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ORIGINAL ARTICLE

Gross Measurements of the Digestive Tract and Visceral Organs of Addax Antelope (*Addax nasomaculatus*) Following a Concentrate or Forage Feeding Regime

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Summary

Differences in macroscopic measurements of the gastrointestinal tract have been hypothesized to correlate with the browser–grazer continuum in the natural diet of ruminants. However, to what extent these characteristics represent species-specific traits, or respond to the actually ingested diet, remains to be investigated. Twelve surplus addax antelope (*Addax nasomaculatus*) were divided into two groups and fed, for 3 months, either their usual diet, consisting of a concentrate feed with a limited amount of hay, or a diet of unlimited hay only. After culling, macroscopic measurements were compared between groups. The macroscopic anatomy of the addax showed many characteristics considered typical for grazing or 'cattle-type' ruminants. While both diet groups had mesenteric, pericardial and perirenal adipose tissue, these depots were subjectively more pronounced in concentrate-fed animals. Hay-fed animals had significantly heavier filled forestomach compartments, with corresponding significantly longer linear measurements. Masseter muscles and the surface of first-order omasal leaves were significantly more prominent in hay-fed animals, reflecting possible adaptations to overcome resistance of grass forage and to reabsorb fluid from increased rumination, but differences were not as distinct as reported between 'cattle-type' and 'moose-type' ruminants. Some measurements such as reticular crests and empty foregut mass remained stable between groups, indicating possibly genetically pre-defined characteristics less prone to change in adult life. The results emphasize the adaptability of ruminant digestive tract anatomy in adult animals even after a short period of time, but also suggest limits to this adaptability that reveals a species-specific anatomy regardless of the diet actually consumed.

Introduction

Species-specific differences in wild ruminant digestive anatomy have been linked to differences in natural diet between these species (Hofmann, 1973, 1988, 1989; Clauss et al., 2008b). However, seasonal changes in the physical form and nutrient content of the diet have a significant influence on the digestive tract of free-ranging ungulates (Hofmann, 1973; Jiang et al., 2003; Lane et al., 2014; Arnold et al., 2015), and studies in domestic ruminants show a certain degree of flexibility in the digestive tract in response to diet (Bailey, 1986; Beharka et al., 1996; McClure et al., 2000). This raises the question whether observed differences in wild ruminants represent unchangeable species-specific traits, or have formed as responses to the actually ingested diet in the course of an animal's life.

A large number of species of wild-ranging ruminants have currently been classified according to their particular anatomical and physiological traits into concentrate selectors (browsers), grass and roughage eaters (grazers) and opportunistic mixed feeders (intermediate) (Hofmann, 1989). More recently, a similar classification based on the digestive morphophysiology (as contrasted to the natural diet) has proposed that 'moose-type' ruminants are mainly browsers in the wild, whereas 'cattle-type' ruminants are both grazers and intermediate feeders (Clauss et al., 2010b). Here, we attempt to categorize the Addax antelope (*Addax nasomaculatus*) in terms of its digestive anatomy. Addax are dry region variable grazers with an estimated 80% monocot material in their natural diet (Gagnon and Chew, 2000). Their digestive physiology has been characterized by a distinct rumen content stratification (Clauss et al., 2009a) and a distinctively faster fluid versus particle throughput through the rumen (Hummel et al., 2008), corresponding to a 'cattle-type' rumen physiology (Clauss et al., 2010b). Addax are adapted to very dry environments (Silanikove, 1994; Hummel et al., 2008) and excrete faeces of a high dry matter content (Clauss et al., 2004). The digestive anatomy of the addax has only been hitherto described based on a single, 1-month-old captive specimen (Pérez and Lima, 2006); these results cannot be used to test expectations for the species. We expected addax to show typical grazer or 'cattle-type' macroanatomical features of the digestive tract such as small salivary glands, a large rumen with prominent pillars, a distinct heterogeneity in the intraruminal papillation pattern, prominent reticular crests and a large omasum (Clauss et al., 2006, 2009b, 2010a; Hofmann et al., 2008). As an adaptation to the desert environment, we also expect a particularly long colon for water reabsorption (Woodall and Skinner, 1993).

In domestic ruminants, the dietary regime has an effect on gastrointestinal organ mass (McLeod and Baldwin, 2000; Hersom et al., 2004). The increase in mass of individual foregut compartments is reported to be variable, with the reticulorumen being most likely to increase in mass (due to a denser papillation) but decrease in linear measurements and content mass with a concentrate feeding regime (Fluharty and McClure, 1997; McClure et al., 2000; Amaral et al., 2005). Omasum mass and size generally increase with the roughage content in the diet (Johnson et al., 1987; Sainz and Bentley, 1997; Fluharty et al., 1999), and are also influenced by the size of roughage particles (Beharka et al., 1996; Greenwood, 1997). With respect to the abomasum, two studies have produced conflicting results, showing either an increase in organ mass with increasing dietary fibre (Fluharty et al., 1999) or no effect of dietary fibre (Bailey, 1986). Intestinal mass has shown to predominantly increase with a high fibre diet (Fluharty et al., 1999; McClure et al., 2000), although one study noted no effect of dietary fibre (Bailey, 1986). The total body mass and mass of visceral organs other than the gastrointestinal tract are inconsistently affected by diet roughage, with energy intake often being the more determinative factor (Fluharty and McClure, 1997; McClure et al., 2000; Amaral et al., 2005). Parotid gland mass increased in response to mechanical stimulation of the food via roughage in sheep and decreased once this stimulation was removed (Wilson and Tribe, 1961).

In addition to characterizing the digestive anatomy of addax, we used the opportunity to investigate two addax groups fed different diets, hypothesizing that digestive tract content, organ mass and linear measurements, and salivary gland mass will increase with a high roughage diet, whereas the empty body mass and mass of other internal organs will increase in response to a concentrate feeding regime. Based on a study in cattle (Nakamura et al., 2007), we did not expect an effect of the feeding regime on the mass of the masseter muscle.

