Water absorption in relation to fermentation in the colon of the ostrich (*Struthio camelus*)

C. MUSARA[1], J.P. CHAMUNORWA[1], K. HOLTUG[2] and E. SKADHAUGE[3]

ABSTRACT

MUSARA, C., CHAMUNORWA, J.P., HOLTUG, K. & SKADHAUGE, E. 2002. Water absorption in relation to fermentation in the colon of the ostrich (*Struthio camelus*). *Onderstepoort Journal of Veterinary Research*, 69:315–320

The colon is a major site for fermentation and water absorption in the ostrich. Water absorption along the colon was evaluated and its relationship to osmolality, Na^+ concentration, short chain fatty acid (SCFA) concentration and carbohydrate content of digesta analysed. Mean water content decreased from 5.30 ± 0.99 to 2.51 ± 0.13 mℓ/g dry mass in the first 5 m of the colon. Correspondingly, mean carbohydrate content fell from 529.85 ± 46.61 to 434.99 ± 29.89 mg/g dry mass. A significant correlation was shown between the decreases in mean carbohydrate and water content along the colon ($r^2 = 0.997$, $P < 0.05$). Changes in mean osmolality (± 10 mOsm/kg) and SCFA concentration (± 7mmol/ℓ) were minimal in comparison to the change in Na^+ concentration (–54 mmol/ℓ). These findings reflect a close coupling between SCFA production and absorption on the one hand and water absorption on the other.

Keywords: Colon, osmosis, ostrich, SCFA, water

INTRODUCTION

Total body water content in the ostrich ranges from 57 % to 69 % depending on the amount of fat reserves in the bird (Whithers 1983; Degen, Kam, Rosentrauch & Plavnik 1991; Williams, Seigfreid, Milton, Adams, Dean, Du Plessis, Jackson & Nagy 1993). In nature, the ostrich has a predilection for arid and semi-arid geographical regions. Body water homeostasis in the ostrich has therefore been a subject of considerable ecological interest (Louw 1971; Whithers 1983; Williams *et al.* 1993). Foraging adult birds are capable of surviving on water contained in herbage without the need for drinking (Williams *et al.* 1993). Nevertheless, an adult ostrich can consume up to 15 ℓ a day if water is provided *ad libitum*. As a result, the lack of water often associated with domestication no longer poses a serious threat to life. Large volumes of water from the diet and gastrointestinal secretions enter the colon each day. The bulk of this water is subsequently absorbed such that faecal water loss is usually low. The objective of this study was to analyse the physiological basis of water absorption in the ostrich colon in terms of the principles of osmosis, particularly the contribution of short chain fatty acids (SCFA) as osmolytes. This basic knowledge should contribute to an understanding of the pathophysiology and management of gastrointestinal water and electrolyte losses in the ostrich as a species.

[1] Faculty of Veterinary Science, University of Zimbabwe, P.O. Box MP 167, Mount Pleasant, Harare, Zimbabwe

[2] Department of Medicine, Frederikssund Sygehus, DK-3600 Frederikssund, Denmark

[3] Institute of Anatomy and Physiology, Royal Veterinary and Agricultural University, Bulowsvej 13, DK-1870, Denmark

Accepted for publication 7 August 2002—Editor

MATERIALS AND METHODS

Experimental animals

A total of 12 healthy, growing ostriches between 2 and 6 months of age, and weighing between 10 and 40 kg, were used in the study. The birds were housed in communal pens where feed and water were provided *ad libitum*. The feed was a commercial preparation the composition of which is shown in Table 1. The birds were adapted to the feed and water regimens for at least 3 weeks prior to experimentation to attain steady state conditions.

Collection of samples

One bird was slaughtered by decapitation each week. Slaughter time was standardized to fall between 09:00 and 10:00 to minimize variation resulting from the diurnal feeding pattern. Immediately after slaughter, the colon was isolated and divided into 1-m segments, starting at the caecocolic junction. Representative samples of intestinal contents were collected in triplicate from each segment into Universal™ bottles (2 x 30 g) and 100 mℓ centrifuge tubes (1 x 80 g) respectively. The vessels were tightly closed with metal caps and placed in crushed ice, i.e. at 0 °C, to minimise evaporation and further fermentative activity. Samples were labelled 1–5 in accordance with the segment of the colon from which they were taken, i.e. from proximal to distal. The entire collection procedure was undertaken within 30 min of slaughter.

