



Protozoa and digestive tract parameters of the impala

Authors:

Dirk G. Booysse¹
Burk A. Dehority²

Affiliations:

¹Department of Anatomy and Physiology, University of Pretoria, South Africa

²Department of Animal Sciences, Ohio State University, USA

Correspondence to:

Dirk Booysse

Email:

dbooysse@op.up.ac.za

Postal address:

Private Bag X04,
Onderstepoort 0110,
South Africa

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Intestinal contents were collected from eight impala at three different localities during the winter hunting season (2005–2009), as well as from another 24 animals from a one-year trial at a game farm called Ditholo (2003–2004). Gas production, protozoa counts and several other physiological parameters were measured from both rumen and caecum or colon contents. Only higher ophryoscolecids and Isotrichidae species of protozoa were counted and identified. *Ostracodinium gracile* was present in all 32 impala. *Eudiplodinium maggii* was present in 31 animals and *Eudiplodinium impalae* and *Epidinium* (either *ecaudatum* or *caudatum*) in 30 animals. *Dasytricha ruminantium* was present in only 11 of the impala. Concentrations of protozoa were correlated with the season of sample collection and highly correlated with the animals living on the game farm. Gas production (mL/g of wet rumen ingesta) was weakly correlated with protozoa concentration but not with the season of collection.

Introduction

A total of 13 protozoan species have previously been reported from the rumen of the impala. Dogiel (1925) listed six protozoan species from impala in Kenya and later, in 1932, he observed five species in Uganda. Four species were the same in both locations and the fifth belonged to the same genus but was identified as a different species (Table 1). In South Africa, Van der Wath and Myburgh (1941) reported the presence of three species in impala rumens and later Kleynhans (1977) recorded the presence of 11 species. Four of the species observed by Kleynhans, *Isotricha prostoma*, *Entodinium parvum*, *Eudiplodinium maggii* and *Dasytricha ruminantium*, were new host records for impala (see Table 1). Dehority and Odenyo (2003) identified protozoa in impala from Kenya only to the generic level and did not find any genera in which species had not previously been observed.

Apart from the counting and identification of protozoa, several other parameters of the digestive tract of the impala (rumen, small intestine, caecum and colon) were also investigated and are reported here for the first time. These include pH and temperature of contents, length of organ, contents weight per organ, gas production, dry matter and density.

Materials and methods

Animals

One female animal was collected from a farm called Karoobult, 100 km north-west of Pretoria (24°43'49.45"S, 27°33'22.88"E), in July 2009, five animals (three male and two female) were from the Loskop Dam Nature Reserve in Mpumalanga province, 200 km east of Pretoria (25°25'51.91"S, 29°19'39.80"E), and two male animals were from the Rietvlei Nature Reserve, 50 km south of Pretoria (25°52'41.17"S, 28°16'17.83"E). In addition, 24 animals (all male) were from a one-year trial from the Ditholo game farm, 60 km north of Pretoria (25°19'43.95"S, 28°19'04.34"E).

Feed

Impala are mixed feeders and therefore graze as well as browse. Feed available to the impala consists of vegetation types called bushvelds. A bushveld can be described as a heavily grassed plain, dotted by dense clusters of trees and tall shrubs. The grasses found here are generally tall and turn yellow or brown in winter, which is the dry season throughout most of South Africa. Sour bushveld consists of grazing fields, mostly comprising grasses like *Eragrostis curvula*, *Eragrostis chloromelas*, *Hyparrhenia hirta* and *Cymbopogon excavatus*. These grasses are fast-growing plants in the summer, but die rapidly in winter, turning hard and tasteless. Sour grass species can, however, recover quite rapidly from overgrazing. Sweet bushveld generally consists of grasses that grow slowly in summer but tend to stay highly nutritious and palatable during the dry season (winter). Sweet grass species include *Digitaria eriantha*, *Panicum maximum* and

