Physico-chemical chyme conditions and mineral absorption in broilers

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Physico-chemical chyme conditions and mineral absorption in broilers

PROEFSCHRIFT

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STELLINGEN

I

Voor een goed inzicht in factoren, die de verteerbaarheid van voederbestanddelen in het maagdarmkanaal beïnvloeden, is onderscheid in afbraak en absorptie noodzakelijk.

II

De potentiële invloed van fysisch-chemische omstandigheden in het maagdarmkanaal op de absorptie van mineralen dient te worden vastgesteld in die darmdelen waar normaliter de meeste absorptie optreedt. *Dit proefschrift*

III

De bijdrage van het ileum aan de absorptie van mineralen is sterk afhankelijk van de schijnbare absorptie in voorgaande darmdelen. *Dit proefschrift*

IV

De transit tijd is alleen in uitzonderlijke gevallen bruikbaar als voorspelling van verschillen in de gemiddelde verblijftijd van voer in het maagdarmkanaal.

Dit proefschrift

V

Om het effect van fysisch-chemische omstandigheden in het maagdarmkanaal op de absorptie van mineralen goed te kunnen bestuderen, is beïnvloeding van deze omstandigheden door modelstoffen onontbeerlijk. *Dit proefschrift*

VI

Ouders realiseren zich onvoldoende dat een automatische keuze voor het regionaal reformatorisch onderwijs een ernstige bedreiging vormt voor het plaatselijk protestants christelijk onderwijs.

VII

Congregationalisme is ingebed in de grondleggende artikelen van de Verenigde Protestantse Kerk in Nederland.

VIII

Invoering van sociale dienstplicht voor jongeren is een goede basis voor een "zorgzame samenleving".

IX

Veel bezuinigingsmaatregelen in maatschappij en kerk zijn uitsluitend mogelijk door een beroep op het stijgend aandeel van de bevolking dat niet aan het reguliere arbeidsproces deelneemt.

X

De uitspraak van Montesquieu (1687-1755) "De veelheid van wetten is een teken van het verval van een volk" is vandaag de dag zeer actueel.

J.D. van der Klis
PHYSICO-CHEMICAL CHYME CONDITIONS AND MINERAL ABSORPTION IN BROILERS
Wageningen, 16 november 1993

WOORD VOORAF

Dit proefschrift is de weergave van een onderzoek dat is uitgevoerd op het Centrum voor Onderzoek en Voorlichting voor de Pluimveehouderij "Het Spelderholt" te Beekbergen. De resultaten van een viertal experimenten zijn hierin beschreven. Bij de voorbereiding en de uitvoering daarvan heb ik een beroep moeten doen op een groot aantal collega's, die zonder uitzondering hun welwillende medewerking hebben verleend (ook op soms minder gebruikelijke werktijden). Ik wil hen allen daarvoor bedanken.

Ik wil een aantal van hen in het bijzonder noemen: Zonder de snelheid, waarmee Sander van Voorst de kuikens van hun maagdarmkanalen wist te ontdoen, was de uitvoering van deze experimenten vrijwel onmogelijk geweest. De medewerkers van de proevendienst en de technische dienst waren onmisbaar bij de voorbereiding en uitvoering van de experimenten. De slachtdames hebben geassisteerd bij het verzamelen van de inhoud uit de honderden maagdarmkanalen. Na de monstervoorbereiding heeft het laboratoriumpersoneel met veel toewijding de talloze analyses uitgevoerd. Er werd zelfs nauwelijks geklaagd als ik hen weer eens met nieuwe monsters verraste, al kon ik hen niet altijd even snel vinden.

Ik ben Pieter Vereijken erkentelijk voor zijn hulp bij de opzet van de proeven en uitwerking van de resultaten. Ook hen die de uiteindelijke publikaties hebben meegelezen wil ik bedanken. Vooral Kees Kan, die met vaardige hand zijn rode pennetje heeft gehanteerd.

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Mijn ouders, schoonouders en grootmoeder wil ik hartelijk danken voor hun morele en financiële steun. Ik wil afsluiten met Nelleke te danken, die gedurende de tijd dat ik met mijn promotie-onderzoek bezig ben geweest onze kinderen heeft opgevoed en mij door dik en dun heeft gesteund. Jij hebt in mijn functioneren een cruciale rol gespeeld.

Jan Dirk

aan Nelleke

aan Jan Willem, Peter en Mirjam

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Klis, J.D. van der. Physico-chemical chyme conditions and mineral absorption in broilers.
In the Netherlands the efficiency of mineral absorption from the gastro-intestinal tract of farm animals is a topic of interest to reduce the mineral concentration in animal manure. This study was done with broilers. It was focused on physico-chemical chyme conditions. These conditions were related to the absorption of minerals. Carboxy methyl cellulose was used as a model substance to affect the intestinal viscosities. Retention time parameters, pH and osmolalities were recorded. The site of mineral (Na, K, Ca, P, and Mg) absorption and apparent absorption values up to successive gastro-intestinal segments were determined. Effects of the intestinal viscosity were verified using wheat-based broiler diets.
Dietary inclusion of carboxy methyl cellulose (up to 1%) increased the intestinal viscosity, the mean retention time and decreased the ileal pH. The absorption of small osmo-active chyme components was reduced, which was reflected in less variable osmolalities as the chyme moved from the proximal small intestine onwards. The main site of mineral absorption is between the duodenum and the lower jejunum. In these segments the absorption is negatively affected by the inclusion of carboxy methyl cellulose in the diet. It was discussed that the intestinal viscosity was the main cause for this reduction in mineral absorption. Negative effects were partially compensated in the ileum. Similar effects were shown in wheat-based diets, but effects were less pronounced.
PhD. thesis. COVP-DLO "Het Spederholt", P.O.Box 15, 7360 AA Beekbergen, The Netherlands.

CONTENTS

General introduction

Intensive livestock production in the Netherlands resulted in manure surplus areas. In these areas manure production by farm animals exceeds the requirements of the crops grown. As a consequence, animal manure has been supplied to arable and grass land in excess to crop requirements (De Mol, 1989). Among other reasons, this has resulted in environmental pollution problems. To prevent further deterioration, manure application to arable and grass land is limited by legislation to reach an equilibrium between mineral supply and mineral use by the crops grown. In order to achieve this objective, the mineral content in the manure must be decreased, the surplus manure must be transported to manure shortage areas, or the surplus must be processed, to prevent a reduction in the number of animals.

One topic on increasing the efficiency of mineral use by animals, to enable a reduction in the dietary mineral concentration (and consequently in the faeces), is an improvement of the absorption of minerals from the gastrointestinal (GI) tract. Numerous studies have been done on dietary components affecting mineral absorption. Most of these studies were based on a direct relationship (as reviewed in Chapter 1). E.g. it was shown that phytate can bind polyvalent minerals, and that elements, which are chemically similar, can exchange for one another in carrier mediated transport processes through the intestinal mucosa, or compete for bonding sites on organic molecules. So far little attention has been paid to the conditions in the GI lumen that might affect mineral absorption.

The scope of this thesis is to study the effects of the physico-chemical conditions in the intestinal tract on the absorption of minerals. Broilers were used as experimental animals. These fast growing birds have high nutrient requirements and were preferred over adult cock (non-productive) or laying hens (diurnal pattern in mineral absorption, and risk of cease in egg laying due to experimental treatment). In Chapter 1, the site of mineral absorption and the absorption processes are reviewed as well as effects of complexing elements and prevailing physico-chemical conditions in the GI tract. The first experiment (Chapter 2) has been carried out as a method evaluation. In this experiment, estimations of the mean retention time of food dry matter in successive GI segments were done, the usefulness of Cr_2O_3 as an inert marker was studied and the site of mineral absorption was determined. This experiment was done using a "normal" broiler diet. As

the efficiency of mineral absorption is dependent on the dietary mineral concentration, semi-synthetic diets were used in Chapters 3 to 5. The mineral concentrations in these diets were standardized around the mineral requirements of the broilers. Carboxy methyl cellulose (CMC), a soluble polysaccharide, was added to these diets to affect the physico-chemical conditions, with only marginal changes in the dietary composition. The primary aim of dietary CMC addition was to increase the mean retention time in the proximal segments of the small intestine of broilers (the site of effective mineral absorption) by increasing the viscous activity of the diet. Therefore, the effect of the dietary CMC concentration on the mean retention time in successive GI segments was studied. Also other physico-chemical conditions (pH, osmolality and viscosity) were determined, to derive which of those factor(s) actually affect mineral absorption (Chapter 4). These factors were related to the absorption of calcium, magnesium, phosphorus, sodium and potassium (Chapter 5). It was emphasized that the viscosity can have strong negative effects on the absorption of minerals from the intestinal tract. Finally, this emphasis was checked in wheat-based broiler diets (Chapter 6).

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CHAPTER 1

THE ABSORPTION OF MINERALS FROM THE GASTROINTESTINAL TRACT OF POULTRY. MECHANISM AND INTESTINAL CONDITIONS (A REVIEW)

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ABSTRACT Minerals are absorbed from the gastrointestinal (GI) tract mainly as ions or small soluble complexes. Monovalent minerals are absorbed more efficiently than polyvalent minerals. In poultry, the absorption of the latter varies considerably and does generally not exceed 50% of the daily intake, while the absorption of monovalent minerals might even reach 80% of the daily intake. The efficiency of mineral absorption is dependent on the absorption mechanisms available, on the dietary composition, on the presence of complexing agents in the GI lumen, and on the physico-chemical conditions in the intestinal tract. Extensive research has revealed many feed components, that affect mineral absorption through direct complexation in the GI tract. Furthermore, mutual interactions occur between minerals, that are chemically alike. Whether a bird can increase the efficiency of absorption of a specific mineral from the GI tract under suboptimal conditions, is dependent on the presence of active transport processes. Indirect effects on mineral absorption due to changes in the physico-chemical conditions (pH, viscosity, osmolarity) in the GI lumen and the time available for digestion and absorption have only been studied marginally. The prevailing pH in the GI tract (as one of those physico-chemical conditions) affects the stability of mineral complexes in the GI lumen. A high intraluminal viscosity reduces digestion and absorption of (an)organic feed components. The absorption of water and solutes from the GI tract is dependent on the difference in osmotic pressure between the intestinal lumen and blood. The time feed is retained in the GI tract should be just long enough to enable digestion and absorption.

In this review, mechanisms available for absorption of macro-elements and the site of mineral absorption are summarized. Subsequently, the prevailing conditions in the GI lumen, together with the significance of interactions between minerals and organic chyme components are described. Finally, factors that influence chyme characteristics are discussed.

(*Key words*: macro minerals, site of absorption, gastrointestinal conditions, interacting components of (in)organic origin).

1 INTRODUCTION

Minerals are essential nutrients for all living organisms. In poultry, minerals are necessary both for maintenance and for growth and egg production. Minerals accomplish a more or less specific physiological/biochemical function in the body; divided by Underwood (1982) into three categories:

1. Structural components of body tissues (e.g. Ca, P, Mg and S);
2. Constituents of body fluids for regulation of osmotic and acid-base balance and membrane permeability (e.g. Na, K, Cl, Ca, Mg);
3. Catalysts in enzyme and hormone systems (e.g. trace elements).

Mineral deficiencies will, due to the wide variety of functions, cause health problems and reduce productivity (Scott *et al*, 1976). When the gross mineral requirement for maintenance[1] and production[1] are met, sufficient minerals are absorbed from the gastrointestinal (GI) tract to meet the net mineral requirement[2]. The gross and net requirement are related as shown in equation 1:

$$\textit{Gross requirement (g/kg feed)} = 100 \times \frac{\textit{Net requirement (g/kg feed)}}{\textit{Apparent absorption (\%)}} \qquad [1]$$

Although many experiments have been carried out to estimate the mineral requirements[3] of poultry (A.R.C., 1975; N.R.C., 1984, WPSA Nutrition working group, 1985), the values still have to be regarded as 'tentative' (Hill, 1988). The mineral requirements are calculated from dose-reponse relationships. The results of these estimations are dependent on the response characteristics chosen (de Groote, 1983). Furthermore, many factors will influence the mineral requirements (Table 1). These factors are divided into those mainly

[1] *Gross requirement for maintenance and for production* is defined as the minimal amount of *dietary minerals* needed to maintain the body mineral content of a non-growing, non-producing animal or needed for body growth and egg production respectively

[2] *Net requirement for maintenance and production* is defined as the amount of *absorbed minerals* needed for maintenance or production respectively

[3] Mineral requirement is considered to be the gross mineral requirement unless stated otherwise

affecting the net mineral requirement and those mainly affecting the mineral availability in and absorption from the GI tract.

Table 1. Some factors, which can affect the gross mineral requirements[1] of poultry, divided into those which primarily affect the net requirement and those that primarily affect the absorption from the GI tract

	Factor	Reference number[2]
Net requirement:	Age	1, 2, 11, 12, 15
	Housing and management	1, 2, 7
	Production level	1, 2
	Strain	1, 7
	Sex	1, 2
Availability/ Absorption:	Feed/nutrient intake	1, 2
	Chelating agents	2
	Carbohydrates	10
	Fat	6, 9, 14
	Protein	2, 7, 8, 11
	Endogenous secretion	13
	Mineral intake/ ratio	3, 4, 5, 7
	Mineral compound in the feed	2, 16
	Mineral status of the animal	2
	Particle size of mineral compound	7

[1] Definition of gross requirement in text

[2] Literature sources: 1. Simons (1986); 2. Peeler (1972); 3. Johnson and Karunajeewa (1985); 4. Atteh and Leeson (1985a); 5. Atteh and Leeson (1984); 6. Whitehead *et al* (1971); 7. Peeler (1982); 8. Harrold *et al* (1983); 9. Håkansson (1975); 10. Vaughan and Filer (1960); 11. Sandström (1988); 12. Bronner (1987); 13. Weigand and Kirchgessner (1988); 14. Atteh and Leeson (1983a); 15. Morley *et al* (1980); 16. Hurwitz and Bar (1968b)

In broilers, both the mineral retention and the apparent absorption from the GI tract of most macro-elements do not exceed 50% of intake (Table 2). Dietary composition, like protein, fat, carbohydrate and mineral content (Table 1), as well as the prevailing intraluminal conditions (e.g. pH, viscosity) affect the efficiency of mineral absorption from the GI tract (van der Klis *et al*, 1993b). Optimizing nutrient digestibilities (which also implies a reduced concentration of potential chelating components in the GI lumen) and the physico-chemical conditions in the GI tract will, assuming an unchanged net

requirement, improve the mineral absorption and thereby reduce the gross mineral requirement of the bird. As the dietary mineral concentration is generally based on the gross requirement of the animal, a reduction of the latter will result in a decreased mineral excretion.

Table 2. The apparent absorption or the retention of some macro-elements from the GI tract (as % of intake) of layers and broilers, determined by sampling of the lower ileal contents or the faeces

Reference	Materials/methods	Experimental treatment/ Dietary mineral content	Mineral retention/ absorption	Remarks
Whitehead *et al* (1971)	2-4 wk old broilers semi-synthetic diet excreta sampling	Ca: 1.5% Mg: 1.1%	Ca: 38% Mg: 40%	
Guenter & Sell (1973)	mature chickens semi-synthetic diet ileal chyme sampling		Mg: 72% (true absorption) Mg: 0% (apparent absorption)	
Atteh *et al* (1983)	3 wk old broilers corn-soybean meal diet excreta sampling	Ca: 0.9, 1.2% Ca/aP was 1.5	P : 35% Ca: 52% Mg: 22%	
Atteh & Leeson (1984)	2 wk old broilers corn-based diet excreta sampling	Ca: 0.8, 1.2, 1.6%	P: 39% Ca: 49-61% Mg: 20-34%	
Van der Klis *et al* (1990)	6 wk old broilers corn soybean diet ileal chyme sampling	Ca: 1.2% Mg: 0.18% Na: 0.18% K: 0.84%	Ca: 35% Mg: 25% Na:-33% K: 64%	
Hurwitz & Bar (1965)	laying hens corn-soybean-milo diet ileal chyme sampling	Ca: 1.9, 3.6%	Ca: 58-61%	Cumulative absorption was dependent on stage of egg shell calcification
Hurwitz & Bar (1968)	laying hens milo-soybean diet ileal chyme sampling	Ca: 0.6, 1.8, 3.9%	P: 50-84% Ca: 60-82%	
Hurwitz *et al* (1970)	laying hens milo-soybean diet ileal chyme sampling	Na: 0.07, 0.15%	Na: 41-45% K: 76-83%	

Six macro-elements: calcium, phosphorus, magnesium, sodium, potassium and chloride are dealt with in this review. First the mechanism for and the site of mineral absorption are described. Subsequently, nutrient interactions and physico-chemical

conditions, which might affect mineral absorption, are described. And finally, ways to change the physico-chemical conditions are discussed.

2 MINERAL ABSORPTION

2.1 MECHANISM OF MINERAL TRANSFER ACROSS INTESTINAL MEMBRANES

Mineral transfer across the intestinal mucosa, from the intestinal lumen into the body fluids (absorption) and vice versa (secretion), is based on two different mechanisms of transmembrane solute movement: *diffusion* and *active transport*.

Diffusion is the most widespread process for solute transfer across a membrane. It takes place from a high solute to a low solute concentration (Neame and Richards, 1972). Apart from the concentration difference, also an electro potential difference across cell membranes (the intracellular side being electro-negative) will affect the rate of transfer (Macey, 1986). Both processes result in an electro chemical potential difference for an ion A ($\Delta\mu_A^{e-i}$) across a membrane, which can be calculated according to equation 2 (e.g. Aronson, 1990).

$$\Delta\mu_A^{e-i} = RT \ln \frac{[A]_e}{[A]_i} + Z_A F (\psi_e - \psi_i) \qquad [2]$$

Where: [A] = concentration; ψ_e, ψ_i = electrical potential extra- and intracellular; Z_A = charge of ion A; R,T,F = universal gas constant, absolute temperature and Faraday's constant respectively. If $\Delta\mu_A^{e-i} > 0$ diffusion will favour transport of ion A into the cell, while diffusion favours transport of A out of the cell when $\Delta\mu_A^{e-i} < 0$.

Processes, which change the concentration gradient of a solute and/or the potential difference across the membrane, will affect the rate of diffusion of a solute. A reduction in ionic activity at the high solute concentration side of a membrane, e.g. by complex formation between a mineral and a chelating agent, will reduce the rate of solute transfer. Consequently, a reduction at the low concentration side will stimulate this rate. Furthermore, the membrane permeability (number and permeability of channels) will also

affect the rate of diffusion.

Diffusion does not require metabolic energy. Diffusion of ions through a lipid bilayer membrane is considered to take place through specialized ion channels, e.g. Na^+, K^+ and Cl^- channels (Aronson, 1990).

In contrast to diffusion, *active transport* facilitates the movement of a solute across a membrane against the electro chemical potential difference. This process requires metabolic energy. Active transmembrane movement of solutes is carrier mediated and unidirectional. Because the number of carrier molecules is not infinite, active transport is saturable and the number of carrier molecules will limit the rate of transmembrane movement of the solute (Neame and Richards, 1972). The significance of carrier-mediated transport processes is dependent on the physiological and nutritional status of the animal, e.g. the rate of transcellular calcium transport is increased during calcium deficiency (Hurwitz, 1989).

Well known examples of active transmembrane mineral transport processes are the Na/K ATPase, the Ca-ATPase and several cotransport systems, like Na^+/H^+, K^+/H^+ and Cl^-/HCO_3^- (as reviewed by Powell, 1987, and Aronson, 1990). In the latter systems, transport of one ion down its electro chemical potential gradient (downhill), is the driving force for the uphill movement of the cotransported ion(s).

Facilitated diffusion and *solvent drag* are also used to describe transmembrane movement of nutrients. Facilitated diffusion is a carrier mediated, saturable process. The maximum rate of transmembrane movement is dependent on the number of carrier molecules (Birge and Avioli, 1987), as is the case for active transport processes. Like pure diffusion, its driving force is the electro chemical potential gradient of the solute across the membrane, however, the rate of transport is much higher. During solvent drag, solutes are transported across a membrane along with its solvent (Macey, 1986). Solvent drag enables the net transport of a solute against its own favourable electro chemical potential difference (Aronson, 1990).

2.2 SITE AND MECHANISM OF MINERALS AND WATER TRANSFER ACROSS INTESTINAL MUCOSA

In the previous section the mechanisms for solute transfer across membranes were summarized. These principles are used to describe the mechanism for and the site of absorption and/or secretion of minerals (Na, K, Cl, Ca, P and Mg) and water. The available data on the respective mineral concentrations in blood plasma and in the lumen of the GI tract are presented (Tables 3 to 6). The ultrafiltrable fraction, which consits of ions and small mineral complexes is indicated. The relevance of the different processes for mineral transfer is dependent on the cell function (absorptive or secretive) and the intestinal site (Powell, 1987).

2.2.1 SODIUM

Table 3. The site of net sodium absorption or secretion in the GI tract of poultry. The sodium concentration in the blood plasma[1] and in the GI lumen[2] is given

	Absorption/ secretion	Concentration	
		Total[3]	Ultrafiltrable[4]
blood plasma		125-150	92
duodenum	secr.	74	83
upper jejunum	abs.	58	75
lower jejunum	abs.	59	87
upper ileum	abs.	111	79
lower ileum	abs.	112	71

1 Morley *et al* (1980); Hurwitz *et al* (1970); Ames and Sakanoue (1964)
2 Data from laying hens fed 0.15% dietary Na (Hurwitz *et al*, 1970)
3 Total mineral concentration (mM)
4 Ultrafiltrable mineral concentration (% of total)

A large sodium secretion into the crop and the duodenum of laying hens was observed (Hurwitz *et al*, 1970), most likely as saliva and pancreatic/bilary secretion respectively. They used Y^{91} as a non-absorbable reference substance. Starting from the upper jejunum, net sodium absorption from the intestinal lumen was found (Hurwitz *et al*,

1970). The sites of sodium absorption and secretion in the GI lumen are comparable to those in 6 week old broilers (van der Klis *et al*, 1990).

Sodium secretion occurs from a high concentration in blood plasma to a low intraluminal concentration in the GI tract (Table 3). As the sodium concentration in the jejunal and ileal lumen is lower than the concentration in the blood plasma (Table 3), sodium absorption takes place against a concentration gradient.

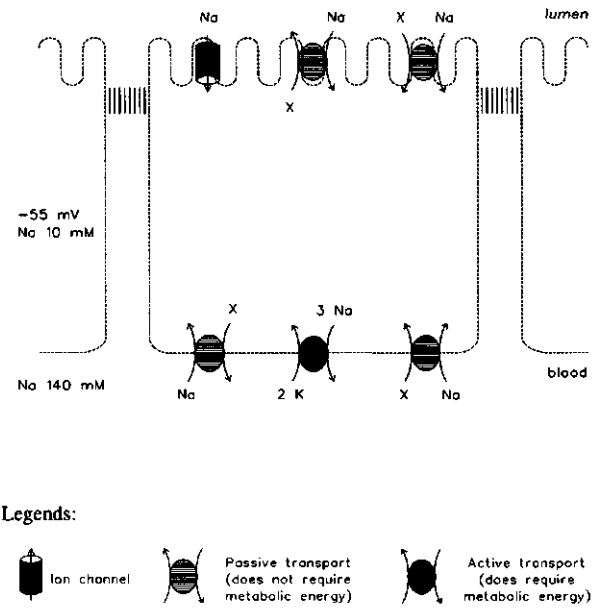

Figure 1. Mechanisms of sodium transfer across the intestinal mucosa.

The intracellular sodium concentration is kept a low level by a Na^+/K^+ ATPase at the basal membrane. Therefore, sodium can enter the cell passively by diffusion or coupled transport processes. In the latter, sodium absorption enables absorption of other minerals (like phosphorus) or organic molecules (like glucose or amino acids) against their own specific concentration gradients.

Mechanisms used for transcellular sodium transfer are shown in Figure 1. The sodium concentration in the cell is kept at a low level by active excretion (Na/K ATPase)

from the cell. Therefore, sodium can enter the cell passively, possibly carrier mediated. Sodium excretion into the intercellular spaces takes place at the basolateral cell membrane (Edmonds, 1974). This process of sodium transfer is discussed in more detail in section 2.2.7, in combination with water absorption processes. The rate of sodium absorption decreased from the lower jejunum to the lower ileum (Hurwitz *et al*, 1970). Sodium is actively absorbed from the colon of the laying hen, as was clearly demonstrated by Holtug and Skadhauge (1982). The rate of sodium absorption is, like other actively absorbed minerals, dependent on the nutritional status of the animal. Its absorption rate is increased in hens fed a low sodium diet (Hurwitz *et al*, 1970). Furthermore, Thomas and Skadhauge (1989b) showed in *in vivo* perfused laying hens, that the sodium absorption from the colon and coprodeum was doubled at a daily sodium intake of 1.8 mg/ kg live weight (LW)/ day compared to hens fed 93.8 mg Na/ kg LW/ day.

2.2.2 POTASSIUM

Table 4. The site of net potassium absorption or secretion in the GI tract of poultry. The potassium concentration in the blood plasma[1] and in the GI lumen[2] is given

	Absorption/ secretion	Concentration	
		Total[3]	Ultrafiltrable[4]
blood plasma		6-7	92
duodenum	secr.	36	82
upper jejunum	abs.	24	52
lower jejunum	abs.	21	53
upper ileum	secr.	35	60
lower ileum	secr.	80	47

1 Hurwitz *et al* (1970); Ames and Sakanoue (1964)
2 Data from laying hens fed 0.9 % dietary K (Hurwitz *et al*, 1970)
3,4 See Table 3

Potassium is secreted into the duodenum of laying hens (Hurwitz *et al*, 1970; De Pont *et al*, 1982) and broilers (van der Klis *et al*, 1990). Its absorption in the jejunum is

rapid, but a net secretion was observed in the ileum (Hurwitz *et al*, 1970). Potassium fluxes in the duodenum and the jejunum occurred from a high to a low concentration and transport was considered to be purely passive (Nys and Mongin, 1982). Secretion of potassium into the ileum, however, has to take place against its concentration gradient (Table 4), which is indicative for active transport. Potassium is also secreted into the integrative segment (caeca, colon and coprodeum). The rate of potassium secretion was related to the rate of sodium absorption from this segment (Thomas and Skadhauge, 1989b) and it was therefore suggested, that potassium transfer might be coupled to the absorption process of sodium (cotransport mechanism). Processes of potassium transfer across the intestinal mucosa are summarized in Figure 2.

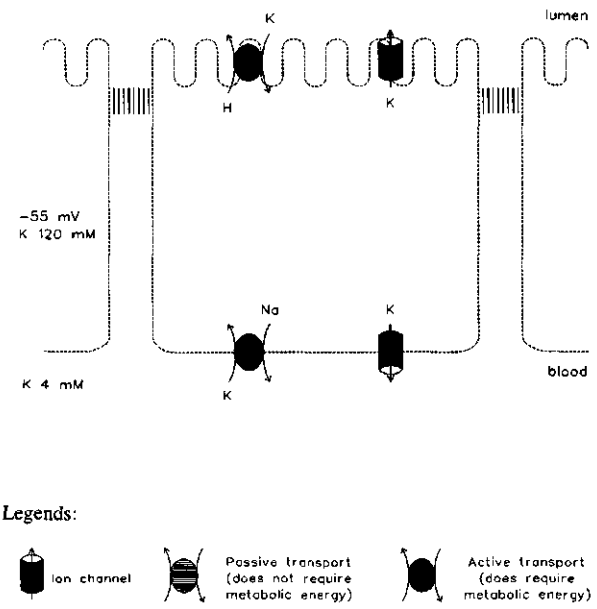

Figure 2. Mechanisms of potassium transfer across the intestinal mucosa.

Potassium absorption is considered to be purely passive. However, a H^+/K^+ATPase at the apical membrane or a Na^+/K^+ATPase at the basal membrane have been proposed. The significance of each of these processes will affect net secretion or absorption.

2.2.3 CHLORIDE

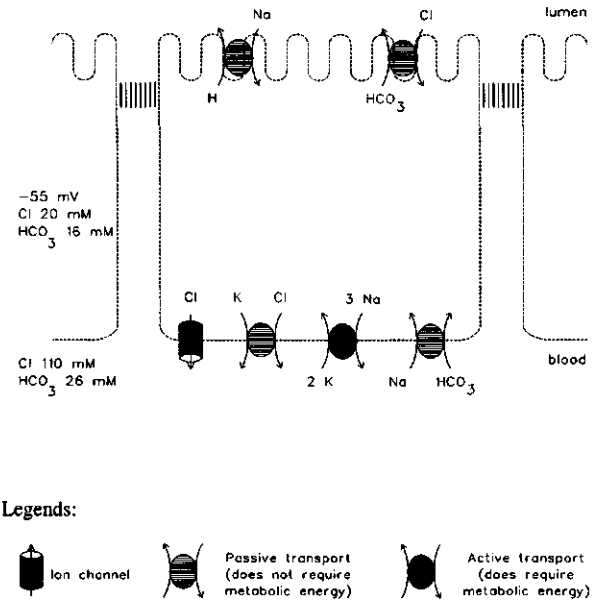

Figure 3. Mechanisms of chloride transfer across the intestinal mucosa.

Chloride is absorbed from the GI lumen as a Na^+/Cl^- cotransport mechanism or a combination of a Na^+/H^+ - Cl^-/HCO_3^- exchange process. Chloride excretion is favoured by its electro chemical concentration gradient (Powell, 1987). Excretion could occur through a chloride channel. The shown Na^+/H^+ and Cl^-/HCO_3^- exchange processes are involved in intracellular pH regulation. The K^+/Cl^- cotransport accelerates Cl excretion from the cell (Aronson, 1992).

Experimental data on the site of intestinal chloride absorption are very limited. The well-known chloride secretion (as hydrochloric acid) into the proventriculus is followed by a secretion in pancreatic fluids (Scratcherd and Hutson, 1982). Chloride transport is based on several cotransport mechanisms (Figure 3). It is likely, that these processes account for chloride absorption from the intestinal lumen. Nys and Mongin (1982) concluded, from their *in vivo* jejunal perfusion experiment with laying hens, that chloride flux was related to the net water flux, and that solvent drag might account for chloride absorption. They

also demonstrated a Na/Cl cotransport mechanism. Thomas and Skadhauge (1989b) found in *in vivo* perfused laying hens a linear positive relationship between sodium and chloride fluxes in the colon and coprodeum. These data seem to conflict with those of Holtug and Skadhauge (1982), who concluded from their *in vitro* experiment using the colon of laying hens, that chloride was absorbed passively from the colon, independently of the sodium flux. Calonge *et al* (1992) demonstrated *in vitro* another process for transmembrane chloride transfer (Cl^-/HCO_3^- cotransport) in brush-border membranes from chickens. This Cl^-/HCO_3^- exchange process is involved in intracellular pH homeostasis and exchanges extracellular Cl^- for intracellular HCO_3^- (Calonge *et al*, 1992).