Materials and Methods

Experimental groups

Six male and six female surplus adult addax antelope were used for this trial, originating from a large breeding herd at the Al Wabra Wildlife Preservation (AWWP), Qatar, in which animals were selected for breeding based on age, external appearance and breeding history. The experiment was approved by the acting director and the veterinary and curatorial departments of AWWP, and was performed 3 months before the intended culling date in 2005 adhering to the NACLAR (2004) guidelines. The twelve animals were kept individually during this period. The enclosures approximated 200 m^2 in size, and each was equipped with a roofed and walled area for protection against direct sunlight and wind. Unrestricted access to drinking water was provided at all times.

Animals were divided into two feeding groups. Each group contained the same number of male and female animals. Group 1 was given the diet usually fed to the species at this facility at that time due to historical feeding tradition (but changed since), consisting of *ad libitum* concentrate feed (wheat bran and barley, 2:1) with a limited amount of supplemented grass hay (Rhodes grass, *Chloris gayana*, at approximately 200 g per animal and day). Group 2 was given *ad libitum* access to the same hay, without any concentrate supplementation. The (estimated) nutrient composition of the individual diet items

Table 1. Nutrient composition (in % dry matter) of the diet items used in the feeding of two addax (*Addax nasomaculatus*) groups; Group 1 received a mixture of wheat bran and barley (2:1) *ad libitum* with approximately 200 g grass hay per animal per day; Group 2 received only the grass hay *ad libitum*

Nutrient	Grass hay[a] (*Chloris gayana*)	Wheat bran[b]	Barley[b]
Crude protein	13	17	12
Total ash	12	6	3
Neutral detergent fibre	71	43	21
Acid detergent fibre	35	16	7

[a] Hummel et al. (2008).
[b] NRC (2001).

is indicated in Table 1. Because the intake of the concentrate mixture was not quantified, the nutrient composition of the actually ingested diet could not be calculated. Animals of Group 2 had been used in additional studies (Hummel et al., 2008; Clauss et al., 2009a).

Macroscopic measurements

Animals were culled 3 months after the beginning of the trial by exsanguination following bolt pistol stunning in a squeeze cage, to facilitate a feeding of their carcasses to the carnivores of the institution. For dissection, carcasses were handled as described previously (Clauss et al., 2009a) to minimize mixing of forestomach contents prior to sampling the contents of the dorsal and ventral rumen, reticulum and omasum for subsequent dry matter analysis by drying to constant weight at 103°C. While these results have been reported previously for the roughage group (Clauss et al., 2009a), those of the concentrate group are reported here for comparison. Following dissection, all animals were weighed and macroscopic measurements as those described in Sauer et al. (2016) were taken. In short, all gastrointestinal tract compartments from the forestomachs to the colon were dissected, freed from mesenteries and adhering adipose tissue, measured and weighed. Following this, all compartments were emptied, rinsed with water, allowed to drip-dry for approximately 10 min, and weighed again. Empty body mass was calculated as body mass minus the contents of the entire gastrointestinal tract. Mass was also measured for liver, spleen, kidneys, heart, and for the parotid and mandibular salivary glands and masseter muscles as previously described (Sauer et al., 2016). Papillae length, width and density were measured for a defined mucosal area at each of the following ruminal areas: dorsal and ventral rumen, atrium and the bottom of the dorsal and ventral blind sac. The surface enlargement factor (SEF) was calculated from these measures as previously described (Clauss et al., 2009b). The surface of the omasal leaves was determined after scanning the dissected leaves as in Clauss et al. (2006).

Statistical analyses

Because our sample comprised animals of both sexes in two experimental groups, measurements were compared by General Linear Models (GLM) with both experimental group (concentrate versus roughage) and sex as co-factors, confirming normal distribution of residuals by Kolmogorov–Smirnov test; in some rare cases indicated in the tables, the data had to be log-transformed to achieve normal distribution of residuals, or if log-transformation did also not achieve this, ranked data were used. Because there was a significant difference in body mass and empty body mass between the sexes, testing for an effect of sex implicitly assessed the effect of different body size irrespective of the influence of experimental treatment on body mass itself. Differences in dry matter concentration in the contents of the forestomach regions, and in the SEF between rumen regions, were assessed using paired t-tests with Sidak adjustment for multiple testing. For the functional interpretations, correlations between the three indices of rumen contents stratification – the difference in dry matter concentration between dorsal and ventral rumen contents, the ratio of the SEF of the dorsal rumen mucosa in % of the atrial rumen mucosa and the ratio of small particle (<2 mm) to solute marker retention time in the rumen – were evaluated by Pearson's correlation coefficient (*r*), and a GLM with faecal dry matter as the dependent variable tested the effect body mass and the length of the large intestine (using log-transformed data). Analyses were performed in SPSS 21.0 (SPSS inc., Chicago, IL, USA). The significance level was set to 0.05. For comparative purposes, data on both digestive tract anatomy and physiology of addax and other ruminant species were taken from the literature (Woodall and Skinner, 1993; Clauss et al., 2004, 2009a; Hummel et al., 2008; Sauer et al., 2016).