**TABLE 1:** Rumen protozoa observed from impala (*Aepyceros melampus*).

Species	Present study, South Africa	Van der Wath and Myburgh (1941), South Africa	Kleynhans (1977), South Africa	Dogiel (1925), Kenya	Dogiel (1932), Uganda
<i>Dasytricha ruminantium</i>	X	-	X	-	-
<i>Isotricha prostoma</i>	-	-	X	-	-
<i>Entodinium dubardi</i>	X	-	X	X	X
<i>Entodinium nanellum</i>	X	-	X	X	X
<i>Entodinium parvum</i>	X	-	X	-	-
<i>Entodinium simplex</i>	-	X	-	-	-
<i>Eudiplodinium impalae</i>	X	-	X	X	X
<i>Eudiplodinium maggi</i>	X	-	X	-	-
<i>Eudiplodinium neglectum</i>	-	-	X	-	-
<i>Ostracodinium gracile</i>	X	-	X	X	X
<i>Epidinium caudatum</i>	X	X	X	-	-
<i>Epidinium ecaudatum</i>	X	X	X	-	-
<i>Epiplastron spinosum</i>	-	-	-	X	X
Total number of species	9	3	11	5	5

Note: Please see the full reference list of the article, Booysse, D.G. & Dehority, B.A., 2011, 'Protozoa and digestive tract parameters of the impala', *Onderstepoort Journal of Veterinary Research* 78(1), Art. #327, 5 pages. doi:10.4102/ojvr.v78i1.327, for more information.

Urochloa mosambicensis. Sweet grasses recover very slowly from heavy impacts like overgrazing. Mixed bushveld is a field composed of both sour and sweet grasses. The veld type in the Rietvlei and Ditholo areas is described as a sourish mixed bushveld, whilst Karoobult represents sour bushveld and the vegetation from the Loskop area is described as a mixed bushveld. In the spring and summer, impala both feed on grass (*Eragrostis*) and browse on trees like *Acacia karoo* and *Acacia caffra*. However, in the winter they are forced to 'low graze' on grass, as little or no browse is available.

Measurements

After shooting, the animals were weighed, the digestive tract removed and the different anatomical sections (rumen, small intestines, caecum and colon) were ligated before separation. Each section was weighed and the temperature, pH and length of the organs were measured and noted. Weights were measured with an electronic scale capable of measuring up to two decimal places and pH was measured with a portable, battery-powered pH meter (Eutech, model EC-PH-10/01N, Singapore). Organ content weights were estimated by weighing the organ full and empty.

Samples for fermentation studies (200 mL) were placed in fermentation vessels and gas production was measured every minute by means of a glass syringe (fitted with a 20-gauge stainless steel needle), inserted through the rubber stopper. Measurements were taken over a period of 45 min. The exact weight of the samples was determined after complete fermentation.

Protozoa

A large sample of ingesta was taken from the rumen and the fluid squeezed out into a 40-mL plastic specimen bottle. For the caecum and colon samples, small aliquots were taken at random from the contents and poured into the 40-mL container. These samples were then transferred to larger marked containers and 100 mL 70% methanol was added

to each. Back at the laboratory, the protozoa samples were washed through sieves as described previously (Booyse, Boomker & Dehority 2010). In essence, the entire sample was washed through a set of sieves with an inner and outer chamber. The sample was poured into the inner sieve (pore size 110 µm) and washed with water. Particulate matter and protozoa passed through to the outer sieve, which had a pore size of 37 µm. The outer sieve was fitted with a draining tap to allow the contents to be drained into a bottle. The washed sample was then allowed to stand for 30 min and the volume adjusted back to 40 mL by decanting the excess water. Two drops of Brilliant Green stain were added to each of the 40-mL samples, which were then allowed to stand for 24 h (Dehority 1984). Three aliquots (0.1 mL each) from each sample were placed onto separate microscope slides and fitted with a cover slip. Protozoa on each slide were counted using a standard light microscope fitted with a Panasonic digital camera (model GP220). Concentrations were determined by multiplying the mean of all three counts by 10, thus giving a count per millilitre.

