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The effect of rumen protozoa on plasma allantoin level and urinary excretion of purine derivatives in sheep

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ABSTRACT

An experiment was carried out to examine the effect of rumen protozoa on feed utilization, nitrogen balance and urinary excretion of purine derivatives (PD) in faunated and defaunated lambs fed a hay and concentrate diets. There was no change in digestibilities of dry matter (DM) and organic matter (OM) with or without protozoa, however the digestibilities of crude protein and crude fibre tended to decrease in defaunated lambs, but not significantly ($P>0.05$). Defaunation decreased nitrogen (N) balance and urinary N excretion. Consequently, retained N was higher ($P<0.05$) in defaunated than in faunated lambs. Mean plasma allantoin concentration was higher ($P<0.05$) in defaunated (41.2 μmol/l) than in faunated lambs (25.5 μmol/l). Total urinary PD excretion and allantoin excretion were higher ($P<0.05$) in the defaunated than in the faunated lambs.

From the above results, it can be concluded that rumen protozoa have an important role in N utilization, plasma allantoin levels and total PD excretion in lambs.

KEY WORDS: rumen protozoa, sheep, purine derivatives, feed utilization

INTRODUCTION

The major microbial species in the rumen are bacteria and protozoa (Leng and Nolan, 1984) with population of 10^9-10^{11} and 10^5-10^6 per ml of rumen fluid

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respectively (Morimoto, 1969). These microbes are major protein sources for ruminants and their proliferation is very closely related to feed degradation and fermentation in the rumen. Measuring their proliferation and their synthesis of microbial protein is very important when assessing the nutritional status of ruminants.

Several methods are used to estimate the microbial protein synthesized in the rumen. They include, microbial markers (2,4 diaminopimelic acid: DAPA and purine bases) (Hutton et al., 1971; Zinn and Owens, 1986), the tracer method using isotopic substance such as ^{15}N (Koenig et al., 2000), ^{32}P and ^{35}S (Stern and Hoover, 1979) and more recently, the indicator method based the use of urinary purine derivatives (PD) (Fujihara et al., 1987; Chen et al., 1990). Nucleic acids from ingested feed are generally completely degraded in the rumen. Therefore, nucleic acids digested in the lower gut are mainly of microbial origin (McAllan, 1982). Nucleic acid N comprises about 20% of the total N content of rumen microbes (Topps and Elliot, 1965) of which 85-95% is digested in small intestine (Coelho et al., 1972a,b).

In the rumen, bacteria and protozoa ferment fibrous feeds as well as starch and soluble carbohydrates into volatile fatty acids (VFAs) and microbial protein, which are in turn utilized by the host animal. Urinary PD is thus used as an index to estimate the amount of microbial protein synthesized in the rumen. It is important to establish the contribution of protozoal protein to total microbial protein yield in ruminants.

According to Eadie and Hobson (1962), Takenaka et al. (1991) and Nakagawa et al. (1992), protozoa in the rumen tend to influence the number of bacteria, ammonia and VFA production. However, there is a little information on effect of protozoa on nucleic acid metabolism in ruminants (Matsumoto et al., 1991; Fujihara et al., 2002).

An experiment was therefore carried out to investigate the effect of protozoa on feed utilization, N balance, plasma PD level and urinary PD excretion using defaunated and faunated lambs. Part of this research work has briefly described by Fujihara et al. (1999).

MATERIAL AND METHODS

Animals and diets

Three crossbred lambs (Japanese Corriedale x Suffolk) of average body weight, 31.6 ± 1.0 kg, were used in this experiment. The lambs were bottle fed and weaned before being introduced to the experimental diets. During the whole experimental period, the lambs were kept in metabolic crates and were fed on a diet

comprised of lucerne hay, wheat bran and soyabean meal (Table 1). The energy content of the diet was calculated as about 1.8 times the maintenance requirement for 30 kg lambs (AFRC, 1994), and dietary protein was also calculated as about 1.5 times the requirement for 200 g daily gain in growing 30 kg lambs (ARC, 1965). Half of the daily ration was given at 09.00 and another at 17.00 h. Fresh water and salt licks containing trace elements were freely available to the experimental animals.

