Digesta retention patterns in geese (*Anser anser*) and turkeys (*Meleagris gallopavo*) and deduced function of avian caeca

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ABSTRACT

Although it is well-known that retrograde transport of urine fills the caeca of birds with fluid and small particles, the function of avian caeca is still not fully understood. We measured mean retention times (MRT) of solute (cobalt-EDTA, Co), small particle (<2mm, chromium-mordanted fibre, Cr) and large particle (8mm, cerium-marked fibre, Ce) markers in geese (*Anser anser*) and turkeys (*Meleagris gallopavo*) fed alfalfa pellets *ad libitum*. Intake did not differ between species. Turkeys had longer MRT for all markers (Co: 10.4 vs. 3.2h; Cr 23.3 vs. 2.9h; Ce 9.5 vs. 2.1h), achieved a higher fibre digestibility, and had a higher calculated dry matter gut fill. Thus, geese and turkeys correspond to the typical dichotomy of good fliers vs. poor fliers/flightless species in avian herbivores. Because uric acid is fermented much faster by microbes than fibre, the ultimate cause of short-MRT digesta retention in avian caeca as in geese is possibly rather uric acid than fibre fermentation. The numerical differences between marker MRT in geese correspond to a colonic separation mechanism that delays the excretion of fluids more than small and again more than large particles. In contrast to geese, turkeys excreted solid and liquid (caecal) faeces. Liquid faeces contained less fibre and more crude protein than solid faeces and accounted for the excretion of 7, 25 and 34% of Ce, Co and Cr markers. Marker excretion patterns and MRT for liquid faeces (Co 15 vs. Cr 50h) suggest that small particles did not simply move in parallel to fluids, but were retained selectively by being trapped in colonic digesta upon expulsion from caeca, with subsequent repeated retrograde transport into caeca with the next batch of urine. Given the absence of coprophagy in birds (in contrast to small mammalian herbivores), such a delay of small (microbial) particle escape from the caeca appears reasonable.

Keywords: colonic separation mechanism, caecum, digesta passage, digestion, intake, gut capacity

1. Introduction

The digestion of plant material typically requires a voluminous gut (Stevens and Hume 1998). In birds, such a structure is generally considered unfavourable for flight performance, which might explain why only 3% of all bird species use fibrous plant material as the primary energy source (Morton 1978; Dudley and Vermeij 1992). Differences in digestive anatomy are often explained by the necessity to minimize body mass for flight. For example, the small caeca of geese (as compared to other herbivorous species such as grouse) have been explained as an adaptation to their long-distance migrations (Sedinger 1997). Nevertheless, even within the Anatids, the size of certain structures of the digestive tract, in particular the caeca, is larger in species with a higher degree of herbivory (Barnes and Thomas 1987).

For many herbivorous, granivorous and omnivorous birds, retrograde transport of urine (and hence, fluids as well as solute markers) in the colon and into the caeca has been described (Björnhag 1989). During this retrograde transport, the opening of the caeca may act as filter structures that prevent the entry of larger particles (Björnhag 1989; Remington 1989). The presumed benefit is the accumulation of microbes, soluble nutrients, small particles with a favourable surface-to-volume-ratio, and nitrogenous compounds in the caeca (Björnhag 1989; Remington 1989). Correspondingly, high microbial counts and high protein levels (Björnhag 1989; Dawson et al. 1989; Jamroz et al. 1998) as well as small particle sizes (Björnhag and Sperber 1977; Potter et al. 2006) have been recorded in caecal contents of various avian species. The physical principle of this process is probably similar to the colonic separation mechanism of the 'wash-back' type in mammalian lagomorphs (Hume and Sakaguchi 1991; Björnhag and Snipes 1999), that also uses a retrograde fluid transport to flush microbes back into the caecum. In mammals, this also leads to an accumulation of microbial protein in caecal contents, which are then excreted as 'cecotrophs' and re-ingested by the animals (Franz et al. 2011). In birds, which generally do not practice coprophagy/cecotrophy (McWhorter et al. 2009), the aim of the retrograde digesta transport into the caeca is not the concentration of microbial protein for later ingestion. Instead, the retrograde transport of urine (incl. uric acid) provides caecal microbes with a source of nitrogen, enhancing their survival and function. Their action is evident in lower digestible-to-indigestible fibre ratios in caecal contents or liquid faeces than in the rest of the digestive tract or solid faeces (Moss and Parkinson 1972; Dawson et al. 1989), and in the high fibre digestibilities achieved in caeca as compared to the rest of the gastrointestinal tract (Duke et al. 1984; Remington 1989). Additionally, the microbial breakdown of uric acid yields volatile fatty acids and ammonia and thus contributes not only to energy gain but also to nitrogen

recycling (Braun and Campbell 1989); amino acid uptake in the avian caeca has been described (McWhorter et al. 2009).

