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Microanatomy of the digestive tract, hooves and some visceral organs of addax antelope (*Addax nasomaculatus*) following a concentrate or forage feeding regime

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Summary

Subacute ruminal acidosis is a common disease in captive non-domesticated ruminants and is mainly diagnosed by rumen fluid pH and rumen histology. Furthermore, differences in ruminant gastrointestinal histology have been hypothesized to correlate with the browser–grazer continuum. Twelve surplus addax antelope (*Addax nasomaculatus*) were divided into two groups, fed either their usual diet, consisting of a concentrate feed with a limited amount of hay, or a diet of unlimited hay only, for 3 months. After culling, descriptive and morphometric histology and pH measurements were compared between groups. Significant variations in cellular subpopulations were noted between groups, with roughage-fed individuals presenting primarily with balloon cells of the *Stratum corneum* and living layer cell vacuolization, whereas parakeratosis and intermediate-type cells were more frequent in the concentrate-fed group. Lesions typical of subacute ruminal acidosis were significantly more pronounced in concentrate-fed individuals. Ruminal pH measurements did not differ significantly, but were more consistent in forage-fed individuals, indicating a more stable reticuloruminal environment. The results indicate that ruminal histology may be more appropriate in assessing ruminal health compared to a single post-mortem pH measurement. It is proposed that *Stratum corneum* balloon cells may indicate cell maturation and not, as previously assumed, hyperfunction. Concentrate-fed individuals scored higher on the presence of inflammatory cells on hoof corium histology. The study further emphasizes the adaptability of ruminant digestive tract microanatomy in adult animals even after a short period of time and the positive effects an increased roughage diet may have in populations of captive grazing ruminants.

1 | INTRODUCTION

The histological structure of the gastrointestinal tract of ruminants can be modified by the diet ingested (Ahmed, Martens, & Muelling, 2013; Dieho et al., 2016; Melo et al., 2013). A variety of changes due to a high concentrate provision have been noted in domestic ruminants, including abnormal ruminal papillation, epithelial thickness, hyperkeratosis, parakeratosis and inflammatory infiltration of the ruminal mucosa (Ahmed et al., 2013; Berg & Edvi, 1976; Cernik et al., 2011; Steele, Greenwood, Croom, & McBride, 2012). Moreover, the physical form or energy content of otherwise similar diets may further result in changes of ruminal microanatomy (Amaral,

Sugohara, Resende, Machado, & Cruz, 2005; Dirksen, Liebich, Brosi, Hagemeister, & Mayer, 1984; Kauffold, Kohler, & Fischer, 1976). Although data have traditionally focused on the ruminal epithelium, anatomical changes due to diet have also been noted further down the gastrointestinal tract (Liu, Xu, Zhu, & Mao, 2014; Tao et al., 2014). To our knowledge, there is no consensus as to which degree such changes should be considered adaptive reactions or indicators of pathologic processes.

Differences in the microscopic appearance of the digestive tract, especially between non-domesticated ruminants, have been described sporadically (Hofmann, 1973, 1989). These traits have been interpreted as a method of species classification along the browser–grazer continuum (Axmacher, 1987; Hofmann, 1988). For example, browsers have a particularly thick fundic *Lamina propria mucosae*, whereas specialized grazers have a thicker muscularis layer in both the small and the large intestines (Axmacher, 1987; Hofmann, 1988). Furthermore, browsers of low body mass have less connective tissue in their liver in comparison to grazers of comparable body mass (Liedtke, 1989). In contrast, no histological differences between the feeding types were noted in comparisons of the parotid and mandibular salivary glands or of the adrenal glands (Bernert, 1981; Thomé, 1989).

Addax antelope (*Addax nasomaculatus*) are dry region grazers (Gagnon & Chew, 2000) that display both morphological and physiological characteristics considered typical for "cattle-type" ruminants (Clauss, Hume, & Hummel, 2010), including stratified rumen contents and an uneven rumen papillation, pronounced reticular crests, a large omasum and a comparatively high rumen fluid throughput (Clauss et al., 2009; Hummel et al., 2008; Tahas et al., 2017). We used the opportunity of the culling of surplus animals at a zoological institution where the animals were traditionally fed with a high proportion of concentrates (that was changed since) to create two experimental groups, and fed the additional group only on grass hay, putatively mimicking the natural diet.

We expected addax fed hay only to more closely approximate the typical histological picture of grazers, as previously described. In this sense, we expected the ruminal epithelium of forage-fed individuals would be thinner and with more prominent sloughing (Hofmann, 1973; Marholdt, 1991). Additionally, we expected the differentiation between epithelial strata to be more difficult to discern in comparison with that of concentrate-fed individuals (Hofmann, 1973; Marholdt, 1991). Based on a previous study that identified corresponding differences between grazers and browsers, we further hypothesized that the abomasal *Lamina propria mucosae* would be thicker in the fundic region for animals on a concentrate diet but the same for both groups in the pyloric region (Axmacher, 1987). Furthermore, we hypothesized that animals fed hay only would have a thicker muscularis layer of all intestines (Fluharty, McClure, Solomon, Clevenger, & Lowe, 1999; Hofmann, 1988; McClure, Solomon, & Loerch, 2000).

Conflicting literature did not allow for an accurate hypothesis with regard to the occurrence of balloon cells in the ruminal *Stratum corneum*. Traditionally, these cells have shown maximum development in the mucosa of browsers and been interpreted as indicative of particularly digestible diets (Hofmann, 1973; Marholdt, 1991). However, a recent study in free-ranging impala (*Aepyceros melampus*) has questioned this interpretation, noting balloon cells of the *Stratum corneum* to be more prominent in animals on a lower-quality diet (Lane et al., 2014). In domestic ruminants, recent studies associate balloon cells with high grain consumption (Beharka, Nagaraja, Morrill, Kennedy, & Klemm, 1996; Steele et al., 2012). However, balloon cells have also been noted in cattle and sheep fed diets of a comparatively lower concentrate component and interpreted as favourable mucosal changes (Berg & Edvi, 1976; Kauffold & Piatkowski, 1971).

With regard to rumen health, we hypothesized that histopathology would be a more accurate diagnostic tool of establishing the presence of potential subacute ruminal acidosis than a single post-mortem pH measurement. Furthermore, we expected that animals fed a concentrate-based diet would develop gastrointestinal pathologies related to subacute ruminal acidosis, such as abnormal ruminal papillae shape, hyperkeratosis, parakeratosis, epithelial sloughing, a thicker *Stratum corneum* and development of epithelium pustules in the rumen (Kauffold & Piatkowski, 1971; Nour, Abusamra, & Hago, 1998; Steele et al., 2012). Finally, due to the negative effects on ruminal and extraruminal health associated with a high concentrate feeding regime, we expected that addax on the concentrate feeding regime would display a higher score of hepatic lipidosis, hoof lesions, as well as a higher adrenal medulla-to-cortex ratio (Boosman, Koeman, & Nap, 1989; Kleen, Hoouer, Rehage, & Noordhuizen, 2003; Thoefner et al., 2004; Wiedner, Holland, Trupkiewicz, & Uzal, 2014).