TABLE 1

Chemical composition of milk replacer and feed ingredients used in the experimental diet, %DM, and daily allowance

Nutrients	Milk replacer	Timothy hay	Soyabean meal	Wheat bran
Crude protein	24.0	7.3	44.8	18.6
Crude fat	18.0	2.5	1.7	4.6
Crude fibre	1.0	32.7	6.6	10.7
Crude ash	10.0	9.4	7.3	5.5
NFE[1]	45.7	48.1	39.6	60.5
Ca	0.8	0.3	0.3	0.1
P	0.5	0.1	0.7	1.1
Diet component, % DM		54.0	6.0	40.0
Daily allowance, gDM/kgBW$^{0.75}$		39.0	4.6	29.0

[1] nitrogen free extractives

Experimental procedure

Defaunation of the animals was accomplished by bottle-feeding them on milk replacer for 2 weeks, followed by feeding on a mixed diet (9:1 to 0:10 DM of MR and solid diet) for 10 days (Figure 1). The animals were then fed on a solid diet alone for 1 week. After confirming there was no protozoa in the rumen contents taken through a stomach tube, the digestion and N balance trial was conducted for a period of five days. Blood samples were collected from the jugular vein just before the morning feed during the digestion trial.

To faunate the animals, 100 protozoa (*Holotrichida and Oligotrichina*, 1:19) were collected from a different animal fed on a hay and concentrate diet and inoculated onto each of the experimental animals. Concurrent to this, sampling of blood from the jugular vein and urine was carried on the faunated animals. As in the defaunated lambs (on 7th days after inoculation) a 5-day digestion and N balance trial was also performed, and blood from the jugular vein collected just before the morning feed.

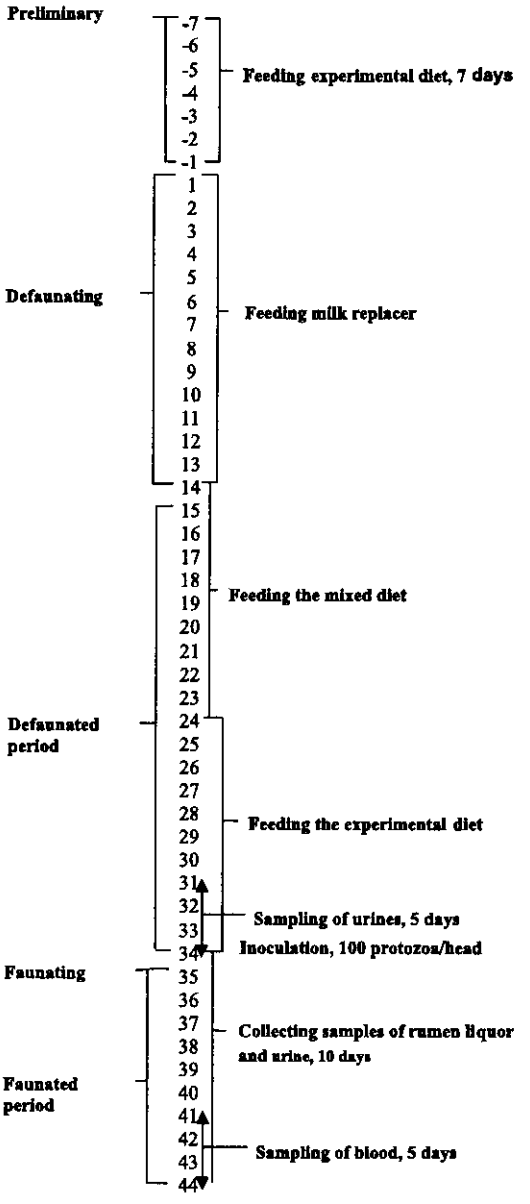

Figure 1. Experimental schedule

* experimental diet (see Table 1) with milk replacer (1st/1.5:8.5-2nd/3.0:7.0-3rd/4.5:5.5-4th/6.0:4.0-5th/7.5:2.5- 6th/9.0:1.0- 7th/10:0)

** days

\# milk replacer (see Table 1)

\## milk replacer and experimental diet (1st/9:1- 2nd/8:2- 3rd/7:3- 4th/6:4-5th/5:5-6th/4:6-7th/3:7-8th/2:8- 9th/1:1- 10th/0:10)

