Fermentative digestion in the ostrich (*Struthio camelus* var. *domesticus*), a large avian species that utilizes cellulose[1]

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The production of volatile fatty acids (VFA) was studied *in vitro* to assess the possible contribution of microbial fermentation to the energy economy of growing ostrich chicks. Structure, capacity and contents of the gastro-intestinal track were examined to identify major sites of microbial activity and VFA energy yield. Radioactive substrates were used to confirm that the products derived from fermentative digestion could provide nutrients to the host animals. In this experiment the theoretical energy contribution of VFA could be as high as 76% of the metabolizable energy intake of the growing ostrich chick. The absorption and oxidative metabolism of end products from cellulose fermentation was demonstrated to contribute to the metabolizable energy requirements of the growing ostrich.

Ten einde die moontlike bydrae van mikrobiese fermentasie tot energieverskaffing by volstruiskuikens vas te stel, is die *in vitro*-produksie van vlugtige vetsure (VVS) in die spysverteringskanaal (SVK) van groeiende volstruiskuikens bestudeer. Die struktuur, kapasiteit en inhoud van die SVK is ondersoek om die vernaamste plekke van mikrobe-aktiwiteit en produksie van VVS-energie aan te dui. Radio-aktiewe substrate is gebruik om te bevestig dat volstruise die eindproduksie van fermentatiewe vertering kan benut. Volgens die resultate van hierdie eksperiment kan die teoretiese energiebydrae van VVS so hoog wees as 76% van die metaboliseerbare energie-inname van die groeiende volstruiskuiken. Daar is gevind dat die absorpsie en oksidatiewe metabolisme van die eindprodukte vanaf sellulose-fermentasie 'n bydrae tot die energiehuishouding van groeiende volstruiskuikens maak.

Keywords: Fermentation sites, VFA energy, volatile fatty acids.

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Introduction

The African ostrich, *Struthio camelus*, is a large (90–120 kg adult body mass) avian herbivore which effectively digests plant fibre, and more specifically hemicellulose and cellulose, that could make a substantial contribution to the apparent metabolizable energy (ME) of the diet consumed (Swart *et al.*, 1993). Vertebrates are unable to synthesize the enzymes necessary to digest plant fibre, but many herbivores, including some birds, have overcome this deficiency by means of a symbiotic relationship with gut micro-organisms (Fuller, 1984; Hungate, 1984; Herd & Dawson, 1984). The host animal is dependent on gut micro-organisms for digestion of both cellulose and hemicellulose (Prins, 1977). The present study was designed to investigate the production of volatile fatty acids (VFA), the principal non-gaseous end product of microbial fermentation in growing ostriches, to assess the contribution of microbial digestion to the energy economy of these birds. The extent of microbial fermentation as influenced by the structure, capacity and contents of the gastro-intestinal tract was examined to identify major sites of microbial activity and VFA energy yield. The production of VFA in the gastro-intestinal tract of the ostrich does not necessarily mean that it is able to absorb and metabolize these acids. Thus, the ability of ostriches to metabolize acetate was determined using ^{14}C-labelled acetate and then compared to the utilization of ^{14}C-labelled glucose. In addition, ostiches were fed ^{14}C-labelled cellulose and the production of $^{14}CO_2$ was measured in order to confirm that the products derived from fibre digestion could provide nutrients to the host animal.

Methods

Animals and housing

Thirteen ostrich chicks, separate from those used in the first experiment, divided into three live mass groups of 6.8 ± 0.6 (n = 6), 20.7 ± 0.6 (n = 3) and 45.8 ± 1.5 (n = 4) kg, were used. They were housed individually in sheep metabolism cages, under natural microbe exposure conditions, and received a dry meal diet (Table 1) containing 19% crude protein and 12.33 MJ/kg ME.

Table 1 Composition of the dry meal diet[a] fed *ad libitum* to ostrich chicks (5–50 kg live mass)

Component (g/kg)	
Maize	530
Lucerne	340
Fish-meal	84
Limestone powder	10
Monocalcium phosphate	26
Salt	10
Composition	
Protein (6.25 × N) (%)	17.3
Metabolizable energy (MJ/kg)	12.33[b]
Fibre constituents (%)	
Cell walls (NDF)	23.0
Hemicellulose	10.7
Cellulose	9.8
Calcium (%)	1.7
Phosphorus (%)	1.0

[a] Milled to pass a 12-mm sieve.
[b] Determined during a 5-day metabolism trial (Swart *et al.*, 1993).

ME intake

Metabolism trials were conducted with the same birds immediately prior to the *in vitro* experiments of the present study, and mean daily ME intake of each live mass group was measured over a 5-day period (Swart *et al*, 1993). At the termination of the metabolism experiment the ostriches were sacrificed to collect morphometric data of the gastro-intestinal tract and to continue experiments with *in vitro* fermentation of gastro-intestinal digesta.