2.2.4 CALCIUM

Many experiments have been carried out to establish the site of and processes responsible for calcium absorption from the intestinal tract. The sites of calcium absorption and secretion in the GI tract of poultry are given in Table 5. Net calcium secretion was observed in the duodenum of broilers (Hurwitz and Bar, 1970), while net absorption was found in layers duodenum (Hurwitz and Bar, 1969). The major part of calcium absorption occurred in the duodenum and the jejunum of broilers and layers (Hurwitz and Bar, 1970, 1971; van der Klis *et al*, 1990). In more posterior segments of the GI tract hardly any absorption was found, while in layers (fed diets containing 3.9% Ca) calcium was secreted into the ileal lumen. In laying hens, absorption and secretion of calcium is dependent on the stage of egg shell formation. Waddington *et al* (1989) observed that birds, which were forced to lay soft-shelled eggs, showed net calcium secretion into the duodenum. This secretive activity was not present in "normal" birds. The latter absorbed more calcium from the upper jejunum, as also found by Hurwitz and Bar (1968b). During shell formation, absorption occurred from the ileum and colon, while at days without shell formation calcium secretion into these segments was found (Hurwitz and Bar, 1965, Waddington *et al*, 1989). In *in vivo* perfused laying hens, Nys and Mongin (1980) showed, that the increased calcium absorption from the jejunum during egg shell formation was not caused by an improved intestinal capacity for absorption, but to an higher ionic strength of calcium in the intestinal lumen. They cited unpublished

Table 5. The site of net calcium absorption or secretion in the GI tract of poultry. The calcium concentration in the blood plasma[1] and in the GI lumen[2] is given

	Absorption/ secretion	Concentration		
		Total[3]	Ultrafiltrable[4]	Ionic[5]
blood plasma		2.5-8.0[6]	20-30	60
duodenum	secr.	68	9	6.1
upper jejunum	abs.	69	10	7.1
lower jejunum	abs.			
upper ileum	abs.	90	7	3.5
lower ileum	≈	107	7	2.0

1 Hurwitz and Bar (1971); Van der Velde *et al* (1986); Shafey *et al* (1990)
2 Data from broilers fed 0.71 % dietary Ca (Hurwitz and Bar, 1971)
3,4 See Table 3
5 Data from broilers fed 0.71% dietary Ca (Hurwitz and Bar, 1970)
6 The calcium concentration in the blood plasma is dependent on the onset of lay, and decreases during egg formation (Van de Velde *et al*, 1986). It is affected by the physiological status of the animal, which explains the large differences between the minimal and maximal plasma calcium level (5.5-8.0 mM) for layers. In broilers fed 1.2 % dietary Ca, a concentration of 2.6 mM was found by Shafey *et al* (1990)

results, showing a higher concentration of Ca^{2+} in the duodenal and jejunal lumen during egg shell calcification, compared to a situation without egg shell deposition.

Calcium is transported across the intestinal membranes by both a saturable and a non-saturable process (Wasserman, 1981; Pansu *et al*, 1981; Bronner, 1987). The former process is considered to occur through the mucosa cells (transcellular), while the latter is paracellular (between mucosa cells: Bronner, 1987). Karbach (1992) estimated the contributions of both processes in the *in vitro* perfused rat small intestine. In there 60-70% of the mucosa-to-serosa flux was considered to be paracellular and the remainder transcellular. The transport of calcium into the mucosa cell may occur along the electrochemical potential difference (Fullmer, 1992). The intracellular concentration is kept at a low level by calcium binding to several calcium binding proteins (e.g. calmodulin and calbindin: Favus, 1992). The excretion of calcium is an active process, mediated by Ca,Na-exchange or a Ca-ATPase at the basolateral membrane (Wasserman *et*

al, 1992), as illustrated in Figure 4.

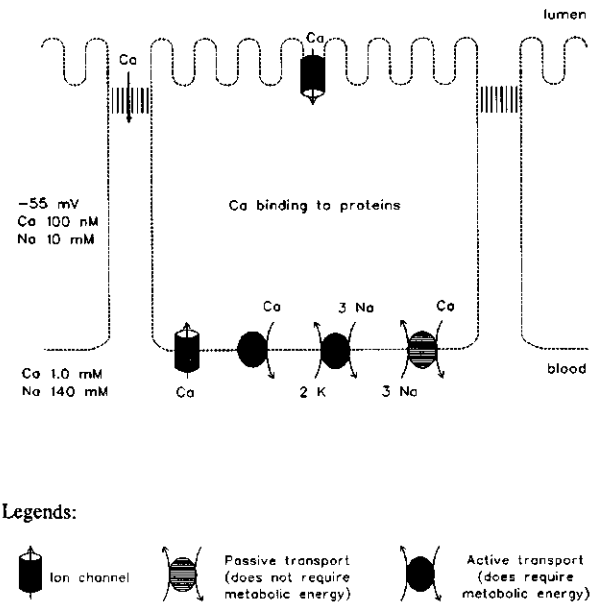

Figure 4. Mechanisms of calcium transfer across the intestinal mucosa.

Calcium entrance into the mucosa cell is a passive process, possibly through calcium channels. Intracellular calcium is bound to proteins (vit D independent calmodulin and vitamin D dependent calbindin) (Favus, 1992). At the basal membrane calcium excretion occurs through a Ca-ATPase or a Na^+/Ca^{2+} exchange, where sodium transport is the driving force for the calcium transport against the calcium concentration gradient. Although transcellular vesicular calcium transport is not illustrated, it might account for some calcium transport (Wasserman *et al*, 1992). Paracellular calcium transport from lumen to blood occurs through the tight junctions along its concentration gradient.

The stimulatory effect of vitamin D_3 (as $1,25(OH)_2D_3$) involves all aspects of calcium transport through the cell: entry across the brush border, intracellular calcium binding and calcium transport and finally excretion across the basolateral membrane (Bronner, 1992).

Bronner (1987) stated, that the active transport process is restricted to the duodenum and the jejunum, while diffusion can take place across the mucosa of the entire small intestinal. Although the electrochemical potential difference between the intestinal lumen and blood plasma is affected by calcium intake, it remains positive in the duodenal and jejunal lumen, allowing diffusion over a wide range of calcium intakes (Hurwitz, 1989). Diffusion is the main driving force for calcium absorption in laying hens, which occurred along its gradient without saturation until a luminal concentration of 20 mM (Nys and Mongin, 1982). The saturable (active) process can be affected by the nutritional and physiological status of the animal. During calcium restriction, the magnitude of active transport increases significantly (Hurwitz and Bar, 1969; Hurwitz, 1989).

2.2.5 PHOSPHORUS

Table 6. The site of net phosphorus absorption or secretion in the GI tract of poultry. The phosphorus concentration in the blood plasma[1] and in the GI lumen[2] is given

	Absorption/ secretion	Concentration	
		Total[3]	Ultrafiltrable[4]
blood plasma		3.5-7.5	-
duodenum	secr.	31	37
upper jejunum	abs.	50	7
lower jejunum	abs.	-	-
upper ileum	≈	72	2
lower ileum	≈	96	1
integrative segment	≈		

1 Atteh and Leeson (1983a); Atteh *et al* (1983)
2 Data from broilers fed 0.56% dietary P (Hurwitz and Bar, 1971)
3,4 See Table 3

Phosphorus absorption in the intestine is closely related to the absorption of calcium, but is not dependent on calcium absorption (Wasserman, 1981). In broilers, no net phosphorus secretion into the lumen of the GI tract was observed anterior to the duodenum (Hurwitz and Bar, 1970). From the duodenum to the upper jejunum

phosphorus was absorbed very efficiently. In the more posterior segments of the intestine, no further net changes were observed by these authors (Table 6). In laying hens, a large inflow of phosphorus into the duodenal lumen was found (Hurwitz and Bar, 1965). In contrast to the broilers, absorption occurred through the whole small intestine, but the rate of absorption decreased in the more posterior segments. Wasserman and Taylor (1973) showed a decreasing rate of phosphorus absorption (P^{32} absorption during 15 min, as a % of the intraluminal dose) from the chick ileum, at higher intraluminal phosphorus concentrations (> 2mM). The absorption of phosphorus was suggested to be a saturable process, which is indicative for active absorption or facilitated diffusion from the GI tract. Based on literature data on human small intestinal mucosa, Wilkinson (1976) concluded, that at low intraluminal concentrations (0.03 mM), phosphorus might be absorbed from the intestinal lumen against its concentration gradient. Favus (1992) indicated the presence of a paracellular and transcellular pathway. Phosphorus enters the cell through the brush border by a Na/P cotransport mechanism, in which the electro chemical potential difference for sodium is the driving force for the cotransport of phosphorus. Phosphorus leaves the cell by (facilitated) diffusion (Figure 5).

2.2.6 MAGNESIUM

Guenter and Sell (1973) observed a net secretion of magnesium into the duodenum of mature male chickens. Using Mg^{28} to label the endogenous magnesium pool, they concluded that the rate of absorption of both dietary and endogenous magnesium was highest in the duodenum and the jejunum. In the ileum and colon hardly any net absorption was observed. These sites of apparent absorption were in also found by van der Klis *et al* (1990). However, Hardwick *et al* (1991) recently reviewed that in humans and rats a significant proportion of magnesium was absorbed from the distal intestine. In a rat perfusion study, magnesium absorption occurred from the duodenal as well as the ileal lumen (Urban and Schedl, 1969). They observed a decreasing magnesium absorption per unit intestinal weight towards the distal intestinal segments. Behar (1974) observed magnesium absorption from the ileal as well as from the colonic lumen in rats. At favourable pH values, Guenter and Sell (1973) demonstrated this capacity for magnesium absorption from these segments also in mature male chickens.

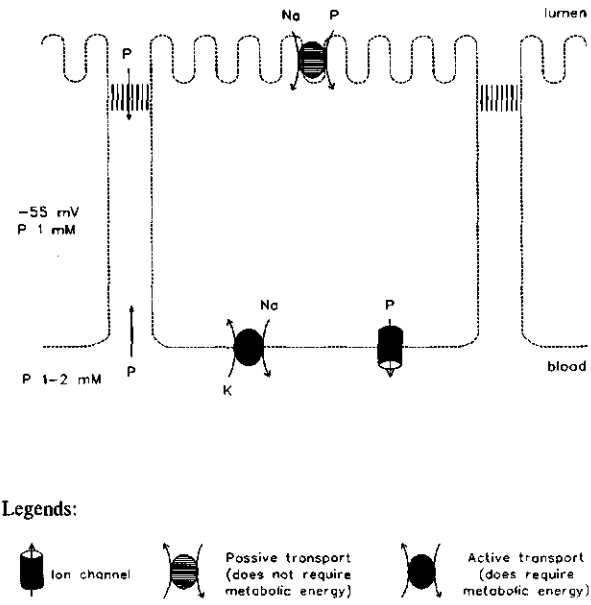

Figure 5. Mechanisms of phosphorus transfer across the intestinal mucosa.

Phosphorus probably enters the cell through a Na/P cotransporter. The transmembrane transfer of sodium is the driving force for the transfer of phosphorus. Phosphorus leaves the cell down its electrical gradient. Paracellular phosphorus transfer might also take place.

Data on the nature of the absorption process of magnesium are scarce. Saturation of the absorption process at a high luminal magnesium concentration occurs (Ebel and Günther, 1981; Anast and Gardner, 1981) and therefore a facilitated diffusion process is assumed. Magnesium was primarily transported by solvent drag in rats, although also passive ionic diffusion might occur additionally (Behar, 1974, 1975). Hardwick *et al* (1991) concluded, that the bulk of magnesium absorption occurs through diffusion and solvent drag, while at low intraluminal concentrations of magnesium also active absorption of magnesium might occur.

2.2.7 WATER

Water is included in this review, because water absorption will affect the absorption of solutes which are partly absorbed by solvent drag (e.g. chloride and magnesium).

In the crop, gizzard and duodenum water is secreted into the GI tract. It is absorbed from the jejunum, the ileum and the integrative segment (Thomas and Skadhauge 1989). In poultry, water is absorbed against the osmotic gradient, as the osmotic pressure in the intestinal contents is higher than the osmotic pressure in the blood plasma (Table 9). This uphill transport is an indication for active transport. Curran (1965), however, has proposed a model which describes a passive process of water absorption against an osmotic gradient. According to this model, water absorption is facilitated by the active transport of sodium. Sodium is transported actively out of the mucosal cell into the intercellular space, in which a local hyperosmotic region is built up. Subsequently water can be transported through the cell into the intercellular space (or paracellular) by simple osmosis. Water and solutes can diffuse from the intercellular space, through the highly permeable connective tissue layer into the blood stream. This process of water absorption is in agreement with data from Thomas and Skadhauge (1989a), who concluded from their data, that the absorption of water from the caeca was sodium linked. Therefore, nutrients absorbed by solvent drag will probably be dependent on sodium transport too, as was observed by Behar (1974) for magnesium absorption in a rat perfusion study. The actual amount of water absorbed from the GI tract, thus depends on the rate of sodium absorption and the time during which chyme is retained in a specific segment of the GI tract.

3 FACTORS AFFECTING MINERAL ABSORPTION

Mechanisms for mineral and water absorption were described in the previous section. Whether intestinal absorption of minerals and water occurs, is dependent on the prevailing intraluminal conditions in the GI tract, as well as on the presence of other minerals and of potentially chelating agents. The next section will be focussed on these

conditions and on the effect of the interacting elements.

3.1 INTRALUMINAL PHYSICO-CHEMICAL CONDITIONS

3.1.1 pH

Minerals are mainly absorbed from the intestinal tract as ions or small soluble complexes (Scott *et al*, 1976). (In)soluble complexes in the GI lumen can be formed with organic chyme components. The stability of these complexes is highly dependent on the pH. At a low pH the stability constants decrease, resulting in dissociation of the complexes (Kratzer and Vohra, 1986). The rate of diffusion across intestinal membranes is dependent on the concentration gradient of the mineral across the membrane. As the ionic activity of a specific mineral is related to the intraluminal pH, the latter will also indirectly affect the rate of absorption.

At the prevailing pH in the proventriculus and gizzard, many dietary mineral compounds are almost completely dissolved (Guenter and Sell, 1975 and Shafey *et al*, 1991). However, it was shown by Honig and Wolf (1987), that even at the low pH in the gizzard other complexes might be formed. When the chyme is transported from the gizzard into the small intestine, the pH increases with the distance to the pylorus (Table 7). These increasing pH values stimulate complex formation and increase complex size. Shafey *et al* (1991) showed a decrease in divalent mineral solubility and an increase in size of dissociated mineral complexes as the chyme was transported from the duodenum into ileum. The lower intraluminal mineral solubility results in a reduced rate of diffusion from lumen to blood (absorption) (Hurwitz and Bar, 1968) or increased rate vice versa (secretion). Nys and Mongin (1980, 1982) showed a positive relationship between the calcium solubility in the intestinal lumen of laying hens and the rate of absorption. An increase in acid production in the proventriculus of laying hens, 12-24 hours post ovulation increased the soluble calcium concentration in the intestinal lumen (Nys and Cabrera-Saadoun, 1986), which stimulated calcium absorption.

A clear relationship between the intestinal pH and the ultrafiltrable mineral contents (free ions and small complexes) was observed for divalent minerals, like calcium (Hurwitz and Bar, 1971; Nys and Cabrera-Saadoun, 1986) and magnesium (Guenter and Sell,

1975), but also for monovalent ones, like sodium and potassium (Hurwitz *et al*, 1970).

Table 7. The pH of the chyme in various segments of the GI tract of laying hens and broilers

	Laying hen	Broiler
Reference[1]	(1,4,6)	(1,2,3,5)
GI segment		
Crop	4.9	4.5-5.3
Proventriculus	5.3	2.0-4.6
Gizzard	4.8	2.6-4.3
Duodenum	5.6-6.9	5.5-6.2
Jejunum	6.2-6.9	5.8-6.9
Ileum	7.2-7.8	6.3-8.0
Caeca	-	5.8-6.8
Rectum	6.6-7.4	6.3-7.7

[1] 1. Guenter and Sell (1975); 2. Wiseman *et al* (1956); 3. Farner (1942); 4. Cheng and Coon (1990); 5. Shafey *et al* (1991); 6. Hurwitz and Bar (1968a); 7. Van der Klis *et al* (1993)

Especially for polyvalent elements, absorption mainly occurs in the duodenum and the jejunum, where the pH is still below 7 (Table 7).

3.1.2 Retention time

The time food is retained in the GI tract determines the time available for digestion and absorption of nutrients. The distribution of the mean retention time (time that an average dry matter particle is retained in a specific segment) over the successive GI segments is especially important, since the conditions favouring digestion and absorption, and the absorptive capacity vary considerably between GI segments. However, measurements on the mean retention time in poultry are scarce. Estimates in successive GI segments are given in Table 8. These values are based on the same principle of measuring (van der Klis *et al*, 1990). Although the transit time (time between intake of a marker and its first appearance in the faeces) is also used as a measure to detect differences in the retention time between diets (Gohl and Gohl, 1977; Mateos *et al*, 1982), it is of limited value (van der Klis *et al*, 1993a).

3.1.3 OSMOLARITY

The effect of the osmolarity of the chyme on the rate of absorption was demonstrated by Pansu *et al* (as cited by Bronner, 1987). They showed, that the transfer of calcium from perfused intestinal loops in rats was increased, when hyperosmolar solutions were used. They assumed, that a high intraluminal osmolarity might alter the cellular junctions of the intestinal mucosa, enabling larger complexes to be absorbed. The influence of the chyme osmolarity on water absorption from the GI lumen has been described in a previous section. Since water is absorbed less efficiently from hyperosmolar solutions (Behar, 1974, Nys and Mongin, 1982), the chyme osmolarity will also affect the absorption of solutes by solvent drag. This implies, that especially chloride and magnesium absorption might be affected by the chyme osmolarity, as these minerals are -at least partly- absorbed by solvent drag (Nys and Mongin, 1982; Hardwick *et al*, 1991).

Table 8. The mean retention time (min) of chyme in successive segments of the gastrointestinal tract of broilers and laying hens

Reference[1]	Laying hen (1,2)	Broiler (2,3)
GI segment		
Crop	48	31- 41
Proventriculus+ gizzard	71	33- 39
Duodenum	4- 7	5- 10
Jejunum	78- 85	71- 84
Ileum	84- 120	90- 97
Small intestine	176- 202	166- 191
Caeca	112	119
Rectum	51	26- 56

[1] 1. Hurwitz and Bar (1966); 2. Shires *et al* (1987). 3. Van der Klis *et al* (1990).The values of Shires *et al* (1987) have been adjusted to broilers weighing 1800 g.

Table 9. The osmotic pressure (mOsm/kg) of the blood plasma and of the chyme in successive segments of the intestinal tract of laying hens and male broilers

	Laying hen	Broiler
Blood plasma	321	312
GI segment		
Crop	537	380
Gizzard	312	338
Duodenum	571	528
Upper jejunum	650	670
Lower jejunum	573	600
Upper ileum	514	469
Lower ileum	451	437

Data from Mongin *et al* (1976)

3.1.4 Viscosity

Increasing the viscosity of the liquid phase of the chyme reduces digestion and absorption of nutrients in broilers (Bedford *et al*, 1991; Salih *et al*, 1991). A higher chyme viscosity increases the thickness of the unstirred water layer, which covers the intestinal mucosa (Johnson and Gee, unpublished observations as cited by Blackburn and Johnson, 1981) and decreases the efficiency of mixing of chyme in axial direction (van der Klis *et al*, 1993a). Less effective mixing will cause a less intensive contact of the feed components with digestive juices (Choct and Annison, 1992), resulting in a lower digestive efficiency. Furthermore, less effective mixing will result in a changed concentration difference of minerals across the intestinal membrane, which in turn will affect the rate of mineral transfer. It was clearly demonstrated by Macey (1986) that the unstirred water layer can be a significant barrier in the rate of diffusion through intestinal membranes. Increasing the thickness of the unstirred water layer will increase the distance for the diffusive transport (Vahouney, 1987). Although the magnitude of this effect depends on the size of the diffusing complex (Dietschy *et al*, 1971), the absorption of all minerals will be adversely affected by increasing intraluminal viscosities.

3.2 INTERACTIONS BETWEEN NUTRIENTS

Numerous factors influence mineral absorption from the GI tract. Quite often these factors interact by complex formation between minerals and other nutrients. Also mutual interactions between minerals may occur. Hill and Matrone (1970) concluded, that mutual interactions between minerals are likely to occur when the minerals have similar physical and chemical properties, which enables them to substitute for each other in all kind of processes. They checked this hypothesis on micro-elements with similar electronical structure.

Interactions between minerals and other nutrients in the GI tract can both decrease and increase the rate of mineral absorption (Frϕlich, 1990). The stability of the complexes formed depends on the pH (Kratzer and Vohra, 1986) and the molar ratio between complexing agents in the chyme (Nolan *et al*, 1987). The nutritional effect of these complexes depends on their stability constants. A weak complex doesn't have practical significance, because it hardly functions as a complexing agent. In a strong complex the mineral will not be available for utilization, unless the complex can be absorbed as such (Kratzer and Vohra, 1986).

3.2.1 MUTUAL MINERAL INTERACTIONS

Several interactions between minerals have been described. Especially the interrelationship between calcium and phosphorus is extensively studied. When calcium or phosphorus are present in excess to one another, rather insoluble calciumphosphates are formed in the intestine. The availability of the deficient mineral is reduced (Harrold *et al*, 1983; Peeler, 1972), while the availability of the abundant element might even increase (Morrissey and Wasserman, 1971). They observed a very efficient absorption of Ca^{47} from *in vivo* ligated duodenal loops (about 80% of the administered Ca^{47} dose was absorbed during 30 min of measurement) at a low dietary phosphorus level (0.25%). The increased level of calcium in the blood serum and the reduction of the tibia ash concentration however indicated, that the excess of absorbed calcium could not be deposited in bone tissue without a proper counter ion, as the Ca/P ratio in bone lies within narrow margins (Shafey *et al*, 1990). Similarly, urinary calcium excretion was increased

at high Ca/P ratios in laying hens (Rao and Roland, 1990).

Calcium and magnesium might interact at intestinal level, based on competition for membrane bonding sites, which influence the rate of carrier mediated transport. Behar (1975) suggested a reduced membrane permeability for magnesium, when the calcium concentration in the perfusion fluid was increased from 0 to 4 mM.

Wu and Britton (1974) have shown, that the magnesium level in the diet required for maximal growth, was increased with increasing levels of dietary phosphorus. On the other hand, excessive dietary magnesium levels increase the animal's requirement for dietary phosphorus and chloride to counteract the effects of hypermagnesemia in broilers (Lee and Britton, 1980). Shafey *et al* (1991) demonstrated a reduced magnesium solubility, when a high calcium, high phosphorus diet was fed to broilers. They suggested the formation of an insoluble compound of calcium, magnesium and phosphorus. Brink *et al* (1992) confirmed the negative effect of high calcium and/or phosphorus on magnesium solubility *in vitro* and *in vivo* in rats. Both experiments didn't show adverse effects on magnesium solubility, if either the calcium or phosphorus content was less than adequate.

Positive interactions are also known. Martin and DeLuca (1969) showed, using a rat everted duodenal segment, that the absorption of calcium was affected by sodium. A lack of sodium in the incubation medium diminished the calcium transport. This interaction is probably based on the Na/Ca-exchange mechanism at the basolateral cell membrane (see Figure 4). As calcium and magnesium have comparable chemical characteristics, both ions can competively bind to macro-molecules and solid particles in the intestinal lumen (Harrison and Harrison, 1974). The ion, which forms a complex with the highest stability constant, will replace the other ion in the complex, thus improving the availability of the liberated ion.

3.2.2 Interactions between minerals and other nutrients

Interactions of monovalent minerals and organic food components are of minor importance, compared to those of di- and polyvalent minerals. Monovalent minerals are generally considered to be present as ions in the intestinal tract, as the stability constants of their complexes are low. Therefore, such complexes will easily dissociate. However,

the high solubility of monovalent mineral complexes doesn't automatically seem to implicate, that the ultrafiltrable fraction of sodium and potassium in the intestinal lumen -as a percentage of the total amount- is high (Tables 3 and 4). Hurwitz *et al* (1970) assumed, that minerals incorporated in endogenous cellular material, originating from epithelial cells, were present in the non-ultrafiltrable fraction.

The effect of organic food components (protein, fat and carbohydrates) on the availability of di- and polyvalent ions has been studied in many experiments. As these minerals can form stable complexes with both undigested and endogenous chyme components in the small intestine, the absorbability of these minerals is usually low. In the following, emphasis will be given to the mineral absorbability in relation to organic compounds.

MINERALS-PROTEIN. Wasserman *et al* (1956) observed an increased retention of calcium in rats, when calcium was fed in combination with arginine or lysine. In their study, other essential amino acids had no effect. The authors could neither explain the effects observed by complex formation between calcium and the amino acid, nor by an increased solubility of calcium. Sandström (1988) reviewed, that trace element absorption is interrelated with protein digestion. Incomplete digestion of protein will decrease the absorption of trace minerals, while the absorption is increased when small peptides and free amino acids are present. The latter will be due to active absorption processes available for amino acids/peptides. This positive effect of amino acids on mineral absorption by complexation might be used to improve the absorption of trace minerals, e.g. zinc (Kratzer and Vohra, 1986).

MINERALS-FAT. Experiments with broilers indicated a negative relationship between the absorption and retention of calcium and magnesium and the inclusion of dietary fat (usually over 8%) (Whitehead *et al*, 1971; Atteh and Leeson, 1983a, 1983b, 1984; Atteh *et al*, 1985). Whitehead *et al* (1971) observed in 2 weeks old broilers, that the detrimental effects of fat on the retention of calcium and magnesium were decreased when fats with a higher digestibility were used. In their study, addition of free fatty acids resulted in a very low absorption of divalent minerals. Sklan (1979) concluded that monoglycerides are needed for efficient micel formation and absorption of fatty acids from the intestinal tract.

A lack of monoglycerides will result in a poor absorption of both free fatty acids and minerals bound to those fatty acids. Atteh *et al* (1989) observed, that fats with a high concentration of saturated fatty acids reduced the absorption of calcium, phosphorus and magnesium more than fats containing mainly unsatured fatty acids. The former resulted in a higher soap (alkali salts of fatty acids) excretion with the faeces. Based on earlier studies (Atteh and Leeson, 1983b, 1984) they concluded, that soaps from unsatured fatty acids can be absorbed efficiently, while salts with satured fatty acids are unabsorbable and will be excreted. Bronner (1987) postulated, that calcium-soaps of medium chain fatty acids might diffuse paracellular, through the tight junctions. This doesn't seem to occur with long chain fatty acids. Also in laying hens faecal soap excretion was increased, when 8% oleic acid was substituted by palmitic acid and calcium retention was reduced by 20% (Atteh and Leeson, 1985b). They did not observe these effects on magnesium retention. In rats it was shown, that the absorption of calcium soaps decreased with increasing chain length and saturation level of fatty acids, as reviewed by Allen (1982).

MINERALS-CARBOHYDRATES. Like proteins, carbohydrates might both increase and decrease mineral absorption. Mono- and disaccharides, like glucose, galactose and sucrose, can stimulate mineral absorption. Vaughan and Filer (1960) concluded from a rat experiment, with ligated duodenal and ileal segments, that several mono- and disaccharides stimulated calcium absorption from the ileum. They did not observe such positive effects in the duodenum. Indigestible polysaccharides (fiber) will reduce the mineral absorption from the intestinal tract (Frϕlich, 1990). Nwokolo and Bragg (1977) have demonstrated, that the availability of calcium, phosphorus and magnesium from plant feedstuffs is negatively related to the fiber and phytate content of that feedstuff. This suggests that the solubility of these minerals is low in the GI tract under these circumstances. Indigestible polysaccharides can also irreversibly bind polyvalent cations in the intestinal tract (e.g. in rats, Harmuth-Hoene and Schelenz, 1980). James *et al* (1978) have shown *in vitro*, that especially the uronic acid concentration (present in the hemi-cellulose fraction) determines the calcium binding capacity. This process is pH dependent, more calcium being bound at higher pH values.

The negative effect of indigestible fibers on the mineral absorbability from the GI

tract can also be due to effects on the endogenous secretion. Oku *et al* (1982) concluded, that the reduction in mineral absorbability might be caused by a higher loss of intestinal mucosa cells, while Ikegami *et al* (1990) have demonstrated an increased secretion of digestive juices. The negative effect of indigestible soluble carbohydrates on mineral absorption depends also on physico-chemical properties in the intestinal lumen, such as the viscosity (van der Klis *et al*, 1993).

In contrast, Thompson and Weber (1981) and Van der Aar *et al* (1983) observed only minor effects of fiber addition to chick diets, on the mineral status of the bird (level of fiber addition was 6% and 4 to 8% respectively). They added fibers from several sources (e.g. cereal byproducts, cellulose and pectin) and used mineral contents in tibia and serum as response characteristics in 4 week old chicks. No mineral digestibilities were reported by these authors, which might account for the apparent discrepancies.

MINERALS-PHYTATE. Although phytate is a part of the crude fiber fraction, it is dealt with seperately, because of its significance in mineral complexation.

Among others, phytate is one of the most widespread complexing agents present in plant feedstuffs. Phosphorus is stored in most seeds as phytate (Frølich, 1990), usually as K-Mg phytate or as K-Mg-Ca phytate (Scheuermann *et al*, 1988). Large complexes can be formed between phytic acid and polyvalent cations, rendering the latter unavailable for absorption.

Phytate has a great *in vitro* affinity for calcium, zinc and iron (Graf, 1983). The amount and tightness of cation binding depends on chemical equilibria and is therefore affected by the pH, temperature, ionic strength and size and valency of the cation. Vohra *et al* (1965) have established an affinity range of phytate for cations. *In vitro* it was shown, that molar ratios between minerals do affect the composition of the complexes formed, due to synergistic effects between cations (e.g. Oberleas and Moody, 1982). They further showed, that the presence of calcium in combination with magnesium, manganese or copper resulted in more precipitation of both elements, than might be expected from the separate precipitation curves. In the GI tract, phytate has a significant effect on the availability of magnesium and calcium. Nelson and Kirby (1987) observed, that increasing amounts of calcium had to be added to a chick diet to obtain maximum bone ash, when

the dietary phytate content was increased. Wu and Britton (1974) made similar observations for magnesium. Except for these direct relationships, minerals can also be mediators for complex formation between phytate, protein and minerals (Nolan *et al*, 1987; Vohra and Kratzer, 1986). This process lowers both the availability of the minerals involved and the complexed protein. Addition of phytase to a diet will improve the availability of phosphorus (Simons *et al*, 1991), but probably also the availability of complexed ions, due to the hydrolysis of phytate (Kaufman and Kleinberg, 1971).

4 FACTORS CHANGING CHYME CHARACTERISTICS

In this review, several parameters in the intestinal tract have been mentioned, which might affect the absorption of minerals from the intestinal tract, directly (by mineral complexation) or indirectly (by affecting the rate of diffusion). Whether food components exhibit significant effects on the absorption of minerals, is dependent on the physico-chemical conditions in the GI tract. Until now, many experiments have been carried out to establish dietary effects on mineral solubility and absorption *in vitro* and *in vivo*, but intraluminal conditions in the GI tract have hardly been a topic of study. E.g. dietary fibers have only been studied as mineral binding agents, while additional effects, due to changing the mean retention time or increasing the intraluminal viscosity have not been included in these experiments. As only little is known about physico-chemical conditions in the intestinal lumen, *in vivo* effects of those conditions on mineral absorption from the GI lumen cannot yet be quantified.

As has been stated earlier, the gastro-intestinal pH is one of the main factors regulating the solubility of mineral complexes. The variation in data, reported in the literature (Table 7) suggest, that there should be ways to change the pH in the intestinal tract. However, results from several experiments indicate, that in fact the pH in the GI tract of poultry is kept rather constant (Heller and Penquite, 1936; Wiseman *et al*, 1956). Hurwitz and Bar (1968) also observed, that the pH regulatory mechanism in the ileum is very efficient. Within 10 minutes of perfusion (pH of perfusion fluids being 4.3, 6.3 and

8.9) the pH was adjusted again to normal values. Shafey *et al* (1991) however, observed an increase in chyme pH in the crop and ileum of broilers, when the dietary calcium content was increased from 1.1 to 2.5%, which also deteriorated mineral solubilities.