Many studies on the retention of digesta in the avian digestive tract rely on a slaughter technique (e.g. Clemens et al. 1975; Björnhag and Sperber 1977; Vergara et al. 1989; Palander et al. 2010), and few comparative marker excretion patterns from live animals have been published (Gasaway et al. 1975; Grajal and Parra 1995; Karasov and Cork 1996; McWilliams and Karasov 1998; Frei et al. 2015b). Based on the observations on the function of the avian caeca outlined above, we expected to see an overall delay in the excretion of a solute as compared to particle markers, as shown previously in grouse (Gasaway et al. 1975) or rhea (*Rhea americana*) (Frei et al. 2015b). If caecal contents are voided separately, then they should have higher concentrations of the solute marker (Duke et al. 1968), indicating its sequestration into the caeca via retrograde fluid transport. In contrast, larger particles are expected to move mainly through the colon without entering the caeca, i.e. be excreted sooner, and mainly via solid faeces. Small particles should either partially or completely move with the solute fraction and hence show either an intermediate excretion pattern or follow the solute marker.

We tested these predictions in two domestic species that are representatives of the two main avian families in which a colonic separation mechanism has been described (Karasov and Martínez del Rio 2007), geese (*Anser anser*) and turkeys (*Meleagris gallopavo*). These species differ in their gastrointestinal anatomy (Fig. 1, with larger caeca in turkeys) as well as in their defecation pattern. Turkeys defecate two easily distinguishable types of faeces, of which the more liquid one represents the 'caecal' faeces (Duke et al. 1969; Duke et al. 1980); these liquid faeces are produced about two times per day (Duke et al. 1980) whereas solid faeces are produced up to 50 times per day (Duke et al. 1969). In contrast, geese do not necessarily always produce two different kinds of faeces (Mattocks 1971; Buchsbaum et al. 1986).

2. Materials and methods

The study was performed in November 2013 in central Switzerland, with approval of the Swiss Cantonal Animal Care and Use Committee Zurich and was carried out under the animal experiment licence no. 142/2011. Ambient temperatures ranged between a maximum of 8°C during the day and a minimum of 2°C at night. Six domestic geese and 6 domestic turkeys were available for this study from a commercial breeder. All animals were subadult. All animals were kept first for an adaptation period of 14 days and then for the subsequent 7-

day experiment, on an exclusive diet of pelleted alfalfa (*Medicago sativa*). The analyzed nutrient composition of the batch used is listed in Table 1. The alfalfa pellets were provided *ad libitum*, with no access to other food items in their enclosures. Drinking water was available *ad libitum*. The animals were weighed at the end of the experiment.

The animals were kept individually for the last 3 days of the adaptation period and the 7-day collection period. They had visual, acoustic and – through the enclosure fencing – also physical contact with conspecifics. Experimental enclosures (formed by sub-dividing the group enclosure) had a size of 4.5 m². They were protected against direct sunlight, rain and wind, were well ventilated, and the floors were covered with a synthetic carpeting to facilitate excreta collection. The carpeting facilitated the collection even of liquid excreta (with the use of plastic dough scrapers) without leaving traces that could contaminate subsequent samples. Enclosures were under constant visual surveillance. All animals were habituated to human presence and did not react overtly nervously to the regular removal of excreta.

Food intake was determined by weighing the offered amount of pelleted food and collecting the leftovers at the next feeding. This took place, at the latest, after 24 h, but sometimes earlier because *ad libitum* feeding conditions were guaranteed by always replacing food before the feeding bowl was empty. Leftovers were dried at 60 °C to constant mass to correct for potential increases in moisture content while on offer. Representative samples of the pellets were taken regularly for a pool sample submitted to standard nutrient analyses (AOAC 1995) for dry matter (DM) and total ash (AOAC no. 942.05), crude protein (AOAC no. 977.02) and ether extracts (AOAC no. 963.15), neutral detergent fibre (NDF, AOAC no. 2002.04), acid detergent fibre and acid detergent lignin (ADF and ADL, AOAC no. 973.18). All fibre values were corrected for ash content. All analyses were performed in duplicate.