Results

Within each group, the males had higher body masses than the females (concentrates: 109.0 ± 4.9 versus 82.3 ± 9.4 kg; roughage: 92.5 ± 3.3 versus 75.0 ± 6.0 kg), and the same pattern held true for empty body mass (concentrates: 99.9 ± 3.5 versus 75.6 ± 7.4 kg; roughage: 65.9 ± 0.6 versus 58.5 ± 6.9 kg). All animals had bezoars in their rumen, consisting of conglomerates of mineralized strings from hay bales; occurrence and mass of these bezoars did not differ between the groups (Table 2). While both groups had mesenteric, pericardial and perirenal adipose tissue, these depots were subjectively more

Table 2. Mean ± SD body mass, empty body mass (without gastrointestinal tract [GIT] contents), GIT contents and organ masses in addax (*Addax nasomaculatus*) fed a concentrate-dominated diet or a hay-only diet

Measurement	Group 1 (Concentrate)	Group 2 (Roughage)	*P* (group)	*P* (sex)
Body mass (kg)	95.7 ± 16.0	83.8 ± 10.5	0.011	<0.001
Empty body mass (kg)	87.8 ± 14.2	62.2 ± 5.9	<0.001	0.004
Content mass (g wet weight)				
Rumen bezoar	751 ± 502	559 ± 660	0.488	0.025
Reticulorumen (w/o bezoars)	4782 ± 1190	16274 ± 4531	<0.001	0.017
Omasum	259 ± 105	736 ± 270	0.002	0.104
Abomasum	332 ± 52	644 ± 384	0.061	0.131
Small intestine	788 ± 500	1234 ± 314	0.035	0.015
Caecum	484 ± 186	712 ± 149	0.047	0.452
Ansa proximalis	185 ± 71	523 ± 249	0.007	0.124
Colon and rectum	335 ± 297	853 ± 349	0.001	0.001
Total GIT (w/o bezoar)	7164 ± 1696	20976 ± 5592	<0.001	0.001
Organ tissue mass (g wet weight)				
Reticulorumen	1594 ± 322	1583 ± 275	0.902	<0.001
Omasum	284 ± 65	278 ± 57	0.730	0.450
Abomasum	297 ± 67	292 ± 70	0.789	<0.001
Small intestine	644 ± 100	462 ± 57	<0.001	0.001
Caecum	83 ± 14	64 ± 7	0.019	0.418
Ansa proximalis	111 ± 9	104 ± 19	0.350	0.080
Colon and rectum	1152 ± 273	777 ± 73	0.004	0.054
Liver	1071 ± 248	723 ± 93	0.001	0.004
Spleen[a]	248 ± 173	125 ± 10	0.031	0.193
Kidneys	230 ± 38	226 ± 48	0.747	0.001
Heart	573 ± 97	452 ± 74	0.007	0.008
Parotis glands	51 ± 11	42 ± 5	0.014	0.004
Mandibularis glands	61 ± 9	50 ± 8	0.032	0.098
Masseter muscles	215 ± 25	262 ± 27	0.013	0.507

P values from a GLM using group and sex as cofactors.
[a]Using log-transformed data to achieve normal distribution of residuals.

pronounced in the concentrate-fed animals (Fig. 1). Organ content measurements differed significantly, with reticulorumen, omasum, small intestine, caecum, *Ansa proximalis coli*, the remaining colon and rectum and total gastrointestinal tract contents being of greater mass in hay-fed animals (Table 2). Nevertheless, concentrate-fed animals had a significantly higher body mass ($P = 0.011$), and also a significantly higher empty body mass ($P < 0.001$). These animals also had higher tissue masses for many organs, such as empty small intestine, empty caecum, empty colon and rectum, liver, spleen and heart; they also had heavier salivary glands (Table 2). In contrast, the masseter muscles had a greater mass in animals fed hay only (Table 2).

Filled organ measurements also differed significantly between hay- and concentrate-fed groups. Hay-fed animals had increased ruminal heights, dorsal and cranioventral ruminal lengths, reticular height and length, and omasal curvature and length (Table 3). Whereas the area of the *Ostium intra-ruminale* did not differ between the groups, that of the *Ostium rumino-reticulare* and the diameter of the *Ostium reticulo-omasale* were greater in hay-fed animals, as was the thickness of the ruminal pillars (Table 3). There was no difference in the height of the reticular crests. The surface of the first-order leaves of the omasum was significantly larger in hay-fed animals ($P = 0.012$), and the total omasal leaf surface area tended to be larger in this group ($P = 0.059$, Table 3). There were no differences in abomasal or intestinal length measurements (Table 3).

There was a significant difference in the dry matter concentration between forestomach regions in the hay-fed animals as reported in Clauss et al. (2009a), but no such differences in the concentrate-fed animals, not even between the omasum and the other regions (Fig. 2). The SEF of the ruminal mucosa only differed between groups at the ventral blind sac (Table 4). While in the concentrate group, there were only significant differences in the SEF of the dorsal rumen and the atrium and the ventral blindsac, respectively, there were more differences in the rumen of the hay-fed animals (Table 4), suggesting a slightly higher degree of contents stratification.

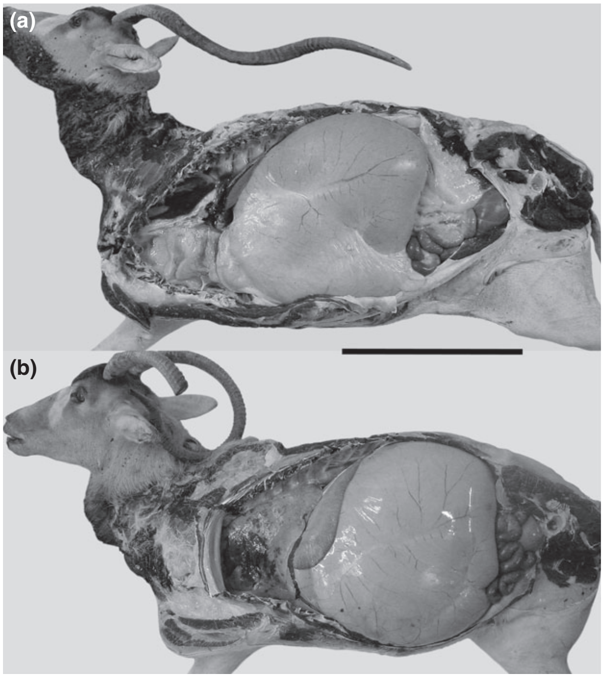

Fig. 1. Examples of whole carcasses of addax (*Addax nasomaculatus*) fed a diet (a) dominated by concentrates or (b) of grass hay only. Note the larger rumen, and the less distinct adipose tissue in mesenteries, in (b). The scale bar represents 40 cm.