Morphometric data collection and digesta sampling from the gastro-intestinal tract

The 13 ostriches were sacrificed in pairs on separate days. Of the two ostriches sacrificed each day, one was killed at 09:00 and the other one at 13:00. Directly after killing, the abdominal cavity was opened and the gastro-intestinal tract was removed by severing the posterior end of the oesophagus and the posterior end of the rectum. The digestive tract was separated by ligatures into eight segments (Figure 1), viz. the proventriculus (P), ventriculus or gizzard (G), two equal segments of the small intestine (S1, S2), the paired caeca (Ca) and three segments of the colon (L1, L2, L3). After measuring and weighing the whole segments, the total digesta contents of the different segments were emptied into separate containers and mixed properly. Special care was taken to prevent contamination of digesta with blood.

The emptied gut segments were washed out and weighed in order to calculate the total digesta content of each segment by subtraction.

In vitro experiments with digesta

Concentration of VFA, lactic acid, NH_3-N, pH, and production rate of individual VFA

Representative digesta samples (*ca.* 25 g) of each segment were accurately weighed into fermentation flasks, 100 ml anaerobic diluent (Meyer & Mackie, 1986) was added, and the

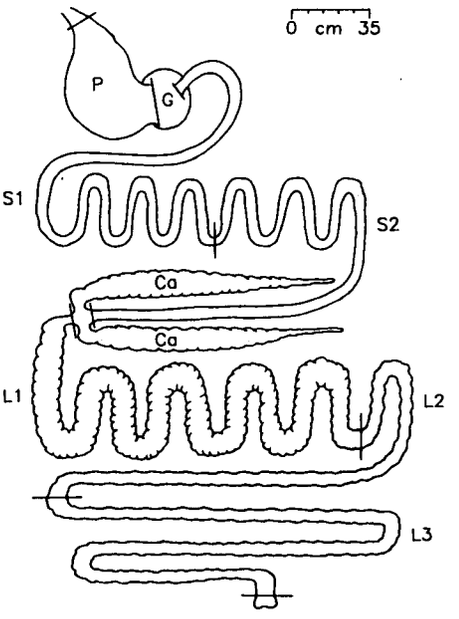

Figure 1 Gastro-intestinal tract of an ostrich chick at 35 kg live mass (P = proventriculus; G = gizzard; S1 = proximal small intestine; S2 = distal small intestine; Ca = caeca; L1 = proximal colon; L2 = mid-colon; L3 = distal colon).

samples were incubated at 39 °C under anaerobic conditions without the addition of other substrate or buffer. This was used to determine production rate of individual VFA by the zero-time incubation method as described by Carrol & Hungate (1954). To calculate VFA increments and thus production rates, samples of the supernatant (9 ml) were taken after 0, 30, 60, 90 and 120 min (Boomker, 1983; Herd & Dawson, 1984). Fermentation in each sample was stopped with 1 ml 5N-NaOH. In acid, VFA exist as volatile free acids (H^+ A). A delay before analysis would therefore result in unknown loss of VFA. This loss tends to occur in plastic storage containers and even into and through rubber stoppers, even under refrigeration. This loss is, however, totally negated by storage in the salt form (Na^+ A) by addition of an alkali (NaOH). According to Swart *et al.* (1993) the pretreatment of ruminal digesta with inorganic acid (HCl) prevented the further release of acetyl groups, suggesting that the low pH in the proventriculus would stabilize the remaining acetyl groups in the digesta. Acetyl groups are very labile, being released by low pH in the proventriculus and gizzard. Addition of an alkali treatment for preservation would therefore lead to little further release.

The concentration and molar proportions of individual VFA were in 5-g samples of whole digesta, representative of each gut segment. In addition, concentrations of lactic acid and NH_3-N were determined.

Analytical

VFA concentrations in the samples were determined by gas chromatography (Carlo Erba 4200, Italy) with a flame ionization detector, using a 2 m glass column (3 mm internal diameter) packed with 60/80 Carbopack C/O, 3% Carbowax 20 M/O, 1% H_3PO_4. Column temperature was programmed to increase from 110 to 140 °C over the 16 min analysis.

Pivalic acid was used as an internal standard (Czerkawski, 1976).

D- and L-lactic acids were analysed enzymically according to the method of Gawehn & Bergmeyer (1974) with biochemicals obtained from Boehringer-Mannheim (West Germany). NH_3-N was determined by the phenolhypochlorite method (Chaney & Marbach, 1962).

The pH of the total mixed digesta of each segment was measured with a portable pH meter (PHM 80 Radiometer: Copenhagen, Denmark) directly after collection, while triplicate samples (*ca.* 5 g) were taken for dry matter (DM) determination.

