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# THE EFFECTS ON CREATININE EXCRETION OF CHANGES IN THE FERMENTATIVE ACTIVITY OF THE HIND GUT IN SHEEP[1]

**Ahmet Öncüer**[2]

**Koyunlardaki kalın barsak fermentasyon aktivitesindeki değişimlerin kreatinin atımı üzerine etkileri**

**Özet:** *Dört dişi koyun sabit ısı ve devamlı ışık altında metabolizma kafeslerinde barındırıldılar. Koyunların hepsine rumen kanülü, abomasum ve ileum infüzyon kateteri takıldı. Koyunlara uçucu yağ asitleri, mineraller, tampon çözelti ve kazein infüzyonu yapıldı. Buna ek olarak değişik seviyelerde kalın barsak fermentasyonu yaratmak için nişasta ve sellüloz terminal ileum'a infüze edildi. Kreatinin atımı hem azotsuz besleme sırasında hemde azotun yaşama payı seviyesinde verildiği dönemlerde ölçüldü. Azotsuz besleme sırasında kreatinin seviyesi ortalama olarak* 52.2 *mg / kg*$^{0.75}$ */ gün bulundu. Bu değer yaşama payı azot ihtiyacı sağlandığı dönemdeki kreatinin seviyesi ile* (52.0 *mg /* kg $^{0.75}$*/ gün) aynı bulundu. Kreatinin atımının kalın barsak fermentasyonunun değişimlerinden etkilenmediği tesbit edildi.*

**Summary:** *Four female sheep were housed indoors in metabolism crates under conditions of continuous lighting and constant ambient temperature. All sheep prepared with rumen cannulas, abomasal and ileal infusion catheters. Sheep were continuously infused with volatile fatty acids, minerals, buffer and casein. In addition these infusates, starch and cellulose were infused into the terminal ileum in order to achieve different levels of hind gut fermentation. Creatinine excretions were measured in the period of nitrogen free regimes and when the maintenance level of nitrogen intake was given. The overall mean value of creatinine excretion for the N-free regime was* 52.2 *mg / kg*$^{0.75}$ */ day and was similar to the value recorded when the maintenance level of nitrogen intake was given* (52.0 *mg / kg*$^{0.75}$ */ d). Creatinine excretion was not affected by the precence or abcence of a hind-gut fermentation.*

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2 DVMS, PhD Lalahan Nuclear Research Institute of Animal Health, Ankara.

## Introduction

Creatinine is the urinary end-product of creatine breakdown in muscle has been used to predict muscle mass. Graystone (5) showed that about 1 g creatinine was excreted daily per 20 kg of muscle mass. Much research since then has been carried out to establish if this relationship is constant in ruminants, in the hope that the endogenous loss of N could be derived from a determination of creatinine excretion.

This relationship has been examined by Orskov&MacLeod (16) and Orskov et al. (17) in fasted cows and by Hovell et al. (9) with lambs. using the procedure of intragastric infusion. Orksov&MacLeod (16) and Orskov et al. (17) reported relatively constant creatinine excretion. Hovell et al. (9) reported however that there was a regular fluctuation in creatinine excretion and could find no evidence of any effect of protein level or of the direction in change of energy level on creatinine excretion.

The effects on creatinine excretion of changes in the fermentative activity of the hind gut do not appear to have been examined. The objective of this study was to measure of creatinine excretion in urine both in N-free regime and the maintenance level of nitrogen intake was given in sheep nourished by intragastric infusion.

## Materials and Methods

Animals and Management: The lambs were 7 months of age used in this experiment. Each animals was fitted with a rumen cannula, an abomasal and ileal infusion catheter as decribed by Orskov et al. (15). Lambs were housed indoors in metabolism crates under continuous lighting. All animals were transferred from solid food to total intragastric nutrition. The methods used to maintain animals by intragastric infusion were essentially those described by Orskov et al. (15), MacLeod et al. (11) and Hovell et al. (8) in which solutions of volatile fatty acids (VFA), buffer and major minerals were infused into the rumen and casein infused into the abomasum. Trace minerals injected daily via the abomasal catheter. Vitamin A, D and E were given by intramuscular injection of Vetrivite.

The calculation of total energy to be supplied was based on the assumption that the maintenance requirement for energy was 450 kJ / $kg^{0.75}$ per day (8). Nitrogen requirement for maintenance were taken to be 350 mg N / $kg^{0.75}$ per day (1). In addition animals in the present

experiment received infusion of starch and cellulose into the terminal ileum. All infusions were achieved by means of peristaltic pumps. Volumes of infusates were delivered continuously over 24 h.

