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Change in body condition of Gobra zebu cattle under different level of feeding. Relationship with body lipids and energy*

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ABSTRACT

Thirty six zebu steers (35 Gobra and 1 Maure), 2.5 years-old on average, were equally alloted in 3 groups fed different crop by-product based diets. For diets 1 and 2, molassed rice straw and molassed millet straw were offered *ad libitum* as a basal diet while the other ingredients were mixed and given (2.5 kg/animal/d) as supplement 1 and 2, respectively. In diet 3, all ingredients were mixed and given as a compound feed. The energy content was higher in diet 3 (0.83 FU/kg DM) than in diet 1 and 2 which were isoenergetic, 0.65 and 0.63 FU/kg DM, respectively. Animals were group-fed twice daily (at 08.00 and 16.00 h) during 3.5 months. Diets offered and refusals were daily measured. Cattle were monthly weighed, their body condition was scored using 2 scales of 6 (BCS_{0-5}) and 9 (BCS_{1-9}) points, and body lipids and energy were estimated. The level of intake of steers was 101, 94.3, and 148.3 g DM/d/$BW^{0.75}$ for the 1rst, 2nd and 3rd groups, respectively. Groups 1 and 2 had average daily gain similar (ADG) (446.7 and 425.5 g BW/animal, respectively) and gain in BCS_{0-5} (+0.8 points) and in BCS_{1-9} (+1.9 points). A spectacular development of the hump was observed in cattle of the 3rd group which was submitted to the richest diet. They also gained more ($P<0.01$) weight (AOG : 1012 g BW/animal), BCS_{0-5} (+1.9 points) and BCS_{1-9} (+3.5 points) than did the others groups. Increased rate of BW gain resulted in an acceleration of body lipids deposits (22.8 kg in the 3rd group vs 11.5 kg in the 1rst and 11.6 kg in the 2nd group) and in gain in body net energy (936.6 MJ in the 3rd group vs 473.9 MJ in the 1rst and 490.3 MJ in the 2nd groups). Each kg of gain in BW contains on average 264.7 g of lipids and 11.4 MJ of net energy.

KEY WORDS: zebu, feeding level, body weight, condition score, lipids, energy

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INTRODUCTION

Earlier studies on zebu Gobra, a lyre-horned zebu raised primarily by Fulani herds have characterized their growth traits (Sow et al., 1988; Diop and Van Vleck, 1998) and their nutritional responses under different environmental conditions like on pasture (Guerin et al., 1988) and in relation to different types of diets using locally available crops and agroindustrial by-products (Denis and Valenza, 1971; Valenza et al., 1971; Calvet et al., 1973; Steyaert et al., 1989). In most of these studies, attention has not been focused on the cattle body condition although it is, in fact, empirically evaluated by traditional farmers. However, the body condition scoring technique is taking increasively importance because of its merit in field situations where extensioners lack facilities for more objective measures of response (e.g., weight changes) to management or feeding practices. Numerous scoring systems have been formulated to quantify the body condition of cattle. Lowman et al. (1976) used a 6 (0-5) points system while Van Niekerk and Louw (1982) suggested a 5 points scale with half points increments. In Africa, Pullan (1978) used a 6 points (0-5) scoring system for Fulani cattle in Nigeria, and Nicholson and Butterworth (1986) recommanded a 9 points (1-9) scale for zebu. The aim of the present study was to examine change in body condition score of Gobra zebu using 2 scoring systems and to quantify them in terms of body lipids and energy.

MATERIAL AND METHODS

Thirty six zebu steers (35 Gobra and 1 Maure), 2.5 years-old (± 0.5) and weighing 165.8 kg (±19.1) on average, were purchased in the sylvopastoral zone in northern Senegal in the dry season. They were equally allotted in 3 groups according to age, liveweight and body condition scored in the systems of 6 and 9 points. Cattle were housed in 3 pens with free-stalls, and were group-fed during 3.5 months different diets based on locally available crops and agroindustrial by-products (Table 1). For diets 1 and 2, molassed rice straw and molassed millet straw (17:63 w/w) were offered *ad libitum* as a basal diet while the other ingredients were mixed and given (2.5 kg/animal/d) as supplement 1 and 2, respectively. In diet 3, all ingredients were mixed and given *ad libitum* as a compound feed. Animals received their diet in 2 equal portions at 08.00 and 16.00 h. Water was available *ad libitum*. Diets offered and refusals were daily measured. Feed and diets chemical composition, digestibility, protein and energy value were given in Fall et al. (1998). At the beginning of the trial, cattle were weighed on 3 consecutive days in the morning before access to feed and water. The average of the three weights was used as the