When the results of the hay-fed addax were added to literature data, several correlations were significant. There was a negative correlation between the ratio of particle versus solute marker retention in the reticulorumen and the intraruminal papillation pattern (Pearson's $R = -0.63$, $P = 0.007$, $n = 17$; Fig. 3a). There was a negative correlation between the difference in dry matter concentration of dorsal versus ventral rumen contents and the intraruminal papillation pattern (Pearson's $R = -0.72$, $P = 0.006$, $n = 13$; Fig. 3b). There was a positive correlation between the difference in fluid versus particle retention to the difference in dry matter concentration of dorsal versus ventral rumen contents (Pearson's $R = 0.91$, $P = 0.034$, $n = 5$; Fig. 3c).

In the GLM using faecal dry matter as the dependent variable and both body mass and the length of the large intestine as co-variables (using log-transformed data), both co-variables were highly significant (body mass: $F_{1,14} = 27.034$, partial $\varepsilon^2 = 0.60$, $P < 0.001$, large intestine length: $F_{1,14} = 21.578$, partial $\varepsilon^2 = 0.54$, $P < 0.001$). In this combined dataset, large intestine length scaled to body mass at an exponent of 0.42 (95% confidence interval: 0.30–0.54). The residuals of this relationship were highly correlated to faecal dry matter content (Pearson's $R = 0.69$, $P = 0.002$, Fig. 4).

Discussion

Comparative ruminant digestive anatomy and physiology

Addax in this study had the typical anatomy of any ruminant (Clauss and Hofmann, 2014). When plotted against existing data on digestive anatomy of other ruminant species, several features of the forestomach make addax group with other 'cattle-type' ruminants irrespective of the feeding regime, in particular the intraruminal papillation gradient (Fig. 3a,b), the height of the reticular crests (Fig. 5) and the omasal leaf surface area (Fig. 6). These characteristics have been shown to be typical for grazing or 'cattle-type' ruminants (Clauss et al., 2006, 2009b, 2010a). Similarly, with respect to salivary glands, addax had parotis glands of a magnitude expected for grazing or 'cattle-type' ruminants (Fig. 7, Hofmann et al., 2008). By contrast, the rumen pillar thickness of addax measured in this study was lower than expected in free-ranging grazing or 'cattle-type' ruminants (Fig. 5; Clauss et al., 2003).

Intestinal length measurements apparently do not reflect the morphophysiological ruminant types (Fig. 8; cf. Pérez et al., 2008, 2009). Addax are not exceptional with respect to large intestine length (Figs 4 and 8), even though they appear particularly adapted to a desert environment and produce very dry faeces. Possibly, the dimension of the large intestine has a certain flexibility to adapt to the actual water provision. If this was the case, then free-ranging animals that have – in contrast to the experimental animals of this study – a limited access to drinking water might display different colonic measurements.

In theory, the degree of rumen contents stratification, the intraruminal papillation pattern and the difference in the ratio of particle versus fluid retention in the rumen (i.e. whether there is a high wash-through of fluid) should be interrelated (Clauss et al., 2009b; Codron and Clauss, 2010). To our knowledge, the addax represents the first ruminant species where all three measures were taken from the same individual animals (Hummel et al., 2008; Clauss et al., 2009a; and the present study). Although there is a relevant scatter in the data, possibly due to the fact that measurements were usually not taken in the same specimens, and that the different measurements have different response lag periods, the corresponding correlations (Fig. 3) support this concept.

Influence of the feeding regime

The most evident effect of the feeding regimes was on body mass measurements. Typically, diets of higher energy density lead to the accretion of adipose tissue and hence a higher empty carcass mass (McClure et al., 2000;

Table 3. Mean ± SD measurements (in cm unless indicated otherwise) of gastrointestinal structures in addax (*Addax nasomaculatus*) fed a concentrate-dominated diet or a hay-only diet

Measurement	Group 1 (Concentrate)	Group 2 (Roughage)	*P* (group)	*P* (sex)
Rumen height[a]	41.3 ± 2.7	51.5 ± 4.5	<0.001	0.047
Rumen length dorsal	40.7 ± 3.4	49.5 ± 3.0	<0.001	0.002
Rumen length cardia - ventral blindsac	42.5 ± 3.4	50.8 ± 3.2	<0.001	0.025
Ostium intra-ruminale (cm^2)	211 ± 68	252 ± 35	0.269	0.154
Ostium rumino-reticulare (cm^2)	26 ± 3	49 ± 17	0.003	0.026
Carnial rumen pillar thickness	0.7 ± 0.0	0.9 ± 0.2	0.020	0.123
Caudal rumen pillar thickness	0.9 ± 0.1	1.0 ± 0.1	0.036	0.012
Reticulum height	16.9 ± 1.3	25.3 ± 2.3	<0.001	0.663
Reticulum length	8.8 ± 1.3	14.5 ± 2.5	0.001	0.272
Reticular crest height	0.7 ± 0.1	0.7 ± 0.1	0.542	0.134
Papillae unguiculiformes length (mm)	1.3 ± 0.5	2.6 ± 1.4	0.086	0.709
Ostium reticulo-omasale diameter	1.6 ± 0.4	2.5 ± 0.4	0.003	0.178
Omasum curvature	23.8 ± 1.5	33.7 ± 3.1	<0.001	0.132
Omasum height[b]	12.2 ± 1.2	14.5 ± 3.6	0.233	0.644
Omasum length	10.3 ± 1.4	12.7 ± 1.2	0.014	0.614
Number of omasal leaves (*n*)				
1st order[a]	15.7 ± 1.4	16.6 ± 0.5	0.077	0.106
2nd order	15.7 ± 1.4	16.5 ± 0.5	0.177	0.177
3rd order	31.2 ± 3.3	34.0 ± 1.9	0.099	0.356
4th order[a]	16.2 ± 9.5	11.3 ± 6.0	0.222	0.433
Surface of omasal leaves (cm^2)				
1st order	1294 ± 240	1703 ± 187	0.012	0.865
2nd order	692 ± 73	755 ± 107	0.286	0.990
3rd order	428 ± 106	429 ± 86	0.986	0.390
4th order	58 ± 73	31 ± 20	0.401	0.508
Total surface	2472 ± 339	2917 ± 342	0.059	0.812
Abomasum greater curvature	41.2 ± 2.5	45.2 ± 5.5	0.086	0.051
Abomasum smaller curvature	31.5 ± 3.7	33.0 ± 4.0	0.530	0.530
Small intestine length	1297 ± 194	1271 ± 170	0.728	0.006
Caecum length	34 ± 3	32.0 ± 3	0.247	0.024
Ansa proximalis length	67 ± 1	70 ± 8	0.222	0.133
Colon and rectum length	688 ± 82	624 ± 104	0.188	0.041