Contribution of VFA production to ME

Energy available from *in vitro* production rates of VFA in the hindgut of the ostriches was calculated assuming combustion energies of 874, 1535 and 2192 (kJ/mol) for acetate, propionate and butyrate, respectively (Hoppe, 1984). The mean daily ME intake of each live mass group, measured during the 5-day metabolism trial period prior to sacrifice, was used in assessing the relative contribution of VFA production in the hindgut to the energy economy of the ostriches.

Digestion experiments with ^{14}C-labelled cellulose, -acetate and -glucose

To confirm that the products (VFA) derived from microbial fermentation of plant fibre could provide nutrients to the host animal, the amount of $^{14}CO_2$ derived from the digestion and metabolism of ^{14}C-labelled cellulose was investigated in two ostrich chicks. In addition the ability to metabolize acetate, quantitatively the most important VFA, was determined using ^{14}C-labelled acetate and then compared to the utilization of ^{14}C-labelled glucose, which normally provides most of the substrate for oxidative metabolism in monogastric animals.

The experiments with ^{14}C-labelled substrates were carried out in an open-circuit indirect calorimeter (Irene Animal Production Institute: Irene, South Africa). Prior to the experiments, the birds were adapted to handling and confinement in dummy calorimeter chambers. At the beginning of the experiments, each ostrich was given a gelatine capsule containing 12 μCi-[U-^{14}C]-labelled cellulose (*Nicotiana tobacum* 34.5 μCi/mg; New England Nuclear, Boston, Massachusetts, USA). The capsule was passed well down the oesophagus via a polythene tube. The amount of CO_2 produced and O_2 consumed was continuously measured and accumulated onto magnetic tape at 4-min intervals via a computerized datalogger (μMAC-4000 model DA85: Johannesburg, South Africa) and microcomputer (Hewlett Packard 85A: USA) as described by Viljoen (1985). Simultaneously, small samples of chamber air were continuously drawn through an absorption train to trap labelled $^{14}CO_2$ in 200 ml of 0.25N-NaOH. Sampling bottles containing trapping solution were changed hourly for 30 h after administration of the ^{14}C-labelled substrate.

Allowing a 7-day period between experiments, the procedure was repeated with 125 μCi ^{14}C-labelled acetate per bird and 250 μCi ^{14}C-labelled glucose per bird. D-[U-^{14}C]glucose (270 mCi/mmol) and [U-^{14}C]-acetate (57.9 mCi/mmol) were obtained from Amersham International, UK.

The specific radioactivity of CO_2 was determined by liquid scintillation counting after 10 ml of the trapping solution was titrated against 0.1N-HCl, using bromophenol blue as indicator.

For ^{14}C-counts, CO_2 was recovered from the trapping solution after adding concentrated HCl and collecting the liberated CO_2 in Carbosorb (Packard Instrument Co., Downers Grove, Illinois, USA). Permafluor (Packard Instrument Co.) was used as scintillant for high counting efficiency (>80%).

Results and Discussion

Anatomy and morphology

The dimensions and contents of the gastro-intestinal tract of ostrich chicks of different live mass (5–50 kg) are shown in Table 2. Compared to the domestic fowl, the gastro-intestinal tract of the ostrich is without a crop, has extremely well developed sacculated caeca and, unlike other ratite birds (Cho *et al.*, 1984; Herd & Dawson, 1984), has an unusually long colon with a wide haustrated proximal part. None the less, the anatomical structure of the intestinal tract is rather similar to that of other birds (Clemens *et al.*, 1975; Gasaway, 1976; Feltwell & Fox, 1978; McLelland, 1979; Herd & Dawson, 1984). A more detailed account of the anatomy of the ostrich gut may be obtained from the study of Swart *et al.* (1987) (see also Cho *et al.*, 1984; Skadhauge *et al.*, 1984; Bezuidenhout, 1986).

The total length of the intestine, in proportion to body mass, decreased from 160 cm/kg at 6.8 kg live mass, to 60 cm/kg at 20.7 kg live mass, and 34 cm/kg at 45.8 kg live mass. The value of 21 cm/kg reported for adult wild ostriches (105–131 kg live mass; Skadhauge *et al.*, 1984) followed this trend and may be compared to 8 cm/kg for the emu (Herd & Dawson, 1984), the Australian counterpart of the ostrich, and 94 cm/kg for the domestic fowl (Feltwell & Fox, 1978). Similarly, the total length of the ostrich colon decreased from 63 cm/kg (6.8 kg live mass) to 19 cm/kg (45.8 kg live mass), compared to 12 cm/kg in adult wild ostriches (Skadhauge *et al.*, 1984) weighing 105–131 kg, 8 cm/kg in the domestic fowl (Feltwell & Fox, 1978) and 1 cm/kg in the emu (Herd & Dawson, 1984). Irrespective of live mass, however, the total length of the colon accounted for 52% of the entire length of the intestinal tract and was in agreement with that of adult wild ostriches (Skadhauge *et al.*, 1984). While the digesta content of the small intestine represented only 11%, the hindgut (colon and caeca) contained 58% of the total wet digesta contents of the entire intestinal tract.

