Short communication

Protozoa population and types in the forestomach of the Arabian camel (*Camelus dromedarius*) associated with diet changes

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Abstract

Enumeration of protozoa of the dromedary camel forestomach and the effect of grain supplement on protozoa counts were investigated. Protozoa were enumerated from four forestomach fistulated Arabian camels (*Camelus dromedarius*) fed either roughage or roughage supplemented with steam flaked barley. The count was performed using a Hawksley counting chamber under light microscopy. The supplementary feeding of roughage diet with steam flaked barley resulted in a decrease in the forestomach protozoal population by 4.9-fold at 0 h, 2.3-fold at 8 h and 5.2-fold at 16 h. *Entodinium* spp. were the predominant species when the camels were fed a roughage diet. Changing the dietary regimen from roughage to roughage plus grains altered the protozoal population in favour of higher *Epidinium* spp., while the *Entodinium* spp. suffered a dramatic decrease. The pH also dropped from 6.4 to 5.4 following the introduction of grain.

The addition of grain to the roughage diet of the camel resulted in significant changes in some protozoa species, which could disrupt the balance and lead to the development of acidosis. The number of protozoa was affected by changing the camel's diet from a roughage diet to roughage plus grain.

Keywords: Arabian camel, *Camelus dromedarius*, protozoa, viable count

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Introduction

Protozoa are present in the gut of various mammals including camelids and ruminants. In cattle the number of protozoa varies from $2.9x10^4$ to $6.3x10^5$ cell ml^{-1} of rumen fluid (Hobson, 1988). The numbers of protozoa in sheep are between $0.7x10^5$ to $3.1x10^6$ cell ml^{-1} (Hungate, 1966). As the diet changes from roughage to concentrate (60% or more concentrate), the number of protozoa in some cases increases, and others decrease, and sometimes lead to the disappearance of some species (Hobson, 1988). In camels, the number of protozoa varies from $1.9x10^4$ per ml in camels fed low fiber diets to $3.3x10^5$ per ml in camels fed high fiber diets (Ghosal *et al.*, 1981; Kayouli *et al.*, 1991). The partition of forestomach protozoa is mainly around 80% *Entodinium* spp. and 20% *Epidinium* spp. with the presence of *Diplodinium* and *Isotricha* genera in small percentages

(Wardeh, 1997). Although there are indications that changes occur in the protozoa population accompanying the change of diet, there are no details about the number of protozoa or about the predominant species when the diet is changed. In an investigation carried out by Wernery and Wensvoort (1992), they reported the absence of protozoa from the forestomach of a young camel when the pH dropped down to <5 following the introduction of grain to the diet. However, the animal recovered 62 h after the introduction of grain.

The objectives of this study are to enumerate and identify protozoa when camels were fed roughage and roughage plus grain diets. We hypothesize that supplementary feeding of grains to camels lowers the forestomach pH and negatively impacts on the protozoa population which may lead to the development of fermentative acidosis.

Material and methods

Sampling, enumeration and identification of protozoa

Forestomach contents were collected from four fistulated camels fed Rhodes grass (*Chloris gayana*) or Rhodes grass + steam flaked barley. The roughage:concentrate (R:C) diet was maintained at a 40:60 ratio for a period of three weeks. This included one week where the grain was gradually increased to 60% of the total diet. The forestomach samples were collected after 14 days at 0, 8 and 16 hours after feeding. The forestomach contents were filtered, pH was measured immediately, and the forestomach fluid sample was preserved in a saline formalin solution (8.1g/l NaCl and 36g/l formaldehyde). The camel forestomach fluid samples were diluted in the saline-formalin solution at a ratio of 1:5 and counted under a microscope fitted with a phase contrast illumination (Nikon, Eclipse E600, Japan) at magnification 200X using a Hawksley counting chamber (Hawksley Cell Counting Chamber, Hawksley Medical and Laboratory Equipment, UK). Identification was performed according to Dehority (1993). For the statistical analysis, the total count was performed using log transformation data and analysed using one-way ANOVA to determine the effect of diet on the protozoal population. Bivariate correlation analysis using SPSS software was used to analyse the effect of the pH on the protozoa count.

