The distribution of mucous secreting cells in the gastrointestinal tracts of three small rodents from the Saudi-Arabian Peninsula: *Acomys dimidiatus, Meriones rex* and *Meriones lybicus*

Olga Johnson[a], Sumine Marais[a], Jacklynn Walters[a], Elizabeth L. van der Merwe[b], Abdulaziz N. Alagaili,[c,d] Osama B. Mohammed[c], Nigel C. Bennett[e], Sanet H. Kotzé,[a]*

[a]*Division of Anatomy and Histology, Department of Biomedical Sciences, Faculty of Medicine and Health Sciences, Stellenbosch University, Tygerberg, 7505, South Africa,*

[b]*Department of Human Biology, Faculty of Health Sciences, University of Cape Town, Observatory, 7925, South Africa, Elizabeth.VanderMerwe@uct.ac.za*

[c]*King Saud University (KSU) Mammals Research Chair, Department of Zoology, College of Science, King Saud University, Riyadh, 11451, Saudi Arabia.*

[d]*Saudi Wildlife Authority, P.O. Box 61681, Riyadh 11575, Saudi Arabia*

[e]*Department of Zoology and Entomology, Mammal Research Institute, University of Pretoria, Pretoria, 0002, South Africa*

The first two authors contributed equally to the study.

* Corresponding author at: Department of Biomedical Sciences, Faculty of Medicine and Health Sciences, University of Stellenbosch, P.O. Box 19063, Tygerberg 7505, South Africa.

Phone: +27 21 938 9428

E-mail address: shk@sun.ac.za (S.H. Kotzé).

Short title:

Intestinal mucous-secreting cells of Saudi Arabian rodents.

ABSTRACT[1]

The proportion of mucin phenotypes (which form the protective biofilm of the gastrointestinal tract) differ between intestinal regions and different species. This study examines the distribution of mucin-secreting cells in the gastrointestinal tracts of the Arabian spiny mouse (*Acomys dimidiatus*), King jird (*Meriones rex*) and Libyan jird (*Meriones lybicus*), which live in the dry and hot climate of Saudi Arabia. Intestinal tract samples were processed to wax and tissue sections stained with Alcian Blue-Periodic Acid Schiff,

[1] ABBREVIATIONS

GIT=gastrointestinal tract; PFA=Paraformaldehyde; SWA= Saudi Wildlife Authority; US= University of Stellenbosch; UP= University of Pretoria; AB-PAS= Alcian Blue-Periodic Acid Schiff; AF-AB= Aldehyde Fuchsin-Alcian Blue; HID-AB= High Iron Diamine-Alcian Blue; E=surface epithelium; C=crypts; ANOVA= Analysis of variance; LSD= Least Significant Difference

Aldehyde Fuchsin-Alcian Blue and High Iron Diamine-Alcian Blue in order to identify the different mucin phenotypes. The distribution of the different mucin-secreting cell types was determined by quantitative analysis. Mixed mucin-secreting cells (combined neutral and acid) was the predominant mucin-secreting cell type observed throughout the gastrointestinal tract in all species with solely neutral mucin-secreting cells found mainly in the glandular region of the stomach and Brunner's glands. Acid mucin-secreting goblet cells were mainly located in the colon. Distribution of sulfo- and sialomucin-secreting goblet cells differed as these were mostly present in the crypts and surface epithelia of the *Meriones* species and *A. dimidiatus*, respectively. The distribution of mucin-secreting cells is therefore similar to previously reported results for small mammals not living under arid conditions.

KEYWORDS:
Acomys, *Meriones*, Mucins, Gastrointestinal tract, Histochemistry

INTRODUCTION

A visco-elastic mucous layer covers the mucosal surface of the mammalian gastrointestinal tract (GIT) from the stomach to the colon. This layer is regarded as an integral part of the barrier between the intestinal luminal contents and the mucosal tissue (Deplancke and Gaskins, 2001). The gel-like layer is constantly changing and consists of various secretions and exfoliated cells (Laux *et al*., 2005; McGuckin *et al*., 2011). The main structural components of the mucous layer are mucins, which are highly glycosylated, high molecular proteins secreted by mucous-secreting cells (mostly specialized goblet cells) (Forstner and Forstner, 1994). The functions of the mucous gel layer include lubrication of the underlying delicate mucosa, protection against the effects of noxious substances and pH changes in the intestinal lumen (Forstner and Forstner, 1994), restriction of the movement of macromolecules across the mucous barrier of the duodenum (Flemstrom *et al*., 1999) and protection against mucosal invasion of bacterial pathogens (Montagne *et al*., 2004; MacFarlane and Dillon, 2007). In addition, this mucous layer plays a role in the formation of an intestinal biofilm which provides the substrate for indigenous microflora and is further supported by the immune system (Bollinger *et al*., 2007, MacFarlane and Dillon, 2007).

Mucins can be classified as neutral or acidic according to the net charge of the molecule. Acid mucins can further be differentiated into sulfate-containing mucins (sulfomucins) or sialic acid containing mucins, referred to as sialomucins (Filipe, 1979). Mucin distribution

throughout the GIT may differ depending on the type of secretory cell, intestinal region, diet, presence of pathological conditions and phylogeny (Scillitani *et al*., 2007).

The three species investigated in this study are the Arabian spiny mouse (*Acomys dimidiatus*), King jird (*Meriones rex*) and Libyan jird (*Meriones lybicus*). All three species are found in semi-arid or desert regions of the Saudi Arabian Peninsula. The diet of the *Meriones* species consists of shoots of bushes, green vegetation, roots, bulbs and fruit with *M. lybicus* consuming seeds in addition to the above (Harrison and Bates, 1991; Nowak and Paradiso, 1983). On the other hand, *A. dimidiatus* is mainly a granivore but also consumes small numbers of invertebrates (Varty, 1990). Over the years, several studies mapped the distribution of intestinal mucin-secreting cells in various mammalian (Sheahan and Jervis, 1976; Filipe, 1979; Coetzee *et al.*, 1995; Kotzè and Coetzee, 1994; Boonzaier *et al*., 2013) and fish species (Tibbetts, 1997; Cao and Wang, 2009). Despite this, mucin distribution in normal tissue is still an area which needs further investigation (Hattrup and Gendler, 2008).

It is hoped that a better understanding of the quality of the mucin layer and the resulting biofilm of the normal GIT may be gained by studying the distribution of mucin-secreting cells in a wide variety of mammalian species from different phylogenies and life histories. The aim of the present study is therefore to use histochemical methods to determine and compare the distribution of various mucin-secreting cells in the GIT of three species of small rodents which live under similarly water scarce, hot desert climates but consume different diets.

MATERIALS AND METHODS

Specimens

Paraformaldehyde (PFA) perfusion fixed intestinal tracts of *A. dimidiatus*, (n=5); *M. rex*, (n=5) and *M. lybicus*, (n=4) were used for the study. These animals had been captured in the wild in Saudi Arabia with permission from the Saudi Wildlife Authority (SWA) and were euthanized to obtain tissues other than the GIT for unrelated studies. The *M. rex* and *A. dimidiatus* species were captured in the Raydah Protected Area near Abha city in the south of Saudi Arabia, while the *M. lybicus* animals were captured in Unizah and Riyadh. Both the animal ethics committees of the University of Stellenbosch (US) and the University of Pretoria (UP) granted ethical approval for the use of these specimens for the present study.