Results for the rate of passage of dry matter through the GI tract, are generally based on the transit time. Mateos *et al* (1982) showed, that high levels of supplemental fat in layer diets could retard the transit time. Dietary addition of 30% fat increased the transit time of Cr_2O_3 from 195 to 270 minutes. Addition up to 15% did not have any effect, which was in accordance with data from Tuckey *et al* (1958). The latter did not observe any effect of the addition of 10% animal fat on the transit time of Fe_2O_3 in pullets. Mateos and Sell (1981) showed, that the effect of dietary fat on the transit time might be dependent on the carbohydrate source. 7% Dietary fat addition increased the transit time in layers, fed sucrose-based diets more than on starch-based diets (32 vs 3 min). Gohl and Gohl (1977) showed, that viscous polysaccharides (dietary level of 1% and 10%) might both increase and decrease the transit time in rats. Van der Klis and van Voorst (1993) observed a decreased transit time, but an increased mean retention time, when 1% and 2% carboxy methyl cellulose were included in semi-synthetic broiler diets. Total fiber addition (oat hulls) did not change the transit time in broilers (Tuckey *et al*, 1958).

Effects of non-food related factors on the transit time were also described. A shortened transit time in aging chicks (7, 14 and 21 days of age) (Golian and Polin, 1984), a retarded transit time in ducks at higher environmental temperatures (18°C vs 29°C) (Wilson *et al*, 1980). Fasting will shorten the period passed from intake of a marker to appearence in the faeces (Mateos and Sell, 1982) and the genotype of broilers also affects the transit time (Cherry and Siegel, 1978).

As discussed in section 3.1.4, the viscosity of the chyme will reduce the rate of absorption of nutrients, probably by an increased distance of diffusion (thickness of the unstirred layer) or a less efficient mixing of intestinal contents. Soluble polysaccharides will increase the intraluminal viscosity (e.g. Hesselman and Åman, 1986, Bedford *et al*, 1991, van der Klis *et al*, 1993a), while dietary enzyme addition might partly alleviate these effects (Hesselman and Åman, 1986, Bedford *et al*, 1991).

The effect of the osmolarity on absorption of minerals is dependent on the available absorption mechanism used, absorption by solvent drag will reduce at higher intraluminal osmotic pressure (Nys and Mongin, 1982), while paracellular diffusion might increase (Pansu *et al*, 1975). The osmolarity however, has to be considered as a result digestion and absorption of feed components, rather than as a factor which can be directly changed by feed composition.

5. REFERENCES

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CHAPTER 2

ABSORPTION OF MINERALS AND RETENTION TIME OF DRY MATTER IN THE GASTROINTESTINAL TRACT OF BROILERS

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ABSTRACT Six-wk-old male broilers (Hybro), on a corn and soybean meal diet, were used to estimate the mean retention time (MRT) of DM in successive parts of the gastrointestinal (GI) tract and to determine the site of absorption of minerals (Na, K, Ca, and Mg). The main site of absorption of these minerals in the GI tract was between the lower duodenum and the lower jejunum. The apparent absorption of Na continued in the ileum and rectum, and secretion of K, Ca, and Mg was observed in the rectum. Between the lower duodenum and the lower jejunum, DM was retained about 70 min (one-quarter of the MRT in the whole GI tract).

(*Key words*: retention time, site of absorption, gastrointestinal tract, broilers, minerals).

INTRODUCTION

In areas with intensive livestock production, a surplus of animal manure is going to be a major problem due to environmental pollution (Jongbloed, 1984). Part of the solution to this problem will be a decrease of the mineral contents in feed and consequently in feces. It is necessary, however, that the animal's nutritional requirements continue to be met.

Properties of the chyme in the gastrointestinal (GI) tract of chickens that effect the absorption of minerals are not well known. A few studies have been published on measurements of parameters of the chyme: pH (e.g., Guenter and Sell, 1975), osmolarity (Mongin, 1976; Mongin *et al*, 1976), electropotential difference between intestinal lumen and blood (Hurwitz and Bar, 1969), and ultrafilterable concentrations of Ca (Hurwitz and Bar, 1966) or Mg (Guenter and Sell, 1975). Data on the mean retention time (MRT) in successive segments of the GI tract of poultry are scarce (Hurwitz and Bar, 1966; Sklan *et al.*, 1975; Shires *et al*, 1987). These data, however, are important as an indication of the time available for digestion and absorption. Time between intake of an indicator and its first appearance in the feces is often used as a parameter for the rate of passage through the whole GI tract (Mateos and Sell, 1981; Lee and Britton, 1987; Kaminska and Summers, 1988).

Knowledge of characteristics of the chyme, such as pH, osmolarity, and ultrafilterable mineral concentrations and ways to influence them can improve mineral absorption in the GI tract. Consequently, smaller amounts of minerals will have to be added to the feed to meet the bird's requirement.

To study the effect of chyme characteristics on the absorption of minerals, the site of absorption in the intestinal tract should be known. A few data have been given on Ca (Hurwitz and Bar, 1970) and Mg (Guenter and Sell, 1973) in broilers and on Ca (Hurwitz and Bar, 1969), Na, and K (Hurwitz *et al*, 1970) in laying hens.

The present study was to determine the site of absorption of Ca, Mg, Na, and K and to estimate the MRT of DM in various parts of the GI tract in broilers.

Materials and Methods

Animals, Housing, and Feeding

Male chicks[1] were reared in litter pens (15 birds per m^2) under standard conditions: the environmental temperature decreased from 32°C for 1-day-old chicks to 24°C for chicks 4 wk of age; for the first 2 days the birds were kept under 24 h light and for the remainder of the rearing period the birds were kept under a schedule of 1 h light alternating with 3 h darkness. At 4 wk of age, 32 chickens were placed in individual battery cages (area per bird was .11 m^2). The birds were kept at 23°C in continuous light. They had free access to water. Pelleted feed was supplied for *ad libitum* access; however, feed was withdrawn during two daily periods (Figure 1). Withdrawal Period 1 (WP 1) lasted from 0830 to 0830 h and Period 2 (WP 2) from 1000 to 1200 h. During WP 1 feed was withdrawn to ensure feed intake from 0930 to 1000 h.

The composition of the diet is given in Table 1. At about 5 wk of age the birds were offered the same pelleted diet, now containing Cr_2O_3 as a nonabsorbable indicator (1 g Cr/kg of feed).

[1] Hybro Euribrid, Boxmeer, The Netherlands

Table 1. The composition of the diet[1]

Feed components	Composition (% of feed)
Corn	54.0
Corn glutenfeed USA (23% CP)	7.8
Soybean solvent extracted (49% CP)	23.9
Herring meal, Danish (73% CP)	2.0
Meat meal, high fat (58% CP)	4.0
Soya oil	5.2
Dicalcium phosphate	.45
Vitamins[2]	.5
Minerals[3]	2.0
DL-methionine (99%)	.1
Amprolium[4]	.05
Total	100.0
Me_n MJ[5]/kg feed	13.18
Dry matter, %	91.08
Crude protein, %	22.1
Crude fiber, %	2.5
Lys (digestible), %	.99
Met + Cys (digestible), %	.71
Calcium, %	1.18
Magnesium, %	.18
Phosphorus (available), %	.52
Potassium, %	.84
Sodium, %	.18

1 During the experimental period Cr_2O_3 was added to this feed at a concentration of 1.02 g Cr/kg of feed.

2 The vitamin premix supplied per kilogram of ration: vitamin A, 12,000 IU; vitamin B_1, 1 mg; vitamin B_2, 5 mg; nicotinic acid, 30 mg; pantothenic acid, 7.5 mg; vitamin B_6, 1 mg; vitamin B_{12}, 15 μg; folic acid, 1 mg; vitamin D_3, 2,400 IU; vitamin E, 15 mg; vitamin K_3, 1.5 mg; choline chloride, 350 mg; and ethoxyquin, 50 mg.

3 The mineral premix supplied per kilogram of ration: dicalcium phosphate, 8 g; $CaCO_3$, 8.8 g; NaCl, 2.5 g; $CuSO_4.5H_2O$, .04 g; $ZnSO_4$, .06 g; $MnSO_4$, .24 g; $FeSO_4$, .26 g; I, .77 mg; K, .23 mg and Se, .1 mg.

4 Merck, Sharp and Dohme (MSD), Haarlem, The Netherlands.

5 1 MJ = .239 Mcal.

Measurements

Feed intake and weight gain were measured in the period from 34 to 41 days of age (Figure 1). On the 5th day after the first intake of indicator-containing feed, the feces were sampled quantitatively for 1 day to determine the apparent absorption of the Ca, Mg, Na, and K at fecal level. The birds were not refed after WP 2 (Figure 1) at the day of sacrifice. That day, during feeding (T1) and at seven different times after closing the feeding trough (T2 to T8), 4 birds were killed per time by an intravenous dose of sodium pentobarbital.

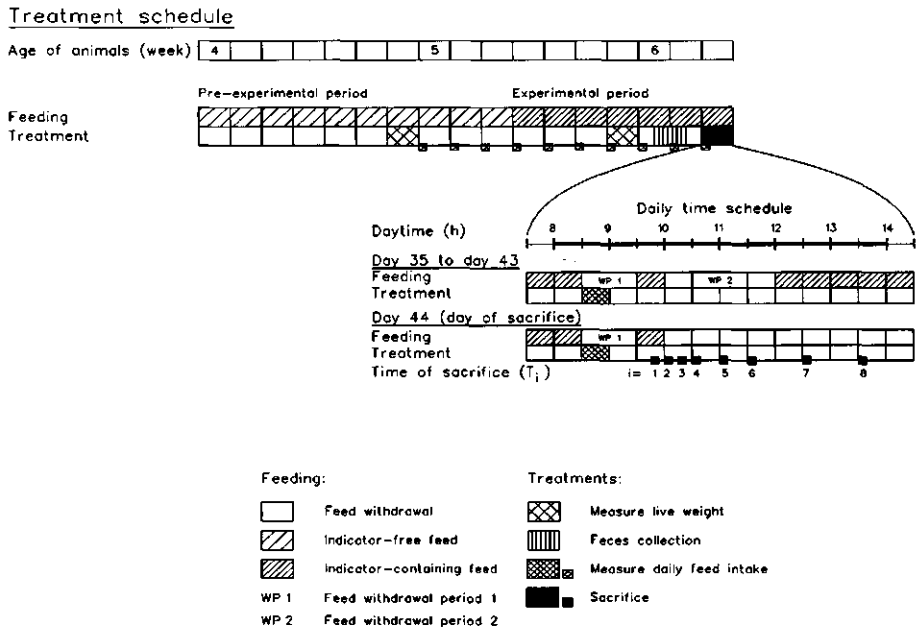

Figure 1. The treatment schedule during the experimental period (including a daily time schedule)

Sampling and Analyses

Immediately after killing a bird, its GI tract was removed. The GI tract was segmented into nine parts by ligation to prevent post-mortem movement of chyme (segments: crop, proventriculus and gizzard, upper duodenum, lower duodenum, upper

jejunum, lower jejunum, ileum, ceca, and rectum). The ligated GI tracts were stored at -20°C. This procedure was carried out within 3 min. After thawing, the chyme were collected and pooled per segment per two birds (two pooled samples per segment, per time). After freeze-drying, the samples were analyzed for DM, Cr, and Mg (by atomic absorption spectrophotometry), Na and K (by flame photometry), and Ca (by colorimetric titration, using calcein as an indicator).

Methods of Estimation

The Mean Retention Time. The MRT was estimated by two different methods. In Method 1, the amount of Cr present in different segments of the GI tract is expressed as a percentage of the daily Cr intake (von Persson and Svensson, 1960). In this method, a steady state is assumed. This assumption implies a constant amount of Cr in each intestinal segment on which the estimate is based. Under that condition, the MRT can be calculated according to Equation 1.

$$MRT_1 = 1440 \cdot \frac{Cr\ in\ segment\ (mg)}{Cr\ intake\ (mg/day)} \qquad [1]$$

where: MRT_1 = mean retention time (min) and 1440 = minutes per day (24 h).

In Method 2, the cumulative amount of Cr present up to the various segments of the GI tract is plotted against time after intake of feed. An exponential curve is fitted to the data (Equation 2). This method is based on a decrease over time of the amount of Cr in the GI tract. For this estimate, the data from animals killed after closing of the feeding troughs (T2 to T8) were used (14 pooled samples of 2 birds each). The model is similar to models of digesta flow in ruminants, given a single pulse dose of a marker (e.g., France *et al*, 1985; Pond *et al*, 1988).

$$Cr_n = A_n \cdot e^{-k_n \cdot t} \qquad [2]$$

where Cr_n = corrected total milligrams of Cr up to the n^{th} GI tract segment at time t; This corrected value was calculated as:

$$Cr_n = Cr_n^{/} \text{ (mg as present)} \times \frac{\text{mean Cr intake (mg/day) of all birds concerned}}{\text{Cr intake(mg/d per bird)}} \quad [3]$$

A_n = total milligrams of Cr up to the n^{th} segment at time T2 (moment of closing of the trough); k_n = mean rate constant (h^{-1}) up to the n^{th} segment; t = time (hours) after closing of the feeding trough; and n = the n^{th} segment ($1 \leq n \leq 9$).

In Equation 2, the cumulative amount of Cr is corrected for differences in the daily Cr intake among birds, because these differences should not affect the regression coefficients estimated.

The MRT_2 values in specific segments of the GI tract have been calculated using the equations given in Table 2. These equations were adapted from France *et al* (1985), who described marker kinetics in the GI tract of ruminants given a single pulse dose of a marker. The major difference between their study and the present situation at time zero was that in the former study, the indicator was present only in that segment in which the indicator was applied ($A_n = 0$; $n > 1$), and in the latter study, the indicator was already present in every segment due to the steady state situation ($A_n > 0$; $1 \leq n \leq 9$).

Validation of the Steady State Assumption

A steady state was defined as the condition in the GI tract in which neither the milligrams of Cr nor its concentration (grams of Cr per kilogram of DM) in the various segments was dependent on the time after feed intake. This implies that the rate of intake of Cr was equal to the rate of excretion throughout the day. After a period of food withdrawal, an established steady state could be disturbed due to the termination of Cr intake and to differences in the rate of passage in the GI tract between Cr and DM. The first cause will result in a decrease of the amount of Cr in the GI tract and the second in a changing Cr concentration and probably in less reliable values for the apparent absorption of DM in various segments of the GI tract.

Table 2. The derivation of the mean retention time (MRT) in successive segments of the gastrointestinal (GI) tract from the estimated total MRT values up to these segments[1,2].

Part of GI tract	n	MRT_2
Crop	1	$t_1 = 1/k_1$
Proventriculus + gizzard	2	$t_2 = 1/k_2 - (p_{1,2} \times t_1)$
Upper duodenum	3	$t_3 = 1/k_3 - (p_{1,3} \times t_1 + p_{2,3} \times t_2)$
Lower duodenum	4	$t_4 = 1/k_4 - (p_{1,4} \times t_1 + p_{2,4} \times t_2 + p_{3,4} \times t_3)$
Upper jejunum	5	$t_5 = 1/k_5 - (p_{1,5} \times t_1 + p_{2,5} \times t_2 + p_{3,5} \times t_3 + p_{4,5} \times t_4)$
Lower jejunum	6	$t_6 = \ldots$
Ileum	7	$t_7 = \ldots$
Rectum	n	$t_n = 1/k_n - (\sum_{i=1}^{i=n-1} P_{i,n} \cdot t_i)$

[1] MRT_{2n} = t_n x 60 min.

[2] k_n = mean rate constant (h^{-1}) up to the n^{th} GI tract segment.
n = the n^{th} segment.
$p_{i,n}$ = ratio of the amount of indicator present up to the i^{th} segment and the amount of indicator present up to the n^{th} segment; $1 \leq i \leq n-1$.
t_n = the MRT (h) in the n^{th} segment.

The validity of the steady state assumption in the intestine, as the main site of interest, was assessed by calculation of the MRT_1 values (Equation 1) for each time of sacrifice (T1 to T8). A MRT_1 value is a measure for the amount of Cr in the intestine, corrected for the daily Cr intake. The steady state assumption was rejected as soon as the mean MRT_1 values at a certain time of killing (Tn), decreased below the mean level up to that time (T1 to Tn). Additionally, the apparent absorption of DM up to the various segments of the GI tract was calculated for T1 to T8 (Equations 3.1 and 3.2). As long as subsequent values at time Tn did not differ systematically from the mean values in a specific part of the GI tract, a steady state was assumed in that part until Tn. This was done for Tn ($5 \leq n \leq 8$), because the probability for a disturbance of an established steady state increases with the duration of food withdrawal. The shortest period (T1 to Tn)

obtained from both calculations, has been taken as the period in which a steady state was present.

The Site of Absorption

In order to determine the site of secretion and absorption of Ca, Mg, Na, and K, a steady state is a prerequisite (Kotb and Luckey, 1972). These authors reviewed the derivation of Equations 3.1 and 3.2 for calculation of the apparent absorption of a specific nutrient.

$$Apparent\ absorption\ (percentage\ of\ intake) = 100\% \cdot (1 - ratio) \qquad [3.1]$$

where:

$$ratio = \frac{\frac{nutrient}{Cr}\ (in\ chyme)}{\frac{nutrient}{Cr}\ (in\ feed)} \qquad [3.2]$$

nutrient and Cr = the concentrations of a specific nutrient (test substance) and the indicator respectively (grams per kilogram of DM).

Statistical Analyses

The regression analysis and the calculations were done using Genstat (Genstat 5 Committee, 1987) as a statistical program. Parameters were estimated by using the Gauss-Newton method to maximize the likelihood function for the non-linear regression model. All results are given as mean and standard errors.

RESULTS

Performance

The mean live weight of the birds was 1,318 g at the start of measurement of weight gain and feed intake. The birds consumed a mean of 148 g of feed per day per

bird, and they gained 72 g weight/day per bird during the period of measurement. The mean feed to gain ratio was 2.04. The standard errors for those data were 17, 2, 1, and .02, respectively.

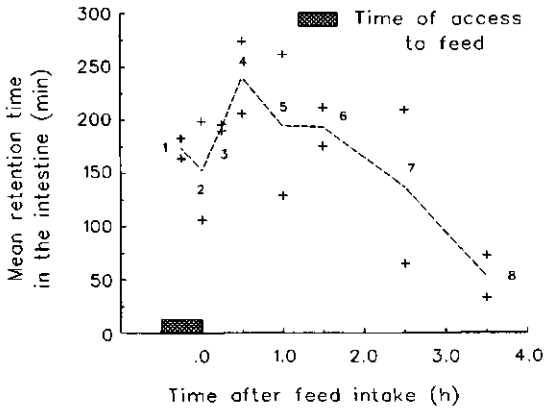

Figure 2. The mean retention time of dry matter in the intestinal tract of broilers, estimated at different times after feed intake. The mean retention time is calculated by Method 1, as described in the text. Individual values are given (+). The line connects the mean values of both pooled samples, per time of sacrifice (T1 to T8). The points numbered correspond with T1 to T8. The box indicates the period in which the birds had access to feed

Steady State

The MRT_1 values in the intestine from the duodenum to the rectum are given in Figure 2. After 0230 h of feed withdrawal (T7), the MRT_1 values were decreased below the mean MRT_1 value from time T1 to T7. The steady state assumption was no longer valid for time T7 and T8. The mean values for the apparent absorption of DM, up to successive segments of the GI tract, are shown in Figure 3. These values are based on all birds. Values per time of sacrifice are given for time T5 to T8. Systematic deviations from the mean line can only be seen for the values obtained with the animals killed at time T8.

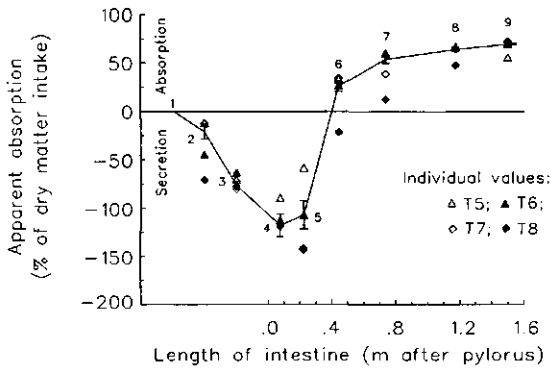

Figure 3. The apparent absorption of dry matter (percentage of daily intake) in successive segments of the gastrointestinal tract of broilers. Values are given as mean (points) and SEM (error bar). For those birds sacrificed from 1 to 3.5 h after closing of the feeding trough (respectively, time T5 to T8) individual values are also shown. The points numbered correspond with the following sites of measurement: 1, feed; 2, crop; 3, proventriculus and gizzard; 4, upper duodenum; 5, lower duodenum; 6, upper jejunum; 7, lower jejunum; 8, ileum; 9, rectum.

From these data it was concluded that the steady state assumption was no longer valid at 0330 h (T8) of feed withdrawal. Measurements needing a steady state must be done within 0130 h after feed intake (T1 to T6), which is the shortest period from both calculations.

Estimates of the Mean Retention Time

The estimates of the MRT_2 in successive segments of the GI tract were obtained by substituting the regression coefficients from Table 3 in the equations given in Table 2.

Table 3. The coefficients for the logarithmic decrease[1] of chromium in the gastrointestinal (GI) tract of broilers cumulative up to the various segments

Part of GI tract	A	k	SE	% Variance[2]
Crop	5.014	1.040	.372	63
Proventriculus + gizzard	7.511	.528	.154	62
Upper duodenum	7.685	.508	.147	61
Lower duodenum	7.936	.494	.138	63
Upper jejunum	10.099	.454	.138	66
Lower jejunum	14.885	.397	.110	64
Ileum	21.393	.301	.109	48
Rectum[3]	24.174	.258	.090	47

[1] Model of estimate

$$Cr = A \cdot e^{-kt} \qquad [2]$$

where:
Cr = corrected cumulative amount of Cr (mg) present at time t

$$\left[correction\text{: } Cr\ (mg\ present) \cdot \frac{mean\ Cr\ intake\ (mg/day)}{Cr\ intake\ (mg/day\ per\ bird)} \right]$$

A = cumulative amount of Cr (mg) present at the moment of closing the feeding trough,
k = mean rate constant (h^{-1}), and

[2] % Variance = percentage of variance accounted for.
[3] The amount of Cr in the ceca is not included.

The estimates of the MRT differed considerably between both methods (Table 4). Using Method 1, the estimated values for the MRT were lower than Method 2. In the whole GI tract, the estimate based on Method 1 was 266 min and on Method 2, 395 min. The MRT of DM in the ceca could not be estimated using Cr as an indicator because only a small fraction of a solid phase marker (Cr_2O_3), passing from the ileum into the rectum, actually enters the ceca (Vergara *et al*, 1989).

Table 4. The mean retention time (MRT, min) in successive segments of the gastrointestinal (GI) tract of broilers estimated by two different methods[1].

Part of GI tract	MRT_1	SEM	MRT_2
Crop	41	8.3	58
Proventriculus + gizzard	33	2.3	75
Upper duodenum	2	.2	7
Lower duodenum	3	.4	7
Upper jejunum	23	2.4	37
Lower jejunum	48	6.2	61
Ileum	90	6.9	94
Small intestine	166		206
Rectum	26	3.3	56
Total[2]	266	16	395

[1] The methods for these estimates (MRT_1 and MRT_2) are given in the text.
[2] The amount of Cr in the ceca is not included.

Site of Absorption and Secretion of Minerals

The mineral concentrations in the feed, in the chyme in various segments of the GI tract, and in the feces are calculated as a ratio to Cr and are divided by the mineral to Cr ratio in the feed (Figures 4 and 5). A value of 1 means that the ratio of mineral to Cr concentration at the site of measurement was equal to the value in feed. When this value was less than 1, apparent absorption of the test substance has taken place up to the site of measurement. A value greater than 1 means apparent secretion (both compared with feed values). An ascending line means that the secretion of the mineral into the GI lumen was greater than its absorption; a descending line means the opposite.

Sodium. In the anterior segments of the GI tract, total sodium secretion exceeded its absorption to a large extent. The latter increased after the lower duodenum (Figure 4). For sodium the highest absorption rate per unit of intestinal length was found between the lower duodenum and the lower jejunum. A small apparent absorption was observed in the

ileum. The rate of absorption increased again in the rectum. Between the ileum and rectum the Na:Cr content in the intestinal lumen became distinctly lower than its content in the feed. The apparent absorption at fecal level was 36 (SEM 1.7)% of the daily Na intake.

Potassium, Calcium, and Magnesium. The data obtained for these minerals were quite comparable (Figures 4 and 5). In the crop, only minor changes were observed. Based on Cr as a reference substance, absorption of Ca and Mg took place in the gizzard. Similar to Na, secretion of K, Ca, and Mg exceeded absorption to a large extent in the duodenum, especially for K and Mg. In the upper jejunum these mineral-to-Cr ratios were reduced below the feed values. From the lower jejunum on, some differences between these three minerals were observed. After this segment, hardly any net absorption of K or Ca was found. For Mg, a slight secretory activity was observed in the ileum and rectum (Figure 5). The fecal concentrations seemed to be higher than the rectal ones, probably because of addition of urine to the feces. For K, Ca, and Mg, the apparent absorption percentages in the whole GI tract were 19 (SEM 1.6)%, 30 (SEM 1.3)% and 12 (SEM 1.8)% of the intake, respectively.

DISCUSSION

Choice of Indicator

In the present experiment Cr_2O_3 was used as a nonabsorbable indicator. The Cr_2O_3 and Cr-mordanted straw particles (particle size .2 to 1.0 mm) were compared as nonabsorbable indicators in a previous experiment (Van der Klis and Verstegen, 1989). Results showed no differences in the recoveries nor in the estimates of the apparent absorption of DM in various segments of the GI tract. These observations concerned 6-wk-old broilers using the same diet as described in Table 1. It was concluded that both indicators tested could be used with the same accuracy. In the present experiment Cr_2O_3 was favored because of its higher Cr concentration (gram of Cr per kilogram of indicator).

Steady State and Apparent Absorption of Dry Matter

A large in-flow of DM into the lumen of the GI tract was observed in the gizzard and the duodenum (Figure 3). In the duodenum, up to 1.25 times the daily DM intake was apparently secreted, which equals 170 g/d of DM. This seems to be highly overestimated, because in the duodenum, apparently 3 to 11 g bile acids and fatty acids were secreted per day in 3-wk-old chicks fed a diet containing 50% heated and raw soybean meal, respectively (Sklan *et al*, 1973). In the same experiment, the maximum protein secretion of 14 g/day was observed using the raw soybean meal diet (Bielorai *et al*, 1973).

The overestimation reported herein might have been caused by a higher rate of passage of Cr than of DM in these segments of the GI tract. Effects of differences in rate of passage are expected to be minimal in broilers, because their meal frequency is about once every 40 min, when housed under continuous light (Classen and Urrutia, 1980). However, some separation between the indicator and DM in these segments seems to occur. This is in agreement with results from a previous experiment (Van der Klis and Verstegen, 1989). Similarly, Sklan *et al* (1975) found differences in the rate of passage of markers for the liquid and the solid phase up to the jejunum. They observed no differences between the rates of passage of these two phases distal to the jejunum. This is in accordance with data presented here in which the SEM values were much smaller after the jejunum (Figure 3). Separation is not likely to occur in these segments of the intestine (Hurwitz and Bar, 1966).

The apparent absorption of DM increased from the duodenum to the jejunum. In the ileum and rectum only small changes were found. At fecal level, 70 (SEM 1.5)% of the daily DM intake was apparently absorbed.

Mean Retention Time

The estimates of the MRT values in successive parts of the GI tract differed considerably between both methods (Table 4). Results of the MRT values from Method 2 were systematically higher than those from Method 1. For Method 2, it was assumed that each particle has an equal probability to leave a GI segment (Pond *et al*, 1988). This assumption can only hold when complete mixing of chyme occurs within each segment.

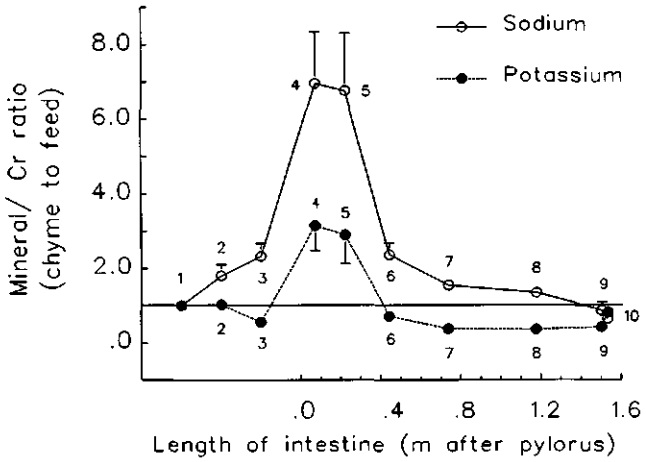

Figure 4. The sodium and potassium ratio to chromium in successive segments of the gastrointestinal tract of broilers (ratio in chyme:ratio in feed). Values are given as mean (points) and SEM (error bars). The points numbered correspond with the following sites of measurement: 1, feed; 2, crop; 3, proventriculus and gizzard; 4, upper duodenum; 5, lower duodenum; 6, upper jejunum; 7, lower jejunum; 8, ileum; 9, rectum; 10, feces

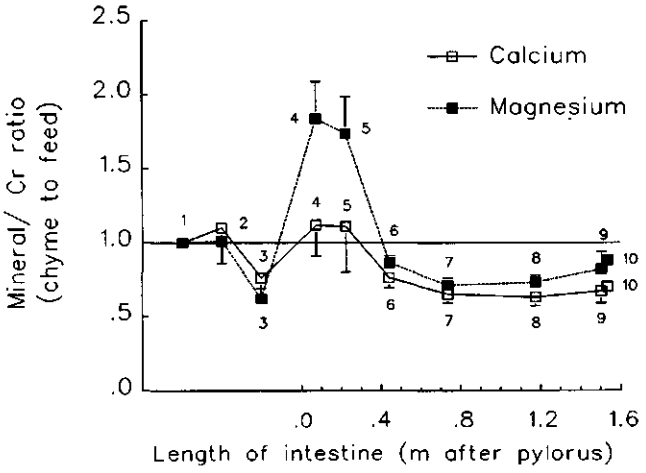

Figure 5. The calcium and magnesium ratio to chromium in successive segments of the gastrointestinal tract of broilers (ratio in chyme: ratio in feed). Values are given as mean (points) and SEM (error bars). The points numbered correspond with the following sites of measurements: 1, feed; 2, crop; 3, proventriculus and gizzard; 4, upper duodenum; 5, lower duodenum; 6, upper jejunum; 7, lower jejunum; 8, ileum; 9, rectum; 10, feces.

Table 5. Estimates of the mean retention time (min) of solid phase markers in successive segments of the gastrointestinal (GI) tract of poultry as published by several authors and from the present experiment

	Source[1]					
			3		Present experiment	
Part of GI tract	1	2	a	b	Method 1	Method 2
Crop	-	246	31	48	41	58
Proventriculus + gizzard	-	54	39	71	33	75
Duodenum	4	3	10	7	5	14
Jejunum	78	23	84	85	71	98
Ileum	120	48	97	84	90	94
Small intestine	202	74	191	176	166	206
Ceca	-	-	119	112	-	-
Rectum	-	27	56	51	26	56

[1] Literature source and animals used: 1) Hurwitz and Bar (1966), laying hens; 2) Sklan *et al* (1975), broilers; 3) Shires *et al* (1987), broilers and laying hens. All values from the literature are based on Method 1. The values from Source 3 have been adjusted for birds weighing 1,800 g.