P values from a GLM using group and sex as cofactors.

[a] Using ranked data because normal distribution of residuals could not be achieved by log-transformation.

[b] Using log-transformed data to achieve normal distribution of residuals.

Amaral et al., 2005). However, a higher proportion of roughage in the diet typically leads to a higher food intake and higher gut fill (Stobo et al., 1966; Nocek et al., 1984; Bailey, 1986; Beharka et al., 1996; Terré et al., 2015), which may also lead to higher overall body mass (Beiranvand et al., 2014; Daneshvar et al., 2015). In the present study, therefore, the difference between the feeding regimes was more evident in the empty than in overall body mass (Table 2).

The larger amount of reticulorumen contents explain the observed differences in linear measurements and differences in the measured areas of the *Ostium rumino-reticulare* and *Ostium reticulo-omasale* (Tables 2 and 3). In particular, potentially in connection with physical characteristics of grass hay and the resulting mechanical stimulation, they are responsible for the difference in ruminal pillar thickness between the feeding regimes (Table 3). These results underline that the measure of pillar thickness should derive from free-ranging animals only when investigating adaptations to the natural diet. To our knowledge, the flexibility of this measure has not been investigated in domestic ruminants.

The concentrate diet resulted in rumen contents that did not show, at dissection, the stratification (as measured by a dry matter gradient) typically reported for grazing ruminants (Clauss et al., 2009a; Hummel et al., 2009). This evident effect (Fig. 2) was not reflected in a similar drastic difference in the intraruminal papillation pattern between the addax groups (Table 4). Because differences in the papillation pattern have been demonstrated between seasons (Hofmann et al., 1988; Mathiesen et al., 2000; Kamler, 2001), we had expected a more distinct difference after a three-month period. However, the SEF differed only in the ventral blindsac between the feeding groups, and the

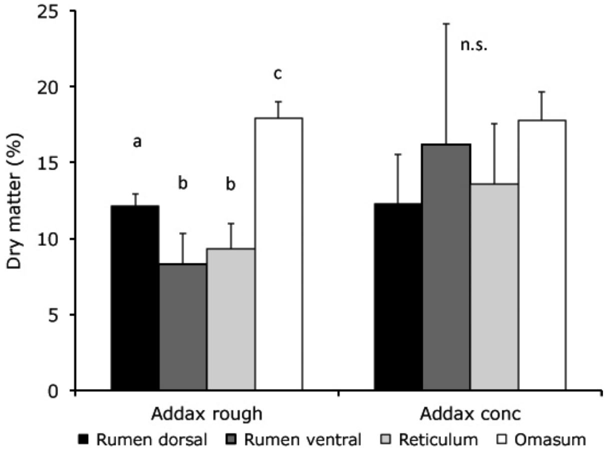

Fig. 2. Dry matter (DM) concentration of the contents of different forestomach regions in addax (*Addax nasomaculatus*) fed a diet dominated by concentrates (conc) or of grass hay only (rough). Superscripts indicate significant differences by paired *t*-test with Sidak adjustment for multiple testing. Results for the hay-fed animals from Clauss et al. (2009a).

Table 4. Mean ± SD surface enlargement factor (SEF) measurements of the ruminal mucosa in different ruminal regions in addax (*Addax nasomaculatus*) fed a concentrate-dominated diet or a hay-only diet

Measurement	Group 1 (concentrate)	Group 2 (roughage)	*P* (group)	*P* (sex)
Dorsal rumen	1.46 ± 0.17^{A}	1.38 ± 0.17^{A}	0.474	0.733
Atrium ruminis	19.28 ± 8.69^{B}	18.18 ± 3.98^{B}	0.793	0.688
Ventral rumen	10.67 ± 5.88^{AB}	7.45 ± 4.29^{AC}	0.323	0.639
Dorsal blindsac	11.77 ± 7.40^{AB}	10.83 ± 2.36^{BC}	0.781	0.649
Ventral blindsac	10.31 ± 4.48^{B}	3.91 ± 3.73^{AC}	0.030	0.771
SEF_{dorsal} ($\%SEF_{Atrium}$)	8.7 ± 3.6	7.8 ± 1.4	0.613	0.597

P values from a GLM using group and sex as cofactors.

^{ABC}Different superscripts within a column indicate significant differences by paired t-test with Sidak adjustment for multiple testing.

general lack of difference in the development of the papillation is also reflected in the similar rumen tissue mass of the two feeding groups (Table 2). This contrasts with findings in developing juvenile ruminants, where differences in diet lead to different papillation patterns and organ tissue masses of the reticulorumen (Johnson et al., 1987; Josefsen et al., 1996; Fluharty and McClure, 1997). The only indication of a more stratified papillation pattern with hay feeding was the fact that in the hay-fed animals, not only the difference between the SEF_{dorsal} and the SEF_{Atrium} but also that between the $SEF_{ventral}$ and the SEF_{Atrium} was significant (Table 4).