We used similar markers as in previous studies with ratites (Fritz et al. 2012; Frei et al. 2015b). Cobalt (Co)-EDTA was used as solute marker for the fluid digesta component. Hay particles of different sizes (either 2 or 8 mm) were mordanted with either chromium (Cr) or cerium (Ce) as particle markers, respectively. Co-EDTA and Cr-mordanted fibres were prepared according to Uden et al. (1980), Ce-mordanted fibres according to Schwarm et al. (2008). The mordanted fibres contained (as analyzed) 36.9 g Cr kg⁻¹ DM and 17.3 g Ce kg⁻¹ DM, respectively. Markers were offered with a defined portion of the pelleted feeds in turkeys and by gavage feeding in geese. The pellets soaked up the Co-EDTA solution. Particle markers were made to stick to the pellets by using carefully dosed small amounts of water. The animals had been habituated to moistened pellets during the adaptation period. Geese and turkeys received 0.1 g of Co-EDTA, and 1 g each of Cr- and Ce-mordanted fibre

as a pulse dose, respectively. Food with markers was offered for 60 min and was then removed after that time and replaced with non-labelled pellets.

Prior to marker feeding, excreta samples were taken for assessing the background levels of Co, Cr and Ce. After marker feeding on day 1 of the experimental period, excreta were sampled at 4 h-intervals during the first 2 days, at 6 h-intervals on day 3, and at 8 h-intervals on days 4 to 7. Due to the expected short retention time in geese, excreta were collected every 20 min for the first 2 h and then every h for another 3 h before changing to the 4 h-intervals in this species. Hard excreta and caecal excreta were collected separately in turkeys; caecal excreta were identified by their different (more fluid) texture. In addition, in order to prevent animals from stepping in their own excreta, excreta were generally removed completely when they were spotted during control. The total mass of all removed excreta was noted. Individual excreta samples were taken for marker analysis, and a representative pooled excreta sample (10% of each individual defecation) was collected for nutrient analysis. During sampling, pasty uric acid excretions were carefully separated from the excreta, but especially in geese, this was difficult; therefore, no protein digestibility data are reported, and we use the term 'excreta' rather than 'faeces' throughout, even though the majority of the uric acid was removed. All samples were immediately dried at 60°C to constant mass and sealed in watertight plastic bags before being ground to 0.75 mm with a centrifuge mill (Retsch GmbH, Haan, Germany).

Marker analysis was performed in a similar way as previously described (Frei et al. 2015b). Briefly, for wet ashing we heated samples with 4 ml nitric acid (HNO₃) and 2 ml hydrogen peroxide with the microwave MLS 'START 1500' (MLS GmbH, Leutkich, Germany). Temperature was increased over 15 min to 170°C, and over 20 min to 200°C, then held at 200°C for 5 min. The wave length was 12.25 cm and the frequency 2.45 GHz. Determination of Co, Cr and Ce in the sample digests was performed using an inductively coupled plasma optical emission spectrometer (model Optima 8000, Perkin Elmer, Rodgau, Germany). Sample introduction was carried out by using a peristaltic pump connected to a Meinhard nebulizer with a cyclon spray chamber. The measured spectral element lines were: Co: 228.616 nm; Cr: 267.716; Ce: 413.764 nm. The RF power was set to 1400 W, the plasma gas was 8 L argon min⁻¹, whereas the nebulizer gas was 0.6 L argon min⁻¹.

Marker excretion patterns were visualized with plots depicting excreta marker concentrations over time (Clauss et al. 2007) expressing concentrations in % of the peak concentration to compensate for differences in absolute concentrations achieved for the

different markers (Matsuda et al. 2015). The mean retention time (MRT) through the whole digestive tract was calculated according to Thielemans *et al.* (1978) as

$$\text{MRT} = \frac{\sum t_i C_i dt_i}{\sum C_i dt_i}$$

with C_i = marker concentration in the excreta samples from the interval represented by time t_i (h after marker administration, using the midpoint of the sampling interval) and dt_i = the interval (h) of the respective sample

$$dt_i = \frac{(t_{i+1}-t_i)+(t_i-t_{i-1})}{2}$$

The marker was assumed to have been excreted completely once the excreta marker concentrations were similar to the background-levels determined in pre-dose excreta samples. For turkeys, MRT were calculated in this manner for hard and caecal excreta either separately or after combining both excreta types, dividing the total amount of marker excreted in a time interval (as calculated by the analysed marker concentration and the measured excreta amount) by the total of excreta matter in that interval. Additionally, the proportions of each marker excreted via solid and liquid excreta were calculated in this way and plotted against time, to contrast the excretion pattern based on concentrations with the actually excreted amounts.

Pooled excreta samples were analyzed for DM, organic matter (OM), NDF, ADL, total ash and crude protein content. The apparent digestibility (aD) for DM, OM and NDF was calculated as the percentage of the respective intake not excreted via excreta (Robbins 1993).