Due to antiperistaltic movement of chyme in the GI tract (Oguro and Ikeda, 1974) some mixing will occur, but complete mixing will not be realized in all cases. The MRT value given for the crop was underestimated by Method 1 of calculation. The crop is the first segment of the GI tract in which the amount of Cr decreases during feed withdrawal. This decrease was observed 30 min after feed intake. The Cr amount in the crop was approximately constant during the period from T1 to T4. Based on this period, the MRT_1 value in the crop was 57 (SEM 8) min. The MRT_1 values in the proventriculus and gizzard were not affected by the duration of feed withdrawal, from time T1 to T6. The MRT values from the present experiment are compared in Table 5 to results published previously by Sklan *et al* (1975), Hurwitz and Bar (1966), and Shires *et al* (1987). All estimates of the MRT values done by these authors are based on Method 1. It is clear from Table 5, that a wide range is given in the literature for the MRT values in successive parts of the GI tract. These variations might be caused by differences in feed composition

and age and type of the birds used (Shires *et al*, 1987). The present estimates from Method 1 fit well into this range. The values from Method 2 especially seem to overestimate the MRT in the small intestine. Therefore, Method 2 does not seem to be an improved alternative for estimating the MRT in broilers.

Site of Absorption of Minerals

Results from the present experiment with regard to the site of absorption and secretion are in agreement with those presented by Hurwitz and Bar (1966, 1970) for Ca, by Hurwitz *et al* (1970) and K and Na, and by Guenter and Sell (1973) for Mg.

Using a nonabsorbable indicator for absorption studies assumes an equal rate of passage in the GI tract for the indicator and the test substance. In Figures 4 and 5, some apparent absorption of K, Ca, and Mg was suggested in the gizzard. This might be due to a higher rate of passage of these minerals than of Cr. This was also concluded by Hurwitz and Bar (1966), who observed a higher outflow rate of Ca compared with a solid phase marker. At the low pH in the gizzard, mineral compounds can dissolve (Hurwitz and Bar, 1971; Guenter and Sell, 1975) and leave the gizzard with the liquid phase at a higher rate than a solid-phase marker. This will cause a higher mineral concentration (ratio to Cr) in the duodenum and possibly in the upper jejunum. Consequently, in these segments the secretion will be overestimated. Results from the jejunum onward will be quite reliable because of a similar rate of passage between the indicator and the test substance, as was discussed earlier.

The in-flow of K, Ca, and Mg in the rectum was probably due to urine excretion (Taylor and Kirkly, 1967). Only Na was still absorbed after this segment. The ability of the cecum, rectum, and the coprodeum to absorb Na has been shown also by Bindslev and Skadhauge (1971).

From results of the present experiment, three conclusions were reached. 1) The main site of apparent absorption of Na, K, Ca and Mg was between the lower duodenum and the lower jejunum. Only Na was apparently absorbed in more distal segments. 2) As can be seen from Table 4, the MRT of DM in the jejunum, as the main site of absorption, was 71 min (approximately 25% of the MRT in the whole GI tract). This estimate was based on a steady state (Method 1). Based on Method 2, the mean retention time in the

jejunum was 98 min (also 25% of the MRT for the entire GI tract). 3) Measurements in the small intestine based on a steady state situation should be made within 1.5 h after the last intake of feed, because the steady state assumption is no longer valid at 2.5 h after feed withdrawal.

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The authors wish to acknowledge Mr. P.F.G. Vereijken for his advice concerning the second method of calculation of the MRT in successive parts of the GI tract.

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CHAPTER 3

THE EFFECT OF CARBOXY METHYL CELLULOSE (A SOLUBLE POLYSACCHARIDE) ON THE RATE OF MARKER EXCRETION FROM THE GASTROINTESTINAL TRACT OF BROILERS

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ABSTRACT Five-week-old broilers were used to estimate the retention time parameters of DM in the gastrointestinal (GI) tract. From 3 to 5 wk of age semisynthetic diets were fed with 0, 1, and 2% carboxy methyl cellulose (CMC, an indigestible soluble polysaccharide). These diets were obtained by exchanging cellulose by CMC. The cumulative excretion curves of a marker (Cr) containing meal were fitted to the data at fecal and intestinal level, using a generalized logistic curve. The transit time (time of first appearance at site of measurement) was estimated as well as some retention time parameters (time of 50% excretion of the Cr intake; time at and rate of maximal Cr excretion). Addition of CMC decreased the transit time from mouth to feces by 60 min (0% CMC versus 2% CMC, respectively). The first differences in transit time were observed in the posterior part of the jejunum. In contrast to transit time, 2% CMC increased the time of 50% Cr excretion by 2 h. Furthermore, CMC reduced the maximal rate of marker excretion, suggesting a more intensive mixing between the Cr-containing meal and the rest of the chyme. Because the transit time was not related to shape of the excretion curves, the former should be used cautiously as an indication of the mean retention time in the GI tract. However, determination of a cumulative excretion curve at fecal level might also be erroneous, because marker entrance into the ceca may depend on dietary composition.

(*Key words*: transit time, rate of excretion, broiler, cumulative excretion curve, soluble polysaccharide).

INTRODUCTION

The time that feed components are retained in successive segments of the gastrointestinal (GI) tract determines the time available for digestion and absorption of nutrients. Time between oral intake of a marker and its first appearance in the feces (transit time) is often used as a parameter for the feed retention time in the GI tract (Hillerman *et al.*, 1953; Mateos *et al.*, 1982; Washburn, 1991). The transit time, however, is determined by the rate of passage of the chyme fraction, which is transported

at the highest rate through the GI tract. Whether it gives any information about the average time available for digestion and absorption is doubtful. The few estimates of the mean retention time in successive GI segments (the time an average DM particle is retained in a specific segment) are based on a steady state situation in the GI tract between a marker and DM (Sklan *et al.*, 1975; Shires *et al.*, 1987; van der Klis *et al.*, 1990). Using this method, the birds have to be killed to collect GI contents.

In ruminant literature, digesta flow studies are based on the rate of fecal excretion of a marker-containing diet, supplied as a pulse dose (Pond *et al.*, 1988). In those experiments with ruminants, deterministic compartmental models were used to describe the transport of digesta through the GI tract. The mean retention time was expressed as a function of the rate constants, which were estimated from these models.

In the current study with broilers, a similar approach was used. The effect of an indigestible soluble polysaccharide (carboxy methyl cellulose; CMC) on the rate of excretion of a marker-containing meal in the feces was examined. Furthermore, the cumulative excretion curves from mouth up to various successive segments of the GI tract were determined. This was the first of a series of experiments that was carried out to determine the relationship between conditions in the GI tract (e.g. retention time parameters) and the absorption of macroelements.

Materials and Methods

Chickens, Housing, and Feeding

Three hundred and thirty day-old male broilers[1] were placed in three-tier battery cages (15 birds per .45 m^2 cage). During the rearing period, from 0 to 3 wk of age, the room temperature decreased from 33 to 24 C. At 3 days of age, the light schedule was changed from continuous light into an alternating schedule of 1 h light and 3 h darkness. A commercial diet was fed; its composition was described by van der Klis *et al.* (1990).

At 3 wk of age (start of the experiment), the light schedule was changed to 24 h

[1] Hybro Euribrid, 5831 JN Boxmeer, The Netherlands.

light again. Two hunderd and seventy birds were distributed randomly over 54 cages (5 birds per cage). Two adjoining cages with the same treatment formed one experimental unit. The experiment was subdivided into three blocks, each containing three tiers of three experimental units.

Three experimental diets, containing 0, 1, and 2% CMC, were prepared by exchanging cellulose for NaCMC.[2] The diets were assigned at random per tier, per block to the experimental units. This experimental design resulted in three replicates per diet per block. The diets were fed for *ad libitum* consumption. Each day, feed was withdrawn from 0815 to 0900 h to ensure feed intake at 0900 h. The composition of the experimental diets is given in Table 1. The birds had free access to water in both periods.

Observations

Feed and water intake and BW gain were recorded from 4 to 5 wk of age. At 5 wk of age, the experimental diets (Table 1) with 1% Cr_2O_3 added as an indigestible marker, were fed for *ad libitum* consumption for a quarter of an hour. This single Cr-containing meal was supplied following the short period without available feed. Directly afterwards, the birds had access to the marker-free diet again. Feces were collected quantitatively at different time intervals after the intake of the Cr-containing meal (Figure 1). The time of feces collection was recorded. After collection, all feces were stored at -20 C. The feces were freeze-dried, ground (over a 1-mm matrix), and analyzed for DM and Cr (atomic absorption spectrophotometry).[3] These data were used to calculate the cumulative excretion curves at fecal level.

The marker-containing meal was given again 2 days later to determine the cumulative excretion curves at GI level. For the excretion data at intestinal level, each cage formed an observational unit. At 18 20-min time intervals (covering 6 h) after the intake of the marker-containing meal, birds were killed with an intravenous injection of

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2 Akzo Chemicals, 1009 AB Amsterdam, The Netherlands.

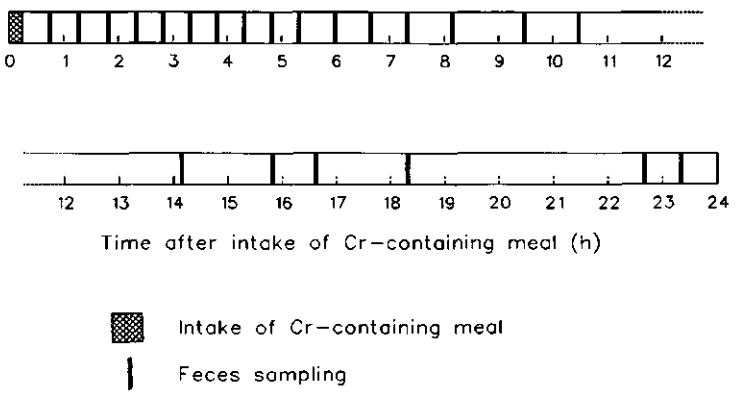
3 Spectraa 400, Varian Nederland BV, 3990 GB Houten, The Netherlands.

Table 1. The composition of the experimental diets

Ingredients and analysis	Composition (g/kg of feed)
Corn starch	538
Glucose syrup G95[1]	100
Soya oil	60
Whey protein, delactosed, spray dried[2]	119
Cellulose	122.1- x[4]
Carboxy methyl cellulose (AF 2985)[3]	x[4]
Vitamins[5]	5.3
Minerals[6]	15.9
$CaCO_3$	8.5
Monocalcium phosphate (anhydrous)	14.3
DL-methionine (99%)	1.3
L-arginine·HCl	10.0
L-isoleucine	.7
L-phenylalanine	3.3
L-threonine	.6
L-valine	1.0
Total	1,000.0
Analysis	
AME_n kcal/kg feed	3,150
Lys, %	1.20
Methionine + Cysteine, %	.85
Calcium, %	.64
Available phosphorus, %	.25

1 Cargill, 4600 AA Bergen op Zoom, the Netherlands.
2 Bio-Isolates Ltd., Glendale Business Centre, Clwyd, CH5 2LR, United Kingdom.
3 AKZO Chemicals, 1009 AB Amsterdam, the Netherlands.
4 The experimental diets contained 0, 10, or 20 g CMC/kg of diet.
5 Vitamins (supplied per kilogram of diet): vitamin A, 12,000 IU; vitamin B_1, 1 mg; vitamin B_2, 5 mg; nicotinic acid, 30 mg; panthotenic acid, 7.5 mg; vitamin B_6, 1 mg; vitamin B_{12}, 15 μg; folic acid, 1 mg; cholecalciferol, 2,400 IU; vitamin E, 15 mg; vitamin K_3, 1.5 mg; choline chloride, 350 mg; ethoxyquin, 50 mg; inositol, .25 g and biotin, .2 mg.
6 Minerals (provided in grams per 100 kg of diet) NaCl, 380; MgO, 150; K_2SO_4, 1,000; $FeSO_4 \cdot 7H_2O$, 25; $MnSO_4 \cdot 4H_2O$, 18; $ZnSO_4 \cdot 7H_2O$, 12; $CuSO_4 \cdot 5H_2O$, 2.5; KI, .050; $Na_2MoO_4 \cdot 2H_2O$, .90; $Co(NO_3)_2 \cdot 6H_2O$, .20.

Figure 1. Distribution of feces collection over a day to determine the excretion curve at fecal level.



T61.[4] The GI tract was exposed and segmented by ligation into crop, proventriculus and gizzard, duodenum, first half of the jejunum, second half of the jejunum, first half of the ileum, second half of the ileum, ceca, and rectum. The GI tracts were stored at -20 C until quantitative emptying. The feces were also collected at the end of the experiment to enable calculation of the total marker intake. These samples were also freeze-dried, ground, and analyzed for DM and Cr. After emptying the intestines, the duodenal, jejunal, and ileal lengths were measured. The procedure for feces and chyme sampling was carried out over 3 consecutive days. Data were collected each day from all cages in one block.

Statistical Analysis

Data for BW gain, feed intake, and water intake (Y) were analyzed according to the following ANOVA:

[4] T61 is a watery solution, which contains (in milligrams/milliliter): embutramide, 200; mebezoniumiodide, 50 and tetracainehydrochloride, 5. Supplied by Hoechst Holland NV, 1100 AZ Amsterdam, The Netherlands.

$$Y_{ijk} = \mu + b_i + t_j + e_{ij} + d_k + t.d_{jk} + e_{ijk} \quad [1]$$

where b = block (i = 1,2,3); t = battery tier (j = 1,2,3); d = inclusion level of CMC (k = 0,1,2); e_{ij} and e_{ijk} are the errors for tier j in block i and for experimental unit k of tier j in block i.

The error terms were assumed to be independently normally distributed with mean equal to 0 and variance to σ^2. In the ANOVA, the sum of squares for diet was partitioned into a linear and quadratic relationship with Y to analyze differences in the magnitude of the effect of the first and the second dietary inclusion level of CMC. Effects of tier and tier by diet interaction never reached significance ($P > .05$) and were therefore omitted from the model.

Cumulative Excretion Curve. The cumulative excretion data at fecal and intestinal level were analyzed by nonlinear regression. The expectation μ of the cumulative excretion data (Y) was described by the generalized logistic function (an S-shaped curve, nonsymmetrical about the point of inflexion).

$$\mu = a + \frac{c}{(1 + h \cdot \exp^{(-b \cdot (t - m))})^{1/h}} \quad [2]$$

where a = lower asymptote (= 0% Cr excretion); c = upper asymptote - a; b = slope parameter; h = power-law parameter (h = 1 represents an ordinary logistic curve); t = time (minutes); and m = point of inflexion (minutes). In Equation [2], time of sampling (t) was log transformed to ensure the fitted curve to approach the lower asymptote (no Cr excretion) as time t tends to zero (start of intake of the Cr-containing meal).

Because the variance of Y was expected to be small at the flat parts at the beginning and the end of the S-shaped excretion curve (data were subject mainly to analytical errors) and larger at the steep central part of the curve (data were subject to analytical errors and biological variation between birds), the error variance is not constant. Equation [3] was taken as a variance function for Y, which is proportional to the binomial variance function. In this function, Y was expressed as a percentage of the Cr intake.

$$Variance\ (Y) = \phi \cdot \mu \cdot (100 - \mu) \qquad [3]$$

For the fecal excretion data, a two-step procedure was used. In the first step, Equation [2] was fitted to the data (milligrams of Cr excreted), assuming variance (Y)= constant, to estimate the upper assymptote of the fitted curve. If the Cr excretion was not completed within the period of measurement, in which case the asymptotic value was higher than the cumulative amount of Cr excreted at the end of the sampling period, Cr intake was estimated as the upper asymptote of the fitted curve.

At chyme level, Cr intake was calculated per cage as the total amount of Cr present in the GI tract at the moment of killing and the amount already excreted in the feces. Subsequently, Cr excreted at fecal and intestinal level was expressed as a percentage of the estimated Cr intake at each time of sampling. In the second step, the data (as a percentage of Cr intake) were analyzed using the nonlinear regression model given by Equations [2] and [3]. The parameters of the model were estimated by maximizing the quasi-likelihood (McCullagh and Nelder, 1989).

From these cumulative curves the transit time (estimated as the time at which .5% of the Cr intake was excreted) and the time of 50% excretion (t_{50}) were calculated. The maximal rate of excretion and the corresponding time (t_{max}) were calculated from the first and second derivate of Y on t (the point of inflexion and the slope of the curve in that point). After log transformation of the time of sampling, the point of inflexion was not simply equal to m (Equation [2]) anymore.

The feces were collected per experimental unit of 10 animals (pooled sample of two cages). A curve was fitted to the respective measured data of each experimental unit, resulting in nine curves at fecal level for each diet. At chyme level, samples from all 18 cages per diet were used to compose the data up to the successive GI segments. Curves were fitted to these data, resulting in one curve per diet at chyme level.

Differences in the retention time parameters at fecal level and length of the intestinal tract were analyzed using the ANOVA Model [1]. The ANOVA and curvefitting were done using the Genstat (Genstat 5 Committee, 1987) statistical program.

RESULTS

Performance

Table 2. Bodyweight gain, feed and water intake, feed:gain ratio, and water:feed ratio of broilers from 4 to 5 wk of age

Variable	CMC content in diet			CMC effect	
	0%	1%	2%	Linear	Quadratic
BW gain, g per bird per day	64	54	33	***	***
Feed intake, g per bird per day	137	130	105	***	*
Water intake, mL per bird per day	198	282	325	***	NS
Feed:gain ratio, g:g	2.14	2.39	3.26	***	**
Water:feed ratio, mL:g	1.45	2.20	3.08	***	NS

[1] carboxy methyl cellulose.
* $P \leq .05$; ** $P \leq .01$; *** $P \leq .001$.

In Table 2, BW gain, feed intake, water intake, feed:gain ratio, and water:feed ratio of the birds are given from 4 to 5 wk of age. Feed intake and BW gain were negatively affected by the level of CMC in the diet. The feed:gain ratio was increased at higher CMC levels. The effect of CMC on these variables showed a significant quadratic component, indicating that the effect of the first percentage of CMC inclusion was smaller than the effect of the second percentage. Water intake was linearly increased by the CMC level in the diet. Due to the lower feed intake and increased water intake at higher dietary inclusion levels of CMC, the water:feed ratio increased linearly from 1.45 to 3.08 at the 0 and 2% CMC level respectively.

Cumulative Excretion Curves

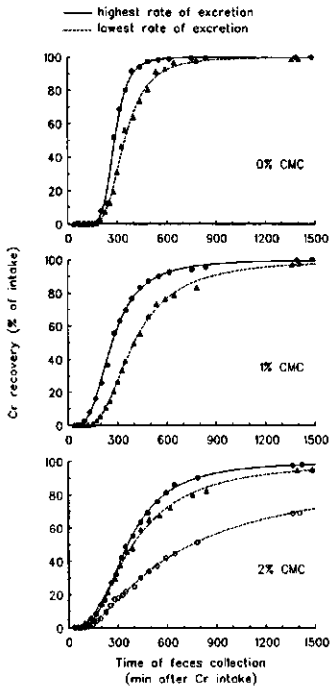


Figure 2. The cumulative excretion curves of Cr at the fecal level, at three inclusion levels of an indigestible soluble polysaccharide, carboxy methyl cellulose (CMC). Experimental units with the lowest (▲) and highest (●) rate of Cr excretion (slope of curve in point of inflexion) are given. Two curves are presented for the lowest rate of excretion at the 2% CMC diet: In these curves the upper segment of the S-shaped curve was (▲) or was not (○) defined by data within the period of measurement.

In Figure 2, the curves with the highest and lowest rate of excretion are given for each diet. From this figure it is clear that the cumulative excretion curves for the 0% CMC diet reached a plateau value within the period of measurement. This resulted in an estimate of total Cr intake. At the 1% CMC diet, Cr excretion was not completed for all experimental units within 24 h after Cr intake, as can be seen from the curve with the lowest rate of excretion. In another experiment at the authors' institute (van der Klis, unpublished data), however, it was shown that a 24-h sampling period was long enough to estimate the Cr intake at the 1% CMC diet accurately, as the asymptotic value based on a 24-h sampling period differed no more than 1.5% from the asymptote based on a 36-h sampling period. In case of the 2% CMC diet, however, the upper segment of four of the nine S-shaped curves was not defined by the data, which probably will result in an

overestimation of the total Cr intake. Consequently, the Cr recovery (as a percentage of the Cr intake) for these four curves might have been too low. Therefore, the results on the rate of Cr excretion on the 2% CMC diet are presented with as well as without these four curves (Figure 3 and Table 3). It should be realized that data calculated for this diet will be over- (e.g., t_{50}) or underestimated (e.g. slope in the point of inflexion) when the four slowest curves were included in the mean values (Table 3) compared to the means calculated without these four curves. From Figure 2, it can be seen that the generalized logistic curve describes the observed data well. To illustrate differences in the rate of excretion between diets, the mean curves are given per diet in Figure 3.

The shortest transit time was observed in the CMC-containing diets. The transit time of the CMC-free diet was about 60 min longer compared with the 2% CMC diet, whereas the t_{50} was about 2 h shorter (Table 3). The maximal rate of excretion, the slope of the curve in the point of inflexion, was the highest at the 0% CMC diet, and decreased with increasing CMC inclusion levels (Table 3). Variable t_{max} was not significantly affected by the CMC level. From Figure 3 and Table 3 it is clear that the transit time gave no

Table 3. Retention time parameters in broilers fed semisynthetic diets differing in carboxy methyl cellulose (CMC) content

	CMC content in diet				
	0%	1%	2%	CMC effect	
Parameter				linear	quadratic
Transit time	2:17	1:33	1:16 (1:11)[2]	***	NS
t_{50}	4:58	5:35	7:20 (6:19)	***	NS
Maximum rate of excretion					
Slope, % of intake/minute	.53	.29	.19 (.22)	***	**
t_{max}	4:31	4:19	4:14 (4:28)	NS	NS

[1] t_{50} = the time of 50% of Cr excretion; t_{max} = time of maximal rate of excretion. Time is given as hour:minute after marker intake.

[2] The value between parentheses is the mean of five curves instead of nine. Significant effects were present in both situations.

** $P \leq .01$; *** $P \leq .001$.

indication for the rate of excretion, nor for the time feed particles are retained in the GI tract as a consequence.

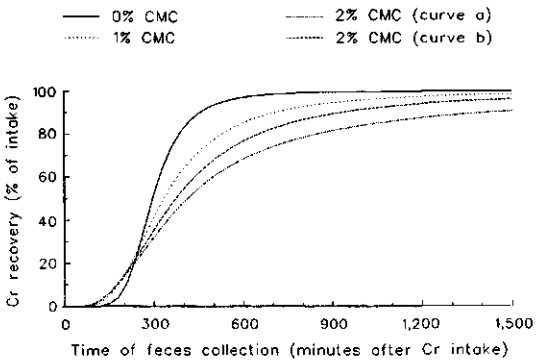


Figure 3. The cumulative excretion curves of Cr at the fecal level, at three inclusion levels of an indigestible soluble polysaccharide, carboxy methyl cellulose (CMC). Each curve is a mean curve of nine experimental units. At the 2% CMC diet two mean curves are given: curve a is based on all observations, including four individual curves in which the upper segment of the S-shaped curve was not defined by the data within the period of measurement; in curve b these four individual curves were excluded.

In Figure 4, the fitted excretion curves are given per intestinal segment. Each time of chyme collection was based on a pooled sample of all birds from one cage. At the 0% CMC diet one observation was omitted (at 200 min after the intake of the Cr containing meal), because of a sampling error. A large variation in rate of excretion between experimental units was shown in Figure 2. Hence, a considerable variation between successive observations in time in Cr distribution over the GI tract segments was noted. From the anterior part of the ileum onwards, no measurements were done within the period of measurement on the upper part of the S-shaped excretion curves.

Directly after the intake of a Cr containing meal, Cr is present in the crop. Some Cr is transported directly into the proventriculus and gizzard and from there into the duodenum. In the posterior part of the jejunum a time delay (transit time) between Cr intake and excretion into the next intestinal segment is observed (Figure 4). Although the

Table 4. The length of intestinal segments of broilers (38 days old) fed semi-synthetic diets differing in carboxy methyl cellulose (CMC) content

Intestinal segment	CMC content in diet			CMC effect	
	0%	1%	2%	Linear	Quadratic
		(cm)			
Duodenum	24.1	27.6	28.6	***	*
Jejunum	57.6	71.6	77.2	***	***
Ileum	57.4	69.8	75.6	***	**
Small intestine	139.2	169.0	181.3	***	***

* $P \leq .05$; ** $P \leq .01$; *** $P \leq .001$.

lengths of the duodenum, jejunum and ileum are increased by dietary CMC addition (Table 4), the time between intake of the Cr-containing meal and the first appearance of Cr in the ileum and more posterior segments is reduced in the CMC-containing diets. Differences in the rate of excretion were already present in the crop. The Cr was almost completely excreted from the crop at the 0% CMC diet, but only 80 to 90% of the Cr intake was excreted when the CMC-containing diets were fed. Differences became more pronounced at more posterior segments. No differences were found between the curves at both CMC containing diets in the ceca and rectum, probably because only a small part of the curve was defined at intestinal level.

No seperate curve could be calculated for the ceca at the 0% CMC diet, because only a negligible amount of Cr was present in that segment (Figure 4). Therefore, the cecal curve is equal to the posterior ileal curve at the CMC-free diet. For the CMC-containing diets, however, Cr was found in the ceca. Despite the large variation in rate of excretion between cages, the cumulative excretion curve up to the rectum fairly well agrees with the cumulative excretion curves at fecal level during the first 360 min (Figure 3).

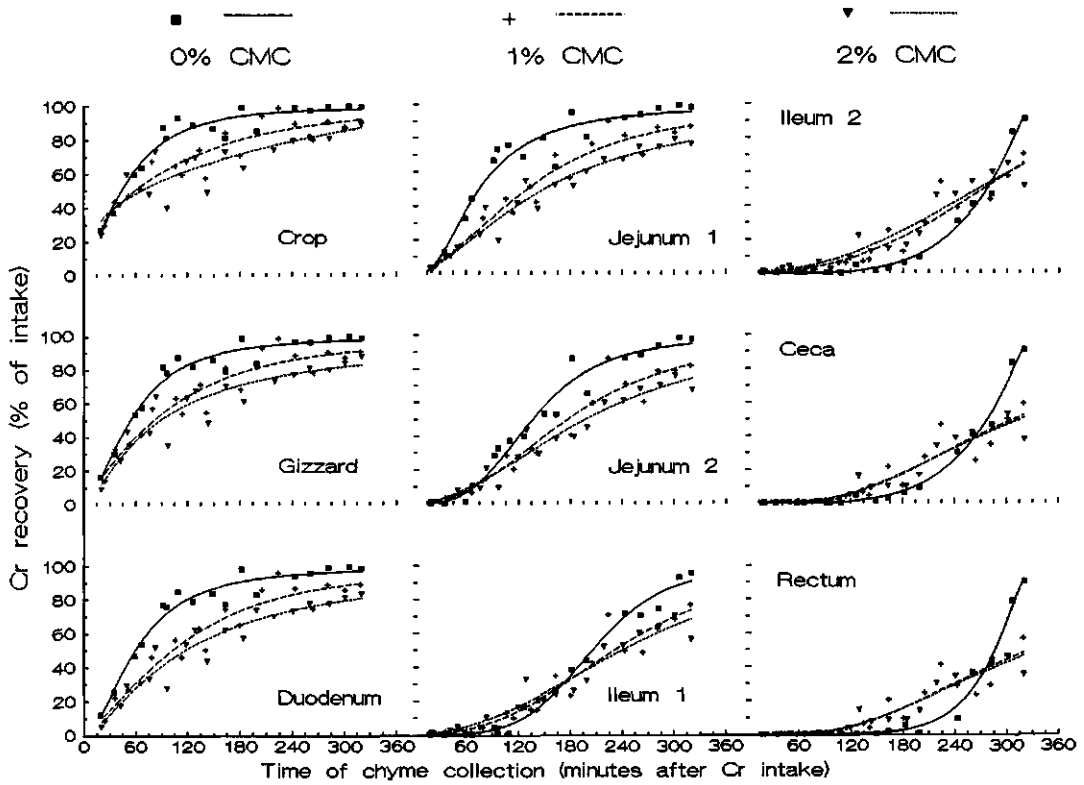


Figure 4. The cumulative excretion curves up to successive segments of the gastrointestinal tract of broilers, fed semi-synthetic diets with three inclusion levels of an indigestible polysaccharide, carboxy methyl cellulose (CMC)

DISCUSSION

Performance

Water intake was significantly increased by increasing levels of CMC in the diet. The higher Na intake, due to the incorporation of CMC as NaCMC into the diet, might have contributed to this effect (Marks and Washburn, 1983), because the diets were not standardized for Na content. The Na content in the diet increased from .15 to .31% at 0 and 2% CMC inclusion respectively, (the NaCMC used contained 81 g of Na/kg of NaCMC). However, it is not likely that the Na intake can account fully for the increase in water intake. Marks and Washburn (1983) observed only 10% increase in water intake in 2-wk-old chicks when the dietary Na content was increased from .16 to .32% by NaCl addition. In the present experiment, an increase in water intake of 65% was observed between the lowest and the highest dietary CMC inclusion level. An additional explanation may be found in the fact that CMC increases the thickness of the unstirred water layer associated with the mucosal surface (as was shown *in vitro* by Johnson and Gee, 1981). This in turn might reduce the rate of water absorption from the GI tract, which may only take place after diffusion through the unstirred water layer. It seems plausible that the bird might have increased its water intake in order to maintain its water balance.

Rate of Chromium Excretion

In this experiment, the intake of the Cr-containing meal was estimated from the fitted curves. The size of this meal could not be measured accurately by weighing due to salivation of the birds into the feeding trough and minor weight changes before and after feed intake (maximal about 1% of the total weight of the feeding trough). During the 15 min that the birds had access to the Cr containing meal, they ate only 7 to 17 g each (estimate based on the cumulative excretion curves).

The transit time in this experiment was not related to the rate of Cr excretion (Figure 3). This might be due to differences in the rate of passage of different feed components through the GI tract. Anterior to the duodenum, some separation between the solid and the liquid phase might occur. This phenomenon has been discussed earlier (Sklan *et al.*, 1975; van der Klis *et al.*, 1990). As Cr is a fine particulate material, it might be

transported with the liquid phase at a higher rate than undissolved larger DM components. This would cause a shortened transit time at a higher water intake. Cherry and Siegel (1978) also observed in an experiment with 8-wk-old chicks, that the transit time from mouth to feces was not a reliable indicator for the time of total disappearance of Fe_2O_3 from the GI tract. Their observation was based on one diet, which was supplied to birds differing in genotype and sex. Differences in water intake will only increase this discrepancy. Transit time of a marker from feed to feces therefore should be used with caution as an indication for the rate of passage through the GI tract.