The size, mass and fill of the omasum typically increase with the roughage content of the diet, both in juvenile

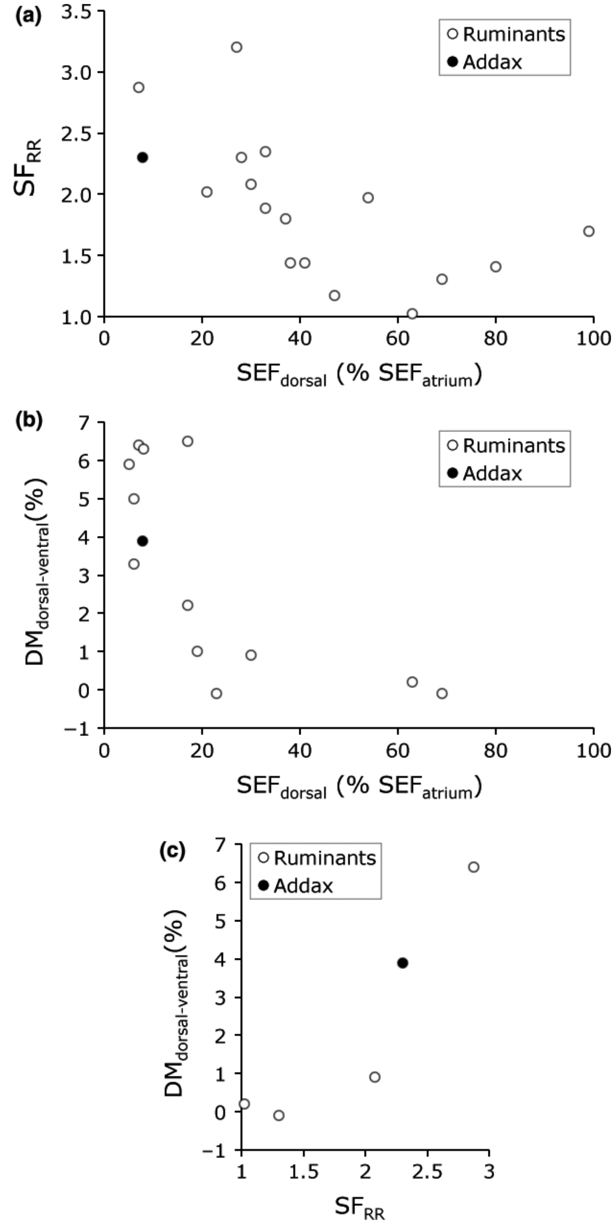

Fig. 3. Relationships between (a) the intraruminal papillation pattern, expressed as the percentage of the surface enlargement factor (SEF) of the dorsal mucosa of the SEF of the *Atrium ruminis*, and the pattern of digesta kinetics in the reticulorumen (RR), expressed as the selectivity factor (SF, the ratio of small particle to solute marker retention), (b) the intraruminal papillation pattern and the difference in dry matter (DM) concentration (in %) between the contents of the dorsal and the ventral rumen, (c) the pattern of digesta kinetics in the RR and the difference in DM between the dorsal and the ventral rumen. Data on addax (*Addax nasomaculatus*) measured in the same (hay-fed) animals from the present study and Hummel et al. (2008), Clauss et al. (2009a). Data collection on the SEF from (Clauss et al., 2009b), on the DM difference from Codron and Clauss (2010) with additional data for cattle DM from Hummel et al. (2009), and on the SF from Hummel et al. (2015).

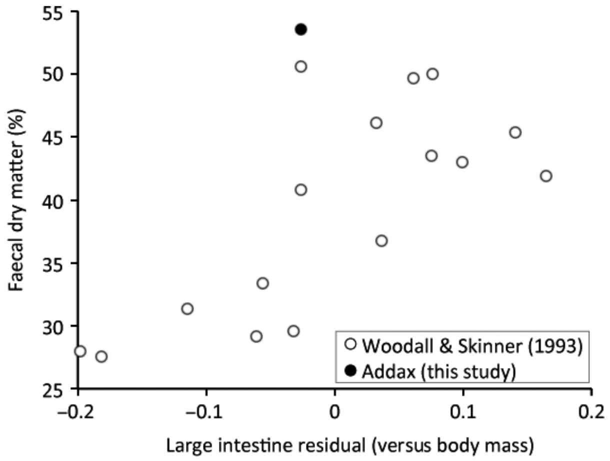

Fig. 4. Relationship of the residuals of large intestine length (versus body mass) and the faecal dry matter content in various wild ruminants (from Woodall and Skinner, 1993) and the addax (*Addax nasomaculatus*). Intestinal measurements for hay-fed animals from the present study, faecal dry matter for addax from Clauss et al. (2004).

and mature domestic ruminants (Stobo et al., 1966; Lauwers, 1973; Hamada et al., 1975; Bailey, 1986; Johnson et al., 1987; McLeod and Baldwin, 2000). The omasum shows significant inter-individual variation in cattle (Becker et al., 1963), which was also evident in the addax in terms of the variability in the number of omasal leaves of different orders (Table 3). The difference between the feeding groups of the present study might partially be sought in such inter-individual variation, as the number of first-order leaves tended to differ between the groups ($P = 0.077$, Table 3), and partially in a reaction to the ingested diet. Interestingly, because omasum tissue mass did not differ between the groups (Table 3), the difference in omasal surface area (Table 4) must be rather the consequence of tissue re-arrangement rather than tissue accretion.