Results

Effect of diet on the numbers of protozoa

The protozoa count when the camels were fed the roughage diet was higher ($P<0.05$) than the roughage plus grain diet (Table 1). The total number of protozoa decreased at all sampling times when the camels were shifted to the roughage diet supplemented with the grain. At 0 h the number decreased 4.9-fold, while at 8 h it decreased 2.3-fold and at 16 h it decreased 5.2-fold. When the camels were fed with the roughage-based diet, the dominant species of protozoa enumerated from the sample was represented by the genus *Entodinium*. They represented 92% at 0 h, 83% at 8 h and 85% at 16 h. However, when the camels changed to the grain diet the percentage of *Entodinium* spp. decreased dramatically; 8.1-fold at 0 h, 6.6-fold at 8 h and 7.4-fold at 16 h.

In contrast, the percentage of *Epidinium* spp. increased and became the dominant species when the camels were fed the roughage + grain diet: 69% at 0 and 8 h and 73% at 16 h. The increase in the percentage from only roughage to the roughage + grain diet was 18.3-fold at 0 h, 13.3-fold at 8 h and 14.7-fold at 16 h.

Eudiplodinium spp. represented approximately 17% at 0 and 8 h and 15% at 16 h when the camels were fed a roughage and grain diet. There was also an increase in the percentage of *Eudiplodinium* spp. by at least 1.5-fold when the camels changed from roughage only to roughage + grain diet.

There were also other species of protozoa found in the forestomach of the camel, which have not been reported before, such as *Dasytricha* spp., *Oligoisotricha* spp. and *Buetschlia* spp. However, they represented <2% of the total protozoa population. These species were all present when the camels were fed a roughage diet, but only the *Dasytricha* spp. were found when the camels were fed the roughage + grain diet at 0 h.

Protozoa numbers were affected by the pH ($P<0.05$) (Table 1). When camels were fed a roughage diet the average pH was 6.3 to 6.5. The dominant species were *Entodinium* spp. and species of *Epidinium* and *Eudiplodinium* were only a small percentage. In contrast the roughage + grain diet caused a decrease in the average pH to 5.2 at 8 h. Species of *Entodinium* decreased in numbers dramatically and species of *Epidinium* increased in numbers and were the dominant protozoa. The percentage of the *Eudiplodinium* spp. increased from 2.8 to 17.5% at 0 h and to an average of 9.5 to 16.3% at 8 and 16 h, but by not as much as the *Epidinium* spp. Also, the *Dasytricha*, *Oligoisotricha* and *Buetschlia* spp. disappeared completely when the pH was below 6.0, except for one camel where some *Dasytricha* spp. were found at 0 h when the pH was 5.8.

Discussion

The influence of the diet on the microbial population in ruminants is well documented. However, not much investigation has been done in relation to the camel forestomach (Caldwell and Bryant, 1966; Hungate, 1966).

The importance of protozoa in the rumen is when the animal is fed a starch-based diet as the protozoa are able to engulf high amounts of starch (Williams and Coleman, 1992). The numbers of protozoa were higher ($P<0.05$) when the camels were on a roughage diet than on the roughage + grain diet (Table 1). In many reports, protozoal counts in cattle and sheep were higher when the animal was on a concentrate diet in the form of corn grain than when they were on a roughage diet (Grubb and Dehority, 1975; Dennis *et al.*, 1983; Varel and Dehority, 1989). In other reports, the number of rumen protozoa decreased when the animals were fed barley grain and the pH was less than 6 (Hristov *et al.*, 2001). Fermentation of the barley, particularly steam flaked barley, is faster than corn, and the rate of the starch degradation in corn is one-third lower than barley over a 12-hour period (Herrera-Saldana *et al.*, 1990). The availability of starch in the rumen will proliferate the bacteria which produce high amounts of lactic acid. The accumulation of lactic acid will result in a drop in the pH and, at pH < 6, the balance of protozoa is disrupted (Purser and Moir, 1959; Hristov *et al.*, 2001). In camels, the total number of protozoa decreased from around 2 to 5-fold depending on the time after feeding. The pH decreased at a range 0.7 to 1.1 units per hour. The dominant protozoa when the camel was on a roughage diet was *Entodinium* spp. This is in agreement with results reported by Kayouli *et al.* (1993), when they fed camels a diet consisting of low-quality roughage (vetch-oat hay) ad lib and limited amount of concentrate (500 g per head per day).

