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Comparative methane emission by ratites: Differences in food intake and digesta retention level out methane production

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ABSTRACT

Ratites differ in the anatomy of their digestive organs and their digesta excretion patterns. Ostriches (*Struthio camelus*) have large fermentation chambers and long digesta retention, emus (*Dromaius novaehollandiae*) have a short gut and short retention times, and rheas (*Rhea americana*) are intermediate. A recent study showed that ostriches produce as much methane (CH_4) as expected for a similar-sized, non-ruminant mammalian herbivore. We hypothesized that emus and rheas produce less CH_4 than ostriches. We individually measured, by chamber respirometry, the amount of O_2 consumed as well as CO_2 and CH_4 emitted from six adult rheas (body mass 23.4 ± 8.3 kg) and two adult emus (33.5 and 32.0 kg) during 23-hour periods on a pelleted lucerne diet. In contrast to previous studies, which classified emus as non-producers, we measured CH_4 emissions at 7.39 and 6.25 L/day for emus and 2.87 ± 0.82 L/day for rheas, which is close to values expected for similar-sized non-ruminant mammals for both species. O_2 consumption was of a similar magnitude as reported previously. Across ratites, CH_4 yield (L/kg dry matter intake) was positively correlated with mean retention time of food particles in the gut, similar to findings within ruminant species. In ratites, this relationship leads to similar body mass-specific CH_4 production for a high intake/short retention and a low intake/long retention strategy. Therefore, when investigating CH_4 production in herbivorous birds, it is advisable to consider various CH_4 measures, not only yield or absolute daily amount alone.

1. Introduction

Animals differ in many characteristics of digestive physiology, including the amount of methane (CH_4) they emit per day (Crutzen et al., 1986; Miller and Wolin, 1986; Jensen, 1996). The best known example is the general difference between ruminants and non-ruminant herbivores (Franz et al., 2010, 2011), but why ruminants produce generally more CH_4 is not completely understood. Current explanations include general differences in the composition of the microbiome in the digestive tract (Jensen, 1996; Morvan et al., 1996) or differences in the time that digesta is retained in the digestive tract (El Oufir et al., 1996; Goopy et al., 2014). In addition to this difference in the magnitude of CH_4 production, current concepts also include the possibility that some herbivore species are non-producers (Hackstein and Van Alen, 1996).

Due to the enormous differences in their digestive tract anatomy and physiology, ratites are an interesting group of herbivores in this respect (reviewed in Frei et al., 2015b). Ostriches (*Struthio camelus*) have long paired caeca and a large colon, a capacious digestive tract, digesta retention times of a magnitude comparable to mammalian non-ruminant hindgut fermenters, and a moderate food intake level. Emus (*Dromaius novaehollandiae*) are characterised by a less capacious digestive tract without prominent colon and with short caeca, extremely short digesta retention times, and very high food intake levels. Rheas (*Rhea americana*) are intermediate, with capacious paired caeca but a short colon. Given the common concept that a long digesta retention time is required for a significant CH_4 production, ostriches would be expected to produce most, and emus to produce least, if any, CH_4. Consistent with this, CH_4 emission from the faeces measured in captive animals was higher for ostriches compared to rheas, with only very low levels measured in emus (Hackstein and Van Alen, 1996). Therefore, the authors of that study classified emus as non-producers. This is in contrast to the estimation of CH_4 production of the Australian National Inventory Report (ANIR, 2009), where the same daily amount of CH_4 is assumed for ostriches and emus. Recent methane measurements in adult ostriches documented a much higher CH_4 production than previously assumed in the literature, to the effect that adult ostriches produce similar amounts of CH_4 as expected for similar-sized non-ruminant mammalian herbivores (Frei et al., 2015a).

Based on these findings and the considerations outlined above, we hypothesized that rheas produce less CH_4 than expected for similar-sized ostriches and, in general, non-ruminant mammals, and that

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emus produce even less. For that purpose, an experiment was conducted where ratite species were compared based on the same diet and with the same respiration chamber equipment.

2. Materials and methods

2.1. Experimental design, feeding, housing and sample collection

The experiments took place after approval by the Swiss Cantonal animal care and use committee (animal experiment license no. 142/2011). Details of measured food intake, digestibility and digesta retention in ostriches, rheas and emus have been reported in Frei et al. (2015b). The experiment was performed in summer 2013 in central Switzerland, with ambient temperatures ranging between 8 °C at night and 32 °C during the day. Six adult rheas (body mass [BM] 23.4 ± 1.9 kg) and two adult emus (BM 33.5; 32.0 kg) were available for the present study from a private collection. All animals received a diet exclusively consisting of pelleted lucerne (*Medicago sativa*). Additions of minerals and vitamins were made before pelleting, which was achieved under steam-heating. The nutrient composition of the pellets as analysed during this study (see Frei et al., 2015b for methods) is listed in Table 1. Pellets and water were provided ad libitum in any experimental phase, and no access to other food items was given. The animals were weighed once at the end of the experiment on a mobile scale.

The experiment consisted of an adaptation period of 14 days (on enclosures covered with soil and woodchips but without vegetation that the animals could consume in addition to the offered diet), 7 days of collection and 1 day of respiration measurements. For the last 3 days of the adaptation period and the 7-day collection period, the animals were kept individually in sheltered outdoor enclosures of a size of 12 m^2. Although kept individually, they had access to visual, acoustic and—through the enclosure fencing—also physical contact with conspecifics. The enclosures were protected against direct sunlight, rain and wind, and the floors were covered with fabric carpets to facilitate faecal collection. All animals were habituated to human presence.