2 | MATERIALS AND METHODS

The animals, the experimental design, the diets used and the macroscopic anatomy have been described previously (Tahas et al., 2017). Six male and six female surplus adult addax antelopes (all more than 4 years of age, non-senile but of unknown exact birth date) 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 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 were culled 3 months after the beginning of the trial. For dissection, carcasses were handled as described previously (Clauss et al., 2009). Approximately 15 min following each animal's death, forestomach content pH was measured using a 206-pH1 set (Tesco, Lenzkirch, Germany). Following dissection, histological samples were taken from five reticulorumen areas (*Atrium ruminis*, ventral and dorsal rumen, ventral and dorsal blind sac), omasum, abomasum, small and large intestines, liver, adrenal gland and all hooves fixed in buffered 3.5% formaldehyde. These were processed routinely. Following fixation, all tissues were embedded in paraffin wax, sectioned at 3.5 μm and stained using haematoxylin–eosin stain.

All slides were observed first by standard light microscopy. Following this, all slides were scanned and measurements were taken from these digital images using NDP.view2 viewing software (Hamamatsu Photonics KK, Hamamatsu, Japan). Epithelial layers were determined as defined by Steele et al. (2011). The *Stratum corneum* was defined as the uppermost layer of the epithelium, stained highly eosinophilic, normally comprising mono- or multilayered thin keratinized nonnucleated cells. Living layers were separated from the *Stratum corneum* proximally by a thin highly eosinophilic line and distally by the *Lamina propria*. All measurements were taken using the shortest linear distance between selected initial and final point of measured objects, as previously described (Cernik et al., 2011). With regard to cells not described in standard anatomical textbooks, balloon cells were identified as superficial, highly differentiated, keratinized, enucleated cells of the *Stratum corneum*, with a vesiculized/vacuolated appearance, based on previous publications (Hofmann, 1973; Kauffold & Piatkowski, 1971; Kauffold, Voigt, & Piatkowski, 1975; Lane et al., 2014; Marholdt, 1991). Instead of the typical flat and thin morphology of *Stratum corneum* keratinized cells, these cells appear extensively enlarged, with abundant clear to lightly eosinophilic cytoplasm.

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 and 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).

Increased observations of a third population of cells of the *Stratum corneum* led to the definition of intermediate cells of this stratum. These were identified as cells with signs of degeneration such as a retained, degraded or fragmented nucleus, with eosinophilic cytoplasm and often multiple basophilic keratohyalin granules. They were typically smaller than ballooning cells yet larger than keratinized epithelial cells and were typically oval, with the minor axis being perpendicular to the epithelium.

The thickness of the epithelial layer was determined for all reticulorumen areas under high power field (HPF) magnification (400x). Ten measurements of the epithelial living strata and 10 measurements of the *Stratum corneum* were taken from five randomly selected papillae, equating to 500 measurements per individual. In addition, the amount of cutaneous pustules in the same five randomly selected papillae was recorded. Furthermore, on every measurement, the number of cells per row of the *Stratum corneum* and *Stratum granulosum* of the respective papillae was counted.

For omasal areas, 10 measurements of the epithelial living strata and 10 measurements of the *Stratum corneum* at random HPFs of the mucosa were taken. Furthermore, 20 measurements per site were taken at random HPFs of the mucosa to measure the thickness of the *Lamina propria mucosae* at the abomasal fundus and pylorus, equating to 40 measurements per individual. The *Lamina propria mucosae* of the abomasum was determined as defined by Axmacher (1987) thus consisting of connective tissue, mucosal glands and lymphoid tissue and extending from the epithelial basement membrane to the *Lamina muscularis* of the mucosa. Abomasal *Lamina propria mucosae* measurements were plotted against data from previous studies on abomasal fundic and pyloric *Lamina propria mucosae* from wild ruminants (Axmacher, 1987). To assess differences in the composition of the *Lamina propria mucosae* between groups, foveolar, parietal and chief cell concentrations were calculated from histological sections measuring 100 μm in width and of variable height to reflect the average height of the *Lamina propria mucosae* in each individual.

To assess the thickness of the intestinal *muscularis* layer, 20 measurements were taken from the small and large intestine, equating to 40 measurements per individual. These measurements were also plotted against previous thickness measurements of large intestine muscularis (colon) for wild ruminants (Ludwig, 1986). Finally, the width of the whole adrenal gland, medulla and cortex were measured and the medulla-to-cortex ratio calculated at the height of the largest diameter of the gland.

Histological mounts of the ruminoreticulum, omasal, hepatic and hoof tissue were described using scores for the following values. Seven variables were assessed for ruminoreticulum tissue: the physiological shape of mucosal papillae (0–3), sloughing of

mucosal epithelium (0–4), presence of balloon cells (0–4), presence of intermediate cells (0–4), extent of parakeratosis (0–4), presence of vacuolated cells in the *Stratum granulosum* and *Stratum spinosum* (0–4) and ease of strata differentiation (0–3). The same values were assessed for the omasal tissue with the exception of the papillae shape. Hepatic tissue was assessed for signs of lipidosis (0–3) and leucocyte number and population (0–3). All measurements and assessments were made by one observer (ST). Additionally, hooves were assessed by another observer for signs of inflammatory cell proliferation (0–8), hyperaemia and congestion (0–8) and vessel proliferation (0–8). The pathological changes were scored from 0 (no pathological changes) to 8 (severe) modified from a scoring system used formerly by Boosman et al. (1989). The final value for each individual was calculated by adding scores for anterior and posterior hooves.

For quantitative data, comparisons between the two experimental addax groups were made using parametric (two-sample *t* test) or nonparametric (Mann–Whitney) tests as indicated after testing for normality by Anderson–Darling test. For qualitative data (scores), nonparametric tests (Mann–Whitney) were applied. The Spearman correlation test was used to evaluate the correlation between balloon cell scorings and *Stratum corneum* thickness for the dorsal and ventral rumen, as well as the *Atrium ruminis*. Pearson's correlation test was used to evaluate the correlation between small and large intestine muscularis thickness and empty intestinal mass, which had been reported for these animals in a previous publication (Tahas et al., 2017). Analyses were performed in Minitab 17.0 (Minitab Inc. State College, PA). The significance level was set to 0.05, with values up to 0.1 considered as trends.

3 | RESULTS

3.1 | Histological findings in relation to previous reports on ruminant feeding types

3.1.1 | Abomasum

The abomasal mucosa consisted of a mucin-covered single columnar epithelium and a densely connected *Lamina propria mucosae*, which was measured as part of this experiment. The *Lamina propria mucosae* consisted primarily of mucosal gland cells, with sparse lymphatic and connective tissue. Gastric pit foveolar cells were the predominant cell type identified in both experimental groups, followed closely by basophilic chief cells. These were subjectively more prominent in addax in the roughage feeding group; however, comparisons between concentrations of all three major cell types of the abomasal *Lamina propria mucosae* showed no statistical difference between groups (Table 2). However, there was a significant difference between groups in the thickness of the *Lamina propria mucosae* at the level of the abomasal fundus but not of the pylorus (Table 3). The fundic *Lamina propria mucosae* of forage-fed addax was on average 1.5 times thicker than that of concentrate-fed addax. When plotted against measurements from other ruminants, addax spanned the range from browsers to grazers, with animals on the roughage diet reaching further into the grazer range (Figure 1).