The indigestible DM gut content (indDMC, kg) and the total DM gut content (DMC, kg) were estimated according to Holleman and White (1989):

$$\text{indDMC} = F \times \text{MRT}$$

with F (faeces output, kg DM h^{-1}) = total daily faeces output 24 h^{-1} and with MRT (h; MRT_{Cr} was used here). Total DMC was calculated as the sum of digestible DMC and indDMC. This was done assuming a linear absorption of ingested food with time spent in the digestive tract (note that this does not mean linear absorption along the digestive tract):

$$\text{DMC}_{\text{lin}} = \text{indDMC} + [(\text{indDMC} \times (\text{aD DM} \times 100^{-1})) \times (2 (1 - (\text{aD DM} \times 100^{-1}))^{-1})].$$

Intraspecific comparisons (such as between different markers) within geese or turkeys were evaluated using repeated-measurements ANOVA with Sidak post hoc tests.

Comparisons between species were made with independent sample t-test. Statistical analyses were performed in SPSS 22.0 (IBM, Armonk, NY, USA), with the significance level set to $P < 0.05$. For comparison of our results with previously reported data for mammals and birds, the data summarized in Fritz et al. (2012) and Frei et al. (2015b) were used.

3. Results

Although the pelleted food had been produced in one batch, differences between the individual bags led to slight differences in the nutrient composition of the diet fed to geese and turkeys (Table 1). Turkeys were significantly heavier than geese, but absolute (in g per day) and relative food intake (in g per unit metabolic body mass and day) did not differ significantly between the species (Table 2). In turkeys, distinct liquid ('caecal') excreta were evident with a significantly lower DM content than solid excreta (Table 3). Liquid excreta represented 5.4 % of total fecal output.

The peak of marker excretion was within the first 1 to 4 h in geese and 4 to 8 h in turkeys (Fig. 1). Whereas excretion patterns showed a simple peak and subsequent continuous decline in geese, suggesting a monophasic digesta movement, there were recurrent peaks for all markers in turkeys, indicating more complex digesta movements. Considering the total excreta output, turkeys had significantly longer MRTs than geese (Table 2). This difference in MRTs was also significant for solutes ($P = 0.049$) and large particles ($P = 0.037$) if only the solid excreta of turkeys were considered, but not for the small particles ($P = 0.075$).

In geese, solutes were retained longer than small particles, which in turn were retained longer than large particles (Fig. 2); these differences were not significant but tended towards significance for the solute-small particle comparison ($P = 0.096$) and the solute-large particle comparison ($P = 0.059$) (Table 2). These differences occurred within a very short time period after marker ingestion.

In turkeys, small particles had numerically much higher MRT than solutes and large particles, but the differences were not significant for complete excreta (Table 2), and were even less distinct in solid excreta only (Table 3). Marker concentrations were generally higher in liquid compared to solid excreta (Fig. 3). For the solute and small particle markers, concentrations remained high for a longer period of time in liquid than in solid excreta. The resulting excreta-specific MRT were higher in liquid than in solid excreta for small particles ($P = 0.002$), not significantly higher but tending towards significance for solutes ($P = 0.066$), and similar for large particles ($P = 0.946$; Table 3). In liquid excreta, small particles were retained longer than large particles ($P = 0.007$), and the difference between small particles

and solutes was not significant but tended towards significance ($P = 0.059$), with large particles tending to be excreted sooner (Table 3).

The large particle marker was mainly excreted via solid excreta, whereas the solute and especially the small particle marker were excreted also via liquid excreta (Table 3). This was also evident in marker excretion patterns where the solute marker was excreted with a delay in liquid as compared to solid excreta (Fig. 3A, Fig. 4); but in particular, the small particle marker was continuously excreted at nearly constant concentrations over long periods (Fig. 3B, Fig. 4). In turkeys, liquid excreta contained more crude protein ($P < 0.001$), less NDF ($P < 0.001$) and less ADL ($P = 0.001$) than solid excreta (Table 3). The proportion of ADL in NDF was higher in liquid ($52.7 \pm 9.0 \%$) than in solid excreta ($20.7 \pm 0.5 \%$, $P < 0.001$); the values observed in solid excreta were actually nearly identical to that in geese ($20.9 \pm 0.4 \%$, $P = 0.615$).

Dry matter and OM digestibility was similar in both species ($P = 0.271$ and 0.170 , respectively), but NDF digestibility was higher in turkeys ($P = 0.010$, Table 2). Regardless of whether dry matter gut fill was calculated with MRT_{Cr} or MRT_{Ce} , turkeys had higher absolute or relative values ($P \leq 0.041$).