TABLE 1

Diet composition (% DM) and nutritive value

	Diets		
	1	2	3
Rice straw	56	-	-
Rice bran	18	-	-
Peanut cake	13	11.5	5
Peanut hulls	-	-	18
Cotton seeds			25
Millet straw	-	58	-
Molasses	11.5	11.5	20
Millet bran	-	17.5	-
Maize grain	-	-	9.5
Senal[1]	-	-	20
Mineral mixture	1.5	1.5	2.5
Ca	5	4.5	5
P	3	3	3
Feed unit/kg DM[2]	0.64	0.6	0.8
Digestible crude protein, g/kg DM	72	67	90

[1]senal: manufactured concentrate wheat bran (90% DM) + molasses (10% DM)
[2]feed unit: Net Energy (INRA, 1978)

starting weight. Cattle were then monthly weighed on 2 days successively until the end of the experiment. Their body condition was scored at the same time by 3 experts using the scale of 9 points (Nicholson and Butterworth, 1986) and the one of 6 points described in Table 2. Photograps of each cow (side and rear views) was taken on the day of scoring. The trial was prolonged for one additional month for 12 steers, 4 in each group, the others being used for another experimental purpose. At the end of the trial, cattle were fasted for 24 h and were then slaughtered the following morning. The carcass and non-carcass parts (head, feet, blood and viscera) were weighed separately. Relationships between steers BW and condition scores were established by linear regression. Body lipids and energy were estimated using following equations (Cissé et al., 1999):

Body lipids (kg) $= 13.2\ BCS_{0-5} - 14.6$, $r^2 = 0.70$, rsd = 8.4
$= 6.34\ BCS_{1-9} - 5.24$, $r^2 = 0.61$, rsd = -5.2
Body energy (MJ) $= 541.645\ BCS_{0-5} + 106.45$, $r^2 = 0.6$, rsd = 426.8
$= 266.525\ BCS_{1-9} + 464.4$, $r^2 = 0.60$, rsd = 427.74,

and data were analyzed by standard ANOVA procedures.

TABLE 2

Scale of scoring body condition of Gobra zebu

Score	Condition	Features
0	Very thin	Animal extremely emaciated and condemned at ante-mortem examination. Ribs are highly visible and sublumbar fossa very deep. Transverse and spinal processes are prominent and dorsal spines sharp. Croupe is highly prominent and bony.
1	Thin	Animal is thin. Ribs are visible and sublumbar fossa deep. Transverse processes are prominent and easily felt at palpation Croupe is very prominent.
2	Little thin	Animal is little thin. Ribs are visible and sublumbar fossa is less deep than in thin animals. Transverse processes are easily felt at palpation Croupe is little prominent.
3	Little fat	Animal is little fat and ribs are little covered and not easily seen The sublumbar fossa is lightly deep, only before meal. The hump is low developped and orsal spines can be felt with firm pressure. Low concavity of the muscular mass between the hooks *(tuber coxae)* and pins *(tuber ischii)*.
4	Fat	Animal is fat and well covered, and ribs are not visible. The sublumbar fossa is not deep and the hump is developped. Dorsal spines are rounded and transverse processes are not visible nor palpable. Convexity of the muscular mass between the hooks and pins. Croupe is well covered.
5	Very fat	Animal is very fat and smooth Heavy fat deposits on tail head, on hump, and cod. Ribs, transverse and spinal processes can not be felt even with great palpation. Croupe is very rounded.

RESULTS

Feed intake and growth performance

During adaptation to each diet, the level of dry matter intake did not significantly differ among groups and cattle lost body weight (Table 3). Throughout the trial, cattle offered the straw-based diets ate about 46.8% less food than for those given the compound feed. Cattle receiving diet 3 grew faster and their final body weight was heavier ($P<0.01$) than those of the straw-based diets which had similar ADG and finishing weights. During the period of feeding after adaptation, the additional body mass gain was 25.4, 24.3 and 58.7% for cattle eating diet 1, 2 and 3,

respectively. In terms of feed conversion, cattle fed the compound feed were better converiers than the others. A spectacular development of their hump was also observed (Photograph 1).