From the cumulative excretion curves at the fecal level, it is clear that dietary CMC inclusion reduced the rate of Cr excretion. The maximum rate of excretion was reduced at the higher CMC levels. This suggests that the intensity of mixing of the Cr-containing meal with the rest of the chyme increases with the CMC level. Theoretically, if no mixing occurred at all, the Cr-containing meal would have been transported through the GI tract as a pulse. In that particular case, the rate of Cr excretion will be entirely dependent on the rate of Cr intake. Mixing will result in a flattened curve. This implies that the time an average feed particle is retained in the GI tract (the mean retention time) will be increased by the CMC content in the diet. It is likely that CMC affected the significance of mixing of the Cr containing meal with the rest of chyme by increasing the water content of the chyme. In this specific experiment, the water content of the chyme was not measured. In another experiment (van der Klis, unpublished data), however, it was shown that the ileal water content was increased from 74 to 86% by 1% of CMC inclusion. This higher water content might enable a higher grade of mixing in the intestine due to peristaltic movement, and thereby reduce the rate of excretion. The length of the intestinal tract may have also contributed to this effect, as CMC increased the intestinal length significantly (Table 4). However, the fact that three quarters of the difference in intestinal length between the 0 and 2% CMC diet was already present at 1% CMC inclusion, is not reflected in the time needed to excrete 50% of the marker intake, because only a linear CMC effect for t_{50} was shown (Table 3). This implicates, that the CMC effect on the rate of excretion is more complex than a simple effect on intestinal length. Furthermore, the increase in Cr retention in the ceca of birds on the CMC-containing diets contributed to the decreased rate of excretion. It was observed that Cr didn't enter the ceca of birds on

the CMC-free diet but that some Cr was found in the cecal contents of birds on the CMC-containing diets. Vergara *et al.* (1989) showed, that only a small fraction of the particles passing from the ileum into the rectum enters the ceca. The entrance of Cr into the ceca may have been facilitated by the dietary CMC content.

The duodenum and the jejunum are the sites of the small intestine in which the highest secretive and absorptive activity is found (van der Klis *et al.*, 1990). Therefore, the period in which the birds were killed was chosen such that the retention time parameters of DM in these two segments could be estimated reliably. These results however, show that 6-h sampling was still too short, because for the CMC-containing diets only 80 to 90% of the total marker intake passed through the duodenum and the jejunum during the period of sampling.

From the present experiment it is concluded that: 1) transit time gives no indication for rate of Cr excretion, as the shape of the excretion curves was affected by the diet. It can only be used as an index for the rate of passage of the chyme fraction, which passes at the highest rate; 2) differences in transit time occur in the jejunum and become more pronounced in more posterior segments of the GI tract; 3) the rate of marker (Cr) excretion is greatly affected by the CMC content of the diet; 4) the entrance of particles into the ceca is dependent on dietary composition and this affects the rate of marker excretion at fecal level.

ACKNOWLEDGMENTS

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CHAPTER 4

EFFECT OF A SOLUBLE POLYSACCHARIDE (CARBOXY METHYL CELLULOSE) ON THE PHYSICO-CHEMICAL CONDITIONS IN THE GASTROINTESTINAL TRACT OF BROILERS

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ABSTRACT 1. The effects of an indigestible soluble polysaccharide (carboxy methyl cellulose: CMC) on broiler performance (body weight gain, food and water intake) and on chyme characteristics (moisture content, viscosity, pH, osmolality and retention time) in broilers were studied.

2. In semi-synthetic diets 0, 5.0 and 10.0 g/kg of cellulose was replaced by CMC on weight basis. These diets were fed to male broilers from 3 to 5 weeks of age.

3. When 10.0 g/kg of CMC was included in the diet, food intake and body weight gain were reduced, compared to the 0 and 5.0 g/kg CMC diets. Food:gain ratio and water intake were increased at each CMC concentration. The significant quadratic response showed an increased response per 5.0 g/kg dietary CMC at the higher CMC concentration.

4. The viscosity in the supernatant of the chyme was linearly increased in all intestinal segments by CMC. A quadratic increase was observed in the crop. In the lower ileum, differences between the 5.0 and 10.0 g/kg CMC diet were not significant.

5. CMC increased the mean retention time of chromium in the duodenum and in the upper jejunum, and reduced the maximal rate of marker excretion. The transit time (first appearance of the marker in the excreta), however, was significantly reduced at the highest CMC concentration.

6. The osmolality of the intestinal fluid decreased less as the chyme moved from the duodenum into the lower ileum, with increasing concentrations of CMC.

7. The ileal pH was reduced linearly by the CMC content of the diet.

8. Based on a higher moisture content of the chyme in the CMC-fed birds, and the higher ileal osmolalities in those birds, it was concluded, that the efficiency of both digestion and absorption was reduced by CMC inclusion in broiler diets.

INTRODUCTION

Digestion and absorption of nutrients in the gastrointestinal (GI) tract are affected by the physico-chemical conditions in the successive GI segments. Burnett (1966) already suggested that the viscosity in the intestinal lumen of chickens might be responsible for

lower feeding values of barley than expected on the basis of its chemical composition. Recently, some studies demonstrated a relationship between intestinal viscosity and digestion and absorption of organic dietary components (Hesselman and Åman, 1986; Salih *et al*, 1991). Although these studies were focused on the viscosity, other parameters related to the viscosity may have been changed as well. Van der Klis and van Voorst (1993) have shown that the indigestible soluble polysaccharide, carboxy methyl cellulose (CMC) affected diet retention time parameters in chickens, e.g. the maximal rate of marker excretion.

The pH is thought to be very important in relation to mineral absorption. Shafey *et al* (1991) showed that the pH affects the solubility of minerals as well as the size of mineral complexes. In addition, other factors will affect mineral absorption too. It is very likely that the viscosity might not only affect mineral absorption indirectly, by reducing the digestibility of organic nutrients (potential complexing agents), but also directly by reducing the rate of mineral absorption. Furthermore, the time available for solubilisation of minerals, their absorption from the intestine and the osmolality in successive GI segments might affect mineral absorption.

In the present experiment the effect of 0, 5.0 and 10.0 g/kg dietary CMC on the pH of the chyme, the osmolality and viscosity of the intestinal fluid and the rate of marker excretion was measured in broilers. These changes were also related to the absorption of macro-minerals (van der Klis *et al* , 1993).

MATERIALS AND METHODS

Three trials were carried out simultaneously. The first was done to determine the effect of CMC on gastrointestinal pH and digesta retention time, the second to measure characteristics of the liquid phase of the chyme and the last to measure the moisture content of the chyme.

Animals, housing and feeding

In each trial 168 1-day-old male broilers (Hybro, Euribrid, 5831 JN Boxmeer, The Netherlands) were placed in 3-tier battery cages (14 birds per 0.45 m^2 cage). During the 3-week rearing period, the room temperature was gradually decreased from 33°C at day 1 to 23°C at 5 weeks of age. The relative air humidity was maintained at 55% (minimum). Until day three, the light was on continuously. Subsequently a light schedule was used, in which 1 h light was alternated with 3 h of darkness. The birds had free access to a commercial broiler diet (van der Klis *et al*, 1990) and water during the rearing period.

At the start of the experimental period at 3 weeks of age, 126 birds were distributed over 18 cages in the 3-tier batteries (7 birds per cage). The pelleted experimental diets (Table 1) and water were supplied for *ad libitum* intake. These diets were allotted at random per tier to the cages, with the restriction that two adjoining cages received the same diet (experimental unit). The room was lit continously during the whole experimental period, to ensure food intake took place as frequently as possible, and was equally distributed over a day.

Observations

From 25 to 32 d of age body weight gain, feed and water intakes were measured per experimental unit in all trials; all the other measurements were done per trial. Trial 1 involved retention time parameters (transit time, time of 50% of Cr excretion and the maximum rate of Cr excretion, as well as its corresponding time) and the pH in successive GI segments. For determination of retention time parameters, food was withdrawn daily from 07.15 to 08.00 h to ensure food was consumed at 08.00 h. At 35 d of age the experimental diets, now containing 10 g Cr_2O_3/kg as an unabsorbable marker, were supplied for 15 min, directly following the food-withdrawal period. After 08.15 h birds had access to the Cr-free diets again. The rate of Cr excretion was determined at faecal level (van der Klis and van Voorst, 1993). Droppings were collected quantitatively for 36 h, according to the time schedule in Figure 1, and pooled per experimental unit.

After collection of droppings, experimental diets containing 1.5 g Cr_2O_3/kg were fed for 2 d, to determine the mean retention time in successive intestinal segments (van der

Table 1. The composition of the experimental diets

Ingredients and analysis	Composition (g/kg of diet)
Ingredients	
Maize starch	540
Glucose syrup G95[1]	100
Soya oil	60
Whey protein[2]	120
Cellulose	100. - x[4]
Carboxy Methyl Cellulose (AF 2985)[3]	x[4]
Vitamins[5]	5.3
Minerals[6]	32.9
Calcium carbonate	5.3
Dicalcium phosphate dihydrate	19.6
DL-Methionine (99%)	1.3
L-Arginine HCl	10.0
L-Isoleucine	0.7
L-Phenylalanine	3.3
L-Threonine	0.6
L-Valine	1.0
Total[7]	1000.0
Analysis	
AME_n (MJ/kg diet)	13.2
Lysine (g/kg)	12.0
Methionine + cysteine (g/kg)	8.5
Calcium (g/kg)	7.7
Available phosphorus (g/kg)	3.2

1 Cargill, Amsterdam, the Netherlands

2 Bio-Isolates Ltd., Glendale Business Centre, CLWYD, CH5 2LR, United Kingdom

3 AKZO Chemicals, Arnhem, the Netherlands

4 The experimental diets contained 0, 5 or 10 g CMC per kg diet. CMC was exchanged for cellulose

5 Vitamins (supplied per kg of diet): retinyl acetate, 4.1 mg; thiamine mononitrate, 1 mg; riboflavin (80%), 5 mg; d calcium panthotenate (99%), 7.5 mg; choline chloride, 350 mg; nicotinic acid, 30 mg; pyridoxine-HCl, 1 mg; folic acid (80%), 1 mg; cobalamin (1%), 15 μg; cholecalciferol, 60 μg; d-α tocoferol acetate, 15 mg; netrazene, 1.5 mg and ethoxyquin, 50 mg. Furthermore 0.25 g inositol and 0.2 mg biotin were added per kg of ration

6 Minerals (g/100 kg of diet) *independent of dietary CMC content*: MgO: 91.4; $FeSO_4.7H_2O$: 35.6; $MnSO_4.4H_2O$: 21.0; $ZnSO_4.7H_2O$: 17.6; $CuSO_4.5H_2O$: 2.75; KI: 0.050; $Na_2MoO_4.2H_2O$: 0.90; $Co(NO_3)_2.6H_2O$: 0.20. Minerals (g/100 kg of diet) *dependent of dietary CMC content (x)*: NaCl: 230.8 -205.6 *x; KCl: 21.0 +262.3 *x; K_2SO_4: 836.2 -306.6 *x. The mineral premix also contained 2032.5 +250 *x g cellulose per 100 kg of ration.

7 For determination of the mean retention time in GI-segments, Cr_2O_3 was added to this diet as a non-absorbable marker (see text).

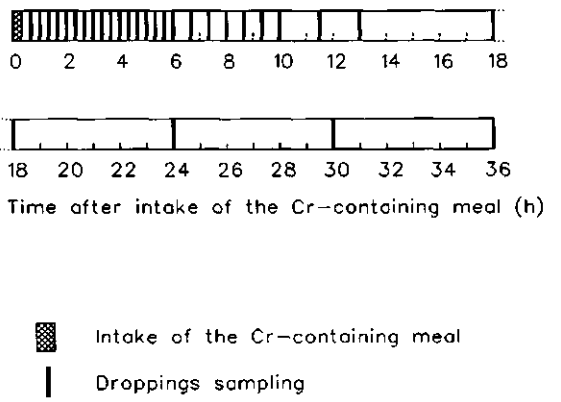

Figure 1. Timing of faeces collection during the sampling period to determine the excretion curve at fecal level

Klis *et al*, 1990). Subsequently the birds were killed by an intravenous injection of T61[1]. The chest cavity and abdomen were opened and the GI tract was ligated into nine segments: crop, proventriculus and gizzard, duodenum, jejunum (proximal and distal halves), ileum (proximal and distal halves), caeca and rectum. The GI tract was removed and the pH of the GI contents was recorded by inserting a micro pH-electrode[2] through an incision in the wall of the specific segment. The incision was tied off immediately after removal of the electrode. Finally the GI tracts were stored at -20°C. After thawing, the GI tracts were emptied quantitatively by hand and the samples were freeze-dried and analysed for Cr (van der Klis *et al*, 1990). The mean retention time was calculated

1 T61 is a watery solution, which contains (in mg/ml): embutramide, 200; mebezoniumiodide, 50 and tetracainehydrochloride, 5, supplied by Hoechst Holland NV, 1100 AZ Amsterdam, The Netherlands.

2 Electrode LoT 440-M3; Dr. W. Ingold AG, 8902 Urdorf, Germany.

according to "method 1" described in detail by these authors.

In trial 2 the viscosity and osmolality of the fluid phase of the GI contents were measured. The same Cr-free diets used in the first trail were fed. At 35 d of age the birds were killed and intestinal segments ligated as described above. After ligation the GI tracts were removed and emptied. The contents were pooled per segment per cage. Directly after collection, the pooled GI contents were centrifuged at 6000 *x g* for 15 min at 15°C and the supernatant was decanted. The viscosity was measured in the supernatant with a rotation viscosimeter[3]. The osmolality measurement by an osmometer[4] was based on freezing point depression.

Finally in trial 3 the moisture and sodium contents of the chyme were determined. The intestinal contents were sampled as in experiment 2. The moisture content of the chyme was measured after freeze-drying.

Statistical analysis

Body weight gain, food and water intakes, food:gain ratio and water:food ratio were analysed (with two adjoining cages as an experimental unit) according to the following ANOVA:

$$Y_{ijk} = \mu + \text{trial}_i + \text{tier}_j + e_{ij} + \text{diet}_k + e_{ijk}$$

The measurements on GI contents and retention time parameters were analysed (with each cage as an experimental unit) by the following ANOVA:

$$Y_{jkl} = \mu + \text{tier}_j + \text{diet}_k + e_{jkl}$$

where: i = number of trials (3); j = number of tiers (3); l = replicates per block, per diet (2); diet = inclusion content of CMC (k=0, 5.0, 10.0); e_{ij}, e_{ijk} and e_{jkl} are the errors for tier j in trial i, diet k of tier j in trial i and observation l on diet k in tier j.

The errors were assumed to be independently and normally distributed with a mean

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3 Rotovisko RV2; Haake, 4707 XZ Roosendaal, The Netherlands.

4 Gonotec Osmomat 030; Salm & Kipp, 3620 AB Breukelen, The Netherlands.

equal to zero and variance to σ^2. In both ANOVA's the sum of squares for diet was partitioned into a linear and quadratic relationship with Y, to analyse differences in the magnitude of the effect of the first and second step of CMC inclusion. The cumulative excretion curves were analysed by non-linear regression, decribed in detail by van der Klis and van Voorst (1993). The ANOVA and curve fitting were done using the Genstat (Genstat 5 Committee, 1987) statistical program. Differences between treatments were considered to be non-significant at $P \geq 0.05$.

RESULTS

Performance

Table 2. Food and water intakes (g/bird/day), body weight gain (g/bird/day), food:gain and water:food ratios in broilers from 25 to 32 d of age

	CMC[1] content in diet (g/kg)					
	0	5.0	10.0	Sed[2]	CMC effect[3]	
					Linear	Quadratic
Body weight gain	64	62	45	1.5	***	***
Food intake	122	122	109	3.6	**	NS
Water intake	163	194	267	5.7	***	***
Food:gain ratio	1.91	1.99	2.45	0.060	***	**
Water:food ratio	1.35	1.60	2.45	0.053	***	***

1 Carboxy methyl cellulose; an indigestible soluble polysaccharide
2 Standard error of difference between mean values for dietary treatment
3 Significance of a linear and quadratic effect: NS, non significant; ** $P<0.01$; *** $P<0.001$

Table 2 shows body weight gain, food and water intakes, food:gain ratio and water:food ratios of the broilers from 25 to 32 d of age. The 5.0 g/kg CMC inclusion did not affect body weight gain nor food intake, while 10.0 g/kg CMC inclusion reduced both significantly. Food:gain ratio was significantly increased with increasing concentrations of CMC inclusion, although the increase at 5.0 g/kg CMC was not significant. Water intake

and water:food ratio were clearly increased by dietary CMC. CMC affected all performance responses quadratically, indicating that the effect of the first 5.0 g/kg CMC inclusion was smaller than the effect of the second 5.0 g/kg.

Rate of marker excretion from the GI tract

Table 3. Retention time parameters[4] in broilers, estimates based on faeces sampling

	CMC[1] content in diet (g/kg)					
	0	5.0	10.0	Sed[2]	CMC effect[3]	
					Linear	Quadratic
tt, min	104	108	66	6.2	**	*
t_{50}, min	261	282	296	18.3	NS	NS
t_{max}, min	238	233	216	15.6	NS	NS
maximum rate, %/h	0.57	0.43	0.32	0.059	*	NS

[1,2] See Table 2

[3] Significance of a linear and quadratic effect: NS, non significant; * $P<0.05$; ** $P<0.01$

[4] tt: transit time, time between marker intake and first appearance in the excreta; t_{50}: time at 50% marker recovery; t_{max}: time at maximum rate of marker excretion

The retention time parameters deducted from the cumulative excretion curves in the faeces are given in Table 3. The transit time, the time from intake of the marker-containing meal to the first marker appearance in the faeces, was decreased by the 10.0 g/kg CMC diet. The maximum rate of marker excretion (percentage of Cr-meal excreted per hour) was linearly reduced by the CMC concentration, so the marker was retained longer in the GI tract. Although the time needed to recover half of the marker (t_{50}) was increased and the time at maximal rate of excretion (t_{max}) was decreased when the CMC diets were fed, differences were not significant. Table 4 shows that the mean retention time of Cr was prolonged in the duodenum and the first part of the jejunum, when CMC was included in the diet. CMC had no significant effect on the mean retention time in more posterior segments of the small intestine.

Table 4. The mean retention time (min) of Cr in successive segments of the small intestine of broilers

	CMC[1] content in diet (g/kg)					
	0	5.0	10.0	Sed[2]	CMC effect[3]	
Intestinal segment					Linear	Quadratic
Duodenum	4	7	11	0.7	***	NS
Upper jejunum	16	16	23	1.8	**	NS
Lower jejunum	40	44	43	3.2	NS	NS
Ileum	79	75	81	9.2	NS	NS
Small intestine	144	142	158	9.4	NS	NS

[1,2] See Table 2

[3] Significance of a linear and quadratic effect: NS, non significant; ** $P<0.01$; *** $P<0.001$

Conditions of the chyme

Data on viscosity and osmolality of the supernatant of the chyme and the pH in the total chyme are given in Table 5. The viscosity was increased linearly by CMC addition in each intestinal segment. In the crop the effect of the first 5.0 g/kg CMC inclusion was smaller than the effect of the second 5.0 g/kg (significant quadratic effect). In the ileum no viscosity measurement was done for the birds fed the CMC-free diet, as the total amount of chyme sampled from those birds was too small.

In the crop, proventriculus and gizzard and in the duodenum, differences in osmolality were not significant between diets. The osmolality was significantly reduced in the jejunal segments by the CMC content of the diet. In the ileum, however, the osmolality of the chyme supernatant was higher in the birds fed the CMC-containing diets. In both ileal segments, the effect of the 5.0 g/kg CMC content was much larger than the effect of the diet containing 10.0 g/kg CMC. The concentrations of small osmo-active molecules in the intestinal lumen was considerably reduced, when chyme passed from the upper jejunum into the lower ileum on the CMC-free diet. This reduction was smaller with increasing amounts of dietary CMC. With the CMC-free diet, the osmolality was reduced by 280 mOsm/kg, while this reduction was only 140 mOsm/kg at the 5.0 g/kg CMC diet and 80 mOsm/kg at the 10.0 g/kg CMC diet.

Table 5. The viscosity and osmolality in the supernatant of the chyme and intraluminal pH in gastrointestinal segments of broilers

	CMC[1] content in diet (g/kg)					
	0	5.0	10.0	Sed[2]	CMC effect[3]	
Gastrointestinal segment					Linear	Quadratic
			Viscosity (mPa.s)			
Crop	1.5	5.7	20.0	1.95	***	*
Proventriculus+gizzard	1.2	2.9	4.7	0.48	***	NS
Duodenum	1.4	3.5	7.6	0.53	***	NS
Upper jejunum	1.2	3.7	8.1	0.76	***	NS
Lower jejunum	1.2	5.0	9.9	0.90	***	NS
Upper ileum	-	8.4	17.1	1.96	**	-[4]
Lower ileum	-	17.3	23.7	3.71	NS	-
			Osmolality (mOsm/kg)			
Crop	569	592	559	97	NS	NS
Proventriculus+gizzard	432	446	414	31	NS	NS
Duodenum	661	626	612	27	NS	NS
Upper jejunum	594	551	517	16	***	NS
Lower jejunum	541	521	489	8	***	NS
Upper ileum	380	455	464	10	***	**
Lower ileum	314	411	437	8	***	***
			pH			
Crop	4.8	5.0	5.1	0.32	NS	NS
Proventriculus+gizzard	4.0	4.2	4.4	0.25	NS	NS
Duodenum	5.9	5.8	5.9	0.07	NS	NS
Jejunum	6.0	5.8	5.8	0.04	*	NS
Ileum	7.2	6.7	6.0	0.19	***	NS

[1,2] See Table 2

[3] Significance of a linear and quadratic effect: NS, non significant; * $P<0.05$; ** $P<0.01$; *** $P<0.001$

[4] Too small sample to perform viscosity measurement at the CMC-free diet; only linear CMC effect was tested

The pH decreased as the chyme passed from the crop into the proventriculus and gizzard. In the small intestine the chyme became alkaline and reached a maximum value of 7.2 in the ileum of the birds fed the CMC-free diet (Table 5). The jejunal and ileal contents of the birds fed the CMC-containing diets were more acidic.

Table 6. The moisture content (kg water/ kg dry matter) in successive segments of the gastrointestinal tract of broilers

	CMC[1] content in diet (g/kg)					
	0	5.0	10.0	Sed[2]	CMC effect[3]	
Gastrointestinal segment					Linear	Quadratic
Crop	1.57	1.94	1.76	0.468	NS	NS
Proventriculus+Gizzard	5.62	5.66	6.53	0.738	NS	NS
Duodenum	6.70	6.62	7.38	0.477	NS	NS
Upper jejunum	5.09	6.41	7.46	0.314	***	NS
Lower jejunum	3.61	5.04	7.14	0.171	***	*
Upper ileum	3.17	4.42	6.62	0.188	***	*
Lower ileum	2.67	3.88	5.57	0.145	***	NS
Caeca	3.79	2.71	2.08	0.298	***	NS
Rectum	3.16	4.22	4.45	0.272	***	NS

[1,2] See Table 2

[3] Significance of a linear and quadratic effect: NS, non significant; * $P<0.05$; *** $P<0.001$

In Table 6 the moisture content in successive segments of the GI tract is shown. The moisture content (given as water:dry matter ratio) in the jejunal and ileal contents was significantly increased by CMC addition to the diet. In the caeca, however, the moisture content was lower in the birds fed the CMC-containing diets.

DISCUSSION

Performance

The effects of dietary CMC inclusion on performance of the male broilers were similar to those reported earlier (van der Klis and van Voorst, 1993). In the present experiment, the water intake was increased by increasing concentrations of dietary CMC. Based on the results from this experiment, it was concluded that the water intake of the broilers was probably increased simply in order to satisfy their water requirement. The intestinal osmolality in hypertonic to the blood plasma, which is around 320 mOsm/l (Mongin *et al*, 1976). This implies that the broilers absorb water from the intestinal tracts against an osmotic gradient. Curran (1965) proposed a mechanism for this phenomenon, based on the active transport of sodium from the mucosal cells into the intercellular space. This process results in local hyperosmotic regions, which enable water transport by simple osmosis. The rate of water transport through the intestinal mucosa is thus related to the rate of sodium transport (Curran, 1965). Johnson and Gee (1981) have shown *in vitro*, that a higher viscosity at the luminal side of the intestinal wall reduces the rate of glucose transport, as a result of an increase in the thickness of the unstirred water layer. The thickness of this layer, which is covering the mucosa cells, influences both passive and active transport processes in the gut (Thomson and Dietschy, 1977). In the present experiment, the intestinal viscosity was increased (Table 5), and the net sodium absorption from the intestinal lumen was reduced (van der Klis *et al*, 1993) in those broilers fed on the CMC-containing diets. This in turn resulted in a lower rate of water absorption, which was supported by data on the moisture content of the chyme (Table 6). The bird therefore had to increase its water intake to satisfy its water requirements. The broilers fed the 5.0 g/kg CMC diet seemed to be able to do so simply by increasing their water intakes. Those fed the 10.0 g/kg CMC diet apparently were not able to compensate for the reduced intestinal water absorption completely, as food intake and body weight gain were reduced by 20-25%.

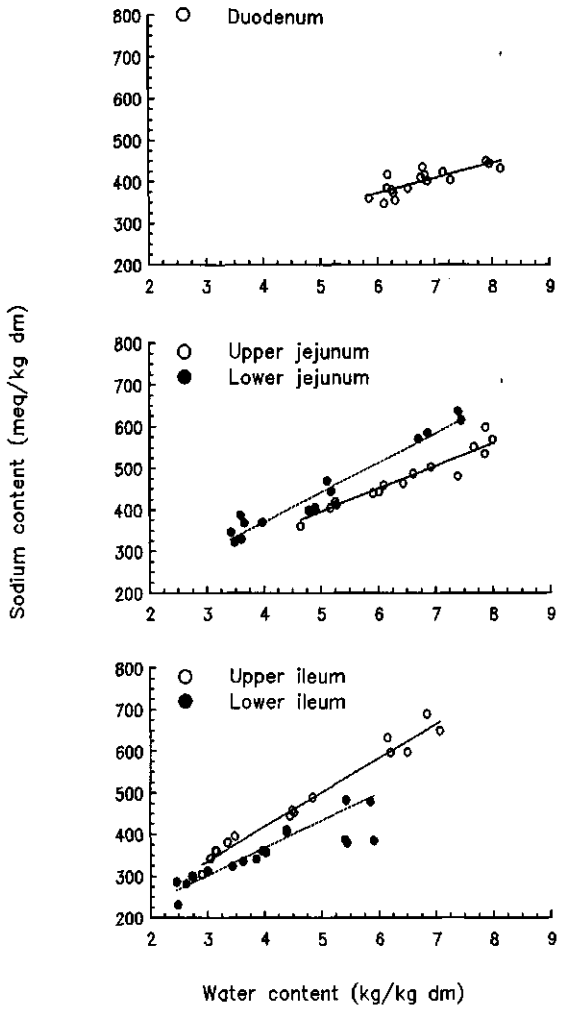

Figure 2. The relationship between the sodium and water concentration in the chyme sampled from the successive intestinal segments.
The percentage of variance accounted for by linear regression models were respectively:

Duodenum: Y= 92.8 (se 46.1)+ 45.5 (se 6.8) * X	76%
Upper jejunum: Y= 130.9 (se 16.7)+ 53.4 (se 2.7)* X	97%
Lower jejunum: Y= 86.4 (se 25.3) + 71.2 (se 4.9)* X	94%
Upper ileum: Y= 89.5 (se 20.0)+ 82.3 (se 4.1)* X	96%
Lower ileum: Y= 175.3 (se 24.3)+ 43.0 (se 6.0)* X	77%

In Figure 2 the relationships between the sodium concentration (van der Klis *et al*, 1993) and water concentration in the duodenal, jejunal and ileal contents are shown. In all segments close relationships between the water and sodium concentrations was observed and this supports the theory that water absorption is dependent on the efficiency of sodium absorption. The suggestion (Choct and Annison, 1992) that the high intestinal osmolality might be the cause for the moist intestinal contents, might be valid for the ileal contents, but cannot hold for more anterior intestinal segments. In those segments a higher osmolalilty and a lower moisture content were observed at the same time (Tables 5 and 6).

Unlike the small intestine, the moisture content in the caeca was decreased in the CMC-containing diets (Table 6). It is well known, that the efficiency of water absorption from the integrative segment (caeca, rectum and coprodeum) is increased in birds during dehydratation (Thomas, 1982, Thomas and Skadhauge, 1989). As cellulose degradation might occur by microbial activity in the caeca, the viscosity there may have been reduced, thereby facilitating water absorption from the integrative segment in the birds given the CMC-containing diets. Also, the fact that the large differences between diets in cumulative sodium absorption, which were observed in the ileum, were reduced in the faeces (van der Klis *et al*, 1993) supports this conclusion.

Rate of marker excretion

The reduction in maximal rate of marker excretion in the faeces by the dietary CMC content (Table 3) was similar to that found in a previous study (van der Klis and van Voorst, 1993). This effect was partially caused by an increased mean retention time in the duodenum and proximal jejunum, which was almost doubled at the high dietary concentration of CMC (Table 4). Furthermore, it was observed that CMC facilitated the entrance of Cr into the caeca (van der Klis and van Voorst, 1993). As the caeca are emptied only 2 to 3 times a day, the entrance of Cr into the caeca will contribute to the reduced maximal rate of excretion in the faeces. Therefore, cumulative excretion curves in the faeces have to be interpreted with caution. The mean retention time of dry matter in the small intestine cannot be derived simply from the cumulative marker excretion curves observed in the faeces.

On the 10.0 g/kg CMC diet, a lower maximal rate of marker excretion and a reduced transit time were observed at the same time. Anterior to the duodenum, some separation between the solid and the liquid phases of the chyme was observed in chickens (Sklan *et al*, 1975). This implies that in the crop, proventriculus and gizzard some of the fine particulate Cr_2O_3, fed in the single Cr-containing meal, might have been transported with the liquid phase at a higher rate than that of the larger paticulate material. The high water water intake (per kg diet) on the 10.0 g/kg CMC diet (Table 2) will have increased the significance of this phenomenon, resulting in a faster appearance of the marker in the faeces (van der Klis and van Voorst, 1993).

Intestinal conditions

The pH measured in the gizzard was somewhat higher than values reported from elsewhere (Guenter and Sell, 1975, Shafey *et al*, 1991). This might be caused by the composition of the diets used. From Table 5 it is clear that the pH in the gizzard was somewhat increased, while the pH in the ileum was decreased by higher dietary CMC inclusion. The latter result was very surprising if we assume that the pH at the surface of the mucosa cells is related to the intraluminal pH, because Hurwitz *et al* (1968) showed that the pH is regulated very efficiently in the ileum of laying hens. As the dietary buffering capacities of the 0 and 10.0 g/kg CMC diets were similar (456 and 438 mmol H^+/g diet was needed respectively to reduce the pH *in vitro* from 6 to 3), the higher pH in the gizzard must therefore be a consequence of the fact that less hydrochloric acid per g chyme was secreted into the proventriculus. Furthermore, the reduced transit time of the marker from mouth to faeces suggests that there is a higher rate of passage of liquid compared to the solid phase. The increased water:food ratio might have contributed to the fact that the pH course in the successive GI segments was less extreme as a result of a higher dilution rate. A higher dilution rate, at a similar rate of secretion, implies that the pH in the gizzard is increased, as less H^+ is secreted per gram water, and the pH in the ileum is decreased, because of a dilution of neutralizing HCO_3^-. Furthermore, dietary CMC might might have affected the amount HCO_3^- needed to increase the pH from 6 to 7.5 (350 mmol and 430 mmol OH^- were needed respectively per gram chyme to increase the pH of the freeze-dried ileal contents *in vitro* at the 0 and 10.0 g/kg CMC diets). A

third possibility is that the secretion of HCO_3^- is actually reduced. In the small intestine, the pH is regulated by the exchange of HCO_3^- for Cl^- and the exchange of H^+ for Na^+ (Charney and Feldman, 1989). An increased thickness of the unstirred water layer will result in a slower exchange between intraluminal and intracellular ions, a result which reduces both the HCO_3^- and H^+ secretion. This might explain why the birds were not able to adapt the intraluminal pH as fast as observed by Hurwitz *et al* (1968). The process most affected by an increased thickness of the unstirred water layer will determine whether the pH increases or decreases by dietary CMC. Finally, the high intestinal viscosity will have reduced the rate of digestion and absorption of organic dietary components (Hesselman and Åman, 1986). Microbial fermentation of highly degradable, unabsorbed nutrients (e.g. starch) cannot be excluded in the ileum. Fermentation products therefore, like volatile fatty acids, might also have caused the reduced ileal pH.