Scientific and common names, mean body weight and sex of the specimens are described in table 1.

Table 1. List of species used for this study, including common names, sample size, mean body mass and the sex of the animals (±Standard deviation).

Species	Common name	n	Mean body weight (g)	Number of male specimens	Number of female specimens
A. dimidiatus	Eastern spiny mouse	5	34.46 (±12.99)	3	2
M. rex	King jird	5	168.80 (±35.64)	3	2
M. lybicus	Libyan jird	4	64.75 (±21.22)	3	1

Sample collection and histochemical methods used

Sample collection from the GIT, histochemical procedures, measurements, quantification of the mucin cell types and statistical analyses, are based on the procedures as described in Boonzaier et al. (2013). Briefly, tissue samples taken from the glandular region of the stomach, the proximal area of the duodenum (in order to visualize Brunner's glands), the jejunum, the ileum, the caecum and the proximal and distal colon were subjected to routine histological processing through to wax. For each region of the GIT, serial sections were mounted in sequence onto three separate slides (one for each staining method) with four sets of serial cross-sections per slide. This ensured that individual sections on any one slide were approximately 16–32 μm apart. For the Alcian Blue-Periodic Acid Schiff (AB-PAS) staining technique, 5 μm thick sections were cut while sections of 8 μm were used for the High Iron Diamine-Alcian Blue (HID-AB) stain.

Slides were stained with AB-PAS in order to detect neutral (magenta), acid (blue), mixed (purple), and HID-AB to distinguish between sialo (blue–green) and sulfomucin (black) secreting cells (Bancroft and Gamble, 2008). For AB-PAS staining, exclusively neutral and acid mucous secreting goblet cells will from here on be referred to as “neutral” and “acid”, while neutral and acid mucus in a single cell are referred to as “mixed”. The different phenotypes were identified by comparing the colour of the cells to a colour wheel on the same computer that was used to count the different mucin secreting cells (Fig. 1). Exclusively sialic acid-containing mucous secreting goblet cells will be referred to as “sialomucins”, exclusively sulfate-containing mucous secreting cells as “sulfomucins” and sulfo- and sialomucins in a single cell are referred to as “mixed”.

Fig. 1. Colour wheel used in the present study to identify and compare the colours of the different mucins phenotypes.

Histochemical methods used

All components used for both stains were freshly made prior to staining. The combined AB-PAS stain can differentiate between neutral and acid mucin secreting cells (Spicer and Meyer, 1960 and Spicer, 1960). Briefly, sections were deparaffinising in xylol, rehydrated to water and immersed in 1% Alcian Blue in 3% Acetic Acid (1% Alcian Blue in 3% Acetic Acid, pH 2.5, (8GX, Colour Index 74240, product 34089, Gurr Microscopy Materials, BDH Chemicals Ltd., Poole, England)) for 15 min. Thereafter, sections were rinsed in running tap water for 2 min, further rinsed in distilled water, and oxidized in 1% Periodic Acid solution (SAARCHEM 4946180, UNIVAR, Muldersdrift, RSA) for 10 min. After rinsing in distilled water, sections were immersed in Schiff's reagent for 15 min, rinsed in lukewarm tap water for 8 min, counterstained in Mayer's Haematoxylin solution (SAAR2822001LC, Merck Chemicals (Pty.) Ltd., Gauteng, RSA) for 1 min, washed in tap water for 3 min, dehydrated and mounted in DPX.

The HID-AB stain differentiates between sulfo- and sialic acid-containing mucous secreting goblet cells (sialomucins), which stain black and blue–green, respectively. HID-AB staining was unsuccessfully performed several times using the protocol described by Spicer and Meyer (1960) and Spicer (1960) on rat colon as control tissue and tissue samples from the species studied here. The lack of Alcian Blue staining might have been due to the fixation procedure used for these samples or masking by the long immersion in HID. For this reason, the original protocol was adapted by introducing a second Alcian Blue staining step and shortening of the HID staining period to avoid masking of cells. The adapted method was as follows: sections were deparaffinized and hydrated to distilled water, stained in the Alcian Blue solution (1% Alcian Blue in 3% Acetic Acid, pH 2.5, (8GX, Colour Index 74240,

product 34089, Gurr Microscopy Materials, BDH Chemicals Ltd., Poole, England)) for 30 min, and rinsed in running water. Slides were immersed in the High Iron Diamine solution (120 mg N, *N*-dimethyl-meta-phenylenediamine dihydrochloride (21922-5G, 13614 TE, Sigma–Aldrich, Switzerland), 20 mg N, *N*-dimethyl-para-phenylenediamine dihydrochloride (GA 21629, D4139 10 g, Sigma–Aldrich, Switzerland), 50 ml distilled water and 1.4 ml ferric chloride (SAAR2340530EM, UN2582, Merck Chemicals (Pty.) Ltd., Gauteng, RSA) (60% BDH solution)) solution for 30 min at room temperature, followed by quick washing with running water. This was followed by staining with the Alcian Blue solution for 30 min. Slides were washed in tap water and nuclei stained with Neutral Red solution (0.5% Aqueous Neutral Red, Vital & Fluorochrome, 15622, Gurr Ltd., London, England) for 30 s, followed by washing, dehydrating and mounting in DPX-Aldrich.

Imaging and quantitative analysis

In the stomach and Brunner's glands, mucous secreting cells were not quantified due to the fact that individual cells were not easily distinguishable. Therefore, only the general staining characteristics are described. In the rest of the intestinal tract, the relative proportions and distribution of mucous secreting goblet cells were quantified in the following manner: tissue cross-sections were studied with 2.5× and 20× objective lenses on a Zeiss Axioxskop2 light microscope equipped with a Axiocam digital camera and AxioVision (AxioVs40) software (Version 4.7.2.0). The images taken with the 2.5× objective lens were used to examine the whole tissue section and to measure the outer circumference of each intestinal region. This was used to ensure that the total area of the images taken at a higher magnification would cover at least 50% of the tissue section. Using the 20× objective, images were taken from every alternative section on the slide, and each section was divided into four equal quadrants where representative images were taken. In each quadrant, images were taken from the surface epithelium (SE) towards the crypts (C) of the tissue and were stitched with Hugin stitching software (Version 2011.0.0.0fd3e119979c) to show the full thickness of the mucosa. Furthermore, every second field of view across the entire length of the tissue section was photographed. The image analysis software, NIS Elements Basic Research program (Version 3.10) was used to outline and measure the SE and C areas, respectively. Within each demarcated area (SE and C) in all regions of the GIT (except the stomach), mucin secreting goblet cell types were counted and the data normalized to reflect the relative cell numbers per mm^2 of each intestinal region. To determine the specific mucin cell types for the entire intestine for each animal, the cell counts and areas from each demarcated region were totaled separately and the data normalized to reflect the total numbers of each mucin cell type per

mm^2. The total area (SE + C) was calculated as the sum of the demarcated areas in the SE and C areas.