TABLE 2 Average (±*SD*) density of fundic *Lamina propria mucosae* cell types in addax antelope (*Addax nasomaculatus*) fed a concentrate or roughage diet. *p* values from *t* tests and Mann–Whitney tests (asterisk) are given

Measurement	Concentrate	Roughage	*p*
Cell density (number of cells per mm^2)			
Foveolar cells	880 ± 294	1339 ± 609	.141
Chief cells	1032 ± 537	1147 ± 289	.659
Parietal cells	663 ± 513	751 ± 394	.873

3.1.2 | Intestines

Two layers of smooth muscle fibres were identified in all sections of the small and large intestines. These layers were subjectively easier to differentiate in sections of the small intestine. The outer longitudinal layer was occasionally absent in its full length from large intestine sections. The thickness of both small and large intestinal muscular layers was greater in the concentrate-fed group (Table 3). There was a significant positive correlation between previously reported empty intestinal mass (Tahas et al., 2017) and intestinal muscularis thickness in all addax (small intestine: $R = .71$, $p = .01$, large intestine: $R = .68$, $p = .02$). When plotted against previous data, large intestine muscularis thickness again spanned the range from browsing to grazing ruminants (Ludwig, 1986) (Figure 2).

3.2 | Histological findings related to diet-induced changes and pathology

3.2.1 | Ruminal mucosa

Animals in the hay-only group displayed a *Stratum corneum* with multifocal to diffuse extensive accumulation of balloon cells, often in multiple rows, causing mild orthokeratosis (Figures 3 and 4, Table 4). Balloon cells intermittently presented with debris of pyknotic nuclei. Intermediate cells were present but sparse. Hydropic, vesicular or ballooning changes were commonly noted in the cytoplasm of *Stratum granulosum* and *Stratum spinosum* cells, often extending in rows throughout papillae (Figure 3, Table 4).

The *Stratum corneum* of individuals in the concentrate-fed group consisted in its majority of intermediate cells (parakeratosis) (Figures 3 and 4, Table 4). Balloon cells were present but sparse, as were vacuolated changes in cells of the *Stratum granulosum*. There was no difference in the number of cell rows in either the *Stratum corneum* or *Stratum granulosum* between feeding groups (Table 4). However, the *Stratum granulosum* of concentrate-fed individuals appeared more dense and compact in comparison to that of hay-only animals (Figure 3).

Measurement	Concentrate	Roughage	*p*
Lamina propria mucosae thickness (μm)			
Abomasal fundus	488.2 (287.3–772.9)	**733.6 (296.3–1250.8)**	**<.001**
Abomasal pylorus	610.2 (370.6–1311.1)	637.6 (429.8–996.4)	.376
Tunica muscularis thickness (μm)			
Small intestine	**594.0 (224.6–3857.0)**	496.1 (257.5–1855.5)	**<.001**
Large intestine	**623.3 (153.7–2497.9)**	496.2 (66.4–2448.7)	**.001**

TABLE 3 Medians for abomasal *Lamina propria mucosae* and intestinal *Tunica muscularis* thickness in addax antelope (*Addax nasomaculatus*) fed a concentrate or roughage diet. *p* values from Mann–Whitney tests are given. In case of significant difference, the group with the higher value is indicated in bold lettering

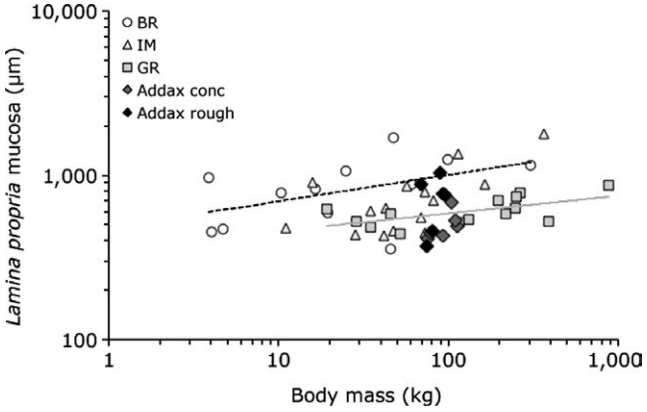

FIGURE 1 Comparison of abomasal *Lamina propria mucosae* thickness of addax (*Addax nasomaculatus*) fed a diet dominated by concentrates or by roughage (hay only) to literature data on grazing (grey regression line) and browsing (dashed regression line) wild ruminants (from Axmacher, 1987). BR, browser; GR, grazer; IM, intermediate feeder; addax conc = addax on a concentrate feeding regime, addax rough = addax on a forage feeding regime

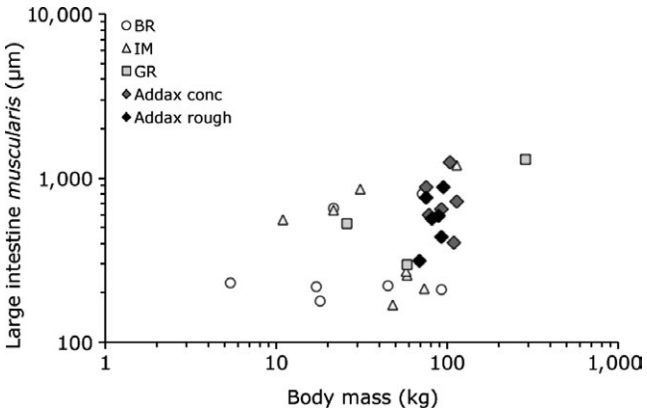

FIGURE 2 Comparison of large intestinal *muscularis* thickness of addax (*Addax nasomaculatus*) fed a diet dominated by concentrates or by roughage (hay only) to literature data on grazing, intermediate and browsing wild ruminants (from Ludwig, 1986). BR, browser; GR, grazer; IM, intermediate feeder; addax conc = addax on a concentrate feeding regime, addax rough = addax on a forage feeding regime

Minor epithelial sloughing was present in animals of both groups and did not differ in any of the examined areas. However, once data for all areas were combined, this proved to be significantly increased in animals of the concentrate group (Table 4). There were numerous

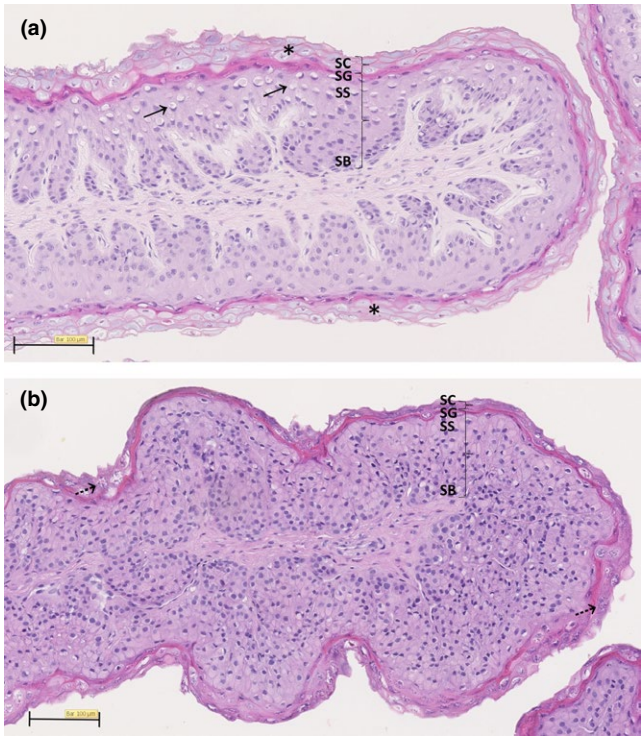

FIGURE 3 Papillae from addax antelope (*Addax nasomaculatus*) on a roughage (a) and a concentrate (b) feeding regime. Note the diffuse presence of balloon cells (asterisks) in the *Stratum corneum* in image (a), as opposed to the much denser and compacter *Stratum corneum* in image (b), primarily consisting of intermediate cells (dashed arrows). Also, note the vacuolization of cells (arrows) in the *Stratum spinosum* of the roughage-fed individual. Finally note the difference in papilla shape, with the papilla in image B being thicker and irregular in comparison with the one in image (a). Haematoxylin–eosin. SC, *Stratum corneum*; SG, *Stratum granulosum*; SS, *Stratum spinosum*; SB, *Stratum basale*. The braces separately indicate the *Stratum corneum* and living layers. Bar = 100 μm

