78B: 777-783.

Chiu, Y.U., Austic, R.E. and Rumsey, G.L. 1988. Effect of feeding level and dietary electrolytes on the arginine requirement of rainbow trout (*Salmo gairdneri*). Aquaculture 69: 79-91.

Cho, C.Y. and Kaushik, S.J. 1985. Effects of protein intake on metabolizable and net energy values of fish diets. In: Cowey, C.B., Mackie, A.M. and Bell, J.G. (Ed), Nutrition and Feeding in Fish. Academic Press, London, UK. pp. 95 – 117.

Chwalibog, A., Jakobsen, K., Henckel, S. and Thorbek, G. 1992. Estimation of quantitative oxidation and fat

retention from carbohydrate, protein and fat in growing pigs. J. Anim. Physiol. Anim. Nutr. 68: 123-135.

Dennis, J.E., Gay, D.M. and Welsch, R.E. 1981. An adaptive nonlinear least - squares algorithm. ACM. Trans. Math. Softw., 7,3.

Dersjant-Li, Y., Verreth, J.A.J., Evers, F., Tijssen, P.A.T., Booms, R. and Verstegen, M. W. A. 1999. The influence of dietary cation-anion differences on acid-base balance, food intake, growth and nutrient utilisation of juvenile African catfish *Clarias gariepinus* (Burchell). Fish Physiol. Biochem., 20: 305-311.

Fauchon, C., Seoane, J.R. and Bernier, J.F. 1995. Effects of dietary cation-anion concentrations on performance and acid-base balance in growing lambs. Can. J. Anim. Sci. 75: 145-151.

Heisbroek, L.T.N. 1987. Effects of body weight, feeding level and temperature on energy metabolism and growth in fish. In: Verstegen, M.W.A. and Henken, A.M. (Ed), Energy Metabolism in Farm Animals. Martinus Nijhoff Publisher, Dordrecht, The Netherlands. pp. 478-500.

Heinsbroek, L.T.N., van Thoor, R.M.H. and Elizondo, L.J. 1990. The effect of feeding level on the apparent digestibilities of nutrients and energy of a reference diet for the European eel, *Anguilla anguilla* L. and the African catfish, *Clarias gariepinus* (Burchell). In: Takeda, M. and Watanabe, T. (Ed), The Current Status of Fish Nutrition in Aquaculture. Toba: Japan. pp. 175-188.

Heinsbroek, L.T.N., Tijssen, P.A.T., Flach, R.B. and de Jong, G.D.C. 1991. Energy and nitrogen balance studies in fish. In: Kaushik, S.J. and Luquet, P. (Ed), Fish Nutrition in Practice. Biarritz: France. pp. 375-389.

Henken, A.M., Lucas, H., Tijssen, P.A.T. and Machiels, M.A.M. 1986. A comparison between methods used to determine the energy content of feed, fish and faeces samples. Aquaculture 58: 195-201.

Hogendoorn, H. 1983. Growth and production of the African catfish, *Clarias lazera* (C. & V.) III. Bioenergetic relations of body weight and feeding level. Aquaculture 35: 1-17.

Hogendoorn, H., Van Korlaar, F. and Bosch, H. 1981. An open circuit balance respirometer for bioenergetic studies of fish growth. Aquaculture 26: 183-187.

Huisman, E.A. 1974. Optimalisering van de groei bij de karper *Cyprinus carpio L.* Ph.D dissertation, Wageningen University, Wageningen, The Netherlands.

Ketelaars, J.J.M.H. and Tolkamp, B.J. 1991. Toward a new theory of feed intake regulation in ruminants. Ph.D dissertation, Wageningen University, Wageningen, the Netherlands.

Moreau, Y., Medale, F., Boujard, T. and Luquet, P. 1991. Indirect calorimetry method to study energy metabolism of an air-breathing fish, *Hoplosternum littorale* (Siluriform, callichthydae). J. Aqua. Trop. 6: 193-199.

Patience, J. F. and Chaplin, R. K. 1997. The relationship among dietary undetermined anion, acid-base balance, and nutrient metabolism in swine. J. Anim. Sci. 75: 2445-2452.

Ross, J.G., Spears, J.W. and Garlich, J.D. 1994. Dietary electrolyte balance effects on performance and metabolic characteristics in growing steers. J. Anim. Sci. 72: 1842-1848.

Van Waversveld, J., Addink, A.D.F., Van den Thillart, G. and Smit, H. 1988. Anaerobic heat production measurements: a new perspective. J. Exp. Biol. 138: 529-533.

Wieser, W. 1994. Cost of growth in cells and organisms: general rules and comparative aspects. Biol. Rev. 68:

1-33.

Winberg, G.G. 1956. Rate of metabolism and food requirements of fish. Res. Board Can. Transl. Ser. 194.

CHAPTER 5

Effect of dietary cation anion difference on metabolic rate and energy balance in pigs

Yueming Dersjant-Li[1 3], Johan W. Schrama[2], Marcel J. W. Heetkamp[2], Johan A. Verreth[3] and Martin W. A. Verstegen[1]

[1] *Animal Nutrition Group,* [2] *Adaptation Physiology Group,* [3] *Fish Culture and Fisheries Group, Wageningen Institute of Animal Sciences, Wageningen University, Wageningen, The Netherlands*

Submitted to Journal of Animal Science

Abstract: The effect of two dietary cation-anion difference (CAD, Na + K - Cl, mEq kg^{-1}) levels (-100 and 200 mEq kg^{-1} diet) on heat production, energy and nitrogen retention in piglets was assessed. The experiment consisted of an 8-d adaptation period in cages and two balance study periods of 6 and 7 days, respectively, in two open-circuit climate respiration chambers. Nine groups of three (4-wk old) crossbred borrows were assigned to the one of two diets (5 and 4 groups for –100 and 200 mEq kg^{-1} CAD diets respectively). During the adaptation period, piglets had *ad libitum* access to feed. In the balance study period, diets were provided at 2.3 times the energy requirement for maintenance twice daily. Total heat production for each group was determined every 9 minutes from the exchange of CO_2 and O_2. Feces and urine mixture was quantitatively collected in each balance period to measure energy and nitrogen balance. During the adaptation period, weight gain was numerically higher ($p > 0.10$) in the 200 mEq kg^{-1} CAD group (113 g pig^{-1} d^{-1}) compared to the –100 mEq kg^{-1} CAD group (74 g pig^{-1} d^{-1}). In the first balance study period, energy and nitrogen balance were similar in the two CAD groups. In the second balance study period, however, total heat production and metabolizable energy costs for maintenance tended ($p < 0.10$) to be higher in the 200 mEq kg^{-1} CAD group (681 and 443 kJ $kg^{-0.75}$ d^{-1} respectively) than in the –100 mEq kg^{-1} CAD group (660 and 412 kJ $kg^{-0.75}$ d^{-1} respectively). Differences in total heat production between two CAD groups mainly occurred in the light period ($p < 0.01$) and were not related to physical activity. The respiratory quotient (RQ) and energy retention as fat were numerically lower in the 200 mEq kg^{-1} CAD group compared to –100 mEq kg^{-1} CAD group. In conclusion, energy balances were similar in both treatments. However in the light period, piglets needed more energy for maintenance after ingesting a diet with a CAD level of 200 mEq kg^{-1} compared to a diet with a CAD level of –100 mEq kg^{-1} at a restricted feeding level.

Keywords: energy metabolism, electrolyte balance, heat production, maintenance requirements, piglets

Introduction

Literature showed that a low (negative) dietary cation anion difference (CAD, Na + K - Cl mEq kg^{-1}) can increase plasma Cl^- concentration, cause metabolic acidosis and consequently reduce feed intake and nutrients utilization of pigs (Yen *et al.*, 1981; Patience and Wolynetz, 1990; Park *et al.*, 1994). Haydon and West (1990) observed that in growing pigs N retention as percentage of N intake increased linearly with CAD level from –50 to 400 mEq kg^{-1}. In a previous study with African catfish, a dietary CAD level of –100 mEq kg^{-1} increased energy requirement for maintenance (Dersjant-Li *et al.*, 2000). However whether dietary CAD affect maintenance energy requirement in pigs so far is not being studied. Several studies showed that increasing dietary CAD improved feed efficiency in pigs (Patience and Wolynetz, 1990; Park *et al.*, 1994). This may suggest that dietary CAD can also influence energy metabolism in pigs. The hypothesis of this study is that an undesired dietary CAD will result in high energy costs for acid base balance regulation (i.e. maintenance of homeostasis). An optimal dietary CAD would reduce these costs and thereby improving food consumption of animals. The objective of this study was to assess the effect of dietary CAD on energy partitioning in young pigs.

Materials and methods

Experimental procedure

The experiment was conducted using two open-circuit climate respiration chambers (1.0m × 0.8m × 0.97m in length × width × height) as described by Verstegen *et al.* (1987). The experiment was carried out in 5 trials. Each trial consisted of an 8-d of adaptation period in cages outside the respiration chambers, followed by two consecutive balance periods of 6 and 7 days, respectively, inside the respiration chambers. Each group of 3 pigs was used as one experimental unit. Two dietary CAD treatments (–100 and 200 mEq kg^{-1} diet) were tested in 10 groups of pigs. In each trial two groups of pigs were allocated to one of the two treatment diets respectively. In trial 1, however, one pig was sick (in 200 mEq kg^{-1} CAD group) and was removed during the second balance period, therefore this group was excluded. Thus 9 groups of pigs were used for data analysis.

Diets, animals, housing and feeding

The basal diet consisted of barley, wheat, rye and soybean isolate. This basal diet had the same composition as the diet used in the growth trial of a previous study (Dersjant-Li *et al.*, 1998). The basal diet was supplemented with $CaCl_2.2H_2O$ and $NaHCO_3$ alternately to formulate the treatment diets with a CAD level of –100 and 200 mEq kg^{-1} respectively. $CaCO_3$ was added to maintain equal Ca levels and diamol was used to maintain ash content constant in the two diets (Table 1).

Thirty weaning barrows (F1 of female of Hypor × male of Piětrain, Hypor is offspring of female of [(Large white × Hampshire) boar line × (Dutch Landrace × Swedish Landrace) sow line] and male of Dutch York)) at age of about 4 weeks and with a mean body weight of 6.2 ± 0.66 kg (mean ± sd) were used. Animals were obtained from a commercial farm immediately after weaning. Upon arrival the pigs were randomized into two groups and allocated to two cages, in the experimental facility of Wageningen University, the Netherlands. The experimental diets were provided starting from the adaptation period. For the first 7 days of adaptation the pigs had *ad libitum* access to feed and water. At day 8 (one day before transferring to the chambers) the pigs were weighed and the feeding level was changed to 2.3 times the energy requirement for maintenance (assumed 458 kJ of metabolizable energy $kg^{-0.75}$ d^{-1}, NRC, 1988), given in two meals a day. Also during the subsequent balance periods in the respiration chambers, pigs were fed at 2.3 times the energy requirement for maintenance, 2 times a day (7.50 and 15.50 h). The ration was equally distributed in two meals. Water was freely available through two single-nipple drinkers. In the respiration chambers, the temperature was kept at 26°C on days 0-3; 25°C on days 3-6; 24°C on days 6-13. The relative humidity maintained at 65-70%. Air velocity was less than 0.20 m/s. A light dark cycle was controlled at 12L : 12D, with lights on from 0700 to 1900 h.

Sampling and measurements

Pigs were weighed upon arrival, day 8 and day 9 (start of the first balance period), day 14 (end of the first balance period and start of the second balance period) and day 21 (end of second balance period). Feed intake was determined from feed allowance and corrected for feed residuals. In the first balance period feed refusal occurred. In the second balance period no feed residuals were found. Feces and urine mixture was collected quantitatively from each chamber in each balance period. Fecal and urinary mixed samples were taken to determine the nitrogen and energy balance. Total heat production (Htot) for each group was determined

every 9 min from measurement of exchange of CO_2 and O_2 (Verstegen *et al.*, 1987) continuously throughout the experiment. The metabolizable energy intake (ME) was calculated from the energy content of feed, feces and urine. Energy retention (ER), energy retained as protein (ER_p) and energy retained as fat (ER_f) were calculated for each group in each balance period, as described by Henken *et al.* (1991). The ME required for maintenance (MEm) was calculated as described by Gentry *et al.* (1997), according to the equation:

$$MEm = ME - (ER_p / 0.54) - (ER_f / 0.74) \quad (1)$$

The values of 0.54 and 0.74 were used as the efficiency of utilization of ME for protein and fat retention respectively (ARC, 1981).

Physical activity was continuously monitored by Doppler-radar activity meters (Radar MD5; Suther, Vierpool, Amsterdam, The Netherlands) (Verstegen *et al.*, 1987) and recorded for the same intervals as Htot. Activity related heat production (Hact) and non-activity related heat production (Hcor) were calculated in the same manner as described by Gentry *et al.* (1997).

Nitrogen content of diet, urine and feces mixture was analyzed by the Kjeldahl method (ISO 5983, 1979). Dry matter and ash content were measured according to ISO procedures (ISO 6496 (1983) and ISO 5984 (1978) respectively). Energy content was determined by bomb-calorimetry (IKA Calorimeter C 7000). Sodium and potassium content in the diet were measured according to ISO procedure (ISO 6869, 1997). Sodium and potassium content of feed, urine and feces mixture were measured by using an atomic absorption spectrometry (SpectrAA 300, Varian Australia Pty Ltd, Mulgrave Victoria, Australia). Chloride content in the diets was determined based on the method described by Fauchon *et al.* (1995). Chloride in the feed and feces plus urine mixture was measured by using a chloride meter (PCLM digital chloride meter, Jenway Ltd. Dunmow, England). Na, K and Cl intakes were calculated from feed consumption data. Because intake of these minerals from water consumption took account for less than 0.1% of the intake from feed, the mineral intake from water was neglected. The Na, K and Cl retention was estimated as intake of those minerals subtracted by those minerals in feces and urine mixture, corrected for the minerals content of water used for cleaning the chambers (less than 0.1%).

Ammonia in the outgoing air from the chambers was removed by passing the airflow through 25% H_2SO_4 solution. At the end of each balance study period ammonia (nitrogen) concentration in the solution was measured. Ammonia emission from the air was calculated from the ammonia concentration in the H_2SO_4 solution and the airflow rate data.

Table 1. Basal and test diets composition (as fed)

Composition of basal diet (g kg^{-1})	
Barley	416
Wheat	200
Rye	200
Soybean isolate	130
Tallow	20
Limestone	8
Monocalciumphosphate	8
Salt (KCl)	3
L-lysine HCl	3
DL-methionine	1
Premix[1]	10
L-threonine	1

	Dietary CAD (mEq kg^{-1})	
Test diet composition (g kg^{-1})	-100	200
Basal diet	970	970
$CaCl_2.2H_2O$	19.3	
$NaHCO_3$		3.1
$CaCO_3$		13.6
Diamol[2]	10.7	13.3
Analyzed composition (g kg^{-1})		
Dry matter	891.5	897.6
Ash	57.5	60.9
Nitrogen	32.9	33.1
Gross energy (kJ g^{-1})	16.6	16.7
Na	1.59	2.28
K	5.34	5.28
Cl	12.1	3.2
CAD (mEq kg^{-1})[3]	-135	145

[1] The vitamin and mineral premix supplied per 1 kg of the basal diet 9000 IU of vitamin A, 1800 IU of vitamin D_3, 40 mg of vitamin E, 5 mg of riboflavin, 30 mg of niacin, 12 mg of d-pantothenic acid, 350 mg of choline chloride, 40 µg of vitamin B_{12}, 3 mg of vitamin K, 50 mg of vitamin C, 1 mg of folic acid, 0.1 mg of biotin, 0.52 mg of Co from $CoSO_4.7H_2O$, 0.06 mg of Se from $Na_2SeO_3.5H_2O$, 0.12 mg of I from KI, 80 mg of Fe from $FeSO_4.7H_2O$, 170 mg of Cu from $CuSO_4.5H_2O$, 44 mg of Mn from MnO_2, 73 mg of Zn from $ZnSO_4.H_2O$. [2] Diamol is diatomaceous shell powder (Biakon N.V., Grobbendonk, Belgium). [3] Calculated from Na, K and Cl content.

Total ammonia emission was calculated as ammonia in the air plus ammonia in the condense water coming from the de-humidifiers in the chambers. The percentage of ammonia nitrogen over nitrogen intake was also calculated.

Statistical analysis

Data were analyzed by a one way ANOVA, according to the general linear model procedure by SAS (SAS 1990), using dietary CAD as independent variable.

Results

Performance of piglets during adaptation period in cages

Dietary CAD did not significantly influence feed intake and growth. However feed intake and growth were numerically higher in 200 mEq kg^{-1} CAD group (171 and 113 g pig^{-1} d^{-1}, respectively) compared to –100 mEq kg^{-1} CAD group (146 and 74 g pig^{-1} d^{-1}, respectively). Feed conversion (feed consumption / weight gain) tended ($p < 0.10$) to be lower in 200 mEq kg^{-1} CAD group (1.6) than in –100 mEq kg^{-1} CAD group (2.3).

Energy metabolism in the first balance period

In the first balance period, some feed residuals occurred. No dietary CAD effect on feed intake, growth, heat production and energy retention was observed. The gross energy and metabolizable energy were similar in both CAD groups (1103 and 919 kJ $kg^{-0.75}$ d^{-1} respectively in –100 mEq kg^{-1} CAD group and 1105 and 918 kJ $kg^{-0.75}$ d^{-1} respectively in 200 mEq kg^{-1} CAD group). Average daily gain was similar in both groups (241 and 232 g pig^{-1} d^{-1} at 200 and –100 mEq kg^{-1} CAD respectively). Total heat production and MEm were also comparable in both CAD groups (622 and 425 kJ $kg^{-0.75}$ d^{-1} respectively at 200 mEq kg^{-1} CAD; 612 and 412 kJ $kg^{-0.75}$ d^{-1} respectively at –100 mEq kg^{-1} CAD).

Energy metabolism in the second balance period

In the second balance period animals ate all the feed offered. Dietary CAD did not significantly affect weight gain, metabolizable energy intake and energy retention (Table 2). However total heat production and MEm tended to be higher in 200 mEq kg^{-1} CAD group than in –100 mEq kg^{-1} CAD group ($p < 0.10$, Table 2). The higher Htot in 200 mEq kg^{-1}

group was associated with non-activity related heat production. Energy retention as protein was comparable for both groups. Energy retention as fat was numerically lower in 200 mEq kg^{-1} CAD group than in –100 mEq kg^{-1} CAD group. Ammonia emission was significantly higher in the 200 mEq kg^{-1} CAD group (Table 2).

Table 2. Daily weight gain, energy intake, heat production and energy retention in piglets in the second balance period

	Dietary CAD (mEq kg^{-1})		SEM	Pvalue
	-100	200		
No. groups per treatment	5	4		
No. pigs per treatment	15	12		
Average daily gain (g pig^{-1} d^{-1})	278	287	13.5	0.64
GE intake (kJ $kg^{-0.75}$ d^{-1})	1239	1237	17.5	0.93
ME intake (kJ $kg^{-0.75}$ d^{-1})	1057	1050	13.1	0.69
ME/GE	0.853	0.849	0.63	0.63
Heat production (kJ $kg^{-0.75}$ d^{-1}):				
Total (Htot)	660	681	7.86	0.10
Activity related (Hact)	120	124	12.7	0.84
Nonactivity related (Hcor)	540	557	13.0	0.38
Hact as percentage of Htot	18.2	18.2	1.88	0.99
RQ (mol CO_2 / mol O_2)	0.996	0.985	0.005	0.18
Energy retention (kJ $kg^{-0.75}$ d^{-1}):				
Total (ER)	397	369	13.3	0.17
Protein (Erp)	218	217	3.20	0.83
Fat (ERf)	180	152	13.2	0.18
MEm	412	443	10.1	0.06
N-emission (g pig^{-1} d^{-1})	0.45	0.91	0.06	0.01
N-emission as % of N intake	3.43	6.90	0.36	0.001

Total heat production, activity and non-activity related heat production were clearly associated with meals. The highest heat production occurred around feeding time. The differences in total heat production between two dietary groups occurred mainly during daytime (Figure 1). Total heat production was significantly ($p < 0.01$) higher in 200 mEq kg^{-1} CAD group than in –100 mEq kg^{-1} CAD group in the light period (daytime) (Table 3). No significant differences were found for total heat production in the dark period (night). Only in the morning feeding moment the piglets in 200 mEq kg^{-1} CAD group showed a tendency of high activity (Figure 2).

Table 3. Heat production (kJ $kg^{-0.75}$ d^{-1}) during light and dark period in the second balance week

	Dietary CAD (mEq kg^{-1})		SEM	P value
	-100	200		
Htot:				
Day	727	763	7.64	0.01
Night	593	599	11.8	0.76
Hact:				
Day	160	179	16.9	0.47
Night	75	68	10.2	0.66
Hcor:				
Day	567	585	12.1	0.33
Night	518	530	10.5	0.45
RQ:				
Day	1.00	0.990	0.0037	0.0897
Night	0.9996	0.988	0.0043	0.0995

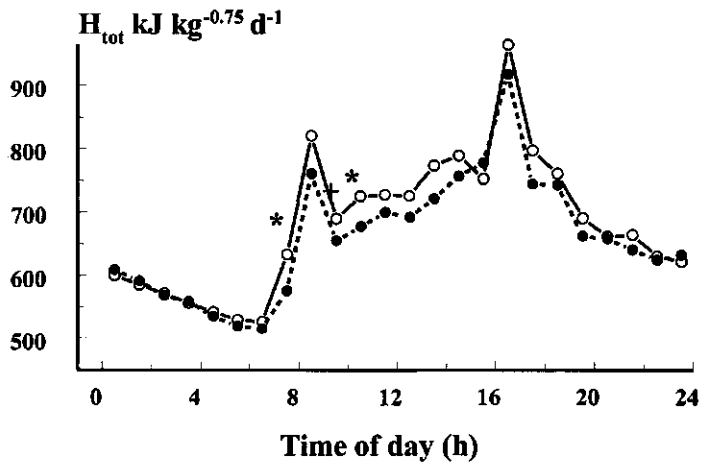

Figure 1. Daily total heat production (kJ $kg^{-0.75}$ d^{-1}) in –100 mEq kg^{-1} (•) and 200 mEq kg^{-1} (O) dietary CAD groups. (*) indicate significant difference and (+) refer to tendency on comparing two CAD groups.

Activity corrected heat production (Hcor) was numerically higher in 200 mEq kg^{-1}

CAD group mainly during daytime and in the postprandial period. In the night the Hcor was similar for both groups (Figure 3).

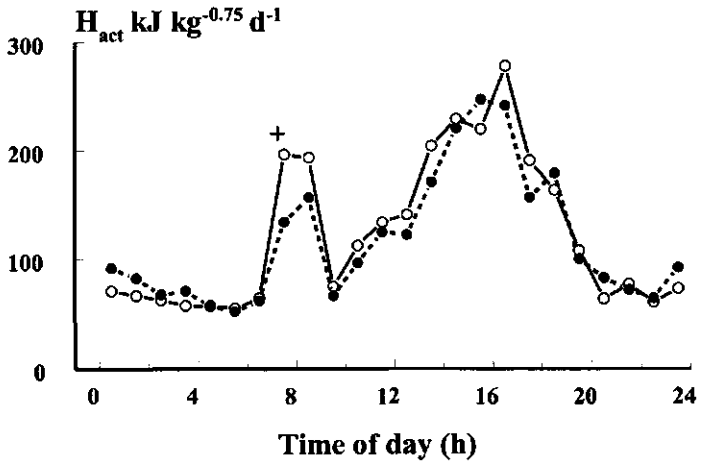

Figure 2. Daily heat production for activity (kJ $kg^{-0.75}$ d^{-1}) in –100 mEq kg^{-1} (•) and 200 mEq kg^{-1} (O) dietary CAD groups. (+) refers to tendency on comparing two CAD groups.

