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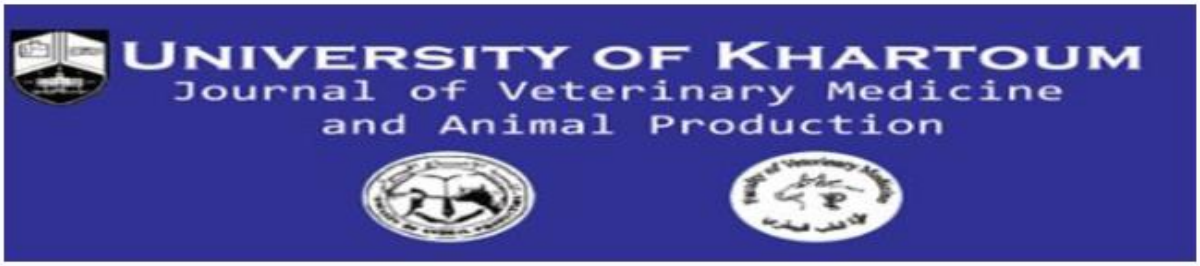

Effect of Fasting on Rumen Ecology in Feed Restricted Male Nubian Goat Kids

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Abstract

This study was designed to investigate the effect of fasting for 4 days on rumen ecology of Nubian goats after two levels of feed restriction (30% and 60% of *ad libitum*) for 6 weeks. Twelve male Nubian goat kids were divided into three groups according to the level of feed restriction; A (control; *ad libitum* feed consumption), B (30% feed restriction of *ad libitum*) and C (60% feed restriction of *ad libitum*) for 6 weeks. Thereafter, they were exposed to fasting (100% feed restriction) for 4 days. During fasting phase, the three groups showed a significant ($p<0.05$) decrease in rumen sort-chain fatty acids (SCFA) and osmolality. They exhibited no significant reduction in rumen protozoa count, rumen pH and rumen calcium concentration in response to complete fasting. The concentration of rumen magnesium was not significantly affected by fasting in all the experimental groups. The concentrations of rumen Na^+ and K^+ were not significantly affected by fasting in group B, while, Group C showed a significant ($p<0.05$) increase in rumen sodium concentration and a significant decrease in rumen potassium concentration in response to fasting. No serious problems have been observed in the rumen milieu during starvation for 4 days

Key Words: Goat, Feed restriction, Fasting, Rumen environment.

المستخلص

تم تصميم هذه الدراسة لمعرفة تأثير الصيام لمدة 4 أيام على بيئة الكرش في الماعز النوبي، باستعمال مستويين من تقييد التغذية (30 ٪ و 60 ٪) لمدة 6 أسابيع. تم استخدام أثنا عشر من صغار ذكور الماعز النوبي في هذه التجربة. تم تقسيمهم إلى ثلاث مجموعاتوفقًا لمستوى تقييد التغذية A (مجموعه التحكم، استهلاك علف طبيعي) B تقييد تغذية بنسبة 30 ٪ من libitum) وC تقييد تغذية 60 ٪ من libitum) لمدة 6 أسابيع. بعد ذلك، تعرضوا للصيام (تقييد التغذية 100 ٪) لمدة 4 أيام. خلال مرحلة الصيام، أظهرت المجموعات الثلاث انخفاضًا ملحوظًا ($p<0.05$) فيالأحماض الدهنية قصيره السلسلة في الكرش وايضاالاسموزية. لم يظهروا أي انخفاض كبير في عدد البروتوزوا في الكرش، درجة الحموضة الكرش وتركيز الكالسيوم الكرش إستجابة للصيام. لم يتأثر تركيز المغنيسيوم في الكرش بشكل كبير نتيجة للصيام في جميع المجموعات التجريبية. لم تتأثر تركيزات الصوديوم والبوتاسيوم في الكرش بشكل كبير بالصيام في المجموعة B ، بينما أظهرت المجموعة C زيادة معنوية ($p <0.05$) في تركيز الصوديوم في الكرش وتراجع كبير في تركيز البوتاسيوم في الكرش استجابة للصيام. لم يلاحظ وجود مشاكل فى الكرش أثناء التصويم لمدة 4 ايام.

الكلمات المفتاحية: الاغنام، تقييد التغذية ، التصويم، بيئة الكرش .