Samples collected in centrifuge tubes were used for harvesting supernatant immediately after collection. The tubes were appropriately balanced and centrifuged at 14 000 rpm (24 542 x *g*) and 0 °C for 30 min in a refrigerated centrifuge (Model Europa 24M, MSE, Germany). About 5 mℓ of supernatant were pipetted in duplicate into Biju™ bottles, one set of which was immediately frozen at –20 °C for subsequent measurement of SCFA and Na^+ concentrations. The other set was kept at 0 °C for measurement of osmolality soon after harvesting.

TABLE 1 Composition of ration fed to ostriches

Component	Content (g/kg)
Crude protein	164.15
Fat	29.51
Crude fibre	99.65
Acid detergent fibre	93.68
Neutral detergent fibre	224.15
Starch	120.10
NaCl	3.48
Total Ash	63.16

Water and carbohydrate contents were determined from samples collected into Universal™ bottles. The samples for measurement of carbohydrate content along with the corresponding supernatant for analysis of SCFA and Na^+ concentrations were frozen at –20 °C. Samples on which water content was determined were kept at 0 °C until analysis.

Analysis of samples

Water content was determined by oven drying 4–6 g of colonic contents at 101 °C to a constant mass. The difference between the initial and final mass, expressed in mℓ/g dry mass, was taken as a measure of the water content. Osmolality was determined on a 50-µℓ sample of supernatant in an Eppendorf™ tube by freezing point depression (Osmomat 030, Gonotec, Germany).

The supernatant samples for measurement of SCFA and Na^+ concentrations, together with the corresponding samples of intestinal contents for measurement of carbohydrate content, were transported on dry ice for analysis in Denmark. The Na+ concentration was analysed on a flame photometer, model 143 (Instrumentation Laboratory Inc, Boston, MA). Total SCFA concentration was determined by steam distillation, followed by gas-liquid chromatography as described by Mortensen (1992). The amount of total carbohydrate in colonic contents was measured by acid hydrolysis (12 M H_2SO_4) followed by colorimetry at 530 nm (Englyst & Cummings 1990).

Statistics

The results were analyzed statistically and plotted graphically using the software program SigmaStat version 2.0, Windows '98. SigmaPlot version 1.02 was used for the regression analysis of water content against carbohydrate content. Results are expressed as mean ± S.E. (standard error of mean).

RESULTS

The length of the colon ranged from 5.3–8.3 m. Due to progressive dehydration of contents, harvest of supernatant beyond the fifth segment was inconsistent. As a result, these segments were excluded from further analysis, except for faecal water content. As shown in Fig. 1A, the water content of colonic contents declined progressively