4. Discussion

The present study confirms the general magnitude of intake, digesta retention times and digestibility previously measured in geese and turkeys. Our goose data on intake, retention time and apparent digestibility of dry matter when fed pelleted lucerne (Table 1) was nearly identical to values of $114 \text{ g kg}^{-0.75} \text{ day}^{-1}$, 1.3 h and 26% measured in Cape barren geese (*Cereopsis novaehollandiae*) fed on dry lucerne chaff (Marriott and Forbes 1970). In turkeys, digesta passage times of 1.5 to 13 h (Duke et al. 1969) and a certain degree of fibre digestion with an estimated utilization of 10% of dietary cellulose (Duke et al. 1984) have been reported. In the context of measurements in other avian herbivores, the geese and turkeys of the present study are not exceptional in terms of intake (Fig. 5A), but represent different strategies in terms of digestive tract capacity (Fig. 5B). In addition, the retention of particles (Fig. 6C) and solutes (Fig. 6D) correspond to the flying abilities of their nondomestic ancestors, with the geese having less capacious guts with shorter digesta retention times. With respect to the relationship between food intake and digesta retention, our two species also correspond to the two groups (good vs. limited/non-fliers) in avian herbivores (Fig. 5E). With the exception of one turkey with a particularly long particle retention time, they also

match the pattern that in contrast to many mammals, distinct aboral washing of particulate digesta by fluids (Müller et al. 2011) hardly occurs in birds (Fig. 5F).

Several limitations of the present study must be considered. Given the well-known responsiveness of the avian gastrointestinal tract to changes in diet (Redig 1989), a longer adaptation period than 14 days would have been desirable. A more detailed fractionation of nitrogenous compounds in the excreta (e.g., Terpstra and De Hart 1973) was similarly beyond the logistical scope of our study. But most of all, it has to be kept in mind that even though the particle markers used were of different sizes when fed, they were subjected to mechanical comminution in the birds' gizzards, and the question which particle size they represented in the lower digestive tract or excreta was not assessed. The use of plastic markers that allow an estimation of the degree of comminution in mammals (e.g. Lauper et al. 2013) does not appear to be feasible in birds, as these materials seem to be retained in the gizzard for unnaturally long periods (Clemens et al. 1975; Ferrando et al. 1985; Bloch et al. 2010). The interpretation of the distinctively different excretion patterns of the small and large particle marker therefore hinges on the assumption that the difference in particle size is retained to an unknown degree after passing through the gizzard.

Even though our interpretation of the movement of the small particle fraction focuses on the caeca, in the absence of control via slaughter we cannot exclude the theoretical possibility that selective retention of these small particles occurred in the crop or the gizzard, even though relevant effects on the MRT measurements appear unlikely in our study. Especially with respect to the gizzard, it would appear logical if larger particles were retained in there for a longer time period (until they are reduced in size) as demonstrated in chicken (see below), yet the results of our experiments did not yield indication for that. In the discussion of our results, species-specific characteristics of birds most likely prevent generalized conclusions, which is an important realization in itself. For example, in pigeons (*Columba livia*), a solute marker was excreted slightly faster than a particle marker (Hatt 2006). The comparative excretion patterns of small and large particles - though markers of the same batch were used - between the two species with prominent caeca, the turkeys of the present and the rheas of a preceding study (Frei et al. 2015b), differ distinctively. In chicken, larger particles are generally retained longer in the gastrointestinal tract than smaller particles (Ferrando et al. 1987), not shorter as in the geese and turkeys of the present study, due to a longer retention of larger particles in the chicken gizzard (Vergara et al. 1989). However, Vergara et al. (1989) also observed in chicken that not only a solute marker, but also a powdered particle marker (<0.2 mm) entered the caeca, in contrast to larger particulate plant-

based markers. This could resemble the observations of our study in both geese and especially turkeys.

The different excretion patterns of large particles, small particles and solutes in the geese of our study (Fig. 2) match the concept that these markers enter the caeca with increasing probability, and are therefore correspondingly delayed. In geese, the question about the ultimate aim of this process is especially evident. Digesta retention is, in the literature, mostly related to the extent of digestion of plant fibre (Stevens and Hume 1998). Geese typically achieve a very low fibre digestibility (Durant 2013), which is not surprising given their short digesta retention times and the time usually required for fibre fermentation (Hummel et al. 2006). However, when considering the microbial degradation of uric acid from retrogradely transported urine into acetate, ammonia and CO₂ to be the main function of avian caeca (Mead 1989), then even very short retention times in the caeca appear feasible, given that uric acid fermentation occurs rapidly (Mortensen and Tindall 1981; Karasawa 1989). This fermentation could also represent the triggering step for subsequent methane production in geese (Chen et al. 2003), rheas and emus (*Dromaius novaehollandiae*) (Frei et al. 2015a). In geese, cecotomy significantly reduces methane emissions (Chen et al. 2003).