aggregations of neutrophilic granulocytes, interpreted as papillary pustules, in animals of both groups (Figure 5, Table 4). The average number of pustules per papilla is noted in Table 4. Only one individual (of the forage group) presented without any papillary pustules in any of the anatomical locations examined.

The thickness of both the *Stratum corneum* and the living layers of the ruminal epithelium was greater in forage-fed individuals (Table 5). When anatomical areas were compared individually, the *Stratum corneum* was thicker in forage-fed animals at the levels of the dorsal rumen and omasum. This was also the case for the living layers

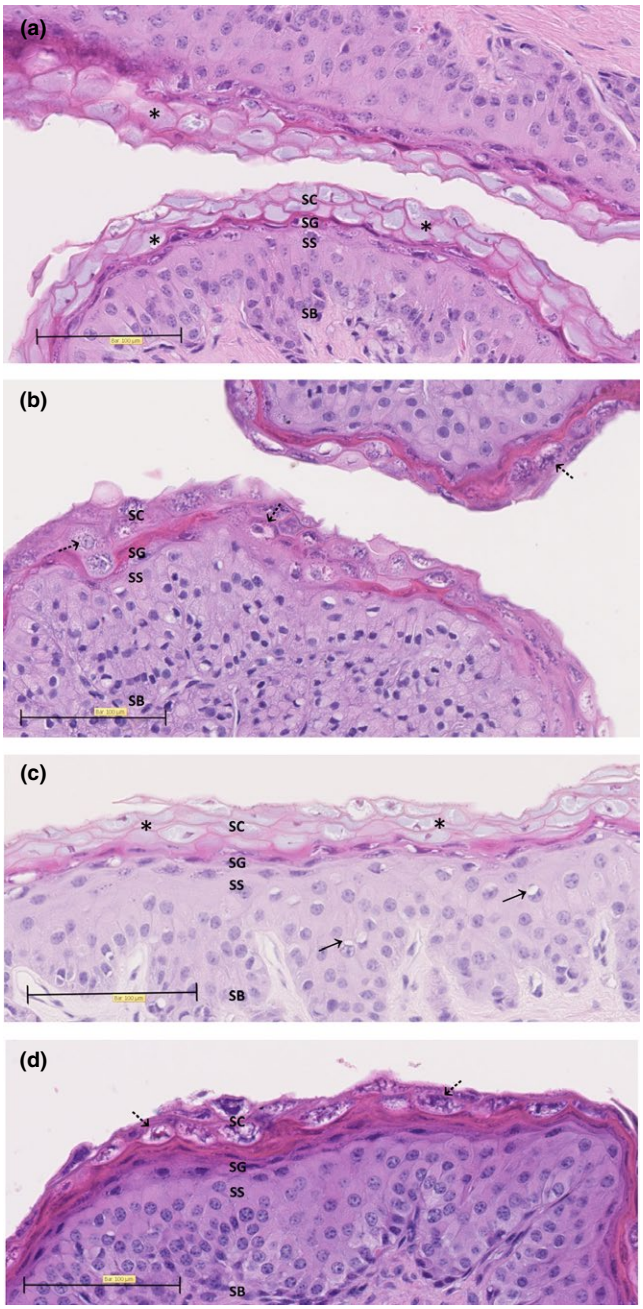

FIGURE 4 Details from papillae of addax antelope (*Addax nasomaculatus*) on a roughage (a,c) and concentrate (b,d) feeding regime. The *Stratum corneum* of addax on a roughage-only diet consisted mainly of lightly eosinophilic vacuolated balloon cells (asterisks) with occasional pycnotic nuclei. The *Stratum corneum* of addax on a concentrate diet consisted of many eosinophilic cells with nuclei, nucleic debris and granules (dashed arrows). Also, note the vacuolizations (arrows) in the *Stratum spinosum* in 4c. Haematoxylin and eosin. SC, *Stratum corneum*; SG, *Stratum granulosum*; SS, *Stratum spinosum*; SB, *Stratum basale*. Bar = 100 μm

at the dorsal and ventral blind sacs. A positive correlation between *Stratum corneum* thickness and balloon cell score was identified for the dorsal rumen (*Rho* = 0.82, p = .001) but not for the ventral rumen (*Rho* = 0.47, p = .12) or *Atrium ruminis* (*Rho* = 0.13, p = .68) (Figure 6, Tables 3 and 4).

3.2.2 | Omasal mucosa

The omasum displayed a stratified keratinized epithelium as previously described (Steele, Penner, Chaucheyras-Durand, & Guan, 2016). Balloon and intermediate-type cells were sparse, and no differences were detected between groups. However, the *Stratum corneum* of the omasum was thicker in forage-fed animals. In contrast, the living layers of the omasal epithelium were thicker in the concentrate group (Table 5).

3.2.3 | Liver

Hepatic microstructure was organized in lobules, as previously described for ruminants (Liedtke, 1989). Scarce to mild lymphoplasmacytic perichoangular inflammation was present in all but one individual, in which this was graded as mild to moderate (Table 6). Hepatic abscesses were not observed, and hepatic lipidosis was minimal in both groups. Only a small amount of cells contained variably sized, well-delineated, clear cytoplasmatic vacuoles that mildly displaced the nucleus to the periphery of the cell and were consistent with lipidosis (Table 6). Minimal connective tissue was present in the liver of animals from either group.

3.2.4 | Hooves

Layers of hoof anatomy were easily distinguishable in all animals, consisting of a cornified wall (epidermis), corium and subcutis, as previously described for the distal limb of ruminants (Desrochers & Anderson, 2001). Hoof lesions were present in both groups but varied in severity, with animals in the concentrate group scoring higher in inflammatory cell presence (Table 6).

3.2.5 | Adrenal

The adrenal medulla and cortex were easily distinguishable. The adrenal width and adrenal cortex-to-medulla ratio did not differ between groups (Table 7).

3.3 | Rumen pH

Ruminal fluid pH did not differ between the two groups (p = .937); however, whereas the forage group had a range of pH values between 6.2 and 6.5 (with a mean of 6.4 and a standard deviation of 0.1), the concentrate group had a wider range between 5.8 and 7.1 (with a mean of 6.4 and a standard deviation of 0.5) (Table 7).