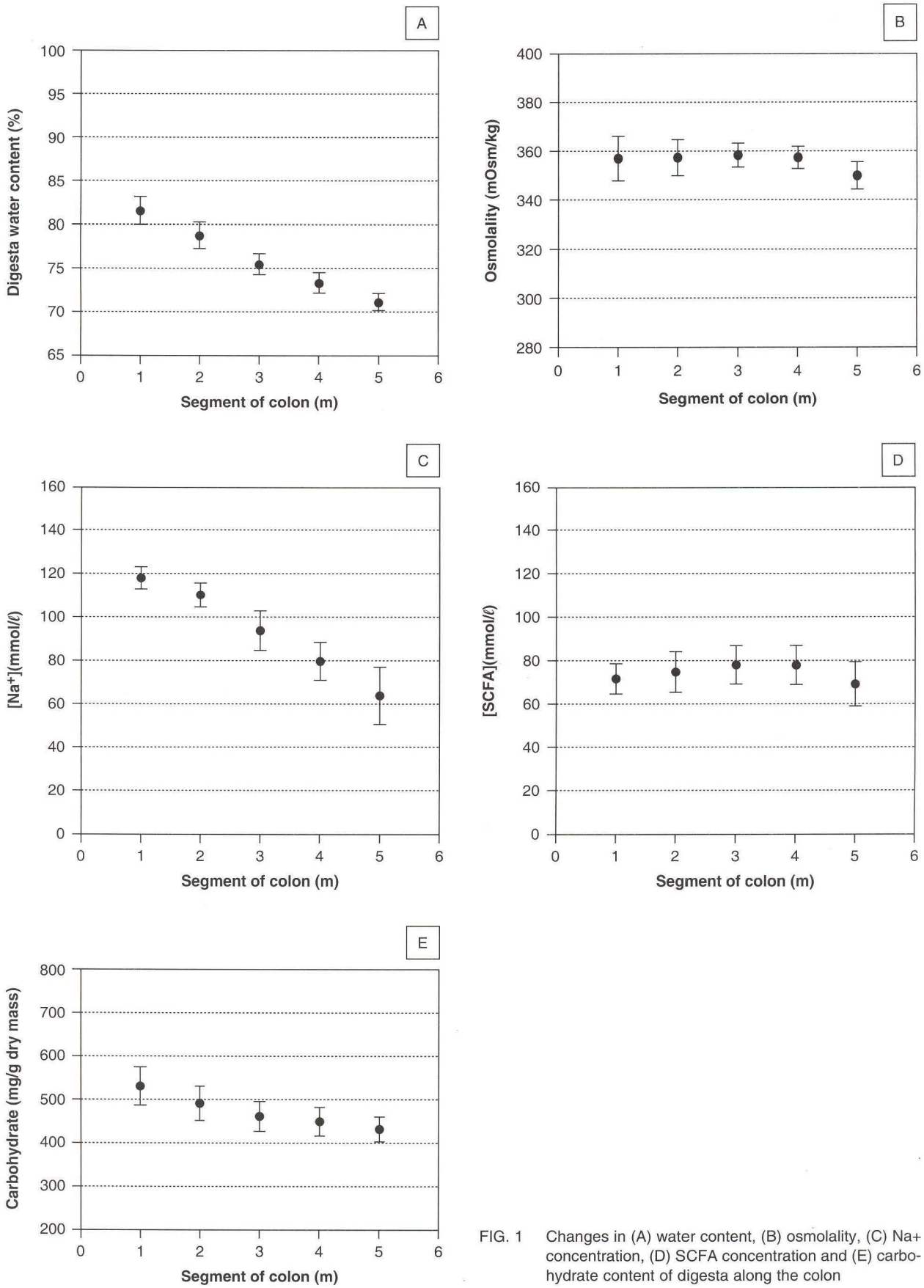

FIG. 1 Changes in (A) water content, (B) osmolality, (C) Na+ concentration, (D) SCFA concentration and (E) carbohydrate content of digesta along the colon

along the length of the colon, indicating net water absorption. Large individual variations in water content along the colon were noted, especially in the first 2 m. Faecal water content ranged from 1.20–3.74 mℓ/g dry mass reflecting a variation of more than 300 %. The osmolality of fluid along the colon is depicted in Fig. 1B. The osmolality of fluid from corresponding segments of different birds was characterised by large individual variations. However, the changes in mean osmolality along the colon did not exceed 10 mOsm/kg.

Fig. 1C illustrates the Na^+ concentration in luminal fluid along the colon. The progressive decline in concentration indicates that net Na^+ absorption was higher than the absorption of water. As a result, fluid in the fifth segment contained on average 54 mmol/l less Na^+ than that in the first segment.

Total SCFA concentration along the colon is shown in Fig. 1D. Similar to osmolality, the SCFA levels were characterised by large individual variations. Changes in the mean SCFA concentration along the colon, however, did not exceed 7 mmol/ℓ. Fig. 1E illustrates the carbohydrate content of dry matter in the colon. The decrease in carbohydrate content along the colon is a reflection of the progressive fermentative digestion of carbohydrate from the proximal to the distal colon.

To explore the relationship between fermentation and water absorption, the interaction of the mean water content against the mean carbohydrate content along the colon was assessed by regression analysis. The relationship conformed to a linear regression pattern (Fig. 2), which is mathematically described by the following equation:

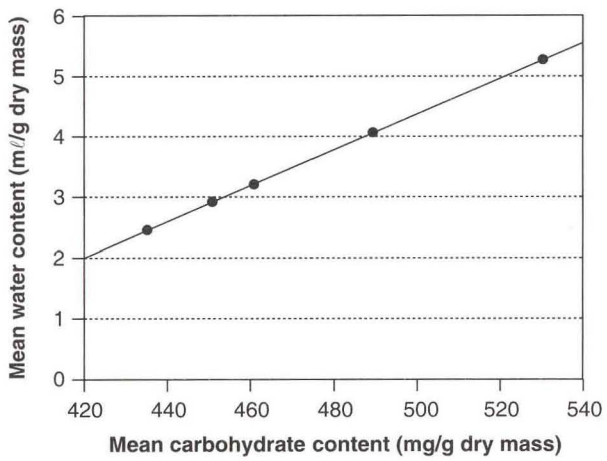

FIG. 2 Correlation between the decreases in water content and carbohydrate content of digesta from the proximal to the distal colon

$$Y = 0.03x - 10.68 \ (r^2 = 0.997, \ P < 0.05)$$

Where y = water content (mℓ/g dry mass)
x = carbohydrate content (mg/g dry mass)

DISCUSSION

Capacity of the colon for water absorption

The fermenting mass in the ostrich colon exists in association with a large volume of water, which is then progressively absorbed along the length of the colon. A wide variation in the magnitude of water absorption by individual birds was noted, underscoring the large adaptive capacity of the colon for water absorption. The range of faecal water content observed in the present study is in close agreement with the findings of Whithers (1983), in which the faecal water content varied from 55% (1.22 mℓ/g dry mass) in water-deprived birds to 72% (2.57 mℓ/g dry mass) in birds drinking water *ad libitum*. By virtue of the large volume of fluid in the colon, varying the water content of faecal matter endows the ostrich with a way of regulating total body water. Therefore, in addition to its role in fermentative digestion of plant fibre, the colon of the ostrich performs a vital function in both constitutive and regulated water absorption from the gastrointestinal tract. In most avian species, including another ratite, the emu (*Dromaius novaehollandiae*) (Dawson, Herd & Skadhauge 1985), the colon is relatively short and colonic water absorption is maximised by retrograde flow of urine into the lower gastrointestinal tract. In the absence of post-renal water absorption in the ostrich (Duke, Degen & Reynhout 1995), the voluminous capacity of the colon and its characteristic mucosal folds are important anatomical adaptations contributing to maximal absorption of water.

Water absorption in relation to osmolality

Normal values for plasma osmolality in 8–26-week-old ostriches were reported to be 303 ± 26 mOsm/kg (Brown & Jones 1996). Thus in the present study, the osmolality of contents of the large intestine was always hyperosmotic to plasma. The hyperosmolality of luminal fluid of the ostrich colon has also been recorded by Skadhauge, Warui, Kamau & Maloiy (1984). In contrast, the osmolality of contents of the large intestine of the pony are approximately isotonic to plasma (Argenzio & Stevens 1975). The existence of hyperosmotic colonic contents in the ostrich demonstrates the capacity of the colon to prevent net water shifts from the extracellular water compartment into the colon down an osmotic gradi-

ent. This widespread phenomenon amongst the avian species depends on the asymmetry of the osmotic reflection coefficient of the colon (Bindslev & Skadhauge 1971a), which favours luminal-to-serosal flow and minimises serosal-to-luminal flux.

The presence of hyperosmotic contents in the ostrich colon also demonstrates that water absorption occurs against an osmotic gradient. Similarly, domestic fowl have been shown to achieve net water absorption against a lumen-to-plasma osmotic gradient of 180 mOsm/kg (Bindslev & Skadhauge 1971b). According to the standing osmotic gradient hypothesis, the absorption of water against an osmotic gradient occurs secondary to transport of solute from the luminal to the serosal side. But exactly which solute (or solutes) drives the uphill absorption of water in the ostrich colon?

Water absorption in relation to Na^+

The average concentration of Na^+ ions in ostrich plasma is in the range of 144–156 mmol/ℓ (Whithers 1983; Skadhauge *et al.* 1984). In the present study, Na^+ was transported from the luminal to the serosal side against a concentration gradient, a feat that demands the expenditure of energy. The active transport of Na^+ ions is known to drive the absorption of water against an osmotic gradient (Bindslev & Skadhauge 1971b; Argenzio, Miller & Engelhardt 1975; Rice & Skadhauge 1982a). In principle, the uphill transport of Na^+ in the ostrich colon may contribute to water absorption as well. However, as has been demonstrated, a large osmotic dissociation exists between Na^+ and water absorption along the colon. In contrast, the comparatively small changes in osmolality along the colon reflect a closer coupling between solute and water absorption. Clearly, this points to the involvement of other solute(s) in addition to Na^+.