In turkeys, the comparison of liquid and solid excreta, in conjunction with the distinct excretion pattern of the small particle marker, leads to a new hypothesis about the processes regulating caecal emptying and re-filling. As expected, the solute marker was retained longer, probably in the caeca, with a decreasing excretion pattern indicative of a dilution process as the liquid was expelled during caecal contractions and new fluid (from urine) re-filled the caeca (Fig 3A, 4). In the rock ptarmigan (*Lagopus mutus*), 81%-86% of a solute marker was recovered in liquid faeces compared to 13%-18% of a particle marker (Gasaway et al. 1975; Gasaway et al. 1976), indicating an even more intensive solute segregation into the caeca than in our turkeys.

For particles, we assume that similar to observations in chicken (Vergara et al. 1989), the small particle marker leaves the gizzard of turkeys faster than, or at least as fast as, the large particle marker - which also corresponds to the observation that its majority is excreted in solid faeces of the first collection interval after marker ingestion (Fig. 3B, 4). From then on, the small particle marker is found nearly exclusively in liquid faeces, and any occurrence in solid faeces could stem from a contamination of colon contents with caecal digesta. In contrast, the large particle marker hardly occurred in liquid faeces (Fig. 3C), indicating that it hardly entered the caeca. The difference suggests the presence of a filtering mechanism that only admits liquids and small particles into the caeca (Björnhag and Sperber 1977).

Typically, therefore, caecal content of turkeys contains less dry matter and less fibre than solid faeces (Zubair et al. 1996; Leeson et al. 1997), as also indicated in our study (Table 3). Because the movement of fluids typically washes along microbes, these are accumulated in the caeca, making caecal contents the site of highest protein accumulation in the lower digestive tract (Björnhag and Sperber 1977). As a side-effect of their fermentative action, the ratio of digestible-to-indigestible fibre (measured here as neutral detergent fibre and acid detergent lignin) decreases (Table 3). Other characteristics, such as the total ash content, do not vary systematically between caecal and colonic digesta (Potter et al. 2006), as also suggested by the similarity of solid and liquid faeces in this respect in our (Table 3) and other studies (Dawson et al. 1989; Dawson et al. 2000). In contrast, the high crude protein content in the caecum typically translates into liquid faeces that have also a higher crude protein content than solid faeces, as demonstrated in red grouse (*Lagopus lagopus*) fed on heather (Moss and Parkinson 1972; Moss 1977), and the turkeys of our (Table 3) and previous studies (Zubair et al. 1996; Leeson et al. 1997).

From after entering the caeca on, one would expect fluids - represented by the solute marker - and small particles to move in parallel. However, the small particle marker did not show the 'diluting' decline over time as the solid marker (Fig. 3, 4) but was retained longer (Table 3). Given that evidence for the re-ingestion of liquid faeces by birds is basically non-existent (reviewed in detail by McWhorter et al. 2009), and that in contrast to mammals, an efficient uptake of caecal faeces by birds is more difficult to imagine (due to the combination of a beak, the unformed consistency of liquid faeces and the apparent lack of a behavioral mechanism to ingest these faeces directly during their defecation), retaining some of the caecal microbes rather than excreting them completely might be a valid strategy in some avian herbivores. Under the assumption that the behaviour of our small particle marker reflects the fate of other small particulate matter, e.g. a part of the caecal microbes, its prolonged retention would make sense.

In our view, there are two hypothetical mechanistic explanations for these observations. First, a filtering mechanism could selectively limit the escape of particles from the caeca; this would mean that there is an interplay of morphological valve-like structures and contraction patterns that selectively retains small particles directly at this location. No corresponding observations have been published so far to our knowledge. Nevertheless an intermittent complete emptying would appear necessary to prevent a clogging of the caeca. This hypothesis would appear more likely if one could assume that, prior to caecal emptying, the colon would be also emptied (as suggested by Duke et al. 1980).