Change in body condition and composition

At the end of the trial, cattle of the 3rd group were in better ($P<0.01$) condition than the others as shown in Photograph 1 and Table 3. When the 6 points scoring system (BCS_{0-5}) is used, gain in BCS_{0-5} averaged 0.9 (±0.4), 0.8 (±0.3), and 1.8 (±0.6)

TABLE 3

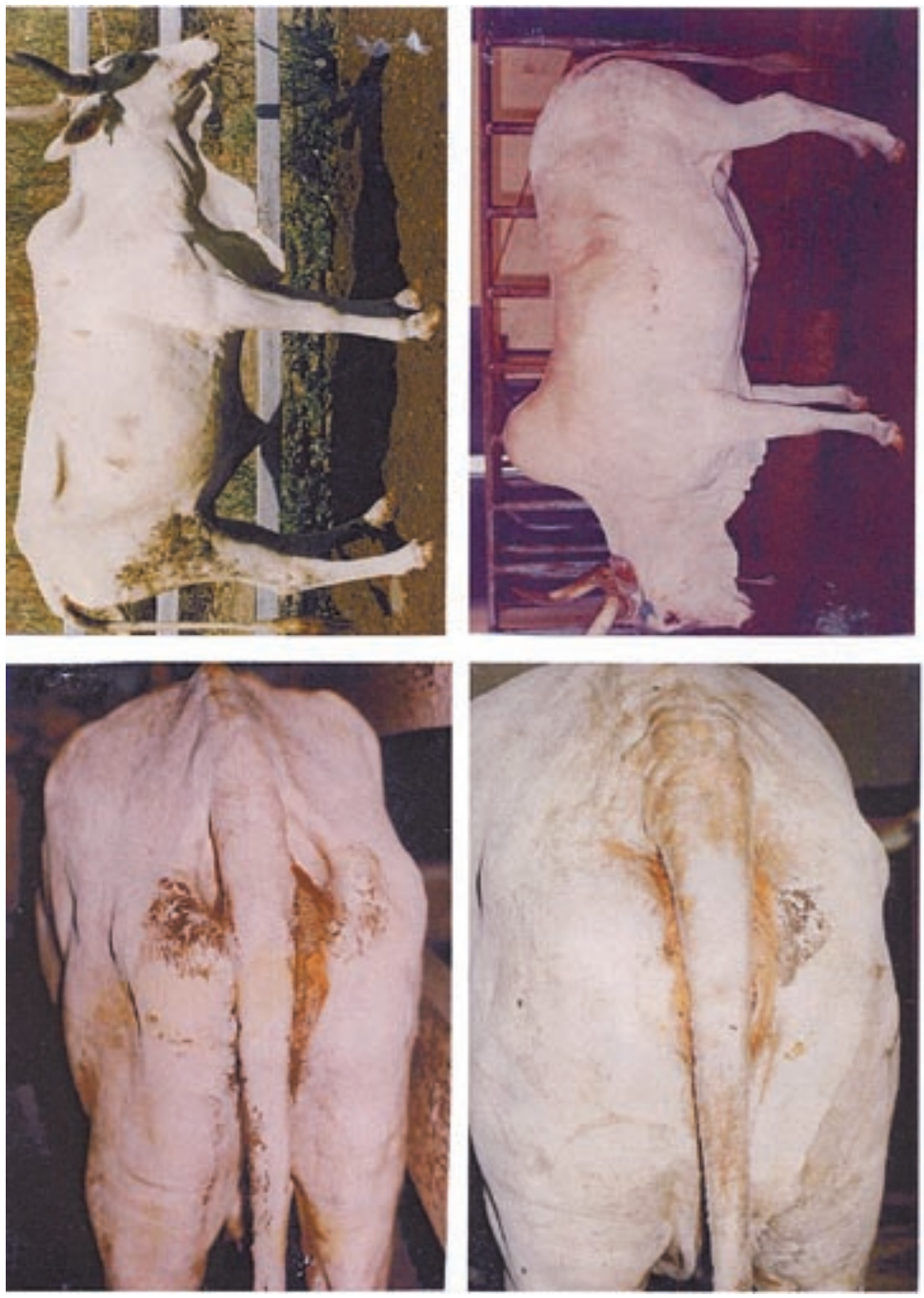
Dry matter intake (DMI) and change in body weight (BW), condition score (BCS) and composition[1,2]