Design and Treatment: The treatment of the experiment involved the infusion at the terminal ileum of 3 levels of nutrient input to achieve different levels of hind-gut fermentation. A 3×3 Latin square design was used with treatment periods of 3 weeks duration in which the treatments were (1) water infusion into terminal ileum, (2) 25 g / d starch and 50 g / d cellulose (air-dry weights) infusion into terminal ileum, (3) 50 g / d starch and 50 g / d cellulose (air-dry weights) infusion into terminal ileum.

The quantities of starch and cellulose selected for treatments 2 and 3 were based on estimates in the literature for the amounts of these constituents of normal diets, which might be expected to reach the terminal ileum in sheep given conventional feeds (13, 14).

In each treatment the total volume of solution infused into the ileum was 2 litres / d.

In order to help establish the initial fermentation, a inoculation of rumen fluid (approximately 50 ml) obtained from a conventionally fed ruminant (cow) was given into the hind-gut via ileal infusion catheter at the start of each period in which starch / cellulose was given into the ileum. No inoculations were given when the control (water) infusion was given into the ileum.

In each 3 week period, the first 7 days served as the preliminary period, in which animals were adjusted to the prescribed level of hind-gut infusion. Days 8—12 inclusive (5 days) were used for quantitative collection of faeces and urine for digestibility and N-balance measurements.

Days 15—19 inclusive (5 days) constituted the N-free period, when casein infusion into the abomasum was discontinued and faeces and urine were collected and analysed on a daily basis to establish endogenous N excretion. Body nitrogen stores were then repleted over days 20 and 21 and days 1. 2 and 3 of the next reatment period by increasing the casein allowance to 1.0, 2.0, 2.5, 2.5 and 2.0 times maintenance on each successive days. From day 4 of the next treatment period nitrogen intake was returned to the standard level of 1 times maintenance.

Measurements and Sampling Procedures: Total urine and faeces were collected over 5 days (8—12 day of periods). Urine samples were analysed on a daily basis for nitrogen, urea and creatinine. Faeces were bulked over 5 days and analysed for ash, dry matter (DM), organic matter (OM), nitrogen (N), starch and acid detergent fibre (ADF).

Faeces for bacterial examination were assessed for aerobic and anaerobic organisms.

The metabolism crates were fitted with a PVC-coated, expanded metal floor. Urine and faeces were caught in a fibre glass separator funnel which covered the entire floor area. Urine passed directly into a collection tray containing 10 % sulphuric acid (300 ml per 12 h collection) to prevent loss of ammonia. Urine volume was weighed, sampled and stored at -20 °C until analysed. Faecal material was separated from the urine by means of a nylon mesh covered the urine collection tray. The faeces sample was collected over 5 days and bulked together.

Daily samples of urine were collected over 5 successive days (15—19 day of periods) on N-free regime. Urine was analysed for N, creatinine and urea on a daily basis. Urine samples were analysed for total nitrogen using the automated Kjeldahl method of Davidson et al. (4). For creatinine the automated technique of Technicon Instruments Co. Ltd. (18) which utilizes the Jaffe reaction were used (6). For urea in urine sample was determined by the automated methods of Marsh et al. (12).

Faecal samples was dried by freeze-drying and grinding for nitrogen (4) and dry matter and ashed at 600 °C for organic matter determination (2). Faeces were analysed for starch as described by Bergmeyer (3) and for ADF as described by AOAC (2). Estimation of the number of total viable bacteria were made as described by Hobson (7). Aerobic bacteria were counted using plate count agar as decribed by Leininger (10). pH was determined electrometrically and osmotik pressure by freezing point depression.

The experimental design was treated as a randomized block and 12 observations were subjected to an analysis of variance for non-orthogonal data which allowed treatment means to be adjusted for animal and period effects.

## Results and Discussion

The health of the experimental animals remained good throughout the experiment. The abomasal and ileal catheters and rumen cannulas were, in all cases, trouble free.

Nutrient intakes and apparent digestibilities: Mean energy and nitrogen intakes of animals are shown in Table 1. Intakes of energy and nitrogen remained constant throughout the experiment (approx. 450 kJ / $kg^{0.75}$ / d and 0.450 g N / $kg^{0.75}$ / d) and there was no difference between treatments.