4 | DISCUSSION

This study aimed to characterize the qualitative and quantitative differences in the gastrointestinal microanatomy of addax in a captive setting on two different diets. It further aimed to evaluate differences in liver, hoof and adrenal histology and measurements on these two diets. The current results suggest that various histological and morphometric characteristics of the gastrointestinal and myoskeletal systems

TABLE 4 Qualitative scoring of reticuloruminal and omasal epithelium in addax antelope (*Addax nasomaculatus*) fed a concentrate (C) or roughage (R) diet. Medians for both groups are given with *p* values (from Mann–Whitney tests) below the measurements in brackets. In the case of a significant difference, the group with the higher values is indicated in bold lettering; in the case of *p* values between .05 and .10, the values are set in italics

	All ruminal areas		Dorsal rumen		Ventral rumen		Atrium ruminis		Dorsal blind sac		Ventral blind sac		Omasum	
Measure	C	R	C	R	C	R	C	R	C	R	C	R	C	R
Papillae shape (score 0–3)	2	2	2.5	2	2	1.5	1	2	1.5	1	2	1	N/A	N/A
	(0.273)		(0.640)		(0.383)		(0.437)		(0.281)		(0.559)			
Epithelium sloughing (score 0–4)	**1**	1	1	1	1	1	1	0.5	1	1	1	1	1	1
	(0.017)		(0.673)		(0.113)		(0.476)		(0.594)		(1)		(1)	
Balloon cells (score 0–4)	1	**2.5**	1	**2.5**	1	**3**	0.5	**2**	1	**2**	1	**3**	0	0
	(<0.001)		**(0.003)**		**(0.021)**		**(0.009)**		**(0.003)**		**(0.019)**		(1)	
Intermediate cells (score 0–4)	**2.5**	1	***2***	1.5	***3***	1.5	***2***	1	**3**	1	**3**	1	2	2
	(<0.001)		***(0.056)***		***(0.054)***		***(0.069)***		**(0.031)**		**(0.006)**		(0.491)	
Parakeratosis (score 0–4)	**2**	1	***2***	1	**2**	1	2	1.5	1	1	1.5	1	1.5	2
	(<0.001)		***(0.081)***		**(0.008)**		(0.640)		(0.386)		(0.213)		(0.682)	
Cell vacuolization (score 0–4)	1	**2**	1	***3***	0.5	**2**	1.5	2	1.5	**2.5**	1	**2.5**	2	2
	(<0.001)		***(0.050)***		**(0.044)**		(0.151)		**(0.025)**		**(0.015)**		(0.920)	
Ease of strata differentiation (score 0–3)	1	1	1	1.5	2	1	1.5	1.5	1	0	0.5	0.5	1	1
	(0.277)		(0.583)		(0.108)		(0.675)		(0.437)		(0.858)		(0.762)	
Str. corneum cell rows (number)	2	2.5	3	***3.5***	2.5	2.5	2.5	2	2	2	2.5	2	2	**3**
	(0.530)		***(0.054)***		(1)		(0.284)		(1)		(0.282)		**(0.048)**	
Str. granulosum cell rows (number)	1	1	1	1	1	1	1	1	1	1	1	1	1	1
	(0.401)		(1)		(1)		(1)		(1)		(1)		(0.396)	
Cutaneous pustules (number)	0	0.5	0	0	1	1	0	1.5	1	0.5	0	1	0	0
	(0.288)		(1)		(1)		(0.143)		(0.932)		(0.300)		(1)	

Scores:
0: No occurrence/no abnormalities/relative ease of layer differentiation.
1: Sparse occurrence (less than 25% of epithelium surface sloughing or cells involved)/minor abnormalities/places where layer differentiation questionable.
2: Notable occurrence (up to 50% of epithelium surface sloughing, or cells involved, cell type common but not in full rows)/Moderate abnormalities/layer differentiation moderately difficult.
3: Moderate to high occurrence (up to 75% of epithelium sloughing or cells involved, cell type extending in full rows over multiple fields of view)/severe abnormalities/layer differentiation difficult.
4: Extensive occurrence (up to 100% of epithelium sloughing or cells involved, cell type extensive throughout all fields of view, sometimes in double layers).

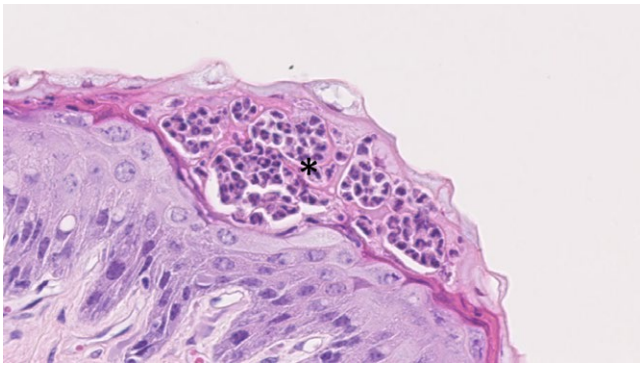

FIGURE 5 Papillary pustule (asterisk) in the ruminal epithelium of an addax antelope (*Addax nasomaculatus*). Haematoxylin and eosin

in addax are under the influence of the ingested diet, as is the case for other ruminants (Neiva, da Mota, Batista, & Sousa-Rodrigues, 2006).

4.1 | Histological findings in relation to previous reports on ruminant feeding types

When comparing the results of our study to previously reported characteristics of ruminants of different feeding types, it is important to remember that albeit the common practice of calling browsing ruminants "concentrate selectors" (Hofmann, 1973, 1989), browse should not be confused in its nutrient composition with "concentrate feeds" used in animal nutrition, and the use of the term "concentrate selector" is therefore discouraged (Clauss et al., 2010; Robbins, Spalinger, & Van Hoven, 1995).

TABLE 5 Median thickness of keratinized (*Stratum corneum*) and non-keratinized (living) (*Str. granulosum*, *Str. spinosum*) epithelium layers in addax antelope (*Addax nasomaculatus*) fed a concentrate or roughage diet. Ranges are given below medians in brackets. *p* values from Mann–Whitney tests are given. In case of significant difference, the group with the higher value is indicated in bold lettering. In the case of *p* values between .05 and .10, the values are set in italics

Measurement	Concentrate	Roughage	*p*
Stratum corneum thickness (µm)			
All rumen locations	15.9 (3.0–53.0)	**16.6 (4.1–80.8)**	**<.001**
Dorsal rumen	16.6 (4.3–48.4)	**24.3 (9.3–80.8)**	**<.001**
Atrium ruminis	15.0 (4.3–53.0)	14.6 (4.1–40.4)	.509
Ventral rumen	17.1 (4.3–52.7)	16.1 (6.7–69.4)	.115
Dorsal blind sac	15.1 (4.1–44.0)	13.9 (4.3–50.0)	.139
Ventral blind sac	15.4 (3.0–43.7)	16.1 (6.4–51.6)	.225
Omasum	12.4 (3.5–26.0)	**18.2 (9.3–32.5)**	**<.001**
Living layer thickness (µm)			
All rumen locations	98.9 (22.6–478.9)	**108.6 (27.9–340.9)**	**<.001**
Dorsal Rumen	97.4 (22.6–214.0)	100.5 (35.7–272.2)	.470
Atrium ruminis	106.4 (34.8–315.6)	104.0 (27.9–267.6)	.483
Ventral Rumen	100.4 (33.2–478.9)	*107.6 (41.3–277.6)*	***.088***
Dorsal Blind Sac	92.3 (23.6–210.4)	**109.2 (36.6–303.4)**	**<.001**
Ventral Blind Sac	96.7 (32.4–234.0)	**124.5 (34.8–340.9)**	**<.001**
Omasum	**81.9 (39.7–192.6)**	63.3 (34.1–117.5)	**.005**