The osmolality of the chyme is affected by the efficiency of digesta degradation into small osmo-active components and their subsequent rate of absorption. With the CMC-free diet the osmolality of the chyme supernatant is decreased much more in going from the duodenum to the lower ileum compared to the CMC-containing diets (Table 5). As the moisture content of the chyme is increased by dietary CMC, the higher ileal osmolarities suggest a less efficient absorption of osmo-active nutrients from the small intestine, when CMC is included in the diet. However, the difference in the ileal pH will have influenced the solubility of minerals (osmo-active components) in the intestinal lumen (Shafey *et al*, 1991) too, which will have contributed to the effect of CMC on the osmolality.

The results from the present study suggest that addition of an indigestible soluble polysaccharide (CMC) does affect the intestinal viscosity throughout the GI tract. In the small intestine, however, other characteristics are also affected, and these might influence the efficiency of nutrient digestion and absorption, such as the mean retention time of dry matter, the osmolality and the pH in the small intestinal contents.

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CHAPTER 5

EFFECT OF A SOLUBLE POLYSACCHARIDE (CARBOXY METHYL CELLULOSE) ON THE ABSORPTION OF MINERALS FROM THE GASTROINTESTINAL TRACT OF BROILERS

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ABSTRACT 1. The effect of an indigestible soluble polysaccharide (carboxy methyl cellulose: CMC) on the absorption of some macro-elements (sodium, potassium, calcium, phosphorus and magnesium) from different segments of the small intestine of broilers was determined.

2. In semi-synthetic diets 0, 5.0 and 10.0 g/kg cellulose was replaced by CMC on weight basis. These diets were fed to male broilers from 3 to 5 weeks of age.

3. CMC inclusion reduced the rate of mineral absorption throughout the small intestine. The effect of CMC on sodium absorption was more pronounced than the effects on the absorption of the other minerals.

4. The cumulative absorption of all minerals up to the lower jejunum was reduced by dietary CMC. This negative effect of CMC on the absorption of minerals was alleviated in the lower ileum, except for potassium.

5. The concentrations of sodium and magnesium in the chyme supernatant were clearly decreased, while those of calcium and phosphorus were increased by dietary CMC inclusion. Taking the mineral concentrations in the total chyme into account, the solubilities of calcium, phosphorus and magnesium in the ileum were increased by dietary CMC. The solubilities of sodium and potassium were not increased.

6. The reduced cumulative absorption of minerals from the gastrointestinal tract with increasing dietary concentrations of CMC, was probably caused by the higher intraluminal viscosities in the small intestine. It is not likely that either the intestinal pH, or the time feed was retained in successive gastrointestinal segments, will have affected mineral absorption negatively in any segment.

INTRODUCTION

The physico-chemical conditions in the gastro-intestinal (GI) tract can affect the digestion of food and absorption of nutrients. It was shown by Hesselman and Åman (1986) and Fengler and Marquardt (1988), that the digestibility of the organic dietary components was reduced with increasing intestinal viscosity. Viscosity might also reduce the absorption of minerals, because the concentration of potential complexing agents in the

intestinal lumen is increased. Furthermore, the absorption of minerals might be affected directly, through a decreased rate of diffusion, as was shown for glucose (Blackburn and Johnson, 1985). Results (Lee and Campbell, 1983) have indicated an increased sodium requirement of young broilers fed on rye-based diets. It was suggested that this increase was the result of decreased sodium absorption from the intestinal tract caused by the dietary fibre component of rye. These fibres - arabinoxylans - increase the intraluminal viscosity (Bedford *et al*, 1991). The solubility of minerals is dependent on the prevailing pH in the GI tract segments (Shafey *et al*, 1991). A pH change will affect mineral absorption, as minerals are absorbed as ions or small soluble complexes (Scott *et al*, 1976). Also the time food is retained in the successive GI tract segments can influence complexation and absorption.

In the experiment presented here, the indigestible but soluble polysaccharide, carboxy methyl cellulose (CMC) was used to affect the physico-chemical conditions (pH, osmolality and viscosity) in the GI tract (van der Klis *et al*, 1993), and the retention time parameters of the digesta (van der Klis and van Voorst, 1993). Different conditions were achieved by substituting dietary cellulose by CMC on weight basis, thereby keeping changes in the chemical composition of the diet to a minimum. The effect of CMC on the absorption of five macro-elements (sodium, potassium, calcium, magnesium and phosphorus) was determined, and their cumulative absorption was related on the prevailing conditions in the GI-lumen.

MATERIALS AND METHODS

Two trials were carried out simultaneously. In the first, the cumulative absorption of some macro-minerals from the GI tract of broilers was determined. The second trial was carried out to measure the mineral concentration in the supernatant of the intestinal contents to calculate the mineral solubility. Methods used and measurements recorded are given below.

Animals, housing and feeding

The rearing conditions of the broilers from 0 to 3 weeks of age are described in detail by van der Klis *et al* (1993). At three weeks of age, 126 birds were distributed over 18 three-tier battery cages (7 birds per cage). Cr_2O_3 (1.5 g/kg; an indigestible marker) was added to the experimental diets, which only differed in CMC content (0, 5.0 and 10.0 g/kg CMC). The composition of these diets, and the way they were allotted to the cages, are described elsewhere (van der Klis *et al*, 1993). The pelleted diets and water were available *ad libitum*. The light was on continuously during the experimental period to ensure frequent food intake.

Sampling and chemical analyses

At 5 weeks of age, the birds were killed by an intravenous injection of T61[1] into the wing vein. The chest cavity and abdomen were opened and the GI tract was ligated into eight segments: crop, proventriculus and gizzard, duodenum, jejunum (proximal and distal halves), ileum (proximal and distal halves), and rectum. After ligation, the GI tract was removed and each segment emptied immediately by squeezing gently between thumb and finger. The contents from each segment of all 7 birds from each cage were pooled and either frozen at -20°C until freeze-drying for mineral analysis (trial 1) or centrifuged directly after sampling at 6000 *x g* for 15 min at 15°C (trial 2). After centrifugation the supernatants were decanted, their viscosities and osmolalities measured (van der Klis *et al*, 1993), and the samples stored at -20°C. In trial 1 one day before killing, the dropping in each cage were sampled between 12.00 and 15.00 h to allow calculation of the mineral retention (mineral intake - mineral excretion).

Dry matter and chromium analyses (by atomic absorption spectrofotometry) were carried out on samples of the diets and freeze-dried chyme. All samples (diets, chyme and supernatant) were analysed for calcium and magnesium (by atomic absorption spectrophotometry), sodium and potassium (by flame photometry), and phosphorus (by spectrophotometry). In Table 1 the analyzed dietary mineral concentrations are given.

[1] T61 is a watery solution, which contains (in mg/ml): embutramide, 200; mebezoniumiodide, 50 and tetracainehydrochloride, 5, supplied by Hoechst Holland NV, 1100 AZ Amsterdam, The Netherlands.

Table 1. The mineral and chromium content (g/kg dm) in the experimental diets

	CMC content (g/kg)			
	0	5.0	10.0	Mean
Phosphorus	4.21	4.15	4.16	4.17
Calcium	7.58	7.53	7.52	7.54
Magnesium	0.62	0.58	0.54	0.58
Potassium	4.37	4.16	4.26	4.26
Sodium	1.66	1.69	1.63	1.66
Chromium	1.04	1.01	1.02	1.02

Statistical analysis

All data were analysed according to the following ANOVA:

$$Y_{jkl} = \mu + \text{tier}_j + \text{diet}_k + e_{jkl}$$

where: j=1,2,3; diet = concentration of CMC (k=0, 5.0, 10.0); l=1,2; e_{jkl} is the error for observation l of the k^{th} diet in block j.

The errors were assumed to be independent and normally distributed with mean equal to zero and variance σ^2. In the ANOVA the sum of squares for diet was partitioned into a linear and quadratic component, to analyse differences in the magnitude of the response on the first and the second step of CMC inclusion. The ANOVA was done using the Genstat statistical program (Genstat 5 Committee, 1987). Differences between treatments were considered to be non-significant at $P \geq 0.05$.

RESULTS

Cumulative absorption of dry matter and minerals

The cumulative absorption of dry matter, sodium and potassium are given in Figure 1. Data are cumulative up to the specified GI segment. If the cumulative absorption in any segment is lower than the value in the preceeding segment, this is the result of net secretion into the intestinal lumen, between the sites of measurement. If the cumulative absorption increases as the chyme moves into the lower intestinal segment net absorption has occurred.

In the upper jejunum, the apparent absorption of dry matter was higher on both CMC-containing diets. In the more posterior segments dry matter absorption from the GI lumen was always linearly, and sometimes also quadratically, reduced by the dietary CMC content. The negative effect of CMC on the dry matter absorption was still obvious in the faeces. Based on the droppings data, the small negative response increased with higher CMC contents, as indicated by a significant quadratic response in the faeces. On the CMC-free diet, net absorption of dry matter was almost completed in the upper ileum, while some dry matter absorption occurred even in the lower ileum, when the CMC-containing diets were fed. In Figure 1, a large sodium secretion was demonstrated anterior to the upper jejunum for all treatments. The amount of sodium secreted into the intestinal lumen was 2-2.5 times the daily sodium intake. From the jejunum onwards, net sodium absorption occurred. As was shown for dry matter, the rate of sodium absorption was reduced by increasing CMC contents. On the 0 and 5.0 g/kg CMC diets the cumulative sodium absorption exceeded its total secretion into the upper and lower ileum respectively. On the highest CMC inclusion level (10.0 g/kg), the cumulative sodium absorption remained negative throughout the small intestine. In the faeces, no differences were present, indicating a large sodium absorption occurred posterior to the lower ileal segment. The cumulative absorption of potassium was negatively affected by increasing dietary CMC contents at all sites of measurement. The rate of absorption was highest on the CMC-free diet, resulting in a cumulative absorption value of 0.87 of the daily potassium intake in the upper ileum. These values were only 0.75 and 0.60 respectively on the 5.0 and 10.0 g/kg CMC diet. In the ileum, potassium secretion occurred on all

diets; this was concluded from the reduced cumulative absorption between the upper and the lower ileum. The apparent absorption of potassium in the faeces varied significantly between 0.28 and 0.19 respectively on the 0 and 10.0 g/kg CMC diets.

In Figure 2, the cumulative absorptions of phosphorus, calcium and magnesium are given. Differences between diets in cumulative absorption values of phosphorus and calcium were less pronounced than observed for sodium and potassium. The cumulative absorption of phosphorus up to the upper jejunum was not affected by the dietary CMC content. In more posterior segments, phosphorus absorption decreased was linearly by increasing the dietary CMC content. On the CMC-free diet, phosphorus absorption was complete in the upper ileum, while its absorption continued throughout the small intestine on the CMC-containing diets. At the lower ileum the cumulative absorption of phosphorus was reduced with 0.05 on the 10.0 g/kg CMC diet compared to the CMC-free diet. Phosphorus secretion was observed between the lower ileum and the faeces on all diets. The lower cumulative absorption values in the faeces indicates phosphorus excretion in the urine. The difference in the cumulative phosphorus absorption in the ileum between the 0 and 10.0 g/kg CMC diets was increased in the faeces. The cumulative absorption of calcium from the intestinal tract of birds fed the CMC-free diet and the 5.0 g/kg CMC diet was only slightly different. Calcium absorption in birds fed on the 10.0 g/kg CMC diet was reduced in all intestinal segments, but differences were not significant in the lower ileum. Only small differences in cumulative calcium absorption between the lower ileum and the faeces were observed, indicating minor calcium excretion in the urine. Like sodium, magnesium secretion into the duodenal lumen exceeded its absorption from that segment. This resulted in a net magnesium secretion on the CMC-containing diets in the upper jejunum, while the cumulative absorption on the CMC-free diet was only slightly above even. The reduced rate of absorption by higher dietary CMC concentrations was also significant for magnesium. Although magnesium absorption was completed in the lower jejunum on the CMC-free diet, it continued in the ileum of birds fed on the CMC-containing diets. On the CMC-free diet, magnesium was excreted between the lower ileum and the faeces. There was a low cumulative absorption (0.21 of the daily intake) in the faeces. The cumulative absorption of magnesium on the 5.0 g/kg CMC diet between the lower ileum and the faeces was not changed, while net magnesium absorption occurred in

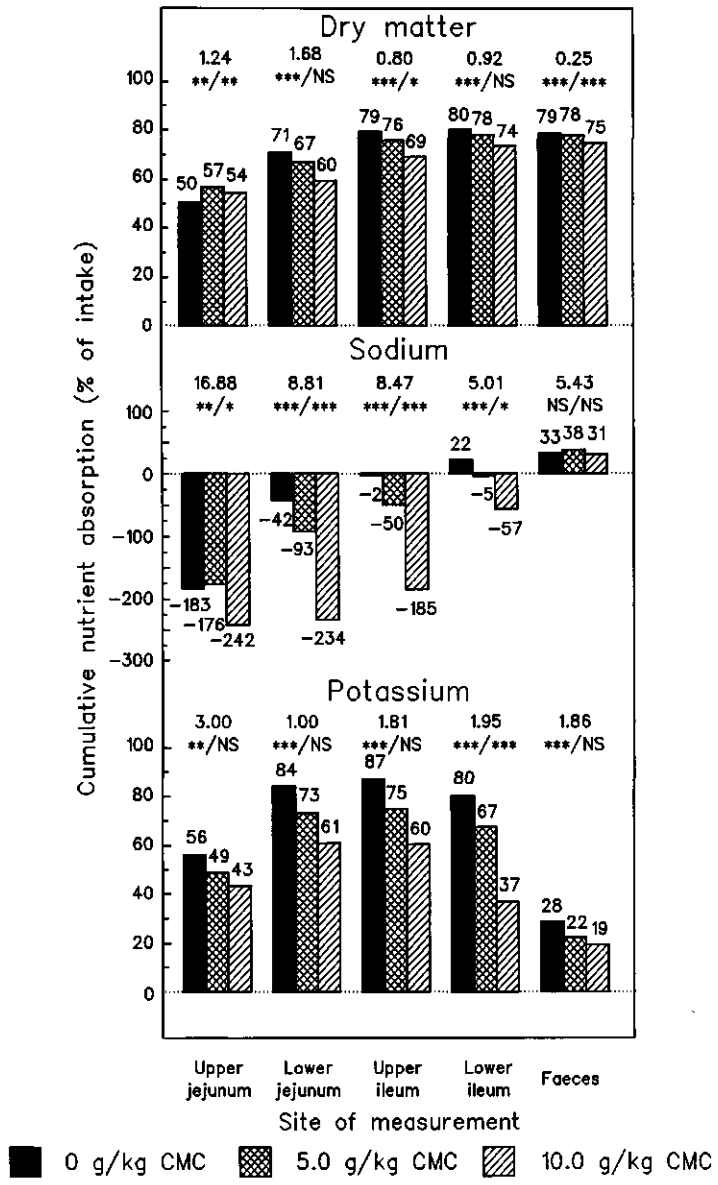

Figure 1. The cumulative absorption of dry matter, sodium and potassium up to successive intestinal segments and the retention in faeces.
The Standard error of difference (Sed) is given above the bars. The CMC effect was partitioned into a linear and quadratic component. The significance of this effect for both the linear/quadratic component is given underneath the Sed value.

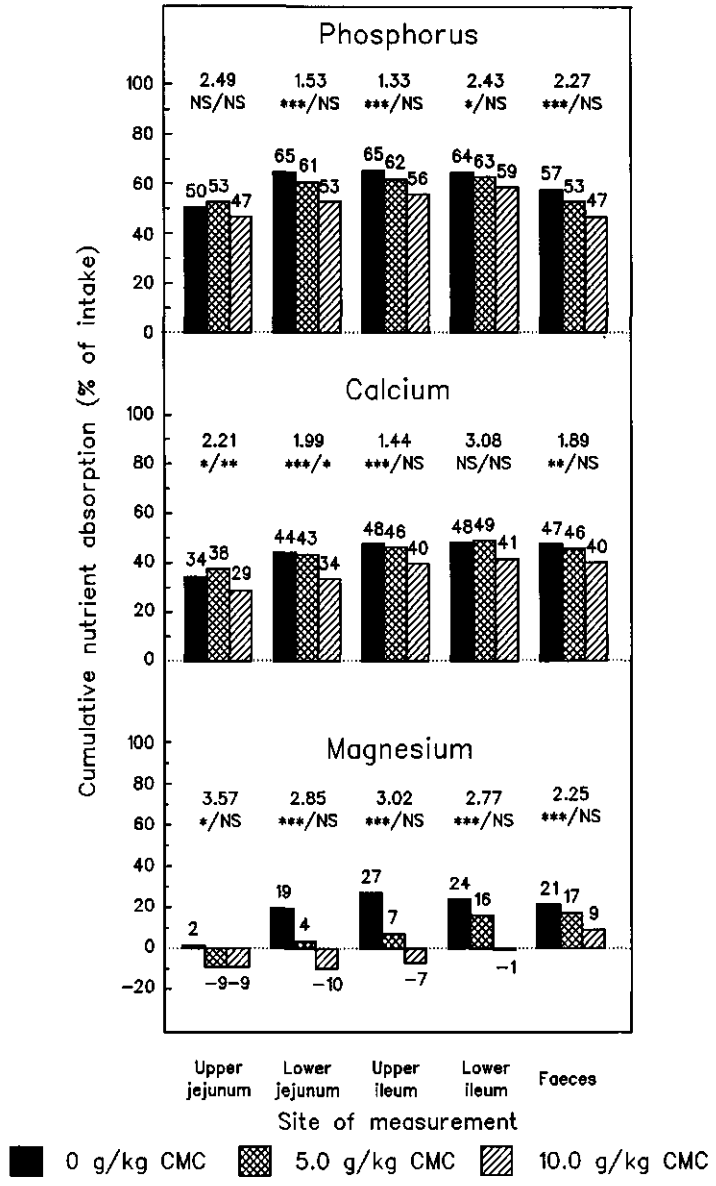

Figure 2. The cumulative absorption of dry matter, sodium and potassium up to successive intestinal segments and the retention at faecal level.
For remarks see Figure 1.

Table 2. The mineral concentrations of the chyme supernatant (g/kg) from different segments of the small intestine of broilers[1]

	CMC content (g/kg)				CMC effect	
	0	5.0	10.0	Sed	Linear	Quadratic
Segment			SODIUM			
Duodenum	1.50	1.38	1.40	0.054	NS	NS
Upper jejunum	1.85	1.60	1.54	0.075	**	NS
Lower jejunum	2.19	1.83	1.76	0.042	***	**
Upper ileum	2.46	2.13	1.96	0.066	***	NS
Lower ileum	2.26	1.79	1.68	0.081	***	*
			POTASSIUM			
Duodenum	1.21	1.16	1.13	0.117	NS	NS
Upper jejunum	0.69	0.70	0.72	0.042	NS	NS
Lower jejunum	0.60	0.57	0.50	0.032	**	NS
Upper ileum	0.87	0.91	0.61	0.068	**	*
Lower ileum	1.40	1.53	1.19	0.115	NS	*
			PHOSPHORUS			
Duodenum	0.70	0.60	0.65	0.040	NS	NS
Upper jejunum	0.38	0.37	0.37	0.023	NS	NS
Lower jejunum	0.24	0.26	0.28	0.014	*	NS
Upper ileum	0.07	0.19	0.36	0.021	***	NS
Lower ileum	0.08	0.26	0.46	0.024	***	NS
			CALCIUM			
Duodenum	0.67	0.69	0.68	0.038	NS	NS
Upper jejunum	0.81	0.77	0.75	0.022	*	NS
Lower jejunum	0.78	0.69	0.73	0.036	NS	*
Upper ileum	0.42	0.48	1.06	0.068	***	**
Lower ileum	0.18	0.50	1.27	0.075	***	***
			MAGNESIUM			
Duodenum	0.16	0.16	0.14	0.004	***	NS
Upper jejunum	0.19	0.16	0.15	0.006	***	NS
Lower jejunum	0.28	0.20	0.16	0.011	***	*
Upper ileum	0.39	0.28	0.23	0.020	***	NS
Lower ileum	0.38	0.35	0.30	0.040	*	NS

[1] The minerals were analysed in the superantant of the chyme sampled from the birds in trial 2.

Table 3. The mineral concentrations in the chyme (g/kg) in different segments of the small intestine of broilers[1]

	CMC content (g/kg)				CMC effect	
	0	5.0	10.0	Sed	Linear	Quadratic
Segment			SODIUM			
Duodenum	1.19	1.20	1.18	0.039	NS	NS
Upper jejunum	1.56	1.44	1.45	0.035	*	NS
Lower jejunum	1.76	1.61	1.69	0.069	NS	NS
Upper ileum	1.97	1.87	1.94	0.050	NS	NS
Lower ileum	1.79	1.67	1.54	0.051	***	*
			POTASSIUM			
Duodenum	0.94	0.95	0.93	0.072	NS	NS
Upper jejunum	0.63	0.65	0.63	0.023	NS	NS
Lower jejunum	0.55	0.55	0.51	0.021	*	NS
Upper ileum	0.66	0.80	0.72	0.031	NS	*
Lower ileum	1.20	1.29	1.56	0.080	***	NS
			PHOSPHORUS			
Duodenum	0.81	0.74	0.89	0.094	NS	NS
Upper jejunum	0.69	0.60	0.58	0.038	*	NS
Lower jejunum	1.16	0.81	0.60	0.061	***	NS
Upper ileum	1.63	1.18	0.78	0.059	***	NS
Lower ileum	2.07	1.46	1.07	0.067	***	NS
			CALCIUM			
Duodenum	1.07	1.18	1.26	0.055	***	NS
Upper jejunum	1.71	1.46	1.40	0.078	**	NS
Lower jejunum	3.29	2.13	1.53	0.125	***	*
Upper ileum	4.42	3.03	1.94	0.133	***	NS
Lower ileum	5.46	3.64	2.62	0.178	***	*
			MAGNESIUM			
Duodenum	0.16	0.16	0.14	0.005	***	NS
Upper jejunum	0.20	0.20	0.16	0.007	***	*
Lower jejunum	0.38	0.28	0.18	0.010	***	NS
Upper ileum	0.52	0.38	0.24	0.017	***	NS
Lower ileum	0.65	0.46	0.33	0.026	***	NS

[1] The minerals were analysed in the chyme samples from the birds in trial 1.

the integrative segment (caeca, rectum and coprodeum) at the 10.0 g/kg CMC diet.

Mineral concentration in the chyme

Minerals are absorbed as ions or small soluble complexes (Scott *et al*, 1976). Therefore, they are linked to the liquid phase of the chyme. Assuming that the mineral composition in the liquid phase of the chyme resembles that in the supernatant, the potential mineral availability for absorption (mineral solubility) can be derived as the ratio between the mineral concentration in the supernatant (Table 2) and the respective values in the total chyme (Table 3).

The mineral concentration in the chyme (Table 3) is related to its dry matter content. Both water intake and the moisture content of the chyme were increased in broilers fed CMC-containing diets (van der Klis *et al*, 1993). This was the main reason for the reduced concentrations of phosphorus, calcium and magnesium in the chyme samples. The moisture content was doubled from the lower jejunum onwards by 10.0 g/kg CMC inclusion (van der Klis *et al*, 1993), while the concentration of those minerals was halved (Table 3). Changes in sodium and potassium concentrations were less extreme or their concentrations were even higher at increasing dietary CMC contents. This implies that the moisture content of the chyme has increased as a result of dietary CMC and was accompanied by a considerable increase in the intraluminal amount of sodium and potassium.

Mineral solubility

The sodium concentration in the chyme supernatant was increased (Table 2), as the chyme moved from the duodenum into the upper ileum. In the lower ileum the sodium concentration was reduced. This was observed in all birds. In the jejunal and ileal segments, the sodium concentrations in the supernatant was reduced by increasing dietary CMC. However, the sodium concentration in the total jejunal and ileal chymes were less affected (or not affected at all) by the dietary CMC content (Table 3) compared to the concentrations in the supernatants. This resulted in similar intestinal sodium solubilities (ratio supernatant/chyme) when CMC was included in the diet (Figure 3). Unlike sodium, the potassium concentration in the chyme supernatant was reduced from the duodenum

onwards, to reach a minimum in the lower jejunum (Table 2). As the chyme was transported into the ileum, the potassium concentration increased again to reach its maximum value in the lower ileum. This occurred on all diets. In all segments the potassium concentration in the supernatant was similar to or higher than the concentration in the total chyme (Table 3), except in the ileum of the birds fed the 10.0 g/kg CMC diet. This indicates a reduced ileal potassium availability on the 10.0 g/kg CMC diet, while the other ratios were similar (Figure 3).

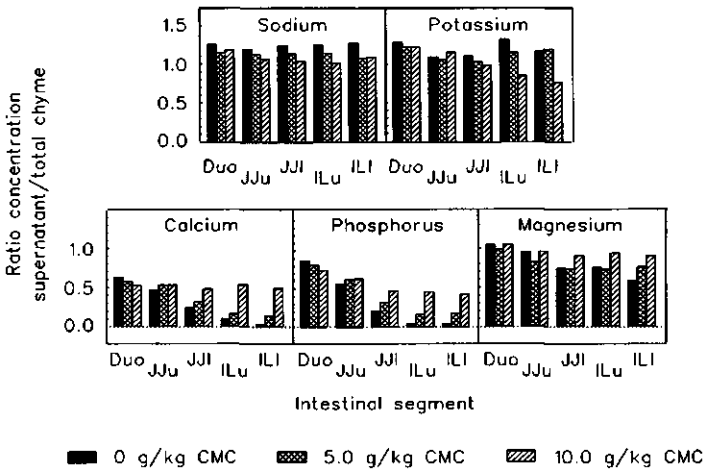

Figure 3. The mineral solubility (ratio concentration in supernatant/total chyme) in successive intestinal segments.
The mineral solubility is calculated from the respective data from Tables 2 and 3. Duo: duodenum, JJu: Upper jejunum, JJl: Lower jejunum, ILu: Upper ileum, ILl: Lower ileum.

The phosphorus concentration in the chyme supernatant decreased as the chyme moved from the duodenum into the upper ileum of the birds fed on the CMC-free diet. It did not change further from the upper to the lower ileum (Table 2). On the 5.0 and 10.0 g/kg CMC diets, respectively, the phosphorus concentration increased after the upper ileum and lower jejunum. From the lower jejunum onwards, its concentration in the supernatant was increased linearly as the dietary CMC content increased, while the concentration in the total chyme decreased lineraly from the upper jejunum onwards (Table 3). The phosphorus solubility in the jejunum and ileum was thus increased by CMC addition (Figure 3). The calcium solubility followed a similar pattern to that

described for phosphorus (Figure 3). The calcium concentration in the supernatant of the jejunal chyme was decreased by increasing concentrations of dietary CMC, and increased in the ileal chyme (Table 2). Its concentration in the jejunal chyme was decreased to a far greater extent by CMC (Table 3) than the decrease observed in the chyme supernatant. As the decrease of calcium in the ileal chyme was accompanied by an increase in the supernatant, the calcium solubility was increased by dietary CMC in the lower jejunum and ileum (Figure 3). The course of the magnesium concentration in the supernatant was different from those of the previous two elements. The magnesium concentration increased from the duodenum onwards to reach a maximum value in the lower ileum. On the CMC-free diet, the magnesium concentration was maximal in the upper ileal supernatant and remained at that level in the lower ileum. In all intestinal segments the magnesium concentrations in both the supernatant (Table 2) and total chyme (Table 3) were reduced by dietary CMC addition. However, it is clear that the solubilities in the jejunum and ileum were positively affected by CMC inclusion (Figure 3).

Discussion

Factors affecting dry matter and mineral absorption

Figures 1 and 2 show that the rate of absorption of dry matter, as well as those of all the minerals studied was decreased by dietary CMC inclusion. The prevailing intestinal conditions, under which digestion and absorption of nutrients took place in this experiment, have been described elsewhere (van der Klis *et al*, 1993). The intraluminal pH is the predominant factor changing mineral solubility. In the jejunum a slight linear pH reduction by the increasing dietary CMC content was observed, while the ileal pH was considerably reduced from 7.2 on the CMC-free diet to 6.0 at the 10.0 g/kg CMC diet. Under normal physiological conditions the pH in the ileal segments of broilers is too high for efficient mineral absorption, as divalent minerals will then be complexed with undigested organic residues (Shafey *et al*, 1991). Thus it was expected that the reduced ileal pH, caused by dietary CMC inclusion, might increase mineral absorption as the result of increased mineral solubility. Dietary CMC indeed resulted in a smaller fraction

of calcium, phosphorus and magnesium being attached to the solid phase of the chyme in the lower jejunal and ileal segments (Figure 3). The reduced pH might have contributed to the prolonged occurrence of mineral absorption in the ileal segments. As expected, the solubility of monovalent cations was higher than the solubility of the other minerals (Figure 3). For some reason, the potassium solubility seemed to be reduced in the ileum of the birds fed the 10.0 g/kg CMC diet. The mean retention time of chyme in the duodenum and proximal jejunum was increased by dietary CMC addition (van der Klis *et al*, 1993). Hurwitz and Bar (1970), Hurwitz *et al* (1970) and Guenter and Sell (1973) showed that in broilers the upper small intestine is the main site of absorption of respectively calcium and phosphorus, sodium and potassium and magnesium. Based on the increased mean retention time of the chyme in the upper small intestine, and the similar mineral solubilities (Figure 3), a positive rather than a negative effect of CMC on the cumulative mineral absorption was expected. Furthermore, it is not likely that a reduced rate of mineral absorption will be a direct result of cation binding to the soluble CMC, because *in vitro* neither the CMC:cellulose ratio (0, 0.05 and 0.10), nor the incubation pH (5 and 6.5), increased the sodium or potassium concentration linked to the supernatant after 3 h of incubation of the CMC:cellulose mixture. The concentrations of calcium and magnesium in the supernatant were even slightly reduced (van der Klis, unpublished results). The higher intraluminal viscosity (van der Klis *et al*, 1993) might explain why the absorption of minerals did not respond. An increase in the intraluminal viscosity can reduce the rate of nutrient absorption, by increasing the thickness of the unstirred water layer covering the mucosa cells (Johnson and Gee, 1981). The thickness of this layer affects both passive and active transport processes (Thomson and Dietschy, 1977). Mixing of the chyme might also be poorer at a higher intraluminal viscosity (Edwards *et al*, 1988). This was considered to affect the rate of digestion (degradation of food particles to absorbable nutrients) negatively. However, our results did not support the hypothesis (Edwards *et al*, 1988) that a higher intraluminal viscosity reduces the mixing of the chyme. The reduced maximal rate of marker excretion at higher CMC concentrations, in combination with the prolonged time at which the rate of marker excretion was maximal (van der Klis *et al*, 1993), indicates better mixing between the marker and the chyme in the small intestine, as was discussed by van der Klis and van Voorst (1993). However,

this improved mixing in a longitudinal direction (which in fact determines the rate of passage of the marker through the intestinal tract) does not imply, that the exchange of fluid from the centre of the intestinal lumen to the surface of the mucosa (mixing in an axial direction) is also improved. A reduced efficiency of axial mixing lowers the concentration gradient between the mucosal and serosal sides of the intestinal wall. This in turn results in a reduced rate of diffusion through the mucosa cells. Viscosity is the only factor studied which can potentially reduce the rate of absorption from all intestinal segments, thus its effect will have been partially alleviated by other conditions which favour absorption. Even at the high intestinal viscosity, however, considerable sodium absorption occurs in the jejunum and the ileum, as more than twice the daily oral intake of this mineral is absorbed from the GI lumen.