Results of the present experiment revealed the presence of other protozoa in the forestomach of the camels, but they

represented <10% of the total population *Diplodinium*, *Oligoisotricha*, *Dasytricha* and *Buetschlia* spp. The last four species of protozoa represented ≤2% of the total count. Kayouli *et al.* (1993) reported that *Epidinium* represented 15% of the total population while the predominant *Entodinium* spp. were 82.6%. The remaining 2.4% was *Eudiplodinium* spp. but neither *Polyplastron*, *Ophryoscolex*, nor *Isotricha* spp. were present. When the camels changed to a roughage + grain diet, *Epidinium* species became dominant and increased in number while the percentage of *Entodinium* spp. were lower. In many reports, *Entodinium* spp. was higher when the animals were fed a roughage + grain diet than when the animals were fed a roughage diet only (Dennis *et al.*, 1983; Franzolin and Dehority, 1996; Goad *et al.*, 1998; Towne *et al.*, 1998). This could be due to the fact that *Entodinium* spp. need more time to adapt to a roughage plus grain diet.

The diurnal variation of the protozoa depended on the diet. On a roughage diet, the number of protozoa decreased in the rumen of the camel after 0 and 8 h but and included *Epidinium*, *Eudiplodinium*, increased at 16 h. However, when the camels were on a roughage + grain diet the number of protozoa decreased at 0 h, then reached the peak after 8 h before they decreased again at 16 h and at 0 h the next day. This was due to the availability of the substrates for the protozoa. When the roughage diet is supplemented with grain, the fermentation processes are faster than when camels are on a roughage diet alone. Rumen bacteria proliferate faster on a starch-based diet than on a roughage-based diet (Allison *et al*., 1975; Mackie and Gilchrist, 1979; Al Jassim *et al.*, 2003). The increase in the number of bacteria provides more substrate for protozoa which feed on bacteria.

In conclusion, the protozoal population of the camel's forestomach is susceptible to dietary change. When a camel's diet shifts from roughage to roughage plus concentrate the normal protozoal population, which is sensitive to low pH, was disrupted and a new balance was created. Such change may contribute to the development of acidosis in the forestomach of the camel.

Table 1. Forestomach protozoa population (total number) and protozoa species (% of total) in camels fed roughage (R) and roughage + grain (R+G) diets

			Protozoa spp. (% of total)							
Diet	Sampling time (h)	Total number ± SE (x10^4 ml $^{-1}$) *	*Entodinium*	*Epidinium*	*Eudiplodinium*	*Diplodinium*	*Dasytricha*	*Oligoisotricha*	*Buetschlia*	pH**
R	0	9.8 ± 2.33	92.1	3.8	2.8	0.6	0.7	-	-	6.3
	8	6.8 ± 1.56	82.3	5.2	9.2	1.4	-	1.4	-	6.5
	16	13.1 ± 4.36	84.5	4.9	9.8	0.5	0.1	0.1	0.1	6.3
R + G	0	2.0 ± 1.67	11.3	69.3	17.5	1.3	0.6	-	-	5.6
	8	3.0 ± 2.55	12.4	68.5	17.1	2.0	-	-	-	5.2
	16	2.5 ± 2.08	11.3	73.3	15.4	-	-	-	-	5.5

*Differences in protozoa numbers between diets are significant ($P<0.05$) at all sampling times; **The pH was lower ($P<0.05$) in the R + G diet at all sampling times.

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