Water absorption in relation to SCFA

The SCFA (also known as VFA, volatile fatty acids) acetate, propionate and butyrate are the primary products resulting from the fermentation of carbohydrate. The role of SCFA in enhancing gastrointestinal water absorption through facilitating Na^+ absorption has been documented in a number of species including the goat (Argenzio *et al.* 1975), pig (Crump, Argenzio & Whipp 1980), calf (Demigne, Remesy, Chartier & LeFaivre 1981) and domestic fowl (Rice & Skadhauge 1982b). Well over 5 mol/day of SCFA are produced and absorbed in 20–22 kg ostriches (Swart, Mackie & Hayes 1993), making SCFA the most abundant solute absorbed from the ostrich colon. It was therefore postulated that SCFA absorption *per se* contributes to the luminal-to-serosal movement of water in accordance with the standing osmotic gradient hypothesis.

The SCFA concentration in luminal fluid reflects the difference between the rate of production and the rate of absorption. In addition, utilization by microbes, oral-to-anal passage and dilution may also affect the levels of SCFA (Hodgson & Thomas 1975). Furthermore, SCFA production is a continuous process, as shown by the increase of SCFA concentrations with time after slaughter (Holtug, Rasmussen & Mortensen 1992). Not surprisingly, attempts at measuring the SCFA concentrations in the ostrich colon have yielded variable levels but within the range 65–200 mmol/ℓ (Skadhauge, Prys-Jones & Swart, unpublished data 1983; Skadhauge *et al.* 1984; Swart *et al.* 1993). The absolute levels of SCFA recorded in the present study were in the lower confines of the normal range. Of greater significance are the relatively small changes in mean SCFA concentration along the colon in the face of continuous production, suggesting that absorption of water is osmotically coupled to the absorption of SCFA.

A role for SCFA in water absorption was further elucidated by analysing the relationship between fermentation and water absorption. *In vivo,* the decrease in carbohydrate content from the proximal to the distal colon is a reflection of the progress of fermentative digestion and hence SCFA production. The present study revealed a significant correlation between the decrease in mean carbohydrate content and that of water along the colon. This finding is an indication of a close coupling between SCFA production and water absorption. The abundance of absorbable solute in the form of SCFA may promote water absorption in the ostrich colon, in as much as the presence of an excess of non-absorbable solute curtails water absorption and causes osmotic diarrhoea.

The presence of large volumes of water in the ostrich colon appears to be related to the universal requirement for an aqueous environment for fermentative digestion of plant fibre. Net water absorption would then occur in association with the absorption of products of fermentative digestion, namely SCFA. The correlation between fermentation and water absorption may have far-reaching consequences in pathophysiological states. In humans the suppression of fermentative digestion during antimicrobial therapy frequently results in a

clinical syndrome known as antibiotic-associated diarrhoea (Clausen, Bonnen, Tvede & Mortensen 1991). The size of the fermenting mass and volume of water in the colon of the ostrich outweigh those in the human colon by far, which would make the ostrich more vulnerable to gastrointestinal water loss during disturbances in fermentative digestion. Further research effort is required before the implications and applications of water absorption in the ostrich colon can be fully understood.

ACKNOWLEDGEMENTS

Analyses of SCFA concentration, Na^+ concentration and carbohydrate content were carried out in the Department of Medicine A, Rigshospitalet, Copenhagen.

REFERENCES

ARGENZIO, R.A. & STEVENS, C.E. 1975. Cyclic changes in ionic composition of digesta in the equine large intestine. *American Journal of Physiology*, 228:1224–1230.

ARGENZIO, R.A., MILLER, N. & ENGELHARDT, V.W. 1975. Effect of volatile fatty acids on water and ion absorption from the goat colon. *American Journal of Physiology*, 229:997–1002.

BINDSLEV, N. & SKADHAUGE, E. 1971a. Salt and water permeability of the coprodeum and large intestine in the normal and dehydrated fowl (*Gallus domesticus*): *in vivo* perfusion studies. *Journal of Physiology*, 216:735–751.