Introduction

The rumen is considered as the main part of the digestive system for nutrient and electrolytes absorption (Kuzinski *et al.,* 2011). Rumen absorptive capacity is influenced by the rumen internal environment, which is affected by the level of nutrition and the type of feed (Doreau *et al*, 1997). The rumen pH is considered as efficient fermentation indicator of feed stuffs (Hobson, 1972). Rumen pH has been observed to vary regularly manner depending on the nature of the diet and the time of measured after ingestion (Phillipson, 1942). Reduction in feed particle size, which would also increase the rate and probably completeness of fermentation, tends to cause a reduction in rumen pH (Cheng and Irpnaka, 1973). Ruminal osmolality normally ranges from 240 to 265mOsmol/l when animal fed roughage diets and 280−300 mOsmol/L when animal fed concentrate diets (Garza *et al*., 1989). Osmolality is quite variable, and does not appear to be a good index of fasting (Galyean *et al.,* 1981). However, the rate and extent of the rise in ruminal fluid tonicity depends on the diet (Bennink *et al.,* 1978), the amount of feed consumed in a given time (Warner and Stacy, 1968), the activity of the ruminal microbes (Schwartz and Gilchrist, 1975) and water intake (Carter and Grovum, 1990).

About 65% to 85% of the short-chain fatty acids (SCFA) produced by intra-ruminal carbohydrate fermentation are absorbed across the rumen epithelium (Remond *et al.,* 1995). The production of SCFA in the rumen is controlled by many factors such as physical and chemical nature of diet, feeding regimes and before total amounts of organic matter digested (Bath and Rook, 1963; Weston and Hogan, 1968). In small ruminants, SCFA concentrations are directly proportional to rumen osmolality when there is no addition of minerals, in order that minerals concentration has a major effect in the rumen ecology (Focant, 1986). The findings of the studies, which investigated the effect of fasting on the concentration of SCFA is contradictory; some of them showed decline in SCFA during fasting (Cole and Hutcheson 1981; Gäbel *et al*., 1993) and other showed increase (Galyean *et al.,* 1981).

Normal rumen fluid contains up to 10^6 protozoa /ml (Franzolin and Dehority, 2010). Feed level is known as one of the factors, which influence the ciliate protozoal population in the rumen. Protozoa population seems to be highest at medium feed intake; however, an interaction between response to feed intake and concentrate proportion in the diet may occur (Kreikemeier *et al.,* 1990; Punia and Leibholz, 1994). Noteworthy, the low energy intake has been shown to affect the number of rumen protozoa negatively (Dehority, 2003).

The absorption of Na^+ from the rumen is mediated by an active transport mechanism (Chien and Stevens, 1972; Harrison *et al*., 1975; Martens *et al.,* 1991). Higher transport rates are thought to be a result from morphological transformations (Dirksen *et al.,* 1984) leading to an increase in the size of the rumen epithelium papillae and consequently, an enlargement of the available absorptive surface area (Shen *et al.,* 2004). However, physiological adaptation by increasing Na^+/H^+-exchanger (NHE) could occur (Lodemann and Martens, 2006). Feed deprivation in sheep increases the Na^+ concentration in the rumen fluid and decreases the K concentration (Holtenius and Dahlborn, 1990). It has been suggested that Na^+ absorptive capacity of the rumen is impaired

during feed deprivation (Gäbel *et al*., 1993), which results in hyponatraemia and activation of the renin-angiotensin-aldosterone system (Holtenius, 1990).
Nubian goat is one of the well adapted species to semi-arid region where the feed is restricted due to the low nutritional resources. Therefore, the present study was tackled to investigate the effects of fasting for four days on the rumen ecology and electrolytes concentration in Nubian goats after two levels of feeding regime (feed restriction; 30 and 60% *ad libitum*).

Materials and Methods

Study area

This study was conducted in the Department of Physiology, Faculty of Veterinary Medicine, University of Khartoum (Shambat), Sudan.

Experimental Animals and Management

Twelve male Nubian goat kids were used in this study. The kids were 2-3 months old (determined by dentition) with an average body weight of 9.5 kg. Animal were kept in a closed shed with adequate ventilation to facilitate the dissipation of sensible heat and disposal of water vapour. The housing system was provided with appropriate facilities for feeding and watering. The animals were kept individually, each animal in a separate cage.

Experimental Design

The animals were randomly assigned to three experimental groups. Group **A**, served as control and fed *ad libitum* (voluntary intake), group **B** (30% feed-restricted) was offered 70% of food offered to the control group and group **C** (60% feed-restricted) was offered 40% of food offered to the control group. The experiment was performed to study the effects of starvation for 4 days directly after different levels of feed restriction for 6 weeks. Rumen samples were collected after 4 days of starvation and after 1 week of re-feeding *ad libitum*.