Statistical data analysis

All data collected in the NIS Elements Basic Research program (Version 3.10) was exported into Microsoft Excel sheets (Version 7) and statistical analysis was done in Statistica data analysis software system (Statistica Version 11. Copyright © StatSoft, 2007 Southern Africa - Research (Pty.) Ltd.). The number of each type of mucin-secreting goblet cell was counted in the E, C and E+C areas of the different regions of the GIT (excluding the stomach). For all data, the normal probability tests were performed to calculate the mean, standard deviation and standard error. Analyses were done using F-test ANOVA (Analysis of variance) and Fisher's Least Significant Difference (LSD), which is used for multiple comparisons. Statistically significant differences were determined at $p \leq 0.05$ and p-values ≤ 0.01 was deemed highly significant (Reed *et al.*, 2007).

RESULTS

Descriptive results of mucin secreting cells in the stomach and Brunner's glands

In AB/PAS stained sections, the surface epithelium (SE), mucous neck cells and gastric glands of the stomach of all three species showed only mixed mucins (mixture of acid and neutral mucins) (Fig. 2A). Cells containing both sulfo- and sialomucins were only present in the proximal parts of the gastric glands for all three species (Fig. 2B). Their proportion was similar to that of the mixed mucin phenotype seen in the AB-PAS stained sections. Cells secreting exclusively sialomucins in the deep parts of the gastric glands were more common in *M. libycus* compared to the other two species (not shown).

Mixed (neutral and acid) mucin secreting cells were the most prominent cell type in Brunner's glands in all three species (Fig. 2C). A small number of cells containing mixed sulfo- and sialomucins were present in *M. rex* and to a lesser degree in *M. libycus*. Although cells in Brunner's glands of *A. dimidiatus* contained mixed (neutral and acid) mucins, no sulfo- or sialomucins were detected in this species.

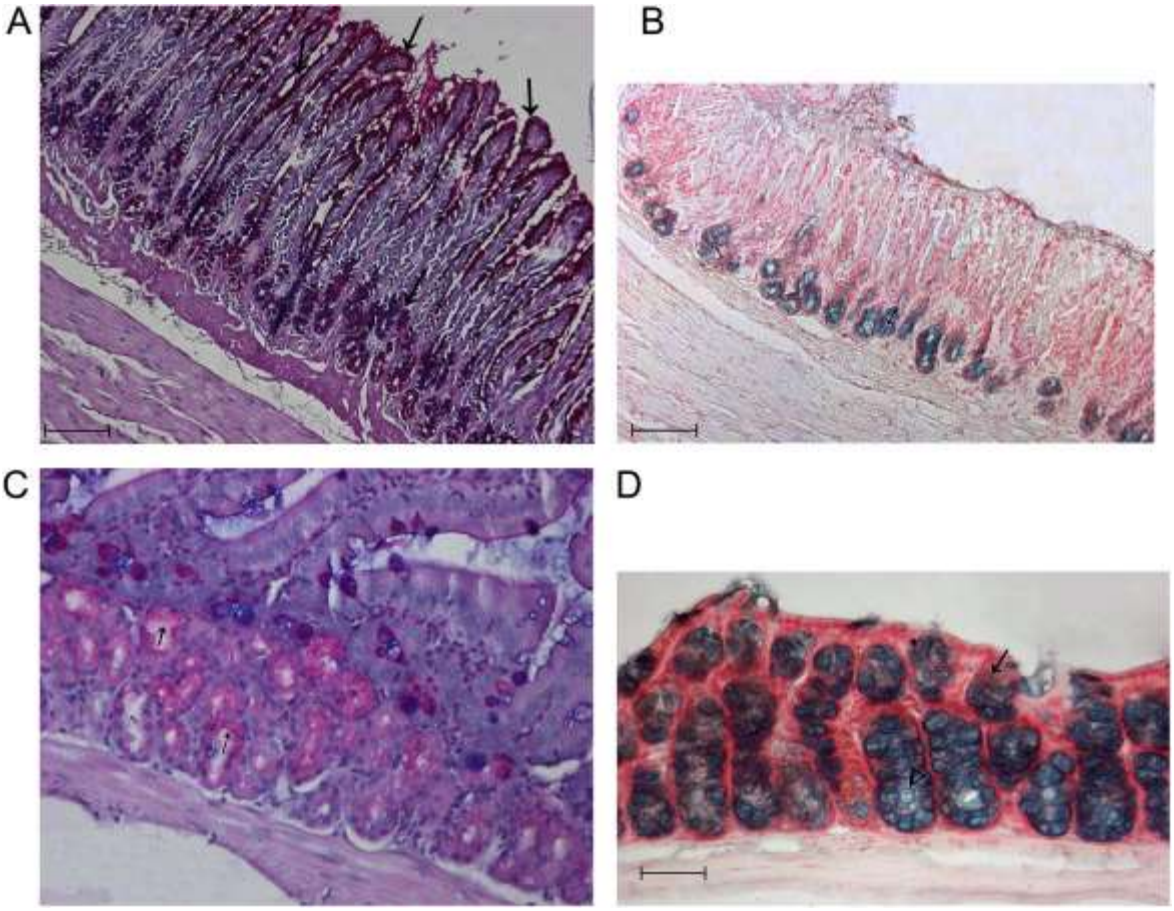

Fig. 2. Composite images of mucin distribution in different regions of the GIT determined by AB-PAS (images A, C), and HID-AB (image B, D) staining, respectively. (A) Fundus of the stomach of *M. rex* showing surface mucous cells containing mixed (neutral and acid) mucins stained purple (arrows), mucous neck cells containing neutral and mixed mucins (arrowhead) and mixed mucins in the gastric glands (chevron) (Bar = 100 µm). (B) Fundus of the stomach of *M. rex* where the gastric glands contained a mixture of sulfo- and sialomucins and stained black/brown (arrowhead) with occasional cells containing sialo-acid mucins exclusively (arrow) (Bar = 100 µm). (C) Brunner's glands of *A. dimidiatus* containing neutral (stained magenta) mucins (Bar = 50 µm). (D) Proximal colon of *M. libycus* where sialo- (arrowhead) and sulfomucin (arrow) secreting cells stained blue and black/brown, respectively and mixed sialo- and sulfomucins (chevron) (Bar = 20 µm).

Quantification of goblet cells

The relative numbers (expressed as log 10) of the different mucin secreting goblet cells in the surface epithelium (SE) and crypt (C) areas as well as the total number of goblet cells in entire epithelium (SE + C area) of each region of the GIT (the stomach is excluded) are shown in Table 2 and Table 3 for AB-PAS and HID-AB staining, respectively. In addition, the total number of mucin secreting goblet cells (all mucin types) per mm^2 is shown for the different GIT regions from all three species as well as the total number of each mucin type of goblet cell in the entire GIT. This illustrates the general distribution of mucin secreting goblet cells in each region of the GIT's of all three species.cells

Distribution of neutral and acid mucins

The The LSD and *post hoc* tests show that the total number of goblet cells in sections stained by AB-PAS in the entire GIT is significantly different between all species with *A. dimidiatus* having the greatest number of cells mm^2 (6.24 ± 0.05 *vs* 5.75 ± 0.04 and 4.66 ± 0.05 for *M. rex* and *M. libycus* respectively) ($p < 0.001$ between all species) (Table 2).