Results and discussion

Protozoa

Initial examination of the protozoa samples revealed – unexpectedly – a complete absence of *Entodinium* species. Subsequent investigation revealed that these species were washed out through the 37-µm pores. The samples were washed primarily to remove most of the larger plant debris, which interferes with microscopical observation. Because all the samples had been washed, we were able to enumerate only Isotrichidae and the subfamilies Diplodiniinae and Ophryoscolecinae in the family Ophryoscolecidae (larger ophryoscolecid protozoa). However, we were able to shoot an additional impala, collect rumen contents, stain and count protozoa in a 0.1-mL sample directly, without washing through sieves. Our examination revealed the presence of numerous *Entodinium* species in this last animal, which were identified and included with our previous data in Table 1.

TABLE 2: Occurrence and concentration of *Dasytricha ruminantium* and larger ophryoscolecoid protozoa in rumen contents of the impala.

Site	Ophryoscolecoid protozoa								
	Number	Age ^a	Sex ^b	<i>Dasytricha ruminantium</i>	<i>Ostracodinium gracile</i>	<i>Eudiplodinium impalae</i>	<i>Eudiplodinium maggii</i>	<i>Epidinium ecaudatum</i>	<i>Epidinium caudatum</i>
Karoobuld	1	AD	F	-	-	-	-	-	-
Occurrence (%)	-	-	-	-	100	100	-	-	-
Concentration (Protozoa/mL)	-	-	-	-	1200	1600	-	-	-
Rietvlei	2	AD	M	-	-	-	-	-	-
Occurrence (%)	-	-	-	-	100	100	100	50	100
Concentration (Protozoa/mL)	-	-	-	-	3940±820 ^c (3360-4520) ^d	4800±169 ^c (4680-4920) ^d	1350±240 ^c (1180-1520) ^d	80	3660±707 ^c (3160-4160) ^d
Loskop	3	AD	M	-	-	-	-	-	-
	2	AD	F	-	-	-	-	-	-
Occurrence (%)	-	-	-	20	100	100	100	-	80
Concentration (Protozoa/mL)	-	-	-	200	1280±784 ^c (400-2320) ^d	520±440 ^c (80-1120) ^d	450±405 ^c (80-1040) ^d	-	750±810 ^c (40-1880) ^d
Ditholo	24	AD	M	-	-	-	-	-	-
Occurrence (%)	-	-	-	33	100	92	100	63	100
Concentration (Protozoa/mL)	-	-	-	1212±1826 ^c (40-4160) ^d	6469±5420 ^c (230-16640) ^d	1827±1720 ^c (60-6440) ^d	945±751 ^c (40-2760) ^d	200±143 ^c (40-520) ^d	5371±5183 ^c (20-17560) ^d

^a, AD, adult.^b, M, male; F, female.^c, Mean value ± STD.^d, Range of values.

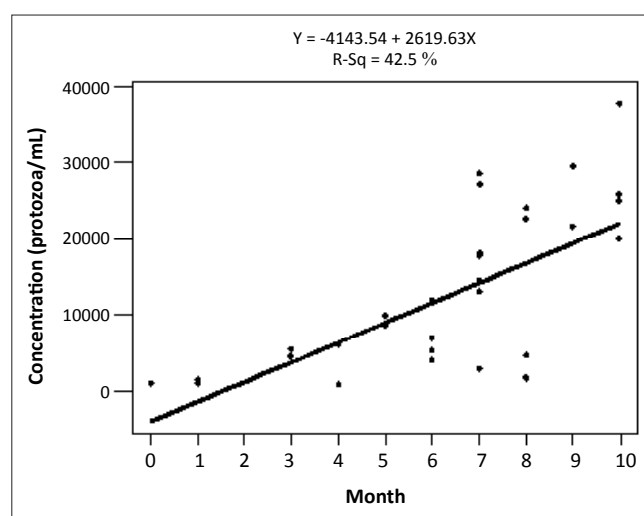
Four earlier reports on protozoa in the impala are also included in Table 1 for comparison. To date, 13 species of protozoa have been observed in the impala rumen. In South Africa, 11 of these were reported by Kleynhans in 1977, nine in the present study and three by Van der Wath and Myburgh in 1941. Dogiel reported a total of five species from impala in Kenya (1925) and Uganda (1932).