Sampling and analytical procedure

Urine was daily collected with 100-150 ml of 10% H_2SO_4 solution to adjust pH value below 3.0 before the animals were fed in the morning throughout the entire experimental period and hourly on the final day in each digestion period. During the 1st-2nd days after inoculation, urine was collected at a 30 min intervals for 48 h into a container with 10% H_2SO_4 solution with a pH value of below 3.0. This prevented the decomposition of PD in urine as described by Fujihara (1986) and Fujihara et al. (1991). The samples were then frozen to -20°C until analysis. Blood was sampled by collecting 10 ml from the jugular vein, centrifuged (1400 x g for 15 min) and the plasma obtained stored in deep freezer (at -40şC) until analysis. Blood sampling was conducted at 0, 1, 3, 5 and 7 h after the morning feeding on the final day of each digestion trial. This also was repeated on the 1st-7th days after inoculation, before every morning feed. However, blood sampling was conducted at 0, 2, 4, 6, and 8 h after morning feed on the 1st and 2nd days after inoculation,

Nitrogen (N) in feed, faeces and urine was analyzed using the Kjeldahl method, while the contents of crude fat, crude fibre and crude ash in the feed and faeces were determined by AOAC method (AOAC, 1960). The PD in urine and plasma was analyzed by the methods of Young and Conway (1942) and Fujihara et al. (1987).

Test for significance of difference between the two groups (faunated and defaunated) was done using the t-test.

RESULTS AND DISCUSSION

Nutrient digestibility and N balance

Table 2 shows apparent digestibility and nitrogen balance in lambs fed hay and concentrate diet. The digestibilities of dry matter (DM) and organic matter (OM) were almost the same in both defaunated and faunated lambs. The digestibilities of crude protein (CP) and crude fibre tended to be slightly lower in defaunated than in faunated lambs. The fact that CP digestibility tended to increase in faunated lambs would be probably due to the effect of proteolytic activities of protozoa, and also due to an ability of protozoa to engulf feed particles in the rumen (Coleman, 1983). The decrease in crude fibre digestibility in defaunated lambs might be due to the decrease in ruminal pH values and also in ruminal ammonia concentrations caused by defaunation.

N balance data in Table 2 shows that faecal N excretion tended to slight increase in the defaunated lambs, but not significantly ($P>0.05$). Similar trend was also reported by Itabashi et al. (1984) using the defaunated goats.

TABLE 2

Apparent digestibility of feed and nitrogen balance of lambs in defaunated and faunated period

Indices	Defaunated		Faunated	
	mean	s.e.	mean	s.e.
Digestibility, %				
dry matter	62.50[1]	2.05	62.18	0.36
organic matter	64.10	1.88	64.41	0.37
crude protein	71.27	0.99	73.88	0.38
ether extract	63.32	2.00	60.87	2.27
crude fibre	56.29	3.41	60.20	0.70
N balance, g/d/$BW^{0.75}$				
intake	1.52	0.03	1.46	0.03
faeces	0.44	0.02	0.38	0.01
urine	0.50[a]	0.02	0.57[b]	0.02
absorbed	1.08	0.01	1.08	0.02
retained	0.58[a]	0.02	0.51[b]	0.01
absorbed/intake, %	71.27	0.99	73.88	0.38
retained/intake, %	38.26	1.67	35.22	1.13
retained/absorbed, %	53.65	1.79	47.68	1.48

[1] mean of 3 lambs

[a,b] values In the same column with different superscripts differ significantly ($P<0.05$)

The urinary N excretion was markedly low ($P<0.05$) in defaunated as compared to that in faunated lambs. Consequently, retained N was higher ($P<0.05$) in the former than in the latter. These findings clearly show that the utilization of absorbed N was more efficiently done in defaunated than that in faunated animals. Matsumoto et al. (1991) also reported urinary N excretion was smaller in defaunated goats than in faunated one when they were fed on mixed hay (800 g/d/head) and concentrates (500 g/d/head) including 1% of urea.