Indices	Groups			SE
	1	2	3	
Initial BW, kg	163.7[a]	163.8[a]	164.2[a]	
Feeding days	14	14	14	
DMI, kg/d/animal	3.02[a]	2.92[a]	2.91[a]	0.9
BW after 14 days, kg	157.8[a]	157.6[a]	155.3[a]	17.6
Feeding days	90	90	90	
DMI, g/d/$BW^{0.75}$	101[a]	94.3[a]	148.3[b]	8.2
DMI, kg/d DM/100 kg BW	2.78[a]	2.59[a]	3.92[b]	0.6
Concentrate intake, g DM/d/animal	2.3[a]	2.2[a]		
Forage intake, g DM/d/animal	3.5[a]	3.1[a]	9.6[b]	0.7
Final BW, kg	198.0[a]	195.9[a]	246.5[b]	34.1
Average daily gain in BW, g/animal	446.6[a]	425.6[a]	1013.3[b]	201.4
Feed conversion, kg DMI/kg BW gain	10.9[a]	10.7[a]	8.0[b]	
Initial BCS_{0-5}	2.4[a]	2.5[a]	2.4[a]	
Final BCS_{0-5}	3.3[a]	3.3[a]	4.2[b]	0.2
Gain in body lipids, kg[3]	11.5[a]	11.6[a]	22.8[b]	5.1
Gain in body energy (NE), MJ[3]	473.9[a]	490.3[a]	936.6[b]	138.4
Lipids, g/kg BW gain[3]	287.2[a]	303.7[a]	250.4[a]	380.6
Energy (NE), MJ/kg BW gain[3]	11.8[a]	13.8[a]	10.3[a]	9.7
Initial BCS_{1-9}	4.1[a]	4.2[a]	4.0[a]	
Final BCS_{1-9}	5.8[a]	6.0[a]	7.5[b]	0.3
Gain in body lipids, kg[4]	11.1[a]	11.6[a]	22.2[b]	1.8
Gain in body energy (NE), MJ	466.4[a]	488.6[a]	932.8[b]	88.3
Lipids, g/kg BW gain[4]	275.8[a]	303.6[a]	243.5[a]	179.7
Energy (NE), MJ/kg BW gain[4]	11.6[a]	12.7[a]	10.2	6.5

[1] means within each row not having common superscript (a,b,c) are different at $P<0.01$

[2] body condition is scored in the 6 (BCS_{0-5}) or 9 (BCS_{1-9}) points scale

estimates in the 6 points[3] or 9 points[4] scoring scale (Cissé et al., 1999)



Photograph 1. A view of the hump and croupe of cattle from the group receiving the richest diet at the beginning and the end of the trial

points in the three respective groups. BCS_{0-5} ranged from 1 to 3.5 in the 1rst and 2nd groups, and from 1 to 5 in the 3rd group. Pooled observations from all animals and over the whole period [(36 cattle x 4 scores) + (12 cattle x 1 score)] provided a relationship between body weight (BW) and body condition score as follows : $BW = 49.8\ BCS_{0-5} + 73$, r=0.78, n=156. In the 9 points scoring scale (BCS_{1-9}), gain in BCS_{1-9} averaged 1.8 points (±0.6) in the 1rst group, 1.8 points (±0.5) in the 2nd group, and 3.5 points (±0.9) in the 3rd group. Body weight is related to condition score according to this linear equation:

$$BW = 23.98\ BCS_{1-9} + 58.7,\ r = 0.78,\ n = 156$$

The weight of digestive contents of the 12 slaughtered steers was higher (15% BW) in animals fed straw based diets, when compared to those of the 3rd group (7% BW) (Table 4). The weight of the head, heart, lungs and bone did not significantly vary among groups, in contrast for the liver, spleen, kidney, digestive tracts and visceral fat that were heavier ($P<0.05$) for cattle fed the high nutritive diet.