Source of water and feed

The animals were fed on alfalfa (Berseem hay) and they were offered quantities of feed according to the experimental groups and they had free access to fresh tap water.

Collection of rumen liquor

The rumen liquor was collected by rumenocentesis technique as described by Nordlund and Garrett, (1995). The samples were collected after 14 hrs from feeding. Immediately after collection, the ruminal pH and the osmolality of the rumen liquor were measured as following: The rumen liquor pH was determined using pH meter (HANNA HI-8424 Europe, Romania). Rumen liquor osmolality was determined by freezing point depression method using an osmometer (Cryoscopic osmometer osmomat 030 D-10823 Berlin, Germany).

Laboratory analysis

One volume of rumen liquor sample was added to 1 volume of formaldehyde and stored in a plastic tube at -18°C for protozoa count as described by (Galyean, 1989). A 5 ml of rumen liquor samples were centrifuged at room temperature (25°C) at 2700 RPM for 15 min, and the supernatant was stored in plastic tubes at -18°C for the determination of minerals concentration (Grace *et al.,* 1988). Another 5 ml of rumen liquor sample was added to 5 ml of 0.1% N hydrochloric acid and prepared for the determination of SCFAs concentration (Kroman *et al.,* 1967).
The rumen SCFAs were measured according to the method described by Kroman *et al.,* (1967). The rumen

protozoa count was counted using a light microscope (×10 lens) as described by Galyean, (1989). The concentration of Ruminal Na^+ and K^+ was determined by flame photometer technique as described by Wootton (1974). Rumen liquor Mg^{2+} concentration was determined using a kit (Liner chemical- Spain). Rumen liquor Ca^{2+} concentration was determined using commercial kit (Spinreact-Spain).

Statistical analysis

The obtained data was statistically analyzed using analysis of variance (ANOVA) model to determine level of significance ($P<0.05$) between different treatments (Treated vs. Control). General Linear Method (GLM) procedure of Statistical Analysis System (SAS, 2000) was used to perform the analysis. Data was analysed using SPSS version 20.

Results

Table 1 showed that there was no significant difference in the rumen pH between the three experimental groups during the three experimental phases (feed restriction, fasting and re-feeding). Nevertheless, during the fasting phase, the three experimental groups showed a slight increase in rumen pH compared to feed restriction (pre-fasting) and re-feeding phases (Table 1). There was no significant difference in rumen osmolality between the three experimental groups during "pre-fasting", fasting and re-feeding (Table 1). However, fasting caused non-significant decrease in rumen osmolality compared to (pre-fasting and re-feeding phases in all experimental groups (Table 1). All experimental groups did not show any significant difference in rumen protozoa count during the same experimental phases (Table 1). In contrast, group A showed a significant ($p<0.05$) decrease in rumen protozoa count during fasting and re-feeding phases compared to pre-fasting phase (Table 1). On the other hand, groups B and C showed a significant ($p<0.05$) decrease in rumen protozoa count during fasting phase compared to pre-fasting phase and a numerical decrease compared to re-feeding phase (Table 1). Although, there was no significant difference in the concentration of SCFA between the three experimental groups of animals during "pre-fasting", fasting and re-feeding (Table 1), fasting decreased the SCFA concentration in all experimental groups when compared to the other two phases. Within one week of ad libitum re-feeding, the animals in the three experimental groups restored the rumen SCFA concentration compared to pre-fasting phase (Table 1).

During fasting phase, Group C (60% feed-restricted) showed a significant ($p<0.05$) increase in rumen Na+ concentration compared to the control group (group A) and a slight increase compared to the group B (30% feed-restricted) (Table 2). Moreover, fasting caused a significant ($p<0.05$) increase in rumen Na+ concentration in group C (60% feed-restricted) and a slight increase in group B (30% feed-restricted) compared to the feed restriction (pre-fasting) phase (Table 2). The three experimental groups of animals showed a significant ($p<0.05$) decrease in rumen Na+ concentration during the re-feeding phase compared to the fasting phase (Table 2). The fasting phase exerted a significant ($p<0.05$) decrease in rumen K concentration in groups C (60% feed-restricted) and A (control) compared to the feed restriction (Pre-fasting) and the re-feeding phases (Table 2). Meanwhile, group B (30% feed-restricted) showed a slight non-

significant decrease in response to fasting compared to the pre-fasting and re-feeding phases (Table 2). During the three experimental phases there was no significant difference in rumen Mg^{2+} concentration between the three experimental groups of animals (Table 2). Fasting did not significantly affect rumen Mg^{2+} concentration. Nevertheless, fasting significantly ($p<0.05$) decreased the rumen Ca^{2+} concentration in the three experimental groups compared to pre-fasting phase (Table 2). Interestingly, the three experimental groups of animals did not show any significant difference in rumen $Ca2^{+}$ concentration during the fasting phase (Table 2), and they restored the concentration of Ca2+ during re-feeding phase compared to pre-fasting.