The other possible explanation, requiring the presence of colonic digesta during caecal emptying, is that the filtering of small particles occurs in this digesta matrix, similar to the filter-bed effect that retains small particles in the forestomach of ruminants (Clauss et al. 2011). In this scenario, the caecal contents are pressed through and around a colonic digesta plug. In doing so, the solute marker does not meet resistance and is simply excreted; the fluid fraction of the caecal contents may even wash particles out of this plug. Evidence for this encounter of caecal contents with colonic digesta comes from studies with turkeys in which liquid faeces consistently contained higher fibre levels than caecal contents (Zubair et al. 1996; Leeson et al. 1997). In return, small particles in the expelled caecal contents may themselves be caught in the colonic digesta, and re-transported into the caeca during the ensuing re-commencing retrograde urine transport. Given the evidence for contact of caecal contents with colonic digesta during caecal emptying, we consider this second explanation the more plausible scenario that will have to be tested in future studies. One possible avenue of further testing this mechanistic hypothesis is to compare the nitrogenous compounds in liquid faeces and caecal contents of turkeys, as we would expect a higher proportion of non-particulate, i.e. non-microbial nitrogenous compounds passing through the colonic digesta plug as liquid faeces. Another possible avenue is to compare the microbiome of the caecum, the colon, the soft and the hard faeces of turkeys. If our scenario were correct, one would expect the microbiome of soft faeces to be somewhat intermediate between the microbiome of the caecum and the colon, whereas hard faeces should mainly be identical in their microbiome to the colon. To our knowledge, although similar comparisons have been made for chicken (Pauwels et al. 2015), in turkeys the microbiome has only been characterised in the ileum and caecum contents (Danzeisen et al. 2015) but not in soft vs. hard faeces.

The avian digestive tract has shown, in a variety of digesta passage studies in species with a fundamentally similar digestive anatomy (Gasaway et al. 1975; Vergara et al. 1989; Hatt 2006; Frei et al. 2015b), a degree of inter-specific variety that may appear surprising. Both the mechanisms determining selective particle retention in the gizzard and lower digestive tract, and the relevance of caecal fermentation, remain to be understood in a broad comparative context. In particular, in spite of fundamental similarities in digestive functions within vertebrates, basing concepts of avian digestive physiology on our knowledge derived from mammals may unduly limit our understanding of avian evolutionary adaptations.

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Table 1. Nutrient composition of the alfalfa pellets* used in the present study (in g kg⁻¹ dry matter).

Species	Goose	Turkey
Organic matter	882	882
Crude protein	150	153
Ether extract	15	17
Neutral detergent fibre	444	454
Acid detergent fibre	374	372
Acid detergent lignin	90	97

*No. 2805, Provimi Kliba SA, Kaiseraugst, Switzerland (pellets produced as one batch, bagged; differences reflect variation in composition between individual bags).

Table 2. Average ($\pm$ standard deviation) measures in geese (*Anser anser*) and turkeys (*Meleagris gallopavo*) describing body mass, intake, digestion and excretion. Values for turkeys represent measures using all excreta (solid and liquid).

	Goose (n=6)	Turkey (n=6)
Body mass (BM; kg)	5.0 $\pm$ 0.9 ^a	6.3 $\pm$ 0.6 ^b
Dry matter (DM) intake		
g day ⁻¹	402 $\pm$ 172	571 $\pm$ 77
g BM kg ^{-0.75} day ⁻¹	118 $\pm$ 44	144 $\pm$ 16
Total DM excreta*		
g day ⁻¹	284 $\pm$ 122 ^a	414 $\pm$ 54 ^b
g BM kg ^{-0.75} day ⁻¹	83 $\pm$ 30	104 $\pm$ 12
Excreta DM (%) (total)	17.4 $\pm$ 0.9 ^a	19.9 $\pm$ 0.6 ^b
Mean retention time (h)		
Solutes (Co)	3.2 $\pm$ 1.4 ^a	10.4 $\pm$ 3.5 ^b
2 mm particles (Cr)	2.9 $\pm$ 1.3 ^a	23.3 $\pm$ 14.3 ^b
8 mm particles (Ce)	2.1 $\pm$ 0.6 ^a	9.5 $\pm$ 6.4 ^b
Apparent digestibility (%)		
DM	29 $\pm$ 3	28 $\pm$ 2
Organic matter	32 $\pm$ 3	34 $\pm$ 2
Neutral detergent fibre	10 $\pm$ 4 ^a	17 $\pm$ 3 ^b
Gut fill (DM)**		
Cr kg	0.033 $\pm$ 0.010 ^a	0.447 $\pm$ 0.308 ^b
% of BM	0.7 $\pm$ 0.2 ^a	7.1 $\pm$ 4.5 ^b
Ce kg	0.026 $\pm$ 0.010 ^a	0.184 $\pm$ 0.137 ^b
% of BM	0.5 $\pm$ 0.1 ^a	2.9 $\pm$ 2.1 ^b

Within lines, superscripts (a,b) indicate significant differences ($P < 0.05$) between species.