Previous studies on the effect of diet on intestines and internal organs reported mass increases with higher energy intakes, often related to concentrate feeding (Johnson et al., 1987; Burrin et al., 1990; Arnold et al., 2015). For example, it has been previously confirmed that liver mass at slaughter is closely related to energy intake (Johnson et al., 1987; Fluharty and McClure, 1997; McClure et al., 2000) and therefore usually closer related to concentrate as opposed to roughage intake (McLeod and Baldwin, 2000).

The heavier parotis and mandibularis glands in the concentrate group were unexpected. In growing ruminants, a higher roughage intake, usually combining a higher overall food intake with increased stimulus for rumination and hence saliva production, leads to an increase in salivary gland size (Wilson and Tribe, 1961; Wilson, 1963). In adult animals, the size of the salivary glands can vary between seasons. For reindeer (*Rangifer tarandus*), Mathiesen et al. (1999) found larger salivary glands in summer as compared to winter, and interpreted this as an effect of the higher food intake and necessary saliva production in summer. In contrast, Jiang et al. (2003) found larger salivary glands in Mongolian gazelles (*Procapra gutturosa*) in winter than in spring. Given that it is very probable that the hay-fed animals of the present study had to chew more during ingestion and rumination, and hence produced more saliva, a direct relationship between the size of the salivary glands and the secreted saliva volume is questionable, similar to considerations in Hofmann et al. (2008). The other factor typically associated with larger salivary glands in browsing/'moose-type' ruminant species is the production of salivary tannin-binding proteins (Hofmann et al., 2008);

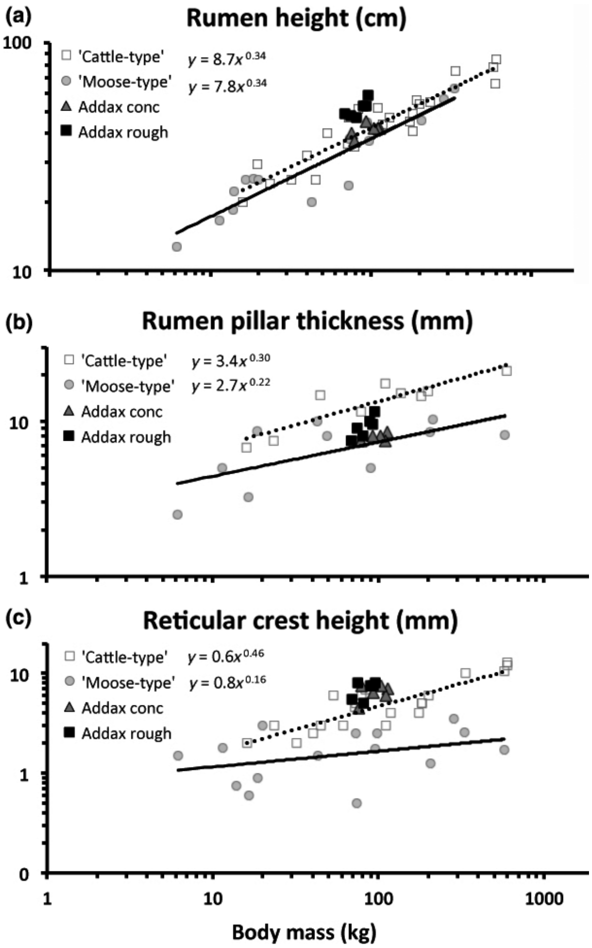

Fig. 5. Comparison of ruminoreticular measures of addax (*Addax nasomaculatus*) fed a diet dominated by concentrates or by roughage (hay only) to literature data on 'cattle-type' (dashed regression line) and 'moose-type' (full regression line) ruminants (from Sauer et al., 2016).

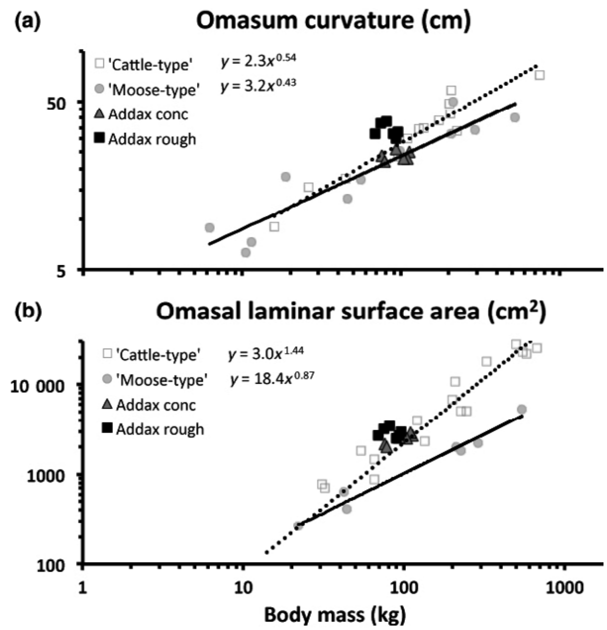

Fig. 6. Comparison of omasal measures of addax (*Addax nasomaculatus*) fed a diet dominated by concentrates or by roughage (hay only) to literature data on 'cattle-type' (dashed regression line) and 'moose-type' (full regression line) ruminants (from Sauer et al., 2016).