Regression analysis of the data in Table 2 revealed a log-linear relationship between wet fermentation contents (F) in the gastro-intestinal tract and body-mass (W) of the growing ostriches (5–50 kg live mass), which could be described by the equation:

$$\log F = 0.431W - 0.186 \quad (n = 13; r = 0.99).$$

This may be compared to the interspecies relationship (Figure 2) derived for African wild herbivores, including hindgut- and both ruminant and non-ruminant foregut-fermenters (Van Soest, 1982; Prins *et al.*, 1984), which had a slope of 1.03. When compared to the adult ostrich (avian herbivore, 100 kg live mass), mammalian herbivores have more than twice the fermentative capacity (Figure 2). It is important to note that in the growing ostrich chick, the relative capacity of the gut (per kg live mass) decreased as body size increased (calculated

Table 2 Morphometric measurements and wet digesta contents of the gastro-intestinal tract of ostrich chicks at different live mass (mean values ± *SE*)

		Measurement		Gut contents	
Live mass (kg)	Gut segment	Length (cm)	Relative length (%)	Weight (g)	Relative weight (%)
6.8 ± 0.6	*Stomach*				
n = 6	proventriculus	16 ± 3	1.5	340 ± 45	22.2
(5–9 kg)	gizzard	11 ± 1	1.0	168 ± 24	10.9
	Small intestine				
	proximal	211 ± 8	19.4	85 ± 16	5.5
	distal	211 ± 8	19.4	109 ± 20	7.1
	Caeca (paired)	101 ± 3	9.3	159 ± 14	10.4
	Colon				
	proximal	196 ± 26	18.0	481 ± 65	31.3
	middle	172 ± 18	15.8	116 ± 3	7.6
	distal	172 ± 18	15.8	77 ± 5	5.0
20.7 ± 0.6	*Stomach*				
n = 3	proventriculus	19 ± 2	1.5	505 ± 24	22.5
(20–22 kg)	gizzard	12 ± 1	1.0	125 ± 10	5.6
	Small intestine				
	proximal	220 ± 16	17.8	108 ± 22	4.8
	distal	225 ± 16	18.2	140 ± 31	6.3
	Caeca (paired)	106 ± 2	8.6	235 ± 25	10.5
	Colon				
	proximal	268 ± 33	21.7	834 ± 132	37.2
	middle	193 ± 26	15.6	166 ± 25	7.4
	distal	193 ± 26	15.6	127 ± 15	5.7
45.8 ± 1.5	*Stomach*				
n = 4	proventriculus	20 ± 1	1.3	864 ± 113	24.5
(43–50 kg)	gizzard	12 ± 1	0.8	312 ± 43	8.9
	Small intestine				
	proximal	288 ± 13	18.4	139 ± 37	3.9
	distal	255 ± 15	16.3	129 ± 40	3.7
	Caeca (paired)	129 ± 12	8.3	413 ± 45	11.7
	Colon				
	proximal	273 ± 13	17.5	1031 ± 71	29.2
	middle	305 ± 15	19.5	401 ± 55	11.4
	distal	280 ± 24	17.9	237 ± 24	6.7

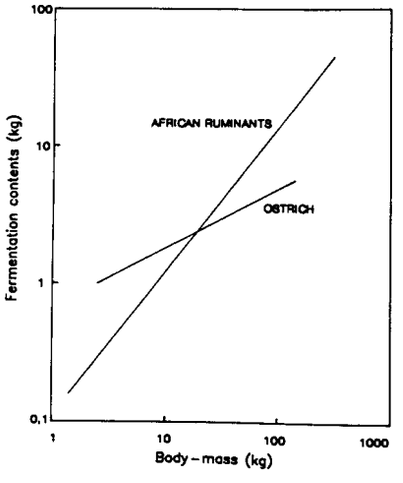

Figure 2 Relationship between log of wet fermentation contents and log of body-mass for ostrich chicks (this study) compared to that for adult African mammalian herbivores (after Prins *et al.*, 1984).

from Table 2), probably placing the young growing herbivore in a more favourable position to balance its energy requirements compared to that of the mature adult as suggested by McBee (1977).

Gastro-intestinal dry matter (DM) and pH

The DM content and pH remained constant within segments of the intestinal tract ($P > 0.05$) for different live mass groups. The mean values are presented in Figure 3. The digesta contents in the caeca were fluid (9.1% DM), containing finely ground fibrous material. The colon contained soft faecal matter (17.8% DM) in the wide haustrated proximal part while the distal part, free of sacculations, contained firm faecal matter (27.8% DM) in the form of pellets.