Table 1. Effect of fasting for 4 days on rumen pH osmolality (mOsm/kg), protozoa count (X10^3/dL) and short chain fatty acids (meq/dL) in feed-restricted male Nubian goat kids.

Parameters	(Pre-fasting)			Fasting			Re-feeding		
	A	**B**	**C**	**A**	**B**	**C**	**A**	**B**	**C**
Rumen pH	6.93±0.15^a	6.68±0.05^a	6.90±0.14^a	7.18±0.17^a	7.15±0.06^a	7.18±0.09^a	6.95±0.06^a	6.88±0.05^a	6.88±0.26^a
Rumen osmolality (mOsmol)	243.30±11.53^a	238.0±12.88^a	259.75±15.44^a	224.00±13.00^a	221.00±14.60^a	225.00±8.54^a	267.80±36.68^a	259.30±45.74^a	269.00±7.96^a
Rumen protozoa count (organisms/ml)	165.00±44.35^a	135.00±19.15^{ac}	112.50±18.93^{ac}	17.50±5.00^b	45.00±19.15^{bd}	40.00±23.09^b	80.00±28.28^{bcd}	100.00±16.33^{ad}	75.00±34.16^{ad}
Short Chain FattyAcids (mM)	6.45±0.61^a	8.05±0.01^a	6.13±1.44^a	2.80±0.57^b	2.58±0.29^b	2.58±0.15^b	4.500±1.50^a	5.68±0.54^a	7.10±3.46^a

A:*ad libitum* feed consumption.

B: 30% feed restriction.

C:60% feed restriction.

^{abcd}Means in the same row bearing different superscript are significantly different at ($p<0.05$).

Table 2. Effect of fasting for 4 days on the concentration of rumen Sodium (mg/dL), Potassium (mg/dL), Magnesium (mg/dL) and Calcium (mg/dL) in feed-restricte male Nubian goat kids.

Parameters	(Pre-fasting)			Fasting			Re-feeding		
	A	B	C	A	B	C	A	B	C
Rumen Sodium (mg/dL)	139.50±15.02^{ab}	120.00±6.68^{ac}	128.50±10.34^{acd}	138.80±7.27^{ad}	140.50±12.04^{bd}	160.25±5.44^{b}	112.80±16.92^{c}	113.80±16.46^{c}	104.00±5.23^{c}
Rumen Potassium (mg/dL)	23.40±4.09^{ae}	22.04±3.69^{abe}	26.48±0.34^{a}	13.10±4.33^{bc}	16.50±4.73^{bce}	9.65±1.14^{c}	29.50±1.10^{a}	29.83±4.29^{a}	30.65±2.45^{d}
Rumen Magnesium (mg/dL)	2.15±0.10^{a}	2.15±0.10^{a}	2.10±0.12^{a}	2.18±0.13^{a}	2.10±0.12^{a}	2.10±0.12^{a}	2.18±0.13^{a}	2.10±0.12^{a}	2.10±0.12^{a}
Rumen Calcium (mg/dL)	3.75±2.10^{a}	3.00±1.29^{a}	3.63±1.38^{a}	1.00±0.58^{b}	1.00±0.71^{b}	0.88±0.48^{b}	2.38±0.48^{ab}	2.38±1.03^{ab}	2.50±1.68^{a}

A:*ad libitum* feed consumption.

B: 30% feed restriction.

C:60% feed restriction.

^{abcd}Means in the same row bearing different superscript are significantly different at ($p<0.05$).

Discussion

The animals of the three experimental groups showed a slight non-significant increase in the rumen pH in response to fasting. This is in consistent with the findings of several authors, who reported that feed deprivation increases rumen pH in fasted sheep (Coop, 1949; Juhász*et al*., 1978) and beef cattle (Rumsey, 1978; Cole and Hutcheson, 1981; Galyean *et al*., 1981). Noteworthy, a rapid decline in the rumen pH in fasted steers during re-feeding phase has been reported (Rumsey, 1978; Cole and Hutcheson, 1981). Increasing rumen pH during feed deprivation could be attributed to a decrease in the concentration of rumen SCFA.