It was previously shown that a high intestinal viscosity might cause a reduced rate of absorption of dry matter from the intestinal lumen (e.g. Hesselman and Åman, 1986). They observed a reduced rate of starch and nitrogen degradation and absorption from the duodenum up to the lower ileum of 3-week-old broiler chickens, when a high viscosity barley was compared to a low viscosity variety.

Although the rate of absorption was reduced, absorption also occurred in the more posterior segments. This partially compensated the large negative effects of CMC on mineral absorption up to the lower jejunum (Figures 1 and 2). The birds fed on the CMC-containing diets still absorbed sodium and magnesium from the lower ileum and the integrative segment. Thomas (1982) has shown that, in birds, the efficiency of sodium absorption from the integrative segment is increased in case of sodium deprivation or dehydratation. The prevailing conditions in the intestinal lumen should of course facilitate such an absorptive mechanism. In birds urine is excreted into the cloaca, and subsequently moved into the rectum and ceaca. The dilution of chyme with urine in the integrative segment might have reduced the viscosity as well as the CMC degradation by the microbial population in the ceaca and rectum. The reduced viscosity, resulting from both processes, will have enabled absorption of these minerals.

The effect of the different physico-chemical conditions on the absorption of minerals from the intestinal lumen cannot be directly evaluated from this experiment. It is most likely that the intestinal viscosity was the major factor reducing the cumulative mineral

absorption in the CMC-containing diets. The physico-chemical parameters studied might even have favoured mineral absorption in the CMC diets. The viscosity probably exhibits its effect on nutrient absorption through an increased thickness of the instirred water layer. Inefficient mixing in axial direction (from the centre of the intestinal lumen up to mucosal surface) will cause a difference in the composition of the chyme sampled from the centre of the lumen and the chyme fluid which is actually in contact with the mucosa cells. Reduced axial mixing will cause a lower concentration gradient over the intestinal mucosa and inhibit the rate of diffusion of minerals.

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CHAPTER 6

ENDOXYLANASE ADDITION TO WHEAT-BASED BROILER DIETS AND THE ABSORPTION OF MINERALS

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ABSTRACT Four wheat varieties, differing in in vitro viscosity and water soluble arabinoxylan content, were added to a basal diet on a fifty-fifty basis. These wheat-based diets were fed to male broilers from 3 weeks of age on, with and without the addition of 75 mg endoxylanase per kg feed. The broilers were killed at 4.5 weeks of age, and the jejunal and ileal contents were sampled. The absorption of sodium, potassium, calcium, phosphorus, and magnesium was calculated, based on the contents of the last 10 cm of these intestinal segments, using Cr_2O_3 as a reference substance. The pH, osmolality and viscosity were measured in the remaining jejunal and ileal contents.

Dietary addition of high viscosity wheats (HVW) resulted in higher intraluminal viscosities, than on the low viscosity wheat (LVW) diets. The osmolality of the chyme supernatant of the birds fed the HVW diets declined less, as the chyme moved from the jejunum into the ileum, compared the LVW diets. This indicates a less efficient absorption of osmo-active soluble chyme components at higher intraluminal viscosities, as complexation in the intestinal lumen could not account for this effect. Linear regression analysis revealed a clear negative relationship between the viscosity of the chyme supernatant and the absorption of dry matter and minerals in either one or both of the intestinal segments. Addition of endoxylanase lowered the viscosity of the chyme supernatant to similar levels on all wheat-based diets. It also showed an improved absorption of minerals from the jejunal lumen. Neither phosphorus absorption, nor the absorption of minerals -except magnesium- from the ileal lumen, was significantly improved by dietary endoxylanase addition.

(*Key words:* wheat arabinoxylans, endoxylanase, intestinal viscosity, male broilers, mineral absorption)

INTRODUCTION

The absorption of minerals from the gastro-intestinal (GI) tract of broilers is influenced by the prevailing physico-chemical conditions, like the luminal pH (Shafey *et al*, 1991) and the chyme viscosity (van der Klis *et al*, 1993b). In the latter experiment

semi-synthetic diets were used, with different amounts of a soluble polysaccharide (carboxy methyl cellulose) exchanged for cellulose. It was found, that the intestinal absorption of all five macro-elements studied (calcium, phosphorus, magnesium, potassium and sodium) was reduced at higher intestinal viscosities.

Native soluble polysaccharides from feedstuffs can similarly affect the intestinal conditions, and thereby alter the rate of mineral absorption. Soluble indigestible polysaccharides in cereals, like ß-glucans in barley (Hesselman and Åman, 1986) and oats (Campbell *et al*, 1987), and arabinoxylans in wheat (Choct and Annison, 1992a,b) and rye (Bedford *et al*, 1991) reduce the digestibility of organic nutrients in chickens by increasing the intestinal viscosity.

In the present experiment, the effect of four wheat varieties, differing in water soluble arabinoxylan (WSA) content and in vitro viscosity, on the absorption of dry matter and minerals (calcium, phosphorus, magnesium, potassium and sodium) from the small intestine of broilers was examined. These wheat-based diets (containing 50% wheat) were fed to broilers with and without dietary addition of endoxylanase, in order to eliminate the viscosity increasing effect of wheat arabinoxylans.

MATERIALS AND METHODS

Chickens, housing and feeding

1080 Male broilers (Ross, EPI, Roermond, The Netherlands) were housed in 3-tier battery cages (15 birds per .45 m^2 cage) in a climate-controlled poultry house. The room temperature was gradually decreased from 33°C for 1-day-old chicks to 21°C at 4.5 weeks of age. The light was continuous during the first three days. Subsequently a light schedule was used, in which one hour light alternated with three hours darkness. The relative air humidity was kept at minimal 55%. The birds had free access to a standard broiler diet (van der Klis *et al*, 1990) and water during the first three weeks.

At the start of the experimental period at three weeks of age, lighting was continuous again to enable the birds to eat as frequently as possible. The number of birds was standardized at 12 birds per cage. The experimental diets were prepared by adding four

Table 1. The composition of the basal diet component, and its analysed mineral concentrations

Ingredient	g per kg
Corn	460
Corn glutenmeal (65% CP)	70
Soybean solvent extracted (49% CP)	200
Meat meal	85
Soya oil	25
Blended animal fat	25
Corn starch	79
Vitamins[1]	10
Minerals[2]	30
Monocalcium phosphate	10
Synthetic lysine	3.3
Synthetic DL-methionine	2.7
Calculated nutrient concentrations	
ME_n, MJ per kg feed	12.50
Crude protein, %	22.8
Crude fat, %	8.4
Crude fiber, %	1.9
Lysine, %	1.40
Methionine + cysteine, %	1.05
Analyzed mineral concentrations	
Calcium, %	1.92
Phosphorus, %	1.10
Magnesium, %	0.17
Sodium, %	0.25
Potassium, %	0.70

1 The vitamin premix supplied per kg of ration: vitamin A, 12,000 IU; vitamin B_1, 1 mg; vitamin B_2, 5 mg; nicotinic acid, 30 mg; panthotenic acid, 7.5 mg; vitamin B_6, 1 mg; vitamin B_{12}, 15 μg; folic acid, 1 mg; vitamin D_3, 2,400 IU; vitamin E, 15 mg; vitamin K_3, 1.5 mg; choline chloride, 350 mg; and ethoxyquin, 50 mg.

2 The mineral premix supplied per kg of ration: monocalcium phosphate, 8.0 g; $CaCO_3$, 17.2 g; NaCl, 3.8 g; $CuSO_4.5H_2O$, 0.06 g; $ZnSO_4.7H_2O$, 0.09 g; $MnSO_4.4H_2O$, 0.36 g; $FeSO_4.7H_2O$, 0.39 g; KI, 1.5 mg; and Se, 0.15 mg.

different wheat varieties (Taurus, Arminda, Minaret and Apollo, numbered I to IV) to the basal diet component (Table 1) on a fifty:fifty weight basis. The wheat varieties differed in in vitro viscosity and the content of WSA (Table 2). Increasing numbers correspond with increasing contents of WSA. The mineral composition of the wheat varieties is also given in Table 2. Each diet was supplied to the broilers with and without 75 mg endoxylanase per kg diet (Lyxasan®, Gist-brocades, Delft, The Netherlands), resulting in 8 experimental diets. Lyxasan® is an enzyme preparation derived from Aspergillus niger and standardized at an endo-1,4-ß-xylanase activity (EC 3.2.1.8) of 70.000 EXU per gram. 1 EXU is defined as the amount of enzyme that liberates one μmol of reducing sugars per minute from a 1% oat spelt xylan suspension at pH 3.5 and temp. 40°C. All diets contained 0.15% Cr_2O_3 as a non-absorbable marker. The experimental diets were pelleted. Feed and water was available continously. A randomized block design was used, each of three blocks containing three cages per diet. Per block, the diets were assigned at random to the cages. Birds from different blocks were housed in different rooms.

Table 2. The mineral composition and the in vitro viscosity of 4 wheat varieties

Wheat variety[1]	In vitro viscosity (mPa.s)[2]	Water Soluble AX[3] (g/kg dm)	Mineral concentration (g per kg dm)				
			Ca	P	Mg	K	Na
I	1.43	0.60	0.58	3.88	1.29	4.50	0.32
II	1.71	0.69	0.69	4.31	1.35	4.90	0.13
III	2.06	0.74	0.60	3.80	1.32	4.06	0.13
IV	2.15	0.81	0.51	4.19	1.25	4.33	0.12

1 Increasing numbers correspond with higher *in vitro* viscosities and water soluble arabinoxylan concentrations.

2 The *in vitro* viscosity was measured in the supernatant of wheat suspended in water (wheat:water= 1:2) for 1 hour. The suspension was centrifuged at 6000 G at 15°C for 15 min.

3 The concentration of water soluble arabinoxylans (AX) in wheat were analyzed according to Annison (1991).

Chyme sampling and measurements

At 4.5 weeks of age, the birds were killed by an intravenous injection of T61 (a

watery solution containing (in mg per ml) embutramide, 200; mebezoniumiodide, 50 and tetracainehydrochloride, 5, Hoechst Holland NV, Amsterdam, The Netherlands). Subsequently, the chest cavity and the abdomen were opened and the small intestine was ligated and removed from the bird. The 10 cm of the jenunum preceeding the Meckel's diverticulum and the 10 cm of the ileum, preceeding 1 cm proximal to the ileo-cecal junction, were emptied for mineral analysis (van der Klis *et al* 1993). This procedure of chyme sampling was approved by the Animal Care and Ethics Committee of the Spelderholt Intitute. The chyme samples were pooled per intestinal segment per cage and stored at -18°C. After freeze-drying, jejunal and ileal samples were pooled per diet per block (pooled samples of three cages) and grinded (particle size ϕ 1 mm). The feed and chyme samples were analyzed for dry matter, chromium, calcium, phosphorus, magnesium, sodium, and potassium. The apparent dry matter digestibility and mineral absorptions were calculated (van der Klis *et al*, 1990). As this method can only refer to the apparent digestibility and apparent absorption, the single terms digestibility and absorption are used throughout the results and discussion section.

One of the three cages per diet per block was randomly chosen for viscosity and osmolality measurements in the chyme supernatant and one for pH measurements in total chyme respectively.

Analytical methods

Pre-weighed samples (2 g diet or 1 g chyme for mineral analysis and 0.5 g sample for chromium analysis) were dried (105°C, 5 hours), and weighed for dry matter analysis and ashed (550°C, 5 hours).

Chromium. Ashed samples were heated gradually to 230°C, with 6 ml $KBrO_3$ solution (30 g $KBrO_3$ per liter demineralized water), 3 ml phosphoric acid (82.5 g per 100 g), and 6.8 mg $MnSO_4.H_2O$. This temperature was kept for 2 hours. After cooling, the volume was standardized with demineralized water. Subsequently, the chromium concentration was determined by atomic absorption spectrophotometry (SpectrAA 400, Varian Nederland BV, Houten, The Netherlands), using an acetylene/air flame, after addition of 3.7 mg $CaCl_2.2H_2O$ per ml sample solution, as a reagens. This method was adapted from Williams *et al* (1962).

Mineral analysis. The ashed samples were boiled for 10 min with 12 mol per liter HCl to solubilize the mineral compounds. The sample was cooled and its volume was standardized with demineralized water.

Phosphorus. The pH of the sample solution was set at pH 4 by adding ammonium hydroxyde (25 g per 100 g). Subsequently, 5 ml reagens (containing 20 g $(NH_4)_6Mo_7O_{24}.6H_2O$, 2 ml NH_4OH (25 g per 100 g), 0.48 g NH_4VO_3, and 135.4 ml HNO_3 (70 g per 100 g) per liter) was added and the volume standardized with demineralized water. The phosphorus concentration was measured using a spectrophotometer (Beckman DU-64, Beckman Instruments Inc., Fullerton, USA).

Calcium, magnesium. The calcium and magnesium concentration were measured by atomic absorption spectrophotometry (SpectrAA 400, Varian Nederland BV, Houten, The Netherlands), after addition of 3.5 mg KCl per ml sample solution or 6.6 mg $La(NO_3)_3.6H_2O$ per ml sample solution respectively, as a reagens. For calcium analysis a nitrous oxide/acetylene flame was used and for magnesium analysis a acetylene/air flame.

Sodium and Potassium. The concentration of these minerals was measured by flame photometry (Instrumentation Laboratory 743, Wilten, Etten Leur, The Netherlands) using 3000 mmol per liter lithium solution as an internal standard.

Viscosity. The jejunal and ileal contents, not sampled for mineral analyses, were taken from all birds from each allotted cage, for viscosity and osmolality determinations in the chyme supernatant. The chyme was centrifuged at 6000 G for 15 min at 15°C and the supernatant was decanted and filtered through a 50μm filter to remove floating particles. In the supernatant the viscosity was measured at 40°C, using a rotation viscosimeter (Rotovisko RV2, Haake, Roosendaal, The Netherlands).

Osmolality. The osmolality was also measured in the chyme supernatant, using an osmometer (Gonotec Osmomat 030, Salm & Kipp, Breukelen, The Netherlands). The osmolality determination was based on freezing point depression.

pH. The intestinal pH was measured in four birds from each cage, allotted for pH measurement. The pH was measured at the beginning, half and at the end of the remaining segment of the jejunum and ileum, after chyme sampling for digestibility determinations, by inserting a micro pH-electrode (LoT 440-M3, Dr. W. Ingold AG, Urdorf, Germany) into the chyme through an incision in the intestinal wall at the site of

measurement. The pH was measured within 15 min after removal of the intestine.

Statistical analysis

Data for intestinal parameters and mineral absorption were analysed according to the following ANOVA:

$$Y_{ijk} = \mu + B_i + V_j + E_k + VxE_{jk} + e_{ijk}$$

Where B= block (i= 1,2,3); V= wheat variety (j= 1...4); E= endoxylanase (k= 0, 75 mg per kg); VxE= interaction; e_{ijk} is the error for the j^{th} diet with the k^{th} level of endoxylanase in the i^{th} block.

The errors were assumed to be independently and normally distributed with a mean equal to 0 and variance to σ^2. Statistical analyses were done using GENSTAT (Genstat 5 Committee, 1987) as a statistical program. Differences between treatment means were considered to be significant at $P \leq 0.10$.

RESULTS

Dry matter digestibility and mineral absorption

The digestibility of dry matter, and the absorption of minerals at the jejunal and ileal level are given in Tables 3 and 4, respectively. Proximal to the jejunal site of sampling, the major part of dry matter was digested. Also the absorption of calcium, phosphorus and magnesium was almost complete. In the ileum only a minor net absorption was observed for these minerals. Sodium secretion into the GI lumen exceeded its absorption proximal to the jejunal segment, resulting in a negative absorption. Total sodium secretion was at least equal to the daily sodium intake. Potassium was highly available and its absorption up to the jejunal segment reached 85-90% of the daily intake. In the ileum, sodium was absorbed from and potassium secreted into the intestinal lumen.

The digestibility of dry matter was significantly reduced in birds fed diets with the high viscosity wheat (HVW) varieties (variety III and IV), compared to those fed the low

Table 3. The apparent digestibility of dry matter and absorption of calcium, phosphorus, magnesium, sodium, and potassium (% of daily intake) at the jejunal level in broilers

	Dry matter		Calcium		Phosphorus		Magnesium		Sodium		Potassium	
	Added endoxylanase		*Added endoxylanase*		*Added endoxylanase*		*Added endoxylanase*		*Added endoxylanase*		*Added endoxylanase*	
Variety[1]	*0 mg/kg*	*75 mg/kg*	*0 mg/kg*	*75 mg/kg*	*0 mg/kg*	*75 mg/kg*	*0 mg/kg*	*75 mg/kg*	*0 mg/kg*	*75 mg/kg*	*0 mg/kg*	*75 mg/kg*
I	67.0	66.5	37.8	39.2	51.5	48.8	25.1	23.1	-91.2	-47.9	89.4	88.3
II	64.1	66.5	35.4	40.4	49.9	51.9	23.2	26.2	-104.1	-71.8	89.7	89.0
III	58.0	65.0	35.3	38.6	51.6	51.7	21.8	25.5	-129.2	-68.0	84.8	88.5
IV	59.7	65.2	34.1	40.2	48.5	51.3	20.8	25.7	-102.2	-91.7	85.6	90.5
MSr[2]	1.17		1.17		14.43		8.24		82.61		1.72	
Level of significance												
P (*Wheat variety*)	<.001		0.884		0.549		0.843		<.001		0.018	
P (*Endoxylanase*)	<.001		0.027		0.592		0.062		<.001		0.008	
P (*Wheat x endox*)	<.001		0.732		0.209		0.229		0.003		0.003	

[1] Referring to a wheat-based diet containing 50% wheat. Increasing numbers correspond with higher *in vitro* viscosities and water soluble arabinoxylan concentrations.

[2] Residual mean squares. The standard error of difference between two means is calculated as

$$SED = \sqrt{\frac{2 \times MSr}{rep.}}$$

Where MSr = residual mean squares; and rep = number of replicates (Variety, 6; Enzyme, 12; Variety x enzyme, 3).

Table 4. The apparent digestibility of dry matter and absorption of calcium, phosphorus, magnesium, sodium, and potassium (% of daily intake) at the ileal level in broilers

	Dry matter		Calcium		Phosphorus		Magnesium		Sodium		Potassium	
	Added endoxylanase		*Added endoxylanase*		*Added endoxylanase*		*Added endoxylanase*		*Added endoxylanase*		*Added endoxylanase*	
Variety[1]	*0 mg/kg*	*75 mg/kg*	*0 mg/kg*	*75 mg/kg*	*0 mg/kg*	*75 mg/kg*	*0 mg/kg*	*75 mg/kg*	*0 mg/kg*	*75 mg/kg*	*0 mg/kg*	*75 mg/kg*
I	74.3	75.0	39.5	41.9	55.0	54.4	27.5	27.4	-70.4	-44.8	70.2	71.5
II	72.6	74.4	37.1	37.5	50.8	49.8	20.9	28.6	-76.6	-70.2	65.9	72.6
III	67.5	73.7	32.3	39.0	47.5	53.4	19.1	33.5	-89.6	-77.9	72.6	69.9
IV	67.0	72.7	32.8	38.9	48.5	51.1	23.7	26.9	-93.4	-66.3	70.1	74.8
MSr^2	4.06		35.88		7.88		15.96		950.4		41.43	
Level of significance												
P (*Wheat variety*)	0.004		0.466		0.040		0.677		0.481		0.854	
P (*Endoxylanase*)	<.001		0.135		0.166		0.002		0.133		0.361	
P (*Wheat x endox*)	0.083		0.770		0.175		0.043		0.967		0.614	

[1,2] See Table 3

Table 5. The pH of the chyme, and the osmolality and viscosity of the chyme supernatant in the jejunal and ileal contents of 4.5 week old broilers

	Jejunum						Ileum					
	pH[3]		Viscosity (mPa.s)		Osmolality (mOsm/kg)		pH[3]		Viscosity (mPa.s)		Osmolality (mOsm/kg)	
	Added endoxylanase		*Added endoxylanase*		*Added endoxylanase*		*Added endoxylanase*		*Added endoxylanase*		*Added endoxylanase*	
Variety[1]	*0 mg/kg*	*75 mg/kg*	*0 mg/kg*	*75 mg/kg*	*0 mg/kg*	*75 mg/kg*	*0 mg/kg*	*75 mg/kg*	*0 mg/kg*	*75 mg/kg*	*0 mg/kg*	*75 mg/kg*
I	5.93	5.98	2.50	2.47	453	454	6.36	6.58	3.98	3.50	361	382
II	5.90	6.02	2.83	2.77	428	455	6.50	6.86	4.88	3.87	354	366
III	5.88	6.07	3.40	2.65	440	444	6.45	6.71	5.23	3.30	383	368
IV	5.95	6.03	3.50	2.43	428	426	6.52	6.63	5.70	3.33	383	374
MSr[2]	0.0126		0.0374		410.2		0.0458		0.0476		84.8	
Level of significance												
P (*Wheat variety*)	0.946		0.001		0.209		0.443		<.001		0.020	
P (*Endoxylanase*)	0.034		<.001		0.357		0.016		<.001		0.574	
P (*Wheat x endox*)	0.728		<.001		0.612		0.766		<.001		0.017	

[1,2] See Table 3

[3] pH is the mean value of four birds and three sites of measurement in each intestinal segment.

viscosity wheat (LVW) diets -LVW (variety I and II) exhibited lower in vitro viscosities compared to HVW (Table 2)-. This reduction in dry matter digestibility was observed on both intestinal segments. No significant effects of the wheat varieties were found on mineral absorption, except for sodium and potassium at the jejunal and phosphorus at the ileal level. In all of these cases the mineral absorption was equal or lower, when HVW varieties were included in the diet, compared to the diet with the lowest in vitro viscosity (wheat I diet).

Addition of 75 mg endoxylanase per kg diet improved the dry matter digestibility. The magnitude of the endoxylanase effect in the jejunum was related to the wheat variety used, as indicated by the significant wheat variety x endoxylanase interaction (Table 3). No effect of endoxylanase was found for the wheat I diet, while the jejunal absorption of dry matter on all the other diets with endoxylanase was improved to a that level. This pattern was also evident in the ileum. Furthermore, the addition of endoxylanase increased the absorption of calcium, magnesium, sodium and potassium at jejunal level and magnesium at ileal level. The significant wheat variety x endoxylanase interaction, for jejunal sodium and potassium absorption and ileal magnesium absorption, indicated that the response to endoxylanase in the specific intestinal segments for those minerals depended on the wheat variety used. However, only for potassium absorption up to the jejunal segment, the magnitude of the endoxylanase effect seemed to be directly related to the in vitro viscosity of the wheat variety used. No significant effects of endoxylanase on phosphorus absorption were observed in either intestinal segment.

Intestinal conditions

In Table 5, the pH of the chyme, and the viscosity and osmolality of the chyme supernatant are given. The intestinal viscosity of the supernatant obtained from the jejunal and ileal chyme increased from birds fed the wheat I to those fed the wheat IV diets. The viscosity of the chyme supernatant was reduced by endoxylanase addition in both intestinal segments. In the jejunum all viscosities were similar irrespective of the wheat variety, when endoxylanase was added to the diets. At ileal level, the viscosity on the wheat II diet remained significantly higher, compared to the ileal contents of the birds fed the other endoxylanase containing diets. The chyme pH was increased by endoxylanase addition by

0.10 and 0.25 pH units in the jejunum and the ileum respectively. The values of the osmolality of the ileal chyme supernatant in the birds fed the HVW diets were decreased by endoxylanase addition, while they were increased by endoxylanase addition to the LVW diets.

DISCUSSION

The viscosity of the supernatant of the jejunal and ileal chyme was related to the in vitro viscosity of the wheat varieties and to their WSA content (Table 2). Addition of endoxylanase to the diets reduced the viscosity of the chyme supernatant. This endoxylanase effect was larger for HVW than for LVW diets, the former containing the higher concentrations of WSA. Choct and Annison (1992b) showed, that high levels of WSA increased the intestinal viscosities in broilers fed wheat-based diets. Furthermore, especially high molecular weight carbohydrates (> 500.000 D) were responsible for increased chyme viscosity (Bedford *et al*, 1991). Endoxylanase reduces the chain length of the arabinoxylans and thereby reduces its viscosity increasing effect (Bedford and Classen, 1992).

The effect of the intestinal viscosity on the absorption of minerals has been studied by van der Klis *et al* (1993a, 1993b). They added 0%, 0.5% and 1% carboxy methyl cellulose (CMC) to a semi-synthetic diet. The digestibility of dry matter and the absorption of calcium, phosphorus, magnesium, sodium and potassium were decreased on diets with a high chyme viscosity. The digestibilities of dry matter and the absorption minerals were therefore plotted against the intestinal viscosity (Figures 1 and 2). The regression equations (Table 6) are based on the diets without endoxylanase addition. From Figures 1 and 2 and Table 6 it is clear, that the digestibility of dry matter and the absorption of calcium, magnesium and potassium at jejunal level and the dry matter digestibility and absorption of calcium, phosphorus, and sodium at ileal level were reduced concurrent with increasing intraluminal viscosities (Table 6). This is in accordance with results on CMC containing diets (van der Klis *et al*, 1993b). A reduced mineral absorption by higher intestinal viscosities was observed, even though the mineral solubility was not affected or even increased by dietary CMC inclusion. It was therefore

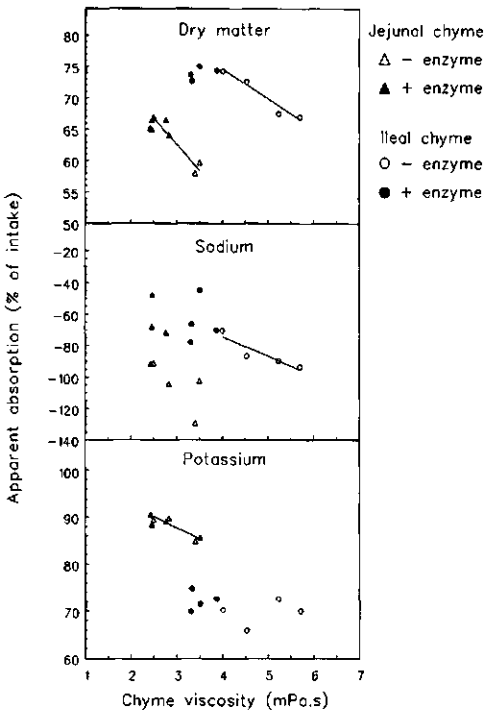

Figure 1. The apparent digestibility of dry matter and absorption of sodium and potassium cumulative up to the jejunum and ileum of broilers fed wheat-based diets, with and without dietary endoxylanase addition.

Regression analyses are based on the wheat-based diets without added endoxylanase. Each point is a mean value of three replicates. The chyme viscosity was measured in the chyme supernatant. The regression lines are not shown when $P > 0.15$. The corresponding regression equations are given in Table 6.

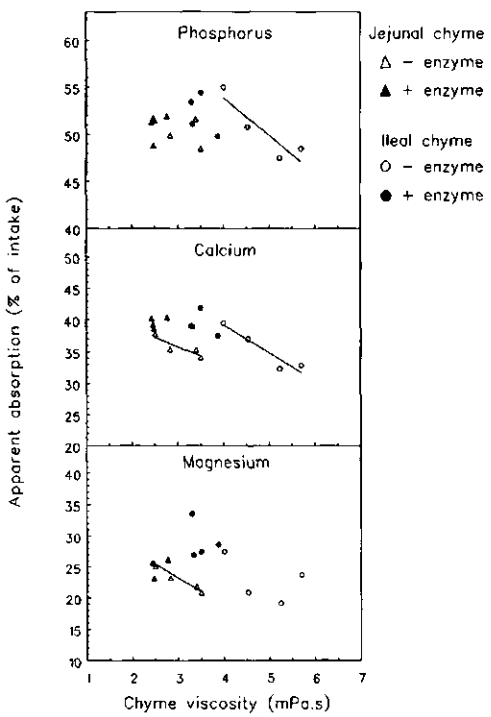

Figure 2. The apparent absorption of calcium, phosphorus and magnesium cumulative up to the jejunum and ileum of broilers fed wheat-based diets, with and without dietary endoxylanase addition.

Footnote see Figure 1.

unlikely, that the reduced mineral absorption was simply due to complexation by undigested food residues and they concluded that the absorption process itself was negatively affected by the higher intestinal viscosities. In the semi-synthetic diets used in their experiments, the major part of the minerals was added as inorganic salts. In the present experiment, however, also native minerals from wheat were present in the diets. From Tables 1 and 2 it can be calculated, that these native minerals contributed for 3% calcium, for 27% phosphorus, for 43% magnesium, for 6% sodium and for 39% potassium. It is therefore possible, that the differences in mineral absorption (especially for those with high levels in wheat: phosphorus, magnesium and potassium) between wheat-based diets are partly due to differences in availability of native mineral compounds in wheat.

Table 6. The regression coefficients of the linear relationship[1] between the intestinal viscosity and the apparent digestibility of dry matter and absorption of minerals

Digestibility/ absorption of	intestinal segment	intercept	a[1]	pva[2]	Level of significance (P)
Dry matter	jejunum	87.8 ± 4.78	-8.4 ± 1.55	90.4	0.033
	ileum	93.4 ± 3.85	-4.7 ± 0.78	92.2	0.026
Calcium	jejunum	44.5 ± 3.35	-2.9 ± 1.09	66.8	0.118
	ileum	56.8 ± 5.01	-4.4 ± 1.02	85.4	0.050
Phosphorus	jejunum	54.6 ± 6.07	-1.4 ± 1.97	-	0.557
	ileum	69.9 ± 6.71	-4.0 ± 1.37	71.6	0.100
Magnesium	jejunum	36.7 ± 2.61	-4.5 ± 0.85	90.1	0.034
	ileum	34.4 ± 14.8	-2.4 ± 3.01	-	0.511
Sodium	jejunum	-39.0 ± 55.8	-22.1 ± 18.1	14.1	0.346
	ileum	-25.2 ± 19.2	-12.3 ± 3.92	74.7	0.088
Potassium	jejunum	102.4 ± 4.54	-4.9 ± 1.47	77.3	0.079
	ileum	63.3 ± 12.0	1.3 ± 2.45	-	0.644

[1] Digestibility or absorption (%)= intercept + a * viscosity (mPa.s)
[2] Percentage of variance accounted for by the linear model

The osmolality of the chyme supernatant is dependent on the concentration of osmo-active chyme components. Digestion or solubilization of food components into small absorbable nutrients will result in an increased osmolality. Absorption or complexation of these nutrients will lower the osmolality again. It was shown by van der Klis *et al* (1993a)

that the osmolality of the chyme supernatant decreased less when higher intestinal viscosities occurred. This phenomenon was also obvious in the present experiment. The osmolality of the chyme supernatant decreased by 82, 81, 66 and 48 mOsm per kg (LSD ($P<0.05$) 25 mOsm per kg) as the chyme moved from the jejunum into the ileum of the birds fed the wheat I to wheat IV diets respectively. It is likely, that these differences were due to a less efficient nutrient absorption from the intestinal lumen at the higher intestinal viscosities and not to complexation, as the pH was not affected significantly by the wheat varieties. Furthermore, van der Klis and Geerse (1993) showed, that the digestibility of fat and starch was also negatively affected by these HVW diets. This reduction in digestibility of organic nutrients results in higher concentrations of potentially complexing agents. Complexation would result in lower intestinal osmolalities, while higher values were found.