The effect of ileal infusion of starch and cellulose on the organic matter (OM) intake and faecal excretions of dry matter (DM), starch, cellulose and OM are given in Table 1. Differences between treatments in OM intake were significant ($P<0.01$) but the excretion of DM and OM in faeces differed significantly only between the zero level of hind gut infusion (Treatment 1) and the other two treatments ($P<0.01$). On all three treatments only small quantities of starch appeared in faeces whereas considerable amounts of cellulose were present in the faeces of these animals given cellulose infusions at the terminal ileum. These findings were reflected in the calculated values for apparent digestibility of these constituents, which were high for starch (0.94—0.98) and comparatively low for cellulose (0.11—0.18) and did not differ significantly between the two higher levels of hind gut infusion.

Although fairly high numbers of both aerobic and anaerobic organisms were recorded in the faeces (Table 1) there were so significant differences between treatments in these measurements. The rather poor digestibility coefficient recorded for cellulose on these treatments was confirmed by a virtual absence of cellulolytic bacteria in faecal material: out of the 12 samples examined asignificant count of cellulolytic organisms ($3.5 \times 10^8$) was found in only one (Trearment 2, Period 2).

Nitrogen intakes, excretion and retentions: Mean values for N intakes and excretions are given in Table 2 expressed as g N / d and in Table 3 as mg N / $kg^{0.75}$ / d. N intake remained constat across treatment groups with an overall mean value of 6.8 g / d (453 mg / $kg^{0.75}$ /d). On all 3 treatments most of the N excretion was via the urine and there were no significant differences between treatments in urinary N excretion: On average 0.77 of total N intake was excreted in urine. Faecal N excretion was low on the control group but showed an inc-

Table 1. Mean intakes and faecal excretion of DM, OM, starch and cellulose, apparent digestibility coefficient and faecal bacterial counts in sheep given infusions of starch and cellulose into the terminal ileum (each value is the mean of 4 observations)

| Treatments† | Mean liveweight ($kg^{0.75}$) | Intakes | | | | | Faecal Excretion | | | | Apparent digestibilities | | | Faecal bacteria | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | Energy (kJ /$kg^{0.75}$ /d) | Nitrogen (g /$kg^{0.75}$ /d) | Starch (g /d) ≠ | Cellulose (g /d) ≠ | OM (g /d) | DM (g / d) | Starch (g / d) | Cellulose ø (g / d) | OM (g / d) | Starch | Cellulose | OM | Aerob (log count /g) | Anaerob. (log count /g) |
| 1 | 14.98 | 437.7 | 0.448 | — | — | 398.7 | 12.2 | 0.08 | 1.70 | 5.90 | — | — | 0.98 | 8.17 | 8.69 |
| 2 | 14.96 | 443.5 | 0.654 | 19.35 | 43.79 | 467.7 | 58.9 | 0.96 | 35.70 | 50.10 | 0.94 | 0.18 | 0.89 | 9.00 | 9.83 |
| 3 | 14.83 | 450.6 | 0.446 | 41.29 | 45.92 | 497.4 | 65.5 | 0.55 | 37.50 | 54.70 | 0.98 | 0.11 | 0.88 | 9.49 | 10.12 |
| SED | 0.240 | 14.69 | 0.011 | — | — | 11.09 | 8.38 | 0.25 | 3.73 | 6.28 | 0.003 | 0.052 | 0.015 | 0.482 | 0.912 |
| Statistical significance | NS | NS | NS | — | — | ** | ** | NS+ | ** | ** | NS | NS | ** | NS | NS |

DM : Dry matter

OM : Organic matter

NS : Not significant

NS+ : $P<0.1$

* : $P<0.05$

** : $P<0.01$

† : Treatments 1, 2 and 3 refer to the levels of starch and cellulose infused: 1 — nil; 2 — 25 g starch, 50 g cellulose; 3 — 50 g starch, 50 g cellulose

≠ : Infused at terminal ileum: intakes expressed on DM basis

ø : Estimated as acid-detergent fibre (ADF)

Table 2. Mean nitrogen intakes, excretion, retention and apparent digestibility coefficient in sheep given infusions of starch and cellulose into the terminal ileum (each value is the mean of 4 observations)

| Treatment † | Mean liveweight (kg) | N intake (g / d) | Faecal N (g / d) | Urinary N (g / d) | N-Retention (g / d) | Apparent N digestibility |
|---|---|---|---|---|---|---|
| 1 | 36.96 | 6.730 | 0.319 | 5.372 | 1.03 | 0.95 |
| 2 | 36.92 | 7.178 | 0.815 | 5.418 | 0.73 | 0.88 |
| 3 | 36.48 | 6.634 | 1.096 | 5.123 | 0.35 | 0.82 |
| SED | 0.770 | 0.234 | 0.114 | 0.228 | 0.282 | 0.017 |
| Statistical significance | NS | NS | ** | NS | NS | ** |

N : Nitrogen

NS : Not significant

** : $P < 0.01$

† : See Table 1 for description of treatments.

rease with each increase in the level of hind gut infusion ($P<0.01$). The apparent digestibility of N showed a similar trend and the increased faecal N excretion was reflected in decreases in total N retention in progressing from Treatment 1 to Treatment 3: these differences in N retention however failed to reach significance.