For neutral mucins, few significant differences were shown in the number of goblets cells per mm^2 throughout the GIT (SE and C) with the greatest number in *M. libycus*(2.42 ± 0.22) and the least in *M. rex* (1.44 ± 0.20) ($p = 0.003$). In the entire epithelium (SE and C) the ileum of *M. libycus* contained significantly more neutral mucins than *M. rex*($p = 0.026$) (Table 2 and Fig. 3B). The greatest number of neutral mucin-containing goblet cells in any one region of the GIT occurred in the SE of the distal colon and the C region of the caecum in *M. libycus* (2.66 ± 0.41 and 2.82 ± 0.50, respectively) (Table 2).

In A. dimidiatus, acid mucin secreting goblet cells formed the greatest proportion in the SE + C and C regions of the distal colon and duodenum. By contrast, acid mucins formed the greatest proportion of goblet cells in the proximal and distal colon of in *M. libycus* and*M. rex*, respectively (Table 2). For acid mucin secreting cells in the SE area, *A. dimidiatus*(1.11 ± 0.21) contained significantly more cells than *M. rex* (0.26 ± 0.20) ($p = 0.008$).

Mixed mucin secreting goblet cells were shown to be the predominant cell type in all three species when using the AB-PAS stain as significantly greater numbers of mixed than either exclusively acid or neutral mucin secreting cells were observed in the SE ($p < 0.001$ for all three species), C ($p < 0.001$ for all three species) and SE + C ($p < 0.001$ for all three species) (Table 2; Fig. 3C). The number of mixed mucin secreting goblet cells for the SE + C areas increased steadily towards the ileum and decreased in the caecum in all three species, although mixed mucin secreting goblet cells still dominated over acid mucin secreting goblet cells in the caeca of all three species (Fig. 3C; Table 2). Significant differences were noted in the distribution of mixed mucin secreting cells in the caeca of the *Meriones* species and *M. libycus* and *A. dimidiatus.* In all three areas measured, the distal colon of *M. libycus*, *M. rex* and *A. dimidiatus* contained the greatest number of mixed mucin secreting cells.

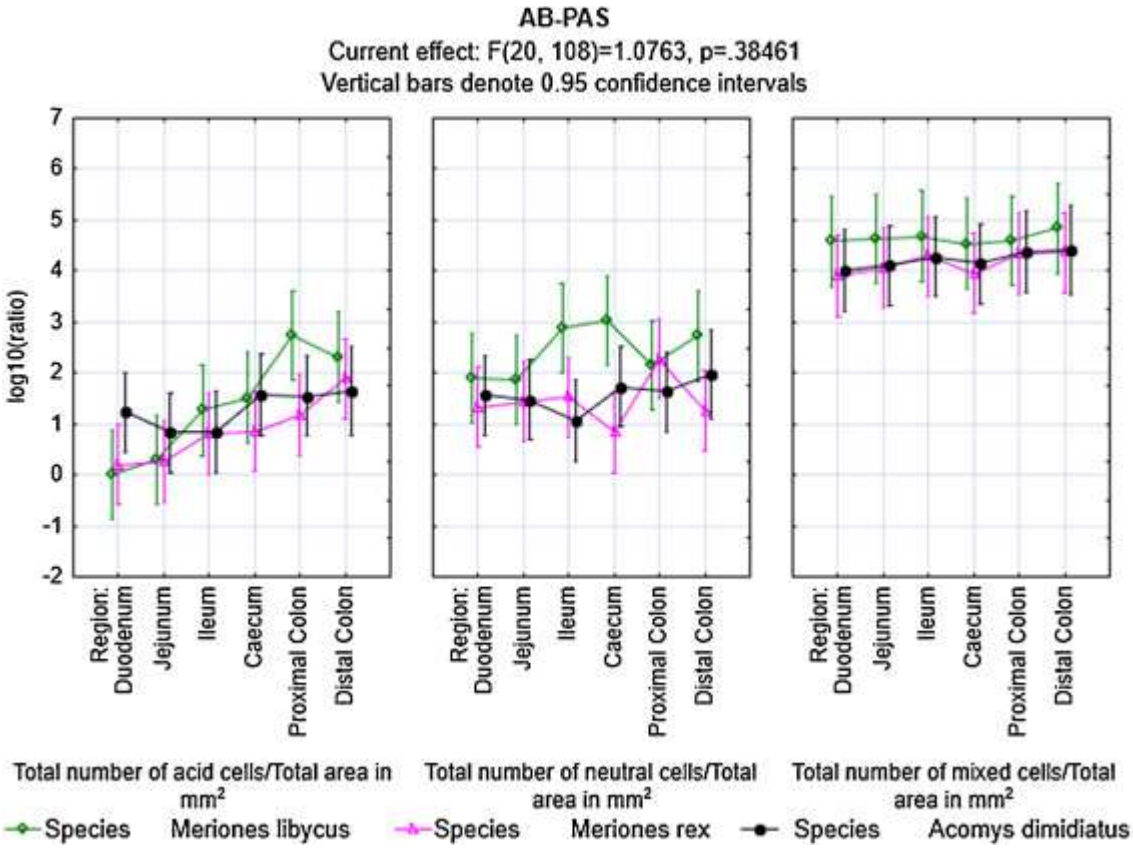

Fig. 3. The distribution of acid, neutral and mixed mucin secreting goblet cells as determined by AB-PAS staining in the GIT of *A. dimidiatus*, *M. rex* and *M. libycus.* Cell counts have been normalized to the same unit area (1 mm^2) for all samples analysed. The data points represent the mean of the total number of cells in both the surface epithelium and crypt combined per unit area (mm^2). Error bars indicate the standard error of the mean. All values were normalized.

(A) The number of acid mucin secreting goblet cells per mm^2 in the surface epithelium and crypts.

(B) The number of neutral mucin secreting goblet cells per mm^2 in the surface epithelium and crypts and

(C) The total number of mixed (combined neutral and acid in the same cell) mucin secreting goblet cells per mm^2 in the surface epithelium and crypts.

Distribution of sialo- and sulfomucins

A significant difference was noted between the total numbers of acid mucin secreting cells (ALL types) between the duodenum to the distal colon of all three species studied here ($p = 0.049$ for *M. libycus* and $p < 0.001$ for both *M. rex* and *A. dimidiatus*) when using HID-AB staining (Fig 2D).