TABLE 4

Body weight and components at slaughter[1,2]

Diets	Groups			SE
	1	2	3	
BCS_{0-5}	3.4[a]	3.6[a]	4.3[b]	0.2
BCS_{1-9}	6.0[a]	6.3[a]	7.7[b]	0.3
Finishing BW, kg	227[a]	212[a]	270[b]	28.6
BW after fasting, kg	212[a]	199[a]	261[b]	20.3
Empty BW, kg	180[a]	173[a]	243[b]	33.5
Warm carcass weight, kg	105[a]	102[a]	150[b]	19
Dressing percentage, %	49.5[a]	50.6[a]	57.7[b]	1.6
Fresh bone, kg	18.1[a]	15.9[a]	20.0[a]	2.1
Non carcass parts, kg				
head, kg	16.6[a]	15.7[a]	17.0[a]	1.9
feet, kg	5.0[a]	4.1[a]	6.1[a]	0.5
spleen, kg	0.6[a]	0.6[a]	0.8[b]	0.1
heart, kg	1.0[a]	0.8[a]	1.2[a]	0.2
liver, kg	3.1[a]	2.7[a]	4.0[b]	0.5
lungs and trachea, kg	1.7[a]	1.5[a]	1.6[a]	0.2
kidney, kg	1.2[a]	1.0[a]	3.2[b]	0.3
viscera[1] fat depots, kg	1.9[a]	1.5[a]	7.5[b]	1.5
digestive contents, kg	33[a]	26[a]	18[b]	3.4
empty digestive tract, kg	13[a]	12[a]	15[b]	2
Gain in body lipids, kg[3]	11.9[a]	13.2[a]	23.7[b]	2.3
Gain in body lipids, kg[4]	120[a]	13.3[a]	23.4[b]	1.5

[1,2,3,4] see Table 3 for the legend

The amount of lipids retained within the body during the trial approximated 11.5 kg on average in both the 1rst and 2nd groups, against 22.8 kg in the 3rd group, corresponding to an increase of 53.7, 54.8, and 108.8% in the three groups, respectively. Body energy (NE) increased by 473.9 MJ (+30%) in group 1, 490.3 MJ (+31.0%) in group 2 and 936.6 MJ (+60.5%) in group 3 (Table 3). Concerning the change in body lipids and energy, similarities were found on estimated values, irrespective of scoring scale, and no significant difference between groups was detected in the content of lipids and net energy per kg of body weight gain. On pooled data, each kilogram of gain in BW contained on average 264.7 g of lipids and 11.4 MJ of net energy.

DISCUSSION

During the period of adaptation, short term change in BW that occured are mainly due to gut fill variations (Kabré and Petit, 1994). When intake is stabilized, these variations more reflect change in organ and tissue masses (Chilliard et al., 1995). As expected, more feed was consumed by cattle offered the richest diet, which resulted in a heavier final weight. Body condition scores and BW increased at a similar rate in the 1rst and 2nd groups, with regard to the same feeding level and energy content in their diets. Their lower growth performance seems to arise both from the concentrate given in fixed amount and the limited intake of fibrous feeds like cereal straws. The ADG obtained with the compound feed approximated the maximum value reported for Gobra zebu in intensive fattening trials (Valenza et al., 1971). The low initial body condition recorded from cattle bought in March for this trial showed that they were underfed in the middle of the dry season (Guérin et al., 1988; Cissé et al., 1994). The quantity and quality of pasture, their main source of feed, vary considerably during the year. Short overfeeding periods due to abundance of herbage which ideally should enable cattle to improve both BW, milk yield (Cissé et al., 1996) and BCS are followed by 9 months of feed scarcity. One point gain in $BCS_{1\text{-}9}$ corresponded to a gain of 24 kg in BW, in agreement with findings of Nicholson and Butterworth (1986) in Boran zebu cattle. Concerning the equivalent (BW) of one point change in $BCS_{0\text{-}5}$, similarities were observed with previous results in Gobra zebu cows (Cisse et al., 1999). In contrast for estimates of body lipids, BCS may not allow a good accuracy of the prediction of body proteins deposits whose physiological priority is in favour of body structural and functional roles, like this the body lipids are extensively used (40-80%) in underfed animals, while the range of protein utilization is limited (15-20%) (Chilliard et al., 1995).