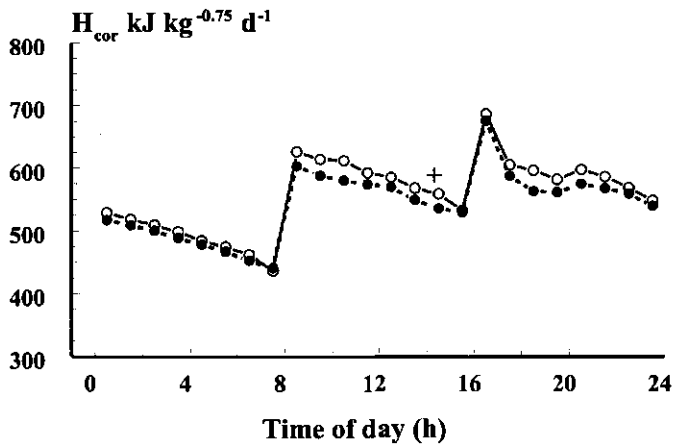

Figure 3. Daily heat production after correction for activity (Hcor, kJ $kg^{-0.75}$ d^{-1}) in –100 mEq kg^{-1} (•) and 200 mEq kg^{-1} (O) dietary CAD groups. (+) refers to tendency on comparing two CAD groups.

The respiration quotient (RQ, mol CO_2 / mol O_2) values were almost constantly lower

($p < 0.09$) in 200 mEq kg^{-1} CAD group than in –100 mEq kg^{-1} CAD group (Figure 4).

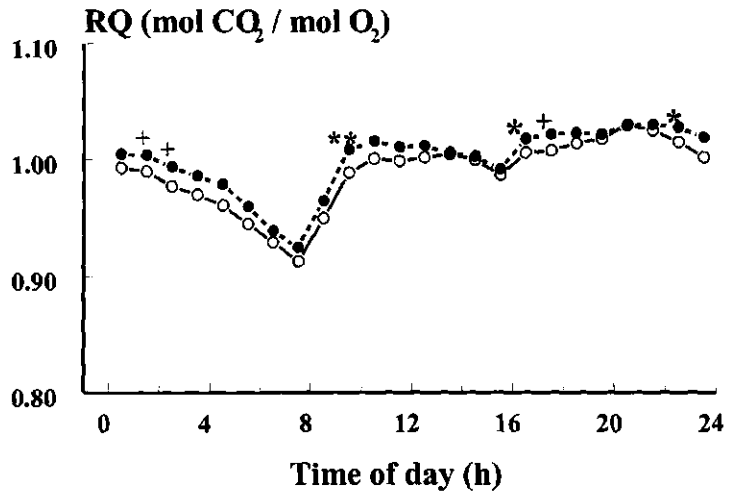

Figure 4. Daily respiration quotient (RQ) in –100 mEq kg^{-1} (•) and 200 mEq kg^{-1} (O) dietary CAD groups. (**) indicates $P < 0.01$, (*) indicates $P < 0.05$, (+) refers to $P < 0.10$ on comparing two CAD groups.

Sodium, potassium and chloride retention

Sodium intake was higher in 200 mEq kg^{-1} CAD group than in –100 mEq kg^{-1} CAD group (0.16 and 0.11 g $kg^{-0.75}$ d^{-1} respectively), however the sodium retention was similar in both dietary CAD groups (0.078 and 0.073 g $kg^{-0.75}$ d^{-1} respectively). Potassium intake from consumed diets and retention were the same for both groups (average value of 0.37 and 0.17 g $kg^{-0.75}$ d^{-1} respectively). Both chloride intake and retention were higher in –100 mEq kg^{-1} CAD group (0.85 and 0.15 g $kg^{-0.75}$ d^{-1} respectively) than in 200 mEq kg^{-1} CAD group (0.23 and 0.07 g $kg^{-0.75}$ d^{-1} respectively).

Discussion

Growth performance

When access feed was provided to piglets in the adaptation period, growth rate of piglets increased by 50% in 200 mEq kg^{-1} CAD group compared to –100 mEq kg^{-1} CAD

Implication

Dietary CAD level of 200 mEq kg^{-1} resulted in higher heat production than of –100 mEq kg^{-1} CAD at a restricted feeding level. This high heat production occurred mainly during daytime. Pigs needed more energy for maintenance after ingesting a diet with a CAD level of 200 mEq kg^{-1} compared to a CAD level of –100 mEq kg^{-1}. The results of this study imply that the effect of dietary CAD on performance of pigs is mainly through altered feed consumption. The poor performance in low CAD diet is related to a reduced feed intake, when feed is provided at *ad libitum*. Further experiments are needed to test maintenance costs at different dietary CAD levels with access feed consumption.

Acknowledgement

The authors sincerely thanks J. M. van der Linden for his skilful technical assistance; Tamme Zandstra for all the arrangement and management work in the chambers; Peter Vos, Ries Verkerk, Andre Jansen, Truus van der Wal and all the people of the experimental unit 'de Haar Varkens' for their help during the experiment; Jane-Martine Muijlaert and Marian van't End for the chemical analysis and all the support for chemical analysis from Dr. Huug Boer.

References

ARC. 1981. The Nutrient Requirements of Pigs. Commonwealth Agric. Bureaux, Slough, UK.

Brouwer, E. 1965. Report of sub committee on constants and factors. In: 3rd Symposium on Energy Metabolism. Troon Scotland EAAP publ. 11: 441-443.

Canh, T. T. 1998. Ammonia emission from excreta of growing-finishing pigs as affected by dietary composition. Ph.D. dissertation. Wageningen University, Wageningen, The Netherlands. P163.

Curtis, S. E. 1983. Environmental Management in Animal Agriculture. IOWA University Press. Ames. IOWA.

Dersjant-Li, Y., Schulze, H., Verreth, J. and Verstegen, M. 1998. Effects of dietary cation-anion differences (CAD) on food intake, growth and nutrient utilization in piglets. In: Proc. Int. Conf. on Pig Prod. 6-8 July. Beijing, China. pp323-326.

Dersjant-Li, Y., Verreth, J. A. J., Tijssen, P. A. T., Booms, R., Verstegen, M. W. A. and Huisman, E. A. 2000. Metabolic costs of changing the cation-anion difference in the diet of juvenile African catfish *Clarias gariepinus* (Burchell). Aqua. Nutr. 6: 39-45.

Fauchon, C., Seoane, J. R. and Bernier, J. F. 1995. Effects of dietary cation-anion concentrations on performance and acid-base balance in growing lambs. Can. J. Anim. Sci. 75: 145-151.

Gentry, J. L., Swinkels, J. W. G. M., Lindemann, M. D. and Schrama, J. W. 1997. Effect of hemoglobin and immunization status on energy metabolism of weanling pigs. J. Anim. Sci. 75: 1032-1040.

Haydon, K. D. and West, J. W. 1990. Effect of dietary electrolyte balance on nutrient digestibility determined at the end of the small intestine and over the total digestive tract in growing pigs. J. Anim. Sci. 68: 3687-3693.

Heisler, N. 1984. Acid-base regulation in fishes. In: Hoar W.S., Randall, D.J. and Brett, J.R. (Ed), Fish Physiology, Vol. XA, Anatomy, Gas Transfer, and Acid-Base Regulation. Academic Press, New York, pp 315-401.

Henken, A. M., van der Hel, W., Brandsma, H. A. and Verstegen, M. W. A. 1991. Differences in energy metabolism and protein retention of limit-fed growing pigs of several breeds. J. Anim. Sci. 69:1443-1453.

ISO 5984. 1978. International Organization for Standardization. Animal feeding stuffs – Determination of crude ash. Genève, Switzerland.

ISO 5983. 1979. International Organization for Standardization. Animal feeding stuffs – Determination of nitrogen content and calculation of crude protein content. Genève, Switzerland.

ISO 6496. 1983. International Organization for Standardization. Animal feeding stuffs – Determination of moisture content. Genève, Switzerland.

ISO 6869. 1997. International Organization for Standardization. Animal feeding stuffs – Determination of the calcium, copper, iron, magnesium, manganese, potassium, sodium and zinc contents – Atomic absorption spectrometric method. Genève, Switzerland.

Moughan, P. J. and Smith, W. C. 1984. The effect of the dietary $Na^+ + K^+ - Cl^-$ balance on the short-term energy and nitrogen metabolism of the growing pig. J. Sci. Food. Agric. 35: 1183-1185.

NRC. 1988. Nutrient Requirements of Swine (9th Ed.). National Academy Press, Washington, DC.

Park, J. H., Han, I. K. and Kim, I. B. 1994. Effects of electrolyte balance (EB: Na+K-Cl) on the growth, blood parameters, urine parameters and nutrient availabilities of weaning pigs. Korean J. Anim. Nutr. and Feed Stuffs 18: 147-154.

Patience, J. F., Austic, R. E. and Boyd, R. D. 1987. Effect of dietary electrolyte balance on growth and acid-base status in swine. J. Anim. Sci. 64: 457-466.

Patience, J. F. and Wolynetz, M. S. 1990. Influence of dietary undetermined anion on acid-base status and performance in pigs. J. Nutr. 120: 579-587.

Patience, J. F. and Chaplin, R. K. 1997. The relationship among dietary undetermined anion, acid-base balance, and nutrient metabolism in swine. J. Anim. Sci. 75: 2445-2452.

SAS. 1990. SAS User's Guide: Statistics (Version 6 Ed.). SAS Inst. Inc., Cary, NC.

Sijben, J. W., van Vugt, P. N. A., Swinkels, J. W. G. M., Parmentier, H. K. and Schrama, J. W. 1998. Energy metabolism of immunized weanling piglets is not affected by dietary yeast. J. Anim. Physiol. a. Anim. Nutr. 79: 153-161.

Verstegen, M. W. A., van der Hel, W., Brandsma, H. A., Henken, A. M. and Bransen, A. M. 1987. The Wageningen respiration unit for animal production research: A description of the equipment and its possibilities. In: M. W. A. Verstegen and A. M. Henken (Ed), Energy Metabolism of Farm Animals: Effects of Housing, Stress, and Disease. Martinus Nijhoff, Dordrecht, The Netherlands. p 21-48.

Yen, J. T., Pond, W. G. and Prior, R. L. 1981. Calcium chloride as a regulator of feed intake and weight gain in

pigs. J. Anim. Sci. 52: 778-782

PART 3

The effect of dietary cation anion difference on acid base balance in the blood and in the digestive system in fish and pigs

CHAPTER 6

Postprandial blood oxygen and pH in response to dietary cation anion difference in pigs

Yueming Dersjant-Li[1, 2], Martin W. A. Verstegen[1], Alfons Jansman[4], Hagen Schulze[5], Johan W. Schrama[3] and Johan A. Verreth[2]

[1] Animal Nutrition Group, [2] Fish Culture and Fisheries Group, [3]Adaptation Physiology Group, Wageningen Institute of Animal Sciences, Wageningen University, Wageningen, The Netherlands. [4] TNO-Nutrition and Food Research Institute, Department of Animal Nutrition and Physiology (ILOB), Wageningen, The Netherlands. [5] Finnfeeds International Ltd., Marlborough, UK, Present address: Provimi B.V., P.O.Box 5063, 3008 AB Rotterdam, The Netherlands

Submitted to Journal of Animal Science

Abstract: The effect of dietary cation anion difference (CAD, Na + K - Cl, mEq kg^{-1}) on postprandial arterial and portal blood oxygen content, blood pH and acid base status in pigs was studied using a cross over experimental design. Four pigs with a mean weight of 45 kg were surgically fitted with catheters in the carotid artery and portal vein. Two dietary CAD levels (-100 and 200 mEq kg^{-1}) were evaluated in two periods of one-week each. Feed was given at 2.6 times the maintenance requirement for energy in two meals per day. Water was freely available. Blood samples were taken at 0, 0.5, 1, 1.5, 2, 3, 4, 6 and 9 h after feeding through catheters. Blood hemoglobin (Hb), O_2 pressure (kPa), O_2 saturation (%), O_2 content (mmol L^{-1}), pH, Pco_2, HCO_3^-, base excess (BE), Na^+, K^+ and Cl^- content were measured. Oxygen content (mmol L^{-1}) in arterial and portal blood was significantly lower in the –100 mEq kg^{-1} CAD group (5.78 and 4.82 respectively) compared to the 200 mEq kg^{-1} CAD group (6.18 and 4.99 respectively). This was related to the low Hb content in the blood at –100 mEq kg^{-1} CAD. Arterial and portal blood pH was significantly lower at –100 mEq kg^{-1} dietary CAD (7.46 and 7.37) than at 200 mEq kg^{-1} dietary CAD (7.49 and 7.43). During the postprandial period, however, blood pH was maintained at a relatively constant level in each CAD group. The average values of arterial and portal blood for base excess and HCO_3^- concentration were significantly higher at the 200 mEq kg^{-1} dietary CAD (12.54 and 35.9 mmol L^{-1} respectively) than the -100 mEq kg^{-1} dietary CAD (6.96 and 31.0 mmol L^{-1} respectively). After a meal the concentration of these parameters in the blood increased especially in the 200 mEq kg^{-1} CAD group. Na^+ concentration in the blood increased and K^+ and Cl^- concentrations decreased significantly by increasing dietary CAD from –100 mEq kg^{-1} to 200 mEq kg^{-1}. Blood CAD level is higher in 200 mEq kg^{-1} CAD group than in –100 mEq kg^{-1} CAD group. In conclusion, dietary CAD changed blood oxygen content and pH level and influenced the acid base buffer system in pigs. Pigs maintained a constant postprandial pH level in the blood within each CAD group.

Keywords: base excess, blood oxygen content, blood pH, chloride, electrolyte balance, pigs

Introduction

Dietary cation anion difference (CAD, Na + K - Cl in mEq kg^{-1} diet) in a diet can significantly alter the performance of pigs (Patience *et al.*, 1987; Park *et al.*, 1994). The physiological mechanisms of this effect are, however, not fully understood. Some studies revealed that a low dietary CAD decreased blood pH around feeding (Patience and Chaplin, 1997) and 5 hours post feeding (Haydon and West, 1990). It was suggested that low dietary CAD resulted in metabolic acidosis and consequently reduced feed intake and feed utilization (Yen *et al.*, 1981; Patience *et al.*, 1987; Patience and Wolynetz, 1990). The low feed utilization suggests that the level of energy metabolism may be increased at low dietary CAD. This suggests an increased oxygen consumption. On the other hand, there is an interaction between oxygen and hydrogen ions in the blood (the Bohr effect and the Haldane effect, Siggaard-Andersen, 1974). The interaction may be expressed by the equilibrium of '$HbH_n^+ + O_2 \rightleftharpoons HbO_2 + n \cdot H^+$'. At acidic situations, the binding of hydrogen ions by hemoglobin will cause liberation of oxygen from oxyhemoglobin. Therefore the oxygen content may alter with the acid base status of the blood. The objective of this study is to investigate the postprandial change in arterial and portal blood parameters at different dietary CAD levels.

In this study we measured oxygen content in both arterial and portal blood simultaneously during the postprandial period in pigs fed two different levels of CAD's. Additionally, arterial and portal concentration differences in blood with regard to pH, Pco_2, HCO_3^-, base excess (BE), Na^+, K^+, Cl^- and their postprandial changes were assessed.

Materials and methods

Diets and feeding

Two treatment diets with a CAD level of -100 and 200 mEq kg^{-1} were tested. Two dietary CAD levels were formulated by supplementation of different amount of $CaCl_2 \cdot 2H_2O$ to a maize and soybean meal based diet. The calcium content in both diets was maintained constant by adding a variable amount of $CaCO_3$ to the diets. The ash content was equalized in both diets by varying diamol content in the diets (Table 1). Feed was given at 2.6 times the maintenance requirement for energy (419 kJME $kg^{-0.75}$ d^{-1}). Feed was administrated as pellets two times per day in equal portions (9.00 and 21.00h) by an automatic feeder. Water was freely available.

Table 1. Composition of the treatment diets as fed

	Dietary CAD (mEq kg^{-1})	
	-100	200
Basal ingredients (g kg^{-1})		
Soybean meal (46.7% cp)	370	370
Maize	150	150
Maize starch	324.9	324.9
Cellulose	60	60
Soy oil	15	15
DL-methionine	1	1
Premix[1]	10	10
NaCl	3.5	3.5
Monocalcium phosphate	8.8	8.8
Additives (g kg^{-1})		
$CaCO_3$	4.8	20.6
$CaCl_2.2H_2O$	24	2
Diamol[2]	28	34.2
Analyzed composition (g kg^{-1})		
DM	893	869
Nitrogen	31.2	33.1
Ash	76.7	81.6
Gross energy (kJ g^{-1})	15.4	15.7
Na	1.61	1.63
K	7.91	8.07
Cl	13.1	3.4
pH	5.18	5.85
Buffer capacity (mEq kg^{-1})	568	832
Measured CAD (mEq kg^{-1})[3]	-97	182

[1] The vitamin and mineral premix supplied per 1 kg of the basal diet 5000 IU of vitamin A, 1000 IU of vitamin D_3, 7.5 mg of vitamin E, 3.5 mg of riboflavin, 20 mg of niacin, 5 mg of d-pantothenic acid, 200 mg of choline chloride, 15 μg of vitamin B_{12}, 0.4 mg of vitamin K, 0.21 mg of Co from $CoSO_4.7H_2O$, 0.06 mg of Se from $Na_2SeO_3.5H_2O$, 0.12 mg of I from KI, 80 mg of Fe from $FeSO_4.7H_2O$, 20 mg of Cu from $CuSO_4.5H_2O$, 25 mg of Mn from MnO_2, 46 mg of Zn from $ZnSO_4.H_2O$. [2] Diamol is diatomaceous shell powder (Biakon N.V., Grobbendonk, Belgium). [3] Calculated from Na, K and Cl content.

Pigs and experimental procedure

Four pigs (Crossbred of (((FL×NL) × GY) × GY)) with a mean initial weight of 45 kg were used. They were housed individually in metabolic cages with tenderfoot floor (0.9 × 1.8m). Room temperature was maintained at about 20 °C, light was on from 8.00 to 22.00h. After two weeks of adaptation to the experimental facility, the pigs were surgically fitted with catheters in the carotid artery and portal vein respectively, according to the procedures as described by van Leeuwen *et al.* (1995). After surgery pigs were allowed to recover for 10 days, before blood sampling started. Feed supply slowly increased after surgery. Four days after surgery, the full ration was fed. In the pre-experimental period and the recovering period a commercial diet was fed.

A cross over experimental design (two treatments in two periods) was applied. Each period lasted for six days with one day (day 6) of sampling. In the first period two pigs were randomly assigned to each of the two treatments, in the second period the assignment of treatments to animals was reversed. The commercial diet was gradually replaced by the treatment diets in three days. In each period the blood samples were taken three days after the pigs received the full experimental diets.

Sampling and measurements

At each sampling day the blood samples were taken 0, 0.5, 1, 1.5, 2, 3, 4, 6, and 9 h after the morning meal. At each sampling point about 2 ml of blood was taken from both the carotid artery and the portal vein through the catheters.

Immediately after sampling, using an i-State Portable Clinical Analyzer (HP iSTAT WR052, Hewlett Packard) with EG6+ cartridges, blood pH, Pco_2, PO_2, Na^+, K^+ and hematocrit (Hct) were measured, HCO_3^-, base excess, Hb and sO_2 (% saturation) were calculated and read out on the Analyzer. Blood base excess (BE) is defined as the strong acid (or base) needed to titrate the blood to pH = 7.40 at Pco_2 = 40 *mm* Hg and t_c = 37°C (Siggaard-Andersen, 1974) and expressed as mmol L^{-1}.

Blood Cl^- concentration was measured directly after sampling by using a chloride meter (PCLM digital chloride meter, Jenway Ltd. Dunmow, England). Nitrogen content of diet was analyzed by the Kjeldahl method (ISO 5983, 1979). Dry matter and ash content were measured according to ISO procedures (ISO 6496 (1983) and ISO 5984 (1978) respectively). Energy content was determined by bomb-calorimetry (IKA Calorimeter C 7000, Fa. IKA-

Analysentechnik, Weitersheim, Germany). Sodium and potassium content in the diet were measured according to ISO procedure (ISO 6869, 1997). Sodium and potassium were measured by using an atomic absorption spectrometry (SpectrAA 300, Varian Australia Pty Ltd, Mulgrave Victoria, Australia). Chloride content in the diets was determined based on the method described by Fauchon *et al.* (1995), by using the same chloride meter as for blood Cl^-. The buffer capacity of the diets was measured according to the procedure as described by Prohaszka and Baron (1980). Dietary pH was measured as described by Tacon and De Silva (1983).

Data analysis

Whole blood O_2 content was defined as the concentration of total oxygen in the blood, calculated as the sum of dissolved oxygen (O_2) and oxyhemoglobin, using the following equation:

$$O_2 \text{ (mmol } L^{-1}) = Hb \times SAT \text{ (SI)} + 0.00136 \times PO_2$$

Where Hb is blood hemoglobin (mmol L^{-1}), SAT is percentage of O_2 saturation, 0.00136 is solubility coefficient of oxygen in the blood (mmol L^{-1} × mmHg). PO_2 is O_2 pressure in mmHg.

Statistical analysis

Data were analyzed for each sampling point and for the average value of repeated measurements in time. The effect of dietary CAD, blood type and their interaction were tested by ANOVA using SAS program (SAS 1990). Additionally pig number was included in the model to account for the random variation between animals. The model was:

$$Y = \mu + Pn_i + CAD_j + B_k + (CAD \times B)_{jk} + e_{ijk}$$

Where Y = dependent variable, μ = the over all mean, Pn_i = pig number (i = 1..4), CAD_j = the effect of dietary CAD (j = 1..2), B_k = Blood type, i.e. arterial and portal blood (k = 1..2), $(CAD \times B)_{jk}$ = interaction term, e_{ijkl} = residuals.

Results

Mean values

Comparison of two CAD groups

The treatment means of arterial and portal blood parameters was summarized in Table 2. No dietary CAD and blood type interaction was found. Dietary CAD did not influence oxygen pressure (kPa) and oxygen saturation (%). Oxygen content (mmol L^{-1}) in both arterial and portal blood was significantly lower in the –100 mEq kg^{-1} CAD group compared to 200 mEq kg^{-1} CAD group. Hemoglobin (Hb) content was significantly lower in the –100 mEq kg^{-1} CAD group.