The overall trend observed with sialomucin distribution in the GIT of *M. libycus* and C area of *M. rex* indicated an increase towards the distal GIT, except in the proximal colon where a decrease of sialomucin secreting goblet cells was observed (Table 3). *A. dimidiatus* (4.66 ± 0.29) presented with a significantly greater total number of sialomucin secreting goblet cells in the SE + C area compared to *M. libycus* (1.59 ± 0.32) and *M. rex*(1.03 ± 0.29) ($p < 0.001$ in both instances) (Fig. 4A, Table 2). In the *Meriones* species, sialomucin secreting cells were more common in the proximal (*M. rex*) and distal (*M.*

Table 2. The relative numbers of neutral, acid and mixed mucin-secreting goblet cells per mm^2 in the intestines of *A. dimidiatus*, *M. rex*, and *M. lybicus* determined by AB-PAS staining. All values were normalized. The values reflected in the table is the mean of the total number of cells counted for all animals in each group for *A. dimidiatus* (n=5), M. *rex* (n=5), and *M. lybicus* (n=4).

Species	**GI region**	**Total cells/epithelial area in mm^2**			**Total cells/crypt area in mm^2**			**Total cells/area in mm^2**		
		Neutral	Acid	Mixed	Neutral	Acid	Mixed	Neutral	Acid	Mixed
A. dimidiatus	Duodenum	78,92	38,92	7823,10	491,82	140,57	16286,98	170,55	75,65	10342,82
	Jejunum	221,24	45,81	11924,30	336,32	153,83	16627,01	237,15	104,98	13047,22
	Ileum	417,40	88,18	17622,24	613,3	16,97	24883,26	466,53	52,37	19729,14
	Caecum	145,18	141,59	10347,35	199,65	180,94	21683,96	156,22	172,27	14202,81
	Proximal Colon	81,92	51,27	18129,06	1060,96	15,98	31694,70	524,67	39,28	24048,64
	Distal Colon	142,25	87,75	20618,29	1141,11	279,63	32766,68	596,87	203,06	26287,40
M. rex	Duodenum	42,37	1,85	6361,76	94,78	0,00	16264,93	46,03	1,74	8190,11
	Jejunum	33,38	0,00	9987,44	113,29	14,30	19610,40	52,67	3,68	11499,77
	Ileum	133,95	18,16	18136,84	29,49	67,21	29253,19	98,97	40,58	20343,40
	Caecum	8,11	305,71	6502,24	45,62	189,88	19074,45	15,45	198,41	9449,95
	Proximal Colon	157,22	0,00	19055,54	430,78	196,87	29669,00	293,13	84,59	23310,03
	Distal Colon	158,30	11,36	22353,95	45,02	2221,26	28763,53	95,27	1138,50	24636,25
M. lybicus	Duodenum	211,65	0,00	11170,66	205,53	0,00	27434,19	417,18	0,00	38604,85
	Jejunum	445,12	3,92	17495,19	412,91	0,00	26758,23	858,04	3,92	44253,42
	Ileum	956,02	41,89	19727,13	1146,99	126,25	28965,08	2103,01	168,15	48692,22
	Caecum	398,80	0,00	10385,75	668,31	691,39	25043,98	1067,11	691,39	35429,73
	Proximal Colon	100,03	137,91	23594,20	110,42	473,10	39831,02	210,45	611,01	39831,02
	Distal Colon	470,78	211,68	29108,22	66,97	1470,80	40474,61	537,75	1682,48	69582,82

* Regions that have less than 0.001 cells/mm^2 are stated as 0.00 in the table

Table 3. The relative numbers of sialo-, sulfated and mixed sulfo- and sialomucin-secreting goblet cells per mm^2 in the intestines of *A. dimidiatus*, *M. rex* and *M. lybicus* determined by AF-AB and HID-AB staining. All values were normalized. The values reflected in the table is the mean of the total number of cells counted for all animals in each group for *A. dimidiatus* (n=5), M. *rex* (n=5), and *M. lybicus* (n=4).

Species	GI region	Total cells/epithelial area in mm^2			Total cells/crypt area in mm^2			Total cells/area in mm^2		
		Sialo	Sulfo	Mixed	Sialo	Sulfo	Mixed	Sialo	Sulfo	Mixed
A. dimidiatus	Duodenum	24,69	672,52	8896,66	0,00	1662,32	20214,84	206019,00	913,27	11287,03
	Jejunum	14,62	905,98	12499,24	0,00	805,01	18457,48	204615,00	788,89	13650,02
	Ileum	25,16	1570,15	19002,74	0,00	1304,50	24068,85	410054,00	1512,41	20188,93
	Caecum	0,00	1686,03	10704,48	0,00	1374,01	21880,82	451709,00	1529,56	15012,46
	Proximal Colon	82,81	1017,30	21220,30	0,00	966,01	35357,56	196415,00	981,37	28295,61
	Distal Colon	79,85	2107,50	20907,24	0,00	875,36	27709,65	527054,00	1391,00	24708,68
M. rex	Duodenum	7,07	335,05	5785,87	0,00	828,66	9419,51	6,00	421,16	6045,72
	Jejunum	38,19	389,68	9948,05	20,83	717,72	9060,11	33,00	436,48	9663,56
	Ileum	15,64	1062,46	19372,36	31,76	1042,76	17349,91	21,00	948,48	17446,28
	Caecum	29,25	694,04	6635,66	130,71	1415,01	22376,47	75,00	1018,28	12627,77
	Proximal Colon	39,74	1279,47	15130,90	116,98	1418,03	24956,31	83,00	1252,50	19205,35
	Distal Colon	134,54	1391,47	21455,54	335,11	643,96	24548,13	258,00	1012,70	22641,49
M. lybicus	Duodenum	18,48	869,47	8378,93	0,00	1853,23	13184,65	18,00	2722,70	21563,58
	Jejunum	42,19	1046,83	15499,33	26,43	1189,28	17316,62	69,00	2236,10	32815,95
	Ileum	51,57	1651,96	17173,90	26,40	2149,21	20309,25	78,00	3801,17	37483,14
	Caecum	83,01	1752,23	5332,15	103,21	1947,69	20963,95	186,00	3699,92	26296,10
	Proximal Colon	115,25	1809,93	19071,24	170,33	1200,93	38970,16	286,00	3010,85	58041,40
	Distal Colon	443,11	2778,53	26163,53	993,07	1932,06	30819,97	1436,00	4710,58	56983,50

* Regions that have less than 0.001 cells/mm^2 are stated as 0.00 in the table.

libycus) colon than in the small intestinal regions of these species. Throughout the GIT of the three species studied here, the highest number of sialomucin secreting cells in the SE + C area was present in the distal colon. In the SE + C area of the caeca of the*Meriones* species, sulfomucins were more abundant than sialomucins, although this difference was only statistically significant in *M. libycus* ($p = 0.008$). Interestingly, a significantly greater number of sialo- and not sulfomucin secreting cells were noted in the SE + C area of *A. dimidiatus* ($p < 0.001$). The number of sialomucin secreting cells was significantly less than the numbers of other acid cell types in the SE areas for all species studied in all regions of the GIT ($p < 0.001$ for all instances for all three species).