Although Nicholson and Sayers (1987) reported a higher sensibility of the 9 points scoring system, the highest precision of body lipids estimates was obtained with the 6 points scoring system (Cissé et al., 1999). In our study, the lipid deposition

rates varied in a higher proportion than did BW, and the lipid content per kg of BW gain approximated those (310 g of lipids/kg BW gain) reported by Robelin (1979) on Charolais x Salers heifers, with, nevertheless, differences within genotypes related to the sex of animals (160 and 195 g of lipids/kg BW gain in Charolais x Salers and Holstein Pie-Noires steers, respectively). Body lipids variations of steers used in our trial ranged within the average extreme values reported in zebu cattle by Buvanendran et al. (1982), Ehoche et al. (1992) and Schlecht (1995). However it is important to note that the equations we used to estimate body lipids were established in Gobra cows. Some biases may be occured in this estimate due to sex (Mukhoty and Berg, 1971) and age (Jones et al., 1985) of animals. Increased rate of BW gain due to elevating dietary energy intake above needs for maintenance and lean growth resulted in an acceleration in fat deposition (Rompala et al., 1985), but steers were still growing in our trial and their lipid deposits could be probably lower than in old animals.

From a practical point of view, the 6 points scoring scale is easier to use for farmers and gives more precise body lipids prediction. However, more research is needed to determine the optimum slaughter score for marketing time appropriate and carcass fat level and desirability for zebu Gobra. In addition no information exists about change in body reserves of zebu cattle on natural pastures in Sahel. In this area, the extent of body reserves mobilization for survivals requirements and BCS may be useful in providing information on which management and feeding decisions can be made.

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STRESZCZENIE

Zmiany w kondycji zebu Gobra w zależności od poziomu żywienia. Związek z zawartością tłuszczu i energii

Trzydzieści sześć wolców zebu, 2,5 letnich, podzielono na 3 grupy, i żywiono przez 3,5 miesiąca różnymi dawkami, których głównym składnikiem były produkty uboczne. W dawce 1 podstawową paszą była melasowana słoma ryżowa, w dawce 2 - melasowana słoma z prosa, podawane do woli; pozostałe składniki były mieszane i podawane jako uzupełnienie dawek 1 i 2 w ilości 2,5 kg/zwierzę/d. Dawka 3 podawana była w formie pełnoporcjowej mieszanki, w której paszą objętościową były łuski orzecha ziemnego. Wartość energetyczna dawki 3 była większa (0,83 FU/kg s.m.) niż dawek 1 i 2 (0,65 i 0,63 FU/kg s.m., odpowiednio). Zwierzęta żywiono grupowo, podając paszę 2 razy dziennie, o 8.00 i 16.00. Codziennie kontrolowano ilość podawanej paszy i niewyjady. Zwierzęta ważono co miesiąc i określano ich kondycję na podstawie dwóch skali: 6-cio (BCS_{0-5}) i 9-cio (BCS_{1-9}) punktowej; oznaczano także zawartość tłuszczu i energii w ciele.

Ilość pobieranej paszy wynosiła 101; 94,3 i 148,3 g s.m./d/ciężar ciała^{0,75} w 1, 2 i 3 grupie, odpowiednio. Średnie dzienne przyrosty w grupach 1 i 2 były podobne (446,7 i 425,5 g, odpowiednio), a przyrost wyrażony w BCS_{0-5} wynosił +0,8 punkta, w BCS_{1-9} 1,9 punkta. Wyraźny rozwój garbu u zebu grupy 3 był następstwem podawania mu najbogatszej dawki. Zwierzęta te miały też większe ($P<0,01$) dzienne przyrosty (1012 g; BCS_{0-5} +1,9 punkta, BCS_{1-9} +3,5 punkta) niż z pozostałych grup. Następstwem większych przyrostów było zwiększenie odkładania tłuszczu i energii netto w ciele zwierząt grupy 3 (22,8 kg i 936,6 MJ) w porównaniu z zebu z grup 1 i 2 (11,5 i 473,9 oraz 11,6 kg i 490,3 MJ, odpowiednio). W każdym kg przyrostu zawartość tłuszczu wynosiła średnio 264,7 g oraz 11,4 MJ energii netto.

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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.