Table 2. Mean arterial (A) and portal (P) blood parameters in different dietary CAD groups

Parameters	Dietary CAD (mEq kg^{-1})				SEM	Effect[1]	
	-100		200			CAD	Blood type
	A	P	A	P		P values	
O_2 (kpa)	13.4	6.89	13.0	6.44	0.091	0.13	0.001
O_2 (%)	97.4	79.6	97.2	78.4	0.265	0.18	0.001
O_2 (mmol L^{-1})	5.78	4.82	6.18	4.99	0.039	0.008	0.001
Hb (mmol L^{-1})	5.79	5.95	6.22	6.29	0.041	0.001	0.26
pH	7.459	7.370	7.491	7.426	0.004	0.003	0.001
BE (mmol L^{-1})	6.6	7.3	11.8	13.3	0.23	0.001	0.15
HCO_3^- (mmol L^{-1})	30.0	32.0	34.6	37.2	0.202	0.001	0.002
Pco_2 (kPa)	5.71	7.51	6.15	7.71	0.065	0.14	0.001
Na^+ (mmol L^{-1})	141.6	140.6	142.1	141.7	0.153	0.003	0.007
K^+ (mmol L^{-1})	4.24	4.55	4.08	4.25	0.031	0.02	0.01
Cl^- (mmol L^{-1})	89.9	89.9	86.7	85.8	0.261	0.001	0.5
CAD (mEq kg^{-1})	56.0	55.3	59.5	60.2	0.37	0.001	0.99

[1] No significant CAD and blood type interaction was found.

Blood pH was significantly lower in both arterial and portal blood in the low CAD treatment group (–100 mEq kg^{-1}) than in 200 mEq kg^{-1} CAD group. The portal blood pH was 7.37 in –100 mEq kg^{-1} CAD group compared to 7.43 in the 200 mEq kg^{-1} CAD group (Table 2). Mean value of arterial and portal blood base excess was significantly higher in the 200

mEq kg^{-1} CAD group compared to -100 mEq kg^{-1} CAD group (12.54 mmol L^{-1} and 6.96 mmol L^{-1} respectively). HCO_3^- content showed a similar pattern as base excess, which had a mean value of 31.0 mmol L^{-1} for –100 mEq kg^{-1} CAD group and of 35.9 mmol L^{-1} for 200 mEq kg^{-1} CAD group. Dietary CAD did not significantly affect Pco_2 (kPa).

Dietary CAD of 200 mEq kg^{-1} resulted in significantly higher Na^+ concentration and significantly lower K^+ and Cl^- concentrations in the blood compared to dietary CAD of –100 mEq kg^{-1}. Blood CAD level was also higher in 200 mEq kg^{-1} CAD group than in –100 mEq kg^{-1} CAD group.

Comparison of arterial and portal blood

Oxygen pressure, saturation and oxygen content, as well as blood pH were significantly higher in arterial blood than in portal blood. HCO_3^- concentration and Pco_2 were lower in arterial blood than in portal blood. Na^+ concentration was low in portal blood and K^+ concentration was high in portal blood. No significant differences were observed between arterial and portal blood for the content of BE, Hb and Cl^- (Table 2).

Postprandial CAD effect

CAD did not affect oxygen pressure (kPa) and oxygen saturation (%) throughout the sampling period (data not shown). Dietary CAD significantly lowered blood O_2 content (mmol L^{-1}) in –100 mEq kg^{-1} CAD group between 0.5 and 6 h after feeding, but no significant differences were found between the two CAD groups before and 9 h after feeding. O_2 content (mmol L^{-1}) was constantly higher in the arterial blood than in the portal blood. An interaction between CAD and blood type was found for oxygen content (mmol L^{-1}) at 0.5, 1, 1.5 h after feeding. This was caused by a rapidly decreased oxygen content (mmol L^{-1}) in the arterial blood in –100 mEq kg^{-1} CAD group (Figure 1). This decrease was mainly due to the decrease in Hb content in arterial blood in this treatment group.

The effect of dietary CAD on blood Hb content mainly occurred between 0.5 and 2 h after feeding (Figure 2). Blood pH remained relatively constant after feeding in both arterial and portal blood in both CAD groups. Statistical analysis of the data showed significant dietary CAD effects on blood pH at 0, 4 and 9 h after feeding and a tendency of this effect 0.5, 1 and 3 h after feeding. No significant difference was found 2 and 6 h after feeding (Figure 3).

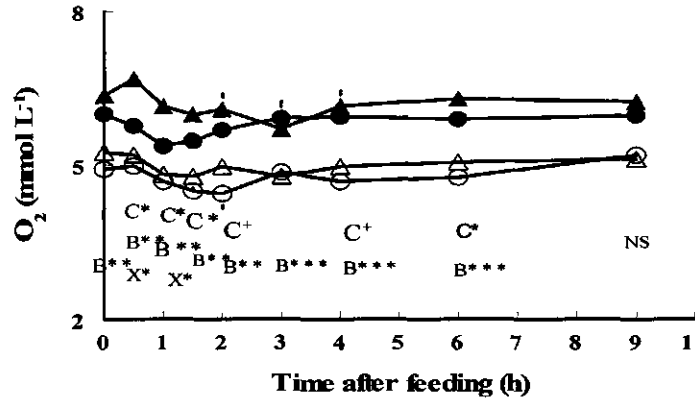

Figure 1. Postprandial changes of oxygen content (mmol L^{-1}) during sampling period. Keys: arterial (●) and portal (O) blood of –100 mEq kg^{-1} CAD; arterial (▲) and portal (Δ) blood of 200 mEq kg^{-1} CAD. C refers to CAD, B refers to blood type, X refers CAD and blood type interaction. NS = non significant, + = $p < 0.10$, * = $p < 0.05$, ** = $p < 0.01$, *** = $p < 0.001$.

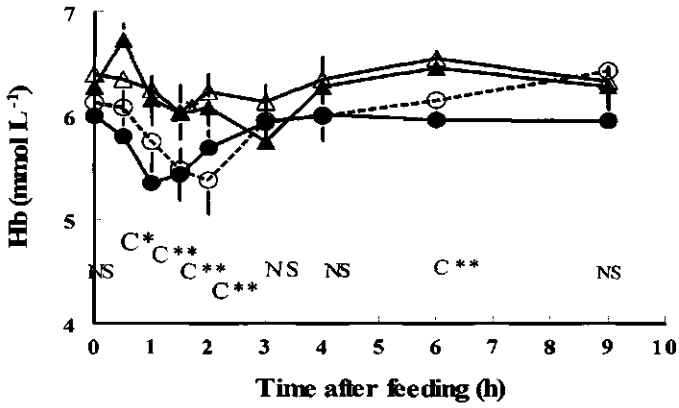

Figure 2. Postprandial changes of hemoglobin content (mmol L^{-1}) during sampling period. Keys: arterial (●) and portal (O) blood of –100 mEq kg^{-1} CAD; arterial (▲) and portal (Δ) blood of 200 mEq kg^{-1} CAD. C refers to CAD, B refers to blood type. NS = non significant, * = $p < 0.05$, ** = $p < 0.01$.

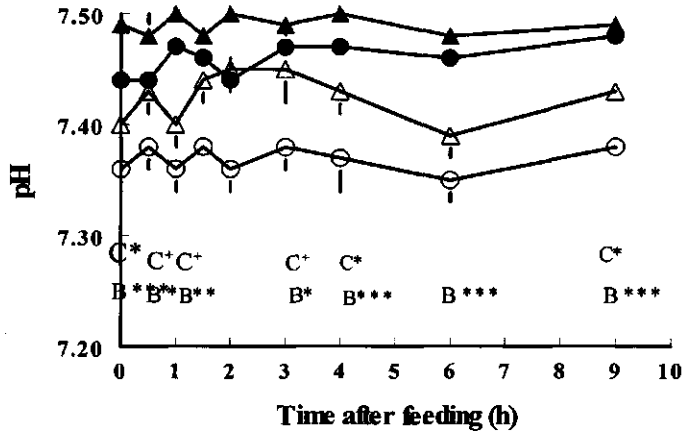

Figure 3. Postprandial changes of blood pH during sampling period. Keys: arterial (●) and portal (O) blood of −100 mEq kg^{-1} CAD; arterial (▲) and portal (Δ) blood of 200 mEq kg^{-1} CAD. C refers to CAD, B refers to blood type. + = $p < 0.10$, * = $p < 0.05$, ** = $p < 0.01$, *** = $p < 0.001$.

Dietary CAD showed a highly significant effect on blood base excess (mmol L^{-1}) throughout the sampling period. Although base excess (mmol L^{-1}) increased more rapidly in portal blood of 200 mEq kg^{-1} CAD group, no CAD and blood type interaction was found (Figure 4). No arterial and portal blood differences were found in base excess content except 9 h after feeding.

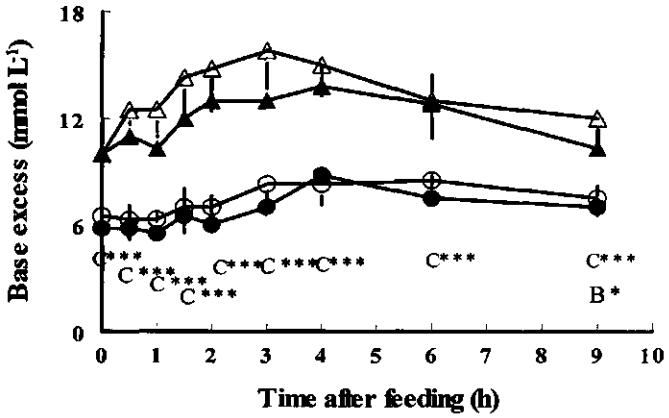

Figure 4. Postprandial changes of base excess (mmol L^{-1}) during sampling period. Keys: arterial (●) and portal (O) blood of −100 mEq kg^{-1} CAD; arterial (▲) and portal (Δ) blood of 200 mEq kg^{-1} CAD. C refers to CAD, B refers to blood type. * = $p < 0.05$, ** = $p < 0.01$, *** = $p < 0.001$.

HCO_3^- content (mmol L^{-1}) was constantly higher ($p < 0.05$) in 200 mEq kg^{-1} CAD group than in –100 mEq kg^{-1} CAD group during the sampling period. It was always higher in portal blood than in arterial blood (Figure 5). Pco_2 (kPa) was also higher in arterial blood compared to portal blood. No dietary CAD effect was found on Pco_2 for each individual sampling point (data not shown).

Significant dietary CAD effect on chloride concentration (mmol L^{-1}) occurred between 1 and 6 h after feeding. No CAD effect was found 0, 0.5 and 9 h after feeding (Figure 6). Chloride concentration (mmol L^{-1}) increased in –100 mEq kg^{-1} CAD group after feeding. No arterial and portal differences were observed for chloride concentration (mmol L^{-1}) during the sampling period. The effect of dietary CAD on potassium concentration (mmol L^{-1}) in the blood was not constant, the significant differences were found 0.5, 3 and 9 h after feeding (Figure 7). Portal blood potassium concentration (mmol L^{-1}) increased rapidly in the first 3 h after feeding, especially for the –100 mEq kg^{-1} CAD group. Arterial and portal differences were found between 0.5 and 2 h after feeding.

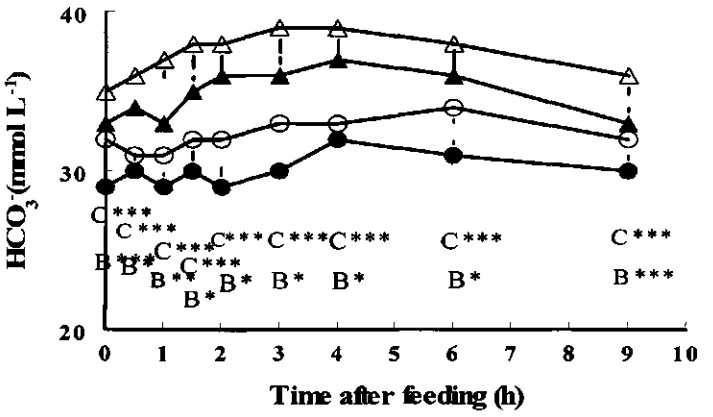

Figure 5. Postprandial changes of HCO_3^- content (mmol L^{-1}) during sampling period. Keys: arterial (●) and portal (O) blood of –100 mEq kg^{-1} CAD; arterial (▲) and portal (Δ) blood of 200 mEq kg^{-1} CAD. C refers to CAD, B refers to blood type. * = $p < 0.05$, ** = $p < 0.01$, *** = $p < 0.001$.

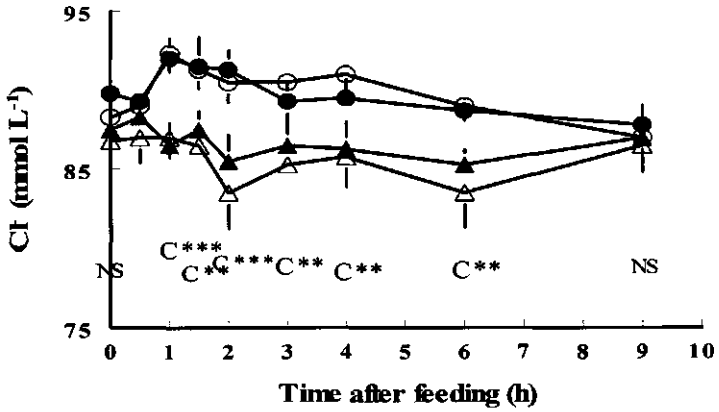

Figure 6. Postprandial changes of chloride content (mmol L^{-1}) during sampling period. Keys: arterial (●) and portal (O) blood of –100 mEq kg^{-1} CAD; arterial (▲) and portal (Δ) blood of 200 mEq kg^{-1} CAD. C refers to CAD, B refers to blood type. NS = non significant, ** = $p < 0.01$, *** = $p < 0.001$.

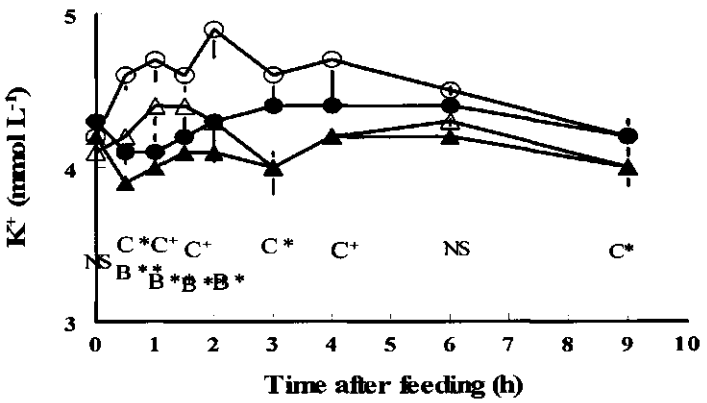

Figure 7. Postprandial changes of potassium content (mmol L^{-1}) during sampling period. Keys: arterial (●) and portal (O) blood of –100 mEq kg^{-1} CAD; arterial (▲) and portal (Δ) blood of 200 mEq kg^{-1} CAD. C refers to CAD, B refers to blood type. NS = non significant, + = $p < 0.10$, * = $p < 0.05$, ** = $p < 0.01$.

Only at 4 and 9 h after feeding dietary CAD showed significant effect on Na^+ concentration in the blood, it was higher in 200 mEq kg^{-1} CAD group than in –100 mEq kg^{-1}

CAD group. Na^+ concentration in arterial blood was significantly higher than in portal blood only 1.5 and 3 h after feeding. The CAD level in the blood showed similar pattern as Cl^-, the significant dietary CAD effect was observed in the postprandial period, between 1 and 6 h after feeding. Which was high at high CAD level and low at low CAD level.

Discussion

Comparison of treatment means

A low dietary CAD level of –100 mEq kg^{-1} significantly decreased Hb content in both arterial and portal blood when compared to a dietary CAD level of 200 mEq kg^{-1}. This is in agreement with the observations by Patience and Wolynetz (1990), who found in pigs a linear decrease in blood hematocrit content when dietary CAD decreased from 248 to –176 mEq kg^{-1}. Hb bound oxygen was more than 97% of the calculated total oxygen content in the blood. In literature, oxygen content was only calculated from Hb content, and the free oxygen was ignored (Burrin *et al.*, 1989). In the present study, the low oxygen content in the portal blood of –100 mEq kg^{-1} CAD group is associated with the low Hb concentrations. As a consequence oxygen supply in the GI tract might become a constraint. This could explain the low feed intake as observed at low dietary CAD levels in literature (Patience *et al.*, 1987; Park *et al.*, 1994). On the other hand, the low oxygen content in the arterial blood of pigs fed –100 mEq kg^{-1} CAD diet might lead to an increased portal blood flow rate in order to increase the oxygen supply. In the present study, the portal blood flow rate was not measured. In literature, many studies reported an increase in portal blood flow rate after feeding (Huntington, 1984; Yen *et al.*, 1989). Burrin *et al.* (1989) showed that portal blood flow rate was also influenced by feeding levels. It may be expected that the acid base status of a diet (i.e. CAD) may also influence the portal blood flow rate.

The blood pH was related to dietary pH, i.e. high dietary pH resulted in high blood pH. Animals can probably cope with different dietary pH and thus maintain a blood pH level at which the energy requirement for homeostasis is minimal. With a low dietary pH, the animal may keep the blood pH at the bottom margin of the normal range. Blood pH was related to BE and HCO_3^- concentration. A dietary CAD of –100 mEq kg^{-1} resulted in low BE and HCO_3^-, leading to a lower blood pH. In the portal blood of –100 mEq kg^{-1} CAD group the mean blood pH was 7.37, this is at the lower margin of the normal blood pH range (Siggaard-

Andersen, 1974). For man a normal value of plasma pH is between 7.36 and 7.45 (Siggaard-Andersen, 1974) at a temperature of 37°C. Scott and McIntosh (1975) reported a normal value of 7.455 for arterial blood pH and 7.392 for venous blood pH obtained by use of catheterized pigs of 25 kg. In the present study animals in the –100 mEq kg^{-1} CAD group may be subject to a slight acidosis. However, the arterial blood pH in 200 mEq kg^{-1} CAD group had a high value of 7.49, which may suggest a slight alkalinity. Apparently this may help animals to cope with the acid load after feeding. The results in the present study are comparable to those of Patience and Chaplin (1997), who found an average blood pH (sampled from vena cava) of 7.48 at a dietary CAD of 163 mEq kg^{-1} and 7.42 at a dietary CAD of –20 mEq kg^{-1}. They also found similar changes in base excess and HCO_3^- content in different dietary CAD groups as in our study. In the present study, the arterial and portal blood pH (7.49 and 7.43 resp.) at 200 mEq kg^{-1} CAD compares well with the observations by van Leeuwen *et al.* (1995). They found a mean value of 7.48 for arterial blood pH and 7.39 for portal blood pH. The measured value of Pco_2 in arterial (6.15 kPa) and portal blood (7.71 kPa) in 200 mEq kg^{-1} group is also similar to the observations of van Leeuwen *et al.*, (1995), who gave a value of 6.07 kPa for arterial blood Pco_2 and 7.90 kPa for portal blood Pco_2. The higher Pco_2 in portal blood explains also why portal blood pH is lower than arterial blood pH.

Plasma Cl^- concentration in both arterial and portal blood was significantly higher in the -100 mEq kg^{-1} CAD group, this is in agreement with some other studies (Yen *et al.*, 1981; Patience and Chaplin, 1997). The K^+ concentration in both arterial and portal blood was also higher in this group. This may indicate that animal need to maintain K/Cl ratio in order to maintain a constant electrolyte balance in the blood. The decrease in Na^+ concentration in portal blood in the –100 mEq kg^{-1} CAD group may be related to the secretion of extra $NaHCO_3$ from pancreas into the small intestine to maintain or reach an optimal intestinal pH (van der Klis *et al.*, 1993). The higher blood CAD level in the dietary CAD of 200 mEq kg^{-1} group compared to the dietary CAD of –100 mEq kg^{-1} group was due to the low Cl^- concentration in the blood of animals receiving this diet.

Postprandial changes:

The decrease in Hb content in –100 mEq kg^{-1} CAD group is not fully understood. At each sampling point only 4ml blood was taken, which may not explain the decrease in Hb. Therefore the decrease in Hb may be due to changes in dietary CAD.

During the first two hours after feeding, the oxygen content in both arterial and portal blood rapidly decreased in –100 mEq kg^{-1} CAD group. This decrease was associated with a decreased blood Hb. The rapid decrease in oxygen content in arterial blood may lead to a low oxygen supply to the GI tract in the first two hours after feeding in –100 mEq kg^{-1} CAD group. The low oxygen content may be a consequence of a high oxygen demand and/or an increased blood volume. In the 200 mEq kg^{-1} group, oxygen content after the meal was less reduced in the arterial blood, this may indicate a better oxygen status of the animals in this group.

Arterial and portal blood pH remained fairly constant after feeding at both dietary CAD levels. However the significant CAD effect was only observed 0, 4 and 9 hours after feeding. This is related to the large within treatment variations. This result implies that a single measurement on blood pH may not be sufficient to test the treatment effect when the sample size is small. The highly significant CAD effects on HCO_3^- and base excess content in the blood were observed during whole sampling period. This indicated that these two parameters were strongly influenced by dietary CAD. The HCO_3^- and base excess content in the blood also increased after feeding both in arterial and portal blood, mainly from 0 to 4 hours after feeding. It was suggested that the rise in blood base excess concentration could be used as an indicator of the amount of gastric acid secretion (Rune cited by Siggaard-Andersen, 1974). The rapid increase in base excess and HCO_3^- concentrations in the blood after feeding in 200 mEq kg^{-1} CAD group would consequently increase postprandial blood pH. However the postprandial blood pH was constant indicating that animals' buffer system actively regulated acid base balance, and therefore remained a constant blood pH after feeding.

The changes in blood CAD levels after feeding were clearly related to the Cl^- concentration in the blood. The dietary CAD effects on Cl^- concentration were found only during postprandial period. This was clearly related to the feed consumption. At 9 h after feeding pigs maintained the Cl^- concentration and CAD level in the blood to a preprandial level. The CAD effect on blood K and Na concentrations was not constant, this might be related to different behavior of individual animal.

Conclusion

Dietary CAD has an impact on arterial and portal blood oxygen and Hb status. This effect was more pronounced between 0.5 and 2 h after feeding. Low dietary CAD lowered both arterial and portal blood Hb and oxygen contents, this may be related to the reduced performance as observed in literature. Pigs can maintain their postprandial blood pH relatively constant within each dietary CAD group. However, this pH level depends on dietary CAD levels, i.e. a low dietary CAD results in a low portal blood pH and animals are maybe more susceptible for metabolic acidosis. Dietary CAD effect on Cl and K concentrations in the blood was related to feed consumption.

Acknowledgement

The experiment was conducted in the facility of TNO Nutrition and Food Research Institute, Department of Animal Nutrition and Physiology (ILOB), Wageningen, the Netherlands. The authors would like to express their thanks to Mr. Piet van Leeuwen, Mr. Dick van Kleef, Mr. Kasper Deuring and Ms Baukje Schat (DVM) for operating the pigs, stable management, health control, sampling and measurements. Thanks to Mr. Gerard Beelen and Mr. Johan de Jong for formulating the experimental diets. Thanks to Mr. Piet Roeleveld and his colleagues for producing the experimental feeds.

References

Burrin, D. G., Ferrell, C. L., Eisemann, J. H., Britton, R. A. and Nienaber, A. J. 1989. Effect of level of nutrition on splanchnic blood flow and oxygen consumption in sheep. Br. J. Nutr. 62: 23-34.