Effect of protozoa on urinary N excretion

Figure 2 shows the daily urinary N excretion during the defaunation and faunation periods in g per kg metabolic body weight. Urinary N excretion decreased markedly during the 10 to 14 days (1.26-0.58 g/d/$BW^{0.75}$) after the defaunated lambs were fed on milk replacer (MR) and reached peak level on the 16 day (1.34 g/d/$BW^{0.75}$). After that there was a gradual decrease in daily urinary N excretion until inoculation (on 34 day) and gradually increased up to the 3 to 6 days reaching almost constant level at the end of the digestion trial. Results in the present study are in agreement with that of Matsumoto et al. (1991), and are opposite with that of Fujihara et al. (2002) using goats. Therefore from the above evidence, there is

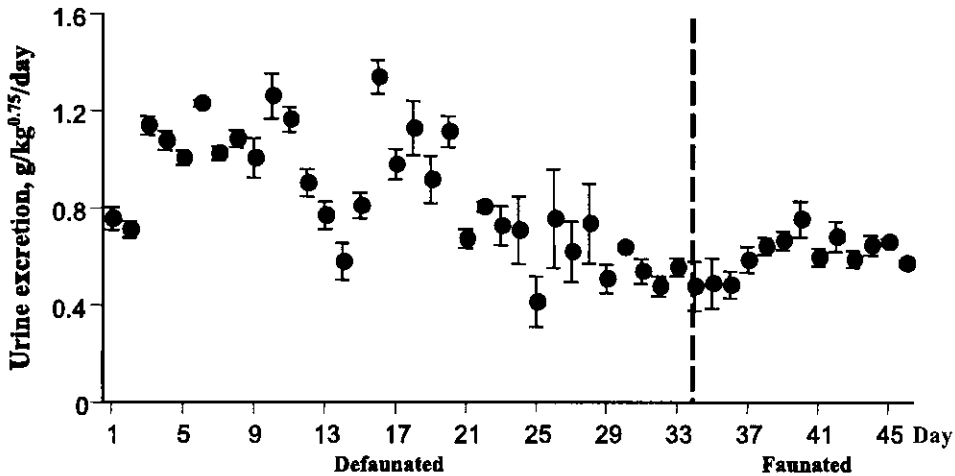

Figure 2. Daily urinary nitrogen excretion during the experimental period

no relationship between protozoa population in the rumen and urinary N excretion when there is a lapse in time after inoculation.

Effect of protozoa on plasma allantoin concentration

Mean values of plasma allantoin concentration were higher ($P<0.05$) in defaunated than faunated lambs (Table 3). This clearly indicates that the amount of nucleic acid metabolized to be higher in defaunated than in faunated lambs. In our previous experiment using goats, contrarily, there was no clear difference in defaunated and faunated periods (Fujihara et al., 2002).

TABLE 3

Plasma allantoin levels in lambs in defaunated and faunated periods

Lambs	Allantoin, μmol/l
Defaunated	$41.20^{a} \pm 1.02^{1}$
Faunated	$25.50^{b} \pm 1.04$

[1] mean ± S. E. of 3 lambs

[a, b] values In the same column with different superscripts differ significantly ($P<0.01$)

Figure 3 shows the daily changes in plasma allantoin levels with time after inoculation, and there were no clear trend as same as the result of our previous experiment, in which there were also no increases or decreases in plasma allantoin level

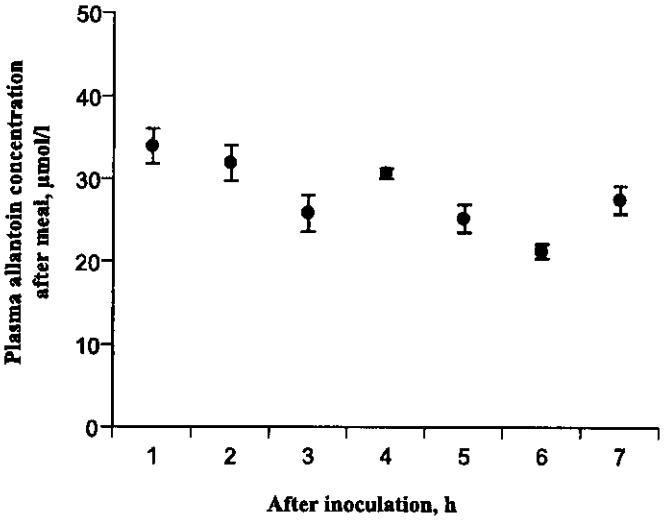

Figure 3. Daily changes in plasma AN levels with a decrease in plasma AN with time after inoculation