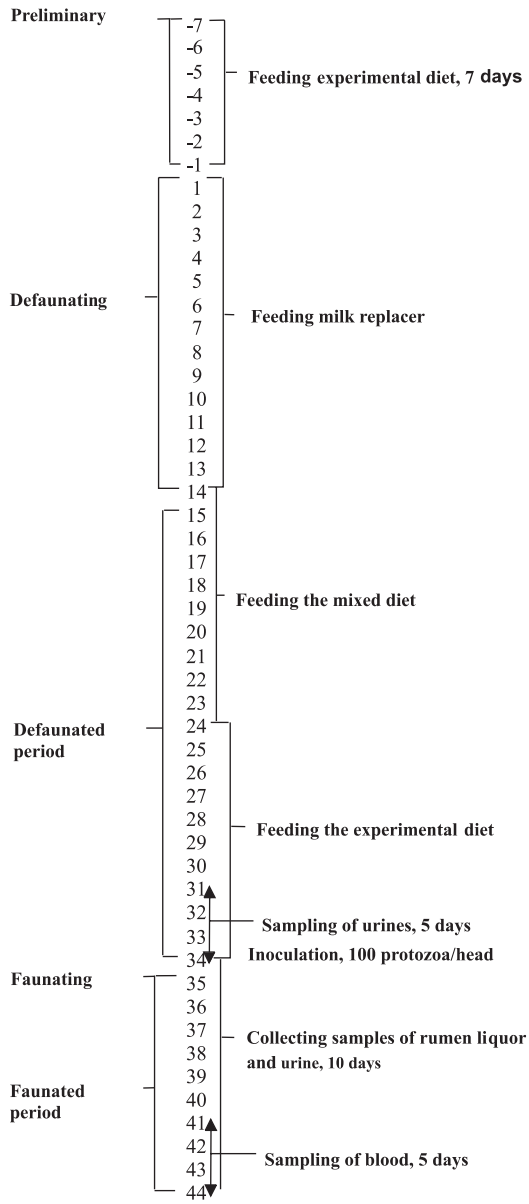

Figure 1. Experimental schedule

* 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

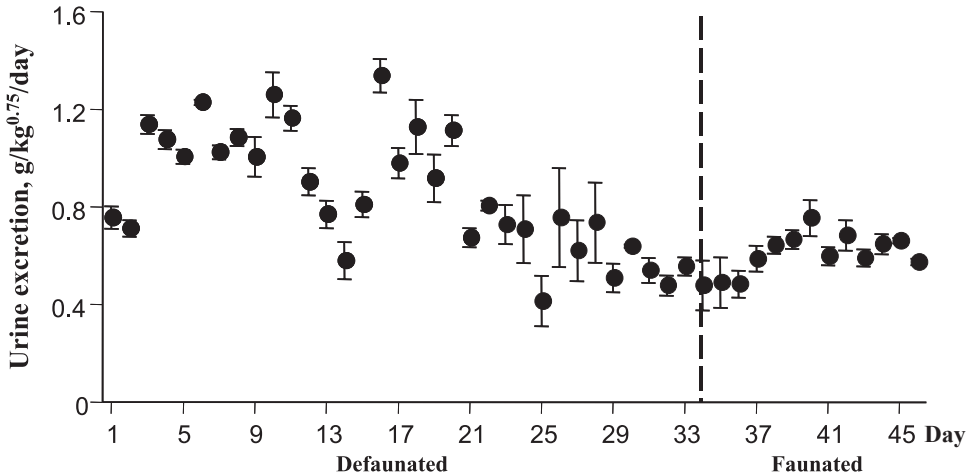

Figure 2. Daily urinary nitrogen excretion during the experimental period

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

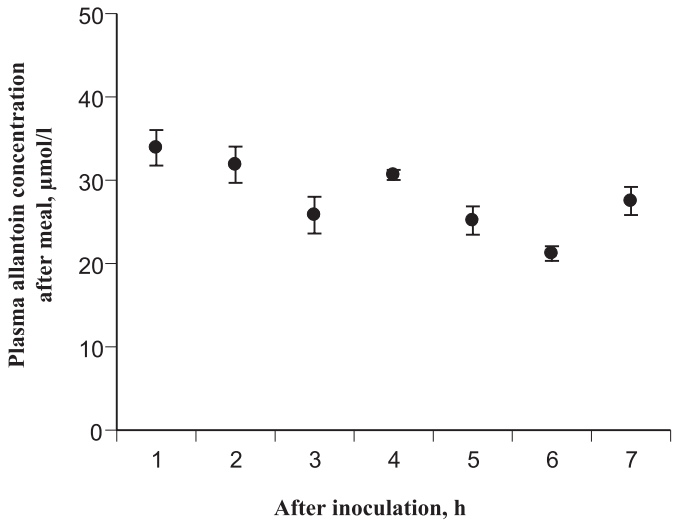

Figure 3. Daily changes in plasma AN levels with a decrease in plasma AN with time after inoculation