Fauchon, C., Seoane, J. R. and Bernier, J. F. 1995. Effects of dietary cation-anion concentrations on performance and acid-base balance in growing lambs. Can. J. Anim. Sci. 75: 145-151.

Huntington, G. 1984. Relationship of portal blood flow to metabolizable energy intake of cattle. Can. J. Anim. Sci. 64 (Suppl.): 16-17.

Haydon, K. D. and West, J. W. 1990. Effect of dietary electrolyte balance on nutrient digestibility determined at the end of the small intestine and over the total digestive tract in growing pigs. J. Anim. Sci. 68: 3687-3693.

ISO 5984. 1978. International Organization for Standardization. Animal feeding stuffs – Determination of crude ash. Genève, Switzerland.

ISO 5983. 1979. International Organization for Standardization. Animal feeding stuffs – Determination of nitrogen content and calculation of crude protein content. Genève, Switzerland.

ISO 6496. 1983. International Organization for Standardization. Animal feeding stuffs – Determination of moisture content. Genève, Switzerland.

ISO 6869. 1997. International Organization for Standardization. Animal feeding stuffs – Determination of the calcium, copper, iron, magnesium, manganese, potassium, sodium and zinc contents – Atomic absorption spectrometric method. Genève, Switzerland.

Van der Klis, J. D., Verstegen, M. W. A. and Van Voorst, A. 1993. Effect of a soluble polysaccharide (carboxy methyl cellulose) on the absorption of minerals from the gastrointestinal tract of broilers. Br. Poultry Sci. 34: 377-389.

van Leeuwen, P., Leuvenink, H. G. D., Haarsbroek, W. M., Priem, G., Bosch, M. and van Kleef, D. J. 1995. A portal-vein-catheterization technique in pigs and sheep, and postprandial changes of pO_2, pCO_2, pH, urea, ammonia, and creatinine and proteins in portal and arterial blood measured in pigs. J. Anim. Physiol. a. Anim. Nutr. 73: 38-46.

Park, J. H., Han, I. K. and Kim, I. B. 1994. Effects of electrolyte balance (EB: Na+K-Cl) on the growth, blood parameters, urine parameters and nutrient availabilities of weaning pigs. Korean J. Anim. Nutr. & feed stuffs 18: 147-154.

Patience, J. F., Austic, R. E. and Boyd, R. D. 1987. Effect of dietary electrolyte balance on growth and acid-base status in swine. J. Anim. Sci. 64: 457-466.

Patience, J. F. and Wolynetz, M. S. 1990. Influence of dietary undetermined anion on acid-base status and performance in pigs. J. Nutr. 120: 579-587.

Patience, J. F. and Chaplin, R. K. 1997, The relationship among dietary undetermined anion, acid-base balance, and nutrient metabolism in swine. J. Anim. Sci. 75: 2445-2452.

Prohaszka, L. and Baron, F. 1980. The predisposing role of high dietary protein supplies in Eneteropathogenic E. Coli infections of weaned pigs. Zbl. Veterinary Medicine B 27: 222-232.

SAS. 1990. SAS User's Guide: Statistics (Version 6 Ed.). SAS Inst. Inc., Cary, NC.

Scott, D. and McIntosh, G. H. 1975. Changes in blood composition and urinary mineral excretion in the pig in response to acute acid-base disturbance. Q. J. Exp. Physoil. 60:131-140.

Siggaard-Andersen, O. 1974. The acid-base status of the blood. Fourth edition. Munksgaard, Copenhagen.

Tacon, A.G.J. and De Silva, S.S. 1983. Mineral composition of some commercial fish feeds available in Europe. Aquaculture, 31: 11-20.

Yen, J. T., Pond, W. G. and Prior, R. L. 1981. Calcium chloride as a regulator of feed intake and weight gain in pigs. J. Anim. Sci. 52: 778-782.

Yen, J. T., Nienaber, J. A., Hill, D. A. and Pond, W. G. 1989. Oxygen consumption by portal vein-drained organs and by whole animal in conscious growing swine. P.S.E.B.M. 190: 393–398.

CHAPTER 7

Digesta pH and buffer capacity of African catfish, *Clarias gariepinus* as affected by dietary cation anion difference

Y. Dersjant-Li[1,2], S. Wu[1], J. W. Schrama[3], M. W. A Verstegen[2] and J. A. J. Verreth[1]

[1] *Fish Culture and Fisheries Group,* [2] *Animal Nutrition Group,* [3] *Adaptation Physiology Group, Department of Animal Sciences, Wageningen University, P.O.Box 338, 6700 AH Wageningen, The Netherlands*

Submitted to Comp. Biochem. Physiol.

Abstract: The impact of dietary cation anion difference (CAD, Na + K - Cl, mEq kg^{-1}) on digesta pH and buffer capacity was investigated in juvenile African catfish, *Clarias gariepinus*. A 2×2 factorial arrangement was applied, which consisted of two dietary CAD levels (-100 and 700 mEq kg^{-1}) and two postprandial measurement times (0.5 and 3 h). At the end of a 10-day feeding period, fish were killed with overdose TMS. Stomach and small intestine digesta pH was measured and buffer capacity of stomach digesta was analyzed. A dietary CAD level of 700 mEq kg^{-1} resulted in significantly high stomach digesta pH (6.39) and buffer capacity compared to a dietary CAD level of -100 mEq kg^{-1} (4.55). Dietary CAD did not affect the small intestinal digesta pH. At 3 h after feeding, the stomach digesta pH decreased and small intestinal digesta pH increased compared to 0.5 h after feeding. In conclusion, fish could adjust the pH of ingested diets shortly after feeding in the stomach and actively regulate acid base balance in the gastro-intestinal tract after ingesting diets with different CAD levels.

Keywords: acid base balance, African catfish, digesta pH, stomach, small intestine

Introduction

In African catfish (*Clarias gariepinus)*, a negative (-100) dietary cation anion difference (CAD, Na + K - Cl, mEq kg^{-1} diet) depressed feed intake and growth, while a dietary CAD level of 700 mEq kg^{-1} improved performance and nutrient utilization (Dersjant-Li *et al.*, 1999). African catfish maintained blood pH with different CAD diets, which is probably realized by adjustment of Na^+ and Cl^- concentrations in the blood (Dersjant-Li *et al.*, 1999). A dietary CAD level of 700 mEq kg^{-1} gives a high dietary pH (8.4), while a CAD level of –100 mEq kg^{-1} results in a low dietary pH (5.6). However, in an animal's digestive system, the optimal pH for digestion is about 3 in the stomach and about 6-7 in the small intestine. Therefore, the animal has to correct the pH of the ingested diets when these contain sub-optimal CAD levels. However, to what extent African catfish can maintain pH in the digestive system has not been investigated so far. The hypothesis of this study is that ingesting diets with different CAD levels may alter pH and buffer capacity of chyme in the stomach shortly after feeding, but animals can adjust this pH rapidly during the postprandial period. Therefore in this study we measured pH in the stomach and small intestine at 0.5 and 3 h after feeding respectively, at two deviating dietary CAD levels. The results could provide information as to what extent the animal can regulate the acid base balance in the digestive system.

Materials and methods

Experimental design

A 2×2 factorial arrangement was applied, which consisted of two dietary CAD levels (-100 and 700 mEq kg^{-1}) and two sampling times (0.5 and 3 h post feeding). Each treatment diet was randomly assigned to 6 tanks, from them 3 tanks were sampled at 0.5 h after feeding, and the other 3 tanks were sampled at 3 h after feeding. Sampling occurred at the last day of a 10-day feeding period.

Diets and feeding

The basal diet consisted of wheat, wheat gluten, soybean meal, meat meal, fishmeal and premix (Table 1). To obtain a CAD level of 700 mEq kg^{-1}, Na_2CO_3 was added to the basal diet. The –100 mEq kg^{-1} CAD diet was made by supplementation of $CaCl_2 \cdot 2H_2O$, in replacement of $CaCO_3$, to the basal diet (Table 1). By doing so, Ca content was equalized in

these two test diets. Diets were pelleted and then oil coated (4%). Diets were provided two times a day to satiation for a 10-day feeding period. Meal was given manually in about one hour period.

Table 1. Composition of the treatment diets

	Dietary CAD (mEq kg^{-1})	
	-100	700
Basal ingredients (g kg^{-1})		
Fish meal	360	360
Wheat	196.5	196.5
Wheat gluten	100	100
Soybean meal	75	75
Meat meal	30	30
Feather meal	75	75
Herring oil	60	60
Premix[1]	10	10
Binder	25	25
Monocacium phosphate	7	7
Additives (g kg^{-1})		
$CaCO_3$	20	35
Na_2CO_3		26.5
$CaCl_2$. $2H_2O$	22.5	
Diamol[2]	19	
Analyzed composition (g kg^{-1})		
Dry matter	893	897
Crude protein	453	456
Crude fat	114	108
Ash	129	135
Gross energy (kJ g^{-1})	185	185
Na	2.2	12.7
K	8.6	8.9
Cl	15.2	4.3
pH	5.6	8.4
Buffer capacity (mEq kg^{-1})[3]	1221	1938
Measured CAD (mEq kg^{-1})[4]	-114	657

[1] Premix was provided by Coppens International BV, Helmond, the Netherlands, [2] Diamol is diatomaceous shell powder (Biakon N.V., Grobbendonk, Belgium), [3] Quantity of 1.0N HCl required for the gastric digestion pH (3.0) of 1 kg feed, [4] Calculated from Na, K and Cl content.

Fish and facilities

Prior to the start of the experiment, African catfish (*Clarias gariepinus*) were reared for five weeks with a commercial diet to obtain a sufficiently large body size for collecting digesta. 144 fish with a mean initial body weight of 272g were distributed at random to 12 aquaria (70L). Two dietary treatments were randomly assigned to the aquaria. Fish were reared at a temperature of 27 ± 0.5°C in a recirculation system with aeration in each aquarium, under a 12L: 12D light regime. O_2 concentration was maintained above 6.0 mg L^{-1}. To avoid bacterial infections, the water salinity was maintained between 2-2.5g L^{-1} by adding agriculture salt into the system storage tanks.

Sampling procedure

The separated tanks were additionally separated by cardboard to avoid that the sampling activity in one tank would disturb the fish in the other tank. To assure that the fish were sampled at the desired time, feeding and sampling was done in 30 min intervals for each tank. Samples were taken alternately between the two treatment groups. At the desired feeding time the fish were fed for 5 min *ad lib*. Twenty minutes after this meal, about 70% of the water was discharged quietly from the aquarium. Tricaine methane sulphonate (TMS, Crescent Research Chemicals, Phoenix, AZ, USA) was added (0.3 g L^{-1}) to the remaining water to anaesthetize the fish. Five minutes later the fish were collected and killed in an overdose TMS (0.6g L^{-1}) solution. Because of limited time schedule, 10 of the 12 killed fish were randomly taken for dissection. Immediately after dissection, the stomach and the small intestine were separated.

Analytical method

The pH of stomach digesta was measured immediately after dissection by inserting a pH electrode (ORION pH meter, 920A) into the stomach. Small intestinal (SMI) digesta was collected and well mixed. Then the pH was measured by inserting the pH electrode into the well-mixed digesta. The collected chyme from the stomach was stored at –20 °C before analysis of buffer capacity. For measuring buffer capacity, the stomach digesta of all sampled fish were pooled per aquarium. The buffer capacity of small intestinal digesta was not measured because of the pH in the intestinal digesta did not vary with treatments. Buffer capacity was defined as the amount of 1.0N HCl needed to bring the pH of the digesta down to pH=3 (mEq kg^{-1}). The buffer capacity was measured according to the following procedure:

about 1-2 g of sample was incubated in 20ml 0.1N HCl solution in 37°C water bath for one hour. Afterwards the suspension was titrated to pH=3 with 0.1 N NaOH solution. Diet composition was analyzed in the same manner as in chapter 3.

Data and statistical analysis

The average value from each aquarium was used as one experimental unit. Data were tested by two way ANOVA using SAS program (SAS 1990). Effect of dietary CAD, sampling time and their interactions were tested.

Results

Stomach and intestinal digesta pH

A significant effect of dietary CAD, sampling time and their interaction on stomach digesta pH was found (Table 2). Stomach digesta pH was significantly higher in dietary CAD of 700 mEq kg^{-1} group than in dietary CAD of -100 mEq kg^{-1} CAD group both 0.5 and 3 h after feeding. The pH level in the stomach was also significantly lower 3 h after feeding (4.96) compared to 0.5 h after feeding (5.97) for both CAD groups. The dietary CAD and sampling time interaction was caused by a rapidly decreasing stomach digesta pH in 700 mEq kg^{-1} dietary CAD group. Dietary CAD did not affect small intestinal digesta pH neither 0.5 h nor 3 h after feeding. The small intestinal digesta pH significantly increased at 3 h (7.20) after feeding compared to 0.5h (6.86) after feeding for both CAD groups (Table 2). No dietary CAD and sampling time interaction effect was observed for small intestinal digesta pH.

Table 2. Effect of dietary CAD and feeding time on digesta pH and buffer capacity

Dietary CAD (mEq kg^{-1})	-100		700		SEM	Effect[1]
Time after feeding (h)	0.5	3	0.5	3		
Stomach digesta pH	4.66	4.44	7.29	5.48	0.14	C***, T***, C×T***
SMI[2] digesta pH	6.80	7.17	6.91	7.23	0.08	T**
BC[3] of stomach digesta	388	299	607	492	32.6	C***, T**

[1] Statistical analysis: C refers to dietary CAD, T refers to sampling time, C×T represents the interaction between CAD and sampling time. NS = non significant, * = $P < 0.05$, ** = $P < 0.01$, *** = $P < 0.001$, [2] SMI- small intestine, [3] BC- buffer capacity (mEq kg^{-1}).

Buffer capacity

Buffer capacity of stomach digesta was significantly higher at a dietary CAD level of 700 mEq kg^{-1} than at a dietary CAD level of –100 mEq kg^{-1}. It decreased significantly 3 h after feeding compared to 0.5 h after feeding. No dietary CAD and sampling time interaction was observed (Table 2).

Discussion

Stomach and intestinal digesta pH

The stomach digesta pH is clearly related to dietary pH. Fish that ingested a diet with high pH showed higher stomach digesta pH than a diet with low pH. In both CAD groups, at 0.5 h after feeding, the stomach digesta pH was about one unit lower compared to the original dietary pH. This indicated that fish actively secreted HCl to lower the pH of the ingested diets. At three hours post feeding, the pH of the stomach digesta in dietary CAD of 700 mEq kg^{-1} group was almost 3 units lower than the pH level of this diet. In the –100 mEq kg^{-1} CAD group it was only 1.2 units lower than the original pH level of the diet. After ingesting a high pH diet, fish secreted more HCl to correct for the high dietary pH and tried to reach an optimal pH in the chyme of the stomach.

The small intestinal digesta pH was similar in the two dietary CAD groups, both at 0.5 h and 3 h after feeding. This implied that fish could maintain the desired pH level in the small intestine after ingesting diets with different pH levels. It also indicated that in the –100 mEq kg^{-1} CAD group, fish had to secrete more $NaHCO_3$ from the pancreas to increase the pH of the digesta that entered the intestine from the stomach. At 3 h after feeding the pH of the small intestinal digesta was significantly higher than 0.5 h after feeding. This indicated that fish could actively regulate acid base balance in the small intestine.

The results are comparable to the observations by Newton and Burtle (1995). They found a cecal pH of 7.36 in channel catfish fed at 3% of body weight and reared at high temperature. However their observed gastric pH (3.59) was lower than our observations. In their study the pH was measured at 5 h after feeding. This may explain the low gastric pH.

The buffer capacity of the stomach digesta

The high buffer capacity of the stomach digesta in dietary CAD of 700 mEq kg^{-1} group was a consequence of the high buffer capacity of this diet. The high buffer capacity in this CAD group means that more HCl was needed to lower stomach digesta pH compared to the situation in animals receiving a dietary CAD of –100 mEq kg^{-1}. At 3 h after feeding fish have already compensated the high pH of the ingested diet and therefore, the buffer capacity was significantly lower than the values of 0.5 h after feeding.

Conclusion

Dietary CAD can affect the pH and buffer capacity of stomach digesta within a short period after feeding. After ingesting a high CAD diet fish need to secrete more HCl to lower the pH of the ingested diet in the stomach. With a low CAD diet fish need to secrete more HCO_3^- in the small intestine to neutralize the low pH digesta received from the stomach. However African catfish can adjust the pH of ingested diets in the stomach and maintain acid base balance in the small intestine with different CAD levels.

Acknowledgements

The authors thank to M. ter Veld, R. Booms and T. Leffering for their assistance in sampling and measurements, S. Leenstra and A. Hutten for their help in system management.

References

Dersjant-Li, Y., Verreth, J. A. J., Evers, F., Tijssen, P. A. T., Booms, R. and Verstegen, M. W. A. 1999. The influence of dietary cation-anion differences on acid-base balance, food intake, growth and nutrient utilization of juvenile African catfish *Clarias gariepinus* (Burchell). Fish Physiol. Biochem. 20: 305-311.

Newton, G. L. and Burtle, G. J. 1995. Water temperature and feed intake influence gastric pH of channel catfish. Annual Report. Animal and Dairy Science Department, College of Agricultural and Environmental Science, The University of Georgia [online]. http://www.ads.uga.edu/annrpt/1995/95-toc.htm. p. 18-19.

SAS. 1990. SAS User's Guide: Statistics (Version 6 Ed.). SAS Inst. Cary, NC.

CHAPTER 8

Performance, digesta characteristics, nutrients flux, plasma composition and organ weight in pigs as effected by dietary cation anion difference and non starch polysaccharide

Yueming Dersjant-Li[1,2], Martin W. A. Verstegen[1], Hagen Schulze[4], Tamme Zandstra[1], Huug Boer[1], Johan W. Schrama[1,3] and Johan A. Verreth[2]

[1] *Animal Nutrition Group,* [2] *Fish Culture and Fisheries Group,* [3] *Adaptation Physiology Group, Wageningen Institute of Animal Sciences, Wageningen University, Wageningen, The Netherlands.* [4] *Finnfeeds International Ltd., Marlborough, United Kingdom. Present address: Provimi B.V., P.O.Box 5063, 3008 AB Rotterdam, The Netherlands*

Submitted to J. Animal Science

Abstract: Two dietary cation anion difference (CAD, Na + K – Cl, mEq kg^{-1}) levels (–100 and 200 mEq kg^{-1}) and two dietary nonstarch polysaccharide (NSP) levels (10 and 15%) were used in a 2 × 2 factorial arrangement to evaluate performance, digesta pH and buffer capacity, apparent digestibility, plasma composition and organ weight in pigs. Seven pigs with a mean initial weight of 7.5 kg were used in each treatment. Piglets had *ad lib* access to feed and water during the 3-wk experimental period. At the end of the experiment, all pigs were euthanized approximately 2.5 h after addition of fresh feed. Liver, kidney and small and large intestine were removed and weighed immediately. Gastric and small intestinal (divided into two equal parts) digesta were collected. Dry matter (DM), pH, buffering capacity, viscosity, acid insoluble ash, mineral content (Na, K and Cl), nitrogen and gross energy content (only in the second part of the small intestine) of the digesta were measured. An interaction between dietary CAD and dietary NSP was observed for feed intake, growth, apparent digestibility of DM, gross energy and N in the distal small intestine, and chloride in the stomach and duodeno-jejunal part of the small intestine. Increasing dietary NSP content increased 'apparent digestibility' of DM, chloride, sodium and potassium in the first part of small intestine. Lowering dietary CAD levels significantly increased 'apparent digestibility' of DM in the stomach and chloride flux in the stomach and in the small intestine. No significant differences were found for pH, buffer capacity, and viscosity of digesta. Plasma urea decreased in the low dietary CAD groups. Liver and large intestine weights were significantly lower at a CAD of –100 mEq kg^{-1} compared to CAD of 200 mEq kg^{-1}. The results showed that the effect of CAD on feed intake and growth depends on the dietary NSP levels.

Keywords: digestibility, electrolyte balance, digesta characteristics, piglets, nonstarch polysaccharides

Introduction

In pigs, it is generally accepted that negative dietary cation anion difference (CAD, Na + K - Cl, mEq kg^{-1} diet) can result in depressed voluntary feed intake, whereas dietary CAD in a positive range will optimize the growth of pigs. The dietary CAD for optimal performance however, varied in different studies (Austic *et al.*, 1983; Patience *et al.*, 1987; Haydon *et al.*, 1989; Park *et al.*, 1994). This may imply an interaction between dietary CAD and other dietary components. The studies which investigated dietary CAD, were mostly done by alteration of mineral content in a basal diet. Although it is relatively well known that dietary CAD and nonstarch polysaccharide (NSP) levels may effect feed and (or) water intake (Yen *et al.*, 1981; Bach Knudsen and Johansen, 1995; Mroz *et al.*, 1995), scarce information is available on the interactive effects of these dietary factors in the body of pigs. The physiological roles of NSP and electrolytes in the gut lumen are complex and not always fully understood (Heitzmann, 1991). Both factors contribute to intraluminal acid base balance, nutrient passage rate, gut morphology, digestion (including pancreatic secretions), metabolism and bi-directional fluxes of organic and inorganic compounds. Van der Klis *et al.* (1993) observed that an indigestible soluble polysaccharide (carboxy methyl cellulose) inclusion in the diet reduced the rate of mineral absorption throughout the small intestine, especially for sodium, in broilers. The hypothesis of our study is that a change in NSP content of the diet may affect mineral absorption in pigs and consequently may interact with dietary CAD.

Materials and methods

Experimental design and treatment diets

A 2 × 2 factorial experiment, with two dietary NSP levels (10 and 15%) and two dietary CAD levels (–100 and 200 mEq kg^{-1}) was conducted. The 10% NSP diet consisted mainly of corn and soybean meal. The 15% NSP diet consisted of corn, soybean meal and 15% wheat middling (Table 1). Both diets had a calculated CAD level of 200 mEq kg^{-1}. The –100 mEq kg^{-1} dietary CAD level was achieved by adding $CaCl_2$ $2H_2O$ (2.3 to 2.6%) to the diet in replacement of $CaCO_3$. In this way the calcium content was equalized between –100 and 200 mEq kg^{-1} CAD diets. Ash and energy contents were maintained constant between the –100 and 200 mEq kg^{-1} CAD diets by altering the diamol content. The experiment was conducted

in two trials, trial 2 was done one week after the first trial was finished. A total of 12 pigs (3 per treatment) were used in the first trial and 16 pigs (4 per treatment) were used in the second trial, each pig was used as an experimental unit. The pigs were randomly assigned to dietary groups. The experimental period for each trial was three weeks with *ad lib* access to feed and water. Every morning non-consumed feed was collected and fresh feed was offered.

Animals and husbandry

Female pigs (offspring of female of [(Large white × Hampshire) × (Dutch Landrace × Swedish Landrace)] and male of Netherlands York) with a mean initial weight of 7.5±1.2 kg (sd) were housed individually in pens (1.10 × 2.75m, with smooth surfaced BOLIDT synthetic floor based on concrete, cement and sand). The pigs were allowed to a commercial feed for three days before the experiment started. Feed was given in a wooden feeding trough. The room temperature was maintained at 22 °C, with an average humidity of about 60%.