The contents of the stomach was highly acid and pH tended to be lowest in the proventriculus (pH 1.6) and highest in the gizzard (pH 2.1), which indicates incomplete mixing of digesta between the two stomach regions. Gastric proteolysis most probably commences in the proventriculus (Streicher *et al.*,

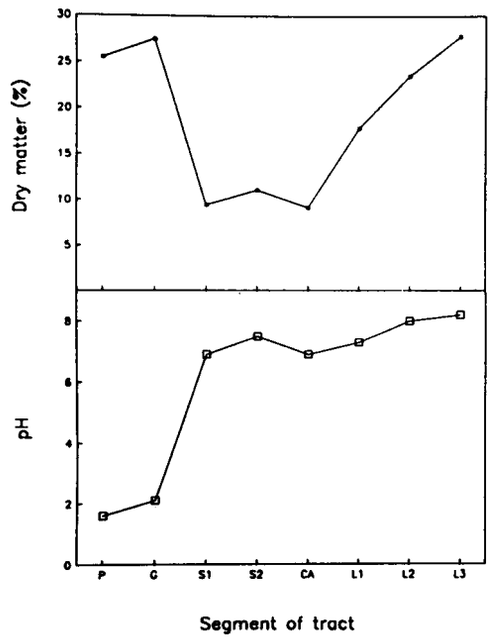

Figure 3 Dry matter and pH of digesta contents along the gastro-intestinal tract of the ostrich chick (P = proventriculus; G = gizzard; S1 = proximal small intestine; S2 = distal small intestine; CA = caeca; L1 = proximal colon; L2 = mid-colon; L3 = distal colon). Values are averaged mean values of three groups of ostriches (7, 21 and 46 kg live mass, respectively). Differences between groups were negligible ($P > 0.05$).

1985) and continues in the gizzard. The pH increased markedly to 6.9 in the proximal small intestine and then increased gradually to 8.2 in the distal colon. The caeca can be described as an independent 'off-set chamber', the pH being 6.9 - a value lower than that of the anterior small intestine (pH 7.5) or the posterior colon (pH 7.3).

Gastro-intestinal VFA, lactic acid and NH_3-N concentrations

Concentrations of VFA and their molar proportions were measured together with concentrations of lactic acid as an index of microbial fermentation of carbohydrates (fibre) in the different segments of the gastro-intestinal tract of growing ostriches. In addition, concentrations of NH_3-N were measured as an index of microbial degradation of protein plus urea (Wrong & Vince, 1984). The results were not influenced by live mass ($P < 0.05$) and mean values are shown in Figure 4. The mean concentrations of total VFA in the proventriculus and gizzard were high, viz. 139.3 and 158.8 mM, respectively. These high acetate values in the proventriculus and gizzard samples, though difficult to explain, are comparable to the values reported by Skadhauge *et al.* (1984). In view of the highly acidic environment (Figure 3), it is questionable whether this is of microbial origin; other sources are, however, unknown at this stage. The concentration decreased to between 65 and 78 mM in the small intestine and then increased again in the hindgut. The mean concentration in the caeca was 140 mM and increased further, progressing from the proximal colon to the distal colon (171 to 195 mM). This was closely related to the increase in DM (Figure 3). Quantitatively, acetate was the most important of the individual VFAs and was the only VFA present in the proventriculus, gizzard and

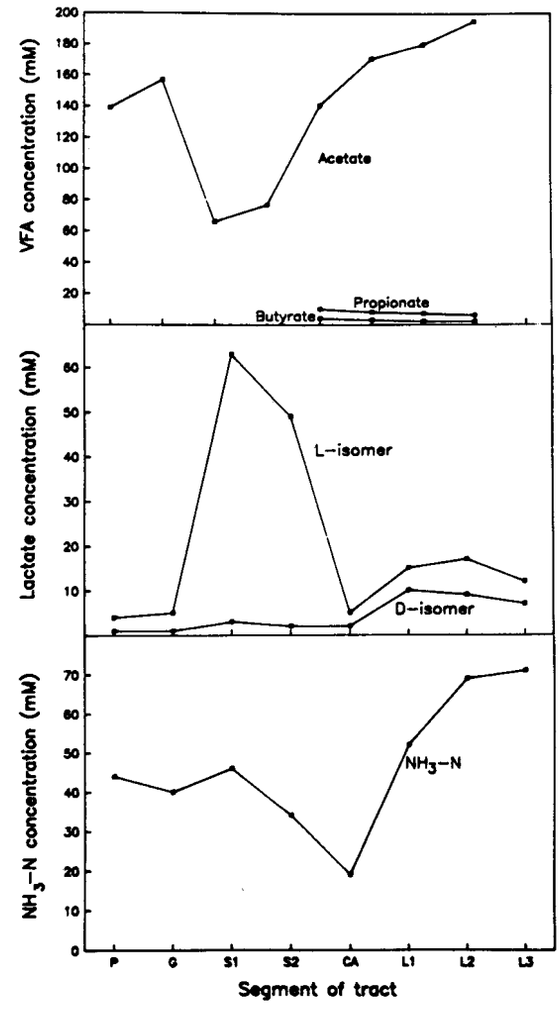

Figure 4 Mean NH_3-N, lactate and VFA concentration in different segments of the gastro-intestinal tract of ostrich chicks (P = proventriculus; G = gizzard; S1 = proximal small intestine; S2 = distal small intestine; CA = caeca; L1 = proximal colon; L2 = mid-colon; L3 = distal colon).