Urea-N made up a high proportion of total urinary N excretion and the total quantity of urea -N excreted was significantly lower on the high level of starch infusion (Treatment 3) than on the other two treatments ($P<0.05$) (Table 3). As a proportion of total urinary N excretion, urea-N accounted for 0.78 on Treatment 1 and 2 and 0.73 on Treatment 3 ($P<0.05$). Creatinine excretion in urine in contrast was remarkably constant and did not differ significantly between treatment groups (Table 3).

Mean values for urinary urea-N excretion and urinary creatinine excretion during each day of the 5-day N-free period are given in Table 4.

Creatinine excretion did not change day to day and between treatments on any day. The overall mean value for the N-free regime was 52.2 mg / $kg^{0.75}$ / d and was similar to the value recorded when the maintenance level of nitrogen intake was given (52.0 mg / $kg^{0.75}$ / d). It can be seen that values are very similar and there was no fluctuation from day to day as reported by Hovell et al. (9). These results also agree with Orskov & MacLeod (16) and Hovell et al. (9) that creatinine is not a useful index of basal metabolism of the animal.

It is concluded from the results of this experiment that creatinine excretion in urine was similarly unaffected by the presence of a hindgut fermentation and appeared aslo to be independent of the level of N intake.

Table 3. Nitrogen intakes, excretion and retention expressed per unit metabolic bodyweight ($kg^{0.75}$) and excretion of urea and creatinine in urine (each; value is the mean of 4 observations)

| Treatment† | Mean liveweight ($kg^{0.75}$) | N-intake (mg/$kg^{0.75}$/d) | Faecal N (mg/$kg^{0.75}$/d) | Urinary N (mg/$kg^{0.75}$/d) | N retention (mg/$kg^{0.75}$/d) | Urine urea-N (mg/$kg^{0.75}$/d) | Urea-N as proportion total urine N | Urine creatinine (mg/$kg^{0.75}$/d) |
|---|---|---|---|---|---|---|---|---|
| 1 | 14.98 | 448.4 | 21.8 | 357.5 | 65.3 | 278.9 | 0.78 | 50.95 |
| 2 | 14.98 | 465.0 | 54.3 | 361.9 | 48.0 | 284.1 | 0.78 | 53.04 |
| 3 | 14.83 | 446.9 | 74.7 | 342.8 | 25.0 | 252.3 | 0.74 | 52.06 |
| SED | 0.240 | 11.93 | 8.29 | 16.41 | 19.80 | 7.29 | 0.01 | 2.29 |
| Statistical significance | NS | NS | ** | NS | NS | * | * | NS |

N : Nitrogen

NS : Not signification

* : $P<0.05$

** : $P<0.01$

† : See Table 1 for description of treatments.

Table 4. Mean daily excretion of urea-N and creatinine in urine over 5 successive days in sheep maintained by infusion under N-free conditions and given infusions of starch and cellulose into the terminal ileum. Each value is the mean of 4 observations (Treatments 1 and 3) or 3 value (Treatment 2)

| Treatments† | Mean liveweight ($kg^{0.75}$) | Urine urea N (mg / $kg^{0.75}$ / d) | | | | | Urine creatinine (mg / $kg^{0.75}$ / d) | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|
| | | Days | | | | | Days | | | | |
| | | 1 | 2 | 3 | 4 | 5 | 1 | 2 | 3 | 4 | 5 |
| 1 | 14.94 | 195 | 172 | 149 | 135 | 121 | 50.8 | 54.1 | 51.6 | 51.4 | 49.5 |
| 2 | 14.90 | 288 | 190 | 134 | 136 | 138 | 55.1 | 55.4 | 47.2 | 53.6 | 53.4 |
| 3 | 14.78 | 179 | 114 | 103 | 104 | 099 | 53.7 | 51.8 | 52.3 | 52.2 | 50.7 |
| SED | 0.371 | 0.032 | 0.014 | 0.018 | 0.017 | 0.021 | 5.360 | 2.949 | 3.840 | 2.401 | 4.44 |
| Statistical significance | NS | NS+ | * | NS | NS | NS | NS | NS | 5S | NS | NS |

N : Nitrogen

NS : Not significant

NS+ : $P<0.1$

* : $P<0.05$

† : See Table 1 for description of treatments.

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