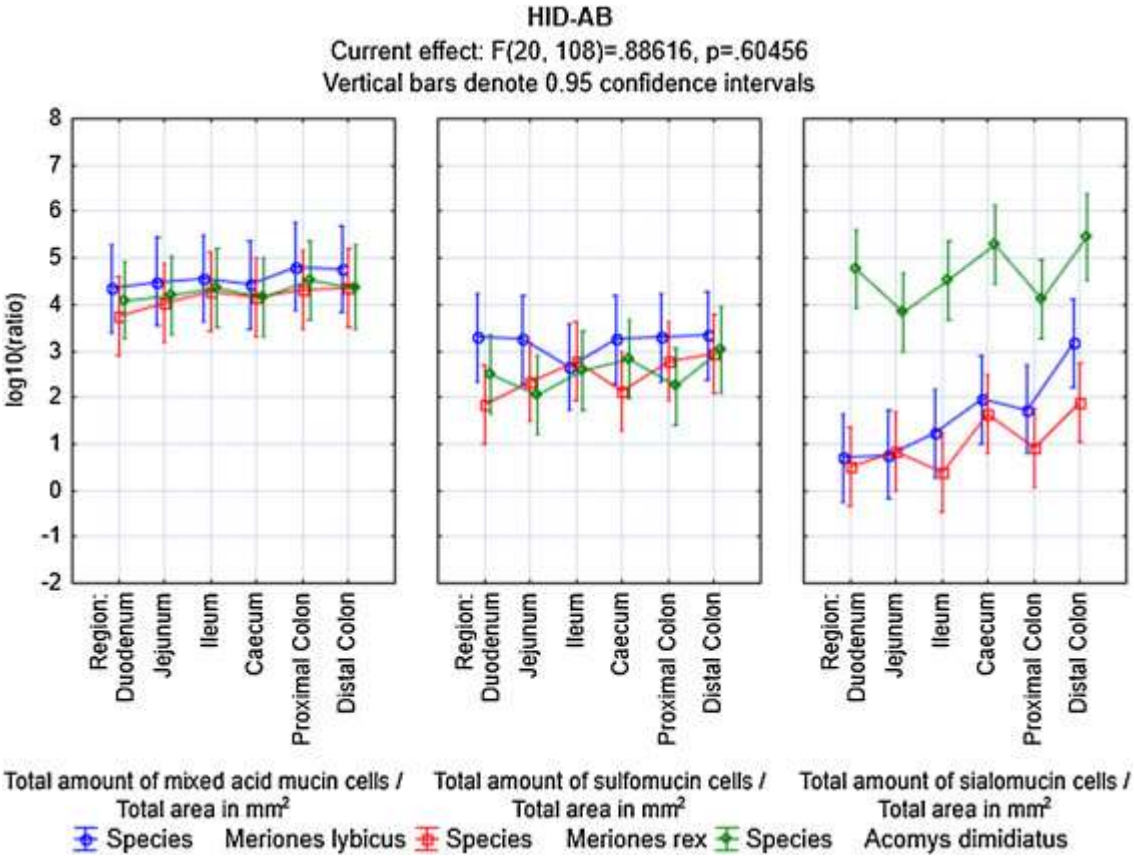

Fig. 4. The distribution of sialo-, sulfo-, mixed and total acid mucin secreting goblet cells were determined by HID-AB staining in the GIT of *A. dimidiatus*, *M. rex* and *M. libycus*. Cell counts have been normalised to the same unit area (1 mm^2) for all samples analysed. The total number of cells per unit area is the mean number of cells per unit area (mm^2). Error bars indicate the standard error of the mean. The total area includes the surface epithelial and crypt regions. All values were normalised.

(A) The number of sialomucin secreting goblet cells per mm^2 in the surface epithelium and crypts,

(B) The number of sulfated mucin secreting goblet cells per mm^2 in the surface epithelium and crypts,

(C) The number of mixed (combined sialo and sulfated mucins in the same cell) mucin secreting goblet cells in the surface epithelium and crypts per mm^2.

In both the SE and SE + C areas of *M. rex* sulfomucin secreting goblet cells increased towards the distal GIT, evident by the significantly greater number of these cells in the distal colon than the duodenum ($p = 0.006$ for SE and $p = 0.025$ for SE + C) (Fig. 4B, Table 3). The

only statistically significant difference for the distribution of sulfomucin secreting goblet cells was present in the C ($p = 0.014$) and SE + C ($p = 0.024$) areas of the duodenum of *M. rex* and *M. libycus* (Table 3). In all three species, the smallest number of total sulfomucin secreting goblet cells were present in different regions of the small intestine (duodenum (*M. rex*), jejunum (*A. dimidiatus*) and ileum (*M. libycus*)).

Goblet cells containing a combination of sialo- and sulfomucins was the predominant acid cell type in all three areas of both *Meriones* species ($p < 0.001$) (Table 3). For the SE + C area, the proximal colon of *A. dimidiatus* and *M. libycus* contained the greatest number of cells containing mixed sialo- and sulfomucins, whereas this was true for the distal colon of *M. rex* (Fig. 4C).

DISCUSSION

Investigation and comparison of the histochemical distribution of mucous secreting cells in the GIT of various mammalian species of different diets and life histories can assistant in the understanding of the quality and composition of the resulting mucous layer and biofilm formed on the surface of the GIT mucosa (Sheahan and Jervis, 1976; Filipe, 1979; Bollinger et al., 2007).

The gastric surface epithelium contained predominantly mixed mucins secreting cells in all species of the current study as well as several rodents and humans (Table 4). In the surface epithelium of the gastric cardia region, traces of sialomucins were present in several rodents (Sheahan and Jervis, 1976) and humans (Filipe, 1979) which is similar to our findings in *M. libycus* (Table 5). However, an absence thereof was noted in the gastric surface epithelium of the other two species studied here. Mucous neck cells were almost exclusively neutral mucous secreting in several species studied by, whereas *A. spinosissimus* and the species of the current study contained both neutral- and mixed mucins. The acid type mucin secreting cells were scarce in the mucous neck cells, in both the present study and in humans (Table 5). The secretion of neutral mucins in the stomach creates a pH gradient that protects the gastric surface against luminal gastric acid and proteolytic digestion by luminal pepsin (Allen & Flemström, 2005).

Table 4. Comparison of the results for neutral, acid and mixed mucins from the present study with relevant literature.