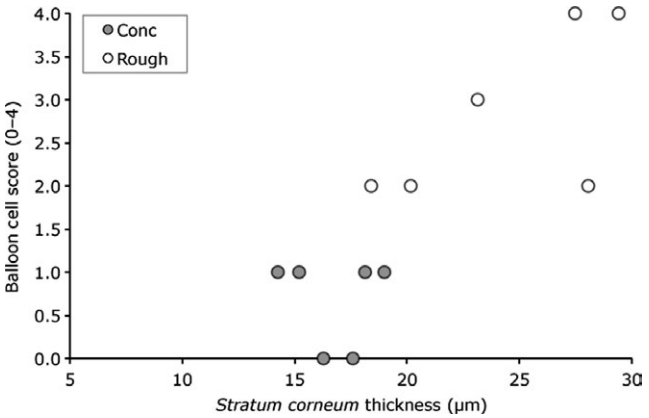

FIGURE 6 Relationship of *Stratum corneum* thickness and balloon cell score in addax (*Addax nasomaculatus*) fed a diet dominated by concentrates or by roughage (hay only)

4.1.1 | Abomasum

Previous work has shown that the abomasum's fundic *Lamina propria mucosae* thickness varies between ruminant feeding types, whereas pyloric *Lamina propria mucosae* thickness does not (Axmacher, 1987). It has been suggested that a higher thickness in the fundic *Lamina propria mucosae* could be a reaction to the higher viscosity of the rumen fluid in browsers that potentially entraps more carbon dioxide, thus requiring the production of more hydrochloric acid by a thicker mucosa (Clauss, Kaiser, & Hummel, 2008; Clauss et al., 2009). On the other hand, it has been suggested that the mechanical stimulation ("scratch factor") brought on by the physical characteristics of grass hay has an effect on multiple gastrointestinal organ measurements in animals on a roughage diet (Tahas et al., 2017;). In contrast to the literature (Axmacher, 1987), addax on the roughage diet had a higher fundic *Lamina propria mucosae* thickness. However, no differences in the density of any of the primary cell types of the *Lamina propria mucosae* (i.e. an increased density of acid-producing chief cells) were noted between addax on the roughage or concentrate diet (Table 2). It is therefore likely that the increased filling of the GIT of roughage-fed individuals (Tahas et al., 2017) and the increased amount of digesta reaching the abomasum in forage-fed individuals might have triggered the development of a thicker fundic *Lamina propria mucosae* due to mechanical stimulation from grass hay.

TABLE 6 Qualitative scores for hooves and liver in addax antelope (*Addax nasomaculatus*) fed a concentrate or roughage diet. Medians and range in brackets are given. The *p* value is provided by a Mann–Whitney test for each score. In the case of a significant difference, the group with the higher values is indicated in bold lettering

Measurement	Concentrate	Roughage	*p*
Hooves			
Inflammatory Cells (0–8)	**4.5 (4–6)**	2 (1–5)	**.047**
Hyperaemia and Congestion (0–8)	5 (4–6)	4.5 (4–6)	.553
Vessel proliferation (0–8)	3 (0–5)	0 (0–5)	.344
Liver			
Hepatic Lipidosis (0–3)	1 (0–2)	1 (0–1)	.673
Hepatic Inflammation	1	1 (1–2)	.317

Scores for hoof histology from 0 (no occurrence) to 8 (severe/extensive changes). Scores of 3 or above were interpreted as pathological.
Scores for hepatic lipidosis and inflammation from 0 (no occurrence) to 3 (severe).

4.1.2 | Intestines

While no published measurements for the small intestinal muscularis layer of non-domesticated ruminants exist, data from addax in our

Measurement	Concentrate	Roughage	*p*
Rumen fluid pH	6.5 ± 0.5 (5.8–7.1)	6.4 ± 0.1 (6.2–6.5)	.937
Adrenal measurements			
Width (cm)	1.62 ± 0.38 (1.31–2.17)	1.40 ± 0.29 (1.10–1.93)	.372
Cortex:Medulla ratio	2.27 ± 0.68 (1.17–2.88)	2.64 ± 1.37 (1.12–4.67)	.588

TABLE 7 Means and standard deviations of ruminal pH measurements, adrenal width and adrenal cortex-to-medulla ratios in addax (*Addax nasomaculatus*). *p* values from *t* tests are given

study spanned the range of thickness measurements of large intestine muscularis (colon) previously described for grazing and browsing ruminants (Ludwig, 1986) (Figure 2), again suggesting that no clear-cut thresholds may exist in this histological measure. However, in disagreement to our initial hypothesis, the average thickness of the *Tunica muscularis* of both small and large intestines was thicker in individuals on the concentrate diet, even though these gut sections had to process more material in the roughage group (Tahas et al., 2017). Although in some experiments overall intestinal mass appears to increase with a high fibre diet (Fluharty et al., 1999; McClure et al., 2000), in others this appears to be more affected by the energy content of the diet ingested (Fluharty et al., 1999). This may also be the case in our study, with the overall mass of empty intestinal compartments being significantly less in addax on the roughage feeding regime and there being a significant positive correlation between empty intestinal mass and muscularis layer thickness (Tahas et al., 2017). Indeed, previous findings state that the energy-sparing effects of restricted caloric intake on visceral organs occur primarily through reduction in organ size (Burrin, Britton, Ferrell, & Bauer, 1992). Reductions or increases in intestinal mass have traditionally not been attributed to any particular microanatomical intestinal layer. Although our analysis clearly shows a positive correlation between empty intestinal mass and muscularis layer thickness, more investigations are necessary to elucidate the possible effect of diet on microanatomical layers thickness.

4.2 | Histological findings related to diet-induced changes and pathology

4.2.1 | Ruminal mucosa, Stratum corneum: Balloon cells

Within the rumen, animals fed a roughage-based diet presented with vacuolizations of cells in all epithelial strata excluding the *Stratum basale*. Such vacuolization has previously been noted in the literature and has been interpreted in conflicting ways (Hofmann, 1973; Lane et al., 2014; Marholdt, 1991; Schilcher, Baumgartner, Geyer, & Liesegang, 2013; Steele et al., 2012). The term "balloon cells," coined by (Hofmann, 1973), are often used to describe vacuolized keratinised cells of the epithelial *Stratum corneum* and have subsequently been used in many other publications (Kauffold & Piatkowski, 1971; Kauffold et al., 1975; Lane et al., 2014; Marholdt, 1991; Schilcher et al., 2013). However, similar cells have often been described with different names by various authors (Bacha & Bacha, 2012; Berg & Edvi, 1976; Steele et al., 2012; Thompson, Kintner, & Pfander, 1958). The conflicting interpretations of the presence of these cells do not allow for any reliable association with diet or function so far.