BINDSLEV, N. & SKADHAUGE, E. 1971b. Sodium chloride and solute-linked water flow across the epithelium of the coprodeum and large intestine in the normal and dehydrated fowl (*Gallus domesticus*). *Journal of Physiology*, 216: 753–768.

BROWN, C.R. & JONES, G.E. 1996. Some blood chemical, electrolyte and mineral values from young ostriches. *Journal of the South African Veterinary Association*, 67:111–114.

CLAUSEN, M.R., BONNEN, H., TVEDE, M. & MORTENSEN, P.B. 1991. Colonic fermentation to short chain fatty acids is decreased in antibiotic-associated diarrhea. *Gastroenterology*, 101:1497–1504.

CRUMP, M.H., ARGENZIO, R.A. & WHIPP, S.C. 1980. Effects of acetate on absorption of solute and water from the pig colon. *American Journal of Veterinary Research*, 41:1565–1568.

DAWSON, T.J., HERD, R.M. & SKADHAUGE, E. 1985. Osmotic and ionic regulation during dehydration in a large bird, the emu (*Dromaius novaehollandiae*): an important function of the cloaca-rectum. *Quarterly Journal of Experimental Physiology*, 70:423–436.

DEGEN, A.A., KAM, M., ROSENTRAUCH, A. & PLAVNIK, I. 1991. Growth rate, total body water volume, dry matter intake and water consumption of domesticated ostriches (*Struthio camelus*). *Animal Production*, 52:225–232.

DEMIGNE, C., REMESY, C., CHARTIER, F. & LEFAIVRE, J. 1981. Effect of acetate or chloride anions on intestinal absorption of water and solutes in the calf. *American Journal of Veterinary Research*, 42:1356–1359.

DUKE, G.E., DEGEN, A.A. & REYNHOUT, J.K. 1995. Movement of urine in the lower colon and cloaca of ostriches. *The Condor*, 97:165–173.

ENGLYST, H.N. & CUMMINGS, J.H. 1990. Non-starch polysaccharides and resistant starch. *Advances in Experimental Medicine and Biology*, 270:205–211.

HODGSON, J.C. & THOMAS, P.C. 1975. A relationship between molar proportions of propionic acid and the clearance rate of the liquid phase in the rumen of sheep. *British Journal of Nutrition*, 33:447–453.

HOLTUG, K., RASMUSSEN, H.S. & MORTENSEN, P.B. 1992. An in vitro study of short chain fatty acid concentrations, production and absorption in the pig (*Sus scrofa*) colon. *Comparative Biochemistry and Physiology* 103A:189–197.

LOUW, G.N. 1971. Water economy of certain Namib Desert animals. *South African Journal of Science*, 67:119–123.

MORTENSEN, P.B. 1992. The effect of oral-administered lactulose on colonic nitrogen metabolism and excretion. *Hepatology*, 16:1350–1356.

RICE, G.E. & SKADHAUGE, E. 1982a. The in vivo dissociation of colonic and coprodeal transepithelial transport in NaCl depleted domestic fowl. *Journal of Comparative Physiology*, B146:51–56.

RICE, G.E. & SKADHAUGE, E. 1982b. Caecal water and electrolyte absorption and the effects of acetate and glucose in dehydrated, low NaCl hens. *Journal of Comparative Physiology*, B147:61–64.

SKADHAUGE, E., WARUI, C.N., KAMAU, J.M.Z. & MALOIY, G.M.O. 1984. Function of the lower intestine and osmoregulation in the ostrich: preliminary anatomical and physiological observations. *Quarterly Journal of Experimental Physiology*, 69:809–818.

SWART, D., MACKIE, R.I. & HAYES, J.P. 1993. Fermentative digestion in the ostrich (*Struthio camelus* var *domesticus*), a large avian species that utilizes cellulose. *South African Journal of Animal Science*, 23:127–134.

WHITHERS, P.C. 1983. Energy, water and solute balance of the ostrich. *Physiological Zoology*, 56:568–579.

WILLIAMS, J.B., SEIGFREID, W.R., MILTON, J., ADAMS, N.J., DEAN, W.R.J., DU PLESSIS, M.A., JACKSON, S. & NAGY, K.A. 1993. Field metabolism, water requirements and foraging behaviour of wild ostriches in the Namib. *Ecology* 74: 390–404.