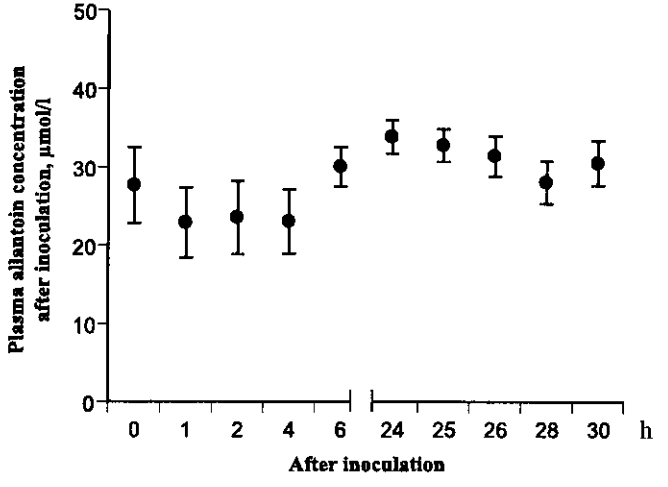

Figure 4. Changes in plasma AN levels during 30 h after inoculation

with time after inoculation in goats (Fujihara et al., 2002). As shown in Figure 4, the changes in plasma allantoin levels for the three lambs during 30 h after inoculation was low at one hour reaching peak levels at 6 h after inoculation and gradually decreasing thereafter.

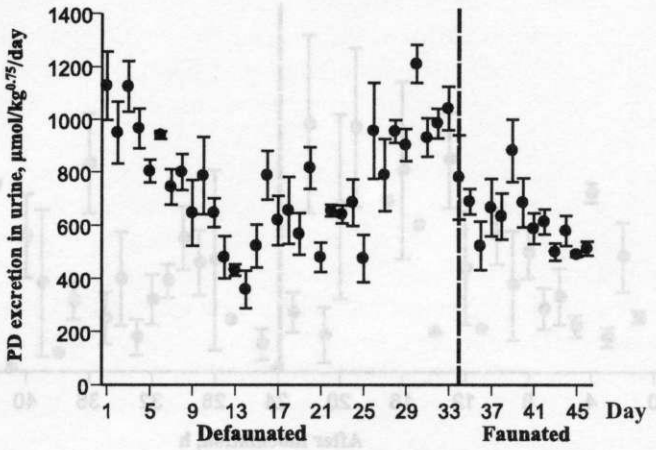

Figure 5. Changes in urinary PD excretion throughout the experimental period

The above findings show that an effect of protozoa on plasma allantoin level is not clear, however, it should be that plasma allantoin level is closely related to metabolism of nucleic acid in the rumen and/or lower gut caused by the changes of microbial population in the rumen as protozoa feed by engulfing bacteria (Koenig et al., 2000).

Urinary PD excretion through entire experimental period

Figure 5 shows the changes in urinary PD excretion throughout the experimental period. There was a gradual decrease in urinary PD excretion to the 14 day after which it increased until the 30 day after initiation of the experiment. These findings show that the changes in urinary PD excretion rate were influenced by the feeding regimes used. Lambs were initially fed on MR followed by feeding on the mixed diet. The metabolism of nucleic acids thus differed depending on the concomitant changes in microbial population in the rumen due to diet influence. Evidence from literature using lambs nourished by intragastric nutrition (Fujihara, 1986; Chen et al., 1997), show the existence of a direct relationship between nucleic acid supply and urinary PD excretion.

Changes in urinary PD excretion after inoculation

Figure 6 shows hourly PD excretion into urine during 2 days after inoculation. PD excretion increased up to the 19-22 h, and then decreased gradually until 48 h. This is because there was "a leading period" until 19-22 h after inocu-

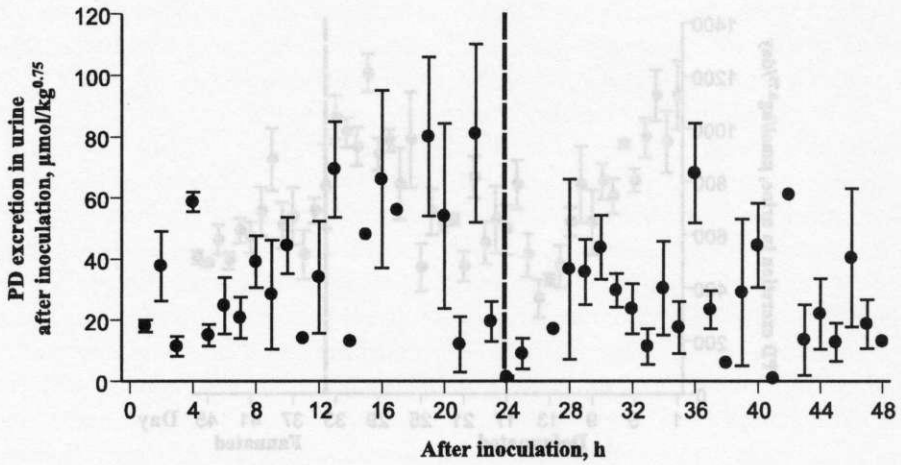

Figure 6. Changes in hourly PD excretion into urine during 2 days after inoculation