The majority of literature on domestic ruminants suggests balloon cells to be present following an increase in dietary fibre (Alhidary et al., 2016; Berg & Edvi, 1976; Kauffold, 1975; Kauffold & Piatkowski, 1971; Kauffold et al., 1975) or decrease in the nutritional (energy) value of the diet (Liebich, Dirksen, Arbel, Dori, & Mayer, 1987), similar to the findings of the present study. Some authors go as far as interpreting the presence of balloon cells as favourable (Kauffold & Piatkowski, 1971) or associating them with healthy areas as opposed to epithelial sites suffering from inflammation (Berg, Bartke, & Kaatz, 1976). However, a proliferation of translucent cornified cells has recently been proposed to be associated with grain-induced hyperkeratosis in lambs (Steele et al., 2012). Vesicular changes have been further noted in the cytoplasm of rumen epithelial cells in goats with experimentally induced rumen acidosis, without, however, stating whether these changes were present in cells of the *Stratum corneum* or living layers of the ruminal epithelium (Nour et al., 1998). Finally, the presence of balloon cells has in some cases not been associated with either the roughage or concentrate percentage of the diet in sheep (Neiva et al., 2006).

In wild ruminants, balloon cells have historically been associated with a high-quality diet coinciding with the onset of the rainy season and the availability of lush grass (Hofmann, 1973), and have been described in free-ranging duiker species (*Cephalophus monticola*, *C. natalensis*; Faurie & Perrin, 1995). In a study of zoo ruminants, they have been noted to be present in sika deer (*Cervus nippon*) fed a diet unusually high in easily digestible carbohydrates but not in three other species displaying histological abnormalities compatible with rumen acidosis (Schilcher et al., 2013). However, a recent study in free-ranging impala (*Aepyceros melampus*) has questioned the traditional association of balloon cells with a higher energy diet, finding them to be more prevalent in individuals on a low-quality diet (Lane et al., 2014). This latter study puts forward the case that Hofmann (1973) studied animals at the onset of the rainy season, so that the *Stratum corneum* status might still have reflected the lower-quality food of the preceding season. Given the fact that most ruminal morphological adaptations to diet appear to be significantly established within 4–6 weeks (Ahmed et al., 2013; Etschmann, Suplie, & Martens, 2009), this does not seem to be an unlikely hypothesis.

In the present study, balloon cells were significantly more prominent in addax on roughage as opposed to a concentrate diet. A thicker *Stratum corneum* was also noted in roughage-fed animals. Given the fact that balloon cells have also been interpreted as a normal component of ruminal epithelial mucosa yet often absent due to sloughing,

it makes sense to assume that their presence may be an indication of slower cell turnover, giving the cells time to mature and increase in size (Bacha & Bacha, 2012; Dobson, Brown, Dobson, & Phillipson, 1956; Lane et al., 2014). This assumption would match the observation that often, on grain-dominated high-energy diets in domestic ruminants, signs of increased cell turnover (sloughing, hyperkeratosis) and the presence of immature cells (parakeratosis) indicate a high metabolic state of the epithelium, often linked with an absence of balloon cells (Kauffold & Piatkowski, 1971; Nour et al., 1998; Steele et al., 2012). We therefore support the hypothesis that balloon cells may in fact be a proxy of cell maturation, as opposed to cell hyperfunction or resorption as previously proposed (Hofmann, 1973; Marholdt, 1991; Schilcher et al., 2013). In further opposition to the theory that balloon cells are indicators of an increased metabolic state of the rumen is the negative association between epithelial thickness and ruminal absorption (Melo et al., 2013), as balloon cells are more prominent in thicker epithelia (Hofmann, 1973, and the results of the present study). The variability of interpretations regarding balloon cells does, at the current time, not allow for these structures to act as a reliable proxy for rumen health or diet quality. However, given their consistency as markers between different dietary groups (including the present study), it is recommended that further experimentation that includes a measure of epithelial cell turnover should be employed so as to elucidate their exact role in ruminal physiology.

4.2.2 | Ruminal mucosa, Stratum granulosum and Stratum spinosum: Vacuolizations

Cytoplasmic vacuolizations of the *Stratum granulosum* have not received an interest equal to balloon cells (Berg & Edvi, 1976; Lane et al., 2014). They appear to have a low level of support as a proxy of diet in free-ranging impalas (Lane et al., 2014). However, vacuolizations of the *Stratum granulosum* appeared to be more prominent in Merino sheep fed roughage in addition to their concentrate ration as opposed individuals fed only concentrate (Berg & Edvi, 1976). This was also the case in this study with addax on a roughage diet showing significantly more vesicular changes in the cytoplasm of the *Stratum granulosum* and *Stratum spinosum* as opposed to addax on a concentrate diet. Again, we hypothesize that these vacuolizations are indicators of a slow epithelial cell turnover. Future observations are required to conclude whether such cells are an indication of a healthy rumen and in fact possibly a precursor of balloon cells of the *Stratum corneum*.

4.2.3 | Ruminal mucosa: Parakeratosis

In contrast to roughage-fed individuals, the ruminal epithelium of concentrate-fed addax consisted of predominantly parakeratotic intermediate-type cells with multiple keratohyalin granules. Intermediate-type cells have been previously noted as cells at various stages of cellular degeneration found between *Stratum granulosum* and differentiated (compact) keratinized cells (Steele et al., 2012, 2015). Similar-looking cells are termed "cells with primary swelling" by Hofmann (1973) who did not associate their presence with any dietary or seasonal factors in game ruminants. In the present study, differentiated keratinized cells occurred only sporadically and in thin monocellular layers, making intermediate cells the predominant cell type of the *Stratum corneum* of concentrate-fed individuals. The frequent absence of a differentiated compact epithelial layer may be interpreted as due to the increased sloughing noted in concentrate-fed addax in this study and might be linked to increased epithelial cell turnover. Nuclear retention (parakeratosis) is associated with ruminal acidosis and a high grain diet in domestic and captive wild ruminants (de Campeneere et al., 2005; Gattiker et al., 2014; Liu et al., 2014; Zitnan et al., 1998). Furthermore, the retention of keratohyalin granules in a thickened *Stratum corneum* has been interpreted as pathologic and termed granular hyperkeratosis (Wallace, Pichardo, Yosipovitch, Hancox, & Sangueza, 2003). Both conditions have been associated with accelerated or anomalous mucosal differentiation (Wallace et al., 2003). The association of similar cells with an increase in the grain content of the diet in sheep and cattle (Steele et al., 2011, 2012) further supports the hypothesis that this cellular component is indicative of mucosal pathology.

4.2.4 | Ruminal mucosa: Microabscesses

The presence of epithelial pustules and leucocyte aggregations is characteristic of chronic subacute ruminal acidosis in wild and domestic ruminants (Jensen, Deane, Cooper, Miller, & Graham, 1974; Schilcher et al., 2013). Surprisingly, leucocyte aggregations and epithelial pustules did not differ between treatment groups in this study. This is cautiously interpreted as changes compatible with a long-term digestion of a concentrate-based diet and subsequent subacute ruminal acidosis (Kleen et al., 2003; Manson & Leaver, 1989; Zebeli, Ghareeb, Humer, Metzler-Zebeli, & Besenfelder, 2015) prior to the onset of the experiment, when the animals of the hay-only group had also received the diet of the concentrate group.