Sampling and measurements

Feed intake was recorded daily. Body weight gain was measured weekly. At the end of the 3-wk experimental period, all pigs were euthanized with an intravenous injection of T61 (Hoechst Roussel Vet, containing 200 mg embutramide, 50 mg mebezoniumjodide and 5 mg tetracaïnehydrochloride per ml), at approximately 2.5 h after addition of fresh feed in the morning. Fresh feed was added to assure sufficient chyme in the stomach. Blood samples were taken from the vena cava before euthanization and were centrifuged at 1500×g for 10 min to obtain plasma. The plasma samples were stored on dry ice and sent directly to a laboratory for measuring plasma urea and ammonia. Liver and kidney were removed and weighed immediately.

Plasma ammonia and urea concentrations were determined on a Vitros dry chemistry analyzer (Ortho-Chemical Diagnose Rochester (NY), USA) in accordance with the manufacturer recommendation, using Vitros AMON Slides and Vitros BUN/UREA Slides respectively.

Table 1. Diet compositions (as-fed basis)

Diet	10% NSP		15% NSP	
CAD (mEq kg^{-1})	200	-100	200	-100
Ingredient (g kg^{-1})				
Corn (8.5 % CP)	655.35	650.15	510.8	505.55
Soybean meal (46.7% CP)	250	250	250	250
Wheat middlings			150	150
Fish meal (>68 % CP)	50	50	50	50
Lysine HCl	1.85	1.85	1.85	1.85
DL – Methionine	0.85	0.85	0.85	0.85
L – Threonine	0.35	0.35	0.35	0.35
Limestone	12.8		13.5	
Dicalcium phosphate	10		9	
Monocalcium phosphate		8		7.5
Salt	3	3	3	3
Trace mineral - vitamin	2.5	2.5	2.5	2.5
$CaCl_2.2H_2O$	0.4	22.5	3.45	25.5
Diamol[2]	12.9	10.8	4.7	2.9
Analyzed composition (g kg^{-1})				
Dry matter	874	872	875	872
Nitrogen	33.4	33.5	35.4	35.4
Gross energy (kJ g^{-1})	15.8	15.7	16.1	16.0
Ash	68.4	68.4	70.5	67.6
Crude fiber	37.7	38.2	46.0	46.6
NSP[3]	108	108	150	149
Viscosity (cP[4])	0.93	0.99	0.97	0.98
pH	6.3	5.3	6.1	5.3
Buffer capacity (mEq kg^{-1})	749	486	793	523
Na	2.16	2.12	2.25	2.18
K	7.71	7.80	9.24	9.27
Cl	3.5	14.8	5.9	15.5
CAD (mEq kg^{-1})[5]	192	-125	168	-104

[1] The vitamin and mineral premix supplied per 1 kg of the diet: 9000 IU of vitamin A, 1800 IU of vitamin D_3, 40 mg of vitamin E, 5 mg of riboflavin, 30 mg of niacin, 12 mg of d-pantothenic acid, 350 mg of choline chloride, 40 µg of vitamin B_{12}, 3 mg of vitamin K, 50 mg of vitamin C, 1 mg of folic acid, 0.1 mg of biotin, 0.52 mg of Co from $CoSO_4.7H_2O$, 0.06 mg of Se from $Na_2SeO_3.5H_2O$, 0.12 mg of I from KI, 80 mg of Fe from $FeSO_4.7H_2O$, 170 mg of Cu from $CuSO_4.5H_2O$, 44 mg of Mn from MnO_2, 73 mg of Zn from $ZnSO_4.H_2O$. [2] Diamol is diatomaceous shell powder (Biakon N.V., Grobbendonk, Belgium). [3] Calculated as organic matter – crude protein – crude fat – starch - suger, based on calculated diet composition. [4] cP = centiPoise (1 cP = 1/100 dyne sec/cm^2 = 1 mPa.s). [5] Calculated from measured Na, K and Cl contents.

The small and large intestine were removed, separated at the ileal-cecal valve. The chymes from stomach and small intestine (divided into two equal parts) were collected by gently squeezing. The stomach, small and large intestine were rinsed, dried with paper towel and weighed.

The digesta pH was immediately measured by inserting a pH electrode (pH 300, HANNA Instrument) into the well-mixed digesta. Afterwards, 10 g of digesta was sampled for measuring viscosity. The remaining digesta samples were kept at –20°C for later analysis of DM, buffering capacity, acid insoluble ash, mineral content, nitrogen and energy content (only in the second part of the small intestine).

For measuring viscosity of digesta, about 10g of digesta was centrifuged at 12900×g for 10 min. to obtain the liquid fraction. The viscosity of the liquid was immediately measured after centrifugation, using a viscometer (model DV-II+ Viscometer, Brookfield). Gastric digesta were diluted 1:1 with demineralized water before centrifugation because of its high dry matter content. The liquid after centrifugation was also used for measuring chloride content. Chloride in the diets was determined based on the method described by Fauchon *et al.* (1995) with some modifications, by using a chloride meter (PCLM digital chloride meter, Jenway Ltd. Dunmow, England).

Nitrogen content of digesta and diet was analyzed by the Kjeldahl method (ISO 5983, 1979). Dry matter contents of feed and digesta, ash content of feed, and acid insoluble ash contents of feed and digesta were measured according to ISO procedures (ISO 6496 (1983), ISO 5984 (1978) and ISO 5985 (1978) respectively). Crude fiber content of feed was analyzed according to NEN 5417 (1988). Energy contents of the feed and digesta were determined by bomb-calorimetry (IKA Calorimeter C 7000). When measuring acid insoluble ash content of the digesta, the ashed digesta was boiled in hydrochloride acid (3mol L^{-1}), followed by filtering to collect the acid insoluble ash. The filtrate with acid insoluble ash was thoroughly washed with demineralized water and the liquid after filtration was carefully collected and used for measuring sodium and potassium in the digesta. Sodium and potassium were measured by atomic absorption spectrometry (SpectrAA 300, Varian Australia Pty Ltd, Mulgrave Victoria, Australia). Sodium and potassium contents in the diet were measured after dissolving the ash samples in 3N HCl acid. Acid binding capacity was measured by the method as described by Prohaszka and Baron (1980). Dietary pH was measured using the same method as in chapter 1.

Data analysis

Digestibility of nutrient was calculated using acid insoluble ash as the indicator, according to the following equation:

$$Di\% = 100\text{-}100\times[(\%AIA\ feed \times \%Nutrient\ digesta)/(\%AIA\ digesta \times \%Nutrient\ feed)]$$

Where Di% is the digestibility of nutrient in percentage and AIA is the acid insoluble ash.

Statistical analysis

Data were analyzed by a three way ANOVA, according to the general linear model procedure of SAS (SAS, 1990), CAD, NSP, trial effect and CAD and NSP interaction were tested as factors.

Results and discussion

Feed intake and growth

An interaction ($p < 0.05$) between dietary CAD and dietary NSP levels was observed for feed intake and growth (Table 2). Reducing dietary CAD from 200 to –100 mEq kg^{-1} increased feed intake and growth in pigs fed 10% NSP diet but decreased feed intake and growth in pigs fed 15% NSP diet. Feed / gain ratio was not affected either by dietary CAD or by NSP. This indicates that the detrimental effect on growth was caused by a reduction in feed intake but not efficiency of feed utilization.

Table 2. Feed intake, growth (g d^{-1}) and feed / gain in different dietary treatment groups

Diet	10% NSP		15% NSP		SEM	Effect[1]
CAD (mEq kg^{-1})	200	-100	200	-100		
Feed intake	395	503	519	430	41.0	CAD×NSP*
Growth	268	319	330	269	24.5	CAD×NSP*
Feed / gain	1.47	1.58	1.57	1.60	0.13	NS

[1] Statistical analysis: NS = $P > 0.10$, * = $P < 0.05$

Patience and Chaplin (1997) observed that weight gain of pigs tended to increase on a diet with a CAD of –20 mEq kg^{-1} compared to a diet with a CAD of 163 mEq kg^{-1}, when

animals were fed a barley and soybean meal based diet at a restricted feeding level. A linear decrease in growth with increasing dietary CAD from 238 to 399 mEq kg^{-1} was found by Wondra *et al.* (1995) in *ad lib* fed pigs with a corn and soybean meal based diet. In another experiment by the same authors, the growth of pigs was not affected by changing dietary CAD from 134 to 222 mEq kg^{-1} with a wheat and soybean meal based diet. On the other hand, with a corn and soybean meal based diet, a linear and quadratic increase in feed intake and weight gain was obtained by increasing dietary CAD levels from –90 to 172 mEq kg^{-1} (Patience and Wolynetz, 1990). These inconsistent effects of dietary CAD on the performance of pigs probably were influenced by other dietary components. Van der Klis *et al.* (1993) reported that high NSP content reduced cation absorption. Therefore an increase in dietary CAD may increase cation availability for a high NSP diet. This may explain the better performance of pigs fed 200 mEq kg^{-1} CAD diet compared to –100 mEq kg^{-1} CAD diet, at a high NSP level.

Digesta pH and buffer capacity

Digesta pH and buffer capacity were not significantly affected by dietary CAD or NSP levels (Table 3). This indicates that the pigs could adjust the pH in the stomach and in the small intestine after ingesting diets with different CAD's. Patience *et al.* (1986) found no effect of dietary supplementation of $NaHCO_3$ on gastrointestinal pH, although gastric pH tended to increase and pH in the colon tended to decrease. In the present study, we provided fresh feed 2.5 h before dissection, so the animals could eat up to the moment of sacrifice and have chyme in all parts of GI tract. This feeding procedure apparently allowed the animal to adjust acidity of intragastric content. With 10% NSP diet, the stomach digesta pH was numerically lower in the –100 mEq kg^{-1} CAD group than the one in 200 mEq kg^{-1} CAD group. In a study with African catfish, the stomach digesta pH was significantly lower in -100 mEq kg^{-1} CAD group compared to 700 mEq kg^{-1} CAD group 0.5 and 3 h after feeding. The stomach digesta pH was significantly reduced 3 h after feeding compared to 0.5 h after feeding in 700 mEq kg^{-1} CAD group (Dersjant-Li *et al.*, chapter 7).

Similar to digesta pH, the buffer capacity of digesta in the stomach and in the small intestine was not significantly influenced by dietary CAD (Table 3).

Table 3. Digesta pH and buffer capacity of stomach, small intestine section 1 (SMI-1) and small intestine section 2 (SMI-2) in different dietary treatment groups

Diet	10% NSP		15% NSP		SEM	Effect[1]
CAD (mEq kg^{-1})	200	-100	200	-100		
Digesta pH						
Stomach[2]	4.33	3.78	4.17	4.15	0.28	NS
SMI-1	5.94	5.82	5.89	5.78	0.06	NS
SMI-2	6.56	6.64	6.58	6.78	0.10	NS
Buffer capacity (mEq kg^{-1})						
Stomach	108	87	105	104	15.3	NS
SMI-1	106	107	112	117	9.65	NS
SMI-2	203	181	192	189	12.15	NS

[1] Statistical analysis: NS = $p > 0.10$. [2] Measured in 1:1 diluted digesta.

Apparent digestibility of DM, N and energy

The 10% NSP diet had lower ($p < 0.001$) 'digestibility' of DM (negative) in the stomach than the 15% NSP diet, measured by using acid insoluble ash as an indicator (Table 4). The different apparent 'digestibility' of DM in the stomach between the two diets may be associated with two aspects. Firstly, it may be related to a difference in secretion (acid and enzymes) in the stomach. Secondly, it may be associated with a different distribution between the liquid and solid phases of the digesta regarding the emptying rates of these phases. The latter will result in different passage rates. The 15% NSP diet gave higher stomach 'digestibility' of DM than the 10% NSP diet, this may be a consequence of a difference in passage rate between phases. Rodriguez *et al.* (1988) observed that addition of hay increased passage rate of the non-fibrous digesta in pigs. Dietary CAD did not significantly affect 'digestibility' of DM in the stomach (Table 4).

In the first part of the small intestine, both NSP and CAD levels influenced apparent digestibility of DM significantly. The apparent digestibility of DM was higher ($p < 0.01$) for 15% NSP diet than for 10% NSP diet. A dietary CAD level of -100 mEq kg^{-1} had a higher ($p < 0.05$) apparent digestibility of DM than a dietary CAD level of 200 mEq kg^{-1} (Table 4).

In the second part of the small intestine, an interaction was found between dietary CAD and NSP for apparent digestibility of DM. For apparent digestibility of N and energy, only a tendency of such an interaction was observed (Table 4). In the case of the 10% NSP diet, low dietary CAD (-100 mEq kg^{-1}) decreased apparent digestibility of DM, N and energy. For the

15% NSP diet, higher digestibility values for DM, N and energy were found at a low dietary CAD (-100 mEq kg^{-1}) compared to a high dietary CAD (200 mEq kg^{-1}).

'Digestibility' of minerals

Both dietary CAD and NSP ($p < 0.001$) influenced chloride 'disappearance' in the stomach and in the first part of the small intestine (Table 4). An interaction between dietary CAD and NSP was also found. Chloride 'disappearance' was significantly lower in the 200 mEq kg^{-1} CAD groups (negative values) compared to the –100 mEq kg^{-1} CAD groups. The high chloride 'disappearance' in the stomach of the –100 mEq kg^{-1} CAD groups may indicate that less HCl was secreted by stomach. On the other hand, the 'disappearance' ('digestibility') of chloride is expressed as a percentage of chloride intake. The chloride content of –100 mEq kg^{-1} CAD diet is about 3-4 times higher than that of the 200 mEq kg^{-1} CAD diet (Table 1), making the chloride intake in these groups much higher than in the 200 mEq kg^{-1} groups. 10% NSP diet had a lower chloride digestibility than the 15% NSP diet. The 'disappearance' of chloride in the first part of the small intestine was lower than in the stomach, this might be a consequence of receiving stomach digesta which containing large amounts of HCl. The pancreas will secrete $NaHCO_3$ to neutralize the HCl in the small intestine, therefore the digesta pH of this part of intestine was not affected. The 'disappearance' of chloride in the first part of the small intestine showed that more chloride was secreted (or needed) for the 10% NSP diet than for the 15% NSP diet. In this situation, a high chloride content in the diet would help the 'digestive' process in the stomach. This might explain why a low dietary CAD (-100 mEq kg^{-1}) showed a high feed intake with the 10% NSP diet.

Dietary CAD did not affect sodium 'disappearance' in the small intestine (Table 4). A negative 'disappearance' of sodium in the small intestine was found regardless of dietary NSP and CAD levels (Table 4). The results agreed with Jørgensen *et al.* (1985), who observed that the amount of sodium which passed the terminal ileum was 3-7 times that of intake. Most of the sodium was reabsorbed in the hind gut. Van der Klis *et al.* (1993) also observed an intraluminal influx of sodium in jejunum and ileum in broilers. In the present study, the 'digestibility' values for sodium were significantly higher for the 15% NSP diet than the 10% NSP diet in the first part of the small intestine. This indicated that more sodium was secreted (or needed) in the small intestine of pigs ingesting the 10% NSP diet. This extra sodium secretion is related to the high chloride content in the digesta. Which means when the

chloride content is high in the small intestine animals will probably secrete more sodium bicarbonate to maintain the electrolyte balance. In the second part of the small intestine, no difference in sodium digestibility was found between both diets.

Table 4. Apparent digestibility (%) of dry matter, nitrogen and minerals measured in stomach, small intestine section 1 (SMI-1) and small intestine section 2 (SMI-2)

Diet	10% NSP		15% NSP		SEM	Effect[1]
CAD (mEq kg^{-1})	200	-100	200	-100		
DM						
Stomach	-15.7	-13.8	4.17	17.8	5.57	NSP***
SMI-1	19.0	25.2	31.7	48.3	5.03	NSP**, CAD*
SMI-2	49.8	43.1	41.1	54.7	4.30	CAD×NSP *
N						
SMI-2	46.9	42.5	44.8	58.6	4.51	CAD×NSP†
Energy						
SMI-2	49.4	43.1	41.0	58.6	4.45	CAD×NSP†
Cl						
Stomach	-128.6	48.55	-30.7	57.2	13.9	CAD***, NSP ***, CAD× NSP **
SMI-1	-422.1	-34.9	-165.1	4.82	29.7	CAD***, NSP ***, CAD× NSP ***
SMI-2	-50.9	37.8	-1.98	68.6	20.1	CAD***, NSP †
Na						
SMI-1	-395.9	-407.5	-303.5	-201.1	57.7	NSP*
SMI-2	-247.8	-337.7	-306.3	-173.4	88.3	NS
K						
SMI-1	43.8	41.2	63.5	61.3	5.38	NSP**
SMI-2	77.6	71.1	77.3	82.2	4.39	NS

[1] Statistical analysis: NS = $p > 0.10$, † = $p < 0.10$, * = $p < 0.05$, ** = $p < 0.01$, *** = $p < 0.001$.

In contrast to the 'digestibility' of sodium, potassium was absorbed in the small intestine (Table 4). In the first part of the small intestine, the absorption of potassium was significantly higher for 15% NSP diet than for 10% NSP diet. This is related to the higher potassium concentration in the feed. The absorption of potassium in the second part of the small intestine was comparable for both diets. Changing of dietary CAD levels did not significantly influence potassium absorption in the small intestine (Table 4).

Viscosity, plasma urea and ammonia, organ weight

Viscosity of stomach and small intestine digesta was not significantly influenced by dietary NSP levels or by changes of dietary CAD (Table 5). This is in contrast to findings by Dusel *et al.* (1997), who found that increasing dietary NSP content increased digesta viscosity.

Table 5. Viscosity (cP[1]) of stomach digesta, small intestine section 1 (SMI-1) digesta and small intestine section 2 (SMI-2) digesta in different dietary treatment groups

Diet	10% NSP		15% NSP		SEM	Effect[2]
CAD (mEq kg^{-1})	200	-100	200	-100		
Stomach	0.85	0.84	0.85	0.81	0.029	NS
SMI-1	1.27	1.43	1.45	1.41	0.085	NS
SMI-2	1.75	1.98	1.76	1.83	0.116	NS

[1] cP = centiPoise (1 cP = 1/100 dyne sec/cm^2 = 1 mPa.s). [2] Statistical analysis: NS = $p > 0.10$.

Dietary CAD significantly affected plasma urea concentration (Table 6). It was higher in the 200 mEq kg^{-1} CAD groups than in the –100 mEq kg^{-1} CAD groups. This is in agreement with Coffey *et al.* (1985), who found that blood urea decreased with increasing dietary chloride in pigs. Plasma ammonia was significantly lower in the 10% NSP diet than 15% NSP diet.

Table 6. Plasma urea and ammonia concentrations in different dietary treatment groups

Diet	10% NSP		15% NSP		SEM	Effect[1]
CAD (mEq kg^{-1})	200	-100	200	-100		
Urea, mmol L^{-1}	4.43	3.09	3.60	2.84	0.228	CAD***, NSP†
Ammonia, umol L^{-1}	40.7	54.1	91	68.1	12.0	NSP*

[1] Statistical analysis: † = $p < 0.10$, * = $p < 0.05$, *** = $p < 0.001$.

Decrease of dietary CAD from 200 mEq kg^{-1} to -100 mEq kg^{-1} significantly decreased liver and large intestine weights (as a percentage of body weight) (Table 7). The decreased liver weight may imply a reduced protein deposition rate. The low liver weight in the –100 mEq kg^{-1} CAD group may also be associated with a low urea concentration in the plasma. Patience *et al.* (1987) suggested that dietary $NaHCO_3$ increased lysine uptake by the liver and decreased lysine uptake by the kidney. The decrease in large intestinal weight in the –100

mEq kg^{-1} CAD group may be related to the water absorption rate. It was observed in a earlier study (Dersjant-Li *et al.*, unpublished data) that pigs fed a 200 mEq kg^{-1} CAD diet consumed more water than pigs fed a –200 mEq kg^{-1} CAD diet. No significant difference in kidney weight was observed for the different dietary CAD groups (Table 7).

Table 7. Proportion (% of body weight) of the gastrointestinal tract (stomach, small and large intestine), liver and kidney of pigs as affected by dietary CAD and NSP contents

Diet	10% NSP		15% NSP		SEM	Effect[1]
CAD (mEq kg^{-1})	200	-100	200	-100		
Liver	2.71	2.45	2.74	2.65	0.097	CAD*
Kidney	0.60	0.54	0.59	0.59	0.021	NS
Stomach	0.90	0.83	0.96	0.91	0.036	NSP†
Small intestine	4.79	4.43	4.79	4.80	0.228	NS
Large intestine	1.76	1.55	1.79	1.68	0.070	CAD*

[1] Statistical analysis: NS = $p > 0.10$, † = $p < 0.10$, * = $p < 0.05$.

Implications

Gastric and intestinal digesta of pigs fed different CAD and NSP levels diets did not differ with regard to acidity, buffering capacity and viscosity. Pigs can maintain intraluminal acid base homeostasis after ingesting a wide range of CAD levels in the diets. The response of pigs to dietary CAD is clearly related to the other dietary components. The different responses are mainly associated with differences in feed intake. The interaction of CAD and dietary NSP for feed intake may be a consequence of the interaction of CAD and NSP for 'digestibility' of Cl and DM. The effect of dietary CAD on pigs seems to be dependent upon dietary NSP content.

Acknowledgement

The authors thank Ries Verkerk, Andre Jansen and all the people of the experimental unit 'de Haar Varkens' for their help during the experiment; Truus van der Wal, Arie van den Dool, Peter van der Togt, Marlou Bosch and Marian van't End for dissecting the piglets and collecting samples; Jane-Martine Muijlaert and Marian van't End for the chemical analysis.

References

Austic, R. E., Boyd, R. D., Klasing, K. C. and Riley. W. W. 1983. Effect of dietary electrolyte balance on growth performance in swine. J. Anim. Sci. 57 (Suppl. 1): 236 (Abstr.).

Bach Kundsen, K. E. and Johansen, H. N. 1995. Plant carbohydrates: Enzymatic hydrolysis and microbial fermentation in the gastrointestinal tract of pigs. In: Proc. 46 th Annual Meeting of the EAAP, Prague, Czech Republic.

Coffey, T., Combs, G. E. and Campbell, D. R. 1985. Effect of dietary sodium, potassium and chloride levels on performance of weanling swine. J. Anim. Sci. 61 (suppl. 1): 307.

Dusel, G., Schulze, H., Kluge, H., Simon, O. and Jeroch, H. 1997. The effect of wheat quality measured by extractviscosity and dietary addition of feed enzymes on performance of young pigs. J. Anim. Sci. 75 (Suppl. 1): 200.

Fauchon, C., Seoane, J. R. and Bernier, J. F. 1995. Effects of dietary cation-anion concentrations on performance and acid-base balance in growing lambs. Can. J. Anim. Sci. 75: 145-151

Haydon, K. D., West, J. W., Newton, G. L. and Dove, C. R. 1989. Effect of varying dietary electrolyte balance on performance and blood components of growing -finishing swine fed in high ambient temperatures. J. Anim. Sci. 67 (Suppl. 1): 254 (abstr.).

Heitzmann, W. 1991. Gehalte an Natrium, Kalium und pufferenden Komponenten im Caecumchymus des Schweines in Abhängigkeit von der Rohfaser-, Natrium- und Wasserzufuhr. In: Diss. Med. Vet. Tierärztliche Hochechule Hannover, Germany.