*Excluding most, but not necessarily all uric acid.

**Estimated according to Holleman and White (1989) based on food intake, digesta retention (MRT_{Cr} or MRT_{Ce}) and digestibility; by definition, this excludes grit.

Table 3. Average ($\pm$ standard deviation) measures in turkeys (*Meleagris gallopavo*) for solid and liquid excreta.

	Solid excreta	Liquid excreta
Dry matter (DM) excretion		
g day ⁻¹	392 $\pm$ 58 ^a	21 $\pm$ 5 ^b
g body mass kg ^{-0.75} day ⁻¹	99 $\pm$ 13 ^a	5 $\pm$ 2 ^b
% total excretion	94.6 $\pm$ 1.9	5.4 $\pm$ 1.9
Composition		
DM (%)	21.1 $\pm$ 0.7 ^a	18.0 $\pm$ 1.5 ^b
Total ash (% of DM)	14.6 $\pm$ 0.4	15.2 $\pm$ 0.4
Crude protein (% of DM)	12.6 $\pm$ 0.9 ^a	32.7 $\pm$ 1.9 ^b
Neutral detergent fibre (% of DM)	54.3 $\pm$ 1.2 ^a	15.7 $\pm$ 4.1 ^b
Acid detergent lignin (% of DM)	11.3 $\pm$ 0.4 ^a	8.0 $\pm$ 0.9 ^b
Mean retention time (h)		
Solutes (Co)	7.4 $\pm$ 4.4	15.4 $\pm$ 5.9 ^{AB}
2 mm particles (Cr)	8.8 $\pm$ 6.5 ^a	50.3 $\pm$ 20.8 ^{bA}
8 mm particles (Ce)	9.6 $\pm$ 6.6	9.8 $\pm$ 6.0 ^B
Excretion of marker (% of total)		
Co	74.8 $\pm$ 15.3 ^{aAB}	25.2 $\pm$ 15.3 ^b
Cr	66.0 $\pm$ 17.6 ^A	34.0 $\pm$ 17.6
Ce	93.2 $\pm$ 8.7 ^{aB}	6.8 $\pm$ 8.7 ^b

Within lines, superscripts (a,b) indicate significant differences ($P < 0.05$) between type of faeces; for MRT and marker excretion, capital superscripts (A,B) indicate significant differences ($P < 0.05$) between markers within a type of faeces.

Figure legends

Figure 1. Anatomical drawings of the digestive tract of A) goose (*Anser anser*), B) turkey (*Meleagris gallopavo*) as well as exemplary marker excretion patterns for solute (Co), 2 mm particle (Cr) and 8 mm particle (Ce) markers in a representative individual of each species determined in the present study (scaled to the same time interval).

Figure 2. Marker excretion patterns for solute (Co), 2 mm particle (Cr) and 8 mm particle (Ce) markers in two geese (*Anser anser*). A) the same animal as in Fig. 1 with three distinct, separate marker peaks; B) two separate marker peaks for solutes and particles, with a faster drop in Ce concentration indicating the faster excretion of the larger particles.

Figure 3. Marker excretion patterns for solid and liquid excreta in a turkey (*Meleagris gallopavo*) (the same animal as in Fig. 1) for A) the solute (Co) marker, B) the 2 mm particle (Cr) marker, and C) the 8 mm particle (Ce) marker. On the left, excreta marker concentrations are expressed as % of the highest concentration of that marker in any sample; on the right, the amount of marker excreted in all the excreta collected in the respective interval is expressed as % of the total marker excretion.

Figure 4. Marker excretion patterns for a solute (Co) and a 2 mm particle (Cr) marker in two turkeys (*Meleagris gallopavo*) in solid and liquid excreta. A) Note the clear delay in marker concentration peaks in liquid excreta, and the subsequent decline in the solid but not the small particle marker; B) a similar pattern but with a biphasic peak in solid excreta.

Figure 5. Interspecific comparison of the results found in the present study for geese (*Anser anser*, n=6) and turkeys (*Meleagris gallopavo*, n=6) with data from herbivorous mammals and birds (from sources given in Fritz et al. 2012; Frei et al. 2015b). A) dry matter intake (DMI), B) dry matter gut fill, C) particle (8mm) mean retention time (MRT), and D) solute (fluid) MRT as a function of body mass; E) particle MRT as a function of relative dry matter intake; F) selectivity factor (SF, ratio of MRTs of two different markers) of particles vs. fluid as a function of particle MRT.

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