4.2.5 | Ruminal mucosa: Thickness measurements

Between groups, significant differences were obtained in thickness of the ruminal and omasal mucosa which confirms that gastrointestinal epithelium thickness responds to variations in the physical and metabolic form of a diet (Dirksen et al., 1984; Melo et al., 2013; Steele et al., 2012). This finding of increased ruminal epithelial thickness in the roughage group is in agreement with studies noting a thicker epithelium in grazing as opposed to concentrate-fed Jersey cows (Melo et al., 2013). On the other hand, an increase in mucosal thickness has been noted as a result of energy-rich feeding in cattle, in offering more browse-type as opposed to hay-type roughage in sheep, and in fallow deer (*Dama dama*) receiving supplemental winter feeding of lucerne, apples and beets as opposed to grazing naturally in summer (Dirksen et al., 1984; Kocisova, Tomajkova, & Kocis, 1995; Kouakou, Goetsch, Patil, Galloway, & Park, 1997).

In particular with regard to *Stratum corneum* thickness, studies in cattle are more in agreement with our results that the *Stratum corneum* appears to be thicker as a result of roughage-fed diets

(Cernik et al., 2011; Melo et al., 2013). Instead, in small ruminants, a high grain and low roughage diet appears to induce thickening of the *Stratum corneum* (Alhidary et al., 2016; Berg & Edvi, 1976; Kauffold & Piatkowski, 1971; Liu, Xu, Liu, Zhu, & Mao, 2013; Steele et al., 2012). The coarseness of the diet in calves has also been negatively associated with thickness of the ruminal *Stratum corneum* (Greenwood, Morrill, Titgemeyer, & Kennedy, 1997). It is currently uncertain whether the above conflicting findings may be explained by species-specific reactions to diet alterations, or whether the measurements are confounded by the presence of different cell types at various stages of *Stratum corneum* hyperkeratosis (Steele et al., 2012; Wallace et al., 2003). Interestingly, throughout the literature, it appears that the range of *Stratum corneum* thickness is much greater in ruminant groups receiving a predominantly concentrate diet (from 9.1 μm in Melo et al. (2013) to 51.0 μm in Steele et al. (2012) as opposed to control or treatment groups on higher fibre diets (from 14.2 μm in Liu et al. (2013) to 20.7 μm in Berg and Edvi (1976)). In the addax, this was not the case, with animals fed the roughage diet showing a greater absolute range (Table 5). With regard to the median measurements of each individual, these showed a similar range between groups (from 13.4 to 19.4 μm in the concentrate group and from 14.3 to 20.9 in the roughage group). Also, though important in establishing differences in thickness of the *Stratum corneum*, balloon cells cannot be presented as the sole explanation for this phenomenon due to the fact that their presence does not necessarily correlate with an increased *Stratum corneum* thickness in all rumen regions.

4.2.6 | Hooves

Subacute ruminal acidosis has negative implications on hoof health in domestic and captive non-domesticated ruminants (Kleen et al., 2003; Wiedner et al., 2014; Zenker, Clauss, Huber, & Altenbrunner-Martinek, 2009). It is assumed that the long-term provision of high-energy concentrates in addax of this study negatively affected hoof health and influenced the increased score of inflammatory cells in hooves of addax in the concentrate group, despite not manifesting as clinical laminitis (Table 6). Interestingly, in a recent study on dairy cattle, it was not a high level of crude protein but a low level of dietary fibre which was negatively associated with a higher prevalence of subclinical laminitis (Pilachai et al., 2013). The findings of this and our study suggest that when feeding zoo ruminants with concentrates, mixing with a substantial part of roughage may be an important strategy in preventing subclinical laminitis (Pilachai et al., 2013).

4.2.7 | Adrenal gland

Adrenal width and medulla-to-cortex ratio did not differ between groups of this study. In captive cheetah (*Acinonyx jubatus*), the medulla-to-cortex ratio of the adrenal is increased with age and the number of pathological diagnoses (Gillis-Germitsch et al., 2017). The absence of a difference in the present study might indicate that neither the roughage-only diet (which still allowed animals to maintain a certain amount of body fat stores, Tahas et al., 2017) nor the concentrate diet represented conditions that triggered different degrees of chronic stress.

4.3 | Rumen pH

Despite the histological changes between groups, the study failed to identify significant changes in the ruminal pH of both groups. It is worth noting that none of the animals in this study presented with ruminal pH values under the cut-off point for diagnosis of subacute ruminal acidosis by rumenocentesis in cattle (5.5) (Cooper & Klopfstein, 1996; Garrett et al., 1999; Kleen & Cannizzo, 2012), yet one animal of the concentrate group had a rumen pH below the cut-off of 6.0 mostly considered as acidotic in small domestic ruminants (Behrens, Gantner, & Hiepe, 2001; Bostedt & Dedié, 1996). However, due to the single post-mortem measurements taken in this study, a nadir measurement could not be defined. As previously reported in cattle and moose (*Alces alces*) (Ritz et al., 2014), the provision of forage lead to more consistent values with a very low standard deviation and a narrow range of values (6.2–6.5), well within the typical pH range found in free-ranging wild ruminant species (Ritz et al., 2013). In contrast, concentrate-fed animals values presented with a higher standard deviation and a larger range (5.8–7.1), with three individuals showing higher values (>6.7) than typically reported for free-ranging ruminants (Ritz et al., 2013), suggesting abnormal rumen function, and one animal close to the 5.5 threshold. The stability of rumen pH has previously been positively associated with ruminal epithelium function (Wang et al., 2009).

Subacute ruminal acidosis is often cited as a prevalent non-infectious disease that is widespread within ungulates of zoological institutions, mainly due to a suboptimal feeding regime (Gattiker et al., 2014; McCusker, Shipley, Tollefson, Griffin, & Koutsos, 2011; Schilcher et al., 2013; Whitehouse-Tedd, Hebbelmann, Strick, Vercammen, & Dierenfeld, 2016; Zenker et al., 2009). However, the disease has rarely been investigated systematically, in particular with regard to diagnostics. Given that ruminal pH did not differ between treatment groups, it appears that ruminal histology is possibly more sensitive than a single post-mortem pH measurement in diagnosing subacute ruminal acidosis (Cernik et al., 2011; Lane et al., 2014).

5 | CONCLUSION

In conclusion, all addax in this study conformed to previous literature regarding ruminant anatomy, but the range of individual measurements spanned values given previously for both browsing and grazing ruminants. Roughage-only animals displayed a significantly thicker ruminal *Stratum corneum* and marked cell ballooning whereas concentrate-fed animals displayed significantly higher levels of parakeratosis and the presence of intermediate-type cells. We propose that balloon cells are an indication of cell maturation and not cell hyperfunction as previously proposed. We further conclude that ruminal histology as opposed to a single post-mortem forestomach pH measurement was more adequate in assessing ruminal health. Therefore, histological screening may be required for assessing the nutritional adequacy of

diets in ruminant collections. Despite persisting pathologies such as mucosal pustule presence, the change to an ad libitum roughage diet appears to provide a more stable ruminal environment and positively affects hoof health. The data presented herein indicate the adaptability of ruminant anatomy in adult animals even after short periods of time and therefore the opportunity for zoo clinicians and managers to improve animal health and welfare by the incorporation of roughage into zoo ruminant diets.

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CONFLICT OF INTEREST

The authors declare they have no conflicts of interest in relation to this work.

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