2.2. Respiration measurements

At the end of the 7-day collection period, animals were moved individually for 23 h into respiration chambers (1.7 × 1.3 × 1.7 m). The two chambers were custom made on site out of wood, with a fabric carpet flooring. Any gaps were covered with construction tape or sealed off with silicon. Windows of a size of 17 × 42 cm, made of acrylic glass, allowed the observer to constantly monitor the animals in the chambers. Water and pelleted lucerne were provided ad libitum and ambient temperatures ranged from 14 °C to 32 °C, which corresponds to the thermoneutral zone of emus (Maloney and Dawson, 1994) and is close to that of ostriches (Crawford and Schmidt-Nielsen, 1967); to our knowledge, the thermoneutral zone of rheas has not been determined. Chambers were constantly and unidirectionally ventilated by a pull through system. Ambient air entered the chamber through a series of air inlets at the bottom, mixed with the air within the chamber and was then pulled out through a series of air outlets on the roof by a pump (Flowkit 100, Sable Systems, Las Vegas, USA) which generated a constant airflow of 21 to 30 L/min for rheas and 86 to 90 L/min for emus. Flow and composition of outgoing air and composition of ambient air (as baseline) were alternately measured in 90 s intervals. Gas concentrations were measured by O_2 and CO_2 analysers (Turbofox, Sable Systems) as well as by a CH_4 analyser (MA-10, Sable Systems). Data were adjusted for barometric pressure, water vapour pressure and air flow rates, which were constantly recorded during respirometry (Turbofox, Sable Systems). The gas analysers were calibrated prior to each measurement by using pure N_2 gas and a span gas (PanGas, Dagmarsellen, Switzerland; 19.91% O_2, 0.51% CO_2 and 0.49% CH_4 dissolved in N_2). While gas recovery could not be tested due to the nature of the on-site chambers, measurements taken with this system showed a high degree of correspondence to literature data for oxygen consumption in various species (Dittmann et al., 2014; Frei et al., 2015a; Hagen et al., 2015; Vendl et al., 2015), supporting reliability of the data. In particular, a putative restriction in gas recovery would mean that O_2 consumption measurements represent over-, and CH_4 emission measurements represent underestimates, which would not change the qualitative relevance of our findings. Data obtained by the respiratory system were analysed with the software ExpeData (Sable Systems) for O_2 consumed and CO_2 as well as CH_4 emitted after correcting for gas concentrations in ambient air. To calculate the overall metabolic rate (MR) per individual, the amount of O_2 consumed (in L) was multiplied by 20.08 kJ/L (based on McNab, 2008). This approach accounted for the entire time the animals spent inside the respiration chamber and therefore includes all activities by the animals inside the chamber (e.g. standing, resting, feeding). The resting metabolic rate (RMR) was estimated by selecting the 20 lowest O_2 measurement data points of each animal within the 23-h period (adapted from Derno et al., 2005). Volume measures of CH_4 were transformed into energy using the conversion factor 39.57 kJ/L (Brouwer, 1965).

Table 1
Nutrient composition of the lucerne pellets[a] used in the present study.

Nutrient	Unit	
Organic matter	[g/kg DM]	883
Crude protein		177
Ether extract		21
Neutral detergent fibre		418
Acid detergent fibre		330
Acid detergent lignin		77
Gross energy	[MJ/kg DM]	18.0

DM dry matter.
[a] Product no. 2805, Provimi Kliba SA, Kaiseraugst, Switzerland.

2.3. Comparative data sources and statistical analyses

Comparative data on the O_2 consumption of rheas and emus were taken from the literature (Crawford and Lasiewski, 1968; Taylor et al., 1971; Calder and Dawson, 1978; Maloney and Dawson, 1993, 1994). Ostrich data were taken from Frei et al. (2015a). For further comparisons, the regression equations for ruminant and non-ruminant mammalian herbivores described by Franz et al. (2011) were used. For the evaluation of the influence of digesta retention and relative food intake on measures of CH_4 production, measurements in the same bird individuals from Frei et al. (2015b), and data for sheep and ponies from Franz et al. (2010) were added. Simple correlations were tested by Spearman's rho (ρ). A General Linear Model was performed to analyse whether body mass, relative dry matter intake (DMI) and mean retention time (MRT) influenced the daily CH_4 output; normal distribution of residuals was ascertained to validate the approach. Analyses were performed in SPSS 21.0 (SPSS Inc., Chicago, IL). The significance level was set to $P < 0.05$, with values up to 0.01 considered as trends.

3. Results

The daily pattern of O_2 consumption and CO_2 production, as displayed exemplarily for one rhea and emu each (Fig. 1A,B), indicates a high activity soon after the beginning of respiratory measurements, with a concomitant increase in CH_4 emission. The plateau indicating a night-time resting period was more distinct in the rheas than in the two emus (Fig. 1A); a change of the respiratory quotient during the night time period supports behavioural observations made during the digestion study that animals did not feed at night. Although emus had higher absolute levels of CH_4 emission, rheas had higher levels per unit food and energy intake (Table 2). The resting metabolic rates for