ISO 5984. 1978. International Organization for Standardization. Animal feeding stuffs – Determination of crude ash. Genève, Switzerland.

ISO 5985. 1978. International Organization for Standardization. Animal feeding stuffs – Determination of ash insoluble in hydrochloride acid content. Genève, Switzerland.

ISO 5983. 1979. International Organization for Standardization. Animal feeding stuffs – Determination of nitrogen content and calculation of crude protein content. Genève, Switzerland.

ISO 6496. 1983. International Organization for Standardization. Animal feeding stuffs – Determination of moisture content. Genève, Switzerland.

Jørgensen, H., Just, A. and Fernandez, J. A. 1985. The influence of dietary supply of minerals on apparent absorption and retention of minerals in growing pigs. In: Proc. 3th Int. Sem. on Dig. Physiol. In the Pig. Copenhagen. Denmark. P 160-163.

Van der Klis, J. D., Verstegen, M. W. A. and Van Voorst, A. 1993. Effect of a soluble polysaccharide (carboxy methyl cellulose) on the absorption of minerals from the gastrointestinal tract of broilers. Bri. Poul. Sci. 34: 377-389.

Mroz, Z., Jongbloed, A. W., Lenis, N. P. and Vreman, K. 1995. Water in pig nutrition: physiology, allowances and environmental implications. Nutr. Res. Rev. 8: 137-164.

NEN 5417. 1988. Cereals, pulses and animal feeding stuffs – Determination of crude fibre content by the abbreviated method. Standards of the Netherlands Normalization Institute, Delft, The Netherlands.

Park, J. H., Han, I. K. and Kim, I. B. 1994. Effects of electrolyte balance (EB: Na+K-Cl) on the growth, blood parameters, urine parameters and nutrient availabilities of weaning pigs. Korean J. Anim. Nutr. and Feed Stuffs 18: 147-154.

Patience, J. F., Austic, R. E. and Boyd, R. D. 1986. The effect of sodium bicarbonate or potassium bicarbonate on acid base status and protein in energy digestibility in swine. Nutr. Res. 6: 263-273.

Patience, J. F., Austic, R. E. and Boyd, R. D. 1987. Effect of dietary electrolyte balance on growth and acid-base status in swine. J. Anim. Sci. 64: 457-466.

Patience, J. F. and Wolynetz, M. S. 1990. Influence of dietary undetermined anion on acid-base status and performance in pigs. J. Nutr. 120: 579-587.

Patience, J. F. and Chaplin, R. K. 1997. The relationship among dietary undetermined anion, acid-base balance, and nutrient metabolism in swine. J. Anim. Sci. 75: 2445-2452.

Prohaszka, L. and Baron, F. 1980. The predisposing role of high dietary protein supplies in Eneteropathogenic E. Coli infections of weaned pigs. Zbl. Veterinary Medicine B 27: 222-232.

SAS. 1990. SAS User's Guide: Statistics (Version 6 Ed.). SAS Inst. Inc., Cary, NC.

Rodriguez, N., Boucourt, R. and Martinez, G. 1988. Digestive indicators in pigs consuming high final molasses levels with the addition of fibre. 2. Digesta distribution, pH and digestibility of the different sections of the gastrointestinal tract. Cuban J. of Agri. Sci. 22: 171-177.

Wondra, K. J., Hancock, J. D., Behnke, K. C. and Hines, R. H. 1995. Effects of dietary buffers on growth performance, nutrient digestibility, and stomach morphology in finishing pigs. J. Anim. Sci. 73: 414-420.

Yen, J. T., Pond, W. G. and Prior, R. L. 1981. Calcium chloride as a regulator of feed intake and weight gain in pigs. J. Anim. Sci. 52: 778-782

GENERAL DISCUSSION

Dietary cation anion difference and the animal's performance response: possible mechanisms

Introduction

Dietary cation anion difference (CAD, Na + K – Cl, mEq kg^{-1}) determines the pH and buffer capacity of a diet. Changes in dietary CAD will affect the acid base balance in the blood of animals and indirectly affect feed intake and growth. In this thesis, the effect of dietary CAD on growth performance, energy metabolism, acid base balance in the blood and in the digestive system was examined in African catfish (*Clarias gariepinus*) and pigs. The results show that dietary CAD influences feed intake and growth both in fish and pigs. The possible mechanism of this effect will be discussed in this section.

The concept of dietary CAD

The main dietary cations are sodium, potassium, magnesium and calcium. Major dietary anions are chloride, phosphorus, and inorganic sulfur (Patience *et al.*, 1987b). From these, sodium, potassium and chloride have the biggest influence on acid base metabolism.

A diet with a low CAD contains a higher Cl equivalent than the sum of Na and K (in mEq kg^{-1}), while a diet with high dietary CAD contains more Na + K equivalent than Cl. A decrease in dietary CAD is normally obtained by replacing $CaCO_3$ by $CaCl_2 2H_2O$. In this way, the Ca content is maintained at the same level between treatment diets. Increasing dietary CAD is mainly obtained by addition of $NaHCO_3$ or Na_2CO_3. This approach means that additional HCO_3^- (CO_3^{2-}) is added to the diets to maintain electrical neutrality of the diets. The additional HCO_3^- (CO_3^{2-}) in the diet may have a profound (indirect) effect on the animal's performance, as HCO_3^- (CO_3^{2-}) is an important buffer base in animal body fluids. Figure 1 summarizes the relationship between dietary HCO_3^- (CO_3^{2-}) content and blood HCO_3^- concentration in pigs. With a low dietary HCO_3^- (CO_3^{2-}) content (<5g kg^{-1}), there is a large variation in blood HCO_3^- concentration. With increasing dietary HCO_3^- (CO_3^{2-}) content, above 5g kg^{-1}, pigs maintain the blood HCO_3^- concentration within a narrow range. The relationship between dietary HCO_3^- (CO_3^{2-}) content and blood HCO_3^- concentration is less clear as the relationship between dietary CAD and blood HCO_3^- concentration (see Figure 2, and discussion in the following section). This implies that dietary CAD gives better description of changes in blood HCO_3^- concentration than dietary HCO_3^- (CO_3^{2-}) levels.

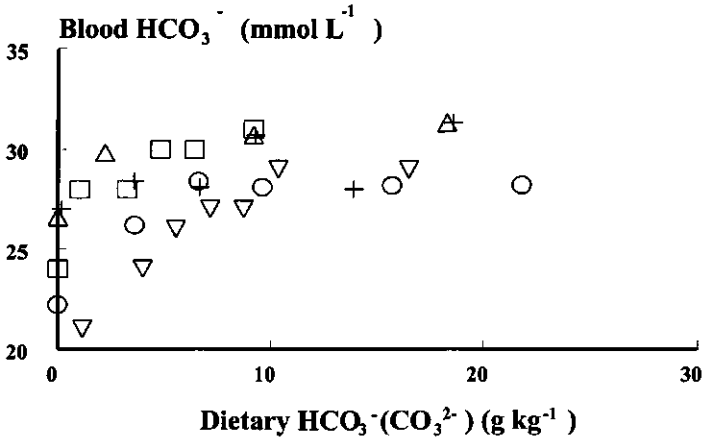

Figure 1. Dietary HCO_3^- (CO_3^{2-}) content in relation to blood HCO_3^- concentration in pigs, data derived from Patience *et al.* (1987a) (O), Patience and Wolynetz (1990) (□ and ∇ for exp. 1 and 2 respectively), Haydon *et al.* (1990) (+) and Haydon and West (1990) (Δ).

Possible mechanisms behind the animal's response to dietary CAD

Evidence from our study and literature indicates that a change in dietary CAD can affect both feed intake and growth of animals. A common response is a depressed feed intake and growth at a negative dietary CAD and a stimulated feed intake and growth at a positive dietary CAD. Generally, the possible mechanism behind this response can be associated with one of the following aspects:

- Acid base balance
- Energy metabolism
- Interaction with other dietary components.

Dietary CAD and the acid base balance in the blood and the digestive system

Many studies have shown that dietary CAD influences blood pH, HCO_3^-, Pco_2 and base excess concentrations. Lowering the dietary CAD can decrease blood pH, HCO_3^- and base excess, thereby causing metabolic acidosis (reduced buffer base concentration in the blood) and/or acideamia (lowered blood pH). Consequently feed intake is reduced and indirectly growth is depressed. An increasing in dietary CAD will increase HCO_3^- concentration and increase acid buffer capacity in the blood (Kemme-Kroonsberg, 1993).

This will increase feed intake and growth of animals. This effect has been shown in many different animal species. Blood pH and HCO_3^- concentration increased in a linear and/or quadratic manner with increasing dietary CAD in growing steers (Ross *et al.*, 1994), in cows (West *et al.*, 1991) and in lambs (Fauchon *et al.*, 1995). The influence of dietary CAD on blood pH and HCO_3^- concentration in pigs is shown in Figure 2, derived from data of Patience *et al.* (1987a), Patience and Wolynetz (1990), Haydon *et al.* (1990) and Haydon and West (1990).

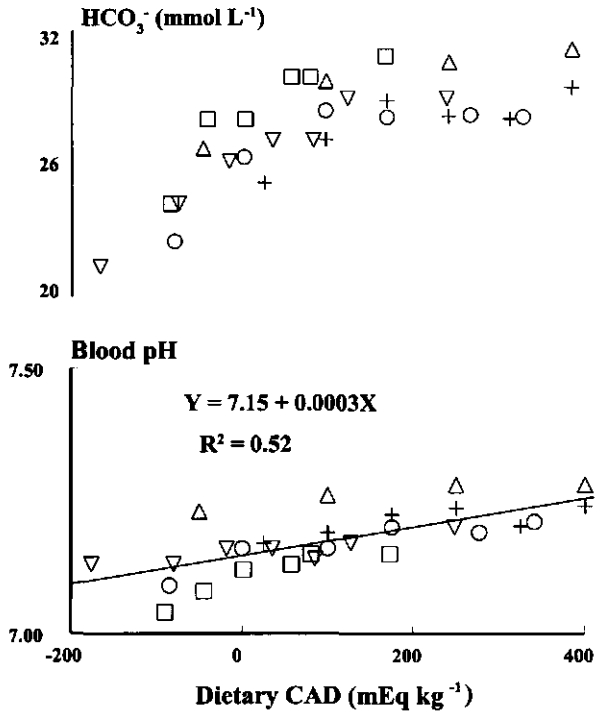

Figure 2. The changes of blood pH and HCO_3^- concentration as affected by dietary CAD in pigs, data derived from Patience *et al.* (1987a) (O), Patience and Wolynetz (1990) (□ and ∇ for exp. 1 and 2 respectively), Haydon *et al.* (1990) (+) and Haydon and West (1990) (Δ).

These summarized data give a clear pattern of a linear increase in blood pH and a curvilinear increase in HCO_3^- concentration in the blood with increasing dietary CAD. Our study (chapter 6 and Dersjant-Li *et al.*, unpublished data) also showed that pH and HCO_3^- concentration in both arterial and portal blood linearly increased with increasing dietary CAD

from –214 to 182 mEq kg^{-1} in 45 kg pigs (Table 1). During the postprandial period, blood HCO_3^- concentration increased rapidly and reached maximum at 3-4 h after feeding in the 200 mEq kg^{-1} CAD group (see chapter 6).

Table 1. Dietary CAD in relation to blood pH and HCO_3^- concentration in arterial and portal blood in 45 kg pigs (average values of 9 repeated measurements from present study)

Dietary CAD (mEq kg^{-1})		-214	-97	171	182
Dietary HCO_3^- (CO_3^{2-}) g kg^{-1}		0	1.92	8.24	8.24
Blood pH	Arterial	7.39	7.46	7.47	7.49
	Portal	7.35	7.37	7.39	7.43
Blood HCO_3^- (mmol L^{-1})	Arterial	27.2	29.9	32.3	34.6
	Portal	29.6	32.1	35.8	37.2

The increase of blood pH and HCO_3^- concentration, as shown in Table 1, was not proportional to the increase of dietary HCO_3^- (CO_3^{2-}) content. The pH values measured in our study are higher than most values mentioned in literature (see Figure 2). This may be the result of the different size of pigs and/or different diet compositions used in our study.

With increasing dietary CAD, feed intake showed a similar pattern as the changes of HCO_3^- concentration in the blood (Figure 3).

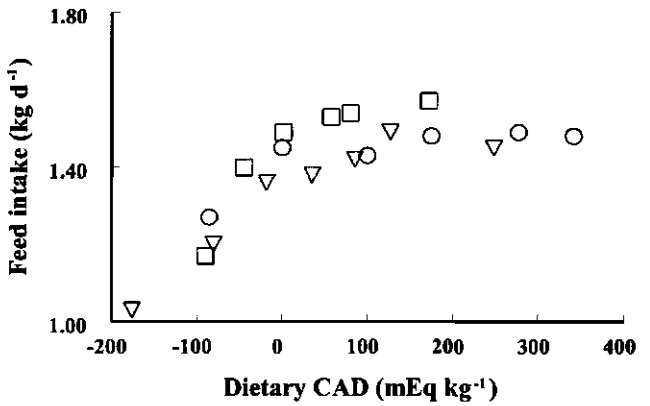

Figure 3. Feed intake in relation to dietary CAD in pigs. Data derived from Patience *et al.* (1987a) (O, mean initial weight of 15.1kg) and Patience and Wolynetz (1990) (□refers data from exp. 1, mean initial weight of 15.1kg; ∇ refers data from exp. 2, mean initial weight of 14.4 kg)

Data, derived from Patience *et al.* (1987a) and Patience and Wolynetz (1990), showed a linear relationship between feed intake and blood HCO_3^- concentration (Figure 4).

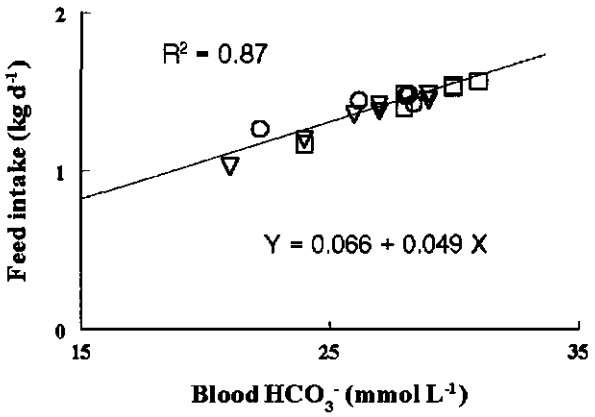

Figure 4. The linear relationship between feed intake and HCO_3^- concentration in the blood in pigs. Data derived from Patience *et al.* (1987a) (O, mean initial weight of 15.1kg) and Patience and Wolynetz (1990) (□refers data from exp. 1, mean initial weight of 15.1kg; ∇ refers data from exp. 2, mean initial weight of 14.4 kg).

Compared to pigs, little work has been done in determining the dietary CAD effect on blood pH and acid base status in fish. Available data illustrate that fish can maintain a stable blood pH with changes of dietary CAD. In our study (chapter 1), we did not notice differences in blood pH (mean value of 7.25) in African catfish when dietary CAD increased from –100 to 700 mEq kg^{-1}. Chiu *et al.* (1984) found that blood pH in rainbow trout did not vary with changing dietary CAD from –200 to 0 mEq kg^{-1}. Wilson *et al.* (1985) reported that in rainbow trout, blood pH did not change with dietary CAD ranging from –90 to 322 mEq kg^{-1}. However, in the latter study, a dietary CAD level of 638 mEq kg^{-1} decreased blood pH significantly compared to dietary CAD levels of –90 and 155 mEq kg^{-1}. This decrease in blood pH was associated with increasing Pco_2 levels in the blood. Table 2 compares blood pH, HCO_3^- and Pco_2 in fish and pigs. Fish have a low HCO_3^-, a low Pco_2 and a low base excess concentration in the blood. This results in a low CO_2 - HCO_3^- buffer capacity in the blood of fish compared to pigs. Our study (chapter 1) showed that electrolyte balance

changed in the plasma of African catfish with changing dietary CAD. This implies that African catfish maintain it's acid base balance by adjustment of Na^+ and Cl^- concentrations in the body at different dietary CAD levels. For fish the acid base balance is mainly regulated by ion exchange, while in air breathing animals, ventilation plays an important role in acid base regulation. The differences in acid base regulation between fish and pigs will be discussed in the following section.

Table 2. Blood pH, HCO_3^- (mmol L^{-1}), Pco_2 (mmHg) and base excess [BE, the strong acid (or base) needed to titrate the blood to pH = 7.40 at Pco_2 = 40 mm Hg and t_c = 37°C (Siggaard-Andersen, 1974) and expressed as mmol L^{-1}] in fish and pigs

Species	pH	HCO_3^-	Pco_2	$[HCO_3^-]/[Pco_2]$	BE	Sources
Rainbow trout[1]	7.35	11.0	14.4	25.6	-17.8	Wilson *et al.* 1985
Rainbow trout[2]	7.69	11.7	7.66	58.5	-13.4	Chiu *et al.* 1984
Pigs[3]	7.21	27.7	70.8	13.2	-1.53	Haydon *et al.* 1990
Pigs[3]	7.17	26.9	73.7	12.2	-2.7	Patience *et al.* 1987a
Pigs[4]	7.40	34.7	57.1	20.3	10.3	Chapter 6

[1] Sampled from caudal vein, [2] Sampled by heart puncture, [3] sampled by venapuncture (which blood vessel was not reported). [4] Sampled from portal blood via catheters.

Our study (chapter 7) showed that pH of stomach digesta is clearly related to the pH of the diet. African catfish fed a 700 mEq kg^{-1} CAD diet resulted in a significantly higher pH in the stomach digesta compared to a –100 mEq kg^{-1} CAD diet, both at 0.5 and 3 h after feeding. The pH of stomach digesta was significantly decreased 3 h after feeding compared to 0.5 h after feeding. Dietary CAD did not affect pH of small intestine digesta both 0.5 and 3 h after feeding. In pigs (chapter 8), we found that dietary CAD levels of –100 and 200 mEq kg^{-1} did not alter stomach and small intestine digesta pH 2.5 h after feeding. It is possible that at 2.5 h after feeding, pigs have already adjusted the pH of ingested feed in the stomach. On the other hand, the pig diets with CAD levels of –100 and 200 mEq kg^{-1} had a pH level of 5.3 and 6.3 respectively. There is only one unit difference in dietary pH levels between these two diets. In contrast, the fish diets with CAD levels of –100 and 700 mEq kg^{-1} had a dietary pH level of 5.6 and 8.4 respectively. This may explain why the effect of dietary CAD on stomach digesta pH was found in fish while this effect was not observed in pigs. The results indicate that animals can actively adjust the pH in the stomach and maintain an optimal pH in the small intestine after ingesting diets with different CAD levels, but to a different extent in fish and pigs and also related to dietary pH levels.

Absorption of ingested cation and anions will affect the acid base balance in the blood and other body compartments. Dietary CAD will therefore not only have a postprandial (short term) effect on the acid base balance in the digestive system of animals, but also (and most importantly) have a long term effect on the acid base balance of the blood and other body compartments of animals. Our study (chapter 6) clearly illustrates that during the postprandial period, both portal and arterial blood pH remained more or less constant within each CAD group in pigs. However, this pH level depends on the dietary CAD. A dietary CAD level of 200 mEq kg^{-1} resulted in a consistently higher pH in both arterial and portal blood than a dietary CAD level of -100 mEq kg^{-1}. In the portal blood, the pH level was maintained at the lowest margin of normal pH range with a dietary CAD level of –100 mEq kg^{-1} (chapter 6). By allowing different pH, pigs minimize the required energy necessary for acid base regulation.

Dietary CAD and energy metabolism

Our study in African catfish (chapter 4) indicated that dietary CAD is related to maintenance costs. We found that maintenance costs for homeostasis was minimized at a dietary CAD of 700 mEq kg^{-1}. This level also allowed an optimal performance. This finding confirms our hypothesis that, at an optimal dietary CAD level, less energy is needed to maintain homeostasis.

However, the results of the energy metabolism in pigs (chapter 5) did not confirm our hypothesis. The results in chapter 5 show that, at a restricted feeding level, dietary CAD of 200 mEq kg^{-1} tended to result in a higher metabolizable energy requirement for maintenance compared to a CAD level of –100 mEq kg^{-1}. However, with the same diet composition, the CAD level of 200 mEq kg^{-1} was optimal for feed intake and growth of pigs when feed was provided *ad libitum* (chapter 2). The housing system and feeding levels may have affected the response of pigs to dietary CAD. The ammonia emission in 200 mEq kg^{-1} CAD group was almost two fold of the one in the –100 mEq kg^{-1} CAD group in the respiration chambers. This high ammonia emission may be associated with the high maintenance costs, observed in this group. Furthermore, pigs fed 200 mEq kg^{-1} CAD diet may release more CO_2 to the air as a result of the high HCO_3^- content in the diet. That may contribute to the high maintenance requirement in this group. Within the same experiment, during the adaptation period before the balance study started, growth (numerically, with 50% increase) and feed efficiency (tendency) were higher in 200 mEq kg^{-1} CAD group compared to –100 mEq kg^{-1} CAD group.

During this adaptation period and in our growth trial (chapter 2), pigs were housed in cages and pens, respectively, with more space per pig and a higher air exchange rate and *ad libitum* feeding. Therefore we believe that the housing system may interfere with the dietary CAD effect on the energy metabolism in pigs. Furthermore, the *ad libitum* feeding may have caused a different energy response to dietary CAD compared to that observed with restricted feeding. This hypothesis is based on the fact that the acid load is related to feeding levels.

As discussed earlier, our data and the results from the literature proved that dietary CAD could influence the acid base regulation of animals. A high dietary CAD provides more buffer base and helps animals to compensate for metabolic acidosis. Thus maintenance costs for acid base regulation may be reduced. When feed is provided *ad libitum* to pigs, it is questionable whether dietary CAD affects maintenance costs, as was observed in African catfish. Several studies showed that increasing dietary CAD did improve feed efficiency in pigs (Patience and Wolynetz, 1990; Park *et al.*, 1994). The improved feed efficiency can be associated with one or more of the following responses: increased digestibility, increased metabolizable energy intake, reduced heat production and reduced maintenance costs. Haydon and West (1990) observed that digestible energy and metabolizable energy intake decreased linearly in pigs with increasing dietary CAD from –50 to 400 mEq kg^{-1}. With similar feed composition, a linear increase in feed intake and growth was reported by Haydon *et al.* (1990) with increasing dietary CAD from 25 to 400 mEq kg^{-1}. In the latter study dietary CAD did not affect feed efficiency. In case a high dietary CAD resulted in a low digestible and metabolizable energy intake combined with a high growth rate, it might have been caused by a low heat production and a low energy requirement for maintenance.

However, according to literature information, the effect of dietary CAD on feed efficiency in pigs is inconsistent. Some studies showed that dietary CAD affected feed intake but did not significantly affect feed efficiency (Patience *et al.*, 1987a; Haydon *et al.*, 1990). The latter data suggest that growth response to dietary CAD is mainly related to increased feed intake.

In conclusion, in African catfish, dietary CAD affects maintenance costs for maintaining homeostasis. The mechanism of this effect will be discussed in the following section. In pigs, however, the result seemed contrary to our hypothesis. More experiments should be done to ascertain energy metabolism in pigs at *ad libitum* feeding level.