Region	Neutral, acid and mixed mucin cell types					
	Present study		Other studies			
	Cell types[a]	Species	Cell types	Staining method	Species	Reference
Gastric surface epithelium	Mostly mixed	All 3 species	Mostly mixed	AB-PAS	Mouse, rat, hamster, gerbil, guinea pig	Sheahan and Jervis (1976)
			Mostly mixed	AB-PAS	Human	Filipe (1979)
			Mostly mixed	AB-PAS	*Acomys spinosissimus*, *Crocidura cyanea*	Boonzaier et al. (2013)
Gastric mucous neck cells	Neutral and mixed	All 3 species	Neutral	AB-PAS	Mouse, rat, hamster, gerbil, guinea pig	Sheahan and Jervis (1976)
			Neutral	AB-PAS	Human	Filipe (1979)
			Neutral and mixed	AB-PAS	*Acomys spinosissimus*,	Boonzaier et al. (2013)
Cardiac and pyloric glands	Mostly mixed	All 3 species	Neutral	AB-PAS	Human	Filipe (1979)
			Neutral	AB-PAS	Mouse, rat, hamster, gerbil, guinea pig	Sheahan and Jervis (1976)
Brunner's glands	Neutral	All 3 species	Mostly neutral	AB-PAS	Gerbil, guinea pig, rat, hamster, dog, cat, macaques, raccoon, human	Sheahan and Jervis (1976) Schumacher et al. (2004)
			Mostly neutral	AB-PAS	*Acomys spinosissimus*, *Crocidura cyanea* *Amblysomus hottentotus*	Boonzaier et al. (2013)
Small intestine	Mostly mixed	All 3 species	Neutral	AB-PAS	*Acomys spinosissimus*, *Crocidura cyanea*	Boonzaier et al. (2013)
			Neutral	AB-PAS	Humans	Subbuswamy (1971)
	Mostly mixed	All 3 species	Mixed	AB-PAS	Human	Subbuswamy (1971)
			Mixed	AB-PAS	*Acomys spinosissimus*	Boonzaier (2012)
Caecum	Neutral	*M. libycus*	Mostly neutral	AB-PAS	Guinea pigs	Sheahan and Jervis (1976)
			Mostly neutral	AB-PAS	Mice and rats	Sakata and Engelhardt (1981)
Colon	Mostly mixed	All 3 species	Mostly mixed	AB-PAS	*C. cyanea*, *A. hottentotus*, *A. spinosissimus*	Boonzaier et al. (2013)
			Neutral	AB-PAS	Cat	Sheahan and Jervis (1976)
			Acid	AB-PAS	Rabbit	Sheahan and Jervis (1976)

[a]Staining method used: AB-PAS.

Table 5. Comparison of the results for sulfo-, sialo and mixed acid mucins from the present study with relevant literature.

Region	Sialo, sulfo and mixed acid mucin cell types					
	Present study		Other studies			
	Cell types[a]	Species	Cell types	Staining method	Species	Reference
Gastric surface epithelium	Traces of sialomucins	*M. lybicus*	Traces of sialomucins	HID-AB	Mouse, rat, hamster, gerbil, guinea pig	Sheahan and Jervis (1976)
Gastric mucous neck cells	Traces of sialomucins	All 3 species	Traces of sialomucins	PB/KOH/PAS, HID-AB	Human	Filipe (1979)
Cardiac and pyloric glands	Sialomucins	*M. lybicus*	Traces of sialomucins	PB/KOH/PAS, HID-AB	Human	Filipe (1979)
Brunner's glands	Small number of mixed acid	*Merioness*species	Sulfated and sialomucins	Lectin histochemistry	Bison, deer, guinea pig, vole and rabbit	Schumacher et al. (2004)
			Sulfated and sialomucins	HID-AB	African elephant	Kotzé and Coetzee (1994)
	Sulfomucins	*M. lybicus*	Sulfomucins	HID-AB	Mouse, guinea pig, rabbit	Sheahan and Jervis (1976)
	Sialomucins	*A. dimidiatus*	Sialomucins	HID-AB	Gerbil, human	Sheahan and Jervis (1976)
Small intestine	Mixed acid	*M. rex*	Sialomucins	AF-AB and HID-AB	*C. cyanea*, *A. hottentotus*	Boonzaier et al. (2013)
	Sialomucins	*A. dimidiatus*	Equal numbers of sulfo- and sialomucins	AF-AB and HID-AB	*A. spinosissimus*	Boonzaier (2012)
Caecum	Mixed acid	*Merioness*species	Sialomucins	Alcian blue	Mice, rats	Sakata and Engelhardt (1981)
	Sialomucins	*A. dimidiatus*	Sulfomucins	AF-AB and HID-AB	*A. spinosissimus*	Boonzaier et al. (2013)
			More sulfomucins than sialomucins	HID-AB	African elephant	Kotzé and Coetzee (1994)
			Sialomucins	AF-AB and HID-AB	*A. hottentotus*	Boonzaier et al. (2013)
Colon	Mixed acid	*Merioness*species	Mixed acid	AF-AB and HID-AB	*C. cyanea*	Boonzaier et al. (2013)
			Sulfomucins	HID-AB	Mouse, rat, hamster, gerbil, guinea pig, rabbit, cat, dog, rhesus monkey, baboon, human	Sheahan and Jervis (1976)

[a] Staining method used: HID/AB.

The three species chosen for this study have hemiglandular stomachs where the non-glandular portion of the stomach is separated from the glandular part by a limiting fold or ridge (Walters et al., 2014). The pyloric glands of the species of the present study contained mostly mixed mucins. This is in contrast to the results on rodents and humans where neutral mucins predominated in the gastric glands (Table 4).

Flemström et al. (1999) reported that the mostly neutral mucous secretions of Brunner's glands are responsible for the maintenance of a neutral pH to protect the duodenal epithelium from gastric hydrochloric acid. In contrast, mixed mucins were the predominant type in the Brunner's glands in the three species under study. The Brunner's glands of both *Meriones* species indicated a small number of mixed acid (sulfo- and sialo) type mucin secreting cells (Table 5), similar to Schumacher et al. (2004) who reported both sulfated and sialomucin secreting goblet cells in the bison, deer, guinea pig, vole and rabbit (Table 5). Both Schumacher et al. (2004) and Kotzé and Coetzee (1994) found exclusively acid mucins to be more abundant than neutral mucins in the Brunner's glands of herbivorous species. As *Meriones* species are herbivores, there seems to be no direct correlation between dietary preferences such as herbivory and the presence or absence of acid mucin secreting cells in the Brunner's glands.

The small intestine of *A. spinosissimus*, *Crocidura cyanea* (Boonzaier et al., 2013) and humans (Subbuswamy, 1971) contains predominantly neutral mucin secreting goblet cells, but this was not the case in the species in the current study, as mixed mucin secreting cells was the predominant type in the small intestine. A trend of exclusively acid mucins secreting cells increasing steadily from the duodenum to the distal colon was noted in *A. spinosissimus* (Boonzaier, 2012) and *M. rex* (present study). Interestingly this trend was not observed in the closely related *A. dimidiatus* of the present study and may be related to the differences in diet between these two species. Sharma and Schumacher (1995) found that the quantity and composition of mucus in the small intestine of the rat is influenced by diet.

Sialomucins are more vulnerable to bacterial degradation than sulfomucins in the small intestine, possibly due to differences in the optimum pH levels of these glycosylated proteins (sulfomucins pH 5.0 and sialomucins pH 7.8). Due to the neutral pH in the small intestine, degradation of sulfomucins is reduced in rats (Hino et al., 2012). Therefor sulfomucins is expected to predominate in the small intestine, as seen in some species studied by Sheahan and Jervis (1976) (Table 5). By contrast, sialomucins dominated in the small intestine of *A. dimidiatus*, human, gerbil, *C. cyanea* and *A. hottentotus* (Table 5), whereas mixed acid mucins predominated in the small intestine of *M. rex.* The distribution of sialomucin secreting cells in the SE + C differed between the *Meriones*species as *M. libycus* presented with more sialomucin secreting cells compared to *M. rex*, which might be due to the dietary differences between these two species as Hino et al. (2012) found that fiber intake increases the production of sialomucins in the small intestine of rats. Both *M. libycus* and *A. dimidiatus* consume mostly seeds, whereas *M. rex* consumes an herbivorous diet (Nowak and Paradiso, 1983, Varty, 1990 and Harrison and Bates, 1991).