Irrespective of the regular level of previous feeding regime, the current finding showed that fasting caused a slight decrease in rumen osmolality compared to the (pre-starvation) and re-feeding phases. The present result agrees with previous findings, which indicated that fasting leads to a reduction in rumen osmolality in feed-deprived goats (Dahlborn and Karlberg, 1986) and feed-deprived sheep (Holtenius and Dahlborn, 1990).

The three experimental groups of animals showed a significant decrease in rumen protozoa count during fasting and re-feeding phases compared to pre-fasting phase. The present finding is in the line with the finding of Galyean *et al.,* (1981) that feed deprivation causes a decrease in the total counts of rumen protozoa in fasted calves. Noteworthy, the number of protozoa decreases as the duration of fasting period increases, and this is related to a reduction in dry matter intake (Fluharty *et al.*, 1996).

In the present study, fasting caused a significant decrease in the rumen SCFA concentration in the three experimental groups, and interestingly, there was no significant difference between the feed restricted groups and the control ones. Studies in feed-deprived sheep (Holtenius and Dahlborn, 1989) and calves (Harmon *et al*., 1999) showed a similar decline in the SCFA during fasting phase. Short-term feed deprivation (48 h) has been shown to cause a reduction in SCFA absorption rates across the reticulo-rumen in sheep (Gäbel *et al*., 1993). This could be attributed to a decrease in the rumen microbial populations that caused by feed deprivation, which diminishes the ability of the animal to digest cellulose, and therefore, decreases the formation of SCFA (Galyean *et al*., 1981).

Fasting led to a significant increase in rumen Na^+ concentration in animal subjected to 60% feed restriction (group C) and a slight increase in animal subjected to 30% feed restriction (group B) compared to the feed Pre- fasting phase. Feed-deprived goats for 28 hrs develop hyponatraemic hypovolaemia (Dahlborn and Karlberg, 1986), while Na^+ concentration in the rumen fluid increases (Holtenius and Dahlborn, 1990). Therefore, it has been suggested that the Na^+ absorption from the rumen is impaired in response to feed deprivation (Holtenius and Dahlborn 1989). Moreover, Sasaki *et al.,* (1988) have reported that cows, which are feed-deprived for 3 days, exhibit an impaired ability to absorb Na^+ and water from the rumen. This is in contrast to the fact that Na^+ is readily absorbed from the rumen in fed animals (Dobson, 1959).

Fasting significantly decreased rumen K^+ in groups C (60% feed-restricted) and A (fed at libitum) compared to feed restriction (Pre-fasting) and re-feeding phases. Meanwhile, group B (30% feed-restricted) showed a slight, non-

significant decrease in response to fasting compared to feed restriction (Pre-fasting) and re-feeding phases. Generally, similar results have been obtained by Holtenius and Dahlborn (1989, 1990) who reported that feed deprivation in sheep is associated with lower concentration of rumen K^+ and higher concentration of rumen Na^+. Scott (1967) claimed that the drop in the intra-ruminal K^+ concentration leads to a reduction in the absorption of Na^+ from the rumen. This could be an explanation of why feed deprivation led to increased ruminal Na^+ concentration. Therefore, a drop in the rumen K^+ concentration due to fasting is expected (Holtenius and Dahlborn, 1990).

There was no significant difference in rumen Mg^{2+} concentration between the three experimental groups subjected to different levels of feed restriction during fasting and re-feeding phases. There is a positive relationship between the ruminal SCFA concentration and ruminal Mg^{2+} absorption (Scharrer and Lutz, 1992). Nevertheless, the present study showed that fasting significantly affect the ruminal SCFA, but it did not affect the Mg^{2+} concentration. Irrespective of the levels of feed restriction to which animals were subjected, the fasting significantly decreased the rumen Ca^{2+} level. There is no available data supporting this finding, however, the decrease in rumen Ca^{2+} could be due to a decrease in the uptake of Ca^{2+} in the feed.

In conclusion, the outcomes of this study revealed that the previous experience to feed restriction (30% and 60%) for 6 weeks in male Nubian goat kids significantly affected the rumen Na^+ and K concentrations during complete starvation. Fasting significantly decreased rumen protozoal count, SCFA concentration and Ca ion concentration. Generally, no serious problems have been observed in the rumen milieu during starvation for 4 days and the animals were apparently normal after one week of re-feeding *ad libitum*.

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Conflict of interest

The authors declare that there was no conflict of interest

Compliance with ethical standards

All procedures described in this experiment were approved by the Faculty Research Ethics Committee at University of Khartoum.

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