Interaction of dietary CAD and other dietary components

Our study (chapter 8) showed that the response of feed intake and growth of pigs to dietary CAD depends on diet composition. An interaction between dietary CAD and non starch polysaccharide (NSP) content was observed for feed intake and growth. With a high NSP diet, feed intake and growth was higher in pigs fed a 200 mEq kg^{-1} CAD diet than in pigs fed the –100 mEq kg^{-1} CAD diet. With a low NSP diet, however, a CAD level of –100 mEq kg^{-1} resulted in higher feed intake and growth compared to a CAD level of 200 mEq kg^{-1}. This result indicates that optimal dietary CAD for feed intake and growth of animals may alter with changes in diet composition, as a result of an interaction between dietary CAD and other feed ingredients or components. Patience *et al.* (1987b) suggested that the dietary electrolyte balance could influence each class of nutrients (vitamins, minerals, amino acids and energy) to a different extent. Since most enzyme systems require an optimum pH for catalyzing metabolic reactions (Heisler, 1984), changes in dietary CAD may influence enzyme activity through changes in pH and thus affect nutrient metabolism. Many studies have shown that dietary CAD can influence utilization of amino acids. Forsberg *et al.* (1987) found that a low dietary CAD depressed liver lysine oxidation and caused less lysine deposition in the liver, whereas an elevated dietary CAD had no effect on lysine metabolism. Our results showed that liver weight was significantly lower in pigs receiving a –100 mEq kg^{-1} CAD diet compared to a 200 mEq kg^{-1} CAD diet (chapter 8). This supports the findings by Forsberg *et al.* (1987). In pigs, Patience *et al.* (1987a) suggested that the response of lysine–deficient pigs to sodium bicarbonate depends on the electrolyte balance of the diet. Madubuike *et al.* (1980) reported that supplementation of a lysine deficient diet with sodium or potassium bicarbonate increased growth rate and feed intake in growing pigs. However, when dietary lysine was adequate, this effect was not observed. Wahlstrom *et al.* (1983) observed an interaction between potassium and lysine for weight gain in pigs. It is suggested that $NaHCO_3$ increased lysine uptake by the liver and decreased uptake by the kidney (Patience *et al.*, 1984 as cited by Kemme-Kroonsberg, 1993). In fish, Chiu *et al.* (1984) found in rainbow trout, that dietary CAD had a marked effect on the metabolism of histidine. Free histidine concentrations in the muscle were higher and lysine concentrations were lower in trout receiving 0 mEq kg^{-1} than in those receiving –200 mEq kg^{-1} CAD diet. The arginine requirement tended to be higher when trout fed a diet containing 200 mEq kg^{-1} CAD compared to trout fed a diet containing –200 mEq kg^{-1} CAD (Chiu *et al.*, 1988). Chiu *et al.* (1984, 1987 and 1988) observed an inconsistent response of trout to dietary CAD for growth

and feed utilization. The interaction between dietary CAD and other feed components may explain the inconsistent response of animals to dietary CAD in different studies.

Specific differences in the response to dietary CAD between fish and pigs

In general, both African catfish and pigs showed a similar growth response to dietary CAD. In both animals a negative dietary CAD depressed growth, while an increase of dietary CAD at a certain level improved growth. However, in more details, African catfish responds to dietary CAD differently from pigs in two aspects. Firstly, the optimal CAD for growth was higher for African catfish than for pigs. Secondly, changing dietary CAD had a larger effect on the energy metabolism in African catfish than in pigs. This different response may be explained by the difference in the acid base regulation mechanism and the different living environment of fish and pigs. The buffer value (defined as the amount of H^+ ions required to change pH by one unit) in the body compartments is much smaller in fish compared to mammals (Table 3, Heisler, 1986).

Table 3. Buffering properties of various body compartments of fish and mammals expressed by non-bicarbonate (β_{NB}) or bicarbonate buffer values (β_{BIC}, values for steady state pH and constant Pco_2). The buffer capacity (κ) of body compartments is the product of buffer value and fluid volume (V_{rel}) of the respective compartment, referred to 1 kg body water

		Buffer values		Buffer capacity	
	V_{rel}	β_{NB}	β_{BIC}	κ_{NB}	κ_{BIC}
Fish					
Blood	~0.04	5-15	5-22	0.2-0.6	0.2-0.6
Extracellular space	~0.22	1-2	7-30	0.2-0.4	1.5-6
Skeletal muscle	~0.50	35-50	1-10	17.5-25	0.5-5
Mammals					
Blood	~0.08	20-30	40-50	1.6-2.4	3.2-4.0
Extracellular space	~0.20	2-4	50-60	0.4-0.8	10.0-12.0
Skeletal muscle	~0.35	65-78	15-20	22-27	5.2-7.0

β expressed as meq/ (pH L body compartment water); κ expressed as meq/pH. Source: Heisler (1986)

In higher vertebrates, non-respiratory acid base disturbances are often compensated by changes in Pco_2 induced by changes in pulmonary ventilation. Water-breathing species, however, are handicapped in applying this mechanism because of the low oxygen content of water as compared to air. Transmembrane and transephithelial ion transfer processes are the

only mechanisms capable of permanent elimination of H^+-equivalent ions produced as non-volatile metabolic end-products (Heisler, 1986). Acid-base relevant ionic transfer is most probably performed at a one to one ratio for HCO_3^-/Cl^-, H^+/Na^+ and H^+/NH_4^+ ion exchange. Ionic exchange processes are sensitive to the availability of appropriate exchange ions. The lack of these exchange ions might be one of the potentially limiting factors of branchial acid base relevant ion transfer. Increasing dietary CAD (by increasing Na or K) may increase the available Na from feed and this will help fish to maintain the acid base balance in the body. On the other hand, uptake of Na^+ from freshwater is against a Na^+ gradient and, therefore, costs energy. When a high concentration of Na is supplied in the diet, the required Na can be obtained from the GI tract and reducing thereby the energy costs for Na uptake from the water. The Na absorbed from the GI tract may help fish to maintain their intracellular pH. Hence, an increase in dietary CAD will consequently increase the acid buffer capacity and help fish to compensate for metabolic acidosis in the body compartments. This may explain the low maintenance costs as observed in chapter 4.

In higher vertebrates, the efficiency of ion transfer is low compared to fish. In fish, ions are mainly exchanged via the gills, although the kidney can play a miner role in ion exchange as well (Cameron, 1980). In pigs, the excretion and re-absorption of ions is only done in the kidney. Furthermore, fish can directly exchange the ions with the water environment. This enables fish to maintain ion balances in the body in spite of large changes in dietary ion balance. At the same time, this may explain why fish showed a higher optimal dietary CAD level than pigs.

Final remarks and conclusions

The results of this study confirm that dietary CAD can influence feed intake and growth of African catfish, as well as of pigs. The mechanism of the dietary CAD effect is related to acid base regulation, maintenance costs and/or interaction with other dietary components. In African catfish, the improved growth rate at optimal dietary CAD levels is related to increased feed intake and reduced maintenance costs. In pigs, the improved growth at an optimal dietary CAD seems mainly associated with increased feed intake. Feed intake is clearly related to the acid base status of the diet and of the animal's body compartments.

The central hypothesis of this thesis, e.g., feed intake is regulated by maintenance energy requirements was proven in fish. More experiments are needed to assess the effect of dietary CAD on the energy metabolism in pigs.

Dietary CAD can significantly influence the acid base balance of the blood in higher vertebrates. Lowering dietary CAD will decrease blood pH and reduce base excess concentration in the blood. However, this effect was not observed in fish. This may be due to the fact that fish have a high ion exchange ability with their living environment (water). This characteristic enables fish to maintain constant blood pH and electrolyte concentrations in the body after ingesting a wide range of dietary CAD's (chapter 1). As a result, fish can adapt to higher dietary CAD than pigs. In African catfish, the ions exchange system for acid base regulation apparently benefits from changes in dietary CAD and, therefore, maintenance costs for homeostasis was reduced.

After ingesting diets with different CAD levels, animals can adjust the pH of the ingested diet to enable optimal pH for digestion in the GI tract (chapter 7, 8). In addition, changes in dietary CAD can influence large intestine and liver weight. The response of feed intake and growth of pigs to dietary CAD depended also on NSP levels in the diet.

Excess potassium can have a negative effect on feed intake and growth of African catfish. Based on our study (chapter 3) and on literature information, we may conclude that for dietary CAD formulation, the ratio between sodium and potassium should be between 1 to 2.5 (mol/mol).

Together with the knowledge from literature about other species, it can be concluded that a negative dietary CAD will depress feed intake and growth of animals. Dietary CAD in a given positive range will improve the performance of animals. However, this effect is species dependent. It is also possible that other fish species (i.e. marine fish) may respond differently to dietary CAD. This study provides valuable information on how and to what extent dietary CAD affects animal's performance, it's acid base balance and energy metabolism. Therefore, when formulating an animal feed, dietary CAD should be taken into consideration.

Reference

Cameron, J. N. 1980. Body fluid pools, kidney function, and acid-base regulation in the freshwater catfish Ictalurus punctatus. J. Exp. Biol. 86: 171-185.

Chiu, Y. U., Austic, R. E. and Rumsey, G. L. 1984. Effect of dietary electrolytes and histidine on histidine metabolism and acid-base balance in rainbow trout (*Salmo Gairdneri*). Comp. Biochem. Physiol. 78B: 777-783.

Chiu, Y. U., Austic, R. E. and Rumsey, G. L. 1987. Interactions among minerals, arginine and lysine in rainbow trout (*Salmo Gairdneri*). Fish Physiol. Biochem. 4: 45-55.

Chiu, Y. U., Austic, R. E. and Rumsey, G. L. 1988. Effect of feeding level and dietary electrolytes on the arginine requirement of rainbow trout (*Salmo Gairdneri*). Aquaculture 69: 79-91.

Fauchon, C., Seoane, J. R. and Bernier, J. F. 1995. Effects of dietary cation-anion concentrations on performance and acid-base balance in growing lambs. Can. J. Anim. Sci. 75: 145-151.

Forsberg, N. E., Austic, R. E. and Boyd, R. D. 1987. Influence of dietary electrolyte balance and extracellular bicarbonate concentration on lysine metabolism. Nutr. Reports Int. 35: 453-469.

Haydon, K. D. and West, J. W. 1990. Effect of dietary electrolyte balance on nutrient digestibility determined at the end of the small intestine and over the total digestive tract in growing pigs. J. Anim. Sci. 68: 3687-3693.

Haydon, K. D., West, J. W. and McCarter, M. N. 1990. Effect of dietary electrolyte balance on performance and blood parameters of growing-finishing swine fed in high ambient temperature. J. Anim. Sci. 68: 2400-2406.

Heisler, N. 1984. Acid-base regulation in fishes. In: Hoar W.S., Randall, D.J. and Brett, J.R. (Ed), Fish Physiology, Vol. XA, Anatomy, Gas Transfer, and Acid-Base Regulation. Academic Press, New York, pp 315-401.

Heisler, N. 1986. Mechanisms and limitations of fish acid-base regulation. In: Nilsson S. and Holmgren S. (Ed), Fish Physiology: Recent Advance. Dover, New Hampshire. pp 24-49.

Kemme-Kroonsberg, C. 1993. Nutrition and acid-base balance of pigs: a review. Research Institute for Livestock Feeding and Nutrition (IVVO-DLO), Lelystad, Netherlands. No. 243. P69.

Madubuike, F. N., Calvert, C. C. and Austic, R. E. 1980. Lysine-cation interrelationships in the pig. J. Anim. Sci. 51 (suppl. 1): 210.

Park, J. H., Han, I. K. and Kim, I. B. 1994. Effects of electrolyte balance (EB: Na+K-Cl) on the growth, blood parameters, urine parameters and nutrient availabilities of weaning pigs. Korean J. Anim. Nutr. and Feed Stuffs 18: 147-154.

Patience, J. F., Austic, R. E. and Boyd, R. D. 1987a. Effect of dietary electrolyte balance on growth and acid-base status in swine. J. Anim. Sci. 64: 457-466.

Patience, J. F., Austic, R. E. and Boyd, R. D. 1987b. Acid-base homeostasis in swine: Nutritional perspective. Feedstuffs, July 6: 13-19.

Patience, J. F. and Wolynetz, M. S. 1990. Influence of dietary undetermined anion on acid-base status and performance in pigs. J. Nutr. 120: 579-587.

Ross, J. G., Spears, J. W. and Garlich, J. D. 1994. Dietary electrolyte balance effects on performance and metabolic characteristics in growing steers. J. Anim. Sci. 72: 1842-1848.

Siggaard-Andersen, O. 1974. The acid-base status of the blood. Fourth edition. Munksgaard, Copenhagen. P229.

Wahlstrom, R. C., Siyoto, S. L. and Libal, G. W. 1983. Effect of potassium and lysine supplementation on performance of young pigs fed low potassium diets. Nutr. Reports Int. 28: 1159 – 1163.

Wilson, R. P., Cowey, C. B. and Adron, J. W. 1985. Effect of dietary electrolyte balance on growth and acid-base balance in rainbow trout (*Salmo gairdneri*). Comp. Biochem. Physiol. 82A: 257-260.

West, J. W., Mullinix, B. G. and Sandifer, T. G. 1991. Changing dietary electrolyte balance for dairy cows in cool and hot environments. J. Dairy Sci. 74: 1662-1674.

SUMMARY

When formulating animal feeds, normally the main considerations are protein and energy content and their respective constituents, amino acids, lipids and carbohydrates. The feed is formulated to meet the animal's requirements for the different nutrients. In the same manner, minerals are added to the diets. Many dietary compounds, especially mineral ions, carry electrolytic charges which determine the dietary pH and acid base buffering capacity of a diet. This electrolyte balance, further called the dietary cation anion difference (CAD, Na + K - Cl, mEq kg^{-1}), may affect the animal's metabolism in different ways. A non-optimal dietary CAD can disturb the acid base balance in the GI tract and upon absorption, also in the blood and other body compartments of the animal. Upon ingestion of non-optimal dietary CAD, the animals need to correct for the dietary pH to regulate their acid base balance. It is believed that this will occur shortly after ingestion, when the chyme enters the stomach and intestine. In the stomach the proper pH has to be obtained. In the small intestine, the acid base balance is adjusted by pancreatic secretion of sodium bicarbonates. In addition to these short term effects, the absorbed cations and anions will have also a long term effect on the acid base balance in the blood and other body compartments (i.e, intra- and extracellular fluids). It is hypothesized that these long-term effects are most strongly expressed by extra energy needs for acid base regulation.

In the present study, these energetic costs to adjust the acid base balance are considered as the energetic costs for maintaining homeostasis and have to be considered as extra maintenance costs. Dietary CAD is based on the electrolytic balance of $Na^+ + K^+ - Cl^-$ (mEq kg^{-1}) because these three ions are most important cations and anions in determining dietary pH. In an animal's acid base regulation system, ion exchange (consists of a Na^+/H^+ exchanger, a Cl^-/HCO_3^- exchanger and the Na^+/HCO_3^- co-transporter) plays an important role in maintaining the acid base balance. When acidosis occurs, H^+ will be excreted and replaced by Na^+, thereby increasing the pH. In alkaline disturbed condition, HCO_3^- or OH^- will be exchanged by Cl^- and the pH will decrease. A low dietary CAD will contribute more Cl^- equivalent than the sum of $Na^+ + K^+$. This will contribute more acids to the diet and, upon ingestion of the diet, to the body compartments of the animals. An optimal (high) dietary CAD will increase the base buffer capacity of the diet and help animals to compensate for metabolic acidosis. The hypothesis is that if dietary CAD is optimal, no extra energy will be

needed for the acid base regulation, the metabolic costs of homeostasis will be minimal and this will lead to better feed intake, growth, feed utilization and animal welfare.

This study aimed to investigate the effect of dietary CAD on feed intake and growth, acid base balance and maintenance costs of animals. To approach this study in a comparative way, African catfish (a freshwater fish) and young pigs (air breathing animals) were chosen as models. By doing so, the results obtained from these two species may enable to draw some general conclusions. This study consisted of 3 parts. In the first part, feed intake and growth response to dietary CAD and dietary Na/K ratios was investigated. In part 2, the energetic response to dietary CAD was determined in African catfish and pigs. In part 3, the acid base balance in the blood and in the digestive system in response to dietary CAD was investigated.

The first step of this research aimed to determine an optimal dietary CAD for African catfish and pigs. We found that dietary CAD significantly affected feed intake and growth for both African catfish (*Clarias gariepinus*) and young pigs. Increasing dietary CAD levels from –100 to 700 mEq kg^{-1} resulted in a linear increase in feed intake and growth. African catfish maintained a constant blood pH with changing dietary CAD. However a quadratic relationship between Na^+, Cl^- and CAD level in plasma and dietary CAD was found, which indicated that African catfish regulate their acid base balance by adjustment of electrolytes concentrations in the blood.

Pigs fed dietary CAD levels of 200 and 500 mEq kg^{-1} showed higher feed intake and growth than pigs fed –100 mEq kg^{-1} CAD diet. A dietary CAD level of 500 mEq kg^{-1} resulted in a higher fecal apparent digestibility of dry matter and nitrogen than dietary CAD levels of –100 and 200 mEq kg^{-1}. Supplementation of xylanase (0.1% xylanase derived from *Trichoderma longibrachiatum*) did not affect the performance of pigs. Xylanase addition in the diet significantly increased apparent fecal digestibility of dry matter and tended to increase apparent digestibility of nitrogen. Although xylanase inclusion showed a numerical increase in feed intake and growth in –100 and 500 mEq kg^{-1} CAD groups, no interaction between dietary CAD and xylanase was found.

We also investigated the optimal dietary Na/K ratio for dietary CAD formulation in African catfish. Excess K concentrations in the diet depressed growth and reduced body fat and protein deposition. Results suggest that the optimal dietary Na/K ratio is 1.5 and 2.5 (mol/mol) for African catfish, *Clarias gariepinus*.

The central hypothesis 'that dietary CAD will affect maintenance costs for homeostasis' was tested in part 2. The measured total heat production and estimated metabolizable energy requirement for maintenance was minimized at a dietary CAD level of 700 mEq kg^{-1} for African catfish. Consequently metabolizable energy utilization efficiency (MEU, percentage of retained energy over metabolizable energy) quadratically ($p < 0.05$) increased and reached a maximum at a dietary CAD of 700 mEq kg^{-1}. In pigs, the total heat production and metabolizable energy requirement for maintenance tended to be higher in 200 mEq kg^{-1} CAD group compared to –100 mEq kg^{-1} CAD group. Ammonia emission was higher ($p < 0.05$) in the 200 mEq kg^{-1} CAD group.

In the third part of study, the question how and to what extent animals can maintain pH in the blood and in the digestive system in response to dietary CAD was answered. In pigs, dietary CAD level of –100 mEq kg^{-1} resulted in significantly low blood pH, Pco_2 (kpa), oxygen content, Hb, HCO_3^-, base excess, Na^+ concentration (mmol L^{-1}) and CAD level (mEq L^{-1}) compared to a dietary CAD level of 200 mEq kg^{-1}. Chloride and K^+ concentrations in the blood were higher in –100 mEq kg^{-1} CAD group. After feeding, base excess and HCO_3^- concentrations in the blood significantly increased especially in 200 mEq kg^{-1} CAD group. During the postprandial period, pigs maintained a constant high blood pH in 200 mEq kg^{-1} CAD group and a constant low blood pH in –100 mEq kg^{-1} CAD group. During the first two hours after feeding, the oxygen content in both arterial and portal blood decreased rapidly in the –100 mEq kg^{-1} CAD group. The oxygen content was less affected by feeding in the 200 mEq kg^{-1} CAD group and this may indicate a better oxygen status in this group after feeding. The result confirmed that an optimal dietary CAD (200 mEq kg^{-1}) can increase the buffer base concentrations in the blood and dietary CAD can indeed have a long term effect on acid base regulation in animals.

Dietary CAD can affect digesta pH in the stomach. African catfish fed a diet with a CAD level of 700 mEq kg^{-1} resulted in a significantly high stomach digesta pH and buffer capacity compared to a diet with CAD level of –100 mEq kg^{-1} at 0.5 and 3 h after feeding. Dietary CAD, however, did not affect the pH of the small intestinal digesta pH. At 3 h after feeding the stomach digesta pH significantly decreased and small intestinal digesta pH significantly increased compared to the levels at 0.5 h after feeding. These changes were more pronounced in 700 mEq kg^{-1} CAD group. The result showed that African catfish could adjust the pH of ingested diets shortly after feeding in the stomach and maintain optimal pH

in the gastro-intestinal tract with different CAD levels. In pigs, stomach and small intestine digesta pH of pigs was not altered 2.5 h after ingesting -100 and 200 mEq kg^{-1} CAD diets.

We additionally tested if pigs would respond differently to dietary CAD when dietary composition changes. Indeed, an interaction between dietary CAD and NSP content was observed for feed intake, growth, and digestibility of dry matter. The results indicated that the effect of CAD on feed intake and growth depends on the dietary NSP content. Furthermore the result of this study showed that liver and large intestine weights were significantly lower at a CAD level of –100 mEq kg^{-1} compared to a CAD level of 200 mEq kg^{-1}.

In conclusion, dietary CAD significantly influenced performance of both African catfish and pigs. A negative dietary CAD depressed feed intake and growth of both studied animals. A positive dietary CAD range improved feed intake and growth. However optimal dietary CAD varies with different animal species. The mechanism of this dietary CAD effect is mainly related to three aspects: altered acid base regulation, altered metabolizable energy requirement for maintenance and the interaction between dietary CAD with other dietary compositions. Dietary CAD should be considered as an important aspect for animal feed formulation.

SAMENVATTING

Bij de formulering van diervoeders zijn met name het eiwit en het energiegehalte belangrijke factoren die de samenstelling bepalen. Voeders worden zo geformuleerd, dat in de nutriëntbehoefte (aminozuren, vetten, koolhydraten) van het dier kan worden voorzien. Om deze reden worden ook mineralen aan voeders toegevoegd. Mineralen bepalen in belangrijke mate de pH en buffercapaciteit van een voeder. De pH en buffercapaciteit van een voeder zijn met name afhankelijke van hoeveelheid en samenstelling van kationen en anionen: de elektrolyten balans van een rantsoen. In dit proefschrift wordt met het verschil tussen kationen en anionen (CAD) gedefinieerd als Na + K – Cl (uitgedrukt in mEq kg^{-1}). Het metabolisme van een dier kan op verschillende wijzen door CAD van het voer beïnvloed worden. Een sub-optimale CAD kan zowel het zuur-base evenwicht in het maagdarmkanaal, in het bloed alsmede in andere lichaamscompartimenten van het dier verstoren. Het dier moet de pH van het voer corrigeren om haar eigen zuur-base evenwicht te kunnen handhaven. Verondersteld wordt dat deze correcties vrijwel direct plaatsvinden na de voeropname, als het voer de maag en darmen binnen komt. De pH in de maag wordt gereguleerd door afscheiding van zoutzuur. In de dunne darm wordt de pH gereguleerd door afgifte van natriumbicarbonaat door de pancreas. Naast deze korte termijn effecten kunnen ook lange termijn effecten van een verstoord zuur-base evenwicht worden verwacht. De hypothese is dat lange termijn effecten van een sub-optimale CAD leiden tot een verhoogd energieverbruik voor de handhaving van het zuur-base evenwicht.