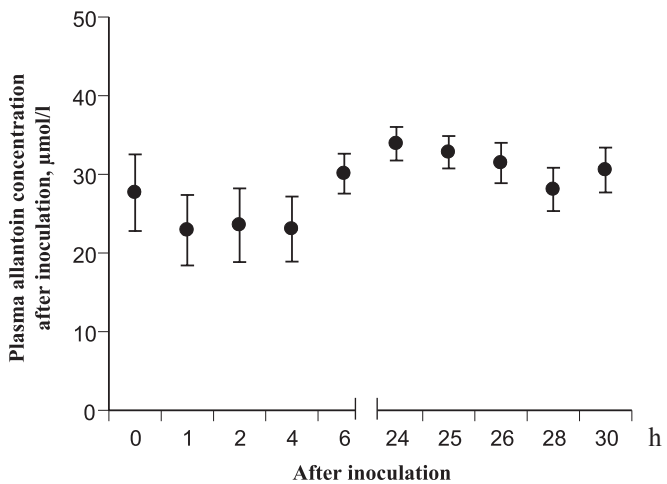

Figure 4. Changes in plasma AN levels during 30 h after inoculation

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.

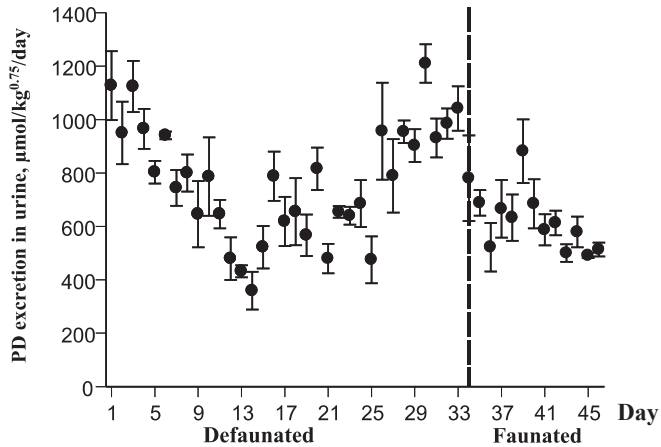

Figure 5. Changes in urinary PD excretion throughout the experimental period

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-

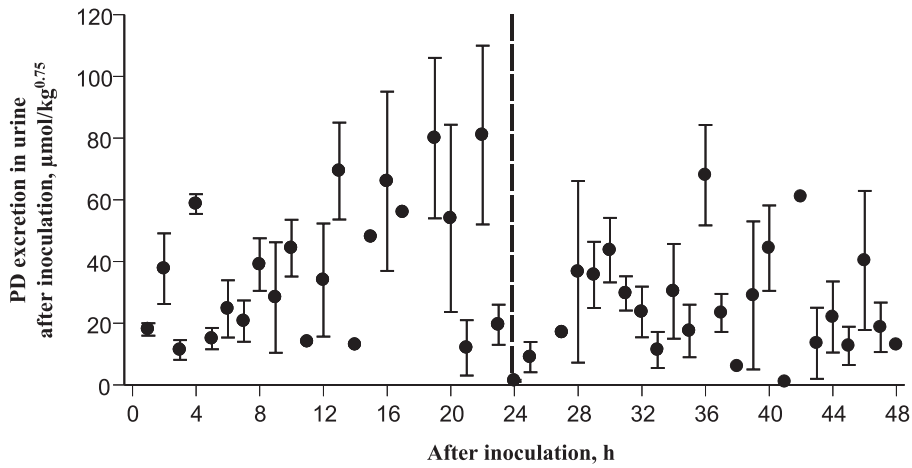

Figure 6. Changes in hourly PD excretion into urine during 2 days after inoculation

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.