small intestine. In the hindgut, propionate and butyrate were also present, together with trace amounts of isobutyrate, isovalerate and valerate. The molar proportions of propionate and butyrate in the caeca were 6.3 and 2.0%, respectively, decreasing in the colon. High proportions of acetate and low proportions of propionate are indicative of fermentation of fibre or material high in plant cell walls (Hungate, 1984; Prins *et al.*, 1984).

The concentrations of lactic acid in the proventriculus and gizzard were low, viz. 5.0 and 6.0 mM respectively, but increased dramatically to 66 and 51 mM, 95–96% being L-isomer, in the small intestine (Figure 4). In the caeca the concentration dropped to 7.0 mM, although the proportion of the D-isomer increased to *ca.* 30%. In the colon the concentration of lactic acid ranged from 19–26 mM with 35–40% as the D-isomer. Although lactic acid may be secreted by the gastric mucosa, it is also produced by microbial fermentation of carbohydrates and at lower pH, bacteria-producing lactic acid would be at an advantage over those producing VFA (Fuller, 1984). The presence of the L-isomer as the predominant form in the small intestine indicates that the gastric mucosa was the source of most of the acid (Hänninen, 1986),

while the high proportion of the D-isomer in the caeca and colon indicates the contribution being mainly of microbial origin. If one assumes that bacteria produce the D- and L-forms in equimolar amounts, microbial fermentation would account for some 60–80% of the lactic acid present in the hindgut.

Ammonia (NH_3-N) concentrations (Figure 4) were highest in the colon (52–71 mM) and lowest in the caeca (19 mM). High concentrations of ammonia are indicative of bacterial proteolytic activity, deamination of amino acids and urease activity. These results also indicate higher fermentative activity in the haustrated colon than in the caeca.

Fermentation in the ostrich, with a pH range of 6.9–7.3 being maintained in the capacious hindgut chambers, is thus characteristic of fermentation in mammalian herbivores (Argenzio & Stevens, 1984).

VFA production rates

It is generally agreed that the concentration or molar proportion of VFA within the gut compartment is not a reliable index of the production rate thereof (Hungate *et al.*, 1961; Kay *et al.*, 1980; Clemens *et al.*, 1983).

VFA concentrations are the combined result of production (fermentation), dilution, absorption, utilization (microflora) and passage to the lower tract (Hodgson & Thomas, 1975). Hence, when production exceeds removal (absorption + utilization + passage), VFA concentration increases. If removal exceeds production, concentration declines. In the present study the VFA production rate was determined *in vitro* by taking samples from the different segments of the tract, thus 'absorption' and 'passage' could play no part in the 'removal' of VFA.

The pooled data for all three groups (5–50 kg live mass) are presented in Figure 5. As expected there was no production of VFA in the small intestine, but surprisingly the caecal samples showed net utilization of acetate. This can be ascribed to faster utilization than production, most likely for biosynthetic processes in bacterial cells. Microbial acetate oxidation was unlikely to account for acetate dissimilation, since studies in the calorimeter showed that CH_4 production in whole intact ostriches was negligible. Methanogenesis was, however, not measured in the *in vitro* incubations.

VFA production in the large intestine was relatively high, ranging from 26–32.5 mmol /h/100 g DM. The differences in VFA production rates between groups of ostriches were not significant ($P > 0.05$) and were thus not influenced by live mass.

The VFA production rates in the hindgut (65–81.3 mmol/l/h) are considerably higher than those reported for the ileum (14.0) and rectum (16.8 mmol/l/h) of the emu (Herd & Dawson, 1984). In general, they are much higher than the values obtained from the hindgut of mammals including sheep (18 mmol/l/h) (Hume, 1977), wallabies (28 mmol/l/h) (Hume, 1977) and pigs (45 mmol/l/h) (Farrell & Johnson, 1970). These levels are characteristic of those found in the rumen of African wild herbivores as compiled by Prins *et al.* (1984).