In dit proefschrift wordt aangenomen dat de energetische kosten van de regeling van het zuur-base evenwicht deel uitmaken van de energetische behoefte voor onderhoud van een dier. Het CAD van een voer wordt berekend als $Na^+ + K^+ - Cl^-$ (in mEq kg^{-1}) omdat deze drie ionen de belangrijkste kationen en anionen zijn die de pH van een voeder bepalen. Bij de handhaving van het zuur-base evenwicht in het dier speelt ion uitwisseling een belangrijke rol. Bij acidose zullen H^+ ionen worden uitgescheiden en vervangen door Na^+ ionen waardoor de pH zal stijgen. Bij alkalose zullen HCO_3^- of OH^- ionen worden uitgewisseld door Cl^- ionen waardoor de pH zal dalen. Een voeder met een laag CAD bevat relatief meer Cl^- ten opzicht van de som van Na^+ en K^+. Een optimale CAD van het voer gaat samen met een toename in buffercapaciteit van het voer en ondersteunt het dier bij het compenseren van metabole acidose. De hypothese is dat bij een optimale CAD in het voer geen extra energie nodig is

voor het regelen van het zuur-base evenwicht. De energetische kosten van homeostase zullen dan minimaal zijn en zullen leiden tot een hogere voeropname, groei, voederbenutting en welzijn van de dieren.

In dit proefschrift wordt het effect van het CAD van voer op voeropname, groei, zuur-base evenwicht en onderhoudsbehoefte aan energie bestudeerd. Voor het vergelijken van diersoorten zijn de Afrikaanse meerval (*Clarias gariepinus*) (een zoetwater vis) en het varken als model gekozen. Het proefschrift bestaat uit drie delen. Het eerste deel is gericht op het bestuderen van de effecten van het CAD en de ratio van Na/K in het voer op voeropname en groei. In Deel 2 worden de effecten van het CAD in het voer op het energie metabolisme van de meerval en het varken bestudeerd. In Deel 3 wordt de invloed van het CAD in het voer op het zuur-base evenwicht in het bloed en in het verteringkanaal onderzocht.

In Deel 1 werd gekeken naar het optimale CAD niveau in het voer van de Afrikaanse meerval en jonge biggen. Het CAD van het voer had een significant effect op de voeropname en groei bij zowel de meerval als bij jonge biggen. Toename van het CAD in het voer voor meervallen van –100 naar 700 mEq kg^{-1} leidde tot een lineaire toename in voeropname en groei. De Afrikaanse meerval was in staat haar bloed pH constant te houden bij deze range aan CAD niveaus. Echter een kwadratische relatie tussen Na^+, Cl^- en CAD niveau in het bloed en het CAD gehalte in het voer werd gevonden. Hieruit blijkt dat de Afrikaanse meerval zijn zuur-base evenwicht reguleert door wijziging van elektrolyt concentraties in het bloed.

Biggen op zowel een voer met een CAD van 200 als van 500 mEq kg^{-1} hadden een hogere voeropname en groei dan biggen op een voer met een CADvan –100 mEq kg^{-1}. Een CAD niveau van 500 mEq kg^{-1} resulteerde in een hogere verteerbaarheid van droge stof en stikstof ten opzicht van de CAD niveaus van –100 en 200 mEq kg^{-1}. Toevoeging van xylanase (0.1% xylanase afkomstig van *Trichoderma longibrachiatum*) had geen invloed op de zootechnische prestatie van de biggen. Xylanase toevoeging aan het voer leidde tot een significante toename van de schijnbare fecale droge stof verteerbaarheid en er was een tendens voor stijging van de schijnbare fecale stikstof verteerbaarheid. Ondanks de numeriek hogere voeropname en groei bij de –100 en 500 mEq kg^{-1} behandelingsgroepen was er geen interactie tussen CAD niveau en xylanase toevoeging aan het voer aanwezig.

De optimale Na/K verhouding in voeders voor meervallen werd tevens onderzocht. Hoge K concentraties in het voer leidden tot een verminderde groei, lagere eiwit- en lagere

vetaanzet. De resultaten suggereren dat de optimale Na/K verhouding in voeders voor de Afrikaanse meerval tussen 1.5 en 2.5 (mol/mol) ligt.

De centrale hypothese dat het CAD niveau van het voer invloed heeft op de energetische kosten voor homeostase werd getoetst in Deel 2. De gemeten totale warmteproductie en geschatte onderhoudsbehoefte aan energie waren het laagst voor Afrikaanse meervallen op het 700 mEq kg^{-1} CAD rantsoen. De efficiëntie waarmee metaboliseerbare energie benut werd voor energieretentie, nam kwadratisch toe en bereikte een maximum bij een CAD niveau van 700 mEq kg^{-1}. Bij biggen was er een tendens aanwezig dat de totale warmteproductie en de onderhoudsbehoefte aan energie hoger waren bij het rantsoen met een CAD van 200 mEq kg^{-1} ten opzichte van het rantsoen met –100 mEq kg^{-1}. De ammoniak emissie was hoger ($p < 0.05$) bij het rantsoen met een CAD van 200 mEq kg^{-1}.

In Deel 3 werd onderzocht hoe en tot welk niveau dieren hun pH in het bloed en hun pH in het maagdarmkanaal constant kunnen houden onder invloed van CAD in het voer. Biggen op een voer met een CAD niveau van –100 mEq kg^{-1} hadden een significant lagere bloedwaarde voor pH, Pco_2 (kpa), zuurstofgehalte, Hb, HCO_3^-, base overschot , Na^+ concentratie (mmol L^{-1}) en CAD niveau (mEq L^{-1}) ten opzichte van een voer met een CAD niveau van 200 mEq kg^{-1}. Cloride en K^+ concentratie in het bloed waren hoger bij biggen op het voer met een CAD niveau van –100 mEq kg^{-1}. Direct na het voeren steeg het base overschot en de concentratie van HCO_3^- in het bloed met name bij biggen van de 200 mEq kg^{-1} behandeling. Tijdens de post prandiale fase konden biggen hun bloed pH constant houden bij beide CAD niveaus (–100 en 200 mEq kg^{-1}). De bloed pH van biggen van 200 mEq kg^{-1} behandelingsgroep was hoger dan van biggen in de –100 mEq kg^{-1} CAD groep. De eerste twee uur na voeren daalde het zuurstofgehalte sterk in zowel het arteriële als portale bloed bij de biggen op het –100 mEq kg^{-1} CAD voer. Het zuurstofgehalte van het bloed werd minder beïnvloed door het voeren bij biggen op het 200 mEq kg^{-1} CAD voer. Dit kan een indicatie zijn voor een betere zuurstof status van deze groep direct na het voeren. De resultaten bevestigden dat een optimale CAD in het voer (200 mEq kg^{-1}) het base overschot in het bloed kan verhogen en dat het CAD niveau in het voer een lange termijn effect kan hebben op de regulatie van het zuur-base evenwicht in dieren.

Het CAD niveau in het voer beïnvloedt de pH van digesta in de maag. Bij de Afrikaanse meerval leidde een verhoging van het CAD van –100 naar 700 tot een verhoging van de pH en buffercapaciteit van digesta in de maag op zowel 0,5 als 3 uur na het voeren. De pH van digesta in de dunne darm werd niet beïnvloed door het CAD niveau van het rantsoen bij meervallen. Drie uur na het voeren was de pH van digesta in de maag significant lager en in de dunne darm hoger ten opzichte van 0,5 uur na het voeren. Deze pH verandering in de tijd waren sterker bij de meervallen op het 700 mEq kg^{-1} CAD rantsoen. De resultaten geven aan dat de Afrikaanse meerval de pH van de digesta aan kan passen korte tijd na de voeropname en dat de pH in het maagdarmkanaal constant kan worden gehouden bij de verschillende CAD niveaus van de rantsoenen. Bij biggen waren geen verschillen aanwezig in pH van digesta in maag en dunne darm 2,5 uur na het voeren tussen rantsoenen met een CAD van –100 en 200 mEq kg^{-1}.

Bij biggen werd tevens onderzocht of het effect van CAD in het voer afhankelijk was van de voersamenstelling. Een interactie effect tussen CAD niveau en het gehalte aan niet zetmeel koolhydraten (NSP) in het rantsoen werd waargenomen ten aanzien van voeropname, groei en drogestof verteerbaarheid. Dit geeft aan dat de effecten van CAD in het voer op voeropname en groei afhankelijk zijn van het NSP gehalte van het voer. Verder werd er in het betreffende experiment gevonden dat gewichten van de lever en de dikke darm significant lager waren bij biggen op het –100 mEq kg^{-1} CAD voer dan op het 200 mEq kg^{-1} CAD voer.

Concluderend kan gesteld worden dat het CAD niveau in het voer de prestaties van zowel Afrikaanse meervallen als van varkens beïnvloedt. Een negatieve CAD in het voer verlaagt de voeropname en groei bij beide diersoorten. Het optimale CAD niveau in het voer is echter verschillend bij deze twee diersoorten. Het mechanisme van dit effect van het CAD niveau in het voer is hoofdzakelijk gerelateerd aan (i) een veranderde regulatie van het zuur-base evenwicht; (ii) een veranderde onderhoudsbehoefte aan energie en (iii) de interactie tussen het CAD niveau in het voer met overige voersamenstelling. Het CAD niveau van een voer is een belangrijk aspect dat meegenomen moet worden bij de formulering van diervoeders.

饲料中 阴阳离子差对非洲鲶鱼和猪生长的影响: 一项对比研究
(中文摘要)

在动物营养领域中，营养学家们往往注重于动物对饲料蛋白质和脂肪的需求。但是最近营养学研究结果表明饲料的代谢后果也同样重要。饲料中的阴阳离子差 (Na + K - Cl, mEq/kg) 会影响动物体内的酸碱平衡。阴阳离子差过低会增加代谢酸，导致动物体内的酸积累。一个合理的阴阳离子差会减少代谢酸的积累，提高动物体内抗酸能力，帮助动物维持酸碱平衡，减少能量消耗，从而提高摄食量和生长速度。

本论文的主要目的是调查饲料中的阴阳离子差对于非洲鲶鱼和猪摄食与生长，体内酸碱平衡和能量代谢的影响。

本论文由三部份组成。第一部份的主要目的是确定鱼和猪饲料中最合理的阴阳离子差。第二部份研究了饲料中的阴阳离子差对于能量代谢的影响。第三部份调查了饲料中的阴阳离子差与动物血液和消化道中酸碱平衡的关系。

本项研究结果表明饲料中的阴阳离子差对于非洲鲶鱼和猪摄食与生长都有很大的影响。负的阴阳离子差抑制了两种动物的摄食与生长; 正的阴阳离子差促近了动物的摄食与生长。对于非洲鲶鱼来说，最合理的阴阳离子差介于500 至700 之间。对于仔猪来说，最合理的阴阳离子差介于200 至500 之间。

对于非洲鲶鱼来说，负的阴阳离子差的确增加了热能消耗。当阴阳离子差是700 mEq/kg时，非洲鲶鱼需要最少的能量来维持基础代谢。然而仔猪的实验结果表明当饲料供给保持在限定的水平，一个负的阴阳离子差倾向于减少热能消耗。

虽然摄入不同阴阳离子差的饲料会在短期内（几小时内）影响非洲鲶鱼消化道内的酸碱平衡，但非洲鲶鱼能够调解消化道内的pH值从而维持适应于消化媒需要的酸碱度。

仔猪血液的pH值明显的受到了饲料中的阴阳离子差的影响。负的阴阳离子差能够降低了血液的pH值。而正的阴阳离子差能够提高血液的pH值。在短期摄食后，正的阴阳离子差能使血液中碳酸氢根离子的含量增加，表明动物摄取这个饲料后提高了体内的抗酸能力。

总体来说，研究结果表明了饲料中的阴阳离子差会改变动物体内的酸碱平衡，影响能量代谢，从而改变动物的摄食和生长。

本中文摘要是特别为我亲爱的母亲，姐姐，哥哥们和家人而作.

ACKNOWLEDGEMENTS

In a bit more than 4 years time, this thesis was realized with direct and indirect help from many people, who I own many thanks.

First of all, I want to say, my husband, Frank Dersjant's encouragement and support assured me to start and to progress with this study. I enjoyed all the helpful discussions with him and he shared all the happiness with me with any progress and good results and comforted me when I met with problems. I am proud to say, I have a sweet daughter, Wenzhuo, she never complains when she has to baby sit for her young sister and brother allowing me to work on my manuscripts.

Very special gratitude is given to my supervisors, also as co-promotor and promotor: Johan Verreth and Martin Verstegen, you both enabled me to start and finish my PhD study. All the help from both of you are beyond any words. I really appreciated all the instructions and suggestions from you and discussion with you. Johan, you have spent many weekends reading and correcting my manuscripts. Martin, I am always surprised with your quick actions. The encouragement and enthusiasm of both of you are the stimulus for my PhD study. Many thanks Johan and Martin!

I would like to express my thanks to Johan Schrama, my co-promoter, thank you for being critical. I appreciate your sharp comments on my manuscripts and all your support during my experiments especially on energy metabolism study in pigs. Thank you also for editing the Dutch translation of the summary. I am grateful to my promoter, Bram Huisman, for his warm support, reviewing the manuscripts and his trust. To Prof. Seerp Tamminga, for all the necessary support when we needed it most.

Wu Sheng, thank you for joining part of this PhD project as your MSc thesis, with your help, I could conduct the fish and pigs experiments at the same time. Thank you for your hard work and friendship.

Peter Tijssen, when I had to leave in the middle of the experiment because of necessary medical care for pregnancy reasons in the hospital, you (together with Johan Verreth) took over and finished the experiment. You assisted me further with data analysis and sample measurement. Thanks Peter. Ronald Booms, I very much appreciate all the technical support from you, from taking blood samples to the patient help in the lab. Menno ter Veld and Tino Leffering, for the same reasons, please accept my thanks. Ep Eding, I have bothered you with many questions and you always try to help me, thanks. Sietze Leenstra and Arts Hutten, thank you for being friendly and kind in helping me in the hatchery.

Tamme Zandstra, I really want to thank you for all the arrangements during my pig experiments. You helped me with details in planning the experiment and preparing the experimental material, so that I could shift from fish to pig experiments without confronting any problems.

Huug Boer, without your kind support for the sample's analysis and all the *in vitro* lab work, my study would take longer time. I own you many thanks. The same to Jane-Martine Muylaert and

Marian van't End, I am very grateful for your help in the chemical analysis of samples. I also want to thank Truus Post and Meijke Booij for kindly helping whenever I needed it.

Marcel Heetkamp and Koos van der Linden, your skilful technical assistance in the respiration chambers allowed me to establish the energy metabolism study of pigs. Marcel, thank you for helping me, checking the data and all the data analysis of that experiment. I would like to thank Ries Verkerk, Andre Jansen, Peter Vos and all the people of the experimental unit 'de Haar Varkens' for their help during my pig experiments. Thanks to Truus van der Wal, Arie van den Dool, Peter van der Togt, Marlou Bosch and Marian van't End for dissecting the piglets and collecting samples. I'd like to thank Thomas van de Poel and Tamme Zandstra, for helping me in oil coating the pellets.

Chapter 6 of this study was conducted in the facility of *TNO-ILOB*. Alfons Jansman, thank you for all the detailed advice and discussions during this experiment. I would like to thank Mr. Piet van Leeuwen, Mr. Dick van Kleef, Mr. Kasper Deuring and Ms Baukje Schat for operating on the pigs, stable management, health control, sampling and measurements. Thanks to Mr. Gerard Beelen and Mr. Johan de Jong for formulating the experimental diets. Thanks to Mr. Piet Roeleveld and his colleagues for producing the experimental feeds. Sincere thanks are given to Rolf Coolen and Anne-Marie van den Driessche, for their help in mineral analysis whenever I needed it.

Sincerely thanks are also given to Dr. Gert Flik from Nijmegen University for reviewing the manuscript of chapter 3. I should also thank Wiebe Koops and Marcel Machiels for patiently explaining to me the statistical methods.

I would like to express my thanks to all my fellow PhDs and colleagues in Fish Culture Group and Animal Nutrition Group, for all your friendship and help. Rodrigo Ozorio, thank you for your kind offer in taking blood samples, Pablo Almazan Rueda, thank you for helping me to draw a figure. Neil Ruane, thank you for checking the English in my manuscript. Gerrie van Eck, Henriette Bal, Nieske Ankersmit, Ana Viveiros, Karin van der Braak, Armondo Ortega, Hans and Nelly van Weerd, Michel Tanck, Jules van Rooij, Marc Verdegem, Ekram-UI-Azim, Josien Bos, Maria Lippelt-Rutten, René Kwakkel, Barbara Williams, Marianne Bruining, Berry Diekema, Walter Gerrits, Wilbert Pellikaan, Sandra Rodrigues, Martin Rijnen and many other persons whom I may forget to mention. I would like to apologize for any bother I might have incurred and thank you for the pleasant working environment.

The warmest thanks are given to my family in China and in Holland and to my friend YuTong for their support. I, of course, should not forget to thank to my friend, Lie Hong, for being always ready to look after my children when needed. For the same reason I would like to thank to Xiaoyan Sun.

This study was financially supported partly by Finn Feeds Int. and Provimi bv., Hagen Schulze, Howard Simmins and France Evers, all the financial support from you gave me the opportunity to carry out the experiments. Many thanks.

LIST OF PUBLICATIONS

Peer-reviewed papers

Conceição, L. E. C., Dersjant-Li, Y. and Verreth, J. A. J. 1998. Cost of growth in larval and juvenile African catfish (*Clarias gariepinus*) in relation to growth rate, food intake and oxygen consumption. Aquaculture 161: 95-106.

Dersjant-Li, Y., Verreth, J. A. J., Evers, F., Tijssen, P. A. T., Booms R. and Verstegen, M. W. A. 1999. The influence of dietary cation-anion differences on acid-base balance, food intake, growth and nutrient utilization of juvenile African catfish *Clarias gariepinus* (Burchell). Fish Physiology and Biochemistry 20: 305-311.

Dersjant-Li, Y., Verreth, J. A. J., Tijssen, P. A. T., Booms, R., Verstegen, M. W. A. and Huisman, E. A. 2000. Metabolic costs of changing the cation-anion difference in the diet of juvenile African catfish *Clarias gariepinus* (Burchell). Aquaculture Nutrition 6: 39-45.

Dersjant-Li, Y., Schulze, H., Schrama, J. W., Verreth, J. A. J. and Verstegen, M. W. A. Feed intake, growth, digestibility of dry matter and nitrogen in young pigs as affected by dietary cation anion difference and supplementation of xylanase. Submitted to J. Anim. Physiol. Anim. Nutr.

Dersjant-Li, Y., Verstegen, M. W. A., Schulze, H., Zandstra, T., Boer, H., Schrama, J. W. and Verreth, J. A. J. Performance, digesta characteristics, nutrients flux, plasma composition and organ weight in pigs as effected by dietary cation anion difference and non starch polysaccharide. Submitted to J. Anim. Sci.

Dersjant-Li, Y., Verstegen, M. W. A., Jansman, A., Schulze, H., Schrama, J. W. and Verreth, J. A. J. Postprandial blood oxygen and pH in response to dietary cation anion difference in pigs. Submitted to J. Anim. Sci.

Dersjant-Li, Y., Schrama, J. W., Heetkamp, M. J. W., Verreth, J. A. J. and Verstegen, M. W. A. Effect of dietary cation anion difference on metabolic rate and energy balance in pigs. Submitted to J. Anim. Sci.

Dersjant-Li, Y., Wu, S., Verstegen, M. W. A., Schrama, J. W. and Verreth, J. A. J. The impact of changing dietary Na/K ratios on performance and nutrients utilization in juvenile African catfish, *Clarias gariepinus*. Submitted to Aquaculture.

Dersjant-Li, Y., Wu, S., Schrama, J. W., Verstegen, M. W. A. and Verreth, J. A. J. Digesta pH and buffer capacity of African catfish, *Clarias gariepinus* as affected by dietary cation anion difference. Submitted to Comp. Biochem. Phyiol.

Dersjant-Li, Y., Verreth, J. A. J., Schrama, J. W., Huisman E. A. and Verstegen, M. W. A. The impact of the dietary electrolyte balance on growth performance, energy metabolism and acid-base balance: a comparison between African catfish (*Clarias gariepinus)* and pigs. Submitted to Aquaculture Research.

Proceeding papers and abstracts

Dersjant-Li, Y., Verreth, J., Evers, F., Tijssen, P. and Verstegen, M. 1997. Effects of dietary cation-anion concentrations on food intake, growth, energy and nitrogen balance in juvenile African catfish (*Clarias gariepinus*). First (Cost 827) Workshop Voluntary Food Intake in Fish, pp 48 (abstract). 3-6 April, Department of Zoology, Aberdeen University, Scotland.

Dersjant-Li, Y., Schulze, H., Verreth, J. and Verstegen, M. 1998. Effects of dietary cation-anion differences (CAD) on food intake, growth and nutrient utilisation in piglets. Proceedings of international conference on pig production, pp323-326. 6-8 July. Beijing, China

Dersjant-Li, Y., Schulze, H. and Verstegen, M. 1998. Effect of dietary cation-anion differences on food intake, growth and nutrient utilisation in piglets. J. Anim. Sci. 76 (suppl. 1): 189.

Dersjant-Li, Y., Verreth, J. A. J., Evers, F. and Verstegen, M. W. A. 1998. Effects of dietary cation-anion differences on food intake, growth, maintenance costs and energy utilisation in juvenile African catfish *Clarias gariepinus* (Burchell). Recent advance in finfish and crustacean nutrition, VIII international symposium on nutrition and feeding of fish P 159. June 1-4, Las palmas, Spain.

Dersjant-Li, Y., Verstegen, M., Jansman, A., Verreth, J., Schrama, J. and Schulze, H. 1999. Postprandial blood pH and acid base balance in pigs as affected by dietary cation-anion difference. J. Anim. Sci. 77 (suppl. 1): 198.

Dersjant-Li, Y., Schrama, J. W., Heetkamp, M., Verstegen, M., Simmins, H. and Verreth, J. Effect of dietary cation-anion differences on energy partitioning in pigs. Proceeding paper submitted to 15th Symposium on energy metabolism in animals. September 10-16, 2000 in Elsinore, Denmark

CURRICULUM VITAE

Yueming Dersjant-Li was born on 7th of August, 1958, in Harbin, Heilongjiang Province, China. She obtained her BSc degree in Aquaculture in January 1982 at Dalian Fisheries University (*cum laude*). After her graduation, she was employed by Fisheries School of Heilongjiang Province as a lecturer. She was responsible with some teaching and research tasks. She gave courses mainly in Hydrochemistry, Freshwater biology and Aquatic Ecology. The research was mainly done in the field of fish nutrition and pond management. She was selected as a municipal teaching expert by the Harbin adult education committee in 1989. In 1992, she, as a visiting scholar, did some research work in the field of fish nutrition in the Netherlands Institute of Ecology, Centre for Limnology, Nieuwersluis, the Netherlands. In 1993, she enrolled as MSc student in Fish Culture and Fisheries Group of Wageningen University. In 1995, she graduated with distinction in Aquaculture. From 1996 she started her PhD study in Animal Nutrition. She is married with two daughters and one son.