lation after which there was a remarkable proliferation of protozoa. The mean value of urinary PD excretion up to 22 h after inoculation was 596, 696 and 730 μmol/$BW^{0.75}$/d and the corresponding values for the 23-48 h was 341, 622 and 646 μmol/$BW^{0.75}$/d for the three lambs, respectively. Thus urinary PD excretion decreased markedly on 2 day compared to that in first day after inoculation.

On the changes in daily urinary PD excretion during 12 days after inoculation, there was quite big variation in urinary PD excretion up to the 6 day, however the changes in urinary PD excretion was relatively small between the 7 and 12 day, that might have been reflected a stable condition as a whole in the rumen microbial population (see Figure 5).

Urinary PD excretion in defaunated and faunated period

Table 4 shows the mean values of urinary PD in the defaunated and faunated lambs. Urinary allantoin and total PD excretion were higher ($P<0.05$) in the defaunated than in the faunated animals, whereas the excretion of uric acid into urine was almost the same in both periods. Similar results were also reported by Fujihara et al. (2002) using goats. These findings clearly show that protozoa have a negative effect on urinary PD excretion due to reduced nucleic acid synthesis in the rumen. Protozoa engulf bacteria in the faunated animals resulting in low levels of PD excretion, especially that of allantoin. There is no effect on the excretion of uric acid both in faunated and defaunated lambs.

TABLE 4

Urinary PD excretion of lambs in defaunated and faunated periods

Lambs	Allantoin	Uric acid[2]	Total PD
		μmol/d/kgBW$^{0.75}$	
Defaunated	726.5[a] ± 48.5[1]	263.9 ± 15.6	989.5[a] ± 52.2
Faunated	334.6[b] ± 25.1	204.7 ± 34.4	539.3[b] ± 19.3

[1] mean ± S.E. of 3 lambs

[2] uric acid, xanthine plus hypoxanthine

[a, b] values In the same column with different superscripts differ significantly ($P<0.05$)

CONCLUSIONS

From the results obtained in the present study, it can be concluded that protozoa have an effect on nitrogen utilization, in particular purine metabolism in lambs. To obtain detailed results on the proliferation of protozoa and their effect on purine metabolism after inoculation, frequent checks on the number of rumen protozoa and urinary PD level is necessary within a few days after inoculation.

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STRESZCZENIE

Wpływ pierwotniaków żwacza na poziom alantoiny w plazmie krwi oraz wydalanie pochodnych purynowych w moczu owiec

Celem doświadczenia było zbadanie wpływu pierwotniaków żwacza na wykorzystanie paszy, bilans azotu oraz wydalanie w moczu pochodnych purynowych (PD) przez faunowane i defaunowane jagnięta otrzymujące dawki złożone z siana i paszy treściwej.

Strawność suchej masy (s.m.) i substancji organicznej (OM) nie różniła się między jagniętami z lub bez pierwotniaków, natomiast stwierdzono tendencję obniżenia strawności białka ogólnego i włókna u defaunowanych owiec, lecz nie potwierdzonej statystycznie ($P>0,05$). W wyniku defaunacji obniżył się bilans azotu (N) oraz wydalanie N w moczu, a w konsekwencji ilość zatrzymanego N u defaunowanych zwierząt była większa ($P<0,05$) niż u faunowanych. Średnie stężenie alantoiny w plazmie krwi było większe ($P<0,05$) u defaunowanych niż faunowanych owiec, 41,2 vs 25,5 µmol/l. Suma wydalonych PD oraz alantoiny była większa ($P<0,05$) u defaunowanych jagniąt.

Na podstawie otrzymanych wyników można stwierdzić, że pierwotniaki żwacza odgrywają ważną rolę w wykorzystaniu N przez jagnięta, wpływają na poziom alantoiny w plazmie krwi oraz wydalanie PD w moczu.