In the caeca of all three species studied, mixed mucin secreting goblet cells were most prevalent, similar to that described in humans and *A. spinosissimus* (Table 4). Sakata and Engelhardt (1981) found neutral mucins to be more common than acid mucins in the caeca of rats, guinea pigs and mice, similar to results for *M. lybicus* of the current study. The present study found that the caecum of *A. dimidiatus* and *M. rex* contained equal numbers of acid and neutral mucin secreting goblet cells, as was the case for the rabbit caecum (Sheahan and Jervis, 1976). Boonzaier (2012) reported equal numbers of sulfo- and sialomucin secreting goblet cells in the caecum of *A. spinosissimus*. Sialomucin secreting goblet cells predominated in the caecum of mice and rats studied by Sakata and Engelhardt (1981), the Rhesus monkey studied by Sheahan and Jervis (1976) and*A. dimidiatus* studied here. Contrary to these findings, sulfomucins were more common than sialomucins in the caeca of both *Meriones* species studied here, although mixed acid mucins predominated in the caeca of these species. The high viscosity and acidity of acid mucus enables resistance against the attack of bacterial enzymes (Fontaine et al., 1996 and Deplancke and Gaskins, 2001). This is important in the caecum as it is regarded as a site for bacterial fermentation in herbivores (Forstner, 1978).

The quantities and composition of mucin secreting cells differ between the small- and large intestines due to differences in the commensal GIT microbiota which was reported to increase drastically from the duodenum (ranging from 10^4–10^5 g^{-1}) to the colon (10^{11}–10^{12} cells g^{-1}) in mammalian species. (Machado-Neto et al., 2013 and Tims et al., 2011).

The large intestine contains the greatest number of mucin secreting goblet cells and its secretion protects the intestinal epithelium against harm done by intestinal microorganisms (Machado-Neto et al., 2013). Atuma et al. (2001) found that the human colonic mucosa contained a significantly thicker mucus layer compared to the rest of the GIT. Although the present study did not determine the thickness of the mucous layer a significant increase in the total number of goblet cells per total area was observed in all three species for both staining methods. Similarly, an increase in numbers of mucus secreting goblet cells is present in the colon of the human (Forstner, 1978) and three insectivorous mammalian species (Boonzaier et al., 2013).
Due to the vulnerability of sialomucins to bacterial degradation, sulfomucin secreting cells are expected to dominate in the large intestine where the bacterial load is high (Fontaine et al., 1996, Deplancke and Gaskins, 2001, Hino et al., 2012 and Machado-Neto et al., 2013). This was true in *M. rex* in the present study where the number of sulfomucin secreting goblet cells increased significantly towards the distal GIT. In accordance, Boonzaier et al. (2013) observed significantly higher numbers of sulfomucin secreting cells in the large intestine than the duodenum in *C. cyanea* and *A. spinosissimus*.

Sulfomucins are a crucial source of sulfate for sulfate-reducing bacteria and play a prominent protective role by increasing mucus viscosity (Croix et al., 2011). It is suggested that sulfomucins are able to reduce the rate at which microflora degrade mucins (Robertson and Wright, 1997) as the high sulfate content of sulfomucins decrease its susceptibility to bacterial glycosidases, limiting the rate and extent of mucin degradation (Hino et al., 2012). Sulfomucins predominated over sialomucins in the colon of the African elephant, *A. spinosissimus*, cat, dog, some primates and rodent species, whereas mixed acid mucins predominated in the colon of *C. cyanea* species (Table 5) in accordance to the Meriones species of the present study.

In *A. dimidiatus* (Table 5) and *A. hottentotus* (Boonzaier et al., 2013) sialomucins were abundant in the colon. Negatively charged sialomucins provide protection against the damaging effects of bacteria in the colon by forming a thick and viscous mucus layer (Nikumbh et al., 2012). In *M. libycus* the overall trend observed was a significant increase in the total number of sialomucin secreting goblet cells in the SE + C and SE areas towards the distal GIT, with the exception of the proximal colon where a decrease of sialomucin secreting goblet cells was observed. Both *A. dimidiatus* and *M. libycus*contained significantly greater numbers of total sialomucin secreting goblet cells than *M. rex.* The similarity of sialomucin distribution in these species may possibly be attributed to similarity in diet as both these species mainly consume seeds.

Boonzaier et al. (2013) found an overall increase in the number of total acid (sum of sulfo- and sialomucins) mucin secreting cells from the duodenum to the large intestine in *A. hottentotus*, similar to that seen in *M. rex*. Mixed acid mucin secreting cells were the predominant acid cell type in the GITs of both *Meriones* species studied here. The greatest number of mixed acid mucin secreting cells was present in the proximal colon of three insectivorous species studied by Boonzaier (2012) and *A. dimidiatus* and *M. libycus*studied here. However, for *M. rex*, the caecum contained the greatest number of mixed acid mucin secreting cells.

Dietary composition plays a role in the distribution of different mucin secreting cells (Sharma et al., 1995). Moré et al. (1987) studied the effect of different diets on the composition of jejunal and colonic glycoproteins in pigs and found that even short term dietary changes affected the biosynthesis and chemical composition of mucin molecules. (Montagne et al., 2004 and Sharma et al., 1995). The animals of the present study, namely *A. dimidiatus* (granivore) (Varty, 1990, Nowak and Paradiso, 1983 and Walters et al., 2014) and two *Meriones* species (herbivores) (Harrison and Bates, 1991 and Walters et al., 2014) consumed different diets and as a consequence differences in mucin distribution would therefore be expected. When comparing the distribution of the total number of sialomucin

secreting goblet cells, the greatest number of cells was present in the distal colon of *A. dimidiatus*, as opposed to the duodenum of *A. spinosissimus* (Boonzaier et al., 2013).

Only a small number of sialomucin secreting goblet cells were located in the large intestine of *A. spinosissimus,* the opposite of that seen in *A. dimidiatus*. Although both of these species belong to the subfamily *Acomys*, differences in diet may explain the differences in mucin distribution.

CONCLUSION

The distribution of mucin secreting goblet cells in the GIT of the species studied presented some similarities when compared to other animal species, but variations to the patterns noted by other researchers were also present. The variation seen in the distribution of mucous secreting cells provides further evidence that several factors individually or in combination can influence the distribution of mucins, namely phylogeny, diet, but also the size of the microorganism population in a specific GIT area (Sharma et al., 1995 and Sharma and Schumacher, 1995).

Distribution of mucin secreting cells in the present study is similar to results reported for small mammals not living under particularly dry conditions. As found in this study and others on distantly related species, Sheahan and Jervis (1976) and Boonzaier et al. (2013) found that mixed (neutral and acid) mucin secreting goblet cells were the predominant cell type present in the GIT. It therefore seems that these differences cannot be related to only phylogeny, diet, or the conditions in which these rodents live but rather a combination of these factors.

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