The mean difference between the proportional production rates of acetate, propionate and butyrate in the colon (83:10:7; Figure 5) and the molar proportions in digesta

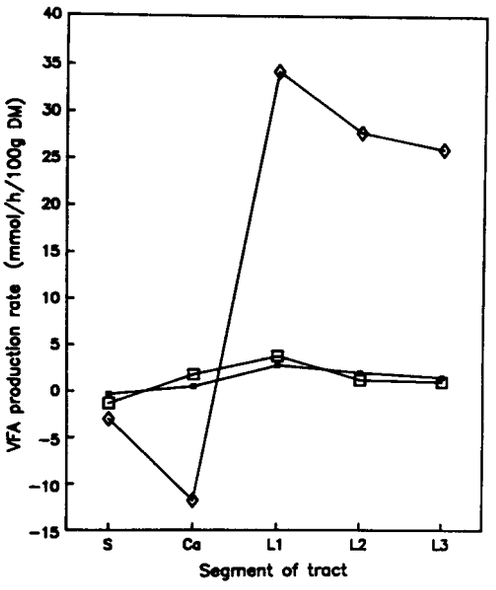

Figure 5 Mean production rates of acetate (◇), propionate (■), and butyrate (□) along the gastro-intestinal tract of ostrich chicks (n = 13; 5–50 kg live mass). Differences between birds were negligible ($P > 0.05$). Negative values suggest *in vitro* net utilization, indicating minimum net amount substrate available to host (see text).

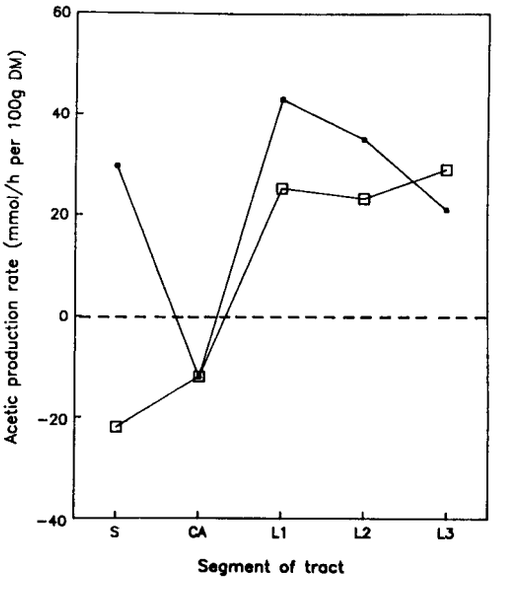

Figure 6 Mean production rate of acetic acid before 10:00 (■) and after 13:00 (□) in different gut segments of ostrich chicks (5–50 kg live mass). Differences within segments were significant ($P < 0.05$).

samples (92:5:3; calculated from mmol/100 g DM) suggest that preferential absorption of individual VFA may occur *in vivo* in the order butyrate > propionate > acetate. A similar trend has been found in the rabbit (Hoover & Heitmann, 1972), koala (Cork & Hume, 1983), horse (Glinsky *et al.*, 1976) and rock ptarmigan (Gasaway, 1976). In both the ruminant forestomach and the equine hindgut, the relative rates of absorption of individual VFA depend not only on the concentration of the acids in the lumen, but also their relative

rates of metabolism in the gut wall (Stevens & Stettler, 1966; Argenzio *et al.*, 1974). Another possible explanation for the difference between proportional production rates and initial molar proportions of VFA is *in vitro* end product inhibition of acetate production (Cork & Hume, 1983), as well as *in vitro* net utilization of acetate as observed for the caecal incubations in this study.

Furthermore, changes in the *in vitro* production rates of acetic acid (but not for propionic or butyric acid) were observed in the gastro-intestinal tract of ostriches killed at different times (09:00 and 13:00) of the day (Figure 6). The most striking change was a marked decrease ($P < 0.001$) in net available VFA (29.0 mmol/100 g DM/h) in the distal small intestine (ileum) from 09:00 to 13:00. Although smaller changes ($P < 0.050$) occurred, the production rates of VFA in the rectum were relatively stable. Furthermore, VFA production in the caeca was not influenced by time of sampling.

Relative contribution of VFA to energy economy

The mean ME intake of the ostriches was 7150; 9150 and 11200 kJ/day for the live mass groups of 6.8 ± 0.6, 20.7 ± 0.6 and 45.8 ± 1.5 kg, respectively. The VFA energy yield from the hindgut of the ostriches (Table 3) was calculated using the method of Allo *et al.* (1973). The values thus obtained were in agreement with those reported for the ruminal contents of wildebeest (Van Hoven & Boomker, 1981) and waterbuck and mountain reedbuck (Hoppe, 1984) but were, however, much higher than the values reported for smaller African ruminants.

Table 3 Volatile fatty acid energy yield (kJ/day) from the hindgut of ostrich chicks at different live masses

Live mass range (kg)	VFA production[a] (mol/day)	Total VFA energy yield[b] (kJ/day)
5–9	3.622	3753
20–22	5.408	5604
43–50	8.220	8518

[a] VFA production rate × digesta contents in DM.