Table 2 reflects the occurrence and concentration of *D. ruminantium* and the larger ophryoscolecids (five species) as grouped by the collection site. *Ostracodinium gracile* was present in all 32 animals, *E. maggii* was present in 31 animals, whilst *Eudiplodinium impalae* and *Epidinium caudatum* were found in 30. In contrast, *Epidinium ecaudatum* was present in only 16 animals and *Dasytricha* species were found in nine. Thus, the majority of the larger ophryoscolecids were represented by four species, namely *O. gracile*, *E. maggii*, *E. impalae* and *Ep. caudatum*. The total concentration of all six species averaged about 1.6×10^4 per mL in the 24 animals from Ditholo, 0.32×10^4 per mL in the five from Loskop, 1.38×10^4 per mL in the two impala from Rietvlei and 0.28×10^4 per mL in the single animal from Karoobult.

The caecum and colon contents were mixed, washed as described above, and examined microscopically for protozoa. The results in Table 3 show that only very low concentrations were present in the ingesta from these organs. Presumably, the protozoa would be digested as they move through the abomasum and small intestine; however, some cells are obviously able to pass through unharmed. It is possible that they are embedded in larger food particles and are not exposed to the acidic and enzymatic conditions in those organs. These concentrations are considerably higher than what one author (B.A.D.) has previously observed in domestic ruminants (unpublished).

A possible relationship between the time (i.e. the month when the animal was harvested) and the concentration of the larger protozoa was investigated, taking all 32 animals into consideration. A correlation coefficient (r) of 0.65 was obtained ($r^2 = 0.42$). However, when only the 24 animals from a single location (Ditholo) were used, the correlation coefficient was 0.865 ($r^2 = 0.75$). Figure 1 shows the regression of protozoa concentration for all 32 animals, according to time of harvesting. The first sample was obtained in December and labeled as 0, followed by January as 1, February as 2, etc. This was based on the assumption that the start of the summer growing season would begin in December, when pasture and browse were low, and that feed would become increasingly available during the following months.

It is also of interest that only two genera of the subfamily Diplodiniinae, *Eudiplodinium* and *Ostracodinium*, have been



Months shown on x-axis run from December (0) to October (10).

FIGURE 1: Regression of concentration of the larger protozoa in impala rumen contents according to the month of collection.

**TABLE 3:** Occurrence and concentration of *Dasytricha ruminantium* and larger ophryoscolecoid protozoa in the caecum or colon contents of impala.

Site	Ophryoscolecoid protozoa								
	Number	Age ^a	Sex ^b	<i>Dasytricha ruminantium</i>	<i>Ostracodinium gracile</i>	<i>Eudiplodinium impalae</i>	<i>Eudiplodinium maggii</i>	<i>Epidinium ecaudatum</i>	<i>Epidinium caudatum</i>
Karoobuld	1	AD	F	-	-	-	-	-	-
Occurrence (%)	-	-	-	-	100	100	-	-	-
Concentration (Protozoa/mL)	-	-	-	-	180	50	-	-	-
Rietvlei	2	AD	M	-	-	-	-	-	-
Occurrence (%)	-	-	-	-	-	-	100	-	-
Concentration (Protozoa/mL)	-	-	-	-	-	-	40±0 ^c	-	-
Loskop	3	AD	M	-	-	-	-	-	-
	2	AD	F	-	-	-	-	-	-
Occurrence (%)	-	-	-	-	60	60	60	-	60
Concentration (Protozoa/mL)	-	-	-	-	50±26 ^c	63±29 ^c	83±31 ^c	-	163±132 ^c
	-	-	-	-	(30-80) ^d	(30-80) ^d	(50-110) ^d	-	(20-280) ^d
Ditholo	24	AD	M	-	-	-	-	-	-
Occurrence (%)	-	-	-	4	75	42	42	8	50
Concentration (Protozoa/mL)	-	-	-	60	88±84 ^c	61±70 ^c	38±30 ^c	15±7 ^c	58±50 ^c
	-	-	-	-	(30-360) ^d	(10-220) ^d	(20-120) ^d	(10-20) ^d	(10-160) ^d