Conclusion

The absorption of minerals from the GI tract of broilers is lowered and intraluminal viscosities in the small intestine increased when HVW diets are fed. The intraluminal viscosity was reduced by dietary endoxylanase, and the absorption of minerals improved, indicating a direct negative relationship between the intestinal viscosity and mineral absorption. It was discussed that a high intraluminal viscosity might have resulted in a less efficient mineral absorption. It was unlikely that mineral complexation in the intestinal lumen, due to a reduced digestibility of organic nutrients, was the only cause for the lower mineral absorption.

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General discussion

In this thesis, studies on the effect of physico-chemical conditions in the GI lumen on the absorption of some macro-minerals (sodium, potassium, calcium, magnesium and phosphorus) are reported. The validity of the results from the experiments reported herein depends on the presence of a steady state situation in all GI segments of interest. It also depends on representative chyme sampling. Both conditions were verified in the first experiment.

METHOD EVALUATION

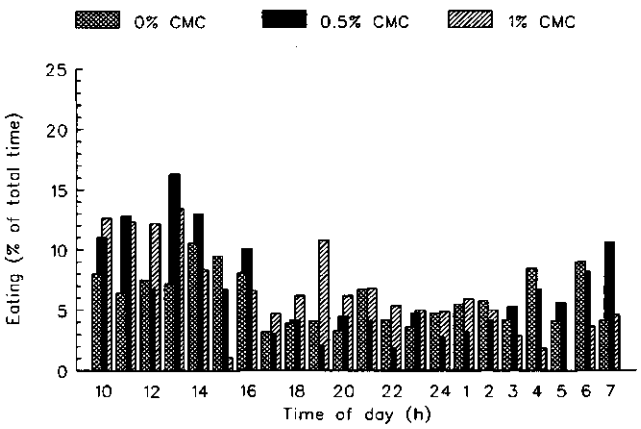
Steady state assumption

The validity of the results of experiments to determine the cumulative absorption of nutrients from the gastrointestinal (GI) tract, is a.o. dependent on the properties of the marker used. A marker should mimic the specific nutrient of interest, it should have similar flow characteristics, and it should be completely recoverable in chyme or faeces (Chapter 2). When these conditions are met, the digestibility of the nutrient can be calculated from the ratio between the nutrient and the marker concentrations as shown in Chapter 2. However, no ideal markers are known from the literature, which fulfill of these necessities. Especially the asssumption of similar flow characteristics is difficult to check experimentally. The preferable flow characteristics of the marker vary with the nutrient it should represent. This problem can be overcome by creating a steady state situation in the GI lumen. In Chapter 2, steady state was defined as both a constant amount of a marker in relation to time and a constant marker concentration in each GI segment of interest, irrespective of the time of feed intake. It was concluded from Chapter 2, that a steady state situation is approached when the birds eat once every 1:30 hours.

Thus the frequency of feed intake influences a steady state between the marker and dry matter in the GI tract. This frequency was used to obtain and justify the steady state situation on semisynthetic diets (Chapters 3 to 5). The eating pattern of all seven birds in a cage per diet, was determined by continuous observations during 22 hours. The results are given in Figure 1 as mean values per 7 birds. From this figure, it is clear that broilers eat frequently throughout the day, although the total time spent eating (given as a

percentage of the 24 hours) is somewhat lower during night than during daytime. The variation in eating pattern within a day, may cause some variation in the amount of Cr_2O_3 present in the GI tract. The sequence of the cages, from which the birds were killed, was therefore chosen in random order. Any effect of a diurnal rhytm in eating frequency will thus increase the residual sum of squares of the nutrient digestibility values and the mean retention time (both based on Cr) in the statistical models given in Chapter 3 to 5.

Figure 1. The eating pattern of broilers fed semi-synthetic diets containing 0%, 0.5% or 1% carboxy methyl cellulose (CMC). Data are mean values of seven broilers from one cages per diet.



Method of chyme sampling

The chyme was sampled directly after the birds were killed by an intravenous injection of sodium pentobarbital (Chapter 2) or T61 (Chapter 3 to 6). The chest cavity and the abdomen were opened after killing, the GI tract was ligated at pre-determined sites and removed from the bird. Subsequently, each segment was emptied by gently squeezing between thumb and finger. The whole content was used as a sample. This procedure was finished within two minutes after the bird was killed. The method of

chyme sampling did not cause any damage to the intestinal mucosa, as was shown by microscopical examination (Nabuurs (1992), personal communication). Furthermore, post mortal movement of the chyme is unlikely due to the method of killing (T61 does prevent muscle contraction).

In the work described in Chapter 2, the ligated GI tracts were stored at -20°C. The chyme was sampled after thawing. Due to this procedure, the mucosa cells may have been damaged, and the intracellular fluid, or even complete mucosa cells may have been sampled together with the chyme. If this really happened, it would have increased the amount of minerals from endogenous origin in the GI lumen contents, and thereby it would have lowered the apparent absorption values. This effect would have been larger for those minerals present in relatively high concentrations in the intestinal mucosa (potassium), than for those present in only low concentrations (sodium). Furthermore, this effect would have been more pronounced in the duodenum compared to the more posterior intestinal segments, due to the relatively large duodenal villi. The results on the site of apparent mineral secretion or absorption were, however, in accordance with the literature data (Chapter 2).

Retention time parameters

The time food is retained in the GI tract, determines the time available for digestion of food and absorption of nutrients. Especially the distribution of the total retention time over the successive GI segments is important in this respect. The mean retention time (the time an average dry matter particle is retained is a specific GI segment) was estimated by several methods (Table 1).

In Chapter 2, two different methods were used to estimate the mean retention time. The first was based on the time related decrease of the amount of Cr present in the GI segments during feed withdrawal. Measurements were carried out on pooled chyme samples of two birds. Fasting of birds, however, might result in an overestimation of the mean retention time, as no new feed is offered. Furthermore, using this method it was assumed, that each chyme particle has an equal probability of leaving the specific GI segment. This is only true in the case of ideal mixing. Therefore, a second method was

Table 1. Different methods for the determination of the mean retention time or retention time characteristics in broilers.

Chapter	Method	Assumption(s)
2	Rate of emptying of the GI tract after food withdrawal	Ideal mixing of marker and chyme
2,4	Determination of the amount of Cr present in GI lumen, related to the daily Cr intake	Steady state situation
3,4	Rate of passage of a Cr containing meal through the GI tract (faeces sampling) and through successive GI segments (chyme sampling)	- Rate of marker transport through the GI tract is similar to that of dry matter - Ideal mixing

also used. This method is based on a steady state assumption, which implies that the marker/nutrient ratio in the GI segments is constant throughout the day and is not affected by the time of food intake. Additionally, the amount of Cr has to be constant, in order to be able to calculate the mean retention time in the successive GI segments. The GI segments were emptied quantitatively. The mean retention time was calculated from the total amount of Cr present in the lumen of each GI segment and the daily Cr intake (Chapter 2). A steady state situation is a prerequisite in this case, as the time of killing (time of sampling of the GI contents) should not interfere with the amount of chyme present in the GI tract. The estimates are dependent on the correct measurement of the daily Cr intake (Chapter 2).

In Chapter 3, a third method was used to estimate the mean retention time. This method is based on the rate of excretion of Cr after consumption of a Cr containing meal (Table 1). Feed was withdrawn for 0:45 hours prior to feeding this meal, to ensure intake of the Cr containing meal at time 0. Based on faeces sampling (a pooled sample of 7 birds per cage), retention time parameters were calculated (time of first marker appearance in the faeces, time of 50% marker excretion, and time of and maximal rate of marker excretion). This procedure was repeated two days after the faeces sampling, to determine

the same characteristics in successive GI segments. The latter curves were based on birds from different cages. The amount of Cr in the GI segments was related on the total Cr intake (sum of Cr present in the GI tract and Cr excreted in the faeces). As Cr was supplied as a pulse dose (Cr containing meal, offered for 15 minutes), a steady state between the marker and dry matter was not present. The results indicated, that some separation between the solid and liquid phase in the proximal GI segments took place. This separation increased the rate of passage of the marker, compared to that of other dry matter components. Furthermore, it should be kept in mind, that entrance of a solid phase marker into the caeca might be food dependent (Chapter 3). Entrance of Cr into the caeca would also affect the rate of its excretion in the faeces. It was shown, that the transit time (time between intake and first appearance of the marker at the site of measurement) was not related to the shape of the excretion curves. Thus data on transit time should be interpreted carefully, when they are used as an indication for differences in the time that food was retained in the GI tract.

The estimates of the mean retention time, which were based on the steady state assumption (Chapters 2 and 4) are probably more accurate than the other methods: the steady state assumption was verified in Chapter 2 and broilers eat frequently throughout the day, which garantuees a continuous flow of nutrients through the intestinal tract of the birds. The validity of the other methods strongly depends on an equal flow of both the marker and the dry matter. As was discussed in the respective chapter, the marker used and the ratio between water and feed intake of the broilers will influence the results.

FACTORS AFFECTING MINERAL ABSORPTION

Food components, that affect mineral absorption are generally considered to act through mineral complexation (Chapter 1). Recently, it was shown, that an increased intraluminal viscosity reduces the digestibility of organic nutrients in broilers (Chapter 1). This reduced digestibility will result in higher levels of undigested, potentially complexing components in the intestinal lumen. Mineral complexation to undigested food components will lower mineral absorption. The viscosity might also directly reduce the rate mineral

absorption by an increased thickness of the unstirred water layer, covering the intestinal mucosa cells (as discussed in Chapter 5 and 6). Nutrients must pass this water layer before they can be absorbed para- or transcellularly. Mineral absorption values in broilers fed semi-synthetic diets (Chapter 5), are calculated as mg absorbed/day (Table 2). The relevance for absorption in the respective GI segments is indicated, to illustrate a possible compensatory mineral absorption. In this calculation, it is assumed that the apparent mineral absorption (% of daily intake: Chapter 5), remains constant throughout the day.

Inclusion of CMC in the diet clearly reduces the apparent absorption of all minerals from the GI tract between mouth and the upper jejunum. The effect of 0.5% CMC inclusion on the apparent absorption of calcium, phosphorus, sodium and potassium was smaller than the effect of the additional 0.5% CMC. As the apparent absorption is the net result of secretion and absorption, this may be due to both an increased secretion as well as a reduced absorption. Ikegami *et al* (1990) have studied the effect on endogenous secretions in rats. They found, that addition of 5% of several fibers to a diet (indigestible viscous polysaccharides) increased the pancreatic-bilary secretion. This effect was dependent on the kind of fiber used. If low CMC levels cause similar effects in chicks, the increase in endogenous secretions might account for a reduction in the cumulative absorption values between mouth and the upper jejunum. Furthermore, the concentrations of sodium, calcium and magnesium in the chyme supernatant (fluid phase of the chyme) in the upper jejunum were reduced by dietary CMC inclusion. Lower intraluminal mineral concentrations will negatively affect the apparent absorption of these minerals, by a reduction of the rate of diffusion -from lumen to blood- through the mucosa. However, the magnitude of the effect of these small reductions in the mineral concentrations in the upper jejunal chyme supernatant on the apparent absorption is questioned. It was discussed in Chapter 5, that the high intraluminal viscosities of the chyme supernatant in birds fed the CMC containing diets most likely accounted for these reduced apparent absorption values up to the upper jejunum. Differences in apparent absorption of all minerals studied up to the lower jejunum were even more pronounced.

The apparent absorption of calcium, magnesium, sodium and potassium up to the lower jejunum in broilers fed wheat-based diets, was improved by the inclusion of endoxylanase (75 mg/kg diet) in the diet. This effect was accompanied by a decreased

Table 2. The daily mineral absorption from the gastrointestinal segments of broilers fed semi-synthetic diets

Diet (CMC level)	Mineral absorption[1] mouth to jj1[3]	jj1[3] to il2[3]	Contribution of respective GI segments[2] jj1[3] to jj2[3]	jj2[3] to il1[3]	il1[3] to il2[3]
			Calcium		
0	288	119	71	29	0
0.5	322	93	46	27	27
1	219	91	42	50	8
			Phosphorus		
0	234	65	107	0	-7
0.5	248	47	80	10	10
1	197	51	50	25	25
			Magnesium		
0	1.3	15	78	35	-13
0.5	-5.9	16	52	12	36
1	-5.2	4.6	-12	38	74
			Sodium		
0	-340	382	68	20	12
0.5	-328	318	49	25	26
1	-403	308	4	27	69
			Potassium		
0	268	115	117	12	-29
0.5	235	86	133	11	-44
1	184	-26	not calculated[4]		

1 In mg/day. Positive values indicate apparent absorption, negative ones indicate apparent secretion

2 Mineral absorbed in segments as % of total apparent absorption between upper jejunum and lower ileum

3 jj1, jj2 first and second half of jejunum; il1, il2, first and second half of ileum

4 Not calculated due to the extremely low cumulative absorption at the lower ileal level.

viscosity of the jejunal supernatant. The effects of wheat arabinoxylans on mineral absorption, however, were less pronounced than observed by addition of 0.5% CMC to the semisynthetic diet. The major portion of the macro-minerals in the wheat-based diets originated from the organic feedstuffs, while these minerals were almost entirely added as salts in the experiments with semisynthetic diets. The mineral compounds in the wheat-based diets is unknown, but was probably less soluble/available than the salts used in the semisynthetic diets. The probable difference in mineral solubility/availablity, was reflected in the lower mineral absorption values up to the lower jejunal level in birds fed the wheat-based diets, compared to those fed the semisynthetic diets.

The reduced cumulative mineral absorption up to the lower jejunum of birds fed CMC containing diets, was not entirely compensated in the rest of the ileum. Normally, the duodenum and the proximal jejunum are the major sites for mineral absorption (Chapter 1). The relevance of the proximal small intestine in mineral absorption is obvious in birds fed the CMC free diet, as the major fraction of the minerals is absorbed in the upper small intestine. When CMC is included in the diets, the mineral absorption in the proximal segments is reduced, despite the prolonged retention time of dry matter in the duodenum and the upper jejunum (Chapter 4). This reduced absorption from the proximal segments is partly compensated by an increased mineral absorption from the more posterior segments. The mechanism for this compensatory absorption is not clear. Our studies indicated, that the following causes may account for this compensatory mechanism:

- Higher mineral solubility in the ileum (observed for calcium, phosphorus and magnesium), due to the lower ileal pH, resulting in higher mineral concentrations in the ileal chyme supernatant (calcium and phosphorus). As the minerals have to be absorbed from the fluid phase of the chyme, this increased concentration may stimulate the rate of absorption/diffusion -transport through both the unstirred water layer and the intestinal mucosa-.
- The length of the small intestine is increased when CMC containing diets are fed (Chapter 3). This will increase the absorptive surface, assuming that the gut wall morphology has not changed. For conclusive evidence, however, the morphology of the gut wall has to be studied.

When the mineral absorption from the intestinal tract is too low the meet the bird's requirement, active absorption processes might also be stimulated/induced (e.g. for sodium).

RECOMMENDATIONS FOR FUTURE RESEARCH

The effect of different physico-chemical conditions in the GI tract on the absorption of minerals cannot be quantified in absolute terms by the experimental methods used in this thesis. As all conditions in the GI tract studied were affected by the dietary treatments, separate effects could not be singled out. No data are available on the absorption processes, that were used by the bird to absorb minerals from the intestinal lumen. The total effect of the dietary treatments was studied, not the processes that were the basis for this result. To estimate the effects of each parameters separately in different GI segments, perfusion studies must be carried out, in which each condition can be set in the fluid perfusing the intestinal lumen. To predict the mineral absorption from intraluminal conditions, or even feedstuff characteristics, such basic mechanisms and underlying relationships have to be quantified.

IMPLICATIONS OF RESULTS

Scientifical implications

Mineral absorption from the intestinal lumen is generally considered to be largely dependent on the ionic mineral concentration or the concentration of absorbable mineral complexes in the intestinal lumen. These concentrations are affected by the occurrence of intraluminal mineral complexation, which is a.o. dependent on the prevailing pH. It was shown in this thesis, that also the viscosity of the chyme supernatant affects mineral absorption. Increasing viscosity in the intestinal lumen reduce mineral absorption in the proximal GI segments. This effect is most likely due to an increased thickness of the unstirred water layer covering the intestinal mucosa cells. The ionic mineral concentration

on the mucosa side of this layer (micro-environment), is probably different from the intraluminal concentration (macro-environment), as the unstirred water layer is a barrier between this macro- and the micro-environment.

The intraluminal viscosity exhibits an effect on both the absorption of the divalent and the monovalent ions.

A reduced cumulative absorption of minerals in proximal GI segments, was partly compensated in more posterior segments.

The transit time, which is commonly used as a indicator for differences in the time food is retained in the intestinal tract, is not necessarily closely related to mean retention time. It was shown, that the shape of the cumulative excretion curves may also be affected by the dietary treatment, which implies that the transit time is no good indicator for differences in the time food was retained in the gastrointestinal tract, and time available for digestion and absorption.

Practical implications

Viscosity increasing feedstuffs will reduce the absorption of highly soluble mineral compounds. The negative relationship between viscosity of the chyme supernatant and the absorption of minerals from the intestinal lumen implies, that the gross mineral requirement of the birds might be increased when these feedstuffs are included in the diet.

REFERENCES

Ikegami, S., F. Tsuchihashi, H. Harada, N. Tsuchihashi, E. Nishide and S. Innami (1990). Effect of viscous indigestible polysaccharides on pancreatic-bilary secretion and digestive organs in rats. J. Nutr. 120: 353-360.

In the Netherlands, mineral[1] application to arable and grass land should be balanced with the mineral requirements of the crops grown. The amount of manure, that can be applied to the land, is limited by its mineral concentration. A manure surplus has to be transported to manure shortage areas, or has to be processed at high costs. An unnecessary high mineral concentration in animal manure should therefore be avoided. A low efficiency of mineral utilization by the animals results in high mineral excretion. Improvement of the mineral absorption from the gastrointestinal (GI) tract is a topic of interest to raise the efficiency of utilization. It enables a reduction of the dietary mineral content without jeopardizing the health and production of the animals.

So far little attention has been paid to the effect of the conditions in the GI tract on the absorption of minerals. In this thesis, several experiments are reported, in which this relationship was studied. The effect of physico-chemical conditions in the GI lumen was studied on the absorption of minerals (K, Na, Ca, Mg, and P) in broiler chickens. These conditions (mean retention time, pH, viscosity, and osmolality) were affected by the addition of carboxy methyl cellulose (CMC) to semisynthetic diets, with minor changes in the dietary composition. Minerals were added almost entirely originating from salts, added to meet the birds' mineral requirements. CMC is a viscosity increasing soluble polysaccharide.

Preceeding these experiments, focused on the relationship between the physico-chemical conditions and the absorption of minerals, an experiment was done 1) to evaluate the method of chyme sampling; 2) to determine the major site of mineral absorption from the GI tract; and 3) to estimate the mean retention time (MRT) of dry matter in successive GI segments (MRT= the time an "average" dry matter is retained in a specific segment). In this experiment, a commercial broiler diet was used. It was shown that the major fraction of K, Na, Ca and Mg was apparently absorbed from the jejunum. The MRT in that segment was 70 min (approximately 25% of the MRT in the total GI tract). Only apparent Na absorption was found in more posterior segments.

Subsequently, the effect of dietary CMC addition (0%, 1% and 2%) to broiler diets was examined on the rate of marker (Cr_2O_3) excretion. It was shown, based on the cumulative excretion curves of a single marker containing meal, that CMC addition

[1] At this moment, phosphate and nitrogen application to the land is already limited by legislation.

lowered the transit time (time between marker intake and first appearance at the site of measurement viz. the faeces) from 137 to 76 min. These differences became obvious from the lower jejunum onwards. The reduced transit time, however, did not correspond with the shape of the cumulative excretion curves. These curves indicated a prolonged retention of the major fraction of the marker containing meal. Differences already existed in the crop and became more pronounced in more posterior segments.

In the next experiment the levels of dietary CMC addition were reduced (0%, 0.5% and 1%). In this experiment, all previously mentioned physico-chemical conditions were studied. These conditions were related to the mineral solubilities in and the mineral absorption from the GI lumen. It was observed that CMC addition reduced the apparent absorption of all minerals studied. It was emphasized that this effect was mainly due to increased intraluminal viscosities in the small intestinal segments. The MRT in the proximal small intestine (duodenum and upper jejunum) was almost doubled by 1% CMC inclusion (from 20 to 34 min). Therefore, based on the CMC effect on the MRT in these segments with a high absorptive capacity, a positive rather than a negative effect of CMC on the mineral absorption would be expected. The ileal pH was clearly decreased (from 7.2 to 6.0), which resulted in an increased solubility of the polyvalent minerals in the ileum. The higher concentrations of calcium and phosphorus in the fluid phase of the ileal chyme, enabled some compensatory absorption of these elements. Also some compensation in Na and Mg absorption was observed.

Finally, the hypothesis that the mineral absorption is reduced by the intraluminal viscosity was verified in wheat containing diets. Four wheat-based diets contained 50% wheat with variable water soluble arabinoxylan contents. These soluble polysaccharides increase viscosity. The viscous activity of these wheat arabinoxylans was reduced by dietary addition of endoxylanase. Dietary enzyme implementation improved the absorption of K, Na, Ca and Mg up to the lower jejunum. This indicated, that also viscosity increasing arabinoxylans reduce mineral absorption. The positive effect of the enzyme was disappeared at the terminal ileum. Linear regression analysis showed the the cumulative absorption of K, Ca, and Mg up to the terminal jejunum and of Na, Ca, and P up to the terminal ileum was reduced by dietary inclusion of wheat varieties with increasing viscosities.

SAMENVATTING

Als gevolg van de ontwikkeling van de intensieve veehouderij in Nederland zijn er gebieden ontstaan, waarin de mestproductie groter is dan de hoeveelheid die op bouw- en grasland zou kunnen worden uitgereden op basis van een evenwichtsbemesting. Jarenlange overbemesting (overmaat aan mineralentoevoer ten opzichte van de mineralenonttrekking door de geteelde gewassen) heeft geleid tot verzadigde gronden. Dit brengt zelfs de kwaliteit van het grondwater in gevaar. Inmiddels is in de wet geregeld, dat in het jaar 2000 de toevoer aan fosfaat en nitraat via bemesting de onttrekking door de geteelde gewassen niet meer mag overschrijden. De hoeveelheid mest, die op het land mag worden aangewend is daardoor afhankelijk van het fosfaat- en nitraatgehalte. Een onnodig hoog mineralengehalte in de mest is ongewenst. Een overmaat mest dient te worden getransporteerd naar tekortgebieden of moet worden verwerkt, om een inkrimping van de veestapel te voorkomen. Het mineralengehalte in de mest kan worden verlaagd, door de efficiëntie van het mineralengebruik door de dieren te verbeteren. Indien deze efficiëntie wordt verbeterd, kan het mineralengehalte in het voer worden verlaagd, zonder dat de gezondheid of de produktiviteit van de dieren in gevaar komt.

In dit proefschrift zijn experimenten met slachtkuikens beschreven. In deze experimenten is de absorptie van mineralen (K, Na, Ca, Mg en P) vanuit het maagdarmkanaal (een facet van de efficiëntie van het mineralengebruik) bestudeerd, in relatie tot een aantal fysisch-chemische omstandigheden (pH, viscositeit, osmolaliteit en de gemiddelde verblijftijd), die daarop van invloed kunnen zijn. Deze omstandigheden in het maagdarmkanaal zijn beïnvloed door de toevoeging van carboxy methyl cellulose (CMC) aan een semi-synthetisch voer. CMC is een niet-afbreekbaar oplosbaar polysaccharide, dat de viscositeit (dikvloeibaarheid) verhoogd. Mineralen zijn als zouten aan deze voeders toegevoegd om in de mineralenbehoefte van de kuikens te voorzien.

Voorafgaand aan deze proeven is een experiment gedaan, waarin: 1) de methodiek voor de verzameling van de inhoud van het maagdarmkanaal (chymus) onder de loep is genomen; 2) de plaats van mineralenabsorptie (K, Na, Ca en Mg) in het maagdarmkanaal is bepaald en 3) de gemiddelde verblijftijd van droge stof in de opeenvolgende delen van het maagdarmkanaal -tijd dat een gemiddeld droge stof deeltje in het darmdeel verblijft- is bepaald. In dit experiment is een "normaal" slachtkuikenvoer gebruikt. Het grootste deel van de in het maagdarmkanaal aanwezige K, Na, Ca en Mg werd uit het jejunum

geabsorbeerd. De gemiddelde verblijftijd in dit darmdeel was 70 minuten (ca. 25% van de gemiddelde verblijftijd in het hele maagdarmkanaal). Alleen voor Na bleek ook schijnbare absorptie in het ileum en het rectum op te treden.

In de daaropvolgende proef is het effect van 0%, 1% en 2% CMC toevoeging aan een semisynthetisch voer gemeten op de passagesnelheid van een chroom (Cr_2O_3) houdende maaltijd door het maagdarmkanaal -Cr_2O_3 is een niet uit het darmkanaal opneembare merkstof-. Het verloop over de opeenvolgende delen van het maagdarmkanaal is eveneens bepaald. Door 2% CMC toevoeging bleek de transit tijd 60 min verkort te zijn (*daling van 137 tot 71 min*). De transit tijd is de tijd, die is verstreken tussen het verstrekken van het voer en het eerste verschijnen ervan op de plaats van meting (bijv. de mest). De transit tijd bleek lineair afgenomen met de CMC dosis. Het tijdstip waarop 50% van de merkstof in de mest was verschenen, werd door CMC toevoeging echter aanzienlijk vertraagd (lineair effect). Dit kwam eveneens tot uiting in de verlaagde maximale uitscheidingssnelheid van deze merkstof (0.53% tot 0.22% van de dosis/minuut). In dit experiment is duidelijk geïllustreerd, dat de relatief makkelijk te bepalen transit tijd geen waarde had voor het voorspellen van verschillen in de tijd die beschikbaar is voor vertering van het voer en absorptie van nutriënten. Het geeft slechts informatie over de snelst passerende fractie. De cumulatieve uitscheidingscurves t/m de opeenvolgende darmdelen geven aan, dat de verschillen die zijn geconstateerd op mestnivo reeds in de krop optraden. Tijdens passage door het maagdarmkanaal werden deze verschillen duidelijker. De periode, waarover de chymus is verzameld, was echter te kort voor schattingen van voornoemde parameters op darmnivo.

Vervolgens is de CMC dosis verlaagd (0%, 0.5% en 1% CMC in het voer), omdat de kuikens, die het voer met 2% CMC kregen, duidelijk in groei en voeropname achter zijn gebleven bij de andere groepen kuikens. In deze proef zijn naast de meting van de gemiddelde verblijftijd, ook de pH in het darmlumen, en de viscositeit en osmolaliteit van het supernatant van de chymus gemeten. Het voornoemde effect van CMC toevoeging aan de voeders op de passagesnelheid door het gehele maagdarmkanaal bleek eveneens in deze proef, echter alleen verschillen in de transit tijd en de maximale uitscheidingssnelheid bleken significant aantoonbaar. De gemiddelde verblijftijd van de merkstof was in het duodenum en het eerste deel van het jejunum vergroot van 20 tot 34 minuten. Verschillen

in de gemiddelde verblijftijd in de andere darmdelen waren niet aanwezig. Zoals werd verwacht, was de viscositeit door de toevoeging van CMC aan het voer duidelijk verhoogd. De pH was in het jejunum licht verlaagd (5.8 vs. 6.0) en in het ileum zelfs sterk (6.0 vs. 7.2) door het CMC gehalte in het voer. De osmolaliteit bleek als gevolg van CMC veel minder sterk af te nemen bij transport van de chymus door het darmkanaal.

De absorptie van K, Na, Ca, Mg en P is bepaald in de afzonderlijke darmdelen, alsmede de concentratie in het supernatant (de vloeibare fase van de chymus). De omstandigheden in het darmlumen zijn vervolgens aan de mineralenabsorptie gerelateerd. Door de opname van CMC in het slachtkuikenvoer werd de cumulatieve absorptie van K, Na, Ca en Mg t/m het eerste deel van het jejunum duidelijk verslechterd. Na de passage door het tweede jejunumsegment waren de verschillen tussen de rantsoenen nog extremer geworden. In het ileum trad gedeeltelijke compensatie op in absorptie van Na, Ca, Mg en P in de kuikens, die gevoerd zijn met de CMC houdende voeders. Met name de Ca en P concentratie in de vloeibare fase van de ileale chymus bleek sterk vergroot in de CMC houdende voeders, hetgeen samenhangt met de lagere pH. Dit concentratieverschil kan een deel van de compensatoire absorptie van deze mineralen in het ileum verklaren. Daarnaast zal een verhoogde actieve absorptie (m.n. voor Na) verantwoordelijk zijn geweest voor compensatie. Van de vergrote verblijftijd in het duodenum en het eerste deel van het jejunum werd evenmin een negatieve invloed verwacht. Dit heeft geleid tot de conclusie dat met name de viscositeit de oorzaak geweest zal zijn van de vertraagde absorptie.

De hypothese dat de viscositeit een sterke negatieve invloed kan hebben op de absorptie van mineralen uit het maagdarmkanaal, is tenslotte nagegaan bij een aantal tarwehoudende slachtkuikenvoeders. In een viertal voeders is 50% van vier verschillende tarwerassen opgenomen. Deze rassen verschilden in het gehalte wateroplosbare arabinoxylanen en daardoor in de *in vitro* viscositeit. Het viscositeitsverhogende effect van deze wateroplosbare polysacchariden is geëlimineerd door toevoeging van 75 ppm endoxylanase aan het voer. De absorptie van Na, K, Ca en Mg bleek in het terminale jejunum duidelijk verbeterd door de toevoeging van endoxylanase aan het voer. Dit enzymeffect was in het terminale ileum alleen nog aanwezig voor Mg. Uit lineaire regressie analyse bleken de cumulatieve schijnbare absorptie van K, Ca en Mg t/m het

terminale jejunum en die waarden voor Na, Ca en P in het terminale ileum verlaagd door het verwerken van hoger visceuze tarwerassen in het voer.

Curriculum vitae

Jan Dirk van der Klis is op 14 juli 1962 geboren in Oost Kapelle. Na het doorlopen van de lagere school en het Pieter Groen College te Katwijk, behaalde hij in 1980 zijn Atheneum diploma. In september van hetzelfde jaar begon hij aan een studie Zoötechniek aan de toenmalige Landbouw Hogeschool te Wageningen. Tijdens zijn studie is hij getrouwd met Nelleke Hovius. Hij is in juni 1987 afgestudeerd en direkt aansluitend begonnen met een promotie-onderzoek op het COVP-DLO "Het Spelderholt" te Beekbergen. In 1991 is hij in vaste dienst van het Ministerie van Landbouw, Natuurbeheer en Visserij aangesteld als wetenschappelijk onderzoeker op het gebied van de Pluimveevoeding, op eerder genoemd instituut.