[b] VFA production (a) × sum of molar % calculated from *in vitro* total VFA production rates of C_2, C_3 and C_4 (1036 kJ/mol), assuming combustion energy of 874, 1535 and 2192 kJ/mol for C_2, C_3 and C_4, respectively (after Hoppe, 1984).

Calculated as a percentage of *ad libitum* daily ME intake, VFA production in the hindgut of the growing ostrich could provide 52% (7 kg live mass) to 76% (46 kg live mass) of the daily intake of ME. This is comparable to a relative contribution of VFA to energy requirements, of 50% in the rock hyrax (calculated from data of Fairall *et al.*, 1983), 12–30% in the rabbit (Hoover & Heitmann, 1972; Marty & Vernay, 1984), 9% in the koala (Cork & Hume, 1983), 8% in the porcupine (Johnson & McBee, 1967) and 4–9% in the pig (Imoto & Namioka, 1978), while 60–70% is reported for ruminants (Bergman *et al.*, 1965). Acetate in particular contributed 38–53% of the total VFA energy supply from the hindgut of ostriches.

Digestion experiments with ^{14}C-labelled substrates

The radioactivity in CO_2 resulting from the metabolism of ^{14}C-labelled acetate, glucose and cellulose, respectively, was plotted against time (Figure 7). Products of the oxidation of ^{14}C-labelled acetate and glucose were detectable in the respired CO_2 within 1 h after dosing. Acetate appeared to be more rapidly metabolized than glucose, although this is more likely to have occurred as a result of absorption in the small intestine followed by oxidative metabolism. Glucose may be absorbed more slowly than acetate, resulting in a broader peak. Furthermore, some glucose could have been metabolized to VFA and CO_2 by intestinal flora. Thus, some $^{14}CO_2$ may have arisen from microbial fermentation (Annison *et al.*, 1968; Annison *et al.*, 1969). Little $^{14}CO_2$ was released from these substrates 12 h after administration of the dose. In contrast, the release of $^{14}CO_2$ from ^{14}C-labelled cellulose was slow, continuing for as long as 28 h after dosing. Although it was not possible to quantitate the contribution of the substrates to the ME requirements of the birds, the results show that acetate produced from hindgut fermentation could qualitatively make an important contribution. Based on digestibilities of cellulose (38%) and hemicellulose (66%) as reported earlier (Swart *et al.*, 1993), the contribution from cellulose *per se* would be considerably less than than from hemicellulose.

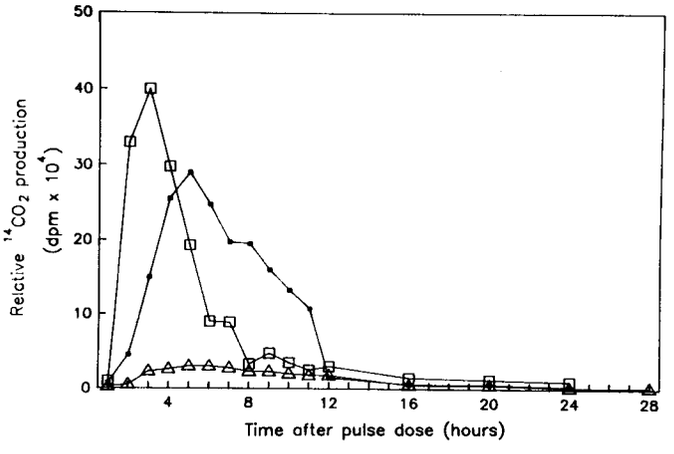

Figure 7 Appearance of ^{14}C-labelled CO_2 from pulse doses of ^{14}C-labelled glucose (■), acetate (□) and cellulose (Δ) administered to ostriches confined in a calorimeter.

Conclusions

This study demonstrates that the ostrich is a unique avian herbivore with large chambers in the hindgut, specialized for fermentative digestion as indicated by large capacity and digesta contents, neutral pH and high concentration of VFA, low lactic acid and high NH_3-N. Furthermore, the long retention time of fibrous feed in the gastro-intestinal tract ensures exposure of feed particles to microbial digestion for extended periods. This is supported by the high digestibilities of cell walls (47%), hemicellulose (66%) and cellulose (38%) (Swart *et al.*, 1993) as well as the net production of VFA, mainly acetate. VFA production rates in the hindgut of the growing ostrich were typical of those reported for the forestomach of ruminants and ruminant-like herbivores. The absorption and oxidative metabolism of end products from

cellulose fermentation were demonstrated to contribute to the ME requirements of the growing ostrich. In this experiment the theoretical energy contribution could be as high as 76% of the ME requirement of the growing ostrich and is in agreement with that reported for adult ruminants above 100 kg live mass.

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