^a, AD, adult.^b, M, male; F, female.^c, Mean value ± STD.^d, Range of values.**TABLE 4:** Physiological parameters of the impala digestive tract and gas production by rumen, caecum and colon contents.

Site	Organ	Physiological parameters							
		Average animal weight (Kg)	Number	Age ^a	Sex ^b	pH	Total organ contents (grams)	Dry matter (%)	Average gas production in wet organ content (mL/g)
Karoobuld		43	1	AD	F	-	-	-	-
	Rumen	-	-	-	-	6.43	1764	12.40	0.18
	Small intestine	-	-	-	-	5.95	901	-	-
	Cecum	-	-	-	-	7.50	200	13.90	0.15
	Colon	-	-	-	-	7.13	202	12.80	0.40
Loskop		77.8±8.61 ^c	4	AD	M	-	-	-	-
		(69.19-86.41) ^d	1	AD	F	-	-	-	-
	Rumen	-	-	-	-	5.94±0.11 ^c	4890±1238 ^c	8.54±1.84 ^c	0.17±0.14 ^c
		-	-	-	-	(5.83-6.05) ^d	(3651-6128) ^d	(6.70-10.38) ^d	(0.03-0.31) ^d
	Small intestine	-	-	-	-	6.99±0.3 ^c	950±356 ^c	-	-
		-	-	-	-	(6.69-7.29) ^d	(594-1306) ^d	-	-
	Cecum	-	-	-	-	6.66±0.22 ^c	483±364 ^c	8.25±1.89 ^c	0.09±0.06 ^c
		-	-	-	-	(6.44-6.88) ^d	(119-846) ^d	(4.87-8.25) ^d	(0.03-0.16) ^d
	Colon	-	-	-	-	6.74±0.05 ^c	360±194 ^c	2.96±4.08 ^c	0.08±0.06 ^c
		-	-	-	-	(6.69-6.79) ^d	(166-554) ^d	(1.88-4.04) ^d	(0.02-0.14) ^d
Rietvlei		42.5±3.54 ^c	2	AD	M	-	-	-	-
		(39-46.04) ^d	-	-	-	-	-	-	-
	Rumen	-	-	-	-	6.6±0.13 ^c	5587±1756 ^c	12.89±1.40 ^c	0.18±0.10 ^c
		-	-	-	-	(6.46-6.73) ^d	(3828-7345) ^d	(11.49-14.29) ^d	(0.09-0.28) ^d
	Small intestine	-	-	-	-	7.85±1.34 ^c	778±219 ^c	-	-
		-	-	-	-	(6.51-9.18) ^d	(558-997) ^d	-	-
	Cecum	-	-	-	-	7.46±0.57 ^c	236±11 ^c	13.18±2.37 ^c	0.20±0.06 ^c
		-	-	-	-	(6.88-8.03) ^d	(225-247) ^d	(1.18-15.54) ^d	(0.13-0.26) ^d
	Colon	-	-	-	-	7.75±0.37 ^c	291±83 ^c	3.78±1.45 ^c	0.11±0.02 ^c
		-	-	-	-	(7.38-8.12) ^d	(208-374) ^d	(2.33-5.22) ^d	(0.08-0.13) ^d
Ditholo		61.33±8.39 ^c	24	AD	M	-	-	-	-
		(52.94-69.72) ^d	-	-	-	-	-	-	-
	Rumen	-	-	-	-	5.74±0.32 ^c	4007±1169 ^c	11.23±2.19 ^c	0.38±0.08 ^c
		-	-	-	-	(5.24-6.37) ^d	(1800-6234) ^d	(9.04-13.41) ^d	(0.24-0.57) ^d
	Small intestine	-	-	-	-	7.16±0.71 ^c	988±837 ^c	-	-
		-	-	-	-	(6.45-7.86) ^d	(162-1835) ^d	-	-
	Cecum	-	-	-	-	6.81±0.26 ^c	212±84 ^c	8.71±2.45 ^c	0.27±0.21 ^c
		-	-	-	-	(6.55-7.07) ^d	(127-296) ^d	(6.26-11.15) ^d	(0.06-0.48) ^d
	Colon	-	-	-	-	6.72±0.51 ^c	189±86 ^c	7.70±3.28 ^c	0.25±0.08 ^c
		-	-	-	-	(6.21-7.23) ^d	(102-275) ^d	(4.44-10.97) ^d	(0.16-0.33) ^d