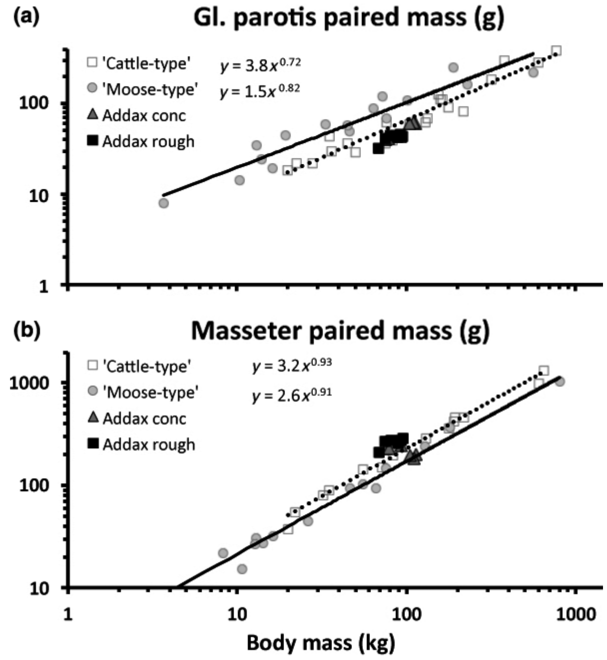

Fig. 7. Comparison of parotis gland and masseter muscle mass of addax (*Addax nasomaculatus*) fed a diet dominated by concentrates or by roughage (hay only) to literature data on 'cattle-type' (dashed regression line) and 'moose-type' (full regression line) ruminants (from Clauss et al., 2008a; Sauer et al., 2016).

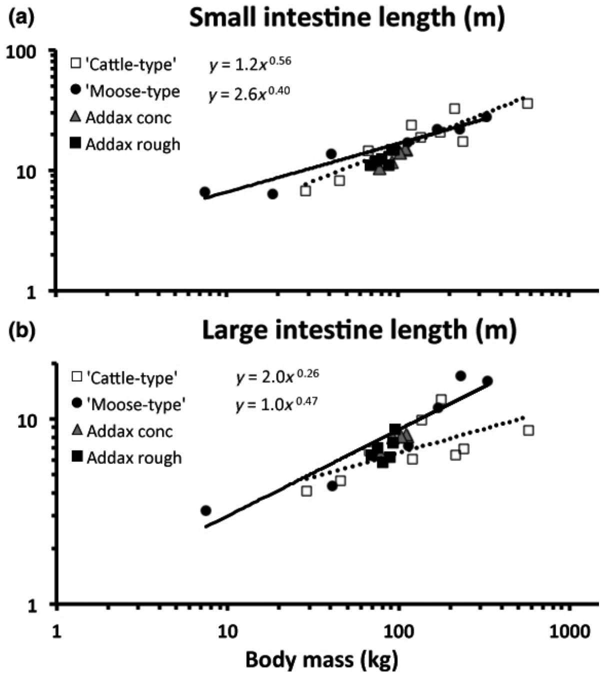

Fig. 8. Comparison of small and large intestine length of addax (*Addax nasomaculatus*) fed a diet dominated by concentrates or by roughage (hay only) to literature data on 'cattle-type' (dashed regression line) and 'moose-type' (full regression line) ruminants (from Sauer et al., 2016).

however, the concentrate diet of wheat bran and barley can hardly be invoked to trigger an increase in these proteins. The larger salivary glands in the concentrate-fed animals thus remain unexplained.

The assumption that hay-fed animals chewed more is supported by the finding of significantly heavier masseter muscles in this group (Table 3). This contrasts with a similar study in Japanese black cattle, where no difference in masseter mass had been found between animals fed unspecified concentrates at either 1.5% or 3% of body mass (with grass hay ad libitum in both groups) for five months, although the daily mastication time was significantly larger in the group receiving less concentrates (Nakamura et al., 2007). However, when re-analysing the results of that study, expressing masseter mass as a percentage of body mass (Clauss et al., 2008a), the cattle with the lower proportion of concentrates in their diet had a higher relative masseter mass (0.12% of body mass) than the cattle with the higher proportion of concentrates (0.10%). In the present study, with a more pronounced difference between the two experimental diets, the difference in relative masseter mass was more pronounced (Group 1: 0.23 ± 0.06%; Group 2: 0.31 ± 0.03%). Given that the difference in masseter mass appeared to reflect the reported difference between the ruminant feeding types (Fig. 8), differences in masseter mass between species may allow conclusions about the masticatory work required by the diets the individual animals consumed.

Given these effects of the feeding regime on the magnitude of various anatomical measures, the question arises to what degree observed differences between species (Hofmann, 1988) represent differences due to the genetically determined morphology, i.e. represent convergences or homologies, or effects of the natural diets consumed by these species. On the one hand, some characteristics, such as the reticular crest height, appear completely unaffected by diet, and some others, such as the omasal leaf surface area or the size of the parotis gland, although affected by diet, nevertheless retain a distinct signal with respect to the ruminant classification. On the other hand, the diets used in the present study, although not characterized by nutrient analyses of the actually consumed diets, represent extremes. While the hay used had levels of neutral and acid detergent fibre of 71 and 35% (dry matter), respectively, these values are typically 43 and 16% for wheat bran, and 21 and 7% for barley (NRC, 2001). Using these data, the hay, wheat bran and barley contained estimated crude fibre levels (using the equation of Kamphues et al., 2004) of 27, 10 and 2.5% (dry matter). By contrast, when comparing the crude fibre levels in the rumen contents of 16 different African wild ruminants, no significant difference was evident between browsers (mean ± SD 22 ± 5%), grazers (22 ± 1%) or intermediate feeders (25 ± 2%) (Woodall, 1992). At least with respect to fibre levels, differences in diet between free-ranging ruminants should therefore have a reduced influence on digestive tract anatomy compared to the present study. In other words, our study provides indirect evidence for the assumption that whereas the ruminant digestive tract shows a certain degree of flexibility, anatomical differences reported between browsing, grazing and intermediate ruminants (see Introduction) are not mainly the effect of a 'blueprint' common to all species that reacts ontogenetically to their respective diets, but the effect of different 'blueprints' (sensu Hofmann, 1998) acquired by the different species during their evolution. This conclusion matches the experience that in captivity, some ruminants appear unable to adapt to certain diets suitable for other ruminant species, such as giraffe that cannot be kept on grass hay (Foose, 1982). To which degree differences in digestive tract anatomy within a wild ruminant species can be intensified by already raising weaned individuals on different diets remains to be investigated.

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