^a, AD, adult.^b, M, male; F, female.^c, Mean value ± STD.^d, Range of values.



found in the impala. Species of *Diplodinium*, *Metadinium*, *Enoploplastron*, *Elytroplastron* and *Polyplastron* occur in many of the other wild ruminants in Africa (Dehority & Odenyo 2003; Dogiel 1925, 1932; Kleynhans & Van Hoven 1975; Van Hoven 1975, 1978, 1983).

Physiological parameters

Table 4 presents information on the collection site, age, sex, organ pH, weight of the organ contents, percentage dry matter, density and gas production from organ contents, incubated *in vitro* (gas production was not measured with small intestine ingesta). Except for the animal from Karoobult and the two from Rietvlei, rumen pH values averaged below 6.0. This value would be expected in grazing ruminants, as demonstrated in the study by Dehority and Tirabasso (2001), where sheep were fed 1, 6, or 24 times per day. Rumen dry matter values fell within the normal reported range of 10% – 13% (Dehority 2003).

The aim of measuring gas production was to evaluate whether this parameter was associated with protozoal concentrations. When gas production was correlated with numbers of larger protozoa, the correlation coefficient was 0.31, indicating little or no association. This is not entirely unexpected, because a number of factors would be involved. Probably the most critical would be the amount of available substrate in the rumen contents, regardless of the concentration of protozoa and bacteria. Gas production from rumen, caecum and colon contents was greater in the animals on the game farm, suggesting that more substrate was available in the ingesta from these animals than from those living in the bushveld. In future studies it would be desirable to measure fermentation capacity as described by El-Shazley and Hungate (1965), where additional substrate is added.

Temperature of organ contents ranged from 30 °C to 41 °C for the rumen, between 11 °C and 28 °C for the small intestine, between 17 °C and 31 °C for the caecum and between 13 °C and 30 °C for the colon. Length of the organs ranged from 281 mm to 570 mm for the rumen, 6218 mm to 13 852 mm for the small intestine, 162 mm to 652 mm for the caecum, and 190 mm to 1411 mm for the colon (data not shown).

Conclusion

The number of genera and species of protozoa occurring in the impala is limited compared to other African wild

ruminants and domesticated cattle and sheep (Booyse & Dehority in press; Dehority & Odenyo 2003; Dogiel 1932; Kleynhans & Van Hoven 1976; Van Hoven 1975, 1978, 1983). Only four species of *Entodinium*, two genera of Diplodiniinae and two species of *Epidinium* have been observed.

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Authors' contributions

D.G.B. collected the samples, was responsible for all the measurements and conducted the microscopic studies. B.A.D. assisted with identification of the species, compilation of the data and writing the manuscript.

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